



Division of Medicinal Chemistry
Scientific Abstracts
for the
253rd National Meeting and Exposition

April 2-6, 2017
San Francisco, CA

American Chemical Society
Division of Medicinal Chemistry
253rd ACS National Meeting, San Francisco, CA, April 2-6, 2017 Spring Meeting

A. Stamford, Program Chair

SUNDAY MORNING

Macrocycles & Cyclopeptides in Medicinal Chemistry

M. J. Blanco, Organizer; M. Blanco, Presiding Papers 1-5

General Orals

A. W. Stamford, Organizer; I. M. Bell, Presiding Papers 6-16

SUNDAY AFTERNOON

General Orals

A. W. Stamford, Organizer; A. W. Stamford, Presiding Papers 17-25

Medicinal Chemists' Toolbox: Factors Influencing Oral Bioavailability & Case Studies

N. A. Meanwell, Organizer; P. M. Scola, Organizer; K. Yeung, Organizer; N. A. Meanwell, Presiding; P. M. Scola, Presiding; K. Yeung, Presiding Papers 26-30

SUNDAY EVENING

General Posters

A. W. Stamford, Organizer; Papers 31-299

MONDAY MORNING

Actually it does Work: Success with Allosteric Kinase Ligands & Phosphatase Modulators

L. Lombardo, Organizer; R. Moslin, Organizer; J. B. Schwarz, Organizer; D. S. Weinstein, Organizer; R. Moslin, Presiding; J. B. Schwarz, Presiding Papers 300-305

Residence Time: Not Just Affinity for Drug Design

B. Blagg, Organizer; P. J. Tonge, Organizer; P. J. Tonge, Presiding Papers 306-310

MONDAY AFTERNOON

Kinase Inhibitors for Immuno-Inflammatory Diseases

J. Ramanjulu, Organizer; J. Ramanjulu, Presiding; L. Krim Gavrin, Presiding Papers 311-316

Misfolded Proteins in Neurodegenerative Diseases

A. M. Walji, Organizer; A. M. Walji, Presiding Papers 317-322

MONDAY EVENING

Sci-Mix

A. W. Stamford, Organizer; Papers 111-112, 117, 136, 148, 154, 177, 182, 216, 234, 242, 245, 258, 266, 277, 290, 452, 457, 462, 473

TUESDAY MORNING

MEDI Awards Symposium

A. W. Stamford, Organizer; W. B. Young, Presiding Papers 323-326

Antibiotic Drug Discovery: The Next Frontier

E. D. Brown, Organizer; C. L. Freel Meyers, Organizer; E. D. Brown, Presiding; C. L. Freel Meyers, Presiding Papers 327-332

TUESDAY AFTERNOON

Drug Discovery for ALS: Putting the Ice Bucket to Work

G. M. Dubowchik, Organizer; L. Bruijn, Organizer; G. M. Dubowchik, Presiding; L. Bruijn, Presiding Papers 333-337

General Orals

A. W. Stamford, Organizer; A. Ali, Presiding Papers 338-346

WEDNESDAY MORNING**First Time Disclosures**

J. B. Schwarz, Organizer; J. B. Schwarz, Presiding Papers 347-354

Targeting Epigenetic Writers & Erasers

J. Jin, Organizer; J. Jin, Presiding Papers 355-360

WEDNESDAY AFTERNOON**First Time Disclosures**

J. B. Schwarz, Organizer; J. B. Schwarz, Presiding Papers 361-368

General Orals

A. W. Stamford, Organizer; J. Ramanjulu, Presiding Papers 369-379

WEDNESDAY EVENING**General Posters**

A. W. Stamford, Organizer; Papers 380-509

MEDI 1

Simple ADME rules for complex molecules and life beyond the rule of 5

Scott Lokey, *slokey@ucsc.edu*. Chemistry & Biochemistry, University of California Santa Cruz, Santa Cruz, California, United States

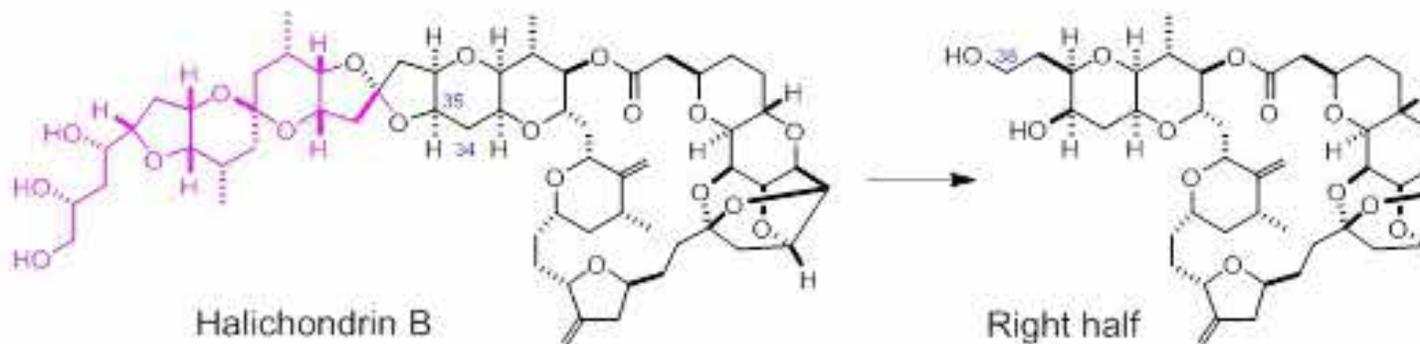
Synthetic and natural cyclic peptides provide a testing ground for studying membrane permeability in non-traditional drug scaffolds. Cyclic peptomers, which incorporate peptide and N-alkylglycine (peptoid) residues, combine the stereochemical and geometric complexity of peptides with the functional group diversity accessible to peptoids. I will present structure-property relationships in cyclic peptomer libraries synthesized by split-pool techniques permuting side chain functionality and backbone geometry, and will report on trends that relate lipophilicity (both calculated and measured) to membrane permeability as well as metabolic clearance. I will also present the synthesis of a >250,000-member cyclic peptide library using one-bead-one-compound techniques, and our experimental and computational methods for decoding individual beads selected for target binding.

MEDI 2

Discovery of oral and BBB permeable eribulin analogs by taking advantage of its macrocyclic properties

Wanjun Zheng, *wanjun_zheng@eisai.com*. Integrated Chemistry, Eisai AiM Institute, Londonderry, New Hampshire, United States

Eribulin mesylate (HalavenTM) is a fully synthetic macrocyclic analog of the right half of the marine natural product Halichondrin B. It is first-in-class, non-taxane microtubule dynamics inhibitor that is currently approved in over 40 countries (including Japan, the US and European Union) based on results of Phase 3 clinical trial for treatment of patients with late-stage metastatic breast cancer. To date, this agent is the only chemotherapeutic drug to have demonstrated an increase in overall survival in this patient population. It was also approved for the treatment of advanced liposarcoma by US FDA in January 2016. In addition, other trials evaluating non-small cell lung cancer, prostate cancer and sarcoma are under way. In light of its distinct antitubulin mechanism of action, high potency and a wide therapeutic window as well as the potential physicochemical properties provided by the unique macrocyclic structure, a discovery effort of second generation of eribulin analogs with oral and CNS-penetrant properties targeting brain cancer are undertaken. The analogs generated showed not only highly potent activity against U-251 and SF-295 human brain tumor cell lines in culture, but also showed oral bioavailability and high levels of exposure in brain and CSF. Finally, in vivo activity in multiple orthotopic brain tumor xenograft models was also observed, suggesting that these novel analogs may have utility in the treatment of brain and other CNS cancers.



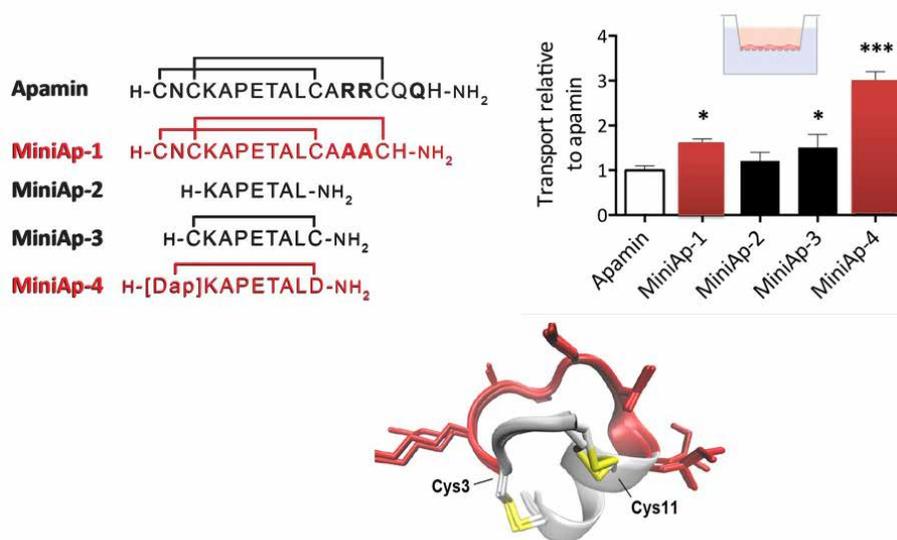
MEDI 3

Peptides as shuttles for drug delivery to the brain

Ernest Giralt^{1,2}, ernest.giralt@irbbarcelona.org. (1) Institute for Research in Biomedicine (IRB Barcelona), Barcelona Institute of Science and Technology (BIST), Baldri Reixac 10, 08028 Barcelona, Spain, Barcelona, Spain (2) Department of Organic Chemistry, University of Barcelona, Martí Franquès 1-11, 08028 Barcelona, Spain, Barcelona, Spain

Treatment of CNS disorders is severely hampered by the presence of the blood-brain barrier (BBB). These last years some peptides have emerged as 'privileged' structures to cross the BBB efficiently enough to be used as BBB-shuttles for drug delivery into the brain. Degradation by proteases is, however, an important limitation for this approach. Due to their capacity to reach the CNS without causing inflammation, venoms emerge as a potentially rich source of novel BBB-shuttles. In our laboratory, recently, we have explored the use of cyclic peptides derived from venoms as protease-resistant BBB-shuttles. Apamin is an 18-mer peptide from bee venom that accumulates in significant amounts in the brain and spinal cord. Starting from apamin, we have designed a series of simplified peptides stabilized *via* cyclization either *via* a disulphide bridge or through lactamization (see figure). Among these molecules, MiniAp-4 has proved to be the most permeable and, accordingly to preliminary studies, it is able to promote the translocation of proteins and nanoparticles both in a human-cell-based assay and *in vivo*. Furthermore, MiniAp-4 is much less toxic and immunogenic than the parent peptide apamin.

MiniAp-4 translocates BBB endothelial cells, most probably *via* receptor-mediated transcytosis and, so, is just an example of peptides able to interact with protein surfaces. In the second part of my talk I will present some other examples of the use of cyclic peptides in protein-surface recognition and, more precisely, on the design of peptides with the capacity to disrupt protein-protein interactions under the control of light.



MEDI 4

Macrocyclic peptides as potential treatments for pain and drug abuse

Jane V. Aldrich², janealdrich@ufl.edu, **Sanjeewa Senadheera**¹, **Thomas F. Murray**³, **Jay P. McLaughlin**⁴. (1) Medicinal Chemistry, University of Kansas, Lawrence, Kansas, United States (2) Medicinal Chemistry, University of Florida, Gainesville, Florida, United States (3) Pharmacology, Creighton University, Omaha, Nebraska, United States (4) Pharmacodynamics, University of Florida, Gainesville, Florida, United States

Since the discovery of the endogenous opioid peptides a variety of peptide analogs have been synthesized and characterized as opioid receptor ligands, but the development of these peptides for therapeutic applications has been limited by their metabolic lability and poor bioavailability. In contrast, the macrocyclic peptide CJ-15,208 (*cyclo*[Phe-D-Pro-Phe-Trp]) and its analogs are stable to proteases and are active after oral administration. Pharmacokinetic analysis of the kappa opioid receptor antagonist [D-Trp]CJ-15,208 demonstrated CNS penetration and high oral bioavailability (>50%). These peptides are metabolized by hepatic cytochrome P450 enzymes with a range of half lives that are affected by the configuration of amino acids in the peptides. While a number of modifications are tolerated with retention of opioid receptor affinity, the impact of structural modification on *in vivo* opioid activity is more complex, with minor structural changes resulting in different opioid activity profiles in mouse antinociception assays. Further evaluation of selected peptides for serious side effects associated with standard narcotic analgesics (tolerance, sedation, respiratory effects, rewarding properties/drug-seeking behavior) has led to the identification of analogs exhibiting improved safety profiles compared to standard narcotic analgesics. Several promising peptides have also been identified with activity in rodent models relevant to their potential use for the treatment of substance abuse. These results suggest that such

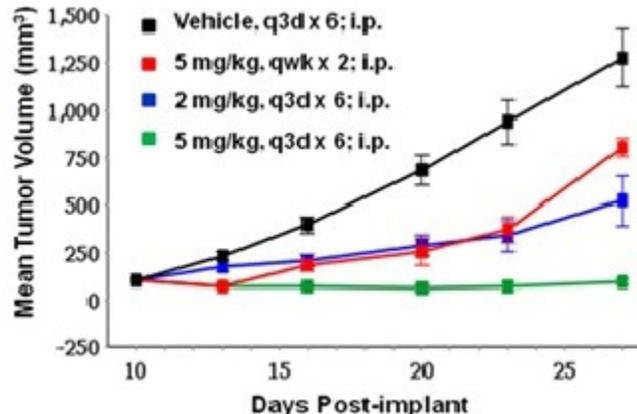
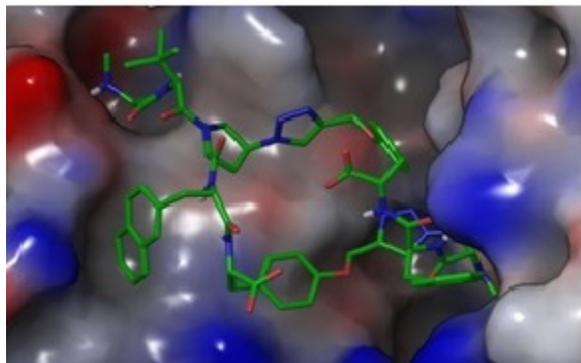
macrocyclic peptides have potential therapeutic application as analgesics and in the treatment of drug abuse.

MEDI 5

Milla molecule inhibitors of IAP proteins

Yong Zhang, *yong.zhang@bms.com*. Bristol-Myer Squibbs, Princeton, New Jersey, United States

Affinity selection screening of macrocycle libraries derived from DNA-programmed chemistry allowed for rapid identification of dual cIAP/XIAP antagonists. Further optimization of cellular potency and ADME properties of the initial lead macrocycles resulted in the discovery of an advanced compound with in vivo activities in multiple tumor xenograft models.



MEDI 6

Development of a potent and selective bromodomain chemical probe

Moses Moustakim¹, *moses.moustakim@chem.ox.ac.uk*, **Paul Brennan**², **Darren Dixon**¹. (1) University of Oxford, Oxford, United Kingdom (2) TDI NDMRB, University of Oxford, Oxford, United Kingdom

Bromodomain containing proteins are therapeutic targets for multiple disease areas such as cancer, autoimmune diseases and inflammation. Understanding the therapeutic relevance of individual bromodomain containing proteins (>45 known expressible bromodomain containing human proteins) by the generation of high affinity, selective small molecule inhibitors or 'probes' can allow for individual target validation for drug discovery. The p300/CBP associated factor (PCAF) bromodomain containing protein has been linked to a variety of disease states including cancer, HIV and inflammation. The development of a potent and selective chemical probe for PCAF bromodomain will allow for early validation of this protein as a target for drug discovery efforts. The primary aim of this work was to develop a potent ($K_D/IC_{50} \sim 100$ nM), selective (>30-fold

over other families) and potentially cell active small molecule probe of the PCAF bromodomain.

A combination of *in silico* computer aided ligand design and iterative cycles of synthesis and biophysical characterisation (Differential Scanning Fluorimetry and Isothermal Titration Calorimetry) were used to develop a focussed library of chemical compounds (>70 compounds) bearing good potency (μM -nM) and selectivity for the PCAF bromodomain.

This work describes the first potent (K_D 126 nM) and selective (>4500-fold over BRD4) small molecule inhibitor of the PCAF bromodomain. The lead compound shows good resistance to *in vitro* metabolism (human and mouse liver microsomes) and a clean toxicity profile. The lead candidate compound is currently being investigated *in vivo* for its effect in a neuroinflammation based animal model.

MEDI 7

Structure-based computer-aided IL-6/GP130 Protein-Protein Interaction (PPI) inhibitor design

Guqin Shi^{2,3}, shi.293@osu.edu, **Liguang Mao**^{2,3}, **Vandana Kumari**¹, **Chenglong Li**³. (1) MCL, NCI, Frederick, Maryland, United States (2) Pharm, Med Chem, The Ohio State University, Columbus, Ohio, United States (3) Pharm, Med Chem, University of Florida, Gainesville, Florida, United States

IL-6 is a pro-inflammatory cytokine which mediates inflammation and immune response. It participates in multiple biological activities by binding to membrane receptor GP130 and triggering Jak/STAT3 trans-signaling pathway. Excessive induction of this pathway has been observed in various diseases like neuro-inflammation and cancers. Blockade of IL-6/GP130 signaling became a novel therapeutic approach. However, few small molecule inhibitors have been reported so far. More importantly, a structure-based rationale for IL-6/GP130 inhibitor design was absent. This poster will present how we have been utilizing structure-based computational methods to develop effective IL-6/GP130 inhibitors.

Through computational analyses on IL-6/IL6-R α /GP130 complex, we have identified two free energy of binding "hot spots" on GP130-D1 domain which contribute most to the hexamerization step. Then a series of small molecules were designed in order to disrupt these key "hot spots". Under the guidance of molecular dockings and dynamic simulations, we further explored the GP130-D1 domain interaction surface and re-arranged the inhibitors into new scaffolds capable of capturing more interactions on GP130. These inhibitors showed successful blockade on IL-6 trans-signaling in biochemical and cancer cellular assays. Computational free energy analyses were also performed to compare with experimental binding energy results to guide our iterative design and to understand the binding mechanism, gaining insights on challenging PPI design.

MEDI 8

Disclosure of a development candidate to treat severe acute pancreatitis through a drug discovery partnership between GlaxoSmithKline and the University of Edinburgh

John Liddle, john.2.liddle@gsk.com. GlaxoSmithKline, London, United Kingdom

Discovery Partnerships with Academia (DPAc) was established in late 2010 as a new mechanism to combine disease insight from the academic community with GSK's drug discovery engine in order to translate academic innovative ideas into medicines that meet the needs of patients. The collaboration with Mr Damian Mole, Clinical Senior Lecturer and Honorary Consultant Surgeon, at The University of Edinburgh was one of the first DPAc collaborations to be established. Damian's work uncovered a novel link between 3-hydroxykynurenine (3HK), a tryptophan metabolite, and the development of severe acute pancreatitis suggesting that inhibition of the enzyme responsible for the generation of 3HK, kynurenine monooxygenase (KMO), could represent a revolutionary new approach to the treatment of acute pancreatitis.

The presentation will describe the medicinal chemistry strategy which utilised substrate knowledge to discover KMO inhibitors with the required properties commensurate with intravenous dosing. Low molecular weight inhibitors with excellent aqueous solubility were rapidly identified and shown to have a clear protective effect in a rat model of acute pancreatitis when dosed therapeutically. Subsequent structural knowledge enabled the fine tuning of key interactions with the protein and ultimately led to the identification of the development candidate, a highly potent and selective KMO inhibitor with outstanding physicochemical properties.

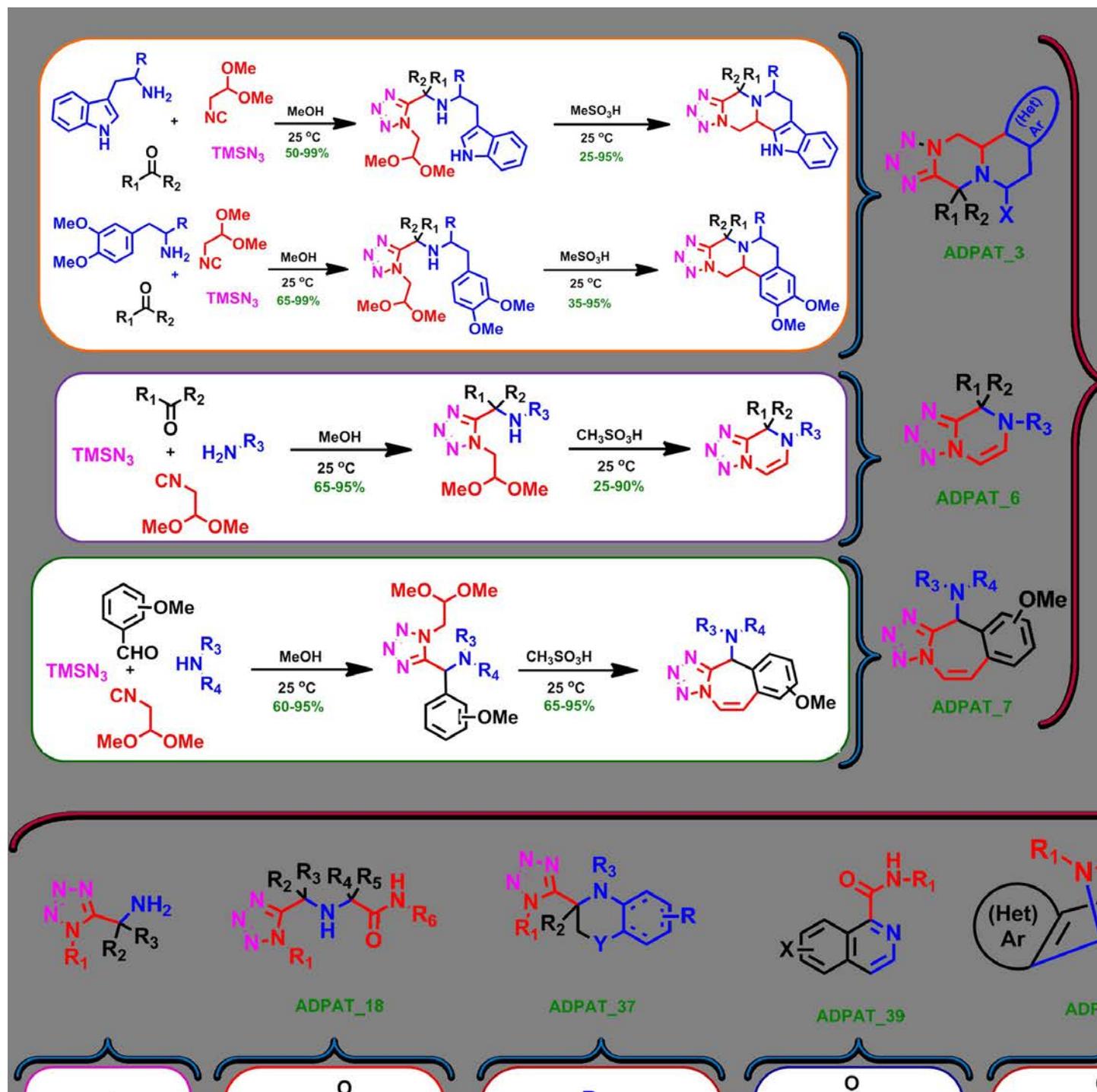
MEDI 9

Expanding screening decks by innovative MCR scaffolds

Alexander Doemling¹, a.s.s.domling@rug.nl, Pravin Pati², Rudrakshula Madhavachary¹. (1) Department of Drug Design, University of Groningen, Groningen, Netherlands (2) Innovative Chemistry, Telesis Pharma, Groningen, Netherlands

HTS is the mainstay in current preclinical drug discovery efforts. The quality of hits and hence leads is directly related to the diversity and quality of the screening decks' content. The European Lead Factory (ELF) is a public private partnership aiming to create a 500K screening library and to perform multiple HTS campaigns on this library. In an unprecedented joint-force approach, seven members of the European Federation of Pharmaceutical Industries and Associations (EFPIA) and a Public Chemistry Consortium collaborate to build a high quality Joint European Compound Library (JECL) to boost drug discovery within Europe (<https://www.europeanleadfactory.eu/#>). The construction principle for innovative scaffolds and derived compound libraries besides molecular properties and structural features include novelty, diversity potential, synthetic tractability and innovative library design. The University of Groningen Domling group is part of ELF and has contributed multiple innovative scaffold design over the last 4 years

and many compounds of JECL are derived from these designs. We will illustrate the scaffold designs which includes multiple small molecule structures up to artificial macrocycles with high 3D character while not jeopardizing PKPD properties. Synthesis, scope and limitations, physicochemical properties, 3D structures and potential applications will be highlighted.



MEDI 10

Metamorphosis of paroxetine into a highly potent and selective GRK2 inhibitor via structure-based drug design

Helen Waldschmidt¹, hwaldsch@umich.edu, Kristoff Homan², Osvaldo Cruz - Rodriguez^{2,3}, Marilyn Cato², Renee Bouley², Michael Wilson¹, Alessandro Cannavo⁴, Jianliang Song⁴, Joseph Cheung⁴, Paul Kirchhoff¹, Walter Koch⁴, John Tesmer², Scott D. Larsen¹. (1) Department of Medicinal Chemistry, Vahlteich Medicinal Chemistry Core, University of Michigan, Ann Arbor, Michigan, United States (2) Life Science Institute, Departments of Pharmacology and Biological Chemistry, University of Michigan, Ann Arbor, Michigan, United States (3) PhD Program in Chemical Biology, University of Michigan, Ann Arbor, Michigan, United States (4) Center for Translational Medicine, Temple University, Philadelphia, Pennsylvania, United States

In the failing heart the sympathetic nervous system (SNS) elevates catecholamine levels leading to stimulation of the β -adrenergic receptors (β ars). Due to this increased signaling the β ars become overstimulated resulting in desensitization via G-protein coupled receptor kinases (GRKs) and severe uncoupling. Specifically, GRK2 is elevated in heart failure and inhibition of GRK2 interrupts the internalization of the β ars allowing the heart to regain stimulation from the SNS. Our lab identified the FDA approved serotonin reuptake inhibitor paroxetine as a GRK2 inhibitor. Furthermore, paroxetine was shown to reverse cardiac remodeling, renormalize levels of catecholamines and β ars in the heart, and have cardio-protective effects up to two weeks post treatment in mice. Utilizing the structural motifs in combination with the previously reported potent and selective GRK2 inhibitor Takeda103A a library of hybrid inhibitors was designed and synthesized. This structure based drug design campaign produced several compounds with impressive potency and selectivity for GRK2 over other AGC kinases. The most selective inhibitor was CCG258208, with an IC_{50} for GRK2 of 30 nM and >230-fold selectivity vs other GRK subfamilies and AGC kinases. At a dose of 0.1 μ M CCG258208 resulted in a significant increase in mouse cardiomyocyte contractility. Short pharmacokinetic studies have revealed plasma level concentrations up to seven hours higher than the IC_{50} of CCG258208. This series of paroxetine based hybrid inhibitors show promise as potent and selective GRK2 inhibitors with potential *in vivo* utility.

MEDI 11

Discovery of novel potent and selective agonists of the serotonin (5-HT) 5-HT_{2C} receptor as neurotherapeutics

Helmut Mack¹, helmut.mack@abbvie.com, Gisela Backfisch², Guenter Blaich³, Wilfried M. Braje¹, Thomas Erhard¹, Andreas Haupt¹, Andreas Kling¹, Viktor Lakics⁴, James J. Lynch⁵, Manolo Mugnaini⁴, Frauke Pohlki¹, Ana L. Relo⁶, Kai Schaefer³. (1) Medicinal Chemistry, Abbvie Deutschland GmbH & Co.KG, Ludwigshafen, Germany (2) Drug Metabolism, Abbvie Deutschland GmbH & Co. KG, Ludwigshafen, Germany (3)

Toxicology, Abbvie Deutschland GmbH & Co. KG, Ludwigshafen, Germany (4) Biology, Abbvie Deutschland GmbH & Co. KG, Ludwigshafen, Germany (5) Development Science, Abbvie Inc., North Chicago, Illinois, United States (6) Pharmacology, Abbvie Deutschland GmbH & Co. KG, Ludwigshafen, Germany

Decreased signaling at the 5-HT_{2C} receptor appears to play a major role in schizophrenia, bipolar depression, psychosis in Alzheimer's Disease, drug addiction and also in obesity. Stimulation of the 5-HT_{2C} receptor has been shown to be efficacious in preclinical animal models of psychosis, substance abuse disorders and food intake. Selectivity versus the closely related 5-HT_{2A} and the 5-HT_{2B} receptor is crucial, since 5-HT_{2A} agonism has hallucinogenic properties and stimulation of the 5-HT_{2B} receptor increases the risk of heart valve damage.

Starting from a published lead compound with good potency and selectivity but with a poor ADME profile including low oral bioavailability, systematic investigation of the site of metabolism and subsequent dedicated structural modification of the metabolic hotspot led to compounds with significantly improved oral bioavailability across species, while maintaining a favorable activity and selectivity profile. Moreover, improving brain penetration by additional modifications led to advanced lead candidates with an exciting preclinical profile regarding dose-dependent in vivo efficacy in a number of animal models, cardiovascular safety and acute and repeat-dose toxicology, yielding promising candidates with the potential for an improved first-in-class neurotherapeutic.

MEDI 12

Assessment of MCHR1 target engagement in the brain using PET imaging

Anders Johansson¹, *anders.m.johansson@astrazeneca.com*, **Madeleine Antonsson**¹, **Anders Hogner**¹, **Marléne Fredenwall**¹, **Martin Hayes**¹, **Peter Johnstrom**², **Magnus Schou**². (1) *Cardiovascular and Metabolic Diseases, Innovative Medicines and Early Development Biotech Unit, AstraZeneca Gothenburg, Molndal, Sweden* (2) *AstraZeneca Translational Science Centre at the Karolinska Institute, Stockholm, Sweden*

The MCH receptor 1 (MCHR1) is a peptide receptor associated with caloric intake. It is located in the hypothalamic region of the brain and has been extensively investigated as a target for weight management. As a part of our internal drug discovery program culminating in the discovery of AZD1979, extensive efforts were made to discover a PET ligand for the MCHR1 for assessment of target engagement in the clinic. For only a minority of the CNS targets investigated a PET ligand is available and for MCHR1 this is extremely challenging. The MCH receptor 1 has a low expression in the brain and known MCHR1 antagonists possess far from ideal properties to be useful as PET ligands. Our work focused on the AstraZeneca chemical series and a screening cascade was set up to identify the most promising compounds with a focus on unbound fraction in the brain and potency. The presentation will show the outcome of this work and subsequent PET studies on potential candidate ligands. We will also disclose PET

microdosing studies with ^{11}C -labelled AZD1979 in Rhesus monkeys confirming a 1:1 free brain to plasma ratio.

MEDI 13

Redox-responsive hyaluronic acid-taxoid nanoconjugate for CD44-targeted cancer chemotherapy

Yaozhong Zhang¹, yaozhong.zhang@stonybrook.edu, Iwao Ojima². (1) Stony Brook University, Stony Brook, New York, United States (2) Chem Dept/ICBDD, Stony Brook University, Stony Brook, New York, United States

Cancer is the second leading cause of mortality in the US and various chemotherapeutic drugs have been developed for cancer treatment. However, chemotherapy usually causes undesired side effects, which is attributed to the lack of specificity of drugs. Therefore, it is crucial to develop a strategy that selectively targets tumors without affecting healthy cells.

Hyaluronic acid (HA), a primary natural ligand of cancer cell biomarker CD44 receptor, enables tumor-specific drug delivery by taking advantage of both passive targeting through enhanced permeation and retention (EPR) effect, and active targeting via CD44 receptor-mediated endocytosis. Thus, redox-responsive HALT(SS)s (HA-disulfide linker-taxoid) were designed and synthesized by conjugating taxoid SB-T-1214 to 5k, 20k, and 60k HA respectively. HALTs underwent self-assembly and formed nanosized micelles in aqueous solution. The structure, percentage of loading, size and morphology of HALTs were characterized by ¹H-NMR, FTIR, GPC and TEM.

Our imaging study on the HA-FITC probe using confocal microscopy (CFM) disclosed that HALT would be highly specific towards CD44-overexpressing cancer cells (i.e., MDA-MB-231, HCT116, NCI/ADR) as opposed to seven other cancer cell lines and normal cells (i.e., NIH-3T3, WI-38) where the number of CD44 receptors was found to be very low. Besides, by performing the time-dependent internalization study of three different sizes of HA-FITC, we gained insight into the effects of particle sizes and CD44 levels on the internalization process.

In addition, the redox-responsive drug release mechanism was validated via MTT assay where HALT(SS) was treated with glutathione ethylester (GSOEt) and the enhancement of potency was observed by comparing with HALT(suc) (suc = succinate linker). Also, drug release profile monitored via HPLC in the presence of GSOEt further confirmed this mechanism.

The potency of HALTs against CD44-overexpressing cancer cells, the size effects on internalization as well as redox-responsive drug release will be presented.

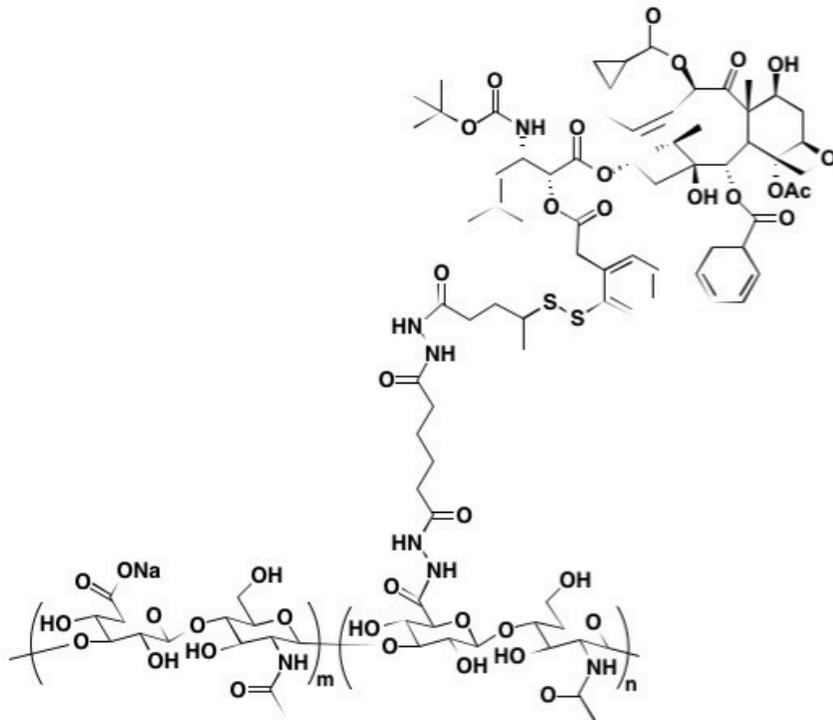


Figure 1. Structure of HALT(SS)

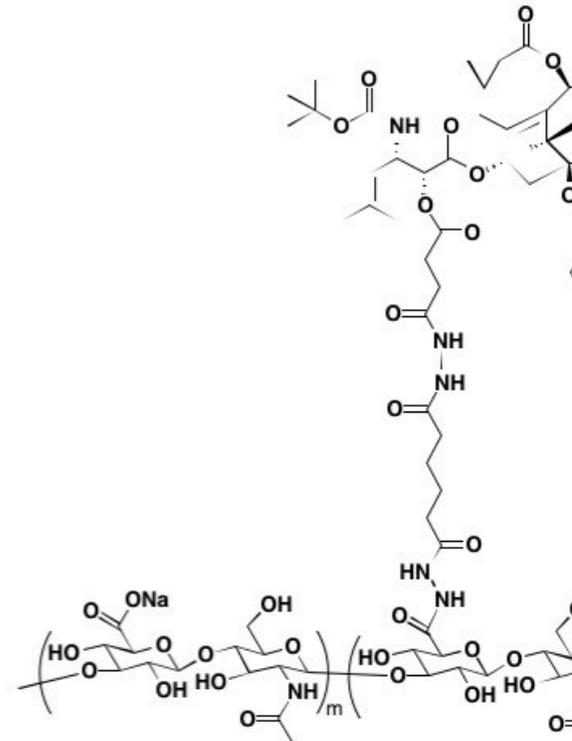


Figure 2. Structure of HALT(suc)

MEDI 14

Targeting ubiquitin pathway enzymes for cancer immunotherapy

Jian Wu, wu@progenra.com, Hui Wang, Suresh Kumar, Feng Wang, Ivan Sokirniy, Christopher Riling, Michael Mattern, Joseph Weinstock. Progenra, Inc, Malvern, Pennsylvania, United States

Immune evasion is a hallmark feature of tumors as they employ various strategies to suppress the immune system's ability to recognize and destroy cancer cells. Significant advances in the understanding of the mechanisms of cancer immune evasion have led to the successful development of immunotherapies that exhibit clinical efficacy against hematological as well as solid tumors. Due to the underlying complexity of cancer immune evasion, however, several different approaches will likely be necessary for effective therapeutic management. Progenra is developing novel small molecule cancer immunotherapeutics by understanding and exploiting the role of ubiquitin in immune regulation. We have identified inhibitors of a deubiquitylase (DUB) that is highly expressed in regulatory T (Tregs) cells and plays a critical role in promoting Treg functions. The unique mechanism of action was demonstrated by a combination of NMR spectroscopy and mass spectrometry. In addition, we have identified inhibitors of an E3 ubiquitin ligase that negatively regulates T-effector cell function. These inhibitors could be combined with other cancer immunotherapies to achieve durable clinical responses.

MEDI 15

Synthesis and biological profiling of 2-azabicyclo[2.1.1]hexane-based proline isosteres as antagonists of TRPA1 ion channel

Daniel Shore¹, shore.daniel@gene.com, Matthew Volgraf¹, Brian Safina¹, Vishal A. Verma¹, Elisia Villemure¹, Huifen Chen¹, Lan Wang¹, Anthony A. Estrada², Joseph P. Lyssikatos², Aleksandr Kolesnikov¹, Steven Do¹, Shannon Shields³. (1) Discovery Chemistry, Genentech, Inc, Oakland, California, United States (2) Denali Therapeutics, South San Francisco, California, United States (3) Genentech Inc., South San Francisco, California, United States

Antagonism of the Transient Receptor Potential cation channel A1 (TRPA1) has been reported to be potentially therapeutic for pain and immunology indications *in vivo*. A variety of proline-based small molecule antagonists of TRPA1 have been discovered by various groups including our own. However, these molecules often exhibit poor physical properties. To attempt to address these issues, we designed and synthesized a series of bicyclic proline isostere cores. Molecules incorporating a 2-azabicyclo[2.1.1]hexane in place of a monocyclic pyrrolidine were shown to have excellent potency, pharmacokinetic properties and *in vivo* target engagement. The design, synthesis and biological properties will be described.

MEDI 16

Cyclophilin D inhibitors rational and fragment based design: From 7 mM to 7 nM potency

Catherine Jorand-Lebrun¹, catherine.jorandlebrun@emdserono.com, Xuliang Jian², Theresa Johnson², Ulrich Graedler⁴, Daniel Schwarz³, Birgitta Leuthner³, Andreas Marx⁵, Didier Roche⁶, Marine Gilardone⁶, Hugues Lemoine⁶, Santosh Kulkarni⁸, Frederic Bernard⁷. (1) Medicinal Chemistry, EMD-Serono, Billerica, Massachusetts, United States (2) Structure and Digital Drug Design, EMD-Serono, Billerica, Massachusetts, United States (3) Molecular Pharmacology, Merck KGaA, Darmstadt, Germany (4) Drug Structure, Prediction & Design, Merck KGaA, Darmstadt, Germany (5) Analytics, Merck KGaA, Darmstadt, Germany (6) Chemistry, Edelis, Lyon, France (7) Global R&D Strategy & Business Operations, EMD-Serono, Billerica, Massachusetts, United States (8) Synthetic Chemistry, Syngene, Bangalore, India

Cyclophilins are folding helper enzymes member of the Peptidyl Proline Isomerases (PPI) superfamily. PPI are extremely challenging targets and the druggability of this target class has been partly demonstrated in the 1990's with Cyclosporin A (CsA) which is still used in the prevention of transplant rejection. The immunosuppressive activity of CsA is due to its calcineurin binding domain, however several analogues have been reported as potent cyclophilin inhibitors with reduced affinity for calcineurin. Some of them have reached clinical phase (Debio-25 for HCV), but very few low MW inhibitors have been reported. Our research was focused on cyclophilin D (CypD) because of its

implication in mitochondrial function. CypD regulates pore opening of the mitochondrial permeability transition pore (MPTP) and therefore plays a significant role in the pathological process driving mitochondria dysfunction. To avoid immunosuppressive activity and obtain improved drug-like properties, we aimed to develop low MW inhibitors exclusively. For that purpose, we set up a unique platform for robust testing of small molecules with potency ranging from mM to nM in CypD binding and enzymatic assays. After the HTS campaign (using a fluorescent based binding assay) did not return any confirmed positives, we decided to base our hit discovery strategy solely on SPR Fragment Based Screening combined with knowledge based design. The screening of the EMD-Serono fragment collection provided 58 confirmed positives with moderate to low Ligand Efficiency (0.1 to 0.3) from which only six crystal structures in CypD were solved. Fragment growing and linking work from two proprietary 3D fragments originated from the Edelris collection produced nM inhibitors after only two to three optimization cycles.

MEDI 17

Discovery of AZD9977: A non-steroidal mineralocorticoid modulator for treatment of diabetic kidney disease

Kenneth L. Granberg¹, kenneth.granberg@astrazeneca.com, Zhong-Qing Yuan¹, Bo Lindmark¹, Karl Edman², Krister Bamberg¹, Anders Hogner¹, Johan Kajanus¹, Marcus Malmgren³, Christian Löfberg¹, Anneli Nordqvist¹, Jan Å. Lindberg¹, Jonas Brånalt¹, Gavin O'Mahony¹, Michael Kossenjans², Dongmei Liu⁴, Anna Aagaard², Martin Billger⁵, Stefan Bäckström², Philip Cornwall³, Hans Ericsson⁷, Fredrik Erlandsson⁶, Eva L. Hansson², Ahlke Hayen¹, Majlis Hermansson¹, Ida Ivarsson², Rasmus Jansson Löfmark¹, Ulrika Johansson¹, Ulla Karlsson², Xueqing Li¹, Grigorios Nikitidis³, Peter Nordberg¹, Andreas Nordin¹, Britta Polentarutti⁵, Nidhal Selm², Andrew Turner³, Lena William-Olsson¹, Caroline E. Wingolf³, Judith Hartleib¹. (1) Cardiovascular and Metabolic Diseases, Innovative Medicines and Early Development Biotech Unit, AstraZeneca, Gothenburg, Sweden (2) Discovery Sciences, Innovative Medicines and Early Development Biotech Unit, AstraZeneca, Gothenburg, Sweden (3) Pharmaceutical Sciences, Innovative Medicines and Early Development Biotech Unit, AstraZeneca, Gothenburg, Sweden (4) Chemistry, Pharmaron Beijing, Beijing, China (5) Discovery Safety & Metabolism, Innovative Medicines and Early Development Biotech Unit, AstraZeneca, Gothenburg, Sweden (6) Global Medicines Development, AstraZeneca, Gothenburg, Sweden (7) Early Clinical Development, Innovative Medicines and Early Development Biotech Unit, AstraZeneca, Gothenburg, Sweden

Diabetic kidney disease (DKD) is a major cause of renal failure and is associated with increased CV mortality and morbidity. The marketed mineralocorticoid receptor (MR) antagonists, spironolactone and eplerenone, slow disease progression but their potassium sparing effects increases the risk for hyperkalemia which can be lethal. Patients with advanced chronic kidney disease struggle to excrete sufficient amounts of potassium, and diabetics are more prone to hyperkalemia. Consequently, patients with DKD are currently not treated with MR antagonists.

AZD9977, a neutral and selective MR modulator, was discovered through use of structure-based drug design. During lead optimization focus was set at achieving good physicochemical properties in combination with a unique pharmacological profile relative the currently known MR antagonists on the market or in clinical development. In both rat and mouse in vivo models of developing or established chronic kidney disease, four weeks repeated dosing of AZD9977 delayed and reversed deteriorating kidney function. AZD9977 reduced urinary albumin secretion and improved renal and cardiac pathology scores. Importantly, when administered acutely to normal rats, AZD9977 had a minimal effect on urinary sodium secretion and attenuated the substantial natriuresis caused by eplerenone. AZD9977 is currently undergoing phase 1 clinical trials.

MEDI 18

Discovery of BMS-962212 a highly potent, selective inhibitor of coagulation FXIa

Donald Pinto, donald.pinto@bms.com, Michael J. Orwat, Leon Smith II, Shefali Shrivastava, Mimi L. Quan, Patrick Y. Lam, Karen Rossi, Atsu Apedo, Jeffrey Bozarth, Yiming Wu, Joanna Zheng, Baomin Xin, Nathalie Toussaint, Paul Stetsko, Olafur Gudmundsson, Earl Crain, Pancras Wong, Zhen Lou, Timothy Harper, Silvi Chacko, Joseph Myers, Steven Sheriff, Huiping Zhang, Xiaoping Hou, Arvind Mathur, Dietmar Seiffert, Joseph Luetzgen, Ruth R. Wexler. Bristol Myers Squibb, Princeton, New Jersey, United States

Factor XIa (FXIa) is a key factor in the intrinsic pathway of the coagulation cascade that is involved in the amplification of the procoagulation signal. Mounting evidence suggests that direct inhibition of FXIa can block pathologic thrombus formation while preserving normal hemostasis and thus has emerged as a major target for anticoagulant therapy. Animal studies using direct inhibitors of FXIa from Bristol-Myers Squibb have demonstrated good antithrombotic efficacy and with low bleeding. Based on the potential efficacy and safety of inhibiting FXIa, we targeted the discovery of an intravenous agent for the treatment of cerebrovascular ischemic events in the hospital setting. This presentation will focus on a structure based drug design effort that culminated in the discovery of a clinical candidate BMS-962212, suitable for intravenous administration. Key insights, structure activity relationships (SAR), and pharmaceutical properties will be presented. BMS-962212 is a potential first in class, reversible, direct, and highly selective intravenous small molecule inhibitor of FXIa (FXIa K_i = 0.7 nM)

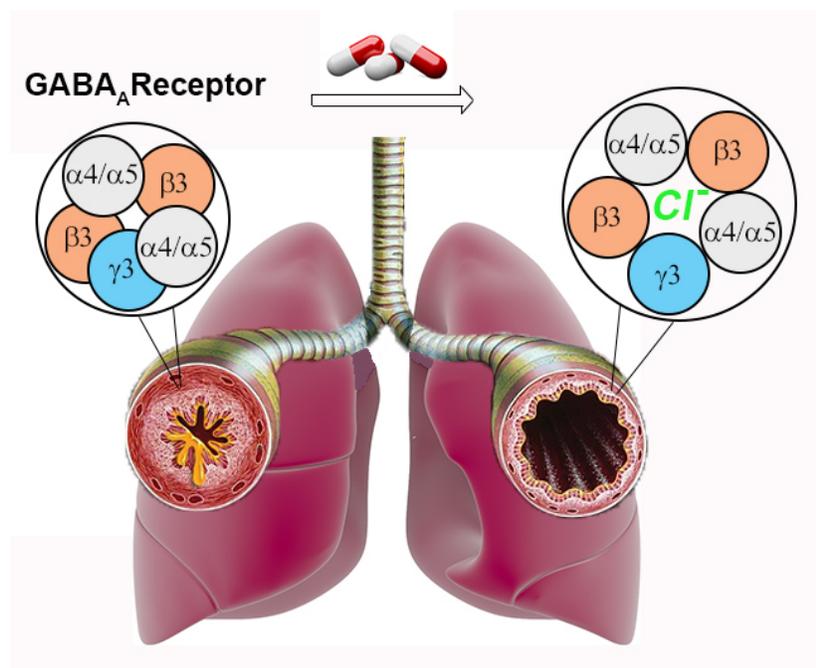
MEDI 19

Development of new therapies for asthma based on compounds that specifically target GABA_A receptors in the lung

Leggy Arnold^{1,3}, arnold2@uwm.edu, Gloria S. Forkuo¹, Nina Y. Yuan¹, Revathi Kodali¹, Olivia B. Yu¹, Nicolas M. Zahn¹, Rajwana Jahan¹, Guanguan Li¹, Michael R. Stephen¹, Margaret Guthrie¹, Amanda N. Nieman¹, Michael M. Poe¹, Gene T. Yocum², Charles W. Emala², Douglas C. Stafford^{1,3}, Douglas A. Steeber¹, James M. Cook^{1,3}. (1)

Chemistry and Biochemistry, University of Wisconsin Milwaukee , Milwaukee, Wisconsin, United States (2) Anesthesiology, Columbia University, New York, New York, United States (3) Milwaukee Institute for Drug Discovery, Milwaukee, Wisconsin, United States

Our goal to develop a novel oral drug to alleviate the hallmark asthma symptoms of airway hyperresponsiveness and lung inflammation has led to the synthesis of positive allosteric modulators for specific GABA_A receptors (GABA_ARs). This approach is based on recent findings that lung cell types involved in asthma pathology (airway smooth muscle (ASM) and immune/inflammatory cells) express discrete GABA_ARs, expression of which is well known in CNS. Starting with positive modulators designed for CNS disorders, such as anxiety or schizophrenia, we engineered new molecules that retain GABA_AR selectivity but do not cross the blood brain barrier, and distribute to the lung to relax constricted ASM and reduce asthma inflammation. Compounds with desired pharmacodynamic performance were optimized further for oral availability, metabolic stability, pharmacokinetics (including extended plasma half-life and good lung exposure), and limited CNS distribution. Immunohistochemistry was used to verify the expression of GABA_ARs on lung cell types, including white blood cells, and electrophysiology to demonstrate functional GABA_AR activity in these cell. Finally, we present flow cytometry and immunochemistry data that show anti-inflammatory leucocyte population and cytokine effects of GABA_AR modulators in the asthmatic mice. Taken together, these results demonstrate for the first time the feasibility of targeting asthma with an orally available GABA_AR ligand.



MEDI 20

Discovery of AGN-241689: A potent, orally-acting CGRP receptor antagonist for migraine prophylaxis

Ian M. Bell, ian_bell@merck.com. Merck & Co., Inc, Harleysville, Pennsylvania, United States

Chronic migraine, defined as migraine headache occurring on at least 15 days each month, is a highly disabling disorder, yet there are no disease-specific treatments for migraine prevention. Calcitonin gene-related peptide (CGRP) has been shown to play an important role in migraine headache and small molecule CGRP receptor antagonists, including telcagepant, have demonstrated clinical efficacy for the acute treatment of migraine. More recently, several monoclonal antibodies that target either CGRP or the CGRP receptor were shown to be effective for migraine prevention. A study evaluating BID dosing of telcagepant (140 and 280 mg) for 12 weeks suggested that this small molecule antagonist was also efficacious for migraine prevention, although the trial was terminated early due to hepatotoxicity concerns. In order to identify a development candidate with reduced risk of liver injury, we focused on compounds with differentiated structure and metabolism from earlier compounds associated with hepatotoxicity. Our strategy also involved structure modifications designed to reduce bioactivation, increase potency, and lower the projected dose. These considerations led to the discovery of AGN-241689 (MK-8031), which combines good oral pharmacokinetic properties with exquisite potency and selectivity. The presentation will discuss the discovery and preclinical profile of AGN-241689 in detail. The compound is currently being studied in the clinic as a novel therapeutic for migraine prophylaxis.

MEDI 21

Discovery of BMS-986142: A reversible inhibitor of Bruton's Tyrosine Kinase (BTK) conformationally constrained by two locked atropisomers

Scott H. Watterson, scott.watterson@bms.com, George V. De Lucca, Qing Shi, Charles M. Langevine, Douglas G. Batt, Qingjie Liu, Myra Beaudoin Bertrand, Hua Gong, Jun Dai, Henry Yip, Peng Li, Dawn Z. Sun, Dauh-Rung Wu, Chunlei Wang, Yingru Zhang, Sarah C. Traeger, Mark A. Pattoli, Stacy Skala, Lihong Cheng, Mary T. Obermeier, Rodney Vickery, Lorell N. Discenza, Celia J. D'Arienzo, Yifan Zhang, Elizabeth Heimrich, Kathleen Gillooly, Tracy L. Taylor, Claudine Pulicicchio, Kim McIntyre, Michael A. Galella, Andrew J. Tebben, Jodi K. Muckelbauer, ChiehYing Chang, Luisa Salter-Cid, Joel C. Barrish, Percy H. Carter, Aberra Fura, James Burke, Joseph A. Tino. Bristol-Myers Squibb, Princeton, New Jersey, United States

Bruton's tyrosine kinase (BTK), a non-receptor tyrosine kinase, is a member of the Tec family of kinases. BTK plays an essential role in B cell receptor (BCR)-mediated signaling as well as Fcγ receptor signaling in monocytes and Fcε receptor signaling in mast cells and basophils, all of which have implications in the pathophysiology of

autoimmune disease. As a result, inhibition of BTK is anticipated to provide an effective strategy for the clinical treatment of autoimmune diseases such as rheumatoid arthritis and lupus. This presentation will detail the structure-activity relationships (SAR) leading to a novel series of highly potent and selective carbazole and tetrahydrocarbazole based, reversible inhibitors of BTK. Of particular interest is that two atropisomeric centers were rotationally locked to provide a single, stable atropisomer, resulting in enhanced potency and selectivity as well as a reduction in safety liabilities. With excellent in vivo efficacy and a very desirable tolerability profile, BMS-986142 has advanced into clinical studies.

MEDI 22

Hydrogen bond interaction geometries in protein-ligand complexes: From large-scale statistics to single cases

Eva Nittinger², nittinger@zbh.uni-hamburg.de, Therese Inhester², Gudrun Lange¹, Robert Klein¹, Matthias Rarey². (1) Crop Science Division, Bayer AG, Frankfurt, Germany (2) University of Hamburg, Hamburg, Germany

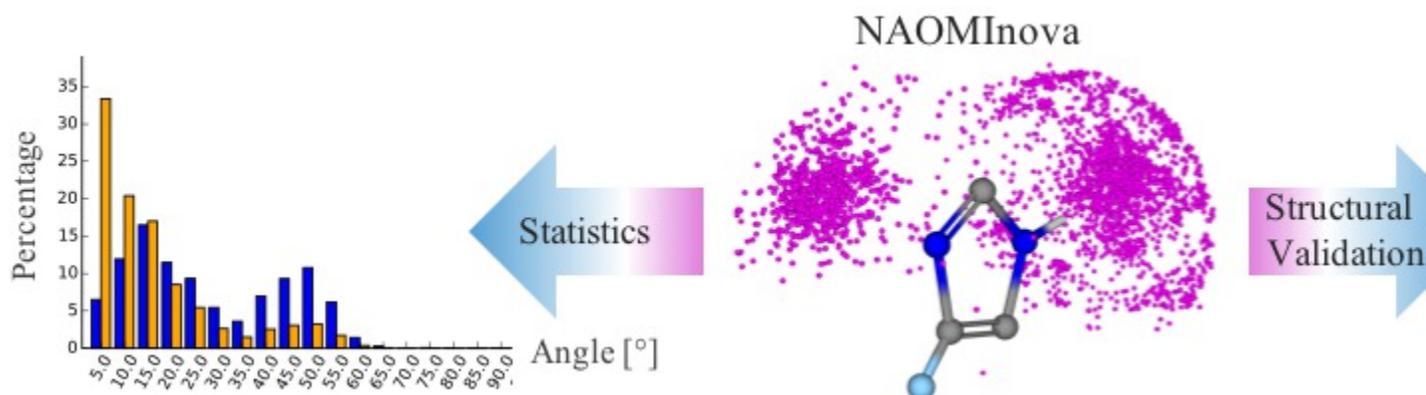
Hydrogen bonds (H-bonds) are a major driving force of protein-ligand recognition. A profound understanding of these interactions – why they form and which geometrical preferences they have – is of interest in various life science applications. Analyzing statistically relevant data, quantitatively as well as qualitatively, leads to chemical meaningful conclusions with implications for medicinal chemistry.

Here, we present a new study on a large experimental data collection. The data set contains about 9,000 high resolution protein-ligand complexes extracted from the Brookhaven Protein Data Bank (PDB). Using this data set, we thoroughly investigated the geometric and functional properties of H-bonds and their dependence on the chemical environment. We defined an extensive range of functional groups relevant for protein and ligand interactions and included diverse structural and chemical properties of their surroundings.

Accompanying the statistical analysis was the development of the software NAOMInova, which allows non-computational scientists to search for and browse through interaction geometries. NAOMInova allows the definition of a functional group of interest and presents the distribution of potentially interacting partner atoms found in crystal structures. Additionally, NAOMInova provides a backlink to the underlying structure enabling an easy retrieval and visualization of the original protein-ligand complex. This way, outliers as well as further structural properties can be analyzed and conclusion for ligand optimization can be derived.

In this talk, we will present results from our statistical study, including unexpected geometric patterns and newly derived optimal H-bonds interaction geometries. We show that the application of NAOMInova intuitively aids the identification of common and untypical interaction geometries. In this way, the medicinal chemist is supported in

understanding three-dimensional binding motifs and optimizing small molecules for protein interaction.



MEDI 23

Discovery of selective SETD8 inhibitors via structure-based approach

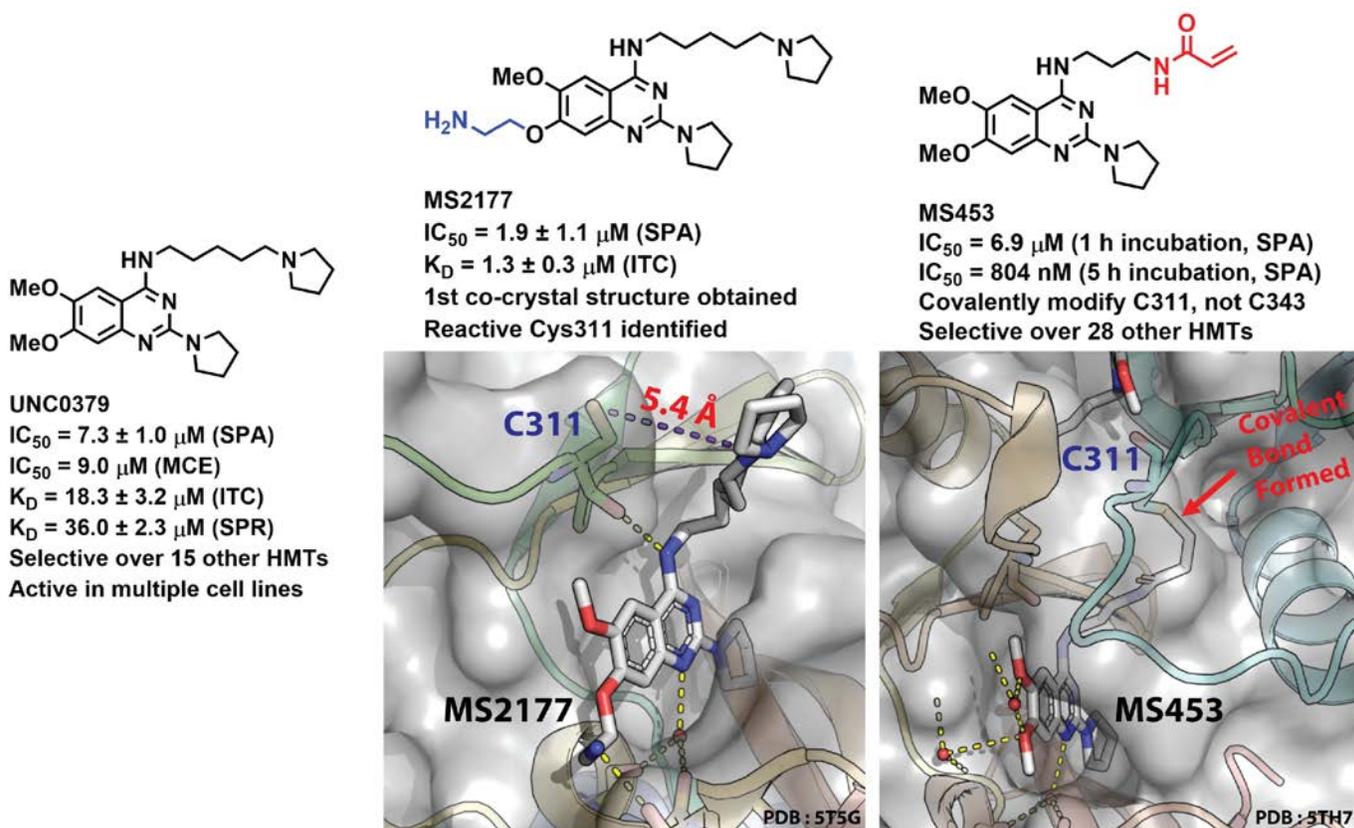
Anqi Ma¹, *a.m.andrewma@gmail.com*, **Wenyu Yu**², **Kyle Butler**¹, **Fengling Li**², **Wolfram Tempe**², **Nicolas Babault**¹, **Pittella-Silva Fabio**³, **Jason Shao**¹, **Junyi Wang**³, **Minkui Luo**³, **Masoud Vedad**², **Peter Brown**², **Cheryl H. Arrowsmith**², **Jian Jin**¹. (1) Icahn School of Medicine at Mount Sinai, New York, New York, United States (2) University of Toronto, Toronto, Ontario, Canada (3) Memorial Sloan Kettering Cancer Center, New York, New York, United States

SET Domain-Containing Protein 8 (SETD8) is the sole enzyme that catalyzes mono-methylation of histone 4 lysine 20. SETD8 plays important roles in gene transcription, genome integrity, cell cycle progression and DNA damage and repair. Overexpression of SETD8 has been reported in many cancers, including lung cancer and bladder cancer. SETD8 also contributes to epithelial–mesenchymal transition (EMT) in breast cancer. Therefore, SETD8 is a potential therapeutic target and inhibitors of SETD8 would be valuable tools to study its biological roles.

After screening our quinazoline-focused compound library, we identified UNC0379 as the first selective small molecule inhibitor of SETD8, which was characterized in scintillation proximity assay (SPA) and microfluidic capillary electrophoresis assay (MCE). Its binding affinity to SETD8 has also been confirmed in a battery of biophysical assays. Importantly, UNC0379 is selective for SETD8 over 15 other histone methyltransferases (HMTs). Subsequent studies have demonstrated UNC0379 is active in multiple malignant diseases, both *ex vivo* and *in vivo* (in press in *Cancer Cell* and unpublished results).

In addition, our structure-activity relationship (SAR) studies resulted in the discovery of compound MS2177 as a more potent SETD8 inhibitor ($IC_{50} = 1.9 \pm 1.1 \mu M$). We successfully obtained the first co-crystal structure of SETD8 with this inhibitor, which revealed a reactive cysteine residue (C311) nearby the inhibitor binding site. Taking advantage of C311, we developed the first targeted covalent inhibitor (TCI) for SETD8,

MS453, displaying improved potency ($IC_{50} = 804 \text{ nM}$). Furthermore, mutagenesis studies confirmed MS453 selectively forms covalent bond with C311 but not with C343 which is also on the surface of SETD8. MS453 is selective for SETD8 over 28 other HMTs (in press in *J. Med. Chem.*). As ongoing effort to develop chemical probe for SETD8, we have developed the next generation SETD8 inhibitor with IC_{50} of 285 nM. This new inhibitor has displayed robust activity in reducing H4K20me1 level in multiple cell lines at low micromolar concentrations (unpublished results).



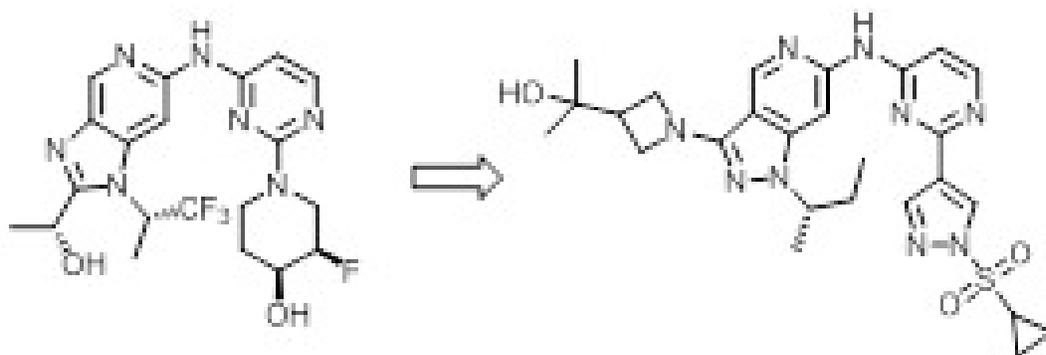
MEDI 24

Discovery of a noncovalent, mutant-selective epidermal growth factor receptor inhibitor

Bryan K. Chan⁴, bkichan@gmail.com, **Emily J. Hanan**⁴, **Krista Bowman**¹, **Marian C. Bryan**¹, **Daniel Burdick**¹, **Emily Chan**¹, **Yuan Chen**¹, **Saundra Clausen**¹, **Trisha Dela Vega**¹, **Jennafer Dotson**², **Charles Eigenbrot**¹, **Richard Elliott**³, **Robert Heald**³, **Philip Jackson**³, **Jamie Knight**³, **Hank La**⁴, **Michael Lainchbury**³, **Shiva Malek**⁴, **Hans E. Purkey**⁴, **Gabriele Schaefer**⁴, **Stephen Schmidt**⁴, **Eileen Seward**³, **Steve Sideris**⁴, **Lily Shao**⁴, **Shumei Wang**⁴, **Siew Kuen Yeap**³, **Ivana Yen**⁴, **Christine Yu**⁴, **Timothy P. Heffron**⁴. (2) Genentech, Belmont, California, United States (3) Charles River Laboratories, Harlow, United Kingdom (4) Genentech, South San Francisco, California, United States

Inhibitors targeting the activating mutants of the epidermal growth factor receptor (EGFR) have found success in the treatment of EGFR-mutant positive non-small cell lung cancer. A secondary point mutation (T790M) in the inhibitor binding site has been linked to the acquired resistance against the first generation therapeutics. Recently, a number of inhibitors designed to covalently inhibit both the activating and T790M resistance EGFR mutants have been reported to be effective in the clinic. Unfortunately, cases of a cysteine-to-serine (C797S) point mutation that conferred resistance to those covalent inhibitors have emerged. In this presentation, we describe the lead optimization of a series of *noncovalent*, pan-mutant selective EGFR inhibitors that should complement the covalent EGFR inhibitors.

Using a noncovalent T790M resistant mutant selective EGFR inhibitor as a starting point, activities against the activating mutants (L858R and del₇₄₆₋₇₅₀) were introduced through a series of structure-guided modifications to give a set of novel, pan-mutant inhibitors. The in vitro ADME-PK properties of the lead molecules were further optimized through a number of rational structural changes. The resulting inhibitor exhibited excellent cellular activity against both the activating (L858R and del₇₄₆₋₇₅₀) and resistance mutants (T790M/L858R and T790M/del₇₄₆₋₇₅₀) of EGFR, demonstrated target engagement in vivo and ADME-PK properties that are suitable for further evaluation.



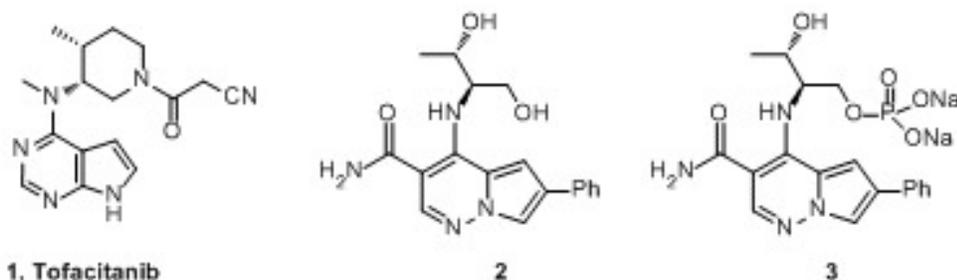
MEDI 25

Discovery of 4-(((2R,3R)-1,3-dihydroxybutan-2-yl)amino)-6-phenylpyrrolo[1,2-b]pyridazine-3-carboxamide (BMT-1438), a potent JAK1/3 inhibitor and the use of a phosphate prodrug in demonstrating efficacy in a rheumatoid arthritis model

Steven H. Spergel, *steven.spergel@bms.com*, Michael Mertzman, James Kempson, Junqing Guo, Sylwia M. Stachura, Lauren Haque, Jonathan Lippy, Rosemary Zhang, Michael A. Galella, Sidney Pitt, Guoxiang Shen, Abbera Fura, Kathleen Gillooly, Kim McIntyre, Vicky Tang, John S. Tokarski, Jack Sack, Javed Khan, Percy H. Carter, Joel C. Barrish, Steven Nadler, Luisa Salter-Cid, Gary L. Schieven, Steve Wroblewski, William J. Pitts. Bristol-Myers Squibb, Princeton, New Jersey, United States

Tofacitinib (1), a JAK family kinase inhibitor has been approved in the United States for rheumatoid arthritis (RA). However a lack of JAK family selectivity is believed to result in

dose limiting side effects. As part of our efforts to identify an improved profile, we prepared compound **2**, which was a functionally selective JAK1/3 inhibitor, however progression to *in vivo* efficacy models was prevented by dose limiting solubility. Identification of a suitable phosphate pro-drug (**3**) enabled advancement to a murine collagen induced arthritis model. High levels of efficacy were observed at doses which showed no effect on hematopoiesis. These results suggest a JAK1/3 inhibitor profile may have utility in the treatment of rheumatoid arthritis and other autoimmune diseases.



MEDI 26

Drug absorption and disposition influencing oral bioavailability: An industrial perspective and literature review

Christine Huang, christine.huang@bms.com. *Metabolism and Pharmacokinetics, Pharmaceutical Candidate Optimization, Bristol-Myers Squibb, Princeton, New Jersey, United States*

Drug absorption and disposition (distribution, metabolism, and excretion), known as ADME, plays a critical role in pharmaceutical research as it governs systemic exposures of a drug. Understanding these processes aids selection of new chemical entities and enables assessment of their safety. Pharmacokinetics describe the ADME processes and concentration-time relationship. Investigation of factors that affect these processes of new chemical entities helps to tackle challenges of improving oral bioavailability.

Oral absorption involves passing a drug through enterocytes. In addition to the influences from formulations and drug metabolizing enzymes (e.g., cytochrome P450 enzymes, UDP-glucuronosyltransferase), intestinal efflux transporters (e.g., P-glycoprotein, BCRP) on the apical side of enterocytes decrease oral absorption. In contrast, uptake transporters (e.g., peptide transporter 1) facilitate uptake of di- and tripeptides and increase oral absorption. Transporters (efflux and uptake) located in hepatocytes also impact drug absorption and disposition. Incorporating active hepatic uptake improves clearance prediction for statins and HIV protease inhibitors. While active hepatic uptake may decrease oral exposures, saturation may be achieved at a high dose, which increases oral bioavailability. Other factors, such as enterohepatic circulation increases systemic exposures by reabsorbing the drug excreted in the bile. To improve efficacy and limit toxicity, drug distribution to the target organ can be optimized by the knowledge of uptake transporters. For example, for statins and HCV

inhibitors, the target organ is the liver. High liver uptake reduces systemic exposures and off-target toxicity.

In summary, holistic approaches improve our understanding of the complex interplay of absorption and disposition processes affecting the oral bioavailability and bridge translation of ADME properties from preclinical studies to the clinic including selection of appropriate doses for clinical trials.

MEDI 27

First-pass intestinal glucuronidation as a potential obstacle for oral absorption of small molecule drug candidates: When should we worry?

Amit S. Kalgutkar, *amit.kalgutkar@pfizer.com*. Pfizer, Waltham, Massachusetts, United States

Uridine 5'-diphosphate (UDP)-glucuronosyl transferases (UGTs) are important drug-metabolizing enzymes, which contribute to the elimination of numerous marketed drugs that contain alcohol, phenol, amine or carboxylic acid functionalities. Currently, 19 UGT proteins have been identified in humans, which are divided into three subfamilies—UGT1A, 2A, and 2B. UGT mRNAs have been detected in various tissues, with particularly abundant expression in the liver and intestine. Because UGTs are also located in the small intestine (similar to the presence of oxidative enzymes such as cytochrome P4503A4), there is a general concern that intestinal glucuronidation will have a pronounced effect on oral bioavailability (F). For example, the extremely low oral F (~ 2%) of raloxifene in humans has been attributed to significant intestinal first-pass glucuronidation. With the increasing awareness of the importance of intestinal first-pass metabolism by P4503A4 and UGTs in humans, *in vitro* and *in vivo* studies in animals and humans are considered important in projecting human oral absorption for investigational drug candidates. Limited information is available, however, on the intestinal glucuronidation capacity (including mRNA expression of relevant UGT isoforms and species differences in glucuronidation) in animals relative to humans. This presentation will highlight tactics deployed in studying intestinal glucuronidation in a preclinical discovery setting using *in vitro* reagents and animal pharmacokinetics studies. Using literature examples and a recent case study with an AMP kinase activator PF-06409577, data will be presented to demonstrate the perils in extrapolating animal oral F to humans without a thorough mechanistic understanding of species differences in intestinal glucuronidation including the role of tissue-specific UGT isoforms.

MEDI 28

Role of drug metabolizing enzymes in oral bioavailability

Cyrus Khojasteh, *pars@gene.com*. DMPK, Genentech, South San Francisco, California, United States

Oral bioavailability of a drug is influenced by several factors including the role that drug-metabolizing enzymes (DME) play at the intestinal and hepatic regions. These enzymes perform reactions such as Phase I oxidative, reductive and hydrolytic reactions to Phase II conjugative reactions. The list could even be expanded to include microbiome potential involvement in metabolizing the drug or its metabolites. In this talk we will cover several examples to demonstrate the role of the enzymes plus the tools that could be used for this purpose to assess oral bioavailability.

MEDI 29

Role of early solubility measurements in predicting bioavailability challenges and subsequent formulation strategies to enable compounds

W P. Wuelfing, pete_wuelfing@yahoo.com. Discovery Pharmaceutical Sciences, Merck & Co., Inc., Kenilworth, NJ, USA , West Point, Pennsylvania, United States

Small molecule solubility profiling measurements whether from high throughput or traditional shake-flask means lead to a reasonable prediction of development risk with minimal investment. These measurements should be considered in light of other physicochemical properties such as lipophilicity and crystallinity to decide on proper formulation development for toxicology and clinical development. The talk will be aimed at rapidly identifying examples of detection of favorable and unfavorable properties in drug discovery and then practical formulation examples to overcome bioavailability issues.

MEDI 30

Mesenteric lymph: A conduit to enhanced oral bioavailability and immune cell targeting

Christopher Porter^{1,2}, chris.porter@monash.edu. (1) Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, Victoria, Australia (2) ARC Centre of Excellence in Convergent Bio Nano Science and Technology, Parkville, Victoria, Australia

The lymphatic system comprises a network of vessels, nodes and aggregated lymphoid tissues distributed throughout the vascular regions of the body. The lymphatics are responsible for the maintenance of fluid balance, and play a significant role in the intestinal absorption and transport of neutral fats. The lymphatics are also implicated in the maintenance of an effective immune defense mechanism, the metastatic spread of some cancers and the development of metabolic disease. Promotion of drug delivery to the lymphatic system therefore provides a number of pharmacokinetic and pharmacodynamic advantages including a reduction in first pass metabolism (and an increase in oral bioavailability) and lymphatic exposure to drug concentrations orders of magnitude higher than that attained in systemic blood. After oral administration, lymphatic access is facilitated by drug association with colloidal lipoproteins in the

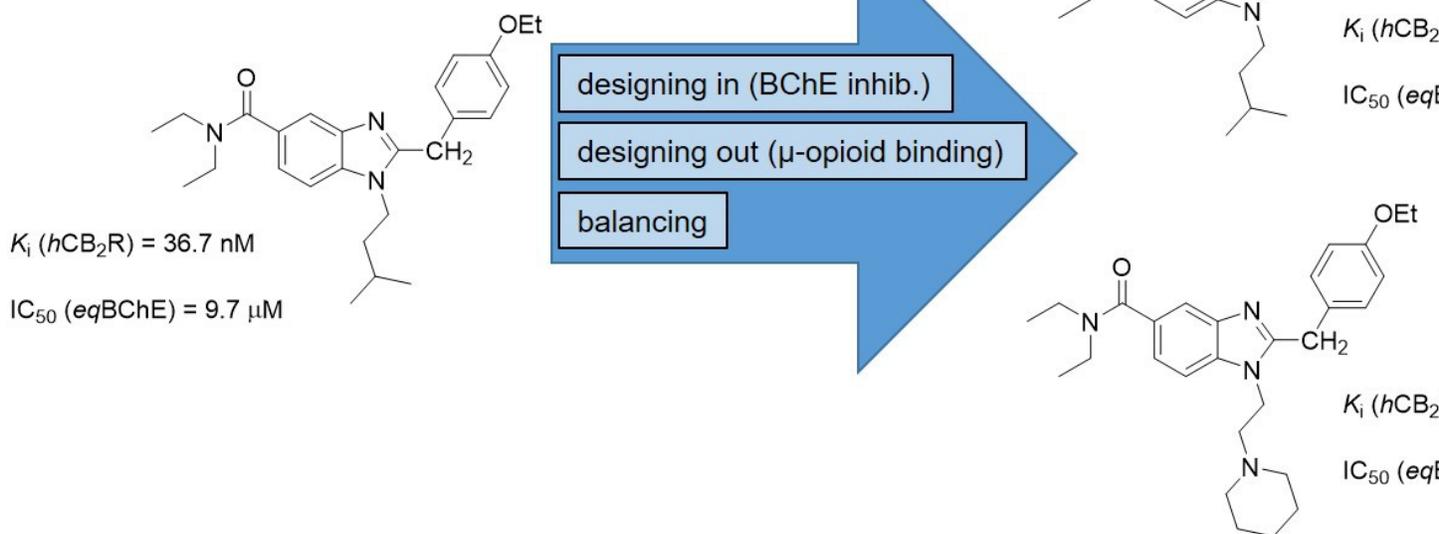
enterocyte, the size of which precludes ready diffusion into the blood, but allows transport across the (more highly permeable) lymphatic endothelium. Lymphatic transport is therefore dependent on lipoprotein association and co-administration with a lipid source to promote lipoprotein formation. This presentation will firstly review the routes and mechanisms of drug access to the lymphatic system and illustrate the factors that affect the extent of lymphatic access with a series of case studies. Secondly, recent data will be presented describing the use of lipid prodrugs that integrate into the biochemical pathways of lipid absorption and significantly enhance lymphatic transport and lymphocyte uptake after oral administration.

MEDI 31

Aminobenzimidazoles and structural isomers: Design, synthesis, pharmacological evaluation and computational studies of novel dual-acting butyrylcholinesterase inhibitors and hCB_2 receptor ligands for the treatment of Alzheimer's disease

Dominik Dolles¹, *dominik.dolles@uni-wuerzburg.de*, Edgar Sawatzky¹, Jan Möller², Massimo Nabissi³, Antonios Drakopoulos¹, Andrea Strasser⁴, Hans-Joachim Wittmann⁴, Martin Lohse², Michael Decker¹. (1) Pharmaceutical and Medicinal Chemistry, Julius-Maximilians Universität Würzburg, Würzburg, 97074, Germany (2) Pharmacology and Toxicology, Julius-Maximilians-Universität Würzburg, Würzburg, 97074, Germany (3) Scuola di Scienze del Farmaco e dei Prodotti della Salute, Università di Camerino, Camerino, Marche, Italy (4) Pharmaceutical and Medicinal Chemistry II, Universität Regensburg, Regensburg, Bavaria, Germany

The multifactorial nature of most neurodegenerative diseases makes the development of multifunctional / dual-active drugs an emerging field of interest. Dual-active compounds are small molecules that stick to Lipinski's rule of five in which merged entities are able to address two distinct biological targets. The development follows three common paradigms: designing in of desired pharmacophores, designing out of interactions with unwanted target and balancing of the activities. We hereby present a set of novel dual-acting compounds that act both as selective hCB_2 receptor agonists and butyrylcholinesterase (BChE) inhibitors for the treatment of Alzheimer's disease. All compounds show high selectivity over hCB_1 receptor and acetylcholinesterase (AChE) and balanced submicromolar activities at both targets. Furthermore, activities in the micromolar range at the μ -opioid receptor were determined. The application of the three paradigms and the interplay of design, synthesis, pharmacological evaluation and computational studies led from first-generation leads – a set of benzimidazole-based molecules – to a second-generation leads with aminobenzimidazole core structure. The application of molecular dynamics and docking of both the first and second generation leads on both targets helps to understand their binding mode and pave the way for further investigations.



MEDI 32

Development of small molecules to modulate Apoe and Abca1/Ldlr levels for Alzheimer's therapy

Nisha John¹, nishamj5@mail.fresnostate.edu, **Irina Boginski**¹, **Amanda Voigt**², **Rafael Remotigue**¹, **Jaekwang Kim**³, **Jungsu Kim**³, **Santanu Maitra**¹. (1) Chemistry Dept MS SB 70, California State University Fresno, Fresno, California, United States (2) University of California, San Francisco, SFO, California, United States (3) Department of Neuroscience, Mayo Clinic, Jacksonville, Florida, United States

The abnormal proteolysis of amyloid precursor protein (APP) causes the deposition of oligomerized Ab plaques. Genetic isoform apolipoprotein E4 (Apoe4) has been shown to be a risk factor for Alzheimer's disease. We had earlier identified triarylmethylamine analogs as hit to inhibit Apoe in human brain cells. Further modification and optimization led to sulfonamide analogs as second-generation lead molecules. *In vitro* biological studies exhibited that sulfonamides acted as Abca1 inducer and/or Ldlr agonist in addition to Apoe inhibition in transgenic mouse brain cells. Our preliminary results led us to hypothesize that this ability for Apoe inhibition in conjunction with Abca1 induction/Ldlr agonism has the potential for Ab plaque clearance in diseased mouse brain. Thus, small molecules with such activity profile could potentially benefit in not only understanding the complex mechanism of Alzheimer's disease, but also in its therapy. Structure-Activity Relationship (SAR) studies are continuing to improve upon efficacy, potency, and selectivity in order to gain a deeper insight.

MEDI 33

Design, synthesis and biological evaluation of 3-hydroxy-4H-pyranone derivatives as potent MTDLs for Alzheimer's disease

Rong Sheng, shengr@zju.edu.cn, Jiacheng Liu, Liu Jiang, Li Tang. College of Pharmaceutical Sciences, Zhejiang University, Hangzhou, China

Alzheimer's Disease is the most common form of dementia, with etiology remained elusive due to the multiple pathogenic mechanisms. The multi-target-directed ligands (MTDLs) exhibit promising therapeutic potential. Among all the factors related, the A β aggregation, metal ion dyshomeostasis, and production of reactive oxygen species demonstrated close relation to each other.

A rational pharmacophore combination strategy was used by connecting phenoxy-propyl amino moiety (H₃ receptor antagonism pharmacophore) with 3-hydroxy-4-pyranone group (metal chelating and anti-oxidation moiety) to get novel derivatives. With the modification of terminal amine groups and substituents, a novel series of MTDLs were designed and synthesized as potential AD therapeutic agents, which were evaluated for *in vitro* biological activities. All target compounds (Table 1) demonstrated good to excellent H₃ receptor antagonistic activity, potent A β ₁₋₄₂ aggregation inhibitory activity, and good antioxidant activity, indicating the rationality of our design strategy. The most promising MTDLs **I-a** and **I-b** were selected for further biological evaluation.

Our research results revealed that these novel compounds can display multiple functions related to AD, with good to excellent H₃ receptor antagonistic activity, potent A β ₁₋₄₂ aggregation inhibitory activity, and good antioxidant activity, providing solid basis for the development of novel MTDLs for AD therapy.

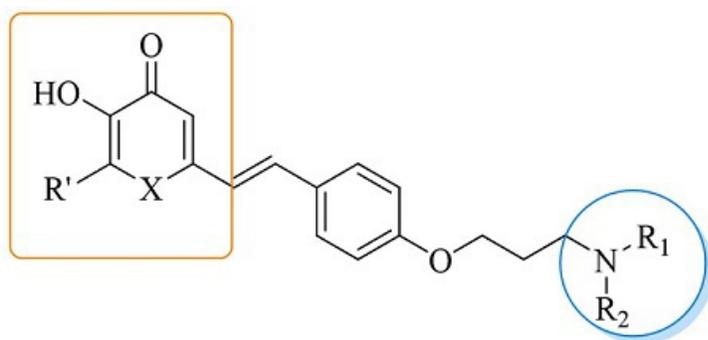


Fig.1 The basic structure of target compound and modification

Compd.	hH ₃ R IC ₅₀ (nM) ^b	Aβ ₁₋₄₂ IC ₅₀ (μM)	ABTS ⁺ IC ₅₀ (μM)
I-a	8.25	2.24 ± 0.07	2.74 ± 0.07
I-b	2.74	3.26 ± 0.05	2.61 ± 0.06
I-c	68.8	4.12 ± 0.10	3.10 ± 0.25
I-d	16.9	3.95 ± 1.70	2.68 ± 0.15
I-e	3.37	3.29 ± 1.72	10.61 ± 0.24
I-f	4.74	2.57 ± 0.33	11.05 ± 0.27
I-g	30.1	5.32 ± 0.17	10.08 ± 0.13
I-h	7.06	5.38 ± 0.13	9.52 ± 0.37
I-I	85.4	6.18 ± 0.01	10.16 ± 0.31
I-j	2.18	2.07 ± 0.07	9.53 ± 0.07
I-k	5.07	3.05 ± 0.02	7.65 ± 0.11
I-l	4.91	7.01 ± 0.47	6.41 ± 0.50
I-m	2.65	6.26 ± 0.26	7.82 ± 0.40
Curcumin	n.t. ^c	8.27 ± 1.51	12.24 ± 0.64
Trolox	n.t. ^c	n.t. ^c	13.62 ± 0.11
Clobenpropit	1.32	n.t. ^c	n.t. ^c

Table 1 H₃R antagonist activities, Aβ₁₋₄₂ inhibition activities and antioxidant activities of target compounds

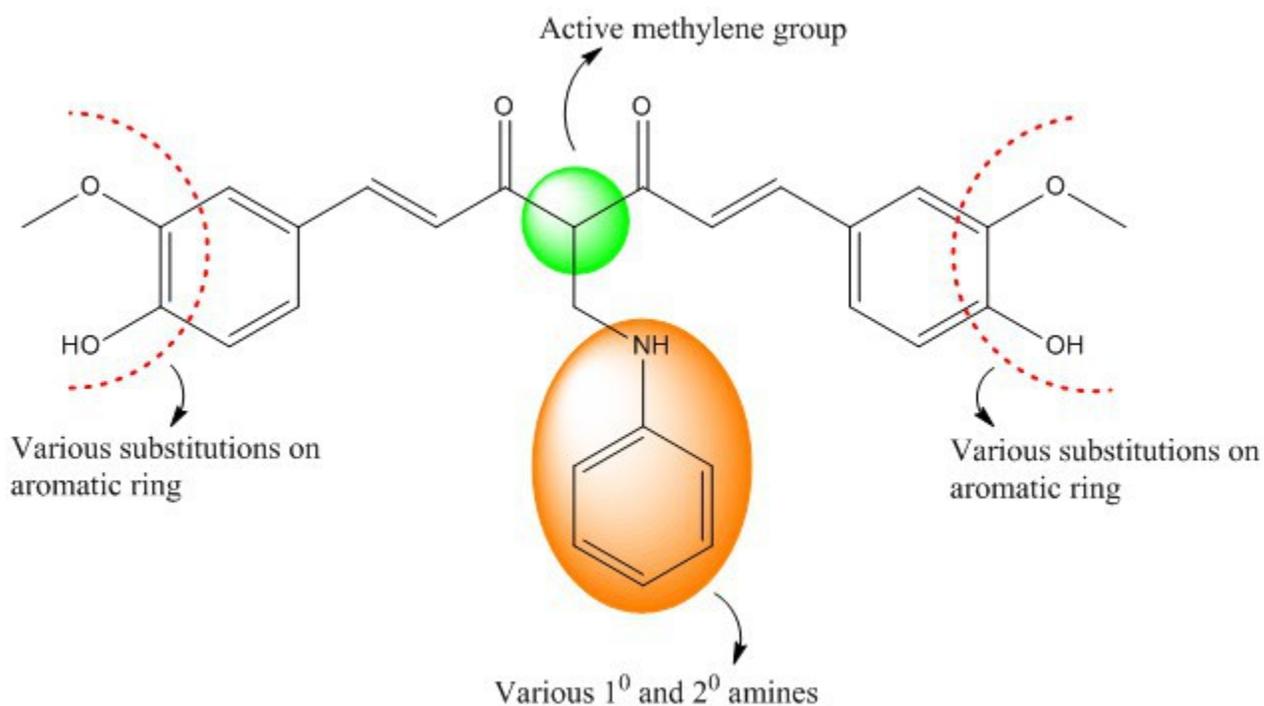
MEDI 34

Acetyl cholinesterase inhibitory and toxicity profiles of tacrine-curcumin hybrids

Rajasekhar Alavala^{2,3}, *sekhar7.pharm@gmail.com*, *Ganapathi Tipparapu*⁴, **Shireesha Boyapati**^{1,2}, *sirimedchem@gmail.com*, *Umasankar Kulanthaivelu*², *Rajanna Ajmeera*⁴, *Bhagavan Raju Mantriprgada*⁵. (1) Department of Pharmaceutical Chemistry, Telangana University, Nizamabad, Telangana, India (2) Department of Pharmaceutical Chemistry, Vaagdevi College of Pharmacy, Warangal, Telangana, India (3) Research

and Development Division, Jawaharlal Nehru Technological University, Hyderabad, Telangana, India (4) Stem Cell Research Division, National Institute of Nutrition, Hyderabad, Telangana, India (5) Sri Venkateshwara College of Pharmacy, Hyderabad, Telangana, India

Application of tacrine in Alzheimer's therapeutics is limited by multiple dosing and its hepatotoxic metabolite 7-hydroxytacrine. On the other hand, curcumin, a traditional Indian herb has demonstrated activities of scavenging radicals, blocking A β aggregation, acetyl cholinesterase inhibition and chelation of metal ions. In the present study, some new hybrid molecules of curcumin analogues and tacrine were designed as prodrugs with reduced toxicity profiles in comparison with simultaneous administration of curcumin and tacrine. A synergistic action was observed, due to simultaneous release of curcumin and tacrine. Ten hybrid molecules were synthesized through selective Mannich reaction of the active methylene group of curcumin with the primary amine of tacrine analogues. The molecules were evaluated *in vitro* for cholinesterase inhibition, antioxidant activity, A β aggregation inhibition and compared with curcumin as well as tacrine. The binding modes and toxicity of the molecules were initially predicted by *in silico* approaches, and selected molecules were subjected to metabolic and toxicity studies to probe their liver toxicity profiles and tumorigenicity.



MEDI 35

Design, synthesis, and in vitro evaluation of novel sigma-2 receptor modulators: An opportunity in Alzheimer's disease therapy

Kevin Blattner¹, *tuf28307@temple.edu*, **Daniel J. Canney**^{1,2}, **Rong Gao**², **Richie Bhandare**², **John C. Gordon**¹, **Magid Abou-Gharbia**¹, **Nicholas J. Izzo**³, **Nicole Knezovich**³, **Colleen Silky**³, **Kelsie Mozzoni**³, **Susan Catalano**³, **Gilbert Rishton**³, **Benjamin E. Blass**^{1,2}. (1) Moulder Center for Drug Discovery Research, Temple University, Philadelphia, Pennsylvania, United States (2) Department of Pharmaceutical Sciences, Temple University, Philadelphia, Pennsylvania, United States (3) Cognition Therapeutics Inc., Pittsburgh, Pennsylvania, United States

Alzheimer's disease (AD) continues to be a significant unmet medical need. According to the CDC, in 2015, AD was the 5th leading cause of disease-related deaths in people over age 65. Current therapies such as Aricept (donepezil), Namenda (memantine), and Razadyne (galantamine) are palliative treatments that slow cognitive decline by regulating neurotransmitters, but there are no clinically approved disease modifying therapies for the treatment of AD. The lack of valid targets is a major factor contributing to the paucity of available AD treatments. One of the early hypotheses on the pathophysiology of AD stated that the formation of A β protein plaques and Tau protein tangles resulted in synaptotoxicity in neurons leading to significant cognitive defects. An alternative hypothesis, the oligomer hypothesis of AD, states that soluble A β oligomers (A β O) are the pathogenic agent in AD. According to this theory, A β O bind specifically and saturably to a receptor site on neurons, rapidly inhibiting long term potentiation (LTP) by altering glutamate receptor trafficking to the plasma membrane. This leads to a transient synaptic spine regression and inhibition of learning and memory. Recent published work has strongly supported the oligomer hypothesis via demonstrating that the sigma-2/PGRMC1 (sigma-2) receptor modulates the binding of A β O to receptors on neurons and that this protein can be targeted with small molecules to inhibit the binding and synaptotoxic effects of A β O. This study established that small molecules directed towards the sigma-2/PGRMC1 receptor can prevent and displace binding of A β oligomers to neurons. These first-in-class small molecule drug candidates blocked downstream synaptotoxicity and restored memory in aged transgenic mouse models of AD. Recently, we have identified a novel series of sigma-2 binders that have the significant potential for further advancement as part of drug discovery program. To date, over 100 compounds have been prepared in this new series, and many examples exhibit potent sigma-2 binding (IC₅₀ < 100nM). The synthesis, biological activity and *in vitro* ADME of this series will be discussed.

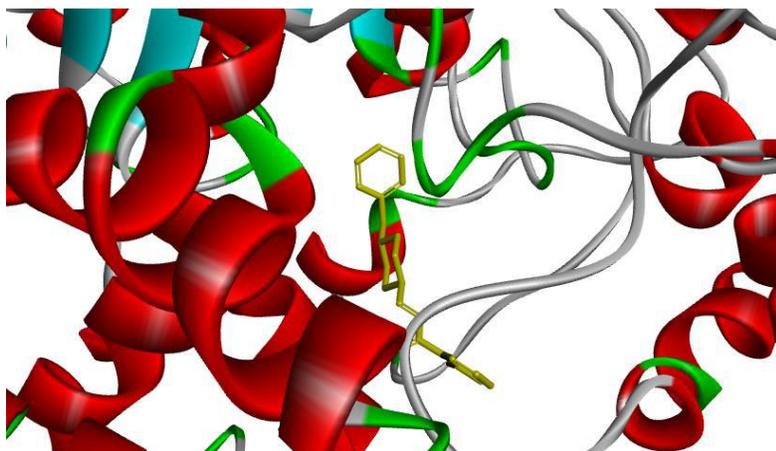
MEDI 36

Discovery of multi-target-directed ligands for the treatment of Alzheimer's disease

Wenhai Huang, *cyj@zju.edu.cn*, **Zhengrong Shen**, **Chuansheng Li**, **Qin Li**, **Xiaoliang Zhen**, **Zhen Ma**, **Meihao Liang**. *Zhejiang Academy of Medical Sciences, Hangzhou, China*

Alzheimer's disease (AD), the leading cause of dementia in the elderly, is a neurodegenerative disorder. The multiple and complexity etiologies of AD make single-

target strategy difficult to get desirable therapeutic effect. Thus Multi-Target-Directed Ligand (MTDL), which is rationally designed to hit multiple targets for a particular disease, raises as a potentially more effective strategy for AD treatment. Until now, most drugs approved for AD treatment are AChE inhibitors, which improve the ACh level in the brain by decreasing the hydrolysis of ACh. On the other hand, recent evidence indicated that dyshomeostasis of biometals (Fe, Cu, Zn) in the brain may contribute to AD pathology. Experiments also found that the levels of metal ions in AD patients are 3-7 folds higher than that of healthy individuals. Therefore, decreasing the level of metal ions in brain by using metal chelator represents another rational therapeutic approach for the treating of AD. Furthermore, both AChE and metal ions are associated with Amyloid- β ($A\beta$), which plays a central role in the pathogenesis of AD. Considering the above, we focused on multi-target-directed ligands integrated AChE inhibitors and metal chelators, which not only reduce hydrolysis of ACh and decrease the levels of metal ions in brain but also slow down the aggregation of $A\beta$. Acetophenone derivatives were designed by hybridizing AChE inhibitor rivastigmine with metal chelator. Compound BM-101 with suitable AChE inhibitory activity had been picked out to study the protein binding pattern using molecular docking model of AChE inhibitor which was built in previous work. Subsequently, it was also tested for their inhibition of $A\beta$ aggregation. Interestingly, the metabolic product of compound BM-101 by AChE also showed inhibition of $A\beta$ aggregation and metal chelating ability.



The protein binding pattern of BM-101/AChE

MEDI 37

Biased agonism at CB2 cannabinoid receptors: Implications for drug development

Rachel Hutchison^{1,2}, rachel.d.hutchison@gmail.com, Paul Prather². (1) Chemistry, University of Arkansas at Little Rock, Maumelle, Arkansas, United States (2) Pharmacology and Toxicology, University of Arkansas for Medical Sciences, Little Rock, Arkansas, United States

CB2 cannabinoid (CB2) receptors are found through out the human body, concentrating in the immune system. Activating these receptors can result in anti- and pro-inflammatory effects, analgesia, and anti-cancer effects. These effects are modulated through the recruitment of beta-arrestin. While most drugs also activate the g-protein, which has a possible psychosis link, finding a drug that has a bias toward the recruitment of beta-arrestin could increase the anti-cancer effects and decrease any adverse effects that might be cause though g-protein activation.

The bias for the drug tested, PNR-4- 20 (PNR), was determined by measuring its g-protein activation in CHO-H2- CB2 membrane and the beta-arrestin recruitment in CHO-H2- CB2 cells. The same assays were preformed on a classical non-biased cannabinoid, CP-55,940 (CP), for a comparison. To ensure the beta-arrestin recruitment internalized the receptors, down-regulation assays were preformed.

PNR activated g-protein less potently and efficaciously compared to CP. PNR recruited beta-arrestin more potently, but with equal affinity compared to CP. Chronic treatment of cells with PNR produced a more rapid down regulation of the CB2 receptor compared to CP. The bias of PNR to recruit beta-arrestin could aid in producing anti-cancer effects, while minimizing adverse effects that g-protein activation might cause, such as psychosis.

MEDI 38

Synthesis and biological evaluation of dual-target peripheral CB₁R antagonists

Malliga R. Iyer, malliga.iyer@gmail.com, Resat Cinar, Alexis R. Katz, George Kunos. National Institute on Alcohol Abuse and Alcoholism (NIAAA), Germantown, Maryland, United States

Polypharmacological approaches to drug design has become important in the recent years, This approach can be favorable for treating chronic, complex disorders where single target- based drugs can have limited benefits. Cannabinoid-1 receptor (CB₁R) and the endocannabinoid signaling system is involved in regulating many physiological functions both in the CNS and the periphery. A series of dual-target compounds based on selective, peripheral blocking of the cannabinoid-1 receptor (CB₁R) and iNOS was developed by our group recently. The first-generation, dual-target compounds revealed key structure-activity relationships and high efficacy in *in vivo* models of liver fibrosis. Building on this, a more soluble series of compounds were synthesized and evaluated in *in vitro* CB₁ receptor binding and functional assays. The structure-activity relationship in this series will be discussed. Additionally, the development of a chromatography-free synthesis of a peripherally restricted, dual-target lead compound will also be discussed.

MEDI 39

Profiling signaling bias of synthetic cannabinoid New Psychoactive Substances (NPS) at the Cannabinoid Type 1 Receptor (CB₁R)

Samuel Banister¹, *samuelb2@stanford.edu*, Kaavya K. Kumar², Brian K. Kobilka², Sanjay V. Malhotra¹. (1) Dept of Radiation Oncology, and Radiology, Stanford University, Palo Alto, California, United States (2) Department of Molecular and Cellular Physiology, 157 Beckman Center, Stanford University, Stanford, California, United States

Since the appearance of the first two examples in 2008, synthetic cannabinoids (SCs) have emerged as the most rapidly growing and dynamic chemical class of new psychoactive substances (NPS), with 177 different SCs reported to the United Nations Office on Drugs and Crime (UNODC) Early Warning Advisory (EWA) in 2014 alone. These recreational drugs are intended to emulate the effects of the low efficacy cannabinoid type 1 receptor (CB1R) agonist Δ^9 -THC, the principal bioactive component of cannabis. However, most SCs function as high efficacy CB1R agonists and, unlike Δ^9 -THC, are frequently associated with severe adverse reactions.

The toxicity of emergent SCs does not appear to be correlated to potency or efficacy of parent structures or their metabolites at CB1R. For example, cumylamine-derived SCs like 5F-CUMYL-PICA appear prevalent (based on law enforcement seizures) but are rarely observed in clinical toxicology cases, while amino acid-functionalized SCs such as MDMB-CHMICA and MDMB-FUBINACA have caused dozens of fatalities across the globe. Although these SCs demonstrate analogous potency and efficacy at CB1R in many in vitro assays, it is possible that the toxicity of SCs can be attributed to signaling bias of particular chemotypes at CB1R.

To explore the agonist trafficking of SCs at CB1R, we have employed a GTPase-Glo assay. This assay is capable of quantifying SC agonist-induced G protein-mediated activity in purified CB1R protein. We have investigated the ligand bias of several libraries of the most prevalent SCs in the presence of different G-proteins (Gi/Go family) in order to systematically characterize differences in signal transduction induced by various SC chemotypes. We have also evaluated the effect of these SCs on β -arrestin coupling to CB1R. Profiles of the functional selectivities of systemically toxic and relatively non-toxic SC NPS will be presented.

MEDI 40

Synthesis and SAR studies of somatostatin subtype-4 agonists for the treatment of Alzheimer's disease

Mahsa Minaeian, *mahsa.minaeian@gmail.com*, Albert M. Crider, Iman Daryaei, Maria Kontoyianni, William M. Kolling, William L. Neumann. Pharmaceutical Sciences, Southern Illinois University Edwardsville, Edwardsville, Illinois, United States

Alzheimer's disease (AD) is the most common form of dementia and affects an estimated 35 million people worldwide. Current medications approved for the treatment of AD (acetylcholinesterase inhibitors and NMDA antagonists) are only palliative and do not change the course of disease progression. Thus, new therapeutic strategies that are

disease modifying are desperately needed. In addition to strategies which prevent the formation of A β -derived pathologies, pathways which can enhance the degradation of A β oligomers and peptides are particularly exciting. The neuropeptide somatostatin is known to enhance the degradation of A β in the CNS through the downstream up-regulation of the endopeptidase neprilysin. Unfortunately, somatostatin levels in the CNS are significantly lowered in the aged and in AD patients. To this end, selective somatostatin subtype 4 (SST4) agonists represent a novel class of therapeutic candidates for treating AD. Herein we report lead identification and initial optimization studies of a novel and druggable class of small molecule SST4 agonists that rescue learning and memory deficits in transgenic mouse models of AD through this potential disease modifying mechanism of action. Synthesis, characterization, physiochemical properties, receptor binding/selectivity, receptor activity/function and in vivo proof of concept studies will be described.

MEDI 41

Discovery of tetrahydroquinoline-containing CXCR4 antagonists with improved ADMET properties

Eric J. Miller², ericjmiller3986@gmail.com, Edgars Jecs², Valarie Truax², Brooke Katzman², Katie Kuo², Michelle B. Kim², Robert J. Wilson², Huy H. Nguyen², Yesim A. Tahirovic², Manohar Saindane², Tao Wang¹, Chi Sum¹, Jing Chen¹, Mary E. Cvijic¹, Ding R. Shen¹, Neil Burford¹, Cliff Chen¹, Haiying Zhang¹, Andrew J. Tebben¹, Lawrence J. Wilson², Gretchen M. Schroeder¹, Dennis Liotta². (1) Research & Development, Bristol-Myers Squibb, Princeton, New Jersey, United States (2) Chemistry, Emory University, Atlanta, Georgia, United States

CXCR4 is a class A 7-transmembrane receptor that is endogenously expressed on the surface of a variety of hematopoietic cells, including T lymphocytes, which are hijacked by T-tropic HIV particles in a CXCR4-dependent manner. While this emphasizes the therapeutic potential of small molecule CXCR4 antagonists against HIV infection, CXCR4 is also upregulated by >20 different tumor types, highlighting an opportunity to utilize these inhibitors to treat cancer. CXCL12 (SDF-1), the endogenous chemokine agonist of CXCR4, is secreted by stromal cells in the tumor microenvironment, a process that has been suggested to confer CXCR4⁺ tumor cell evasion of the immune system, not only by stimulating pro-survival signaling, but also via the recruitment of CXCR4⁺ regulatory T cells that suppress immunoreactivity. In principle then, small molecule CXCR4 antagonists can inhibit proliferative signal transduction and also recondition the leukocyte/lymphocyte content of the tumor microenvironment to boost the anti-tumor immune response. Despite these implications for the development of small molecule CXCR4 antagonists against cancer, many of the CXCR4 antagonists disclosed in the literature are not orally bioavailable and demonstrate promiscuous off-target activity. Accordingly, a series of tetrahydroquinoline-containing CXCR4 inhibitors were designed, synthesized, and evaluated biologically with the goals of improving intestinal absorption, eliminating off target effects, and retaining relative on-target

potency. Structure activity relationships, as well as implications for developing small molecule CXCR4 antagonists to treat cancer, will be presented.

MEDI 42

Synthesis of novel TIQ-15 analogs with improved drug properties

Edgars Jecs¹, *ejecs@emory.edu*, **Eric J. Miller**¹, **Robert J. Wilson**⁶, **Huy H. Nguyen**⁷, **Yesim A. Tahirovic**⁸, **Michelle B. Kim**⁹, **Brooke M. Katzman**¹⁰, **Valarie T. Truax**¹¹, **Katie Kuo**¹², **Jing Chen**³, **Mary Ellen Cvijic**³, **Ding R. Shen**³, **Cliff Chen**³, **Haiying Zhang**³, **Andrew J. Tebben**³, **Chi Sum**⁴, **Tao Wang**¹³, **Neil Burford**¹³, **Gretchen M. Schroeder**³, **Lawrence J. Wilson**⁵, **Dennis Liotta**². (1) Chemistry, Emory University, Decatur, Georgia, United States (2) Chemistry Dept, Emory University, Atlanta, Georgia, United States (3) Research & Development, Bristol-Myers Squibb, Princeton, New Jersey, United States (6) Department of Chemistry, Emory University, Atlanta, Georgia, United States (13) Research & Development, Bristol-Myers Squibb, Princeton, Georgia, United States

Despite enormous progress in the development of cancer treatments such as surgery, radiation therapy and chemotherapy, cancer metastasis is a common reason for the failure of these current cancer strategies. Metastasis is a multistep cancer cell translocation from the primary source of cancer to distant organs. Chemotaxis is one of the steps in this process and is regulated by chemokines and their receptors such as **CXCL12** and **CXCR4**. G protein-coupled receptor **CXCR4** is a transmembrane protein which transmits signals mediated by its natural chemokine ligand **CXCL12**, inducing several physiological responses: chemotaxis, cell survival and proliferation, and gene transcription. The **CXCR4** receptor is over-expressed on a wide variety of cancer cell surfaces and contributes to cancer metastasis to organs with high **CXCL12** concentration. Moreover, the **CXCR4/CXCL12** axis also affects tumor growth within the primary tumor microenvironment via stimulation of pro-survival signaling cascades. Previously, the Liotta group reported **TIQ-15** - a potent **CXCR4** antagonist for HIV therapy – that can, in principle, be alternatively utilized to treat cancer. However, **TIQ-15** is not orally available, a property that is desirable to simplify management of cancer therapy. To improve the oral bioavailability of **TIQ-15**, novel analogs of **TIQ-15** with enhanced hydrophobicity were synthesized and characterized for **CXCR4** activity (Ca^{2+} flux), cell permeability, and metabolic stability. Structure-activity relationships and strategies to improve the drug properties and drug delivery will be discussed.

MEDI 43

Synthesis and SAR of TIQ-15 based CXCR4 antagonists: Identification of tetrahydroquinoline replacements

Robert J. Wilson², *r.wilson.j@gmail.com*, **Eric J. Miller**², **Edgars Jecs**², **Valarie T. Truax**², **Lawrence J. Wilson**², **Huy H. Nguyen**², **Yesim A. Tahirovic**², **Dennis Liotta**², **Gretchen M. Schroeder**¹, **Tao Wang**¹, **Haiying Zhang**¹, **Michelle Kim**². (1) Bristol Myers

Squibb, Ewing, New Jersey, United States (2) Chemistry Dept, Emory University, Atlanta, Georgia, United States

CXCR4 is the most common chemokine receptor expressed on the surface of many cancer cell types. In comparison to normal tissues, CXCR4 is overexpressed by cancer cells and correlates with cancer cell metastasis, angiogenesis and tumor growth. CXCR4 antagonists can potentially diminish the viability of cancer cells by interfering with CXCL12-mediated pro-survival signaling and by inhibiting chemotaxis. Over the last several years some academic groups and companies have developed small molecule CXCR4 antagonists that are being investigated as potential cancer therapeutics. Our group has discovered a series of novel tetrahydroisoquinoline based small molecules that are potent CXCR4 antagonists. Herein is presented recent structure-activity studies to improve upon target specificity and metabolic stability.

MEDI 44

Synthesis of 2,4,6-trisubstituted pyridines using palladium-catalyzed cross-coupling reactions and in vitro anticancer evaluation

Alicia Hernandez Campos², hercam@unam.mx, Pedro J. Trejo², Ignacio González³, Lilián Yépez-Mulia⁴, Jaime Pérez-Villanueva³, Marco A. Cerbón-Cervantes⁵, Rafael Castillo-Bocanegra¹. (1) Farmacia, Div De Estudios De Posgrado, Mexico, Mexico (2) Departamento de Farmacia, Universidad Nacional Autónoma de México, Mexico, Mexico (3) Unidad de Sistema Biológicos, Universidad Autónoma Metropolitana-Unidad Xochimilco, Mexico City, Ciudad de México, Mexico (4) Unidad de Investigación Médica en Enfermedades Infecciosas y Parasitarias, IMSS, Mexico City, Ciudad de México, Mexico (5) Facultad de Química, Departamento de Biología, Universidad Nacional Autónoma de México, Ciudad de México, Ciudad de México, Mexico

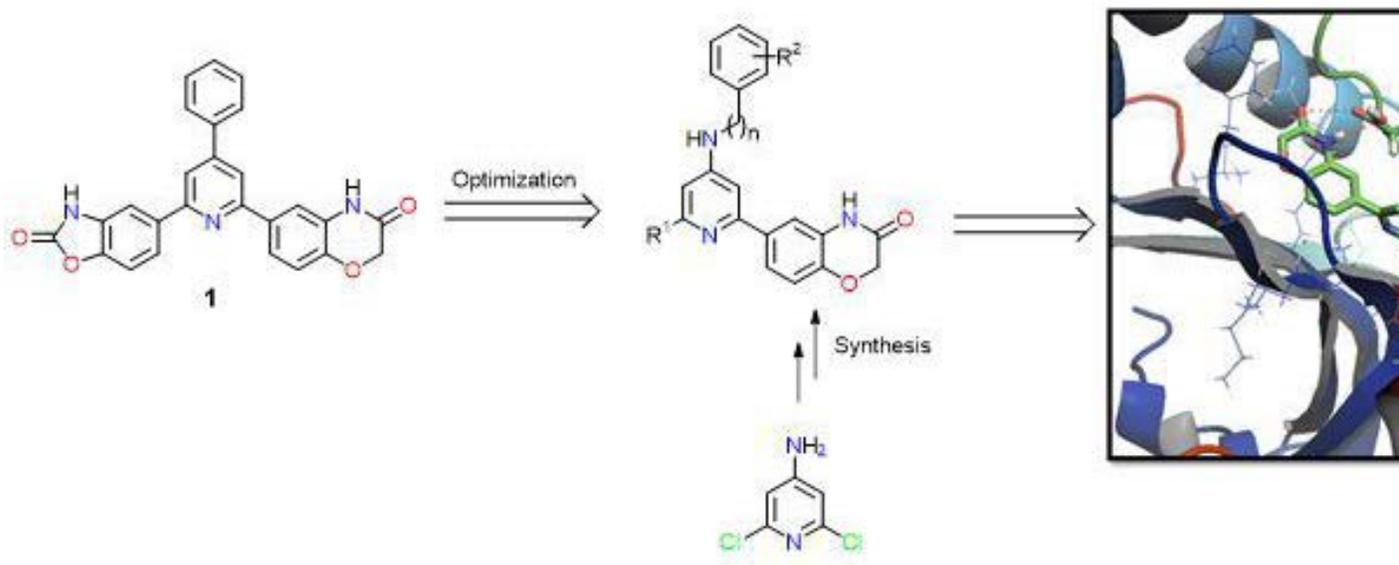
Pyridine is likely both, the simplest and the most popular *N*-azaheteroaromatic ring in the chemistry field. Polysubstituted pyridines has been widely described in the medicinal chemistry area as a convenient scaffold. In the course of our works, we proposed two series of novel 2,4,6-trisubstituted pyridines as inhibitors of the subtypes serine-threonine kinase B (AKT1, AKT2, AKT3). In this work we report the synthesis of nineteen 2,4,6-trisubstituted pyridines through the use of sequential and regioselective palladium-catalyzed Suzuki-Miyaura cross-coupling reactions, starting from versatile 2,6-dichloro-4-iodopyridine for the synthesis of compounds of Series 1, or starting from 2,6-dichloro-4-pyridinecarboxylic acid for the synthesis of compounds of Series 2. Compounds were obtained with moderate yields. In addition, their potential as anticancer agents were evaluated under cancer cell lines that overexpressed AKT subtypes.

MEDI 45

First steps in hit-to-lead optimization towards AKT inhibition

Elkin E. Sanabria-Chanaga, *eduardo48_6@hotmail.com*, **Rafael Castillo-Bocanegra**, **Alicia Hernandez Campos**. *Departamento de Farmacia, Universidad Nacional Autónoma de México, Mexico, Mexico*

Recent studies report the overexpression of protein kinase B PKB/AKT in many human cancers, making AKT a promising and attractive therapeutic target in anticancer drug development. A new ATP-competitive AKT inhibitor **1**, active in micromolar concentration, has recently been found. This compound presents a different scaffold with respect to other published AKT inhibitors. In order to identify new compounds with biological activity based on **1**, we started a hit-to-lead optimization, the first step in this process was a computational analysis of a designed database, then, a series of 2,4,6-trisubstituted pyridines were proposed using docking-based virtual screening and molecular dynamics simulation, once computational hits were identified, compounds with safe pharmacological profile were synthesized. To begin this process, 4-amino-2,6-dichloropyridine was used as starting material and a Buckwald-Hatwig coupling was carried out using different compounds containing bromine, then, a Suzuki-Miyaura coupling was performed with the appropriate boronic ester and the designed computational hits were obtained with good yields. The evaluation of the selected compounds against different cell lines and directly on AKT will be presented

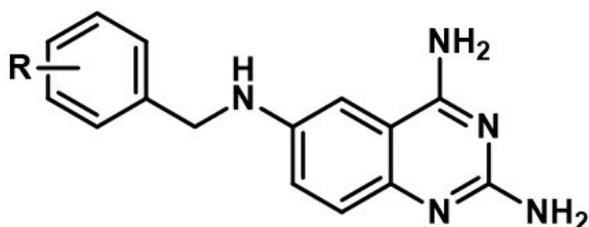


MEDI 46

Design, synthesis and biological evaluation of quinazoline derivatives as cytotoxic molecules of breast cancer triple-negative

Audifas-Salvador S. Matus-Meza¹, *audi_matus@hotmail.com*, **Francisco Hernández-Luis**¹, **Marco A. Velasco-Velázquez**². (1) *Pharmacy, Universidad Nacional Autónoma de México, Distrito Federal, Mexico, Mexico* (2) *Pharmacology, Universidad Nacional Autónoma de México, Distrito Federal, Mexico, Mexico*

Epidermal Growth Factor Receptor (EGFR) belongs to the family of tyrosine kinase receptor protein. EGFR overexpression is present in many types of tumors, being breast cancer one of the major in overexpress it and is associated with a poor prognosis, although EGFR is observed in all subtypes of breast cancer, EGFR is more commonly found in breast cancer triple-negative, which are more aggressive. Although there are EGFR inhibitor drugs that showing health benefits such erlotinib and vandetanib, most have drawbacks such as skin and gastrointestinal toxicity. In this context, it is necessary to develop new molecules that present an alternative treatment to existing drugs. A series of ten molecules analogues to the erlotinib were synthesized with different substituents of the phenyl ring, and these analogues were evaluated against the cell line MDA-MB-231. Among the molecules evaluated, the compound with substituent trifluoromethoxy presents the best antiproliferative activity with a IC_{50} of 7.50 μ M to 24 hours of exposition.



MEDI 47

Targeting specific interactions to improve EGFR-ligand binding

Chris Williams¹, Alain Ajamian¹, Nels Thorsteinson¹, **Nadia Li**¹,
private.nli@chemcomp.com, Bertrand Jean-Claude². (1) Chemical Computing Group,
Montreal, Quebec, Canada (2) McGill University, Montreal, Quebec, Canada

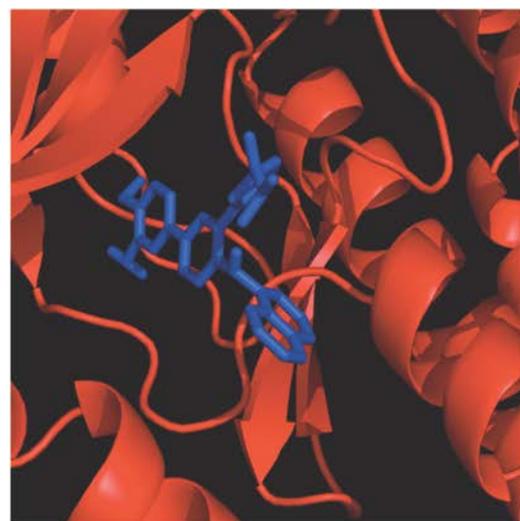
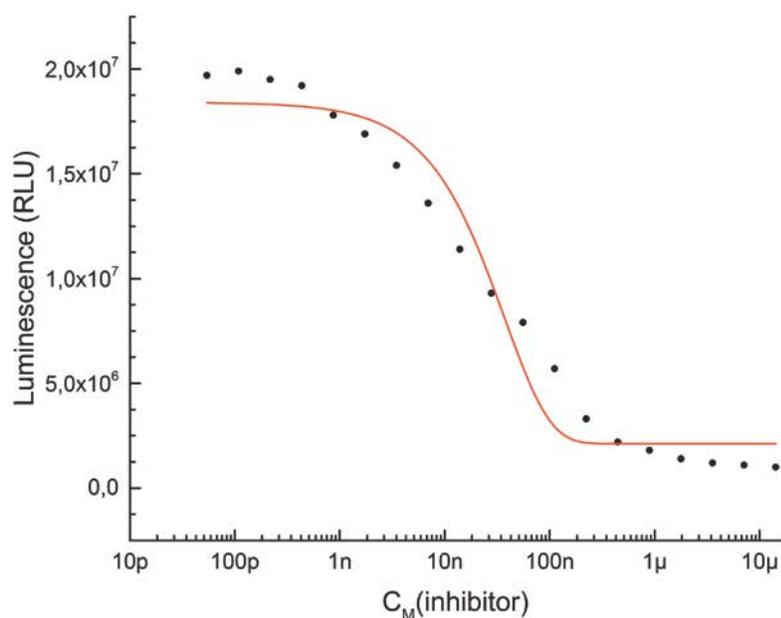
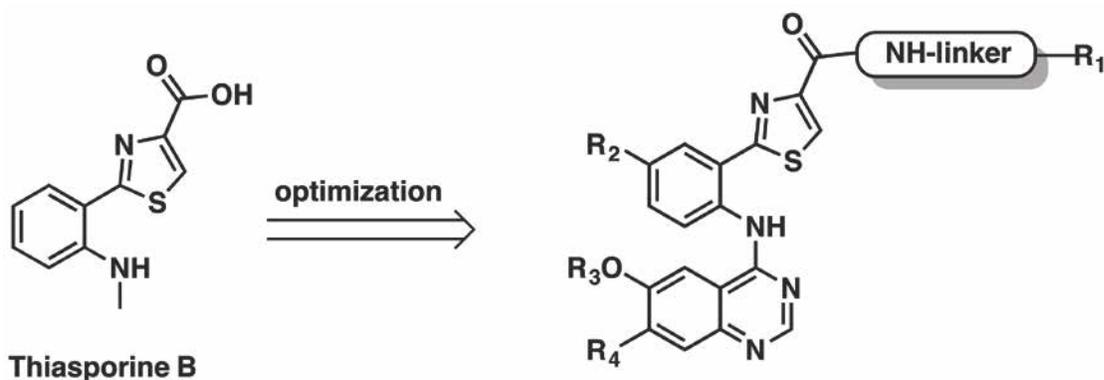
The epidermal growth factor receptor (EGFR) is implicated in many cancers, and its kinase activity is the target of commercial anti-cancer agents such as Tarceva and Iressa. However, despite their effectiveness, EGFR kinase inhibitors often show only moderate anti-proliferative activity against certain tumor types in the clinic. This inspired the investigation of dual action therapeutic agents directed not only at EGFR kinase but also at divergent targets such as Src kinase or DNA, with the purpose of producing single compounds termed “combi-molecules”, with greater potency than the single-mode EGFR inhibitor. A structure-based drug design modeling program, combined with PDB data-mining and protein structural fingerprints was used to help identify and characterize inhibitor design motifs for the development of combi-molecules. The resulting combi-molecules showed EGFR inhibitory potency in the low micromolar to nM range as well as DNA cross-linking activity.

MEDI 48

Molecular design and synthesis of inhibitors of EGFR kinase: New quinazoline derivatives

Alexander S. Bunev, Elena V. Sukhonosova, **Sergey Sokov**,
s.a.sokov.tltsu@gmail.com. Chemicals, Chemical Processes and Technologies,
Togliatti State University, Togliatti, Russian Federation

Design and synthesis of new qinazoline derivatives possessing Thiasporine B moiety are described. Their in vitro cytotoxicity activities against of one human non-small cell lung cancer were tested. Its IC₅₀ value over A549 cells and EGFR kinase were 0.098 μmol and 78.4 nM, respectively.

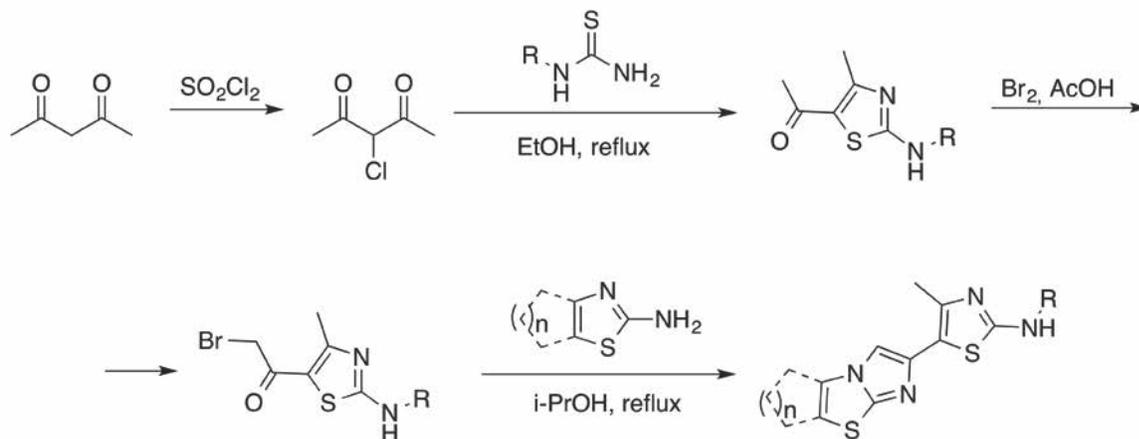


MEDI 49

Derivatives of 5-(imidazo[2,1-b]thiazol-6-yl)-4-methylthiazol-2-amine new effective EGFR-kinase inhibitors

Alexander S. Bunev, Elena V. Sukhonosova, **Kristina Talina**, *brglab@tltsu.ru.*
Chemicals, chemical processes and technologies, Togliatti State University, Togliatti,
Russian Federation

In this report will be present the main results of the synthesis derivatives of 5-(imidazo[2,1-*b*]thiazol-6-yl)-4-methylthiazol-2-amine and *in vitro* investigation their inhibiting activity against certain tyrosine-kinase (EGFR).



R = Ar, Het; n = 1-2

MEDI 50

Discovery of atropisomeric quinolinone sulfonamide (AM-0466), a potent and selective Na_v1.7 inhibitor with robust *in vivo* analgesic activity

Russell Graceffa, russellg@amgen.com. Medicinal Chemistry, Amgen, Cambridge, Massachusetts, United States

The voltage-gated sodium channel Na_v1.7, which serves as a primary driver of action potential firing and neuronal excitability, has received considerable attention for its involvement in the pain processing pathway. Genetic evidence supports the role of Na_v1.7 in a range of inherited pain syndromes and as such, selective inhibition of Na_v1.7 represents a potential method for the management of pain. Herein, we report the discovery of a novel series of atropisomeric quinolinone sulfonamides, which demonstrates nanomolar inhibition of Na_v1.7 and exhibits high levels of selectivity over the other sodium channel isoforms. Pursuant to the optimization of a number of metabolic and pharmacokinetic properties this series was advanced into *in vivo* target engagement and efficacy models. AM-0466 demonstrated robust activity in a mouse histamine-induced pruritus (itch) model of Na_v1.7 inhibition and a mouse capsaicin-induced nociception model of pain.

MEDI 51

Discovery of non-zwitterionic aryl sulfonamides as Na_v1.7 inhibitors with efficacy on preclinical behavioral and translational measures of pain

Yong-Jin Wu, *yong-jin.wu@bms.com*, Jason Guernon, Andrea McClure, Guanglin Luo, Ramkumar Rajamani, Alicia Ng, Amy Easton, Amy Newton, Clotilde Bourin, Dawn Parker, Kathleen Mosure, Omar Barnaby, Matthew Soars, Ronald J. Knox, Michele Matchett, Rick Pieschl, James Herrington, Ping Chen, D.V. Sivarao, Linda J. Bristow, Nicholas A. Meanwell, Joanne J. Bronson, Richard E. Olson, Lorin A. Thompson, Carolyn D. Dzierba. Bristol-Myers Squibb Research and Development, Wallingford, Connecticut, United States

Zwitterionic benzenesulfonamide Na_v1.7 inhibitors suffer from poor permeability, and we sought to eliminate the zwitterionic character by replacing the basic moiety with non-basic bicyclic acetals and monocyclic ethers. These efforts led to the discovery of a series of non-zwitterionic aryl sulfonamides as isoform-selective Na_v1.7 inhibitors that showed increased membrane permeability. One of the cyclic ether analogs showed robust oral activity in two mouse models of pain, suggesting that Na_v1.7 inhibitors may have potential for the treatment of pain. Lastly, the robust modulation of the nociceptive flexion reflex by Na_v1.7 inhibitors, a clinically translatable measure, identifies an objective biomarker for early clinical development.

MEDI 52

Synthesis and structure-activity relationships of morpholine-based aryl sulfonamide Na_v1.7 inhibitors

Jason M. Guernon, *guernon@comcast.net*, Andrea McClure, Ramkumar Rajamani, Ronald J. Knox, Michele Matchett, Rick Pieschl, James Herrington, Linda J. Bristow, Nicholas A. Meanwell, Richard E. Olson, Lorin A. Thompson, Carolyn D. Dzierba, Yong-Jin Wu. Bristol Myers Squibb, Wallingford, Connecticut, United States

Zwitterionic moieties, which are well known to contribute to poor pharmacokinetic properties, are prevalent in small molecule Na_v1.7 inhibitors such as piperidine-based benzenesulfonamides. One approach to mitigate zwitterionic properties is to reduce the basicity of the amino moiety. Thus, we replaced the piperidine ring with a weakly basic morpholine core to obtain a series of potent Na_v1.7 inhibitors. Structure-activity relationship studies revealed that the aryl group at C3 of the morpholine core played an important role in both activity and isoform selectivity, and optimization of the aryl group culminated in the identification of a highly potent and isoform-selective Na_v1.7 inhibitor.

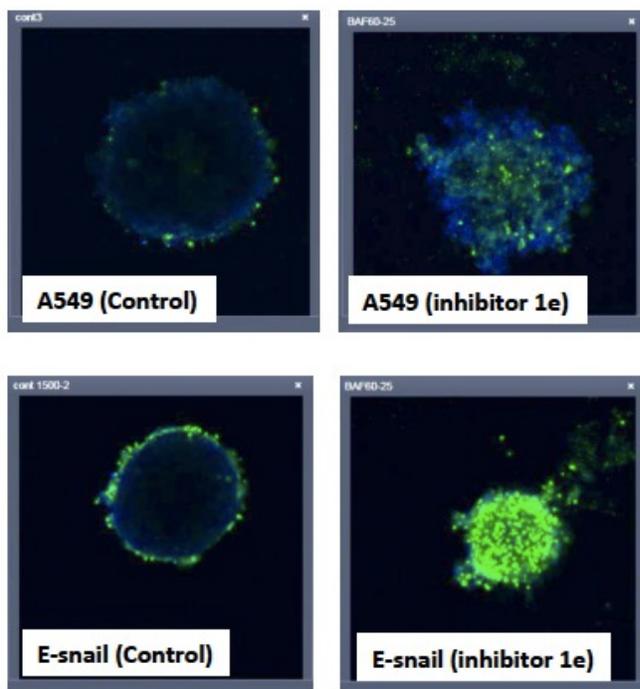
MEDI 53

Structure-based design of ATP citrate lyase inhibitors and their anticancer activities

Finith E. Jernigan^{1,2}, Steffi K. Koerner¹, Jun-ichi Hanaï³, Vikas P. Sukhatme³, **Lijun Sun**¹, *sun_lj@hotmail.com*. (1) Center for Drug Discovery, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts, United States (2) Silicon Therapeutics, Boston, Massachusetts, United States (3) Medicine, Beth Israel

Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts, United States

ATP citrate lyase (ACL) plays a critical role in generating cytosolic acetyl CoA, a key building block for *de novo* fatty acid and cholesterol biosynthesis. ACL is overexpressed in numerous cancer cells and considered as an attractive target for developing anticancer drugs. Structural analyses and MD simulation of the cocrystal structure of citrate-ACL led to our identification of a hydrophobic cleft adjacent to the charged citrate-binding domain of ACL. We implemented a structure-based approach to identify and optimize novel furan and emodin types ACL inhibitors that target specifically this previously unrecognized allosteric binding site. In the emodin series, we synthesized halogenated derivatives, aryl and amine derivatives for structure-activity relationship (SAR) studies. Among them, compound **1e** (1,3,8-trihydroxy-2,4-dibromo-6-methyl-anthraquinone) showed IC₅₀ of 2.9 μM in the ACL enzymatic activity assay and K_d of 0.65 μM in the ACL binding assay. Further, **1e** dose-dependently inhibited the proliferation of A549 lung cancer cells and significantly reduced the stemness of cancer cells as measured by 3D tumorsphere and FACS analyses. In the furan series, substituted 2-furoic acids and 3-benzofuroic acids were identified as ACL inhibitors via a virtual high-throughput screening (vHTS) of a focused library of 2,000 compounds. Remarkably, the hit rates of the vHTS protocol are 45% - 11 out of the 24 compounds selected from the vHTS potently inhibited ACL enzymatic activity. The IC₅₀ of the most potent furan derivatives ranged from 2 to 10 μM. We will discuss in details the SAR results, in silico protocols, biophysical characterizations, and DMPK analyses of the lead compounds. In addition, we will also provide an update on our on-going efforts in the identification of the first-known ATP-competitive ACL inhibitors and the co-crystallization of ACL in complex with a citrate or ATP competitive inhibitor.

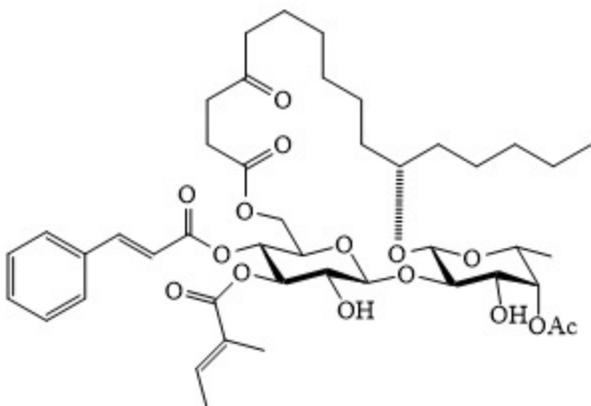


MEDI 54

Unique natural macrocycles of ipomoeassin glycoresins with potent cytotoxicity

Wei Shi, *weishi@uark.edu*. University of Arkansas, Fayetteville, Arkansas, United States

Bioactive natural products are a rich source of drug candidates as well as important tools for investigating biological systems. Ipomoeassin F is a flagship congener of a plant-derived macrolide resin glycoside family with an embedded disaccharide core. It possesses potent cell growth inhibition activity with IC_{50} values in the single-digit nanomolar range. In the NCI 60-cell line screen, ipomoeassin A—a structural homolog of ipomoeassin F—demonstrated a unique cytotoxicity profile; however, its mode of action remains largely unexplored. We recently accomplished the gram-scale production of ipomoeassin F and have been carrying out systematic studies to understand its structure-activity relationship. To further harness scientific values of this special chemical space, we report here the first studies towards target deconvolution of ipomoeassin F using cell imaging and proteomics. This work represents a significant step forward in moving the ipomoeassin family of glycolipids into the field of chemical biology for drug discovery.



MEDI 55

Bioassay protocols: Semantic annotation to enable informatics

Alex Clark, aclark.xyz@gmail.com. Research Informatics, Collaborative Drug Discovery, Burlingame, California, United States

Bioassay protocols are conspicuously absent from the informatics of drug discovery: current best practices have not progressed beyond using scientific English text, which is intractable to software. We will present our solution which draws from the rich semantic web vocabularies of the BioAssay Ontology, Drug Target Ontology, Gene Ontology, and others. On their own these ontologies are not friendly to experimental scientists, and so we have created the Common Assay Template, which turns the massive hierarchies of the underlying ontologies into useful guidelines. This has been supplemented by machine learning infrastructure to help translate existing text into suggestions, with the help of natural language analysis. Using our new web based interface, a small team of biologists were able to annotate 3500 MLPCN screening assays that were extracted from the PubChem database, which consumed approximately 3 weeks FTE.

These semantically annotated protocols are fully machine readable, which imparts many new capabilities that apply at all scales. Searching can be done using precise specific terms, which is far more effective than keyword searching. In conjunction with electronic lab notebooks, the annotations serve as a facile way to classify and organize experiments, and keep tabs on the activities of colleagues. These well defined annotations can also serve as an alternative to long-winded text.

Applied to a large scale, the machine readability of these annotations enables a diverse array of algorithms to be applied to assay databases, such as clustering and selection of groups of compatible assays for model building, or analysis of the protocol designs and their effects on structure-activity relationships. Large numbers of annotated assay protocols from open data such as PubChem and ChEMBL are available for novel analyses for big pharma, biotechnology companies, academics and consortia. We have explored a number of techniques for utilizing large quantities of data, including the

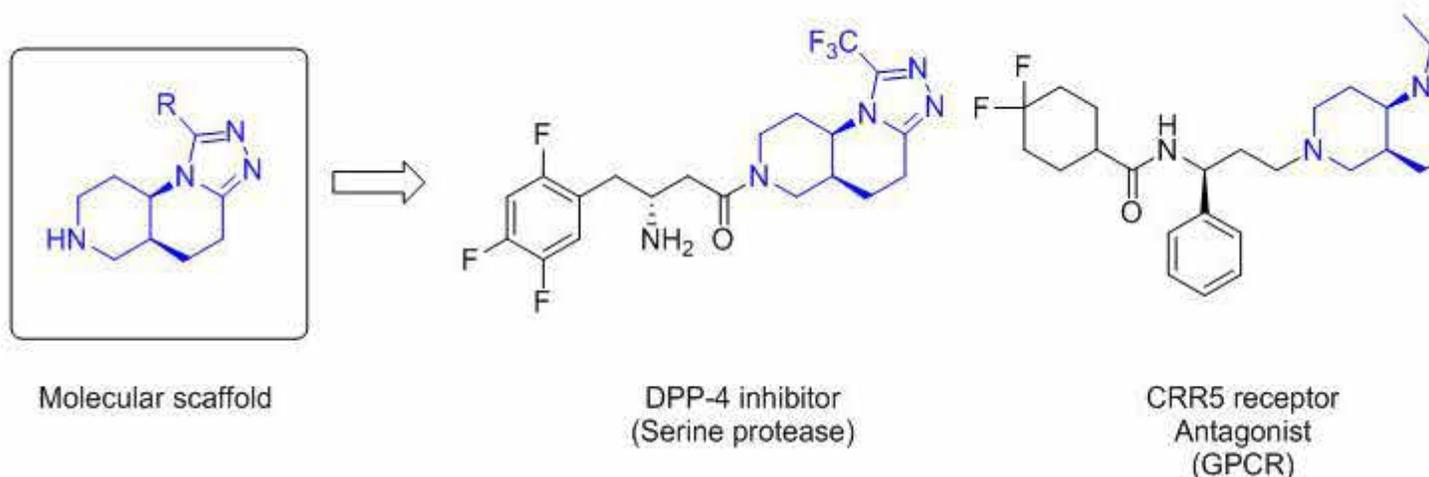
development of searching interfaces, visualization modes, and methods for extracting related data and creating models. We have also studied specific trends within public screening data which we have elucidated with the help of our own curated content, and investigated some of the characteristics of specific projects, such as the NIH Molecular Libraries Probes, which can be analyzed retrospectively given the much larger amount of information that is now available.

MEDI 56

Design, synthesis, and evaluation of a new privileged scaffold for use in drug discovery

Michael J. Stocks², *michael.stocks@nottingham.ac.uk*, **Carolyn Schwehm**², **Barrie Kellam**², **Aimie Garces**², **Nicholas Kindon**², **Tracey Bradshaw**², **Jin Li**³, **Simon J. MacDonald**¹, **James Rowedder**¹. (1) GlaxoSmithKline, Stevenage, United Kingdom (2) School of Pharmacy, University of Nottingham, Nottingham, United Kingdom (3) HitGen, Chengdu, China

A new tricyclic molecular scaffold has been synthesized and its incorporation to generate novel analogues of known drugs across multiple target classes is presented. We highlight 3 case histories where the scaffold has been used to synthesize a series of potent reversible antagonists of the C-C chemokine receptor type 5 (CCR5), potent inhibitors of the serine dipeptidyl peptidase-4 (DPP-4), and highly potent and selective phosphatidylinositol-3-kinase δ isoform (PI3K δ) inhibitors. We will compare the biological activity and physicochemical properties of the resulting inhibitors to the known drugs, demonstrating the utility of the novel privileged molecular scaffold to synthesize lead-like compounds.



MEDI 57

From chemical similarity to rational polypharmacology

Matthew J. O'Meara³, mattjomeara@gmail.com, Xi-Ping Huang⁴, Bryan L. Roth², Brian Shoichet¹. (1) Univ of Calif San Fran, San Francisco, California, United States (2) Univ of North Carolina CHPL HI, Chapel Hill, North Carolina, United States (3) Pharmaceutical Chemistry, University of California, San Francisco, Toronto, Ontario, Canada (4) Department of Pharmacology School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, United States

Sometimes functionally-related proteins recognize similar small molecules. To explore these as targets for polypharmacology, we used functional genomic networks to prioritize proteins linked by the Similarity Ensemble Approach (SEA) method. We focused on sequence dissimilar proteins pairs not previously known to share even a single ligand. On testing, we found eight pairs of proteins where a newly predicted ligand modulated the activity of both proteins, binding in the 5 nM to 13 μ M range. We further investigated in depth targeting a pair of proteins involved in synaptic dopamine release. Such functionally relevant bridging ligands may be broadly sought by this approach.

MEDI 58

Quantum chemistry calculation-aided optimization of novel microtubule-targeting agents binding to colchicine site

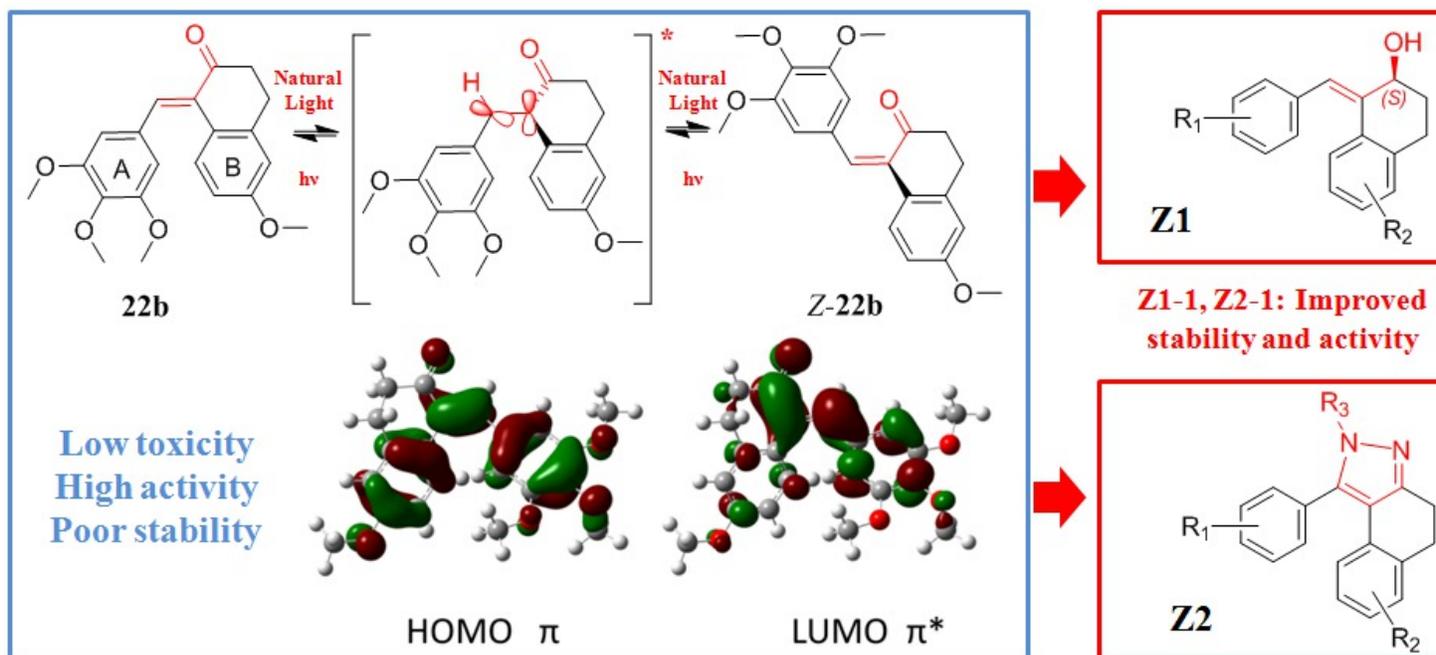
Canhui Zheng, canhuizheng@smmu.edu.cn, Junhang Jiang, Jia Liu, Ju Zhu, Youjun Zhou. Second Military Medical University, Shanghai, China

Microtubule-targeting agents (MTAs) binding to Taxol site and Vinca site have been largely used for cancer treatment. The Colchicine binding site inhibitor (CBSI) combretastatin A-4 (CA-4) was found to have vascular-disrupting effects and its prodrugs are currently being investigated in clinical trials. We recently found a novel CBSI **22b** with strong antitumor activity and lower toxicity. However, it was found to easily undergo *cis-trans* isomerization under natural light, and the resulting decrease in activity limits its further applications.

To solve this problem, time-dependent density functional theory (TD-DFT) calculations were used to explore the molecular basis of its instability. The results showed that the wavelength required for the $\pi \rightarrow \pi^*$ electron excitation of **22b** is in the visible spectrum, and the carbonyl group and conjugated double bond linking the phenyl moieties contribute most to the π^* orbital.

Aided by the calculations, two types of structural optimizations of **22b** were conducted. Firstly, the carbonyl group was changed to groups without double bond, such as a hydroxyl, which could increase the excited state energy and shorten the excitation wavelength to UV spectrum. Among the synthesized inhibitors, compound **Z1-1** was confirmed to have higher stability, and activity *in vitro* and *in vivo* than **22b**. Secondly, the carbonyl group was cyclized with the conjugated double bond, and this restricts the conformation in its active form. Among the synthesized inhibitors, compound **Z2-1** showed better activity *in vitro* than **22b** and **1** in NCI-60 human tumor cell line

anticancer drug screen. This study provides novel molecular scaffolds for the further development of antitumor agents that target tubulin, and further studies are ongoing.



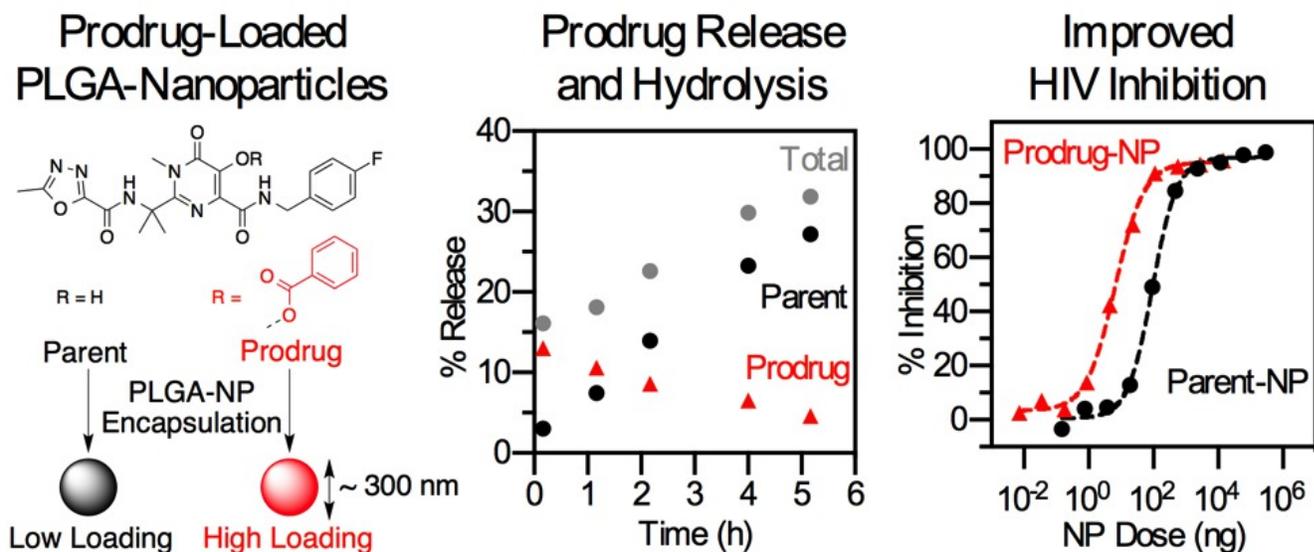
MEDI 59

Enhanced delivery of HIV integrase inhibitors with prodrugs designed for polymeric nanocarriers

Wilma E. Afunugo¹, *wilma4live@yahoo.com*, **Mikaela E. Ebner**¹, **Alaina M. Bever**¹, **Shijie Cao**², **Yongzhou Jiang**², **Kim A. Woodrow**², **Ian T. Suydam**¹. (1) Department of Chemistry, Seattle University, Seattle, Washington, United States (2) Department of Bioengineering, University of Washington, Seattle, Washington, United States

Polymeric nanocarriers have been extensively used to improve the delivery of hydrophobic drugs, but often provide low encapsulation efficiency and percent loading for hydrophilic compounds. In particular, insufficient loading has limited the development of sustained-release therapeutics against HIV, where many of the most promising drug combinations include a hydrophilic member. To address the low encapsulation observed for the HIV integrase inhibitor raltegravir (RAL) we developed a prodrug strategy where the loading, release and subsequent hydrolysis can be tuned by promoiety selection. Prodrugs with large partition coefficients increased the encapsulation efficiency by as much as 25-fold relative to RAL, leading to significant dose reductions in antiviral activity assays. The differential hydrolysis rates of these prodrugs also led to distinct patterns of RAL availability and observed antiviral activity, likely reflecting changes in the temporal distribution of both prodrug and RAL in cellular compartments. Our results suggest that the design of prodrugs for specific polymeric

nanocarrier systems could provide a more generalized strategy to formulate physicochemically diverse hydrophilic drugs with a number of biomedical applications.



MEDI 60

Synthesis and biological evaluation of sulfonyl piperazine derivatives for LpxH inhibition

Minhee Lee¹, ml216@duke.edu, **Jinshi Zhao**¹, **Jae Cho**¹, **Do-Yeon Kwon**¹, **Pei Zhou**¹, **Jiyong Hong**². (1) Duke University, Raleigh, North Carolina, United States (2) Chemistry/Box 90346, Duke University, Durham, North Carolina, United States

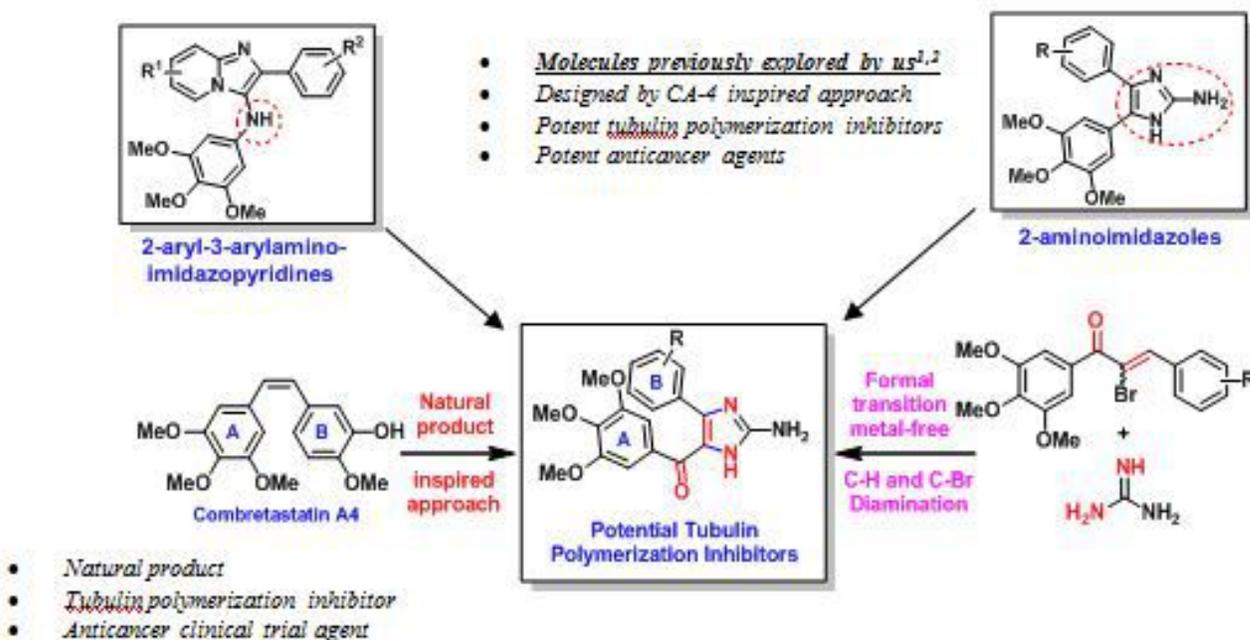
The outer leaflet of the outer membrane of Gram-negative bacteria consists of the lipid A component of lipopolysaccharide (LPS). Lipid A plays a critical role in eliciting the host response to bacterial infection. As lipid A biosynthesis is required for the survival and fitness of most Gram-negative bacteria, essential lipid A biosynthetic enzymes, including LpxH that is found in the vast majority of Gram-negative human pathogens, are attractive targets for the development of novel antibiotics. Here, we describe the chemical synthesis and LpxH inhibition of a series of sulfonyl piperazine derivatives to establish the structure-activity relationship and binding mode of these novel compounds.

MEDI 61

Creating new from clinical agents: Discovery of Combretastatin A-4 inspired heterocycles as antitubulin anticancer agents

Neha Hura, nehahura31@gmail.com, **Sankar K. Guchhait**. Niper, Mohali, India

“The most fruitful basis for the discovery of a new drug is to start with an old drug”- Sir James Black, Nobel Laureate in Physiology and Medicine said. Natural products have inspired the discovery of new drugs. Combretastatin A-4 (CA-4) is a natural product that has led to the discovery of several tubulin polymerization inhibitors. As our interest of medicinal chemistry research on anticancer drug discovery, in the present study, 2-aminoimidazole with carbonyl linker as key scaffold was considered as replacement of the double bond of CA-4. Other important pharmacophoric features like 3,4,5-trimethoxy substitution on the ring A, relevant substituted aryl as ring B, cis configuration between rings A and B and an additional linker like carbonyl group to provide the optimal dihedral angle for binding at the colchicine binding site were also considered in the design of novel 2-aminoimidazole-bridged analogs. A new and efficient approach for the synthesis of 4-aryl-5-aryl substituted 2-aminoimidazoles via previously unknown formal diamination of guanidine with α -bromoalkene has been developed. Utilizing this method, 2-aminoimidazole analogs with relevant substitutions, were synthesized. Most of the compounds were found to exhibit pronounced antiproliferative activities in nanomolar concentrations. Compared to CA-4, they were also found to be significantly potent in tubulin polymerization inhibition. Single crystal X-ray analysis confirmed the required geometry/orientation present in the structure. The details of development of synthetic method, mechanistic aspects, biological activities and SAR will be presented.



MEDI 62

Matching medium characterization for microwave brain stroke imaging

Tuba Yilmaz², Gozde A. Eken¹, Emine Yildirim¹, **Metin H. Acar¹**, macar@itu.edu.tr, Ibrahim Akduman². (1) Chemistry, Istanbul Technical University, Istanbul, Turkey (2)

Electronics and Communication Engineering, Istanbul Technical University, Istanbul, Turkey

Due to the high dielectric properties of brain tissues, the matching medium is necessary to ensure the necessary power transfer into the tissues for imaging of the stroke. Preliminary analysis of the matching medium dielectric properties are presented in the literature. It was reported that the medium can have a permittivity between 10 to 40 and the recommended permittivity was 40 between 0.6 to 1.5 GHz band. It was also reported that the conductivity of the material did not have a significant effect.

This paper presents a matching medium where the antennas can be immersed. Dielectric property measurements of the material is performed with broadband contact probe technique and a probe with an attached standard RF cable is used to ensure to minimize the connection errors. The mixture with the desired dielectric properties is obtained by mixing of distilled-water (DI), ethylene glycol (EG), and carboxymethyl cellulose (CMC). From 0.5 to 2 GHz measured permittivity and conductivity of the material is ranging from 44 to 25 and 0.2 (S/m) to 2 (S/m), respectively. In this work, the goal is to obtain a rather smooth variance along the frequency band. The matching medium will also be tested with antennas and with a brain phantom.

MEDI 63

Synthesis of functionalized benzofulvenes and their possible application towards thioredoxin reductase inhibition and cancer treatment

Adam Glass, Katherine Caspary, casparke@plu.edu. Pacific Lutheran University, Tacoma, Washington, United States

The thioredoxin system, comprised of thioredoxin (Trx) and thioredoxin reductase (TrxR), is a key component for oxidative stress control within the intracellular environment. This system has been linked to potential oncogenic events through the manipulation of reactive oxygen species (ROS). Benzofulvenes have been shown, via docking software, to bind to the active site of TrxR. Implications of active inhibition of TrxR would result in Trx not being reduced, meaning neither would the ROS within that transformed cell, leading to apoptosis. Eight functionalized benzofulvenes have been synthesized for testing against TrxR. Reaction yields for the benzofulvenes have been improved remarkably through the use of lanthanum chloride as a key carbonyl activator in the first synthetic step. Future research for this project includes in-vitro assay testing of the benzofulvenes against TrxR to determine the level of inhibition.

MEDI 64

Hologram QSAR and structure-based design of novel small-molecule inhibitors of choline acetyltransferase

Rajnish Kumar, *rajnishjangra@gmail.com*, Taher Darreh-Shori. Dept. of Neurobiology, Care Sciences and Society, Center for Alzheimer Research, Division of Translational Alzheimer Neurobiology, Karolinska Institutet, Stockholm, Sweden

Dementia is a leading cause of death affecting more than 47.5 million people worldwide with additional 7.7 million new cases every year and expected to increase to 75.6 million by 2030. Alzheimer's disease (AD) alone accounts for nearly 60-70 % cases of dementia. Other forms include vascular dementia, dementia with Lewy bodies (DLB) and frontotemporal dementia and Down syndrome (DS). Decrease in the activity of acetylcholine synthesizing enzyme has been observed with progression of these dementia disorders especially AD. Therefore, pharmacokinetically acceptable, water soluble, nonquaternary, irreversible inhibitors/ligands of ChAT could be developed as PET tracers and used to interrogate the cholinergic neurodegeneration observed in AD and other dementia disorders. In the present study a specific strategy is reported for the design of novel ChAT ligands based on Hologram Quantitative Structure Activity Relationship (HQSAR) and molecular docking analyses on a dataset of 26 ChAT inhibitors with reported IC_{50} . The results provide insight into the contribution of specific structural moieties of the compounds towards their activity on ChAT, which allowed us to design novel ligands using lead optimization strategy implemented in Muse[®] Invent[™] molecular design workflow. The novel findings may provide useful insights for the development of potential ChAT PET probes.

MEDI 65

Virtual hit-to-lead drug development program FRESH and success across carbonic anhydrase II and phosphatidylinositol 3-kinase α

Thomas Kaiser, *t.m.kaiser@emory.edu*, Qi Shi, Zackery Dentmon, Pieter Burger, James Snyder, Dennis Liotta. Chemistry Dept, Emory University, Atlanta, Georgia, United States

As costs of medicine development continue rise, the early identification of compounds with a high probability of clinical success is an area of major interest. A chemoinformatics/machine learning protocol (FRESH) that dramatically increases the ease of quality candidate identification will be presented. This method should, as a consequence, reduce the attrition rate of compounds entering the clinic and increase the cost-effectiveness of drug development. Compounds identified and synthesized by FRESH were universally found to have nanomolar or subnanomolar activity (10 out of 10). Five of these compounds were inhibitors of human carbonic anhydrase II, and five compounds inhibited phosphatidylinositol 3-kinase α . As a result, we found that FRESH can be used as lead development tool for reducing the effortfulness of synthetic preparation required in lead-optimization.

MEDI 66

Dopamine transporter ligands with short and long residence time

Siim Kukk, *siim.kukk@ut.ee*, Jaak Jarv. Chair of Organic Chemistry, University of Tartu, Tartu, Tartumaa, Estonia

Kinetic constants, or derivatives thereof, are probably the second most important characteristics, besides the inhibition constant, of a drug molecule. The reciprocal value of the drug-protein complex dissociation rate constant (k_{off}) or drug residence time is a very popular metric with which to describe the kinetic effect of a drug molecule binding to the target protein. Depending on the application, both short and long residence time ligands have their merits. It has been common practice to regard IC_{50} or K_i values as sufficient to describe the binding of a ligand to a protein. Although the equilibrium dissociation constant ($K_d = k_{\text{off}}/k_{\text{on}}$) is a function of the off-rate (k_{off}), one usually cannot evaluate kinetic parameters from typical displacement studies, where all ligands are subjected to a standard incubation time.

Affinity and the drug residence time are both functions of the off-rate (k_{off}). As affinity is also a function of molecular structure, there are structural features which determine the rate of the protein-ligand complex off-rate and whether the ligand has a slow step in the binding process. The drug residence time can be extended in two ways: by decreasing the off-rate (k_{off}) of a drug-protein complex; or by introducing a second, slowly dissociating step. Dopamine transporters (DAT) have ligands that exhibit either a fast off-rate (e.g. 2 α -PE2I) or a slow off-rate (e.g. PE2I) when binding to DAT. The core structure is identical; the ligands differ only in the stereochemistry of one carbon atom. This small difference is responsible for inducing the slow off-rate, which can be determined via kinetic analysis of the effects of the unlabelled ligand concentrations on the binding of a labelled, slowly dissociating ligand.

MEDI 67

Structure function studies of silent agonists of the alpha 7 nicotinic acetylcholine receptor

Marta Quadri¹, *marta.quadri@chem.ufl.edu*, Clare Stokes², Roger Papke², Ciara Sanon¹, Nicole Horenstein¹. (1) Dept of Chem Box 117200, Univ of Florida, Gainesville, Florida, United States (2) Pharmacology & Therapeutics, University of Florida, Gainesville, Florida, United States

Alpha7 nicotinic acetylcholine receptor (nAChR) silent agonists show little or no ionotropic activity in the absence of a positive allosteric modulator (PAM). However, they can induce metabotropic-like signal transduction, showing effectiveness in suppressing acute inflammation in mouse models. This activity is associated with desensitized states of the receptor. Our laboratories seek to define structure function relationships for these compounds. We express the receptor in *Xenopus* oocytes and use two-electrode voltage clamping to detect silent agonism by application of compounds at 30 mM (agonism \leq 0.1 times ACh controls) and co-application of 30 mM compound with 10 mM PNU-120596, a tool to reveal the PAM-sensitive desensitized (D_s) state of the receptor by rendering it conductive. The lead silent compound diEPP (*N,N*-diethyl-*N'*-phenylpiperazine) showed virtually no agonism but PNU co-application

enhanced responses (0.002 ± 0.003 vs. 1.3 ± 0.3 , respectively relative to responses evoked by control applications of $60 \mu\text{M}$ ACh). It was successfully modified to generate more effective silent derivatives. The *p*-CF₃-diEPP displayed one of the best silent profiles (0.032 ± 0.003 ; 61.8 ± 7.7 , relative to ACh controls), by strongly inducing the D_s state. A 3-fold decrease in silent activity observed for *p*-F-diEPP (0.010 ± 0.002 ; 22.9 ± 5.8) was ascribed to greater fluorine bond contribution for the CF₃ group in the ligand binding pocket. However, *p*-SF₅-diEPP retained the silent profile (0.055 ± 0.013 ; 1.94 ± 0.52), preferentially favoring the alpha7 PAM insensitive desensitized state (D_i) over D_s. Replacement of the *N,N*-diethyl group of diEPP with a spirocycle derived from 1,5-diodopentane produced a potent alpha7 silent compound (0.00 ± 0.06 ; 23.9 ± 2.7). However the *N*-spirocycle substituent and *p*-CF₃ group were not synergetic, as including these two groups in the same phenyl piperazine moiety resulted in conversion to a partial agonist, with loss of the silent profile (0.14 ± 0.01 ; 6.27 ± 1.66), suggesting the possibility of different binding modes for the disubstituted compound relative to its parents.

MEDI 68

Dual soluble Epoxide Hydrolase (sEH) and Fatty Acid Amide Hydrolase (FAAH) inhibitors for treating

Sean D. Kodani, sdkodani@ucdavis.edu, Karen Wagner, Sung Hee Hwang, Kin S. Lee, Christophe Morisseau, Bruce D. Hammock. Entomology and Nematology, University of California, Davis, Davis, California, United States

Soluble epoxide hydrolase (sEH) and fatty acid amide hydrolase (FAAH) regulate lipid mediators responsible for mediating pain, inflammation and other biological processes. They act primarily by controlling titers of their bioactive substrates epoxyeicosatrienoic acid (EETs) and arachidonoyl ethanolamide (AEA). Concurrent inhibition of these targets is synergistic in the treatment of inflammatory and neuropathic pain, although the exact mechanism for this synergy is unknown. To develop tools to study this mechanism of synergy and to understand other applications of dual sEH/FAAH inhibition, we developed dual inhibitors with high potency towards both FAAH and sEH. Using an integrated pharmacophore approach, where a urea functional group is incorporated as the pharmacophore for both sEH and FAAH, we identified inhibitors with low nanomolar potency towards both targets. These inhibitors are selective for their targets and do not inhibit related serine hydrolases or epoxide hydrolases. In this presentation, challenges in developing dual inhibitors will be discussed including species selectivity, engaging multiple targets *in vivo* and demonstrating efficacy.

MEDI 69

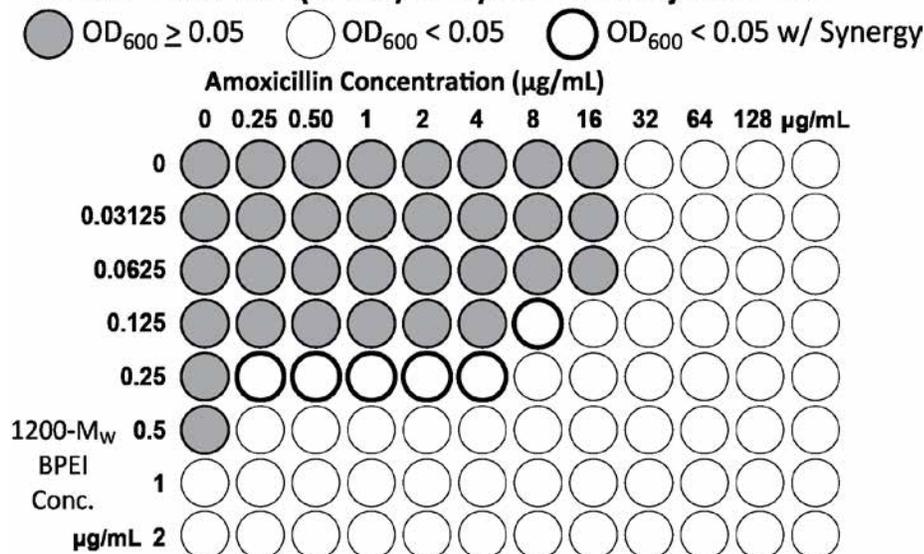
Low-cost high-impact route to kill MRSA with beta-lactam antibiotics

Charles V. Rice, *rice@ou.edu*, Melissa Foxley, Min Xiao, Summer Wright, Stoffel Strange. Chemistry and Biochemistry, University of Oklahoma, Norman, Oklahoma, United States

New low-cost antibiotics that kill both susceptible and resistant bacteria will improve patient outcomes and reduce health care costs. However, antibiotic development is time consuming (>10 years) with a small chance of success. It may be possible to overcome these barriers with a discovery made in our laboratory. Low-cost β -lactam antibiotics that kill methicillin-susceptible *S. aureus* also prevent the growth of methicillin-resistant *S. aureus* (MRSA) if administered with a readily available and low-cost polymer: branched poly(ethylenimine), BPEI. We envision β -lactam + BPEI combinations as a potential low-cost antibacterial treatment. We have been able to demonstrate efficacy and low cytotoxicity. We have filed a full patent application that brings numerous off-patent antibiotics under patent protection.

MRSA Growth Assay with Amoxicillin and 1200-M_w BPEI

S. aureus 700787 (MRSA/VISA) in Cation Adjusted MHB



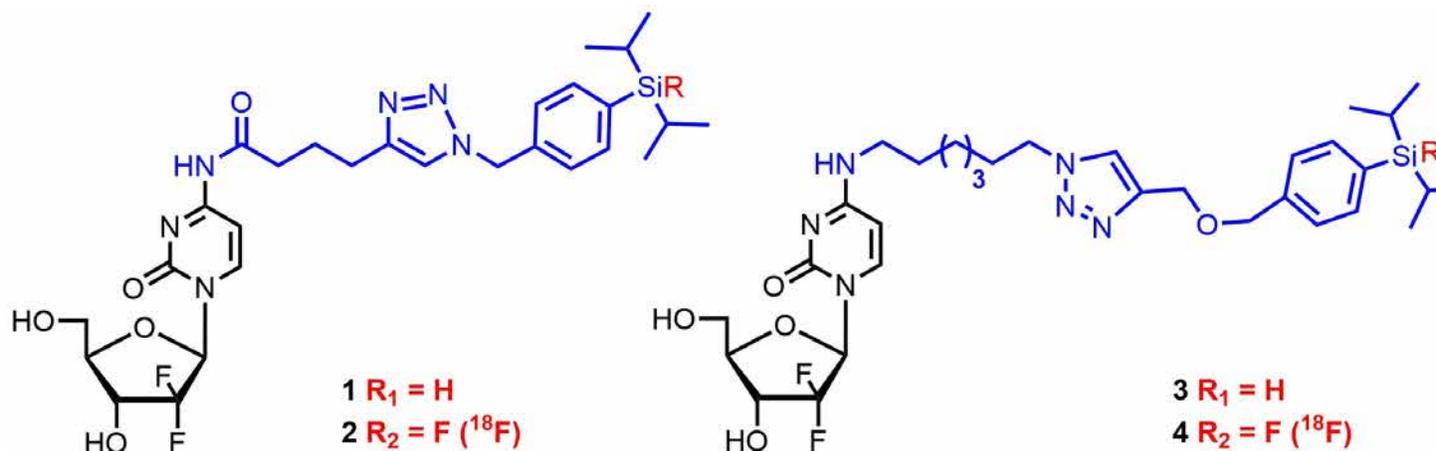
MEDI 70

Clickable 4-N-alkanoyl and 4-N-alkylgemcitabine analogues with silicon-fluoride acceptors

Cesar Gonzalez, *cgonz042@fiu.edu*, Andersson Sanchez, Stanislaw F. Wnuk. Florida International Univ, Miami, Florida, United States

Gemcitabine (dFdC) is an effective chemotherapeutic nucleoside analog in the treatment of cancers and solid tumors. Synthesis of lipophilic gemcitabine analogues via click reactions with silicon-fluoride-acceptor building blocks suitable for ¹⁸F labeling has been accomplished. The coupling of gemcitabine with carboxylic acids using peptide coupling conditions afforded 4-N-alkanoyl analogues with a terminal alkyne or azido

moiety. Click reaction of these compounds with dialkylsilyl building blocks afforded 4-*N*-alkanoylsilanegemcitabine analogue (e.g., **1**). Reaction of 4-*N*-tosylgemcitabine with functionalized azidoalkyl amines provided 4-*N*-alkylgemcitabine with a terminal azido group. Coupling of the latter with 4-(di-*iso*-propylsilyl)-*O*-propargylbenzylalcohol provided 4-*N*-alkylsilanegemcitabine (e.g., **3**). Treatment of these trisubstituted silane derivatives with KF/18-crown-6 resulted in efficient fluorination to give **2** or **4** under conditions that are compatible with protocols for positron emission tomography (PET) ^{18}F labeling. The cytotoxicity and antiproliferative activities against several cancer cell lines will also be discussed.



MEDI 71

Novel 5-nitroimidazole and 5-nitrothiazole piperazine derivatives and their antiparasitic activity

Haythem A. Saadeh^{1,2}, hasaadeh@yahoo.com, **Mohammad Khasawneh**¹, **Youssef Abou-Zeid**¹, **Ismail El-Haty**¹, **Sylvain Nsangou**³, **Kapil Goyal**⁴, **Rakesh Sehgal**⁴, **Abdelouahid Samadi**¹. (1) Chemistry, United Arab Emirates University, Al-Ain, Abu Dhabi, United Arab Emirates (2) Chemistry, The University of Jordan, Amman, Amman, Jordan (3) Department of Biochemistry, University of Yaounde, Yaounde, Cameroon (4) Department of Medical Parasitology, Postgraduate Institute of Medical Education and Research, Chandigarh, India

A novel series of 5-nitroimidazole- and 5-nitrothiazole-piperazine derivatives have been prepared in good yields. The 2-(2-methyl-5-nitroimidazolyl) ethylamine hydrochloride (**1**) was treated with bromoacetyl chloride to give 2-bromo-N-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)acetamide (**2**) which then coupled with aryl piperazines (**3**) to give 5-nitroimidazole-piperazines (**4a-j**). Similarly, 2-amino-5-nitrothiazole (**5**) was treated with bromoacetyl chloride in presence of a base to give 2-bromo-N-(5-nitrothiazol-2-yl)acetamide (**6**) then reacted with aryl piperazines (**3**) to give 5-nitroimidazole-piperazines (**7a-j**). Structures of the newly prepared compounds were confirmed through different spectroscopic methods such as $^1\text{H-NMR}$, $^{13}\text{C-NMR}$, mass spectrometry and also by elemental analyses. The anti-giardial and antitrichomonal

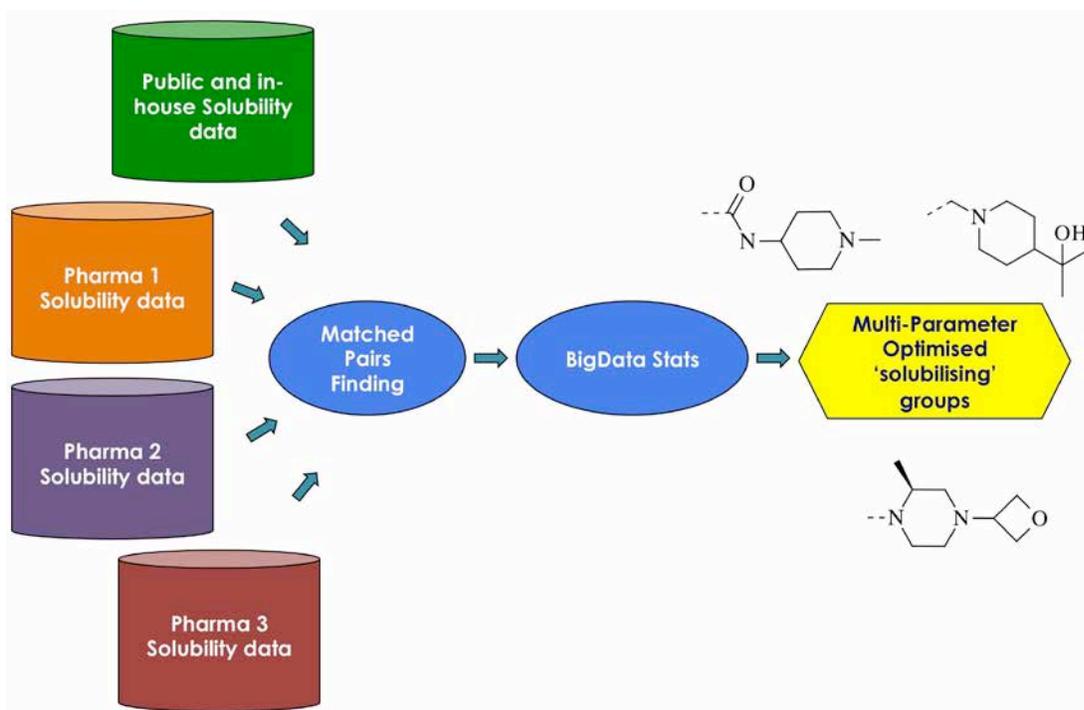
activities of the prepared compounds were evaluated *in vitro* at incubation for 24 and 48 hours. Majority of the compounds showed less IC₅₀ as compared to metronidazole at 24 and 48 hours of incubation, except for compound **7c** that showed almost similar activity against *Girardia lamblia* at 48 hours of incubation in MTT assay. However, these compounds showed better anti-parasitic activity against *T. vaginalis*. Compounds **7e** and **7j** showed slightly better activity against *T. vaginalis* as compared to metronidazole at 24 hours of incubation in MTT assay. The antitrichomonal activity increased significantly for the compounds **4c**, **7c**, **7e**, **7i** and **7j** at incubation of 48 hours. Compounds **4c** and **7e** were the most potent among the prepared compounds and were with 6.6 and 7.2 times better activity than standard drug metronidazole respectively.

MEDI 72

Matched molecular pair analysis and collaboration: Finding rules to the age-old problem

Alexander Dossetter¹, *al.dossetter@medchemica.com*, **Edward J. Griffen**¹, **Andrew G. Leach**^{2,1}, **Shane Montague**¹. (1) MedChemica Limited, Macclesfield, United Kingdom (2) Liverpool John Moore University, Liverpool, United Kingdom

The challenge of designing aqueous soluble organic molecules in drug discovery is acutely felt in many projects. The approach of adding solubilizing groups appears attractive but often leads to its own problems, such as increasing hERG binding and reducing permeability. Matched Molecular Pair Analysis (MMPA) provides an attractive mechanism to compare molecules and gauge the true effect of functional groups to improve solubility. MedChemica worked in collaboration with three pharmaceutical companies and analyzed solubility data using MMPA. Standardisation of chemical information and statistical methods allow multi-parameter optimization knowledge to be gained. For example we can find which solubilizing basic side chains will improve solubility further and also reduce hERG binding and metabolism. A recent collaboration with Cambridge Crystallographic Data Centre (CCDC) to analyze the data further and also incorporate pKa measurement to tackle the 'age-old' solubility prediction problem. The talk will discuss the solubility knowledge sharing process, MMPA "Big Data" analysis and recently published results.



MEDI 73

Diketo acids and their hybrid bioisoster derivatives as bacterial biofilm and Methionine Aminopeptidase (MetAP) inhibitors

Abid Mohammad¹, *mabid@jmi.ac.in*, Phool Hasan^{1,2}, Vijay Pillalamarry³, Mohamamad Irfan¹, Ahmad Perwez¹, Belal Ahmad², Umesh Yadava⁴, Moshahid Rizvi¹, Constantin Daniliuc⁵, Ronan Maguire⁶, Kevin Kavanagh⁶, Anthony Addlagatta³. (1) Biosciences, Jamia Millia Islamia University, New Delhi, Delhi, India (2) Chemistry, T.N.B. College, Tilka Manjhi Bhagalpur University, Bhagalpur, Bihar, 812007, India, Bhagalpur, Bihar, India (3) Centre for Chemical Biology, Indian Institute of Chemical Technology, Hyderabad, Telangana, India (4) Physics, Deen Dayal Upadhyay Gorakhpur University, Gorakhpur, UP, India (5) Organisch-Chemisches Institut, Westfälische Wilhelm-Universität, Munster, Munster, Germany (6) Biology, Maynooth University, Co. Kildare, Co. Kildare, Ireland

Bacteria prefer to exist in biofilms that are composed of specialised non-replicating cells encased within an extracellular matrix of biomolecules and demonstrate tolerance towards every class of antibiotic therapy. The cleavage of *N*-terminal methionine by MetAP is an important event during protein synthesis and maturation, therefore it is an ideal target for designing new inhibitors against bacterial pathogens. Here, we disclose various diketo acids and their bioisosteres as potential bacterial biofilm and MetAP inhibitors. In the biochemical assays against purified MetAPs from *Streptococcus pneumoniae* (SpMetAP), *Mycobacterium tuberculosis* (MtMetAP), *Enterococcus faecalis* (EfMetAP) and *human* (HsMetAP), compounds 2, 8, 11 and 13 showed about >70% Inhibition of all the MetAPs at 100µM inhibitor concentration. Moreover, compound 27 found selective inhibitor of MtMetAP by 88% while compound 26 showed HsMetAP inhibition by 93% at 100 µM inhibitor concentration. Selective potent inhibitors were also subjected to cell proliferation assay on CHO cells line and were found to be non-toxic. Growth curve studies for lead inhibitors 11 and 13 indicated their bacteriostatic nature. Significant inhibition of biofilm formation was observed in treated bacterial cells exposed to sub MIC concentrations of inhibitor 13 by Scanning Electron Microscopy (SEM). Transmission Scanning Electron Microscopy (TEM) analysis of bacterial cells exposed to 13 clearly showed morphological changes and intracellular damage as its possible mode of action. Further computational docking studies were performed to investigate the mode of interaction of the most active compounds with the MetAPs active site. The lead inhibitor 13 did not cause an alteration in the hemocyte density in *Galleria mellonella* larvae thereby not stimulating an immune response. In addition, it was non-toxic up to a concentration of 3.0 mg/ml. The results suggested that 13 could be considered as lead inhibitor and a suitable core for further structural optimization for better and safe antibacterial agents.

MEDI 74

Studies aimed at the synthesis of Hsp90 inhibitors as antileishmaniasis agents

Linda Barбето¹, *aquilina@mail.usf.edu*, **James Leahy**², **Dennis Kyle**³. (1) Chemistry, University of South Florida, Brandon, Florida, United States (2) Chemistry, University of South Florida, Tampa, Florida, United States (3) University of South Florida, Tampa, Florida, United States

Leishmaniasis is a widespread parasitic disease prevalent in less developed countries for which few effective treatments are available. Studies have shown that compounds active against Hsp90 are also active against *Leishmania donovani* cells. Our lab is currently investigating Hsp90 inhibitors with the goal of discovering new antileishmaniasis agents. Our project consists of making analogs to the tetrahydroindazole core of the Hsp90 inhibitor SNX 2112 in order to explore increased binding within the active site in an attempt to increase antileishmanial activity.

MEDI 75

Synthesis and biological activity studies of C1-substituted carbapenem antibiotics

Thu Nguyen¹, *nguyentq@smu.edu*, **Jean Kim**¹, **Paulin Nguyen**¹, **Melina Cox**¹, **Maricka Bennett**¹, **Byron Meshram**¹, **Linda Phung**¹, **Chelsea Watanabe**², **Angela Shi**², **Maha Alqurafi**¹, **John D. Buynak**¹. (1) Southern Methodist Univ, Dallas, Texas, United States (2) The Hockaday School, Dallas, Texas, United States

With the rise in antimicrobial resistance, and the evolution and dissemination of a wide array of resistance factors, the structural features of selected classes of antibiotics (e.g. the β -lactam antibiotics) which were developed in the 20th century may no longer be relevant to treating 21st century infections. The carbapenems represent the most potent and broad spectrum of the Λ -lactams. We will present an update of our recent work on the stereospecific synthesis and biological evaluation of new members of this class.

MEDI 76

2-Nitrobenzenesulfonyl fluoride is a novel small molecule pharmacophore for the development of new antibiotics

Bora Park, *bora0225@gmail.com*. Bioengineering, UC Berkeley, Berkeley, California, United States

The development of new classes of antibacterial agents is a central challenge in medicinal chemistry. Sulfonyl fluorides have tremendous potential as lead fragments for generating new antibacterial agents because of their ability to covalently modify potential drug targets and permanently inactivate proteins. However, the ability of sulfonyl fluorides to act as antibacterial agents has never been examined. In this report we generated a sulfonyl fluoride library and investigated their ability to kill bacteria via high throughput screening. We identified 2-nitrobenzenesulfonyl fluoride as a lead fragment for future antimicrobial drug development, and demonstrate that this small

molecule is active against bacteria, and exhibited an MIC value of approximately 5 mg/mL. In this presentation we will present the design and synthesis of new 2-nitrobenzenesulfonyl fluoride derivatives, their target identification and the mechanism by which 2-nitrobenzenesulfonyl fluoride inhibits bacterial growth. 2-Nitrobenzenesulfonyl fluoride has great promise as a new scaffold for next generation antibiotics, given its small size and high potency against bacteria.

MEDI 77

Gold-phosphines and gold-phosphine-modified human serum albumin as potent inhibitors of T-cell proliferation

Tyler C. Dean¹, *deantc13@wfu.edu*, **Mu Yang**¹, **Mingyong Liu**², **Paul K. Langston**², **Jingyun Lee**³, **Cristina M. Furdui**⁴, **Jason Grayson**², **Ulrich Bierbach**¹. (1) Department of Chemistry, Wake Forest University, Winston-Salem, North Carolina, United States (2) Department of Microbiology and Immunology, Wake Forest School of Medicine, Winston-Salem, North Carolina, United States (3) Comprehensive Cancer Center, Wake Forest School of Medicine, Winston-Salem, North Carolina, United States (4) Department of Internal Medicine, Wake Forest School of Medicine, Winston-Salem, North Carolina, United States

The ultimate goal of this research is to introduce gold(I)-based compounds as therapeutic payloads into serum albumin as a passive delivery vehicle to target autoimmune diseases, such as systemic lupus erythematosus (SLE). A small combinatorial library of 90 gold(I) complexes was generated and pre-screened in a 96-well-plate format (MTS assay) for their inhibitory effects on CD8⁺ T cell proliferation. Two low-molecular-weight compounds [Au(ACRAMTU)PEt₃](NO₃)₂ (**1**) and [Au(ACRAMTU)JohnPhos](NO₃)₂ (**2**) (ACRAMTU = 1-[2-(acridin-9-ylamino)ethyl]-1,3-dimethylthiourea), JohnPhos = (*t*-Bu)₂-*o*-biphenylphosphine) identified using the modular platform were re-synthesized with good yield. Both compounds were characterized by ¹H NMR spectroscopy and X-ray crystallography. The reactivity of both compounds with cysteine sulfur was studied using glutathione (GSH) as a model nucleophile prior to reactions with human serum albumin (HSA). The compounds were found to react effectively with glutathione under basic pH conditions but not in acidic conditions due to the protonation of the cysteine. Selective substitution of the ACRAMTU ligand by cysteine was confirmed by ³¹P NMR spectroscopy and electrospray mass spectrometry (ESI-MS). Analogous reactions with HSA resulted in HSA-Au-PEt₃ and HSA-(Au-JohnPhos)₁₋₃ conjugates, which could be observed in direct injection (whole protein) ESI-ToF mass spectrometry. The ligand exchange reaction using ACRAMTU as a water-soluble gold transfer ligand is a novel, highly efficient means of attaching a known therapeutic payload to protein cysteine residues. When CD8⁺ T cells from naïve C57BL/6 mice were treated with compound **1** or HSA-Au-PEt₃ for 60 min at 37 °C, and activated with αCD3 and αCD28 antibodies, both carrier-free and protein-bound gold showed complete blockage of T cell activation. The results provide insight into the immunosuppressive mechanism of gold(I) pharmaceuticals. The

data suggest that HSA may have applications as a suitable carrier that enhances the safety and pharmacological properties of gold(I)-based therapies.

MEDI 78

Insulin: Its structure, function, and interaction in model cell membranes

Kate Saulcy², *ksaulcy@monmouthcollege.edu*, **Debbie C. Crans**¹, **Audra Sostarecz**².
(1) Colorado State University, Fort Collins, Colorado, United States (2) Chemistry, Monmouth College, Monmouth, Illinois, United States

Insulin is a polypeptide hormone that is created and used by a healthy human body to regulate blood sugar. The specific conformation that an insulin molecule adopts affects the stability and functionality of the hormone. Interactions with lipids, specifically Dipalmitoylphosphatidylcholine (DPPC) causes conformational changes in the shape of the insulin molecule and its state of aggregation. The Langmuir technique can be used to create monolayers of insulin, both human recombinant and bovine. Samples of insulin were mixed with lipid, at varying ratios, to determine any molecular interactions between the two compounds and to determine to what extent they interact. At a 25% Insulin/75% DPPC ratio, insulin makes a monolayer of DPPC more fluid. At a 75% Insulin/25% DPPC ratio, DPPC orders the insulin molecules. Subcutaneous injection exposes insulin molecules to lipid and this interaction appears, due to the disappearance of a phase transition on the mixed isotherm, to cause the insulin molecules to adopt a hexameric conformation. As the most active form of insulin is the monomer, this may account for less effective or incorrect dosing of insulin. In the presence of zinc, insulin is known to bind into hexamers. This is how long-acting medical insulin is stored. The Langmuir technique, with a multi-well plate, can be used to investigate changes in pressure that correspond with changes in molecular area. These changes indicate a conformational change in the insulin molecule.

MEDI 79

Synthetic and mechanistic studies of cyclotriazadisulfonamide (CADA) down-modulators of human CD4

Thomas W. Bell, *twb@unr.edu*. Dept of Chemistry 216, Univ of Nevada, Reno, Nevada, United States

Cyclotriazadisulfonamide (CADA) compounds are macrocycles that down-modulate human CD4 by selectively inhibiting co-translational translocation of the nascent protein across the ER membrane, thus acting as HIV entry inhibitors. This ability is also of interest for developing potential immunomodulatory drugs. CADA is the first small molecule known to selectively down-modulate any protein by targeting its signal sequence during translation of the nascent protein. We have synthesized potent CADA analogs ($IC_{50} < 0.1 \mu M$) having electron donating groups on one of the arenesulfonamide side arms and found that CD4 down-modulating potencies correlate

with the electric dipole moment of this side arm. This suggests that one arm makes an attractive interaction with one or more critical amino acid residues of the signal sequence. Our mechanistic hypothesis is that the small molecule stabilizes a folded conformation of the nascent protein within the transmembrane channel, blocking CD4 translocation into the ER lumen. Current advances on modifying properties of CADA compounds and on identifying molecular details of the mechanism of action are described.

MEDI 80

Facile synthesis of Benzalkonium Chloride (BAC)-derived mesoporous silica nanoparticles as antibacterial material

Hongwang Wang¹, *hongwang@k-state.edu*, **Tej B. Shrestha**³, **Jose Covarrubias**¹, **Aruni P. Malalasekera**¹, **Sebastian O. Wendel**³, **Jing Yu**¹, **Prem Thapa**², **Lauren Chlebanowski**¹, **Obdulia Covarrubias Zambrano**¹, **Deryl L. Troyer**³, **Stefan H. Bossmann**¹. (1) *Chemistry, Kansas State University, Manhattan, Kansas, United States* (2) *1041 Haworth Hall, The University of Kansas, Lawrence, Kansas, United States* (3) *Anatomy & Physiology, Kansas State University, Manhattan, Kansas, United States*

In spite of the rapid advances of novel technologies, we are still losing the battle against infectious diseases. Due to the extensive use of antibiotics and antimicrobials, more and more antibiotic resistant bacteria emerge. They pose the most serious threat to human health. To overcome these challenges, various nanoparticle based antibacterial materials have been studied. Mesoporous silica nanoparticles (MSNs) are promising candidates for drug delivery due to their biocompatible and biodegradable properties. Their unique porous structure and versatile surface functionality allows carrying high payloads of drugs through encapsulation or attachment. However, in order to entrap or deposit active drugs into the channels of MSNs, the pre-existing surfactants have to be removed first, which usually involves ion exchange, multi-step washings, and finally, the addition and incubation of the active drug at higher concentration. Herein, we report a simple one-pot synthesis of self-containing mesoporous silica nanoparticle using benzalkonium chloride (BAC) as both structure-directing surfactant, and antibacterial agent. BAC is a commonly used amphiphilic antibacterial agent, mainly acting by damaging the pathogen's membrane. Using BAC as structure-directing agent, tetraethyl orthosilicate (TEOS) and (3-Aminopropyl)triethoxysilane (APTES) as silica precursors, NH₂-functionalized BAC containing MSNs have been synthesized. We have incorporated vancomycin on the surface of the MSNs through amide bond formation between the carboxylic group of vancomycin and the MSNs' surface NH₂-groups. Vancomycin is not only an antibacterial agent, but also able to specifically target Gram-positive bacteria, through multiple H-bonding between the heptapeptide backbone of vancomycin and the D-alanyl-D-alanine dipeptide of the bacteria cell wall. We anticipate that this additional targeting function will increase the efficacy of the antibacterial effect. This is also a viable way to selectively recognize and kill bacteria, and at the same time, to limit damage of macrophages.

MEDI 81

Observational therapeutics and systems approaches for chemical biology, drug discovery -Solving the problems of dearth of NCEs

Mukund Chorghade^{1,2}, chorghade@verizon.net. (1) 13 Sheffield Court, Chorghade Enterprises, Somerset, New Jersey, United States (2) Chemistry, THINQ Pharma, Somerset, New Jersey, United States

While biotechnological advances, genomics and high throughput screenings or combinatorial and asymmetric syntheses have opened new vistas in drug discovery, the industry is facing a serious innovation deficit. Critics suggest that “we have become high throughput in technology, yet have remained low throughput in thinking” leading to a significant shift in favor of single to multi targeted drugs and affording greater respect to traditional knowledge. Typical reductionist approach of modern science is being revisited over the background of systems biology and holistic approaches of traditional practices. Scientifically validated and technologically standardized botanical products may be explored on a fast track using innovative approaches like reverse pharmacology and systems biology, which are based on traditional medicine knowledge. Indian Ayurvedic and traditional Chinese systems are living ‘great traditions’. Ayurvedic knowledge and experiential database can provide new functional leads to reduce time, money and toxicity - the three main hurdles in the drug development. We begin the search based on Ayurvedic medicine research, clinical experiences, observations or available data on actual use in patients as a starting point. We use principles of systems biology where holistic yet rational analysis is done to address multiple therapeutic requirements. Since safety of the materials is already established from traditional use track record, we undertake pharmaceutical development, safety validation and pharmacodynamic studies in parallel to controlled clinical studies. Thus, drug discovery based on Ayurveda follows a ‘Reverse Pharmacology’ path from Clinics to Laboratories. Herein we describe such approaches with selected examples based on previous studies.

We aim to reconfigure products into chemical hybrid “Molecular Legos” and to screen the deck of diverse compounds against targets. A significant disadvantage of natural products is the draconian organic synthesis/medicinal chemistry effort required for commercialization. In many cases, the availability of the natural product compound is not sufficient for various biological assays, thereby limiting their exploration. We offer unique and elegant solutions to these twin challenges by bringing together structure guided drug design and hybrid molecule synthesis.

MEDI 82

Advanced carbapenem antibiotics: The synthesis and activity studies of C6 substituted carbapenems

Maha Alqurafi², malqurafi@mail.smu.edu, Jean Kim³, Duyen Le², Maureen Lohry⁶, Chelsea Watanabe⁴, Angela Shi⁵, Thu Nguyen⁶, John D. Buynak¹. (1) Southern Methodist Univ, Dallas, Texas, United States (2) Chemistry, Southern Methodist University, Dallas, Texas, United States (4) The Hockaday School, Dallas, Texas, United States

The 21st century has seen the evolution and dissemination of carbapenem-resistant Gram-negative pathogens. The carbapenem class represents the most potent and broad spectrum of the b-lactams. We will discuss our work involving redesign of these valuable drugs to counter the evolution of highly resistant strains.

MEDI 83

Impact of pH on solubility, hydrolysis kinetics, and pharmacokinetics of tenofovir disoproxil fumarate delivered from intravaginal rings

John A. Moss¹, j.moss@oak-crest.org, Marc M. Baum¹, Manjula Gunawardana¹, Christine S. Miller¹, Irina Butkyavichene¹, Sandrine Calvez¹, Flora Yang¹, Kathleen L. Vincent², Richard B. Pyles². (1) Oak Crest Institute of Science, Monrovia, California, United States (2) University of Texas Medical Branch, Galveston, Texas, United States

The majority of pre-exposure prophylaxis regimens that have demonstrated clinical efficacy toward preventing HIV-1 infection are based on the nucleoside analogue reverse transcriptase inhibitor (nRTI) tenofovir (TFV) or its disoproxil prodrug (TDF), alone or in combination the nRTI emtricitabine. In prior studies of TDF delivery from intravaginal rings (IVRs), the pH dependent dissolution and hydrolysis steps prior to intracellular phosphorylation to form the active TFV diphosphate moiety have resulted unpredictable release kinetics *in vivo*. To better understand this lack of correlation between *in vitro* and *in vivo* behavior, a series of studies were undertaken to characterize the pH dependence of TDF solubility and hydrolysis *in vitro*, and pod-IVR formulations designed to increase the *in vivo* TDF release rate were developed. Solubility of TDF varied three-fold over the range pH 2.5 to pH 9. The kinetics of *in vitro* TDF hydrolysis across a broad pH range were measured. *In vivo*, esterase catalyzed TDF hydrolysis rapidly forms TFV at all relevant pH values. The hydrolysis state of TDF is an important factor in understanding drug partitioning and transport across the vaginal compartments. Five IVR formulations of TDF with excipients including citric acid, glucose, glycogen, and emtricitabine were evaluated in an *in vitro* system and in a sheep model using a crossover design. *In vivo* and *in vitro* release rates were correlated; however, vaginal fluid and tissue concentrations did not increase with higher *in vivo* TDF release from IVRs, suggesting that involvement of a complex, multi-compartment model, saturation effects, or active transport is involved in the pharmacokinetics of TDF vaginal delivery. Understanding these pH dependent effects is important for preclinical and clinical studies to determine TDF concentrations in the relevant compartments (vaginal fluids, tissues, blood, and associated CD4+ cells) that are effective in preventing HIV infection.

MEDI 84

Designing drugs for environmental degradability

J. Samuel Arey², *samuel.arey@eawag.ch*, Jennifer J. Guerard³, Peter R. Tentscher², Daniela Trogolo¹. (1) EPFL, Lausanne, Switzerland (2) Environmental Chemistry Department, Eawag, Dübendorf, Switzerland (3) Chemistry, University of Alaska Fairbanks, Fairbanks, Alaska, United States

Diverse pharmaceutical products are increasingly found at low-level concentrations in lakes, rivers, and treated drinking water in the United States. This occurs because many pharmaceuticals administered to humans pass from patient to toilet and subsequently persist through wastewater treatment facilities, thereby entering the natural aquatic environment, including drinking water supplies. Similarly, many pharmaceuticals fed to livestock are passed from animal to pasture and thereby enter natural waters. The observed persistence of drugs “from toilet to tap” raises concerns about the potential impacts on human health and aquatic ecosystems from the unintended, long-term exposure to an admixture of bioactive substances. In this talk, I will present an overview of strategies for evaluating candidate drug structures for degradability in the environment based on molecular modeling approaches. This will include case studies from our laboratory, as well as a broader perspective on the potential toolkit of computational approaches that could be implemented to evaluate the environmental degradability of existing pharmaceutical products and candidate chemical structures in clinical evaluation. Our vision is to lay the technical groundwork upon which environmental researchers and the pharmaceutical industry will build innovative partnerships that address the potential impact of pharmaceutical compounds in aquatic environments, evaluate pharmaceuticals for their degradability in aquatic environments, and engender environmentally conscious drug design.

MEDI 85

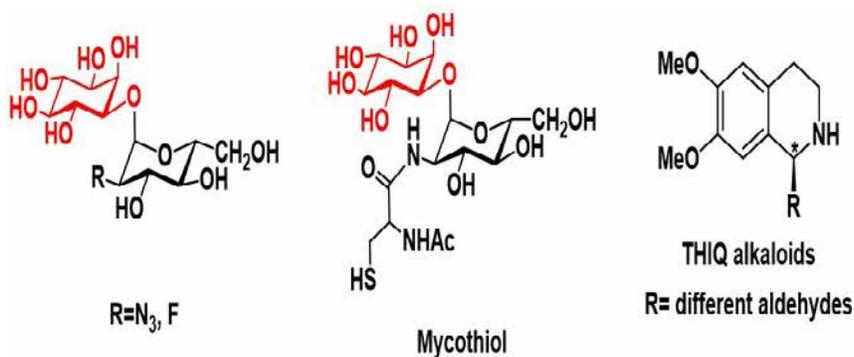
Use of substrate analogues and Tetrahydroisoquinolines (THIQ) as potential inhibitors for *Mycobacterium tuberculosis* enzyme MshC and synthesis of mycothiol

Krishnakant Patel¹, *krishnakant.patel@rockets.utoledo.edu*, Amarendar Reddy Maddirala¹, Peter R. Andreana². (1) Chemistry and Biochemistry, The University of Toledo, Toledo, Ohio, United States (2) MS 602, The University of Toledo, Toledo, Ohio, United States

Tuberculosis (TB) is an airborne infectious disease that has long been preventable and curable. Caused by bacillus *Mycobacterium tuberculosis*, TB remains to be a global health problem heightened by recently identified multidrug resistant TB (MDR-TB) strains. *Mycobacterium smegmatis* MshC catalyzes the ATP-dependent condensation of GlcN-Ins and L-cysteine to form L-Cys-GlcN-Ins, the penultimate step in mycothiol biosynthesis. Mycothiol facilitates the detoxification process by removing alkylating

agent bound L-cysteine. By inhibiting the MshC enzyme, the detoxification process lies in an arrested state. In order to design the inhibitors we focused on substrate analogues and natural product based small molecules. We proceeded by designing fluoro and azido based substrate analogues. The synthesis of the desired target molecule begins with partial protection of myo-inositol to give 2,3:4,5-di-O-cyclohexilidene-D/L-myoinositol. Treatment of myo-inositol with 1-(*R*)-menthylchloro-formate, followed by benzylation and resolution gives the protected D-isomer of myo-inositol in exceptionally good yields and purity. Deprotection under basic conditions led to the acceptor. Glycosylation with the required glucosyltrichloroacetimidate donors, followed by global deprotection gave the desired molecule. Heterocyclic molecules have the potential of being anti-bacterial molecules and Tetrahydroquinolines (THIQ) are one of them. They represent one of the important “Privileged Scaffolds” amongst natural products. But the synthesis of THIQ has proved to be tricky over the years. We aim at the use of well-known reaction, **Pictet Spengler** to achieve cyclization with better yields and diastereoselectivity against use of Asymmetric reduction or Chemical or Enzymatic resolution.

We also attempted the synthesis of Mycothiol, the substrate for the enzyme MST (mycothiol S-transferase). During this process few hurdles were encountered, due to which we had to opt for alternative synthetic scheme. These molecules will be helpful in better understanding the roles of the enzymes MshC and MST.



MEDI 86

MRI probe loaded HDL mimicking nanoparticles for diagnosis and therapy of atherosclerosis

Bhabatosh Banik¹, bbanik@med.miami.edu, **Shanta Dhar**^{1,2}. (1) Biochemistry and Molecular Biology, University of Miami, Miami, Florida, United States (2) Sylvester Comprehensive Cancer Center, University of Miami, Miami, Florida, United States

Among all forms of Coronary Heart Diseases (CHDs), atherosclerosis is the leading cause of deaths in the United States. The scenario is further worsened by the fact that a vast majority of Americans have Diabetes, a disease that accelerates atherosclerosis by driving atherosclerosis related inflammation and slowing down the blood flow. Typical hallmarks of atherosclerosis include impaired Reverse Cholesterol Transport (RCT),

inefficient lipid metabolism and deposition of fat-laden macrophages along the inner lining of the arteries to form plaques. An important aspect of efficient anti-atherosclerotic treatment is the early detection of plaque formation. Magnetic resonance imaging (MRI) is easy and minimally invasive technique for diagnosis of atherosclerosis. We, in an effort to design a therapeutic-diagnostic combo tool, have synthesized iron oxide loaded high density lipoprotein (HDL) mimicking nanoparticles that enable MR imaging of atherosclerotic plaques in addition to cholesterol and triglyceride removal from the body. Suitable surface functionalities impart macrophage targeting property to these nanoparticles and thus can enhance MRI contrast. To tackle atherosclerosis associated inflammation, we loaded these NPs with CoQ₁₀ and the resultant NPs showed significant anti-inflammatory properties in angiotensin-II (Ang-II) or oxidized LDL (OxLDL) stimulated Smooth Muscle Cells (SMCs).

MEDI 87

Copper responsive gadolinium-based MRI contrast agents

Namini N. Paranawithana¹, nnp130330@utdallas.edu, Andre F. Martins¹, Gabriele Meloni¹, Sara Chirayif², Piyu Zhao¹, A. Dean Sherry^{1,2}. (1) Department of Chemistry, University Of Texas at Dallas, Richardson, Texas, United States (2) Advanced Research Imaging Center, University of Texas Southwestern Medical Center, Dallas, Texas, United States

Copper is the third most abundant transition metal in the body. Normally bound to important biomolecules and metalloproteinases, copper is an essential redox cofactor in several enzymatic reactions. Disruption of copper homeostasis is implicated in a number of diseases including Alzheimer's, Parkinson's, Menkes, and Wilson's disease. Local concentration of copper ions can vary from a few micromolar to several millimolar in these diseases. Early versions of copper responsive MRI agents demonstrated that Zn²⁺ interferes with the Cu²⁺ response, limiting its use in MR diagnostic applications. Here, we report the synthesis and MR properties of a series of novel copper responsive contrast agents for magnetic resonance imaging (MRI). The MR sensors consist of DO3A gadolinium-based contrast agents attached to bis(benzoic-acid)methylamine copper-selective recognition motif. The sensors respond selectively to copper ions and bind more tightly to Cu²⁺. Our copper sensors exhibit high relaxivities upon binding to 1 equivalent of Cu²⁺. Interestingly, only when fully bound to Cu²⁺, the sensors presents up to 3-fold increase in relaxivity (~24mM⁻¹.s⁻¹) in the presence of physiologic concentrations of human serum albumin (HSA) with high affinity constants (K_A), as uniquely reported for zinc sensors. These results demonstrate that it is possible to design a functional MRI contrast agent responsive to copper at low concentrations, paving the way for the possible translation to pre-clinical imaging.

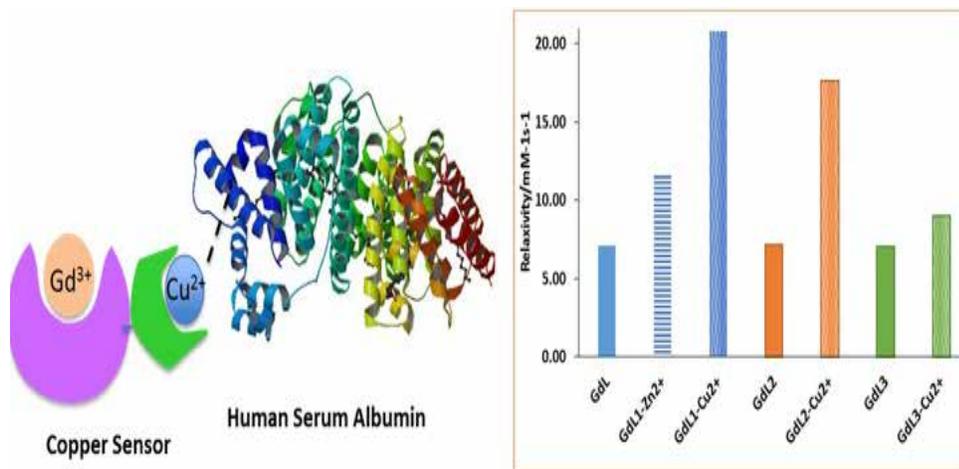


Figure 1. Longitudinal relaxivity (r_1) of the copper sensor in the presence of Cu^{2+} and Human Serum Albumin (HSA) at 0.6mM, 310K, pH=7.4 in MOPS buffer

MEDI 88

Novel, real-time analysis of nanomaterial inhibition of *E. coli*

Paul A. Sermon, paul.sermon@brunel.ac.uk. Wolfson Centre, Brunel University, Uxbridge, United Kingdom

Jennings used IR scattering to confirm the size (0.65mm x 1.35mm) and weight (0.17pg) of *E.Coli*. Now imaging of living cells on PDMS with a motorized stage fluorescence microscope, bacterial colonies on agar and silane-silica by SEM and biofilms and *E.coli* and *S.Aureus* growth in a petri dish using confocal microscope is possible, but with modest time (Dt) and spatial (Dx-Dy-Dz) resolution and in the case of SEM, unrealistic bioassay conditions.

The authors have explored traditional bioassays of the activity and photoactivity of biomimetic nanomaterials based on Portobello mushroom spores (PMS) at inhibiting the growth of *Eschericia coli*, *Staphylococcus aureus*, *Asperillus* and *Candide*. Antibacterial activity was determined in duplicate using Mueller Hinton agar. The plates were incubated for 24 h at 309K, under aerobic conditions. The diameter of the zone of inhibition was measured in mm; tests were performed in duplicate.

TiO_x /PMS, Ag- TiO_x /PMS, Au- TiO_x /PMS and Ag/PMS were active in inhibition towards *Eschericia coli* and *Staphylococcus aureus*, but Au/PMS was not active (suggesting a strong Au-PMS interaction). TiO_x /PMS, Ag/PMS and Ag- TiO_x /PMS were equally active in an antibacterial and an antifungal sense when tested against *Asperillus* and *Candide*. We report on the process of fine-tuning these antibacterial properties, progress on making these nanomaterials optically self-indicating and movement towards optical control of their antibacterial activity. The future of such green bio-nanomaterials is

strong.

Here we report on the results of high-speed optical profiling that may provide a rapid and accurate bioassay at the patient-clinician interface and in the antibiotic drug discovery forums, providing a high-throughput screening route to measuring, understanding and then overcoming microbial resistance.

MEDI 89

Organizing 3D project data for structure-based drug design

*Howard J. Feldman², Alain Ajamian¹, **E Metwally¹**, emetwally@chemcomp.com. (1) Chemical Computing Group, Orange County, California, United States (2) Chemical Computing Group Inc, Montreal, Quebec, Canada*

It is often desirable to organize disparate crystallographic project data into a common homogeneous format, ready to use for modelling. We present a web-based application that permits users to specify numerous options controlling superposition and alignment of structures in a family or project, ligand specification, and whether electron densities or other grids are to be included. The final result is a project database containing superposed structures all in the same frame of reference. From here, structures can be dynamically regrouped, for example by scaffold class, for easy management, and can be easily browsed and used as a starting point for further research. The system is able to handle multi-subunit complexes, including structures which may be missing subunits, by using a novel algorithm to determine which subunits of each complex correspond to each other.

MEDI 90

Fast generation of novel leads using virtual screening and fast MCR chemistry

***Alexander Doemling¹**, a.s.s.domling@rug.nl, Carlos J. Camacho². (1) Department of Drug Design, University of Groningen, Groningen, Netherlands (2) University of Pittsburgh, Pittsburgh, Pennsylvania, United States*

Screening virtual libraries can be very useful in the hit and lead generation if high resolution structural target information is accessible. However, for the general use of virtual in drug discovery, several issues have to be solved: 1) academic groups mostly rely on the ZINC database to perform virtual screening exercises. Although freely accessible and compounds commercially available, its chemical diversity is rather limited as its size is; 2) virtual screening is predicting hits often with high rate of false positives. In case of ZINC approach purchasing compounds then can become quite expensive. In case of a de novo synthesis approach a lot of expensive synthesis time can be wasted.

Thus we have introduced the pharmacophore screening approach ANCHOR.QUERY, which can screen very large libraries of virtual compounds for receptor binding to any

PDB structure. The virtual chemistry space used for virtual screening is based exclusively on multicomponent reaction chemistry (MCR). Thus any virtual hit can be instantaneously de novo synthesized and the corresponding docking hypothesis can be tested fast. In ANCHOR.QUERY (freely available @ <http://anchorquery.csb.pitt.edu/>) the concept of *anchors*, amino acid residues that bury a large amount of solvent accessible surface area at the protein-protein interface is used. Currently ANCHOR.QUERY and the related databases NUCLEO.QUERY and TPP.QUERY harbor a chemical space of >50 million molecules based on >20 different MCR scaffolds.

We will discuss the discovery of PPI antagonists for p53/mdm2 and allosteric PDK1 antagonists incl. the design principle and discuss the discovered molecules including synthesis, protein cocrystal structures and biological activities.

MEDI 91

Tumor suppressor P27^{Kip1} regulation by tissue transglutaminase and its potential application in cancer therapeutics

Lei Zhang, lez972@utulsa.edu, Robert J. Sheaff. Chemistry and Biochemistry, The University of Tulsa, Tulsa, Oklahoma, United States

Cyclin dependent-kinases (Cdks) are main regulators of the cell cycle. Cdk inhibitor 1B (p27^{Kip1}) is a member of the cdk inhibitory family with an important role in negatively controlling cell cycle progression at G1 phase by inhibiting cyclin E-Cdk2 and cyclin D-Cdk4 complexes. P27 is a tumor suppressor protein commonly deregulated in aggressive human cancers. Because p27 is deregulated at the protein rather than gene level, it is a strong candidate for targeted cancer therapy. However, before considering p27 as a target, it is important to fully describe the mechanisms by which p27 is deregulated during cancer formation.

The enzyme transglutaminase (TG) catalyzes formation of a covalent bond between a free amino group and the acyl group in glutamine, which typically generates intermolecular crosslinks between a lysine and glutamine residue on different protein monomers. Tissue transglutaminase (tTG) is one of the most versatile TG enzymes due to its involvement in multiple biological processes. tTG has been found to be upregulated in some types of cancers and involved in cancer metastasis.

We found that both microbial TG and human tTG utilize human wide type p27 as substrate in a special way. Rather than covalently linking p27 monomers as predicted, TG forms an intramolecular bond between one p27 monomer. This modification creates a more compact structure of p27 as suggested by its faster migration on SDS-page compare to unmodified p27. This modification alters p27 functions, preventing its binding to Grb2 complexes but does not affect its binding ability with cyclin E-Cdk2. These results suggest TG modification of p27 may be a novel regulatory mechanism affecting its tumor suppressing function, which could be targeted in cancer therapeutics.

MEDI 92

Toward the synthesis of hydroxytyrosol polyphenol

Emmanuel Onobun¹, *onobun@goldmail.etsu.edu*, **Ismail Kady**². (1) Department of Chemistry, East Tennessee State University, Johnson City, Tennessee, United States (2) Dept of Chemistry, East Tennessee State Univ, Johnson City, Tennessee, United States

Hydroxytyrosol, 2-(3,4-dihydroxyphenyl)ethanol, a naturally occurring polyphenol most common in olive tree (*Olea europaea*), is one of the most effective member of the polyphenols family, because of its remarkable antioxidant activity, its ability to inhibit oxidation of low-density lipids (LDL), and its protection against DNA oxidative damage. Hydroxytyrosol, which is widely used in cosmetics and food supplements industries, can be purchased as an olive oil extract that contains low concentration of hydroxytyrosol besides other polyphenols. The price and low natural abundance of hydroxytyrosol make alternative synthetic sources very attractive. This research aims to develop a novel method for the synthesis of pure hydroxytyrosol from commercially inexpensive precursor such as catechol; this can satisfy the increasing market demand and provide a more economical alternative source for this valuable polyphenol.

MEDI 93

Dual targeting of the cancer antioxidant network with redox active gold(I) N-heterocyclic carbene complexes

Rebecca McCall⁵, **Jannet Kocerha**⁵, **Jonathan L. Sessler**², **Vinoth Sittaramane**¹, **Kuppuswamy Arumugam**³, **Jonathan F. Arambula**⁴, *jarambula@georgiasouthern.edu*. (1) Biology, Georgia Southern University, Statesboro, Georgia, United States (2) Univ of Texas, Austin, Texas, United States (3) Chemistry, Wright State University, Dayton, Ohio, United States (4) Chemistry, University of Texas, Austin, Texas, United States (5) Chemistry, Georgia Southern University, Statesboro, Georgia, United States

To achieve a systems-based approach to target the antioxidant pathway, ferrocene and quinone functionalized N-heterocyclic carbene Au(I) complexes were designed, synthesized, and biologically tested in a series of human cancer cells. Redox reversible complexes were revealed by electrochemical and spectroelectrochemical studies. Complexes containing both a redox active moiety (to accentuate exogenous ROS via redox cycling) centered around a Au(I) atom (to irreversibly inhibit thioredoxin reductase (TrxR)) significantly inhibited cancer cell proliferation compared to controls (i.e. Au(I)-NHC alone, naphthaquinone alone, ferrocene alone). Treatment of A549 lung cancer cells with redox active Au(I)-NHCs led to increased levels of exogenous reactive oxygen species (ROS) via flow cytometry. Inhibition of TrxR, an essential mediator of ROS homeostasis, was also achieved in the same cell line. TrxR inhibition by a bis-naphthaquinone-Au(I)-NHC was comparable to that of auranofin, a Au(I) complex known to irreversibly inhibit TrxR. The complexes presented herein were found to

induce ER stress related mechanisms (via RNA microarray), activate cell death via an apoptotic mechanism (confirmed by annexin-V), and were shown to be efficacious in zebrafish embryos containing A549 human lung xenografts. These results illustrate that the dual targeting approach of reducing ROS tolerance while increasing ROS production leads to antioxidant homeostasis being perturbed from both ends, thus overwhelming the network and promoting cell death.

MEDI 94

Fluorescent chemosensing for chloride and nucleotides based on artificial receptors in water

Alejandro Dorazco Gonzalez, *alex.dorazco@ciencias.unam.mx*. Centro Conjunto de Invest. en Química Sustentable, Universidad Nacional Autónoma de México, Toluca city Estado de México, México

A series of fluorescent cationic compounds derivatives of *N,N*-bis(*N*-benzylquinolinium)pyridine-2,6-dicarboxamide triflate have been synthesized, characterized structurally by X-ray diffraction and studied in detail as fluorescent probes for inorganic anions and nucleotides in buffered aqueous solutions by NMR and fluorescence spectroscopy. In general, all the compounds display fluorescent quenching by additions of inorganic anions and nucleotides. For inorganic anions, Stern-Volmer quenching constants were determined in the range of 10-380 M⁻¹ with a very high affinity for chloride in the case of chemosensor based on *N,N*-bis(*N*-benzylquinolinium)pyridine-2,6-dicarboxamide triflate ($K_{SV} = 380$ and $K_A = 5050$ M⁻¹). A photoinduced electron transfer quenching mechanism with simultaneous receptor-anion complexation both in the excited and the ground state is proposed for inorganic anions and nucleotides. On basis of ¹H NMR and UV-Vis titrations, ESI-MS experiments, crystal structure and DFT calculations, the binding mode of Cl⁻ is proposed involving two hydrogen bonding interactions N-H ⋯ Cl⁻ with simultaneous formation of two short C-H ⋯ Cl⁻ contacts inside the cleft of receptor formed by pyridine-dicarboxamide motif. The receptor for chloride allows the fluorescence detection in water with a detection limit of 33 mmolL⁻¹ and good selectivity over other common anions. On the other hand, dicationic *N*-methylated at quinolyl moieties derivatives of three isomers of *N,N'*-bis(quinolyl)pyridine-2,6-dicarboxamide and respective *N*-methylquinolinium benzamides as reference compounds have been studied as chemosensors for nucleotides. In all chemosensors studied, the fluorescence quenching by nucleoside triphosphates is much more efficient than by inorganic anions. On basis of correlations of binding and quenching effects with structures of host and guest molecules as well as ¹H and ³¹P NMR titration results a model of nucleotide binding is proposed in which the terminal phosphate group is bound to bisamide cleft and nucleobase forms a stacking contact with quinolinium groups of receptors. Efficient binding of even simple inorganic anions by neutral amide N-H donors in water is attributed to high acidity of amides and preorganized rigid structure of the receptors.

MEDI 95

D-peptides as inhibitors of Proteinase 3-membrane interactions

Ksenia Maximova¹, Tom Venken², Nathalie Reuter², Joanna Trylska¹, joanna@cent.uw.edu.pl. (1) Centre of New Technologies, University of Warsaw, Warsaw, Poland (2) Department of Molecular Biology, University of Bergen, Bergen, Norway

Proteinase 3 (PR3) is a neutrophil enzyme that has been identified as a drug target for chronic inflammatory diseases. PR3 is expressed in cytoplasmic granules but also at plasma membranes where it mediates the inflammatory effects. The membrane interface binding site (IBS) of PR3 is distinct to its catalytic site therefore, targeting IBS would provide a more specific way to inhibit only the membrane-expressed PR3. We have designed D-peptides to target IBS by applying docking and molecular dynamics simulations. Next, we verified if these D-peptides bind to PR3 using isothermal titration calorimetry and fluorescence spectroscopy. Further, we performed surface plasmon resonance experiments to determine if these D-peptides inhibit the binding of PR3 to lipids.

We verified that the designed D-peptides did not affect the catalytic activity of PR3. A few peptides bound to PR3 hydrophobic pockets and inhibited PR3 binding to lipid vesicles. However, the (KFF)₃K peptide (both in the L- and D-form) bound also to the membrane. One D-peptide with the SAKEAFFKLLAS sequence inhibited the PR3-membrane binding site with IC₅₀ of about 40 μM and did not bind to the membrane itself. This work provides a direct connection between the computational studies and experimental verification of D-peptides. We also suggest possible inhibition pockets in PR3 other than the catalytic site.

MEDI 96

Molecular investigation of cyclic β- and γ-peptoids

Robert Grams, justing4@vt.edu, Daniel Marron, Mintesinot Kassu, Jatinder S. Josan. Chemistry, Virginia Tech, Blacksburg, Virginia, United States

A new generation of synthetic peptides, called foldamers, has emerged to provide bioinspired materials with unique structural properties. Foldamers, or oligomers that adopt specific secondary structures, provide endless possibilities in terms of their material and biological applications and tunability. When properly designed, foldamers can mimic the secondary structures of biologically active peptides with potentially higher affinity, greater specificity, and better physiological stability. Over the last two decades, this field has rapidly expanded from α-peptoids to linear and cyclic forms, as well as β- and γ-peptides. In particular, α-, β-, and γ-peptides adopt secondary structures, often helices, with greater stability than naturally occurring peptides. In a serendipitous discovery, we identified the unique sensitivity of cyclic β- and γ-peptoids towards a chiral environment. Cyclic, achiral β- and γ-peptoids were synthesized using SPPS,

characterized by LC/MS, and their secondary structure was evaluated by FRET, CD, and NMR. When these achiral residues were used, the presence of one chiral amino acid induced organization of the backbone in a structure reminiscent of a helix. It is our strong contention that the molecular understanding of cyclic β - and γ -peptoids in foldamer chemistry is critical in determining their utility in material and biomedical applications.

MEDI 97

Mapping of cell surface receptors using silver nanodiscs

Jatinder S. Josan, jsjosan@vt.edu, Mintesinot Kassu, Guoliang Liu, Assad Khan, Robert Grams. Chemistry, Virginia Tech, Blacksburg, Virginia, United States

Cell phenotype and function are correlative with changes in its surface, including the dynamic expression and/or distribution of cell surface receptors (CSRs). Innovative approaches are needed to uncover membrane proteins dynamics, and their potential effect on current biomedical approaches. Many multivalent ligand architectures, including polymers, dendrimers, and nanoparticles, are synthesized *a priori* and then evaluated for activity. However, the assumption that binding of such an architecture will be optimal is naïve at best, and possibly worse in many scenarios, particularly for targeting of a multi-protein system. Nano-patterning of silver nanodiscs (Ag NDs) with CSR-bound ligands acts as a “nano-camera”, thus providing a “snapshot” of receptor distribution on the cell surface. This image is then “revealed” through atomic force microscopy (AFM), with a functionalized cantilever that is sensitive towards tagged CSR ligands. As a proof-of-concept study, we synthesized melanocortin receptor 1 (MC1R) agonists and agonists with biorthogonal chemistries that allow immobilization into Ag NDs and AFM imaging. Our patterning of MC1R distribution on WM-266-4 melanoma cell line with this nano-camera technology will be discussed.

MEDI 98

Late-stage functionalization of complex molecules

Rashad Karimov, karimov.rashad@gmail.com, Ankit Sharma, John F. Hartwig. Chemistry, University of California, Berkeley, Berkeley, California, United States

Complex molecules are underrepresented in modern biological screening efforts due to challenges in accessing these types of molecules. Isolation of new natural products is slow, thus, alternative approaches to access natural product like compounds is required. Late stage functionalization is a useful strategy for creating analogues of complex small molecules for structure-activity relationship studies and for creating libraries of structurally complex small molecules. We have developed new strategies based on C-H and C=C bond azidation, as well as C-H bond silylation, that can be used for late-stage functionalization of natural product derivatives. Azides and silanes obtained from these reactions can be converted into alcohols, amines, amides and

triazoles. These C-H bond functionalization strategies and guidelines for their applications toward late-stage complex molecule functionalization will be discussed.

MEDI 99

Synthesis of novel imidazobenzodiazepine oxazole bioisosteres as potential alpha 2, 3 subtype selective GABA(A) receptors agonists with excellent metabolic stability, pharmacokinetics, and anxiolytic efficacy

Guanguan Li^{1,2}, *guanguan.li@yahoo.com*, **Kashi R. Methuku**^{1,2}, **Michael M. Poe**^{1,2}, **Jeffrey M. Witkin**³, **Jeffrey M. Schkeryantz**³, **Margot Ernst**⁴, **James M. Cook**¹. (1) Univ of Wisconsin, Milwaukee, Wisconsin, United States (2) Milwaukee Institute Drug Discovery (MIDD), University of Wisconsin-Milwaukee, Milwaukee, Wisconsin, United States (3) The Lilly Research Labs, Eli Lilly and Company, Indianapolis, Indiana, United States (4) Molecular Neurosciences, Medical University of Vienna, Vienna, Vienna, Austria

Benzodiazepines (BZD) are commonly used in the treatment of mental disorders such as anxiety disorders, schizophrenia, depression and neuropathic pain which affect over 18% of the US population. However, many patients respond poorly to the classical BZD's by suffering a variety of adverse effects including sedation, ataxia, tolerance, and addiction. Hz-166 (Zeilhofer et al.), a previously characterized 1,4-Benzodiazepine was identified as an alpha 2/alpha 3 GABA(A)R subtype-selective ligand (Rivas et al.), with significantly reduced ataxia/sedative effect, but with anticonvulsant, anxiolytic and antinociceptive properties. The ester moiety in Hz-166 was quickly metabolized in rodents to its corresponding carboxylic acid, which resulted to a suboptimal pharmacokinetics and precluded further study of it in rodents. A series of new heterocyclic bioisosteres at C-3 with various substituents were then designed, synthesized, and evaluated to improve brain penetrability and increase plasma and brain exposure. A novel lead compound, the 1,3-oxazole KRM-II-81 (Poe et al., J. Med. Chem., 2016) was the most promising candidate of the bioisostere series as a potent, alpha1 sparing GABA(A)R ligand with excellent PK, and drug stability in rodent anxiety models. In addition, KRM-II-81 exhibited increased anticonvulsant potency and efficacy in rodent seizure-induction models. Moreover, it exhibited the anxiolytic-like effects in a Vogel conflict test and a mouse marble-burying assay compared to the parent Hz-166, with no sedative effects. Thus, KRM-II-81 represents a significant advance in the pharmacology of these anxiolytic and anticonvulsant imidazobenzodiazepines.

MEDI 100

Study and isolation of chemical compounds of *Guaiaecum sanctum* for cytotoxicity and antitumor activities

Ashley A. Laureano, *ashley.laureano@upr.edu*. Natural Sciences, UPR Cayey, Vega Baja, Puerto Rico, United States

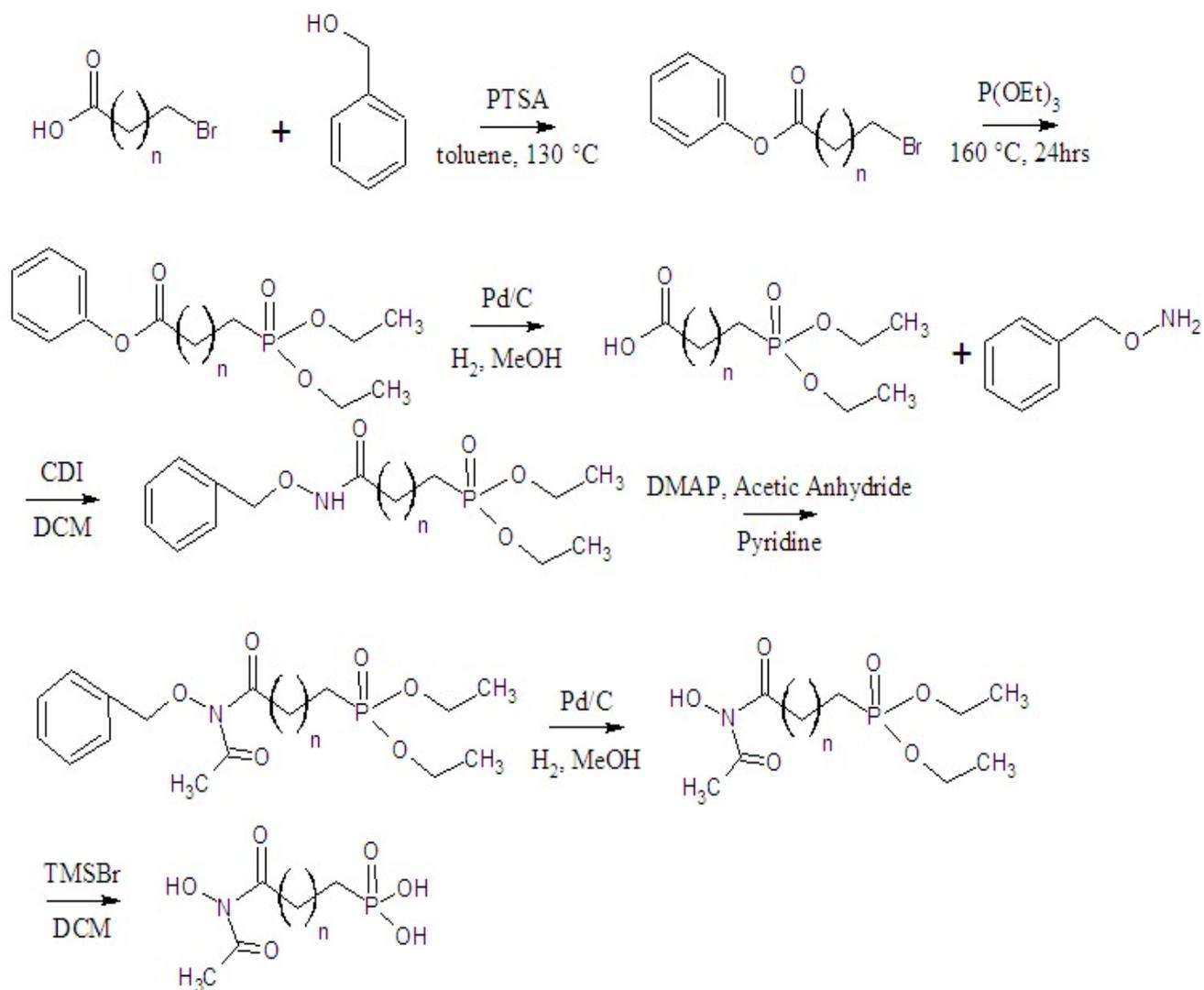
Guaiacum sanctum plant, commonly called holy wood or wood of life, has been used since the 1500's as a medical remedy as treatment for several conditions and diseases including syphilis and rheumatoid arthritis. In the year of 2011, a group of scientists published the results of a research where they isolate four spirocyclic lignans, which induce cell death via apoptotic mechanisms and exhibit cytotoxic activity against human breast cancer cell lines with an IC₅₀ value of 18 μM. *Guaiacum sanctum* can be found in many tropical zones in America including Puerto Rico. In a preliminary study, our research group analyzed other species of this genus, *Guaiacum officinale* showing strong activity with values of lethality in the chloroform and ethyl acetate extracts below 10 μg/mL and exhibited the ability to inhibit the cellular proliferation of breast cancer cells. At the present, extracts in different solvents of the *Guaiacum sanctum* cortex have been made. The purification of the hexane extract through column chromatography and the cytotoxicity tests of the solvent and crude extracts are in process. The cytotoxic activity of all extracts had been tested against different cell lines derived from solid tumors including ovarian (A2780, SKOV3), breast (MCF-7, MDA-MB-435), prostate (PC-3, LNCAP), and mammary epithelial cells (MCF-10A). The bioactivity results will be presented.

MEDI 101

Synthesis and evaluation of increased binding affinity analogues of fosmidomycin as inhibitors of the non-mevalonate isoprenoid biosynthesis pathway

Bryan C. Figula¹, *bfigula@mail.usciences.edu*, **John W. Tomsho**², **James M. Gamra**².
(1) Chemistry, University of the Sciences, Brick, New Jersey, United States (2)
Department of Chemistry and Biochemistry, University of the Sciences in Philadelphia, Philadelphia, Pennsylvania, United States

Many infectious diseases, including malaria and M. Tuberculosis continue to ravage the world. These pathogens use the non-mevalonate isoprenoid biosynthesis (MEP) pathway to synthesize isopentyl pyrophosphate to remain alive. Fosmidomycin is the most potent inhibitor to the MEP pathway known to date. Fosmidomycin, however, has issues with keeping the pathway blocked due to the fact that it does not bind strongly to the magnesium ion present in the enzyme, 1-deoxyxylulose-5-phosphate reductoisomerase (IspC). This project investigates the synthesis of analogues of fosmidomycin with modifications to the metal chelating functionality and evaluation as inhibitors of IspC. If this inhibitor gives favorable results, other modifications to fosmidomycin will be made, including the use of boronic acid to keep fosmidomycin neutral at physiological pH. If successful, these modifications will be incorporated together, to see if both aspects of the molecule can give significant improvements to the pharmacological properties of fosmidomycin.



Proposed reaction scheme for the synthesis of fosmidomycin analogues

MEDI 102

Design and synthesis of alpha-helix mimetics for treatment of high-risk human papillomavirus infection

Stephanie Rendon, *stephrendon@csu.fullerton.edu*. Chemistry & Biochemistry, California State University, Fullerton, Fullerton, California, United States

α -Helices are common secondary structural elements forming key parts of protein–protein interactions. These protein–protein interactions (PPIs) are key drug targets due to their significance in regulating and deregulating cellular signaling pathways. Targeting these interactions by creating inhibitors in that pathway have lead to small molecular α -helical mimetic design. The challenges that arise with α -helical drug targeting include size, limited residue mimicking, and creating an amphipathic molecule. The Orchard

Group has designed a library of α -helical compounds that vary in size and residue mimics to use as potential therapeutic treatment against viruses such as the Human Papilloma Virus (HPV). By mimicking the viral binding protein E6AP that inhibits the tumor suppressor protein P53, we hope to disrupt the viral cycle, thus promoting apoptosis of infected viral cells. Our library of molecules has been composed through using the computational molecular docking aid Molsoft ICM Pro to predict binding abilities and α -helical compounds. A synthesis of these compounds has been proposed and is currently being pursued as well as continually increasing the library of compounds by computational docking.

MEDI 103

Design of ER beta selective agonists for hippocampal memory enhancements

Karannagoda Perera¹, *karannagodaliyanage.perera@mu.edu*, **William A Donaldson**¹, **Alicia Schultiz**², **Daniel Sem**², **Jaekyoon Kim**³, **Karyn Frick**³. (1) Chemistry, Marquette University, Milwaukee, Wisconsin, United States (2) School of Pharmacy, Center for Structure-Based Drug Design and Drug Development, Concordia University, Mequon, Wisconsin, United States (3) Department of Psychology, University of Wisconsin Milwaukee, Milwaukee, Wisconsin, United States

Estrogens (17 β -estradiol, E2) have been garnered considerable attention over past decades in influencing cognitive process in relation to phases of the menstrual cycle, aging and menopausal symptoms. However, hormone replacement therapy can have deleterious effects leading to breast and endometrial cancer, predominantly mediated by estrogen receptor-alpha (ER α) the major isoform present in the mammary gland and uterus. Conversely, accumulating evidence supports for a dominant role of estrogen receptor-beta (ER β) for improved cognitive effects such enhanced hippocampal signaling and memory consolidation via ERK/MAPK signaling cascade. Thus, an exciting new direction in drug discovery is designing of ER β selective agonists. To date, several ER β agonists have been developed (e.g. DPN, 70-fold selective). The research presented herein, focus towards the design of two types of ER β selective dihydroxyl analogues based on phenolic and cycloheptane hydroxymethyl core. ER β agonist potency was initially evaluated a TR-FRET assay; and compounds having higher potency were re-evaluated in cell based assays for potency and with ER β vs. ER α selectivity. The lead compound thus obtained was further tested for CYP450s metabolism and its *in vivo* efficiency was evaluated using ovariectomized mice with object recognition (OR) and object placement (OP) tasks.

MEDI 104

Rational design, synthesis, and *in vitro* evaluation of mPGES-1 inhibitors as next-generation of anti-inflammatory drugs

Kai Ding³, *kai.ding@uky.edu*, **ziyuan zhou**³, **Yaxia Yuan**³, **Fang Zheng**¹, **Chang-Guo Zhan**². (1) University of Kentucky, Lexington, Kentucky, United States (2) Dept of

Pharm. Sciences, University of Kentucky, Lexington, Kentucky, United States (3)
Pharmaceutical Sciences, University of Kentucky, Lexington, Kentucky, United States

Purpose

Microsomal prostaglandin E synthase-1 (mPGES-1), functionally coupled with COX-2, is an inducible prostaglandin E synthase in response to pro-inflammatory stimuli and, therefore, represents a novel target for the development of anti-inflammatory drugs for therapeutic treatment of acute and chronic inflammatory disorders. The aim of this study is to rationally design and synthesize new mPGES-1 inhibitors that could become next generation of inflammatory drugs.

Methods

Starting from our recently discovered new inhibitors identified from structure-based virtual screening, we carried out *de novo* design of new compounds as possible inhibitors against mPGES-1. The computational design was followed by organic synthesis and *in vitro* activity assays for their inhibitory efficacies against mPGES-1 and COX enzymes (COX-1 and COX-2).

Results

A series of new compounds as potent inhibitors against mPGES-1 have been successfully designed, synthesized, and tested. Most of these compounds were capable of inhibiting mPGES-1 with sub-micromolar potency ($IC_{50} = 20$ to 900 nM). Further *in vitro* assays against other off targets including COX-2 indicated that these new compounds can selectively inhibit mPGES-1.

Conclusion

The structure-based computational design, followed by synthesis and *in vitro* assays for evaluating both potency and selectivity, has led to the discovery of a set of novel, potent and selective mPGES-1 inhibitors.

MEDI 105

Delivery of flavonol-based photoCORMs to mitochondria

Tatiana Soboleva¹, *tatiana.soboleva@aggiemail.usu.edu*, **Hector Esquer**², **Stacey Anderson**¹, **Abby Benninghoff**², **Lisa M. Berreau**¹. (1) Department of Chemistry and Biochemistry, Utah State University, Logan, Utah, United States (2) Department of Animal, Dairy, and Veterinary Science, Utah State University, Logan, Utah, United States

Mitochondria are the powerhouses of cells. These organelles are involved in modulating an array of cellular responses ranging from proliferation to apoptosis. Mitochondrial interactions with carbon monoxide (CO) are known to affect respiration, metabolism and biogenesis, and pathways involved in cell death. Currently, the distinction between pathways leading to cell proliferation and apoptosis that depend on mitochondrial CO homeostasis are poorly understood. To date, experiments to probe the quantitative

effects of CO on mitochondrial function have been performed using either CO gas or metal carbonyl-based CO-releasing molecules (CORMs), which cannot be controlled in terms of the precise timing and location of CO release. To enable dose-dependent CO studies on mitochondrial function, we are developing phosphonium-appended flavonols that can be targeted to mitochondria and used as visible light-induced CO-releasing molecules. In this contribution, we report the results of synthetic and aerobic visible light-induced CO-releasing studies of the phosphonium-appended flavonols. These include investigations of the quantum yield for CO release and product identification. Toxicity assays (MTT) performed for the phosphonium-appended flavonols and their CO release products indicate that the structure of the phosphonium appendage affects the toxicity. Fluorescence microscopy studies show that the functionalized flavonols are taken up by HUVECs cells and localize in the mitochondria.

MEDI 106

Exploring the mechanism of action of a newly-discovered therapeutic peptide for blinding retinal diseases by identifying its receptors

Dan Zhou, dzhou@caltech.edu, Zixuan Shao, Julia A. Kornfield. California Institute of Technology, Pasadena, California, United States

Diabetic and age-related retinopathies, both associated with growth of abnormal blood vessels, are leading causes of blindness in the developed world. Current treatments, such as Laser Photocoagulation and anti-Vascular Endothelial Growth Factor (VEGF) drugs, have limited efficacy and undesirable side effects. A recently discovered therapeutic peptide Luminate[®] (Allegro Ophthalmics, LLC) has proven to be effective in human clinical trials. Over half of the patients in phase I human clinical trial demonstrated vision improvement with 4 lines or over, and in some patients, the macular thicknesses was reduced back to the healthy state. It has shown significantly longer lasting benefits than anti-VEGF treatments and shows synergistic effects when used with them. Thus, the peptide appears to act through a different pathway than anti-VEGF agents. Finding that pathway may provide new insights into the retinopathies and their managements. Therefore, we are using the peptide as a tool to find the molecular mechanism of its clinically observed therapeutic effects.

The peptide and its scrambled counterparts are used to prepare fluorophore-peptide conjugates and peptide-directed coupling reagents. Results will be presented from *in-vitro* and *ex-vivo* experiments to visualize the distribution of Luminate binding using fluorophore-peptide conjugates. Progress toward enrichment of binding using ligand-directed receptor “pull down” will be described. Our goal is to enrich and identify the associated receptors by drug-directed crosslinking and immunoprecipitation. If successful, identification of the receptor that binds Luminate could provide further insight into the molecular basis of retinopathies, which could guide novel therapeutic agents to prevent vision loss.

MEDI 107

Design of new irreversible arginase inhibitors

Xuefeng Guo, xuefeng_guo@brown.edu, Christopher Seto. Chemistry, Brown University, Providence, Rhode Island, United States

Arginase is a manganese metalloenzyme that catalyzes the hydrolysis of arginine to form ornithine and urea. In addition to its fundamental role in the urea cycle, it also influences the immune systems in humans and mice. Certain parasites can evade human immune responses by activating the hosts' arginase which results in decreasing the synthesis of nitric oxide. Therefore, small-molecule arginase inhibitors are currently described as promising antiparasitic chemotherapeutics for the treatment of several diseases, including Leishmaniasis, Chagas' disease and sleeping sickness. We have designed and synthesized two irreversible arginase inhibitors with good inhibition and inactivation constant. We envision that this mechanism-based irreversible inhibition strategy can be developed into probes to further monitoring the activity of arginase in living cells.

MEDI 108

Development of biodegradable microneedles using dexamethasone and Poly Lactic-co-Glycolic Acid (PLGA) for cochlear drug delivery

Carlos Bravo, cbrav021@fiu.edu. Biochemistry and Molecular Biology, University of Miami, Miami, Florida, United States

Inner ear disorders are accountable for the vast majority and severity of diseases causing sensorineural hearing loss (SNHL), affecting more than 25 million people in the United States alone. Providing effective strategies to treat hearing loss disorders is still a continuing issue among physicians. Anatomical features of the inner ear, like the blood-labyrinth barrier (BLB) and the round window membrane (RWM), hinder the process for successful drug delivery. In the past decade, interest in the microneedle technology has increased significantly because of its capability to prevent pain and distribute a precise drug load to hair cells in the Organ of Corti (OC). This project is targeted to provide non-ototoxic, biodegradable and biocompatible microneedles using dexamethasone, an anti-inflammatory steroid, and Poly Lactic-co-Glycolic Acid (PLGA), a biodegradable polymer. Rhodamine B needle fabrication and analysis is conducted for comparative reasons by mimicking dexamethasone release. For smaller scale microneedle production and further studies, a wafer with micron-sized structures was prepared using SU-8 by photolithography.



In depth look of microneedle structures prepared using SU-8 on silicone wafer substrate.

MEDI 109

Alpha-helix mimetics as potential drugs for Human Papillomavirus (HPV)

Van Do, juliedo95@csu.fullerton.edu. Chemistry, California State University, Fullerton, Fullerton, California, United States

High-risk strain of human papillomavirus (HPV) have been identified as the main cause of cervical cancer by inducing infected epithelial cells. Specifically, the viral E6 protein associates with the human E6AP protein to form the E6-E6AP protein-protein complex, which causes the degradation of tumor suppressing p53 and subsequently, survival of the virus. Protein-protein interactions (PPIs) such as the E6-E6AP interaction, have been identified in many cellular processes as potential drug targets. New classes of drug-like compounds are needed for successful inhibition of such interactions. The Orchard lab has designed novel, amphiphilic, small molecule α -helix mimics that may be capable of inhibiting the E6-E6AP interaction by mimicking consecutive amino acid sequences from both faces of the E6AP α -helix which have been shown previously to be important for this binding event to occur. Successful inhibition of this PPI would allow infected cells to undergo apoptosis, avoiding any complex invasive clinical treatments. These compounds will be tested for their ability to inhibit the E6-E6AP interaction and characterized to determine their conformation during binding and in solution.

MEDI 110

Discovery of a novel irreversible and potent FLT3 inhibitor FF-10101 under clinical investigation

Masaru Takasaki, masaru_takasaki@fujifilm.co.jp, Atsushi Hirai, Daisuke Terada, Megumi Okubo, Toshiaki Tsujino, Kimihiko Sato, Shintaro Tanabe, Shinsuke Inuki,

*Shinsuke Mizumoto, Norie Fujikawa, Daisuke Hirano, Takeshi Yamaura.
Pharmaceutical and Healthcare Research Laboratories, Fujifilm Corporation,
Kanagawa, Japan*

Mutationally activated fms-like tyrosine kinase 3 (FLT3) causes aberrant growth of leukemia cells in acute myeloid leukemia (AML) patients. In particular, internal tandem duplication (ITD) mutation of FLT3 is identified in approximately 30% of AML patients and known to be a poor prognostic factor. Therefore, mutated FLT3 is considered to be an attractive and promising target for the treatment of AML.

To date, a number of FLT3 inhibitors have been developed, and several compounds are being evaluated for clinical efficacy in late phase trials. Recently, it has been reported that ITD mutations with the secondary point mutations at positions 691 (gatekeeper region) and 835 (activation loop) of FLT3 are likely to be drug resistance mutations corroborated by identification of such mutations in relapsed or refractory AML patients treated with Quizartinib.

To overcome these issues, we have developed a series of novel irreversible FLT3 inhibitors which formed a covalent bond with a cysteine residue at position 695 of FLT3 molecule. These inhibitors showed potent inhibitory activities against both FLT3 and mutated FLT3, and FF-10101 was selected as a drug candidate for clinical trials. The covalent bond formation was proved by X-ray crystallography, and the irreversibility was confirmed by using washout experiment in FLT3 kinase assay.

FF-10101 showed a strong inhibitory activity for FLT3 with IC₅₀ value of 0.2 nM, as well as antiproliferative activities against FLT3/ITD positive leukemia cell lines MV4-11 and MOLM13 with GI₅₀ values of 0.83 and 1.1 nM, respectively. Furthermore, FF-10101 exhibited potent inhibitory effects in a mouse xenograft model.

In conclusion, FF-10101 is a promising agent for the treatment of AML and the Phase 1/2a clinical trial will be conducted. Here, we describe chemical features of FF-10101 and its derivatives in terms of molecular design and optimization in medicinal chemistry.

MEDI 111

Discovery of CCT251236 from an HSF1 phenotypic screen: A pirin ligand with efficacy in an ovarian adenocarcinoma model

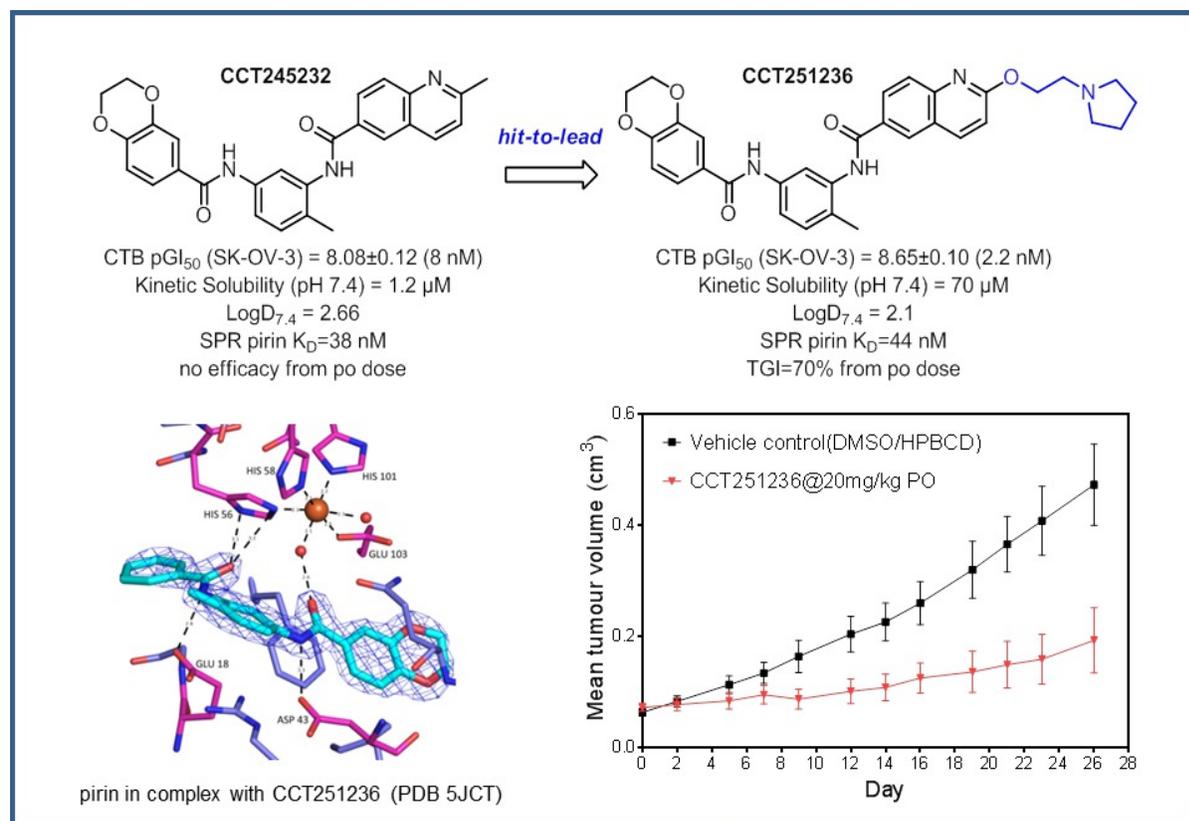
Elisa Pasqua¹, Elisa.Pasqua@icr.ac.uk, Matthew Cheeseman¹, Nicola E. Chessum¹, Carl Rye¹, Michael J. Tucker¹, Birgit Wilding¹, Lindsay E. Evans¹, Susan Lepri¹, Meirion Richards¹, Swee Y. Sharp¹, Salyha Ali^{1,2}, Martin Rowlands¹, Lisa O'Fee¹, Asadh Miah¹, Angela Hayes¹, Alan T. Henley¹, Marissa Powers¹, Robert te Poele¹, Emmanuel De Billy¹, Loredana Pellegrino¹, Florence Raynaud¹, Rosemary Burke¹, Rob L. van Montfort^{1,2}, Suzanne A. Eccles¹, Paul Workman¹, Keith Jones¹. (1) Cancer Therapeutics Unit, The Institute of Cancer Research, London, United Kingdom (2) Division of Structural Biology, The Institute of Cancer Research, London, United Kingdom

Heat-shock factor protein 1 (HSF1) is the master regulator of the heat-shock response and a key transcription factor that enables cancer cells to deal with oncogenic stress. As such, the inhibition of HSF1-mediated transcription represents a viable strategy in cancer treatment.

In this work, we describe the discovery of the bisamide series, identified using an unbiased, cell-based, high throughput, phenotypic screen to detect inhibitors of the HSF1 stress pathway. The hit compound, CCT245232, possessed poor solubility and consequently was ineffective in in vivo models following a po dose. Cell-based SAR and multi-parameter optimization of the solvent-exposed vector led to the discovery of the chemical tool CCT251236. This bisamide possessed good mouse pharmacokinetic properties and demonstrated in vivo efficacy in a human ovarian adenocarcinoma xenograft model. Therefore, this work represents an important advance in inhibition of the HSF1 pathway showing in vivo activity for the first time with an orally bioavailable compound.

We also present the target ID strategy based on a chemoproteomics approach, which identified the protein pirin, a metal-dependent putative transcription regulator, as a molecular target. The high affinity of the bisamide series for pirin was confirmed by SPR. Additionally, CCT251236 demonstrated the reported anticancer phenotype of pirin ligands by inhibiting the migration of WM266.4 melanoma cells in vitro at low nanomolar concentrations.

The discovery of CCT251236 provides a novel potent chemical tool to investigate the biological role of inhibiting the HSF1 pathway and binding to pirin, in vitro and in vivo.

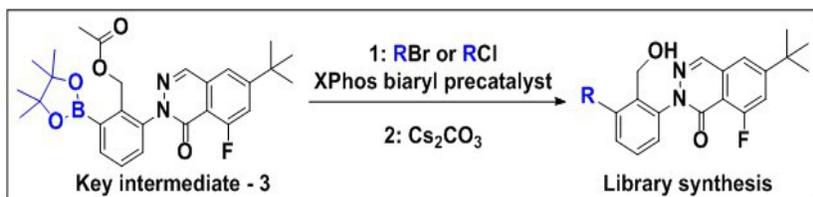
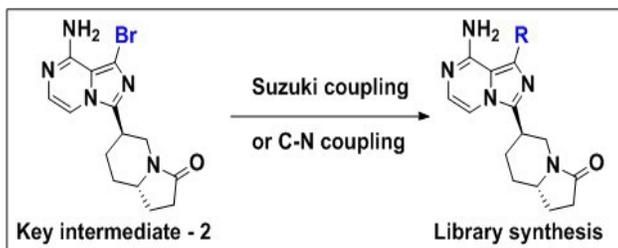
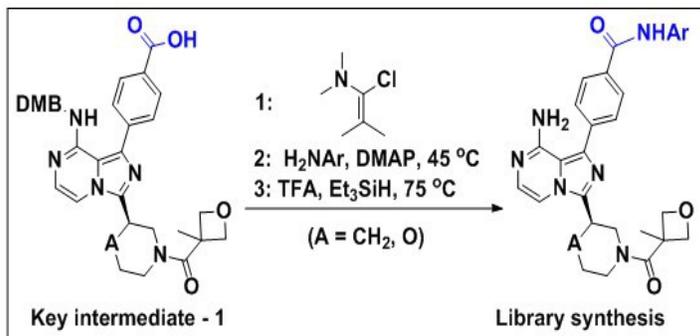


MEDI 112

Bruton's Tyrosine Kinase (BTK) program: Quick systematic SAR development utilizing parallel synthesis

Younong Yu¹, Rajan Anand¹, Deyou Sha¹, Henry A. Vaccaro¹, Milana Maletic¹, Rob Mazzola¹, Zhi-Cai Shi¹, Mo Abdul-Basit Alhassan¹, Sobhana B. Boga¹, Joseph L. Duffy¹, **Xiaolei Gao**¹, xiaoleigao2016@gmail.com, Deodial Guiadeen¹, Joseph Kelly¹, Arto Krikorian¹, Jian Liu¹, Kevin Maloney¹, Oleg Selytin¹, James Wang¹, Jiayi Xu¹, Wensheng Yu¹, Michael Altman¹, Brian M. Andresen¹, Yuan Liu¹, Michelle Martinez¹, Tony Siu¹, Shilan Liu², Chundao Yang², Hao Wu², Jiaqiang Cai², Ying-Duo Gao¹, Thierry Fischmann¹, Jeremy Presland¹, My Mansueto¹, Zangwei Xu¹, Erica Leccese¹, Jie Zhang-Hoover¹, Ian Knemeyer¹, Charles Garlisi¹, Nathan Bays¹, Peter Stivers¹, Philip Brandish¹, Alexandra Hicks¹, Ronald Kim¹, Joseph A. Kozlowski¹. (1) Merck & Co., Inc, Kenilworth, New Jersey, United States (2) WuXi PharmaTech Co. Ltd, , Shanghai, China

Bruton's Tyrosine Kinase (BTK) is a nonreceptor cytoplasmic tyrosine kinase belonging to the Tec kinase family, critical for B-cell proliferation, differentiation and signaling. Inhibition of BTK may be an effective way to treat autoimmune diseases such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). In this poster, we will describe (1): the quick systematic back-pocket SAR development in the imidazolpyrazine series using library approach; (2): Quick hinge screening in the H3 binder series utilizing parallel synthesis, and hit optimization through a close collaboration between ACE, Med Chem groups, CM&I, and structure chemistry.



MEDI 113

Design, synthesis, and evaluation of small molecule inhibitors of human *N*-myristoyltransferase

Mengjie Shen, *ms913@ic.ac.uk*. Chemistry, Imperial College London, London, United Kingdom

N-myristoylation is a ubiquitous protein modification which is potentially correlated with cancer. The enzyme catalyses this modification is NMT. A Recent study shows that inhibition of NMT by small molecules can kill cancer cells in a week. Several oncogenes including Src are NMT substrates. NMT inhibition induces ER stress, which is proved to kill cancer cells.

Based on a developing series of Plasmodium NMT inhibitors and one crystal structure, 8 compounds with a new linker were designed, synthesised and evaluated. Among them, the best compound showed an IC_{50} of 257 nM. Little potency loss comparing to the original series ($\text{IC}_{50} = 61.3$ nM) indicates the flexibility of the linkage part, just as the crystallography data suggested (No interaction with NMT in the linker area). This compound is also showing excellent penetration rate ($\text{EC}_{50}/\text{IC}_{50} = 13$) comparing to the same structure with the old linker ($\text{EC}_{50}/\text{IC}_{50} = 99$). YnC12 (an alkyne version of Myristic acid) tagging assay showed inhibitor-concentration-dependent labelling of the myristoylated NMT substrates, proved that this compound acts on target. HPG (an

alkyne version of Methionine) tagging assay done in parallel ruled out the possibility of comprehensive protein-synthesis-inhibition from cell toxicity.

Crystal structures of ligand-protein complexes (12 inhibitors from this series and 6 from another series) including this compound with human NMT1 has been solved at 1.49A-2.45A, which revealed that these 12 inhibitors with similar structures adopt two different conformations in the binding pocket. More crystallography work is undertaking to understand the two conformations and a re-design and modification of compounds are underway.

MEDI 114

Leucine's role of dry powder inhaler performance of salbutamol sulphate containing spray dried mannitol

Carlos Molina, *camolina4@me.com. Chemistry, University of Sussex, Brighton, United Kingdom*

Through the use of spray-drying, which allows for the modification and optimization of a particle's physicochemical properties along with their surface morphology, particle size, size distribution, particle morphology, density, and polydispersity index, L-Leucine was introduced into mannitol formulations of varying concentrations (0.1%, 0.5%, 1%, 5%, and 10%; w/w) and then compared to physical mixtures of similar concentrations to determine aerosolization performance of salbutamol sulphate. To analyze each formulation, numerous analytical techniques were implemented such as particle size distribution analysis, high-pressure liquid chromatography (HPLC), differential scanning calorimetry (DSC), scanning electron microscope (SEM), powder X-Ray diffraction (PXRD), and fourier transform infrared (FT-IR) spectroscopy. It was, then, determined that the efficacy of salbutamol sulphate's aerosolization performance was, in part, due to the introduction of L-Leucine in the formulation, prior to being spray-dried, that accounted for an increase in the fine particle fraction (FPF) of the 10% L-Leucine spray-dried formulation that surpassed all other formulations (both physical and spray-dried). It was also determined that all of the formulation carriers, when undergoing solid-state characterization, were spherical in their morphology with the exception being 0.1% L-Leucine, which was needle-like; α -, β -, and gamma-polymorph characterization was also determined.

MEDI 115

Effects of varying H₂S concentrations on sulfhemoglobin complex formation

Natalia E. Crespo Rosado, *natalia.crespo2@upr.edu. Industrial Biotechnology Department, University of Puerto Rico-Mayagüez, Mayagüez, Puerto Rico, United States*

Hydrogen sulfide, H₂S, a known toxic gas has recently been discovered to be synthesized endogenously in humans at low concentrations by the enzymatic pathways of cystathionine β synthase (CBS), cystathionine γ lyase (CSE) and 3-mercaptopyruvate (MPST). A highly lipophilic molecule, it is capable of penetrating a cell by simple diffusion where it interacts with various cellular components. This molecule has been identified as biologically significant and is thought to be associated with some essential bodily functions and various illnesses such as neuro degenerative disorders, respiratory disorders and some cardiovascular functions. However it has yet to be studied in full due to the lack of a reliable biomarker. Using the sulfheme derivatives formed by anaerobically reacting heme proteins with H₂S in the presence of H₂O₂ within 6Q cuvette, the sulfheme compounds obtained were studied using UV-Vis spectrophotometry where the bands of interest were the 620nm and 720nm bands. From the observed data, a positive correlation between sulfheme compound formation and variable H₂S concentration could be effectively suggested, which could be indicative of the sulfheme compound's potential as a biomarker for endogenous H₂S concentration.

MEDI 116

Discovery of novel inhibitors of NF-κB Inducing Kinase (NIK) from an FBDD approach

Lily Kwok, lkwokj@gmail.com. Medicinal Chemistry, Takeda California, San Diego, California, United States

(NIK, MAP3K14), is a key regulator in the non-canonical NF-κB pathway which is central to multiple autoimmune and inflammatory disorders biology. Accordingly, modulation of this pathway and inhibition of NIK is an attractive strategy that has generated substantial interest for pharmaceutical companies including Takeda. Our method to targeting NIK was centered around fragment-based approaches aimed at producing highly efficient inhibitors with superior physicochemical properties. A fragment-based screen against the target was performed using STD NMR and thermal shift assays which resulted in identification >90 hits. The subsequent optimization of a prioritized fragment to a novel lead compound with high ligand efficiency, adequate cellular potency and good physicochemical properties will be presented.

MEDI 117

Utilizing the message-address concept to develop selective mu opioid receptor antagonists as potential treatment of opioid addiction

Samuel Obeng¹, obengs@vcu.edu, Abdulmajeed Jal², David Stevens², William L. Dewey², Dana E. Selley², Yan Zhang¹. (1) Department of Medicinal Chemistry, Virginia commonwealth University, Richmond, Virginia, United States (2) Department of Pharmacology & Toxicology, Virginia commonwealth University, Richmond, Virginia, United States

Substance abuse is a big problem in the US with 47,055 deaths reported due to drug overdose. Substance abuse costs the US over \$600 billion annually. The opioid receptor agonist methadone and the partial agonist buprenorphine are the major agents used in opioid addiction treatment, but 40-60% of these patients relapse. Opioid receptor antagonists such as naltrexone have been shown to block relapse and curb drug craving in opiate addicts, but some severe side effects of these antagonists have been reported. Studies have shown that in mu opioid receptor (MOR) knock-out mice, the addictive/abuse liability, respiratory depression and constipation associated with opioids are abolished. Thus, blocking the MOR by opioid antagonists may lead to treatment of addiction relapse, whereas blocking the kappa opioid receptor (KOR) and delta opioid receptor (DOR) may result in side effects including depression and dysphoria. Therefore, selective MOR antagonist could be optimal for the treatment of opioid addiction. Utilizing the "message-address" concept and previous data from our lab, we have designed and synthesized a series of MOR selective antagonists. 18 compounds were synthesized and their binding affinities were determined by radioligand binding assay using Chinese hamster ovary (CHO) cells stably expressing the mouse MOR. All the compounds tested bound with high affinity (≤ 5 nM). *In vitro* functional studies were then conducted using the [35 S]-GTP γ S assay. In this assay, two compounds produced the highest stimulation of G-protein activity (60% - 88%) relative to DAMGO (a known opioid agonist). All other compounds produced low stimulation ($\leq 25\%$) relative to DAMGO, with VZMN106 having the lowest stimulation of DAMGO. *In vivo* studies were conducted to test for agonism and antagonism, 10mg/kg of compounds and morphine were used. Two compounds were identified as agonists and partial agonists respectively with % maximum possible effects of 80% and 59%. In the screen for antagonism, VZMN106 was identified as the most potent antagonists and it antagonized morphine's effect by 93%. Several compounds also antagonized morphine's antinociception effect by more than 50%. Our molecular design strategy has been successful in producing very high affinity mu opioid receptor ligands. We have identified a MOR opioid antagonist that has the potential to be used in opioid addiction treatment.

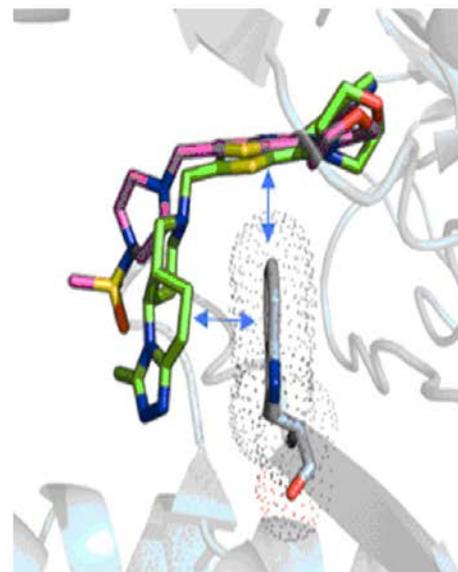
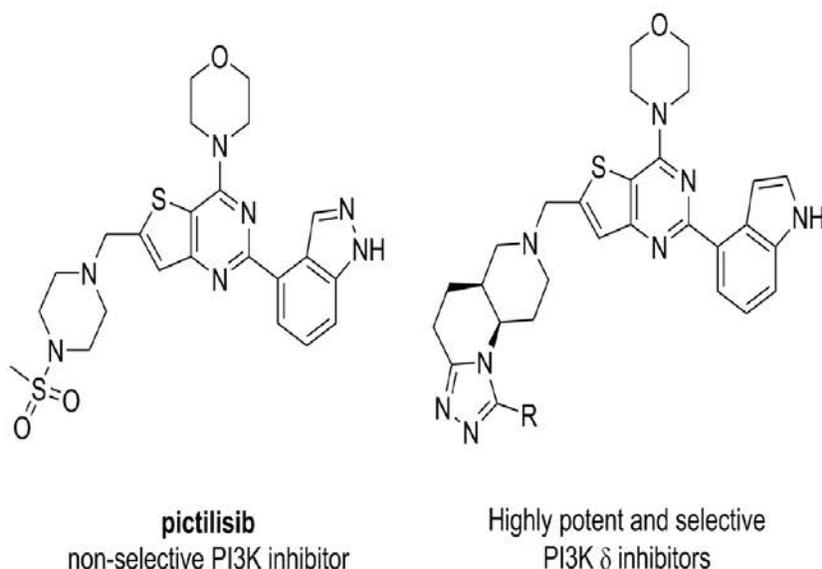
MEDI 118

Structure-activity relationships of phosphatidylinositol-3-kinase δ inhibitors for the treatment of inflammatory respiratory diseases

Aimie Garces², paxaeg@nottingham.ac.uk, **Carolin Schwehm**², **Michael J. Stocks**², **Nicholas Kindon**², **Simon J. MacDonald**¹, **James Rowedder**¹, **Tracey Bradshaw**². (1) *GlaxoSmithKline, Stevenage, United Kingdom* (2) *Pharmacy, University of Nottingham, Nottingham, United Kingdom*

Phosphatidylinositol-3-kinases (PI3K) are a family of kinases that phosphorylate phosphoinositides. Class I consists of four structural isoforms, PI3K α , PI3K β , PI3K γ , and PI3K δ which have differing biological roles. The PI3K δ isoform is required for cytokine production and proliferation in T-cells and allergen induced de-granulation of mast cells via the IgE, ultimately leading to inflammation. PI3K δ inhibitors are therefore

in a position to treat inflammatory respiratory diseases such as allergic asthma and chronic obstructive pulmonary disease. Work will be presented relating to the design, synthesis and evaluation of highly potent PI3K δ isoform-selective inhibitors. Rationalisation of the unique selectivity observed is by considering the novel inhibitors docked into the known X-ray crystal structure of PI3K δ suggesting the high isoform selectivity may result from a synergistic combination of key interactions within the PI3K δ active site.



MEDI 119

Analysis of the binding parameters of the reversible inhibitor Argyrin B at the active sites of the constitutive and immunoproteasomes

Duncan Allardyce, Celia Bell, Erika Loizidou, e.loizidou@mdx.ac.uk. Natural Sciences, Middlesex University, London, United Kingdom

The reversible, non-covalent inhibitor Argyrin B is investigated for active site selectivity and binding interactions between the constitutive (CP) and immuno-proteasomes (IP), using purified enzyme assays alongside computational molecular modelling. The proteasome pathway degrades >90% of cytosolic proteins deemed redundant, misfolded or toxic, thereby influencing key regulatory pathways such as: cell cycle control, DNA repair and apoptosis. Existing CP inhibitors have shown great success for multiple myeloma and mantle cell lymphoma treatment however currently lack specificity for active sites, associated with severe toxicity. Upon stimulation by inflammatory cytokines, CP active sites are replaced with corresponding β 1i, β 2i and β 5i subunits; forming the immunoproteasome (IP) with key structural differences recently identified. As such, there are high levels of IP reported in various disease states requiring increased protein degradation.

Recently, the naturally derived Argyrin cyclic peptides have emerged as an exciting

family of compounds among which, Argyrin A and F exhibit reversible, strong proteasome inhibitor effects with mechanisms distinct to existing therapeutics. Argyrin B molecular docking at the CP and humanised IP active sites revealed favourable binding energies at $\beta 1i$ over $\beta 1c$, due to specific amino acid substitutions. These create a more hydrophobic $\beta 1i$ S1 pocket, increased interactions with Trp moieties, and different spatial arrangements for increased Thr1 and Ser129 Hydrogen bonding. $\beta 2$ and $\beta 5$ active site inhibitions were predicted similar affinities between the CP and IP, although with distinct orientations and intermolecular bonding. Kinetic analysis of Argyrin B inhibition was determined through purified 20S CP & IP assays. Using active site specific substrates, AMC liberation was measured over time at varied inhibitor and substrate concentrations. Inhibition at $\beta 1i$ exhibited an approximately 20-fold increase compared to $\beta 1c$. Whilst $\beta 5c$ and $\beta 5i$ revealed similar binding affinities, at a low micromolar range.

Active site selectivity combined with understanding of binding interactions and conformation allows potential enhancement of targeted actions and development of highly selective therapeutics.

MEDI 120

Flavonol-based CO-releasing molecules: Tunability and albumin binding properties

Marina Popova, storozhenko.marina@gmail.com, Tatiana Soboleva, Lisa M. Berreau. Department of Chemistry and Biochemistry, Utah State University, Logan, Utah, United States

The exploration of CO-releasing molecules (CORMs) has been driven by the discovery of several beneficial health effects associated with the controlled administration of small amounts of CO. These include anti-inflammatory, anti-apoptotic, anti-hypoxia and anti-proliferative effects, as well as the promotion of vasodilation and protection of tissues against reperfusion injury. Metal-free CORMs that release CO upon illumination with visible light (photoCORMs) are of particular current interest. Our laboratory has previously reported a new family of organic photoCORMs that are based on the well-known biological structural motif found in flavonols. These compounds are highly tunable in terms of their light absorption properties and CO-releasing efficiencies. In the research to be presented, we outline results of synthetic and CO release reactivity studies of azaflavonol-based photoCORMs. We will also describe the results of fluorescence quenching studies which show that the non-covalent interaction of flavonol and azaflavonol-based photoCORMs with albumin can be tuned over a wide range of binding constants through structural modification of the flavonol. These results are of particular interest as albumin is known as a versatile carrier protein for therapeutic and diagnostic agents due to its natural transport function, multiple ligand binding sites and cellular interactions.

MEDI 121

Identification of ligand-efficient inhibitors of *Trichomonas vaginalis* adenosine/guanosine preferring nucleoside ribohydrolase using NMR-based fragment screening

Samantha N. Muellers¹, smuellers21@gmail.com, Annie L. Benzie¹, Dean G. Brown³, Scott Cowen³, David W. Parkin¹, Brian J. Stockman². (1) Adelphi University, Smithtown, New York, United States (2) Department of Chemistry, Adelphi University, Garden City, New York, United States (3) AstraZeneca Pharmaceuticals, Waltham, Massachusetts, United States

Trichomoniasis is caused by the parasitic protozoan *Trichomonas vaginalis*, and is the most prevalent, non-viral sexually transmitted disease. The parasite has shown increasing resistance to the current 5-nitroimidazole therapies indicating the need for new therapies with different mechanisms. *T. vaginalis* is an obligate parasite in that it is incapable of the de novo synthesis of purine and pyrimidine rings. It must scavenge nucleosides from host cells and then use salvage pathway enzymes to obtain the nucleobases. The first step in this pathway is the hydrolysis of nucleosides to release the nucleobases. The adenosine/guanosine preferring nucleoside ribohydrolase was screened against a 2,000-compound subset of the AstraZeneca fragment library using a ¹H NMR-based activity assay to monitor substrate hydrolysis. Three classes of inhibitors with more than five representatives were identified: bis aryl phenols, amino bicyclic pyrimidines, and aryl acetamides. Several other classes of inhibitors with more than three representatives were also identified. Included among the active fragments were six compounds with IC₅₀ values < 10 μM and ligand efficiency values greater than 0.5. Several identified and validated chemical templates are presently serving as the basis for medicinal chemistry efforts aimed at discovering < μM compounds that can be tested in vitro for target validation against both 5-nitroimidazole-sensitive and 5-nitroimidazole-resistant *T. vaginalis* strains.

MEDI 122

Design, synthesis and employment of Flupirtine-derivative chemical probes for neuroprotective target identification

Nihar Kinarivala³, nihar.kinarivala@ttuhsc.edu, Fadi Saadeh², Joelle Makoukji², Rose-Mary Boustany², Paul C. Trippier¹. (1) Department of Pharmaceutical Sciences, Texas Tech University Health Sciences Center, Amarillo, Texas, United States (2) Department of Biochemistry and Molecular Genetics, American University of Beirut Medical Center, Beirut, Lebanon (3) Department of Pharmaceutical Sciences, Texas Tech University Health Science Center, Amarillo, Texas, United States

Batten disease is a rare, autosomal recessive neurodegenerative disorder, affecting around 1 in 12,500 children in the US. It is the most common form of a group of disorders called neuronal ceroid lipofuscinosis (NCLs). Various genes have been

identified (CLN1-14) in which mutations can lead to Batten disease. However, the common pathway underlying these genes and their resulting proteins has not yet been identified. Currently, there is no cure for Batten disease; stem cell, gene therapy and enzyme replacement therapy have been attempted but failed to show efficacious results. Current treatment is symptomatic and supportive but does not target the underlying disease, accelerated rate of death of neurons.

We and our collaborators have shown that the small molecule Flupirtine exerts neuroprotective effect in both isolated neurons and crucially, in Batten Disease patient lymphoblasts under pro-apoptotic conditions. Flupirtine was never designed as an NCL treatment, yet indicates potential in this disease state. No medicinal chemistry structure-activity relationship (SAR) studies have been conducted to investigate the potential of this compound as a hit for Batten disease.

We have established a synthetic route suitable for the generation of diverse analogues of flupirtine to permit SAR determination of neuroprotective activity. Our preliminary screening in PC12 cells have identified several analogues which provide enhanced neuroprotective effect as compared to flupirtine. These results translate to SH-SY5Y cells, human neurons differentiated from iPSCs and NCL phenotypic siRNA knockdown PC12 cells. We have demonstrated that the lead molecules act, at least in part, by upregulating the antiapoptotic protein Bcl-2. Appendage of affinity-capable moieties to these enhanced activity compounds will provide chemical probes to allow identification of protein targets.

MEDI 123

Development of imidazopyridines and anti-proliferative effect against castration resistant prostate cancer cells

Idris Wazeerud-Din^{1,2}, wazeerud@gmail.com, Ana Millena², Shafiq Khan², James Bu¹. (1) Chemistry, Clark Atlanta University, Atlanta, Georgia, United States (2) Center for Cancer Research Therapeutic Development, Atlanta, Georgia, United States

Prostate cancer (PCa) is a complex multifaceted and biologically heterogeneous disease. PCa is identified as the second leading cause in cancer death in men. Statically, PCa is found more commonly in African-American men. Moreover, they are more likely to be diagnosed at an advanced stage, and are nearly 2.5 times as likely to die from the disease compared with Caucasian men. Not only are African American men more susceptible to prostate cancer based on statistics, they are developing the disease at a younger age.^{1, 2} The category of men presenting with either a high risk localized cancer or with metastatic disease is usually treated aggressively with any of the followings: prostatectomy, radiation therapy and/or androgen deprivation therapies (ADT), which have been expanded in recent years to include novel substantially more efficient drugs.² ADT has been the mainstay of treatment towards patients with metastatic PCa. The idea of hormone therapy (also commonly referred to androgen deprivation therapy (ADT) or androgen suppression therapy) is to reduce levels of male hormones, namely testosterone, which stimulates prostate cancer cells to grow. However, this treatment eventually leads to develop castration-resistant prostate

cancer. Serum testosterone concentration is less than 20 ng/dl (0.69 nmol/l) at castrate level (normal serum testosterone levels are about 500 to 600 ng/dl (17.3-20.8 nmol)). The androgen-deprivation therapy is considered effective if the testosterone level is lowered to a threshold of 50 ng/dl (1.73 nmol/l). Therefore, the development of new drugs is critical for the treatment of castration-resistant prostate cancer. A recent study of imidazopyridines (which are developed in our lab) reveals potential antiproliferative properties against castration-resistant prostate cancer. The inhibitory activity is partly due to the induction of apoptosis. The mechanistic study reveals that imidazopyridines inhibit both AR and PI3K/Akt signaling via the inhibition of phosphoinositide 3-kinase (PI3K). In this study, we have reduced the imidazopyridine moiety that is comparable in molecular structure to the unreduced imidazopyridine and pose as a structural insight in the development of novel imidazopyridines for castration-resistant chemotherapy.

MEDI 124

Novel chemotype of histone demethylase family KDM4 inhibitors

Marton Siklos, *martonimre.siklos@ucsf.edu*, Magdalena Korczynska, Timothy A. Bates, Brian Shoichet, Danica G. Fujimori. UCSF, San Francisco, California, United States

Posttranslational chromatin modifications play a key role in regulation of gene expression. Chromatin methylation at lysine residues is one such modification, dynamically regulated via opposing activities of histone methyltransferases and demethylases. Changes in lysine methylation caused by aberrant expression or mutation of these enzymes have been shown to promote tumorigenesis in several cancer models. In several types of cancer, overexpression of KDM4 isoforms promotes tumor growth and metastasis, therefore, KDM4 isoforms are promising targets for potential targeted cancer therapy. We intend to identify selective, cell permeable small molecule inhibitors of enzymes belonging to the KDM4 subfamily in order to enable pharmacological validation of these enzymes as potential therapeutic targets for cancer. Rationally-designed derivatives of our first generation pyridyl carboxylate-derived biaryl demethylase inhibitors, which show excellent potencies but have limited subfamily selectivity and lack cellular activity, were evaluated for target binding via molecular docking. Derivatives that showed favorable docking poses were synthesized and subjected to iterative optimization. Obtained derivatives were evaluated for their potency by *in vitro* enzyme inhibition assay. The cellular activity of most potent derivatives was assessed by high-content fluorescent imaging cellular assay that monitors disappearance of the methylated substrate. *In vitro* potencies of lead compounds were in the 1 - 30 μ M range. Cellular activity was assessed in U2OS cell line expressing FLAG-KDM4C. Modest recovery of substrate methyl mark was observed, and no substantial improvements were gained through derivatization. Our findings indicate that carbamate derivatives of the initial scaffold are potent *in vitro* inhibitors of KDM4 demethylases. Despite favorable *in vitro* potencies, cellular activity of these molecules and their pro-drug ester derivatives was modest.

MEDI 125

Progress towards a novel class of immune modulators: Covalent toll-like receptor-7 agonists

Alfred C. Chon, *acchon@uci.edu*, Aaron Esser-Kahn. *Chemistry, University of California, Irvine, Irvine, California, United States*

Toll-like receptors (TLRs) are interesting targets for immunotherapies and their agonists are currently being explored for anti-viral activity, cancer immunotherapies, and vaccine adjuvants. One particular challenge is making TLR agonists more specific, so that they do not contribute to general activation. Targeted covalent inhibitors have been shown to have irreversible binding with the receptors of interest, leading to high selectivity and potency. These properties are very attractive for TLR agonists. In addition, it is currently not known what effect continuous activation of TLRs will have on immune activation. By applying the design principles of covalent inhibitors to agonists of TLR, we have developed several potential covalent agonists of TLR7 and evaluated them in biological assays.

MEDI 126

Novel prodrugs of transition state inhibitors of norovirus 3CL protease

Anushka Galasiti Kankanamalage¹, *axgalasitikankanamalage@shockers.wichita.edu*, Yunjeong Kim², Kevin Alliston¹, Athri D. Rathnayake¹, Michelle Butler³, Setven Cardinale³, Terry L. Bowlin³, Kyeong-Ok Chang², William Groutas¹. (1) *Chemistry, Wichita State University, Wichita, Kansas, United States* (2) *Department of Diagnostic Medicine & Pathobiology, Kansas State University, Manhattan, Kansas, United States* (3) *Microbiotix Inc, Worcester, Massachusetts, United States*

Human noroviruses are the principal cause of non-bacterial acute gastroenteritis worldwide, consequently, they have a major impact on public health. It is estimated that noroviruses are responsible for 19-21 million infections, 56000-71000 hospitalizations, and 700-800 deaths annually in the USA. Morbidity is particularly high among the young and elderly, as well as immunocompromised individuals. In developing countries, the mortality rate among children <5 years old due to diarrheal disease caused by noroviruses is estimated to account for 71 000 deaths annually. The problem is further exacerbated by the high infectivity, genetic diversity, copious virus shedding, and environmental stability of noroviruses. Other factors that compound the problem and hamper drug discovery efforts include the lack of a robust animal model that recapitulates all aspects of the disease and a sub-optimal understanding of norovirus biology and pathogenesis. Collectively, the management of norovirus infections presents a challenge because no effective vaccines or norovirus-specific therapeutics or prophylactics are currently available.

As part of an ongoing research program focused on the discovery of antiviral therapeutics and prophylactics for norovirus infections, we have recently reported the

structure-guided design, synthesis, and *in vitro* biochemical evaluation of peptidyl aldehydes, α -ketoamides, and their corresponding bisulfite adducts, as inhibitors of norovirus 3CL protease. These inhibitors were found to potently inhibit norovirus in a cell-based replicon system and to exhibit efficacy in a small animal model of norovirus infection. Importantly, the generated bisulfite adducts were found to display pharmacological activity *in vitro* and in a cell-based replicon system comparable to the precursor aldehydes and α -ketoamides. In order to further optimize the PK characteristics of the bisulfite adducts and identify an orally-bioavailable drug candidate, we report herein the design, synthesis, and evaluation of a series of ester and carbamate prodrugs of aldehyde bisulfite adduct inhibitors of NV 3CL protease.

MEDI 127

Cyclopropylthiazines as potent BACE1 inhibitors for Alzheimer's disease: Challenging chemistry and *in-vivo* data of this new pre-clinical series

Aaron Siegmund², *asiegmund@gmail.com*, *ke kong*², *Mike Frohn*², *Nobuko Nishimura*², *Alexander pickrel*², *Michael D. Bartberger*¹, *Dean Hickman*³, *Jennifer R. Allen*^{3,2}, *Stephen Wood*³, *Matthew P. Bourbeau*². (1) Molecular Structure and Characterization, Amgen, Thousand Oaks, California, United States (2) Medicinal Chemistry, Amgen, Inc., Ventura, California, United States (3) Amgen, Inc., Newbury Park, California, United States

Abstract: β -Site amyloid precursor protein cleaving enzyme 1 (BACE1) is an aspartyl protease whose function is essential for the production of the neurotoxic β -amyloid (A β) peptide. A β is widely considered to play a critical role in the etiology of Alzheimer's disease, and consequently the inhibition of BACE1 activity is a compelling target for this chronic neurodegenerative disease. Previous BACE inhibitors have utilized thiazine warheads as substrates to engage the bi-aspartate binding pocket of the BACE1 enzyme. Our team targeted the synthesis of cyclopropylthiazines as an alternative aspartate binding chemotype. The resulting molecules demonstrated significant activity on both biochemical and cellular inhibition of BACE1 and lowered brain and CSF A β levels in rat pharmacodynamic studies. The chemistry required to access the cyclopropylthiazines was challenging to develop, and this poster will present both the successful and unsuccessful synthetic approaches. Additionally, the poster will include an x-ray co-crystal structure of a lead inhibitor with BACE1 that supports the assigned stereochemistry of these new inhibitors.

MEDI 128

Threading the needle: Exploiting a P1-P3 ether linkage in the development of novel BACE inhibitors

Shawn P. Walsh¹, *shawn_walsh@merck.com*, *Aurash Shahripour*¹, *Esther Kim*¹, *Wei Li*¹, *Jack D. Scott*¹, *Diane Rindgen*², *Lynn Hyde*³, *Peter Orth*⁴, *Hongwu Wang*⁴, *Matthew Kennedy*³, *Jared Cumming*¹. (1) Lead Optimization Chemistry, Merck Research

Laboratories, Kenilworth, New Jersey, United States (2) Pharmacokinetics, Pharmacodynamics and Drug Metabolism, Merck Research Laboratories, Kenilworth, New Jersey, United States (3) Neuroscience, Merck Research Laboratories, Kenilworth, New Jersey, United States (4) Structural Chemistry, Merck Research Laboratories, Kenilworth, New Jersey, United States

Alzheimer's disease (AD) is a leading cause of mortality in the US and is characterized by neuronal cell death and cognitive decline. Aggregation of β -amyloid (A β) peptides, formed through processing of amyloid precursor protein by beta secretase (BACE1) and gamma secretase, is one of the key hallmarks of the disease. The amyloid hypothesis posits that the accumulation of oligomeric forms of A β and the subsequent formation of plaques are central to the development of AD. Considerable effort has been focused on discovery of BACE inhibitors in the hope of altering the course of the disease, most notably resulting in the disclosure of a number of iminoheterocycle based P1-P3 amide inhibitors. Replacement or deletion of the P1-P3 amide linker while maintaining a favorable overall profile of these molecules has posed a significant challenge. In this presentation, the design and exploration of iminoheterocycle BACE inhibitors featuring a novel P1-P3 ether linker will be described. These efforts have produced potent BACE inhibitors with high selectivity over related aspartyl proteases including Cathepsin-D. The presentation will describe *in vitro* SAR as well as include a discussion of the *in vivo* PK/PD relationship in this series.

MEDI 129

Novel ROS activated prodrugs as kinase inhibitors: A strategy to improve selectivity

Puruji N. Gurjar, gurjarpn@mail.uc.edu, Safnas Farwin Abdul Salam, Edward J. Merino. Chemistry, University of Cincinnati, Cincinnati, Ohio, United States

Reactive Oxygen Species (ROS) is a phrase used to describe a number of reactive molecules and free radicals derived from molecular oxygen. These species, predominantly produced as byproducts during the mitochondrial electron transport have a vital role in cell signaling, including; apoptosis; gene expression; and the activation of cell signaling cascades. Recent evidence has shown that compared to normal healthy cells, ROS levels are overexpressed in bulk cancer cells, including cancer stem cells where they promote many aspects of tumor development and progression. Elevated ROS levels interfere with the redox homeostasis and promote tumor formation by initiating an abnormal induction of signaling pathways that can cause tumorigenesis. To translate this finding into a therapeutic strategy we designed a self-cyclizing scaffold which can be attached to a drug, an antibody or a nanoparticle; which is activated only in the presence of high oxidative stress to eject the molecule of interest in the high ROS containing environment. Many potent kinase inhibitors with anticancer activity have failed clinical trials for not showing selectivity towards cancer cells over normal cells. We hypothesize that, attaching these molecules of interest to our scaffold will act as a prodrug which will be activated only in the high ROS containing cancer cells; thus by

improving their selectivity without losing potency. The structure activity relationship (SAR) of our scaffold was explored to investigate the rate of oxidation and self-cyclization to yield an efficient release of molecule of interest attached to it. Here, the synthesis and strategies of our ROS-activated scaffold attached to a kinase inhibitor will be discussed. Furthermore, how ROS activation can be used as a strategy for development of novel prodrugs for efficient and selective treatment of cancers is shown.

MEDI 130

Design and synthesis of selective HDAC6 inhibitors as potential agents against glioblastoma

Sheang Tze Fung³, *issa87belle@hotmail.com*, Amit A. Sadani⁴, Kuen-Da Wu¹, Ji-Wang Chern². (1) National Taiwan University, Taipei City, Taiwan (2) Natl Taiwan Univ Sch Pharma, Taipei, Taiwan (3) National Taiwan University, Taipei, Taiwan

Glioblastoma multiforme (GBM), the most common brain tumors which are very hard to prognosis, with high proliferative activity, and highly resistive to chemotherapeutic agents. Apart from that, the survival rate of GBM patients are very poor, there are about 3.3% of average 2-years survival, 1.2% of average 3-years survival and the mean survival time are approximately 12 months. Therefore, a significant unmet medical need is urgent to develop new drug which fulfill the effective requirement for GBM treatment. Glioblastoma cells proliferation is associated with HDAC6. Histone deacetylases (HDACs) are involved in different physiological function, and also found involved in tumorigenesis. HDAC6, a class IIb HDACs family which observed overexpression in different cancer cell lines, including lymphoma, leukemia, breast cancer, lung cancer and brain cancer.

Herein, this poster reports the design and synthesis of a tricyclic naphthalimide, a well-known pro-fluorophore, as the HDAC6 selective inhibitors for the potential GBM treatment.

To design and synthesize HDAC6 inhibitors, we employed naphthalimide as the core structure which connected with hydroxamate group through p-toyl linker, and several target compounds were synthesized for SAR study. Compound with methoxy derivative (HDAC6 IC₅₀ = 0.1 nM, HDAC1 IC₅₀ = 108 nM) showed sub-nM inhibition activity against HDAC6 and 1080 times of selectivity against HDAC6 over HDAC1, and U87MG IC₅₀ = 0.92 μM. From SAR analysis, O-substitution or N-substitution at para-position provided far better activities. However, substitutions at meta-position lost inhibition against HDAC and cancer cell lines.

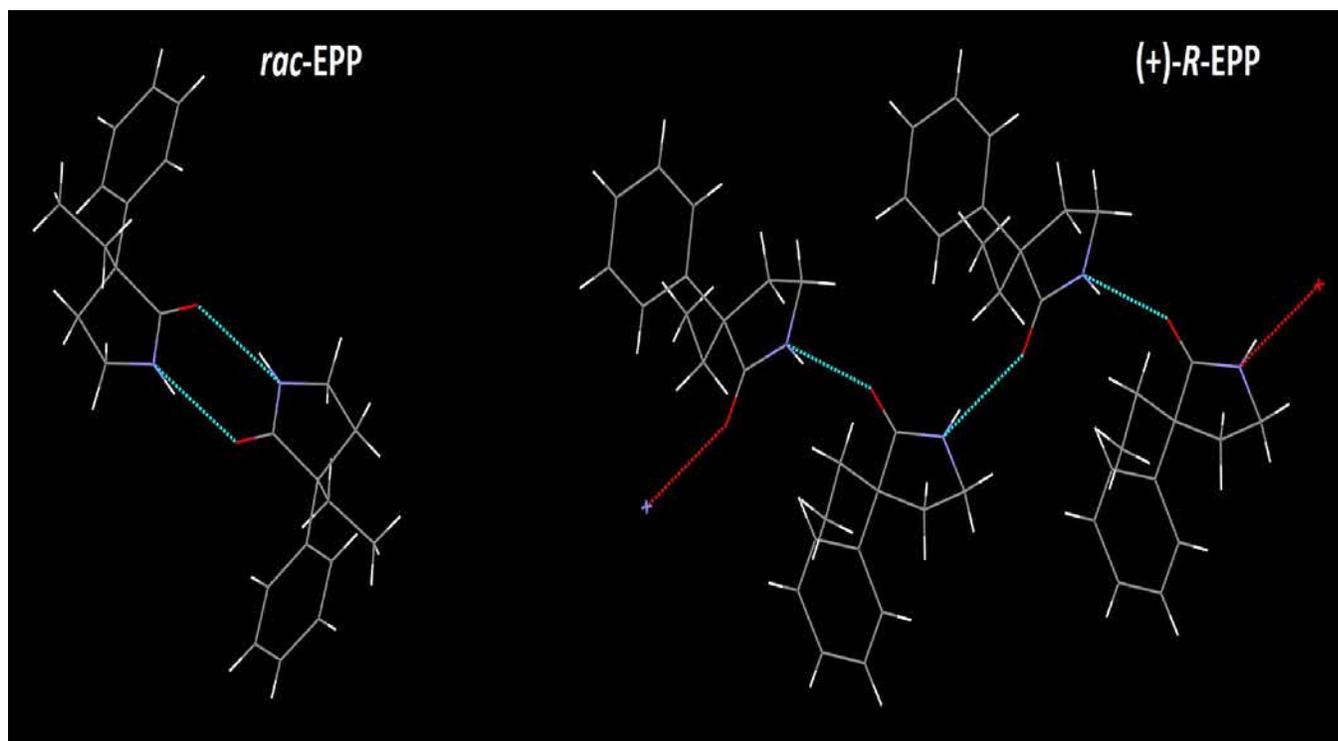
To sum up, the naphthalimide scaffolds of HDAC6 inhibitors were developed for the potential therapeutic agents against glioblastoma in this study. Further studies on the improvement of pharmacokinetic properties are undergoing.

MEDI 131

Solid-state structure and absolute configuration of enantiomers of 3-ethyl-3-phenylpyrrolidin-2-one

Arcadius V. Krivoshein¹, krivoshein@uhcl.edu, **Sergey V. Lindeman**², **Victor N. Khrustalev**³, **Tatiana V. Timofeeva**⁴. (1) Physical & Applied Sciences, University of Houston - Clear Lake, Houston, Texas, United States (2) Chemistry, Marquette University, Milwaukee, Wisconsin, United States (3) Inorganic Chemistry, Peoples' Friendship University of Russia, Moscow, Russian Federation (4) Chemistry, New Mexico Highlands University, Las Vegas, New Mexico, United States

3-Ethyl-3-phenylpyrrolidin-2-one is a novel experimental anticonvulsant based on the α -substituted amide group pharmacophore. In order to understand pharmaceutically relevant properties of such compounds, we are conducting an extensive investigation of their structures in the solid state. In this presentation, we report chiral HPLC separation, crystal structures, and absolute configuration of enantiomers of 3-ethyl-3-phenylpyrrolidin-2-one determined using single crystal X-ray diffraction at 100 K. We demonstrate that the homochiral and racemic forms of this anticonvulsant have considerable differences in their supramolecular organization and in IR spectra in crystals. These structural differences can be related to the differences in melting points and solubility.



MEDI 132

Chemical correction of cellular dysfunction caused by progranulin deficiency in frontotemporal dementia

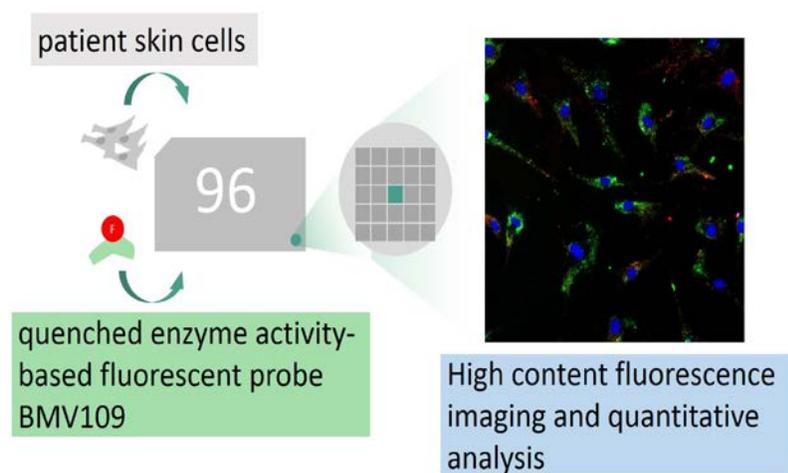
Maria Telpoukhovskaia¹, maria.telpoukhovskaia@gmail.com, **Kai Liu**², **Connor Ludwig**^{1,3}, **Jon Iker Etchegaray**¹, **Faten Sayed**¹, **Yungui Zhou**¹, **Matthew S. Bogoy**⁴,

Sheng Ding², Li Gan¹. (1) Neurological Disease, Gladstone Institutes, San Francisco, California, United States (2) Cardiovascular Disease, Gladstone Institutes, San Francisco, California, United States (3) Neurology, University of California, San Francisco, San Francisco, California, United States (4) Stanford Univ, Palo Alto, California, United States

Frontotemporal dementia (FTD) is the second leading cause of dementia in adults under 65 years of age. About 20% of genetic cases of FTD are caused by mutations in the gene that codes for the protein progranulin (PGRN). The role of PGRN in neurodegenerative diseases is not completely understood. We use fluorescent probes to study patient cells deficient in PGRN to uncover the underlying dysfunction linked to disease pathology. As well, we perform compound screens to discover chemical PGRN mimics to correct cellular dysfunction.

First, this report will present data from our study using a fluorescent probe that measures enzyme activity in FTD patient-derived cells. Second, discovery of compounds that mimic progranulin protein and correct cellular function is presented. We use a multiplex approach with RASL-seq (RNA-mediated oligonucleotide Annealing, Selection, and Ligation with Next-Gen sequencing) technology, which allows us to do a multiplex screen for hundreds of compounds in a 384-well plate format, with multiple gene expression profiles evaluated in each well. Once the hits from this screen are obtained, structure-activity relationship (SAR) analyses and modifications of compounds are done to elucidate and improve their activity.

Overall, this research project aims to develop compounds able to correct cellular dysfunction caused by PGRN deficiency, with the ultimate goal of discovering drugs to cure FTD.



Brazil (2) Institute of Chemistry, University of Campinas, Campinas, Brazil (3) Department of Biochemistry and Immunology, Federal University of Minas Gerais, Belo Horizonte, Brazil

Chagas' disease is endemic in Latin America and affects about 10 million people worldwide. Caused by the protozoan *Trypanosoma cruzi*, it is classified as a neglected tropical disease of high priority for which no effective and safe treatment is available. The two approved drugs (nifurtimox and benznidazole) are obsolete compounds discovered in the 1970s, which are characterized by several shortcomings, including lack of efficacy in the chronic phase, and high toxicity. In this scenario, the development of novel therapeutic agents for Chagas' disease is an urgent need. In this study, 3D quantitative structure-activity relationship (QSAR) models were generated for a series of cruzain inhibitors using the Comparative Molecular Field Analysis (CoMFA) method. The data set used in the chemometric analyses consists of 27 compounds bearing a benzimidazole ring as the common core, for which the concentration required to achieve 50% of enzyme inhibition (IC_{50}) was determined against recombinant cruzain (IC_{50} values ranging from 210 nM to 173.6 μ M). The training and test sets for model development and external validation, respectively, were selected by applying a principal component analysis over the complete data set. The molecular alignment was obtained by a structure-based strategy, whereby each inhibitor was docked into the active site of cruzain (PDB ID: 3KKU, resolution of 1.28 Å). The final CoMFA models, refined by the standard deviation*coefficient (Stdev*Coeff) region focusing method, displayed high statistical consistency ($r^2 = 0.97$ and $q^2 = 0.71$) and predictive power for untested inhibitors ($r^2_{pred} = 0.94$). The Stdev*Coeff 3D contour maps highlighted essential features of the data set compounds that are strongly correlated with the antiparasitic activity. These results validate the CoMFA models to be used as valuable tools to guide the design of new and optimized cruzain inhibitors structurally related with the investigated benzimidazole series.

MEDI 135

Improving new molecule design using electrostatics

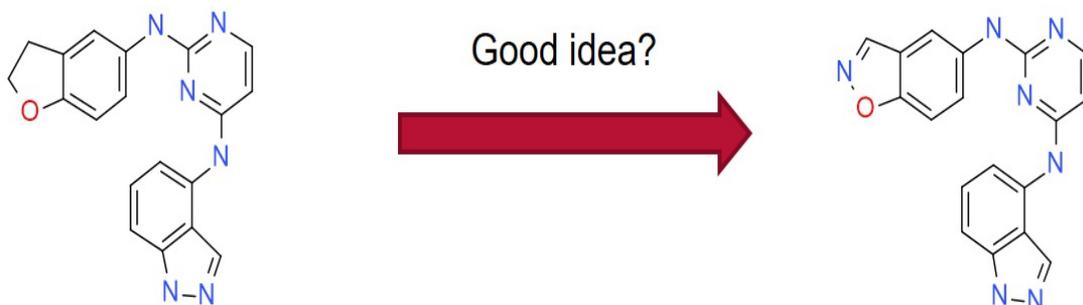
Tim Cheeseright, *tim@cresset-bmd.com*, Mark D. Mackey. Cresset, Cambridgeshire, United Kingdom

Electrostatics are critical to ligand binding and yet largely overlooked in new molecule design due to the difficulty in calculation and visualization of meaningful potentials. We have previously shown how electrostatics can be used effectively for scaffold hopping, virtual screening, ligand alignment and SAR interpretation.

In this poster we will focus on ligand and protein electrostatics. We will show how considering the changes in ligand electrostatics improves the outcome for new molecule design. Going beyond traditional H-bonding based pharmacophore descriptors enables designers to map the effect of molecular changes on the full electrostatic potential of their molecules. Full exploitation of aromatic dipole moments, C-H hydrogen bonding,

halogen bonds, and pi-pi interactions is only possible by understanding the electrostatic basis of these effects.

Consideration of the protein electrostatics can inform ligand design to generate complementary patterns of electron rich and electron poor regions.



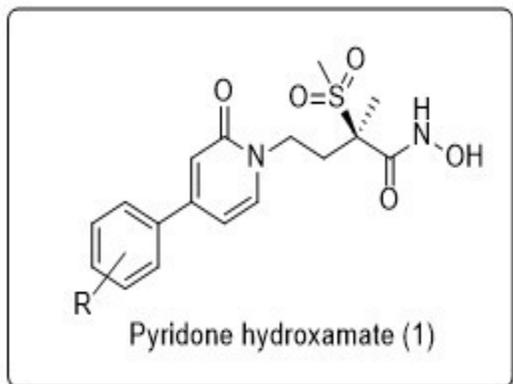
In proposing a new molecule the electrostatic changes on attached groups are rarely considered. In this case the change to an benzisoxazole is expected to change the electrostatics on the left side, but what happens to the amino-pyrimidine?

MEDI 136

Structure-kinetics relationships for LpxC inhibition

Brendan Lichtenthal¹, lichtenthal.b@gmail.com, **Chendi Gu**¹, **Fereidoon Daryaei**¹, **Rajeswari Basu**¹, **Mustafa Babar**¹, **Peter J. Tonge**². (1) Chemistry, State University of New York at Stony Brook, White Plains, New York, United States (2) Chemistry, Stony Brook University, Setauket, New York, United States

The zinc metalloamidase LpxC catalyzes the first committed step in the biosynthesis of lipid A, a key constituent of the lipopolysaccharide in the cell wall of Gram negative bacteria. LpxC is thus a target for the discovery of novel antibacterial agents. Previously we discovered a correlation between the residence time of inhibitors on LpxC and time-dependent antibacterial activity of the compounds towards *Pseudomonas aeruginosa* LpxC (paLpxC), and described a mechanistic PK/PD model that predicted the activity of a pyridone hydroxamate paLpxC inhibitor (1) in a mouse model of infection. To provide a platform for the rational design and synthesis of paLpxC inhibitors with altered residence times, we have now conducted a structure-kinetic analysis for LpxC inhibition by analogs of the pyridone hydroxamate lead. Both the thermodynamics and kinetics for LpxC inhibition by the compound series will be presented, together with their time-dependent antibacterial activity at the whole cell level (post-antibiotic effect). The structure-kinetic relationship for LpxC inhibition will enable the development of compounds with extended target engagement at low drug concentration which we hypothesize can be dosed less frequently leading to an improved therapeutic index.



MEDI 137

Problem based learning with MOE

Audrey Bonin, *conferences@chemcomp.com*. Chemical Computing Group, Montreal, Quebec, Canada

Problem-Based Learning (PBL)¹ is a pedagogical method which incorporates hands-on, active learning centered on the investigation and resolution of difficult, real-world problems. Some of the defining characteristics of PBL include:

A guided learning process with challenging open-ended problems where there are multiple solutions.

An environment where students work as self-directed, active investigators and problem-solvers.

Here we demonstrate the effectiveness of the Molecular Operating Environment (MOE) in a PBL setting to teaching students about the advantages and limitations of the modeling tools that are used in the forefront of early stage drug design.

MEDI 138

Exploiting solvent effects in drug design and optimization

Raul Alvarez, **Alain Ajamian**, **Chris Williams**, *cwilliams@chemcomp.com*. Chemical Computing Group, Montreal, Quebec, Canada

There is significant interest in understanding the behavior of water molecules as it relates to ligand-receptor interactions. In specific cases, ambiguous and counterintuitive SAR seems to be linked to solvent effects. Ligand affinity and specificity appear to be influenced by the action of water molecules on the solvated ligand-receptor complex. As such, a deeper analysis of solvent effects would expose potential ligand design opportunities that were previously not conceivable. Here we report the application of the 3D Reference Interaction Site Model as a potential method to account for such solvent effects.

MEDI 139

Synthesis of novel efflux pump inhibitors which target the AcrAB-TolC multidrug efflux system in *Escherichia coli*

Keith M. Haynes¹, 30150168@acs.org, Narges Abdalr², Jerry Parks^{3,4}, Julie L. Chaney², Adam T. Green³, David Wolloscheck², John K. Walker¹, Natalie Wood¹, Valentine V. Rybenkov², Jerome Y. Baudry^{3,4}, Jeremy Smith^{3,4}, Helen I. Zgurskaya². (1) Pharmacology and Physiology, Saint Louis University, St. Louis, Missouri, United States (2) Department of Chemistry and Biochemistry, University of Oklahoma, Norman, Oklahoma, United States (3) UT/ORNL Center for Molecular Biophysics, Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee, United States (4) Department of Biochemistry and Cellular and Molecular Biology, University of Tennessee, Knoxville, Knoxville, Tennessee, United States

The increase of bacterial resistance to common antibiotics is recognized as a global health issue. In *E. coli*, the efflux pump AcrAB-TolC is responsible for multidrug resistance. The synthesis of compounds which bind this system and shut down the efflux of common antibiotics are investigated. Initial screening hits were used as a starting point to establish structure-activity relationships (SAR) and guide synthesis toward more potent analogs.

MEDI 140

Design of mGlu2 negative allosteric modulators for the treatment of neuropsychiatric disorders

Susana Conde, sconddec@its.jnj.com. Janssen Pharmaceuticals, Toledo, Spain

Glutamate is the main excitatory neurotransmitter in the brain. It acts on two distinct classes of receptors, the ionotropic (NMDA, AMPA, kainate) and metabotropic glutamate (mGlu) receptors. The metabotropic glutamate receptors play an important modulatory role in neurotransmission and are closely involved in a variety of physiological functions. Preclinical data support the therapeutic potential of negative allosteric modulation of the mGlu2 receptor in neuropsychiatric disorders such as depression and improvement in cognitive function in disorders like Alzheimer Disease. A high throughput screening (HTS) campaign resulted in attractive pyrazole hits with moderate potency as negative allosteric modulators of the metabotropic glutamate 2 receptor (mGlu2 NAM's). A focused medicinal chemistry optimization effort led to the identification of mGlu2 NAM compounds with nanomolar potency. Structure-activity Relationships (SAR), ADME properties and *in vivo* data will be presented and discussed more in detail.

MEDI 141

Phosphoramidate inactivators of *Mycobacterium tuberculosis* (Mtb) BlaC

Dawanna S. White, *dawanna.white@email.wsu.edu*, Cindy Choy, Clifford E. Berkman.
Chemistry, Washington State University, Pullman, Washington, United States

The increasing frequency of multidrug-resistant (MDR) and extremely drug-resistant (XDR) strains of *Mycobacterium tuberculosis* (*Mtb*) remains a challenge to current therapeutic strategies for effectively treating tuberculosis. The drug resistance of MDR and XDR is attributed to *Mtb*'s ability to express a β -lactamase (BlaC), which exhibits broad specificity for hydrolyzing traditional β -lactam-type antibiotics. Currently, BlaC is the only known β -lactamase in *Mtb*, and a recent *BlaC* knockout revealed that strains lacking this enzyme were more sensitive to β -lactam antibiotics. This presentation will focus on our progress on the design, synthesis, and evaluation of phosphoramidate esters as potential irreversible inactivators. We will evaluate the potency and selectivity of an initial library of analogs from a new class of phosphoramidate esters designed around known β -lactam substrates of BlaC. Our small library of compounds are phosphoramidate ester analogs of known β -lactamase BlaC inhibitors. The results are expected to reveal that phosphoramidate analogs of β -lactam antibiotics can serve as novel, inactivators of serine β -lactamases. In addition, it is expected that the preliminary data from this study will guide a more focused subsequent SAR study.

MEDI 142

Development of covalent caspase-6 inhibitors derived from disulfide trapping (tethering)

Kurt S. Van Horn², *ksvanhor@gmail.com*, Dongju Wang², Clifford Bryant², Daniel Medina-Cleghorn³, Priya Jaishankar³, Peter Lee², Michelle Arkin², Adam R. Renslo¹. (1) UCSF, San Francisco, California, United States (2) University of California, San Francisco, San Francisco, California, United States

Tauopathies are neurodegenerative diseases characterized by the formation of tau protein neurofibrillary tangles in the brain. Tau is a substrate of the protease caspase-6, which has been found to be associated with tau tangles in post-mortem brain tissue of people with Alzheimer's disease. Caspase-6 inhibition may suppress Alzheimer's disease progression, but selective inhibitors of caspase-6 have not yet been identified. Utilizing a small molecule-cysteine disulfide trapping (tethering) technology, we have discovered specific inhibitors of caspase-6 by targeting a non-active-site cysteine unique to caspase-6. Optimization of the identified electrophile and linker has led to our development of selective, covalent small molecule inhibitors of caspase-6 with more than 10,000-fold greater potency over caspases -2, -3, and -7. Further crystallographic analysis has revealed that inhibition occurs through partial occupation of the substrate binding groove and locking of caspase-6 in a nonfunctional active conformation.

MEDI 143

What do recently approved oral drugs look like? A year-by-year analysis of FDA approved drugs in 2007-2016

Andreas Ritzén¹, *dfrdk@leo-pharma.com*, **Laurent David**². (1) Drug Design, LEO Pharma A/S, Ballerup, Denmark (2) Medicinal Chemistry, H. Lundbeck A/S, Valby, Denmark

Medicinal chemists use guidelines e.g. the Rule of 5 and rules for polar surface area and rotatable bonds to steer the design of new molecules into the region of chemical space in which drugs are known to exist. Thus, it is important to know the boundaries of this region in order to inform decision making. Herein, we present a year-by-year analysis of all orally administered drugs approved by the FDA between 2007 and 2016. Structural and physicochemical parameters, e.g. molecular weight, polar surface area, and log P were compared with common rules of thumb. It was found that the fraction of drugs with structural properties beyond Rule of 5 (bRo5) have increased in recent years. HIV and hepatitis C protease inhibitors, protein-protein interaction inhibitors, and selective kinase inhibitors for the treatment of various cancers represent a large portion of new bRo5 drugs. They show that the region of oral drug-like space can be expanded to accommodate drugs for challenging targets. We performed molecular dynamics (MD) simulations for selected examples of bRo5 molecules to provide a more realistic view of their polarity in water and lipophilic phases. These explicit solvent MD simulations were analyzed to infer distributions of polar surface area and hydrogen bonding in solution.

MEDI 144

μCyclic peptides containing tryptophan and arginine residues: Antibacterial activities and structure-activity relationship

Neda Riahi¹, *riahi103@mail.chapman.edu*, **Taibah Aldakhil**¹, **Sammy Nasser**¹, **Francisco Nunez**¹, **Khalid Zoghebi**¹, **Saghar Mozaffari**¹, **Jason Yamak**², **Keykavous Parang**¹, **Rakesh K. Tiwari**¹. (1) Department of Biomedical and Pharmaceutical Sciences, Chapman University School of Pharmacy, Irvine, California, United States (2) Department of Pharmacy Practice, Chapman University School of Pharmacy, Irvine, California, United States

Amphiphilic cyclic peptide [W₄R₄] showed antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) with the minimum inhibitory concentration (MIC) value of 4 μg/ml. Herein we made an effort to determine the structural requirements for optimal activity using [W₄R₄] template. Five new peptides were synthesized using *N*-methyl tryptophan, D-arginine, and higher numbers of tryptophan residues (5 -7). The antibacterial activities against MRSA revealed that the increasing the number of tryptophan residues improved the activity as MIC values for [W₅R₇], [W₆R₇], [W₇R₇] were 32 μg/ml, 16 μg/ml, and 8 μg/ml, respectively. Furthermore, arginine was replaced with D-arginine to study the effect of stereochemistry on activity.

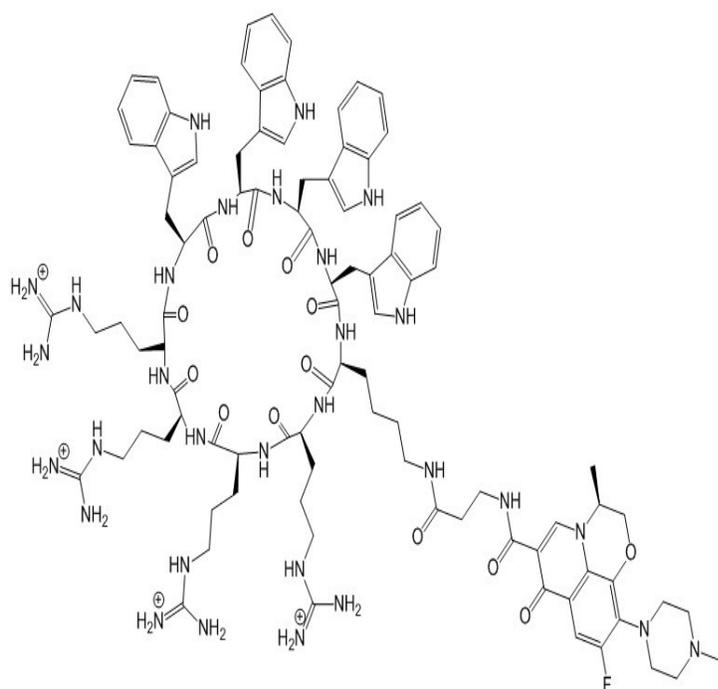
[W₄R₄] containing D-arginine or *N*-methyl tryptophan (MIC = 8 µg/mL) showed 2-fold less activity than [W₄R₄]. These data indicate that R₄, arginine with L configuration, and unmodified tryptophan are required for generating optimal activity. Further structure modification is required to optimize the antibacterial activity of [W₄R₄].

MEDI 145

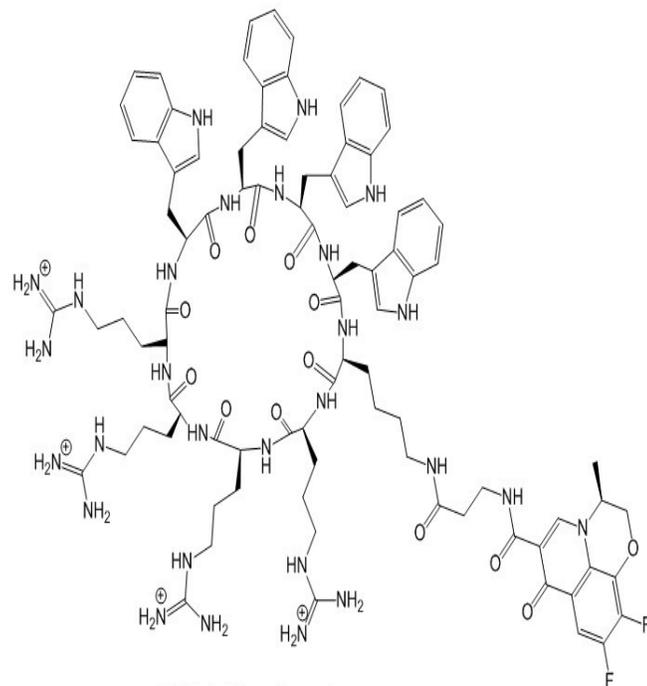
Synthesis and evaluation of antimicrobial activity of levofloxacin-[R₄W₄] and Q-levofloxacin-[R₄W₄] conjugates and comparison with the corresponding physical mixtures

Neda Riahi¹, *riahi103@mail.chapman.edu*, **Kathy Tavakoli¹**, **Jason Yamaki²**, **Rakesh K. Tiwari¹**, **Keykavous Parang¹**. (1) *Department of Biomedical & Pharmaceutical Sciences, Chapman University School of Pharmacy, Irvine, California, United States* (2) *Department of Pharmacy Practice, Chapman University School of Pharmacy, Irvine, California, United States*

Amphiphilic cyclic peptide [W₄R₄] showed antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) with a minimum inhibitory concentration (MIC) of 4 µg/mL. Herein, we hypothesized that conjugation or combination of the lead peptide with levofloxacin could improve the antibacterial activity of levofloxacin. Levofloxacin-[R₄W₄] and Q-levofloxacin-[R₄W₄] were synthesized, and their antibacterial activities were compared with the corresponding physical mixtures and parent compounds against MRSA. Although Q-levofloxacin was inactive even at a concentration of 128 µg/mL, Q-levofloxacin-[R₄W₄] and the corresponding physical mixture showed MIC values of 8 µg/mL, possibly due to the activity of the peptide. On the other hand, levofloxacin-[R₄W₄] conjugate (MIC = 32 µg/mL) and the corresponding physical mixture (MIC = 8 µg/mL) was less active than levofloxacin (MIC = 2 µg/mL). The data showed that the conjugation of levofloxacin with [R₄W₄] significantly reduced the antibacterial activity compared to the parent analogs, while Q-levofloxacin-[R₄W₄] conjugate was more potent than Q-levofloxacin alone.



[W₄R₄]-Levofloxacin



[W₄R₄]-Q-levofloxacin

MEDI 146

Anti-malarials targeting the heat shock 90 protein of *Plasmodium falciparum*

Nikalet Everson, *neverson@calpoly.edu*, Jordan Bach, Tyler Sisley, Michael Walls, Scott C. Eagon. Chemistry & Biochemistry, California Polytechnic State University, San Luis Obispo, California, United States

Malaria remains one of the most deadly infectious diseases on the planet. A recent report by the World Health Organization estimated that nearly half of the world's population is at risk of contracting the disease. In 2013 alone, there were an estimated 198 million cases of malaria resulting in 584,000 deaths with the majority of these deaths occurring in children under the age of 5. Drug resistant strains of malaria are primarily responsible for the continued death toll, as the parasite has developed resistance to several drug classes. Although antimalarial medicines such as chloroquine have been used effectively for more than 50 years, the failure to completely wipe out malaria in the 20th century was due in large part to the parasite evolving resistance to these standard treatments. Today, artemisinin combination therapy remains a highly effective treatment for many drug-resistant cases, but emerging resistance to artemisinin has been reported for several years now. The further spread of artemisinin resistant strains now threatens modern efforts to eliminate the parasite. New antimalarial drugs that are inexpensive and efficacious are a crucial component of any strategy to eliminate the parasite. Moreover, given the ability of the parasite to develop resistance to antimalarials, highly conserved protein targets with a high fitness cost to resistance conferring mutations are high priority targets. In

conjunction with Dr. Dylan Pillai's research group at the University of Calgary, we have identified a library of potential inhibitors of the *Plasmodium falciparum* heat shock 90 protein based on a tetrahydro- β -carboline scaffold. Results from our first generation candidates, as well as synthetic progress toward a second generation library will be presented.

MEDI 147

Synthesis of anti-malarial compounds targeting the ATP4 protein in *Plasmodium falciparum*

*Grant Koch, Julia Tryhorn, Kevin Ahn, Bri Belanger, Kenya Yniguez, **Horacio Lazaro**, hlazaroj@calpoly.edu, Anna Kashtanova, Scott C. Eagon. Chemistry & Biochemistry, California Polytechnic State University, San Luis Obispo, California, United States*

Malaria remains one of the most deadly infectious diseases on the planet. A recent report by the World Health Organization estimated that nearly half of the world's population is at risk of contracting the disease. In 2013 alone, there were an estimated 198 million cases of malaria resulting in 584,000 deaths with the majority of these deaths occurring in children under the age of 5. Drug resistant strains of malaria are primarily responsible for the continued death toll, as the parasite has developed resistance to several drug classes. Although antimalarial medicines such as chloroquine have been used effectively for more than 50 years, the failure to completely wipe out malaria in the 20th century was due in large part to the parasite evolving resistance to these standard treatments. Today, artemisinin combination therapy remains a highly effective treatment for many drug-resistant cases, but emerging resistance to artemisinin has been reported for several years now. The further spread of artemisinin resistant strains now threatens modern efforts to eliminate the parasite. New antimalarial drugs that are inexpensive and efficacious are a crucial component of any strategy to eliminate the parasite. Moreover, given the ability of the parasite to develop resistance to antimalarials, highly conserved protein targets with a high fitness cost to resistance conferring mutations are high priority targets. We report the progress towards the synthesis of anti-malarials targeting the *Plasmodium falciparum* ATP4 protein, which is thought to be a cation transporting ATPase responsible for regulating intracellular sodium levels in the parasite. Based on preliminary screening results, we have proposed a library of more than 50 potential inhibitors. Development of our current methodology will be presented, as well as synthetic progress to date.

MEDI 148

Novel high-affinity dopamine D4 receptor-selective ligands

***Comfort A. Boateng**¹, cboateng@highpoint.edu, Thomas M. Keck³, Benjamin Free⁴, Chun Wu³, Alessandro Bonifazi², Amy H. Newman², David R. Sibley⁴. (1) Basic Pharmaceutical Sciences, High Point University, High Point, North Carolina, United*

States (2) NIDA IRP, Baltimore, Maryland, United States (3) Rowan University, Glassboro, New Jersey, United States (4) National Institute of Neurological Disorders and Stroke, NIH, Bethesda, Maryland, United States

The dopamine D4 receptor (D4R), a G protein-coupled receptor, is predominantly expressed in the prefrontal cortex in which it plays an important role in cognition, attention, and decision making. Previous studies using D4R ligands of varying efficacies have determined that D4R signaling alters behavior in animal models of drug addiction and cognition. Developing novel D4R-selective ligands will allow more detailed investigations into the biological role of D4R signaling in the brain and assist in medications development for neuropsychiatric disorders, including Alzheimer's disease and substance use disorders (SUD). In order to develop new candidate medications for SUD, we have designed, synthesized, and pharmacologically evaluated novel D4R agonist ligands. Starting with the classical D4R agonist A-412997 (2-(4-(pyridin-2-yl)piperidin-1-yl)-N-(m-tolyl)acetamide) as our parent compound, we optimized development of a next-generation compound library by using molecular dynamics computational modelling approaches. We hypothesized that structural modifications of the A-412997 template would produce novel ligands with high D4R binding affinity, receptor subtype selectivity, and high efficacy. In this pursuit, we have produced a library of twenty compounds with varied substitutions on the phenylpiperidinyl (PP) ring and/or the arylamide moieties. These novel ligands were synthesized and their *in vitro* binding affinities were determined using [³H]7-OH-DPAT radioligand binding competition assays in membranes prepared from HEK293 cells expressing human dopamine D2-like receptors. By modifying the phenylpiperidinyl and arylamide moieties, we have identified several high-affinity compounds ($K_i \leq 4.30$ nM) with >100-fold selectivity at the D4R versus D2 and D3 receptors. Based on binding profiles, a subset of analogues was evaluated in functional assays measuring β -arrestin recruitment, cAMP production, and activation of co-expressed GIRK (G-Protein inwardly-rectifying Potassium) channels. These new lead compounds will be further evaluated for effects in animal models of cognition and/or drug addiction.

MEDI 149

Optimized chemical tools to probe the function of thioredoxin-interacting protein as therapeutic target

Olivier Mirguet¹, oliviermirguet@yahoo.fr, Séverine Nicolas¹, Michel-Christophe Aumis¹, Karine Daeron-Courté¹, Françoise Perron-Sierra¹, Sylvain Guizzetti², Cédric Vinson¹, Audrey Caliez¹, Caroline Chesneau¹, Béatrice Cremers¹, Marjorie Sadlo¹, Isabelle Wehrle¹, Ghislaine Zanirato¹, Philippe Delerive¹, Catherine Bernard¹. (1) Institut de Recherches Servier, St Remy Les Chevreuse, France (2) Novalix, Illkirch, France

Thioredoxin-interacting protein (TXNIP) has emerged as an important regulator of key cellular processes including metabolic disorders, cancer and inflammation. Type 2 diabetes mellitus (T2DM) is characterized by a defect in insulin secretion and action and recent studies showed that TXNIP is implicated in pancreatic β -cell glucose toxicity.

Moreover, it has been shown that TxNIP is among the most highly regulated gene in β -cells exposed to high glucose. In this context, TXNIP has emerged as an attractive target for T2DM and for the promotion of endogenous β -cell mass and insulin production. In this poster, we describe the discovery and optimization of orally available small molecule probes that would allow further exploration of the role of TXNIP in T2DM prevention but also reversion.

MEDI 150

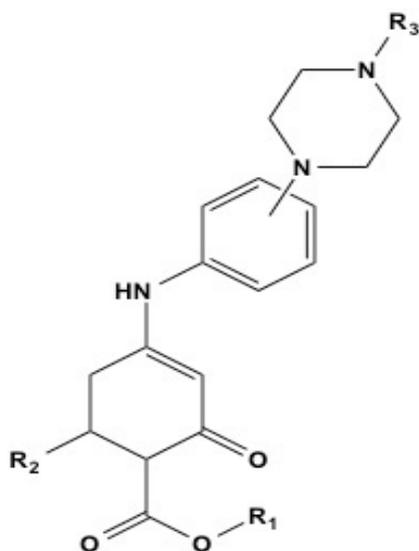
Structure activity relationship of novel piperazino-enaminones (JOAB series) as pro-inflammatory cytokines suppressants

Ashley Bill², Doreen Szollosi¹, Jyothi Dhuguru¹, Ivan Edafiogho¹, Ola M. Ghoneim¹, ola.ghoneim11@gmail.com. (1) Pharmaceutical Sciences, University of Saint Joseph-School of Pharmacy, Hartford, Connecticut, United States (2) Chemistry Department, University of Saint Joseph, West Hartford, Connecticut, United States

TNF-alpha and IL-6 are pro-inflammatory mediators important for the regulation of the immune response when an infection is present, but when overproduced, can be responsible for the development of tissue and organ injury seen in sepsis, as well as severe asthma, and autoimmune diseases such as Crohn's disease and rheumatoid arthritis.

Enaminones are a unique series previously synthesized in our lab. The enaminone E121 is able to significantly reduce the release of TNF-alpha and IL-6 in macrophages stimulated with lipopolysaccharide (LPS). Additionally, functional experiments in a mouse model of asthma have shown that E121 is efficacious in decreasing airway hyperresponsiveness. JODI-18 and 19 were synthesized to test the hypothesis that incorporating N-aryl piperazine motif into the aromatic side of the enaminone would enhance the immunosuppressive activity as anti-inflammatory agent by also acting as a chemokine receptor antagonist. We recently reported that JODI series can suppress TNF-alpha and IL-6 in a dose-dependent manner similar to E-121, and were more effective in reducing TNF-alpha after LPS stimulation when compared to dexamethasone. Studies using MCP-1 suggest that the JODI compounds, and not E121, are able to block CCR2 signaling as evidenced by decreased total ERK1/2.

To further examine the structural components of JODI series that are responsible for this unique activity, a new series of compounds (JOAB) were designed, and synthesized. The JOAB series explored four possible modifications of the JODI series (R1, R2, R3, and the location of the N-arylpiperazine on the aromatic ring; Figure attached). Modifications were done one at a time to systematically determine the essential pharmacophoric elements. The detailed synthetic scheme, and the comparison of the effect on the TNF-alpha and IL-6 release of the JOAB series as compared to the JODI series will be presented.



MEDI 151

Phytochemical evaluation of antimicrobial properties of *Combretum igneiflorum* extracts

Irma Maldonado, *irma_maldonado@dusty.tamtu.edu*, Alfred K. Addo-Mensah. LBVSC 303, TAMU Dept Biology Chemistry, Laredo, Texas, United States

The genus *Combretum* comes from a vast plant family, Combretaceae, of about 600 species and only a few have been studied for biological activities. Natural compounds from this family have been shown to be efficient against bacteria, fungi, inflammation, malaria, tumor formation, enzyme activity, and also to have cytotoxic properties. Thus, it is fundamental to increase scientific knowledge on this family. The search of new treatment options is constant since pharmaceutical drugs become inefficient towards microorganisms. *C. igneiflorum* (*Cl*) is a relatively new discovered species hence it is essential to conduct investigation to find if this plant has similar or enhanced biological properties compared with other *Combretum*. *Cl* was separated into roots, vines/stems, and leaves. Each section underwent sequential Soxhlet extraction with petroleum ether (PE), acetone (Ace.), and 9:1 ethanol/water (E/W). The freeze-dried crude compounds were used to prepare different concentrations and each tested for antimicrobial activity. Gram-positive [*S. aureus* (*SA*), Methicillin-Resistant *S. aureus* (*MRSA*), *B. subtilis* (*BS*), *E. faecalis* (*EF*)] and Gram-negative [*P. aeruginosa* (*PA*), *E. coli* B (*ECB*), *S. flexneri* (*SF*), *S. enteritidis* (*SE*)] were selected based on availability. Antimicrobial activity was successful for all extracts against *SA*, *BS*, and *SE*. *Cl* Ace. Roots and Leaves extracts were active against *EF*, Ace. Stems inhibited growth of *PA*, whereas E/W Roots and E/W Stems against *ECB*. The E/W extracts were effective against *SF*.

MEDI 152

Next generation small molecule inhibitors of 5'-Methylthioadenosinenucleosidase (MTN) as novel antimicrobial agents

John H. Thurston^{2,1}, Ken Cornell¹, **Lacey Wayment²**, lwayment@collegeofidaho.edu.
(1) Boise State University, Boise, Idaho, United States (2) Chemistry, The College of Idaho, Boise, Idaho, United States

Infectious disease, which currently accounts for approximately one-third of the annual worldwide mortality, presents a continuing, pressing threat to the health and well-being of the global population. This challenge is compounded by an increasing rate of emergence of drug resistant and multiple-drug resistant microbial infections, which further underscores the continued need to develop novel antibiotics that are both selective and safe. One potential target for antimicrobial therapies is 5' Methylthioadenosine/S-adenosylhomocysteine nucleosidase (MTN), an enzyme that is unique to microorganisms and which is known to play a central role in processes associated with bacterial quorum sensing including biofilm formation, exotoxin production and the upregulation of drug resistant phenotypes. As part of efforts to validate MTN as a target for antimicrobial therapies, we wish to describe here synthesis, characterization and *in vitro* activity of a series of non-nucleoside small molecule inhibitors (SMIs) of this enzyme.

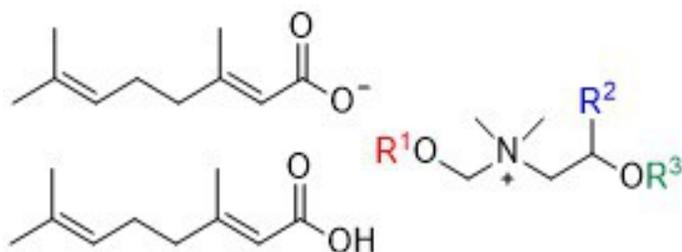
MEDI 153

Synthesis and evaluation of choline-derived Deep Eutectic Solvents (DES) as biofilm eradicating antibiotics

Andrew W. Jemas, ajemas@gmail.com, Danielle Jacobs. Dept of Chem, Rider University, Lawrenceville, New Jersey, United States

Infections caused by biofilm-forming strains of bacteria are responsible for a large number of deaths each year worldwide. Furthermore, these bacterial strains are rapidly developing antibiotic resistance, making the discovery of new antimicrobial substances of utmost importance. One such candidate is the deep eutectic solvent (DES) choline geranate, which has been shown to be an effective antimicrobial and biofilm-removing agent for gram-negative bacteria, able to penetrate deep tissue layers of the skin with relatively little inflammation. Preliminary studies from our laboratory, however, indicated that this DES is not as effective at killing the gram-positive bacterium *Staphylococcus aureus*, possibly due to differences in cell membrane construction as well as choline's intrinsic osmoprotectant properties. With an aim toward the development of DESs with more broad spectrum activity, our laboratory synthesized sixteen novel DESs, which replaced choline with other quaternary ammonium salts possessing known antimicrobial activity against gram-positive bacteria. Kirby-Bauer and MIC testing indicated that five of these DESs, particularly dodecyl-DMAE geranate ($R^1 = C_{12}H_{25}$, $R^2 = H$, $R^3 = H$), exhibited enhanced antimicrobial effects across both gram negative and gram positive bacterial strains, demonstrating their potential as a new class of broad spectrum

antibiotics. Future studies will evaluate the ability of these DESs to eradicate biofilms and penetrate the skin.



16 derivatives

R¹ = C₈H₁₇, C₁₀H₂₁, C₁₂H₂₅, C₁₄H₂₉

R² = H, CH₃

R³ = H, COPh, COCH₃

MEDI 154

Structure Activity Relationship (SAR) studies of EP2 receptor antagonists

Thota Ganesh, *tganesh@emory.edu*, Ray Dingledine. Pharmacology, School of Medicine, Emory University, Atlanta, Georgia, United States

Cyclooxygenase-2 (COX-2) is upregulated in several brain injury models and patients of traumatic brain injury (TBI), status epilepticus (SE), Alzheimer's disease (AD) and epilepsy. It is also upregulated in several peripheral disease models and patients of rheumatoid arthritis, inflammatory bowel disease (IBD) and chronic obstructive pulmonary disease (COPD). Thus, COX-2 has been targeted with inhibitors in the past, and several COX-2 inhibitor drugs were approved by the FDA to reduce the pain and severity of the disease in patients with rheumatoid and osteoarthritis. However, chronic use of these agents resulted in adverse cardiovascular events, as a result, two selective COX-2 inhibitor drugs, Vioxx® and Bextra® have been withdrawn from the United States market, but a low selective COX-2 drug Celebrex® is still being used with a black-box cardiotoxicity warning. COX-2 catalyzes the synthesis of five prostanoids (PGD₂, PGE₂, PGF₂, PGI₂ and TXA₂) which activate 11 prostanoid receptors (DP1, DP2; EP1, EP2, EP3 and EP4; FPα and FPβ; IP; TPα and TPβ). Studies have confirmed that the adverse effects observed by the COX-2 drugs are due to the inhibition of the prostanoid receptor IP, downstream of COX-2 signaling. Thus, the future anti-inflammatory therapy should be targeted through a specific prostanoid receptor, such as EP2. The EP2 receptor has emerged as an important target mediating majority of the proinflammatory effects of COX-2 in a variety of chronic neurodegenerative as well as peripheral disease models. Thus, we recently identified a novel acrylamide class of EP2 antagonists and then conducted SAR studies to identify several amide lead compounds in the class. We will present structure activity

relationship studies that lead to develop highly selective EP2 inhibitors with drug-like properties. We will also present in vitro and in vivo proof-of-concept studies suggesting EP2 antagonism will be a superior therapeutic strategy than global COX-2 inhibition.

MEDI 155

Identification of vacuolar (H⁺)-ATPase modulators by virtual screening process

*Renukadevi Patil¹, Arpita Kulshrestha², Anjali Tikoo², Sara Fleetwood², Gajendra Katara², William Seibel³, Alice Gilman-Sachs², **Shivaputra Patil¹**, shivaputrap@yahoo.com, Kenneth Beaman². (1) Pharmaceutical Sciences, Rosalind Franklin University, North Chicago, Illinois, United States (2) Microbiology and Immunology, Rosalind Franklin University, North Chicago, Illinois, United States (3) Division of Oncology, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, United States*

The vacuolar (H⁺)-ATPases (V-ATPases) are a family of ATP driven proton pumps that couple ATP hydrolysis with translocation of protons across membranes. The V-ATPase plays a major role in the regulation of cellular pH conditions, and its role has been associated with cancer invasion, metastasis and drug resistance. Despite the clear involvement of V-ATPases in cancer, to date therapeutic use of V-ATPase targeting small molecules have not reached the clinic. Thus, V-ATPases are emerging as important targets for the identification of potential novel therapeutic agents. To discover new small molecules as Vacuolar (H⁺)-ATPase modulators, we screened a library of 362,000 compounds from the University of Cincinnati by virtual screening and identified a bisbenzimidazole derivative as initial hit. Initially our hit molecule was screened for selected breast and ovarian cancer cell lines and it exhibited high potency towards breast cancer cell lines (MDA-MB-468: IC₅₀ 0.72±0.08 µM and MDA-MB-231: IC₅₀ 1.02±0.08 µM) and moderate activity against three ovarian cancer cell lines (A2780: IC₅₀ 3.87±0.09 µM, CisA2780: IC₅₀ 3.95±0.33 µM, and PA-1: IC₅₀ 1.70±0.21 µM). We are synthesizing novel bisbenzimidazole analogs and evaluating them against breast and ovarian cancer cell lines. Additionally, these new small molecules will be screened for ATPase activity along with proton pumping assay.

MEDI 156

Identification of a novel chemotype of ASK1 inhibitors for heart failure utilizing structure-based drug design

Alison L. Chambers, alison.chambers@earthlink.net. Medicinal Chemistry, Takeda California, San Diego, California, United States

Apoptosis signal-regulating kinase 1 (ASK1) is a mitogen activated protein family member (MAP3K) that upon stimulation activates the p38- and JNK-pathways, leading to acute ischemia/reperfusion injury. Structure-based drug design (SBDD) in parallel with deconstruction and re-optimization enabled the identification of a novel chemotype

with a unique hinge-binding element. This yielded a series of compounds that were potent ASK1 inhibitors with desirable drug-like properties. Lead compounds from this series showed excellent oral bioavailability in a rat PK study and demonstrated reduction of infarct size in a Langendorff isolated perfused heart model of cardiac injury. Herein we describe the discovery of these novel ASK1 inhibitors and efficacy in the *ex vivo* model.

MEDI 157

Determination of partition and distribution coefficients using ^1H NMR spectroscopy time domain Complete Reduction to Amplitude-Frequency Table (CRAFT) analysis

David P. Soulsby, david_soulsby@redlands.edu, Jeryl Chica. Chemistry, University of Redlands, Redlands, California, United States

Partition coefficients are a key physicochemical characteristic used in determining various pharmacokinetic parameters important in drug development. Many direct and indirect methods to measure partition coefficients have been reported but the spread of values reported for some compounds demonstrates the need to develop new approaches to measuring this important parameter. Herein we report on a simple and efficient method for the determination of partition and distribution coefficients ($\log P$ and $\log D_{7.4}$) using ^1H NMR spectroscopy combined with time domain complete reduction to amplitude-frequency tables (CRAFT) analysis. Partition coefficients measured using this approach for a variety of pharmaceutical compounds gave values that are in excellent agreement with those reported in the literature.

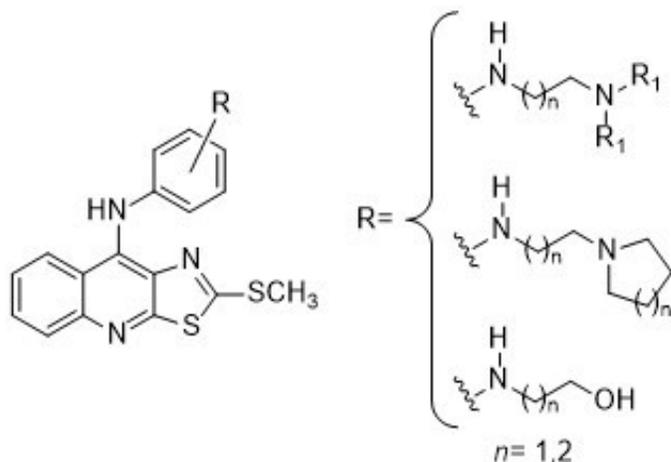
MEDI 158

Synthesis, cytotoxic evaluation and docking studies of novel 9-anilinothiazolo[5,4-*b*]quinoline derivatives bearing polar groups at the anilino ring

Alfonso Lira-Rocha, lira@unam.mx, Veronica Tinajero-Delgado, José Solano-Becerra, jsolanob2000@yahoo.com. Facultad de Química, Depto. de Farmacia, UNAM, Mexico D F, Mexico

Poly-heterocyclic compounds are among the most studied compounds for the treatment of cancer. Our research group has reported the synthesis of several derivatives of 9-anilinothiazolo[5,4-*b*]quinoline (9-ATZQ), which are bioisosteres of the acridines, being the last one the scaffold of several compounds with antitumor activity. 9-ATZQ derivatives have shown good cytotoxic activities against several human cancer cell lines. In the present work several novel compounds having dialkylaminoalkylamino, azacycloalkylaminoalkylamino, or hydroxylalkylamino groups at anilino ring of 9-ATZQ system were prepared by a convergent synthesis and their cytotoxic activities against several human cancer cell lines were evaluated. Compounds with a diamine group were

more active than those with an hydroxylalkylamino group, in spite that the last ones showed better affinity energy values than the first ones in a molecular docking study.



MEDI 159

Identification of R419, an indirect AMPK activator

Simon J. Shaw², simonjshaw@gmail.com, Dane Goff², David C. Carroll², Rajinder Singh¹, David Sweeny¹, Gary Park¹, David Lau¹, Yonchu Jenkins¹, Vadim Markovtsov¹, Tian-Qiang Sun¹, Yingwu Li¹, Alison Pan¹, Yasumichi Hitoshi¹, Kristen Baltgalvis¹, Henry Nguyen¹, Todd Kinsella¹, Donald Payan¹. (1) Rigel Pharmaceuticals Inc, S San Fran, California, United States (2) Chemistry, Rigel Pharmaceuticals, Inc., Oakland, California, United States

The compound R419 was identified through the development of structure-activity relationships around a hit compound using a cell-based AMPK activation assay. A particular focus was to retain the on-target potency while also improving microsomal stability and reducing off-target activities, including hERG inhibition. We were able to show that removing a tertiary amino group improved microsomal stability while increasing the polar surface area of the molecule resulted in a reduction in the hERG inhibition. Optimised compounds maintained AMPK activation activity. The SAR led to a compound R419 that activated AMPK in vivo after oral administration and showed efficacy in animal models investigating activation of AMPK as a therapy for both glucose control (db/db and DIO mouse models) and muscle endurance in mice.

MEDI 160

Effects of propolis on cancer cell membranes and bacterial cell membranes studied with Langmuir monolayers

Brittney Book, bbook@monmouthcollege.edu, Audra Sostarecz. Monmouth College, Monmouth, Illinois, United States

Langmuir-Blodgett Monolayers of propolis and phospholipids, saturated and unsaturated phosphatidylethanolamines and phosphatidylcholines, are examined for antibacterial and anticancer properties. The Langmuir Monolayer technique allows for the analysis of the organization of amphiphilic molecules at an air-water interface and is; therefore, a useful technique for the formation of model cell membranes. Propolis is a green-yellow to red-brown resinous material collected by worker bees from various vegetation around the hive and is used to cover the walls of the hive, keep out intruders, and keep out harmful pathogens. There are different chemical compositions of propolis based on the vegetation at the geological location. As a result, the biological activity of propolis is related to the plants native to the site of collection. In general, both propolis types used, American and Brazilian, increased the molecular area thereby breaking apart the bacterial cell membrane lipids. Specifically, the American propolis not only increased the molecular area but also increased the compression modulus indicating that the propolis molecules penetrated the monolayer and interacted with the tail groups of the dipalmitoylphosphatidylethanolamine (DPPE).

MEDI 161

Langmuir monolayer investigation in to the antibacterial properties of essential oils

Antonetta Axup, netta.axup@gmail.com, Audra Sostarecz. Chemistry, Monmouth College, Monmouth, Illinois, United States

Langmuir monolayers of sweet orange essential oil with the phospholipids dipalmitoylphosphatidylcholine (DPPC), dipalmitoylphosphatidylglycerol (DPPG), and E.coli lipid extract, a model for gram negative bacteria, were used to investigate the antibacterial properties of essential oils. The Langmuir Monolayer technique is useful for the formation of model cell membranes and allows for the analysis of the interactions between phospholipid molecules and and other surfactants for the investigation of their organization capabilities. Monolayers of DPPC using sweet orange oil as a subphase were found to be more fluid, and to be less stable, as indicated by a low surface pressure at low molecular areas, when compared to monolayers with ultra pure water as the subphase. Similarly, monolayers using sweet orange oil as a subphase with the E. coli lipid extract monolayer were found to be more fluid, and to be less stable, as indicated by a low surface pressure at low molecular areas when compared to monolayers with ultra pure water as the subphase. DPPG, a model system for gram positive bacteria, had similar interactions with the essential oil. Further investigations will involve using a simpler gram-negative model, such as 1,2-dipalmitoyl-sn-glycero-3-phospho-L-serine (DPPS), instead of E.Coli lipid extract to investigate the concentration dependence of various essential oils on the breakdown of the lipid membrane.

MEDI 162

Synthesis and antiproliferative activities of doxorubicin thiol conjugates and doxorubicin-S-S-cyclic peptide

Shaban Darwish, *darwish@chapman.edu*, **Neda Sadeghiani**, **Rakesh Tiwari**, *tiwari@chapman.edu*, **Keykavous Parang**, *parang@chapman.edu*. School of Pharmacy, Chapman University, Irvine, California, United States

Myocardial toxicity and drug resistance caused by drug efflux are major limitations of doxorubicin (Dox)-based chemotherapy. Dox thiol conjugates were synthesized to determine whether Dox antiproliferative activity is affected. Dox was reacted with Traut's reagent to generate thiolated doxorubicin (Dox-SH). The thiol group was activated by the reaction with dithiodipyridine to afford the corresponding thiol-reactive Dox-pyridine disulfide (Dox-SS-Pyridine). A cyclic peptide containing a cysteine residue [K(WR)₄C] was prepared using Fmoc solid-phase strategy. Dox-SS-Pyridine was reacted with the free sulfhydryl of cysteine in [K(WR)₄C] to generate Dox-SS-[K(WR)₄C] as cyclic peptide-conjugated Dox. Human leukemia cancer (CCRF-CEM) cancer cells were treated with Dox, Dox-SH, Dox-SS-[K(WR)₄C], and Dox-SS-Pyridine. Cytotoxicity MTS assays were performed after 72 hours at a concentration of 5 μM. Dox, Dox-SH, Dox-SS-[K(WR)₄C], and Dox-SS-Pyridine reduced the growth of CCRF-CEM cells by 69%, 73%, 68%, and 73%, respectively. These data indicate that Dox-SH and Dox-SS-Pyridine were slightly more potent than Dox.

MEDI 163

Radiosynthesis of P2X₇ receptor radioligands [¹¹C]GSK1482160 and [¹¹C]GSK1482160 isomer under different base conditions

Mingzhang Gao, *migao@iupui.edu*, **Min Wang**, **Qi-Huang Zheng**. Department of Radiology and Imaging Sciences, Indiana University School of Medicine, Indianapolis, Indiana, United States

P2X₇ receptor is an adenosine triphosphate (ATP)-gated ion-channel, which is found in the immune, peripheral and central nervous systems, implicated in ATP-mediated cell death, regulation of receptor trafficking and inflammation, and associated with various cancer, neurological and cardiovascular disorders. GSK1482160 ((S)-N-(2-chloro-3-(trifluoromethyl)benzyl)-1-methyl-5-oxopyrrolidine-2-carboxamide) developed by GlaxoSmithKline is a potent P2X₇ receptor antagonist with excellent biological activity (IC₅₀ 3 nM for human P2X₇ receptor). P2X₇ receptor is an attractive therapeutic target as well as imaging target for biomedical imaging technique positron emission tomography (PET). In our previous works, we have synthesized and characterized [¹¹C]GSK1482160 ((S)-N-(2-chloro-3-(trifluoromethyl)benzyl)-1-[¹¹C]methyl-5-oxopyrrolidine-2-carboxamide) for targeting the P2X₇ receptor. During the radiotracer production of [¹¹C]GSK1482160, there was always a minor radiolabeled by-product [¹¹C]GSK1482160 isomer ((S)-N-(2-chloro-3-(trifluoromethyl)benzyl)-N-[¹¹C]methyl-5-oxopyrrolidine-2-carboxamide) formed. In this ongoing study, we re-investigate the radiosynthesis of [¹¹C]GSK1482160 and [¹¹C]GSK1482160 isomer under different base conditions. The precursor desmethyl-GSK1482160 ((S)-N-(2-chloro-3-(trifluoromethyl)benzyl)-5-oxopyrrolidine-2-carboxamide) contains both cyclic amide and side chain amide. N-[¹¹C]methylation under basic condition of desmethyl-GSK1482160

with [^{11}C]CH₃OTf at cyclic amide and side chain amide would form [^{11}C]GSK1482160 and [^{11}C]GSK1482160 isomer, respectively. The different bases would change the ratio of the radiolabeled products [^{11}C]GSK1482160 and [^{11}C]GSK1482160 isomer. If NaOH-Na₂CO₃ (w/w 1:2, solid) was used as a base, [^{11}C]GSK1482160 and [^{11}C]GSK1482160 isomer was formed in a ~10:1 ratio. If NaOH (solid) was used as a base, [^{11}C]GSK1482160 and [^{11}C]GSK1482160 isomer was formed in a ~1:1 ratio. If NaH (95% dry or 60% dispersion in mineral oil, powder) was used as a base, [^{11}C]GSK1482160 and [^{11}C]GSK1482160 isomer was formed in a ~1:10 ratio. The radiochemical yield was 50-60% based on [^{11}C]CO₂ and decay corrected to end of bombardment (EOB), and specific activity at EOB was 370-1110 GBq/μmol.

MEDI 164

Fully automated synthesis of [^{11}C]Me-GDC-0068 as a PET tracer for imaging of Akt in cancers

Mingzhang Gao¹, migao@iupui.edu, Min Wang¹, Harlan Shannon², Barbara Bailey², Karen Pollok², Hamideh Zarrinmayeh¹, Qi-Huang Zheng¹. (1) Department of Radiology and Imaging Sciences, Indiana University School of Medicine, Indianapolis, Indiana, United States (2) Herman B. Wells Center for Pediatric Research, Indiana University School of Medicine, Indianapolis, Indiana, United States

Protein kinase B (PKB or Akt), is a serine/threonine protein kinase. Akt, containing three isoforms Akt1, Akt2, and Akt3, is a downstream target for phosphoinositide 3-kinase (PI3K), a critical regulator of cell growth and transformation. The mammalian target of rapamycin (mTOR) is a key component of PI3K pathway and is also a central regulator of cell growth. The PI3K/Akt/mTOR pathway is an intracellular signaling pathway that is involved in several cell functions including growth, proliferation, apoptosis and autophagy, and is among the most commonly activated pathways in human cancers. Akt is a pivotal node in the PI3K/Akt/mTOR pathway and has emerged as an attractive target for cancer therapeutics, and several Akt inhibitors are currently in human clinical trials. Among these, GDC-0068 {(R)-3-(4-chlorophenyl)-1-(4-((5R,7R)-7-hydroxy-5-methyl-6,7-dihydro-5H-cyclopenta[d]pyrimidin-4-yl)piperazin-1-yl)-3-(isopropylamino)propan-1-one} is a potent Akt inhibitor with IC₅₀ 5, 18 and 8 nM for Akt1, Akt2 and Akt3, respectively. Me-GDC-0068 {(R)-3-(4-chlorophenyl)-1-(4-((5R,7R)-7-hydroxy-5-methyl-6,7-dihydro-5H-cyclopenta[d]pyrimidin-4-yl)piperazin-1-yl)-3-(isopropyl(methyl)amino)propan-1-one} is a derivative of GDC-0068 with similar potency, IC₅₀ 18, 31 and 28 nM for Akt1, Akt2 and Akt3, respectively. Akt has become an interesting target for cancer imaging, however, no specific imaging agents have been developed so far. Carbon-11 and fluorine-18 labeled GDC-0068 compounds may serve as new probes for the biomedical imaging technique PET, and enable non-invasive monitoring of the enzyme Akt in cancers. To radiolabel therapeutic agents as diagnostic agents for imaging of Akt and monitoring of therapeutic efficacy of Akt inhibitors, we have designed and synthesized [^{11}C]Me-GDC-0068 {(R)-3-(4-chlorophenyl)-1-(4-((5R,7R)-7-hydroxy-5-methyl-6,7-dihydro-5H-cyclopenta[d]pyrimidin-4-yl)piperazin-1-yl)-3-(isopropyl(^{11}C methyl)amino)propan-1-one}. The standard Me-GDC-0068 was

synthesized from GDC-0068 with 37% formaldehyde and NaBH(OAc)₃ under DIPEA in 77% yield. The tracer [¹¹C]Me-GDC-0068 was prepared from GDC-0068 with [¹¹C]CH₃OTf under NaH through N-[¹¹C]methylation and isolated by HPLC combined with SPE in 30-45% radiochemical yield based on [¹¹C]CO₂ and decay corrected to EOB. The radiosynthesis was performed in a home-built automated ¹¹C-radiosynthesis module. The radiochemical purity was >99%, and specific activity at EOB was 370-1110 GBq/μmol.

MEDI 165

Natural product-inspired agents and their anticancer activity against glioblastoma multiforme cells

Vineet Kumar¹, kumarvin@stanford.edu, Samuel Banister¹, Hunter Hunter Kaufman¹, Jack Vittimberga¹, Sam A. Jacobo¹, Sanjiv S. Gambhir², Sanjay V. Malhotra^{1,2}. (1) Department of Radiation Oncology, Stanford University School of Medicine, Palo Alto, California, United States (2) Molecular Imaging Program, Department of Radiology, Stanford University, Palo Alto, California, United States

Gliomas account for more than 70% of all neoplasms of the central nervous system. Glioblastoma multiforme (GBM) is the most common, aggressive and fatal malignant glioma in adults. It is characterized by distinct cell proliferation and invasiveness with a rapid progression, resulting in a high grade of recurrence and poor prognosis of tumor. Treatment options for GBM are very limited and are often ineffective. Despite the increasing progress in the field of radiotherapy, chemotherapy, and surgical techniques, the survival rate for these GBM patients remains low with mean survival of about one year. Therefore, there is an urgent need for new and effective therapies for treating GBM tumors. In this regard, natural product-based small molecules can be potential therapeutic alternatives.

In our continuing effort to develop natural products inspired therapeutic agents, we have synthesized a library of chalcone, cyclohexylphenol, and indole-based small molecules. These molecules were tested for their anticancer activities on GBM39 cells using a CellTiter-Blue assay. Initial screening showed several hit compounds with IC₅₀ values ranging from 1 –10 μM, and little cytotoxicity against several non-cancer cell lines. We are expanding these ‘hits’ into synthetic libraries for explore structure-activity relationships, identify new targets and mechanisms-of-action for the development of increasingly potent compounds for the treatment of GBM.

MEDI 166

CDD vault: A modern approach for drug research project team informatics

Whitney W. Smith², wws.smithfamily@gmail.com, Barry A. Bunin¹, Kellan Gregory¹. (1) CDD, Belmont, California, United States (2) Sales, Collaborative Drug Discovery, Santa Rosa, California, United States

Whereas the main focus in Big Data has been its vast size, the main value that can be obtained from it depends on the tools and processes that allow for effective mining of such data. Big Data mining is the capability of extracting useful information that has been historically impossible with the existing technologies. The information management needs of the pharmaceutical industry has been pushing for new ways of mining large and complex databases, as well as for new technologies to identify useful trends and information that can be extracted.

CDD Vault® is a platform that provides a hosted database solution for secure management and sharing of chemical and biological data. It lets you organize chemical structures and biological study data, and collaborate with internal or external partners through a web interface. CDD Vault is differentiated by ease-of-use and superior collaborative data sharing workflows. Within the CDD Vault software, Activity & Registration, Visualization, and Inventory are well integrated for handling the majority of private drug discovery data requirements.

CDD Public is an open database that can be mined through CDD Vault and is freely accessible to scientists at no charge. Importantly, it contains negative data which makes it valuable for those involved in predictive model development. Simultaneous analysis of private and public data in a multi-site multi-disciplinary secure environment makes the collaborative CDD Vault platform uniquely valuable.

In this presentation, we will provide several examples where CDD Vault has been utilized as the central platform for multi-national collaborations including NIH Blueprint (11 organizations), Bill & Melinda Gates foundations (BMGF) Tuberculosis (TB) collaborations (14 organizations including 7 big pharma), and More Medications for Tuberculosis (MM4TB) (25 organizations including 2 big pharma). The search, analysis, and data visualization capabilities of CDD Vault will be presented.

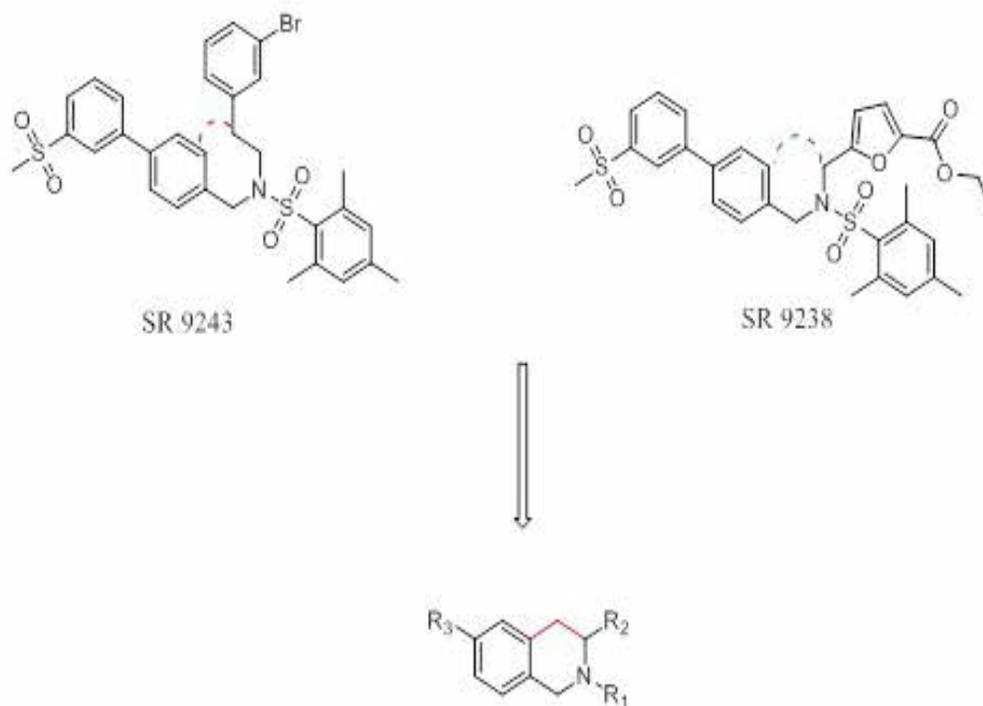
MEDI 167

Novel tetrahydro isoquinoline derivatives as LXR modulators

Arindam Chatterjee, *chatterjee.arindam@yahoo.com*, Thomas P. Burris, Kristine Griffett, Subhashis Banerjee, Zach Doerer, Amer Avdagic, Terri Boehm, **John K. Walker**, *walkerjk@slu.edu*. *Pharmacology and Physiology, Saint Louis University, St. Louis, Missouri, United States*

The nuclear receptors, liver X receptor- α and $-\beta$ (LXR α and LXR β), were originally identified as orphan members of the nuclear receptor (NR) superfamily that function as heterodimers with the retinoid X receptor (RXR). LXR α is primarily expressed in the liver, kidneys, intestines, and adipose tissues while LXR β is widely expressed. Both LXR α and LXR β play important roles in cholesterol homeostasis and lipid metabolism, and have been implicated in the pathology of several diseases including atherosclerosis, cancer, and obesity. Agonists induce a conformational change that results in an increase in transcription of a target gene. An antagonist blocks the action

of an agonist and may cause a shift in potency or efficacy, but causes no change in receptor activity itself. An inverse agonist has the ability to induce a unique conformational change that results in repression of target gene transcription, most likely due to the recruitment of corepressors. Burriss et al. has previously identified novel synthetic LXR Inverse Agonists (SR 9238 and SR 9243) which displayed the ability to suppress NASH and Liver Fibrosis. In this current endeavor, we exploited the structure based computational approach to investigate novel tetrahydro isoquinoline templates as LXR modulators.



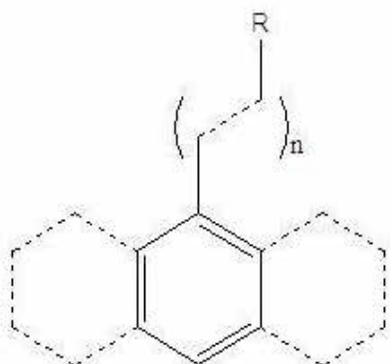
MEDI 168

From photochemically responsive crystals to anticancer compounds: Discovery of novel anthracene analogs as antineoplastic agents

Imadul Islam¹, imadulislam@yahoo.com, Atef Nehdi¹, Rabih O. Al-Kays^{2,1}, Ahmed Alaskar¹, Mohamed Boudjelal¹. (1) King Abdullah International Medical Research Center, Ministry of National Guard-Health Affairs (MNGHA), Drug Discovery Program, Medical Research Core Facility and Platform, Riyadh, Saudi Arabia (2) Department of Chemistry, KSAU_HS, Riyadh, Saudi Arabia

In the quest of new anticancer therapies, structurally diverse compounds have been assayed in selected breast cancer and leukemia cell lines. When some of these compounds are grown into crystals they can be used as photomechanical actuators. These compounds show moderate activity and some selectivity towards breast cancer cell lines. Two analogs were assayed against MDA-MB-231 cell lines. After 48 hours

incubation, one analog shows IC₅₀ of 5.2 μM and other shows the IC₅₀ of 11.2 μM. Synthesis, brief SAR and selectivity data will be discussed.



Where
n=Saturated/ Unsaturated bonds
in conjugation
R= Electron withdrawing/donating groups,
Substituted Heterocycles

General Structure

MEDI 169

Design, synthesis, and biological properties of a fluorescent duocarmycin analog: HxTfA

Konstantinos Kiakos³, Pravin C. Patil⁵, Stephanie Yanow⁴, John A. Hartley³, **Moses Lee**^{1,2}, mosesl@murdocktrust.org. (1) Program Director for Science Grants Programs, M. J. Murdock Charitable Trust, Vancouver, Washington, United States (2) Chemistry, Georgia State University, Atlanta, Georgia, United States (3) UCL Cancer Institute, London, United Kingdom (4) University of Alberta, Edmonton, Alberta, Canada (5) Chemistry, Hope College, Holland, Michigan, United States

Duocarmycins are natural products that target contiguous adenine sequences of DNA, resulting in the formation of covalent bonds between the cyclopropane unit of the drug and adenine-N3 on the floor of the minor groove. Both naturally occurring and synthetic duocarmycins, and their *seco*-prodrugs are endowed with potent *in vitro* and *in vivo* anticancer and antiparasite activities. Recently, our group reported a novel class of *seco*-iso-furano duocarmycin analogs, exemplified by tafuramycin A (TfA), which demonstrated superb stability from nucleophiles under acidic conditions. TfA exhibited potent cytotoxicity against cancer cells (nanomolar range) and *Plasmodium falciparum* (picomolar range). TfA is currently being clinically developed in Phase I as part of vaccine against malaria. This presentation will describe our design and synthesis of HxTfA, a fluorescent analog of TfA. The DNA binding and biological properties of HxTfA and TfA against cancer cells and *Plasmodium falciparum* will be compared, and evidence for HxTfA entering the nucleus of cells as determined by confocal microscopy will also be presented.

MEDI 170

Evaluated as potential hypocholesterolemic agent, the preparation and characterization of steroidal α,β -unsaturated ketones

*Edward J. Parish*¹, **Dong-Chun Ren**⁵, *rendc@ahjc999.com*, *Yu-Chen Lo*⁴, *Gui Ren*³, **Hiroshi Honda**², *chrishonda@yahoo.com*. (1) Chemistry, Auburn University, Auburn, Alabama, United States (3) Bioengineering, Northwestern Polytechnic University, Fremont, California, United States (4) Bioengineering, Stanford University, Palo Alto, California, United States (5) Pharmacy, China Pharmaceutical University, Nanjing, Jiangsu, China

This paper represents novel approaches to the chemical preparation and characterization of steroidal α,β -unsaturated ketones, evaluated as potential hypocholesterolemic agents.

MEDI 171

Novel approaches to the chemical preparation and characterization of kinsenoside

Dong-Chun Ren³, *rendc@ahjc999.com*, *Yu-Chen Lo*⁴, *Edward J. Parish*¹, **Hiroshi Honda**², *chrishonda@yahoo.com*. (1) Chemistry, Auburn University, Auburn, Alabama, United States (2) Bioengineering, Northwestern Polytechnic University, Fremont, California, United States (3) Pharmacy, China Pharmaceutical University, Nanjing, Jiangsu, China (4) Bioengineering, Stanford University, Palo Alto, California, United States

This paper represents the novel discovery of new approaches to the chemical preparation and characterization of kinsenoside

MEDI 172

New compounds for the treatment of *Mycobacterium abscessus* infections

Keith D. Combrink¹, *keithd.combrink@tamiu.edu*, *Florian Maure*², *Kira Elizondo*¹, *Andrea Ramirez-Ramos*¹, *Stephanie Spring*¹. (1) Biology and Chemistry, Texas A&M International University, Laredo, Texas, United States (2) Institute of Medical Microbiology, Virology and Hygiene, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

The emerging pathogen *Mycobacterium abscessus* (*Mab*) is the most pathogenic and resistant to chemotherapy of all of the rapid-growing mycobacterium (RGM). *Mab* has been found in hospital water supplies, traumatic skin infections, post-surgical soft tissue infections, is a leading pathogen in lung transplant infections and is a common pathogen in cystic fibrosis patients. Clinical studies on therapeutic outcome are rare and

cure rates do not exceed 30-50% with the currently available antimicrobials. A standardized treatment regimen has not been established, partially due to the high levels of natural antibiotic resistance and the lack of bactericidal activity of commonly administered compounds. Rifamycins are potent anti-*Mycobacterium tuberculosis* agents but have no activity against *Mab*. *Mycobacterium* have two resistance mechanisms to deactivate rifamycins. Genomic analyses shows that the DNA sequence of the *rpoB* gene of *Mab* (type strain ATCC 19977) is identical to rifampicin-susceptible strains of *M. tuberculosis*. *Mab* harbors an ADP-ribosyltransferase gene (GenBank ID MAB_0591) and preliminary data indicate that the Arr homolog of *Mab* confers high-level resistance to rifampicin.

We have prepared a series of rifamycin derivatives that have potent anti-bacterial activity against *Mab*. We believe our improved anti-bacterial activity is due to insensitivity to Arr enzymatic deactivation. We have identified two series that show very potent antimicrobial activity and we will describe the structure activity relationships of these new series.

MEDI 173

Anticancer activity of exo-cyclic carbohydrate enones containing thiophene and pyrrole moieties

Joanna Sarnik², Anna Czubatka-Bienkowska³, Anna Macieja², **Zbigniew J. Witczak**¹, zbigniew.witczak@wilkes.edu, Roman Bielski⁵, Tomasz Poplawski⁴. (1) Dept of Pharm SCI Sch of Phar, Wilkes Univ, Wilkes Barre, Pennsylvania, United States (2) Department of Molecular Genetics, University of Lodz, Lodz, Poland (5) Department of Pharmaceutical Sciences, Wilkes University, Wilkes-Barre, Pennsylvania, United States

Despite recent advances in understanding cancer biology available therapeutic procedures remain insufficient. Chemotherapy is often accompanied by serious side effects negatively affecting the treatment outcome. Moreover, in view of the commonly occurring phenomenon of resistance to cancer chemotherapeutic agents, there is an acute need for new treatment strategies preventing or reversing the drug resistance. The discovery and development of new, efficient and safe antitumor agents is necessary. We investigated the anticancer activity of derivatives of 4-deoxymonosaccharides equipped with exocyclic enone functionality and coupled to thiophene, bromothiophene and pyrrole.. All novel synthesized compounds (named as FCP28, 29 and 30 and presented on Figure 1) were evaluated for their *in vitro* anticancer activity against variety of cancer cell lines. We used the human breast cancer cell line (MCF7), human epitheloid cervix carcinoma (HeLa), human ovarian carcinoma (A2780) and a variant of ovarian carcinoma resistant to cisplatin (A2780cis). Cancer cells were incubated in the presence of increasing concentrations of tested compounds for 72h. Next, the IC₅₀ value was calculated using the colorimetric cell viability assay CCK-8 and BioDataFit software. The anticancer potential of studied compounds varied - the most promising compound was FCP28. The most sensitive cell line was HeLa (FCP28: IC₅₀=89 μM and FCP29: IC₅₀=102 μM). It was noticed that

FCP28 also killed efficiently human ovarian carcinoma resistant cells (calculated IC_{50} was 197 μ M).

These obtained data clearly show that FCP28 and FCP29 have promising anticancer properties. However, the mechanism of their anticancer action still needs to be evaluated.

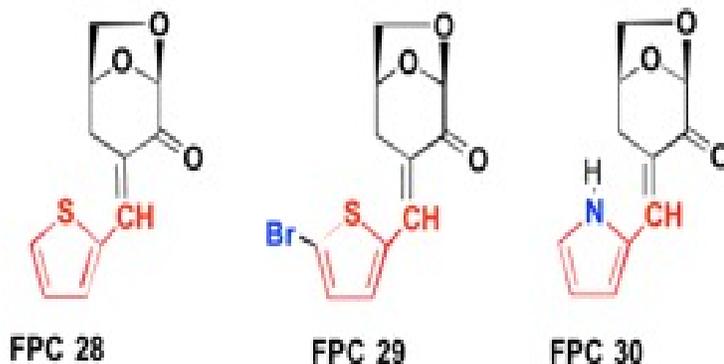


Figure 1. Exo-cyclic carbohydrate enones with thiophene and pyrrole moieties

MEDI 174

Novel carbohydrate functionalized thiosemicarbazides evoked DNA damage in cancer cell lines

Anna Czubatka-Bienkowska², Joanna Sarnik², **Zbigniew J. Witczak**¹,
zbigniew.witczak@wilkes.edu, Tomasz Poplawski². (1) Dept of Pharm SCI Sch of Phar,
Wilkes Univ, Wilkes Barre, Pennsylvania, United States (2) Department of Molecular
Genetics, University of Lodz, Lodz, Poland

Thiosemicarbazides and their analogs has shown potential medical applications as antiviral, antibacterial and anticancer drugs but their main problem are due to their imperfect hydrophilic/lipophilic balance. This factor is crucial for membranes permeability and potential biological activity. We assumed that S-glycosylation of thiosemicarbazides enhanced their anticancer potential. Therefore a series of four novel CARB functionalized thiosemicarbazides (shown on Figure 1) was designed, synthesized and evaluated *in vitro* anticancer activity against ovarian (A2780), cervix (HeLa), colon (LoVo), breast (MCF-7) and brain (MO59J) human cancer cell lines. They consist of thiosemicarbazide core linked via sulfur bond with pentose (FCP20), hexoses (FCP21 and FCP22) and thiodisaccharide (FCP16A). The results of cell viability assay CCK-8 revealed that our compounds displayed moderate cytotoxic activity against tested cancer cell lines. The most potent compound was FCP16A. We also investigated genotoxic properties using Single Cell Gel Electrophoresis assay (Comet assay). We have discovered that FCP16A as well as FCP20 have induced high and concentration depended genotoxic effect. It is worth to mention that thiosemicarbazides CARB functionalized with hexoses did not displayed high genotoxicity. The data obtained suggests that selective and rationale S-CARB functionalization of thiosemicarbazides

may enhanced their anticancer potential. However, their mechanism of anticancer action must be evaluated further.

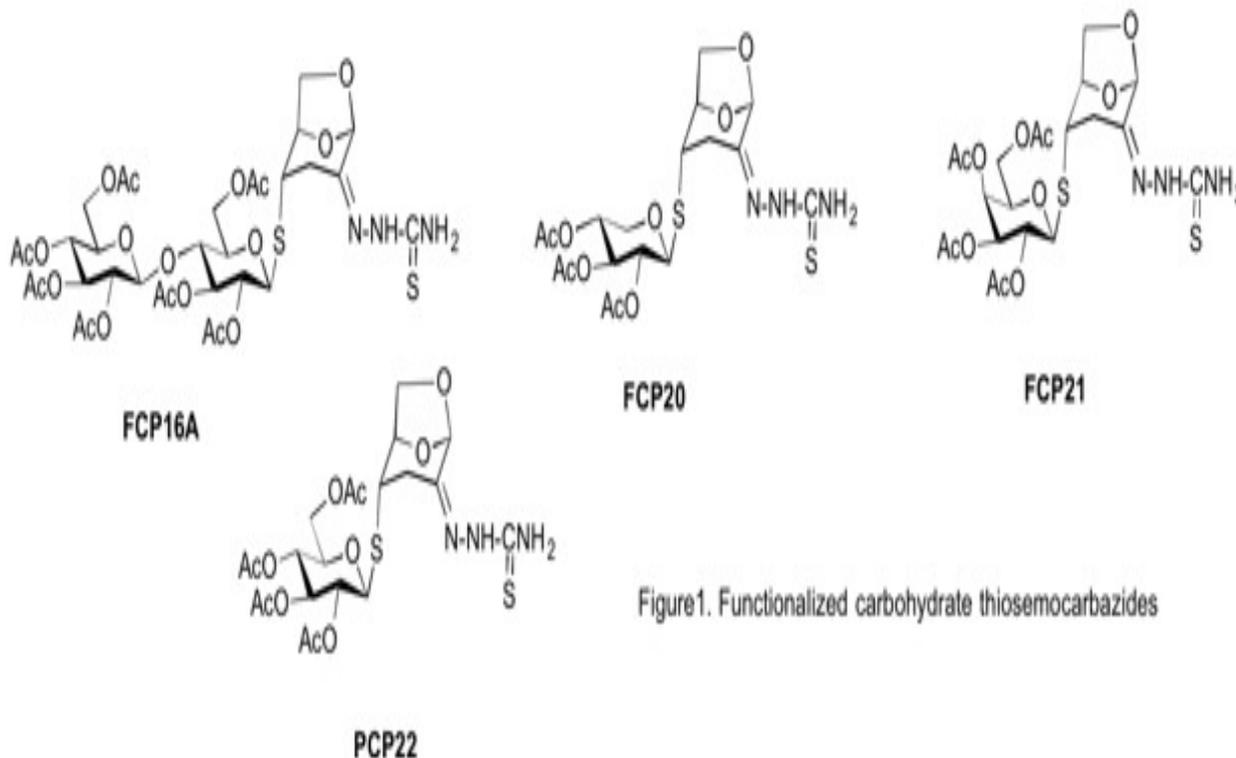


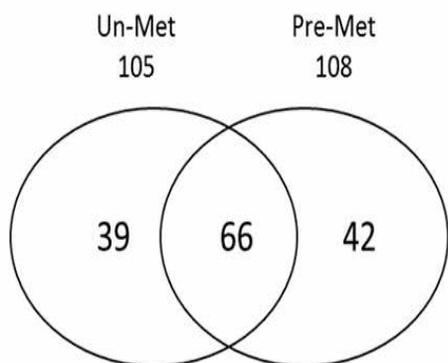
Figure1. Functionalized carbohydrate thiosemicarbazides

MEDI 175

Enhanced chemical diversity through library *in situ* pre-metabolism: Using biological chemistry for novel lead discovery

Navid J. Ayon, **William G. Gutheil**, gutheilw@umkc.edu. SchI of Pharmacy, Univ of Missouri, Kansas City, Missouri, United States

The screening of chemical compound libraries has become an essential component of the drug and bioactive agent discovery process. A key feature of chemical compound libraries is their chemical diversity, which refers to the chemical space encompassed by the library. Nearly all drugs and other xenobiotics are transformed into at least one metabolite, and generally more. Such metabolites frequently have distinct biological activities. The present study was directed toward assessing the potential of *in situ* library pre-metabolism (Pre-Met) as a means to enhance library chemical diversity in a biologically relevant fashion, and to increase the hit rate and informational value of a library screening effort. This demonstration effort focused on whole cell screening for novel antibacterial agents (MRSA), using the NCI Diversity Set V library metabolized using human liver microsomes. Pre-Met library screening provided a substantial number of unique Pre-Met library hits compared to the Un-Met library screen, demonstrating the potential of this approach.



Total Un-Met Hits	105
Total Pre-Met Hits	108
Un-Met U Pre-Met Hits	147
Un-Met \cap Pre-Met	66
Un-Met but not Pre-Met	39
Pre-Met but not Un-Met	42

Using the Pre-Met library increased the number of hits by:
 $100 * 42 / 105 = 40\%$

MEDI 176

Actinomycete antibiotic compounds from soil bacteria at an ancient Roman site

Angela Hoffman, hoffman@up.edu. Chemistry, University of Portland, Portland, Oregon, United States

Antibiotic resistance is a serious public health problem, and there is a growing need for tools and strategies to slow or stop the evolution of antibiotic resistance. A variety of bacteria were recovered from soil samples taken from Pollentia, an ancient Roman archeological site on the island of Mallorca (Spain). Since the bacteria were obtained from soils known to range between about 125 BC to the third century AD, compounds made by the recovered actinomycetes may represent unique antibiotics. Antibiotic activity is being analyzed against gram negative and gram positive bacteria and cell cultures. Structures of compounds with antibiotic activity are being identified.

MEDI 177

Inhibitors of the protein-protein interaction of Class IIa HDACs with MEF2 as potential anticancer agents

Caitlin M. DeAngelo¹, caitlindeangelo@gmail.com, Kevin J. Gaffney^{1,2}, Marcos A. Sainz¹, Jamie A. Jarusiewicz¹, Nimanthi Jayathilaka³, Lin Chen³, Stan G. Louie², Nicos A. Petasis^{1,2}. (1) Dept of Chemistry and Loker Hydrocarbon Institute, University of Southern California, Los Angeles, California, United States (2) School of Pharmacy, University of Southern California, Los Angeles, California, United States (3) Molecular & Computational Biology, University of Southern California, Los Angeles, California, United States

Histone deacetylase enzymes (HDACs) are known to modulate transcription by mediating the removal of acetyl groups from lysine residues of histones. The resulting

increased affinity of the histone lysine tails to the DNA backbone leads to the repression of gene expression. This type of HDAC-mediated epigenetic modulation has prompted the development of inhibitors of the HDAC active site as novel therapeutics for cancer, several of which have been approved for clinical use. However, there are many HDAC isoforms that often have similar deacetylase domains in addition to other functions associated with each HDAC, making the selective inhibition of particular HDAC actions quite challenging. Herein, we report a novel approach for blocking the actions of Class IIa HDACs by inhibiting their protein-protein interaction with the transcription factor MEF2. A series of small molecules were designed, synthesized, and assessed for their ability to inhibit the HDAC/MEF2 interaction, as well as their ability to inhibit the proliferation of human cancer cells.

MEDI 178

Development and screening of new cathepsin B and K inhibitors utilizing substituted thiosemicarbazones

Rose McConnell¹, rm-mcconnell@wiu.edu, Hema Latha Sarepalla¹, Prashanth G. Akula¹, Bharat Guda¹, Durga Yermala¹, Naveen Kadasala², Kelley Sayyar³, Walter Godwin³, Lisa Wen¹. (1) Department of Chemistry, Western Illinois University, Macomb, Illinois, United States (2) Chemistry, Purdue University, West Lafayette, Indiana, United States (3) Mathematics and Natural Sciences, University of Arkansas at Monticello, Monticello, Arkansas, United States

Cathepsins B and K are among the most biologically important proteolytic enzymes. Cathepsin B is clearly involved in the process of tumor invasion and metastasis and Alzheimer's disease, while cathepsin K has been identified a major target in the battle against osteoporosis. Thiosemicarbazones have been utilized as inhibitors of cathepsin L and other cysteine proteases. Additionally, thiosemicarbazones have increased solubility over aldehydes and have versatile chelating ability in metal complexes. The design and synthesis of 20 thiosemicarbazones containing P₁ arginine, lysine, ornithine, citrulline, and leucine sidechains as inhibitors as cathepsin B and cathepsin K based on SAR data has been accomplished. A library of 20 of these C-terminal aldehyde thiosemicarbazones with variations in the P₂ and P₃ positions are described. Several of these compounds have proven to be very potent inhibitors of human cathepsin B and K activity as measured using either spectrophotometric (BANA) or fluorometric assay (Cbz-Phe-Arg-AMC) techniques. The thiosemicarbazones showed mixed and non-competitive inhibition. Compounds showing the weakest inhibition of cathepsin B activity and the strongest inhibition of cathepsin K activity (IC₅₀ values range from 1.2 to 2.4 nM) were those that contain a C-terminal leucine aldehyde or thiosemicarbazone with either a citrulline or glutamine in the P₃ position.

MEDI 179

Novel mPGES-1 inhibitors identified from structure-based virtual screening based on new acting mechanism

Shuo Zhou, *szh238@uky.edu*, Ziyuan Zhou, Yaxia Yuan, Chang-Guo Zhan.
Pharmaceutical Sciences, University of Kentucky, Lexington, Kentucky, United States

Purpose: Microsomal prostaglandin E synthase-1 (mPGES-1) is an inducible prostaglandin E synthase after exposure to pro-inflammatory stimuli and, therefore, represents a novel target for therapeutic treatment of acute and chronic inflammatory disorders. However, in this study, we aimed to identify novel inhibitors of mPGES-1 with new scaffolds through structure-based virtual screening.

Methods: We carried out combined structure-based virtual screening and experimental studies in order to identify novel inhibitors of mPGES-1. Our virtual screening was performed on compounds in the SPECS and NCI libraries, containing a total of ~500,000 available compounds. The virtual screening was followed by *in vitro* activity assays against mPGES-1. The identified active compounds were further tested against COX-2.

Results: Based on the virtual screening, the top-50 compounds were ordered for *in vitro* activity assays against mPGES-1. According to the *in vitro* activity assays, 15 compounds can significantly inhibit mPGES-1 at 10 μ M. Of the 15 compounds, 8 compounds have been determined for their IC₅₀ values (200 nM to 8 μ M). Further *in vitro* activity assays against COX-2 and other off targets indicate that most of these new inhibitors of mPGES-1 do not inhibit COX-2. Starting from these novel inhibitors with new scaffolds, more potent and selective inhibitors of mPGES-1 may be designed in the future.

Conclusion: The structure-based virtual screening, followed by *in vitro* activity assays, has led to discovery of a set of novel inhibitors of mPGES-1 with new scaffolds.

MEDI 180

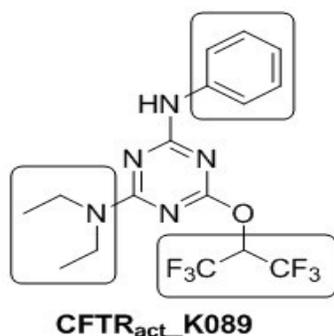
Nanomolar-potency aminophenyl-1,3,5-triazine CFTR chloride channel activators for pro-secretory therapy of dry eye diseases

Sujin Lee, *sujinlee99@gmail.com*, Puay-Way Phuan, Christian Felix, Marc H. Levin, Alan S. Verkman. Department of Medicine and physiology, UCSF, San Francisco, California, United States

Dry eye disorders are a significant health problem for which limited therapeutic options are available. CFTR (cystic fibrosis transmembrane conductance regulator) is a major pro-secretory chloride channel at the ocular surface. Using high-throughput screening platform, we previously identified aminophenyl-1,3,5-triazine **CFTR_{act}-K089** that activated CFTR with EC₅₀ ~250 nM. When delivered topically **CFTR_{act}-K089** increased tear fluid secretion in mice and showed efficacy in an experimental dry eye model. Based on preliminary SAR data from commercially available aminophenyl-1,3,5-triazine analogs, we synthesized novel analogs and conducted functional studies. The most potent analog fully activated CFTR chloride conductance with EC₅₀ ~30 nM, without

causing cAMP or calcium signaling. The potent triazine analog was non-toxic, stable in an ophthalmic vehicle, and rapid metabolized by hepatic microsomes, which supports its topical use in dry eye disease. In vivo studies showed increased tear volume for 8 h in wild type mice following a single topical administration, and no effect in CFTR-deficient mice.

In summary, we designed and synthesized aminophenyl-1,3,5-triazine CFTR activators, which are novel candidates for treatment of dry eye disease.



MEDI 181

Development of novel anti-biofilm compounds for the prevention and treatment of Candidiasis

Matthew Valdez, *mpf162@my.utsa.edu*. Chemistry, University of Texas at San Antonio, San Antonio, Texas, United States

Each year approximately 5% to 10% of all patients admitted to one of 7,000 acute care hospitals in the U.S will develop a health-care associated infection, which translates into a total of 2-4 million nosocomial infections. Although bacterial organisms represent the majority of these infections, fungal infections represent only 10% of all nosocomial infections, yet account for up to 70% of all patient deaths. Of all fungal invasive infections, by far candidiasis remains the most common, now representing the 3rd to 4th most frequent nosocomial infection in hospitals in the US and worldwide, and *Candida albicans* remains the main etiological agent of candidiasis. Biofilm formations are being increasingly recognized as the main virulence factor contributing to the pathogenesis of candidiasis and are linked to most clinical manifestations. As such, biofilm formation represents a high value target for the development of novel antifungal agents. We used high throughput screening (HTS) techniques to identify small molecule compounds from Chembridge's NOVACore™ chemical library, with inhibitory activity on *C. albicans* biofilm formation. Based on their IC₅₀ values (potency), lack of toxicity and efficacy in animal models of infection we identified several lead structures. These structures were then modified through iterative synthesis to improve on their anti-biofilm activity, physical chemical properties, and in an attempt to identify potential underlying pharmacophores. Several SAR (structure-activity relationship) trends were observed through the alteration of these structures and the results are actively aiding current studies for further improvement on “drug-like” properties and biofilm inhibition.

MEDI 182

Anti-bacterial activity of isoxazole rifamycin derivatives against *Mycobacterium abscessus*

Andrea Ramirez Ramos¹, andie.rmz@hotmail.com, **Keith D. Combrink**², **Florian Maurer**³, **Sebastian Schmidl**¹, **Stephanie Spring**¹, **Kira N. Elizondo**¹. (1) Biology, Texas A&M International University, Laredo, Texas, United States (2) Biology and Chemistry, Texas A&M International University, Laredo, Texas, United States (3) University Medical Center Hamburg Eppendorf, Hamburg, Germany

Mycobacterium abscessus (*Mab*) is considered the most pathogenic and resistant out of all the rapid-growing mycobacterium (RGM). It has been found in hospital settings and is leading pathogen in lung transplant infections, as well as common in cystic fibrosis patients. Using the currently available antimicrobials, cure rates for *Mab* infections do not exceed 30 to 50%, and clinical studies on therapeutic outcomes are uncommon. Due to its high levels of natural antibiotic resistance and the lack of bactericidal activity of common antimicrobial drugs, no standardized treatment for this pathogen has been established. While Rifamycins have potent activity against *Mycobacterium tuberculosis*, they have no activity against *Mab*. Mycobacterium have two resistance mechanisms to deactivate rifamycins. Genomic analyses shows that the DNA sequence of the *rpoB* gene of *Mab* (type strain ATCC 19977) is identical to rifampicin-susceptible strains of *M. tuberculosis*. *Mab* harbors an ADP-ribosyltransferase gene (GenBank ID MAB_0591) and preliminary data indicate that the Arr homolog of *Mab* confers high-level resistance to rifampicin.

We have prepared a series of isoxazole rifamycin derivatives with potent anti-bacterial activity against *Mab*. To better understand the permeability of these compounds we used a disk-diffusion test (Kirby-Bauer test) against two different strains of *Mab*. We will describe the structure activity relationships of these new derivatives as well as the results obtained.

MEDI 183

Identical HIV populations and levels of proviral expression in lymph nodes and PBMCs in individuals on antiretroviral therapy

Victoria Musick, vkm2@hood.edu. Hood College, Libertytown, Maryland, United States

HIV attacks the immune system by killing CD4+ T-cells, eventually making infected individuals vulnerable to opportunistic infections. Antiretroviral therapy (ART) prevents the destruction of the immune system by inhibiting the viral enzymes and preventing the spread of HIV to new cells. However, T-cells infected prior to initiating ART undergo massive proliferation despite treatment, creating a renewable source of infected cells during treatment. Although daughter cells can sometimes generate virus particles, ART prevents those particles from infecting new cells in the blood. It has been suggested

that ART has poor penetration into lymph nodes and therefore, viral replication may persist in this compartment during treatment. In this study, we used the Cell Associated HIV RNA and DNA Single Genome Sequencing (CARD-SGS) assay to determine the fraction of infected cells that express HIV RNA and their levels of expression in single PBMCs and in single cells isolated from lymph nodes in two ART-treated donors (5 and 11 years suppressed on ART). We also compared the HIV genetics in the two compartments to determine if viral evolution (due to on-going replication) occurred in the lymph node, making this population divergent from that in the blood. We found the HIV RNA and DNA populations in the lymph nodes and PBMCs to be genetically the same ($p=0.7$ for a difference) and we found no difference in the fraction of infected cells that express HIV RNA (27% vs. 24%) or in the levels of HIV RNA expression in single cells isolated from both compartments (median of 1 RNA molecule per expressing cell). Our results show that new HIV variants did not emerge in the lymph node during ART nor are there more cells in the lymph node expressing HIV RNA or expressing higher levels of HIV RNA than those in PBMCs. We conclude that on-going viral replication in the lymph node during ART did not likely occur in these two individuals.

MEDI 184

Synthesis and evaluation of 1, 3, 5 (10) estratriene aminoalkoxy,16-formyl derivatives of Estrone as potential anti-breast cancer agents

Christopher Sullen, cgsullen@aol.com, John S. Cooperwood. Pharmacy and Pharmaceutical Sciences, Florida A&M University, Birmingham, Alabama, United States

The chance of a woman developing invasive breast cancer in her lifetime is nearly 1 in 8 (12%). Estrogens contribute to the development of secondary sex characteristics and play an essential role in the regulation of a woman's reproductive processes. Tamoxifen, a Selective Estrogen Receptor Modulator (SERM), works by blocking estrogen at its receptor and is used for the treatment of advanced hormone-dependent mammary carcinoma. Unfortunately, it also exerts estrogenic effects within the uterus leading to cancer of the endometrium. Consequently, there is an urgent need to develop more specific drugs for the treatment of breast cancer. In this work, we designed and synthesized a series of Estrone-derived compounds that will act as anti-estrogens blocking the estrogen receptor (ER). Compounds were synthesized by combining a portion of the chemical features of tamoxifen, with the rigid structure of Estrone. These modifications are made at the 3-OH position of the Estrone A-ring. Additionally, formylation at 16-position on the D ring of Estrone was employed. Efficacy studies were performed using MCF-7 (ER⁺), MDA-MB-231 (ER⁻) and Ishikawa Cell (Human endometrial cancer). With an IC₅₀ (μM) of 7.461, CS-010-34-50 was more potent in the Ishikawa cells than both tamoxifen (25.830) and 4-hydroxytamoxifen (10.269). The results showed that CS-010-29-50 was also more potent against MCF-7 cells. Furthermore, the anti-proliferative studies in the MDA-MD-231 cells indicated that CS-010-34-50 was the most active. Molecular modeling studies were used to evaluate structure activity relationship (SAR) of the synthesized compounds.

MEDI 185

Modulation of Glioblastoma tumor area is time dependent on the application of G4-OH PAMAM dendrimer

Mark Jeakle^{1,2}, jeakl1mm@cmich.edu. (1) Chemistry, Central Michigan University, Mount Pleasant, Michigan, United States (2) Neuroscience, Central Michigan University, Mount Pleasant, Michigan, United States

Glioblastoma multiform (GBM), is a form of rapid-onset, hyper-aggressive brain tumor, that develops from oncogenic astrocytes. These astrocytes become malignant and recruit surrounding tissue and cells to form the tumors. The typical treatment is partial resection, followed by chemotherapy and radiotherapy. However, there is no known cure for GBM. The mortality rate is very high, and on an average, the patients survive for about 12-15 months post-chemotherapy and/or radiotherapy. As tumor grows the microglia surrounding the tumor become pressurized and release pro-inflammatory factors as an immune response, which in turn, increases the blood flow, migration and expansion of the tumor. This results in damaged brain paranchyma. The aim of this study was to develop a novel therapeutic strategy using dendrimer nanoparticles to arrest the inflammatory response to the tumor. These dendrimers are 3-dimensional nanoparticles that can be synthesized to contain different surface groups and properties. To achieve our goal, we used a 100% hydroxyl surface generation 4 (G4-OH) dendrimers that are known to exhibit anti-inflammatory properties. To induce tumor in rats, F98 glioma cells (engineered with Lu2 gene to enable the tracking of tumor progression) were unilaterally injected into the basal ganglia of the brain. Post injection, the tumor growth was followed using bioluminescence as measured by *in vivo* imaging system (IVIS) on days 11, 17, 25, and 31. The animals were randomly divided into 4 groups: (I) received no treatment (n=7); (II) received G4-OH dendrimers on day 7 (n=7); (III) received G4-OH dendrimers on day 14 (n=7); and (IV) received G4-OH dendrimers on day 21 (n=7). Histological analysis using Cresyl violet indicated that there was a significant increase in tumor area between the groups I and IV, whereas there was a significant decrease in tumor area between the groups III and group IV. Therefore, our findings reinforce those showing the anti-inflammatory properties of dendrimers on tumor growth, and, further, this effect is dependent on when the dendrimers were delivered.

MEDI 186

Polymorphs and solvates of erlotinib and dasatinib

Taylor Maddox, maddox32@marshall.edu, Rosalynn Quinones. Chemistry, Marshall University, Ashland, Kentucky, United States

Polymorphism is the capacity for a molecule to change its crystalline lattice and have other crystalline forms. Polymorphs of pharmaceutical compounds can have different properties and elicit different responses than their commercial forms. Erlotinib and

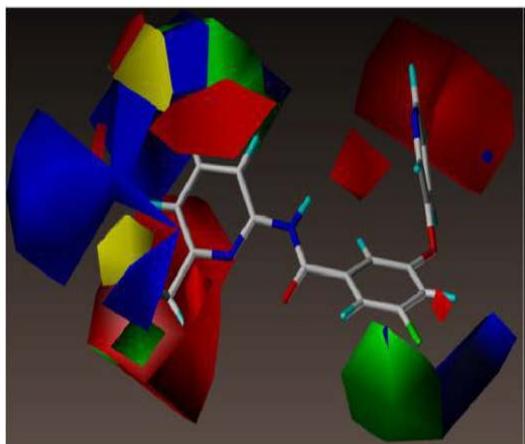
Dasatinib, tyrosine kinase inhibiting chemotherapy drugs, are used to prevent cancer cells from growing and dividing. The focus of this work is to analyze the conformational changes of these drugs. The method used was surface modifications using Self Assembled Monolayers (SAMs) and solvent-base screening. SAMs were prepared by modifying zinc and nickel oxide tiles in one of three organic acids: hexadecane sulfonic acid, 16-phosphonohexadecanoic acid, or n-octadecylphosphonic acid. Then, the pharmaceuticals drugs were recrystallized on the SAMs. The resulting crystals were analyzed and characterized by Reflectance Infrared Spectroscopy, Raman Spectroscopy, Powder X-Ray Diffraction, Thermogravimetric Analysis, Differential Scanning Calorimetry and Solid State Nuclear Magnetic Resonance to determine the conformational flexibility and hydrogen bonding, as these factors influence the ability of a molecule to form polymorphs.

MEDI 187

Computer-aided design of negative allosteric modulators of metabotropic glutamate receptor 5 (mGluR5):CoMFA studies on aryl ether derivatives

*Selvam Chelliah¹, **Brian Jordan**¹, jordanb683@yahoo.com, Ramasamy Thilagavath². (1) Pharmaceutical Sciences, Texas Southern University, Houston, Texas, United States (2) Department of Biotechnology, Karpagam University, Coimbatore, India*

Metabotropic glutamate receptors (mGlu receptors) have emerged as attractive targets for number of neurological and psychiatric disorders. mGluR5 is one of the best characterized receptors and recently negative allosteric modulators (NAMs) of mGluR5 have gained considerable attention in pharmacological research. To understand the 3-D quantitative structure activity relationship of mGluR5 NAMs, a comparative molecular field analysis (CoMFA) was performed on 73 analogs of aryl ether which were reported as mGluR5 NAMs. CoMFA of 73 analogs of aryl ethers produced a statistically significant model with high correlation coefficient and good predictive abilities. The CoMFA method generated contour plots to represent the steric and electrostatic interactions. The resulted contour maps highlight the structural features pertinent to biological activities. The generated model is highly reliable to predict the activities of newly designed molecules and thus the resulted information might be useful to expand the design and development of novel mGluR5 NAMs.



MEDI 188

Chemo-enzymatic approach towards the synthesis of vancomycin and its analogs

Seyma Ozturk, *seyma@princeton.edu*. Chemistry Department, Princeton University, Princeton, New Jersey, United States

Combating multidrug-resistant bacteria is one of the grand health challenges of the new millennium. The Center for Disease Control and Prevention recently reported that annually over 2 million Americans are infected by such pathogens; at least 23,000 infections are fatal. Resistance has been observed to every antibiotic that is currently on the market, even to our most important drug-of-last resort, vancomycin. Traditionally, synthetic tailoring has allowed us to repurpose ineffective antibiotics and reintroduce more effective derivatives back into the clinic. This approach has been successfully employed with nearly all antibiotic families on the market. Because of its structural complexity; however, synthetic tailoring is not feasible with vancomycin. Herein, we propose a new platform for developing useful vancomycin analogs, thus allowing us to repurpose this drug. In this chemo-enzymatic approach, synthetic routes generate the monomeric units, while the macrocycles are formed using the biosynthetic enzymes, thus mirroring the natural biogenesis of these molecules (Figure 1).

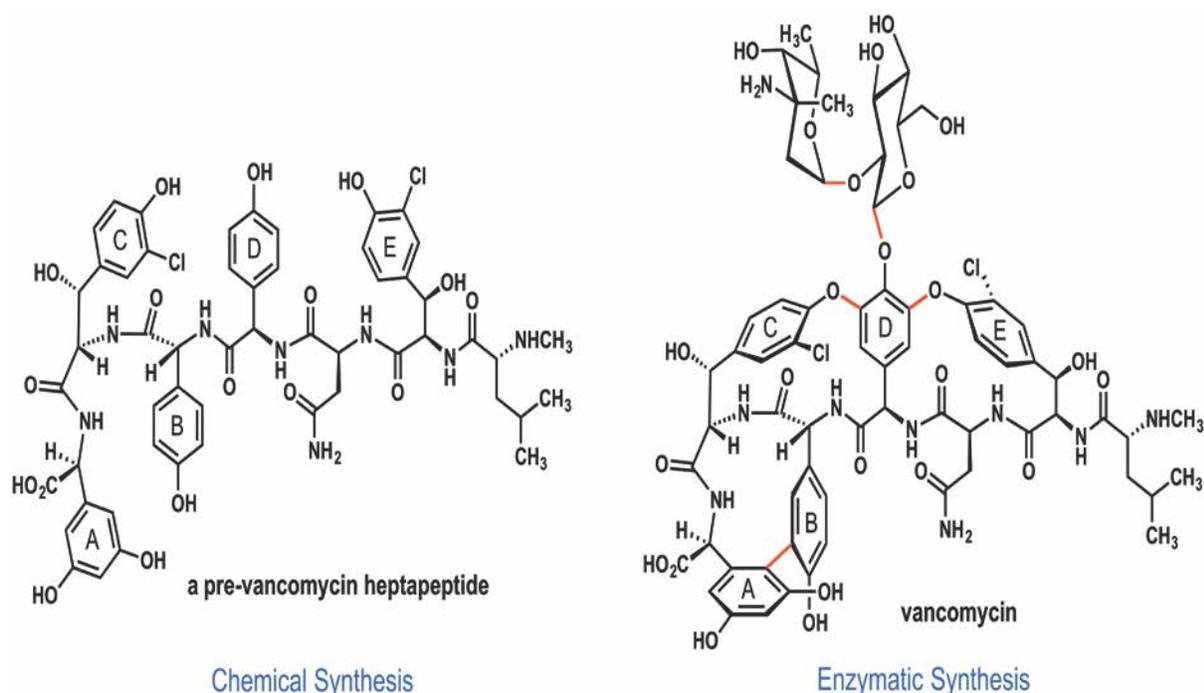


Figure 1. Chemo-enzymatic approach for the synthesis of vancomycin and its analogs.

MEDI 189

EGCG and Silybins as treatment for inherited cardiomyopathies: Binding simulations to cardiac troponin

Juan Eiros Zamora¹, *je714@ic.ac.uk*, **Gil Hoben**¹, **Alice Sheehan**², **Andrew Messer**², **Afnan Chaudhry**², **David Biedermann**³, **Vladimír Kren**³, **Steven B. Marston**², **Ian R. Gould**¹. (1) Chemistry, Imperial College London, London, United Kingdom (2) National Heart & Lung Institute, Imperial College London, London, United Kingdom (3) Institute of Microbiology, Czech Academy of Sciences, Prague, Czech Republic

Many mutations causing the inherited diseases hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM) act by affecting Ca^{2+} -regulation of contraction by troponin. HCM Mutations can increase Ca^{2+} -sensitivity 2-3 fold and most mutations uncouple the Ca^{2+} -sensitivity change from TnI phosphorylation by PKA. Small molecules that bind to troponin and reverse these abnormalities are of therapeutic interest. We have found that Epigallocatechin 3-Gallate (EGCG) is a Ca^{2+} -desensitiser and also re-couples uncoupled mutant troponin. Silybin exists as stereoisomers: we found that Silybin B is a pure recoupler (no effect on Ca^{2+} -sensitivity) whilst Silybin A has no activity. We propose that these compounds act by differentially binding to troponin in the phosphorylated, unphosphorylated, wild type and mutant states.

In this work, we attempt to address the structural and dynamical effects of these compounds with atomistic detail building on the results obtained from our existing

computational model of phosphorylated and unphosphorylated cTn in its Ca²⁺ activated state, totalling 419 amino acid residues. The objective is to study the binding modes of EGCG and Silybins A and B using extensive Molecular Dynamics simulations. Based on the same starting structure as previously, both Autodock and MD simulations indicate two preferred binding sites for EGCG, both are intercalated between the stable TnC N-terminal domain and the disordered N-terminal phosphorylatable peptide of cTnI forming hydrogen bonds with both peptides. According to Autodock calculations, Silybin A and B preferentially bind at the same interface as EGCG but they present a less favourable energy of interaction with cTn as the simulation proceeds, and in some simulations, they only remain bound for a fraction of the simulation time.

Simulations of ligand binding to phosphorylated troponin and to troponin containing HCM and DCM-causing mutations are in progress and should yield valuable data on differential binding that may explain how these potentially therapeutic small molecules function.

MEDI 190

Derivatives of L-tryptophanhydroxamic acid as potential inhibitors of *Burkholderia pseudomallei* IspF

Chante Muller, *chante.a.muller@gmail.com*. Northern Illinois University, DeKalb, Illinois, United States

Burkholderia pseudomallei is the cause of a severe and potentially fatal infectious disease called melioidosis. This bacteria, generally found in tropical and subtropical regions, is labelled as a Tier 1 select agent by the CDC. In order to discover effective treatments for melioidosis, inhibitors of *Burkholderia pseudomallei* IspF are being investigated. The MEP pathway is used by most bacteria to produce IPP and DMAPP. Inhibition of the MEP pathway is desirable since it is not found in humans which may limit side effects. This research focuses on developing inhibitors of the enzyme from the fifth step of the MEP pathway, IspF. Starting with the lead compound, L-tryptophanhydroxamic acid, analogues were designed, synthesized, and assayed for antibacterial activity and binding affinity. This poster will describe the observed Structure Activity Relationships.

MEDI 191

Synthesis and evaluation for biological activity of cyclic guanidine containing natural product analogues

Vamshi Krishna Reddy Sammeta², *sammetavamshikrishna@gmail.com*, **Sivappa Rasapalli**¹, *srasapalli@umassd.edu*. (1) University of Massachusetts Dartmouth, North Dartmouth, Massachusetts, United States (2) Chemistry and Biochemistry, University of Massachusetts Dartmouth, Newbedford, Massachusetts, United States

Guanidine group has a crucial role to play in medicinal chemistry. Because of the presence of basic and highly polar functional group they can make interactions with various other functional groups through strong hydrogen/ionic bonding. Potent biological properties shown by the naturally occurring polycyclic molecules made guanidine group an attractive functional group in medicinal chemistry. In our research we were successful in synthesis of cyclic guanidine through formamidinium chemistry. We are currently focusing on using the same strategy to synthesize natural product analogues containing cyclic guanidine group. A computational approach will be used in synthesizing selective analogues based on analogue and target interactions. All the synthesized analogues will be tested for their biological activity *in vitro* to find the lead molecule(s).

MEDI 192

Exploring the chemical biology of secondary metabolites: Scalable synthesis of majusculamide D

Eduardo J J. Caro-Diaz, ecaro@ucsd.edu, William H. Gerwick. Ctr for Marine Biotech Biomed, La Jolla, California, United States

Secondary metabolites, also known as natural products, are an important source for the discovery of biologically active compounds and new pharmaceutical leads. More than 60% of all drugs are inspired or derived from secondary metabolites isolated from natural sources. Standard extraction-isolation techniques mostly supply very low amount of compounds that may have great potential as therapeutic agents. Synthetic chemistry can be of great value to explore the chemical biology of scarce natural products, especially if careful design and consideration can allow access to multi-milligram quantities of biologically interesting natural product. Herein, we describe our efforts towards the synthesis of majusculamide D, a lipopentapeptide isolated from *Lynbya majuscula* that has shown interesting and potent anti-cancer activity. Our strategy was to target large amounts of compound *via* a scalable synthesis that aimed to: take advantage of a convergent route, reduce the amount of purifications by making use of simple work-ups, and to produce large amounts of synthetic intermediates without the need to begin with decagram quantities of starting materials. Also, we aimed to determine the absolute configuration of the 1,3-dimethyloctanamide motif on the western region of majusculamide D. Our synthetic design will allow for ample amounts of material to be prepared for in depth biological studies of majusculamide D.

MEDI 193

Prostate cancer targeted nanoparticle cocktail for combination therapy

Uttara Basu, ubasu@med.miami.edu. Biochemistry and Molecular Biology, University of Miami, Miller School of Medicine, Miami, Florida, United States

Prostate cancer is the second leading cause of death among the American male population for which the prevalent treatment options are surgery and/or radiation therapy. However, the advanced castration-resistant prostate cancer is difficult to treat with the conventional therapies. Moreover the use of a single therapeutic modality has limited success since several factors *viz.* inflammation, resistance, metastases to bones, and participation of metabolically altered cancer stem cells (CSCs) play integral roles for progression and spread of this disease.

To address the varied features of advanced prostate cancer, our lab has developed a multifunctional polymer-based nanoparticle (NP) technology which can deliver a predefined stoichiometric combination of chemotherapy (cisplatin) and an anti-inflammatory agent (aspirin), in a controlled manner specifically to prostate cancer cells.¹ Polylactic-co-glycolic acid (PLGA) forming block copolymer with polyethylene glycol (PEG) appended to prostate specific membrane antigen (PSMA) (PLGA-*b*-PEG-PSMA) was blended with a polylactic acid (PLA) derivative appended to a cisplatin and aspirin prodrug; (Asp)₄-PLA-(Platin-4)₃. More recently we have developed a more hydrophobic congener (Asp)₄-PLA-(Platin-12)₃.

Cytotoxic activity of T- (Asp)₄-PLA-(Platin-12)₃-GLU-NPs in PC3 and LNCaP cell lines indicated that Platin-12 conjugated NP system is much more active than with Platin-4. IC₅₀ in LNCaP cells was significantly decreased when conjugated Platin-12 was delivered using PSMA targeting ligands strongly supporting the targeting ability of PSMA ligand. We also saw that a low dose of X-ray irradiation further sensitizes the activity of this targeted multifunctional platform towards PSMA expressing advanced prostate cancer cells. Under ionizing radiation, The NPs can alter the mitochondrial metabolism of PSMA expressing prostate cancer cells. Colony survival studies demonstrated that radiosensitization enhances the activity of T-(Asp)₄-PLA-(Platin-12)₃-GLU-NP in LNCaP cells, whereas no significant enhancement was observed for PC3 cells. MitoStress assay in LNCaP and PC3 cells in presence of T-(Asp)₄-PLA-(Platin-12)₃-GLU-NPs in the absence and presence of radiation suggested that mitochondrial activity was hampered in presence of T-NPs when combined with radiation. A tumor reduction study conducted using PSMA expressing LNCaP xenograft model also indicated that T-NPs have significant tumor remission.

MEDI 194

Direct thrombin inhibitors with a novel, reversible, covalent mechanism of action

*Mohan Sivaraja, Sivan Sizikov, David Williams, Chengpei Xu, Daniel Clemens, Subhadra Dash, Kenneth Lin, Mamatha Reddy, Georg Neckermann, Simon Yau, Elaine To, Lev Igoudin, Stephanie Chang, Samuel Keutzer, Piotr Zalicki, Nichole Sandoval, John Zhang, Timothy Shiau, **Kevin Short**, kshort@verseon.com, M. Angels Estiarte, Anirban Datta, David Kita. Verseon Corporation, Fremont, California, United States*

Patients with non-valvular atrial fibrillation or venous thromboembolism typically require anticoagulation prophylaxis/treatment. Direct oral anticoagulants (DOACs) offer certain advantages over warfarin and heparins, but there remains a significant unmet need for anticoagulant agents with better risk-benefit profiles than currently available treatments.

We have identified a new class of highly potent and selective direct thrombin inhibitors (VE-DTIs), which are reversible covalent inhibitors of thrombin. We will present enzyme kinetic, biochemical, and X-ray crystal studies to demonstrate how VE-DTIs interact with Ser195 of the catalytic triad in the active site of thrombin.

The poster will feature data for enzyme inhibition, selectivity, and the thrombin generation assay (TGA). We will also present a comparison of the VE-DTIs to argatroban and dabigatran for the inhibition of thrombin-mediated activation of endogenous substrates such as fibrinogen cleavage, protein C, and thrombin activated fibrinolysis inhibitor (TAFI), and thrombin mediated inhibition of platelet activation.

MEDI 195

Cheminformatics analysis of WNK-inhibitor interactions

Melaine A. Kuenemann^{1,2}, makuenem@ncsu.edu, **Denis Fourches**^{1,2}. (1) *Department of Chemistry, North Carolina State University, Raleigh, North Carolina, United States* (2) *Bioinformatics Research Center, North Carolina State University, Raleigh, North Carolina, United States*

Protein kinases play a central role in a variety of signal transduction cascades. Their dysfunctions or deregulations are at the origin of severe diseases, especially cancer. The human kinome with more than 500 kinases identified represents a wealth of therapeutic targets. The distinct family of With-No-Lysine (WNK) serine/threonine kinases constitutes a unique and distinctive branch of the kinome. The four proteins of this family (WNK1/2/3/4) are involved in blood pressure regulation, body fluid, and electrolyte homeostasis. In fact, it has been shown that key mutations in WNK1 or WNK4 can cause pseudohypoaldosteronism type II.

Interestingly, only a few compounds have been found to bind WNK proteins. A recent study identified the first orally bioavailable and highly selective inhibitor (WNK463) of WNK's catalytic activity. WNK463 is a low-nanomolar binder at all four WNK proteins but has been stopped in preclinical trials due to its safety profile.

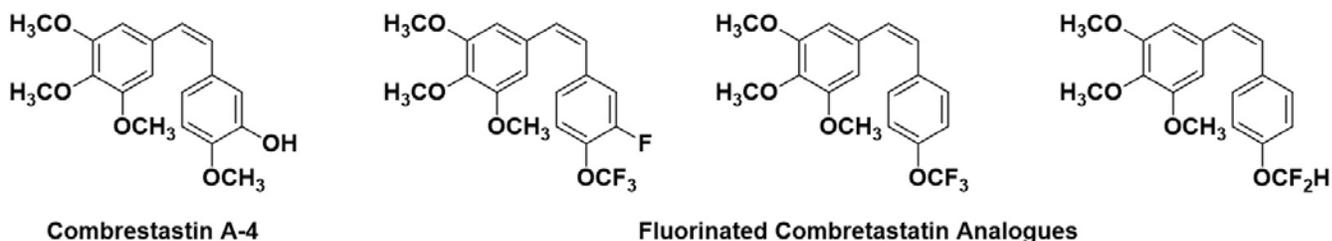
In this study, we aim to analyze and better understand the binding modes of all public-domain small molecules known to be active towards any of the WNK family member. To do so, we relied on cheminformatics approaches including structure-activity relationships, structure-based molecular docking, and molecular dynamics. In particular, we analyzed the (i) molecular selectivity of known inhibitors when docked in the binding site of each WNK family member, (ii) the dynamic WNK-ligand interactions, and (iii) the prediction performances of cheminformatics techniques to discriminate the most potent WNK inhibitors. Finally, we report on the results of a virtual screening of the ZINC library for identifying new potent and selective WNK inhibitors.

MEDI 196

Computational structural-based design and structure and activity study of fluorinated combretastatin analogues

Yao Zong, *yao.zong@stonybrook.edu*. Chemistry, Stonybrook University, Stonybrook, New York, United States

Combretastatin A-4 (CA-4) is one of the most potent antimetabolic agents, showing strong cytotoxicity against a variety of cancer cells. Besides, it is an angiogenesis inhibitor and causes tumor blood vessels shut down. Currently, there are several CA-4 derivatives in clinical trials. Fluorine-containing compounds have been widely used in medicinal chemistry because the incorporation of fluorine often furnishes molecules with unique properties that cannot be attained using any other element. So a small library of fluorinated combretastatin analogs was designed, synthesized and evaluated. Docking studies were carried out to investigate the binding mode and affinity between Combretastatin analogs and tubulin (PDB code: 4O2B). The influence of the fluorine atom on Combretastatin scaffold was also evaluated, *in silico*. Fluorinated analogs were screened and ranked by binding affinity by Autodock and Moe. During screening, several analogs with better energy scores than CA-4 were found. Those fluorine-containing combretastatin analogs with good docking energy score were synthesized and evaluated.



MEDI 197

Benzoate derived pH sensitive phosphoramidate-based linkers for controlled release

Brian Backer, *brian.backer@wsu.edu*, Austen Davis, Cindy Choy, Clifford E. Berkman. Chemistry, Washington State University, Pullman, Washington, United States

We previously discovered a novel pH-sensitive linker based on a phosphoramidate scaffold that can be tuned to release amine-containing drug molecules at various pH conditions. The pH-sensitivity of the P-N bond is due to a proximal acidic group, such as a carboxylic acid. The triggered release of a drug at endosomal pH is attractive in that it does not require intracellular enzymatic action for drug release/delivery. Our continued work in this area is currently focused on a benzoate as the proximal acidic group in which the pK_a of the benzoic acid can be predictably modulated allowing for another degree of tunability. It is envisioned that this scaffold offers the advantage of fine-tuning

of amine-release kinetics through substitution effects on the aromatic ring. The substitution effect of various electron withdrawing groups (EWG) and electron donating groups (EDG) allows for a correlation to be drawn to traditional Hammett effects and allows the predictability and control of drug release. The EWGs on the benzoate are expected to stabilize the linker at mild pH conditions to provide a slower drug release while the EDGs are expected to destabilize the linker at mild pH conditions for more rapid drug release. This technology is advantageous compared to current cleavable linker technologies because of its broad applicability (e.g., prodrug design, drug eluting stents, and targeted therapy).



MEDI 198

New vacuolar-ATPase inhibitors as antiviral therapies

Aaron Lindstrom¹, alindst@purdue.edu, **Dino P. Petrov**¹, **Douglas LaCount**¹, **Robert Davey**², **Vincent J. Davisson**¹. (1) Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, Indiana, United States (2) Department of Virology and Immunology, Texas Biomedical Research Institute, San Antonio, Texas, United States

The Filoviridae family of viruses are highly contagious and lethal pathogens that cause severe hemorrhagic fever in humans and primates. The Ebolavirus genus of the Filoviridae family have been responsible for outbreaks that result in mortality rates of up to 90% fatality. Currently, there are no approved therapeutic measures for the treatment of filovirus infections; which makes their development of paramount importance. A potential target for inhibiting Ebolavirus infection occurs when the virus taken into the cell by macropinocytosis and trapped in early endosomes early in the viral lifecycle. Typically, the virus is released from the endosome via pH-dependent processes driven by vacuolar-ATPase (V-ATPase). V-ATPase is a multiprotein complex present in all eukaryotic cells that couples ATP hydrolysis to the transport of protons across cellular membranes. One of V-ATPase's most important activities is the acidification of endosomes and lysosomes within cells. By blocking endosomal acidification caused by V-ATPase, ebolavirus infection can be stopped before the replication of the virus begins. Thus, inhibition of endosomal acidification by blocking V-ATPase function represents a novel and promising approach to halt ebolavirus infection. The natural product diphyltin was recently discovered to possess V-ATPase inhibitory activity. Diphyltin presents a novel and unexplored chemotype for the development of potential lead compounds. Our lab has focused on synthesizing and testing derivatives containing alterations of the lactone ring. *We have hypothesized that modifications of the lactone ring of diphyltin can be used to increase the potency and physicochemical*

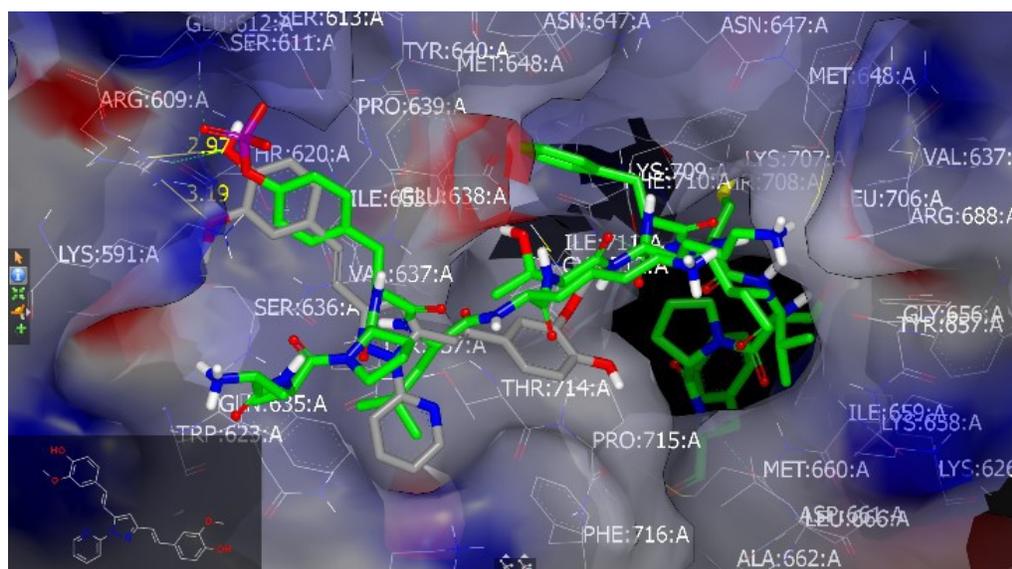
properties of diphyllin. Several primary and secondary amide derivatives of diphyllin have been synthesized that inhibit Ebolavirus infection of primary human macrophages with similar potency to diphyllin. The amide derivatives also exhibit improved physicochemical properties when compared to the parent compound. Several secondary amide derivatives of diphyllin have also demonstrated the ability undergo cyclization under endosomal conditions to reform diphyllin.

MEDI 199

Design and synthesis of curcumin analogues for cytotoxicity against head & neck cancer

Selvam Chelliah¹, *chelliahs@tsu.edu*, **Brian Jordan**¹, **Bhavna Kumar**², **Ramasamy Thilagavathi**³, **Pawan Kumar**². (1) *Pharmaceutical Sciences, Texas Southern University, Houston, Texas, United States* (2) *Department of Otolaryngology-Head and Neck Surgery, The Ohio State University Wexner Medical Center, Columbus, Ohio, United States* (3) *Department of Biotechnology, Karpagam University, Coimbatore, India*

Curcumin is a popular, plant-derived compound that has been extensively investigated for diverse range of biological activities. Anti-cancer activity against various types of cancers and high safety profile associated with curcumin makes it very attractive. In this study, we report the synthesis and evaluation of pyrazole and click chemistry curcumin analogues for Head and Neck cancer. MTT assay against head and neck cancer cell line CAL27 revealed that the synthesized compounds are active. Pyrazole compound showed significant inhibition on pSTAT3 (Tyr 705) phosphorylation at 20 and 30 μ M. As far as other compounds, they showed potent cytotoxicity against CAL27 however these compounds didn't show any activity on pSTAT3 phosphorylation at 10-30 μ M concentration. So the compounds 3 and 5 maybe acting on different signaling pathway involved in HNSCC. Molecular docking studies revealed the possible binding mode of pyrazole compound in the SH2 domain of STAT3.



Overlay of phosphor-tyrosine peptide (green) and pyrazole compound (grey) in the active site of STAT3-SH2

MEDI 200

Indole-2-carboxamide derivatives as G-protein coupled estrogen (GPER/GPR30) ligands

Austin O'Dea, *odeaat@slu.edu*, Christopher K. Arnatt. Chemistry, St. Louis University, St. Louis, Missouri, United States

Classical estrogen receptors (estrogen receptors α and β), have been shown to cause cell proliferation in a variety of breast cancers upon interaction with estrogenic compounds; however, G-protein estrogen receptor (GPER) has also been linked to cell proliferation, when in the presence of estrogens in breast cancers absent Estrogen receptors α and β . Here we demonstrate the ability of small molecules, specifically computationally modeled hits that may interact with GPER, to inhibit estrogen binding to GPER. Interactions of these modeled hits were studied on breast cancer cell lines SKBR3 and MCF7 in an estrogen promoted antiproliferation assays to search for potential activity with the GPER to determine estrogenic interactions upon the breast cancer lines. These interactions may signify that inhibition of GPER to block estrogen is an effective method for curbing estrogen promoted cancer cell proliferation.

MEDI 201

Interactions of isoniazid with model membranes

Allison Groninger², *alli.groninger@hotmail.com*, Benjamin J. Peters³, John Peter B. Hough⁴, Fabio Levi Fontes⁶, Gabriel Cardiff¹, Dean C. Crick⁵, Debbie C. Crans¹. (1) Colorado State University, Fort Collins, Colorado, United States (2) Colorado State University, Colorado Springs, Colorado, United States (3) Chemistry, Colorado State University, Fort Collins, Colorado, United States (4) Department of Biochemistry, Colorado State University, Fort Collins, Colorado, United States (5) Colorado State University, Fort Collins, Colorado, United States (6) Colorado State University, Portimao, Portugal

Despite being a curable disease, tuberculosis (TB) is still one of the world's most common diseases with an estimated one third of the population infected with latent TB. A current first line tuberculosis drug is isoniazid. The mechanism of the drug is known in that it couples with NAD⁺ to form a complex that can bind to the enoyl-AcpM substrate and the action of fatty acid synthesis. This inhibits synthesis of mycolic acids effectively inhibiting growth of mycobacterium tuberculosis. It also inhibits cytochrome P450 system and thus can be a source of free radicals. Though we know the function of the drug, the method of uptake into the cell is unknown. Since isoniazid is a small molecule it may be able to diffuse through the membrane but there is limited information about

the interactions of isoniazid with membranes. To explore this membrane interaction, the interactions of isoniazid with reverse micelles and phospholipid Langmuir monolayers were studied at different pH values. Through this research, it was discovered that isoniazid interacts with various phospholipids differently and the exact placement of isoniazid at a surfactant interface was determined.

MEDI 202

Synthesis and biological evaluation of novel casein kinase 1d inhibitors for treatment of Alzheimer's disease

Vishwajeet Jha², *vjha@xula.edu*, **Cory Gettridge**², **Richard Schroeder**¹, **Melyssa Bratton**³, **Phan Tram**¹, **Jayalakshmi Sridhar**⁴. (1) Chemistry, Xavier University of Louisiana, New Orleans, Louisiana, United States (2) chemistry, Xavier University of Louisiana, New Orleans, Louisiana, United States (3) Xavier University of Louisiana, New Orleans, Louisiana, United States

Alzheimer's disease (AD) is a progressive neurodegenerative disorder known to have notable symptoms like short term memory loss otherwise referred to as dementia. Abnormal hyperphosphorylation (P-tau) of the tau protein leads to the aggregation of amyloid plaques which is the hallmark of AD and several other neurodegenerative disorders. Casein kinase 1 δ (CK1 δ) and casein kinase 1 ϵ (CK1 ϵ) are the two isozymes expressed in the brain among the eight known isozymes that belong to Casein kinase 1 family. CK1 δ plays a critical role in AD through phosphorylation of the tau protein (associated with microtubules) which precedes neuritic lesion formation, involving CK1 δ in the tau fibrillization reaction pathway. CK1 δ has been reported to be associated with pathological accumulation of tau in several neurodegenerative diseases including AD, Down syndrome, progressive supranuclear palsy, and Parkinsonism dementia complex of Guam. Inhibition of CK1 δ has been shown to reduce fibrillar lesions and inhibit A β production.

Our recent studies on quinones as kinase inhibitors revealed one such compound that pharmacologically inhibited CK1 δ and Pim1 kinase preferentially over CK1 γ 2 and 98 other human protein kinases. Several derivatives of the lead compounds were synthesized and preliminary *in-vitro* CK1 δ kinase inhibition assay have shown a few compounds with good potency. Herein, we describe the synthesis and bioassay results of our study.

MEDI 203

Exploring the interactions between the anti-tuberculosis agent, pyrazinamide/pyrazonoic acid, and lipid model membrane systems

John Peter B. Hough¹, *bart@rams.colostate.edu*, **Allison S. Groninger**¹, **Benjamin J. Peters**², **Fabio Levi Fontes**^{4,3}, **Dean C. Crick**^{4,3}, **Debbie C. Crans**^{2,3}. (1) Department of Biochemistry, Colorado State University, Fort Collins, Colorado, United States (2) Department of Chemistry, Colorado State University, Fort Collins, Colorado, United

States (3) Cell and Molecular Biology Program, Colorado State University, Fort Collins, Colorado, United States (4) Mycobacteria Research Laboratories, Department of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, Colorado, United States

Pyrazinamide (PZA) is a drug listed on the World Health Organization's list of essential medicines due to its crucial role in treating tuberculosis. PZA is known to enter the cell and be hydrolyzed into pyrazonoic acid (POA). Once hydrolyzed, it has been shown to do two things: collapse the membrane potential, and inhibit ribosomal rescue. We are interested in the membrane interaction due to this collapsing of the membrane potential. To look at this, NMR studies of reverse micelle microemulsions were used to identify the location of PZA/POA in a model of a membrane leaflet. Interactions between POA/PZA and Langmuir monolayers, prepared from various phospholipids, were also measured using a Langmuir trough. These studies help characterize the drug-membrane interaction, which provides insight into PZA's mechanism of disrupting membrane potential.

MEDI 204

Aza-peptide aldehydes and ketones: A new class of proteasome inhibitors

Thomas Corrigan³, *Corrigan.104@osu.edu*, **Kayla Kasper**³, **Ryan McCauslin**¹, **Ryan J. Yoder**¹, **Christopher M. Hadad**², **Özlem Doğan Ekici**⁴. (1) *Ohio State Marion, Lewis Center, Ohio, United States* (2) *Ohio State University, Columbus, Ohio, United States* (3) *Chemistry, Ohio State University, Columbus, Ohio, United States* (4) *Ohio State University - Newark, Newark, Ohio, United States*

Proteases, enzymes responsible for protein hydrolysis, play important roles in a variety of cellular signaling pathways. The proteasome, an important protease that is a crucial component of the ubiquitin-proteasome pathway (UPP), is responsible for the quality-control of newly synthesized proteins in eukaryotic cells. Many UPP substrates are involved in the regulation of apoptosis and the cell cycle. Defects in the UPP are believed to play a role in a number of disease states, including cancer. Hence, the proteasome has been validated as an important target for the treatment of cancers, particularly multiple myeloma. While numerous examples of proteasome inhibitors have been reported, there remains a demand for potent therapeutics with higher specificity for the target proteasome. We present here the synthesis and kinetic inhibition study of the first examples of aza-peptide proteasome inhibitors, incorporating an aza-P1 amino acid residue and an α -aldehyde or α -ketone moiety as the active site electrophilic warhead. This new class of reversible inhibitors demonstrates competitive inhibition for the 20S proteasome with measured K_i values in the μM range. These novel inhibitors show potential for the development of a new class of protease inhibitors of the aza-peptide variety.

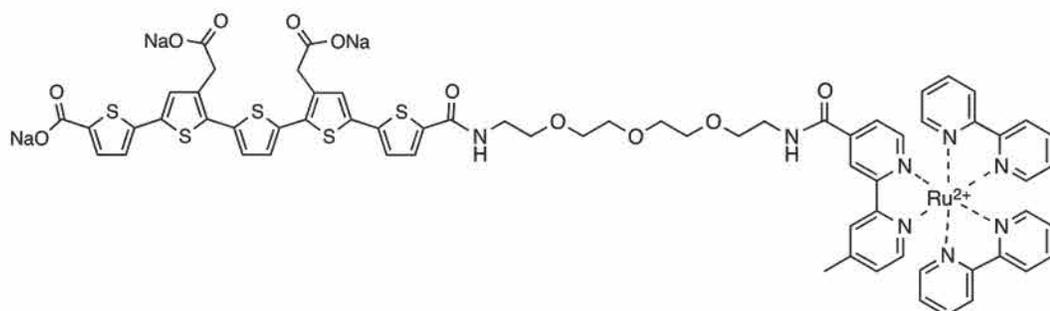
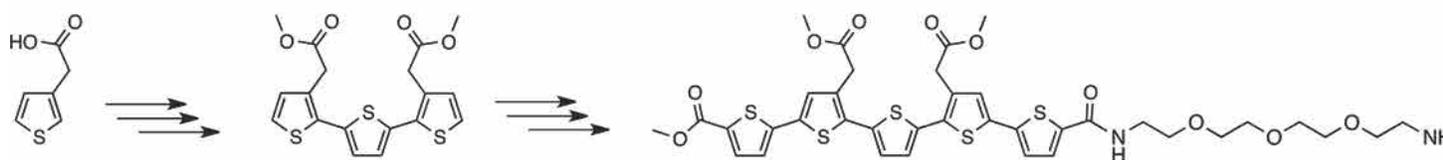
MEDI 205

Synthesis of oligothiophene tris(2,2'-bipyridyl)-type ruthenium (II) conjugate as electrochemiluminescence-luminophore

Linda Lantz, linda_lantz@hotmail.se, Peter Nilsson. Department of Physics, Chemistry and Biology, Linköping University, Linköping, Sweden

In protein aggregation diseases such as Alzheimer's disease, which affect numerous people and are not yet curable, the strive continues to gain better knowledge in the molecular events for the diseases. In research, development of molecular tools and usage of such can aid further insight to these events.

Our functionalized thiophene oligomers have been utilized in studying protein aggregates and distinct cells. The linking of a redox-active ruthenium polypyridyl complex to the oligothiophenes is a prospect for utilization within the method electrochemiluminescence, ECL, with the conjugate operating as a complement for conventional antibodies. ECL is a very sensitive method with analytical and clinical applications, wherein electrical energy is converted into radiative energy. We are synthesizing oligothiophene ruthenium (II) trisbipyridyl conjugates through stepwise synthesis and we will evaluate their photophysical properties and capabilities as ECL-luminophores.



MEDI 206

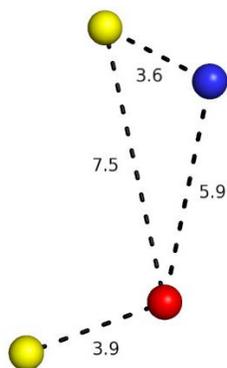
Molecular dynamic simulation and pharmacophore model of parasitic cysteine proteases

Drielli Gomes Vital Fuji², *driellivital@usp.br*, **Glaucio M. Monteiro Ferreira¹**, *gmf@usp.br*, **Juliana da Fonseca Rezende e Mello²**, *foncor@gmail.com*, **Gustavo H. Trossini²**, *trossini@usp.br*. (1) Toxicologia e Análises toxicológicas, Faculdade de Ciências Farmacêuticas - USP, São Paulo, São Paulo, Brazil (2) Pharmay, Faculty of Pharmaceutical Science, São Paulo, Brazil

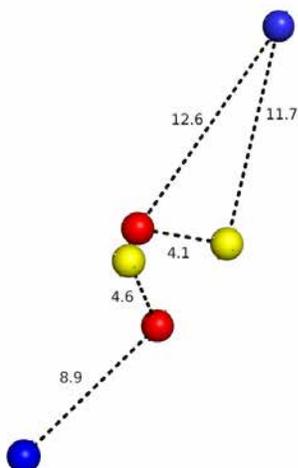
Group of tropical and subtropical diseases caused by parasites and infectious agents is called neglected tropical diseases (NTDs). NTDs are prevalent in 149 countries; more than one billion people are affected with 534,000 deaths every year. Among NTDs we can cite Chagas diseases and Leishmaniasis, they have a high global rate of mortality and morbidity.

Despite this fact, these diseases haven't much attention from pharmaceutical companies. In this context, biochemical pathways important to the survival of the parasite have aroused interest in the study of new drugs. Cysteine proteases are prevalent in parasites and responsible for differentiation, immune evasion, for these reason should be used as target in drug design.

Thereby, in this work we constructed a CPB2.8 model by comparative modeling technique. This model was refined by 100ns of Molecular Dynamic Simulation (MD) and stabilization was about 80ns. The molecular interaction fields (MIFs) were calculated in GRID program to suggest physicochemical properties of cysteine proteases cruzain from *Trypanosoma cruzi* (Chagas Disease) and CPB2.8 of *Leishmania mexicana* (Leishmaniasis). After these studies were proposed a pharmacophore model based on active site of these enzymes and favorable MIFs using SBDD techniques that were validated by ROC curve in order to obtain a new inhibitor against these neglected diseases by virtual screening studies.



Cruzain Pharmacophore model constructed after MD and MIFs studies. Red: hydrogen donor site; blue: hydrogen acceptor site; yellow: hydrophobic site.



CPB2.8 Pharmacophore model constructed after MD and MIFs studies. Red: hydrogen donor site; blue: hydrogen acceptor site; yellow: hydrophobic site.

MEDI 207

Comparison of the efficacy of ester substituents in their specificity and effectiveness for reducing cell growth in certain cancer cell lines

Romie Nguyen², romienguyen@csu.fullerton.edu, Aneta Jelowick², Montgomery Young², Christopher Bunye², Kim Soriano², Emil Guglielmo², Erika Lavassan², Alan Nguyen⁴, Nilay Patel³, Peter De Lijser¹. (1) Chemistry Biochemistry, California State University Fullerton, Fullerton, California, United States (2) Chemistry and Biochemistry, California State University, Fullerton, Fullerton, California, United States (3) California State University, Fullerton, Fullerton, California, United States (4) Biology, California State University, Fullerton, Fullerton, California, United States

Niclosamide, an FDA approved drug for the treatment of parasites, which functions by uncoupling their mitochondria, has recently been repurposed as an effective anti-cancer drug therapy that is currently undergoing clinical trials. Previous research has shown that Niclosamide has great potential in reducing cell growth indiscriminately in cervical, epithelial, and osteosarcoma cancer cell lines. Previous work in our laboratory has indicated that certain ester groups show promise when used on small organic molecules used to reduce cell growth. Using Niclosamide as a starting point, a drug library consisting of these small organic molecules containing at least one type of ester substituent as well as a combination of bulky polar and non-polar groups were synthesized and tested in biological assays on HeLa, HUTU80, and MG63 cancer cell lines. The design of these compounds has taken into account their ability to effectively diffuse through the cell membrane and reach the target of interest as well as their specificity to one cancer cell line rather than all of the cell lines. The biological assays specifically focused on the drugs' ability to reduce cell growth in one or two, but not all of the cell lines. As an initial screening test, the CyQUANT® Cell Proliferation Assay (a

fluorescence-based method for quantifying cells and assessing cell proliferation and cytotoxicity) was conducted using HeLa cells that have been inoculated with 10 μ M of a drug solution and allowed to incubate for a 24 hour period before collecting the data. This assay is utilized as a quick way to quantify drug efficacy in how well cancer growth is reduced and has helped to identify several promising leads. However, further assays and follow-up studies are needed in order to determine the importance of the ester group(s), the substituent(s), as well as their relative positions. This data, in turn, will help improve the design and performance of the new drug library.

MEDI 208

Synthesis of KS15 derivatives as CRY-mediated circadian clock modulators

Youjeong Choi¹, *cyj9475@naver.com*, **Youngeon Son**¹, **Sooyoung Chung**², **Gi Hoon Son**³, **Kyungjin Kim**⁴, **Young-Ger Suh**⁵, **Jong-Wha Jung**¹. (1) Department of Pharmacy, Research Institute of Pharmaceutical Sciences, Kyungpook National University, Daegu, Korea (the Republic of) (2) Department of Brain and Cognitive Sciences, Scranton College, Ewha Womans University, Seoul, Korea (the Republic of) (3) Department of Biomedical Sciences, College of Medicine, Korea University, Seoul, Korea (the Republic of) (4) Department of Brain and Cognitive Sciences, Daegu-Gyeongbuk Institute of Science & Technology, Daegu, Korea (the Republic of) (5) College of Pharmacy, Seoul National University, Seoul, Korea (the Republic of)

Physiological and metabolic processes such as hormonal changes, metabolism, and sleep/wake cycles are attributed to the circadian clock. The autonomous circadian rhythms are orchestrated by transcriptional and translational feedback loops, which is constituted by the clock genes and proteins. Small molecules have been playing prominent roles in understanding the molecular clock machinery. KS15, a novel cryptochrome (CRY) inhibitor, is an ethoxypropanoic acid directly binding to CRY1/2 thereby modulating the molecular circadian clockwork. In spite of the physiological and clinical importance of circadian rhythms, only a part of the structural and functional properties of KS15 has been studied. Considering small-molecules modulating CRY provide unique chemical tools to understand the molecular circadian clock machinery along with numerous applications, it is of importance to have their derivatives evaluated. Hence, we undertook a systematic derivatization of KS15 and evaluation of the derivatives to further understand the depth of chemical perturbation on circadian clock machinery. Herewith, we would like to report our studies on the design, synthesis, and evaluation of KS15 derivatives. Examples will be discussed including varied alternative patterns of 2-ethoxypropanoic acid moiety, varied functional groups at the hydrophobic benzene ring, and conformationally restricted oxazolines and oxazoles in comparison of the oxime linker of KS15 on their molecular clock modulating activities. We believe the derivatization and functional studies based on the KS15 derivatives will pave the way for the development of novel therapeutics with circadian nature.

MEDI 209

Synthesis and evaluation of xanthurenic acid analogs for impeding transmission of *Plasmodium falciparum* from host to vector

Ankush Kanwar¹, *ankush.jar@gmail.com*, James Leahy^{2,3}, Dennis Kyle^{4,5}, Tommy Mcgaha⁵. (1) Chemistry, University Of South Florida, Tampa, Florida, United States (2) Chemistry, University of South Florida, Tampa, Florida, United States (3) Florida Center of Excellence for Drug Discovery and Innovation, Tampa, Florida, United States (4) Department of Global Health, University of South Florida, Tampa, Florida, United States (5) College of Public Health, University of South Florida, Tampa, Florida, United States

The need for new antimalarial drugs is greater than ever before owing largely to the emergence of multidrug resistance in common pathogens as well as the rapid emergence of new infections. Malaria is one of the most common infectious diseases which affect large populations, especially in less developed countries. *Plasmodium* is the parasitic protozoa that cause malaria. The life cycle of *Plasmodium* involves two hosts (mosquito and mammals). During the life cycle, gametocytes (mature sexual stage) are present in the blood of infected vertebrate hosts while gametogenesis (activation of mature gametocytes) and formation of diploid zygotes only occur in the midgut of vector mosquitoes. It has been reported that the process of gametogenesis is induced by various factors such as pH and temperature change. Xanthurenic acid, which is present in the head and midgut of the mosquito, also plays an important role in the differentiation of the parasite. Xanthurenic acid and a series of analogs have been synthesized and evaluated with the aim of impeding the transmission of malaria.

MEDI 210

Design of phosphonate-based Biocompatible Metal-Organic Frameworks (pBioMOFS) as potential drug delivery systems

Gabriel Quinones Velez, *gabrielq4229@gmail.com*, Vilmali López-Mejías. Chemistry, University of Puerto Rico-Rio Piedras, San Juan, Puerto Rico, United States

The design of metal-organic frameworks (MOFs) is gaining popularity due to the many practical applications that arises from their large porosity. Recently, several studies have suggested that MOFs can be employed to encapsulate active pharmaceutical ingredients (APIs) for therapeutic purposes. This research attempts to design biocompatible MOFs for encapsulating APIs, and to employ them for drug delivery. It is proposed to use biocompatible phosphonic acids as organic linkers and biologically relevant metals to form porous structures denominated as phosphonate-based biocompatible metal-organic frameworks (pBioMOFs). The resulting porous material could be potentially employed as a drug delivery system to treat bone related diseases because of the selectivity of the linker to osseous tissue. Synthesis and structural characterization of metal complexes employing phosphonic acids have been challenging due to their propensity to spontaneously form amorphous powders instead

of crystalline networks. After controlling several crystallization parameters, nine pBioMOFs were synthesized using a green hydrothermal method under mild conditions. Characterization of the resulting crystals was performed using powder (PXRD) and single crystal X-ray diffraction (SCXRD), and scanning electron microscope with energy dispersive spectroscopy (SEM-EDS). SEM-EDS was able to detect the presence of all elements involved in the synthesis and confirmed the presence of coordination complexes. Different PXRD patterns indicated that new crystalline arrangements had formed. Structure elucidation of the coordination complexes presented solutions with R-factors between 2.63% and 7.51%. From nine pBioMOFs, five coordination complexes seem feasible for drug delivery applications. Cell viability assays will be performed to determine the cytotoxic effects of these pBioMOFs.

MEDI 211

Magnetite nanoparticles effect on *Escherichia coli* growth

Maria A. Gratacos, *maria.gratacos@upr.edu*, Eduardo Acevedo. Biology, University of Puerto Rico Mayaguez, Mayaguez, Puerto Rico, United States

The excessive use of antibiotics around the world is alarming. Both international and national health organizations are concerned about the lack of new antibiotics or derivatives to treat or prevent future infections. In this work it is proposed the use of rusty nanoparticles called Magnetites (Fe_3O_4) with a modified surface to reduce or control the growth of bacteria. *Escherichia coli*, a Gram (-), is a commonly found bacteria in our environment as well as in the human gastrointestinal system. With a variety of strains and an exponential growth rate; it is considered one of the most diverse bacteria. The preparation method for cultivating *E. coli* was within the culture media Tryptic Soy Broth. This allowed the use of the technique of UV-Visible Spectroscopy to measure growth rates. A medium with a concentration of 2.4g/L of magnetite nanoparticles was used to test the antibacterial effects on *E. coli*. The optical density was tested every 2 hours with a UV Spectrophotometer (600 nm). Results were compared to the optical density of the medium TSB, a medium of TSB containing magnetite nanoparticles and medium with TSB and *E. coli*. Preliminary results show a reduction in the growth of the *E. coli* exposed to the magnetic nanoparticles. With a diameter between 63 nm to 86nm, the magnetite nanoparticles may be facilitating possible interactions between the surface of the modified-magnetite and the bacteria. The effect of this nanoparticles on the growth of *E. coli* showed a possibility of a new form of bacterial control. This may be used to create an antiseptic environment in operation rooms, or even a possible modified antibiotic to combat bacteria, reducing the negative side effects by improving its efficiency.

MEDI 212

Development of naphthalimide DNA intercalators as anticancer therapeutics

Kristin Skubic, *kskubic2@slu.edu*, Miranda Adams, Jill Horn, Anna Priddy, Renae Oelrich, Michael A. Lewis, Brent Znosko, **Christopher K. Arnatt**, *arnattck@vcu.edu*. Saint Louis University, St. Louis, Missouri, United States

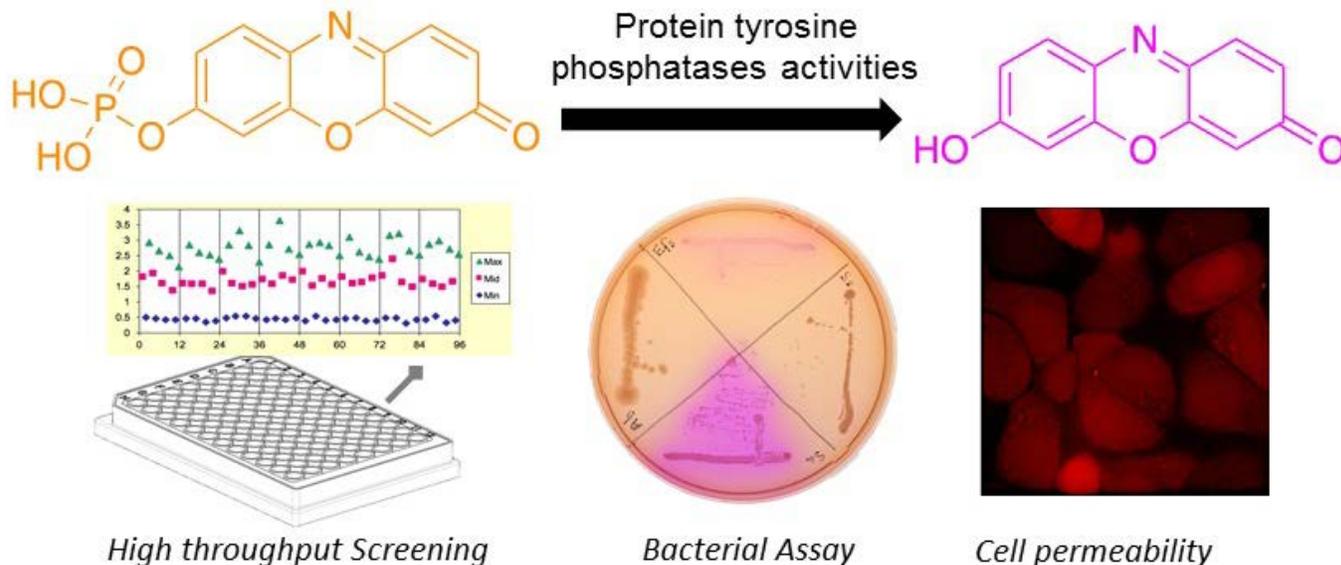
In the past decade, there has been substantial progress in research of small molecule DNA intercalators as antineoplastic agents. Due to specificity of the DNA-intercalator interaction, compounds may be tailored to target specific genes to avoid potential side effects. For this research, derivatives of 3-amino-1, 8-naphthalic anhydride were synthesized (initial research by Braña *et al*) with combination of different amino arms and substituents along the 3- or 4- position generating a series of naphthalimide derivatives to determine if compound structure influenced DNA intercalation (via DNA melt studies), antiproliferative activities (standard breast cancer cell lines SKBR3, MDAMB231, and MCF-7), and topoisomerase II inhibition activity. Calculated LD₅₀ values for the antiproliferation assays (ranging from low nM to low μM), DNA melt studies, and topoisomerase II inhibition activities illustrate a clear structure-activity relationship for this series of naphthalimides.

MEDI 213

Developing dual colorimetric and fluorogenic probes for visualizing tyrosine phosphatase activity and high throughput screening

Suwendu Biswas¹, *sbiswas13@hotmail.com*, Brandon S. McCullough¹, Elena S. Ma¹, Dollie LaJoie², Colin W. Russell³, Garrett Brown³, June L. Round³, Katharine S. Ullman², Matthew A. Mulvey³, Amy M. Barrios¹. (1) College of Pharmacy, University of Utah, Salt Lake City, Utah, United States (2) Department of Oncological Sciences, University of Utah, Salt Lake City, Utah, United States (3) Department of Pathology, University of Utah, Salt Lake City, Utah, United States

Protein tyrosine phosphatases (PTPs) are key signaling enzymes involved in both pathological and physiological processes and have been implicated in numerous human diseases including bacterial infections, autoimmune disorders, diabetes, and cancer. In order to advance our understanding of these important enzymes with the aim of realizing their potential as therapeutic targets, the chemical toolkit available for studying the PTPs must be expanded as many existing probes are incompatible with cellular applications. In this work, a resorufin-based PTP substrate, pRes, has been synthesized. pRes has red-shifted emission and excitation profiles as compared to existing substrates and has been used as a fluorogenic substrate to monitor human PTP activity both *in vitro* and in living cells and validated for use in high throughput screening. In addition, pRes was used for colorimetric visual detection of the pathogenic bacteria *Staphylococcus aureus*. Finally, a fluorinated version of pRes was synthesized and utilized for monitoring the activity of tyrosine phosphatases active at acidic pH.



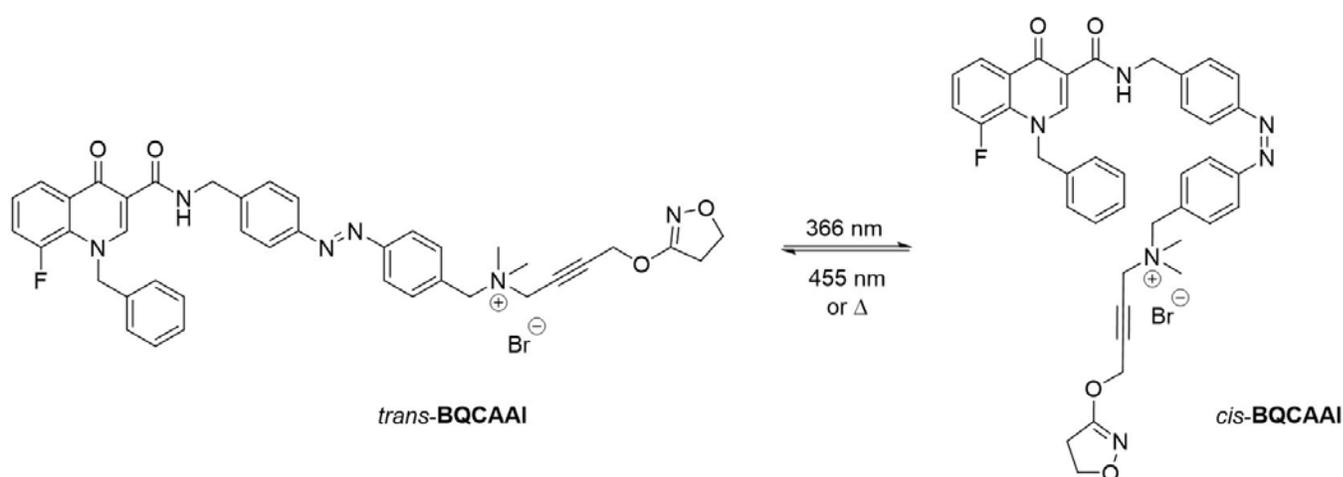
Dual Colorimetric and Fluorogenic Probe for PTPs

MEDI 214

Photoswitchable dualsteric M1 ligand

Luca Agnetta¹, luca.agnetta@uni-wuerzburg.de, **Michael Kauk**², **Maria Consuelo Alonso Caniza**², **Regina Messerer**¹, **Ulrike Holzgrabe**¹, **Carsten Hoffmann**², **Michael Decker**¹. (1) *Pharmaceutical and Medicinal Chemistry, Julius-Maximilians-Universität Würzburg, Würzburg, 97074, Germany* (2) *Pharmacology and Toxicology, Julius-Maximilians-Universität Würzburg, Würzburg, 97074, Germany*

*h*M1 muscarinic acetylcholine receptors are key actuators in learning and memory processes and may hold therapeutic potential in the treatment of cognitive impairments such as schizophrenia and Alzheimer's disease. Dualsteric ligands of G protein-coupled receptors are known to be highly selective as they interact with both orthosteric and allosteric binding sites of the receptor. The modification of the spacer length between the orthosteric and allosteric moieties of the iperoxo (orthosteric agonist)/BQCA-d-type (positive allosteric modulator) hybrids controls the efficacy and leads to different degrees of partial agonism. A molecular photoswitchable azobenzene moiety has been incorporated as a linker between the agonist and the positive allosteric modulator, giving rise to a photoresponsive BQCA-azobenzene-iperoxo (BQCAAI) hybrid compound and the photochromic behaviour has been studied in detail. FRET studies were conducted and the extent of G_q activation measured to test and quantify the mode and extent of receptor activation of this first photoswitchable dualsteric M1 ligand. The ligand allows the change between an inactive purely allosteric and the active orthosteric/allosteric binding pose in a light-controlled fashion and offers the possibility to study the process of receptor activation at the molecular level with high temporal resolution.



MEDI 215

Synthesis of chemical probes for serine/threonine protein phosphatase 5C based on a natural product template

Madison Tuttle¹, *mrt1222@jagmail.southalabama.edu*, **Larry Yet**¹, **Richard Honkanen**², **Mark Swingle**². (1) Chemistry, University of South Alabama, Mobile, Alabama, United States (2) Biochemistry and Molecular Biology, University of South Alabama, Mobile, Alabama, United States

Recent studies have shown that the overexpression of serine/threonine protein phosphatase 5 (PP5) is associated with invasive ductal carcinoma of the breast, cancer cell proliferation, and resistance to apoptosis. However, scientists currently lack the molecular equipment with which to further characterize the biological role of PP5 in tumorigenesis. For the purpose of investigating this role, small molecule inhibitors of the catalytic subunit of PP5 (PP5C) may be useful as chemical probes. LB100 is a small molecule inhibitor that is structurally similar to cantharidin, a natural product of the Mexican bean beetle. LB100 is a potent inhibitor of PP2A that is currently in phase I clinical trials, but our group shows that LB100 is also a potent inhibitor of PP5C at nanomolar concentrations. Based on the structure of LB100 and its similarity to cantharidin, our group has devised a synthetic scheme to create a library of useful chemical probes. Several analogs were synthesized using this scheme, and their activity against PP5C was evaluated by means of a homogenous fluorescence intensity-based assay.

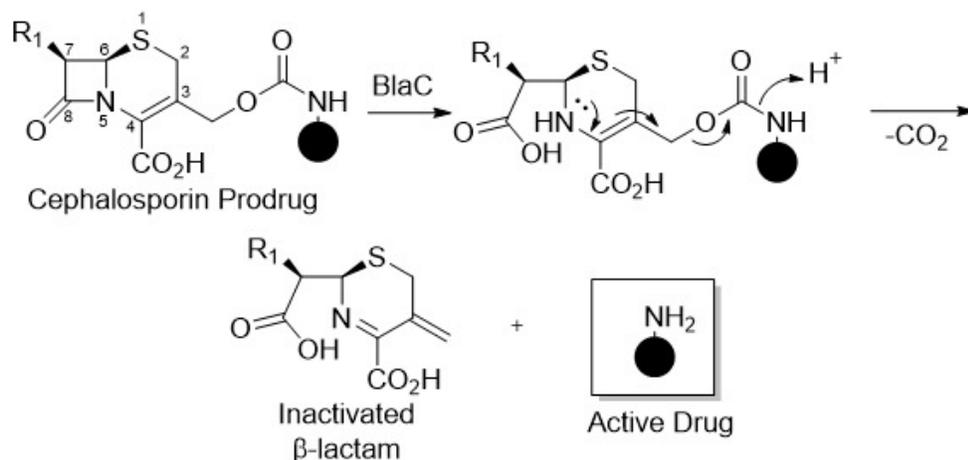
MEDI 216

Development and synthesis of β -lactam prodrugs for tuberculosis

Malcolm Cole, *colex275@umn.edu*, **Joseph Buonomo**, **Courtney C. Aldrich**. Medicinal Chemistry, University of Minnesota, Minneapolis, Minnesota, United States

Tuberculosis has recently surpassed HIV as the leading cause of infectious disease mortality globally. Drug-resistant TB causes an estimated 10% of TB infections, necessitating the use of second-line agents that often have significant adverse effects. We will describe a general prodrug strategy using a cephalosporin pro-moiety for selective release of active drugs that exploits inherent β -lactamase expression in *Mycobacterium tuberculosis* (*Mtb*). Hydrolysis of the β -lactam leads to ejection of the conjugated drug via collapse of an allylic carbamate promoimety (Fig. 1). Our strategy features three key design criteria: the β -lactam promoimety should be **devoid of intrinsic antibacterial activity** to prevent disruption of commensal bacteria; the conjugate should be **physiologically stable and orally bioavailable**; and the active drug should be **selectively released by *Mtb*** to prevent the associated off-target effects that limit its current therapeutic utility. Latent antibacterial activity of the β -lactam scaffold is readily eliminated through modification at C7 of the cephalosporin nucleus. Improved chemical stability is imparted through substitution at C2, and oral bioavailability can be improved through modifications to R1. To validate this strategy, we will describe the synthesis and initial biochemical and biological evaluation of several prodrug conjugates containing known second and third-line TB drugs.

Fig. 1. Prodrug Design and Release Mechanism



MEDI 217

Synthesis and biological evaluation of a novel series of GPER antagonists for the treatment of gallstone formation

Chelsea DeLeon¹, chelseadeleon@sbcglobal.net, **Christopher K. Arnatt**², **David Q.-H. Wang**², **McKenna Wilhelm**¹, **Patrick Sweeney**¹. (1) Chemistry, Saint Louis University, Saint Louis, Missouri, United States (2) Biochemistry, Saint Louis University, Saint Louis, Missouri, United States

Classically, it was believed that 17 β -estradiol (E2) produced both genomic and non-genomic signaling solely through interactions with estrogen receptors α and β (ER α and ER β). More recent discoveries have demonstrated that estrogen response can occur via

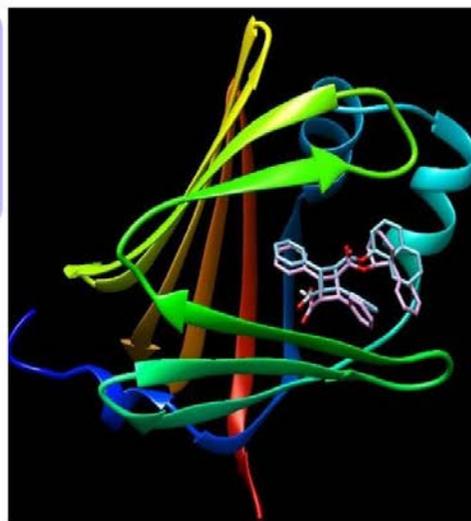
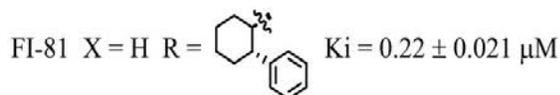
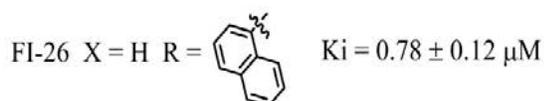
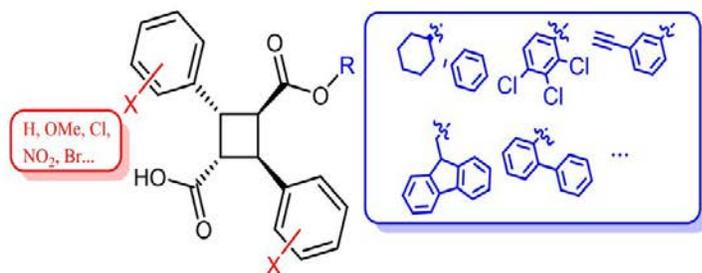
signaling pathways activated by the G protein-coupled estrogen receptor (GPER). Furthermore, binding of known agonists, such as E2 and G-1 (GPER selective agonist), have had implications in the formation of gallstones. In this study a pharmacophore was developed from homology modeling and docking of known agonists and antagonists. From the proposed pharmacophore, a novel series of antagonists were designed and examined with several functional assays. Current efforts involve testing select compounds in an *in vivo* mouse model of gallstone formation.

MEDI 218

Design, synthesis and SAR study of truxillic acid-based fatty acid binding protein inhibitors as anti-nociceptive and anti-inflammatory drugs

Su Yan¹, *yansu19900912@gmail.com*, Kongzhen Hu⁶, Simon Tong¹, Monaf Awwa¹, Qianwen Gan¹, Matthew Elmes⁵, Joseph Sweeney⁵, Hao-Chi Hsu⁵, Martin Kaczocha^{6,4}, Huilin Li^{6,5}, Robert C. Rizzo², Dale Deutsch^{6,5}, Iwao Ojima³. (1) Chemistry Department, Stony Brook University, Stony Brook, New York, United States (2) Stony Brook University, Stony Brook, New York, United States (3) Chem Dept/ICBDD, Stony Brook University, Stony Brook, New York, United States (4) Department of Anesthesiology, Stony Brook University, Stony Brook, New York, United States (5) Department of Biochemistry and Cell Biology, Stony Brook University, Stony Brook, New York, United States (6) Institute of Chemical Biology and Drug Discovery, Stony Brook University, Stony Brook, New York, United States

Anandamide, an endocannabinoid, is linked to the regulation of stress, pain and inflammation. It activates cannabinoid receptors (CB receptors) on the cell surface, leading to the relief of pain and stress. Anandamide can also enter cells by diffusion, carried by fatty acid binding proteins (FABPs) through cytoplasm and degraded by fatty acid amide hydrolase (FAAH). Inhibition of these tissue specific FABPs would arrest the transportation and hydrolysis of the endocannabinoid, resulting in elevated extracellular anandamide levels and anti-inflammatory and anti-nociceptive effects. Targeting on FABP 5 (epidermal) and FABP 7(brain), pioneering work which includes high-throughput virtual screening, fluorescence displacement assay, and high resolution protein X-ray structure determinations, has been done from our laboratories. It demonstrated that α -truxillic acid 1-naphthyl mono-ester (SB-FI-26) exhibits strong binding to FABP5 and potent anti-nociceptive effects in mice. Based on the binding energy score from Autodock, a series of α -/ γ - truxillic esters were synthesized and their binding affinity towards FABP 5 and FABP 7 were evaluated *in vitro*. Based on preliminary *in vitro* data, we explored SAR and developed new inhibitors with high potency and specificity.

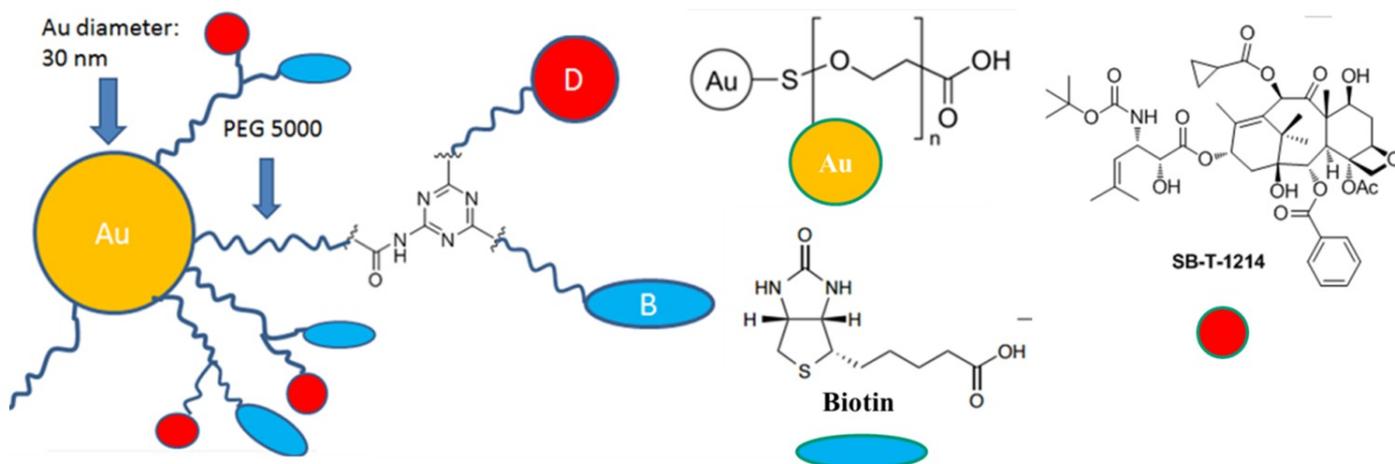


MEDI 219

SB-T-1214 and biotin functionalized gold nanoparticles

Xin Wang¹, xin_wang15@hotmail.com, **Iwao Ojima**². (1) Chemistry, Stony Brook University, Stony Brook, New York, United States (2) Chem Dept/ICBDD, Stony Brook University, Stony Brook, New York, United States

Although SB-T-1214 has shown high potency and anti-MDR activity towards cancer cells, its systemic toxicity limits the application as an anti-cancer drug. To overcome the drawbacks from lack of specificity and relatively low solubility in water, research on hydrophobic drug delivery system has gained a lot of interests. The gold nanoparticles (GNPs) offer a unique set of chemical and physical properties which make it a potential drug delivery system: 1) The gold core can be easily functionalized with Au-S linkage; 2) Monodisperse gold nanoparticles can be formed with core sizes ranging from 1 nm to 150 nm; 3) The gold core is essentially inert. The nano-sized gold nanoparticles preferentially accumulate within tumors due to enhanced permeability and retention (EPR) effect. By introducing the tumor targeting molecules onto the AuPs, we anticipate to utilize receptor-mediated endocytosis effect (active targeting) together with AuPs' EPR effect (passive targeting) to increase selectivity toward cancer cells. Biotin (vitamin H) is a water soluble growth promoter of cells. Biotin-specific receptors are often overexpressed on the cancer cell surface. Accordingly, we have designed the biotin-AuPs drug delivery system. The design, synthesis and characterization of this SB-T-1214 and biotin functionalized Gold nanoparticles drug delivery system will be presented.



MEDI 220

Discovery of a novel series of potent and selective Phosphatidylinositol-3-Kinase delta (PI3K δ) inhibitors for the treatment of inflammatory and autoimmune diseases

Nuria Aguilar¹, nuria.aguilar@almirall.com, Joan Carles Fernandez², Begoña Hernandez¹, Marta Carrascal¹, Patricia Niño², Alba Lopez², Laura Vazquez², Estrella Lozoya¹, Monica Maldonado¹. (1) Medicinal Chemistry and Screening, Almirall, Sant Feliu de Llobregat, Catalonia, Spain (2) Unitat Mixta Almirall-PCB, Barcelona, Catalonia, Spain

Phosphoinositide 3-kinases are a family of lipid kinases involved in the development of a wide range of diseases. Four isoforms (alpha to delta) have been identified in the case of class I PI3Ks. Among them, the delta isoform, whose expression is confined to leukocytes, has shown to play an essential role in specific cell immune functions and may provide a potential therapeutic target for autoimmune and inflammatory diseases. In contrast, the utility of pan-PI3K inhibitors has been limited due to side effects such as GI intolerance, hyperglycemia and maculopapular rash. Therefore, there is a strong need for delta selective PI3K inhibitors.

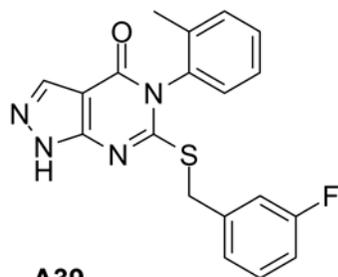
Some years ago, propeller-shaped inhibitors were discovered and the structural rationale for their selectivity was reported. However, there are very few reports on PI3Kdelta selective inhibitors from alternative structural approaches. Herein, we present a novel series of potent and strongly selective PI3Kdelta inhibitors originated following structural-based drug design, that do not belong to the propeller-shape class.

MEDI 221

Development of cancer stem cell depleting ALDH1A selective inhibitors as potential therapeutics for ovarian cancer

Brandt Huddle¹, *bchuddle@umich.edu*, Cameron Buchman², Kun Yang³, Mikhail Chtcherbinine², Ilana Chefetz-Menaker³, Cindy Morgan², Ronald Buckanovich³, Thomas Hurley², Scott Larsen¹. (1) Department of Medicinal Chemistry, University of Michigan, Ann Arbor, Michigan, United States (2) Indiana University School of Medicine, Indianapolis, Indiana, United States (3) University of Michigan, Ann Arbor, Michigan, United States

The presence of a subpopulation of cancer stem-like cells (CSCs) expressing the surface glycoprotein CD133 and the enzyme Aldehyde Dehydrogenase (ALDH) within ovarian tumors has been associated with poorer cancer patient outcomes. ALDH⁺ cells show resistance to cisplatin, and engraftment of fewer than 500 CD133⁺/ALDH⁺ patient tumor cells is sufficient to establish tumors in mice, implicating these CSCs in recurrence metastasis following treatment in ovarian cancer. Administration of ALDH inhibitors can selectively deplete CD133⁺ cells and may represent a mechanistically novel treatment for ovarian cancer. While most evidence suggests ALDH1A1 is most critical for CSC function, the ideal selectivity profile among the 19 ALDH isoforms remains unclear. We have generated a library of analogs based on the ALDH1A1 inhibitor **A39**. Small structural changes have led to substantial improvements in 1A1 potency, widely varying selectivity profiles among the ALDH1A family, and several compounds capable of depleting CSCs in our cellular assay.



A39

ALDH1A1 IC₅₀ = 0.4 μM

ALDH2 IC₅₀ = >20 μM

ALDH3A1 IC₅₀ = >20 μM

MEDI 222

Photoaffinity probes for protein N-terminal methyltransferases

Brianna Mackie, *baxleyb@vcu.edu*, Rong Huang. Medicinal Chemistry, Virginia Commonwealth University, Richmond, Virginia, United States

Protein N-terminal methyltransferases (NTMTs) have recently been implicated in regulating protein-chromatin interactions, mitotic division and DNA damage repair. Recently, we have successfully obtained co-crystal structures of NTMT1 in a ternary complex with both peptide substrates and S-adenosylhomocysteine (SAH). However, knowledge of the NTMT family's functional and structural significance is not entirely understood. Photoaffinity labeling has become a powerful strategy for the use of further

studying enzyme-ligand interactions and elucidating information about the target in a biological context. Herein, we have designed and prepared the first generation of photoaffinity probes that are capable of selectively labeling the NTMTs. Our probes contain three main components: a recognition element, a photocrosslinker, and a propargyl handle. The photoaffinity probe's recognition elements are derived from the N-terminus of NTMT1 substrates: Retinoblastoma1 (RB1), a tumor suppressor protein with an initial sequence of P-P-K-T and oncoprotein TAF1 α , with an initial sequence of A-P-K-R. The recognition portion of probes is vital to determine the probe selectivity. We incorporated benzophenone or diazirine as a photocrosslinker which forms a covalent bond with its interacting partner upon UV irradiation. The propargyl handle can be derivatized with a TAMRA azide through click chemistry to install a fluorescent tag for detection through fluorescence imaging. Our results suggest that our photoaffinity probes exhibit in a dose and time dependent manner to label NTMT1/2. Additionally, the photoaffinity labeling can be competitively inhibited in the presence of RB1-10, but not by SAH, which validates that the labeling is specifically driven by recognition. The designed probes also selectively label NTMT1 and NTMT2 in HeLa cell lysates, verifying specificity of the probe for NTMT1/2. Lastly, the probe was enzymatically methylated by NTMT1 in a MS-based methylation assay, indicating that our probes can be efficiently recognized by NTMT1 and inferring the recognition-driven labeling. In summary, we have successfully developed the first series of photoaffinity probes to specifically recognize protein N-terminal methylation writers. Our developed probes have potential to be valuable tools to explore NTMTs in physiological conditions. Furthermore, our studies will lay a paradigm for the next critical step in identifying future protein writers, readers and erasers.

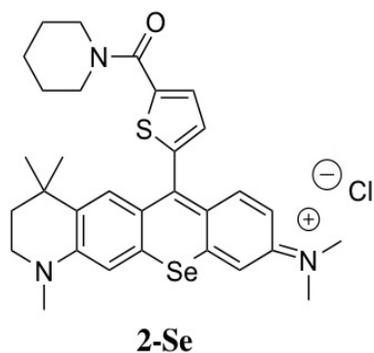
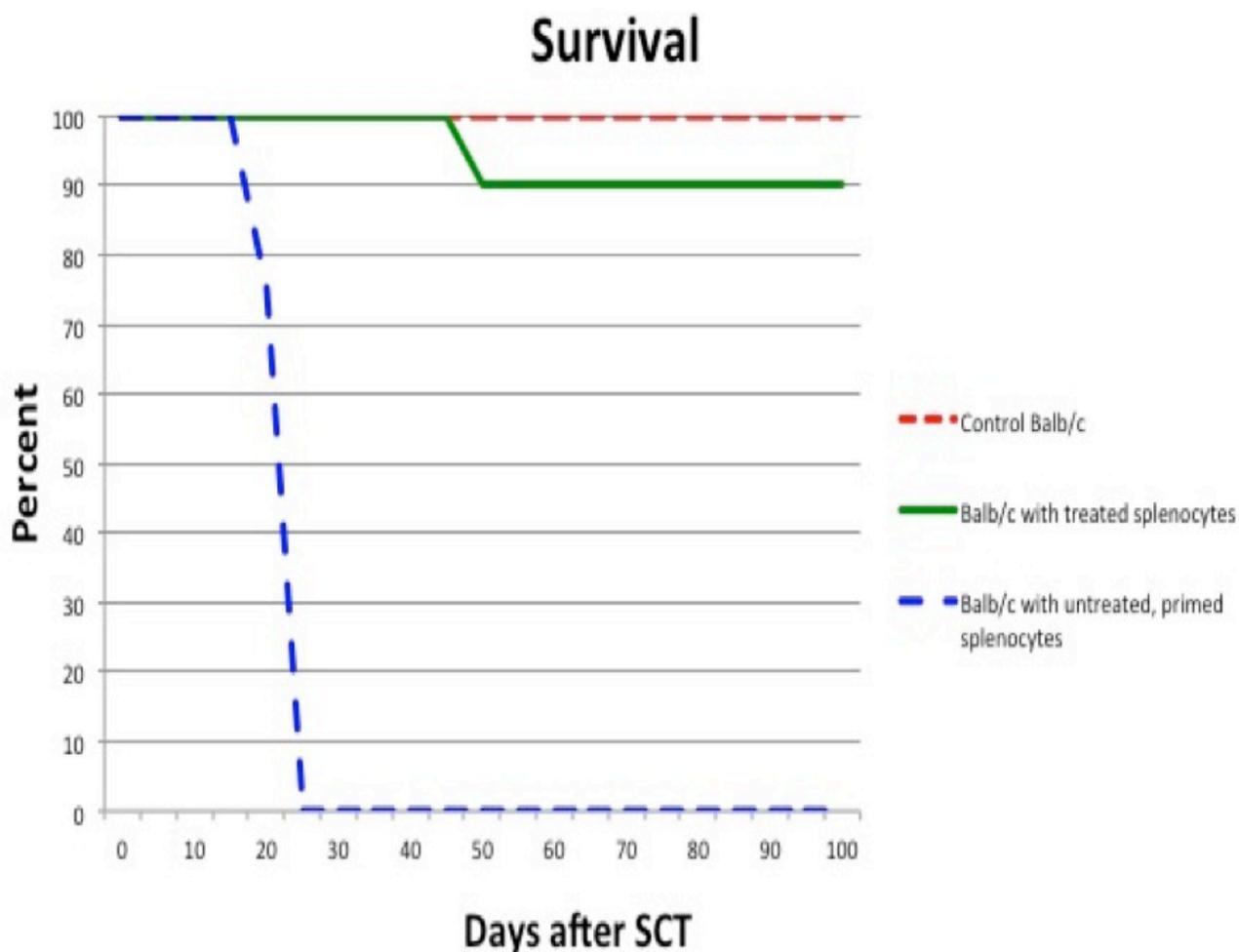
MEDI 223

Novel selenorhodamine dyes as photosensitizers in varying applications

Jacqueline Hill¹, jehill@buffalo.edu, **Mark W. Kryman**¹, **Gregory A. Schamerhorn**¹, **Michael R. Detty**¹, **Zachariah A. McIver**². (1) Chemistry, SUNY University at Buffalo, Buffalo, New York, United States (2) Hematology and Oncology, Wake Forest University School of Medicine, Winston-Salem, North Carolina, United States

The Detty lab has focused on synthesizing analogues of Rhodamine-123 with varying heavy chalcogens (S, Se, and Te), core modifications (half-julolidyl, julolidyl, and bis), as well as amide and thioamide substituents at the 9-position of the core. These compounds have been tested as photosensitizers in the photodynamic therapy (PDT) of cancer and, more recently, in extracorporeal photopheresis (ECP) prior to hematopoietic stem cell transplantations (HSCT) as a preventative measure against acute graft-versus-host disease (GVHD). The preferential uptake of rhodamines in the mitochondria is attributed to their high polarizability and alloreactive T cells, responsible for GVHD occurrence, have been shown to have an increased mitochondrial metabolism coupled with impaired P-glycoprotein function. These characteristics allow for a rapidly transported rhodamine dye to selectively accumulate in the alloreactive T cells while being extruded from, and thus preserving, the resting and memory T cells pertinent to

healthy immune function. A complete MHC antigen-mismatched murine HSCT model study using recipient Balb/c mice showed >90% survival >60 days post-transplantation with no evidence of GVHD following selective photodepletion of donor C57BL/6 splenocytes with selenorhodamine dye **2-Se** whereas control mice died of GVHD 25 days following treatment. Currently, a synthesis focusing on novel selenorhodamine dyes with a bis *gem*-dimethyl julolidine core is underway, described herein, in hopes of improving upon the already highly selective half-julolidyl selenorhodamine **2-Se**.

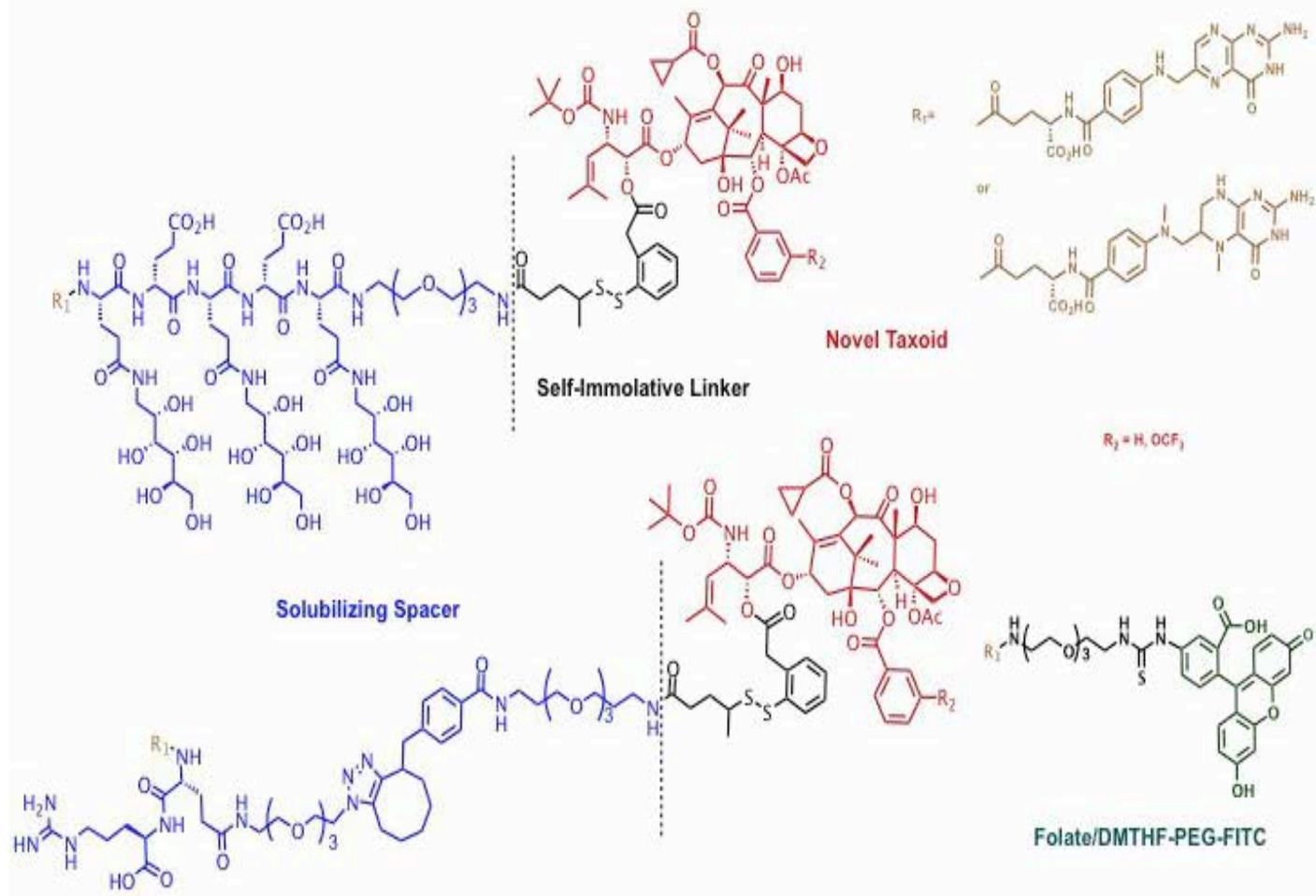


MEDI 224

Design, synthesis and preclinical study of novel taxoid-based Small Molecule Drug Conjugates (SMDCs) using folate/Dimethyltetrahydrofolate (DMTHF) as tumor targeting module

Changwei Wang^{1,2}, changwei.wang@stonybrook.edu, Yican Wang², Micky Tortorella², Iwao Ojima^{1,2}. (1) Chem Dept/ICBDD, Stony Brook University, Stony Brook, New York, United States (2) Drug Discovery Pipeline, Guangzhou Institute of Biomedicine and Health, Guangzhou, Guangdong, China

Not only can Small Molecule Drug Conjugates (SMDCs) kill cancer cells as precision chemotherapy, but they can also take advantage of the CAR-T platform to interfere immunological response. With more than 10 SMDCs already in clinical trial for either therapy or diagnosis, their bright future is indisputable. Based on the design of vintafolide and EC1456, and the highly efficient self-immolative disulfide smart linker, a series of Folate-Linker-Taxoids (FLT)s were designed, synthesized and biologically tested *in vitro* as well as *in vivo*. To deliver attached therapeutic agents selectively to tumor cells, a reduced and alkylated form of folic acid, *N*⁵, *N*¹⁰-dimethyl tetrahydrofolate (DMTHF) that exhibits selectivity for FR- α , is also used as a targeting ligand. Since CAR-T cell binds to FITC extraordinarily well, bispecific SMDC adaptor molecules constructed with a FITC molecule and tumor homing module are designed and synthesized. All of the chemical syntheses and biological data of this study will be presented.



MEDI 225

Structure-based discovery of novel small molecule Wnt/ β -catenin signaling inhibitors

Stacy Guzman¹, staguzman138@csu.fullerton.edu, Alan Nguyen², Peter De Lijser¹, Nilay Pate². (1) Chemistry Biochemistry, California State University Fullerton, Fullerton, California, United States (2) Biological Science, College of Natural Sciences and Mathematics, California State University, Fullerton, Fullerton, California, United States

The canonical Wnt signaling pathway is an essential signal transduction pathway which leads to the regulation of cellular processes such as differentiation and migration. Deregulation of components involved in Wnt/ β -catenin signaling has been implicated in a wide spectrum of diseases including several cancers and degenerative diseases. Recent data suggests pre-cancerous cells overexpress the protein β -catenin when they become cancerous cells by Wnt stabilization and nuclear localization, ultimately activating the expression of β -catenin mediated genes downstream. However, in the absence of Wnt ligand stimulation, several proteins participate in a multiprotein "destruction complex" that targets the proto-oncogene β -catenin for ubiquitin-mediated

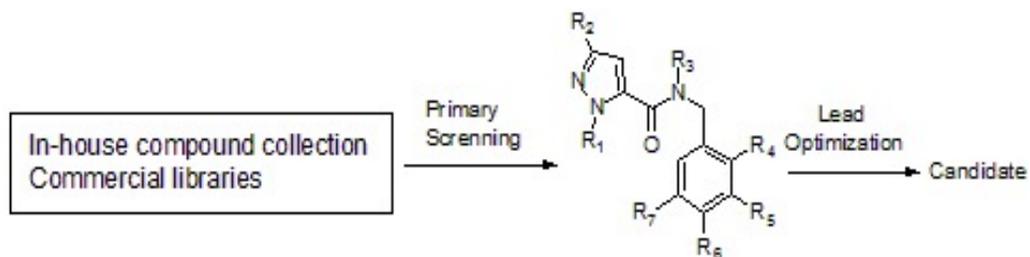
proteolysis, resulting in the inhibition of downstream gene expression. To inhibit β -catenin accumulation, we designed and synthesized a library of novel small molecules that can inhibit Wnt signaling in various cancers by blocking specific protein–protein interactions or the activity of specific enzymes. We hypothesize that small molecules that reduced β -catenin levels can potentially function as anti-cancer drugs. To test this hypothesis, various small molecules with modified structures were synthesized and purified. To assess and quantify cell proliferation, the fluorescence-based CyQUANT assay was used to measure DNA content. Total β -catenin levels that are associated with raised nuclear and cytosolic pools were determined by immunocytochemistry. Our recent findings show that several small molecules contain a specific moiety that exhibit higher inhibitory activities in cell-based assays than known inhibitors and show promising results to reduce β -catenin levels. Recent studies have also indicated that a specific kinase exerting tumor suppressor function has been hampered by the lack of selective inhibitors. A next generation of drug compounds will focus on molecules with similar functionalities but greater rigidity to target this protein to inhibit Wnt signaling and highlight a new strategy for targeted therapeutics directed against the Wnt pathway. Docking studies and cell-based assays will compare the results of the original drugs to those of the new series to determine the best method to increase phosphorylation of β -catenin, thereby generating more potent anti-cancer drugs.

MEDI 226

Identification and lead optimization of pyrazole carboxamides with antiviral activity

Kumar Paulvannan, *kpaulvannan@gmail.com*. Med Chem, Prosetta Biosciences, San Jose, California, United States

Discovery of novel potent and low toxicity antiviral therapeutics targeting host factors, remains an active area in the field of medicinal chemistry. To initiate an in-house antiviral program against host-catalyzed capsid assembly pathways, we started screening our in-house compound collection and commercial libraries/ compounds using our proprietary screening assay involving de novo influenza protein biogenesis and assembly. Initial primary screening resulted in the identification of Pyrazole carboxamides with activities in the high nanomolar ranges against infectious influenza virus in cell culture. Optimization of the initial hit lead to the identification several highly potent antiviral compounds with good Pharmacokinetic properties has been achieved. The synthetic approaches and Structure Activity Relationships (SAR) will be presented.



MEDI 227

Inhibition of arginase from *Leishmania mexicana* by benzimidazole derivatives

Irene Betancourt¹, betancourtirene@gmail.com, Alondra Chaidez-Avila¹, Alejandra G. Vazquez-raygoza¹, Claudia I. Avitia-Domínguez¹, Antonio Romo-Mancillas³, Alicia Hernandez Campos², Alfredo Téllez-Valencia¹. (1) Facultad de Medicina y Nutrición, Universidad Juárez del Estado de Durango, Durango, Durango, Mexico (2) Departamento de Farmacia, Universidad Nacional Autónoma de México, Mexico, Mexico (3) Universidad Autónoma de Querétaro, Querétaro, Mexico

Leishmaniasis is a parasitic disease that is transmitted to the host by the phlebotomine sandfly bite, this disease can involve the skin, mucous membranes and viscera. The drugs that are currently used for the treatment of leishmaniasis are inadequate due to the toxicity, high cost, severe adverse reactions and the emerging drug resistance which limits their use. Therefore it is important to looking for new drugs against leishmaniasis. Polyamines are molecules important for parasite survival and proliferation. Arginase, is the first enzyme in polyamine biosynthesis and catalyzes the hydrolysis of L-arginine to L-ornithine and urea. Here we used this enzyme as target to search inhibitors through a virtual screening strategy. Virtual screening, having the enzyme active site as the binding pocket, was made with Glide software (www.schrodinger.com), using the crystal structure of arginase from *Leishmania mexicana* (LmARG, PDB: 4IU0) and an *in house* library of approximately 450 benzimidazole derivatives. The one hundred molecules with the highest binding energy were selected for inhibition studies. Arginase activity was measured in a coupled assay system. Compounds were evaluated at 200 μM . The concentration that causes 50% enzyme inhibition (I_{50}) was determined by curves at different inhibitor concentrations. Furthermore, Drug-like descriptors of each compound were evaluated. From the one hundred molecules tested *in vitro*, compounds **B6** and **JOULZ-01** showed 100% inhibition of LmARG. Additional characterization showed that **B6** and **JOULZ-01** had an I_{50} of 77.5 μM and 125 μM , respectively. Structural analysis showed that the compound **B6** made hydrogen bonds with Asp141 and Asp143. Finally, compound **JOULZ-01** made a hydrogen bond with Thr257. Predicted drug like properties from these molecules was in the range to be considered as potential drugs. The molecules reported here could serve as starting point in the search of a new leishmanicidal therapy.

MEDI 228

Design and development of a novel class of structurally-enhanced allosteric modulators of hemoglobin for sickle cell disease treatment

Piyusha Pagare¹, pagarepp@vcu.edu, Osheiza Abdulmalik³, Martin K. Safo², Yan Zhang¹. (1) Medicinal Chemistry, Virginia Commonwealth University, Richmond, Virginia, United States (2) Institute of Structural Biology, Drug Discovery and

*Development, Virginia Commonwealth University, Richmond, Virginia, United States (3)
Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, United States*

Intracellular polymerization of deoxygenated sickle hemoglobin (Hb) remains the principal cause of the pathophysiology associated with sickle cell disease (SCD). Naturally occurring and synthetic allosteric effectors of hemoglobin (AEH) have been investigated as potential therapeutic agents for the treatment of SCD. Previous studies have shown that aromatic aldehydes form high-affinity Schiff-base adducts with Hb and inhibit red blood cell sickling by allosterically shifting the oxygen equilibrium curves towards the left. However, challenges related to short half-life and limited bioavailability have severely hampered this line of investigation. To counter these challenges, we designed and synthesized a series of novel compounds based on our previously reported pyridyl derivatives of vanillin. These modifications were expected to increase binding interactions with the protein and thus to stabilize the Schiff-base adduct, as well as lead to perturbation of the surface-located F-helix that would stereospecifically destabilize polymer contacts. We investigated the in vitro pharmacokinetic/pharmacodynamic properties of these newly synthesized compounds to ascertain sustained binding and modification of normal human Hb. Subsequently, we conducted in vitro screening assays to test for inhibition of sickling, modification of Hb to the high-affinity form, as well as for a direct left-shift in oxygen equilibrium curves (OEC).

Our results showed maximal levels of Hb modification (adducts) at 12 h, which were sustained for the entire 24 h experimental period. These findings suggested that our modifications appeared to successfully limit drug metabolism in red blood cells. These compounds showed a dose dependent inhibition of cell sickling, Hb modification and corresponding changes in Hb oxygen affinity. To establish the mode of interaction with Hb, we further conducted x-ray crystallography studies. Our studies showed that these compounds bind in symmetry-related fashion at the α -cleft of Hb to form Schiff-base adducts with the N-terminal Val1 amines and showed water-mediated interactions with the F-helix which overall enhanced interactions with Hb.

Thus, our results establish these compounds as a novel and promising group of potent anti-sickling agents, demonstrate their proposed mechanism of action, and provide proof-of-concept justifications for our structure-based approach to developing potent therapeutics for SCD.

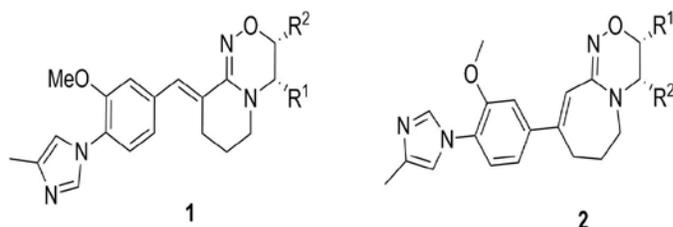
MEDI 229

Generation of novel leads for γ -secretase modulation

Mihirbaran Mandal, mihirbaran.mandal@merck.com. Medicinal chemistry, Merck Research Laboratory, Kenilworth, New Jersey, United States

Using our previously validated oxadiazine moiety as the suitable isosteric replacement of amide functionality, several structurally differentiated leads were designed, synthesized and validated as the modulators of γ -Secretase enzyme for the potential treatment of Alzheimer's disease. The oxadiazines with substitutions at both 3 and 4

positions represented as **1**, designed to limit conformational plasticity, robustly lowered the CSF A β 42 upon oral dosing. To further diversify the structure and to mitigate the concern related to exocyclic double bond presents in **1**, novel endo cyclic β , β -disubstituted framework, represented as **2** was designed for the γ -Secretase modulation in vivo to lower A β 42 in CSF upon oral dosing.



MEDI 230

Synthesis of benzimidazole derivatives for uridine nucleoside ribohydrolase targeting

Angelica S. Leonardo¹, angelicaleonardo@mail.adelphi.edu, **Melissa A. Vanalstine-Parris**². (1) Adelphi University, Island Park, New York, United States (2) Chemistry, Adelphi University, East Meadow, New York, United States

Trichomonas vaginalis is the parasitic agent responsible for trichomoniasis, a common non-viral sexual transmitted disease of worldwide importance. This parasitic infection is normally treated with 5-nitroimidazole drugs which target the parasite's DNA. However, the parasite has developed resistant strains against these drugs and, as a result, a new treatment that works different than the current drug of choice is needed. Castillo *et al.* reported that benzimidazole derivatives can act as good antiparasitic agents; some of them are even more active than 5-nitroimidazole drugs when tested for antiprotozoal activity. Stockman *et al.* identified that proton-pump inhibitor drugs, containing benzimidazole scaffolds, inhibit one of the parasite's enzymes needed for survival, uridine nucleoside ribohydrolase (UNH). For a better understanding on how benzimidazoles affect the parasite, different benzimidazole derivatives are being synthesized using cyclocondensation, methylation, oxidation and amidation reactions. These compounds are then tested for their inhibitory effects on the enzyme rather than testing them with the whole parasite. If these compounds are found to show a similar profile towards UNH as the parasite, it supports our hypothesis that these benzimidazoles work as antiparasitic agents by inhibiting UNH.

MEDI 231

Synthesis and biological evaluation of the first triple inhibitors of human topoisomerase 1, tyrosyl-DNA phosphodiesterase 1 (Tdp1), and tyrosyl-DNA phosphodiesterase 2 (Tdp2)

Ping Wang¹, Mohamed S. Elsayed¹, melsaye@purdue.edu, Caroline Plescia², Evgeny Kiselev³, Christophe Marchand³, Olga Zeleznik³, Keli Agama³, Yves Pommier³, Mark Cushman¹. (1) MCMP, Purdue University, West Lafayette, Indiana, United States (2) Drake University, Des Moines, Iowa, United States (3) NIH, Bethesda, Maryland, United States

Tdp1 and Tdp2 are two tyrosyl-DNA phosphodiesterases that are involved in the repair pathway of damaged DNA resulting from topoisomerase inhibitors and a variety of other DNA-damaging agents. It is believed that Tdp1 and Tdp2 inhibition could hypothetically potentiate the cytotoxicities of topoisomerase inhibitors. A limited number of Tdp1 and Tdp2 inhibitors have been reported in the literature, but many of them are hampered by problems of chemical or metabolic instability and low potency, or by being PAINS (pan-assay interference) compounds. This study reports the successful structure-based design and synthesis of new 7-azaindenoisoquinolines that act as triple inhibitors of Top1, Tdp1 and Tdp2. Enzyme inhibitory data and cytotoxicity data from human cancer cell cultures establish that modification of the lactam side chain of the 7-azaindenoisoquinolines can modulate their inhibitory potencies and selectivities vs. Top1, Tdp1 and Tdp2. A cytotoxic compound with an aminothiazoline side chain showed the best activity profile against all three enzymes. Molecular modeling of selected target compounds bound to Top1, Tdp1, and Tdp2 was used to design the inhibitors and facilitate the structure-activity relationship analysis. Finally, this study adds to the efforts exerted to discover novel inhibitors for Tdp1 and Tdp2 as novel targets for cancer treatment.

MEDI 232

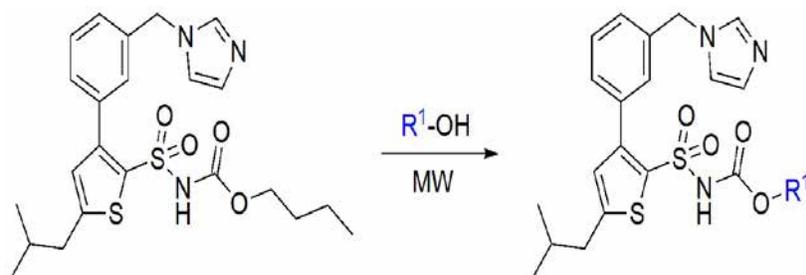
Transcarbamoylation of sulfonyl carbamates to generate new Angiotensin II Type 2 Receptor (AT2R) ligands

Johan Wannberg¹, Rebecka Isaksson², rebecka.isaksson@orgfarm.uu.se, Mathias Hallberg³, Mats Larhed¹. (1) Science for Life Laboratory, Department of Medicinal Chemistry, Uppsala University, Uppsala, Sweden (2) Division of Organic Pharmaceutical Chemistry, Department of Medicinal Chemistry, Uppsala University, Uppsala, Sweden (3) Beijer Laboratory, Department of Pharmaceutical Biosciences, Uppsala University, Uppsala, Sweden

The renin-angiotensin system (RAS) is well-known for its role in blood-pressure regulation and fluid/electrolyte balance. This effect is mediated via the G-protein coupled receptor (GPCR), angiotensin II type 1 receptor (AT1R). The endogenous ligand of this receptor, angiotensin II (AngII), also binds to the GPCR angiotensin II type 2 receptor (AT2R). The function of AT2R has long been debated and it has been suggested to act opposing AT1R, exert wound healing and anti-inflammatory properties, as well as promoting neuroprotection and neuronal regeneration.

Our group has previously reported on the first selective non-peptide agonists of AT2R, compound 21 that has entered clinical trials as a possible treatment for idiopathic

pulmonary fibrosis. We have also reported structurally related antagonists. These structures contain a sulfonyl carbamate motif, and we here report new AT2R ligands generated using the transcarbamylation reaction as previously demonstrated.



Scheme 1: Transcarbamylation of sulfonyl carbamates.

MEDI 233

Development of new novel therapeutics for refractive breast cancer

Kennedi Crosby, *kennedi_crosby@yahoo.com*, **Kamrin Johnson**, *kjohns42@xula.edu*. Xavier University of Louisiana, Stockbridge, Georgia, United States

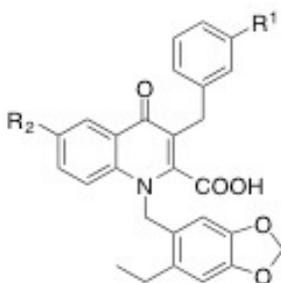
Overexpression of Human Epidermal Growth Factor Receptor (HER2) occurs in around 30% of breast cancers. In HER2 positive resistant breast cancers, three main isoforms are found: WT-HER2, the truncated p95HER2, and HER2 Δ 16. We focus more on the HER2 Δ 16, mainly because HER2 Δ 16 is found in resistant breast cancers and aggressive tumor growth. Of the many growth inhibitors tested in our laboratory, 5,8-dihydroxynaphthoquinones was found to be the most effective growth inhibitors of HER2 Δ 16 overexpressed MCF7 breast cancer cell lines. Currently, the objective is to synthesize new derivatives from our lead compound to improve the inhibition potency. Using a synthetic sequence that involved bromination, formylation, and Wittig reactions, a new derivative was formed from these multiple steps. A Wittig product (1, 4, 5, 8-tetramethoxy-2-vinylnaphthalene) was made and in the future we plan to create further derivatives and test them in bioassay trials.

MEDI 234

Design, synthesis and pharmacological evaluation of 1,3,6-trisubstituted-4-oxo-1,4-dihydroquinoline-2-carboxylic acid derivatives as selective ET_A antagonists

Nikhil S. Khadtare³, *nkhadtare@gmail.com*, **Ralph Stephan**², **Vijaya L. Korlipara**¹. (1) St Johns Univ, Jamaica, New York, United States (2) St Johns University, Jamaica, New York, United States (3) St. John's University, Fresh Meadows, New York, United States

The endothelin axis and in particular the two receptors, ET_A and ET_B, are under investigation for the treatment of various diseases such as pulmonary arterial hypertension, fibrosis, renal failure and cancer. Previous work in our lab has shown that 1,3,6-trisubstituted-4-oxo-1,4-dihydroquinoline-2-carboxylic acid derivatives exhibit noteworthy endothelin receptor antagonist activity. A series of substituted quinolone analogues was designed and synthesized to further optimize the structure activity relationship. Alkylloxy groups of varying chain lengths at position 6 of the quinolone core and an acidic moiety at *meta* position of the side chain benzyl ring were substituted to enhance the inhibitory activity against ET_{A/B} receptors. The potential endothelin receptor antagonist activity was determined by *in vitro* Förster resonance energy transfer (FRET) using GeneBLAzer[®] assay technology. The most potent member of this series exhibited ET_A receptor antagonist activity in the subnanomolar range with an IC₅₀ value of 0.8 nM, and was 1000 fold selective against ET_B receptor.



MEDI 235

Synthesis of 7-sulfonamide indoline inhibitors of the bacterial enzyme DapE

Rachel Torrez¹, rtorrez1@luc.edu, **Tahirah Heath**², **Daniel P. Becker**³. (1) Loyola University Chicago, Chicago, Illinois, United States (2) Chemistry and Biochemistry, Loyola University Chicago, Chicago, Illinois, United States (3) Dept of Chemistry, Loyola University Chicago, Chicago, Illinois, United States

The alarming increase of antibiotic resistant bacterial strains emphasizes an urgent need for research to identify new classes of antibiotics. One promising enzymatic target is DapE (N-succinyl-L,L-diaminopimelic acid desuccinylase), which is found in all Gram-negative and most Gram-positive bacteria. DapE is part of the late stage mDap (meso-diaminopimelate)/lysine biosynthetic pathway. Lysine and meso-diaminopimelate (mDap) are essential in protein synthesis and bacterial peptidoglycan cell wall remodeling. The deletion of the gene DapE is lethal to bacteria, which is very encouraging in support of the hypothesis that inhibitors of DapE will function as antibiotics. Another appealing aspect of targeting DapE is that this enzyme is not found naturally in the human body. Therefore inhibitors that target DapE could potentially provide selective toxicity against bacteria with no mechanism-based toxicity in humans. After the completion of a high throughput screen of over 33,000 compounds, two indoline sulfonamides demonstrated promising inhibition of DapE. Herein, we describe different methods of synthesizing medicinally relevant analogs of the two lead indoline compounds achieved through intramolecular cyclization and the second method being

ortho-directed sulfonylation. Inhibitory data of these analogs will be used to assess structure activity relationship and aid in the design of new analogs.

MEDI 236

Structure-based design of small-molecular inhibitors targeting the Menin-MLL protein-protein interaction

Shilin Xu, xushilin0129@gmail.com, Tianfeng Xu, Ke Zheng, Angelo Aguilar, Liyue Huang, Denzil Bernard, Donna McEachern, Sally Przybranowski, Jeanne Stuckey, Shaomeng Wang. University of Michigan, Ann Arbor, Michigan, United States

Although only 5-10% of adult human acute myeloid leukemias (AML) have MLL chromosomal rearrangements, MLL leukemia patients have very poor prognosis; their 5-year survival rate is 35% with current treatments. Therefore, there is an urgent need to develop new and more effective therapeutic approaches for the treatment of MLL leukemia. The menin-MLL protein-protein interaction (PPI) has been shown to drive leukemogenesis in mouse models of MLL leukemia, and targeting the the menin-MLL PPI has been pursued as a novel therapeutic approach for the treatment of MLL leukemia.

Through structure-based design and extensive medicinal chemistry efforts, we have discovered a class of highly potent small-molecule inhibitors of the menin-MLL PPI. Our most potent compounds bind to menin with K_i values < 1 nM, achieve low nanomolar IC_{50} values in inhibition of cell growth in leukemia cell lines harboring MLL fusion and demonstrate outstanding cellular specificity over leukemia cell lines lacking MLL fusion. Mechanistic studies show that our potent small-molecule inhibitors of the menin-MLL PPI are very effective in suppressing the expression of MEIS1 gene in MLL leukemia cells at low nanomolar concentrations. Further optimization of this class of compounds may ultimately yield a new class of therapy for the treatment of MLL leukemia and potentially other human diseases in which the menin-MLL interaction plays a critical role.

MEDI 237

Novel pirfenidone derivatives: Potent antifibrotic agents

Zhen Ma, mazhen@zjams.com.cn, Chenhuan Yu, Qi Chen, Wenhai Huang, Zunyuan Wang, Chixiao Zhang, Zhengrong Shen. Zhejiang Academy of Medical Sciences, Hangzhou, China

Idiopathic pulmonary fibrosis (IPF) is a typical chronic fibrosing interstitial pneumonia characterized by progressive worsening of dyspnea and lung function with a poor prognosis. With a median survival of 3 to 5 years and a 5-year survival rate of approximately 20%, IPF is an ultimately fatal disease which is considered more lethal than most of the malignant cancers. The pathogenesis of IPF implicated a variety of cellular processes, signaling pathways, and genetics. However, the precision

mechanisms of the disorder are poorly understood.

Pirfenidone is the first orally administered drug that has orphan designation for the treatment of IPF. It is generally thought to be a multiple-targets drug with antifibrotic, anti-inflammatory, antioxidative stress and antiproliferative effects. Beneficial effects have been shown for pirfenidone in the fibrotic disease, including pulmonary, liver, renal, and cardiac muscle fibrosis.

Unfortunately, some side effects including gastrointestinal upset, fatigue and photosensitivity have been observed in clinical practices for pirfenidone. It was speculated that these adverse symptoms might be attributed to the requirement for high therapeutically effective doses of pirfenidone. Therefore, considerable efforts have been made towards the modification of pirfenidone in order to increase the antifibrotic activity. Novel pirfenidone derivatives were designed, synthesized and evaluated for their antifibrotic activity by our group. These compounds showed the remarkable inhibition on cell proliferation compared with pirfenidone *in vitro*. The antifibrotic activity of compound **8d** as the ideal candidate of the novel pirfenidone derivatives *in vitro* and *in vivo* is reported.

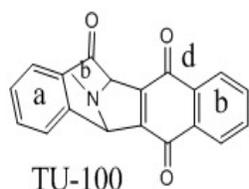
In this study, **8d** effectively inhibits TGF- β 1-induced fibroblast differentiation through inactivation of p38 and Smad3 phosphorylation *in vitro*. Furthermore, **8d** treatment of rats with bleomycin-induced pulmonary fibrosis also shows significantly attenuation of fibrosis. Our findings indicate that **8d** downregulates the levels of TGF- β 1, p38 MAPK and α -SMA protein expression, and markedly inhibit fibroblast activation and collagen deposition in lung tissues of pulmonary fibrosis rats. We demonstrate that this novel pirfenidone derivative attenuates fibrosis probably by inhibiting the key cytokine TGF- β 1 and phosphorylated p38 MAPK. The results of the present study suggest that **8d** might be a potent agent with antifibrotic property for treatment of IPF.

MEDI 238

Synthesis and biological activity of novel TU-100 derivatives

Oladotun J. Alao², oa00866@georgiasouthern.edu, Robert J. Sheaff¹, John C. Dicesare². (1) Chemistry and Biochemistry, University of Tulsa, Tulsa, Oklahoma, United States (2) Chemistry and Biochemistry, Georgia Southern University, Statesboro, Georgia, United States

In an attempt to create more effective chemotherapeutic compounds, the naphthoquinone adduct, 12,13-dihydro-N-methyl-6,11,13-trioxo-5H-benzo[4,5]cyclohepta[1,2 b]naphthalen-5,12-imine (hereafter called TU100) was synthesized. Inspired by its unique and novel mechanism of action, a series of structural derivatives were synthesized to explore structure activity relationships. The analogues exhibited different cytotoxicity profiles, revealing that the indicated regions are important in cell death induction. Furthermore, the analogues had dramatically different effects on cellular ATP production, suggesting different molecular targets. Synthesis, biological activity, and SAR study of these analogues will be presented.



MEDI 239

New α -helix mimetics targeting the E6 protein in the human papillomavirus

Ernest Armenta, *ernest.armenta@csu.fullerton.edu*, Alexandra Orchard. California State University Fullerton, Fullerton, California, United States

Protein-protein interactions (PPIs) are involved in many cellular processes, making them potential drug targets. Many of these interactions involve amino acid residues projecting off of an α -helix. Small molecules have been designed to mimic short amino acid sequences important for binding to target proteins. Many of these small molecules mimic only the hydrophobic face of the α -helix, possibly excluding important interactions arising from polar residues. The Orchard Group has designed novel, amphiphilic, small-molecule α -helix mimics capable of mimicking consecutive amino acid residues on both faces of the helix for treatment of various diseases, such as Human Papillomavirus (HPV) infection. HPV is a small DNA virus that infects epithelial cells. The E6 oncogenic protein in HPV allows for survival of infected cells by binding to the human protein E6AP. Binding allows for degradation of p53, a tumor suppressing protein. Inhibition of E6 should allow for apoptosis of infected cells, clearing the infection in a non-invasive way. Our proposed library of potential α -helix mimetics has been rationally designed with the aid of Molsoft ICM Pro software, and synthesis of these compounds is underway. Herein, we present promising docking results and our proposed synthetic route toward a small subset of compounds capable of mimicking 4 of the 5 residues known to be important for E6 binding. Once synthesis is complete, compounds will be tested for their ability to cause apoptosis in infected cells as well as their ability to specifically inhibit the E6-E6AP interaction.

MEDI 240

Finding hits for designing new antidiabetic drugs. Inhibition of protein tyrosine phosphatase 1B

Marie J. Sarabia-Sánchez¹, Pedro J. Trejo², Erick Sierra³, Mónica Valdez-Solana³, Alicia Hernandez Campos², Claudia I. Avitia-Domínguez¹, **Alfredo Téllez-Valencia**¹, *tellezalfredo@gmail.com*. (1) Facultad de Medicina y Nutrición, Universidad Juárez del Estado de Durango, Durango, Durango, Mexico (2) Departamento de Farmacia, Universidad Nacional Autónoma de México, Mexico, Mexico (3) Facultad de Química, Universidad Juárez del Estado de Durango, Durango, Durango, Mexico

International Diabetes Federation in 2014 reported 382 million of people with diabetes in the world. Type 2 diabetes is the most common disease, it characterizes by impaired glucose tolerance and insulin resistance on their target organs. In the search for new drugs, one of the main strategies is to promote the action of insulin. In this regard, attention has been focused on protein tyrosine phosphatase 1B (PTP1B), a protein whose overexpression or increased activity has been linked in several studies with insulin resistance. With the aim to find new inhibitors of PTP1B, our *in-house* chemical library of small molecules was evaluated to determine their potential inhibition against PTP1B. Kinetics assays were made to characterize the effect of the most potent compounds, as well as docking and molecular dynamics studies. Furthermore, their ADMET properties were determined using FAFDrugs and Molsoft software. From the one thousand compounds tested against PTP1B, ten molecules showed an inhibition percent higher than 50%, then the top three were selected for additional characterization. Kinetic studies showed that these compounds presented a mixed type inhibition with a K_i value in the low micromolar range. Structural data indicated that they bound at the second aryl phosphate-binding site of the PTP1B, allowing selectivity with regard to T-cell Protein Tyrosine Phosphatase (TCPTP), its more closer homologous. Additionally, the inhibitors presented physicochemical characteristics to be considered as potential drugs. The data reported here suggest that these PTP1B inhibitors could be used as hits to design molecules with higher potency and selectivity against PTP1B and represent one step ahead in the search of new antidiabetic drugs.

MEDI 241

Identification of small molecular activators on the unfolded protein response in leukemia cells

Nour Mahmoud, fp8816@wayne.edu, Danielle Garshott, Yue Xi, Amy Brownell, Michael Callaghan, Andrew Fribley. Pediatrics, Wayne State University, Detroit, Michigan, United States

More than 60,000 Americans are expected to be diagnosed with leukemia in 2016, and although survival rates have improved about four fold in the last five decades, relapse and refractory disease continue to provide unacceptably high morbidity and mortality. Our group and others have hypothesized that aberrantly high stress in the secretory pathway in leukemia cells might be exploited pharmacologically. When the burden of unfolded proteins in the ER lumen outpaces peptide processing, folding and post-translational modification, the unfolded protein response (UPR) is activated in an attempt to restore homeostasis or induce cell death if the challenge cannot be overcome. Increased ER stress and UPR is a feature of many solid and hematological malignancies, especially ALL and AML. A pilot high-throughput screen was performed to identify small molecules that could induce the apoptotic (CHOP) arm of the UPR. The MicroSource Spectrum Collection of 2,400 compounds was screened with a K562 cell line stably transfected with a CHOP-luciferase reporter. Twenty primary hits were identified; med chem triage reduced the number of tractable hits to ten compounds that were re-ordered as dry powder stocks and confirmed using the reporter cell line. Three

compounds that could activate the CHOP reporter in a dose dependent fashion were subject to proliferation assays using wildtype or CRISPR-Cas9-deleted (CHOP) K562 cell lines. Cetylpyridinium (a component in various oral rinses) emerged as a promising hit compound that activated the CHOP-luc reporter and reduced proliferation in a CHOP-dependent fashion. RT-qPCR and immunoblot experiments currently underway are focused to determine if/how cetylpyridinium activates the UPR in a panel of human leukemia cell lines.

MEDI 242

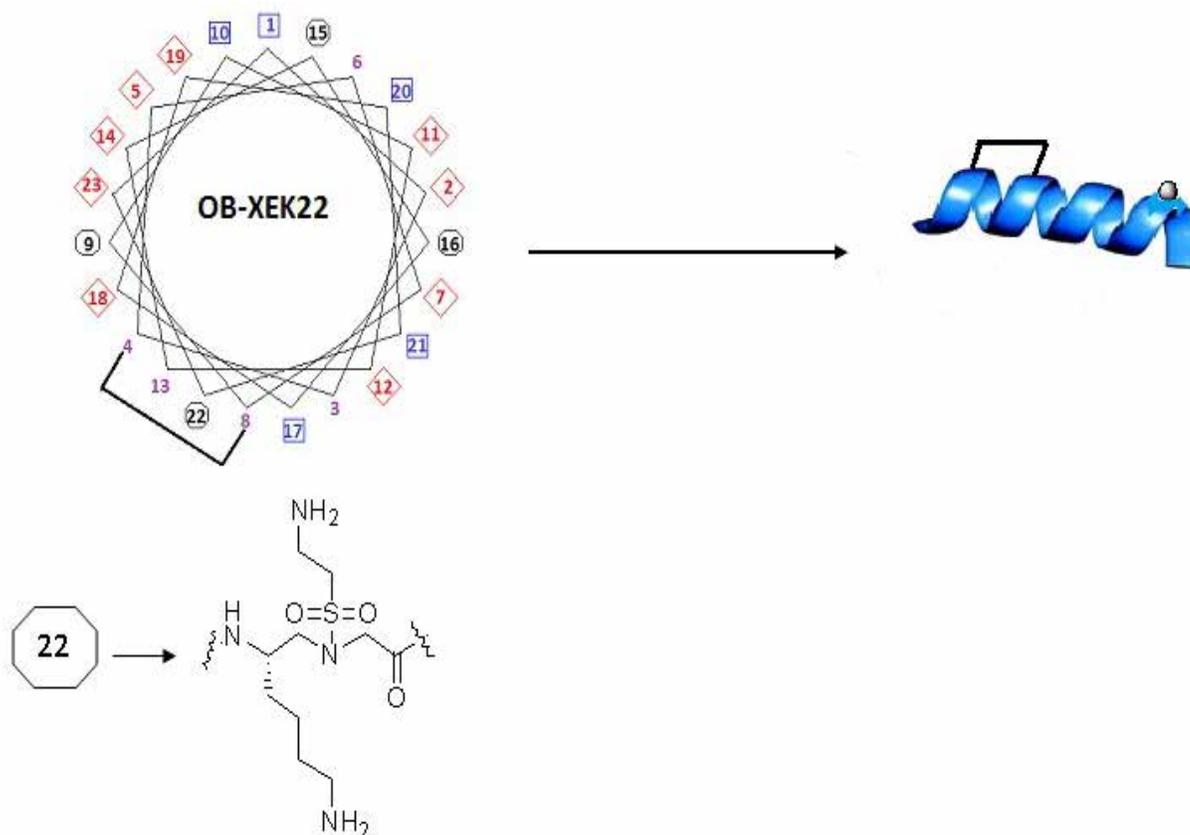
Constrained peptides that inhibit HIV-1 fusion

Olapeju Bolarinwa, ooyesiku@mail.usf.edu, Jianfeng Cai. Chemistry, University of South Florida, Tampa, Florida, United States

The utilization of bioactive peptides in the development of highly selective and potent pharmacologic agents for the disruption of protein-protein interactions has become more appealing for drug discovery. However, this strategy is limited by loss of bioactivity and instability to proteases.

HIV-1 entry into host cell is through a fusion process that is mediated by the trimeric viral glycoprotein gp120/41 which are obtained from gp160 through proteolytic processing. Linear peptides derived from the HIV gp41 C-terminus have proven potent in inhibiting the fusion process. These peptides have shown good interaction and significant binding to the hydrophobic pocket on gp-41 N-terminus which was previously identified as a potential inhibitor site.

In this study, we introduce a 23-residue C-peptide, OB-XEK22 that was optimized for HIV-1 gp-41 N-terminus binding and proteolytic stability through sulfono- γ -AApeptide substitution and all-hydrocarbon stapling. OB-XEK22 inhibited envelope-mediated membrane fusion in cell-cell fusion assays at nanomolar potency and showed improved protease resistance.



Helical wheel diagram of stapled OB-XEK22 and sulfono- γ -AApeptide residue substituted at position 22

MEDI 243

Design and optimization of small molecule inhibitors blocking the menin-MLL interaction

Tianfeng Xu, xu_tianfeng@hotmail.com, Shilin Xu, Ke Zheng, Angelo Aguilar, Liyue Huang, Krishnapriya Chinnaswamy, Denzil Bernard, Donna McEachern, Sally Przybranowski, Jeanne Stuckey, Shaomeng Wang. University of Michigan, Ann Arbor, Michigan, United States

Chromosomal rearrangements of the mixed lineage leukemia gene (MLL) have been found in about 10% of all acute leukemia patients and 70-80% of infant acute lymphoblastic leukemia. Patients with rearranged MLL gene have dismal prognosis and low long-term survival rates. Recent studies demonstrated that the interaction between MLL fusion proteins and menin played an important role in driving leukemogenesis in MLL leukemia. Therefore, drugs directly disrupt the interaction may be used for treating the MLL leukemia patients.

Using a powerful structure-based design approach, we have successfully obtained a series of conformationally rigid, highly potent menin-MLL inhibitors. A representative compound **M-89** binds to menin with a K_d value of 1.4 nM. It specifically inhibits the proliferation of MV4; 11 and MOLM-13 cell lines bearing MLL fusion with $IC_{50} = 25$ nM

and 54 nM, respectively, and displays about 200-fold less potent against HL-60 cell line lacking MLL fusion. **M-89** dose-dependently suppresses *HOX9* and *MEIS1* gene expressions, and effectively induces apoptosis in these cells. We have determined the co-crystal structure of **M-89** in complex with menin. Further optimization based on this crystal structure may give us new menin inhibitors with better drug-like properties, which may be used for the treatment of MLL leukemia.

MEDI 244

Discovery of LLM4 as potent and specific IL-6/gp130 protein-protein interaction inhibitor for potential cancer therapy

Liguang Mao^{1,2}, *mlg861122@gmail.com*, **Guqin Shi**^{1,2}, **Chenglong Li**². (1) College of Pharmacy, The Ohio State University, Gainesville, Florida, United States (2) College of Pharmacy, University of Florida, Gainesville, Florida, United States

IL-6 belongs to the gp130-related cytokine family. It plays an important role in IL-6/gp130/STAT3 signaling pathway, which is found to be protumorigenic in many cancers. IL-6 firstly binds to IL-6 receptor α (IL-6R α), which recruits gp130 on cell membrane; then dimerization of two of the IL6/IL-6R α /gp130 trimers allows signal transduction via gp130 cytoplasmic domains, which activates Jak1 then STAT3 (Tyr705 phosphorylation), leading to STAT3 nucleus translocation, DNA binding, and multiple oncogene transcriptions. To date, very few small molecules have been identified as IL6/gp130 inhibitor. In our lab, by using computational modeling, we were able to find the key binding sites on the interaction surface between IL-6 and gp130 D1 domain. Based on these understanding, we designed novel small molecule IL-6/gp130 inhibitors by implementing MLSL (multiple ligand simultaneous docking) and other structure-based drug design strategies. These small molecule IL6/gp130 inhibitors were synthesized in our lab and preliminary biological tests show that one of these molecules, LLM4 is a novel anti-cancer compound, with IC₅₀ value of 17.0 μ M against breast cancer cell line SUM159 and 3.9 μ M against pancreatic cancer cell line BXPC3. Furthermore, LLM4 is able to specifically inhibit the IL-6/gp130/STAT3 pathway with little/no side-effect on the other gp130-related cytokine signaling pathways.

MEDI 245

ML418: The first selective, sub-micromolar pore blocker of K_{ir}7.1 potassium channels

Haruto Kurata^{1,3}, *h.kurata@ono.co.jp*, **Daniel R. Swale**⁴, **Sujay V. Kharade**⁴, **Jonathan Sheehan**⁵, **Rene Raphemot**⁴, **Karl R. Voigttritter**^{2,3}, **Eric E. Figueroa**⁴, **Jens Meiler**⁵, **Anna L. Blobaum**^{2,3}, **Craig W. Lindsley**^{2,3}, **Jerod S. Denton**^{4,2}, **Corey R. Hopkins**^{2,3}. (1) Medicinal Chemistry, Ono Pharmaceutical Co., Ltd., Mishima, Osaka, Japan (2) Dept of Pharmacology, Vanderbilt University, Nashville, Tennessee, United States (3) Vanderbilt Center for Neuroscience Drug Discovery and the Vanderbilt Specialized Chemistry Center for Accelerated Probe Development, Vanderbilt University, Nashville,

Tennessee, United States (4) Department of Anesthesiology, Vanderbilt University Medical Center, Nashville, Tennessee, United States (5) Department of Chemistry, Vanderbilt University, Nashville, Tennessee, United States

The inward rectifier potassium (K_{ir}) channel $K_{ir}7.1$ (KCNJ13) has recently emerged as a key regulator of melanocortin signaling in the brain, electrolyte homeostasis in the eye, and uterine muscle contractility during pregnancy. The pharmacological tools available for exploring the physiology and therapeutic potential of $K_{ir}7.1$ have been limited to relatively weak and nonselective small-molecule inhibitors. Here, we present the discovery of the first selective, sub-micromolar pore blocker of $K_{ir}7.1$ potassium channels.

VU714, identified by a fluorescence-based high-throughput screen with a quinoline core and a lipophilic 4-benzylpiperidine in the eastern part, was modified to reduce lipophilicity. Initial SAR development revealed that removal of a phenyl ring in the eastern part did not affect inhibitory activity for $K_{ir}7.1$ channel. Subsequent SAR exploration to introduce hydrophilic moieties in the eastern part found a carbamate group has potential to be more potent and less lipophilic. Optimizing a carbamate functionality culminated in the discovery of ML418 which exhibits sub-micromolar activity ($IC_{50} = 310$ nM) and superior selectivity over other K_{ir} channels (at least 17-fold selective over $K_{ir}1.1$, $K_{ir}2.1$, $K_{ir}2.2$, $K_{ir}2.3$, $K_{ir}3.1/3.2$, and $K_{ir}4.1$) except for $K_{ir}6.2/SUR1$ (equipotent). Evaluation in the EuroFins Lead Profiling panel of 68 GPCRs, ion-channels, and transporters for off-target activity of ML418 revealed a relatively clean ancillary pharmacology. While ML418 exhibited low CL_{HEP} in human microsomes which could be modulated with lipophilicity adjustments, it showed high CL_{HEP} in rat microsomes regardless of lipophilicity. A subsequent *in vivo* PK study of ML418 by intraperitoneal (IP) administration (30 mg/kg dosage) revealed a suitable PK profile ($C_{max} = 0.20$ μ M and $T_{max} = 3$ h) and favorable CNS distribution (mouse brain/plasma K_p of 10.9) to support *in vivo* studies. ML418 should be useful for exploring the physiology of $K_{ir}7.1$ *in vitro* and *in vivo*.

MEDI 246

Effect of magnetite chitosan/alginate beads infused with the antibiotic oxytetracycline hydrochloride on *E. coli* growth rates

Rosario M. Zamora¹, rosario.zamora@upr.edu, Alejandro Chardon¹, Faviola Alvarez², Ana Gabriela Zapata², Victor Fernandez-Alos², Felix Roman², Oscar Perales³. (1) Biology, University of Puerto Rico-Mayaguez, Mayaguez, Puerto Rico, United States (2) Chemistry, University of Puerto Rico-Mayaguez, Mayaguez, Puerto Rico, United States (3) Engineering Science and Materials, University of Puerto Rico at Mayaguez, Mayaguez, Puerto Rico, United States

Composite nanoparticles coated with chitosan/ alginate beads were developed to target and deliver chemicals throughout the human body by modifying the particle surface with antibiotics or with nutrient supplements. Numerous reports relating bacteria in humans have increased over the last years. Bacteria resistant to antibiotics and antibacterial

materials are of great interest but high level of complexity in their production. In this paper biocomposites magnetic materials was developed to achieve a surface with antibacterial properties. The synthesis of this matrix was derived with the following proportions 1.5 grams of medium molecular weight chitosan, alginate 1 gram of acid, and 1 gram of magnetite (Fe_3O_4) nanoparticles. This proportion was optimized and analytical techniques were used to link relate the surface with the growth rate of *E. coli* in the petri dish with Tryptic Soy Agar. Analytical techniques such as infrared spectroscopy, liquid chromatography hyper-performance were used to determine the efficiency of absorption of the antibiotic oxytetracycline. Antibacterial activity is related to compounds kill bacteria or that locally their growth slow down, without being in generally toxic to surrounding tissue. Preliminary results show that 78.3% of antibiotics were removed from aqueous solution with the chitosan/alginate magnetic beads. One of the first test it was observed an inhibition halo and its correlation on the growth of *E. coli*. Further studies are required to examine swelling of different beads as the function of inhibition ring and growth rates of *E. coli*.

MEDI 247

Design and solid-phase synthesis of Muramyl dipeptide (MDP) surrogates as NOD2 signaling activating agents

Ivy Kekessie, ivy.kekessie@gene.com, Tanya Goncharov, Jeffrey Tom, Domagoj Vucic, Aimin Song. Genentech Inc., South San Francisco, California, United States

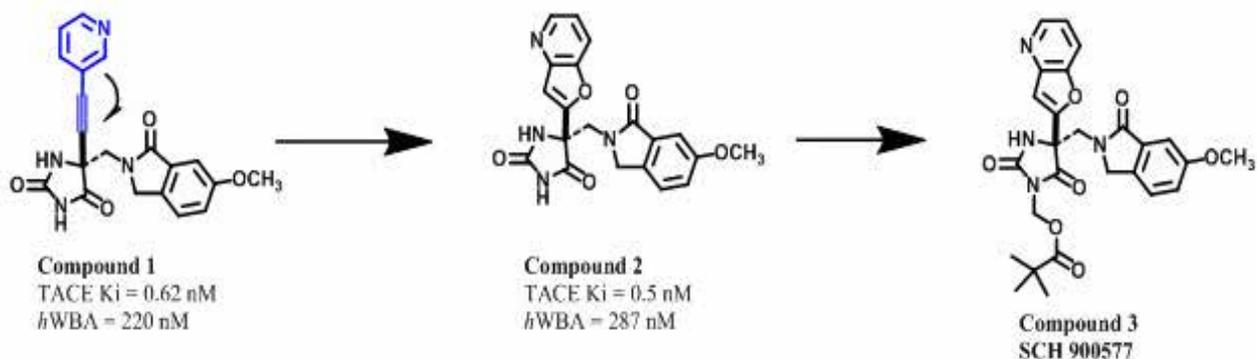
NOD2 is an intracellular pathogen receptor that plays a critical role in the maintenance of intestinal homeostasis and response to Gram positive and negative bacteria. Mutations in NOD2 have been implicated in several immune disorders such as Blau syndrome, sarcoidosis and Crohn's disease. Thus, it is increasingly important to develop appropriate tools for studying this critical pathway. Solution phase syntheses have been applied extensively to obtain several analogues of MDP for probing the interactions with NOD2. Disadvantages of solution phase functionalization are that solution phase requires extensive synthetic steps and also involves several purification steps of the intermediates. These laborious steps can be eliminated with solid-phase synthesis. The existing solid-phase approach does not address modifications of the Muramyl building block at the C-6 position. We have demonstrated that, chemical modifications of the muramyl moiety can be achieved on solid support with orthogonally protected muramyl building block, which allows access to the C-6 position of MDP for tethering various probes to enhance and expand on the available tools for studying interactions of MDP and NOD2. Thus a facile synthetic approach for obtaining several analogues of MDP on solid phase has been described.

MEDI 248

Discovery of preclinical candidate SCH 900577: A prodrug of hydantoin based TACE inhibitor

Ling Tong¹, ling.tong@merck.com, Seong Heon Kim¹, Lei Chen¹, Aneta Kosinski¹, Bandarpalle Shankar¹, Vinay Girijavallabhan¹, De-Yi Yang³, Wensheng Yu¹, Guowei Zhou³, Neng-Yang Shih¹, Kristin Rosner Rosner¹, Dansu Li¹, Chaoyang Dai¹, Janeta Popovici-muller¹, Liping Yang¹, Arshad Siddiqui³, Zhuyan Guo¹, Peter Orth¹, Shiyang Chen¹, Mengwei Hu¹, Daniel Lundell¹, Xiaoda Niu¹, Shelby Umland¹, Joseph A. Kozlowski². (1) Merck & Co., Inc, Kenilworth, New Jersey, United States (2) RY800-C100, Merck and Co, Rahway, New Jersey, United States (3) Discovery Chemistry, Merck & Co., Inc., Kenilworth, New Jersey, United States

Our research on hydantoin based TNF- α converting enzyme (TACE) inhibitors identified fused bi-heteroaryl hydantoin (e.g. compound **2**) as a series that demonstrates sub-nanomolar potency (K_i), excellent activity in human whole blood assay, and reasonable DMPK profiles. Compound **2** was derived from lead **1** by cyclization onto the linking acetylene. However, compound **2** showed less than ideal dose proportionality in rat single rising dose studies and it also posed formulation challenges for further development. A prodrug approach was investigated to address this issue. The pivalate prodrug **3** (SCH 900577) was identified. It demonstrated improved DMPK properties compared with parent compound **2**, provided a stable formulation, and was suitable for further development.



MEDI 249

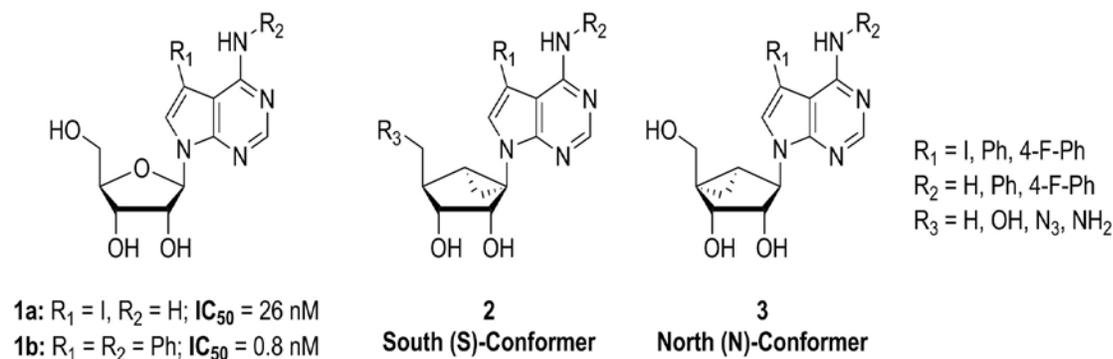
Probing conformational preference: (S)- and (N)-methanocarba 7-deazaadenosine analogues as inhibitors of human adenosine kinase

Kiran S. Toti¹, kiran.toti@nih.gov, Danielle Osborne², Antonella Ciancetta¹, Detlev Boison², Kenneth A. Jacobson¹. (1) Laboratory of Bio-organic Chemistry, NIDDK, National Institutes of Health, Bethesda, 20892, Maryland, United States (2) Robert Stone Dow Neurobiology Laboratories, Legacy Research Institute, Portland, 97232, Oregon, United States

Adenosine kinase (ADK), which converts adenosine (ADO) to adenosine monophosphate, is an important enzyme for maintaining optimum adenosine levels in the brain. An increased extracellular ADO concentration can protect neurons by activating adenosine receptors. Similarly in the nucleus of astrocytes, elevated ADO

concentration reduces DNA-methylation, which is a primary cause of epileptogenesis. Thus, inhibiting the ADO metabolizing enzyme ADK is an effective strategy for both prevention and progression of epileptic seizures. In nature, most nucleos(t)ides adopt either Northern (N)- or Southern (S)-conformation. Also, the biological macromolecular targets, such as enzymes, polymerases, transporters and receptors, prefer nucleos(t)ide in one of these conformations as ligands. There are two isoforms of ADK, ADK-S in the cytoplasm and the long form (ADK-L) in the nucleus. In the crystal structure of ADK-S bound to the nucleoside 5'-deoxy analog of **1a**, the inhibitor adapted an unusual East (E)-conformation, tending towards the Southern (S)- hemisphere ($P = 125.3^\circ$).

To probe the conformational preference of inhibitors of ADK, we synthesized both (S) and (N) locked methanocarpa analogues (**2** and **3**, respectively) of the known potent nucleoside inhibitors **1**. The synthetic protocols, biological evaluation data and rationalization of the results based on molecular modelling will be presented.



MEDI 250

Synthesis and NMDA receptor activity of various ketamine metabolites

Patrick J. Morris¹, patrickjmorris@gmail.com, Panos Zanos², Ruin Moadell³, Carlos A. Zarate⁴, Todd Gould², Craig J. Thomas¹. (1) Division of Preclinical Innovation, National Center for Advancing Translational Sciences, Rockville, Maryland, United States (2) Department of Psychiatry, University of Maryland School of Medicine, Baltimore, Maryland, United States (3) Biomedical Research Center, National Institute on Aging, Baltimore, Maryland, United States (4) Experimental Therapeutics and Pathophysiology Branch, National Institute of Mental Health, Bethesda, Maryland, United States

Depression is a serious medical condition that affects hundreds of thousands of Americans. Ketamine and recently some metabolites of ketamine have been reported to have unique antidepressant activity in humans and mouse models. Here, we report the full synthesis of known ketamine metabolites and assess their activity against the N-methyl-D-aspartate receptor, the primary pharmacological target of ketamine. With this data, we hope to help further the development of ketamine metabolites as potential treatments for depression.

MEDI 251

Discovery of potent glucagon receptor antagonists for the treatment of type 2 diabetes

Guozhang Xu, gxu4@its.jnj.com, Michael D. Gaul, Fengbin Song, Baoping Zhao, Yin Liang, Fuyong Du, Karen Diloreto, Norman Huebert, Brian C. Shook, Dennis Rentzeperis, Rosemary Santulli, Annette Eckardt, Charles Smith, Keith Demarest. Janssen Pharmaceutical R & D, Spring House, Pennsylvania, United States

Type 2 diabetes mellitus (T2DM) is characterized by chronically elevated plasma glucose levels. Both glucagon and insulin are key hormonal agents which mediate homeostatic regulation of the glucose in the blood. Glucagon excess coupled with insulin resistance contributes significantly to the development of hyperglycemia. Glucagon, a polypeptide hormone consisting of 29 amino acids that is produced in the alpha islet cells of the pancreas, stimulates hepatic gluconeogenesis and glycogenolysis upon binding to and activating the glucagon receptor (GCGR). Disruption of glucagon signaling, especially chronic antagonism of hepatic GCGR, is expected to enable improved glycemic control in T2DM. A novel series of indole/indazole-based β -alanine derivatives has been discovered as potent human GCGR antagonists in cAMP assays. Through carefully designed SAR studies, important structural motifs were recognized. Compounds within this class exhibited excellent pharmacokinetic properties in multiple preclinical species. An efficient enantioselective synthesis of the desired enantiomer has been developed.

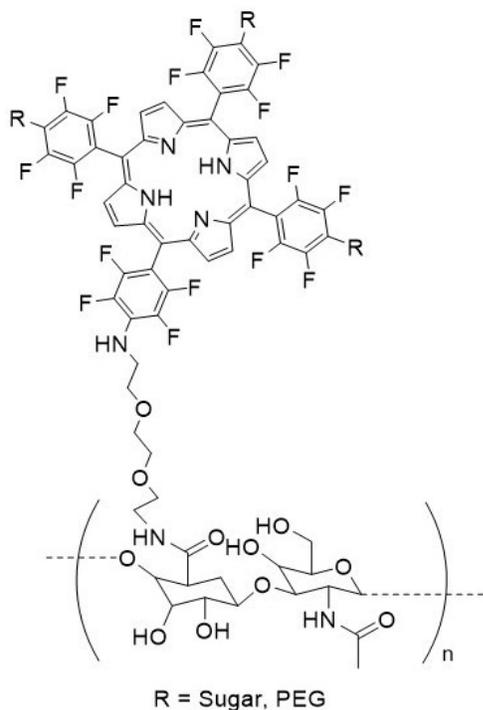
MEDI 252

Synthesis and two- and three-dimensional *in vitro* studies of hyaluronic acid porphyrin conjugates

NVS Dinesh K Bhupathiraju¹, nb216@hunter.cuny.edu, Christopher Farley³, Naxhije Berisha¹, Bibi begum¹, Charles M. Drain². (1) Chemistry, Hunter College, New York, New York, United States (2) Chemistry Dept, Hunter College Cuny, New York, New York, United States (3) Chemistry, Hunter College, CUNY, Westbury, New York, United States

To date, there is no permanent cure for cancer and billions of dollars are spent each year to treat or improve the quality of life of those afflicted. Several drugs are in clinical trials in finding effective treatments and diagnostics for early detection. Photodynamic therapy (PDT) is one of the approved cancer therapy which has the potential to selectively destroy malignant cells while sparing the normal tissues. Hyaluronic acid (HA) is a naturally occurring high molecular weight linear polysaccharide with D-glucuronic acid and N-acetyl-D-glucosamine as repeated units linked by an α -1,3-glycosidic bond and is known to direct drugs to cancers that express CD44 receptors. In our study we conjugated porphyrin to high and low molecular weight HA efficiently and

studied their efficacy in both two dimensional (monolayer) and three dimensional (spheroids) cell culture.



MEDI 253

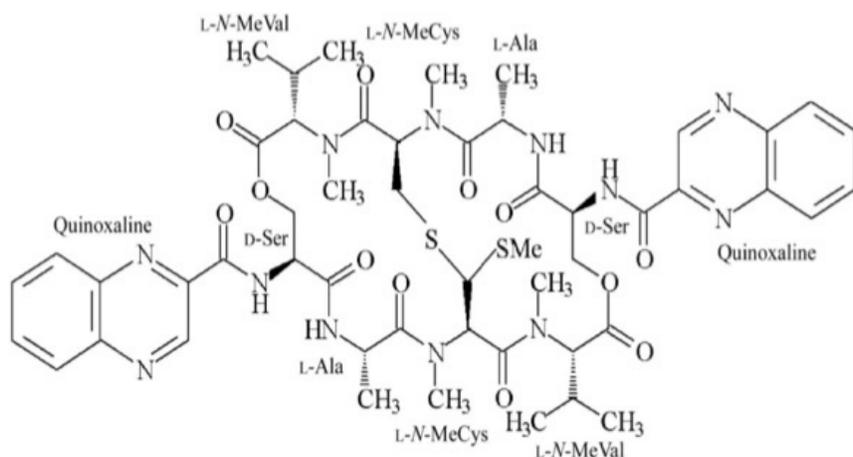
Preformulation analysis and stability of hydrophobic small molecular echinomycin for injection formulation

Jun H. Lee, jun.lee@nih.gov, Marleen Tran, Peng Yuan, Gopal K. Potti. Pharmacy, Clinical Center, National Institutes of Health, Bethesda, Maryland, United States

Echinomycin is quinoxaline-containing depsipeptide antibiotic and proposed to treat acute myeloid leukemia (AML) as molecular targeting HIF-1a in cancer stem cells (CSCs). Through targeting HIF-1a, echinomycin selectively eliminates CSCs in hematological tumors. Formulation of echinomycin for injection was challenging due to its hydrophobic nature. Cyclodextrins (CDs) are a group of cyclic oligosaccharides, consisting of 6 or more 1-4 linked α -anhydroglucose moieties. CDs have hydrophobic cavity and hydrophilic exterior. Here we report preformulation study in development of echinomycin for injection formulation using various CDs. Also stability studies of the drug in the formulation over years using HPLC assay method will be reported. Cavasol and Captisol (CDs) were tested and Cavasol was chosen for further study. Study variations to modify the proper formulation method were including stirring time with magnetic stirrer, type of syringe filter, concentration of solution, and type of diluent. Stress condition analysis performed with echinomycin-cavasol formulation stored in 0.1N HCl, 0.1N NaOH, and 3% H₂O₂ at RT and 60°C for 24 hours. HPLC assay

procedure is a gradient assay using water and acetonitrile as mobile phase. A Waters XBridge BEH C18 Column was used for elution in 40°C and detection was set at 254 nm. The analyses of echinomycin-cavasol formulation under stress conditions indicated that the assay procedure is stability indicating and linearity of the procedure was established with six points over the concentration range of 0.004 to 0.227 mg/mL ($R^2=0.9998$). Echinomycin injection (2mL of 20 mcg/mL in pre-siliconized 2mL clear type I vial) was made and on going stability study performs using products stored in two different temperature conditions.

Echinomycin injections are stable for at least eighteen months when stored in freezer while for only one month in refrigerated condition. Continuous stability monitoring over the course of the clinical trial will be conducted.



Echinomycin

Molecular Formula: $C_{51}H_{64}N_{12}S_2$

MW: 1101

MEDI 254

Characterization of the degradants formed during formulation of rigosertib, a phase III clinical candidate

Muralidhar R. Mallireddigari², mmallireddigari@onconova.us, Venkata Dandu¹, Vijaya Bharathi¹, Balireddy Akula², Venkat Pallela², Stephen C. Cosenza¹, Chen Ren², Manoj Mania², Premkumar Reddy¹, M Reddy^{1,2}. (1) Department of Oncological Sciences, Icahn School of Medicine at Mount Sinai, New York, New York, United States (2) Onconova Therapeutics Inc., Newtown, Pennsylvania, United States

Rigosertib, is a small molecule inhibitor of cellular signaling and acts as a RAS mimetic, which is in Phase 3 clinical trial. The effects of rigosertib appear to be mediated by direct binding of the compound to the RAS-binding domain (RBD) found in many RAS effector proteins, including the Raf kinases and PI3K. The therapeutic focus for rigosertib is myelodysplastic syndromes (MDS), a group of bone marrow disorders characterized by ineffective formation of blood cells that often converts into acute

myeloid leukemia (AML). Clinical trials for rigosertib are being conducted at leading institutions in the U.S., Europe, and the Asia-Pacific region. Both the Intravenous (IV) and oral formulations of rigosertib are being tested in multiple clinical trials. Rigosertib has been awarded Orphan Designation for MDS in the United States, Europe and Japan.

The oral form of rigosertib provides a more convenient dosing for use where the duration of treatment may extend to multiple years. To date, more than 350 patients have been treated with the oral formulation of rigosertib, either as a single agent or in combination with other drugs in hematological malignancies, lower-risk MDS and solid tumors. During the manufacturing and stability studies of oral formulated drug product, we observed formation of some of impurities. In this presentation, we describe the synthesis, characterization and the biological activity of these impurities.

MEDI 255

Design, synthesis and biological evaluation of specific ARK5 inhibitor

M Reddy^{1,2}, *r.reddy@mssm.edu*, Saikrishna Athuluri-Divakar¹, Balireddy Akula², Muralidhar R. Mallireddigar², Stephen C. Cosenza¹, Venkata Dandu¹, Vijaya Bharathi¹, Venkat Pallela², Premkumar Reddy¹. (1) Department of Oncological Sciences, Icahn School of Medicine at Mount Sinai, New York, New York, United States (2) Medicinal Chemistry, Onconova Therapeutics Inc., Newtown, Pennsylvania, United States

We have earlier shown that ON123300 inhibits both CDK4/CDK6 and ARK5 at low nano-molar concentrations. ARK5, a fifth member of the AMPK family has been identified as a transcriptional target of several tumor cells in which it is overexpressed. Our SAR studies and modifications performed on ON123300 backbone led to the identification of ON123790 which specifically inhibits ARK5 at 14nM without interfering with CDK4/CDK6 activity.

In this presentation we show the synthesis, SAR, kinase profile of ON123790 and the effect of 123790 on glutamine uptake and ATP production in three tumor cell lines.

MEDI 256

Discovery of novel class IIa-selective histone deacetylase inhibitors using an in silico virtual screening approach

Hui-Ju Tseng^{1,3}, *d343105007@tmu.edu.tw*, Chang-Yi Liu¹, Chun-Yung Chen¹, Kai-Cheng Hsu², Wei-Jan Huang¹. (1) Graduate Institute of Pharmacognosy, College of Pharmacy, Taipei Medical University, Taipei, Taiwan (2) Graduate Institute of Cancer Biology and Drug Discovery, College of Medical Science and Technology, Taipei Medical University, Taipei, Taiwan (3) Ph.D Program in Biotechnology Research and Development, Taipei Medical University, Taipei, Taiwan

Histone deacetylases (HDACs) are a family of enzymes that regulate epigenetic process by removing acetyl groups from histone. HDACs are divided into four classes based on their sequence homology, catalytic site and tissue distribution. Class IIa HDACs have been considered to play an important role in various diseases, such as diabetic nephropathy, cancers, neurodegeneration and inflammatory disease, thus becoming vital drug targets. Most HDAC inhibitors contain a common hydroxamate moiety that chelates the zinc ion in the catalytic site of HDAC enzymes. However, such inhibitors have two major problems that include poor pharmacokinetics and some undesirable toxicities. Additionally, hydroxamate-based inhibitors often have poor selectivity for the four HDAC classes, which may cause side effects. Herein, we identified six functional groups from the protein data bank by analyzing the moieties that potentially coordinated with the zinc ion to form the protein-ligand complexes. Based on this filter strategy, six new non-hydroxamate compounds that inhibited class IIa HDACs were identified using structure-based virtual screening combined with the assay of a panel of HDAC isoforms. The inhibitors exhibited class IIa HDACs enzyme-inhibitory activities superior to SAHA, a clinically used HDAC inhibitor. In contrast, they showed weak inhibitory activities against HeLa nuclear HDACs, which contain class I HDACs, as well as class IIb HDACs. These results indicated these non-hydroxamates inhibitors displayed a high degree of selectivity for class IIa HDACs over other HDACs.

MEDI 257

Hybridization approach to selective RIPK2 inhibitors by targeting inactive kinase conformations

Chalada Suebsuwong², *csuebsuwong@uh.edu*, Alexei Degterev¹, Gregory Cuny³. (1) Department of Developmental, Molecular & Chemical Biology, Tufts University, Boston, Massachusetts, United States (2) Department of Chemistry, University of Houston, Houston, Texas, United States (3) Department of Pharmacological and Pharmaceutical Sciences, University of Houston, Houston, Texas, United States

Receptor interacting protein kinase 2 (RIPK2) is a caspase recruitment domain (CARD) containing kinase that has been implicated in nucleotide binding and oligomerization domain 1 and 2 (NOD1 and NOD2) signaling. NOD1/2-RIPK2 interactions result in activation of nuclear factor κ B (NF- κ B) and mitogen-activated protein (MAP) kinase pathways to promote transcription of pro-inflammatory cytokines that associate with pathogenesis of inflammatory diseases such as inflammatory bowel disease (IBD), Crohn's disease, ulcerative colitis and multiple sclerosis.

Published RIPK2 crystal structures in complex with different inhibitors reveal that RIPK2 protein can adopt both active (DFG-in/Glu-in) and inactive (DFG-out/Glu-in and DFG-in/ α C-helix-out) kinase conformations. One strategy for improving kinase selectivity is targeting structurally distinct inactive kinase conformations. This presentation will report a hybridization strategy to achieve potent and selective allosteric, α C-helix-displacing inhibitors of RIPK2.

MEDI 258

Design and synthesis of tricyclic and tetracyclic fused coumarin sulfonate derivatives, and their inhibitory effects on LPS-induced nitric oxide and PGE₂ productions in RAW 264.7 macrophages

Mohammed I. El-Gamal^{1,2}, drmelgamal2002@gmail.com, Chang-Hyun Oh³, Kyung-Tae Lee⁴, Daejin Baek⁵. (1) Department of Medicinal Chemistry, University of Sharjah, College of Pharmacy, Sharjah, United Arab Emirates (2) Department of Medicinal Chemistry, University of Mansoura, Faculty of Pharmacy, Mansoura, Egypt (3) Department of Biomolecular Science, Korea Institute of Science and Technology, Seoul, Korea (the Republic of) (4) Department of Biochemistry, Kyung Hee University, College of Pharmacy, Seoul, Korea (the Republic of) (5) Department of Chemistry, Hanseo University, Seosan, Korea (the Republic of)

A new series of 21 fused coumarin derivatives was designed and synthesized. Their *in vitro* antiinflammatory effects as inhibitors of lipopolysaccharide (LPS)-induced nitric oxide (NO) and prostaglandin E₂ (PGE₂) production in RAW 264.7 macrophages have been evaluated. The target compounds **1a-u** were first tested for cytotoxicity to determine a non-toxic concentration for antiinflammatory screening, so that the inhibitory effects against NO and PGE₂ production would not be caused by cytotoxicity. Compounds **1f** and **1p** were the most active PGE₂ inhibitors with IC₅₀ values of 0.89 μM and 0.95 μM, respectively. Western blot and cell-free COX-2 screening showed that their effects were due to inhibition of both COX-2 protein expression and COX-2 enzyme activity. Their IC₅₀ values against COX-2 enzyme were 0.67 μM and 0.85 μM, respectively, more potent than etoricoxib. The selectivity indexes of compounds **1f** and **1p** against COX-2 than COX-1 were 41.1 and 42.5, respectively. Compound **1f** showed strong inhibitory effect at 5 μM concentration on COX-2 mRNA expression in LPS-induced RAW 264.7 macrophages. Moreover, the tricyclic compounds **1l** and **1n** as well as the tetracyclic analogue **1u** were the most potent NO inhibitors with one-digit micromolar IC₅₀ values. They showed dose-dependent inhibition of inducible nitric oxide synthase (iNOS) protein expression. The tetracyclic derivative **1u** was the most potent inhibitor of NO production. It also exhibited a strong inhibitory effect on iNOS mRNA expression in LPS-induced RAW 264.7 macrophages.

MEDI 259

Novel benzamides as capsid assembly modulators for the hepatitis B virus

Nicky Hwang¹, nicky.hwang@bblumberg.org, Matthew Campagna¹, Kelly McGuire², Shuo Wu¹, Ju-Tao Guo¹, Yanming Du¹. (1) Baruch S. Blumberg Institute, Doylestown, Pennsylvania, United States (2) Temple University, Philadelphia, Pennsylvania, United States

Chronic hepatitis B virus infection is a disease of the liver that affects millions of people worldwide, but there is a low cure rate under the current regimen. One promising

approach, which is complementary to the current treatment, is to target the capsid assembly of the virus to block further replication. We present here an identification of a benzamide as an inhibitor of a correct virus capsid formation through a high-throughput screening effort. Optimization of this hit compound through evaluation of structural alterations will be also discussed.

MEDI 260

Computational design, synthesis and characterization of novel mPGES-1 inhibitors

Ziyuan Zhou¹, ziyuan.zhou@uky.edu, Kai Ding², Shuo Zhou⁴, Yaxia Yuan⁴, Fang Zheng¹, Chang-Guo Zhan³. (1) University of Kentucky, Lexington, Kentucky, United States (2) Chemistry, University of Kentucky, Lexington, Kentucky, United States (3) Dept of Pharm. Sciences, University of Kentucky, Lexington, Kentucky, United States (4) Pharmaceutical Sciences, University of Kentucky, Lexington, Kentucky, United States

Purpose: Microsomal prostaglandin E synthase-1 (mPGES-1) inhibitors are recognized as better alternatives of cyclooxygenase (COX) inhibitors, and considered to be the next generation of anti-inflammatory drugs. But none of the reported mPGES-1 inhibitors has been proven clinically useful to date. In this study, we aimed to identify novel inhibitors of mPGES-1 with new scaffolds through structure-based virtual screening.

Methods: A combination of computational and experimental approaches was used to achieve our goal. The methods used include large-scale structure-based virtual screening, molecular dynamic simulation, molecular mechanics-Poisson-Boltzmann surface area (MM-PBSA) binding free energy calculation, molecular docking, organic synthesis, and *in vitro* activity assays. Our virtual screening was performed on compounds in the NCI libraries, containing a total of ~260,000 compounds. The virtual screening was followed by *in vitro* activity assays against human mPGES-1.

Results: Based on the virtual screening, the top-41 compounds were ordered for *in vitro* activity assays against mPGES-1. Based on the results of *in vitro* activity assays, 15 compounds exhibit significant inhibition against mPGES-1 at 10 μ M. Of the 15 compounds, 8 compounds have been determined for their IC₅₀ values (about 300 nM to 8000 nM). The identified active compounds were further tested against COX enzymes (COX-1 and COX-2). Most of these new inhibitors of mPGES-1 do not inhibit COX-1 and COX-2. Starting from the scaffolds of these novel mPGES-1 inhibitors, more potent and selective inhibitors of mPGES-1 have been designed, synthesized, and assayed.

Conclusion: We have successfully identified a set of compounds that can potentially inhibit mPGES-1 without inhibiting COX-1 and COX-2. These results suggest that our combined computational and experimental approach works for rational design and discovery of new mPGES-1 inhibitors.

MEDI 261

Evaluation of 2-amino-pyrimidine derivatives as antimicrobial agents and IspF inhibitors

Sydney Watkins¹, swatkins@niu.edu, **Debarati Ghose**³, **Chanté A. Muller**¹, **Zheng Zhang**¹, **Joy M. Blain**¹, **Zak Lazowski**¹, **Stephanie T. McDonald**¹, **Taylor Riggins-Walker**¹, **James R. Horn**¹, **R. Meganathan**³, **Heike Hofstetter**², **Timothy J. Hagen**¹. (1) Chemistry and Biochemistry, Northern Illinois University, Sycamore, Illinois, United States (2) Chemistry, UW-Madison, Madison, Wisconsin, United States (3) Biological Sciences, Northern Illinois University, DeKalb, Illinois, United States

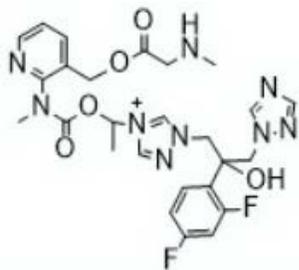
There is a pressing need to discover new antibiotics to combat serious and life threatening infections. An attractive target is the methylerythritol 4-phosphate (MEP) pathway, which is used by most bacteria to produce isoprenoids. The enzyme IspF is the fifth enzyme of the MEP pathway and contains a zinc ion. A series of 2-amino-pyrimidine derivatives were designed to potentially bind to the zinc ion present in the IspF enzyme. Differential scanning fluorimetry, surface plasmon resonance, and saturation transfer difference NMR techniques were used to evaluate binding. Antimicrobial activity was demonstrated by Kirby-Bauer disk diffusion assays against nine strains of bacteria.

MEDI 262

Design and synthesis of 1-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)propyl)-2-(1-((methyl(3-(((methylcarbamoyl)oxy)methyl)pyridin-2-yl)carbamoyl)oxy)ethyl)-1H-1,2,4-triazol-2-ium

Lee Peyton, lpeyton0@gmail.com, **Mehrnoosh Hashemzadeh**, **Scarlett Gallagher**. Pima College, Tucson, Arizona, United States

With the research project we have been performing at Pima College, we have designed and synthesized a novel triazole antifungal we believe has intrinsic properties other triazole antifungals currently on the market are lacking. We have successfully characterized our desired compound, and are performing the necessary *in-vitro* testing to confirm the activity of our molecule. We have reason to believe the compound synthesized may have characteristics necessary to be utilized for intramuscular injection.



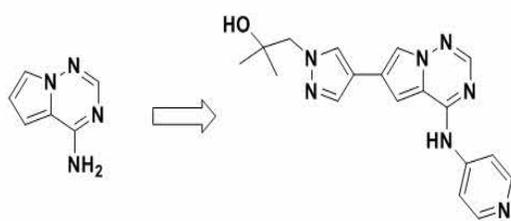
MEDI 263

Fragment-based lead discovery of a novel Map4k4 inhibitor

Lan Wang, lanwang@gene.com. Discovery Chemistry, Genentech, Foster City, California, United States

Mitogen-activated protein kinase kinase kinase kinase 4 (Map4k4), a serine/threonine protein kinase, is upregulated in many types of cancers. To study the function of MAP4K4 *in vivo* for oncology indications, a FBLD approach was initiated to identify a proof of concept tool compound. A screening of 2361 fragments at 100 μM as singletons using Surface Plasmon Resonance (SPR) led to 225 confirmed hits with LE > 0.35. We followed up on multiple fragment hits initially and settled on two fragments for full optimization. Starting from a micromolar pyrrolotriazine fragment hit (**1**), we developed a series of single digit nanomolar inhibitors of MAP4K4 by utilizing crystal structures and molecular modeling. Several compounds in the pyrrolotriazine series also demonstrated favorable *in vivo* bioavailability in mouse. In parallel, we also worked on an oxazole fragment (**2**), and through scaffold hopping approach we obtained a novel and potent naphthyridine series of MAP4K4 inhibitors. These efforts resulted in the identification of compound G-495, which had excellent potency, kinase selectivity and mouse PK profile, providing a robust chemical probe for studying MAP4K4 biological function *in vivo*.

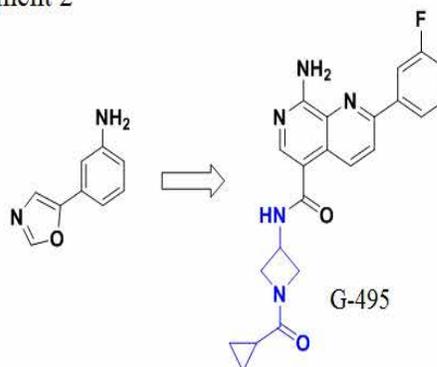
Fragment 1



SPR K_D : 88 μM
LE: 0.56

LC3K IC_{50} : 0.005 μM
LE: 0.44

Fragment 2



SPR K_D : 88 μM
LE: 0.42

LC3K IC_{50} : 0.003 μM
LE/LE = 0.38 / 6

MEDI 264

SDAP substrate analog enantioselective synthesis and ninhydrin-based enzyme assay for the dapE-encoded bacterial enzyme diaminopimelate desuccinylase (DapE)

Tahirah Heath⁴, *theath@luc.edu*, Marlon Lutz^{5,4}, Cory Reidl¹, Boguslaw Nocek⁶, Miguel Ballicora⁴, Richard C. Holz³, Ken Olsen⁴, Daniel P. Becker². (1) chemistry and biochemistry, Loyola University, Franklin Grove, Illinois, United States (2) Dept of Chemistry, Loyola University Chicago, Chicago, Illinois, United States (3) Marquette Hall Rm 208, Marquette University, Milwaukee, Wisconsin, United States (4) Chemistry and Biochemistry, Loyola University Chicago, Chicago, Illinois, United States (5) Regis Technologies, Morton Grove, Illinois, United States (6) Argonne National Laboratories, Lemont, Illinois, United States

The development of resistance to current antibiotics makes the discovery of novel compounds towards new target pathways an urgent challenge. DapE encoded N-succinyl-L,L-diaminopimelic acid desuccinylase is a late stage enzyme in the meso-diaminopimelate (mDAP)/lysine biosynthetic pathway. DapE hydrolyzes N-succinyl-L,L-diaminopimelic acid (L,L-SDAP) to succinate and diaminopimelate which is then converted to mDAP by both gram negative and gram positive bacteria. DapE has been identified as an attractive potential antibiotic target for two main reasons 1) the proliferation of many bacteria is dependent on lysine and DAP, which are essential for the peptidoglycan cell wall synthesis, and 2) both DAP and lysine are synthesized in pathways that do not occur in humans. The structural and functional conservation of DapE among various bacteria suggests the possibility of creating a new broad spectrum antibiotic through the inhibition of DapE. A previous enzyme assay for DapE employed monitoring cleavage of the amide bond in L,L-SDAP at 225nm, which precludes the testing of many organic compounds that strongly absorb in this region. Therefore, we have developed a new assay which employs monitoring a much higher wavelength of 570nm through detection of Ruhemann's purple produced by reaction of ninhydrin with the hydrolyzed primary amine product of a newly synthesized L,L-SDAP substrate analog, produced by a new enantioselective synthesis, which enables identification of new novel inhibitors without substrate interference. Using this new robust enzymatic assay we are able to provide inhibitory data for several analogs synthesized from ligands identified through HiTS.

MEDI 265

Discovery of a new class of highly potent small-molecule degraders of BET bromodomain proteins

Fuming Xu¹, *xuming1948@163.com*, Jiantao Hu³, Bing Zhou⁵, Zhuo Chen⁶, Mei Lin⁷, Longchuan Bai⁸, Chao Y. Yang⁹, Donna McEachern⁴, Sally Przybranowski¹⁰, Ester Fernandez-Salas¹¹, Bo Wen¹², Duxin Sun¹³, Shaomeng Wang². (1) The University of Michigan, Ann Arbor, Michigan, United States (2) Cancer Center Room 3215, Univ of

Michigan, Ann Arbor, Michigan, United States (3) University of Michigan, Ann Arbor, Michigan, United States (5) Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, China

The Bromo- and Extra-Terminal (BET) proteins, including BRD2, BRD3, and BRD4 proteins are epigenetic reader proteins and attractive new therapeutic targets for human cancers and other diseases. Several small-molecule BET inhibitors are currently in clinical development for cancer treatment. Recently, Proteolysis Targeted Chimeras (PROTAC)-based small-molecule degraders of BET (BET degraders) have been developed and demonstrate much improved potency *in vitro* and *in vivo* in different tumor models over small-molecule BET inhibitors. We present herein our design, synthesis, and evaluation of a new class of BET degraders based upon our Carboline-containing BET inhibitors. Our most potent BET degrader ZBC260 can effectively induce rapid degradation of BET proteins in leukemia and solid tumor cells at pico-molar to sub-nanomolar concentrations. ZBC260 achieve IC₅₀ values of pico-molar to sub-nanomolar in inhibition of cell growth against human leukemia and solid tumor cell lines. Significantly, ZBC260 can induce rapid and complete tumor regression in animal models of human cancer at well tolerated dose-schedules in mice. ZBC260 represents the most potent BET degrader reported to date and a promising lead compound for extensive testing as a potential new therapy for cancer and other human diseases.

MEDI 266

Glyoxylate shunt as an antibacterial drug target

Sean Bartlett¹, sb956@cam.ac.uk, Alyssa McVey², Audrey Crousilles², Martin Welch², David R. Spring¹. (1) Department of Chemistry, University of Cambridge, Cambridge, United Kingdom (2) Department of Biochemistry, University of Cambridge, Cambridge, United Kingdom

Pseudomonas aeruginosa is a ubiquitous Gram-negative pathogen and a prominent cause of chronic infections in patients with cystic fibrosis. Under the control of quorum sensing it adapts to the microaerobic and nutrient-deplete conditions in the lung by directing carbon flux through an anaplerotic pathway called the glyoxylate shunt.

The glyoxylate shunt enables growth on acetate, in the form of Acetyl-CoA, derived from oxidation of fatty acids. Normally this carbon would be lost in the tricarboxylic acid cycle as CO₂, meaning that acetate may be used to generate energy but not biomass. The shunt bypasses these decarboxylative steps to provide a route from isocitrate to gluconeogenic precursors, thereby facilitating both anabolism and gluconeogenesis.

It is known that inactivation of the glyoxylate shunt renders *P. aeruginosa* avirulent in a murine burn model; the correct functioning of the shunt is necessary for fitness and virulence. It is therefore a promising target for the study and treatment of chronic infections.

Metabolic regulation of the glyoxylate shunt deviates significantly from the better understood *E. coli* model. Our group is studying the enzymology of the TCA/shunt branch point in *P. aeruginosa* using a combination of techniques. We describe our study of the structure and function of isocitrate lyase and malate synthase, the discovery of small molecules that inhibit them, and their evaluation as antibacterial agents. We also describe the development of new methodology for the synthesis of these leads.

MEDI 267

Synthesis of monomers with enzymes and chemical reagents for preparing polyester

Kyu Ah Kim¹, *kakim803@gmail.com*, *Chiman Song*², *Kyu-Sung Jeong*³, *Chan S. Cheong*². (1) *George Mason University, Manassas, Virginia, United States* (2) *Kist, Seoul, Korea (the Republic of)* (3) *Department of Chemistry, Yonsei University, Seoul, Korea (the Republic of)*

Polyester is a promising material for the various application fields. In order to prepare this polymer, monomer should be selected properly. Lactones were used to polymerize as monomers and selected lactones containing chiral center were used for special applications. These monomers were synthesized with heterocyclic ketones using whole cells of recombinant *E. coli* overexpressing cyclohexanone monooxygenase (CHMO) from *Acinetobacter* sp. NCIMB 9871 strain and *m*-CPBA. The chemoselectivity, regioselectivity and mild condition of this biotransformation, opposite to that observed by chemical oxidation justifies its importance in organic chemistry. These lactones will be polymerized using lipase under the specific condition for further modification to increase potentiality.

MEDI 268

Exploit ionic liquids to significantly improve asymmetric reduction of 3,5-bis(trifluoromethyl) acetophenone catalyzed by *T. asperellum* ZJPH0810 cells

Jun Li, lijun@hzm.edu.cn, Wenting Du. Hangzhou Medical College, HangZhou, China

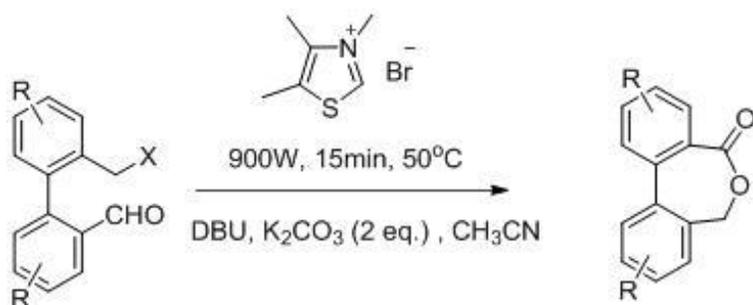
The aim of this study is to exploit a Good's buffer ionic liquid-containing system for efficient production of (*R*)-[3,5-bis(trifluoromethyl)phenyl] ethanol [(*R*)-BTPE] from 3,5-bis(trifluoromethyl) acetophenone (3,5-BTAP) catalyzed by *T. asperellum* ZJPH0810 cells. A new type of quaternary ammonium-based ILs (QAILs) were investigated and compared with more conventional imidazolium or pyridium-based ILs. [N1,1,1,1]⁺ come up as a Good's buffer of cation to the *T. asperellum* ZJPH0810 cell in the bioreduction of 3,5-BTAP to (*R*)-BTPE. After selection and optimization, the highest concentration of substrate and product for the bioreduction observed in [N1,1,1,1][PF₆]-containing system were 75 mM and 65.4 mM, which is 1.5-fold and 1.4-fold increase in contrast to that in monophasic aqueous system, respectively. [N1,1,1,1][PF₆] was considered to be a Good's buffer IL for the *T. asperellum* ZJPH0810-catalyzed bioreduction owing to its good biocatalytic reduction results and excellent cell biocompatibility.

MEDI 269

Microwave-promoted/assisted method for rapid preparation of biaryl seven-membered lactones

Wenting Du, ddwttt@163.com. Hangzhou Medical College, Zhejiang, China

A simple and efficient procedure for the synthesis of biaryl seven-membered lactones under microwave-promoted is reported. The umpolung reaction was promoted by N-heterocyclic carbenes under oxygen, with anhydrous potassium carbonate as base and with 1,4,7,10,13,16-Hexanoxacyclooctadecane (18-crown-6) as a phase-transfer catalyst. The practical protocol was found to be compatible with different structurally diverse substrates. Moderate to excellent yields, operational simplicity are the main highlights.



MEDI 270

Potent but potentially non-cardiotoxic anthracycline anti-cancer analogs

Jerrett A. Holdaway², jerrettholdaway@boisestate.edu, Ariel L. Petty¹, Pete L. Barnes³, Phil Moon², Ken Cornell¹, Don L. Warner². (1) Boise State University, Boise, Idaho, United States (2) Chemistry and Biochemistry, Boise State University, Boise, Idaho, United States (3) Material Science and Engineering, Boise State University, Boise, Idaho, United States

Cancer is the second leading cause of death in the United State, surpassed only by heart disease. In 2016, there will be an estimated 1.6 million new cases along with nearly 600,000 deaths. The anthracycline Doxorubicin (DOX) is an important and effective anti-cancer agent. However, its use is severely limited due to the risk of irreversible dose dependent cardiomyopathy and eventual congestive heart failure, limiting the maximum lifetime cumulative dose to only 550 mg/m². To eliminate this cardiotoxicity, a new analog, GPX150, was synthesized through several steps: removal of DOX's C-13 carbonyl followed by conversion of the quinone moiety to a less reactive iminoquinone species. The alterations, while alleviating the cardiotoxic side effects, decrease the potency of the drug fourfold, translating into a significantly higher cost for treatment. As well, several cancer cell lines, initially quite sensitive to DOX, have shown insensitivity to GPX150. Modification of the 3'-amine of DOX's sugar moiety with electrophilic substituents such as a pyrrolino, propyrrolino, morpholino, and cyanomorpholino have resulted in 100-1000 times increase in observed potency. This drastic increase in potency has been attributed to the electrophilic moieties being able to form covalent aminal adducts between the DOX compound and amino containing groups such as guanine bases of the DNA. Presumably, the covalent DNA linkage is responsible for the observed intensification as an anti-cancer agent. A variety of GPX150 analogs with differing electrophilic moieties have been synthesized with yields up to 90% and purity greater than 95%, as determined by mass spectrometry and NMR spectroscopy. Preliminary results indicate that, compared to GPX150, the new analogs have increased efficacy against a variety of cancer cell lines. In one instance, for example, the new compound is 10-30 times more potent than GPX150 and has activity typically equivalent to DOX. These and related results will be presented.

MEDI 271

Targeting the colchicine binding site in tubulin for cancer treatment: Structural optimization of ABI-231 leads to improved potency and microsomal stability

Qinghui Wang³, qwang17@uthsc.edu, Kinsie Arnst³, Duane D. Miller¹, Wei Lf². (1) Department Pharmaceutical Sciences, College of Pharmacy, Memphis, Tennessee, United States (2) University of Tennessee HSC, Memphis, Tennessee, United States (3) pharmaceutical sciences, university of tennessee, Memphis, Tennessee, United States

Although microtubule have achieved great success in the last decades and was among the most prolific therapeutic targets for developing anticancer drugs, however, the clinical efficacy of these drugs is often limited by the multidrug resistance that is acquired over the course of treatment, and additional research efforts are urgently demanded to address this shortcoming. We have previously reported 2-aryl-4-benzoylimidazole (ABI) scaffolds including ABI-I, II and III showing highly potent antiproliferative effects both *in vitro* and *in vivo* by interacting with colchicine binding site in tubulin. Importantly, these ABI compounds were able to circumvent multidrug resistant mechanisms. As the most potent analog in these series, ABI-231 had an average IC₅₀ value of 5.2 nM against panels of melanoma and prostate cancer cell lines. Molecular modeling studies revealed the existence of unoccupied pockets in the vicinity of the indole moiety that might potentially serve as an opportunity to strengthen the ligand-protein interaction. With the hypothesis in mind, we developed a new synthetic method that was amicable to introduce indole rotation as well as versatile functional groups on the indole moiety. Of these 27 analogs, the 4-methyl-3-indolyl analog showed significantly improved antiproliferative activity in contrast to ABI-231 and its strong inhibitory effect was confirmed through a clonogenic assay. New analogs shared the same mechanism of action as ABI-231 as demonstrated from confocal microscopy, tubulin polymerization assay as well as x-ray crystal structures in complex with tubulin. Wound healing migration assays indicated that the new analogs may potentially induce antimetastatic action through the inhibition of cancer cell motility and migration. Additionally, *in vitro* metabolism studies revealed that our new analogs possess favorable metabolic and chemical properties. Currently, *in vivo* efficacy is being evaluated for the best analogs.

MEDI 272

Novel, highly selective direct thrombin inhibitors: *In vivo* studies demonstrate efficacy with lower bleeding risk

Chengpei Xu, Daniel Clemens, Subhadra Dash, Kenneth Lin, Mamatha Reddy, Georg Neckermann, Simon Yau, Elaine To, Lev Igoudin, Stephanie Chang, Samuel Keutzer, Piotr Zalicki, Nichole Sandoval, Sivan Sizikov, David Williams, Mohan Sivaraja, John Zhang, Timothy Shiau, Kevin Short, M. Angels Estiarte, aestiarte@verseon.com, Anirban Datta, David Kita. Verseon Corporation, Fremont, California, United States

Patients with non-valvular atrial fibrillation or venous thromboembolism typically require anticoagulation prophylaxis/treatment. Direct oral anticoagulants (DOACs) offer certain advantages over warfarin and heparins, but there remains a significant unmet need for anticoagulant agents with better risk-benefit profiles than currently available treatments.

We have identified a new class of highly potent and selective direct thrombin inhibitors (VE-DTIs) with a unique mode of action. VE-DTIs prevent clot formation with efficacy similar to that of the DOACs in *in vivo* rodent models of arteriovenous shunt and tissue-factor venous stasis thrombosis. In addition to their strong anticoagulation properties, the VE-DTIs demonstrate significantly lower bleeding in several rodent bleeding models

compared to the DOACs. In this poster we will present *in vitro* and *in vivo* ADMET and efficacy results for a series of promising lead compounds.

MEDI 273

Synthesis of a known binder of the GRB2 SH2 domain from naphthaldehyde

Alyssa Bowlsby², *alybowsby@gmail.com*, **Carolynn Arpin**¹. (1) Phsc 216, CSU Chico, Chico, California, United States (2) CSU, Chico, Chico, California, United States

GRB2 (Human Growth Factor Receptor Bound Protein 2) is an adaptor protein whose overexpression has been linked to CML (chronic myeloid leukemia). Importantly, GRB2 binds its partners through its SH2 (Src Homology 2) domain and acts as a homodimer. Thus, to enhance GRB2 inhibition, we've set out to link two known monomeric binders of the GRB2 SH2 domain to yield novel dimeric antagonists. These synthesized dimeric antagonists are designed to mimic endogenous phosphotyrosine binding residues and simultaneously bind dual GRB2 SH2 domains, thus blocking the activity of the GRB2 homodimer. This method of GRB2 inhibition has not yet been studied despite the significant potential for increased binding affinity of the antagonists and subsequent enhanced biological activity. The motivation, design, and novel synthesis of our dimeric antagonists will be presented, along with a preliminary evaluation of biological activity.

MEDI 274

Novel synthetic strategies to disarm the myeloid cell leukemia-1 oncoprotein

Samuel J. Hughes¹, *sam_hughes1996@hotmail.co.uk*, **Brandon Drennen**⁴, **Maryanna E. Lanning**², **Steven Fletcher**³. (1) Chemistry, Cardiff University, Cardiff, United Kingdom (2) Pharmaceutical Sciences, University of Maryland Baltimore, Baltimore, Maryland, United States (3) Dept of Pharmaceutical Sciences, University of Maryland School of Pharmacy, Baltimore, Maryland, United States (4) Pharmaceutical Sciences, University of Maryland School of Pharmacy, Baltimore, Maryland, United States

Myeloid cell leukemia-1 (Mcl-1) is an anti-apoptotic member of the BCL-2 family of proteins, and it is often upregulated in a diverse range of tumors, including breast cancer, non-small cell lung cancer, acute myeloid leukemia and multiple myeloma. Importantly, it has been demonstrated that Mcl-1 is essential for the growth of these tumors, hence the inhibition of Mcl-1 potentially affords a new avenue for the discovery of novel chemotherapies. While there have been notable successes in the selective antagonism of Mcl-1's sister proteins Bcl-2 and Bcl-x_L with small-molecules, the analogous selective inhibition of Mcl-1 has proven less fruitful, although clinical candidates are beginning to emerge. As a complementary strategy to our current approach where small-molecules are designed to delve deeply into the p2 pocket on the surface of the protein, we will present our progress on novel synthetic strategies to inhibit Mcl-1. Specifically, validated Mcl-1 inhibitors have been modified with chemical warheads towards the targeted covalent inhibition of the protein, while the grafting of

thalidomide moieties is anticipated to cause the destruction of Mcl-1 through targeted hijacking of the cell's protein degradation machinery.

MEDI 275

Improving the *in vitro* and *in vivo* performance of a ¹⁷⁷Lu-labeled phosphoramidate-based PSMA inhibitor with an albumin-binding motif

Cindy J. Choy¹, *cchoy@cancertargetedtechnology.com*, Xiaoxi Ling², Jonathan J. Geruntho², Sophia Beyers¹, Joseph Latoche², Beatrice Langton-Webster¹, Carolyn J. Anderson², Clifford E. Berkman¹. (1) Cancer Targeted Technology, Woodinville, Washington, United States (2) Radiology, University of Pittsburgh, Pittsburgh, Pennsylvania, United States

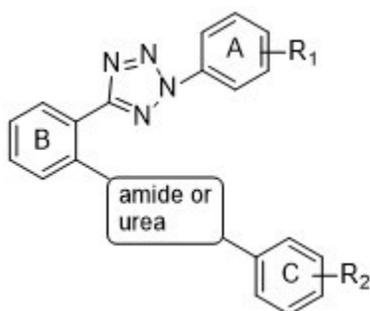
Prostate-specific membrane antigen (PSMA) has been described as an 'ideal biomarker' because of its restricted expression on prostate cancer cells and is increased on late-stage, androgen-independent, and metastatic prostate tumors. Consequently, PSMA remains an active target for the delivery of imaging and therapeutic agents. One of the challenges for small-molecule PSMA inhibitors with respect to delivering therapeutic payloads is their rapid renal clearance. In order to overcome this pharmacokinetic challenge, we outfitted a ¹⁷⁷Lu-labeled phosphoramidate-based PSMA inhibitor (CTT1298) with an albumin-binding motif (CTT1403) and compared its *in vivo* performance with that of an analogous compound lacking the albumin-binding motif (CTT1401). The radiolabeling of CTT1401 and CTT1403 was achieved using click chemistry to connect ¹⁷⁷Lu-DOTA-N₃ to the DBCO-bearing CTT1298 inhibitor cores. A direct comparison *in vitro* and *in vivo* performance was made for CTT1401 and CTT1403; the specificity and efficacy by means of cellular uptake and internalization, biodistribution, and therapeutic efficacy were determined for both compounds. While both compounds displayed excellent uptake and rapid internalization in PC3-PIP cells, the albumin binding moiety in CTT1403 conferred clear advantages to the PSMA-inhibitor scaffold including increased circulating half-life and prostate tumor uptake that continued to increase up to 48-72 h post-injection. This increased tumor uptake translated into superior therapeutic efficacy of CTT1403 in PSMA+ PC3-PIP human xenograft tumor.

MEDI 276

Synthesis and evaluation of phenyl tetrazole derivatives with amide or urea linkers as BCRP/ABCG2 inhibitors

Nehaben Gujarati¹, *nehagujarati@gmail.com*, Leli Zeng^{1,2}, Zhe-Sheng Chen¹, Vijaya L. Korlipara¹. (1) Pharmaceutical Sciences, St. John's University, New York, New York, United States (2) School of Chemistry and Chemical Engineering, Sun Yat-Sen University, Guangzhou, China

Overexpression of breast cancer resistance protein (BCRP/ABCG2) has been shown to play an important role in the development of resistance to various anticancer agents. A series of BCRP/ABCG2 inhibitors with an amide group between rings A and B (benzamide series) and amide or urea linker between rings B and C were previously synthesized and evaluated in our lab. Two of the analogues in the benzamide series exhibited an activity that was comparable to that of the known ABCG2 inhibitor, Fumitremorgin C (FTC), and were selective against ABCB1 overexpressing KB-C2 cells. In the present study, a series of phenyl tetrazole derivatives with an amide or urea linker between rings B and C along with various substitutions at R₁ and R₂ positions has been synthesized. The results of their ABCG2 inhibitory activity using drug resistant H460/MX20 cells and selectivity against ABCB1 protein in KB-C2 cells along with their effects on the ATPase enzyme using ABCG2 overexpressed membranes will be presented.



MEDI 277

Two-faced synthetic α -helix mimetics based on heterocyclic cores as dual BCL-2/MDM2 inhibitors

Ivie L. Conlon⁴, **Brandon Drennen**³, *bdrennen_92@umaryland.edu*, Maryanna E. Lanning¹, Lijia Chen¹, Samuel J. Hughes³, Paul T. Wilder⁴, Steven Fletcher². (1) Pharmaceutical Sciences, University of Maryland Baltimore, Baltimore, Maryland, United States (2) Dept of Pharmaceutical Sciences, University of Maryland School of Pharmacy, Baltimore, Maryland, United States (3) Pharmaceutical Sciences, University of Maryland School of Pharmacy, Baltimore, Maryland, United States (4) University of Maryland, Baltimore, Baltimore, Maryland, United States

Overexpression of the anti-apoptotic proteins of the BCL-2 family, specifically Bcl-2, Bcl-x_L and Mcl-1, leads to tumorigenesis through the sequestration of neutralization of their pro-apoptotic counterparts through their BH3 α -helical "death" domains. Likewise, MDM2 and MDMX sequester and inhibit the tumor suppressor p53 through its α -helical transactivation domain. It is noteworthy that both members of the BCL-2 and MDM2 families are co-upregulated in many cancers, including acute myeloid leukemia. Significantly, the recognition patterns presented by the hydrophobic faces of both the BH3 and p53 α -helical domains are similar; Bak-BH3: Leu62, Ile65 and Phe69; p53: Phe19, Trp23 and Leu26. On the opposing faces, there are conserved aspartates between the *i* and *i*+4 residues; while this plays a functional role in the BH3 α -helices in

the recognition of their target proteins, its role in the p53 α -helix is only to ensure proper folding into the secondary structure. Therefore, towards more efficacious anti-cancer agents, we have designed small-molecule α -helix mimetics based on heterocyclic scaffolds to co-emulate both faces of the BH3 and p53 α -helices. Preliminary evaluations have validated our polypharmacology design rationale, and we will present an investigation into the structure–activity relationships of our novel α -helix mimetics across the BCL-2 and MDM2 families of proteins.

MEDI 278

Kröhnke pyridine synthesis permits facile access to novel Mcl-1 inhibitors

Ivie L. Conlon³, ivie.conlon@umaryland.edu, Daniel Van Eker⁴, Jamal Chauhan¹, Paul T. Wilder³, Steven Fletcher². (1) School of Pharmacy, University of Maryland, Baltimore, Maryland, United States (2) Dept of Pharmaceutical Sciences, University of Maryland School of Pharmacy, Baltimore, Maryland, United States (3) University of Maryland, Baltimore, Baltimore, Maryland, United States (4) Cardiff University, Cardiff, United Kingdom

Protein-protein interactions (PPIs) are central to many different cellular processes, and dysfunctional PPIs have been associated with disorders such as cancer and neurodegenerative diseases. The BCL-2 family of proteins is comprised of pro- and anti-apoptotic members. The PPIs between opposing BCL-2 family members within the cell are tightly regulated to ensure proper cellular function. Particularly, the anti-apoptotic proteins bind and neutralize their pro-apoptotic counterparts via their α -helical BH3 domains. In various cancers, including acute myeloid leukemia and multiple myeloma, there is an imbalance of the BCL-2 proteins, where anti-apoptotic proteins such as Bcl-2, Bcl-x_L, and Mcl-1 are upregulated, leading to cell immortality and tumorigenesis. A validated strategy towards the inhibition of the anti-apoptotic proteins is through specific mimicry of key side chains projected from opposing faces of the BH3 α -helices with non-peptidic scaffolds. However, such rational designs are associated with lengthy synthesis and pan-BCL-2 inhibitory activities. With the emergence of selective Bcl-2 and Bcl-xL inhibitors, the development of selective Mcl-1 inhibitors represents an unmet medical need. Furthermore, expedited access to such inhibitors is desirable. Towards these ends, we will present our on-going work on the discovery of selective Mcl-1 inhibitors through the exploitation of the Kröhnke pyridine synthesis that accelerates access to a compound library of novel two-faced, synthetic α -helix mimetics.

MEDI 279

Dual stimuli-activatable oxidative stress amplifying hybrid anticancer drugs

Joungyoun Noh, wjddusdl34@naver.com, Donghyuck Yoo, Eunkyeong Jung, Dongwon Lee, Junghoon Lee, 0112wjdgns@naver.com. Chonbuk National University, Jeonju, Chonbuk, Korea (the Republic of)

Compared to normal cells, cancer cells have a higher level of reactive oxygen species (ROS) and are under oxidative stress due to aberrant metabolism and disruption of redox homeostasis which drive their proliferation and promote progression and metastasis of cancers. Cancer cells adapt to oxidative stress by up-regulating antioxidant systems such as glutathione (GSH) to counteract the damaging effects of ROS. The altered redox balance and biological difference between normal cells and cancer cells provide a basis for the development of anticancer agents which are able to generate pharmacological ROS insults to kill cancer cells preferentially. We therefore hypothesized that elevation of oxidative stress in cancer cells by depleting glutathione or generating ROS is a logical therapeutic strategy for the development of anticancer drugs. In this work, we present novel hybrid anticancer therapeutic agents which not only weaken antioxidant defense systems but also elevate ROS production simultaneously, leading to massive ROS accumulation preferentially in cancer cells and subsequent ROS-mediated cell death. Two major components of the hybrid anticancer drugs are cinnamaldehyde and quinone methide (QM). QM is known to rapidly alkylate GSH to trigger apoptotic cell death and is therefore regarded as an antioxidant inhibitor with anticancer activity. Cinnamaldehyde is a major component of cinnamon tree and has been known to induce intracellular ROS generation, leading to apoptotic cell death. We coupled QM and cinnamaldehyde in single molecules which are able to generate QM and cinnamaldehyde in a dual stimuli triggered manner. QM and cinnamaldehyde were also expected to act in a synergistic manner to amplify oxidative stress, leading to preferential killing of cancer cells *in vitro* and *in vivo*. The hybrid anticancer drugs significantly elevated oxidative stress in cancer cells, leading to enhanced apoptotic cancer cell death through mitochondrial membrane disruption, cytochrome c release, activation of pro-caspase 3, deactivation of signal transducer and activator of transcription-3 and DNA fragmentation. The hybrid anticancer drugs also significantly suppressed the tumor growth in a mouse model of tumor xenografts without notable side effects. Oxidative stress amplifying hybrid drugs hold tremendous potential as a new anticancer drug and provide a new therapeutic paradigm which can be extended to development of hybrid anticancer drugs.

MEDI 280

Covalent-docking based protocol for the rational design of covalent inhibitors

Xiaobo Wan, xiaobowan2013@gmail.com. Department of Cellular and Molecular Pharmacology, UCSF, San Francisco, California, United States

Targeted covalent inhibition of drug targets has become a powerful methodology in the field of drug discovery and ligands capable of forming a covalent bond with lysine often show enhanced selectivity and potency. However, rational design lysine targeting probes is a challenging problem for which the linker regions connecting the scaffold and warheads need to be optimized. Here, we will describe our effectors to develop a new computational protocol to design new lysine-targeting probes. We applied this method prospectively to discovery new covalent kinase inhibitors.

MEDI 281

Study of the chemical composition, cytotoxic and antitumor activities of *Croton discolor*

Andrea del Mar d. Ramos Vicente, *andrea.ramos4@upr.edu*. Natural Sciences, University of Puerto Rico at Cayey, Cidra, Puerto Rico, United States

From past generations, our grand and great grandparents have been using plant leaves in different ways to treat colds, coughs, headache, and upset stomach among many others. Therefore, folk medicine has opened the doors for the study of a broad world of natural products for the treatment of diseases. Past studies on several specific kinds of native and endemic plants of Puerto Rico have shown to have growth inhibition activity against some breast adenocarcinoma cell lines. One of the most active plants against these cell lines was *Croton discolor* showing a growth inhibition > 84% against MCF-7 and T47D cell lines. *Croton discolor* is a native shrub found in Puerto Rico and US Virgin Islands from the *Euphorbiaceae* family. Its common name is lechecillo and its leaves have been used in tea to treat rheumatism and leukemia. We decided to collect leaves and cortex in greater quantity. The aim of this work is to isolate and identify the chemical compounds in leaves and cortex of *Croton discolor* responsible for the biological activity. The plant was collected, dried and extracted with a mixture of CH₂Cl₂-MeOH (1:1). The resulting crude extract was suspended in water and extracted with solvents of different polarities. Purification by column chromatography of the chloroform extract of the leaves is in process. Extracts and purified fractions are analyzed by nuclear magnetic resonance (NMR) and the purification process is monitored by thin layer chromatography. The cytotoxic activity of all extracts had been tested against different cell lines derived from solid tumors including ovarian (A2780, SKOV3), breast (MCF-7, MDA-MB-231), prostate (PC-3, LNCAP), mammary epithelial cells (MCF-10A), and neuroblastoma (SH-SY5Y). The chloroform extract showed the higher cell viability percent results against MDA-MB-231 and SH-SY 5Y cell lines. Other bioactivity results will be presented.

MEDI 282

***Trypanosoma cruzi* sirtuin-2 construction by modeling threading and molecular dynamics**

Glaucio M. Monteiro Ferreira², *gmf@usp.br*, **Gustavo H. Trossini**¹, **Flávio d. Emery**¹, **Vinicius G. Maltarollo**¹. (1) Faculdade de Ciências Farmacêuticas, Sao Paulo -SP, Brazil (2) Toxicologia e Análises toxicológicas, Faculdade de Ciências Farmacêuticas - USP, São Paulo, São Paulo, Brazil

Currently, 6 to 8 million people are affected by Chagas disease, a parasitosis caused by *Trypanosoma cruzi*. Chagas's disease is endemic in Latin America but has spread to other continents by migratory movements. Every year, there are 56,000 new cases and approximately 12,000 deaths from complications of the disease. Actually, there are only

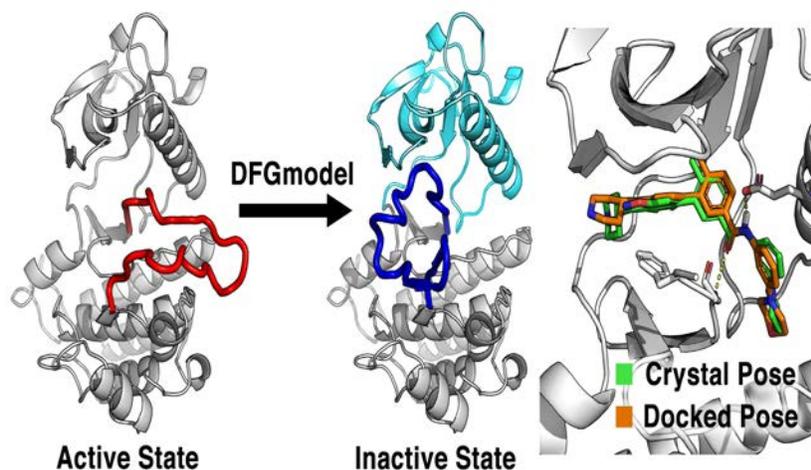
two drugs available to the treatment, Nifurtimox and Benzonidazole, but are effective only in the acute phase, present several side effects and parasite resistance¹. Face of this panorama, it is imminent need to new drugs against *T. cruzi*. One of the most important enzymes for *Trypanosoma cruzi* infection is the Silent information regulator 2 (Sir-2), which is NAD⁺ dependent class III histone deacetylases. In this context we use modeling threading to perform the construction of the *T. cruzi* Sir-2 (tcSir2) and Molecular Dynamics method to refine the model to be used in the strategy of Structure-Based Drug Design (SBDD). The choice of template by Modelling threading (human sirtuin 2 [hSir2] PDB code: 3ZGV) was based on highest degree of identity (46%) and low probability of error (2×10^{-68}). The model construction was performed in two steps: alignment (1D) of the sequences in 4.5.4 SEAVIEW software; and building of the 3D model with MODELLER v9.14 program. The Ramachandran plot of the lowest energy models was presenting 0.5% outliers (Figure 1), as main validation. We used Molecular Dynamic (MD) calculations to refine the model, that employed in Gromacs program using molecular force fields (amber99+ILDN), box water cubic 0,5, ions Cl and Na was added, the concentration used was physiological pH. The MD were performed for 100 ns, and the model was stable after 40ns. The figure below shows the model after MD was observed it was stable and also that the model has the same fold selected template (PDB code: 3ZGV) in finger example zinc residues were forecast in the same conformation is important to keep the fold.

MEDI 283

DFGmodel: Modeling protein kinases in inactive conformations and its applications in drug discovery

Peter Man-Un Ung, peter.ung@mssm.edu, Avner Schlessinger. Pharmacological Sciences, Icahn School of Medicine at Mount Sinai, New York, New York, United States

The catalytic domain of protein kinases exists in equilibrium of active and inactive states, in which in the aspartate-phenylalanine-glycine (**DFG**) motif (DFG-in/-out states) can undergo significant conformational changes. Limited structural data of the inactive, DFG-out conformations of kinases hampers the rational drug discovery and development of drugs targeting these conformations. We developed **DFGmodel**, a method that takes the sequence or the structure of a kinase in active conformation to generate models in DFG-out conformations. Models generated by DFGmodel accurately distinct known inhibitors from likely non-binders, suggesting that they are potentially useful for structure-based discovery of unique kinase modulators with optimal binding profiles. Finally, we applied DFGmodel to identify novel and potent inhibitors for a single kinase target (i.e. Dyrk1A), as well as to develop unique multi-kinase inhibitors which were confirmed in a *Drosophila* cancer model. Our computational approach provides structural framework for chemists and biologists to characterize kinases in the inactive states and explore new chemical spaces with structure-based drug design.



MEDI 284

Virtual screening for potential inhibitors of neuraminidase for influenza treatment

Elsa C. Gomez-Suarez, anilorak95@gmail.com. Universidad La Salle, Mexico City, Mexico

Influenza is an infectious disease caused by *Influenzavirus A*, which belongs to *Orthomyxoviridae* family. This disease results in about three to five million severe cases every year and between 250,000 to 500,000 deaths. In 2009, WHO declared that a new type of influenza A/H1N1 to be pandemic. Some antivirals have been effective to treat influenza, among these, neuraminidase inhibitors are among the most used like oseltamivir and zanamivir. However, there is a growing concern about the emergence of new forms of *Influenzavirus* that become resistant to oseltamivir and zanamivir, thus new inhibitors are needed. We have used *drug-like* ZINC database to identify molecules that could bind to neuraminidase (PDB code: 3CKZ). We identified near 1000 molecules with better docking score than zanamivir. Interestingly, some molecules showed 19.6% better docking score than zanamivir and interacted in a different site of the binding pocket (Figure 1). *In silico* pharmacokinetic properties were calculated and suggested that these compounds could have good ADME profile, including oral absorption. In conclusion, these compounds are an attractive starting point for the development of new neuraminidase inhibitors.

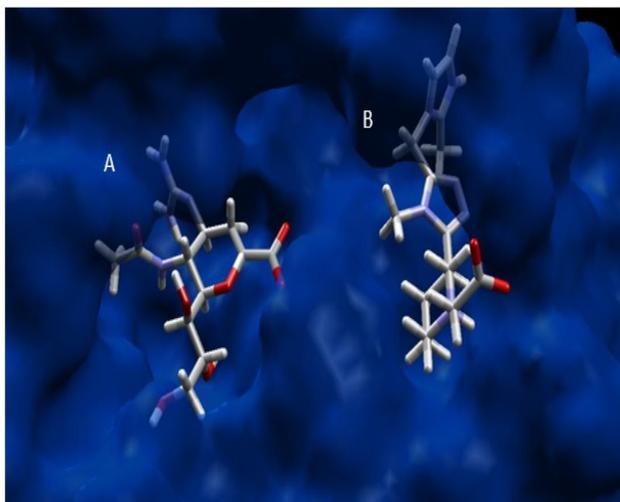


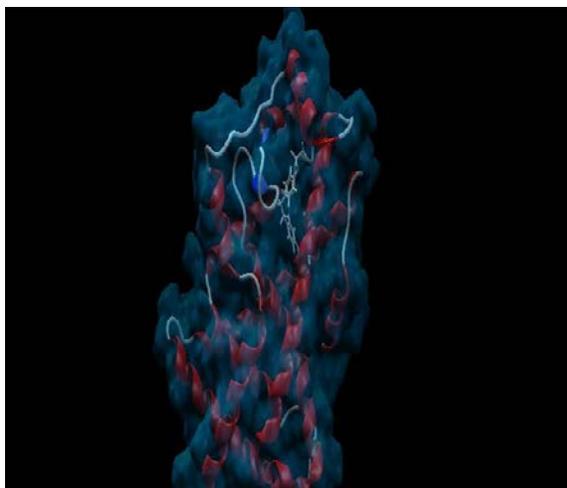
Figure 1. Docking poses of zanamivir and ZINC95362314 in active site of neuraminidase

MEDI 285

Anthranilic acid derivatives as potential multi-target drugs for metabolic syndrome treatment

Astrid Bravo, *astriidjmz@gmail.com*, Héctor González Álvarez, Marco A. Loza-Mejía.
Universidad La Salle, Mexico, Mexico

Metabolic syndrome (MS) is a complex disease in which diabetes, obesity, hyperglycemia, high cholesterol and high blood pressure are the most common disorders. It is estimated that around 20-25 % of the world's adult population have metabolic syndrome and they are twice as likely to die from some of their complications and three times as likely to have a heart attack or stroke compared with people without the syndrome. Currently, the research of multi-target drugs has been a challenging task in medicinal chemistry, and it has been proposed as an interesting approach to develop drugs aimed to treat complex diseases. In our group, we have evaluated some anthranilic acid derivatives *in silico* as potential ligands to some biological targets related to MS. Compound MABJ 1-48 was identified as a potential multi-target drug, as it showed high docking scores against aldose reductase, PDE5, PPAR- α , PPAR- γ and HMG-CoA. Its *in silico* pharmacokinetic profile was calculated, suggesting this molecule may have good ADME-Tox profile, including high oral absorption. This compound was synthesized and fully characterized by IR, ^1H NMR, ^{13}C NMR and MS. Finally, it was biological evaluated in a diet-induced obesity rat model and significantly lowered glucose levels. Hence, MABJ 1-48 is an interesting starting point for the development of new multitarget drugs for MS.



MEDI 286

Design and synthesis of N-substituted acridone derivatives as potential antibacterial and antiviral agents

Liliana J. Jimenez Sanchez, jazmin.js22@hotmail.com, Dante G. Juan-Guadarrama, Marco A. Loza-Mejía. Universidad La Salle, Mexico City, Mexico

Dengue is an important health issue in developing countries. Annually, Dengue Virus infects 390 million infections occur, leading to 500,000 hospitalizations and 20,000 deaths. Some N-allyl acridone derivatives have been studied as potential antivirals and have shown promising bioactivity. Docking studies suggested that they could interfere with viral IMPDH activity. We decide to prepare some isosters of these compounds to test their antibacterial and antiviral activities. In our group, we have developed an easy and direct method for the preparation of 2,3-diaminoquinolin-4(1H)-one, which was used to prepare compounds **1a-d** and **2a-d** as seen in Figure 1 in good yields. Docking studies were carried out in some dengue virus proteins (PDB codes: 1NF7, 5CUQ, 5HMY). Compound **2d** had the lowest docking score in IMPDH, suggesting it could be an interesting lead for new antivirals. In other hand, their antibacterial properties were tested, compound **1b** was active against Gram(+)bacteria.

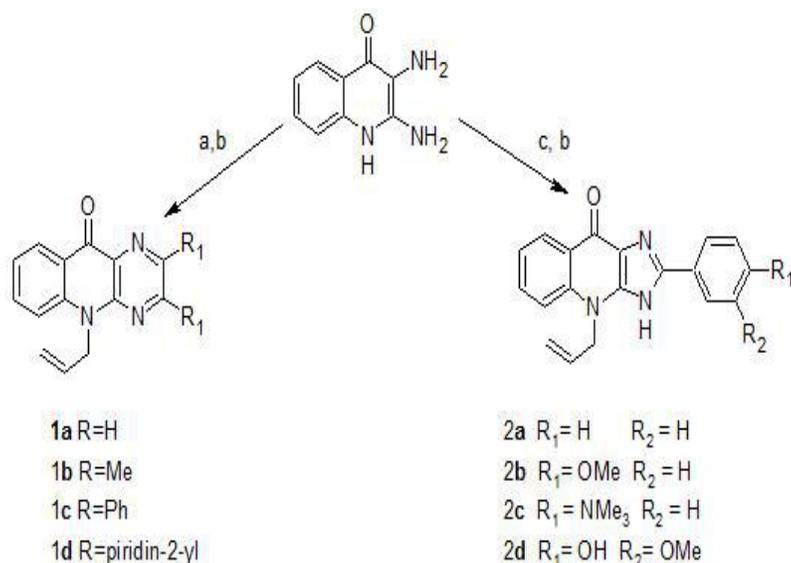


Figure 1. Preparation of *N*-allyl acridone isosters. a. 2,2 dicarbonyl compounds; b. NaOH 15%, CETB, DCM; c. Aryl-CHO *in situ*

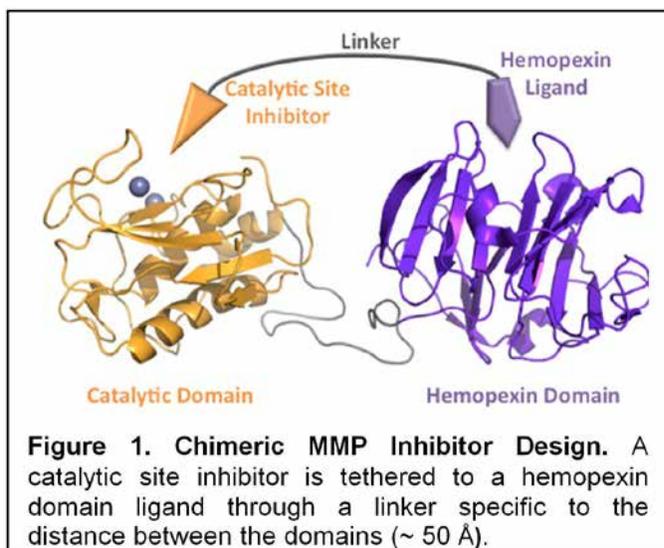
MEDI 287

Developing a chimeric heterobivalent platform as a selective imaging probe for MMP-14

Michael Pun, michael.pun@wsu.edu, Rosa Rios, Clifford E. Berkman. Chemistry, Washington State University, Pullman, Washington, United States

Matrix metalloproteinases (MMPs) play a key role in extracellular matrix remodeling, metastasis, and angiogenesis in cancer. While MMPs are expressed in normal tissue, they are upregulated in proliferating cancer cells. Within the MMP family there is considerable sequence and structural homology in the pro-peptide, catalytic, and hemopexin domains. Membrane-type 1 matrix metalloproteinase (MT1-MMP or MMP-14), is a cell-surface transmembrane protein that catalyzes the hydrolysis of collagen, the main component of extracellular matrix and it is highly correlated with metastasis in breast cancer. Due to the over-expression of MMP-14 on metastatic cancer cells, it has been recognized as an attractive target for both targeted diagnostic and therapeutic applications. A characteristic of MMP-14 as a membrane biomarker is that it undergoes internalization, enhancing its value in targeted molecular imaging applications. The *hemopexin and catalytic domains* of MMP-14, which are essential for collagenolysis, have been shown to be inhibited by small-molecule and peptide ligands. However, due to the highly conserved domain structure across all MMPs, such ligands are prone to lack specificity toward MMP-14 or other MMPs. To address this specificity problem, we have designed a chimeric hetero-bivalent platform targeted to both the catalytic and hemopexin domains. This platform is designed to impart specificity toward MMP-14 by incorporating a PEG spacer equivalent to the length of the linker peptide between the two domains of MMP-14 (~50 Å). The chimeric platform is also outfitted with a near-IR

fluorescent dye allowing for detection by confocal microscopy and *in vivo* optical imaging. Our progress towards the synthesis and initial preclinical imaging results of the platform will be presented.



MEDI 288

Structural characterization, homology modelling and virtual screening studies in shikimate kinase from methicillin resistant *Staphylococcus aureus*

Alejandro Favela¹, alejandro_ifca@hotmail.com, Alfredo Téllez-Valencia¹, Hugo Nájera³, Jorge Cisneros¹, Marcelo Gómez-Palacio⁴, Alicia Hernandez Campos², Claudia I. Avitia-Domínguez¹. (1) Facultad de Medicina y Nutrición, Universidad Juárez del Estado de Durango, Durango, Mexico (2) Departamento de Farmacia, Universidad Nacional Autónoma de México, Mexico, Mexico (3) Universidad Autónoma Metropolitana-Cuajimalpa, Ciudad de México, Mexico (4) Universidad Juárez del Estado de Durango, Durango, Mexico

Staphylococcus aureus is a particularly virulent microorganism; the main impact is due to methicillin resistant strains of *S. aureus* (MRSA), commonly found in hospitals and community, creating an increased necessity to develop a new antibacterial therapy. In this context, shikimate kinase (SK) is an essential enzyme in the shikimic acid pathway, which is an essential route involved in the biosynthesis of aromatic compounds in this bacterium. Here an *in silico* approach was used to characterize the *S. aureus* SK (SaSK) and find potential inhibitors. A 3D model of SaSK was constructed through homology modeling. Structural characterization was made using different programs such as Protparam, NPS Server, CYS_REC, and CASTp. A virtual screening protocol, into the active site of the enzyme, was applied to identify new potential inhibitors using Glide software. The “Drug like” subset of the ZINC small molecules database was used to this end, some physicochemical and drug likeness parameters were determined for these compounds. According to the structural analyses, alpha helices were the most

predominant followed by random coils, extended strand and beta turns and potential disulfide bridges were not detected. Furthermore, a predicted isoelectric point of 5.09 was obtained. Virtual screening results showed that the three compounds with the highest binding energy were ZINC34616948 which made hydrogen bonds with Asp117, Glu57, Arg138 and Arg61; ZINC03162994 that formed hydrogen bonds with Asp37 and Asp117, a salt bridge with Asp37 and a π - π stacking with Phe60; and ZINC70632388 which made hydrogen bonds with Arg61 and Arg138, a salt bridge with Glu57, Arg61 and Arg138. The structural data as well as the potential inhibitors reported for SaSK will serve as a starting point to obtain a new drug against MRSA.

MEDI 289

Selective inactivation of triosephosphate isomerase from *Trypanosoma brucei*

Alejandra G. Vazquez-Raygoza¹, avazquezraygoza@gmail.com, **Irene Betancourt**², **Rafael Castillo-Bocanegra**³, **Claudia I. Avitia-Domínguez**², **Erick Sierra**², **Mónica Valdez-Solana**², **Alfredo Téllez-Valencia**¹. (1) Facultad de Medicina y Nutrición, Universidad Juárez del Estado de Durango, Durango, Durango, Mexico (2) Universidad Juárez del Estado de Durango, Durango, Durango, Mexico (3) Farmacia, Div De Estudios De Posgrado, Mexico, Mexico

Human African Trypanosomiasis (HAT), a disease which affects 36 sub-sahara countries, is caused by *Trypanosoma brucei*. Currently, available treatments are limited to only five drugs that are highly toxic and parasite resistant strains are emerging. Therefore, there is an urgency to find new drugs against HAT. In this context, *T. brucei* depends on glycolysis as the unique source for ATP supply, hence enzymes from this pathway, such as the homodimeric enzyme triosephosphate isomerase (TbTIM) are attractive targets for drug design. In a previous work four benzimidazole derivatives that inactivate at 80% TbTIM activity (IVM-1, IVM-4, LCG-1 and LCG-5) were obtained. Here we presented the characterization of TbTIM inhibition by these compounds. TbTIM activity was determined in a coupled assay using α -glycerol phosphate dehydrogenase, in the direction of glyceraldehyde 3-phosphate to dihydroxyacetone phosphate, the reaction was started with 2 ng TbTIM. Inactivation studies were performed incubating TbTIM at a concentration of 5 μ g/ml during 2 h at 36 °C, and compounds at the indicated concentrations. Compounds were tested against human TIM (HsTIM) under the same conditions described above. Additionally, a molecular dynamics study to compare compounds binding mode, between TbTIM and HsTIM was applied. According to the kinetics study, these compounds showed an I_{50} (concentration that decreases 50% of enzyme activity) value of 115.2 μ M, 105.89 μ M, 102.2 μ M and 73 μ M for compounds IVM-1, LCG-1, LCG-5 and IVM-4, respectively. The four compounds were very selective against TbTIM with respect to HsTIM. Assays at different enzyme concentration indicated that these compounds act at the dimer interface. Molecular dynamics studies showed that they had more affinity for TbTIM than HsTIM. These molecules will serve as a hit to design more potent and selective compounds that could be used to obtain new drugs against HAT.

MEDI 290

Design, synthesis, and biological evaluation of novel C3-sustituted β -carboline-based HDAC inhibitors with potent antitumor activities

Yong Ling, *lyyy111@sina.com*, Jiao Feng, Jiefei Miao, Jing Guo, Yanfu Peng, Yanan Zhang. Nantong University, Nantong, Jiangsu, China

A series of β -carboline-based histone deacetylase (HDAC) inhibitors (**9a–l**) that incorporate the β -carboline structural motif into hydroxamic acids have been designed and synthesized. The effect of the substitution at the C3-amide on the HDAC inhibition and antiproliferative activities was investigated. Most of these compounds displayed significant histone deacetylase inhibitory effects and good antiproliferative activity with IC_{50} 's in the low micromole range. SAR studies show that the size and composition of the substitutions at the C3-amide clearly affect the activities of these compounds. Introduction of a basic nitrogen increased the potencies of these compounds, including compound **9h** which is the most potent of the series. The IC_{50} value of **9h** in HDAC inhibition was five-fold lower than SAHA. Furthermore, **9h** dose-dependently increased acetylation of histone H3 and α -tubulin, and induced DNA damage as evidenced by the hypochromism and the enhanced histone H2AX phosphorylation expression. Finally, **9h** also inhibited Stat3, Akt, and ERK signaling, important cell-growth promoting pathways that are aberrantly activated in most cancers. Our findings suggest that these novel 3-substituted β -carboline-based HDAC inhibitors may hold a great promise as therapeutic agents for the intervention of human cancers.

MEDI 291

Design and development of reversible inhibitors of lysine specific demethylase 1

Daniel P. Mould¹, *daniel.mould@cruk.manchester.ac.uk*, Alison McGonagle¹, Matthias Geitmann², Ulf Bremberg², Allan M. Jordan¹, Donald Ogilvie¹. (1) Drug Discovery, Cancer Research UK Manchester Institute, Manchester, United Kingdom (2) Beactica AB, Uppsala, Sweden

Background: LSD1 plays a key role in maintaining the balance between hematopoietic stem cell characteristics and differentiation to mature myeloid cells. Mechanism-based inhibitors of LSD1, developed from the monoamine oxidase (MAO) inhibitor tranylcypramine, have recently entered clinical trials for acute myeloid leukaemia (AML). While the mechanism and inhibitory potential of these compounds are now well defined, the potential for effective reversible inhibitors of LSD1 as clinical agents is less clear.

Methods: Starting from existing literature series, we employed rational medicinal chemistry and computational design using Cresset software to scaffold-hop into novel chemical space, while retaining activity against LSD1 in biochemical assays and by surface plasmon resonance (SPR). Selected compounds were tested in cellular assays and evaluated for their physicochemical properties *in vitro* and *in vivo*.

Results: Three series of reversible LSD1 inhibitors have been designed and synthesised. These novel series demonstrate a clear pharmacophore for effective inhibition. The most active series displays K_D values of <10 nM by SPR, and IC_{50} values of <300 nM in a CD86 cell assay. Compounds showed good metabolic stability in mouse hepatocytes, and despite displaying high efflux ratios were shown to be moderately bioavailable. Given the paucity of reversible and selective inhibitors of LSD1 available these compounds may be effective tools to drive further research into the use of LSD1 inhibitors as single agents, or in combination with other therapies.

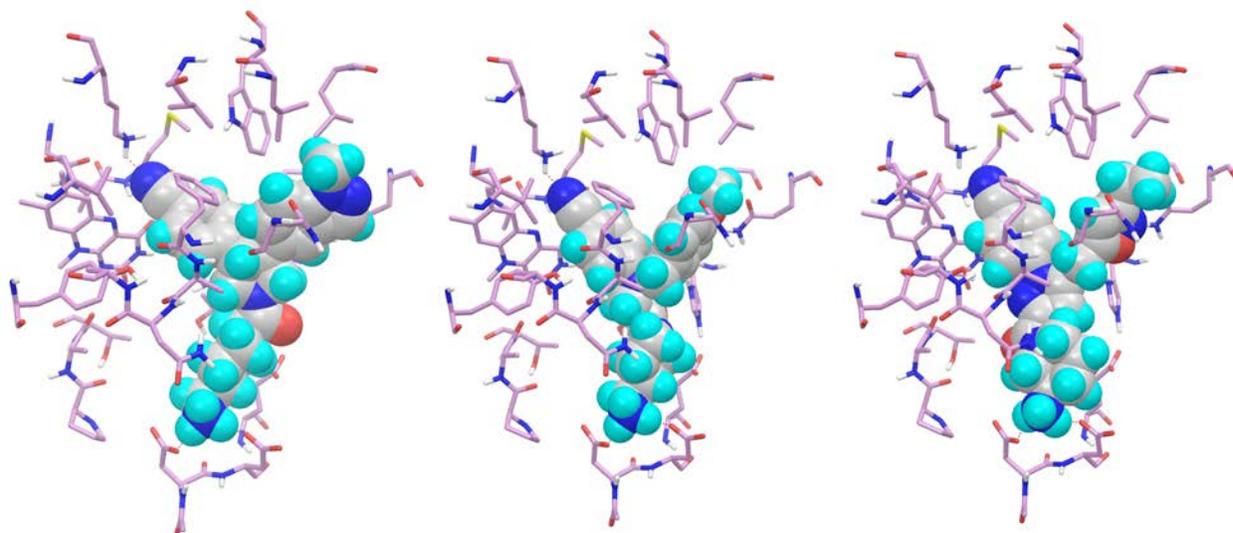


Figure 1. Glide (Schrödinger, New York) docking of reversible LSD1 inhibitors

MEDI 292

Evaluation of a targeted delivery strategy for development of potent trypanocidal peptide conjugates

Heeren Gordhan¹, hg8189@gmail.com, **Stephen Patrick**², **Jim Morris**², **Daniel C. Whitehead**¹. (1) Department of Chemistry, Clemson University, Clemson, South Carolina, United States (2) Microbiology, Clemson University, Seneca, South Carolina, United States

Human African Trypanosomiasis, or African Sleeping Sickness, is a disease caused by *Trypanosoma brucei*, or trypanosomes, and affects sub-Saharan Africa, with the potential to harm millions of people. Treatments have existed for fifty years, but are marginalized due to their poor efficacy or severe side effects. Bloodstream Form (BSF) trypanosomes generate energy currency through glycolysis. The first step of glycolysis involves the formation of 3-phosphoglycerate via the transfer of a phosphoryl group from ATP to the 6' hydroxyl group on glucose and is catalyzed by hexokinases called TbHK1 and TbHK2. Of the two hexokinases, TbHK1 has been chemically and

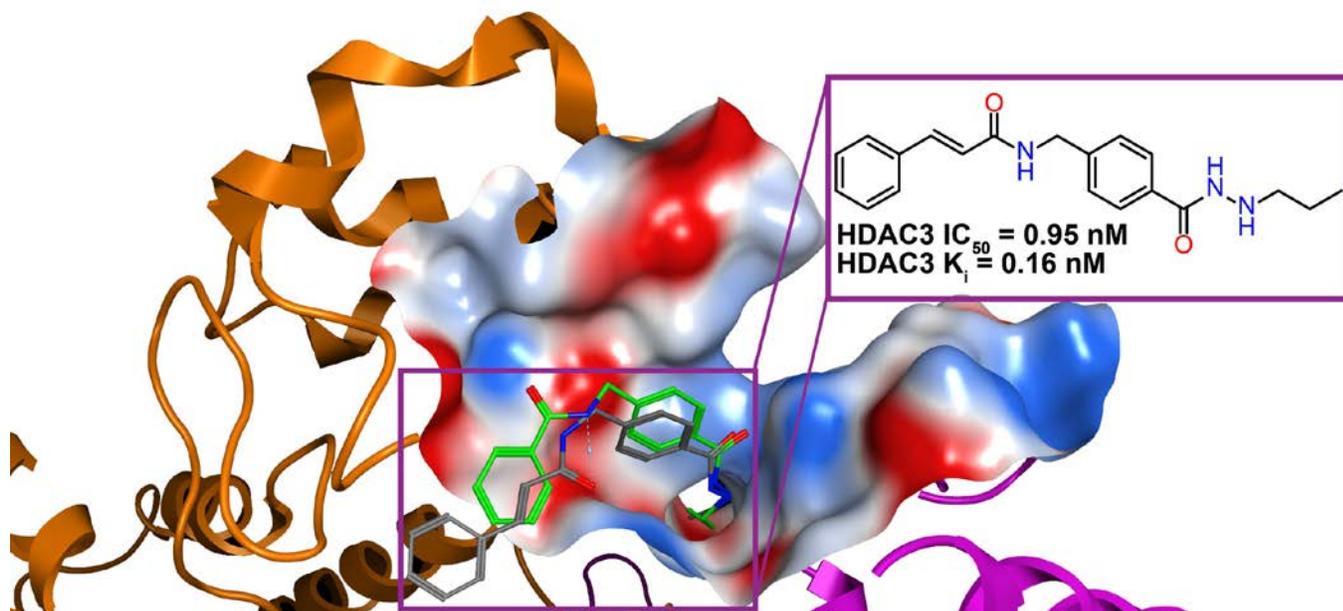
genetically validated as a target for the inhibition of the glycolytic pathway in *T. brucei*. A high-throughput screening (HTS) was conducted that revealed Ebselen, a drug currently in clinical trials for ischemic stroke patients, and benzamidobenzoic acid (*BABA*) as effective inhibitors of the enzyme. Nonetheless, these scaffolds reacted poorly against the whole-cell parasite. Work in our lab has focused on synthesizing derivatives of these two scaffolds in order to produce a highly active kinase inhibitor that can be translated into a potent trypanocide via conjugation to a peptide sequence. A carboxylic acid handle on the Ebselen and *BABA* derivatives was crucial to our strategy as this functionality serves as the handle to which the peptide may be attached via an amide bond. Two sets of Ebselen derivatives, bearing either a selenium or sulfur in the core motif, and one novel *BABA* derivative were synthesized. These inhibitors were first tested *in vitro* against TbHK1 and whole-cell trypanosomes to determine their baseline activity. To improve on their potency as trypanocides, the inhibitors were conjugated to a peptide sequence consisting of a peroxisomal targeting sequence (PTS), a linker, and the drug. Using the peptide-drug conjugate, we hoped that the drug could be selectively targeted to the glycosome of the trypanosome, where glycolysis occurs. This talk will present the syntheses of several novel kinase inhibitors and their peptide-conjugate analogues, followed by a discussion of the legitimacy of our targeted drug delivery of kinase inhibitors to the glycosome of trypanosomes.

MEDI 293

Development of allosteric hydrazide-containing class I histone deacetylase inhibitors for use in acute myeloid leukemia

Jesse J. McClure, mcclurj@musc.edu, Cheng Zhang, Elizabeth B. Inks, Yuri K. Peterson, Jiaying Li, C. James Chou. Drug Discovery and Biomedical Sciences, Medical University of South Carolina, Charleston, South Carolina, United States

One of the biggest hurdles yet to be overcome for the continued improvement of Histone Deacetylase (HDAC) inhibitors is finding alternative motifs equipotent to the classic and ubiquitously used hydroxamic acid. The N-hydroxyl group of this motif is highly subject to sulfation/glucuronidation-based inactivation in humans; compounds containing this motif require much higher dosing in clinic to achieve therapeutic concentrations. With the goal of developing a second generation of HDAC inhibitors, lacking this hydroxamate, we designed a series of potent and selective class I HDAC inhibitors using a hydrazide motif. These inhibitors are impervious to glucuronidation and demonstrate allosteric inhibition. *In vitro* and *ex vivo* characterization of our lead analogs' efficacy, selectivity, and toxicity profiles demonstrate they possess low nanomolar activity against models of Acute Myeloid Leukemia (AML) and are at least 100-fold more selective for AML than solid immortalized cells such as HEK293 or human peripheral blood mononuclear cells.



A novel series of hydrazone based class I HDAC inhibitors with low to sub nanomolar potency is described. These agents are particularly selective and deadly for Acute Myeloid Leukemia cells and represent a novel HDAC inhibitor moiety, being impervious to glucuronidation based inactivation that affects the hydroxamate containing compounds.

MEDI 294

Discovery of PF-06427878: A potent hepatoselective DGAT2 inhibitor evaluated in clinical trials

Kentaro Futatsugi, Kentaro.Futatsugi@gmail.com. Pfizer Inc, Cambridge, Massachusetts, United States

Diacylglycerol acyltransferase 2 (DGAT2) is one of enzymes that involve in the terminal step of the triacylglycerol synthesis. DGAT2 is expressed in liver, adipose, skin, and whole blood and its proposed primary functions include hepatic triacylglycerol synthesis and the production of very low-density lipoprotein (VLDL). While the inhibition of DGAT2 has shown promise for the treatment of several metabolic diseases in the preclinical space, the impact of DGAT2 inhibition in clinical settings remains unknown to date. This presentation will describe (1) the overview of the project strategy to address safety liabilities of the first pre-clinical candidate PF-06424439, and (2) the discovery of PF-06427878, a potent hepatoselective DGAT2 inhibitor originated from the structurally distinct chemical series relative to PF-06424439. PF-06427878 was ultimately advanced to phase 1 clinical trial for the evaluation of this novel mechanism.

MEDI 295

Translational feasibility of novel methionyl-tRNA synthetase inhibitors

Mansour Bassiri, *mbassiri@bioxiness.com*. Research & Development, Bioxiness Pharmaceuticals, Inc., Hercules, California, United States

The emergence of bacterial resistance to available antibiotics has caused great concern in the medical community and has created a need for the discovery of novel antibiotics. Aminoacyl-tRNA synthetases (aaRSs) play a crucial role in protein biosynthesis by catalyzing the binding of a specific amino acid to its corresponding tRNA as substrate for protein synthesis. Although aaRSs are essential enzymes in all living organisms, significant differences exist between bacterial and their eukaryotic aaRSs counterparts, providing an opportunity for developing species-selective inhibitors with potentially reduced host cell toxicity. Mupirocin, a natural product (isolated from *Pseudomonas fluorescens*) that inhibits isoleucyl-tRNA synthetase, is currently marketed as a topical antibiotic. However, overuse of this antibiotic has led to development of bacterial resistance.

Bioxiness has developed a synthetic series of close analogues of methionine (BXN-compounds) that target the bacterial methionyl-tRNA synthetase (MetRS). These compounds contain a nucleophilic “head” group that substitutes for the carboxylate group and is appropriate for adenylation; a “sidechain” that mimics methionine by modifications; and a “tail” group involving the amino group of methionine that can be either a substituted amine, or be aminoacylated to form a di- or oligo-peptide-like appendage to facilitate transport into the bacterial cell.

Our *in vitro* data show that BXN-compounds inhibit bacterial MetRS at IC₅₀= 32 nM and these non-optimized compounds currently possess MIC potency in the range of 30-100 µg/mL (116-388 µM) against representative strains of gram positive and gram negative bacteria, and have safety tolerance greater than 50 mM against eukaryotic cells. Furthermore, BXN-compounds unlike most antibiotics are not subject to the bacterial efflux pump AcrB, a pump that plays major role in antibiotic resistance phenotype.

MEDI 296

Identification and development of small molecule inhibitors of the aggregation of amyloid β

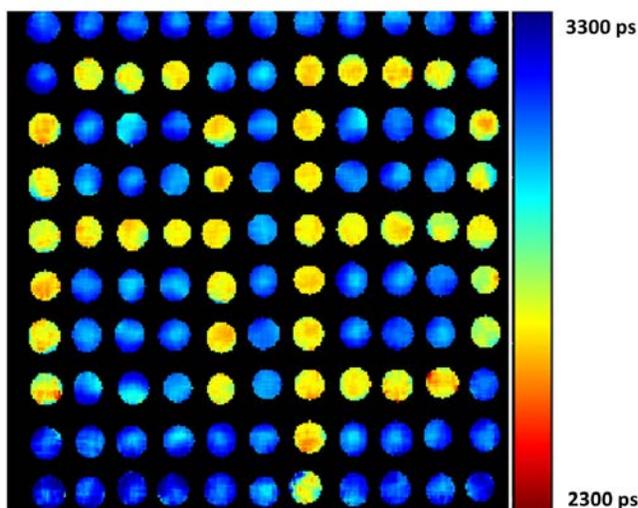
Suil Collins^{1,2}, *sc806@cam.ac.uk*, **Fabrice Gielen**², **Liisa van Vliet**², **Gabriele Kaminski**³, **Florian Hollfelder**², **David R. Spring**¹. (1) Department of Chemistry, University of Cambridge, Cambridge, United Kingdom (2) Department of Biochemistry, University of Cambridge, Cambridge, United Kingdom (3) Department of Chemical Engineering and Biotechnology, University of Cambridge, Cambridge, United Kingdom

Perturbing the process of amyloid aggregation is a key strategy for the development of therapeutic agents in a range of neurodegenerative diseases. A variety of issues with current screening methods, including lack of reproducibility and high reagent consumption, can limit the efficiency of identifying small molecule inhibitors. This project aims to overcome such restrictions and identify new inhibitory molecules, through the

development of a microfluidic based assay, which uses fluorescence lifetime imaging microscopy (FLIM) to monitor amyloid aggregation in up to 100 simultaneous assays.

Aggregation of fluorescently labelled amyloidogenic monomers into larger structures is accompanied with a reduction in the fluorescence lifetime of the attached fluorophore dye, as a result of FRET processes. This phenomenon can be exploited to monitor the aggregation profile of such peptides and the effect that different extrinsic factors or inhibitory compounds can have on the process. The FLIM screening process has been coupled with droplet-on-demand technology and the development of a new microfluidic device, in which 100 precisely ordered droplets containing monomeric peptide together with test compounds can be imaged concurrently over several hours.

Using this assay format, novel chemical libraries have been screened to identify potential inhibitors of the aggregation of amyloid β - the seminal neuropathic agent in Alzheimer's disease. IC_{50} values of hit compounds have been calculated and have identified two molecular scaffolds capable of exerting a strong inhibitory effect on the aggregation process. *In vivo* tests, using adapted FLIM imaging techniques, are currently being developed to validate the potential therapeutic value of the hit compounds.



MEDI 297

Drug-target residence time affects *in vivo* drug efficacy through multiple pathways

Kin S. Lee⁵, sing0621@gmail.com, Jun Yang³, Karen Wagner⁴, Connie J. Ng², Jun Niu², Alex Dickson¹, Bruce D. Hammock². (1) Biochemistry & Molecular Biology, Michigan State University, East Lansing, Michigan, United States (2) Dept Entomology, Univ California Davis, Davis, California, United States (3) University of California, Davis, Davis, California, United States (4) UCD Comprehensive Cancer Center, University of

California at Davis, Davis, California, United States (5) Pharmacology and Toxicology, Michigan State University, East Lansing, Michigan, United States

Lack of *in vivo* efficacy becomes the leading cause that fails drug candidate during clinical trials. Several studies suggested that the difficulties to translate *in vitro* potency to *in vivo* efficacy leads us to select less efficacious drug candidates. Therefore, an alternate method to determine *in vitro* potency of drug is needed in order to facilitate drug development processes.

The drug-target residence time (t_R) is proposed to be a better *in vitro* indicator to predict drug *in vivo* efficacy. Over the years, several reports indicated that compounds with long t_R show extended biological effects and better *in vivo* efficacy. Unfortunately, there is no direct evidence to demonstrate that t_R affects the duration of *in vivo* drug-target interaction.

Here, I used soluble epoxide hydrolase (sEH) as a model system to investigate the effect of t_R on drug efficacy. For the first time, we demonstrate that t_R not only affects the duration of *in vivo* drug-target binding but also affects the metabolism of drugs. The system developed and the data obtained from this study provide a nice platform for the field to develop a new pharmacokinetic/ pharmacodynamic modeling for drugs with different t_R and to better translate *in vitro* data to *in vivo* efficacy.

MEDI 298

Cytotoxicity study of α -hydroxy- β -dicarbonyl bearing synthetic metabolites of poecilosclerid sponge

Sanjay V. Malhotra¹, smalhotra@Stanford.edu, Michael P. Doyle², Phong Truong², Dipali Sharma³, Arumugam Nagalingam³, William C. Reinhold⁴, James R. Alleyn², Yang Yu². (1) Dept of Radiation Oncology, and Radiology, Stanford University, Palo Alto, California, United States (2) Department of Chemistry, University of Texas at San Antonio, San Antonio, Texas, United States (3) The Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University, Baltimore, Maryland, United States (4) Genomics and Bioinformatics Group, Developmental Therapeutics Branch, National Cancer Institute, National Institute of Health, Bethesda, Maryland, United States

The α -hydroxy- β -dicarbonyl moiety is a common structural motif in variety of natural products and pharmaceuticals, such as kjellmanianone, hamigeran A, or doxycycline. Moreover, this functional unit appears in key metabolites of pharmaceutically important natural products obtained from *Hamigera tarangaensis*. Motivated from these observations we have synthesized a library of 20 compounds. These compounds were prepared from 2,3-diketoesters by a highly enantioselective carbonyl-ene transformation using chiral copper (II) catalysts. To evaluate their potential for anticancer activity, we tested these compounds for cell growth inhibition on nine different cancer panels, representing diverse histologies i.e., leukemia, melanoma, and cancers of lung, colon, kidney, ovary, breast, prostate, and central nervous system. This screening identified a lead compound NSC781411 which selectively inhibits the growth of breast cancer cells. To elucidate the underlying molecular mechanisms, we examined the effect of this

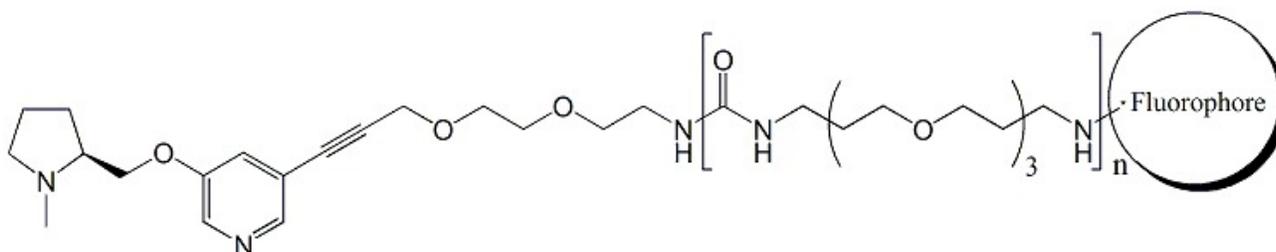
compound on the clonogenic potential using well-established representatives of estrogen-responsive (MCF7) and estrogen-refractory (MDA-MB-231) human breast cancers. These experiments showed that the compound inhibits clonogenicity and alters expression of proteins involved in growth and apoptosis. mRNA analysis of the NCI60 data suggested significant positive correlation of NSC781411 to response to radiation, opening the possibility of an effective complimentary treatment to the commonly used patient therapy (radiation).

MEDI 299

Second-generation fluorescent ligands for nicotinic acetylcholine receptors

Richard W. Fitch, *rfitch@indstate.edu*. Chemistry and Physics, Indiana State University, Terre Haute, Indiana, United States

We report here a second-generation of homologous fluorescent ligands for nicotinic acetylcholine receptors based on (S)-(N-methyl-2-pyrrolidiny)methoxypyridine (A-84543). We had previously prepared homologated non-fluorescent methyl-terminated PEG-homologs of A-84543 and shown high affinity for nicotinic receptors. However, our first generation fluorescent ligands, while competent in labeling receptors, had modest affinity, which we attribute to an unfavorable electrostatic interaction in the binding pocket due to the urea linkage we employed. This second-generation of ligands shifts the linkage further from the binding pocket, thus removing the offending interaction. Here we will discuss the changes in linkage chemistry, homologation and relationship of chain-length to affinity and labeling effectiveness using radioligand binding and fluorescence microscopy, as well as our efforts at developing a plate-based fluorescent binding assay using these ligands.



MEDI 300

Discovery of ARQ 092: A potent, selective allosteric inhibitor of AKT1, 2, 3 and AKT1-E17K in clinical development for cancer and rare diseases

Jean-Marc Lapierre, *jmlapierre@arqule.com*, Sudharshan Eathiraj, Yi Yu, Ronald E. Savage, Giovanni Abbadessa, Brian Schwartz. ArQule Inc, Burlington, Massachusetts, United States

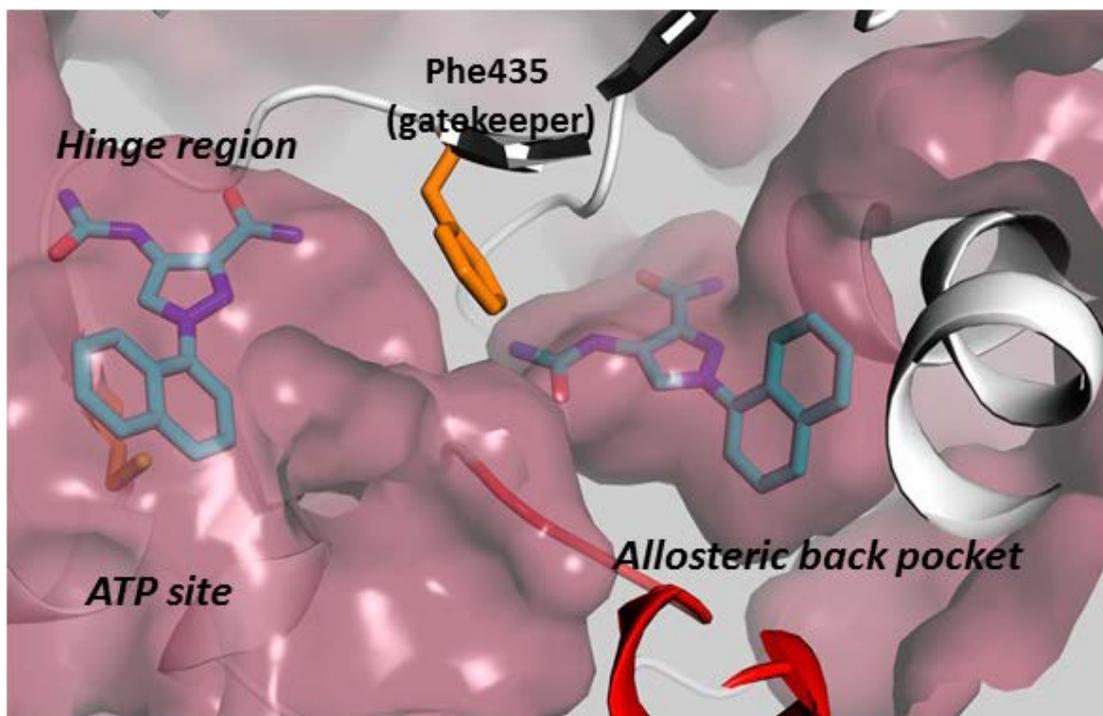
AKT is a pivotal component of the PI3K/AKT/mTOR pathway, attracting significant interest as a therapeutic target for cancer. Herein we describe the discovery of a pan-AKT and AKT1-E17K allosteric inhibitor, ARQ 092. The series of novel derivatives of the core scaffold 3*H*-imidazo[4,5-*b*]pyridine was first identified through biochemical and biophysical screening assays. A lead optimization campaign provided several highly potent allosteric AKT inhibitors, of which ARQ 092 emerged. ARQ 092 demonstrated potent and sustained inhibition of AKT activation and downstream targets including PRAS40 signaling in cancer cells. PK/PD studies demonstrated its effectiveness at inhibiting the AKT phosphorylation as well as additional downstream effectors following oral dosing in mice. ARQ 092 showed strong tumor growth inhibition in a human xenograft mouse model of PI3K/AKT driven endometrial adenocarcinoma. Co-crystallization studies with unphosphorylated form of AKT1 revealed an allosteric binding mode of ARQ 092 and highlighted the role of the key cyclobutyl amine moiety that is critical for pharmacological activity.

MEDI 301

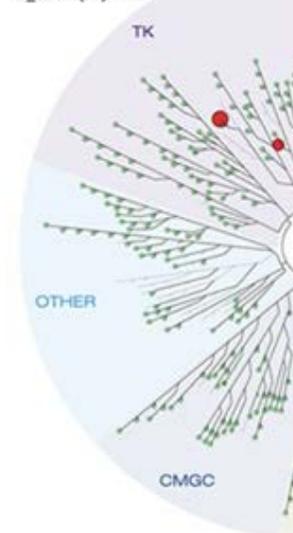
Allosteric inhibitors of Interleukin-2-inducible T cell kinase that selectively target its inactive conformation

Ann E. Aulabaugh, ann.aulabaugh@pfizer.com. Structural Biology Biophysics, Pfizer, Groton, Connecticut, United States

Interleukin-2-inducible T-cell kinase (ITK) is a critical component of signal transduction in T cells and is an attractive target for the treatment of T-cell mediated inflammatory diseases. Allosteric or ATP non-competitive inhibitors provide an opportunity for improved selectivity and biochemical efficiency within the cellular environment. We discovered allosteric Type III inhibitors of ITK that also bound its ATP site. Utilizing classical medicinal chemistry approaches, we have been successful in further optimization of these compounds resulting in selective inhibition of the inactive form of ITK. We describe how an integrated biophysical platform and enzymology studies enabled the discovery and optimization of allosteric ITK inhibitors into a kinome selective ITK allosteric lead that was efficacious in human whole blood and proximal cell-based assays.



451 Kinase Assays Tested
3 Interactions Mapped
S_Score(35) = 0.01



Dual site binding mode of initial lead and kinome selectivity of optimized allosteric inhibitor.

MEDI 302

BCR-ABL allosteric inhibitors targeting the myristoyl pocket: Optimization and pharmacological evaluation of substituted N-(4-(trihalomethoxy)phenyl)nicotinamide derivatives towards ABL001

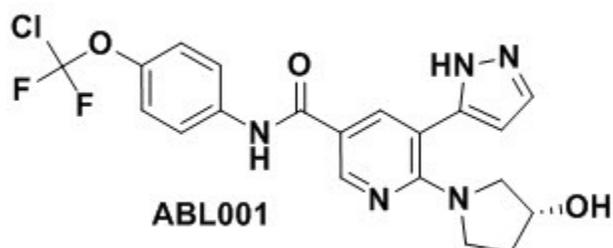
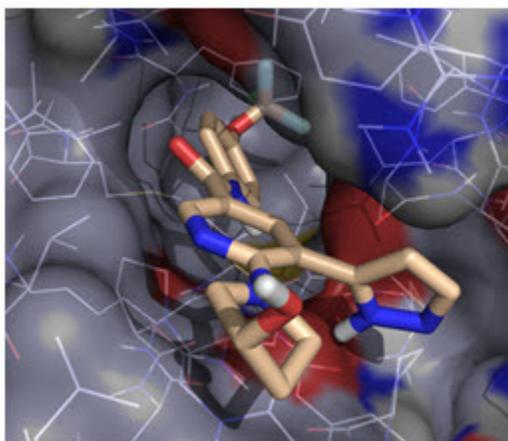
Joseph Schoepfer, joseph.schoepfer@novartis.com. Global Discovery Chemistry, Novartis Institutes for BioMedical Research, Novartis Pharma AG, Basel, Switzerland

Chronic myeloid leukemia (CML) results from a chromosome translocation leading to expression of the chimeric BCR-ABL oncoprotein. In a normal cell setting, the N-terminal cap (NCap) region of the SH3 domain of ABL1 is myristoylated and interacts with the myristoyl binding site within the C-terminal of the catalytic kinase domain (KD) to afford the assembled inactive state of ABL1. This negative regulatory mechanism is absent in the BCR-ABL fusion protein, which lacks the NCap and the ability to be myristoylated, resulting in constitutively active TK activity.

Although drugs that inhibit the TK activity of the BCR-ABL oncoprotein via an ATP-competitive mechanism are effective treatments for CML, some patients relapse due to the emergence of drug-resistant clones, in which mutations in the catalytic KD domain impede inhibitor binding.

We have previously reported the discovery of ABL001, a potent and specific ABL1 kinase inhibitor which inhibits BCR-ABL driven cell proliferation. This allosteric ABL1 inhibitor was identified based on X-ray crystallography, NMR and molecular modeling. ABL001 has been shown to mimic the role of the myristoylated N-terminus of ABL1 by

occupying its empty binding site, thereby restoring the negative regulation of BCR-ABL kinase activity. This presentation will describe the use of structure-based design to optimize potency and the further optimization of the pharmacological properties that culminated in the identification of ABL001. Structure-activity relationships will be described along with *in vivo* evaluation of selected N-(4-(trihalomethoxy)phenyl)nicotinamide derivatives in BCR-ABL dependent tumor xenograft models. ABL001 is currently being investigated in clinical trials in BCR-ABL positive leukemia patients.



MEDI 303

Identifying allostery in signaling enzymes

Wolfgang Peti, wolfgangpeti@email.arizona.edu. Chemistry and Biochemistry, University of Arizona, Tucson, Arizona, United States

Protein function originates from a cooperation of structural rigidity, dynamics at different timescales and allostery. However, how these three pillars of protein function are integrated is still only poorly understood. Here we show how these pillars are connected in the Protein Tyrosine Phosphatase 1B (PTP1B) and the serine/threonine protein Kinase p38, both validated drug target for diabetes and/or cancer. By combining new experimental and computational data on these enzymes and their variants in multiple states, we discovered novel fundamental and evolutionarily conserved conformational and dynamic events that are essential for enzyme function and allostery. Our data show that both conformational rigidity and dynamics are essential for controlling protein activity. Furthermore, both enzymes use both conformational and dynamic allostery to regulate their activity. This connection between rigidity and dynamics at different timescales is likely a hallmark of all enzyme function and can be readily leveraged specific drug design.

MEDI 304

Selective inhibition of a phosphatase to treat neurodegenerative diseases

Anne Bertolotti, *aberto@mrc-lmb.cam.ac.uk*. MRC LMB, Cambridge, United Kingdom

Protein phosphorylation regulates virtually all biological processes. Although protein kinases are popular drug targets, targeting protein phosphatases has been a challenge. We have discovered small molecules that safely and selectively inhibit a regulatory subunit of protein phosphatase 1 (PP1) in cells and in vivo. One of the small-molecule inhibitors is Sephin1, which selectively binds and inhibits the stress-induced PP1 regulatory subunit PPP1R15A, but not the related and constitutive PPP1R15B. Sephin1 thereby prolongs the benefit of an adaptive phospho-signaling pathway and protects cells from otherwise lethal protein misfolding stress. In vivo, Sephin1 selectively inhibits PPP1R15A to safely prevent the motor, morphological and molecular defects of two otherwise unrelated protein misfolding diseases in mice: the neurodegenerative diseases Charcot-Marie-Tooth 1B and amyotrophic lateral sclerosis (ALS). This demonstrates that regulatory subunits of phosphatases are drug targets, a property we exploited to safely prevent two neurodegenerative diseases in mice. We now show, using multiple quantitative readouts, that Sephin1 treatment initiated after disease onset slows down the progression of the disease in an aggressive model of ALS. This demonstrates the therapeutic potential of selective phosphatase inhibition. Because there are hundreds of phosphatases that could in principle be inhibited using the same paradigm, selective inhibition of phosphatases by targeting their regulatory subunits may open up a broad range of possibilities to safely and selectively manipulate cellular functions, perhaps for therapeutic benefit.

MEDI 305

Allosteric inhibition of SHP2 phosphatase

*Jorge Garcia Fortanet¹, Christine Chen¹, Yingnan Chen¹, Zhouliang Chen¹, Zhan Deng⁴, Brant Firestone¹, Peter Fekkes¹, Michelle Fodor¹, Pascal Fortin¹, Cary Fridrich¹, Denise Grunenfelder¹, Samuel Ho¹, Zhao Kang¹, Rajesh Karki¹, Mitsunori Kato^{2,1}, Nick Keen¹, Laura Labonte¹, Jay Larrow¹, Francois Lenoir¹, Gang Liu¹, Shumei Liu¹, Franco Lombardo¹, Dyuti Majumdar¹, Matthew Meyer¹, Mark G. Palermo⁵, Lawrence Perez¹, Minying Pu¹, Timothy Ramsey¹, William Sellers¹, Michael D. Shultz³, Travis Stams¹, Chris Towler¹, Ping Wang¹, Sarah Williams¹, Ji-Hu Zhang¹, **Matthew J. LaMarche¹**, *matthew.lamarche@novartis.com*. (1) Novartis, Reading, Massachusetts, United States (2) Computer Aided Drug Discovery, Novartis Institutes for BioMedical Research, Cambridge, Massachusetts, United States (3) Novartis Institutes for Biomedical Research, Inc., Cambridge, Massachusetts, United States (4) Novartis, Winchester, Massachusetts, United States (5) Novartis, Rindge, Massachusetts, United States*

SHP2 is a nonreceptor protein tyrosine phosphatase (PTP) encoded by the *PTPN11* gene involved in cell growth and differentiation via the MAPK signaling pathway. SHP2 also purportedly plays an important role in the programmed cell death pathway (PD-1/PD-L1). As an oncoprotein associated with multiple cancer-related diseases, as well as a potential immunomodulator, controlling SHP2 activity is of significant therapeutic interest. Recently in our labs, a small molecule inhibitor of SHP2 was identified as an

allosteric modulator that stabilizes the auto-inhibited conformation of SHP2. A high throughput screen was performed to identify progressable chemical matter and X-ray crystallography revealed the location of binding in a previously undisclosed allosteric binding pocket. Structure-based drug design was employed to optimize for SHP2 inhibition and several new protein-ligand interactions were characterized. These studies culminated in the discovery of 6-(4-amino-4-methylpiperidin-1-yl)-3-(2,3-dichlorophenyl)pyrazin-2-amine (SHP099, **1**), a potent, selective, orally bioavailable, and efficacious SHP2 inhibitor.

MEDI 306

Drug-target residence time model: A 10-year retrospective

R Copeland, *rcopeland@epizyme.com*. Epizyme, Cambridge, Massachusetts, United States

The drug-target residence time model was first introduced in 2006 and has been broadly adopted across the chemical biology, biotechnology and pharmaceutical communities. While traditional *in vitro* methods view drug-target interactions exclusively in terms of equilibrium affinity, the residence time model takes into account the conformational dynamics of target macromolecules that affect drug binding and dissociation. The key tenet of this model is that the lifetime, or residence time, of the binary drug-target complex and not the binding affinity *per se*, dictates much of *in vivo* pharmacological activity. Here, we revisit this model and highlight key applications over the past ten years.

MEDI 307

Protein dynamics and allostery in kinase activation

Natalie Ahn, *natalie.ahn@colorado.edu*. Chemistry & Biochemistry, University of Colorado at Boulder, Boulder, Colorado, United States

The MAP kinases, extracellular-regulated protein kinases 1 & 2 (ERK1/2), are important drug targets for cancers caused by oncogenic mutations in RAS and B-RAF. Preclinical studies show that cells from metastatic cancers with acquired resistance to RAF and MKK inhibitors can be effectively killed using small molecule inhibitors of ERK, two of which are in clinical trials. ERK1/2 are activated by dual phosphorylation at Thr and Tyr residues, both which are catalyzed by MKK1/2. We examined ERK2 activation using hydrogen exchange mass spectrometry (HX-MS), which can report localized conformational mobility within folded proteins, where exchange predominantly occurs through low energy fluctuations in structure, allowing transient solvent exposure. Changes in conformational mobility may impact protein function, even when structural changes are unobservable. Phosphorylation of ERK2 leads to changes in local HX, suggesting that kinase activation modulates protein motions. This was corroborated by NMR relaxation dispersion experiments, which revealed substantial changes in the

dynamics of the enzyme upon phosphorylation and activation. Preliminary data suggest a model in which activation of ERK2 leads to global exchange between two conformational states, where phosphorylation-regulated dynamics are coupled to steps in catalytic turnover. We further investigated high affinity ERK inhibitors which belong to families that have been shown to be effective towards cells with acquired resistance. While one inhibitor shifts the equilibrium completely towards one conformational state, a different inhibitor shifts the equilibrium completely to the opposite state. Therefore, ERK inhibitors have properties of conformation selection, in a manner correlating with slow dissociation properties. Remarkably, HX-MS reveals that slow tight binding inhibitors induce changes in hydrogen exchange across many regions of the enzyme beyond the active site, mimicking changes induced by AMP-PNP. By contrast, other inhibitors alter HX only within regions proximal to the active site. Taken together, our findings suggest that ERK2 is activated through the release of protein motions, and that this dynamic behavior may be exploited to improve the kinetic properties of ERK inhibitors.

MEDI 308

Drug target residence time in the early drug discovery phase: HSP90, a model to gain insight into the molecular mechanism of kinetics

Matthias Frech, *matthias.frech@merckgroup.com*. *Molecular Interaction & Biophysics, Merck KGaA, Darmstadt, Germany*

A decade ago target residence time was reconsidered as a parameter to increase the therapeutic index of drug molecules. An idea driven by Copeland, Swinney and others. They suggested that the key determinant of *in vivo* pharmacological activity is not only the binding affinity of a drug for its target but the lifetime, or residence time. The model for that concept seems simple: the pharmacological activity depends on the binding of the drug to its target, and pharmacological activity will usually persist while the drug remains bound.

Having in mind that a biological relevant drug target interaction kinetic is a parameter which could be used to develop improved drugs in terms of efficacy, side effects and even selectivity, the Innovative Medicines Initiative supported a European consortium to work on that topic. Pharma companies and academic institutions work jointly on kinetics for Drug Discovery.

Hsp90 is one of the projects beside many others. As a key protein involved in a variety of pathways including cell signaling, proliferation and survival, protein folding and tumor repression. Many proteins in tumor cells are dependent upon the Hsp90 protein folding machinery for their stability, refolding and maturation and therefore it has emerged as a promising target for the treatment of cancer. During our internal project, we observed a correlation between target occupancy, inhibitor residence time and cellular efficacy in a cell based assay.

In that sense the amino-terminal ATP-binding domain has been used as a model system to study the mechanism of interaction by different biophysical, structural and MD methods. A detailed structure-kinetic relationship study of HSP90 with resorcinol compounds revealed that variations of resorcinol substituents can cause the

compounds to change from fast to slow binding kinetics to elongated residence times of more than two orders of magnitude.

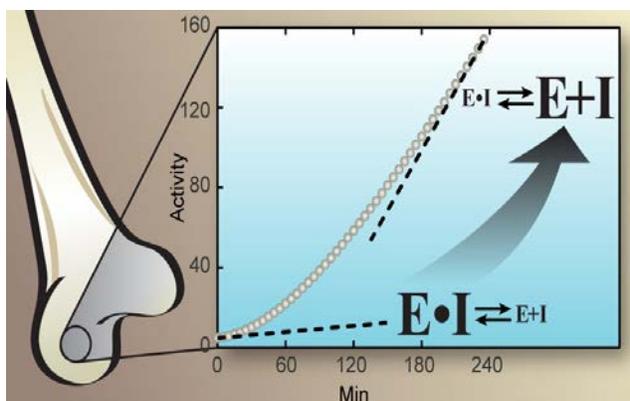
Transition state thermodynamic analysis by SPR in combination with 3D structural information provide first hypothesis on the energetic origins determining association/dissociation processes. The dynamics of the ligand and the protein in the solvent need to be accounted for to get a better understanding of the molecular mechanism and kinetics of inhibitor interaction with the target protein.

MEDI 309

Determination of *in vivo* enzyme occupancy utilizing inhibitor dissociation kinetics

Ming Lai¹, *MingTain_Lai@Merck.com*, **Dennis Murphy**¹, **Yangsi Ou**¹, **Danielle Euler**², **Keith Wessner**², **Sharon Adamski**², **Bin Luo**², **Gregg Wesolowski**³, **Robert Vogel**³, **Helmut Glantschnig**³, **Laura Lubbers**², **Stephen Carroll**¹. (1) *In Vitro Pharmacology, Merck & Co. Inc., West Point, Pennsylvania, United States* (2) *In Vivo Pharmacology, Merck & Co. Inc., West Point, Pennsylvania, United States* (3) *Bone Biology, Merck & Co. Inc., West Point, Pennsylvania, United States*

During drug discovery, assessment of *in vivo* target occupancy by therapeutic candidates is often required for predicting clinical efficacy. Current strategies for determining target occupancy include using radiolabeled or irreversible surrogates, which can be technically challenging and the results are often not sufficiently quantitative. We developed a straightforward method by applying slow-dissociation kinetics to quantitatively determine enzyme occupancy without using specialized reagents. We applied this method to determine occupancy of Cathepsin K inhibitors in bone tissues harvested from rabbit femurs. Tissues from dosed animal were harvested, flash frozen, lysed, then analyzed by a jump-dilution assay with substrate. The rate of substrate turnover was monitored continuously until reaching steady state and progress curves were fit with the equation $[\text{Product}] = v_s \cdot t + ((v_i - v_s) / k_{\text{obs}}) \cdot (1 - \exp(-k_{\text{obs}} \cdot t))$. The initial rate, v_i , represents the residual activity of the enzyme before inhibitor dissociation; v_s is the reaction rate after dissociation of the inhibitor. Occupancy is derived from the ratio of v_i / v_s . A significant benefit of the method is that data from both the occupied and unoccupied state are obtained in the same assay under identical conditions, which provides greater consistency between studies. The Cat K inhibitor MK-0674 (*in vitro* IC₅₀ ~1 nM) was tested in young rabbits (< 6 month old) and showed a dose dependent increase in occupancy, reaching essentially complete occupancy at 1.0 mg per kg. In addition the method enables measurement of the total Cat K in the target tissue. Results confirmed complete occupancy even as the osteoclasts responded to higher doses with increased enzyme production. This effect was evident in PK/PD models of Phase II Clinical data that informed the dosing regimen in patients.



MEDI 310

Linking target engagement and *in vivo* drug activity: Insights into target vulnerability

Peter J. Tonge², peter.tonge@stonybrook.edu, **Fereidoon Daryaei**², **Zhuo Zhang**², **Kayla Gogarty**², **Stewart L. Fisher**^{1,3}. (1) SL Fisher Consulting, LLC, Framingham, Massachusetts, United States (2) Chemistry, Stony Brook University, Setauket, New York, United States (3) C4 Therapeutics, Cambridge, Massachusetts, United States

Predicting drug efficacy in humans remains a major barrier to the development of novel therapeutics. We propose that the kinetics of drug-target interactions, and in particular the life-time of the drug-target complex (residence time), should be included in predictions of drug activity, since drug and target are not at equilibrium *in vivo*. To inform the development of mechanistic PK/PD models that incorporate drug-target kinetics, we are quantifying the molecular factors that modulate the coupling of drug-target residence time to prolonged drug activity following compound washout. This analysis provides direct insight into target vulnerability. Data on three systems will be discussed: reversible inhibitors of two antibacterial targets, the enoyl-ACP reductase from *Staphylococcus aureus*, and the LpxC enzyme from *Pseudomonas aeruginosa*, and a covalent inhibitor of Bruton's tyrosine kinase (Btk), a target for treating diseases stemming from B cell dysregulation.

MEDI 311

Discovery of GDC-0853: A potent & selective BTK inhibitor for the treatment of lupus & rheumatoid arthritis

Wendy B. Young, young.wendy@gene.com. Discovery Chemistry, Genentech, South San Francisco, California, United States

Bruton's tyrosine kinase (Btk) plays a critical role in B cell maturation and survival in addition to regulating myeloid cell inflammatory cytokine production, making it an attractive target for the treatment of immunological disorders such as lupus and

rheumatoid arthritis (RA).

At Genentech, we have invented GDC-0853, a highly potent, selective, non-covalent Btk inhibitor that is currently in Phase II clinical trials for RA and Lupus. In this presentation, we will describe the SAR, preclinical *in vivo* Lupus and RA efficacy results, DMPK and toxicology investigations leading up to the discovery and selection of our lead clinical candidate, GDC-0853. Additionally, the initial results from our Phase 1 clinical trials will be shared.

MEDI 312

Development of the selective, allosteric RIPK1 kinase inhibitors

Malek Najjar¹, Chalada Suebsuwong², Soumya Ray³, Gregory Cuny², Alexei Degterev¹, alexei.degterev@tufts.edu. (1) Tufts University, Boston, Massachusetts, United States (2) University of Houston, Houston, Texas, United States (3) Harvard Medical School, Cambridge, Massachusetts, United States

Necroptosis is a process of a highly regulated necrotic cell death, which has been implicated in the pathogenesis of a variety of poorly treatable diseases including myocardial infarction, brain ischemia, and a variety of inflammatory conditions. Necroptosis is controlled by a "necrosome" complex of two homologous kinases, Receptor Interacting Protein Kinases 1 and 3 (RIP1 and RIP3). Small molecule inhibitors of these kinases attracted significant interest therapeutically. Our previously published cell based screen identified three structurally dissimilar small molecule inhibitors of RIP1 kinase, termed necrostatins. Further work demonstrated that necrostatins displayed potent inhibition of necroptosis through targeting RIPK1 kinase *in vitro* and *in vivo*. Furthermore, these molecules displayed unexpected mono-selectivity towards RIPK1 kinase. We present the analysis of the molecular basis for this unusual selectivity using a combination of site directed mutagenesis, structural biology and molecular modeling approaches. Furthermore, using the conclusions from this analysis, we describe development of the more potent and equally highly selective allosteric inhibitors of RIPK1 kinase.

MEDI 313

Identification of a first-in-class RIP1 kinase inhibitor in phase 2a clinical trials for immuno-inflammatory diseases

Philip A. Harris, philip.a.harris@gsk.com. PRR DPU, GlaxoSmithKline, Wayne, Pennsylvania, United States

Receptor interaction protein 1 (RIP1) kinase activity has been shown to be a critical driver of cell death and pro-inflammatory cytokine production downstream of multiple signaling pathways including TNFR1. Hence inhibitors of this kinase have the potential to result in a broad therapeutic benefit for multiple inflammatory diseases. We identified

a novel and highly kinase selective RIP1 inhibitor series from a DNA-encoded library screen. This presentation will highlight the lead optimization and SAR of this series that led to identification of the development candidate GSK2982772 now under phase 2a clinical evaluation in psoriasis, rheumatoid arthritis and ulcerative colitis patients.

MEDI 314

Targeting NF- κ B pathways in toll-like receptor and antigen receptor signaling

Hao Wu, *wu@crystal.harvard.edu. Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts, United States*

Novel targeted therapies are needed most urgently for the ABC-DLBCL subtype of lymphomas due to their chemotherapy resistance. In this presentation, I will talk about our efforts in developing specific inhibitors for the CARMA1/Bcl10/ MALT1 and the MyD88/IRAK pathways that lead to NF- κ B activation and survival advantage for the lymphomas.

MEDI 315

Approaches to TYK2 pseudokinase: A unique mode of allosteric kinase inhibition

Ryan Moslin⁶, *ryan.moslin@bms.com*, **Steve Wroblewski**⁴, **Yanlei Zhang**⁹, **Shuqun Lin**⁹, **Daniel Gardner**⁹, **Joseph B. Santella**³, **John V. Duncia**², **Chunjian Liu**¹, **James Lin**⁹, **Steven Spergel**⁹, **Michael Mertzman**⁹, **John S. Tokarski**⁸, **Huadong Sun**⁹, **Manoj Chiney**⁹, **Paul A. Elzinga**⁹, **Nelly Aranibar**⁹, **Anjaneya Chimalakonda**⁹, **Joann Strnad**⁹, **Adriana Zupa-Fernandez**⁹, **Lihong Cheng**⁹, **Kathleen Gillooly**⁹, **Kim McIntyre**⁹, **Percy H. Carter**¹⁰, **Louis Lombardo**¹¹, **James Burke**⁹, **John E. Macor**⁷, **David S. Weinstein**⁵. (1) L12-12, Bristol Myers Squibb, Princeton, New Jersey, United States (2) Mail Stop 13-02, Bristol Myers Squibb, Princeton, New Jersey, United States (3) MS L13-02, Bristol Myers Squibb Co, Princeton, New Jersey, United States (4) Pharm Research Inst, Bristol Myers Squibb Co, Princeton, New Jersey, United States (5) Rt 206 E Province Line Rd, Bristol Myers Squibb MS L12 04, Princeton, New Jersey, United States (6) Discovery Chemistry, Bristol-Myers Squibb, Princeton, New Jersey, United States (7) L12-06, Bristol-Myers Squibb, Princeton, New Jersey, United States (8) RM H3812, Bristol-Myers Squibb, Princeton, New Jersey, United States (9) Bristol-Myers Squibb, Princeton, New Jersey, United States

A member of the Janus (JAK) family of non-receptor tyrosine kinases, TYK2 plays a critical role in mediating the signaling of the pro-inflammatory p40 subunit-containing cytokines (IL-12 and IL-23) and type 1 interferon. Owing to the high sequence homology within the JAK family catalytic (JH1) domains, achieving high inhibitor selectivity for TYK2 over other Janus family members has proved challenging. The pseudokinase (JH2) domains of the Janus kinases have been shown to regulate function of the JH1 domains in the cellular context. We have discovered multiple classes of ligands for the JH2 domain of TYK2 that stabilize the inactivated state of the enzyme and thereby

inhibit signaling of the TYK2-dependent pro-inflammatory cytokines. Structure activity relationships and pharmacology of diverse TYK2 JH2 ligands will be presented.

MEDI 316

Design of JAK3 covalent inhibitors for the interrogation of JAK3 signaling in humans

Agustin Casimiro-Garcia¹, *agustin.casimiro-garcia@pfizer.com*, Atli Thorarensen¹, Jean-Baptiste Telliez², Paul Balbo², Mary E. Banker³, Matthew F. Brown³, Ye Che³, Jill Chrencik³, Jotham W. Coe³, Robert Czerwinski², Martin E. Dowty⁴, Adam M. Gilbert³, Matthew M. Hayward³, Martin Hegen², Brian Juba², Jason Jussif², Jonathan Langille³, Louis Leung³, Sidney Liang³, Tsung Lin², Justin I. Montgomery³, Sarah Soucy², John Trujillo³, Ray Unwalla¹, Felix F. Vajdos³, Fabien Vincent³, Xin Yang³. (1) Medicine Design, Pfizer Worldwide Research & Development, Cambridge, Massachusetts, United States (2) Inflammation and Immunology, Pfizer Worldwide Research & Development, Cambridge, Massachusetts, United States (3) Medicine Design, Pfizer Worldwide Research & Development, Groton, Connecticut, United States (4) Medicine Design, Pfizer Worldwide Research & Development, Andover, Massachusetts, United States

The JAK family of tyrosine kinases consists of four enzymes that have a central role in cytokine and growth factor mediated signal transduction. Extensive research efforts towards the identification of JAK kinase inhibitors have led several compounds to enter clinical development for the treatment of various inflammatory and oncology diseases with two agents reaching FDA approval up to now. One area of particular interest has been the identification of highly JAK3 selective inhibitors, but such agent has yet to reach clinical evaluation.

Significant efforts within our research organization have led to the identification of the first orally active JAK3 specific inhibitor that achieves high JAK isoform selectivity through covalent interaction with a unique JAK3 residue (Cys909). The challenge with this approach is the design of JAK3 covalent inhibitors possessing appropriate pharmacokinetic properties, not only due to the relatively rapid resynthesis rate of the JAK3 enzyme, but also because of nontraditional metabolic pathways of direct conjugation with glutathione and/or mediated through glutathione S-transferase, in addition to CYP-mediated metabolism. A novel pharmacokinetic algorithm was developed that took into account these extrahepatic and hepatic mechanisms which facilitated compound optimization, prediction of human pharmacokinetics and dose, and candidate selection. Guided by this framework, and through a structure-enabled program, a series of acrylamides based on a pyrrolopyrimidine scaffold was developed. This effort led to the identification of **PF-06651600**, a potent, JAK3-specific, and low clearance compound with demonstrated in vivo efficacy. The favorable efficacy and safety profile of this compound led to its progression into human clinical studies.

MEDI 317

Targeting intrinsically disordered proteins: Taming alpha-synuclein with small molecules

*Jiahui Tao¹, Amandine Berthet², David Agard¹, **Lisa McConlogue^{1,2}**, daalcm@gmail.com. (1) Biochemistry and Biophysics, UCSF, San Francisco, California, United States (2) GIND, The J. David Gladstone Institutes, San Francisco, California, United States*

The over-expression, mis-folding and aggregation of alpha-synuclein (aSyn) are linked to the onset and pathology of Parkinson's diseases. Nevertheless, the therapeutic targeting of it by small molecules has been a major challenge because of its heterogeneous conformational properties. In this study we aim to identify drug-like small molecules that bind to native states of aSyn and impact pathogenic processes. As an intrinsically disordered protein, aSyn in its native state exists in an ensemble of interconverting structures. We adopted an approach to first screen for molecules that bind to monomeric aSyn, and then further test those binding compounds for impact on mis-folding and mis-function of aSyn. We've also established a screen that targets the formation of early oligomers and its modulation by cellular chaperones. Because the small molecules we identify have the potential to bind to and stabilize a variety of native forms of aSyn, we tested their impact on both aggregation propensity and also on the perturbation of vesicular dynamics by over-expression of aSyn and found molecules impacting both of these malfunctions.

MEDI 318

Current status of the development of PET radiotracers for imaging alpha synuclein aggregates in Lewy bodies and Lewy neurites

***Robert H. Mach**, rmach@mail.med.upenn.edu. Radiology, University of Pennsylvania, Philadelphia, Pennsylvania, United States*

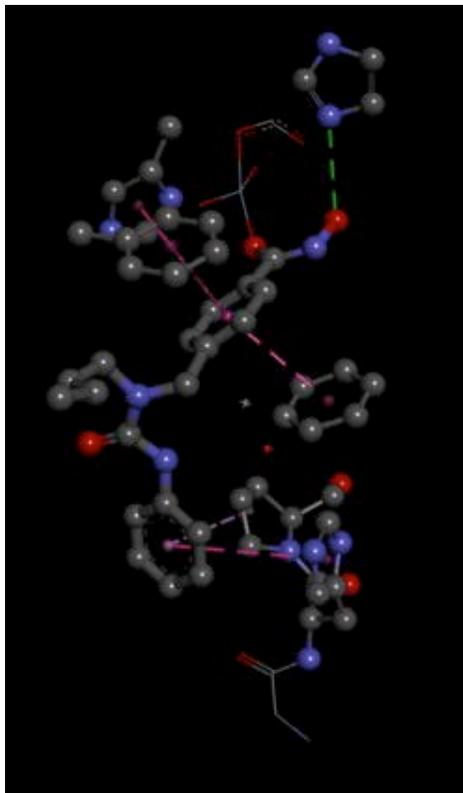
One of the hallmark features of neurodegenerative disorders is the accumulation of misfolded proteins which lead to the formation of insoluble protein aggregates in the CNS. One such protein is alpha synuclein (Asyn), which serves as the major component in Lewy bodies (LBs) and Lewy neurites (LNs). LBs and LNs are the primary lesions in Parkinson's disease (PD), dementia with Lewy bodies (DLB), and multiple system atrophy (MSA). Furthermore, nearly 50% of Alzheimer's disease (AD) patients have LBs at time of autopsy, and AD patients with LBs may have a more rapid rate of cognitive decline than patients not having LB pathology. Therefore, the development of a PET radiotracer capable of imaging Asyn aggregates would provide a valuable tool in the clinical evaluation of a wide panel of neurodegenerative disorders. This presentation will describe the challenges in developing a PET radiotracer for imaging Asyn aggregates, the different strategies being used in accomplishing this goal, and the current status of efforts aimed at developing an Asyn PET radiotracer.

MEDI 319

HDAC6 inhibitors, autophagy, mitochondrial movement, and disease modification

Alan P. Kozikowski, kozikowa@uic.edu. Univ of Illinois Chicago, Chicago, Illinois, United States

Histone deacetylases or HDACs are associated with the removal of acetyl groups from histones and a host of other proteins. Class I, II and IV HDACs require Zn^{2+} as a cofactor for their deacetylating activity and these are often referred to as the conventional HDACs. HDAC6 has garnered significant attention due to its unique structure and activity, and to the finding that HDAC6 knockout animals remain viable. While HDAC6 does not work on nuclear histones, it is involved in the acetylation status of proteins such as cortactin, HSF-1, HSP90, and tubulin. In particular, HDAC6 controls the acetylation status of the microtubule protein α -tubulin, and microtubule-dependent transport rates are more efficient along acetylated α -tubulin than deacetylated α -tubulin. This effect stems from the increased association of the motor proteins kinesin-1 and dynein with acetyl-tubulin, and therefore, affects both anterograde and retrograde transport activities. Thus, in addition to facilitating anterograde transport of new cargo to synaptic zones, acetyl-tubulin also increases the ability of damaged organelles or misfolded proteins to leave synaptic zones. This may be very important for Rett Syndrome, inter alia, as damaged mitochondria and elevated levels of improperly spliced mRNA transcripts have been noted in MeCP2-deficient neurons. In this lecture I will present information on the design, synthesis, and testing of ligands that are highly selective for HDAC6 inhibition and show the effects of these compounds in animal models of diseases such as Rett syndrome, Alzheimer's disease, Charcot Marie Tooth disease, cancer, and stroke.



Nexturastat A in complex with HDAC6; only key interactions shown for clarity.

MEDI 320

Imaging mutant huntingtin aggregates: Development of potential PET ligand

Celia Dominguez, celia.dominguez@chdifoundation.org. Advisors to CHDI Foundation, CHDI Management Inc, Los Angeles, California, United States

Despite the initiation of huntingtin lowering clinical trials, biomarkers that can be used to assess target engagement and evaluate disease modification are currently lacking. A PET ligand that binds mutant HTT aggregates (mHTT) in both pre-clinical HD models and human subjects would allow for monitoring changes in aggregate load in different brain regions over time, and assist with translation from pre-clinical to clinical studies. An ideal PET ligand would penetrate the blood brain barrier and enter brain cells, bind mHTT aggregates with sufficient affinity to provide a signal over background noise, show little binding (signal) in non-diseased brain, be selective over other misfolded proteins, such as beta amyloid, and have suitable brain kinetics, metabolic profile and chemical structure for labelling with ¹¹C or ¹⁸F.

Here we summarize our strategy to develop PET ligands directed to mHTT aggregates and highlight progress made during lead optimization. We will present data from a radioligand binding assay (RBA) developed to detect sub-nanomolar affinities towards recombinant HTT aggregates, and highlight compounds with improved performance in autoradiography (ARG) using HD mouse and human brains. In addition, we will show

examples of compounds with progressively improved metabolite profiles that predict minimal brain exposure of radiolabeled metabolites, selectivity data over beta amyloid binding in both mouse AD model and human post mortem brains. Finally, we will present mPET in Q175 and PET in non-human primates on our clinical candidate, CHDI-180.

MEDI 321

Small molecule modulators of ER stress for the treatment of neurodegenerative diseases

Nicholas D. Cosford, ncosford@sanfordburnham.org, Haixia Zou, Allison Limpert, Jiwen Zou, Anna Dembo, Daniel Grant, Robert Ardecky, Anthony Pinkerton, Gavin Magnuson, Mark Goldman, Juan Rong, Douglas Sheffler, John Reed. Sanford-Burnham Medical Research Institute, La Jolla, California, United States

Endoplasmic reticulum (ER) stress causes neuronal dysfunction followed by cell death and is recognized as a feature of many neurodegenerative diseases. Using a phenotypic screen, we recently identified benzodiazepinone derivatives that reduce ER stress-mediated apoptosis in a rat neuronal progenitor cell line (CSM14.1). Structure-activity relationship (SAR) studies around these screening hits led to compounds that display robust cytoprotective activity against thapsigargin-induced ER stress in SH-SY5Y and H4 human neuronal cell lines. We demonstrate that the most potent of these derivatives inhibits the activation of p38 MAP kinase (p38) and c-Jun N-terminal kinase (JNK), protein kinases that are downstream signal effectors of the unfolded protein response (UPR). ER stress inhibitors specifically protect against thapsigargin-induced cell death and display no protection against other insults known to induce cellular stress or activate p38. However, moderate inhibition of p38 activity stimulated by compounds that disrupt calcium homeostasis is observed. Our data indicate that these probe compounds are valuable small molecule tools that can be used to investigate the effects of ER stress on human neurons. This approach may provide the basis for the future development of therapeutics for the treatment of neurodegenerative diseases such as ALS.

MEDI 322

Discovery of the P7C3 class of neuroprotective compounds

Joseph Ready, joseph.ready@utsouthwestern.edu. UT Southwestern, Dallas, Texas, United States

An aminopropyl carbazole (P7C3) was discovered through an in vivo phenotypic screen for compounds that would promote hippocampal neurogenesis. Subsequent chemical optimization provided analogs with improved safety, stability and efficacy. Lead compounds have proven efficacious in multiple models of neuroprotection. For example, they minimize neuron death in the MPTP model of Parkinson's disease, protect motor

neurons in the SOD mutant model of ALS, and protect learning and memory in models of traumatic brain injury. Initial studies have indicated that the P7C3 class of compounds maintain NAD levels in neurons, providing a general mechanism for neuroprotection.

MEDI 323

Award Address (Alfred Bader Award in Bioinorganic or Bioorganic Chemistry sponsored by the Alfred R. Bader Fund). Merging of chemistry and biology: Molecules with translational function

Kim D. Janda, kdjanda@scripps.edu. Scripps Rsrch Inst, La Jolla, California, United States

Nature contains information to instruct scientists about what is possible. This can serve as an inspiration to probe the frontiers of biology and chemistry. At the same time, chemistry can contribute to our understanding of biology and also to our ability to manipulate complex systems for human health and welfare. The combination of the tools and principles of chemistry, together with the tools of modern biology, allows us to create complex synthetic and natural molecules, comprising processes with novel biological, chemical and physical properties. This lecture will illustrate how we have interfaced chemistry and biology to create platforms enabling "academic" molecules to be transformed into clinical candidates.

MEDI 324

Award Address (E. B. Hershberg Award for Important Discoveries in Medicinally Active Substances sponsored by Merck Research Laboratories). Present and future of antisense technology

Stanely Crooke, scrooke@ionisph.com. Ionis Pharmaceuticals, Carlsbad, California, United States

Antisense technology at Ionis has advanced to the state in which we have 38 drugs in development and three significant programs in different patient populations completing phase 3 studies. The pipeline is comprised of three different chemical classes and exploits several different mechanisms of action and routes of administration which demonstrate the value and versatility of the technology.

Unlike most drug discovery platforms, antisense technology continues to advance. We hope to have drugs with a human ID₅₀ dose of 1–5mg/week for liver targets next year. The longer-term future is even more exciting. As we exponentially add new knowledge about the molecular pharmacology of ASOs, the future is created. The translation of these advances into drugs promises an even more substantial enhancement of therapeutic index and patient convenience.

In this presentation, the exciting present and even the more compelling future of antisense technology will be discussed.

MEDI 325

Award Address (ACS Award for Creative Invention sponsored by ACS Corporation Associates). CPP-115: A novel GABA aminotransferase inactivator and potential new treatment for epilepsy, addiction, and hepatocellular carcinoma

Richard B. Silverman, *r-silverman@northwestern.edu*. Department of Chemistry, Northwestern University, Evanston, Illinois, United States

An imbalance in the levels of the inhibitory neurotransmitter g-aminobutyric acid (GABA) and the excitatory neurotransmitter glutamate can lead to convulsions. Inhibition of g-aminobutyric acid aminotransferase (GABA-AT), the enzyme responsible for the degradation of GABA, increases the GABA levels, which has been shown to produce an anticonvulsant effect. A sharp rise in dopamine release is associated with a variety of addictive behaviors. This dopamine release can be attenuated by an increase in GABA; therefore, inactivation of GABA-AT also has an effect on addictive behavior. Inactivation of a related enzyme, ornithine aminotransferase (OAT) in hepatocellular carcinoma (HCC) has been shown to slow the growth of this cancer. In this lecture the design and mechanism of some of our GABA-AT inactivators will be discussed and how these compounds led to the design and discovery of CPP-115, a potent inactivator of GABA-AT, which has been found to have excellent pharmacokinetic and pharmacological properties for the potential treatment of epilepsy and addiction. CPP-115 also inactivates OAT and has been shown to slow the growth of HCC. A related analogue of CPP-115 was identified that does not inactivate GABA-AT but is a potent inactivator of OAT. Another analogue of CPP-115 has been designed that is 10 times more potent than CPP-115. Enzyme inactivation mechanism studies will be discussed, as well as in vitro and in vivo efficacy and pharmacokinetic results, toxicology studies, including an early clinical trial with CPP-115.

MEDI 326

Bristol-Myers Squibb Smissman award lecture: Receptor structures enable drug discovery

Kenneth A. Jacobson, *kjacobs@helix.nih.gov*. Laboratory of Bioorganic Chemistry, National Institute of Diabetes & Digestive & Kidney Diseases, NIH, Bethesda, Maryland, United States

Edward E. Smissman pioneered an interdisciplinary approach in medicinal chemistry. We take synthetic chemical, pharmacological, and structural approaches to discover and characterize new compounds to modulate purinergic signaling, with the potential for treating chronic diseases. This encompasses 4 G protein-coupled receptors (GPCRs) for adenosine, 8 GPCRs activated by nucleotides (P2YRs), 7 ATP-gated P2X ion

channels, and the associated catabolic and metabolic enzymes that regulate the levels of the native agonists. We use the high-resolution X-ray structures of the adenosine receptors (ARs) and P2YRs to rationally design ligands, either by modification of known agonists and antagonists or by virtual screening to discover novel chemotypes. In collaboration with Ray Stevens and colleagues, we determined A_{2A}R, P2Y₁R and P2Y₁₂R structures in complex with high-affinity ligands, which displayed surprising structural features that could not be predicted by modeling derived from previous GPCR templates. We introduced sterically constrained rings to mimic native ribose in nucleosides and nucleotides, to determine their preferred conformation when bound to protein targets. Chemical tools for related receptors, such as fluorescent probes of the inflammation-related P2Y₁₄R, were designed with the aid of molecular modeling and applied to drug discovery. Novel A₃AR agonists for pain control were designed and screened using an in vivo phenotypic model, which reflected both pharmacokinetic and pharmacodynamic parameters. High specificity (~10,000 fold selectivity for the A₃AR) and clean ADME-tox and off-target properties were achieved. Activation of the A₃AR in peripheral neurons, spinal cord, and brain was found to reduce chronic neuropathic pain in vivo. This protection was dependent on modulation of GABA_A receptors, oxidative pathways, astrocytic activation, and cytokine levels in the spinal cord. Earlier A₃AR agonists are progressing favorably in clinical trials for rheumatoid arthritis and psoriasis (phase 3) and liver cancer (phase 2). These agonists demonstrated clinical efficacy without serious adverse effects. Thus, purine receptor structures and an interdisciplinary approach have enabled the elucidation of their biological role, the conceptualization of future therapeutics and novel ligand discovery.

MEDI 327

Targeting bacterial bioenergetics and central metabolism: Challenges and opportunities

Kevin Pethe, *kevin.pethe@ntu.edu.sg*. Lee Kong Chian School of medicine, Nanyang Technological University, Singapore, Singapore

The rapid emergence and spread of multi-drug resistant *Mycobacterium tuberculosis* and other pathogenic bacteria is a serious concern worldwide that advocates for the development of new classes of antibacterials with a novel mode of action. Current antibiotics derive mainly from natural sources and inhibit a narrow spectrum of cellular processes such as DNA replication, protein synthesis and cell wall biosynthesis. With the spread of drug resistance, there is a renewed interest in the investigation of alternate essential cellular processes, including central metabolic and bioenergetics pathways, as a drug target space for the next generation of antibiotics. However, the validation of those targets is more complex, as essentiality of such targets can be conditional. Interest in targeting central metabolism has also been muted because of a concern about selectivity with human orthologs. Nonetheless, we and others have shown that selective inhibition can be achieved for enzymes that are conserved between bacteria and humans. Oxidative phosphorylation as recently emerged as a relevant target space for the development of new classes of drug for tuberculosis. In this

context, I will discuss the relevance of targeting respiratory terminal oxidases for the development of rational drug combination for tuberculosis and other mycobacterial diseases.

MEDI 328

Targeting bacterial nutrient biosynthesis with natural products

Eric D. Brown, *ebrown@mcmaster.ca*. *Biochemistry and Biomedical Sciences, McMaster University, Hamilton, Ontario, Canada*

When bacteria are grown in media containing only carbon, nitrogen and essential salts, they shift in their metabolic activities to include the synthesis of essential amino acids, vitamins and other cofactors. Only 303 genes, for example, are essential for growth of *E. coli* on rich media and some 119 additional genes are required for growth on nutrient-limited media.

Hence compounds that target bacteria under nutrient-limited conditions could serve as leads for novel antibacterial drugs. In fact, nutrient-limited media probably provide a better proxy for the host environment. There have been many reports of impaired growth and attenuated virulence in pathogens due to mutations in vitamin, nucleobase and amino acid biosynthetic genes. Nevertheless, systematic searches for antibacterial chemicals have overwhelmingly emphasized rich media conditions. Thus, there is a considerable gap in antibacterial chemical space surveyed to date.

With the goal of targeting biosynthetic pathways, we have been screening libraries of structurally diverse synthetic compounds and natural products to find inhibitors of *E. coli* growth in minimal media. In this screening platform, synthetic compounds active in primary screens are subject to the addition of an array of key metabolites and pools thereof to identify suppressors of growth inhibition and provide hypotheses for physiological, genetic and biochemical experiments to elaborate mechanism of action. Natural products extracts on the other hand were found to contain nutrients that confounded this discovery platform. In this presentation, I will provide an account of on-going efforts to discover and characterize novel chemical matter targeting nutrient biosynthesis in bacteria, especially from natural sources.

MEDI 329

Antibacterial agents that target adenylating enzymes in *Mycobacterium tuberculosis*

Courtney C. Aldrich, *aldri015@umn.edu*. *Medicinal Chemistry, University of Minnesota, Minneapolis, Minnesota, United States*

Mycobacterium tuberculosis (*Mtb*) putatively encodes more than 60 adenylating that catalyze a multitude of essential biochemical processes in protein synthesis, glycolysis,

lipid metabolism, and cofactor biosynthesis (biotin, coenzyme A, and nicotine adenine dinucleotide) as well as synthesis of small molecule metabolites including the mycobactins (siderophores for iron acquisition) and mycothiols (a thiol to protect against oxidative stress). Bisubstrate inhibitors of representative adenylating enzymes, inspired by the natural product ascamycin, were prepared by mimicking the native acyl-adenylate reaction intermediate, which in many cases resulted in the preparation of sub-nanomolar enzyme inhibitors with corresponding potent whole-cell activity and excellent on-target activity. In parallel, high-throughput and fragment-based screening (HTS and FBS) was performed against several targets, providing a unique opportunity to directly compare these complimentary approaches (HTS, FBS, and rationale design of bisubstrate inhibitors). Our experience and lessons learned through targeting diverse adenylating enzymes will be described.

MEDI 330

Targeting a branch point in bacterial metabolism through inhibition of DXP synthase

Caren L. Freel Meyers, cmeyers@jhmi.edu. Pharmacology and Molecular Sciences, Johns Hopkins School of Medicine, Towson, Maryland, United States

With the growing public health threat of antibiotic resistance, we are challenged to investigate alternate drug target spaces for development of new antibiotics. Targeting central metabolic pathways required to establish infection and support bacterial growth in host niches is an important antimicrobial strategy. 1-Deoxy-d-xylulose 5-phosphate (DXP) synthase is an emerging antibacterial target functioning in bacterial central metabolism and isoprenoid biosynthesis. The product DXP is a key branch point metabolite that is partitioned into three essential metabolic pathways to produce the 5-carbon isoprenoid bioprecursors dimethylallyl diphosphate and isopentenyl diphosphate, and vitamins pyridoxal phosphate and thiamin diphosphate (ThDP), the latter a cofactor which is also required for DXP synthase catalysis. Thus, inhibition of DXP synthase can potentially introduce multiple, simultaneous blockades in bacterial metabolism. Development of DXP synthase inhibitors is limited, due in part to the challenge of selectively targeting a bacterial ThDP-dependent enzyme. We elucidated a novel mechanism for DXP synthase catalysis and subsequently identified inhibitor classes that exploit the large active site and unique mechanism. Here, I will present findings from our ongoing investigation of DXP synthase mechanism and function, and our efforts to develop selective inhibitors to probe mechanism and establish starting points for antimicrobial agents targeting this essential metabolic branch point.

MEDI 331

Co-therapy strategy to enhance target vulnerability in *Mycobacterium tuberculosis*

Nicole S. Sampson, *nicole.sampson@stonybrook.edu*. Chemistry Dept, Stony Brook University, Stony Brook, New York, United States

The ability of *Mycobacterium tuberculosis* (*Mtb*) to metabolize cholesterol is critical for the maintenance of the *Mtb* infection in the host. We identified two new structural-functional motifs for catalysis of beta-oxidation with steroid substrates in the *igr* operon that function in the metabolic pathway for sterol side-chain cleavage. These enzymes form unusual heterotetramers. A fuller bioinformatic analysis of the *Mtb* genome has identified additional heteromeric complexes that are encoded by cholesterol-regulated genes and enabled further mapping of the pathways. Using a privileged pharmacophore, we have identified lead compounds that show excellent antibacterial activity against *Mycobacterium tuberculosis* under both aerobic and anaerobic growth conditions and that enhance bactericidal activity.

MEDI 332

Discovery of ETX2514, a novel, rationally designed inhibitor of class A, C and D β -lactamases, for the treatment of Gram-negative infections

Thomas F. Durand-Reville, *t.durand-reville@entasistx.com*. Chemistry, Entasis Therapeutics, Waltham, Massachusetts, United States

Background: Multidrug resistant (MDR) Gram-negative infections are of great concern due to high mortality rates and limited treatment options. β -lactamase expression is an important resistance mechanism in these organisms. While the recent development of new β -lactamase inhibitors (BLI), especially around the diazabicyclooctanone (DABCO) scaffold, is encouraging, none of these inhibitors cover all class A, C and D serine β -lactamases. Our aim was to discover a novel broad spectrum BLI that would restore the cellular activity of partner β -lactams in MDR Gram-negative bacteria.

Methods: Innovative synthetic, medicinal and computational chemistry methods were employed to generate novel DABCOs and test their enzymatic activity against a panel of representative serine β -lactamases. *In vitro* antibacterial activity was determined by broth MIC using CLSI standards. *In vivo* efficacy was evaluated using murine neutropenic thigh and lung models of *A. baumannii* infection.

Results: Through lead optimization and structure-based drug design efforts, a novel series of unsaturated DABCOs with broad enzymatic coverage of serine β -lactamases was discovered. ETX2514 exhibits best in class activity vs Class A, C enzymes and for the first time, broad Class D activity with IC_{50} s of 4, 14 and 190 nM for KPC-2, AmpC and OXA-24, respectively. The MIC_{90} of each β -lactam tested in combination with 4 mg/L ETX2514 was ≤ 0.12 mg/L for both *K. pneumoniae* and *E. coli*, including isolates containing Class B β -lactamases or the *mcr-1* gene. Imipenem was the most effective β -lactam partner for ETX2514 against *P. aeruginosa* isolates ($MIC_{90} = 1$ mg/L), while sulbactam was the most potent partner against *A. baumannii* isolates ($MIC_{90} = 2$ mg/L). *In vivo* evaluation of sulbactam-ETX2514 against MDR *A. baumannii* isolates showed dose-dependent activity, leading to >2 -log drop in CFU levels at 24 hours when target exposures exceeded 50% of the dosing interval. ETX2514 exhibited no adverse effects

up to the limit dose in both rat and dog 14-day GLP toxicology studies.

Conclusions: The preclinical safety, unprecedented spectrum and potency vs β -lactamases of the novel diazabicyclooctenone ETX2514 make it an ideal candidate for several combinations with β -lactams to treat MDR Gram-negative infections. ETX2514 has entered Phase 1 clinical studies.

MEDI 333

Challenges and opportunities for drug discovery in ALS

Lucie I. Bruijn, lucie@alsa-national.org. Research, The ALS Association, Washington, District of Columbia, United States

Over the past several years thanks to increased investment and advances in technology, gene discovery for ALS has grown exponentially and with this the opportunities to develop therapies for this devastating disease. Drug development for neurodegenerative diseases remains incredibly challenging and in particular for ALS, the heterogeneous nature of the disease, late diagnosis and a varied pattern of disease progression makes clinical trials extremely difficult. Over the past two years the ALS association research efforts have focused on how to best impact these challenging areas with a focus on improved collaboration, bringing new players to the field, open data sharing and engaging all stake holders in the drug development process more actively. Topics that will be covered in the presentation include:

- a) Development of new therapies,
- b) Search for diagnostic and disease-progression biomarkers,
- c) Clinical trials design,
- d) Role of genes in the pathogeny of familial and sporadic forms,
- e) Potential role of stem cells as therapeutic agents,
- f) The role of Agencies in the support of research.

MEDI 334

Identification and preclinical pharmacology of antisense oligonucleotides targeted to human SOD1 for the treatment of ALS

Eric E. Swayze, eswayze@isisph.com. Ionis Pharmaceuticals, Inc., Carlsbad, California, United States

Compelling evidence supports a toxic gain of function hypothesis for the dominantly inherited form of Amyotrophic lateral sclerosis (ALS) caused by mutations in the Cu/Zn superoxide dismutase (SOD1) gene. Given that the toxic function of mutant SOD1 is the likely driver of disease pathogenesis, suppression of SOD1 expression has the potential to be a disease modifying therapeutic. An attractive approach to reduce gene expression is via the use of Antisense oligonucleotides (ASOs), which have shown success in both preclinical models of diseases and in clinical trials for diseases of the

central nervous system (CNS). ASOs do not cross the blood brain barrier, and must be administered directly into the cerebrospinal fluid (CSF) of the CNS. ASOs then distribute broadly within the CNS, modulate their RNA targets, and display the expected pharmacology. In human patients, ASOs are delivered via intrathecal (IT) injection into the CSF. In order to translate rodent work to the clinic, we initially addressed the kinetics and mechanism of ASO distribution after IT administration in rodents to determine the extent and mechanism of distribution. We then examined the ability of IT administration of ASOs to effect broad distribution and target reduction in non-human primates (NHP). These studies supported the development of an ASO targeting SOD1 for the treatment of SOD1 familial ALS. We first identified lead human SOD1 targeting ASOs in cell culture, then optimized the leads for activity and safety in rodents. In preclinical models of ALS expressing mutant human SOD1, we demonstrated robust inhibition of SOD1 mRNA and protein in multiple CNS cell types. This suppression resulted in a pronounced improvement in survival, as well as preservation of nerve/muscle function as assessed by compound muscle action potentials (CMAP). This data supports ongoing human clinical trials assessing IT delivered SOD1 ASOs for the potentially disease modifying treatment of SOD1 ALS patients.

MEDI 335

Design and study of small molecules targeting r(G₄C₂) repeats in ALS and FTD

Matthew D. Disney, Disney@scripps.edu. Department of Chemistry, The Scripps Research Institute, Jupiter, Florida, United States

An expanded repeat of r(G₄C₂) [r(G₄C₂)]^{exp} in *C9ORF72* is the most common cause of amyotrophic lateral sclerosis and frontotemporal dementia ("c9ALS/FTD"). Small molecules have been developed to target this RNA repeat and affect various modes of toxicity. This includes small molecules that inhibit repeat-associated non-ATG translation, where homopolymeric proteins are synthesized from the repeating transcript, and RNA gain-of-function, where the RNA repeat binds to and sequesters proteins involved in RNA biogenesis. In the present study, we describe mechanistic investigations to study how designer small molecules affect these processes and also report the design of improved small molecules. In addition, we present the development and implementation of various technologies to study target selectivity and potency in cells that have broad implications. The overall goal of this study is to discern factors affecting the druggability of RNA repeats expansions by using the most common cause of familial als as a test case.

MEDI 336

Dual leucine zipper kinase inhibitors for the treatment of neurodegenerative diseases

Michael Siu, msiu1@chemalum.berkeley.edu. Discovery Chemistry, Genentech, South San Francisco, California, United States

Dual leucine zipper kinase (DLK, MAP3K12) activation of the JNK / c-Jun pathway in neurons is essential for stress-induced degeneration; thus, DLK presents an attractive therapeutic target for neurodegenerative diseases such as ALS. Previously, we have disclosed a series of small molecule DLK inhibitors that effectively reduce c-Jun phosphorylation in acute injury mouse models (optic nerve crush and MPTP). These inhibitors were important to our understanding of DLK inhibition *in vivo*. Herein, we describe the discovery of a series of inhibitors with enhanced potency, PK, kinase selectivity, and tolerability.

MEDI 337

Development of small-molecule autophagy inducers that mitigate neurodegeneration in models of ALS and other disorders

Steven Finkbeiner^{1,2}, sfinkbeiner@gladstone.ucsf.edu. (1) Gladstone Institute of Neurological Disease, San Francisco, California, United States (2) Neurology and Physiology, UCSF, San Francisco, California, United States

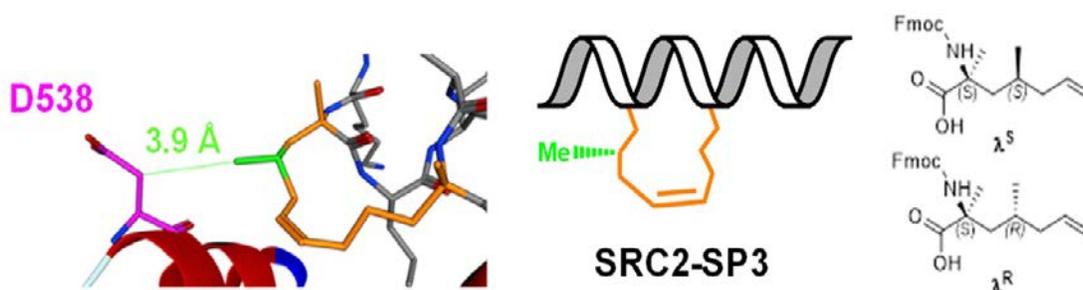
Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease, and the only approved treatment extends life by just a few months on average. The vast majority of patients with ALS exhibit abnormal deposits of misfolded and aggregated TAR DNA binding protein 43 (TDP43) in their brain and spinal cord. These observations suggest that ALS is characterized by a mismatch between the production and clearance of misfolded proteins, similar to other neurodegenerative diseases, such as Alzheimer's, Parkinson's and Huntington's diseases. Autophagy is one of the two major protein clearance pathways in cells and the only pathway thought to be capable of clearing accumulated toxic misfolded proteins. We hypothesized that stimulating the endogenous autophagy pathway in brain cells could be a therapeutic strategy for treating ALS and other disorders characterized by protein misfolding and accumulation. We conducted a candidate screen and identified small molecules that induce autophagy in neurons. Using computational chemistry and the initial structure-activity relationships, we developed a pharmacophore and performed *in silico* screening of a 1M compound library and identified additional small-molecule autophagy inducers that were more potent and had a wider therapeutic window. One of the major challenges of developing autophagy inducers is that many of the conventional assays are slow and insensitive, relying on snap-shot measures of autophagy pathway intermediates to infer flux through the pathway. To overcome this limitation, we developed a new optical pulse-labeling method to directly measure flux through the autophagy pathway, and we adapted it to a high-throughput walkaway robotic microscopy platform. With this platform and medicinal chemistry approaches, we developed potent (nanomolar) compounds with novel chemistry that stimulate autophagy in rodent and human neuron and are protective in rodent and human neuron models of ALS and other neurodegenerative disorders. The molecules have drug-like properties, are orally bioavailable, have excellent blood-brain barrier penetration, and appear to be safe with chronic dosing. We are continuing to develop these molecules with the goal of choosing a clinical candidate for first-in-human trials.

MEDI 338

γ -Functionalized hydrocarbon stapled peptides for inhibiting mutant estrogen receptor/coactivator interaction

Tom Speltz², tspeltz99@gmail.com, Christopher G. Mayne¹, Sean Fanning³, Emad Tajkhorshid¹, Geoffrey Greene³, Terry W. Moore². (1) Beckman Institute, Univ Illinois Urbana Champaign, Urbana, Illinois, United States (2) Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, Chicago, Illinois, United States (3) The Department of Biochemistry and Molecular Biology, University of Chicago, Chicago, Illinois, United States

Hydrocarbon stapled peptides hold much promise as a general platform for inhibiting protein-protein interactions, but, beyond the initial reports, relatively little research has gone into exploring the amino acids used for this technology. Stapled peptides are typically designed to replace two *non-interacting* residues with the constraining, olefinic staple. In contrast to this approach, we recently reported a design strategy to leverage the lipophilic nature of the hydrocarbon staple by preparing new amino acids (λ^R and λ^S) that incorporate a methyl at the γ -position of the stapling amino acid S^5 . When incorporated into a peptide derived from steroid receptor coactivator-2 (SRC2), the resulting γ -methylated stapled peptides disrupt the estrogen receptor (ER) /SRC2 interaction with improved potency compared to the unsubstituted staple. In addition to inhibiting wildtype ER, these stapled peptides show good affinity to estrogen receptor mutants Y537S and D538G. ER-D538G and ER-Y537S have recently been reported to be highly prevalent mutations found in endocrine resistance breast cancer, however, the mechanistic role of these mutations has yet to be fully elucidated. Interestingly, the γ -methyl group of a stapled peptide we have previously prepared (SRC2-SP3) binds within 4 Å of estrogen receptor residue D538. Guided by x-ray crystallography and molecular dynamics studies, we have set out to prepare functionalized stapled peptides for selectively inhibiting ER-D538G. The selective inhibition of this ER isoform will shed new light on its involvement in the progression of breast cancer.



Discovery of BMS-986104: Moving from direct-acting, full agonists to pro-drug, partial agonists in the identification of a differentiated S1P1 modulator with an improved safety profile

Alaric J. Dyckman¹, Alaric.Dyckman@bms.com, Murali Dhar¹, David Marcoux¹, Hai-Yun Xiao¹, Zili Xiao¹, John L. Gilmore¹, Ling Li¹, Arvind Mathur¹, jenny xie¹, Xiaoxia Yang¹, Tracy L. Taylor¹, Rochelle Thomas¹, Kim McIntyre¹, Lois Lehman-McKeeman¹, Hong Shi¹, Paul Levesque¹, Praveen Balimane³, Huadong Sun¹, Anthony M. Marino¹, Zheng Yang¹, Ding Ren Shen¹, Mary Ellen Cvijic¹, Bethanne M. Warrack¹, Georgia Cornelius¹, Celia J. D'Arienzo¹, Luisa Salter-Cid¹, Joel C. Barrish², Percy H. Carter¹. (1) Bristol-Myers Squibb, Pennington, New Jersey, United States (2) Achillion, New Haven, Connecticut, United States (3) FDA, Silver Spring, Maryland, United States

Agonism of the sphingosine-1-phosphate family of G-protein coupled receptors (S1PRs) gained validation as a therapeutic approach for treating multiple sclerosis with the approval of FTY720 (Gilenya) for the relapsing remitting form of this disease. FTY720 is a pro-drug, whose metabolically activated phosphate form (FTY720-P) is a non-selective S1PR agonist. Activation of S1P1 by FTY720-P has been linked to its beneficial immunomodulatory properties, whereas agonism of S1P3 was initially associated with several safety issues, such as cardiovascular and pulmonary effects (transient reduction in heart rate, atrioventricular conduction issues, mild but sustained elevation of blood pressure, and decreased pulmonary function). Subsequent clinical evaluation of S1P3-sparing S1P1 agonists, of both the pro-drug class as well as direct-acting agonists (those which do not require bio-activation), demonstrated that removing activity on S1P3 did not eliminate the liabilities. Our initial research, which focused on selective, direct-acting compounds with full agonism at S1P1, led to the identification of advanced compounds that had preclinical liability profiles similar to FTY720. Herein, we detail our pursuit of compounds with improved safety, in which we focused on S1P1 agonists that showed differentiated G-protein coupled signaling relative to FTY720-P and earlier full agonists. The search for S1P1 modulators that function as biased ligands required a shift from the direct-acting class to the pro-drug class of agonists (active phosphate metabolite) and resulted in the identification of BMS-986104. BMS-986104 produced robust, dose-dependent lymphopenia and maintained efficacy comparable to that of full agonists in animal models of human disease. The compound is readily differentiated from FTY720 and other clinical-stage S1P1 agonists as it shows improved pulmonary and cardiovascular (CV) safety in preclinical models. The human relevance of the preclinical assays was of key importance, particularly for CV effects, as rodent-based predictions had been found to correlate poorly with human CV profiles for S1P agonists. Utilization of a CV safety screen based on cultured human cardiomyocytes derived from inducible pluripotent stem cells eliminated concerns of cross-species differences. Based on the favorable efficacy and safety profile, BMS-986104 was advanced to clinical evaluation.

MEDI 340

Phenotypic screening identifies a small molecule anti-secretagogue of PCSK9 that acts via a novel mechanism of action

*Donna Petersen¹, Julie Hawkins², Wanida Ruangsiriluk², Kimberly Stevens², Bruce Maguire¹, Thomas N. O'Connell¹, Benjamin N. Roche¹, Markus Boehm³, Roger B. Ruggeri¹, Tim Rolph², David Hepworth³, Paula Loria¹, **Philip A. Carpino³**, philip.a.carpino@pfizer.com. (1) Medicine Design, Worldwide Research & Development, Pfizer, Inc., Groton, Connecticut, United States (2) CVMET Research Unit, Pfizer, Inc., Cambridge, Massachusetts, United States (3) Medicine Design, Worldwide Research & Development, Pfizer, Inc., Cambridge, Massachusetts, United States*

Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a secreted protein synthesized by the liver that has been implicated in the regulation of low-density lipoprotein cholesterol (LDL-C), a risk factor for coronary heart disease. Monoclonal antibodies (mAbs) against PCSK9 have been demonstrated to decrease LDL-C in human clinical trials. The discovery of small molecules capable of disrupting the protein-protein interaction (PPI) between PCSK9 and its receptor has proven challenging. We sought new points of pharmacological intervention in the pathway leading from synthesis and secretion of PCSK9 to PCSK9-mediated elevation of plasma LDL-C levels that would be amenable to small molecule modulation and could potentially yield new pharmacotherapies to treat lipid disorders. In this presentation, we will describe the identification and characterization of the first anti-secretagogues of PCSK9 via a phenotypic screen of the Pfizer compound collection. These compounds were shown to recapitulate the effects in cells observed with anti-PCSK9 mAbs. Several post-screen challenges will be discussed, including: (1) separating real active compounds from cytotoxic compounds, both of which produce the same phenotype; (2) building confidence in the lipid lowering pharmacology of the hits; and (3) de-convoluting the molecular target of a selected compound. From a screen of over 2.5 million compounds, only a single compound was identified as a validated PCSK9 secretion inhibitor, becoming the lead structure around which a new project for reducing plasma lipid levels was established. This compound was shown to decrease PCSK9 secretion by an unprecedented mechanism involving binding to the 80S ribosome to inhibit PCSK9 translation.

MEDI 341

Discovery of chemical biology probes inhibiting activation of SGK3 kinase in cancer cells

***Mika Lindvall**, mika.lindvall@novartis.com, Gisele A. Nishiguchi, Cornelia Bellamacina, Wei Shu, Li Tian, Eric J. Martin, Sylvia Ma, Eric Fang, Tatiana Zavorotinskaya, Eunhye Park, David Duhl, Alice C. Rico, Victoriano Tamez, Laura Doyle, Michael Doyle. Novartis Institutes for BioMedical Research, Oakland, California, United States*

Development of selective kinase inhibitors – still a challenge – can be greatly accelerated by machine learning from large bodies of kinase activity data. Here we describe discovery of selective inhibitors of SGK3 from Protein Family Virtual Screening and their rapid further optimization into picomolar activity. We are disclosing for the first time SGK kinase inhibitors targeting the inactive conformation of SGK3 and describing their target modulation in cancer cell lines.

MEDI 342

Unveiling the truth about PAK1 with medicinal chemistry

Joachim Rudolph¹, rudolph.joachim@gene.com, Lesley J. Murray¹, Chudi O. Ndubaku¹, Thomas O'Brien¹, Elizabeth Blackwood¹, Weiru Wang¹, Ignacio Aliagas¹, Lewis J. Gazzard¹, James J. Crawford¹, Joy Drobnick¹, Wendy Lee¹, Xianrui Zhao¹, David Favor², Ping Dong², Haiming Zhang¹, Christopher E. Heise¹, Angela Oh¹, Christy Ong¹, Hank La¹, Paroma Chakravarty¹, Connie Chan¹, Diana Jakubiak¹, Jennifer Epler¹, Sreemathy Ramaswamy¹, Roxanne Vega¹, Gary Cain¹, Dolores Diaz¹, Yu Zhong¹. (1) Genentech Inc, S San Fran, California, United States (2) Chempartner, Shanghai, China

p21-activated kinase 1 (PAK1) is a serine/threonine kinase with an important role in transducing signals in several oncogenic pathways. The concept of inhibiting PAK1 has garnered significant interest over the past decade, particularly for targeting cancers associated with PAK1 amplification. *In vivo* studies with the selective Group I PAK (PAK1, 2, 3) inhibitor G-5555 from the pyrido[2,3-d]pyrimidin-7-one class uncovered acute toxicity with a narrow safety window. To investigate its root cause, we explored structurally differentiated Group I PAK inhibitors that included analogs from the pyrido[2,3-d]pyrimidin-7-one class and two other, chemically distinct, series. Mouse tolerability studies of these compounds revealed persistent toxicity and a correlation of minimum toxic concentrations and PAK1/2 mediated cellular potencies. Our data suggests acute cardiovascular toxicity resulting from the inhibition of PAK2, which may be enhanced by PAK1 inhibition, and cautions against continued pursuit of pan-Group I PAK inhibitors in drug discovery.

MEDI 343

Discovery of the potent and selective, broad spectrum fungal CYP51 inhibitor VT-1598

Christopher M. Yates, cyates@viamet.com, Edward P. Garvey, Robert J. Schotzinger, Sammy R. Shaver, William J. Hoekstra. Viamet Pharmaceuticals, Durham, North Carolina, United States

Current fungal CYP51 inhibitors (i.e., “azoles” such as fluconazole and voriconazole) are effective antifungal agents, yet potently inhibit a broad range of off-target human cytochrome P450 enzymes leading to safety liabilities (e.g., drug-drug interactions, liver

and reproductive toxicities). We report the discovery of a novel, fungal CYP51-selective agent with broad-spectrum activity. Herein, the triazole heme iron binding group of current agents was replaced with novel, less avid heme iron binding groups in concert with homology-model guided, potency-enhancing scaffold modifications. This process generated VT-1598, an orally available inhibitor that demonstrates high potency against a broad range of yeasts, molds, and endemic fungi. Viamet is developing VT-1598 for the treatment of coccidioidomycosis, or Valley Fever, a systemic fungal infection in the SW United States with notable unmet need. In a preclinical model of Valley Fever, VT-1598 was highly effective in treating an infection localized to the CNS. VT-1598 has been granted Qualified Infectious Disease Product (QIDP) and orphan drug designations by the FDA to treat coccidioidomycosis. VT-1598 complements Viamet's yeast- and dermatophyte-active Phase 2B agent VT-1161 under development for both onychomycosis and recurrent vulvovaginal candidiasis.

MEDI 344

Discovery of the brain penetrant Phosphodiesterase 1 (PDE1) inhibitor Lu AF64386

Jan Kehler¹, jke@lundbeck.com, Anna I. Parachikova³, Hanna Lindgren³, Lars K. Rasmussen¹, Morten Langgard¹, Claus T. Christoffersen¹, Christoffer Bundgaard¹, Karsten Juhf², Lasse Skibsbye³, Jeppe Agner³, Jacob Nielsen³. (1) Discovery Chemistry, H- Lundbeck A/S, Valby, Denmark (2) Discovery Chemistry and DMPK, H. Lundbeck, Valby, Denmark (3) H. Lundbeck A/S, Valby, Denmark

PDE1A and PDE1B are highly expressed in neurons in brain regions involved in learning, memory and cognitive processing. PDE1 is an attractive target for the treatment of a number of psychiatric and neurological diseases, since it regulates the levels of cyclic nucleotides cAMP/cGMP, which are critical intracellular signaling molecules that regulate neuronal functions like synaptic plasticity, cognitive functions, neuronal survival, and axonal regeneration.

Here we describe the discovery of the PDE1 inhibitor Lu AF64386 together with the SAR for the related class of quinazolines, including the X-ray structure of Lu AF64386 in complex with the catalytic domain of PDE1B. LU AF64386 is a highly permeable and brain penetrant PDE1 inhibitor with low intrinsic clearance and low propensity for Drug-Drug interactions. The relationship between exposure and PDE1 CNS occupancy was established by the use of a novel tritiated PDE1 ligand. The compound was able to elevate brain levels of cGMP and demonstrate pro-cognitive properties in doses corresponding to PDE1B CNS occupancy 30-60%. The compound has shown an overall profile making it attractive for further studies of PDE1 in vitro and in vivo.

MEDI 345

Impact of P-gp susceptibility on brain penetration for a series of potent, selective, and orally bioavailable TrkA inhibitors

Mark E. Fraley, *mark_fraley@merck.com*. *Discovery Chemistry, Merck & Co., Inc., West Point, Pennsylvania, United States*

Nerve growth factor (NGF) signaling through tropomyosin receptor kinase A (TrkA) mediates the induction and modulation of pain associated with injury, inflammation, and chronic pain states. Activation of TrkA by NGF triggers downstream intracellular processes and protein expression that increase the sensitivity of nociceptors. Inhibition of the NGF/TrkA pathway as a novel approach for the treatment of pain has been clinically validated with NGF-neutralizing monoclonal antibodies. For example, tanezumab (Phase III) has demonstrated efficacy in chronic osteoarthritis and lower back pain. Our approach has focused on the identification of small molecules inhibitors of TrkA for the treatment of pain. We previously described a potent, selective, and orally bioavailable series of benzamides derived from a screening hit. Because of literature reports of CNS cholinergic deficits in rats following direct injection of anti-NGF antibodies in the brain, we sought peripherally selective molecules to mitigate the risk of cognitive dysfunction due to central TrkA inhibition. To achieve peripheral selectivity, we utilized P-glycoprotein (P-gp) susceptibility as a primary design feature. Using TrkA phosphorylation (pTrkA) as functional assay of target engagement, we demonstrated that these compounds exhibited lower plasma IC₅₀ values for pTrkA in skin compared to brain. This presentation will describe the relationship between peripheral selectivity, as measured by the shift in IC₅₀ values, and in vitro P-gp efflux ratio, and limitations discovered for this series of compounds. Also highlighted will be other approaches attempted to increase peripheral selectivity, including reducing permeability, and the subsequent impact on oral bioavailability. The evolution and profiles of representative leading molecules will be discussed. Lastly, the binding site and key interactions for this novel allosteric class of TrkA inhibitors will be illustrated using X-ray crystal structures.

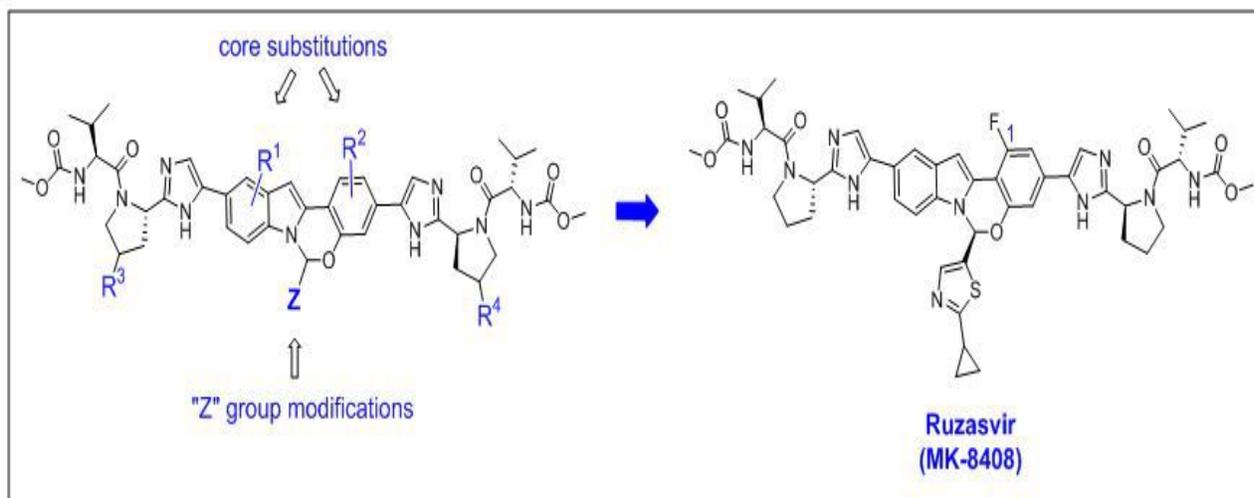
MEDI 346

Discovery of ruzasvir (MK-8408), a 2nd generation HCV NS5A inhibitor

Wensheng Yu¹, *wensheng.yu@merck.com*, **Ling Tong**¹, **Lei Chen**¹, **Oleg Selytin**¹, **Michael P. Dwyer**¹, **Anilkumar G. Nair**¹, **Rob Mazzola**¹, **Jae-Hun Kim**¹, **Deyou Sha**¹, **Jingjun Yin**², **Rebecca Ruck**², **Ian W. Davies**², **Bin Hu**³, **Bin Zhong**³, **Jinglai Hao**³, **Tao Ji**³, **Shuai Zan**³, **Rong Liu**⁴, **Sony Agrawal**⁴, **Ellen Xia**⁴, **Stephanie Curry**⁴, **Patricia Mcmonagle**⁴, **Karin Bystof**⁴, **Fred Lahser**⁴, **Donna Carr**⁴, **Laura Rokosz**⁴, **Paul Ingravallo**⁴, **Shiyong Chen**⁵, **Kung-I Feng**⁶, **Mark Cartwright**⁷, **Ernest Asante-Appiah**⁴, **Joseph A. Kozlowski**¹. (1) *Department of Medicinal Chemistry, Merck Research Laboratories, Kenilworth, New Jersey, United States* (2) *Department of Process Chemistry, Merck Research Laboratories, Rahway, New Jersey, United States* (3) *WuXi AppTec, Shanghai, China* (4) *Department of Discovery Biology, Merck Research Laboratories, Kenilworth, New Jersey, United States* (5) *Department of Pharmacokinetics, Merck Research Laboratories, Kenilworth, New Jersey, United States* (6) *Department of Discovery Pharmaceutical Science, Merck Research Laboratories, Kenilworth, New Jersey, United States* (7) *Department of Drug Safety, Merck Research Laboratories, Kenilworth, New Jersey, United States*

HCV nonstructural protein 5A (NS5A) is essential for HCV viral replication; thus makes HCV NS5A inhibitor an important component in the combo therapy for HCV infection. The discovery of potent and pan-genotypic HCV NS5A inhibitors faces multiple challenges, such as the significant diversity among genotypes and mutants, substantial potency shift conferred on some key resistance-associated variants (RAVs), inconsistent SARs between different genotypes and mutants, and the lacking of X-ray structures of the inhibitor/protein complexes for rational inhibitor design.

Research effort in Merck on HCV NS5A inhibition has led to the discovery of a tetracyclic-indole based inhibitor Elbasvir (MK-8742), one of the two components in ZEPATIER™ which were approved for the treatment of chronic HCV genotype 1 or 4 infections in adults. Herein we present our continued effort on HCV NS5A inhibition and the discovery of a 2nd generation NS5A inhibitor Ruzasvir (MK-8408). Key SARs identified for the discovery of Ruzasvir include multiple structure modifications that could significantly improve the potency against the key RAVs, such as GT2b and GT1a_Y93H simultaneously, and maintain a good potency against the other genotypes and mutants. These structure modifications include the introduction of a relatively large substituent, such as a cyclopropyl or a phenyl group, to the para position of the phenyl “Z” group on the aminal carbon, the introduction of a C1-fluoro substitution on the tetracyclic indole core, and the replacement of the phenyl “Z” group with a thiophene or thiazole moiety. Ruzasvir is a very potent and pan-genotypic NS5A inhibitor which has a “flat” potency profile, with the “flat” been defined by minimum potency shift (<10-fold) from GT1a to other genotypes and RAVs. Ruzasvir is currently in clinical development as a part of an all-oral regimen for the treatment of chronic HCV infection.



The Discovery of Ruzasvir (MK-8408)

MEDI 347

Discovery of highly efficacious potentiators for the treatment of cystic fibrosis

Steven Van der Plas¹, *steven.vanderplas@glpg.com*, **Hans Kelgtermans**¹, **Tom De Munck**¹, **Sebastien Martina**¹, **Linda Tomaskovic**², **Thierry christophe**¹, **Mia Jans**¹, **Ellen Van der Aar**¹, **Monica Borgonovi**³, **Luc Nelles**¹, **Maarten Gees**¹, **Pieter Stouten**¹, **Jan Van Der Schueren**¹, **Oscar Mammoliti**¹, **Martin Andrews**¹, **Katja Conrath**¹. (1) Galapagos NV, Mechelen, Belgium (2) Fidelta ltd, Zagreb, Croatia (3) Galapagos SASU, Romainville, France

Cystic fibrosis (CF) is the most common life-threatening autosomal recessive disorder in Caucasian populations, affecting approximately 70,000 patients worldwide. It is caused by mutations of the gene for the cystic fibrosis transmembrane conductance regulator (CFTR) protein. This results in a decreased chloride transport across epithelial tissues. To address the underlying causes of cystic fibrosis, two biomolecular activities are required, namely correctors to increase CFTR levels at the cell surface, and potentiators to allow the effective opening of the CFTR channel. Combined, these activities allow chloride ion transport, yielding improved hydration of the lung surface and subsequent restoration of mucociliary clearance. We are developing compounds capable of performing both these activities individually, and we report here the identification of novel potentiators able to open the CFTR channel with high efficacy.

An initial screen was performed to identify compounds that modulate CFTR protein activity located at the cell surface, leading to enhanced chloride transport. A set of 589 compounds, selected using 2D structural/electrostatic field similarities to known potentiator compounds were screened. Hit expansion around the most active hits resulted in the discovery of a novel series that showed enhanced channel opening when compared to the disease modifying agent Kalydeco on G551D CFTR. Further medicinal chemistry efforts focused on improving efficacy, ADME and PK, but also the overall drug-drug interaction profile. This resulted in the identification and development of GLPG1837, a novel potentiator that shows enhanced efficacy in primary human bronchial epithelium cells (HBE) derived from CF patients. At the time of submitting this abstract, GLPG1837 is undergoing Phase 2 trials in CF patients with class III mutations.

MEDI 348

Discovery of ABBV/GLPG-2222: A potent, efficacious CFTR corrector for the treatment of cystic fibrosis

Xueqing Wang, *xwa142@yahoo.com*. *Discovery Chemistry and Technology, AbbVie Inc., North Chicago, Illinois, United States*

Cystic fibrosis (CF) is a multisystem disease of the lungs, sinuses, pancreas, and gastrointestinal tract, and is caused by dysfunction or deficiency of the cystic fibrosis transmembrane conductance regulator protein (CFTR), an epithelial anion channel that regulates salt and water balance in tissues and maintains homeostasis of the airway surface liquid layer of the lungs. To address the most prevalent patient population (F508del mutation), two biomolecular modulators are required, correctors to increase CFTR levels at the cell surface, and potentiators to allow the effective opening of the CFTR channel. Despite approved potentiator and potentiator/corrector combo therapies,

there remains high need to develop more potent and efficacious correctors to provide robust clinical efficacy for the large patient population.

We have identified a highly potent series of CFTR correctors. In this presentation, we will describe structure activity relationship (SAR) studies that guided the discovery and selection of ABBV/GLPG-2222. This compound was advanced into clinical trials.

MEDI 349

Discovery of clinical candidate BMS-986158, an oral BET inhibitor for the treatment of cancer

Ashvinikumar V. Gavai, ashvinikumar.gavai@bms.com, Derek Norris, George V. De Lucca, David Tortolani, Daniel P. O'Malley, Yufen Zhao, Claude A. Quesnelle, Wen-Ching Han, Patrice Gill, Wayne Vaccaro, Tram Huynh, Vijay Ahuja, Dharmpal Dodd, Christopher Mussari, Lalgudi S. Harikrishnan, Muthoni Kamau, John S. Tokarski, Richard Rampulla, Dauh-Rurng Wu, Jianqing Li, Huiping Zhang, Peng Li, Dawn Z. Sun, Henry Yip, Chunlei Wang, Yingru Zhang, Arvind Mathur, Haiying Zhang, Christine Huang, Zheng Yang, Asoka Ranasinghe, Celia J. D'Arienzo, Ching Tye, Ching Su, Gerry Everlof, Lisa Zhang, Nirmala Raghavan, Krista Menard, Mei-Li Wen, John T. Hunt, Michael Poss, Gregory Vite, Richard Westhouse, Francis Lee. Bristol Myers Squibb, Princeton, New Jersey, United States

The Bromodomains and extra-terminal domain (BET) is a family of 4 adapter proteins, BRD2, BRD3, BRD4, and BRDT, which bind to specific acetylated lysine residues on the histone tails of chromatin and recruit additional regulatory proteins to regulate gene transcription. The interest in BET as an oncology target was sparked by the observation that patients with NUT-midline carcinoma have a chromosomal rearrangement fusing the NUT gene with BRD4. Based on subsequent studies, BET is now considered as an effective strategy for targeting transcriptional suppression of key oncogenes, such as MYC and BCL2. Super enhancers are large enhancer regions that are densely populated by transcription factors leading to increased transcription of lineage-specific survival genes. BRD4 is enriched in these critical control regions, suggesting that BET inhibition will lead to transcriptional suppression of oncogenic drivers. Taken together, these studies provide a strong rationale for pursuing transcriptional regulation via BET inhibition as a strategy for the treatment of hematologic malignancies and solid tumors. This presentation will describe crystal-structure guided SAR studies in a novel series of BET inhibitors that culminated in identification of BMS-986158 as a highly potent BET inhibitor suitable for oral dosing. Structure-activity relationships will be described along with in vivo evaluation of BMS-986158 in relevant tumor xenograft models. BMS-986158 is currently in Phase I clinical trials for the treatment of cancer.

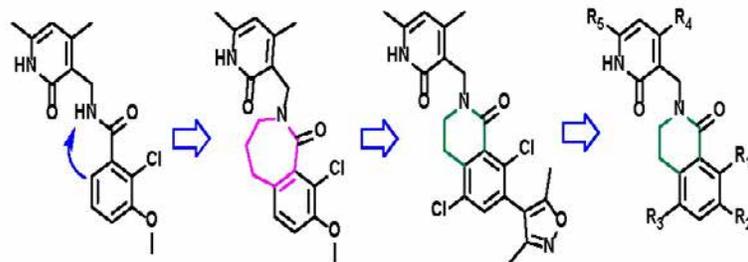
MEDI 350

Discovery of a novel class of potent, selective, and orally bioavailable histone methyltransferase Enhancer of Zeste Homolog 2 (EZH2) inhibitors and the identification of development candidate PF-06821497

*Pei-Pei Kung*¹, *peipei.kung@pfizer.com*, *Simon Bergqvist*², *Patrick Bingham*¹, *John F. Braganza*¹, *Alexei Brooun*¹, *Michael R. Collins*¹, *Wade Diehl*¹, *Ya-Li Deng*¹, *Dac Dinh*¹, *Connie Fan*¹, *Valeria R. Fantin*³, *Hovhannes J. Gukasyan*¹, *Wenyue Hu*¹, *Buwen Huang*¹, *Robert Kania*¹, *Wei Liu*¹, *Susan Kephart*¹, *Manfred Kraus*¹, *Cody Krivacic*², *Robert A. Kumpf*¹, *Gary Li*⁴, *Karen Maegley*¹, *Indrawan J. McAlpine*¹, *Lisa Nguyen*¹, *Sacha Ninkovic*¹, *Martha A. Ornelas*¹, *Dan Richter*¹, *Eugene Rui*¹, *Michael Ryskin*⁵, *Stephanie A. Scales*¹, *Jillian Spangler*¹, *Al Stewart*¹, *Scott C. Sutton*¹, *John Tatlock*¹, *Cheng-Chung Tsao*¹, *Dominique Verhelle*⁶, *Fen Wang*¹, *Hui Wang*¹, *Peter Wells*¹, *Martin Wythes*¹, *Shinji Yamazaki*¹, *Brian Yip*², *Xiu Yu*¹, *Luke Zehnder*¹, *Wei-Guo Zhang*², *Peter Zhu*¹, *Jinjiang Zhu*¹, *Robert A. Rollins*¹, *Shikhar Sharma*¹, *Martin P. Edwards*¹. (1) Pfizer WRD La Jolla, San Diego, California, United States (2) Unknown, San Diego, California, United States (3) Oric Pharma, South San Francisco, California, United States (4) Ignyta, San Diego, California, United States (5) Bank of America, New York, New York, United States (6) Third Rock Ventures, Boston, Massachusetts, United States

The histone methyltransferase EZH2 is the catalytic subunit of the polycomb repressor complex 2 and several EZH2 inhibitors have entered clinical trials. One such molecule (EPZ-6438) has demonstrated promising clinical responses in diffuse large B cell lymphoma (DLBCL) patients and has currently progressed to phase II trials with a recommended dose of 800 mg, twice a day. Our internal discovery program focused on identifying a novel EZH2 inhibitor which displayed potent, selective inhibitory potency as well as desired pharmacokinetic and pharmaceutical properties. We identified an initial benzamide-containing lead (compound **1**) via a high throughout chemistry approach. In the absence of a co-crystal structure of this lead and/or other known EZH2 inhibitors with the protein, we used a ligand-based design strategy to optimize compound potency/efficiency. Accordingly, analysis of the torsional angles present in **1** and subsequent conformational restriction afforded the cell-active 7-membered lactam (compound **2**). Further potency optimization of **2** resulted in a robust tool compound (**3**) which demonstrated proof of concept both in vitro and in vivo for this novel class of EZH2 inhibitors.

Further lead transformation and LipE optimization of **3** resulted in potency, pharmacokinetic, and physical property improvements and provided the clinical candidate, compound **4** (PF-06821497). Several co-crystal structures of the new inhibitors in complex with EZH2 were obtained during the course of these activities, and they helped rationalize the observed potency and LipE improvements. Compound **4** (PF-06821497) demonstrated robust TGI effects and modulation of tumor H3K27Me3 levels in a DLBCL Karpas-422 xenograft model. The molecule also demonstrated favorable pharmaceutical properties and predicted human pharmacokinetic parameters and was therefore selected for subsequent clinical development.



Compound#	1	2	3	4
	Initial lead	cell active	In vivo tool	Dev. Candidate (first disclosure)
Enzyme (WT, nM)	7200 (IC ₅₀)	150 (IC ₅₀)	0.68 (Ki)	0.12 (Ki)
LipE	4.0	5.2	6.1	8
Karpas 422 cell H3K27Me3/prolif. IC ₅₀ (nM)	ND	7900/6300	15/25	4/6
PK/PD	ND	ND	66.4% reduction @ 300 mg/kg	In presentation
TGI (Karpas422)			127% @ 300 mg/kg	In presentation

MEDI 351

Discovery and development of BLU-554: A potent, highly selective covalent inhibitor of Fibroblast Growth Factor Receptor 4 (FGFR4) in development for the targeted treatment of advanced Hepatocellular Carcinoma (HCC) patients with amplified and overexpressed FGF19

Chandra V. Miduturu, cmiduturu@blueprintmedicines.com, Margit Hagel, Mike Sheets, Nooreen Rubin, Weifan Weng, Neil Bifulco, Lucian V. Dipietro, Joseph Kim, Natasja Brooijmans, Brian L. Hodous, Nico Stransky, Klaus Hoeflich, Vivek J. Kadambi, Nancy Kohl, Christoph Lengauer, Timothy Guzi. Blueprint Medicines, Cambridge, Massachusetts, United States

Liver cancer is the second leading cause of cancer-related deaths worldwide, with HCC accounting for most liver cancers. There is a significant need for more effective and targeted therapies to treat HCC. FGF19 regulates bile acid synthesis and hepatocyte proliferation in normal liver through activation of its receptor FGFR4. Activation of the FGF19 signaling pathway is observed in up to 30% of patients with HCC and has been shown to induce liver cancer in genetic mouse models. Selective inhibition of FGFR4

thus represents a targeted strategy to treat this genetically defined sub-group of HCC. BLU-554 is a potent, highly selective covalent inhibitor of FGFR4 currently being evaluated by Blueprint Medicines in a Phase 1 clinical trial for the treatment of patients with advanced HCC. Herein, we will describe the discovery of BLU-554 utilizing iterative structure-based drug design. This presentation will also include the characterization of the mechanistic details of covalent inhibition of FGFR4 and the structure-activity relationships (SAR) development toward the optimization of overall drug properties for BLU-554. The pharmacokinetics and pharmacological activity of BLU-554 in tumor models with genomically amplified and overexpressed FGF19 will also be described.

MEDI 352

Discovery of PRN1371: A highly selective, irreversible inhibitor of FGFR1-4 in clinical development for the treatment of solid tumors

***Kenneth A. Brameld**, ken.brameld@principiabio.com. Principia Biopharma, South San Francisco, California, United States*

Aberrant signaling of the FGF/FGFR pathway occurs in 7% of cancers and is an oncogenic driver in many solid tumors including bladder, breast, endometrial, gastric, hepatocellular, lung, and ovarian cancers. There is a compelling medical need for a highly selective inhibitor of all four FGFR family members, FGFR1-4, to improve clinical responses in patients while minimizing off-target toxicity. Principia Biopharma, a pioneer in covalent drug discovery and development, has addressed this need with the discovery of PRN1371, an oral, low dose, highly selective, covalent irreversible inhibitor of fibroblast growth factor receptor (FGFR) 1-4.

The discovery and development of covalent drugs presents unique challenges. Starting from a 7-oxopyridopyrimidine core, we applied structure-based design to incorporate a Michael acceptor appropriately positioned to engage Cys 488 in a covalent bond. To guide lead optimization, specialized assays were developed to measure FGFR target occupancy and assess the ADME liabilities of Michael acceptors. The final selection of PRN1371 as a clinical candidate was based upon the ideal combination of selectivity, potent and prolonged pharmacodynamic inhibition of FGFR1-4 in vivo and high predicted human oral absorption. PRN1371 is now being investigated in a Phase 1/2 open-label, multicenter clinical trial for solid tumors.

MEDI 353

FGF401: A reversible-covalent inhibitor of FGFR4 for the treatment of hepatocellular carcinoma

***Robin A. Fairhurst**¹, robin.fairhurst@novartis.com, **Thomas Knoepfel**¹, **Pascal Furet**¹, **Nicole Buschmann**¹, **Catherine Leblanc**¹, **Robert Mah**¹, **Michael Kiffe**², **Diana Graus-Porta**², **Andreas Weiss**², **Jacqueline Kinyamu-Akunda**², **Markus Wartmann**², **Joerg Trappe**², **Tobias Gabriel**¹, **Francesco Hofmann**², **William Sellers**². (1) Global Discovery*

Chemistry, Novartis Institutes for BioMedical Science, Basel, Switzerland (2) Novartis Institutes for BioMediacal Research, Basel, Switzerland

The aberrant signaling of fibroblast growth factor 19 (FGF19) through the fibroblast growth factor receptor 4 (FGFR4) in combination with the co-receptor β -klotho (KLB) has been shown to be essential for the initiation and maintenance of a subset of hepatocellular carcinomas (HCC). This presentation will describe the discovery of FGF401 as a highly potent and selective, first-in-class, reversible-covalent inhibitor of the kinase activity of the FGFR4. FGF401 exhibits: a good oral pharmacokinetic profile; high in vivo potency, leading to activity in preclinical FGF19/FGFR4 dependent xenograft models; and a favorable safety profile. A PhI/II study with FGF401 is currently ongoing in patients with solid tumors positive for FGFR4 and KLB.

MEDI 354

Interdiction at a protein-protein interface: Structure-based design of the Mcl-1 inhibitor AMG 176

Sean P. Brown, sebrown@amgen.com. Amgen Inc, San Francisco, California, United States

Mcl-1 is an anti-apoptotic member of the Bcl-2 family of proteins which act via protein-protein interactions between pro- and anti-apoptotic factions to mediate the intrinsic pathway of programmed cell death. Identification of Mcl-1 as a key pro-survival factor in many human cancers and its association with tumor progression and resistance to chemotherapy has made it a highly desirable drug target. Although compelling, targeting disruption of Mcl-1's protein-protein interaction to induce tumor cell death, was previously thought to be "un-druggable," due to the high affinities of Mcl-1 to the pro-apoptotic Bcl-2 proteins and lack of a small molecule binding pocket. This presentation will describe the convergence of structural information and small molecule conformational analysis applied to the optimization of a small molecule high-throughput screening hit culminating in the identification of the Mcl-1 inhibitor AMG 176. A phase 1 clinical trial is currently evaluating AMG 176 in multiple myeloma patients.

MEDI 355

Probing the epigenome for therapeutic targets

Cheryl H. Arrowsmith, carrow@uhnres.utoronto.ca. Princess Margaret Cancer Centre, The Structural Genomics Consortium, and Department of Medical Biophysics, University of Toronto, Toronto, Ontario, Canada

Regulation of gene expression via chromatin associated factors and alterations of the cellular epigenome are fundamental to most biological processes, and many disease mechanism. We are taking a protein family approach to understand how chromatin regulatory proteins recognize specific histone tail sequences and their posttranslational

modifications. Proteins such as histone methyltransferases, demethylases, acetyltransferases and bromodomains and chromodomains mediate nuclear signaling networks that regulate epigenetic cellular states and gene expression programs. Systematic structural and biochemical analyses of these protein families are revealing key features of selectivity and regulation among these factors, enabling structure-based development of potent, selective, cell-active small molecule inhibitors of individual epigenetic regulatory proteins. Such compounds – Chemical Probes - are extremely valuable for understanding epigenetic signaling mechanisms in cells. Chemical probes are highly complementary to genetic methods and more closely mimic strategies for therapeutic translation. We are providing our epigenetic chemical probes as an open access resource to the biological research community to facilitate understanding of epigenetic mechanisms and to more rapidly identify and validate therapeutic targets for cancer and other diseases. I will present our work on Chemical Probes for protein methyltransferases and their characterization in human disease models.

MEDI 356

Tazemetostat, a first-in-class inhibitor of EZH2: From bench to bedside to bench

R Copeland, *rcopeland@epizyme.com*. Epizyme, Cambridge, Massachusetts, United States

The protein methyltransferases (PMTs) constitute a class of enzymes that catalyze the methylation of lysine or arginine residues on histones and other proteins. The enzyme EZH2 provides a representative example of altered PMTs that act as genetic drivers of specific human cancers. Point mutations of EZH2 are found in a subset of non-Hodgkins lymphoma patients; the enzymatic activity of both wild type and mutant EZH2 are required for pathogenesis in these patients. Also, deletion of the INI1 or SMARCA4 subunit of the SWI/SNF chromatin-remodeling complex occur in a number of cancer types. For example, INI1 is deleted in nearly all malignant rhabdoid tumors (MRTs), a cancer found mainly in children that carries a particularly poor prognosis. Similarly, the SMARCA4 subunit of SWI/SNF is deleted, for example, in malignant rhabdoid tumor of the ovary (MRTO, also referred to as small cell carcinoma of the ovary hypercalcemic type), an aggressive cancer affecting young women. An antagonistic relationship has been demonstrated between the biochemical action on chromatin of the SWI/SNF complex and EZH2 that is relieved in MRTs due to the INI1 deletion. We have shown that INI1-deficient MRT and SMARCA4-deficient MRTO are selectively killed by EZH2 inhibition in culture and in mouse xenograft models. Drug discovery efforts have yielded a potent, selective inhibitor of EZH2, tazemetostat (EPZ-6438), that has now transitioned into phase 2 clinical trials. This inhibitor affects the appropriate histone methyl marks in cells, leads to selective cell killing that is dependent on genetic lesions associated with EZH2 activity and effects tumor growth inhibition in xenograft models. Combining tazemetostat with other treatment modalities for non-Hodgkins lymphoma results in dramatic synergy of anti-proliferative activity in preclinical models. Results of preclinical and phase 1 clinical studies of tazemetostat will be presented.

MEDI 357

Sirtuin inhibitors as promising anticancer agents

Hening Lin^{1,2}, hl379@cornell.edu. (1) Cornell University, Ithaca, New York, United States (2) Howard Hughes Medical Institute, Ithaca, New York, United States

Sirtuins are known as nicotinamide adenine dinucleotide (NAD)-dependent deacetylases. They regulate aging, transcription, and metabolism, and are considered important targets for treating several human diseases. There are seven sirtuins in humans, SIRT1-7. Four of them (SIRT4-7) have very weak deacetylase activity, which have caused many confusions and debates in the biological community. My laboratory has recently discovered several novel enzymatic activities, such as desuccinylation and defatty-acylation, for several sirtuins with no robust deacetylase activity. This has led to the identification of previously unknown protein posttranslational modifications (PTMs) and revealed new regulatory mechanisms of biology. Furthermore, this finding has enabled us to develop compounds that can inhibit particular sirtuins selectively. Some of the selective sirtuins inhibitors can kill cancer cells in cell culture and inhibit tumor formation in mouse models at least partly via the regulation of c-Myc and Ras. The roles of sirtuins and the new PTMs in cancer are being elucidated.

MEDI 358

Targeting histone lysine methylation regulatory pathways in cancer

Patrick Trojer, Patrick.Trojer@constellationpharma.com. Constellation Pharmaceuticals, Inc., Cambridge, Massachusetts, United States

Histone lysine methylation is widely recognized as part of the cell's repertoire to alter chromatin structure, and thus to regulate processes that require access to DNA, including the regulation of transcription, DNA damage response and replication. Several protein families of lysine methyltransferases (KMTs), methyl-lysine demethylases (KDMs) and methyl-lysine specific chromatin binders, controlling placement, removal and recognition of histone lysine methylation marks were identified as transcriptional co-regulators to allow for dynamic changes in gene expression in response to a variety of stimuli.

Genomic and transcriptomic sequencing campaigns have revealed that KMTs, KDMs and methyl-lysine binders are frequently dysregulated in cancer, suggesting that cancer cells utilize manipulation of histone lysine methylation patterns as a means to tweak gene expression programs to gain a growth advantage. Multiple KMTs and KDMs have been identified as candidate oncogenic drivers in recent years. Small molecule KMT and KDM inhibitors constitute an attractive therapeutic approach to alter aberrant chromatin states and gene expression programs that cancer cells are dependent on. The discovery of KMT and KDM inhibitors, their application in various oncology contexts, as well as mechanistic consequences of target inhibition will be discussed.

MEDI 359

Exploring novel models of interaction to inhibit protein methyltransferases

Minkui Luo, *luom@mskcc.org*. Box 248, Memorial Sloan-Kettering Cancer Center, New York, New York, United States

Protein methyltransferases (PMTs) play essential roles in many biological processes. Given the implicated roles of many PMTs on cancer malignancy, many efforts have been made to develop PMT inhibitors. In contrast to genetic perturbation, which depletes full-length proteins, small-molecule inhibitors can act via distinct modes of interaction (MOI) and thus lead to different outcomes even if being designed against the same target. My laboratory leveraged multiple approaches to identify PMT inhibitors with novel MOI. Our sinefungin analogues are SAM-competitive inhibitors with the potency and selectivity for multiple PMTs. The selectivity of these PMT inhibitors largely stems from their abilities to target the distinct conformations. We will present our recent progress in this aspect.

MEDI 360

Chemical probes targeting the protein arginine deiminases

Paul R. Thompson, *paul.thompson@umassmed.edu*. Biochemistry and Molecular Pharmacology, UMass Medical School, Worcester, Massachusetts, United States

The Protein Arginine Deiminases (PADs) hydrolyze arginine residues to form citrulline. This post-translational modification is upregulated in numerous cancers and autoimmune disorders including RA, lupus, and Alzheimer's disease. While these enzymes are important regulators of gene transcription, the full spectrum of biological activities is relatively underexplored.

Herein, I will discuss our efforts to develop next generation inhibitors targeting the PADs, focusing on our success in developing inhibitors with improved potency, selectivity, and cellular and *in vivo* activity. These next generation compounds hold therapeutic promise as potential inhibitors of PAD activity.

MEDI 361

Discovery of clinical candidate PF-06648671: A potent, highly brain penetrant gamma secretase modulator for the treatment of Alzheimer's disease

Martin Pettersson, *martin.pettersson@pfizer.com*, Christopher am Ende, Todd W. Butler, Peter H. Dorff, Ivan V. Efremov, Edelweiss Evrard, Shane A. Eisenbeis, Christopher J. Helal, Michael E. Green, John M. Humphrey, Gregory W. Kauffman, Patrick B. Mullins, Christopher J. O'Donnell, Danica A. Rankic, Antonia F. Stepan, Cory M. Stiff, Nandini Patel, Chakrapani Subramanyam, Tuan P. Tran, Edward X. Yang, Longfei Xie, Kelly R. Bales, Eva Hajos-Korcsok, Betty A. Pettersen, Leslie R. Pustilnik,

Stefan J. Steyn, Kathleen M. Wood, Ruolun Qiu, Patrick R. Verhoest. Pfizer Inc., Mystic, Connecticut, United States

Alzheimer's disease is a devastating neurodegenerative disorder where formation and deposition of neurotoxic oligomers of amyloid β 42 ($A\beta$ 42) is believed to play a pivotal role. $A\beta$ 42 is derived from the amyloid precursor protein (APP) via sequential processing by the β -secretase (BACE) and γ -secretase enzymes. Over the past decade, γ -secretase modulators (GSM) have emerged as promising therapeutic agents for selectively reducing brain levels of the neurotoxic $A\beta$ species without inhibiting γ -secretase cleavage of other critical substrates such as the Notch receptor. However, γ -secretase is an intramembrane aspartyl protease, and designing potent modulators within good physicochemical property space to ensure an adequate safety profile for clinical development has proven to be particularly challenging.

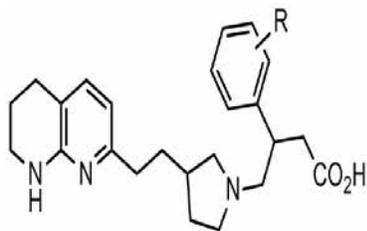
This presentation will describe the medicinal chemistry design strategies that resulted in clinical candidate PF-06648671. Successful lead optimization was enabled by ligand-based design tactics such as development of a pharmacophore model, introduction of rigid structural motifs to lock the molecule into its putative bioactive conformation, and design of a novel, heterocyclic core with increased polarity. Efficient use of parallel medicinal chemistry facilitated rapid evaluation of chemical space. Ultimately, we were able to achieve an optimal balance of in vitro potency and ADME parameters to afford the clinical candidate PF-06648671 with excellent brain penetration and robust in vivo efficacy. This presentation will also highlight human pharmacokinetics data and reduction of $A\beta$ 42 in human cerebrospinal fluid.

MEDI 362

Discovery of a small molecule $\alpha v\beta 6$ inhibitor for idiopathic pulmonary fibrosis

Simon J. MacDonald, *simon.jf.macdonald@gsk.com*, John Pritchard, Niall Anderson. GlaxoSmithKline, Stevenage, United Kingdom

Fibrotic diseases lead to progressive loss of tissue function and organ failure and are estimated to contribute ~45% of deaths in the developed world. Idiopathic pulmonary fibrosis (IPF) is one of these and is poorly treated with a survival rate lower than that of most cancers. It is characterised by excessive scarring - unregulated wound healing - in the lung with transforming growth factor β ($TGF\beta$) thought to play a pivotal role. Integrins are cell surface receptors (involved in cell adhesion *inter alia*) involved in activating $TGF\beta$ and it is thought that their inhibition - and in particular the integrin $\alpha v\beta 6$ - may have a therapeutic benefit in treating IPF. This presentation will focus on our work on the synthesis and properties of $\alpha v\beta 6$ inhibitors which led to a clinical candidate.



- -RGD- mimetic
- nM activity in cell adhesion assay
- 2 chiral centers, 2 basic centers, 1 acidic center

MEDI 363

Identification of AZD9567, an anti-inflammatory glucocorticoid receptor modulator with improved side effect profile

Lena Ripa¹, [lena.e.ripa@astrazeneca.com](mailto:lana.e.ripa@astrazeneca.com), **Matthew Dearman**¹, **Goran Edenro**¹, **Karl Edman**², **Ramon Hendrickx**¹, **Matti Lepistö**¹, **Lisa Öberg**¹. (1) Respiratory, Inflammation & Autoimmunity, Innovative Medicines and Early Development Biotech Unit, AstraZeneca RD, Mölndal, Sweden (2) Discovery Sciences, Innovative Medicines and Early Development Biotech Unit, AstraZeneca RD, Mölndal, Sweden

Synthetic glucocorticoids like prednisolone have been used for more than 50 years to treat inflammatory diseases however their use is limited by side effects including diabetes and osteoporosis. The aim of our oral glucocorticoid receptor (GR) modulator project was to identify an orally available dissociated glucocorticoid receptor modulator with improved therapeutic margins versus prednisolone.

In the chemistry program we focused our design to find potent partial modulators of GR with the potential to sample the GR conformational space differently compared to glucocorticoids currently in use opening for the possibility of differentiated co-factor interactions, signaling and biological effects subsequently leading to the separation of desired and non-desired GR related effects. Utilizing full GR agonist indazole ethers as the starting point for design we have identified structural modifications that lead to compounds with potent but partial profile in GR reporter gene assays. Regardless of the compound profile in the reporter gene assays only a narrow subset of specific structural modifications lead to a beneficial biological response with respect to GR related side effects. This work led to the discovery of AZ9567 a potent anti-inflammatory compound with reduced side effects compared to prednisolone in *in vitro* cell systems and in *in vivo* rat models and currently is evaluated in clinical trials.

MEDI 364

Discovery and early clinical profile of a non-catchol dopamine 1 receptor agonist

David L. Gray, david.l.gray@pfizer.com, **Rouba Kozak**, **Scot Mente**, **Jennifer E. Davoren**, **Deane Nason**, **Steven O'Neil**, **Ivan V. Efremov**, **Anthony Harris**, **Rebecca O'Connor**, **Michelle Salafia**. Pfizer, Cambridge, Massachusetts, United States

Selective agonism of dopamine D1 receptors (D1Rs) has been a therapeutic goal for several important neurologic and psychiatric diseases. This target has received nearly

40 years of medicinal chemistry attention due to the fundamental role of D1Rs in supporting motor function, reward processing, and cognition. To date, selective D1R agonists with good oral pharmacokinetics have remained elusive. All known D1R-selective agonists are catechols, which are rapidly metabolized *in vivo*. Here we disclose for the first time the discovery of a potent, selective, orally bioavailable non-catechol D1R partial agonist and its advancement into clinical study. This compound and close analogs have excellent preclinical efficacy in models of Parkinson's motor symptoms and display distinct binding to the D1R orthosteric site that leads to significantly reduced desensitization, recruitment of b-arrestin, and *in-vivo* tolerance. The clinical candidate was selected by balancing and optimizing a number of *in-vitro* and *in-vivo* parameters including intrinsic activity, brain penetration, pharmacokinetics, and metabolism. Targeting once-daily oral dosing, a modified release formulation was developed and advanced to clinical studies. A favorable pharmacokinetic and Phase 1 safety profile will be presented, and demonstrate that this compound is poised to examine longstanding D1R agonist therapeutic hypothesis in multiple CNS diseases including Parkinson's.

MEDI 365

Discovery and development of BLU-285: A potent, highly selective inhibitor of KIT and PDGFR α activation loop mutants

Brian L. Hodous, *bhodous@blueprintmedicines.com*, Erica Evans, Alexandra Gardino, Alison Davis, Julia Zhu, Douglas P. Wilson, Kevin Wilson, Lucian V. Dipietro, Joseph Kim, Natasja Brooijmans, Vivek J. Kadambi, Adam Shutes, Yulian Zhang, Nancy Kohl, Christoph Lengauer, Timothy Guzi. Blueprint Medicines, Cambridge, Massachusetts, United States

KIT and PDGFR α activation loop mutants are recognized drivers of disease in subsets of patients with systemic mastocytosis (SM), gastrointestinal stromal tumors (GIST) and acute myeloid leukemia (AML). For the patients with primary activation loop mutations in PDGFR α and KIT Exon 17, there are no approved treatments that target these driver mutations. In patients with acquired mutations in the activation loop of KIT, despite advances in treatment options, patients still experience disease progression during or after treatment with available therapies. BLU-285 is a potent, highly selective oral inhibitor that targets KIT Exon 17 and PDGFR α D842 activation loop mutants. Blueprint Medicines is currently conducting Phase 1 clinical trials for BLU-285 in advanced SM and unresectable, treatment-resistant GIST. We will describe the discovery of BLU-285, including an in-depth mechanistic understanding of kinase activation loop mutants. The discussion will include kinome-wide selectivity structure-activity relationships (SAR) and the optimization of overall drug properties. The pharmacokinetics, *in vivo* potency and direct target engagement in i) a KIT D816 mutant *in vivo* model of SM, and ii) a TKI-refractory KIT activation loop-driven GIST patient derived xenograft (PDX) model will also be described.

MEDI 366

NVP-LXS196, a novel PKC inhibitor for the treatment of uveal melanoma

Michael Visser, michael.visser@novartis.com, Julien P. Papillon, Jianmei Fan, Michael Luzzio, Walter Michael, Runmingdavid Wang, Alan zhang, Christopher Straub, Simon Mathieu, Mitsunori Kato, Mark G. Palermo, Christine Chen, Matthew J. LaMarche, Timothy M. Ramsey, Anthony Vattay, Ribo Guo, Vesselina Cooke, Anka Bric, Franklin Chung, Guiqing Liang, Michael Romanowski, Andrew Wylie. Novartis Institutes of Biomedical Research, Cambridge, Massachusetts, United States

Uveal Melanoma (UM) is the most common primary intraocular malignancy of the adult eye, with an incidence of five to six cases per million. Despite aggressive local management of primary UM, the development of metastases is common and occurs in ~50% of patients. There are currently no effective treatment options for metastatic disease and median survival is around nine months. Genetic analysis of UM samples reveals the presence of activating mutations in the Gq alpha subunits, GNAQ and GNA11. One of the key downstream targets of the constitutively active Gq alpha subunits is the protein kinase C (PKC) signaling pathway.

We describe the discovery of NVP-LXS196, a potent, selective PKC inhibitor. The lead series was optimized for kinase and off target selectivity to afford a compound that is rapidly absorbed and well tolerated in pre-clinical species. NVP-LXS196 is currently in Phase I clinical trials to assess the safety, tolerability and pharmacokinetic profile in metastatic uveal melanoma patients.

MEDI 367

Discovery of selonsertib (GS-4997): A first in class, selective inhibitor of apoptosis signal-regulating kinase 1

Gregory T. Notte¹, gnotte@gilead.com, Britton Corkey¹, Eric Lansdon¹, David Breckenridge¹, Oliver L. Saunders³, Michael Graupe¹, Bernard Murray¹, Chandru Venkataramani¹, Juan Guerrero¹, Julie Farand¹, Jeff A. Zablocki¹, Kerim Babaoglu², John Liles¹, Grant Budas¹, Sarah Wise¹, Keith Koch⁴, Laurie Castonguay⁵, Manoj C. Desai¹. (1) Gilead Sciences, Redwood City, California, United States (2) Merck, West Point, Pennsylvania, United States (3) Novartis Institute for Biomedical Research, Emeryville, California, United States (4) University of Colorado Anschutz Medical Campus, Aurora, Colorado, United States (5) Ency2 Consulting, Lafayette, Colorado, United States

Apoptosis signal regulating kinase 1 (ASK1) is a widely expressed redox-sensitive serine threonine kinase that activates inflammatory, fibrotic and apoptotic signaling. Pathological imbalances in cellular redox homeostasis and the resultant activation and dysregulation of downstream ASK1 signaling have been implicated in the pathogenesis of diseases such as diabetic kidney disease (DKD), pulmonary arterial hypertension (PAH), and nonalcoholic steatohepatitis (NASH). This presentation describes the

research effort leading to the identification of Selonsertib, a first-in-class, potent, and highly selective inhibitor of ASK1. In murine models of NASH and liver fibrosis, ASK1 inhibition reduced the amount of steatosis and fibrosis and decreased the circulating levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) as compared to normal controls. In a Phase 1 trial, Selonsertib demonstrated an excellent safety profile and a $t_{1/2}$ amenable to QD dosing.

MEDI 368

Discovery of a selective inhibitor of indoleamine-2,3-dioxygenase for use in the therapy of cancer

Aaron Balog, *aaron.balog@bms.com*. *Oncology Chemistry, Bristol-Myers Squibb, Princeton, New Jersey, United States*

Indoleamine-2,3-dioxygenase 1 (IDO-1) is a heme-containing enzyme that catalyzes the first and rate-limiting step in the conversion of tryptophan to kynurenine. The action of IDO-1 leads to increased levels of kynurenine and reduced levels of tryptophan in the serum and tumor microenvironment of cancer patients, which results in suppression of local immune response and subsequent growth of the tumor. Over-expression of IDO-1 has been reported in many tumor types and has been correlated with poor prognosis. IDO-1 is an attractive immuno-oncology target that is amenable to perturbation by small molecules and has been the focus of efforts by many academic and pharmaceutical company research teams. We set out to identify highly potent small molecule inhibitors of IDO-1 with good selectivity over tryptophan-2,3-dioxygenase (TDO). We investigated numerous chemotypes with differentiated mechanisms of action in their inhibition of IDO-1. Many of these leads were found to interact with IDO-1 in a manner that promotes heme displacement from the enzyme, leading to an inactive holoenzyme-inhibitor complex. This presentation will summarize the preclinical evaluation of a selective IDO-1 inhibitor that induces the loss of heme and has been advanced into Phase I clinical trials for the treatment of cancer.

MEDI 369

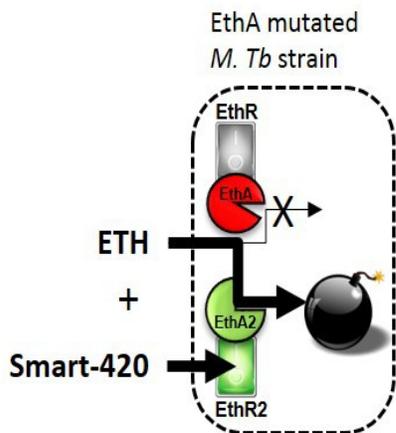
Teaching old drugs new tricks: Reprogramming ethionamide's bioactivation to fight multidrug resistant *Mycobacterium tuberculosis*

Nicolas Willand¹, *nicolas.willand@icloud.com*, **Marc Gitzinger**³, **Benoit Deprez**¹, **Alain Baulard**². (1) *Univ. Lille, Inserm, Institut Pasteur de Lille, U1177 - Drugs and Molecules for living Systems, Lille, France* (2) *Univ. Lille, CNRS, Inserm, CHU Lille, Institut Pasteur de Lille, U1019 – UMR 8204 - CIIL - Center for Infection and Immunity of Lille, Lille, France* (3) *BioVersys AG, Basel, Switzerland*

Antimicrobial resistance (AMR) is a growing public health problem worldwide, that, if left unchecked is predicted by 2050 to annually cause 10 million deaths and cost the world up to 100 trillion USD. Tuberculosis is the bacterial infection most affected by AMR and

the estimated global burden of multi-drug resistant tuberculosis is 450,000 each year. The most alarming figure is that extensively drug resistant *M. tuberculosis* strains have already been reported in more than 92 countries. The increasing burden of multidrug resistant tuberculosis forces us to develop innovative and novel means of killing this organism.

The originality of our approach arises from the peculiar observation that a significant number of anti-TB antibiotics are prodrugs, meaning that they acquire their activity thanks to mycobacterial enzymatic bioactivation pathways that are tightly controlled by transcriptional regulators. Using a combination of phenotypic and molecular assays, we have discovered and optimized a new type of antibacterial compounds called **SMART** for **Small Molecule Aborting Resistance** that are able to wake-up a cryptic alternative bio-activation pathway of ethionamide in *M. tuberculosis* called EthA2, by targeting a transcriptional repressor called EthR2. Treatment of a panel of clinical isolates highly-resistant to ETH because of mutations in *ethA* were tested by respirometry (MGIT960) with the combination of ETH and **SMART-420**. All these strains displayed striking hypersensitivity to ETH with MIC below 0.5 µg/mL. In this experiment, **SMART-420** did not only increase the basal sensitivity of *M. tuberculosis* to ethionamide but also fully reversed ethionamide-acquired resistance. Finally, mice infected with an ethionamide-resistant mycobacterial strain were also successfully treated orally with the combination of ETH and **SMART-420** (@50mpk) and a 4.6 log reduction of the bacterial load in the lungs was observed.



MEDI 370

Preclinical development and characterization of MYC inhibitors

Nicholas Jacob, njacob@scripps.edu, Pedro Miranda, Pedro Serrano Navarro, Jonathan Hart, Peter K. Vogt, Kim D. Janda. The Scripps Research Institute, San Diego, California, United States

The transcriptional regulator MYC was first discovered more than three decades ago, and has since been identified as an oncogenic driver in a wide variety of the most aggressive human malignancies. As a pleiotropic transcriptional regulator, MYC directly

or indirectly controls expression of hundreds of coding and noncoding transcripts. Genes under MYC control affect cell cycle entry, proliferation, differentiation, metabolism, and death/survival decisions in normal and transformed cells. Despite its prevalence, there is no current clinically-available therapeutic strategy to treat tumors with aberrant MYC expression. We have identified a unique and selective inhibitor of the MYC protein that is able to attenuate MYC-driven cellular proliferation and reduce the tumor burden of xenograft mice without acute toxicity. We will present initial SAR studies that have resulted in new equipotent inhibitors, which are of significantly increased solubility and potentially better pharmacokinetics. Also discussed will be biophysical characterization of these inhibitors, which has led to a better understanding of the mechanism of MYC inhibition, and revised strategies for targeting this elusive oncogene.

MEDI 371

Discovery of PF-06748962: A potent and selective lactam-based EP3 antagonist

Kentaro Futatsugi, Kentaro.Futatsugi@gmail.com. Pfizer Inc, Cambridge, Massachusetts, United States

Prostaglandin E receptor 3 (EP3) is a G₇-coupled class-A G-protein coupled receptor (GPCR) that is activated by its endogenous agonist PGE2. EP3 antagonists have been studied for treating several clinical indications such as thrombosis, cardiovascular disease, and type-2 diabetes. The majority of previously reported EP3 antagonists are acidic with high molecular weight and lipophilicity (molecular weight >500, cLogP>5), while recent publications indicate that neutral EP3 antagonists within the rule of five space can be identified for this target. This presentation will describe the optimization of neutral EP3 antagonists based on the lactam scaffold, ultimately leading to the discovery of a potent and selective EP3 antagonist **PF-06748962**. Rigorous property-based optimizations translated into several candidate-quality attributes of **PF-06748962**, including good ADME/safety profile and high lipophilic efficiency. The presentation will also highlight cases where relatively minor structural changes within the same chemical series led to dramatic improvements in potency and *in vivo* safety profile.

MEDI 372

Novel pyrrolomycins as potent antibacterial agents against *ESKAPE* pathogens and biofilms

Rongshi Li^{2,4}, rongshi.li@unmc.edu, Zunhua Yang², Jongsam Ahn³, Yan Liu², Yashpal Singh Chhonker⁵, D.J. Murry^{5,4}, Haizhen A. Zhong^{2,1}, Kenneth W. Bayles³. (1) Department of Chemistry, University of Nebraska at Omaha, Omaha, Nebraska, United States (2) Center for Drug Discovery and Department of Pharmaceutical Sciences, University of Nebraska Medical Center, Omaha, Nebraska, United States (3) Center for Staphylococcal Research and Department of Pathology and Microbiology, UNMC, Omaha, Nebraska, United States (4) Fred and Pamela Buffett Cancer Center, UNMC,

Omaha, Nebraska, United States (5) Pharmacy Practice, College of Pharmacy, UNMC, Omaha, Nebraska, United States

Enterococcus faecium, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter spp.* (ESKAPE) pathogens can readily develop resistance to the biocidal action of existing antibiotics. Multidrug resistance among these organisms is responsible for many serious infections. Infections by methicillin-resistant *Staphylococcus aureus* (MRSA) alone cost U.S. hospitals between \$3.2 and \$4.2 billion annually. Compounding this problem is the propensity of ESKAPE pathogens to form biofilms. To date, no anti-biofilm agents are available for therapeutic use. Thus, it is imperative that we continue to seek new antibiotics for combating both ESKAPE and biofilm infections.

We previously reported marinopyrroles and their fragment derivatives – novel pyrrolomycins, as potent anti-MRSA and anti-staphylococcal biofilm agents. Using fragment-based and bioisostere approaches, we designed, synthesized, tested these pyrrolomycins, and performed quantitative structure-activity relationship (QSAR) analysis. In addition, drug metabolism and pharmacokinetics (DMPK) were investigated. The results of these studies revealed that these natural product derivatives have a minimum inhibitory concentration in the nanogram/mL and a minimum bactericidal concentration in the low microgram/mL range against ESKAPE pathogens and biofilms with a lack of mammalian cytotoxicity and without detectable resistance, and the desirable oral bioavailability and pharmacokinetic profiles. This presentation will discuss the development of these novel pyrrolomycins as potent antibacterial agents against ESKAPE pathogens and biofilms, their QSAR, DMPK, and in vivo efficacy in animal models.

MEDI 373

Development and application of an NMR-based activity and inhibition assay for mycobacterial isocitrate lyase

Ram P. Bhusal¹, rbhu144@aucklanduni.ac.nz, **Krunal Patel**¹, **Brook kwai**¹, **Ghader Bashir**², **Jóhaness Reynisson**¹, **Jonathan Sperry**¹, **Ivanhoe K. Leung**¹. (1) School of Chemical Sciences, The University of Auckland, Auckland, New Zealand (2) School of Biological Sciences, The University of Auckland, Auckland, New Zealand

Tuberculosis (TB) is an infectious disease that is caused by *Mycobacterium tuberculosis*. Fatty acids and lipids are thought to be important sources of carbon and energy for *M. tuberculosis* during infection, in particularly when the bacteria are in the latent phase. The enzyme isocitrate lyase (ICL), which catalyses the formation of succinate and glyoxylate from isocitrate, is the first enzyme in mycobacterial glyoxylate cycle. ICL is important for the survival of latent *M. tuberculosis* as it allows the bacteria to survive with fatty acids as its sole carbon source. Whilst ICL is crucial for the survival of *M. tuberculosis*, it is not present in humans. As such, ICL is an excellent therapeutic target for the development of new anti-TB treatments.

The development of ICL inhibitors for potential anti-TB treatments is hampered by a lack

of reliable and efficient *in vitro* assays for ICL activity. Reported methods included radioactive assay using ^{14}C -labelled isocitrate, which is tedious to set up and is potentially dangerous, or by the use of a chemically-coupled assay with detection by UV/Vis spectroscopy. Herein, we report the development of an NMR-based activity assay that allows the detection of isocitrate consumption and succinate formation in real time. This method was demonstrated by using existing ICL inhibitors, and exemplified by our inhibitor discovery work in tandem with virtual high-throughput screening.

MEDI 374

LEGO[®]-inspired drug design: Discovery of novel fungal Plasma membrane H⁺-ATPase (Pma1) inhibitors from small molecule libraries: An introduction of HFSA-SBS_DOS-RD strategy in drug discovery

Truong Thanh Tung¹, truong.tung@sund.ku.dk, Trong Tuan Dao^{1,2}, Michael B. Palmgren², Anja T. Fuglsang², Søren B. Christensen¹, **John Nielsen**¹, john.nielsen@sund.ku.dk. (1) Department of Drug Design and Pharmacology, University of Copenhagen, Copenhagen, Denmark (2) Centre for Membrane Pumps in Cells and Disease – PUMPKIN, Department of Plant and Environmental Sciences, University of Copenhagen, Copenhagen, Denmark

Fungal plasma membrane H⁺-ATPase (Pma1) has recently emerged as a potential target for the discovery of new antifungal agents. This p-type pump which localized on the surface of fungal cells plays a crucial role in many physiological functions and processes inside the cell. Especially, by pumping proton to extracellular, this enzyme generates a transmembrane electrochemical gradient, as a consequence, fungi can uptake nutrients by secondary transport systems. Until now, only low resolution of protein structure has been reported, and notably there is no report of co-crystal structure of Pma1 with inhibitors. Therefore, we have identified the need for small molecule library of high quality for targeting Pma1. The **LEGO[®]**-inspired hypothesis encouraged us to first develop new strategy from the combination of hypothesis-based fragment selection and assembly (**HFSA**), specific biological relevance scaffold based diversity-oriented synthesis (**SBS_DOS**) and rational design (**RD**), so called **HFSA-SBS_DOS-RD** strategy in drug discovery and development process. Using **HFSA-SBS_DOS-RD**, our group successfully designed, synthesized, and performed SAR studies of novel compounds potent Pma1 inhibitors. An expeditious, high yield and scalable microwave-assisted synthesis was developed and applied for synthesis of library compounds. To our delight, our compound libraries were able to inhibit Pma1 activity and growth inhibitory activity of *C. albicans* and *S. cerevisiae* revealed the most promising example for future development of antifungal drugs on this target.

MEDI 375

Reducing cycle time in medicinal chemistry drug discovery

John S. Wai, john_wai@wuxiapptec.com, Tao Wang. WuXi AppTec, Shanghai, China

Synthesis of newly designed molecules continues to be a serious rate limiting step in drug discovery. To shorten synthetic cycle time means investing only in reactions that are more likely to work throughout the synthetic sequence and that have the least number of steps. Structural diversification, different substitution patterns, unique heterocyclic systems can often adversely impact the crucial step(s) of established synthetic sequences or development of feasible routes. Furthermore, unique arrays of functionalities in novel heterocyclic systems can lead to unexpected reactivities. Retrospective analysis of our collection of interesting and surprising observations with calculation tools showed that we could avoid most of these dead ends and provided insight on how to successfully resolve these synthetic problems. Real-time incorporation of these analytical tools in our route design processes greatly enhances our success rates, reduces cycle times, and improves overall isolated yields of the sequences. This presentation will highlight results from our analysis and leave medicinal chemists with new insights that they can bring back to their own labs.

MEDI 376

Enzymatic tandem carboxylation-amidation as a bioactivity-potentiating strategy in the production of natural and unnatural thiolactomycin antibiotics

Jie Li³, jjl407@ucsd.edu, **Xiaoyu Tang**², **Shaun Mckinnie**⁶, **Takayoshi Awakawa**⁴, **Bradley S. Moore**^{1,5}. (3) Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California, San Diego, San Diego, California, United States (4) Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, Tokyo, Japan (5) Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, San Diego, California, United States

Terminal amide functionality is prevalent in nature and significantly contributes to both biological activity and chemical stability of compounds. Terminal amidation is frequently utilized as a strategy in medicinal chemistry for lead modification to achieve improved bioactivity and bioavailability, thus this functionality is often present in drugs on the market (e.g., Rebetol[®], Viberzi[®], and Adlyxin[®]). Such wide occurrence and pharmaceutical application of terminal amidation drew our attention to thiotetroamide (TTM), a molecule being studied for its biosynthesis as part of our continuing efforts towards antibiotics discovery. TTM is the sole member that bears a terminal amide functionality within the thiolactomycin (TLM) class of antibiotics, and consequently exhibits enhanced bioactivity compared to other TLMs lacking terminal amidation. In addition, bioinformatics analyses suggested that the terminal amide formation in TTM involves a selective carboxylation of a saturated alkyl group, which is a challenging feat in synthetic chemistry and is also distinct from previously established enzymatic amidation processes. Therefore, the enhanced bioactivity and the unusual enzymology motivated the investigation of this unique tandem carboxylation-amidation enzymatic strategy to utilize it for the production of unnatural and potent TTM analogues. First, targeted *in vivo* gene disruption results supported the hypothesis that a unique enzyme pair consisting of a cytochrome P450 and an amidotransferase was involved in the biosynthesis of the terminal amide group. Next, *in vitro* enzymatic analysis showed that

the recombinant cytochrome P450 selectively oxidized a terminal methyl group to ultimately form a carboxylic acid via a rare triple oxidation. This carboxylic acid was isolated and structurally characterized, and was subsequently investigated as a substrate for the specific amidotransferase, which successfully generated the final amide product. Furthermore, these two enzymes were assayed together and demonstrated the coupled carboxylation-amidation process. After the characterization of these two enzymes, structurally diverse TLM analogs are being used to probe the substrate specificity, which has so far resulted in the production of two unnatural TTM analogues with potentiated bioactivity. This suggested a potential of engineering this unique enzyme pair as a useful avenue to modify the drug-like properties of lead molecules in discovery programs.

MEDI 377

Structure-based design of highly potent small-molecule inhibitors of DCN1-UBC12 protein-protein interaction

Haibin Zhou¹, haibinz@umich.edu, Jianfeng Lu¹, Liu Liu¹, Denzil Bernard¹, Jeanne Stuckey², Yi Sun², Shaomeng Wang^{1,2}. (1) Internal Medicine, Medical School, University of Michigan, Ann Arbor, Michigan, United States (2) University of Michigan, Ann Arbor, Michigan, United States

The interaction of E2 enzyme Ubc12 and E3 enzyme Dcn1 in NEDD8 pathway facilitated the transfer of NEDD8 from Ubc12 to cullins, which dramatically accelerated the activity of cullin-RING. Starting from the native, 12-residue peptide derived from UBC12 protein, which binds to DCN1 with a moderate affinity, we have successfully designed a series of highly potent, druglike small-molecule inhibitors of UBC12-DCN1 interaction with low nanomolar binding affinities through structure-guided design and extensive medicinal chemistry campaign. Significantly, employing our lead compound as a chemical probe, we for the first time discovered the specificity and function of DCN1 protein and provide proof of concept for the DCN1 inhibitors as specific modulator of CRLs activity. Our designed compounds serve as an excellent chemical probe for basic research to investigate NEDD8 pathway-related biology.

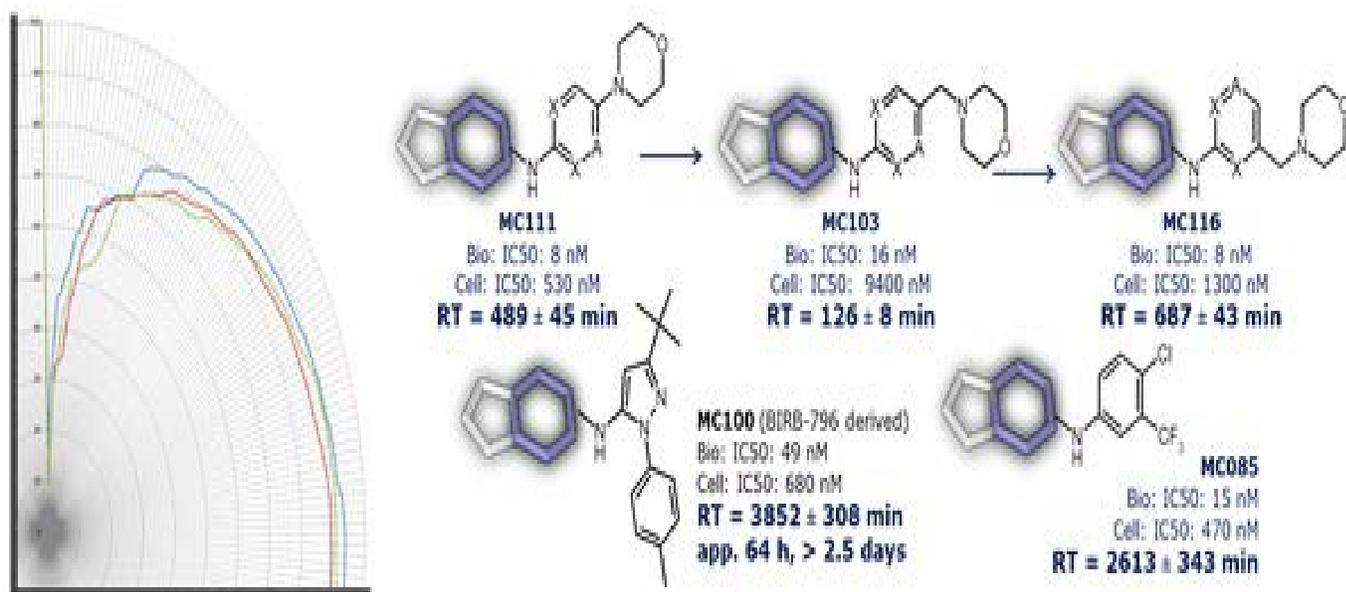
MEDI 378

CDK8 inhibitors with long residence time emerging from a retro-design approach: New opportunities for cancer treatment

Jorg C. Benningshof¹, Bas Aerts³, Gerhard Müller², gerhard.mueller@mercachem.com, Johan Veerman³, Eddy Damen², Michael Kubbutat⁴, Jan Ehler⁴, Holger Holger⁴, Frank Totzke⁴. (1) Parallel Chemistry, Mercachem, Nijmegen, Netherlands (2) Medicinal Chemistry, Mercachem, Nijmegen, Netherlands (3) Mercachem, Nijmegen, Netherlands (4) ProQinase GmbH, Freiburg, Germany

Upregulation of CDK8 has recently been described for colon cancer, gastric cancer, and melanoma, rendering CDK8 as an attractive target for the development of selective and efficacious anti-cancer drugs.

Based on the recent findings that in contrast to almost all other CDK family members, CDK8 is amenable to a type II inhibition mode, we set out to design selective CDK8 inhibitors pursuing a privileged structure-based approach. The employed privileged structures are tailor-made for disrupting the hydrophobic R spine within the N-terminal lobe of a kinase, thereby leading to an induced-fit mechanism of derived inhibitors that will exhibit a pre-engineered binding kinetic signature. This “Retro-Design” approach allows to keep the molecular complexity of inhibitors at a minimum level since the seed scaffold is targeted towards the deep pocket of the conformationally rearranged binding. Here we report on the discovery and optimization of a new class of CDK8 inhibitors. Frontrunner compounds exhibit excellent biochemical inhibition data and a high cellular efficacy in a variety of mechanism-of-action models as well as phenotypic models such as inhibition of anchorage-independent cell growth. The front-runner compounds show superior selectivity over a huge panel of kinases when compared to market approved drugs or to competitor CDK8 inhibitors. This selectivity is attributed to the distinct inhibition mechanism which is corroborated by detailed binding kinetic studies which reveal residence times in the range of several hours. Detailed structure-kinetic relationships will be discussed.



Left – radar plot highlighting the selectivity of front-runner compounds in a 400 membered kinase panel; right – detailed structure-kinetic relationships emerge

MEDI 379

Structure-based design, synthesis, biological evaluation, and x-ray crystallographic analysis of novel, highly potent HIV-1 protease inhibitors to address multi-drug resistant HIV

Arun K. Ghosh², **Heather L. Osswald**², hosswald@purdue.edu, Johnson Agniswamy¹, Yuan-Fang Wang¹, Irene Weber¹, Masayuki Amano³, Hiroaki Mitsuya^{3,4}. (1) Georgia State University, Atlanta, Georgia, United States (2) Purdue University, West Lafayette, Indiana, United States (3) Kumamoto University, Kumamoto, Japan (4) National Cancer Institute, National Institutes of Health, Bethesda, Maryland, United States

Multi-drug resistant (MDR) HIV continues to be a devastating problem for HIV/AIDS patients. Strains of HIV oftentimes confer resistance to drugs used in highly active antiretroviral therapy (HAART), rendering current therapies inadequate. In order to address this need, novel HIV-1 protease inhibitors can be designed that maintain potency against MDR strains of HIV. The structure-based design, asymmetric synthesis, biological evaluation, and X-ray crystallographic analysis of a novel class of HIV-1 protease inhibitors will be described. Utilizing the structural information gained from the X-ray crystal structure of FDA approved darunavir-bound wild type and mutant protease, highly active inhibitors were designed which maintained their potency across many MDR strains of HIV. Modifications were made to the P2 ligand in order to optimize the hydrogen bonding with the S2 pocket and flap region of the enzyme. Important hydrophobic contacts can also be made in the small S1-S2 subpocket. The hydrophobicity of the P1 ligand was also investigated. These inhibitors proved to be potent against HIV in enzymatic and antiviral assays. Selected inhibitors were tested against multi-drug resistant strains of HIV. High-resolution X-ray crystallography provided a structure of the inhibitor-bound protein which revealed key interactions in the active site of the enzyme.

MEDI 380

Inhibition of A β -40 and A β -42 aggregation by piceatannol and cis-piceatannol

James M. Chapman^{1,4}, chapman@sccp.sc.edu, Melissa Moss^{2,3}, Yiyang Wang^{2,3}. (1) Pharm. Chem. and Drug Discovery, University of South Carolina, Columbia SC, South Carolina, United States (2) Biomedical Engineering, University of South Carolina, Columbia, South Carolina, United States (3) College of Engineering, University of South Carolina, Columbia, South Carolina, United States (4) College of Pharmacy, University of South Carolina, Columbia, South Carolina, United States

Polyphenols have been extensively documented to inhibit aggregation of amyloid- β (A β) protein, a key process involved in the pathogenesis of Alzheimer's disease. In this study, five polyphenols were investigated for their ability to mechanistically modulate A β aggregation, including piceatannol, cis-piceatannol and three O-methylated derivatives of piceatannol, rhapontigenin, isorhapontigenin and tetramethoxystilbene. The effects of these compounds on early aggregation events, or oligomerization, as well as the growth of aggregates by both lateral aggregate association and aggregate elongation by monomer addition were studied within assays designed to isolate these mechanistic steps within the aggregation pathway. Both piceatannol and cis-piceatannol dramatically reduced the formation of β -sheet aggregates from A β monomer. However, the presence of methoxy ethers generally resulted in significantly decreased inhibitory activity. Within

late stages of the aggregation process, both piceatannol and cis-piceatannol dramatically reduced A β lateral aggregate association, with piceatannol additionally demonstrating a significant effect on A β aggregate elongation. Transmission electron microscopy of the aggregation end products revealed that these inhibitory capabilities resulted in aggregates with an altered morphology, rather than the abrogation of aggregate material. Within early stages of the aggregation process, cis-piceatannol and tetramethoxystilbene reduced A β oligomerization by 60-70%, suggesting a different mechanism for intervention at this early stage of aggregation as well as the superiority of cis-piceatannol to act at multiple stages within the aggregation pathway. Molecular modification of these compounds may overcome the poor bioavailability and blood brain barrier penetration typically found in polyphenols, allowing therapeutic possibilities in Alzheimer's disease and perhaps other amyloid-associated neurodegenerative disorders.

MEDI 381

Photoelectrocatalytic inhibition of Alzheimer's β -amyloid aggregation in vitro by hole-derived radicals

Kayoung Kim, *kayoungkim@kaist.ac.kr*, Byung Il Lee, You Jung Chung, Woo Seok Choi. Korea Advanced Institute of Science and Technology, Daejeon, Daehak-ro, Yuseong-gu, Korea (the Republic of)

The self-assembly of β -amyloid peptides (A β) into aggregates is a major hallmark of Alzheimer's disease (AD) that affects more than 11% of the population aged over 65. The suppression of A β aggregation is considered to be an attractive therapeutic intervention for treating AD, thus numerous efforts have been made in the past decades to suppress A β aggregation. While the previous reports have focused on organic compound-based inhibitors of A β aggregation, this study is a first example of expanding the scope of traditional A β inhibition strategy to the field of photoelectrochemistry. Here, we demonstrated that the self-assembly of A β peptides can be suppressed effectively by hematite-based photoanode through photoelectrocatalytic mechanism. Hematite is an appealing n-type semiconductor widely used as a photoanode material due to its excellent photocatalytic performance, biocompatibility, and low cost. Upon illumination of a light-emitting diode with anodic bias, we found that hematite photoanode generates reactive radical species such as superoxide ions and hydroxyl radical via the photoelectrocatalytic process. According to our analyses, the hole-derived radicals, in particular hydroxyl radical, played a significant role of oxidizing A β peptides, which effectively blocked further fibrillation, as depicted in **Figure 1**. Furthermore, we verified that hematite photoanode is biocompatible and effective in reducing A β aggregation-induced cytotoxicity. This work hints at the potential of utilizing photoelectrocatalytic approach for inhibition of A β aggregation using visible light-active photoelectrode.

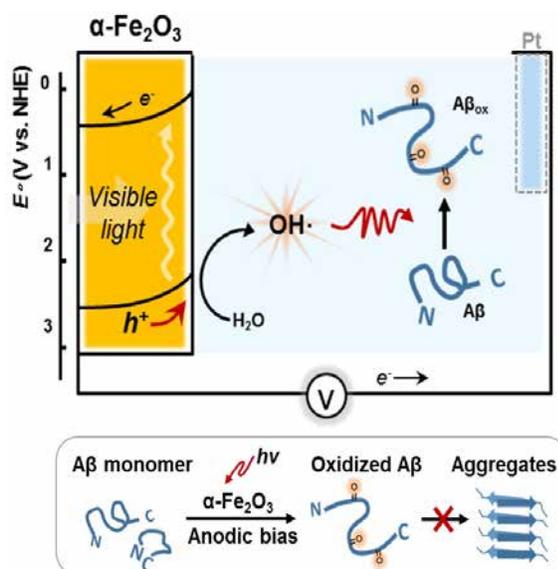


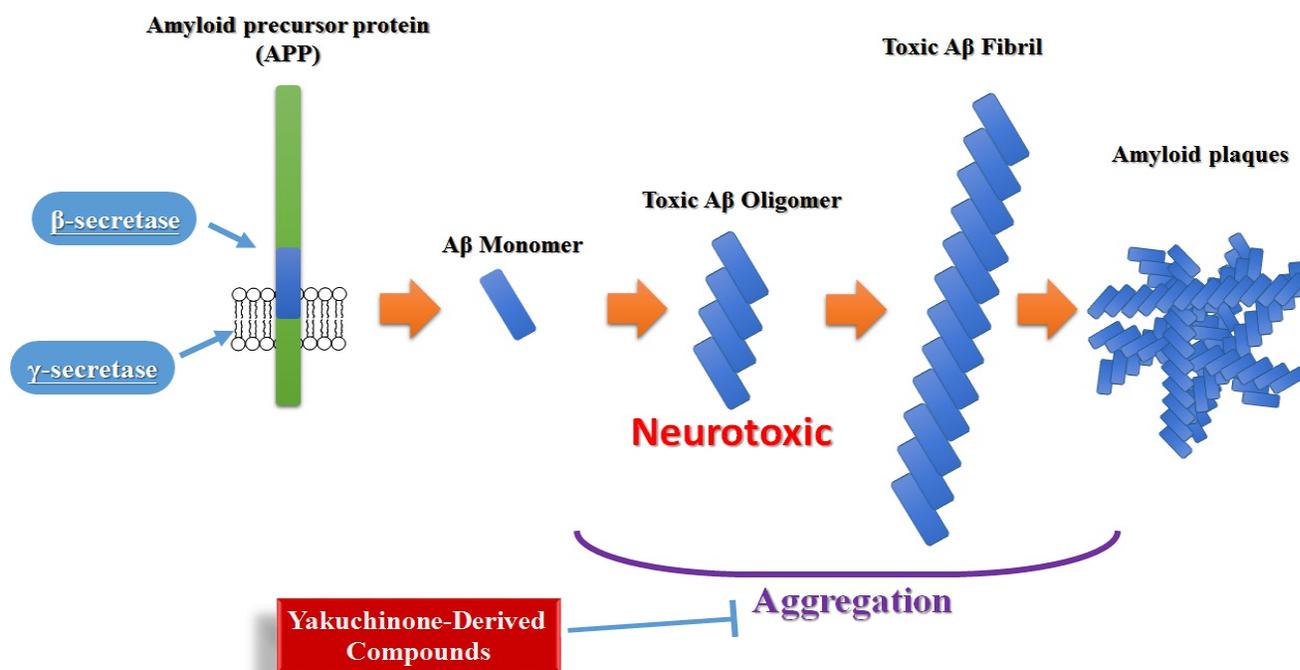
Figure 1. Schematic illustration of the photoelectrocatalytic inhibition of β -amyloid ($A\beta$) aggregation. The hematite ($\alpha\text{-Fe}_2\text{O}_3$)-based photoelectrode generates hole-derived radicals through the photoelectrocatalytic process, which suppress $A\beta$ aggregation by radical-mediated oxidative reaction.

MEDI 382

Synthesis of Yakuchinone-derived compounds that inhibit β -amyloid aggregation

Liang-Chieh Chen^{1,2}, pigjay15@gmail.com, Cheng-Chung Yen², Hui-Ju Tseng^{2,3}, Yun-Yi Huang², Yeh-Lin Lu^{1,2}, Wen-Chi Hou², Kai-Cheng Hsu⁴, I-Horng Pan⁵, Kuo-Kuei Huang⁵, Wei-Jan Huang². (1) School of Pharmacy, Taipei Medical University, Taipei, Taiwan (2) Graduate Institute of Pharmacognosy, Taipei Medical University, Taipei, Taiwan (3) Ph.D Program in Biotechnology Research and Development, Taipei Medical University, Taipei, Taiwan (4) The Ph.D. Program for Cancer Biology and Drug Discovery, Taipei Medical University, Taipei, Taiwan (5) Herbal Medicinal Product Div., Industrial Technology Research Institute, Hsinchu, Taiwan

Aggregation of β -amyloid ($A\beta$) has been regarded as a main cause of Alzheimer's disease (AD). Therefore, developing compounds against the aggregation of $A\beta$ is a therapeutic strategy for AD. A traditional Chinese medicine, *Alpinia oxyphylla* has reportedly had a significant neuroprotective activity. Two diarylheptanoids, Yakuchinone A and B, which were isolated from *Alpinia oxyphylla*, are shown to be the active compounds for anti-Alzheimer's disease. In this study, we synthesized twenty three yakuchinone-derived compounds and screened them for thioflavin T fluorogenic assay. One of these compounds, designated as compound **7c**, is the most potent with the IC_{50} of 2.25 μM . In contrast, it even at the concentration of 10 μM shows no significant cytotoxicity in human neuroblastoma SH-SY5Y cell line. The results suggest that compound **7c** is an effective $A\beta$ -aggregation inhibitor to be further conducted for exploration.



Yakuchinone-derived compounds against the aggregation of A β .

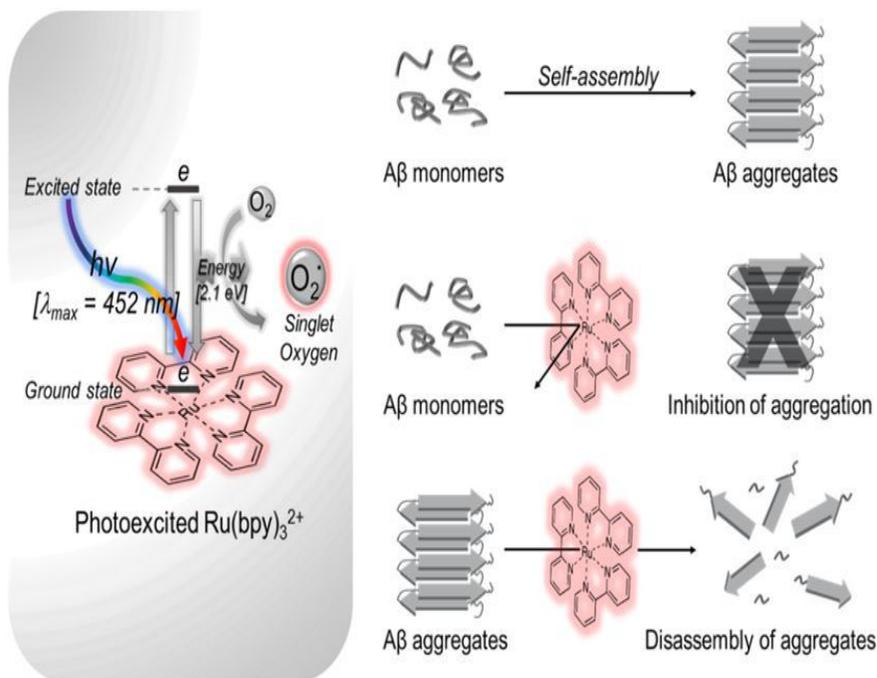
MEDI 383

Photoexcited ruthenium complex for highly sensitive inhibition of β -amyloidogenesis

Giyeong Son, *giyeong.son@kaist.ac.kr*, Chan Beum Park, Byung Il Lee, You Jung Chung. *Materials science and engineering, Korea Advanced Institute for Science and Technology, Daejeon, Korea (the Republic of)*

Ruthenium (II) complexes are visible light-active photocatalyst extensively used for solar energy harvesting and conversion applications. Here, we report our finding that tris(2,2'-bipyridine) ruthenium(II) [$\text{Ru}(\text{bpy})_3^{2+}$] can function as a highly sensitive, biocompatible agent for anti-amyloidogenesis. The abnormal aggregation of β -amyloid (A β) peptides is a major pathological hallmark of Alzheimer's disease (AD). According to our analysis using thioflavin T, circular dichroism, atomic force microscopy, and native gel electrophoresis, visible light-induced $\text{Ru}(\text{bpy})_3^{2+}$ in nanomolar concentration strongly suppresses the conformational changes of A β monomers into β -sheet-rich aggregates. More intriguingly, we verified the possibility of photosensitized disaggregation of preformed A β fibrils into non-fibrillar aggregates by $\text{Ru}(\text{bpy})_3^{2+}$. We also identified that the efficacy of $\text{Ru}(\text{bpy})_3^{2+}$ is derived from photon-energy in visible spectrum. $\text{Ru}(\text{bpy})_3^{2+}$ generates reactive oxygen species such as singlet oxygen upon absorbing photon energy ($\lambda = 452 \text{ nm}$), resulting in inhibition of A β aggregation and disassembly of A β fibrils, as evidenced by using thioflavin T, 2,4-dinitrophenylhydrazine and singlet oxygen sensor green. Furthermore, according to MTT assay using PC 12 cells, photoexcited

$\text{Ru}(\text{bpy})_3^{2+}$ can reduce *in vitro* cytotoxicity of A β aggregates. Our study advances a new insight of ruthenium (II) complexes as therapeutic agents against AD.



A schematic design of inhibition of A β aggregation and disassembly of A β fibrils by using light-induced $\text{Ru}(\text{bpy})_3^{2+}$. Upon absorbing photon energy at 452 nm, $\text{Ru}(\text{bpy})_3^{2+}$ generates reactive oxygen species such as singlet oxygen ($^1\text{O}_2$), via energy transfer, resulting in inhibition of aggregation of A β monomers and disassembly of preformed A β fibrils.

MEDI 384

New hydroxyquinoline-based derivatives as potent modulators of amyloid- β aggregations

Ming Kuan Hu, *hmk@ndmctsgh.edu.tw*. School of Pharmacy, National Defense Medical Centr, Taipei 114, Taiwan

Copper and zinc were found to contribute to the burden of amyloid- β (Ab) aggregations in neurodegenerative Alzheimer's disease. Dysregulation of these metals led to the generation of reactive oxygen species (ROS) and eventually resulted in oxidative damage and accumulation of Ab peptide, which were the key elements of the disease. Aiming to pursue the discovery of new modulators for the disease, we here rationally focused on conjugating the core hydroxyquinoline of the metal-protein attenuating compound PBT2 and the *N*-methylanilide analogous moiety of the Ab imaging agent to build a type of new multi-target modulators of Ab aggregations. We found that the *N,N*-dimethylanilinyll imines **7a**, **8a** and the corresponding amines **7b**, **8b** exerted efficient inhibition of Cu^{2+} - or Zn^{2+} -induced A β aggregations and significant disassembly of metal-mediated Ab aggregated fibrils. Further, **7a** and **7b** also exhibited significant ROC

scavenging effects compared to PBT2. The results suggested that **7a** and **7b** worth to be the lead compounds for the development of the new therapy for Alzheimer's disease.

MEDI 385

Potential multimechanistic therapeutic effects of dihydropyridine calcium channel blockers: Mechanistic study of effects on amyloid-beta aggregation associated with Alzheimer's disease

James M. Chapman^{1,4}, *chapman@sccp.sc.edu*, **Melissa Moss**^{2,3}, **Jui-Heng Tseng**^{2,3}.
(1) *Pharm. Chem. and Drug Discovery, University of South Carolina, Columbia SC, South Carolina, United States* (2) *Biomedical Engineering, University of South Carolina, Columbia, South Carolina, United States* (3) *Biomedical Engineering, College of Engineering, Columbia, South Carolina, United States* (4) *College of Pharmacy, University of South Carolina, Columbia, South Carolina, United States*

The etiology of neurodegenerative diseases that increasingly plague our society involves numerous pathological factors, thereby requiring the co-administration of several different drugs or the development of single compounds with multimechanistic therapeutic effects. While more difficult to develop, single multimechanistic agents have potential advantages in decreased drug interactions and patient compliance. The latter presents a heightened concern in Alzheimer's disease (AD). The incidence of AD is directly correlated to hypertension and increased CNS calcium levels. The dihydropyridine class of antihypertensive calcium channel blockers continues to be extensively utilized in hypertensive patients. This research involves a mechanistic study of the inhibition of amyloid- β ($A\beta$) aggregation, a pathology prominently involved in AD, by six dihydropyridine calcium channel blockers including two agents, nimodipine and nivaldipine, that are known to penetrate the blood brain barrier in therapeutic concentrations. All the compounds had significant efficacy in overall inhibition of $A\beta$ aggregation. Further studies of the effects of these agents on $A\beta$ oligomerization as well as two mechanisms of intermediate aggregate growth, lateral association and elongation, demonstrated differences in inhibitory mechanism. Nicardipine was the most potent at intervening during the earliest stage of aggregation, oligomerization, where therapeutic efficacy may be highest. Amlodipine was the most efficacious of these six agents in inhibiting later aggregation events. An *in silico* derived comparison of the ability of these agents to penetrate the blood brain barrier was conducted in an initial effort to determine the structural characteristics required for CNS accumulation to guide future design of multimechanistic agents for AD.

MEDI 386

Novel cephalosporins selectively active on nonreplicating *Mycobacterium tuberculosis*

Quyên Nguyễn¹, *quyennguyen@unc.edu*, **Jeff Aube**¹, **Ben Gold**². (1) *School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina,*

United States (2) Department of Microbiology and Immunology, Weill Cornell Medical College, New York, New York, United States

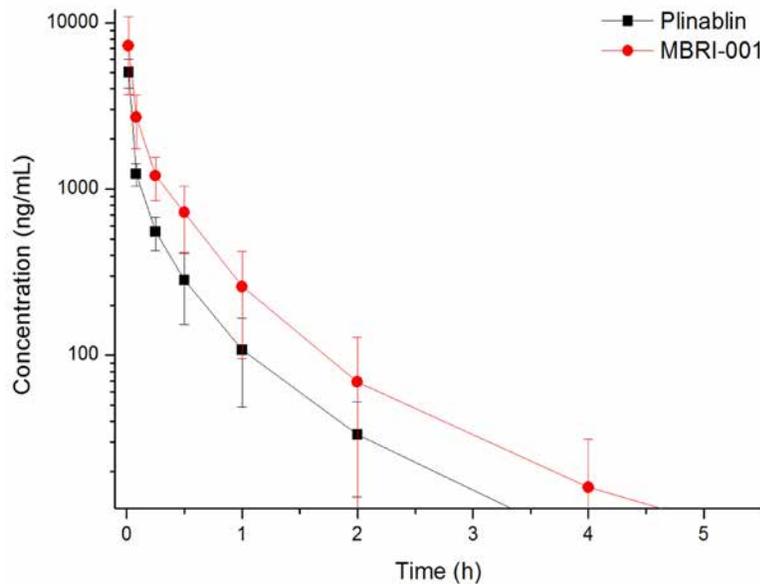
Results from a high-throughput screening campaign revealed cephalosporin derivatives bearing an ester at the C-2 position with activity against *M. tuberculosis* (Mtb) which prompted our interest in developing novel cephalosporins for the treatment of tuberculosis (TB). We report two series of novel cephalosporins bearing an ester or an oxadiazole isostere at C-2, a position that is almost exclusively a carboxylic acid in clinical drugs, to be active against Mtb in non-replicating (NR) condition and inactive in replicating (R) condition. Noticeably, this is the first report of β -lactams that only kill a given bacterium in NR state. Representatives of the series were identified to be stable in cell-free medium, stable at both pH 2 and 7, soluble at pH 7.4, predicted to be membrane-permeable and active in macrophages without toxicity. This result suggests that we reconsider the use of β -lactams which have only recently been viewed as viable drugs to treat TB.

MEDI 387

Development of MBRI-001, a deuterium-substituted plinabulin, as a potent anti-microtubule agent for anticancer

*Zhongpeng Ding¹, Hejuan Cheng¹, Shixiao Wang², Yingwei Hou², Jianchun Zhao², Huashi Guan^{1,2}, **Wenbao Li^{1,2}**, wbli92128@163.com. (1) School of Medicine and Pharmacy, Ocean University of China, Qingdao, Shandong, China (2) Marine Biomedical Research Institute of Qingdao, Qingdao, Shandong, China*

The microtubule system is a common target of many anticancer drugs. To improve the pharmacokinetic properties and overcome the multi-drug resistance, deuterium atom as an isostere was introduced to synthesize MBRI-001 based on the structure of plinabulin which is a clinical candidate drug in phase III. The total synthesis yield was 10% for eight steps. The chemical structures of MBRI-001 was characterized by HRMS, NMR and a single crystal analysis. As showed in the plasma concentration-curves after single intravenous injection administration to Wistar rats, MBRI-001 had the longer $T_{1/2}$ at 1.47 h compared with plinabulin at 1.13 h, and exhibited over 2-fold increase in area under the plasma concentration-time curve AUC compared with plinabulin. The results indicated the pharmacokinetic characteristics of MBRI-001 is significantly better than plinabulin. Also, its antitumor activity was observed at a low nanomolar level for a variety of cancer cell lines, and its anticancer activity against NCI-H460 lung carcinoma has shown a significant efficiency without apparent toxicity by intravenous injection. Therefore, MBRI-001 could be developed as a valuable anti-microtubule agent in clinical oncology.



MEDI 388

β -Sheet propensity of competitive peptide inhibitor's residue is crucial in binding to proteases: PACE4 inhibitors as a case study

Vahid Dianati², vahiddianati@yahoo.com, Azar Shamloo³, Anna Kwiatkowska⁴, Roxane Desjardins⁴, Armand Soldera¹, Robert Day⁴, Yves L. Dory². (1) Univ De Sherbrooke, Sherbrooke, Quebec, Canada (2) Chemistry, Universite de Sherbrooke, Sherbrooke, Quebec, Canada (3) Chemistry, Universite de Sherbrooke, Sherbrooke, Quebec, Canada (4) Surgery/Urology Division, Université de Sherbrooke, Sherbrooke, Quebec, Canada

PACE4 is a prostate and ovarian cancer validated target, which belongs to the proprotein convertase's (PCs) family of serine proteases. We have developed a potent and selective inhibitor, Multi-Leu, with the following structure: Ac-LLLLLRVKR-NH₂. The PCs active site is conserved within the family, which make the designing of the selective inhibitors difficult.

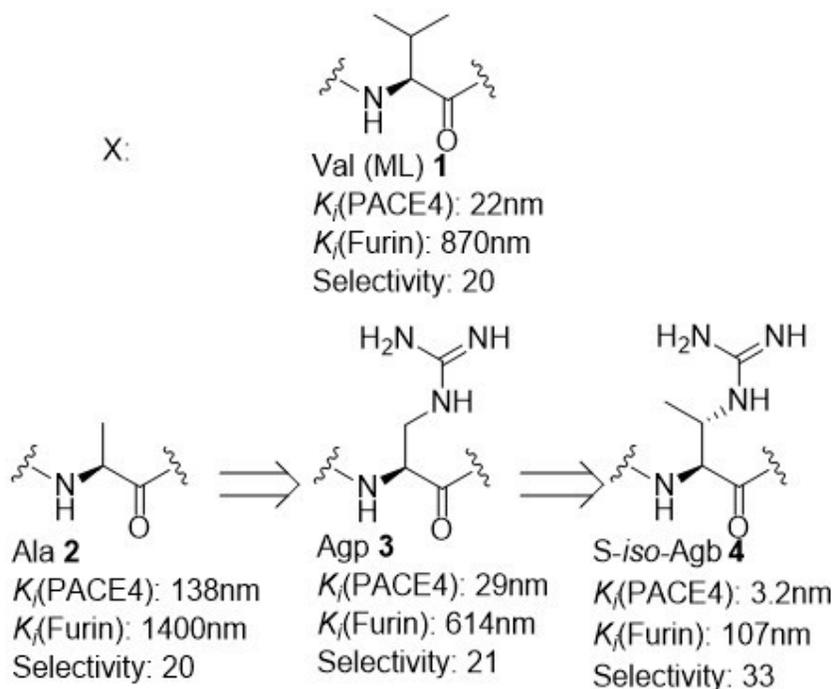
In order to benefit from a difference in S3 pocket of PACE4 and furin (the other member of PC family), we modified the P3 Val of our Multi-leu (ML) inhibitor to target PACE4 Asp160 vs. furin Glu256. The other interaction in S3 is β -sheet like hydrogen bonding of P3 Val with Gly158 of PACE4. This is a general attribute of proteases substrates to form a β -sheet with the active site residues. The β -branched amino acids like Val and Ile are known to enhance this interaction in the β -sheets. We designed β -branched basic amino acids to consider both mentioned interactions in S3.

We examined different basic amino acids in P3 to optimize the side chain length. We found Apg as the most potent in the series so we synthesized our β -branched analogues based on it. With *iso*-Apg in P3, we obtained more selective compound than ML (30fold vs. 20fold) while increasing the potency 7 times.

We analyzed H bonding between Ala or Val backbone and Gly158 PACE4 in S3,

utilizing MD simulations. We found that in the case of Val, the backbone H-bond is shorter and the resulting conformation of the inhibitor is closer to the conformation of antiparallel beta-sheets which results in stronger binding.

In summary, we described the introduction of a basic β -branched residue instead of Val in P3 of a PACE4 peptide inhibitor, ML, which results in better selectivity and increased potency. In addition, we showed that β -branching of P3 is important for formation of β -sheet conformation of peptide inhibitor in the active site.



Binding affinities of selected peptide inhibitors for PACE4 and furin and their selectivity profile toward PACE4

MEDI 389

Hepsin-targeted ligands for prostate cancer imaging and therapy

Youngjoo Byun, byun12@gmail.com, Sang-Hyun Son, Hongmok Kwon. Pharmacy, Korea University, Sejong, Korea (the Republic of)

Hepsin, a type II transmembrane serine protease, is an attractive protein which acts as a potential therapeutic and diagnostic biomarker of prostate cancer because it is highly up-regulated in prostate cancer and promotes the progression and metastasis of prostate cancer. Our efforts to develop novel hepsin inhibitors were based on the reported hepsin inhibitors such as amidine-functionalized indole analogs and guanidine-containing peptides. Novel amidine/guanidine-containing analogs as hepsin inhibitors were designed and synthesized. Most of the synthesized compounds showed *in vitro* inhibitory activity against hepsin in the submicromolar ranges. Based on the structure-

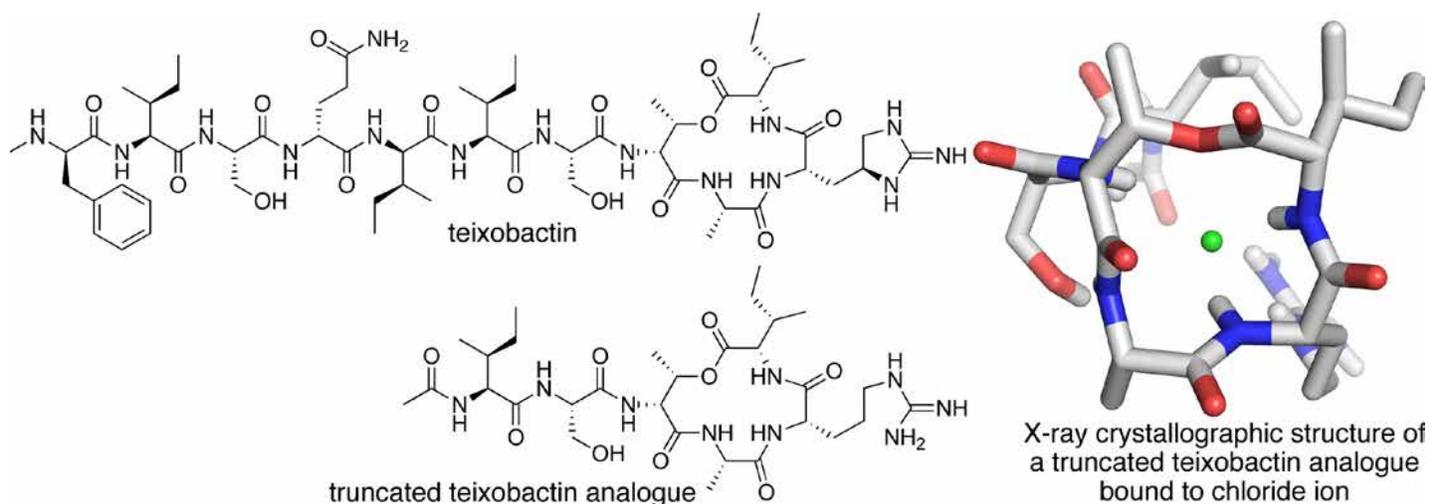
activity relationship (SAR) studies of amidine/guanidine-derived analogs, the most potent hepsin was conjugated with optical dyes such as SulfoCy7 and Bodipy for in vitro and in vivo imaging studies. *In vitro* cell uptake and in vivo preliminary optical imaging experiments exhibited moderately selective binding and retention in high hepsin-expressing cells and tumors as compared with low hepsin-expressing cells. The identified hepsin inhibitors have a potential to be utilized as lead compounds for the development of novel prostate cancer diagnostic/therapeutic agents.

MEDI 390

X-ray crystallographic structures of teixobactin analogues

Hyunjun Yang, yanghj1@uci.edu, Derek R. Du Bois, Joseph W. Ziller, James S. Nowick. University of California, Irvine, California, United States

Teixobactin is a recently discovered antibiotic that inhibits cell wall formation in Gram-positive bacteria by binding to the pyrophosphate group of lipid II and related peptidoglycan precursors. In this presentation, we report X-ray crystallographic structures of teixobactin analogues, with the goal of better understanding the pharmacophore of teixobactin and designing analogues with enhanced pharmacological properties. In the X-ray crystallographic structure of a truncated teixobactin analogue bound to chloride ion, for example, the amide NH groups of the five C-terminal residues (7-11) and the side-chain guanidinium group of residue 10 create a cavity that binds chloride anion. This cavity should also be capable of binding other anions, including the pyrophosphate group of lipid II and related peptidoglycan precursors. Additional X-ray crystallographic structures generated in ongoing studies will be presented, and insights into the mechanism of action of teixobactin will be discussed.



Structures of teixobactin and truncated teixobactin analogue

MEDI 391

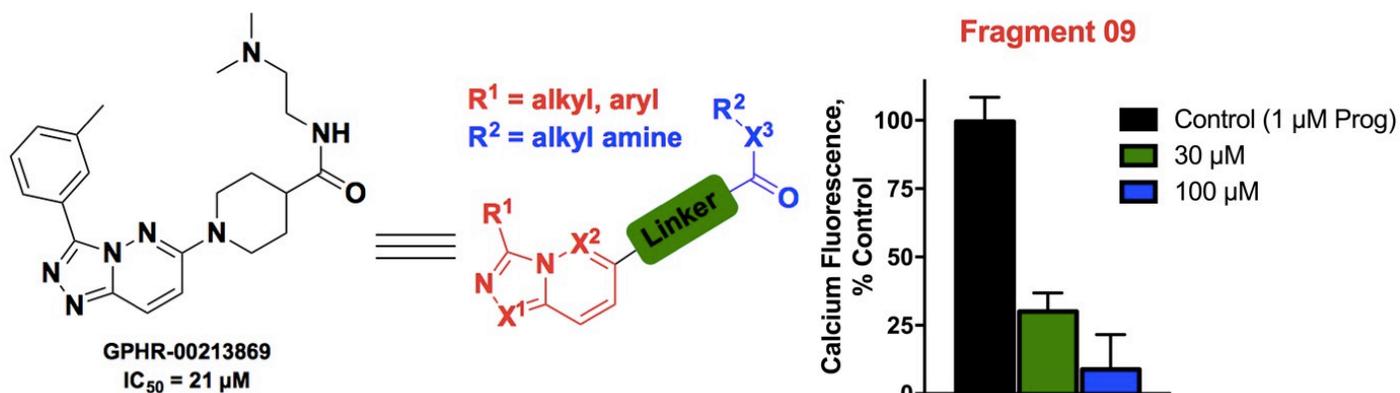
Retrosynthetic fragment evolution and its application towards potent CatSper blockers

Erick J. Carlson^{1,2}, carl2810@umn.edu, Jon E. Hawkinson^{1,2}, Gunda I. Georg^{1,2}. (1) Dept of Medicinal Chemistry Room 452, Univ of Minnesota College of Pharmacy, Minneapolis, Minnesota, United States (2) Institute for Therapeutics Discovery & Development, Minneapolis, Minnesota, United States

Regulation of internal calcium concentration is essential for several processes in sperm, including hyperactivation. This process is essential for proper fertilization and is mediated by calcium flow through the Cation channel of Sperm (CatSper). Mutations within genes encoding human CatSper subunits have been shown to cause infertility in males. Furthermore, CatSper null mice show complete infertility with no other observable phenotypes. Given these two observations, compounds that can block this crucial channel would be promising contraceptives.

In 2011, an HTS campaign conducted in our group revealed a 1,2,4-triazolopyridazine hit compound. The modest potency of this compound in our calcium influx inhibition assay meant that improving the activity of the hit compound was paramount. Inspection of this scaffold reveals several sites at which the SAR of this compound could be studied. Fully exploring the substitution patterns available to this scaffold would require the synthesis of an inordinate number of target compounds. In order to sufficiently probe this scaffold for favorable substitutions while reducing the number of synthesized analogs, an unconventional retrosynthetic fragment evolution strategy is being employed.

In this strategy, a series of fragments resembling the triazolopyridazine moiety of the hit compound were synthesized via modified Bischler–Napieralski indole chemistry and subsequently tested in our developed calcium influx assay; only the most potent fragments were carried on to the next step in the synthetic route. Various heterocycle and boronic acid derivatives were coupled to the best-performing fragments, which was used to explore the internal linker moiety of the scaffold. This strategy simultaneously grew the fragment while exploring the SAR of the linker region. By applying this strategy through each step of the synthetic route, we are approaching compounds of significant potency and have considerably reduced the total number of analogs needed to be synthesized. This strategy seeks to rapidly sample a large area of chemical space with a small number of analogs that have been carefully selected through iterative fragments. The progress of this scaffold's development and the utility of our retrosynthetic fragment evolution strategy will be detailed herein.

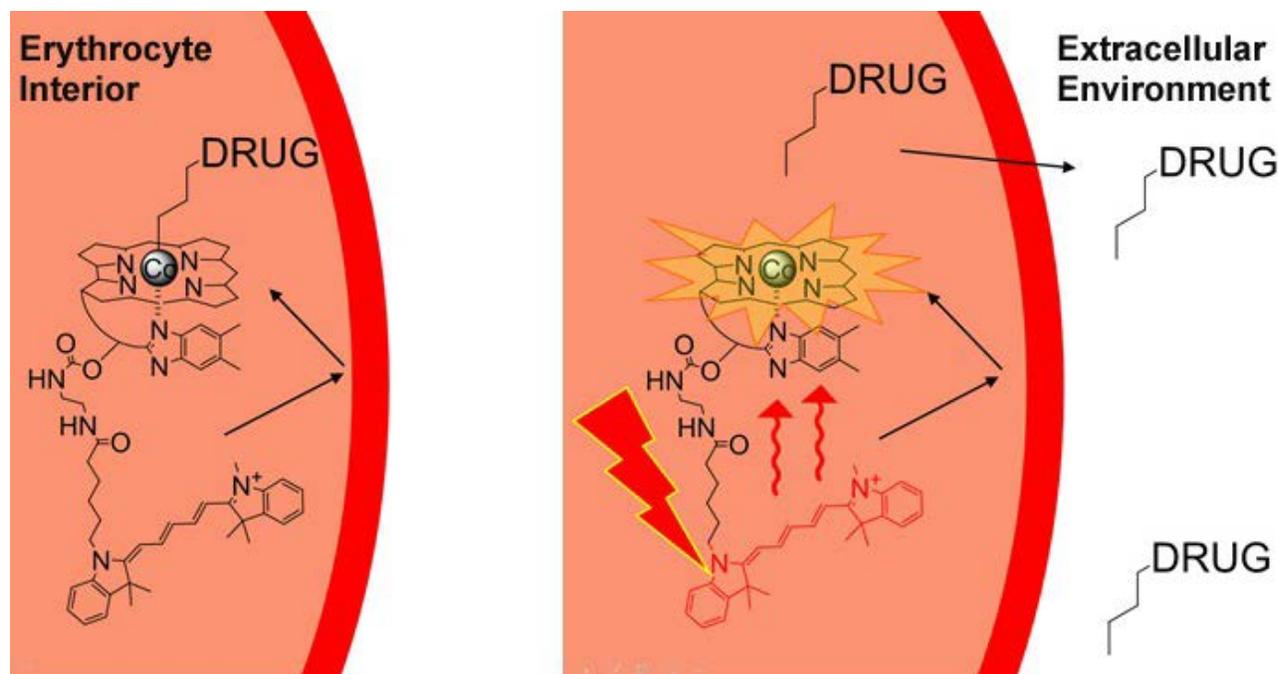


MEDI 392

Long wavelength, orthogonal release of internalized anti-inflammatory compounds from cellular vehicles

Robert M. Hughes¹, hughesrm@gmail.com, **Christina Marvin**³, **Zach Rodgers**³, **Song Ding**³, **Nathan Oien**², **Weston J. Smith**⁴, **David S. Lawrence**^{3,5}. (1) Department of Chemistry, East Carolina University, Greenville, North Carolina, United States (2) Analytical Development, KBI Biopharma, Research Triangle Park, North Carolina, United States (3) Department of Chemistry, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, United States (4) Skaggs School of Pharmacy and Pharmaceutical Sciences, University of Colorado Denver, Denver, Colorado, United States (5) Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, United States

Light-activated prodrugs enable rapid, spatially discrete activation of therapeutic molecules. However, their widespread implementation has been impeded by a number of technical challenges, including toxicity, poor tissue penetrance of short wavelength light, and a deficit of suitable drug delivery vehicles. In this work, we demonstrate a possible solution to many of these challenges, via a cobalamin (vitamin B12)-based methodology that utilizes long wavelength light to trigger the release of therapeutic compounds internalized within erythrocytes. In this study, we initially characterize the quantity of photoproducts released from erythrocyte carriers upon exposure to 520 nm light and subsequently demonstrate the biological impacts of the photo-released anti-inflammatory compounds Methotrexate, Taxol, and Colchicine via CETSA (Cellular Thermal Shift Assay) and microtubule polymerization assays. We then employ a series of fluorophore-linked cobalamin prodrugs to release these anti-inflammatory compounds from within erythrocytes with long wavelength light (650 – 800 nm). Finally, we utilize wavelength-assigned orthogonal release experiments to demonstrate independent release of phototherapeutics from a single pool of erythrocyte carriers.



Membrane-impermeable, light sensitive cobalamin derivatives enable on-command release of therapeutics from within erythrocyte carriers.

MEDI 393

Amino acid and peptide conjugates are potential drug candidates

Siva S. Panda, *sspanda12@gmail.com*. Chemistry & Physics, Augusta University, Augusta, Georgia, United States

N-(Protected α -aminoacyl)benzotriazoles offers many advantages in the preparation of peptides, cyclic peptides, peptidomimetics and peptide conjugates. The conjugates of amino acid or peptides with medicinal important scaffold leads to low toxicity, biocompatibility and enhanced bioactivity. It is known that amino acids can be used as vector for drugs to transport into mammalian tissue and/or to the target sites. The coupling of amino acids and peptide residues to biologically active compounds resulting in enhanced selectivity, pharmaceutical activity and bioavailability has been widely explored.

We have successfully synthesized various amino acid and peptide conjugates using benzotriazole chemistry in excellent yields without losing the chirality integrity. The prepared bio-conjugates showed significant biological properties and less toxicity with respect to parent drugs. The details will be discussed in the conference.

MEDI 394

Development of synthetic novel host defense peptides mimetic as anti-infective agents

Siya Ram, *sram@cellceutix.com*, David Brennan, Karima Chafai-Fadela, Ashok Kumar, Sylvia Holden, Krishna Menon. Cellceutix Corporation, Beverly, Massachusetts, United States

Cationic Host Defense Peptides (HDP) are part of the innate immune response found among all classes of life, which are produced by eukaryotes to defend against infections. These peptides are active against Gram-positive and Gram-negative bacteria, viruses, fungi and even transformed or cancerous cells, and have immunomodulatory activity. Synthetic mimetics are being explored by Cellceutix, and Brilacidin has emerged as a novel small molecule for the treatment of bacterial infections. A successful Phase IIb clinical trial for ABSSSI has been completed, and a Phase III is being planned.

This library of compounds resulted in broad or narrow spectra of activity, less likely to develop resistance, and is being optimized for various pathogens, including Gram-positive, Gram-negative, fungi and other pathogens. Compounds, CC-18116, CC-18117 and CC-18133 have shown very potent antifungal activity against *C.albicans* (CA), *A. fumigatus* and *A.flavus* along with antibacterial activity against *S.aureus* (SA), *E.coli* (EC), *K. pneumoniae* (KP) and *P. aeruginosa* (PA) as shown in Table 1. Lead compounds, CC-18116, CC-18117 and CC-18133 were also tested for cytotoxicity against human fibroblasts, and their IC₅₀ (µg/mL) values were 175, 25 and 120, respectively. Maximum Tolerated Dose for CC-18133 was >5 mg/kg, but it was <5 mg/kg for CC-18117 and CC-18116. In antimicrobial screening assays, aryl\heteroaryldicarboxamides were more potent than triaryl series. The results of this study will be discussed.

ID	Structural Series	Gram-positive	Gram-negative			Fungus		
		SA	EC	KP	PA	CA	A. fumigatus	A. flavus
Brilacidin	Heteroaryldicarboxamide	0.195	3.125	0.78	12.5	50/3.12	100	100
CC-18117	Heteroaryldicarboxamide	< 0.781	100	>100	50	0.625	0.049	0.098
CC-18133	Aryldicarboxamide	0.781	6.25	>100	>100	<0.781	0.312	0.312
CC-18116	Triaryl	6.25	>100	>100	>100	0.625	0.39	1.56

Table 1. Antibacterial & Antifungal Activities of Lead Compounds, MIC (µg/mL)

MEDI 395

Directed immune responses via covalently linked TLR agonist combinations for a Q-fever vaccine

Tyler J. Albin¹, *talbin@uci.edu*, Janine Tom¹, Saikat Manna¹, Adrienne Gilkes², Aarti Jain², Medalyn Supnet², Huw Davies², Aysegul Nalca³, Amanda Burkhardt², Phillip

Felgner², Aaron P. Esser-Kahn¹. (1) Chemistry, University of California, Irvine, Irvine, California, United States (2) Medicine & Institute of Immunology, University of California, Irvine, Irvine, California, United States (3) USAMRIID, Fort Detrick, Frederick, Maryland, United States

Vaccines are a powerful tool of modern medicine. Although empirical vaccine development has been successful to address many diseases, it has failed to combat others, like HIV and malaria, demonstrating the need for more effective, rationally designed vaccines. We are working to better understand the process of immune system stimulation in order to design more effective vaccines, specifically for a novel Q-Fever vaccine. Successful vaccines elicit potent immune responses by activating pattern recognition receptors, including Toll-like receptors (TLRs). We are developing novel immunostimulants by covalently linking synergistic combinations of TLR agonists which can elicit a directed immune response to desired antigens. Recently, we have shown that a covalently linked tri-agonist (TLR 4, 7, and 9 agonists) elicits a distinctive immune response and enhances the antibody response toward vaccinia virus in a vaccination model. Here, we show the synthesis of new TLR tri-agonist combinations, their immune activity *in-vitro*, and their efficacy *in-vivo* as vaccine adjuvants in the development of a Q-fever subunit vaccine.

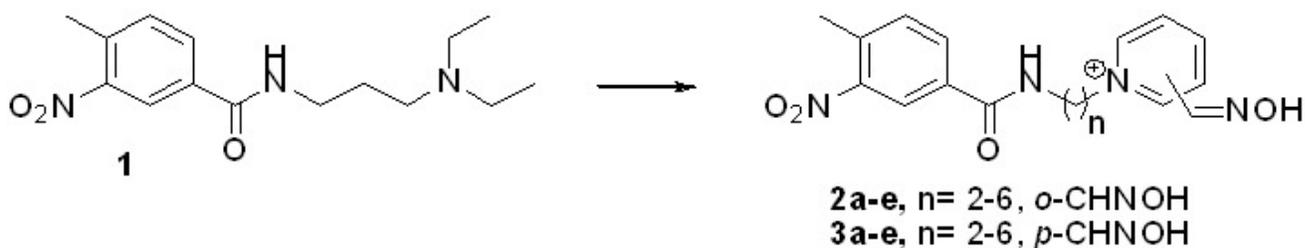
MEDI 396

Development of antidotes against nerve agent inhibited acetylcholinesterase – the transformation of an inhibitor into a reactivator

Cecilia Lindgren¹, cecilia.lindgren@umu.se, Nina Forsgren², Christine Akfur², Lotta Berg¹, David Andersson¹, Mikael Hillgren¹, Weixing Qian¹, Franz Worek³, Fredrik Ekström², Anna Linusson¹. (1) Umeå University, Umeå, Sweden (2) Swedish Defence Research Agency, Umeå, Sweden (3) Bundeswehr Institute of Pharmacology and Toxicology, Munich, Germany

The enzyme acetylcholinesterase (AChE) is responsible for the hydrolysis of the neurotransmitter acetylcholine. In the presence of certain organophosphorous inhibitors (OPs) AChE can be inactivated as a result from phosphorylation of the catalytic serine residue. OPs are used as insecticides but also as chemical warfare agents in the form of nerve agents, for instance the sarin terrorist attack in the Tokyo subway in 1995 and more recently in the civil war in Syria 2013. Today, pyridinium oximes are used as antidotes, cleaving the covalent bond between the OP and AChE, thereby restoring AChE to the active form. However, the efficiency of reactivation is highly dependent on the type of nerve agent and antidotes do not penetrate the blood-brain barrier. Thus, there is a great need for compounds with improved efficacy, selectivity and permeability. Based on a hit molecule identified in a high throughput screening campaign, a subset of analogues was designed with the aid of statistical molecular design. The synthesized and tested analogues were used in SAR modelling. One of the resulting inhibitors, **1**, was thereafter selected as a starting point for the development of reactivators of nerve agent inhibited AChE, aided by IC₅₀ values and X-ray crystal structures. The idea was to

transform the inhibitor, **1**, into a reactivator, **2** or **3**, by attachment of an oxime with the ability to cleave the covalent bond between AChE and a nerve agent. The position of the oxime as well as the length of the linker has been varied. An initial screen confirmed that a number of compounds could efficiently reactivate AChE inhibited by a range of nerve agents. Focus was shifted to compound **2d** that could effectively reactivate AChE inhibited by tabun, which is particularly difficult to do for current reactivators. X-ray crystal data has been collected and compared for **2d** and **3d** in complex with AChE inhibited by various nerve agents. The aim is to investigate the mechanism of reactivation, as well as the effect of the position of the oxime, which would aid in future research of nerve agent antidotes.



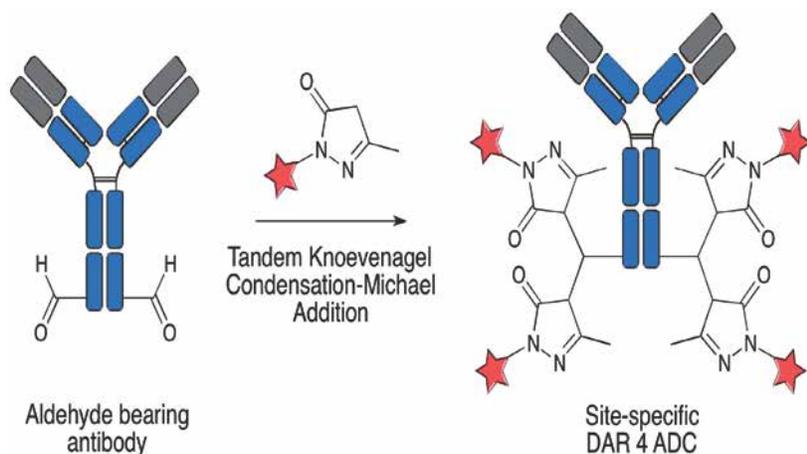
MEDI 397

Generating site-specific antibody-drug conjugates with high drug to antibody ratios using a tandem Knoevenagel condensation-Michael addition

Romas Kudirka, romaskudirka@gmail.com, Robyn Barfield, Jesse McFarland, Penelope Drake, Adam Carlson, Stephanie Banas, Wes Zmolek, Albert Garofalo, David Rabuka. Catalent, Emeryville, California, United States

Antibody-drug conjugates (ADCs) represent a promising new therapeutic option for cancer treatment. By exploiting the targeted specificity of an antibody, ADCs can deliver their toxic payloads selectively to tumors, sparing healthy tissues. This discrimination minimizes systemic toxicity, improving safety and the therapeutic index. Currently, there are two FDA approved ADCs, Adcetris and Kadcyła. At the molecular level, both of these therapeutics are heterogeneous mixtures resulting from the nonselective ligation of drug to cysteine and lysine residues, respectively. The nonselective ligation approaches used to make these two ADCs render it very difficult to optimize their biological, physical, and pharmacological properties. In addition, certain linkages, such as cysteine-maleimide and oximes are unstable in serum over time, complicating the pharmacokinetics of molecules using these ligation strategies. By contrast, our ability to create site-selective, stable linkages allows us to design ADCs with enhanced efficacy, safety, and pharmacokinetics by controlling payload stoichiometry and placement. We create homogeneous ADCs by using a genetically encoded 5 amino acid "aldehyde-tag" sequence, in which a cysteine is co-translationally converted to an aldehyde. This method affords exquisite control over the placement of the reactive aldehyde group, which can be modified using a variety of stable carbonyl bioconjugation methods that we have developed. Here, we introduce a ligation chemistry that allows access to species with higher drug to antibody ratios (DAR). This new ligation chemistry involves

the double addition of a pyrazolone with an aldehyde labeled protein, via a tandem Knoevenagel condensation-Michael addition. This rapid and facile ligation technique is performed under mild conditions in the absence of catalyst to generate new architectures that were previously inaccessible via conventional ligation reactions. Using this ligation, we will present the impact of drug placement on in vitro and in vivo efficacy and highlight the pharmacokinetics of ADCs chemically modified at different locations on the antibody peptide backbone.



MEDI 398

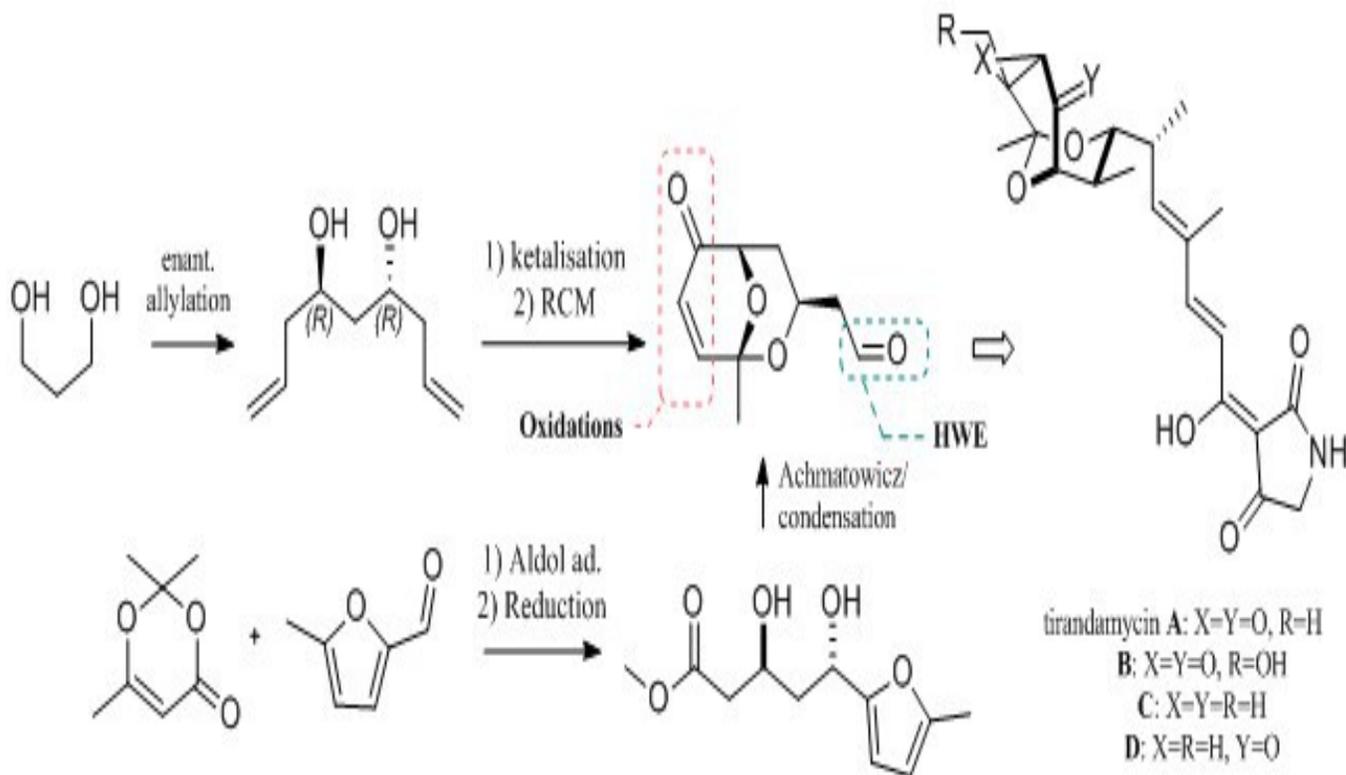
Synthesis of truncated tirandamycin A-D derivatives as new antihelminthic agents

Tania Jimenez, tania.jimenez@chem.gu.se, Morten Grotli, Carl Johan Wallentin.
Department of Chemistry and Molecular Biology, University of Gothenburg, Goteborg, Sweden

Lymphatic filariasis (LF) caused by the parasitic nematodes *B. malayi*, represents a worldwide health crisis with over 200 million people infected and another 20% of the global population at risk for infection. Thus, a top priority of the WHO is to search for new antihelminthic drugs that kill adult parasites, have new mechanisms of action and exhibit fewer side effects than the current medications available.

Tirandamycins A-D (TAMs A-D) derivatives have attracted much attention as potential antihelminthic agents for the treatment of LF, as they inhibit asparagyl-tRNA synthase (AsnRS), an excellent filarial target for *B. malayi*. Due to their pharmacological properties and their intriguing molecular architectures, a handful of total syntheses have been documented in the literature. However, common for all these achievements are lengthy synthetic routes not amenable late stage derivatization and thus, they are not applicable to drug development programs.

Two different synthetic protocols that are able to produce advanced intermediates in 2–3 steps have been pursued, providing a short, robust and scalable route to access key intermediates suitable for library synthesis.



MEDI 399

Beyond IC50 and simple PK models – considerations for discovery chemists

Robert Fraczkiwicz, robert@simulations-plus.com, Michael B. Bolger, Walter Woltosz. Simulations Plus, Lancaster, California, United States

Should medicinal chemists consider the pharmacokinetic (PK) profile of drug candidates? Absolutely. PK concerns can kill even the most promising drug candidates. Classical computational estimators of PK, which are based on assumptions of linearity, are widely used in this arena. We show that these are sufficient to predict multiple dosing profiles at steady state, but only for limited classes of drugs. Significant deviations from this predicted behavior are common, and may remain undiscovered until late in the drug discovery and development cycle. Those cases are often blamed on “nonlinearities in PK” – but what does that really mean? What specific factors contribute to these different outcomes?

Using the Advanced Compartmental Absorption and Transit™ (ACAT™) model, we have performed simulation studies on several drugs that show deviations from linear pharmacokinetics with multiple dosing. For example, a linear model underestimates the steady state PK of digoxin dosed at 24h intervals because it does not take into account intricacies of its colonic absorption. On the other hand, a linear model overestimates the steady state PK of carbamazepine due to too optimistic assessment of its solubility and

dissolution. Full-scale mechanistic oral absorption/PBPK simulations can account for these mechanisms, but are not well-suited for large scale discovery projects. We have developed a fast *in silico* tool for conducting oral absorption/compartamental PK simulations that are faster than full-blown PBPK models and more accurate than simple linear models for initial dose estimations in large discovery compound libraries.

Our work concludes with a set of recommendations for predicting steady state PK in lead optimization.

MEDI 400

Synthesis and evaluation of antitubercular agents 2-aminothiophenes and benzo-1,2-selenazol-3(2H)-ones targeting Pks13 and Ag85C respectively

Sandeep Thanna¹, sandeep.thanna@gmail.com, Susan E. Knudson², Christopher M. Goins¹, Fatma Salem¹, Sunayana Kapil¹, Anna Grzegorzewicz², Mary Jackson², Donald R. Ronning¹, Richard A. Slayden², Steven J. Sucheck¹. (1) Department of chemistry, The University of Toledo, Toledo, Ohio, United States (2) Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, Colorado, United States

Mycobacterium tuberculosis (*Mtb*) is the causative agent of tuberculosis (TB). TB ranks first for the number of deaths in humans caused by an infectious agent. This fact emphasizes the need to identify new drugs to treat TB. Pks13 and Ag85 complex are validated anti-TB drug targets, which are essential for biosynthesis of the unique mycobacterial cell wall. Pks13 in presence of CmrA catalyzes a Claisen-type condensation reaction to produce trehalose monomycolate (TMM), which is utilized by the Ag85 complex to produce mAG, TMM, and TDM (Cord Factor), the key constituents of *Mtb* cell wall. In the present study we share our progress towards synthesis and evaluation of antitubercular agents 2-aminothiophenes and benzo-1,2-selenazol-3(2H)-ones compounds targeting Pks13 and Ag85 complex respectively.

Two library's of 2-aminothiophene-3-carboxylate (2-AT) compound are derivatives of compounds synthesized using classic Gewald reaction. In the first generation of library the compound ethyl 6-ethyl-2-(perfluorobenzamido)-4,5,6,7-tetrahydrothieno [2,3-c]pyridine-3-carboxylate showed remarkable potency against *Mtb* H37RV (MIC = 0.23 μ M) and showed an impressive potency (MIC = 0.20–0.44 μ M) against *Mtb* strains resistant to isoniazid, rifampicin and fluoroquinolones. Second generation compounds showed good ADME properties and compound ethyl 2-(perfluorobenzamido)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylate showed improved potency with an MIC of 67 nM.

For synthesis of benzo-1,2-selenazol-3(2H)-ones compounds, we developed copper catalyzed, C-Se coupling method, which uses KSeCN, Cs₂CO₃ and 2-halo-*N*-alkylbenzamides as starting materials. Sixteen compounds were tested for growth inhibition of *Mtb* H37Rv (MIC = 12.5–50 μ g/mL), followed by percentage of activity (POA) and enzyme inhibition studies (IC₅₀ = 0.54–28.6 μ M) on *Mtb* Ag85C. The allyl derivative showed good MIC of 12.5 μ g/mL and POA of 15%.

In summary, these studies suggest compounds 2-aminothiophenes and benzo-1,2-selenazol-3(2H)-ones represent a promising anti-TB leads which exhibits activity well below toxicity to human monocytic cells.

MEDI 401

Novel pyrimidine compounds as potent JAK inhibitors

Yan Chen, yanchen94010@yahoo.com, Hui Li, Rose Yen, Thilo Heckrodt, Darren McMutrie, Nan Lin, Rajinder Singh, Vanessa Taylor, Meagan Chan, Esteban Masuda, Gary Park, David Lau, Donald Payan. Rigel Pharmaceuticals Inc., South San Francisco, California, United States

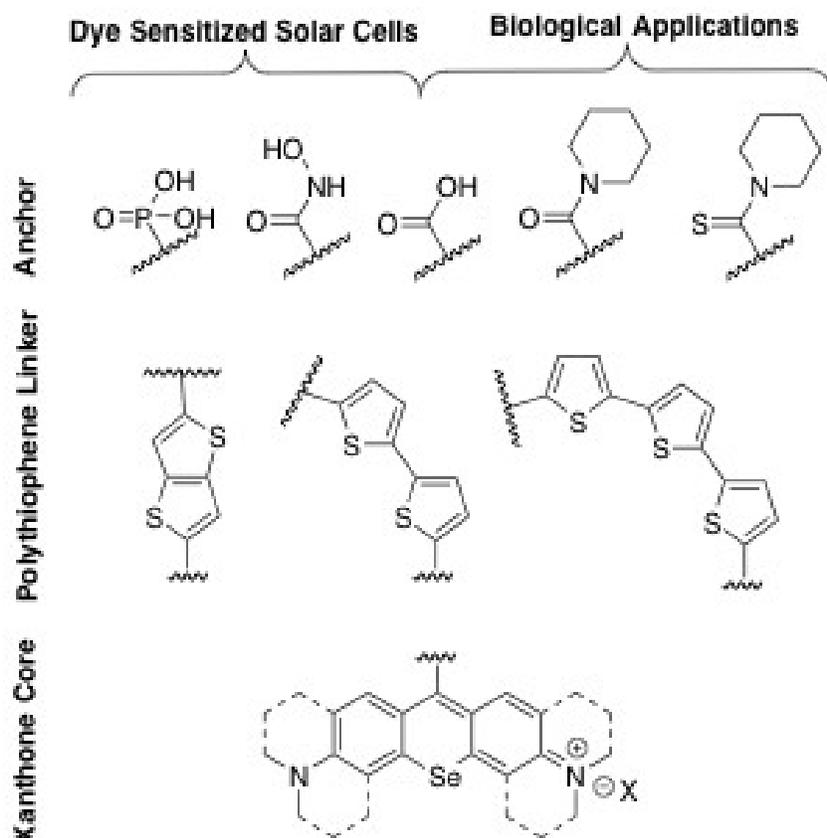
Janus kinases (JAK) play a critical role in JAK/STAT signaling pathways that mediate immune response and cell growth. From HTS hit to lead optimization, a series of Pyrimidine compounds have been identified as potent JAK1/3 inhibitors. These molecules showed good potency in inhibiting cell-growth and phosphorylation of Stat-5, induced by IL-2 which is one of the cytokines that would initiate JAK1/3-STAT process. Further targeted SAR explorations led to molecules with much reduced p450 activities and improved selectivity over JAK2 kinase in cell assays. Some compounds were also evaluated in IL-2 animal model studies and in CIA disease model, good efficacy was observed.

MEDI 402

Synthesis and evaluation polythiophene containing rhodamine dyes for biological and photochemical applications

Michelle K. Linder², mklinder@buffalo.edu, Justin N. Nasca², Kellie S. Gast², Geri Sawada¹, David Watson², Michael R. Detty². (1) Drug Disposition, Eli Lilly and Company, Indianapolis, Indiana, United States (2) Chemistry, University at Buffalo, Buffalo, New York, United States

The synthesis of heavy chalcogen-containing rhodamine dyes, which have applications as photosensitizers for photodynamic therapy of cancer, extracorporeal photopheresis of malignant T-cells, and graft vs. host disease due to their interactions with P-glycoprotein, mitochondrial localization, and their strong absorption have been described by the Detty group. Additionally, dyes bearing carboxylic, hydroxamic, or phosphonic acid functionalities have been shown to function as metal-free alternative photosensitizers for solar cells. To date, rhodamines bearing thienyl moieties located at the 9-position of the xanthylium core have been examined. However, thieno[3,2-*b*]thiophene, 2,2'-bithiophene, and 2,2':5',2''-terthiophene derivatives have yet to be investigated. The synthesis of these new polythiophene derivatives and preliminary biological and photochemical data obtained from collaborators will be described.



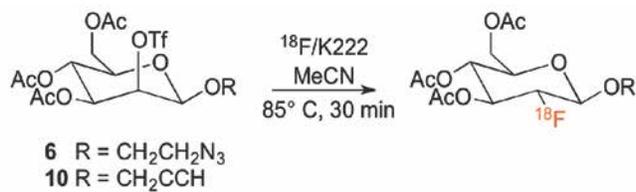
MEDI 403

Synthesis of β -configured clickable [^{18}F]FDGs as novel ^{18}F -fluoroglycosylation tools for PET *in vivo* imaging

Mathias Elgland¹, mathias.elgland.phd@gmail.com, Patrik Nordeman², Timmy Fyrner¹, Peter Konradsson¹, Gunnar Antoni², Peter Nilsson¹. (1) Department of Physics, Chemistry and Biology (IFM), Linköping university, Linköping, Sweden (2) Department of Medicinal Chemistry, Preclinical PET Platform, Uppsala University, Uppsala, Sweden

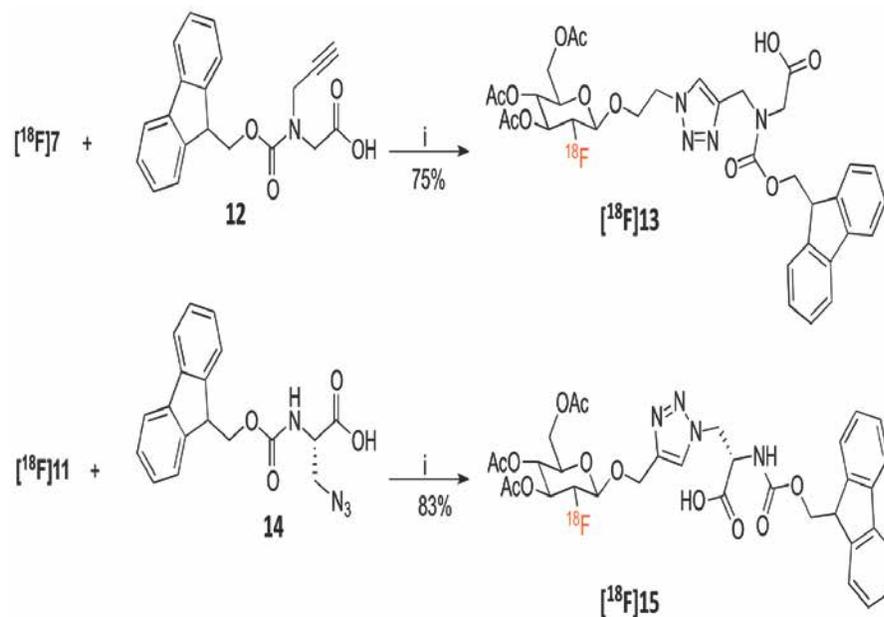
In this work, we have developed a mild and chemoselective ^{18}F -fluoroglycosylation method that may be employed as a general means to provide novel ^{18}F -fluoroglycosylated tracers for positron emission tomography. More specifically, we have devised a synthetic route to β -configured mannopyranosyl triflates (*i.e.*, PET precursors) equipped with a terminal azide or alkyne aglycon (**6** and **10**), which enables further chemical ligation to any click-functionalized ligand by using the copper(I)-catalyzed alkyne-azide 1,3-cycloaddition reaction. Standard ^{18}F -radiolabelling of the precursors, using K^{18}F and the cryptand cryptofix (K.2.2.2), provided the corresponding β -configured [^{18}F]FDGs, [^{18}F]**7** and [^{18}F]**11**, in excellent radiochemical yields of $77\pm 2\%$ and $88\pm 5\%$ respectively (**table 1**). Moreover, the clickability of these [^{18}F]FDGs was investigated by click coupling to the suitably functionalized Fmoc-protected amino acids,

Fmoc-*N*-(propargyl)-glycine (**12**) and Fmoc-3-azido-L-alanine (**14**) which provided the ^{18}F -fluoroglycosylated amino acid conjugates, [^{18}F]**13** and [^{18}F]**15**, in radiochemical yields of 75% and 83% respectively, in a simple one-pot two step protocol (**scheme 1**).



Entry	Precursor	Product	RCY(%)
1	6	 [^{18}F] 7	77±2
2	10	 [^{18}F] 11	88±5

Table 1. ^{18}F -radiolabeling of β -*D*-mannoside precursors to yield clickable [^{18}F]FDGs.



Scheme 1. ^{18}F -fluoroglycosylation of amino acids **12** and **14**. General conditions and reagents: i) aq., CuSO_4 (0.3 eq.), aq., (+)-sodium L-ascorbate (0.6 eq.), 60°C , 20 min, DMF.

MEDI 404

Small-molecule anti-virulence agents for the prevention of dental biofilms

Bhavitavya Nijampatnam¹, *snij@uab.edu*, Hui Wu³, Sadanandan E. Velu². (1) Chemistry, University of Alabama at Birmingham, Birmingham, Alabama, United States (3) Pediatric Dentistry, University of Alabama at Birmingham, Birmingham, Alabama, United States

The oral microbial flora consists of many beneficial species of bacteria that are associated with a healthy condition and control the progression of oral disease. *Streptococcus mutans* is an oral pathogen that has developed multiple mechanisms to proliferate and maintain dominant presence in the oral cavity, and is the primary etiologic causative agent of dental caries. Not only can *S. mutans* form biofilms readily on the tooth surface, but this bacterium rapidly produces lactic acid from dietary sugars which lead to tooth decay. Due to the tenacious biofilms that are resistant to conventional antibiotics, current marketed therapies are not species-specific and kill pathogenic species as well as beneficial species, which are protective against the formation of pathogenic biofilms. In order to develop a species specific therapeutic agent, we have targeted the *S. mutans* glucosyltransferases (Gtfs) for our drug design. These enzymes metabolizes sucrose into water insoluble and soluble glucans, which play a role in mediating irreversible attachment of *S. mutans* to the tooth and also provide an extracellular matrix, shielding the bacteria from the host immune response, and antimicrobial agents. A diverse library of small molecules based on the *in silico* screening against X-ray crystal structure of GtfC, one of the contributing Gtfs, was subjected to a biofilm formation inhibition assay to identify potent small molecules that inhibit *S. mutans* biofilm formation specifically. The emerging lead compound from this study reduced the formation of biofilms by *S. mutans in vitro* and reduced the incidence of smooth-surface caries in rats at low micromolar concentrations. The inhibition of *S. mutans* Gtfs expression was confirmed using zymogram assays and conversely selectivity against *S. mutans* was established by confirming the growth of commensal bacteria was not compromised. Subsequently, we have synthesized a library of compounds to optimize the activity of the lead scaffold and identified novel, selective, anti-biofilm therapeutics for the prevention of dental caries.

MEDI 405

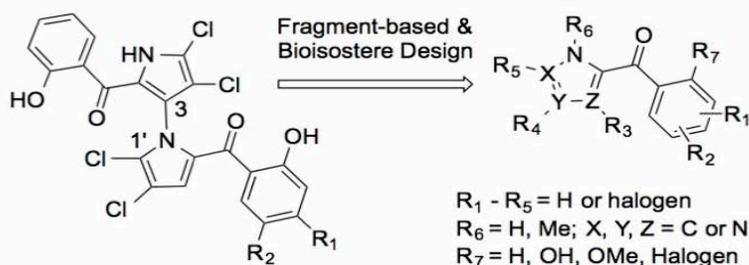
Discovery of novel pyrrolomycins as potential anticancer agents

Yan Liu³, *yan.liu@unmc.edu*, Timothy R. McGuire⁴, Zunhua Yang³, Don Coulter⁵, Yashpal Singh Chhonker⁴, D.J. Murry^{4,2}, John G. Sharp⁶, Haizhen A. Zhong¹, **Rongshi Li**^{3,2}, *rongshi.li@unmc.edu*. (1) Department of Chemistry, The University of Nebraska at Omaha, Omaha, Nebraska, United States (2) Fred and Pamela Buffett Cancer Center,

UNMC, Omaha, Nebraska, United States (3) Center for Drug Discovery and Department of Pharmaceutical Sciences, University of Nebraska Medical Center, Omaha, Nebraska, United States (4) Pharmacy Practice, UNMC, Omaha, Nebraska, United States (5) Department of Pediatrics, UNMC, Omaha, Nebraska, United States (6) Department of Genetics, UNMC, Omaha, Nebraska, United States

We previously reported the design, synthesis and evaluation of marine natural products marinopyrrole derivatives as potent Mcl-1/Bcl-2 inhibitors. Using fragment-based approach, we designed a novel series of pyrrolomycins – the fragment derivatives of asymmetrical marinopyrroles. Upon completion of the chemistry development and synthesis of pyrrolomycins, in vitro evaluation, quantitative structure-activity relationship (QSAR) calculation, drug metabolism and pharmacokinetics (DMPK), and in vivo studies were performed. Our results have revealed that some promising compounds exhibited submicromolar IC₅₀ values against a MYCN amplified neuroblastoma cell line and disruption of ATP production. These active compounds also possess desirable pharmacokinetic profiles. This presentation will discuss fragment-based design, synthesis, evaluation of their anticancer activity, DMPK, QSAR, and in vivo efficacy results in mice model.

Table 1. Physicochemical properties and anticancer activity.



Compds	IC ₅₀ (μM)	logD	logS	TPSA	pKa	LE
1	17.4	5.7	ND	ND	ND	0.20
2	16.5	5.5	ND	ND	ND	0.20
3	11.0	5.3	-5.95	53.1	5.84	0.38
4	4.80	4.4	-4.86	53.1	5.87	0.43
5	50.0	5.1	-5.65	53.1	5.94	0.33
6	1.41	3.3	-3.62	53.1	5.59	0.53
7	0.12	4.0	-4.23	53.1	5.57	0.59
8	0.80	4.2	-4.50	53.1	5.89	0.49

MEDI 406

Design and synthesis of small molecule inhibitors bearing 1,2,3-triazole/sulfonate pharmacophore from natural precursors for the treatment of bacterial infections

Babita Aneja^{1,3}, aneja.babita@gmail.com, Shadab Alam¹, Mudsser Azam¹, Ahmad Perwez⁴, Rizwanul Haque¹, Moshahid Rizvi¹, Ronan Maguire⁵, Kevin Kavanagh⁶, Umesh Yadava⁷, Constantin Daniliuc⁸, Amir Azam³, Abid Mohammad². (1) Biosciences,

Jamia Millia Islamia, New Delhi, New Delhi, India (2) Biosciences, Jamia Millia Islamia University, New Delhi, Delhi, India (3) Chemistry, Jamia Millia Islamia, New Delhi, New Delhi, India (4) Biosciences, Jamia Millia Islamia, New Delhi, New Delhi, India (5) Biology, Maynooth University, Co. Kildare, Ireland (6) Biology, Maynooth University, Co. Kildare, Co. Kildare, Ireland (7) Physics, Deen Dayal Upadhyay Gorakhpur University, Gorakhpur, Uttar Pradesh, India (8) Organisch-Chemisches Institut, Westfälische Wilhelms-Universität, Münster, Münster, Germany

Over the past decades, emergence of resistant bacterial strains has created havoc to the human health resulting in increased morbidity and mortality, including in developed countries. In order to enhance armamentarium against these resistant strains, we synthesized 1,2,3-triazole and sulfonate derivatives of natural bioactive precursors. *In vitro* antibacterial evaluation against six sensitive bacterial strains (*Pseudomonas aeruginosa*, *Salmonella enterica*, *Streptococcus pneumoniae*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Enterococcus coli*) showed very promising results specially **14**, **19** and **22** emerged as potent antibacterial agents against *S. pneumoniae* (IC₅₀: 0.178, 0.021 and 0.104 µM), *E. faecalis* (IC₅₀: 0.104, 0.018 and 0.162 µM) and *E. coli* (IC₅₀: 0.043, 0.037 and 0.060 µM), respectively. Moreover, these lead inhibitors also demonstrated considerable activity against multidrug resistant *E. coli* strain. Growth curve analysis for **14**, **19** and **22** exhibited their bacteriostatic nature. Selected compounds did not show significant cytotoxicity up to 100 µg/mL concentration on HEK293 cell line. TEM micrographs of bacterial cells (*S. pneumoniae* and *E. coli*) exposed to **14** and **22** clearly revealed membrane disruption leading to cell death. Studies on the larvae of *Galleria mellonella* showed that the selected inhibitors **14** and **22** did not cause an alteration in the hemocyte density thereby did not provoke an immune response and were non-toxic up to the conc. of 3.0 mg/ml. Our study revealed that better activity and toxicity profiles of the lead compounds (**14** and **22**) make them suitable candidates for further structural optimization for the development of potent and safe antibacterial agents.

MEDI 407

New N-substituted indazole-5-carboxamides as subnanomolar, selective monoamine oxidase B and dually active MAO-A/B inhibitors with BBB and GI permeability

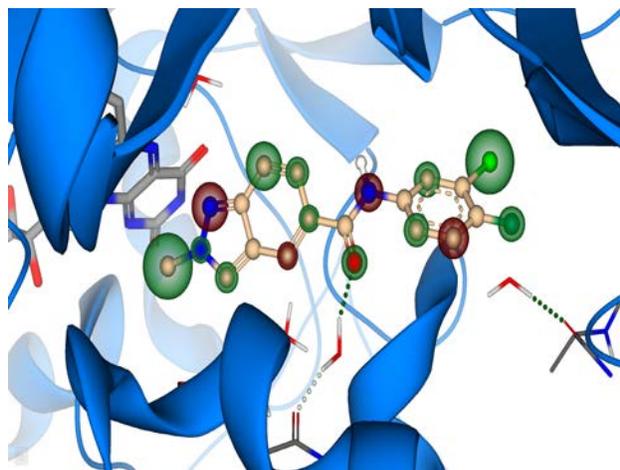
Marcus Gastreich², ghost@biosolveit.de, **Carsten Detering**¹, **Liudmil Antonov**⁵, **Silviya Hristova**⁵, **Hans-Georg Stammler**⁴, **Nikolay T. Tzvetkov**³. (1) BioSolveIT Inc, Bellevue, Washington, United States (2) BioSolveIT, St. Augustin, Germany (3) NTZLab, Sofia, Bulgaria (4) Chemistry, University of Bielefeld, Bielefeld, Germany (5) Inst. of Organic Chemistry with Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria

We report on new, structurally optimized *N*-alkyl-substituted indazole-5-carboxamides, developed as potential drug and radioligand candidates for the treatment and diagnosis of Parkinson's Disease. Recently, we published remarkably potent and *selective* MAO-B and *dually active* MAO-A/B inhibitors. Those compounds had subnanomolar potency

towards MAO-B and moderate activity versus MAO-A, respectively. Computer studies with a novel software tool, SeeSAR, that incorporates dehydration/desolvation and interaction terms consistently indicated paths towards their improvement.

The resulting most promising drug-like derivatives that emerged are *N*-(3-chloro-4-fluorophenyl)-1-methyl-1H-indazole-5-carboxamide that shows pM activity vs. MAO-B and a 15,000-fold selectivity versus MAO-A, and *N*-(3-chloro-4-fluorophenyl)-2-methyl-2H-indazole-5-carboxamide (hMAO-B, IC₅₀ = 8nM, hMAO-A, IC₅₀ = 439nM). Both compounds are predicted to cross both the gastrointestinal tract and the blood-brain barrier in vitro with appropriate drug-like properties required for CNS active drugs.

Further, combined single X-ray-based molecular modeling studies provided insights into the enzyme-inhibitor interactions within both MAO isoforms and a rationale for their inhibitory activity with controlled MAO-A/B selectivity – despite their small structural differences. We propose modeled binding modes and a thorough energetic analysis that are in line with safinamide and harmine's interaction patterns, allowing an outlook to further exploration of the alkyl side chain for next step lead optimization efforts.



MEDI 408

Efflux pump inhibitors which bind and inhibit multidrug efflux pump AcrAB-TolC in *Escherichia coli*

Keith M. Haynes, 30150168@acs.org, John K. Walker. *Pharmacology and Physiology, Saint Louis University, St. Louis, Missouri, United States*

Antibiotic resistance is one of the biggest concerns of modern medicine, and multidrug efflux is the primary mechanism responsible for antibiotic resistance in gram-negative bacteria. In *E. coli*, the efflux pump AcrAB-TolC is able to bind to antibiotics and expel them from the cell. This reduces the concentration of the antibiotic inside the cell and greatly reduces its effectiveness. Our research is focused on the synthesis and screening of efflux pump inhibitors (EPIs) which can bind to the multidrug pump AcrAB-TolC preventing the efflux of known antibiotics.

MEDI 409

Glycosylated porphyrins for use in PET and PDT: Synthesis and characterization

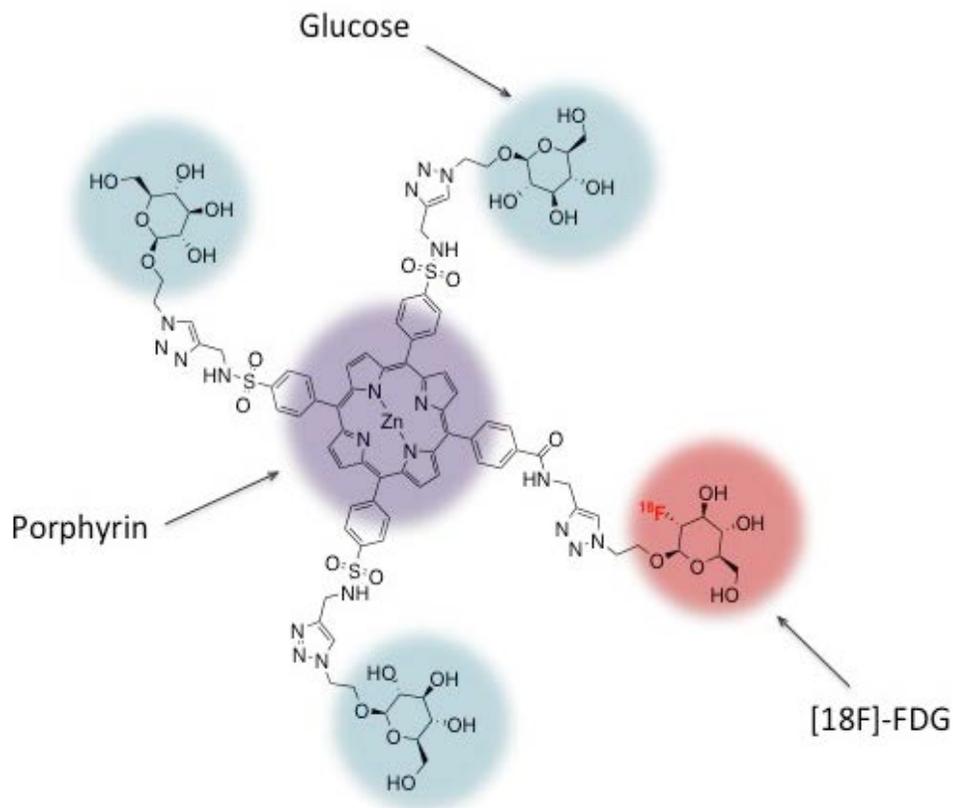
Katriann Arja, katriann.arja@liu.se, Mathias Elgland, Peter Nilsson. Department of Physics, Chemistry and Biology (IFM), Linköping University, Linköping, Sweden

Theranostical tools to both visualize and terminate cancer-diseased tissue are of high interest as cancer is one of the leading causes of death worldwide. Both diagnostics and therapy for this group of deadly diseases are of urgent need for more research. In our current study a series of ^{18}F -labelled porphyrin-based glycoconjugates were synthesized for use in cancer diagnostics with positron emission tomography (PET) and cancer therapy via photodynamic therapy (PDT). Porphyrins' propensity to accumulate in tumor tissue and their property to generate singlet oxygen upon irradiation by light make them good candidates as PDT agents. Furthermore, by conjugating a porphyrin scaffold to various carbohydrates, improved cancer cell selectivity and higher cellular uptake can be achieved as different cancer cell lines generally overexpress various carbohydrate-binding receptors.

Finally, incorporating a radiotracer for PET in these PDT agents, theranostical molecules are achieved that can visualize cancer-affected areas and assist in monitoring the therapy progress.

We have developed a convenient and high-yielding synthetic route to get the desired glycosylated porphyrins. Copper(I)-assisted click-reaction between terminal alkynes on the porphyrin's periphery and azides on carbohydrates is used to conjugate these moieties. To produce a PET-tracer, a novel β -configured [^{18}F]FDG with an azide equipped linker is utilized. As a result, a series of porphyrin glycoconjugates is produced with a varying sugar on the three positions of the porphyrin while the fourth position is occupied by the [^{18}F]FDG.

These molecules are being studied in cell assays for their cellular uptake, selectivity and toxicity, showing promising preliminary results. PDT and PET studies are conducted to evaluate the therapeutic and diagnostic properties of these compounds.



Tri-glucose-porphyrin-[^{18}F]FDG, an example in series of the synthesized PET-PDT agents.

MEDI 410

Selective nicotinic acetylcholine receptor activities from the areca nut

Nicole Horenstein¹, horen@chem.ufl.edu, **Clare Stokes**², **Roger Papke**². (1) Dept of Chem Box 117200, Univ of Florida, Gainesville, Florida, United States (2) Pharmacology and Therapeutics, University of Florida, Gainesville, Florida, United States

The *Areca* nut is well known in southeastern Asia as the source of muscarinic active alkaloids that constitute a popular addictive drug known as the betel quid, in which limed extracts of the nut are rolled in betel vine leaves and in some cases, include tobacco. Of the four known alkaloids in *Areca*, arecoline is the most active muscarinic agonist while guvacoline is less potent. Arecaidine is an M2 selective agonist. Muscarinic activity has not been reported for guvacine, a GABA transport inhibitor. Two electrode voltage clamp electrophysiology experiments in *Xenopus* oocytes expressing muscarinic or nicotinic receptors have revealed that in addition to muscarinic activity, arecoline displays weak nicotinic partial agonism with a selectivity profile favoring $\alpha 4\beta 2$ and $\alpha 6\beta 2\beta 3\alpha 4\beta 2$ subtypes considered as targets for therapeutics aimed at nicotine addiction, while avoiding $\alpha 7$, $\alpha 3\beta 4$, and muscle-type nAChR. This is in contradistinction to the known therapeutics cytisine and varenicline which although capable of partial

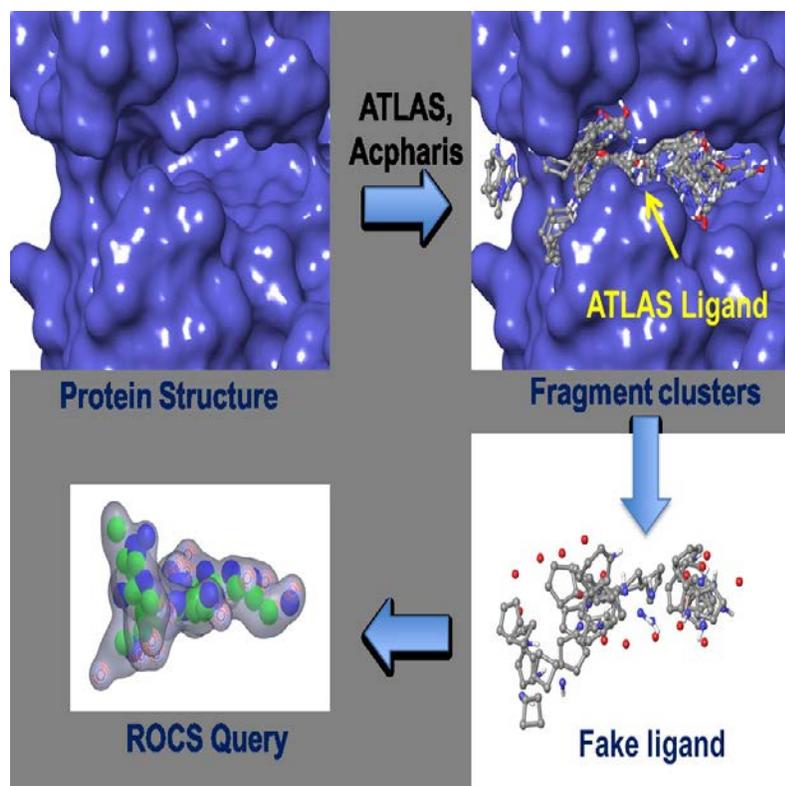
agonism at $\alpha 4\beta 2$ and $\alpha 6\beta 2\beta 3\alpha 4\beta 2$ receptors, are strong agonists of the $\alpha 7$ receptor, and show significant activity at ganglionic $\alpha 3\beta 4$ receptors. The arecoline analog isoarecolone shows an even more pronounced selectivity for $\alpha 4\beta 2$ and $\alpha 6\beta 2\beta 3\alpha 4\beta 2$ receptors compared to arecoline. It is noteworthy that whereas arecoline shows significant muscarinic activity, primarily at M1 mAChR, isoarecolone has nearly abolished activity at M1 and significantly reduced mAChR activity in assays against co-expressed M1, M2, and M3 receptors. Isoarecolone thus represents an interesting template for the development of new agents targeting $\alpha 4\beta 2$ and $\alpha 6\beta 2\beta 3\alpha 4\beta 2$ receptors. Interestingly we have found that *Areca* extracts also demonstrate an inhibitory component acting at nicotinic receptors. Fractionation based on size revealed that while both mAChR and nAChR activities resided in the low molecular weight fraction < 1 kDa, the inhibitory component was large with an estimated size > 10 kDa. These results will be discussed in the context of development of new therapeutics aimed at smoking cessation.

MEDI 411

Using computational solvent mapping to generate a negative template of the binding site

Istvan J. Enyedy², enyedyi@gmail.com, David Hall¹. (1) Acpharis, Allston, Massachusetts, United States (2) Chemistry and Molecular Therapeutics, Biogen, Milton, Massachusetts, United States

Computational solvent mapping has emerged as a tool for determining binding sites on proteins and predicting their druggability. We have extended this method to generate a negative template of the binding site highlighting important interactions for binding. This negative template can be used to guide virtual screening with ROCS, HYBRID, or our custom OEShape-based script that weights the features by predicted importance. This method increases the ROC AUC for separating binders from non-binders across all methods versus using known ligands from crystal structures to guide virtual screening, showing these negative templates more reliably choose those features important for binding.



MEDI 412

Differentiating antiproliferative and chemopreventive modes of activity for electron-deficient aryl isothiocyanates against human MCF-7 cells

Jared R. Mays, jared.mays@augie.edu. Chemistry, Augustana University, Sioux Falls, South Dakota, United States

Dietary consumption of *Brassica* vegetables provides beneficial effects due to organic isothiocyanates (ITCs), a resultant product of the enzymatic hydrolysis of glucosinolate secondary metabolites. The ITC L-sulforaphane (SFN) is the principle agent in broccoli that demonstrates several modes of anticancer action. While the anticancer properties of ITCs like SFN have been extensively studied and SFN has been the subject of multiple human clinical trials, the scope of this work has largely been limited to those derivatives found in nature. Previous studies have demonstrated that structural changes in an ITC can lead to marked differences in a compound's potency to (1) inhibit growth of cancer cells, and (2) alter cellular transcriptional profiles. This study describes the preparation of a library of non-natural ITCs and the development of a bifurcated screening approach to evaluate antiproliferative and chemopreventive properties against human MCF7 breast cancer cells. The results of this study have led to the identification of (1) structure-activity relationships and (2) lead ITCs for continued development.

MEDI 413

Application of the boronic acid as an isostere of the phenolic hydroxyl group in optimization of Selective Estrogen Receptor Downregulators (SERDs)

Jiawang Liu, jliu1@xula.edu, shilong zheng, Shanchun Guo, Qiu Zhong, Melyssa Bratton, Thomas E. Wiese, Guangdi Wang. RCMI Cancer Research Center, Xavier University of Louisiana, New Orleans, Louisiana, United States

Boronic acid has been proved a bioisostere of a phenolic hydroxyl group with superior pharmacokinetic properties. Using this knowledge, we have identified an orally bioavailable steroidal SERD fulvestrant-3 boronic acid (ZB716). Herein, we report the modification of GW7604, a non-steroidal estrogen receptor downregulator, forming a boronated GW7604 (GLL398). It possesses quite a high affinity to ER α in a fluorescence resonance energy transfer binding assay with an IC₅₀ of 1.14 nM, which is 10-fold stronger than GW7604 does. Estrogen receptor reporter gene luciferase assay suggests that GLL398 is an antiestrogen against 17 β -estradiol. *In vitro* cell assays show that GLL398 exhibits comparable potencies as GW7604 in inhibiting breast cancer cell proliferation and degrading ER α protein. Most importantly, GLL398 confers superior oral bioavailability (AUC = 36.9 $\mu\text{g}\times\text{h}/\text{mL}$) in rats as compared to GW7604 (AUC = 3.35 $\mu\text{g}\times\text{h}/\text{mL}$). The strikingly favorable pharmacokinetic property of GLL398 makes it a promising oral non-steroidal SERD suitable for clinical evaluation. These studies disclose the superiority of boronic acid as a pharmacophore enhancing the enrichment of active pharmaceutical ingredients in the circulation.

MEDI 414

Controlled singlet oxygen release photosensitizers in PDT

Mikhail A. Filatov, mihafil@gmail.com, Mathias O. Senge. School of Chemistry, Trinity College Dublin, Dublin, Ireland

Photodynamic therapy (PDT) relies on the generation of highly reactive singlet oxygen ($^1\text{O}_2$) through a photosensitizer (PS) excited state interaction with molecular oxygen ($^3\text{O}_2$), resulting in oxidative stress and cell death through apoptosis or necrosis. However, prolonged expose of tissue to light irradiation causes side-effects which may include pain, burning/stinging sensation and itchiness. To minimize a damage of healthy tissue during light treatment, such techniques as precise delivery of PS into diseased cells, use of two-photon absorption, chemical activation of PS in the target cells are under active development.

In this work we focused on a novel approach towards the control of photocytotoxicity – application of organic endoperoxides as alternative sources of $^1\text{O}_2$.

We explored a new type of PS which incorporates oxygen trapping moieties. The latter can be either chemically bound to the porphyrin or incorporated into supramolecular

systems (Figure 1). Upon light irradiation the system produces singlet oxygen, similarly to conventional PS. The singlet oxygen formed is being bound to the trapping units via cycloaddition process. A fraction of singlet oxygen formed upon direct PS quenching is then responsible for an immediate therapeutic response of the PDT agent. Following this initial response, a “slow” phase of the therapeutic effect occurs, whereby a retro-Diels-Alder process initiates to evolve singlet oxygen from the trapping moieties and this can last up to several days, depending on the chemical structure of the trapping molecules. The outcome of the proposed approach is a reduction of irradiation time required for achievement of therapeutic effect.

Design, synthesis, optical properties and implementation of these new photosensitizer systems in PDT will be discussed.

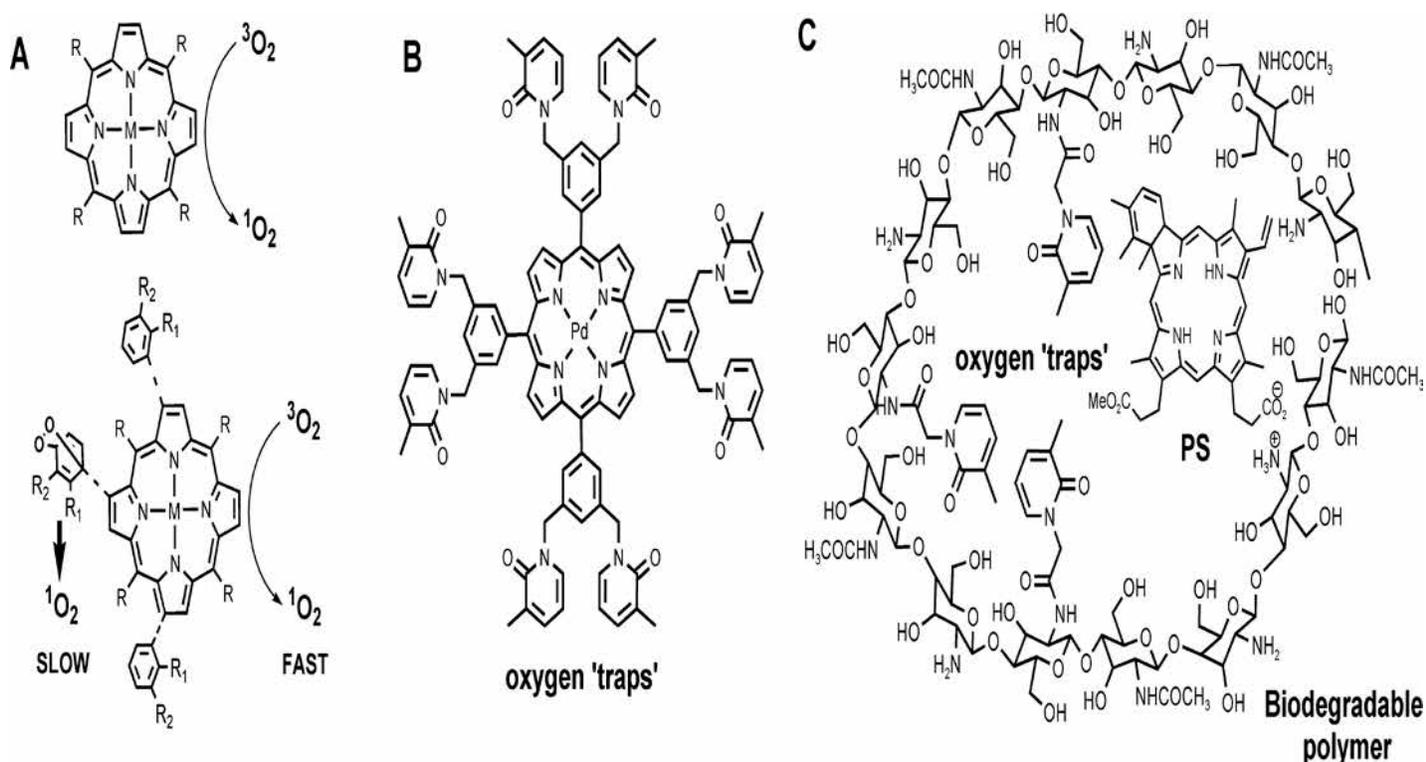


Figure 1. A) Conventional PDT compared with new approach, B) an example of the developed PS containing oxygen traps, C) Biopolymer containing oxygen traps in combination with PS.

MEDI 415

Substituted acylsulfonamides as surrogates of a terminal carboxylic acid: More effective small-molecule blockade of the Mcl-1 oncoprotein

Maryanna E. Lanning¹, mlanning@umaryland.edu, **Steven Fletcher**². (1) *Pharmaceutical Sciences, University of Maryland Baltimore, Baltimore, Maryland, United States* (2) *Dept of Pharmaceutical Sciences, University of Maryland School of Pharmacy, Baltimore, Maryland, United States*

The dysregulation of the anti-apoptotic Bcl-2 proteins, particularly Bcl-2, Bcl-x_L, and Mcl-1, leads to the evasion of apoptosis, which is a hallmark of cancer. In addition to its direct role in promoting tumor growth in a variety of cancers that include acute myeloid leukemia and multiple myeloma, the over-expression of Mcl-1 has been associated with resistance to current chemotherapies. Mcl-1's immortalizing role is manifested through the sequestration of the Bcl-2 pro-apoptotic proteins, such as Bim and Noxa, through their α -helical BH3 domains. This occurs through a hydrophobic groove comprising four binding pockets termed p1 through p4 that recognize four conserved hydrophobic residues projected from one face of the BH3 α -helix, along with a conserved arginine, R263 (Mcl-1) that engages a conserved aspartate on the opposing face of the helix. Recently, we reported on *N*-substituted 1-hydroxy-4-sulfamoyl-2-naphthoic acids as selective, nanomolar inhibitors of Mcl-1. Molecular modeling suggested that only the p2 pocket is engaged by the inhibitors. It is hypothesized that even greater potencies will be realized through the replacement of the carboxylic acid with various acylsulfonamides whose introduced moieties will extend into the p4 pocket. Moreover, this bioisosteric replacement may promote cell penetration and reduce serum binding that is typical of lipophilic inhibitors bearing acidic groups. Our efforts toward this endeavor will be presented along with comprehensive structure activity relationship studies.

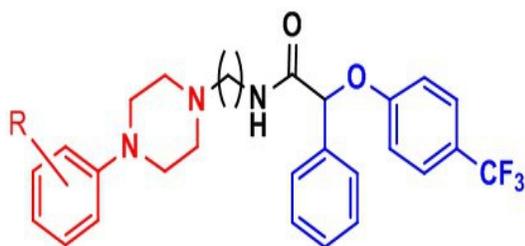
MEDI 416

Novel class of substituted phenoxyacetamide derivatives as serotonin reuptake inhibitors and serotonin autoreceptor antagonists for repetitive behavior modulation in autism spectrum disorder

Victoria M. Gancarczyk¹, Jyothi Dhuguru¹, Ashraf Khaliq², Ola M. Ghoneim¹, ola.ghoneim11@gmail.com. (1) Pharmaceutical Sciences, University of Saint Joseph-School of Pharmacy, Hartford, Connecticut, United States (2) College of Pharmacy, Qatar University, Doha, Qatar

Despite the alarming prevalence rate of 1 in 68 children diagnosed with Autism Spectrum Disorder, only a handful of drugs have been approved by FDA so far. Repetitive behaviors represent one of the main core domains characteristic of Autism Spectrum Disorder. Selective Serotonin Reuptake Inhibitors (SSRIs) are currently the off-label drug of choice for repetitive behaviors. The time taken (4-6 weeks) for SSRIs to become therapeutically efficient can be attributed to the negative feedback inhibition exerted by serotonin autoreceptors.

We designed and synthesized a series of novel hybrid ligands that can act as serotonin autoreceptors antagonists (SAAs) while maintaining the inhibition to the serotonin reuptake. Preliminary results in our lab showed promising lead compounds with our latest series of substituted phenoxyacetamide derivatives. This series is capable of acting as SSRIs and SAAs when tested at the 5-HT uptake inhibition, and the 5-HT_{1B}, 5-HT_{1D} antagonist assays. The design, synthesis of five novel compounds, and their inhibition concentration (IC₅₀) at desired targets will be presented.



MEDI 417

Polymersomes for targeting and eradicating intracellular parasites

Loris Rizzello¹, l.rizzello@ucl.ac.uk, James Robertson², Philip M. Elks², Timothy McHugh³, Stephen A. Renshaw², Giuseppe Battaglia¹. (1) Chemistry, University College London, London, United Kingdom (2) University of Sheffield, Sheffield, United Kingdom (3) Medical Microbiology, University College London, London, United Kingdom

The treatment of intracellular pathogens, such as *Mycobacterium tuberculosis*, represents a global health challenge. These microorganisms are able to survive within immune cells, exploiting them to evade host killing. In this work, we demonstrate that pH-sensitive PMPC₂₅-PDPA₇₀ polymeric vesicles (polymersomes) are ideal candidates for targeting macrophages containing viable intracellular bacteria. In particular, both *in vitro* and *in vivo* experiments confirmed that polymersomes are taken-up by macrophages within minutes of injection. Antimicrobial-loaded nanovesicles were able to significantly reduce and, in certain conditions, eradicate the proliferation of *Staphylococcus aureus*, *Mycobacterium bovis* (Bacillus Calmette-Guérin - BCG), and *Mycobacterium tuberculosis*. Co-localisation image analyses confirmed that our polymeric nano-capsules accumulate within the same intracellular compartment as the pathogens, thus locally enhancing the final dose and effect.

MEDI 418

Two-in-one approach to modulate repetitive behaviors in autism spectrum disorder: N-arylpieprazines as key motifs towards developing bi-functional serotonergic ligands

Ola M. Ghoneim, ola.ghoneim11@gmail.com. Pharmaceutical Sciences, University of Saint Joseph-School of Pharmacy, Hartford, Connecticut, United States

The Diagnostic and Statistical Manual of Mental Disorders (DSM-5) defines Autism Spectrum Disorder with two main domains: communication and language deficits, and repetitive behaviors and restricted interests. The frequency and degree of severity of these repetitive behaviors vary from one individual to another and negatively affect the individual's social and educational experience, and impair the quality of life of the individual and the community as a whole.

Increasing the level of serotonin in the synaptic cleft is capable of relieving/modulating some of these disruptive behaviors via an unknown mechanism. As such, Selective Serotonin Reuptake Inhibitors (SSRIs), a drug class that block the uptake of serotonin, would be an ideal choice. However, the elevated level of serotonin (by SSRIs) also stimulates inhibitory autoreceptors to exert a negative feedback inhibition, and counteract the action of SSRIs. Therefore, it takes 4-6 weeks to SSRIs to become therapeutically efficient, which might be attributed to the time needed for serotonin autoreceptor to be desensitized. Acute blockade of autoreceptors eliminates their negative feedback effect and increases the efficiency of SSRIs.

This established synergistic activity motivated us to incorporate SSRIs and autoreceptor antagonists in a series of bi-functional 'hybrids' to achieve rapid and consistent increase in serotonin concentration and a corresponding quick control over the disruptive repetitive symptoms. Two series of compounds have been designed in our lab to test the two in one approach: fluoxetine derived series and sertraline derived series. We use the tethering technique in our design, and all of our compounds include N-arylpiperazines as a common motif. Of the more than 25 new compounds that have been synthesized and tested, 2 molecules displayed 100% inhibition at both serotonergic targets. The detailed synthetic scheme of both series, the corresponding biological data including the inhibition concentration (IC50), and a comparison of the two series will be presented.

MEDI 419

Zinc(II)-dipicolylamine coordination complexes are strongly active against cutaneous leishmaniasis

Maria Betancourt¹, mbetanco@nd.edu, Douglas Rice¹, Paola Vacchina², Brianna Norris-Mullins², Miguel A. Morales², Bradley D. Smith¹. (1) Chemistry and Biochemistry, University of Notre Dame, Notre Dame, Indiana, United States (2) Biological Sciences, Eck Institute for Global Health, Notre Dame, Indiana, United States

Cutaneous leishmaniasis is a neglected tropical disease caused by a protozoan parasite that produces lesions of the skin and mucous membranes. Standard treatment involves intravenous pentavalent antimonials which are highly toxic drugs and incidents of relapse are increasing, forcing the use of more expensive agents including amphotericin B, isethionate pentamidine, paromomycin or miltefosine. These second-line drugs also exhibit high toxicity and many are accompanied by the emergence of drug resistance. It is clear that the existing therapies for leishmaniasis are inadequate, and new cost-effective drugs are needed. A series of zinc(II)-dipicolylamine complexes (ZnDPA) were prepared and found to exhibit selective toxicity against *Leishmania major* axenic promastigotes, with 50% effective concentration values in the range of 12.7 to 0.3 μ M. Confocal microscopy of the a fluorescent ZnDPA (mSeek) probe revealed a strong distribution through the cytoplasm. One of the ZnDPA complexes was tested for activity against cutaneous leishmaniasis in a mouse footpad infection model. Lesion progression was monitored by imaging the red fluorescence emission of mCherry-L.

major amastigotes. The parasite burden was 70% less in the cohort treated with five foot-pad injections of ZnDPA (0.1 mg/kg) compared to saline-treated animals (Fig. 1A). As shown in Fig. 1B, antimonial treated footpads (also five foot-pad injections) displayed cutaneous necrosis and scabbing, whereas, footpads treated with ZnDPA exhibited no apparent cutaneous reaction. These results demonstrate that ZnDPA coordination complexes are a promising new class of potent antileishmanial agents with potential for clinical translation.

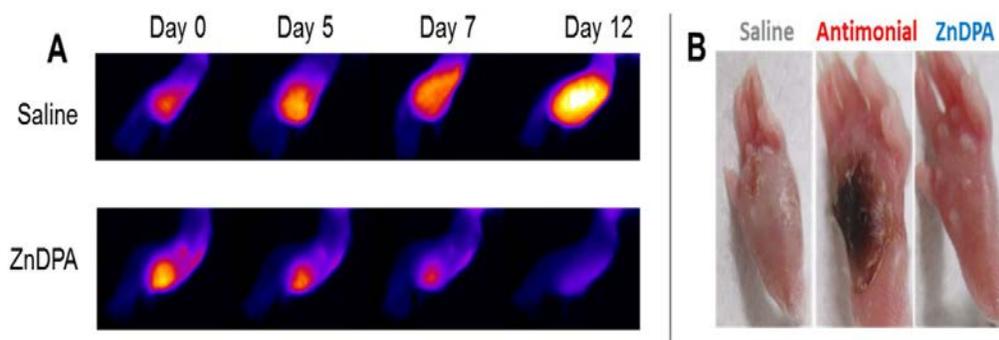


Fig 1. [A] Representative red fluorescence intensity images of BALB/c mouse footpads after inoculation with mCherry-*L. major* promastigotes (10^8) followed by treatment with five foot-pad injections per week of saline (top) or ZnDPA (0.1 mg/kg, bottom). [B] Representative photographs of mouse footpads after 12 days of different treatments.

MEDI 420

Peptide-based nanosponges

Stefan H. Bossmann¹, sbossman@ksu.edu, **Hongwang Wang**¹, **Asanka S. Yapa**¹, **Sebastian O. Wendel**², **Nilusha Kariyawasam**¹, **Tej B. Shrestha**², **Marla Pyle**², **Paul E. Smith**¹, **Deryl L. Troyer**². (1) Chemistry, Kansas State University, Manhattan, Kansas, United States (2) Anatomy&Physiology, Kansas State University, Manhattan, Kansas, United States

We have developed peptide-based nanosponges, which are spontaneously formed by mixtures of multiple lysine- (arginine) and aspartate-containing peptide sequences, which were tethered to trigonal linkers via Michael additions. We have studied the formation of these nanosponges by dynamic light scattering/zeta potential measurements, AFM, and TEM. We have utilized the nanosponges to synthesize novel delivery vehicles to deliver perillyl alcohol to tumors. Perillyl alcohol is a promising anticancer agent especially against Glioblastoma multiforme, one of the most common and deadliest forms of cancer. These nanosponges were not only effective against glioblastoma cell cultures (GL26), but also against melanoma (B16F10) and metastasizing breast cancer cells (4T1). The nanosponges are opened up by the effector caspases 3, 6, and 7. Their consensus sequence DE-VD-GC is built in the peptide sequences of the nanosponges. Perillyl alcohol is released by either protease

activity or acid catalyzed ester bond cleavage, taking advantage of the lower interstitial pH in numerous solid tumors and their cell cultures.

MEDI 421

Heterocyclic mimetics of crinine alkaloids – Novel scaffolds against drug-resistant cancer cells

Liliya V. Frolova, lilfrolova@gmail.com. Chemistry, New Mexico Institute of Mining and Technology, Socorro, New Mexico, United States

Engagement of apoptosis is a common mechanism of action of the current, clinically-used anticancer drugs; unfortunately, resistance to apoptosis and (multi) drug resistance (MDR) evolves with unacceptable frequency. Crinine alkaloids have been shown to express selective cytotoxicity against tumor cells with little toxicity against normal cell lines, are toxic against cancer cells otherwise resistant to apoptosis. Taking advantage of a concise biomimetic route to the crinine skeleton, a collection of crinine analogues were synthetically prepared and evaluated against cancer cells. The compounds exhibited single-digit micromolar activities and retained this activity in a variety of drug-resistant cancer cell cultures. Also it was shown that heterocyclic mimetic, similar to natural crinine alkaloids, inhibit protein synthesis in cancer cells.

MEDI 422

Characterization of histone lysine methyltransferase and discovery of NSD2 inhibitors

Jacek Kwiatkowski¹, jmkwiatkowski@etc.a-star.edu.sg, Alvin Hung¹, Yvonne Tan¹, Nur Huda Ahmad¹, Grace Lin¹, Anna Ngo¹, Yan Li¹, Hui Qi Ng¹, John Wee¹, Xiaoying Koh-Stenta¹, Perlyn Z. Kwek¹, Esther H. Ong¹, Joma K. Joy¹, Anders Poulsen¹, CongBao Kang¹, Jeffrey Hill¹, Thomas H. Keller². (1) Experimental Therapeutics Centre, Biomedical Sciences Institute, A-Star, Singapore, Singapore (2) Experimental Therapeutics Center, Singapore, Singapore

Methylation of lysine 36 on histone H3 (H3K36me2) by histone lysine methyltransferase (NSD2) constitutes one of the major chromatin regulatory mechanisms. Overexpression of NSD2 has been genetically linked to multiple myeloma in various cancers. Despite these findings, no drug candidate has been developed to date.

Our computational analysis of homology model of NSD2 revealed two druggable binding sites – the S-adenosyl-methionine (SAM) and substrate binding pockets. Further biochemical and biophysical characterization of the SAM binding to NSD2 was performed. Subsequent efforts towards developing inhibitors of NSD2 led to the discovery of ligands binding in the low micromolar range. The structure-activity-relationship, as well as proposed binding site and mode will be detailed during presentation.

MEDI 423

Computational rationalisation of ligand specific T-cell activation by the lipid presenting proteins CD1b and CD1c: Different means to the same end?

Christopher Cave-Ayland², *c.i.cave-ayland@soton.ac.uk*, Andrew Chancellor¹, Ivo Tews¹, Salah Mansour¹, Chris-Kriton Skylaris¹, Jonathan W. Essex¹. (1) University of Southampton, Southampton, United Kingdom (2) School of Chemistry, University of Southampton, Southampton, United Kingdom

The CD1 glycoprotein family plays an important role in immune function parallel to that of the peptide presenting major histocompatibility complex but with a focus on antigen presentation of lipid molecules. A structurally diverse range of lipids, including both self and bacterial lipids, are presented by different CD1 isoforms through variations in their intricate and interlinked binding pockets. In this presentation we focus on our recent simulation work exploring at a molecular level the antigen presentation behaviour of CD1b and CD1c. In particular we seek to determine how lipid structure influences T-cell activation, thereby opening the door to the design of novel therapeutics.

Mycolic acids are found in the cell walls of Tuberculosis bacterium (and other members of the mycolata taxon) and are known ligands of CD1b. They demonstrate diversity primarily through changes in hydrocarbon chain length as well as through variation of chemical substituents at multiple positions of the chain. Recent experimental data from Southampton has shown marked differences in T-cell activation based on the position and nature of the chemical substituents of the Mycolic acid long chain. Owing to their considerable size and flexibility, these molecules make extremely challenging targets for study with conventional computational chemistry approaches. We report a comprehensive series of Molecular Dynamics simulations which relate differing ligand activities to structural features of the CD1b binding pocket and a novel ligand binding pose. This provides a molecular-level interpretation for the T-cell response.

In a previous crystal structure CD1c has been shown to bind mannosyl- β 1-phosphomycoketide. We recently solved and published a new crystal structure bound with short spacer lipids and showing a distinct arrangement of the binding site roof structure. Subsequent exploratory simulation work was able to demonstrate a novel mechanism whereby loading of the CD1c binding pocket with ligands of different physicochemical character directly influenced the roof structure of the pocket and consequently the T-cell binding surface.

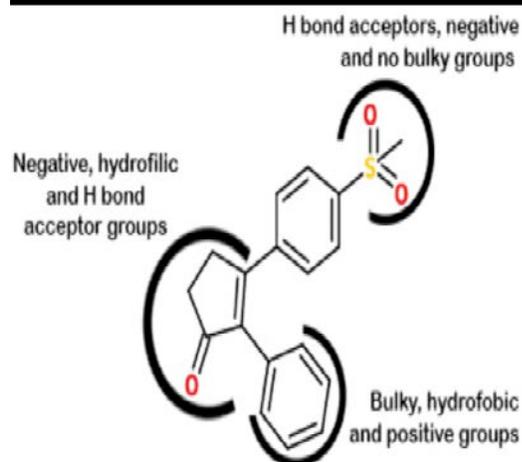
In our work we have successfully demonstrated the value of computer simulation to complement experimental work and explore how bound ligands can influence T-cell activation in CD1 systems. Two very different mechanisms have been identified, one involving ligand chain localisation in CD1b, the other ligand induced binding site restructuring in CD1c.

MEDI 424

Pharmacophore construction of Cyclooxygenase-2 (COX-2) selective inhibitors based on QSAR models

Renan A. Gomes¹, Giovani L. Luiz Genesi¹, Vinicius G. Maltarollo^{1,2}, **Gustavo H. Trossini¹**, *trossini@usp.br*. (1) Faculdade de Ciências Farmacêuticas, Sao Paulo -SP, Brazil (2) Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil

The enzyme cyclooxygenase (COX) has two known isoforms in the human body, COX-1 and COX-2. The inhibition of COX-2 has been shown effective in the reduction of inflammatory processes. From this fact we should realize the clinical relevance of COX-2 inhibitors, since it is possible to relate it to all diseases that result in inflammatory processes in the human body. In this context, the objective of the study was to identify the structures with high influence on the biological activity of selective COX-2 inhibitors by drug design strategies. Quantitative structure-activity relationship (QSAR) studies – HQSAR, CoMFA and CoMSIA, were used for determining physicochemical characteristics (stereochemical and electrostatic fields and hydrogen bonds acceptance) which contributed for the activity of 59 selective inhibitors of COX-2. Measures of IC₅₀ were used as the activity endpoint. HQSAR, CoMFA and CoMSIA models were generated and externally validated employing the test set (HQSAR $q^2 = 0.773$, CoMFA $q^2 = 0.649$ and CoMSIA $q^2 = 0.698$). The benzenesulfonyl group, the central five-membered ring containing heteroatom, functional group or hydrogen bond acceptor substituent, and a third benzene ring with a small substituent group in para position were essential to the inhibition of COX-2, contributing positively with stereochemical fields, electrostatic or hydrogen bonds acceptance. 3D and 2D pharmacophore models were designed using the structural characteristics suggested by QSAR studies and will be used in the virtual screening of the new COX-2 selective inhibitors.



MEDI 425

Perturbing dissimilar biomolecular targets from natural product scaffolds and focused chemical decoration

John Nielsen¹, john.nielsen@sund.ku.dk, **Truong Thanh Tung**¹, truong.tung@sund.ku.dk, Tim Holm Jakobsen², Trong Tuan Dao¹, Anja T. Fuglsang³, Michael Givskov², Søren B. Christensen¹. (1) Department of Drug Design and Pharmacology, University of Copenhagen, Copenhagen, Denmark (2) Immunology and Microbiology, University of Copenhagen, Copenhagen, Denmark (3) Plant and Environmental Sciences, University of Copenhagen, Copenhagen, Denmark

Fungal plasma membrane H⁺-ATPase (Pma1) has recently emerged as a potential target for the discovery of new antifungal agents. This p-type pump plays a pivotal role in many physiological functions and processes inside the cell. Therefore, inhibition of Pma1 could lead to discovery of new antifungal agents. On first attempt, by screening natural product sources we have successfully discovered that curcuminoids as potent inhibitors of p-type ATPases from diverse kingdoms of life including Pma1. On other attempt, the fungal metabolite fusaric acid was reported to reduce stomatal conductance in banana plants infected by *Fusarium spp.* suggesting that the agent might stimulate the H⁺-ATPase. The possibilities that fusaric acid could affect the H⁺-ATPase inspired us to design and synthesize a focused library of structural analogues. However, a number of bioassays revealed no significant effect on the plasma membrane proton pump. To our delight, we took notice of the structure of fusaric acid being homologous to the gram-negative quorum sensing (QS) signal molecules and to some reported quorum sensing inhibitors (QSI). This encouraged us to test the QS inhibitory activity of the fusaric acid library in three cell-based biological screens. Consequently, we identified several compounds showing good QSI activity and a structure-activity relationship has been established. Herein, we present our story from natural product scaffolds to macromolecular biological target via focused chemical synthesis.

MEDI 426

Design of novel GPCR family-targeted scaffolds: Synthetic and cheminformatic exploration of novel medicinal chemistry space

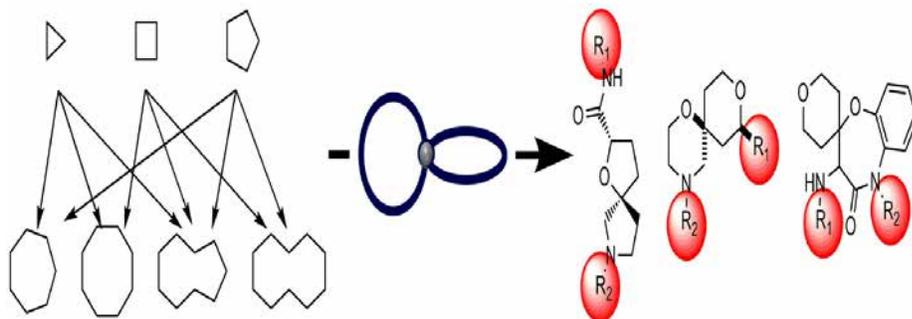
Jorg C. Benningshof², jorg.benningshof@mercachem.com, Gerhard Müller⁴, Tim Berkenbosch⁴, Dagmar Stumpfe¹, Jurgen Bajorath³. (1) B-it, University of Bonn, Bonn, Germany (2) Parallel Chemistry, Mercachem, Nijmegen, Netherlands (3) Life Science Informatics, University of Bonn, B-IT, Bonn, Germany (4) Medicinal Chemistry, Mercachem, Nijmegen, Netherlands

Matching the synthetically accessible chemical space with disease-related biological target space is one of the core activities of current medicinal chemistry. The content of today's compound collections is a reflection of the target families that have been

addressed in the past, and chemical libraries are a reflection of the number and type of chemical reactions we can pursue in e.g. a 2 week chemistry/biology cycle time typically embedded in lead finding campaigns. Hence, there remains a substantial risk that currently populated compound space might not match with the areas of biological target space the pharmaceutical industry will have to focus on in the near future. However, chemical complexity, associated with synthetic challenges prevented medicinal chemists from a systematic exploration of e.g. natural product-related compound space over the last two decades, despite the obvious structural complementarity of natural product-derived analogues to main stream libraries.

Within our design and synthesis work we embark into a systematic exploration of fused, bridged, and spiro-cyclic systems in which a smaller ring (3 to 7 skeleton atoms) is associated with a medium-sized ring (7 to 12 skeleton atoms, figure 1). We will elaborate on the results of a systematic cheminformatics and data mining analysis of the charted bioactive compound space, followed by structure-based designs of novel, thus patentable bicyclic ring topologies. Subsequent synthetic feasibility considerations are then fueling chemical validation of new bicyclic ring systems that qualify as scaffolds for 2D and 3D library expansion.

In pursuit of this concept, we try to achieve an optimal balance between novelty on one hand, and proximity to bioactive compound space, i.e. resemblance of peptide secondary structure elements, and increased 3D skeletal complexity on the other hand. We consider this as a significant contribution to unlock the chemical accessible bicyclic ring system space that is often inaccessible in lead finding and lead optimization campaigns due to the underlying chemical complexity.



Schematic illustration of the design principles for spiro fused bicyclic topologies

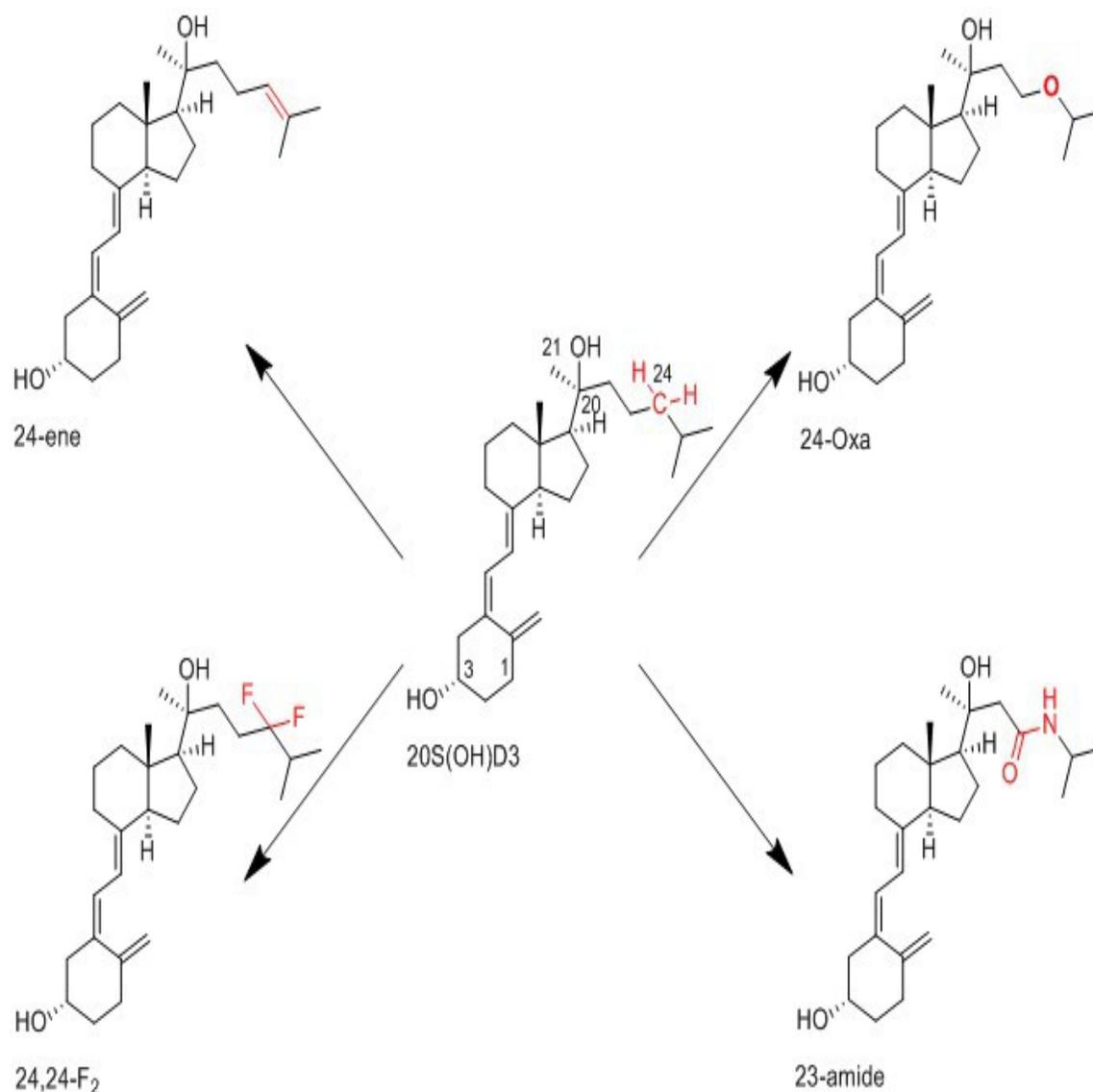
MEDI 427

Synthesis and biological evaluation of C24 20S(OH)D3 analogs as anti-inflammatory agents

Zongtao Lin¹, lin2510.com@163.com, **Srinivasa Marepally**¹, **Emily Goh**³, **Chloe Y. Cheng**³, **Arnold E. Postlethwaite**^{4,5}, **Zorica Janjetovic**⁶, **Tae-Kang Kim**⁶, **Andrzej J. Slominski**^{6,7}, **Robert C. Tuckey**³, **Natacha Rochef**², **Duane D. Miller**¹, **Wei Li**¹. (1)

Department of Pharmaceutical Sciences, University of Tennessee Health Science Center, Memphis, Tennessee, United States (2) Department of Integrative Structural Biology, IGBMC, Illkirch, France (3) School of Chemistry and Biochemistry, University of Western Australia, Crawley, Western Australia, Australia (4) Department of Medicine, University of Tennessee Health Science Center, Memphis, Tennessee, United States (5) Department of Veterans Affairs Medical Center, Memphis, Tennessee, United States (6) Department of Dermatology and Pathology, University of Alabama at Birmingham, Birmingham, Alabama, United States (7) Department of Veterans Affairs Medical Center, Birmingham, Alabama, United States

Anti-inflammatory 20S-hydroxyvitamin D3 [20S(OH)D3], the major CYP11A1 product of vitamin D3, effectively suppressed the collagen-induced arthritis at 2 µg/kg. It is not hypercalcemic (toxic) up to 60 µg/kg in mice, suggesting its potential as a lead compound for the development of anti-inflammatory agents. In this study, four C24 analogs (24-ene, 24-oxa, 24,24-F2 and 23-amide) of 20S(OH)D3 were chemically synthesized and comprehensively tested against different activities. Metabolism of these analogs against CYP27B1 (activation enzyme) and CYP24A1 (catabolism enzyme) *in vitro* were compared with that of 20S(OH)D3. Results suggested that they are better substrate of CYP27B1 than 20S(OH)D3 and can be activated (1 α -hydroxylated) by CYP27B1 except 23-amide which is not a substrate but an inhibitor of CYP27B1. Their 1 α OH derivatives were potent VDR agonists comparable with 1,25(OH)2D3 although they themselves showed weak or none VDR stimulation activity in three cell lines (Jurkat, HaCaT and Caco2). To understand the molecular interactions between these 20S(OH)D3 analogs and the VDR, 24-ene and 23-amide together with 20S(OH)D3, 1,20S(OH)2D3 and 1,25(OH)2D3 were used to produce co-crystals with human VDR. These analogs (with or without 1 α OH moiety) were able to upregulate the mRNA expression of VDR downstream genes including CYP24A1, VDR and TRPV6, suggesting their mechanism of action through VDR, at least partially. In addition, their anti-inflammatory activities have been investigated by measuring their inhibitory effects against INF γ production in splenocytes. This study is of great importance for understanding the differential mechanisms of action of vitamin D3 analogs.



Structures of C24 20S(OH)D3 analogs.

MEDI 428

Multi-omic approach to unraveling the microbial ecology of *Euphorbia* plant latex

Manjula Gunawardana¹, Embriette Hyde², Sofia Rivera¹, Simon Webster¹, Amalia Castonguay¹, Mackenzie Anderson¹, Sean Lahmeyer³, Brian Dorsey³, Taylor La Val³, David VanderVelde¹, Paul Webster¹, Rob Knight², **Marc M. Baum**¹, m.baum@oak-crest.org. (1) Chemistry, Oak Crest Institute of Science, Monrovia, California, United States (2) Pediatrics, University of California, San Diego, San Diego, California, United States (3) The Huntington Library, Art Collections, and Botanical Gardens, San Marino, California, United States

The milky white sap, or latex, that exudes from cut or bruised surfaces of *Euphorbia* plants is often poisonous, or at least highly irritant, and is rich in secondary metabolites. Genomic microbial DNA was isolated from latex collected aseptically from nearly 40 *Euphorbia* species. Deep sequencing of bar-coded amplicons from taxonomically informative gene fragments was used to measure bacterial and fungal species richness, evenness, and composition and revealed unexpected complexity in the latex microbiomes. Metabolomic analysis of paired samples using several complementary targeted and untargeted approaches was employed to establish host-microbiome relationships. Our results suggest that *Euphorbia* plant latex, a putatively hostile antimicrobial environment, unexpectedly supports complex microbial ecosystems that could hold important implications for the discovery of new antimicrobial agents.

MEDI 429

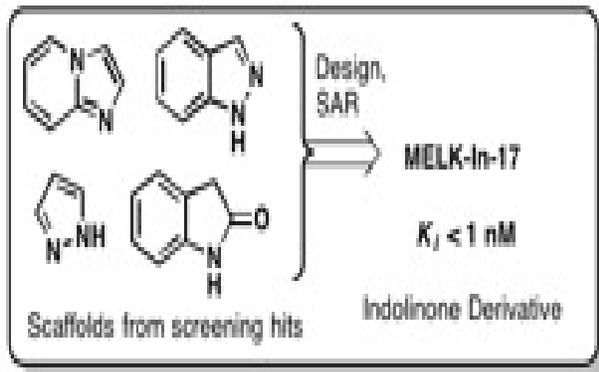
Discovery of novel indolinone derivatives as potent MELK inhibitors

Ramakrishna Edupuganti^{1,3}, ramedupuganti@utexas.edu, Juliana M. Taliaferro¹, Qiantao Wang¹, Xuemei Xie², Eun J. Cho³, Vidhu Sharma², Pengyu Ren⁴, Chandra Bartholomeusz², Eric V. Anslyn⁵, Kevin N. Dalby^{1,3}. (1) Division of Chemical Biology & Medicinal Chemistry, The University of Texas at Austin, Austin, Texas, United States (2) Department of Breast Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, Texas, United States (3) The Targeted Drug Discovery and Development Program, University of Texas at Austin, Austin, Texas, United States (4) Dept of Biomedical Eng, University of Texas Austin, Austin, Texas, United States (5) Chemistry, The University of Texas at Austin, Austin, Texas, United States

Maternal embryonic leucine zipper kinase (MELK) is highly expressed in various types of intractable malignancies, including glioblastoma multiforme (GBM) and breast cancer, in particular triple negative breast cancer (TNBC), where level of overexpression correlates with poor prognosis and aggressive disease course. Although there are some ATP-competitive MELK inhibitors in literature, no inhibitor addressed the challenge of specificity with respect to off-target enzymes closely related to MELK and there are no FDA-approved drugs of MELK at present.

Our discovery approach for finding a novel MELK inhibitor through kinase inhibitor library screening, structure-guided design of a series of novel indolinone derivatives with subnanomolar inhibition will be presented. We identified various micro molar inhibitors that have scaffolds including imidazopyridine, indazole, pyrazole and indolinone through screening; then synthesized a library of novel indolinone derivatives by structure-guided design and discovered three tight binding inhibitors with $K_i < 1$ nM (Graphical Abstract). The most potent compound (MELK-In-17) decreases proliferation of TNBC cells expressing high levels of MELK.

Graphical Abstract



Graphical Abstract

MEDI 430

Spingosine analogues as inhibitors of Spingosine Kinase (SK1)

Adrian Cardona², Margarita Escudero-Casao², Macarena Corro², Jesus Hernandez², Yolanda Diaz², Isabel Matheu², Santiago Garcia-Vallve², Miguel Mulero², Gerard Pujadas¹, **Sergio Castillon**², sergio.castillon@urv.net. (1) Universitat Rovira i Virgili, Tarragona, Spain (2) University Rovira i Virgili, Tarragona, Spain

Sphingolipids, essential components of cell membranes, have emerged as key signaling molecules involved in the regulation of many physiological and pathophysiological functions. Thus, they play an important role in the regulation of cell proliferation, differentiation, survival, trafficking and cell death. They are interconvertible by different metabolic enzymes in a complex lipid signaling system. Modulation of this sphingolipid metabolism is a promising strategy for cancer, and many connections between cancer therapies and sphingolipid metabolism having been implemented. The regulation of sphingolipid metabolism involves deacylation of ceramide (Cer) by ceramidases to generate sphingosine (Sph) and the subsequent conversion of sphingosine to sphingosine 1-phosphate (S1P) catalyzed by sphingosine kinases (SK). The dynamic balance between ceramide and S1P signalling guides the cell toward either an apoptotic process or a survival process.

In this communication we present our recent result in the synthesis of different analogues of sphingosine and their evaluation as inhibitors of SK1.

MEDI 431

Biological screening of *Moringa oleifera* for cytotoxicity and antitumor activities

Elys P. Rodriguez², elys.rodriguez@upr.edu, Claudia A. Ospina¹. (1) Chemistry, University of Puerto Rico at Cayey, San Juan, Puerto Rico, United States (2) University of Puerto Rico at Cayey, Barranquitas, Puerto Rico, United States

Moringa oleifera, also known as the miracle tree, has a vast reputation regarding folk medicine. It is known to relieve symptoms of arthritis, cancer, cardiovascular problems, asthma and many others. In 2013, a study conducted in Ougadou, proved Moringa Oleifera leave powder can be used as a dietary supplement and can contribute against malnutrition in children and adults. Also, according to studies made between 1997 and 2014 isothiocyanites, obtained from the ethanolic Moringa Oleifera leaves extract, found to induce apoptosis in ovarian cancer cells in vitro. But, all of these findings have been through the use of polar extracts, thus leaving non-polar compounds unanalyzed. In the present study, the leaves and cortex were chemically scrutinized in the search of biologically active compounds of all sorts of polarity. The plant material was extracted in chloroform/methanol (1:1 v/v) and subjected to liquid-liquid extraction with solvents of different polarities such as hexane, chloroform and ethyl acetate. The hexane extract was analyzed by Nuclear Magnetic Resonance (NMR) and the NMR data of the extract projected a variety of intense signals of vinylics, alpha-heteroatoms, aliphatic and allylic hydrogens. The hexane extract was chromatographed with a mixture of chloroform/methanol (1:1 v/v) to yield 15 fractions. Fraction 4 was purified by column chromatography with a mixture of hexane/ethyl acetate (8:2 v/v). This fraction produced seven subfractions from which one is being currently analyzed. We evaluated the leaves crude extract against prostate, breast and ovarian cancer cell lines, exhibiting IC₅₀ values of 0.17 µg/mL, 0.25 µg/mL and 0.27 µg/mL respectively. Along with other findings, this data shows that Moringa oleifera leaves and cortex are a potent source of biologically active compounds capable of treating cancer; and is therefore necessary to isolate them.

MEDI 432

Design of a nucleic acid aptamer to achieve localization of a ROS-activated anti-cancer agent

Kaylin G. Earnest, earnesk@mail.uc.edu, Edward J. Merino. Department of Chemistry, University of Cincinnati, Cincinnati, Ohio, United States

Anticancer agents that modify DNA are a mainstay of chemotherapy regimens, but development of new classes of these agents has slowed because of the modification of DNA in non-cancerous cells, known as off-target reactivity, which gives rise to serious side effects and poor selectivity. We have designed a means to achieve specificity by translating the finding that levels of reactive oxygen species (ROS) are elevated in acute myeloid leukemia (AML) cells into a therapeutic modality termed ROS-Activated Cytotoxic DNA-modifying agents. Therefore, only upon ROS activation, DNA modification occurs. Our most effective agent, RAC1, has an IC₅₀ against AML cells of 750 nM. In addition, this compound is exquisitely specific with an order of magnitude lower potency for closely related CD34+ blood stem cells. Data indicate signs of efficacy in a realistic mouse model. This study aims to improve the selectivity and delivery by adding a specifically designed aptamer as a selective transporter. The developed aptamers are selective against AML cancer and not against normal CD34+ cord blood

cells. The aptamer is conjugated to RAC1 via a cleavable hydrolysable linker. The anticancer activity and selectivity of the aptamer conjugate has also been tested.

MEDI 433

Exploring the binding site of GPR119 receptor inverse agonists

Evangelia Kotsikorou, *evangelia.kotsikorou@utrgv.edu*, ***Sarah Kowalski***, *sarah.kowalski01@utrgv.edu*. *Chemistry Department, University of Texas Rio Grande Valley, Edinburg, Texas, United States*

GPR119 is a G protein-coupled receptor shown to be important for the treatments of Type 2 diabetes. Understanding how the receptor works is essential for drug development. One of the tools to study receptors are inverse agonists; these are compounds that bind to the receptor and prevent it from activating. To date few inverse agonists of the GPR119 receptor have been reported in the literature and it is not known how they interact with it. The goal of this project is to study the structure of two different inverse agonists and their mode of binding to the GPR119 receptor. The Schrodinger Molecular Modeling software was used to explore the conformational space of two inverse agonists, AR436352 (long and slender shape) and TM43718 (shorter and wider shape). A conformational search followed by quantum mechanical optimizations was run to locate the global energy minimum conformers of the two molecules as well as other low energy structures and to calculate the electron density distribution of the global energy minimum conformers. The electron density map of the inverse agonists was compared to the electron density map of the binding site to provide insight on how the molecules may fit inside the receptor binding pocket. The program Glide was used to dock the two molecules in the GPR119 receptor binding site. The docked complexes were minimized using a QM/MM method. The docking results indicate that the inverse agonists bind in between the transmembrane helices 2, 3, 6, and 7. One end of the molecule extends to the extracellular loop region and the other end is situated above the toggle switch amino acid W6.48 preventing it from moving. The interaction energies between the binding site amino acids and the inverse agonists were calculated. The analysis suggests that amino acids W7.39, F6.51, T3.33, M3.29, R3.28, as well as the extracellular loop 2 amino acid F157 are important for binding. Site directed mutagenesis will be used to confirm these results.

MEDI 434

Methicillin-resistant *Staphylococcus aureus* becomes vulnerable to β -lactam antibiotics when in combination with branched polyethylenimine

Melissa Foxley, *melissafoxley@ou.edu*, *Min Xiao*, *Summer Wright*, *Stoffel Strange*, *Anh Lam*, *Katherine Grogan*, *Charles V. Rice*. *University of Oklahoma, Norman, Oklahoma, United States*

Methicillin-resistant *Staphylococcus aureus* (MRSA) infections have a high mortality rate in humans due to the bacteria's resistance mechanisms that make treating infections difficult. We found that branched polyethylenimine (BPEI), a non-toxic cationic polymer, makes MRSA susceptible to β -lactam antibiotics. Checkerboard assays were used to establish synergy between BPEI and β -lactam antibiotics on MRSA and determine optimal combination concentrations. Also, checkerboard assays showed that Gram negative bacteria, methicillin-susceptible *S. aureus*, and wall teichoic acid (WTA) knockout MRSA mutants do not have synergy between BPEI and β -lactam antibiotics. Laser scanning confocal microscopy was used to show that BPEI localized on the cell wall of MRSA. Electron microscopy was used to observe altered cell morphologies. Using nuclear magnetic resonance, we found that BPEI binds to WTA thereby causing steric hindrance of the cell wall. We propose that BPEI prevents proper localization of PBP2a by binding to WTA and thereby overcoming MRSA's resistant factor.

MEDI 435

Old and new privileged scaffolds for medicinal chemistry

Petra Schneider, petra@insili.com, Gisbert Schneider. Institute of Pharmaceutical Sciences, ETH, Zurich, Switzerland

We present a comprehensive update on the concept of privileged structures in medicinal chemistry. The term "privileged scaffold" was coined in 1988 when it was discovered that benzodiazepine derivatives serve as effective ligands for a variety of different receptors even from different classes. Current medicinal chemistry efforts seem to pursue rivaling concepts of using privileged structures vs. performing rare chemistry to yield new scaffolds. We have analyzed the scaffolds of bioactive compounds annotated in ChEMBL. We obtained information on the macromolecular targets of both frequently used and underexplored scaffolds and studied their expected target promiscuity. We also attempt to provide an answer to the question whether performing unusual chemistry could deliver new target-selective scaffolds.

MEDI 436

Vienna LiverTox Workspace: Towards predicting liver toxicity

Floriane Montanari¹, floriane.montanari@univie.ac.at, Eleni Kotsampasakou¹, Bernhard Knasmüller¹, Marta Pinto^{1,2}, Melanie Grandits¹, Lars Richter¹, Gerhard F. Ecker¹. (1) Pharmaceutical Chemistry, University of Vienna, Vienna, Austria (2) Evotec, Milton, Oxfordshire, United Kingdom

Transporters expressed in the liver play a major role in drug pharmacokinetics and are key for maintaining bile flow. Inhibition of these transporters may lead to liver toxicity and drug-drug interactions. Predicting inhibition and substrate profiles of small molecules towards these transporters may help medicinal chemists to prioritize compounds in an early phase of the drug development process and thus help to avoid

developing potentially problematic compounds.

The Vienna LiverTox Workspace aims at combining a set of locally developed transporter models to allow fast and easy profiling of compounds. Furthermore, direct hepatotoxicity endpoints are predicted which aid in drawing a predicted liver toxicity picture for a given compound.

The models included in the Workspace were built on public datasets that were collected and assembled in-house at the University of Vienna. Descriptors and fingerprints were computed in RDKit and models were implemented in WEKA and scikit-learn. The models are embedded into the eTOXlab framework (<https://github.com/manuelpastor/eTOXlab>), which allows for chemical structure cleaning and maintenance of the models. New models developed in the future can easily be integrated in the Workspace to broaden its capabilities. Furthermore, new data can be used to retrain and improve the predictivity of existing models.

The Workspace allows computing probabilities for a compound to be an inhibitor or a substrate of a considerable number of liver transporters, such as ABCB1, ABCG2, ABCB11, ABCC1, ABCC2, OATP1B1, OATP1B3, as well as its risk of causing hyperbilirubinemia, cholestasis, and drug-induced liver injury. It is available as a small web application free of charge for non-commercial use.

MEDI 437

Pyrrole-based antitubulin agents at the colchicine site: SAR of C-5 analogues in explicitly solvated models

Ahmad J. Obaidullah^{2,3}, obaidullaha@vcu.edu, Cristina C. Rohena⁴, James A. Sikorski⁵, Susan Mooberry⁴, John T. Gupton¹, Glen E. Kellogg^{2,3}. (1) Department of Chemistry, University of Richmond, Richmond, Virginia, United States (2) Department of Medicinal Chemistry, Virginia Commonwealth University, Richmond, Virginia, United States (3) Institute of Structural Biology, Drug Discovery and Development, Virginia Commonwealth University, Richmond, Virginia, United States (4) Department of Pharmacology and Cancer Therapy & Research Center, University of Texas Health Science Center at San Antonio, San Antonio, Texas, United States (5) Medicinal Chemistry & Drug Discovery, Chesterfield, Missouri, United States

Microtubules have been known as a target for anticancer drugs for several decades. We have been investigating pyrrole-based antitubulin agents as destabilizing agents that target the tubulin colchicine-binding site and thus affect the microtubule stability during mitosis division. In the past decade, a series of pyrrole compounds related to 3,5-dibromo-4-(3,4-dimethoxyphenyl)-1H-pyrrole-2-carboxylic acid have been synthesized, biologically evaluated, and predicted by molecular modeling. Our computational tools include GOLD for docking and HINT (Hydropathic INTeraction) for scoring. The substituents at the pyrrole's C-2, C-3, and C-4 positions have previously been optimized through Structure-activity relationship (SAR)/modeling. At the C-2 position, the alkyl end

of the ester makes hydrophobic interactions while the carboxyl oxygen forms hydrogen bonds with the Val181a backbone, with ideal binding for ethyl ester. The bromine at the C-3 of pyrrole seemingly fits precisely in a small hydrophobic pocket. The microtubule depolymerization activity was optimum when an ideally oriented acceptor for Cys241b was imbedded in an otherwise hydrophobic group at the C-4 position. Our current investigations of C-5 substituents are attempting to optimize the efficacy and physiochemical properties of additional pyrrole analogues by docking them into the colchicine-binding site. Since the C-5 substituents are exposed at the site's entrance, we have solvated the molecular models of these analogues with explicit water in our efforts to establish a predictive SAR.

MEDI 438

Design, synthesis, and biological evaluation of small molecule $G\alpha 2$ -androgen inhibitors in prostate cancer therapy

Subhasish Tapadar¹, stapadar3@mail.gatech.edu, **Silvia Caggia**², **Shafiq Khan**³, **Adegboyega K. Oyelere**⁴. (1) School of Chemistry & Biochemistry, Georgia Institute of Technology, Atlanta, Georgia, United States (2) Center for Cancer Research and Therapeutic Development, Clark Atlanta University, Atlanta, Georgia, United States

Prostate cancer is very common in men and is the second leading cause of death in American men. Small molecule androgen inhibitors are among promising drug candidates for the therapy of androgen-dependent prostate cancer. We envision that conjugation of anti-androgen with $G\alpha 2$ protein inhibitor will disrupt prostate cancer development, progression, and cell migration. Moreover, these conjugates are expected to be active against both early stage hormone positive and castration-resistant prostate cancer. We have so far synthesized a small library of imine-based $G\alpha 2$ protein inhibitors and tested for migration assay in PC3 cell lines. Efforts are on the way to append some of the lead candidates to androgen inhibitors.

MEDI 439

Synthesis of GRB2 SH2 domain inhibitors: Analogues of sclerotiorin

Joshua J. Gladfelder, joshuag1248@gmail.com, **Carolynn Arpin**. Chemistry and Biochemistry, Chico State University of California, Chico, California, United States

GRB2 (Growth Factor Receptor-Bound protein 2) is known to be a critical downstream intermediary in several oncogenic signaling pathways. Significantly, the GRB2 homodimer was recently found to play a major role in the protein tyrosine kinase signaling of these oncogenic pathways. A peptidomimetic antagonist of the GRB2 SH2 domain (Src Homology 2), which has been shown to exhibit high affinity through in vitro studies, was tested on K562 leukemia cells with GRB2 overexpression. Unfortunately, no inhibition of protein activity was observed due to restricted cellular uptake resulting from the polar molecular motif. Thus, we resolve to create a library of compounds based

on the natural product (+)-8-O-methylsclerotiorinamine, which has been shown to significantly inhibit binding between the GRB2 SH2 domain and the phosphopeptide derived from the Shc protein. The motivation, design, and synthesis of our library of antagonists will be presented, along with preliminary biological evaluation.

MEDI 440

Phytoestrogens: New ligands targeting the estrogen receptor domains

Vanrajsinh J. Thakor, *vanraj7777@gmail.com*, Mallehappa Noolvi. Department of Pharmaceutical Chemistry, Shree Dhanvantary Pharmacy College, Surat, Gujarat, India

Estrogen receptor, aromatase and 17- β HSD inhibitors are main target of pharmacological interest for the treatment of estrogen dependent cancers. Proposed ligands having estrogen receptor domains and this rationale has led to work on the development of "multiple targets" class of drugs that would modulate the action of estrogens and thereby interfere with, or even prevent, the proliferation of breast and uterine cancer cells. 4-methoxy Tamoxifen was taken as standard which has shown affinity toward estrogen receptors. Thus, the PDB ID: 1QKU and 3ERT binding site is potentially a good target for new anticancer drugs that will directly inhibit metastasis. Docking studies using the structure of the 1QKU binding domain suggested that 3-methyl-2-phenyl-2,3-dihydrochromen-4-one derivatives substituted at position C-5 and C-6 could be good candidates. Series of flavone derivatives were synthesized and evaluated by Preliminary *in-vitro* cytotoxicity was checked by sulphorhodamine B assay and for promising molecule, five dose assay in prostate cancer cell line PC-3, ER negative cell line (MDA-MB 453) and determination of IC₅₀ by SRB assay. In addition, mechanistic study was done with cytometric analysis and electrophoretic determination of apoptosis. For *In-vivo* activity, evaluation of anti-tumor activity of selected synthetic compounds by Ehrlich Ascites Carcinoma (EAC) model and related studies was performed.

MEDI 441

Structure-based design of macrocyclic tetrapeptides intended to modulate the opioid receptors

Michael J. Ferracane^{2,1}, *michaelferracane@gmail.com*, Jane V. Aldrich¹. (1) Department of Medicinal Chemistry, University of Florida, Gainesville, Florida, United States (2) Department of Chemistry, University of Redlands, Redlands, California, United States

The opioid receptors (μ , δ , and κ) are important targets for the treatment of pain and potentially addiction. Traditionally, peptide-based opioid receptor ligands have had limited therapeutic utility as a result of their poor bioavailability and metabolic stability. The macrocyclic peptide (MTP) CJ-15,208 and its analogs are stable to proteases but have exhibited vastly different opioid activity *in vivo* following oral administration. We are

using molecular modeling to better understand the activity of these compounds. Here, we will describe modeling experiments intended (a) to examine possible active conformations of the MTPs, (b) to understand how the MTPs may bind to opioid receptors, (c) to explain the activity of known MTPs, and (d) to design novel MTPs with improved *in vivo* activity.

MEDI 442

MOEsaic: Making SAR analysis easier through the use of matched molecular pairs and R-group profiling

Alain Ajamian, aajamian@chemcomp.com. Chemical Computing Group, Montreal, Quebec, Canada

The ability to effectively manage the structure activity relationships (SAR) generated in a medicinal chemistry program is of paramount importance to drug discovery. This is not a trivial task as the number of synthesized molecules can grow very rapidly. Additionally, a substantial number of molecules can be routinely tested in multiple biological and physico-chemical assays, leading to the generation of hundreds to thousands of data points for each chemical series. Therefore, distilling the information to a manageable discrete set for guiding ligand design is a serious challenge. Here we describe a new web-based application which is a single framework dedicated to the analysis of SAR data and the design of novel chemical targets. The application can be used to quickly address typical medicinal chemistry workflows aimed at interrogating the SAR data through the use of filters, plots and a versatile structure visualizer. Additionally, the application can be used to smoothly navigate complex SAR data using matched molecular pairs to quickly identify the key SAR trends and investigate if SAR can be transferred between the different templates present in the dataset.

MEDI 443

In search of AKT inhibitors as anticancer agents, an *in silico* approach

Pedro J. Trejo¹, piter_jo@comunidad.unam.mx, Alicia Hernandez Campos¹, Antonio Romo-Mancillas², José L. Medina-Franco³, Rafael Castillo-Bocanegra¹. (1) Departamento de Farmacia, Universidad Nacional Autónoma de México, Mexico City DF, Mexico (2) División de Estudios de Posgrado, Universidad Autónoma de Querétaro, Santiago de Querétaro, Querétaro, Mexico (3) Departamento de Farmacia, Universidad Nacional Autónoma de México, Mexico City, Ciudad de México, Mexico

The serine-threonine kinase B (AKT or also PKB) is a key kinase in tumorigenesis since it favors tumor progression by increasing cell proliferation, angiogenesis, apoptosis prevention and drug resistance. There are three AKT isoforms reported: AKT1, AKT2 and AKT3; these isoforms are overexpressed in some types of cancer cells; however, recent research suggests that AKT isoforms are not functionally redundant; this fact

should be considered for the design of selective AKT inhibitors. Herein in this work we present an in silico approach to find potential selective inhibitors, particularly against AKT1 isoform. Using structure based-design methodology were designed 42 trisubstituted pyridines, then docking calculations were performed in Glide, AutoDock 4.2 and AutoDock Vina to find the most promising inhibitors. Molecular dynamics simulations were also performed to describe the dynamic behavior of complexes. Six molecules shown selectivity on AKT1, where the hydrophobic energy contribution together with AKT kinase domain architecture leads the selectivity.

MEDI 444

Design, synthesis and biological evaluation of Liver X Receptor (LXR) ligands

Rajesh Komati, *rkomati@xula.edu*, Kortney M. Lamark, Kourtney A. Payne, Marilyn Ndukwe, Dominick Spadoni, Jayalakshmi Sridhar, Kevin Riley. Chemistry, Xavier University of Louisiana, New Orleans, Louisiana, United States

Nuclear receptors (NRs) are one of the most abundant classes of transcriptional regulators in animals. The liver X receptors (LXRs) are NRs that act as oxysterol sensors, regulating genes involved in cholesterol and lipid metabolism. Based on the coding genes LXRs are classified as LXR α (NR1H3) and LXR β (NR1H2). LXR α is expressed most highly in the liver and to a lesser extent in the kidney, small intestine, spleen, and adrenal gland. In contrast to the restricted expression pattern of LXR α , LXR β is ubiquitously expressed. Concurrent with our increasing knowledge of the roles of LXRs in lipid homeostasis, development of selective and potent ligands of the hormone receptors has gained significant grounds toward clinical applications of LXR modulations. Here we are presenting our computational approach to develop a new series of LXR ligands based on scaffold replacement studies. We are also presenting the synthesis of a library of designed molecules and their biological evaluation studies towards LXR activity.

MEDI 445

Amido phthalimides as CDKs and VEGF inhibitors

Rajesh Komati, *rkomati@xula.edu*, Veronica C. Miles, Moamen Ismail, Faith Joseph, Harris McFerrin, Jayalakshmi Sridhar. Chemistry, Xavier University of Louisiana, New Orleans, Louisiana, United States

Angiogenesis is the process of vascular growth by sprouting from pre-existing vessels. Angiogenesis is involved in various pathological conditions such as arthritis, psoriasis, diabetic retinopathy, macular degeneration and cancer. Angiogenesis is stimulated by several growth factors, including basic fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), transforming growth factor- α , and vascular endothelial growth factor (VEGF) family. Among these, VEGF is the most potent tumor angiogenic factor, which acts on endothelial cells and plays a central role in their proliferation, migration,

and survival. VEGF is expressed abundantly in most human and animal tumors. Cyclin-dependent kinases (CDK-1,2,3,4,5,6,7,8,and 9) are serine/threonine kinases. In contrast to the cell cycle-related CDKs (e.g. Cdk1, 2, 4 or 6) which regulate the main cell cycle transitions, CDK9 forms the catalytic core of the positive transcription elongation factor b (p-TEFb), which plays a critical role in the angiogenesis. These factors led to a rigorous search for small molecules that are dual inhibitors of the VEGFR and CDK9 for therapeutic purpose. Computational molecular modeling studies conducted by our group on CDK9 and VEGF revealed that amido-phthalimide is a reputable core structure due to its ability to competitively bind CDK9 and VEGFR, in place of ATP, and thus inhibit downstream signaling of angiogenesis. Here in we are presenting the synthesis and biological studies of a library of phthalimide derivatives as CDK and VEGF inhibitors.

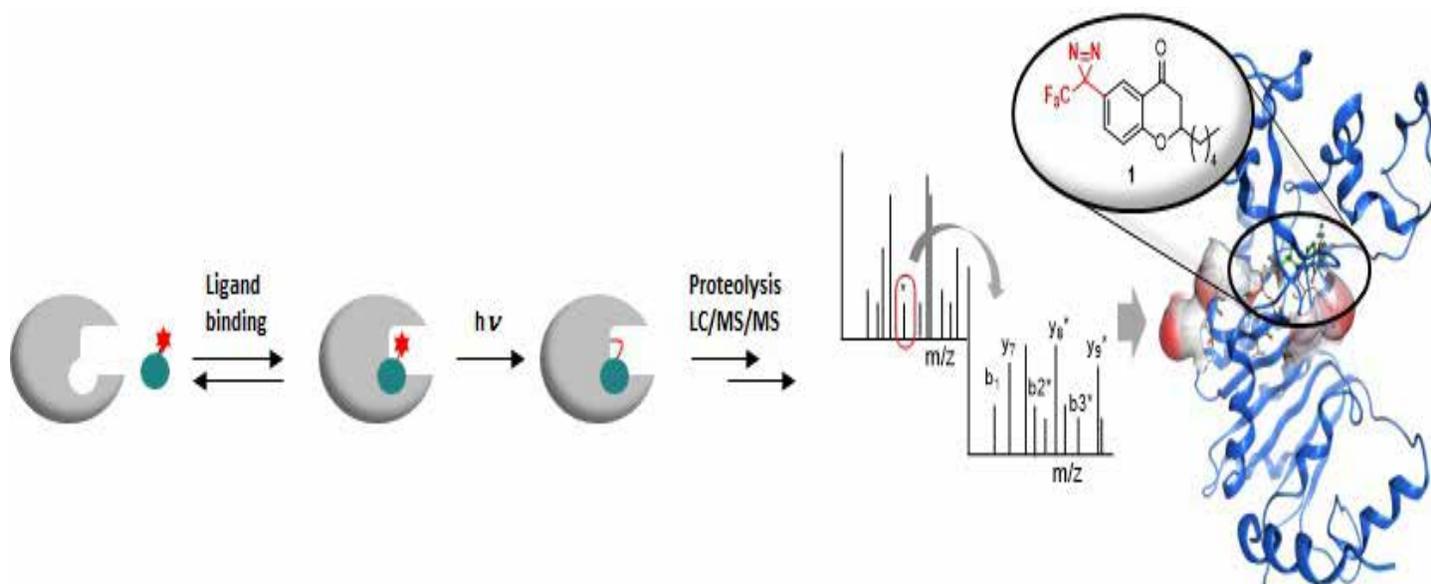
MEDI 446

Photoaffinity labeling approach towards binding site identification of chroman-4-one based sirtuin 2 inhibitors

Tina Seifert¹, tina.seifert@chem.gu.se, Marcus Malo¹, Johan Lenggqvist², Carina Sihlbom², Elina Jarho³, Kristina Luthman¹. (1) Dept of Chemistry and Molecular Biology, University of Gothenburg, Göteborg, Sweden (2) The Proteomics Core Facility, University of Gothenburg, Göteborg, Sweden (3) School of Pharmacy, University of Eastern Finland, Kuopio, Finland

Knowledge regarding the biotarget of bioactives is essential for drug discovery and in-depth understanding of the binding site is important to be able to make appropriate structural modifications to improve both potency and selectivity. Light-induced covalent cross-linking of photoactivatable ligands to biological macromolecules, known as photoaffinity labeling (PAL), is a powerful biochemical approach for locating ligand binding sites as well as target identification of bioactives.

We have used PAL to locate the binding site of our chroman-4-one based sirtuin 2 (SIRT2) selective inhibitors. We have developed two potential PAL probes containing a diazirine and azide moiety based on SAR data, which have been evaluated for their SIRT2 inhibitory activity. The photochemical properties of **1** were investigated in detail using NMR and UV/Vis spectroscopy prior to the application in cross-linking experiments. Using tandem mass spectrometry, a modified tryptic peptide arising from the covalent attachment of photoactivated **1** could be identified. The peptide sequence covers the active site of SIRT2 and the area which is proposed as the binding site of the chroman-4-one-based SIRT2 inhibitors.



Schematic illustration of a PAL approach to identify ligand binding sites.

MEDI 447

Discovery of Lu AF64196 a highly ligand efficient, brain penetrant and selective PDE1 inhibitor

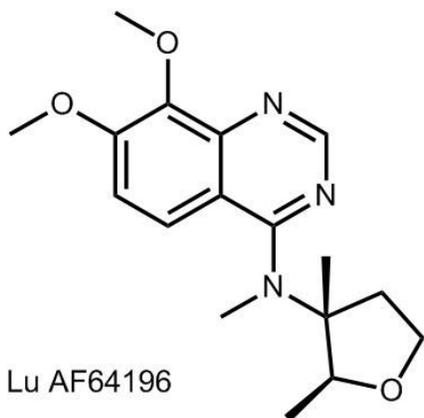
Lars K. Rasmussen², LKRA@lundbeck.com, Morten Langgard², Claus T. Christoffersen², Jacob Nielsen¹, Christoffer Bundgaard², Jan Kehler². (1) *Synaptic Transmission In Vitro*, H. Lundbeck A/S, Valby, Denmark (2) *Discovery Chemistry & DMPK*, H. Lundbeck A/S, Valby, Denmark

Phosphodiesterase 1 (PDE1) is an attractive target for the treatment of a number of psychiatric and neurological diseases.

Here we describe the discovery of the PDE1 inhibitor Lu AF64196 together with the SAR for the related class of quinazolines.

In our lead optimization Lu AF64196 was found to be highly ligand efficient and to have good properties in terms of stability, solubility and permeability. Lu AF64196 was successfully tritiated and used as a radioligand for in vitro and in vivo binding studies.

In conclusion the compound has shown an overall profile making it attractive for further studies of PDE1 in vitro and in vivo.



MEDI 448

Repurposing for G-protein couple receptors by structure-based discovery: Transformation of adenosine derivatives into 5HT_{2B}/5HT_{2C} serotonin receptor antagonists

Dilip Tosh, toshd@mail.nih.gov, Antonella Ciancetta, Eugene P. Warnick, Steven Crane, Zhan-Guo Gao, Kenneth A. Jacobson. NIDDK, Bethesda, Maryland, United States

Adenosine derivatives developed to activate adenosine receptors (ARs) revealed μM off-target activity at serotonin 5HT_{2B} and 5HT_{2C} receptors (5HTRs). We explored the SAR at 5HT₂R_s and modeled receptor interactions in order to optimize affinity and simultaneously reduce AR affinity. Depending on *N*⁶ substitution, a small 5'-alkylamide modification of ribose maintained weak 5HT_{2B}R affinity, which was enhanced upon ribose substitution with a rigid bicyclo[3.1.0]hexane (North (N)-methanocarba) moiety, i.e. *N*⁶-dicyclopropylmethyl 4'-CH₂OH derivative MRS7134 (*K*_i 11 nM). Corresponding 5'-methylamide MRS7185 was 12-fold selective for 5HT_{2B}R vs. 5HT_{2C}R. Alkyl ester MRS7221 potently antagonized 5HT₂R_s with moderate selectivity in comparison to ARs; related 6-*N,N*-dimethylamino analogue MRS7248 was 5HT₂R-selective. 5'- ω - position flexibility of substitution in (N)-methanocarba nucleosides was indicated in the docking mode at 5HT_{2C}R but not ARs. Consistent with predictions, 5'- ω -aminoalkylamides displayed moderate 5HT₂R affinity with reduced AR affinity. Thus, we have used GPCR modeling to repurpose nucleoside scaffolds in favor of binding at an off-target receptor to provide novel 5HT₂R antagonists, which can be explored for cardioprotection, liver protection or CNS activity.

MEDI 449

Discovery of highly selective Itk inhibitors with *in vivo* IL-2 inhibition

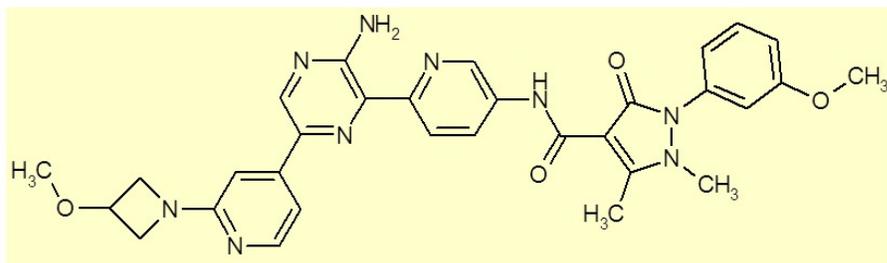
Shigeyuki Takai¹, s.takai@ono.co.jp, Hiroyuki Takeda¹, Akio Watanabe¹, Kazuma Tsuboi¹, Ryo Suzuki¹, Atsushi Hiramatsu¹, Yoko Iyoda¹, Takayuki Inukai¹, Akihiro Kinoshita¹, Hiroshi Kohno¹, Bin Liu², Rupa Shetty², Kevin Moriarty², Masakuni Kurono¹,

Shuheji Umemura¹, Hiromu Egashira¹, Jinming Zou², Zennon Konteatis², Rie Omi¹, Hari Namboodiri², Wako Sawada¹, Masayuki Murata¹, Tomoya Koike¹, Rei Komaki-Nishikawa¹, Nobumichi Yada¹, Toshio Yoshizawa¹, John McCool², Marina Bukhtiyarova², Martha Kelly², Jun Takeuchi¹. (1) ONO Pharmaceutical Co. Ltd., Osaka, Japan (2) Locus Pharmaceuticals, Inc., Blue Bell, Pennsylvania, United States

Inhibition of Itk (Interleukin-2 inducible T-cell Kinase) is related to suppress T cell differentiation, proliferation, chemotaxis and cytokine production. Itk inhibitor can be a novel drug for diseases in terms of T cell function such as inflammatory disorders. Lck is a tyrosine kinase operating upstream of Itk in the TCR cascade and its inhibition significantly affects IL-2 inhibition. We selected Lck as the most important off-target to get signal selectivity in the TCR cascade.

Boehringer Ingelheim (BI) and Nycomed reported orally potent Itk inhibitors but their kinase selectivities are moderate. To the best of our knowledge, there was no Itk inhibitor showing high selectivity and orally *in vivo* IL-2 inhibition.

Itk inhibitory activity of our hit compound (ONO-8810443) was improved by X-ray structure analysis and MD simulation. Then hinge binder replacement led to a lead compound (ONO-2120449) with a great kinase selectivity against Lck (140-fold). Further modifications identified N-[6-[3-amino-6-[2-(3-methoxyazetidino-1-yl)pyridin-4-yl]pyrazin-2-yl]pyridin-3-yl]-1-(3-methoxyphenyl)-2,3-dimethyl-5-oxopyrazole-4-carboxamide (ONO-7790500) with extremely high selectivity in a broad kinase panel (Itk IC₅₀ < 0.004 μM, Lck > 2000-fold, Jurkat T cell IC₅₀ 0.014 μM, broad kinase selectivity 3/311 kinases > 80% at 0.3 μM). In addition, it showed an excellent signal selectivity in mouse CD4⁺ T cell (αCD3/CD28 IC₅₀ 0.074 μM, PMA/Ionomycin IC₅₀ > 10 μM; αCD3/CD28 stimulates upstream of Itk in the TCR cascade while PMA/Ionomycin does downstream) and mouse *in vivo* IL-2 inhibition by oral administration. Design, synthesis and biological activity will be presented.



ONO-7790500

Itk IC₅₀ < 0.004 μM

Jurkat T cell IC₅₀ 0.014 μM

signal selectivity in mouse CD4⁺ T cell

αCD3/CD28 IC₅₀ 0.074 μM PMA/Ionomycin IC₅₀ > 10 μM

Selectivity 3/311 kinases (>80%, 0.3 μM)

including mutant kinases

@0.3 μM

>80%
Itk (96), MLK3 (90), MLK1 (86)

50-80%
TNIK (68), FLT3 (67), KIT(V560G) (61), PDGFRα(T674I) (58), HIPK4 (55), TRKA (54), HGK (52), TRKC (53), MAP4K2 (51)

< 50%



MEDI 450

Decoupling proton motive force to overcome antibacterial resistance

Joseph Buonomo, buono007@umn.edu, Courtney C. Aldrich. Medicinal Chemistry, University of Minnesota, Minneapolis, Minnesota, United States

Bacterial resistance to antibiotics is a continuing concern for clinicians around the globe. In order to overcome this problem, many have attempted to identify new chemical entities with novel mechanisms of action in an effort to combat pre-existing resistance. However, a common mechanism of resistance involves the utilization of multi-drug resistance efflux pumps, many of which are the cause clinical resistance to one or multiple classes of drugs, complicating and extending the course of treatment. These efflux pumps either utilize ATP or proton motive force (PMF) directly as a power source to remove problematic entities. Overcoming the resistance imposed by these efflux pumps would restore the action of many drugs for many pathogens. In order to overcome these multi-drug resistance efflux pumps, we've envisioned decoupling PMF to block bacterial metabolism and efflux.

We are looking into multiple metabolic poisons that disrupt PMF to recover the action of a variety of drugs in multiple pathogens including gram-negative infectious agents and

M. tuberculosis. PMF poisons, such as 2,4-dinitrophenol (DNP), can unselectively disrupt the gradient of protons over a membrane, disrupting cellular metabolism and efflux mechanisms. To overcome this limitation, we have chosen to study the effects of PMF poisons with a chemical handle which can be used to attach a targeting moiety to reduce the side-effects of the poisons. Herein we describe a limited set of PMF poisons which show synergy with FDA-approved drugs in pathogenic bacteria such as *P. aeruginosa* and *M. tuberculosis*. We will also show that β -lactam conjugates of these poisons will offer selectivity for bacteria, with β -lactamase activity, over mammalian cells using a targeted release strategy. Combining our PMF poison with known drugs has shown significant synergy with heavily resisted chemotherapeutics. *In-vitro* testing of our PMF poison in combination with tetracycline in checkerboard assays has shown fractional inhibitory concentrations (FIC) of each compound by 32-fold compared to monotherapies for *P. aeruginosa*. We will present detailed characterization of the nature of this synergy in *P. aeruginosa* and investigate other pathogens with similar efflux machinery.

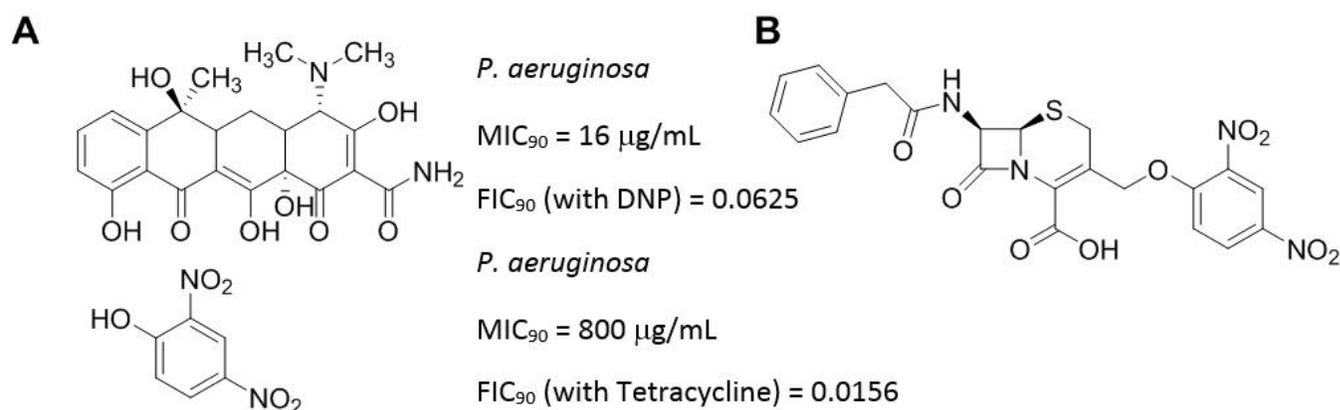


Figure 1. A) Minimum inhibitory concentrations (MIC) and fractional inhibitory concentrations (FIC) of tetracycline and 2,4-dinitrophenol in *P. aeruginosa*; B) 2,4-Dinitrophenol conjugated to a cephalosporin targeting moiety.

MEDI 451

Adverse drug reactions triggered by the common HLA-B*57:01 variant: Virtual screening of DrugBank

George Van Den Driessche¹, georgevdd@gmail.com, **Denis Fourches**^{1,2}. (1) Chemistry, North Carolina State University, Raleigh, North Carolina, United States (2) Bioinformatics Research Center, North Carolina State University, Raleigh, North Carolina, United States

Idiosyncratic adverse drug reactions (ADR) are detrimental effects patients can suffer from after taking a particular medication. Such type of immune-based ADRs are mostly triggered by drugs directly binding to human leukocyte antigen (HLA) proteins. Interestingly, the HLA-B*57:01 variant has been reported to bind with abacavir,

flucloxacillin, and pazopanib inducing either abacavir hypersensitivity syndrome (AHS) or drug induced liver injury (DILI). Recently, we developed a virtual screening model using structure-based molecular docking to forecast a drug's ability to bind with the HLA-B*57:01 variant using three X-ray crystals (PDB: 3VRI, 3VRJ, 3UPR). In this new study, we used our model to screen the DrugBank library containing over 7,000 drugs and drug candidates at the time of this study. Drugs were considered potentially HLA liable if they afforded a Glide docking score (DS) ≤ -7 kcal/mol and eModel score (eM) ≤ -50 kcal/mol. Due to the limited number of known true HLA-B*57:01 actives, two screening approaches were employed referred to as Filter 1 and Filter 2. Filter 1 identified 620 HLA-B*57:01 active compounds that were then analyzed by DS, eM, binding mode, and similarity to abacavir. From our earlier work, it was determined that the co-binding peptide had a significant impact upon a drug's ability to bind with HLA-B*57:01. In order to account for the role of peptide upon binding a second screening method was adopted: Filter 2 used the X-ray crystals 3VRI, 3VRJ, and 3UPR with the peptides P1, P2, and P3, respectively. Filter 2 identified drugs that showed activity in presence of all three peptides using both SP and XP scoring functions. Our presentation will focus on analyzing the top drugs retrieved using both Filters 1 and 2, their binding modes, and overall properties. This work is of high relevance for Precision Medicine.

MEDI 452

Optimization of 4(1H)-quinolone antimalarials for oral bioavailability and in vivo efficacy

Cynthia Lichorowic¹, *clichorowic@gmail.com*, Jordany R. Maignan², Raghupathi Neelarapu², Andrii Monastyrskii², James V. Giarrusso², Tina Mutka³, Lynn Blake³, Debora Casandra³, Alexis LaCrue³, Dennis Kyle³, Roman Manetsch¹. (1) Chemistry and Chemical Biology, Northeastern University, Boston, Massachusetts, United States (2) Chemistry, University of South Florida, Tampa, Florida, United States (3) Global Health, University of South Florida, Tampa, Florida, United States

Malaria is estimated to have caused 584,000 deaths and 198 million cases of the disease globally in 2013. Four strains of *Plasmodium* parasite cause malaria in humans and the disease is transferred by *Anopheles* mosquitos. Though mortality rates are down 47% globally since 2000 and significant progress has been made in the quest for eradication, reported occurrences of resistance against current therapeutics threatens to reverse that progress. Longstanding treatment chloroquine has seen resistance since the 1950's, with resistance becoming widespread in the 70's and 80's. In order to curb further resistance, it is essential that new antimalarial compounds be brought through the pipeline.

To further our efforts on antimalarial 4(1H)-quinolones, we have turned to 4(1H)-quinolone ICI 56,780, an antimalarial with significant potency, yet sub-par properties and bioavailability. By completing a full structure-activity and structure-property relationship study on ICI 56,780, focusing on the improvement of aqueous solubility and microsomal stability, we have ultimately revealed several frontrunner compounds with

superb *in vivo* antimalarial activity. The best compounds were found to be curative with all mice surviving a *Plasmodium berghei* infection after 30 days. Furthermore, frontrunner compounds were also shown to be mitochondrial inhibitors acting against all life cycle stages of *P. falciparum*.

The new discoveries are significant as mitochondrial inhibitors have the potential to advance the malaria elimination campaign by blocking parasite development in the blood and liver, as well as preventing transmission to mosquitoes.

MEDI 453

Design of inhibitors for the human papillomavirus E6 protein

Dino P. Petrov¹, petrov@purdue.edu, Vincent J. Davisson¹, Elliot Androphy², Anne Rietz². (1) Purdue University, W Lafayette, Indiana, United States (2) Indiana University School of Medicine, Indianapolis, Indiana, United States

The Human papillomavirus (HPV) is the leading cause of STIs in the US and a major contributor to cancer worldwide. Of the 30+ HPV types, 16 and 18 are the two most dangerous ones, causing 70% of cervical cancers. Around the globe, close to 79 million people are infected, of which 300,000 are expected to be terminal annually. The situation is particularly grim in Sub-Saharan Africa, Eastern Europe, and Latin America. China has recently joined this group, with nearly 62,000 cases diagnosed and 30,000 mortalities each year. Currently, the only FDA-approved products on the market are Gardasil and Cervarix. While effective, the vaccines are insufficient to treat patients post-infection. *Therefore, a clear and urgent need exists for easy-to-administer, low-cost therapies, capable of preventing disease progression and treating HPV-associated cancers (caused by type 16/18 infections). The goal of this project is to develop potent and selective antagonists for the HPV16-E6 protein, which is required for progression of HPV-associated malignancies.*

To date, insights into the chemical features of the binding pocket on HPV-16 E6 for the E6AP/UE3A protein (E6AP) have defined the LxxLL α -helical motif as being critical for recognition and binding. Our work has further uncovered potential involvement of several highly flexible arginine residues (R102, R129, R131), surrounding the binding pocket, in small-molecule binding by hydrogen-bonding and pi-cation interactions with the ligands. Mutational analyses and molecular dynamics (MD) simulations have shown the propensity of these residues for compensatory actions – removal of one arginine results in a rearrangement in the others to maintain ligand contact. To advance these data into novel ligand development, our research team has taken a dual approach. We have successfully tested over 2M small molecules and fragments *in silico* and arrived at a discrete set to be evaluated in our biochemical assays for HPV16-E6 binding. Further, we have developed an all-hydrocarbon stabilized peptidomimetic of the LxxLL motif, which shows superior α -helicity and improved binding, as compared to the originally reported peptide E6ap18. In conclusion, we have developed a promising peptidomimetic scaffold and identified a diverse set of fragments and complete

molecules which can bind HPV16 E6. Taken together, these tools have the potential to introduce novel highly-potent and very specific ligands for the E6 protein of HPV16.

MEDI 454

Scaffold replacement and 3D ligand optimization applied to the discovery of tyrosine kinase inhibitors

Alain Deschenes, alain.deschenes@unb.ca. Chemical Computing Group, Montreal, Quebec, Canada

Point mutations within the BRC-ABL tyrosine kinase domain give rise to imatinib-resistant mutants. Designing next generation ligands to counteract TK inhibitor resistance remains a challenging problem. Scaffold replacement is applied to the imatinib framework where the 2- amino-pyrimidine fragment is exchanged through a scaffold screen to produce a number of related congeneric series. 3D ligand optimization is subsequently performed on one of the hits yielding a structurally related isomer of ponatinib, a known selective high affinity tyrosine kinase inhibitor.

MEDI 455

Discovery of the first low-molecular-weight *Mycobacterium tuberculosis* MabA (FabG1) inhibitors using a fragment-based approach

*Catalin Pintiala*¹, *Martin Moune*², *Kevin Bourbiaux*¹, *Rosangela Frita*², *Kamel Djaouf*², *Catherine Piveteau*¹, *Benoit Deprez*¹, *Alain Baulard*², *Nicolas Willand*¹, **Marion Flipo**¹, marion.flipo-2@univ-lille2.fr. (1) Univ. Lille, Inserm, Institut Pasteur de Lille, U1177 - Drugs and Molecules for living Systems, Lille, France (2) Univ. Lille, CNRS, Inserm, CHU Lille, Institut Pasteur de Lille, U1019 – UMR 8204 - CIL - Center for Infection and Immunity of Lille, Lille, France

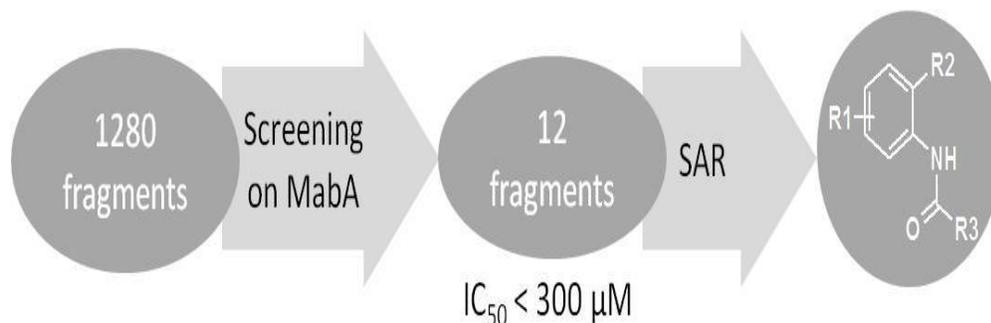
Tuberculosis, a disease caused by *Mycobacterium tuberculosis*, remains a major cause of mortality killing each year 1.4 million people. The treatment of this disease involves multidrug chemotherapy regimen often associated with serious side-effects thus alternative therapies are needed.

The enzyme MabA (FabG1) is involved in the biosynthesis of mycolic acids, which play an essential role in the architecture and permeability of the envelope of *M. tuberculosis*. This gene has been shown to be essential for *M. tuberculosis* and our objective is to discover drug-like inhibitors of MabA active on mycobacteria in order to propose a novel strategy to treat tuberculosis.

To enhance the chance to find low-molecular-weight inhibitors, with adequate physicochemical properties to reach their target inside the mycobacteria, we focused our strategy on a fragment based approach.

A screening of 1280 fragments from our chemical library was carried out on the target using a new enzymatic assay based on tandem mass spectrometry. Several chemical series were identified. In order to optimize one of these series we synthesized a library

of 240 amides on solid support using o-nitrophenol lanterns. This library was tested on MabA to establish structure-activity relationships and to rapidly increase the activity on the target. The mass spectrometry based enzymatic assay, as well as optimization of MabA inhibitors and their biological activities will be presented.

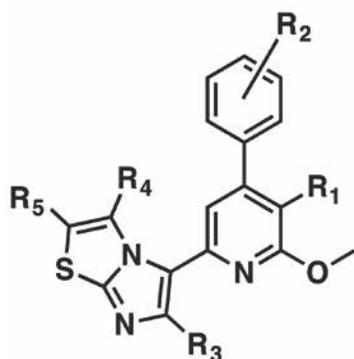


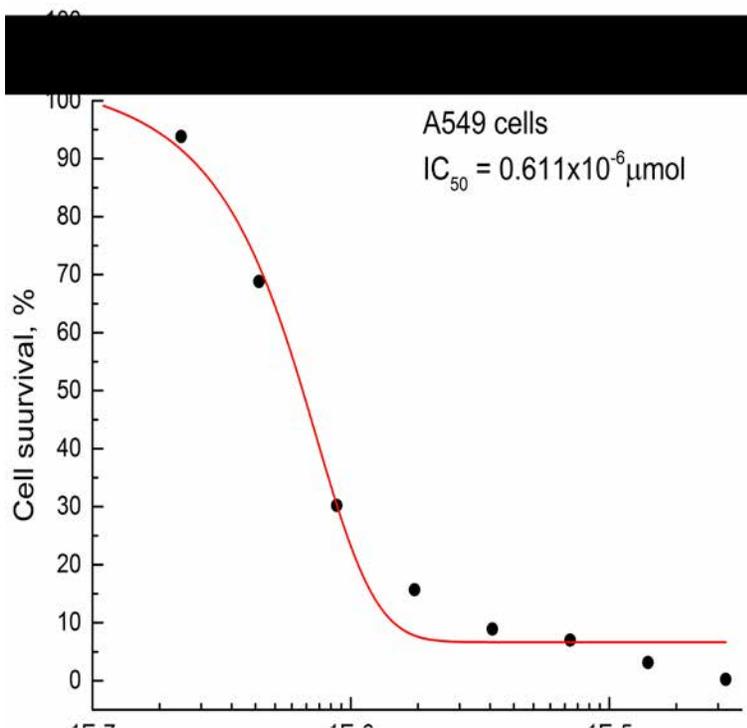
MEDI 456

Structure-based, in molecular design and synthesis of inhibitors of protein kinase family of receptors of epidermal growth factor

Alexander S. Bunev¹, a.s.bunev@gmail.com, **Elena V. Sukhonosova**¹, **Gleb Lisnik**¹, **Nikita Yabbarov**², **Gannady Ostapenko**¹. (1) Chemicals, chemical processes and technologies, Togliatti State University, Togliatti, Russian Federation (2) Bioengineering Center Russian Academy of Sciences, Moscow, Russian Federation

Design and synthesis of new serie 6-(het)arylimidazo[2,1-b]thiazole derivatives possessing pyridine moiety are described. Their *in vitro* cytotoxicity activities against of one human non-small cell lung cancer were tested. Its IC₅₀ value over A549 cells and EGFR kinase were 0.611 μmol and 112.4 nM, respectively.





MEDI 457

In vivo structure-efficacy studies of regioisomeric arterolane-like endoperoxides

Brian R. Blank¹, Brian.Blank@ucsf.edu, **Jiri Gu**², **Philip J. Rosenthal**², **Adam R. Renslo**¹. (1) Pharmaceutical Chemistry, University of California, San Francisco, San Francisco, California, United States (2) Medicine, University of California, San Francisco, San Francisco, California, United States

The artemisinin antimalarials are activated via a Fenton-type reaction of an endoperoxide bond promoted by labile iron(II) in the parasite. The clinical value of these drugs and their novel chemical pharmacology has inspired the investigation of synthetic endoperoxides, including the clinical-stage 1,2,4-trioxolanes arterolane and artefenomel. These compounds share a common pharmacophore, with adamantane and cyclohexane rings flanking the 1,2,4-trioxolane moiety, and possessing a side chain at the 4' position of the cyclohexane. Hypothesizing that the disfavored chair conformer is the iron(II)-reactive one, we predicted that regioisomeric 3'-substituted analogs might exhibit useful properties, including enhanced stability to iron(II), distinct *in vivo* metabolism, and enhanced solubility due to their asymmetric structure. Here we report the first systematic study of 3'-substituted arterolane-like antimalarials, contrasting their *in vitro* and *in vivo* activities with the original 4' derivatives of Vennerstrom and co-workers.

We synthesized arterolane and nine related 4' analogs previously reported to exhibit good *in vivo* efficacy in the *P. berghei* mouse malaria model. Synthesis of the desired 3' congeners was complicated by the fact that these compounds lack the internal symmetry of the 4' analogs and are chiral. We therefore devised a concise synthesis of

the 3' derivatives that permitted full control of relative and absolute stereochemistry. *In vitro* testing revealed that the 3' variants exhibit low nanomolar activity against *P. falciparum* and the SAR trends were similar but not identical amongst the 4' comparators. We then performed extensive *in vivo* studies of the 3'-substituted analogs alongside their 4'-substituted comparators. While many analog pairs exhibited comparable efficacy in mice, selected analog pairs differed dramatically in this regard, consistent with our original hypothesis. A number of 3' analogs with *in vivo* properties superior to arterolane were identified. Overall our results suggest that a re-evaluation of the arterolane pharmacophore with a focus on 3' side chain substitution has the potential to yield new clinical candidates with improved properties.

MEDI 458

Optimization of peptide substrates for conjugate modification of macromolecular mediated RNAi delivery

Jeffrey C. Carlson¹, jeffreyc.carlson@gmail.com, **Jonathan Benson**¹, **Andrei V. Blokhin**², **David Rozema**², **Alexander Sokoloff**². (1) Discovery Chem, Arrowhead Pharmaceuticals, Madison, Wisconsin, United States (2) Arrowhead Pharmaceuticals, Madison, Wisconsin, United States

It has been previously demonstrated that endogenous proteases can be exploited to safely facilitate activation of membrane disruptive polymer conjugates for safe delivery of RNAi to the cytosol. Para-aminobenzyl carbonate (PABC) incorporated in a conjugate as a self-immolative spacer provides fast kinetics of free amino-group liberation following enzyme triggered cleavage of the anilide bond. This approach, originally proposed by Katzenellenbogen, has been utilized in countless pro-drug type motifs. Although implemented clinically, the PABC moiety is considered a source of potential downstream mutagenesis due to the potent electrophilicity of the quinone imine methide generated during 1,6 elimination. Many examples have been described where regeneration of the parent amine resulted from proteolysis of a peptide sequence lacking the PABC modality. Unfortunately the rate of such proteolysis is often not sufficiently expedient to facilitate endosomal escape of the co-delivered RNAi without sufficient degradation in the nuclease rich environment. In this paper we explore novel protease substrates that can rapidly regenerate the conjugate parent polyamine without reliance on the self-immolative based approach. Kinetics of peptide degradation in-vitro and relation to in-vivo knockdown utilizing the aforementioned conjugate platform will be discussed.

MEDI 459

Impact of activation of GPR68 by metal cations on high throughput screening

Chenbo Wang, chenbo_w@hotmail.com, **Siqi Lin**, **Lee D. Fader**, **Alice Granger**. Small Molecule Discovery Research, Boehringer Ingelheim Pharmaceutical Inc, Ridgefield, Connecticut, United States

A high throughput screening against GPR68, a novel GPCR target, was performed. An unusually high hit rate was observed even after filtering with a counter screen and removing pan-assay interference compounds. Metal contamination of compounds in the BI screening collection was found to activate the target and led to false positive hits, which necessitated trace metal analysis on representative compounds from the HTS hit set. A set of structural patterns in the screening compounds were found to be indicative of metal contamination. Based on the discovery, we made recommendations on revising screening approaches and hit analysis.

MEDI 460

Synthesis of 1,2,4-substituted imidazoles for a fragment-based drug discovery library

Tyler Lafferty, tlafferty@rollins.edu, James Patrone. Chemistry, Rollins College, Winter Park, Florida, United States

Fragment-Based Drug Discovery (FBDD) is a powerful drug discovery technique that uses small (<300 amu) molecules (fragments) as a starting point for drug discovery. FBDD is contrasted with other methods of drug discovery through its use of small fragments, rather than fully elaborate molecules, such as those employed in High-Throughput Screening. Vital to this technique of FBDD is a high-quality library full of fragments that cover a broad range of chemical space, can easily be synthesized, and can readily be optimized in a modular fashion in the lead optimization stage. The ideal fragment will satisfy these demands along with possessing good physiochemical properties such as cLogP, number of rotatable bonds, and H-bond donors and acceptors. To meet these criteria, a molecule consisting of a hydrophobic core, a hydrophilic moiety, and a synthetic handle for rapid optimization would be ideal. The synthesis of underrepresented heterocyclic compounds and known heterocyclic structures with novel spatial arrangements requiring synthetic routes is a worthwhile endeavor. One such molecule is 2-ethynyl-1-phenyl-1*H*-imidazole-4-carboxylic acid. This hydrophobic imidazole possesses a hydrophilic carboxylic acid, a phenyl ring, and an alkyne substituent in an underrepresented 3-D spatial arrangement that would provide a high quality, synthetically tractable fragment for a drug discovery library if a known synthesis were available. To this end, a straightforward, modular synthesis of 2-ethynyl-1-phenyl-1*H*-imidazole-4-carboxylic acid and more broadly 1,2,4-substituted imidazoles was explored.

MEDI 461

Identification and optimization of 5-aryl benzimidazolones as AMPA receptor negative modulators selective for TARP-γ8

Suchitra Ravula¹, suchitrarr@hotmail.com, Michael Ameriks⁵, mameriks@its.jnj.com, Brad M. Savall², bsavall@san.rr.com, Jeannie M. Ziff⁵, jeannie.ziff@gmail.com, Brock T. Shireman⁵, bshirema@its.jnj.com, Mark J. Seierstad⁴, mseierst@its.jnj.com,

Nyantsz Wu², *nwu2@its.jnj.com*, **Brian Lord**⁴, *blord@its.jnj.com*, **Michael P Maher**², *mmaher1@its.jnj.com*, **Nicholas I. Carruthers**³, *ncarruth@its.jnj.com*, **Timothy W. Lovenberg**³, *tlovenbe@its.jnj.com*. (1) Chemistry, Janssen Pharmaceuticals, San Diego, California, United States (2) Neuroscience, Janssen Pharmaceutical R&D, San Diego, California, United States (3) Pharm Rsch Dev, Johnson Johnson, San Diego, California, United States (4) Johnson Johnson PRD, San Diego, California, United States (5) Johnson and Johnson, San Diego, California, United States

AMPA receptors (AMPA_Rs) are glutamate-gated ion channels that mediate the majority of fast synaptic transmission within the central nervous system (CNS). Although AMPA_Rs are widely expressed throughout the CNS, their activity can be modulated by auxiliary proteins such as transmembrane regulatory proteins (TARPs), which are often localized in distinct brain regions. Herein, we describe the medicinal chemistry efforts that led to the identification of JNJ-55511118, a selective negative modulator of AMPA_Rs dependent upon TARP-γ8, which is the primary TARP expressed in the hippocampus. Following oral dosing in rodents, JNJ-55511118 exhibited dose-dependent receptor occupancy as determined by *ex vivo* autoradiography and also provided strong seizure protection with minimal impairment of learning, memory, and motor function.

MEDI 462

Design and synthesis of L-neplanocin analogues as antiviral agents

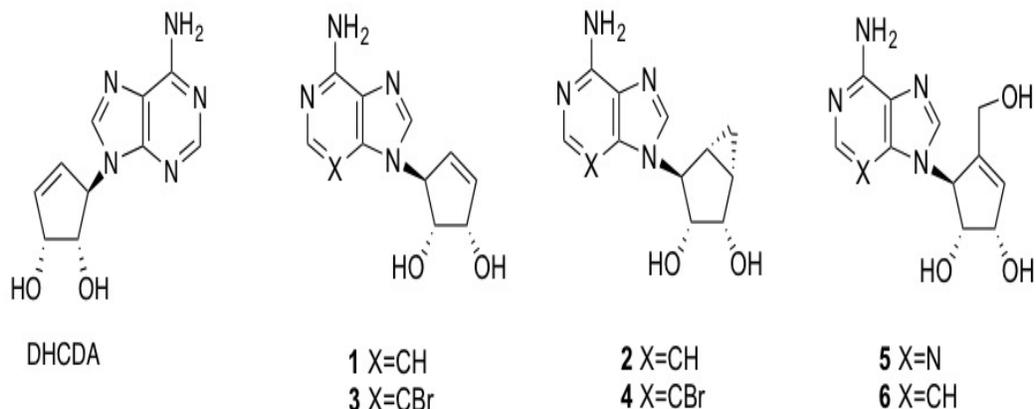
Qi Chen², *qi.chen@sru.edu*, **Chong Liu**³, *Stewart Schneller*¹, **Amber Davidson**², *axd1040@sru.edu*, **Megan Stout**², *mes1024@sru.edu*. (1) Auburn University, Auburn, Alabama, United States (2) Chemistry, Slippery Rock University, Slippery Rock, Pennsylvania, United States (3) Chemistry, Auburn University, Auburn, Alabama, United States

Recent studies have shown that L-like carbocyclic nucleosides, such as L-isonenplanocin analogues, possess broad spectrum antiviral activities, including Ebola, norovirus, vaccinia, HBV, HCMV, measles and Dengue. It is noteworthy that replacing the nitrogen atom to a CH or a CBr group at the N-3 position has significant impacts on their biological properties.

Previous studies have also found that C-4' truncated neplanocin analogue (DHCD_A) is effective against a series of viruses, which is likely due to its inhibition of SAH hydrolase. Its selectivity was even greater than that of neplanocin, particularly against vesicular stomatitis virus (VSV) and rotavirus.

Following the lead of these compounds and as part of a program that is study L-like carbocyclic nucleosides, the L form of DHCD_A (**1**), its 3-Br derivative (**3**), and the conformation restricted methanocarpa (MC) nucleoside analogues (**2** and **4**) were set as target structures. The L form MC nucleoside analogues (**2**, **4**) have similar locked conformation as conventional D-MC nucleosides, but different configuration, which will provide interesting information regarding to their antiviral activities. The L-5'-isonenplanocin (**5**) and its 3-deaza analogue (**6**) targets were also included in the study

of L-like carbocyclic nucleosides. The synthesis of these compounds and their antiviral properties will be described.



MEDI 463

Targeted isolation and simplified structure elucidation of new analogues of dolastatin 10 from marine cyanobacteria using MS/MS molecular networking

Ben Naman¹, bnaman@alumni.cmu.edu, **Jehad Almalit**², **Lena Keller**¹, **Ariana Remmel**¹, **Evgenia Glukhov**¹, **Pieter C. Dorrestein**³, **William H. Gerwick**^{1,3}. (1) Scripps Institution of Oceanography, University of California, San Diego, La Jolla, California, United States (2) Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Jordan, Amman, Jordan (3) Skaggs School of Pharmacy, University of California – San Diego, San Diego, California, United States

Natural products and their synthetic derivatives constitute a significant fraction of the drugs approved for use by regulatory bodies such as the U.S. FDA, and this is particularly true for cancer chemotherapeutic agents. One example is dolastatin 10, a potently cytotoxic cyanobacterial natural product that was originally purified from the sea hare *Dolabella auricularia* and later determined to originate from several species of *Symploca*. Many cytotoxic analogues of dolastatin 10 have also since been discovered and reported. Although dolastatin 10 failed in initial drug clinical trials, it served as the lead molecule for the development of monomethyl auristatin E (MMAE) that has been incorporated into the antibody-drug conjugate brentuximab vedotin. We have utilized the untargeted metabolomics tool Global Natural Products Social molecular networking (GNPS) to interrogate and prioritize cyanobacterial extracts for isolation studies. This data analysis allowed for the dereplication of dolastatin 10 and suggested the presence of several new analogues of this compound. These molecules were targeted using LC-MS, isolated chromatographically, and characterized structurally using MS/MS fragmentation patterns and NMR spectroscopic methods. The structure and biological activity of these new compounds will be presented.

MEDI 464

MELK as a valid cancer therapeutic target? From virtual screening to highly selective in vivo tool compounds

Simon Mathieu², *ssimonx@gmail.com*, **Bakary-Barry Toure**², **John Giraldes**², **Troy Smith**², **Elizabeth Sprague**³, **Yaping Yang**², **Zhuoliang Chen**², **Yuji Mishina**³, **Yun Feng**³, **Yan Yan-Neale**³, **Dongshu Chen**³, **Matthew Meyer**³, **Christopher Straub**³, **David Sage**³, **Kirk Wright**³, **Xin Chen**³, **Sean Kim**³, **Eric J. Martin**¹, **Kristen Hurov**³. (1) Computational Chemistry, Novartis, El Cerrito, California, United States (2) Global Discovery Chemistry, Novartis Institute for Biomedical Research, Cambridge, Massachusetts, United States (3) NIBR, Cambridge, Michigan, United States

Maternal Embryonic Leucine Zipper Kinase (MELK) is a serine/threonine kinase and an atypical member of the SNF1/AMPK family. MELK has been identified as part of a gene signature representing undifferentiated cancers, which are thought to be more aggressive in behavior and linked to poor patient outcome. MELK has been proposed as a potential therapeutic target based on its overexpression in a broad spectrum of cancer types, including breast, ovarian, lung, pancreas, and colorectal. An increase in MELK expression correlates with more aggressive forms of astrocytoma, breast cancer, melanoma, and glioblastoma. Among breast cancer subtypes, MELK is more highly expressed in triple-negative (estrogen receptor (ER)-negative, progesterone receptor (PR)-negative, and no HER2 overexpression) than in ER-positive. A key issue remains our poor understanding of the molecular mechanism underpinning the role of MELK in cancer due to the lack of selective pharmacological agents that inhibit its activity. In this poster, we will discuss the successful use of virtual screening hit finding strategy to uncover selective MELK hits with good physical chemical properties. The optimization of these hits into highly potent, selective, cell permeable MELK inhibitors and their use to understand MELK biology will be presented. Finally, our strategies to overcome PK challenges and enable in vivo dosing will also be discussed

MEDI 465

P(NIPAM) microgel embedding p(AAm) hydrogel interpenetrating polymer networks for controlled drug delivery vehicles

Nurettin Sahiner, *sahiner71@gmail.com*, **Alper Yasar**, **Ferah Onder**, **Mehmet Ay**. Chemistry, Canakkale Onsekiz Mart University, Canakkale, Turkey

Poly(N-isopropyl acrylamide) (p(NIPAM)) microgels were synthesized via emulsion polymerization, and then included within poly(Acrylamide) hydrogels during synthesis as (p(AAm)/p(NIPAM)) hydrogel interpenetrating polymer networks (IPN). The amounts of p(NIPAM) microgels were varied to increase temperature response of the prepared IPNs network films. The swelling behavior of prepared p(AAm)/p(NIPAM) IPN hydrogels were investigated in 20-50 °C range to determine effect lower critical solution temperature (LCST) of p(NIPAM) microgels to p(AAm) based matrices. These

p(AAm)/p(NIPAM) IPN composites were used as controlled drug delivery vehicles for various coumarinyl chalcone derivative as model drugs/drug precursors. New target coumarinyl chalcones were synthesized by Knoevenagel condensation of 6-methoxy-3-acetyl coumarin with substituted aromatic benzaldehydes, and their structures were confirmed by melting point, FT-IR, ¹H-NMR, ¹³C-NMR, LC-MS spectral analysis. The drug release kinetics of coumarinyl chalcones from p(AAm)/p(NIPAM) IPN composites were evaluated for potential topical applications.

MEDI 466

Evaluation of antimicrobial properties of *Combretum laxum* extracts

Jasmin G. Escobar², jasminessobar@dusty.tamtu.edu, **Irma Maldonado**³, irma_maldonado@dusty.tamtu.edu, **Alfred K. Addo-Mensah**¹, addomens@hotmail.com. (1) LBVSC 303, TAMTU Dept Biology Chemistry, Laredo, Texas, United States (2) Biology and Chemistry, Texas A&M International University, Laredo, Texas, United States (3) Biology & Chemistry, Texas A & M International University, Laredo, Texas, United States

Bacterial resistance against current pharmaceutical drugs has increased vastly across a variety of microorganisms, along with allergic reactions to these medications in the human population. This challenge has prompted the search for novel medicine or drug candidates from natural sources that could have higher potency, quality, and safety than what is currently available. Prior studies in our lab on plant extracts from the *Combretum* family have yielded very good results. Thus, natural compounds from *Combretum laxum* (CL) are worth investigating. CL was divided into stems, leaves, and fruits. Each plant section underwent sequential Soxhlet extraction with petroleum ether (P.E.), acetone (Ace.), and 9:1 ethanol/water (E/W). The dry-freeze crude extracts were then tested for preliminary biological activity against *S. aureus* (SA), Methicillin Resistant *S. aureus* (MRSA), *B. subtilis* (BS), *E. coli* B (ECB), and *S. enteritidis* (SE). Preliminary bioassays for the E/W stems and fruits extracts showed positive results against SA, MRSA, BS, ECB and SE. On the other hand, ECB was not inhibited by the Ace. leaves or the E/W stem extracts.

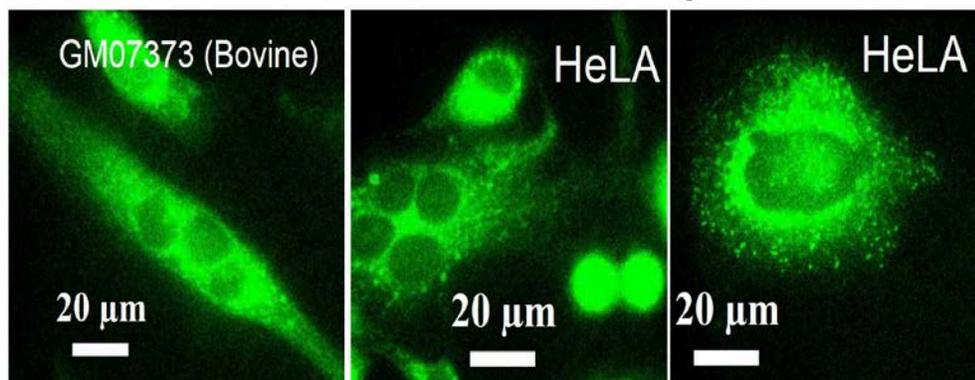
MEDI 467

Development of a new class of fluorophores and their applications as biological imaging agents and chemical sensors

Jake R. Zimmerman, j-zimmerman.3@onu.edu. Chemistry and Biochemistry, Ohio Northern University, Ada, Ohio, United States

This poster will highlight the development of a new class of fluorophores utilizing an inverse-demand hetero-Diels-Alder reaction with silyl enol ethers and substituted 3-formylchromones and coumarins. These compounds yield blue to green fluorescence with quantum yields up to 86% and Stokes shifts up to 143 nm (7003 cm⁻¹). The

synthetic scheme is concise and the overall yields are good to excellent. These fluorophores exhibit good potential for the use of fluorescent imaging agents in biological systems, as they are cell membrane permeable with low cytotoxicity. This new class of fluorophores also show great capability as chemical sensors. In particular, they are excellent at detecting fluoride and they also achieve good selectivity of detecting iron(III) over iron (II). More recently, it has been discovered that this class of fluorophores can detect Pb^{2+} at the low ppb level.



MEDI 468

Inhibition of pilocarpine-induced fluid secretion by ethylatropine bromide

Thota Ganesh, tganesh@emory.edu, Asheebo Rojas, Alec Walker, Ray Dingleline.
Pharmacology, School of Medicine, Emory University, Atlanta, Georgia, United States

Atropine has a broad range of medical applications. For example, it is often used to decrease heart rate, reduce saliva production during anesthetic induced surgery and to treat patients suffering from organophosphorus (OP) agent poisoning. Atropine is a competitive antagonist of the muscarinic acetylcholine receptors (mAChRs). It binds and inhibits all subtypes including M1, M2, M3, M4 and M5. The most common form of atropine used in medicine is atropine sulfate monohydrate. However, analogs of atropine that are unable to cross the blood-brain barrier (BBB) have been synthesized. These quaternary derivatives of atropine include ethylatropine, methylatropine, propylatropine and benzylatropine. Methylatropine is the most popular and potent of these quaternary derivatives and is suggested to be more potent than atropine. Methylatropine was introduced in 1902 by Bayer initially as a pupil dilating medication and then later as a therapeutic for gastrointestinal disorders such as pyloric stenosis. Until recently methylatropine nitrate and methylatropine bromide were available for commercial purchase. However, due to a limited production of methyl bromine (a key starting material in the synthesis) in 2005, methylatropine bromide is no longer available for commercial purchase. As a result, there has been an increase in demand for methylatropine nitrate which is still available for purchase in the United States of America. However, only a few vendors carry methylatropine nitrate and they possess limited quantities. Thus, the cost of methylatropine nitrate has ballooned and it is now

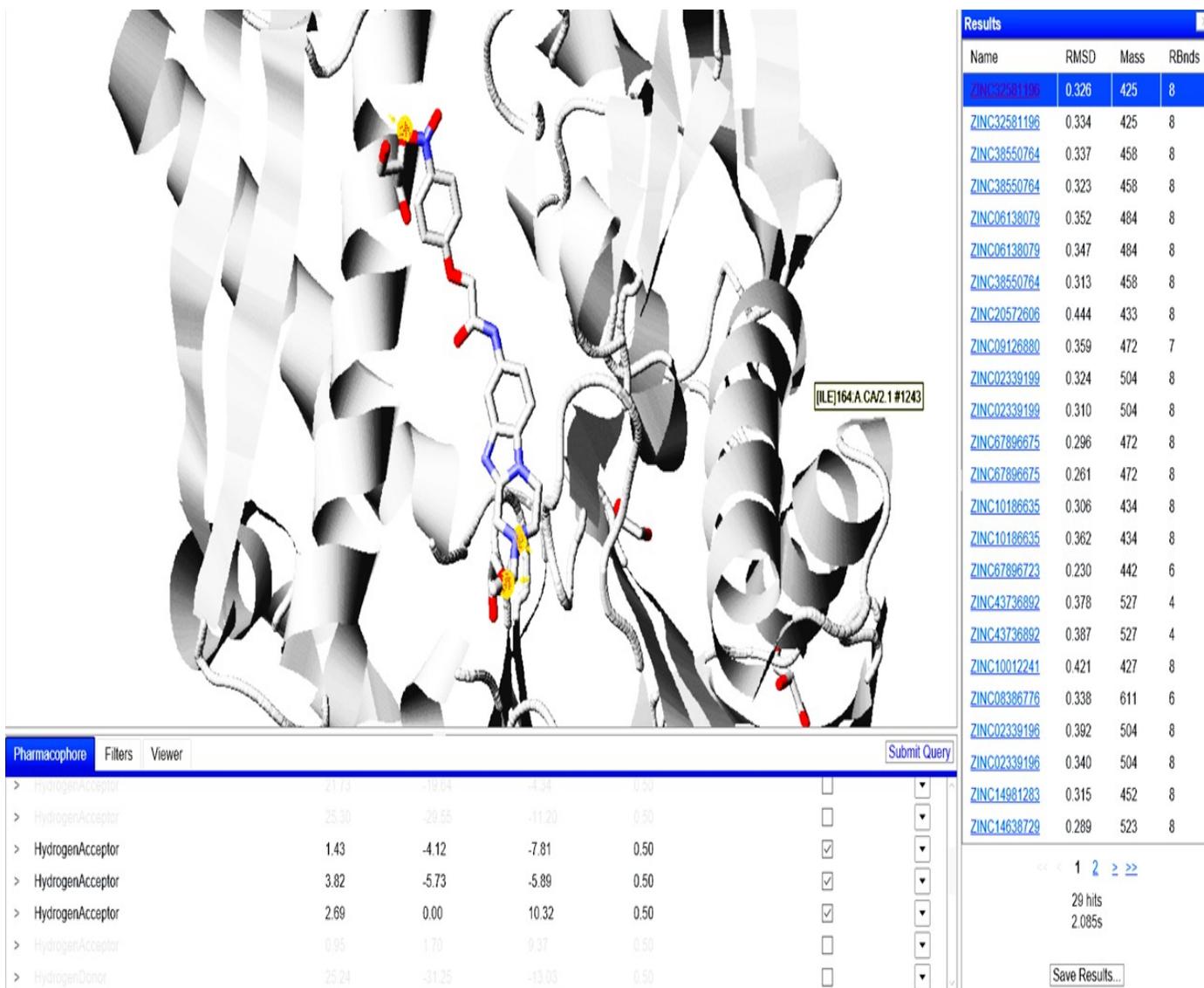
much more expensive than atropine sulfate. We designed an environmentally safe and inexpensive derivative ethylatropine bromide, then characterized its potency and efficacy as a selective antagonist of peripheral muscarinic receptors *in vitro* and *in vivo*. We present these data to suggest ethylatropine bromide as an alternative tool compound to other muscarinic receptor antagonists.

MEDI 469

Structure-Based Drug Design (SBDD) of allosteric inhibitors for HSC-70 using a combination of pharmacophore searching with ZINCPharmer and AutoDock Vina molecular docking

Cristina C. Clement¹, clement.cristina624@gmail.com, Janet Gonzalez², Manfred Philipp³. (1) Pathology Chemistry, Albert Einstein Coll Med CUNY, Bronx, New York, United States (2) Natural Sciences, LaGuardia Community College, Long Island City, New York, United States (3) Chemistry and Biochemistry, Lehman College & Graduate Center, CUNY, Scarsdale, New York, United States

In recent years, the chaperone Hsc-70 has become a target for drug design in anti-cancer therapies. Recently X-Ray crystallography revealed that Hsc-70 has a potential allosteric pocket occupied by glycerol, which is located on the side of the molecule opposite the ATP/ADP binding cavity. Binding and docking experiments predicted that the glycerol-binding site is a promising target for Hsc-70 inhibition (Z. Zhang et al., *Biochimie* 108 (2015), 186-192). A novel method of inhibition was proposed using compounds that bridge the adjacent phosphate and glycerol binding sites. Our research was the logical next step, and aimed to discover and validate new compounds, i.e., reversible allosteric inhibitors of HSC-70, binders to the allosteric tunnel occupied by glycerol, and the phosphate ion. We developed a new screening platform for an *in silico* HTS screening of the purchasable compounds of the ZINC database using the Pharmer-pharmacophore search technology provided by ZINCPharmer's (<http://zincpharmer.csb.pitt.edu>) online interface. This screening was coupled with the docking of sdf files containing selected pharmacophore libraries using *AutoDock Vina* software. Multiple rounds of rigid molecular docking were performed using two target X-ray structures of Hsc-70: 4H5R.pdb and 4H5v.pdb. Compounds predicted to have free energies of interaction lower than "-8.0 kcal/mol" were further selected for the *in vitro* validation of their putative reversible inhibition of Hsc-70 ATP-ase activity using the fluorometric PicoProbe™ ADP Assay Kit (BioVisionInc).



MEDI 470

Bioguided fractionation and isolation of chemical constituents of the chloroform extract from the Puerto Rican plant *Simarouba tulae*

Claudia A. Ospina², *clospina69@hotmail.com*, **Pablo Vivas**³, **Eliud Hernandez**¹. (1) Pharmaceutical Sciences, University of Puerto Rico, San Juan, Puerto Rico, United States (2) Chemistry, University of Puerto Rico at Cayey, San Juan, Puerto Rico, United States (3) Biochemistry, University of Puerto Rico Medical Sciences Campus, San Juan, Puerto Rico, United States

Species of the genus *Simarouba* have been studied because of its anti-malarial, anti-inflammatory, anti-leukemic, anti-feedant and antiviral activities. A group of highly oxygenated terpenes called quassinoids have been isolated from species of the *Simarouba* genus and are thought to be responsible for its therapeutic properties. We

hypothesize that *Simarouba tulae*, an endemic plant species, are a natural source rich on quassinoid compounds and, thus, will inhibit growth of different cancer cells. The objective of this study is to test the biological activity of the secondary metabolites from *Simarouba tulae* against, ovarian (A2780, SKOV3), breast (MCF-7, MDA-MB-435 or -231), prostate (PC-3, LNCAP), and neuroblastoma (SH-SY5Y) cells. The leaves were extracted with a mixture of CH₂Cl₂-MeOH (1:1). The resulting crude extract was suspended in water and extracted with solvents of different polarities. The extracts were preliminary screened using the brine shrimp lethality test. Among all extracts analyzed, the chloroform extract was the most active showing a LC₅₀ value of 157 µg/ml. This extract was chromatographed on Si gel with a 5% of methanol in chloroform to obtain 7 fractions. Fraction 3 was purified by size exclusion chromatography, column chromatography and HPLC reversed phase to afford 11 mg of the quassinoid Simalikalactone D. This compound showed in vitro cytotoxicity with GI₅₀ = 0.1 µM in MDA-MB-231 metastatic cell line and 39.8 µM against neuroblastoma cells. Therefore, this finding demonstrates the possibility of the presence of other quassinoid compounds as constituents of the chloroform and other extracts. Based in our results we concluded that this plant showed anticancer activity and merit a closer investigation.

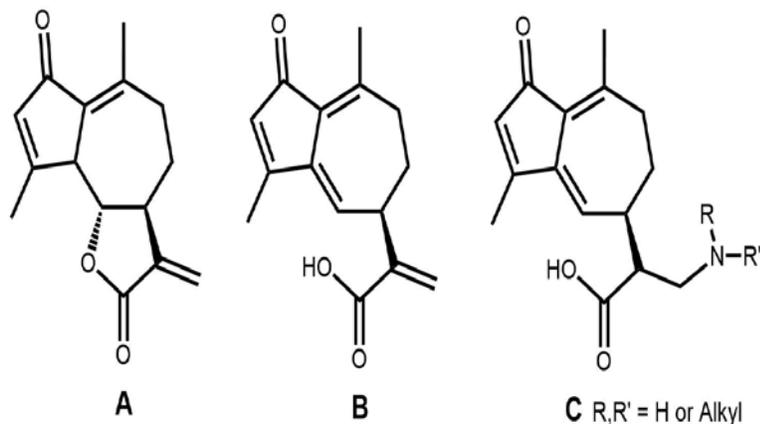
MEDI 471

Synthesis of amino derivatives of dehydroleucodine and dehydroparishin-B as potential anti-proliferative against breast cancer and B16 melanoma cells

Andersson Sanchez¹, *asanc291@fiu.edu*, **Maria-Luisa Veisaga**³, **Stanislaw F. Wnuk**¹, **Manuel Barbieri**³, **Luis A. Lopez**². (1) Department of Chemistry and Biochemistry, Florida International Univ, Miami, Florida, United States (2) Facultad de Ciencias Medicas, Universidad de Cuyo, Mendoza, Argentina (3) Department of Biology, Florida International Univ, Miami, Florida, United States

Dehydroleucodine (DHL, **A**) is a known sesquiterpene lactone that can be isolated from the extraction of *Artemisia douglasiana*, which is a native plant located in the western United States and in Argentina. Another lesser-known guaiane-type sesquiterpene acid, dehydroparishin-B (DHP, **B**), can also be extracted from *Artemisia douglasiana* but as a minor product. These two sesquiterpenes have shown to have anti-cancer properties by inhibiting the proliferation of breast cancer cells and B16 melanoma cells in µM range based on the previous studies. One objective of this study was to develop a synthetic route to DHP (**B**) from DHL (**A**) and the isolation of any possible intermediates between these two sesquiterpene compounds. The synthesis of DHP from DHL was attempted by the simple basic hydrolysis of the lactone group (aqueous NaOH) followed by acid-catalyzed (aqueous H₂SO₄) thermal dehydration of the tertiary hydroxyl group from the intermediate species. The second objective was to prepare the amino derivatives of DHL and DHP (e.g., **C**). Two different routes were taken to synthesize amino derivatives of DHP. One route involves the addition of the several primary or secondary amines (e.g., pyrrolidine, dimethylamine, tyramine) to the *exo*-alkene unit of DHL followed by the basic hydrolysis and dehydration of the resulting amino-substituted DHL derivatives to give desired amino-DHP products **C**. The other route involves the direct Michael

addition of the amines to the *exo*-alkene unit of DHP. The anti-proliferative and anti-migration properties of these synthesized compounds will be discussed.



MEDI 472

Design and evaluation of cyclic peptides containing arginine, lysine and tryptophan residues as a cellular drug delivery system and establishing structure-activity relationship

Saghar Mozaffari, Neda Sadeghiani, Rakesh Tiwari, **Keykavous Parang**,
parang@chapman.edu. School of Pharmacy, Chapman University, Irvine, California,
United States

We have previously shown that cyclic peptide $[WR]_5$ significantly improved the cellular uptake of a number of anti-HIV and anti-cancer drugs. A number of cyclic peptides containing arginine, lysine and tryptophan residues, namely $[RK]_4W_4$, $[W_5K]R_5$, $[WK]_4R_4$, and $[R_5K]W_5$ were synthesized through Fmoc-solid based chemistry and compared with $[WR]_5$. Among all the peptides, $[R_5K]W_5$ significantly improved fluorescence-labeled cell-impermeable negatively charged phosphopeptide (F' -GpYEEI) uptake by 27 folds when compared with F' -GpYEEI and 3 folds when compared with $[WR]_5$ in human leukemia cancer (CCRF-CEM) cells. The peptides were not toxic to the normal kidney cells (LLCPK) at a concentration of 50 μ M. $[R_5K]W_5$ decreased the cell viability in CCRF-CEM cells by 2.5 fold at a concentration of 50 μ M. The presence of positively charged residues on the ring and hydrophobic residues in a sequential linear outside the ring was an optimal approach for generating compounds with more molecular transporter properties.

MEDI 473

Design, synthesis and evaluation of potent inhibitors of PARP-14/ARTD8, a mono-ADP-ribosyltransferase

Matthew Meyers², *mem019@connections.mcdaniel.edu*, **Kristen Upton**², *kau002@connections.mcdaniel.edu*, **Ann-Gerd Thorsell**¹, **Herwig Schuler**¹, **Dana Ferraris**². (1) Karolinska Institutet, Stockholm, Sweden (2) Chemistry, McDaniel College, Eldersburg, Maryland, United States

Mono- and poly-ADP-ribosylation are established post-translational modifications catalyzed by the diphtheria toxin-like ADP-ribosyltransferase family of enzymes (i.e. ARTD or PARP family of enzymes). The founding member of this family, PARP-1, is integral to DNA repair and genomic integrity. As such, PARP-1 is a well-established drug discovery target with seven PARP-1 inhibitors currently in clinical trials. However, some of the clinical PARP-1 inhibitors non-specifically inhibit other members of the PARP superfamily, many of which are mono-ADP-ribosyltransferases. The pharmacological effect of inhibiting these other members of the PARP family is largely unknown. **Thus, specific, potent inhibitors must be designed in order to delineate the effects of this inhibition.** Herein we describe a series of small molecule inhibitors focused on inhibiting PARP-14 (a.k.a. ARTD8), a mono-ADP-ribosyltransferase. PARP-14 is up regulated in multiple myeloma and is associated with disease progression and poor survival rates, implicating this enzyme as a potential drug discovery target. In this poster we present the synthesis and *in vitro* evaluation of a series of (*Z*)-4-(3-carbamoylphenylamino)-4-oxobut-2-enyl amides as PARP-14 inhibitors. Several members of this series exhibit sub-micromolar potency against PARP-14 and possess good physicochemical properties and moderate selectivity over other members of the PARP superfamily.

MEDI 474

Design, synthesis, and evaluation of potent DNA-alkylating agents for use in Antibody-Drug Conjugates (ADCs)

Katie E. Archer, *katie.archer@immunogen.com*, **Emily E. Reid**, **Manami Shizuka**, **Alan Wilhelm**, **Nicholas C. Yoder**, **Chen Bai**, **Nathan Fishkin**, **Megan Bogalhas**, **Paulin Salomon**, **Luke Harris**, **Erin K. Maloney**, **Olga Ab**, **Ravi V. Chari**, **Michael L. Miller**. *ImmunoGen Inc, Waltham, Massachusetts, United States*

A new class of mono imine-containing DNA alkylating agents, indolinobenzodiazepine dimers (termed IGNs) have been designed and synthesized. These compounds are highly cytotoxic *in vitro* towards cancer cell lines, with IC₅₀ values in the picomolar range. IGNs were conjugated using peptidase-labile alanine-alanine linkers to monoclonal antibodies directed against tumor-associated antigens. These antibody-IGN conjugates displayed high antigen-specific potency *in vitro*, and anti-tumor activity *in vivo* at non-toxic doses. The design, synthesis and preclinical evaluation of IGN ADCs linked via all 4 stereoisomeric forms of the alanine-alanine dipeptide will be presented.

MEDI 475

Improved trifluoromethylation via organic electrochemistry

Jinshan Chen, michael.j.chen@pfizer.com, Jeremy Starr. Pfizer, Madison, Connecticut, United States

This poster will disclose a small scale (<0.05 mmole) electrochemical trifluoromethylation based on substantial modifications to the literature gram scale procedure. Key to the miniaturization was using a significantly simplified cell configuration, a safer electrolyte and an alternative trifluoromethyl radical source. Examples of trifluoromethylation of drug compounds, late stage pharmaceutical intermediates, and heterocyclic monomers will be presented.

MEDI 476

R996, an orally-bioavailable and selective activator of nuclear factor (erythroid-derived 2)-like 2 (Nrf2), is active in a Murine model of multiple sclerosis

Matthew A. Duncton, mattducton@yahoo.com, Alexander Owyang, Florentino San Pablo, Allan Torneros, Gary Park, David Lau, Esteban Masuda, Donald Payan, Rajinder Singh. Rigel Pharmaceuticals, South San Francisco, California, United States

Nuclear factor (erythroid-derived-2)-like 2 (Nrf2) is a transcription factor and a key player in the anti-oxidant response. Nrf2 can be activated by alkylating an associated chaperone, Kelch like-ECH-associated protein 1 (Keap1), resulting in lower ubiquitination and degradation of Nrf2. Upon translocation to the nucleus, Nrf2 forms a dimer with a small Maf-protein, and binds an Antioxidant Response Element (ARE), leading to the transcription of antioxidant, or cytoprotective gene products. Activation of the Nrf2 pathway may be useful against a broad-range of conditions (e.g. multiple sclerosis, psoriasis, and sickle-cell disease, among others). For example, monomethylfumarate (MMF), the major metabolite of the approved multiple sclerosis medication, dimethylfumarate (DMF), has been shown to activate the Nrf2-pathway. However, MMF is also an agonist of GPR109A (niacin receptor), which may be responsible for the flushing side-effect seen in some patients taking DMF. In this publication, we disclose a novel small molecule (R996) that acts as a potent activator of Nrf2 (EC50 = 60 uM; MMF EC50 = 127 uM), yet is inactive at the niacin receptor (0% activity at 1500 uM; MMF EC50 ca. 1 uM). Additionally, R996 is selective against a broad-range of G-protein-coupled receptor, ion-channel, and transporter targets. When dosed orally in a murine model of multiple sclerosis, R996 delayed the onset and also suppressed clinical disease. Further exploration with R996 and other selective activators of Nrf2 is ongoing, and may yield a next-generation Nrf2-therapeutic, with a reduced side-effect profile.

MEDI 477

New small molecule inhibitors of Ghrelin O-acyltransferase

John D. Chisholm, jdchisho@syr.edu, Nivedita S. Mahajani, Kayleigh R. McGovern-Gooch, Ariana Garagozzo, Anthony J. Schramm, Lauren G. Hannah, Michelle A.

Sieburg, James Hougland. Chemistry, Syracuse University, Syracuse, New York, United States

Ghrelin signaling is involved in controlling hunger, insulin regulation and glucose metabolism. Influencing this pathway may provide new methods for the treatment of hunger related disorders impacting body energy regulation, including obesity, diabetes and Prader-Willi syndrome. Ghrelin signaling is dependent on the ability of the 28-amino acid peptide hormone ghrelin to bind to and activate its target cognate receptor, growth hormone secretagogue receptor 1a. In order to efficiently signal through this receptor, ghrelin must first be modified with an eight-carbon acyl chain at its serine 3 residue during its maturation prior to secretion into the bloodstream. This acylation reaction is catalyzed by the enzyme ghrelin O-acyltransferase (GOAT). Screening of a library of small molecules for inhibition of GOAT has led to the identification of new small molecule inhibitors of the enzyme. The most potent inhibitors found from this screening exhibits and IC₅₀ values below 10 μ M in an enzyme-based assay. Several of these new inhibitors also provide new insights into the mechanism for GOAT-catalyzed ghrelin acylation, which provides a basis for the further development of these molecules. Initial structure activity relationships on the new inhibitors will be presented.

MEDI 478

Fused imidazole derivatives as TGF- β inhibitors

Jiixin Yu, jyu@rigel.com, Ihab S. Darwish, Marina Gelman, Rong-Xian Ding, Annabelle Frieria, Guillermo Godinez, Wayne Lang, Wei Li, Kelly McCaughey, John McLaughlin, Henry Nguyen, Ira Smith, Kathy White, George Yam, Todd Kinsella, Vanessa Taylor, Sylvia Braselmann, Chrystelle Lamagna, Esteban Masuda, Hong Ren, Lu Chou, Gary Park, Rachel Basile, Bhushan Samant, David Sweeny, Maurice Standlee, David Lau, Allan Torneros, Florentino San Pablo, George Clemens, Donald Payan, Rajinder Singh. Rigel Pharmaceuticals, Inc., South San Francisco, California, United States

Myostatin, also known as Growth and Differentiation Factor-8 (GDF-8), and Transforming Growth Factor- β (TGF- β) are members of the Transforming Growth Factor- β (TGF- β) superfamily of structurally related growth factors. GDF-8 is a negative regulator of skeletal muscle mass and is highly expressed in developing and adult skeletal muscle. GDF-8 null mutation in transgenic mice is characterized by a marked hypertrophy and hyperplasia of the skeletal muscle. TGF- β , which exists in three isoforms, TGF- β 1 through TGF- β 3, controls cell proliferation/differentiation, production of extracellular matrix and immune-suppression. While working towards inhibiting GDF-8 signaling pathway, we recognized that the new therapeutic agents that inhibited the activity of GDF-8 are desirable for treating human diseases associated with muscle and adipose tissue disorders as well as treating diseases related to TGF- β and its ability to modulate immune responses. Herein, we disclose a series of low nanomolar fused imidazole inhibitors of GDF-8 and TGF- β , SAR studies, PK profile and *in vivo* inhibition of GDF-8 and TGF- β data.

MEDI 479

Fused pyrazole derivatives as TGF- β inhibitors

Ihab S. Darwish, darwishis@yahoo.com, Jiaxin Yu, Marina Gelman, Rong-xian Ding, Annabelle Frieria, Guillermo Godinez, Wayne Lang, Wei Li, Kelly McCaughey, John McLaughlin, Henry Nguyen, Ira Smith, Kathy White, George Yam, Todd Kinsella, Vanessa Taylor, Sylvia Braselmann, Chrystelle Lamagna, Esteban Masuda, Hong Ren, Lu Chou, Gary Park, Rachel Basile, Bhushan Samant, David Sweeny, Maurice Standlee, David Lau, Allan Torneros, Florentino San Pablo, George Clemens, Donald Payan, Rajinder Singh. Rigel Pharmaceuticals, Inc., South San Francisco, California, United States

Transforming growth factor- β (TGF- β) receptor I kinase (TGFBR1), also known as Activin-Like Kinase 5 (ALK5) has been investigated as a target for the treatment of various cancers and autoimmune diseases. Although a good number of ALK5 inhibitors have been reported at the preclinical stage, only one inhibitor, LY-2157299, has advanced into clinical development for an oncology indication. The ubiquitous role of TGF- β in the homeostasis of various physiological functions suggests chronic systemic inhibition of TGF- β signaling might lead to harmful toxicity. Nevertheless, assuming one can overcome these shortcomings, the role of TGF- β as an immune system modulator could be harnessed in a number of disease areas including cancer and autoimmune diseases. Herein, we disclose a series of low nanomolar fused pyrazole inhibitors of TGF- β , SAR studies, PK profile and *in vivo* inhibition of TGF- β data.

MEDI 480

Collaborative web-based architecture for fragment-based drug discovery data

Whitney W. Smith², wws.smithfamily@gmail.com, Barry A. Bunin¹. (1) CDD, Belmont, California, United States (2) Sales, Collaborative Drug Discovery, Santa Rosa, California, United States

The Collaborative Drug Discovery CDD Vault™ for private Fragment-Based Drug Discovery (FBDD) data, also contains Fragment-Based Public data (such as the ChemBridge “Astex Rule of 3” compliant Fragment Library). The CDD platform provides a secure, cloud-based database with a web interface that permits users to access and search chemical FBDD collections of structures with SAR data, as well as screening and preclinical study data.

FBDD researchers have a general informatics resource of not only fragments, but also private, published or patented bioactivity data associated with published fragments. This is a unique resource that may be used to answer common questions in the field, such as why certain fragments bind better than others, chemical property trends, 2D vs 3D fragment properties, and other useful similar insights that can only be addressed by combining novel analyses with a critical mass of data. Over 100 Public resources in

CDD Vault complement researchers' private, secure mining of IP-sensitive data. Publications, patents, or data provided for the public good receive free formatting and hosting with attribution.

MEDI 481

Planar aryl triazenes inhibit cytochrome P450 1A1 and 1B1 as a potential means to prevent cancer

Ryan Nakamura², *rnakamur@poets.whittier.edu*, **Rachel Moran**², *rachelmoran01@gmail.com*, **Ralph A. Isovitsch**¹, *risovitsch@gmail.com*, **Devin S. Imoto**², *diimoto@whittier.edu*. (1) Chemistry, Whittier College, Whittier, California, United States (2) Whittier College, Whittier, California, United States

Cytochrome P450 1A1 (CYP1A1) and 1B1 (CYP1B1) metabolically activate polycyclic aromatic hydrocarbons (PAHs) to carcinogenic compounds. Aryl-morpholino triazenes inhibit CYP1A1 and CYP1B1 as a potential means to prevent cancer, but these compounds adopt a chair conformation which may not fit into the active site as well as a more planar molecule. Planar triazenes, 1,3-bis(phenyl)triazene, 1,3-bis(4-bromophenyl)triazene, 1,3-bis(4-cyanophenyl)triazene and 1,3-bis(4-acetylphenyl)triazene, were synthesized from substituted aniline derivatives, such as aniline, 4-bromoaniline, 4-aminobenzonitrile, and 4-aminoacetophenone respectively, using 12 M HCl to generate a highly reactive diazonium ion. This was subsequently treated with NaNO₂ which provided the third nitrogen in the triazene unit. These triazenes and respective starting materials were screened at 100 μM for their ability to inhibit CYP1A1 and CYP1B1. 1,3-bis(4-bromophenyl)triazene and 1,3-bis(4-cyanophenyl)triazene inhibited CYP1B1 to 45% and 36% of the uninhibited CYP1B1 respectively, while their aniline derivatives showed no inhibition of CYP1B1. 1,3-bis(4-acetylphenyl)triazene inhibited CYP1A1 to 40% of the uninhibited activity while its aniline derivative showed no inhibition of CYP1A1. Compounds that inhibit CYP1A1 and CYP1B1 will be analyzed for their IC₅₀ values. The IC₅₀ value of one compound, 1,3-bis(4-bromophenyl)triazene, has been determined to be 92 μM with respect to CYP1B1. This indicates linear triazenes are a new class of compounds that effectively inhibit CYP1A1 and CYP1B1 as a means to prevent cancer.

MEDI 482

Discovery of BPR1K871 – a quinazoline based multi-kinase inhibitor for the treatment of AML and solid tumors: Rational design, synthesis, in vitro and in vivo evaluation

Hsing-Pang Hsieh^{1,2}, *hphsieh@nhri.org.tw*, **Wen-Chieh Wang**¹, **Hui-Yi Shiao**¹, **Yi-Yu Ke**¹, **Wen-Hsing Lin**¹, **John T.-A. Hsu**¹, **Chiung-Tong Chen**¹, **Teng-Kuang Yeh**¹. (1) National Health Research Institutes, Miaoli County, Taiwan (2) Chemistry, National Tsing Hua University, Hsinchu, Taiwan

Acute myeloid leukemia (AML), an aggressive and frequently fatal hematologic malignancy, is one of the most common type of leukemia in children and adolescents. In this work, we report the design and synthesis of a quinazoline-based multi-kinase inhibitor for the treatment of AML and other malignancies. Based on the previously reported furanopyrimidine **3**, quinazoline core containing lead **4** was synthesized with dual FLT3/AURKA inhibition ($IC_{50} = 127/5$ nM) as well as improved physicochemical properties. Detailed structure-activity relationship study in the lead **4** allowed FLT3 and AURKA inhibition to be finely tuned, resulting in AURKA selective, FLT3 selective and dual FLT3/AURKA selective (**BPR1K871**; $IC_{50} = 19/27$ nM) agents. **BPR1K871** showed potent anti-proliferative activities in MOLM-13 and MV4-11 AML cells ($EC_{50} \sim 5$ nM). Moreover, kinase profiling and cell-line profiling revealed **BPR1K871** to be a potential multi-kinase inhibitor. In vivo efficacy in AML xenograft models (MOLM-13 and MV4-11), as well as in solid tumor models (COLO205 and Mia-PaCa2), led to the selection of **BPR1K871** as a preclinical development candidate for anti-cancer therapy. Further detailed studies could help to investigate the full potential of **BPR1K871** as a multi-kinase inhibitor.

MEDI 483

Novel approaches to the chemical synthesis of haloindoles for the development of ergot alkaloids compounds

Gui Ren³, renglauren@gmail.com, **Edward J. Parish**¹, **Dong-Chun Ren**⁴, **Yu-Chen Lo**⁵, **Hiroshi Honda**², chrishonda@yahoo.com. (1) Chemistry, Auburn University, Auburn, Alabama, United States (3) Bioengineering, Northwestern Polytechnic University, Fremont, California, United States (4) Pharmacy, China Pharmaceutical University, Nanjing, Jiangsu, China (5) Bioengineering, Stanford University, Palo Alto, California, United States

This paper represents the facile synthesis of haloindoles for the development of ergot alkaloids compounds for biological study.

MEDI 484

Novel approaches to the structure activity relationship study of citronellol type compounds useful as enzyme inhibitors

Edward J. Parish¹, **Liu-Qiang Lv**⁵, lvliuqiang@163.com, **Gui Ren**³, **Yu-Chen Lo**⁴, **Hiroshi Honda**², chrishonda@yahoo.com. (1) Chemistry, Auburn University, Auburn, Alabama, United States (3) Bioengineering, Northwestern Polytechnic University, Fremont, California, United States (4) Bioengineering, Stanford University, Palo Alto, California, United States (5) Medicine, Benbu Mendical College, Huaibei, Anhui, China

This paper represents new approaches to the chemical synthesis of citronellol type compounds. Certain acyclic terpenoid compounds and their derivatives are potent inhibitors of tyrosinase.

MEDI 485

Novel approaches to the synthetic study of lanost-8-en-3 β -ol-7,11-dione, an inhibitor of cholesterol biosynthesis

Gui Ren³, renglauren@gmail.com, Edward J. Parish¹, Yu-Chen Lo⁴, **Hiroshi Honda**², chrishonda@yahoo.com. (1) Chemistry, Auburn University, Auburn, Alabama, United States (3) Bioengineering, Northwestern Polytechnic University, Fremont, California, United States (4) Bioengineering, Stanford University, Palo Alto, California, United States

This paper represents a compound, lanost-8-en-3 β -ol-7,11-dione has been found to be potent inhibitor of sterol biosynthesis in animal cells in culture.

MEDI 486

Second generation inhibitors of *Porphyromonas gingivalis* biofilm formation

Frederick A. Luzzio², faluzz01@louisville.edu, Pravin C. Patil¹, Donald R. Demuth³, Jinlian Tan⁴. (2) Department of Chemistry, University of Louisville, Louisville, Kentucky, United States (3) Dept. of Oral Immunology and Infectious Diseases, University of Louisville, School of Dentistry, Louisville, Kentucky, United States

Periodontitis and its systemic sequelae remain a major public health problem and the development of a safe, cost-efficient therapy will benefit healthcare worldwide. The adherence of *Porphyromonas gingivalis* to *Streptococcus gordonii* facilitates colonization of the oral cavity by *P. gingivalis* and contributes to the development of periodontal disease. It was previously shown that a synthetic peptide derived from the streptococcal protein potently inhibits this interaction and prevents formation of *P. gingivalis* biofilms. However, peptides are not ideal therapeutic agents due to preparation costs and hydrolytic instability. Consequently, our approach is to rationally-design small-molecule peptidomimetics that inhibit *P. gingivalis* adherence to *S. gordonii*. The inhibitors are based on the principle of click chemistry whereby two inhibitory small-molecule fragments are joined through a click reaction to produce an enhanced inhibitor. Our first generation inhibitors were based on 2-azidomethyl-4,5-disubstituted oxazoles and substituted arylacetylenic click components. We now report on the synthesis and bioassay of a 'second generation' of click biofilm inhibitors based on azidoarylsulfonyloxazoles and acetylenic triazine components. The click products are composed of a new arylsulfonylmethyl spacer and utilize acetylenic triazines as the coupling partners. The synthesis of the inhibitors and the in vitro bioassay studies of the compounds will be presented.

MEDI 487

Preparative method development from analytical columns

Jack E. Silver, *jacksilver27@earthlink.net*, Ronald L. Lewis, Fowler Nancy, Letteney M. Esther. Teledyne ISCO, Lincoln, Nebraska, United States

The determination of an efficient preparative gradient method for C18 from analytical data is not simply scaled up from an analytical scouting run. This is due to the fact that the actual solvent composition eluting the compound during the purification run is delayed from the value observed in the HPLC/UPLC scouting gradient. An algorithm that calculates the focused gradient parameters for a preparative column using the data obtained from an HPLC/UPLC scouting gradient is described. The calculated focused gradient saves time and solvent when compared to the preparative system default gradient conditions. It also eliminates the guesswork in how to produce a fast, efficient gradient manually. The algorithm is also applicable to other adsorption chromatography techniques such as flash chromatography (both normal and reverse phase) as well as supercritical fluid chromatography (SFC).

MEDI 488

Synthesis of organic azides via flow chemistry

Jack E. Silver, *jacksilver27@earthlink.net*, Letteney M. Esther, Ronald L. Lewis, Fowler Nancy. Teledyne ISCO, Lincoln, Nebraska, United States

The use of flow chemistry for the synthesis of an organic azide is described. The apparatus used for the reaction consisted of a system constructed using an inexpensive pulseless continuous flow pump and 316 stainless steel HPLC tubing. There are safety issues with azide synthesis; in protic solvent systems hydrazoic acid can easily form. In order to reduce the possibility of the possibility of hydrazoic acid accumulating during the reaction the reaction temperature is often controlled and maintained below hydrazoic acid's boiling point of 36 °C to avoid accumulation of this toxic and shock-sensitive compound. As flow chemistry has no headspace for hydrazoic acid to accumulate in, reactions can safely be run at higher temperatures allowing shorter reaction time and synthesis of larger amounts of the desired azides than traditional batch methods.

MEDI 489

Development and screening of new cathepsin D inhibitors

Rose McConnell¹, *rm-mcconnell@wiu.edu*, Karthik Malayala¹, Karthika Yarlagadda¹, Kelley Sayya², Carol Trana², Walter Godwin³, Lisa Wen¹. (1) Department of Chemistry, Western Illinois University, Macomb, Illinois, United States (2) Mathematics and Natural Sciences, University of Arkansas at Monticello, Monticello, Arkansas, United States

Cathepsin D is an aspartyl protease similar to the HIV-1 aspartyl protease in substrate specificity. Cathepsin D has emerged in recent years as a prognostic indicator in several types of carcinoma, including bladder cancer, colorectal cancer, breast cancer

and lung cancer. Also, cathepsin D has been associated with the development of Alzheimer's disease. Therefore, protease inhibitors can lead to the development of therapeutic agents for treatment of many types of carcinomas and Alzheimer's disease. Specific proteinase could lead to the development of therapeutic agents for treatment of many types of carcinomas. Described is the design and synthesis of inhibitors containing substituted hydroxyethyl morpholine and substituted hydroxyethyl piperazine isosteres. The synthetic compounds were evaluated as cathepsin D inhibitors by fluorometric assay using as substrate: Ac-Glu-Glu(Edans)-Lys-Pro-Ile-Cys-Phe-Phe-Arg-Leu-Gly-Lys(Methyl Red)-Glu-NH₂.

MEDI 490

Greener reversed-phase flash chromatography using acetone instead of acetonitrile

John R. Bickler, bob.bickler@biotage.com. Biotage, LLC, Hampstead, North Carolina, United States

Reversed-phase flash chromatography is growing in use within medicinal chemistry labs. One reason for this is increasing intermediate polarity which challenges the capabilities of normal-phase purification and the other is that reversed-phase chromatography is greener than normal-phase. However, one of the popular solvents in reversed-phase is acetonitrile which is toxic and expensive.

In this poster we explore the use of acetone as a replacement for acetonitrile in reversed-phase flash chromatography.

MEDI 491

Acetonitrile as a replacement for methanol in normal-phase flash chromatography

John R. Bickler, bob.bickler@biotage.com. Biotage, LLC, Hampstead, North Carolina, United States

The use of methanol/DCM mobile phase has been used for decades to purify polar organic molecules by flash chromatography. However, this solvent system does pose some challenges including silica sloughing and gradient creation.

In this poster we show the advantages and benefits replacing methanol with acetonitrile can provide including more predictable separations and the elimination of silica degradation.

MEDI 492

NCI/NExT discovery HTS resources: Oncology Interrogation Tools Library

Raj N. Misra², *misrara@mail.nih.gov*, **Michael Eckert**¹, **Charles R. Johnson**³, **Christian Laggner**¹. (1) *Evotec (US) Inc, South San Francisco, California, United States* (2) *Rm 4W-118/MSB 9734, NIH/National Cancer Institute, Bethesda, Maryland, United States*

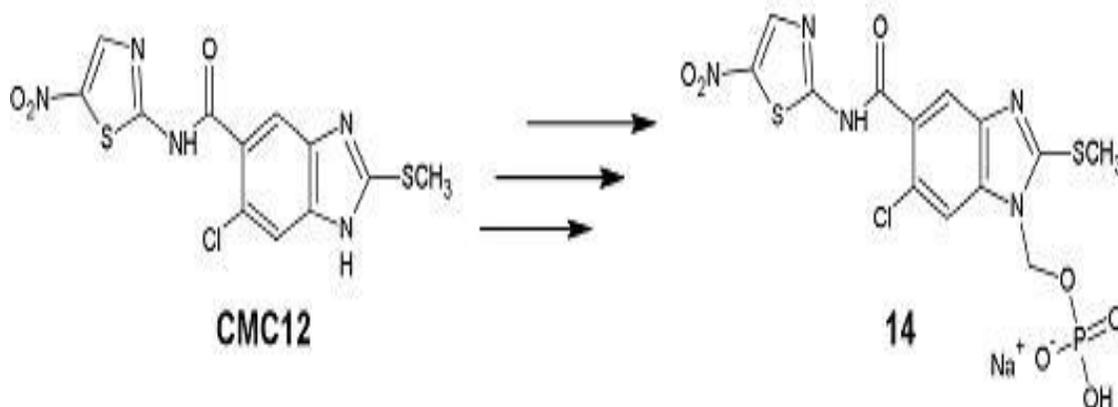
The NCI Experimental Therapeutics Program (NExT) is making available to drug discovery HTS investigators our Oncology Interrogation Tools screening library for research purposes. The library contains ~600 commercially-available compounds that can be used for general screening or as probes to facilitate interrogation of oncology-related biological pathways. Both historical compounds and newer investigational drugs that interact with intracellular signaling, growth factors and nuclear receptors are included. Annotation is provided indicating the relevant molecular target/pathway. The set is arrayed in single-use, 384-well plates. The NExT program (<https://next.cancer.gov/>) is a partnership between NCI's Division of Cancer Treatment and Diagnosis (DCTD) and the Center for Cancer Research (CCR). NExT consolidates NCI's anticancer drug discovery and development resources with the goal of maintaining a robust and balanced therapeutics pipeline. It encompasses tasks from new target validation through Phase III clinical trial evaluation. The Program is designed to streamline development and testing of promising new anticancer drugs and to expedite their delivery to the bedside. As part of the Program to support HTS drug discovery, NCI has previously offered academic oncology investigators access to our full 83K NExT Diversity Library in single-use, 384-well plate format. The diversity set was designed to identify lead small molecules for drug discovery programs. The details of the new set, Oncology Interrogation Tools, and procedures for accessing this resource are presented along with information on other available screening sets.

MEDI 493

Synthesis and solubility determination of a highly water soluble phosphonoxymethyl prodrug

Rafael Castillo-Bocanegra¹, *rafaelc@unam.mx*, **Jorge Victoria-Miguel**², **Alicia Hernandez Campos**², **Helgi Jung-Cook**². (1) *Farmacia, Div De Estudios De Posgrado, Mexico, Mexico* (2) *Departamento de Farmacia, Universidad Nacional Autónoma de México, Mexico, Mexico*

The use of prodrugs is an approach widely used to modify the physical and chemical properties of bioactive compounds such as the solubility. **CMC12** is a novel benzimidazole derivative with potent antiprotozoal activity but poor water solubility, which is the main obstacle to further research in vivo. Herein we present the synthesis and solubility studies of phosphonoxymethyl prodrug of **CMC12**. The new prodrug **14** improved the aqueous solubility of its precursor **CMC12** by 79,200 times.



MEDI 494

Inhibition of *Candida albicans* biofilm formation with biaryl amides

Daniel A. Hinojosa, *daniel.hinojosa@utsa.edu*. Chemistry Department, University of Texas at San Antonio, San Antonio, Texas, United States

Each year approximately 5% to 10% of all patients admitted to one of 7,000 acute care hospitals in the U.S will develop a health-care associated infection, which translates into a total of 2-4 million nosocomial infections. Fungal infections represent 10% of all nosocomial infections, yet account for up to 70% of all patient deaths. Of all fungal invasive infections, by far candidiasis remains the most common, now representing the 3rd to 4th most frequent nosocomial infection in hospitals in the US and worldwide, and *Candida albicans* remains the main etiological agent of candidiasis. Biofilm formations are being increasingly recognized as the main virulence factor contributing to the pathogenesis of candidiasis and are linked to most clinical manifestations. As such, biofilm formation represents a high value target for the development of novel antifungal agents. We used high throughput screening (HTS) techniques to identify small molecule compounds from Chembridge's Diverset™ chemical library, with inhibitory activity on *C. albicans* biofilm formation. Based on their IC₅₀ values (potency), lack of toxicity and efficacy in animal models of infection, we identified a series of biaryl compounds with the desired bioactivities. We hypothesized that specific structural changes guided by structure-activity relationship data would improve physical chemical properties such as lipophilicity (LogD), polar surface area (tPSA) and molecular weight (MW), while simultaneously removing potentially reactive functional groups such as activated olefins. These efforts produced structural analogs with biofilm IC₅₀ values in the 1-10 μM range and favorable "drug-like" physical chemical properties.

MEDI 495

Center for innovative in drug discovery collaborative programs: Structure based drug design, synthesis and evaluation of new antischistosomal agents

Reid Tarpley, *Reid.tarpley@utsa.edu. Chemistry, University of Texas at San Antonio, San Antonio, Texas, United States*

Schistosomiasis is a major human parasitic disease and a major cause of morbidity in 76 countries of the world where it afflicts more than 208 million people. Existing therapies such as Praziquantel (PZQ) or Oxamniquine (OXA) although efficacious, are showing reduced cure rates, while evidence for drug resistant strains is being established. Previous work at UTHSCSA identified a sulfotransferase as the gene responsible for OXA drug resistance and thus the hitherto unknown mode of OXA action. With this information X-ray structural studies helped establish the protein-drug interactions and paved a path for rational design of improved OXA derivatives that kill the three most prevalent species *S. mansoni*, *S. haematobium* and *S. japonicum*. Guided by X-ray crystallographic studies, the OXA structure and mechanism of action, structure-based drug design efforts are focused on the design of OXA-like analogs targeting *S. mansoni*, *S. haematobium* and *S. japonicum*. The design of new analogs, compound synthesis, structure-activity relationships, protein-drug interactions and *in vitro* assay results will be presented.

MEDI 496

Fragment based drug discovery of allosteric FAK inhibitors

Oswaldo Cossio¹, *osvaldocossio@yahoo.com*, **Ramon Campos-Olivas**³, **Clara Santiveri Martín-Varés**². (1) *Chemistry Department, University of Arkansas at Little Rock, North Little Rock, Arkansas, United States* (3) *Spanish National Cancer Research Centre (CNIO), Madrid, Spain*

The Focal Adhesion Kinase (FAK) is a cytoplasmic tyrosine kinase that plays a key role in cell matrix adhesion signals. Here, signaling is essential for cell migration and proliferation. In several advanced-stage solid cancers, FAK is overexpressed and helps to promote tumor invasion and metastasis. This discovery has led to much research on developing small molecule FAK inhibitors that target the FAK Kinase Domain ATP binding site. However, due to the similarity of ATP binding sites between different kinases this raises concern on the undesired toxicities that could result. Therefore, our research focuses on finding allosteric small molecule FAK inhibitors that target the kinase function but not the active ATP-binding site. To find novel allosteric small molecule FAK inhibitors, we employed the fragment-based drug discovery approach. A collection of about 500 fluorinated small molecules was screened against the FAK protein target with 1D 19F Nuclear Magnetic Resonance (NMR). To identify the binding between small molecules and FAK protein targets, the increase in the small molecules' 19F NMR signal linewidth was used. The hits identified in our research represent potential fragments that can be used to further develop them into lead compounds possessing drug-like properties against this protein overexpressed in cancer. Our future studies will involve further characterizing the hits with WaterLOGSY (WL) and saturation transfer difference (STD) NMR experiments, SPR analysis, and crystallography.

MEDI 497

Discovery of novel small molecule inhibitors of oncoprotein EYA2 for breast and ovarian cancers

Bismarck Campos², *bismarck.campos@utsa.edu*, **Stanton F. McHardy**², **Ambrosio Lopez**², **Hua-Yu Wang**², **Davante Wilson**², *Davante.Wilson@utsa.edu*, **Dirk Wristers**², *dirk.wristers@utsa.edu*, **Rong Li**¹, **Yuan Bin**¹, **Sabrina Smith**¹, **Shelby McCowen**³. (1) Department of Molecular Medicine Institute of Biotechnology, UTHSCSA, San Antonio, Texas, United States (2) Chemistry, CIDD-UTSA, San Antonio, Texas, United States (3) Chemistry, University of California, Berkeley, Berkeley, California, United States

Triple negative breast cancer (TNBC) is a subtype of breast cancer that lacks the expression of estrogen receptor α (ER α), progesterone receptor (PR), and HER2. While TNBC constitutes about 15% of all breast cancers, mortality of patients with TNBC is disproportionately higher than those with other subtypes of breast cancer. ER β is expressed in more than half of breast cancer cases across all major subtypes, thus providing the opportunity of stimulating its antitumor activity as a potential therapeutic approach. Previous work in Dr. Rong Li's lab (UTHSCSA) uncovered eye absent 2 (EYA2) as the tyrosine phosphatase that directly dephosphorylates pY36-ER β in vitro and in vivo. EYA2 is a known oncoprotein in ovarian and breast cancers. This work focused on testing the central hypothesis that pharmacological targeting of the oncoprotein EYA2 with small molecule inhibitors can effectively inhibit breast and ovarian cancer cell proliferation. Due to the EYA2 x-ray structural and modeling data available, along with some known small molecule inhibitors of EYA2, the program was perfectly positioned for designing, synthesizing and testing novel EYA2 inhibitors. This poster will highlight our collaborative program results, which has produced several new lead EYA2 inhibitors producing good inhibition (IC₅₀ 1-5 μ M) in the MTT assay using triple negative breast cancer cells MDA-MB-468. New compound design, synthesis, structure-activity relationships and in vitro data on lead analogs will be presented.

MEDI 498

Synthesis of N-functionalized chiral 3-hydroxyphenylpyrrolidines and their evaluation as selective D₃ receptor ligands

Anahid Omran, *aomran@siue.edu*, **Shakiba Eslamimehr**, **Albert M. Crider**, **William L. Neumann**. Pharmaceutical Sciences, Southern Illinois University Edwardsville, Edwardsville, Illinois, United States

Dysfunction of dopaminergic receptor signaling in the brain is a hallmark of a number of neurodegenerative pathologies. Of the five dopamine receptors, the D₃ subtype has emerged as a promising target for treating neurodegenerative diseases, especially Parkinson's disease, due to the specific distribution of this receptor in limbic and nigrostriatal brain regions known to be associated with motor functions. Not only have D₃-selective agonists shown positive effects in re-establishing control of motor activity in

animals, but they also display neuroprotective activity and may be important in reducing the dyskinesia side-effect often seen with current non-selective dopaminergic therapies. Herein we report the extension of our previous studies of racemic 3-hydroxyphenyl pyrrolidines to their chiral analogues. We have used our best racemic D₃ ligand, N-nonyl-3-hydroxyphenyl pyrrolidine **1** (K_i = 13 nM), as starting point. Synthesis, characterization and D₃ receptor affinity of both the R- and S-enantiomer of **1** will be reported. In addition, we will describe SAR studies of new chiral N-functionalized-3-hydroxyphenylpyrrolidines (based upon the steric requirements of compound **1**) in which the N-substituent has been designed to engage key residues in the secondary binding site of the D₃ receptor to enhance affinity and selectivity.

MEDI 499

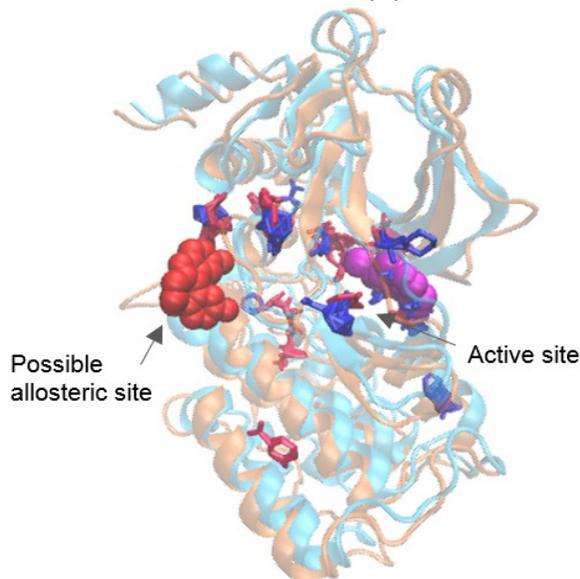
Anti-cancer drug discovery efforts target two kinases on the non-canonical NF-κB pathway

Garrett Chan², garrettjchan@gmail.com, *Ozlem Demir*¹, *Gourisankar Ghosh*³, *Rommie E. Amaro*². (2) *Chemistry and Biochemistry, University of California, San Diego, La Jolla, California, United States* (3) *Chemistry and Biochemistry, University of California, San Diego, La Jolla, California, United States*

The noncanonical NF-κB pathway regulates the transcription of the protein APOBEC3B (A3B), whose overexpression has been linked to cancerous behavior in cells and has been found in over 50% of breast cancer tumors. As such, inhibition of the proteins on the noncanonical pathway would diminish overexpression of A3B and prevent tumor evolution. Our goal is to develop selective inhibitors for two kinases on this pathway, inhibitor of IκB kinase 1 (IKK1) and NF-κB inducing kinase (NIK). Beginning with atomic-resolution crystal structures of our target proteins, we have used molecular dynamics (MD) to elucidate the behavior of these ligand-protein systems and virtual screening to identify leads for small molecule inhibitors. First, we have begun designing an inhibitor of IKK1. Provided with a novel crystal structure of the IKK1 complex, we were able to find druggable hotspots. Through cycles of virtual screening and biochemical assays with our collaborators, we have identified hits from a compound virtual library. From there, we have screened analogs and derivatives of the first round of actives to identify further hits. By focusing on a binding pocket away from the active site and expanding our field of potential actives, we aim to design an inhibitor that will be specific to IKK1.

Second, taking protein-ligand co-crystal structures, we have used MD to model the dynamics of NIK bound to existing but currently unpursued drugs. Analyzing our MD simulations, we have dissected the behavior of the NIK active site by looking at interactions of crucial residues, quantifying changes loop dynamics, and identifying cryptic side pockets. We have also identified a possible allosteric site, and demonstrated that the allosteric site-binding ligand induces similar behavior in NIK to that of the active site-binding ligand. We will use our novel models to improve on the selectivity and potency of NIK inhibitors.

Overlay of NIK crystal and MD structures with FTMap probes



MEDI 500

Novel mitochondrial complex I inhibitors for anti-cancer therapeutics

Jalisa Holmes¹, jholmes19@gsu.edu, **Krishna Damera**¹, **Joy Yancey**¹, **Mengyuan Zhu**¹, **Narra Devi**^{3,4}, **Stefan Kaluz**^{3,4}, **Erwin Van Meir**^{3,4}, **Binghe Wang**². (1) Chemistry, Georgia State University, Atlanta, Georgia, United States (2) Dept of Chem, Georgia State University, Atlanta, Georgia, United States (3) Winship Cancer Institute, Atlanta, Georgia, United States (4) Neurosurgery and Hematology & Medical Oncology, Emory University, Atlanta, Georgia, United States

Inhibition of mitochondrial complex I (NADH:ubiquinone oxidoreductase) has been shown to negatively affect proliferative tissue undergoing aerobic glycolysis due to the Warburg effect. The potential implications in cancer therapeutics led us to develop a diverse class of novel benzhydrol complex I inhibitors. Many of these analogues inhibit mitochondrial complex I as well as the hypoxia inducible factor 1 (HIF-1) pathway, an important transcription factor for cell survival in low oxygen concentrations. We will present qualitative and quantitative structure-activity relationships that may provide some insights into future direction for establishing new analogues as well as to establish the proposed mode of *in vivo* cancer treatment.

MEDI 501

Efforts to expand our antibiotic arsenal to eradicate persistent bacterial biofilms

Robert Huigens, *rhuigens@cop.ufl.edu*. University of Florida, Gainesville, Florida, United States

Bacterial biofilms are surface-attached communities of bacteria that pose a significant threat to human health as 17 million new biofilm-associated bacterial infections occur annually that result in 550,000 deaths in the United States. Biofilms are home to metabolically dormant, non-dividing persister cells encased within a protective extracellular polymeric matrix of biomolecules and display tolerance to every known class of antibiotic. Biofilms are associated with numerous human diseases, including: infections of indwelling devices, burn wounds, catheter infections, endocarditis, cystic fibrosis, urinary tract infections and gingivitis. Despite the urgent need for clinical agents to effectively kill persistent biofilms, no biofilm-eradicating therapeutic exists. With the significant impact that biofilms have on human health, primary interests in anti-biofilm small molecules have focused on biofilm inhibitors/dispersal agents that operate through non-bactericidal mechanisms via the targeting of proteins involved in bacterial communication. Conversely, biofilm-eradicating agents hit bacterial targets critical to non-replicating persister cells and can ideally be used as stand-alone biofilm-killing agents. We have discovered a synthetically tunable series of small molecules inspired by the marine phenazine antibiotic 2-bromo-1-hydroxyphenazine that demonstrates potent, broad-spectrum biofilm-eradicating activities. These small molecules kill persister cells through a unique mechanism of action which is critical in the development of improved biofilm control strategies. Providing new mechanistic insights into persister cell viability and advancing the first biofilm-eradicating therapeutic agent to the clinic would lead to ground-breaking cures for persistent bacterial infections.

MEDI 502

Design, synthesis, and biological evaluation of heteroaryl amine derivatives for anticancer activity

Mousumi Besan, *mousumibesan@gmail.com*, Radhey Shyam Srivastava, Sushant Srivastava. Department of Pharmaceutics, Indian Institute of Technology, Banaras Hindu University, Varanasi, Varanasi, Uttar Pradesh, India

Histone deacetylases are the enzymes which involved in remodeling of chromatin through deacetylation on the surface of histone protein. The reestablishment of the positive charge which is disrupted by the negative charge formed by the acetylases that is catalyzed by HDACs and it is thought to tighten the interaction between histones and DNA. Ultimately leading to blocking the binding sites on protomer thus, inhibiting gene transcription. HDAC inhibitors are proficient to interact with the catalytic domain of histone deacetylases to block the substrate recognition ability of these HDACs resulting in restoration of the expression of relevant genes. Therefore, it was considered of interest to design, synthesis and biological evaluation of 1, 3, 4 - thiazole derivatives which mimic the basic structural feature of MS-275. The designed compounds were synthesized and characterized by using various analytical tools such as fourier transform infrared spectroscopy (FT-IR), proton NMR and mass spectroscopy. Among

all the compounds the MB-3 was most active and good binding affinity which have a meta nitro group in the ring while the MB1 was found inactive, which is without any substitution in the benzene ring. MB-3 was found to have anticancer activity with GI_{50} 35 μ M against HCT116 (Colon) and U251 (Glioma) cells.

MEDI 503

Luminescent Conjugated Oligothiophenes (LCOs) for detection and characterization of disease-associated protein aggregates and cells

Marcus Bäck, *marcus.back@liu.se*. Chemistry, IFM, Linköping University, Linköping, Sweden

Many neurodegenerative diseases including Alzheimer's and Parkinson's are associated with damaged neurological tissues that arise from the occurrence of disease-specific misfolded and aggregated proteins and peptides. Molecules with specific binding properties to such aggregates are highly desired as tools for diagnostics and treatment. We have previously described how specific thiophene based oligomeric structures (Luminescent Conjugated Oligothiophenes, LCOs) can be used for the detection of premature protein fibrils preceding mature amyloids, and for the discrimination between the two main pathological hallmarks of Alzheimer's disease, Ab plaques and neurofibrillary tangles. Also, when using modified LCOs with specific side chains differential staining of various cell types including cancer cells was achieved. Recently the character of a tetramer oligothiophene scaffold has been altered by the introduction of functional groups with different electron donating and withdrawing effects. This resulted in the discovery of a molecule with strikingly high affinity for Alzheimer's disease brain-derived A β .

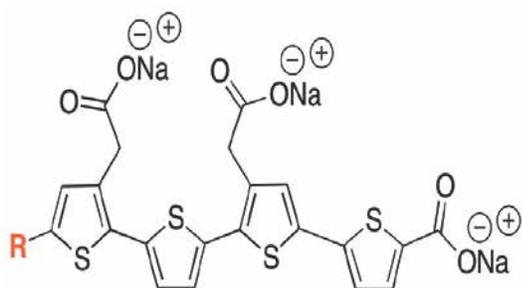


Figure 1. General structure of a tetrameric oligothiophene carrying different substituents at one of the α -terminal positions of the thiophene backbone

MEDI 504

Drugging the undruggable with MCR scaffold manifold: The design and synthesis of covalent inhibitors and macrocycles

Tryfon Zarganes-Tzitzikas¹, *ttzitzikas@yahoo.com*, **Pravin Patil**¹, **Alexander Doemling**^{2,1}, **Konstantinos Neochoritis**¹. (1) TelesisPharma B.V., Thessaloniki, Greece (2) Department of Drug Design, University of Groningen, Groningen, Netherlands

High-throughput screening (HTS) is one of the most utilized methods of identification of chemical probes and drug leads in today's drug discovery of many pharma companies. However the limitations and pitfalls of HTS technologies are more obvious than ever. The discovery of novel scaffolds and exploitation of the still high-degree unknown chemical space for the unmet medical needs is urgent. Companies more than ever need highly customized libraries based on the needs of their in-house biophysical screening and co-crystal structure analysis.

TelesisPharma, based on its unique experience in Multi-Component Reactions (MCRs) chemistry engineers highly functionalized small molecule and macrocyclic scaffolds in 1 to 3 synthetic steps.

Amongst the scaffold manifold that we can easily access and demonstrate the power of MCRs, TelesisPharma reassesses an important class of drugs: Covalent inhibitors. We design and synthesize covalent target binders based on unique and yet underexplored scaffolds equipped with a variety of electrophilic warheads, to target mutant proteins, e.g. *KRAS (G12/13C)*, *p53 (Y220C)*, *IDH1 (R132C)* or *DNMT3a (R882C)*. Moreover, we present an efficient synthesis and virtual screening of macrocyclic libraries. Competent access to hundreds of macrocyclic scaffolds using MCRs will be presented.

MEDI 505

Synthesis of novel agents for the treatment of neurodegenerative diseases

Benjamin J. Eduful¹, *beduful@mail.usf.edu*, **James Leahy**², **David Kang**³, **Melissa Chin**¹, **Arianna Rashedi**¹, **Ousman Jallow**¹. (1) Chemistry, University of South Florida, Tampa, Florida, United States (3) USF Health Byrd Alzheimer's Institute, University of South Florida, Tampa, Florida, United States

Notwithstanding the great advances made by drug discovery scientists in transforming deadly human diseases into curable ones, treatment options for neurodegenerative diseases such as Alzheimer's disease (AD) are either ineffective or not available. AD is the leading cause of dementia and the most prevalent neurodegenerative disease, affecting more than 40 million people worldwide and has been classified as the sixth leading cause of death in the United States. It results from the accumulations of two highly toxic proteins (β -amyloid and tau) in the brain. There is currently no cure for AD, and available treatment options are only symptomatic. To this end, we have identified and synthesized a novel class of agents active against Slingshot (SSH1) - a protein believed to contribute to the formation of β -amyloid peptides in people afflicted with AD.

MEDI 506

Cyclic peptides containing tryptophan and arginine residues for targeting and delivery of anticancer agents to tumor cells

Keykavous Parang, *parang@chapman.edu*, Shaban Darwish, Rakesh Tiwari. School of Pharmacy, Chapman University, Irvine, California, United States

Cyclic peptides containing alternative tryptophan and arginine residues $[WR]_n$ ($n = 4,5$) significantly improved the cellular uptake of a number of anti-HIV and anti-cancer drugs. The cellular uptake of $[WR]_5$ was found to be independent of the endocytotic pathway. The fluorescence-labeled conjugate of the peptide was localized in the nucleus. Cy5.5 labeled $[W_5R_3K_2]$ conjugated with folate preferentially accumulated in folate receptor overexpressing HeLa cells xenograft mice model. Furthermore, folic acid-PEG-cyclic peptide-doxorubicin labeled with VivoTag680XL was synthesized. PEG and folic acid were used for improving the stability and tumor targeting, respectively. A tumor xenograft model, HT-29 colorectal cancer flank implantation in nu/nu mice, was used to provide a folate-receptor-expressing model for assessing a folate-targeted drug delivery. The conjugate was localized in HT-29-RedFLuc (red-shifted luciferase) tumor cells within 1 hour. Thus, this system containing imaging, targeting, and an anticancer drug can be used for active targeting and imaging of tumor cells.

MEDI 507

Artificial macrocycles by multicomponent reactions

Alexander Doemling, *a.s.s.domling@rug.nl*. Department of Drug Design, University of Groningen, Groningen, Netherlands

Macrocycles promise to populate a chemical space in between small molecules and biologics, while combining the best of both worlds. However, for the regular use of synthetic macrocycles in drug discovery, three main problems have to be solved: 1) The extremely difficult access towards a large and diverse macrocyclic chemical space; 2) the majority of MCs does not show sufficient passive membrane permeations, a prerequisite to discover molecules for intracellular targets like protein-protein interactions and to have the option to develop oral medications with drug-like properties; 3) the potential chemical space of MC is very poorly reflected in the current screening collections in terms of numbers and diversity.

We have recently devised multiple novel ways to close macrocyclic rings starting from simple building blocks such as α -amino- ω -carboxylic acids, α -amino- ω -isocyanides, α -formyl- ω -carboxylic acids or α -formyl- ω -isocyanides and using multicomponent reactions (Ugi, Passerini and others). In order to address the issues of diversity, ring size, synthetic generality, ease-of-access (low numbers of sequential steps) and library size (size of chemical space) we recently introduced the concept of 'Union' of MCRs as a synthetic platform to rapidly access diverse MC (Fig. 1). In this concept the ring atom diversity is assembled by a MCR-1 and suitable classical two component reactions in a way that two side-chain functional groups can be used for macro-ring closure via a second MCR-2, etc. Chemistry, 3D structures and permeabilities as well as applications towards demanding targets such as IL17A and PD1-PDL1 will be discussed.



Synthetic platform to rapidly access diverse macrocycles by the union of two MCRs.

MEDI 508

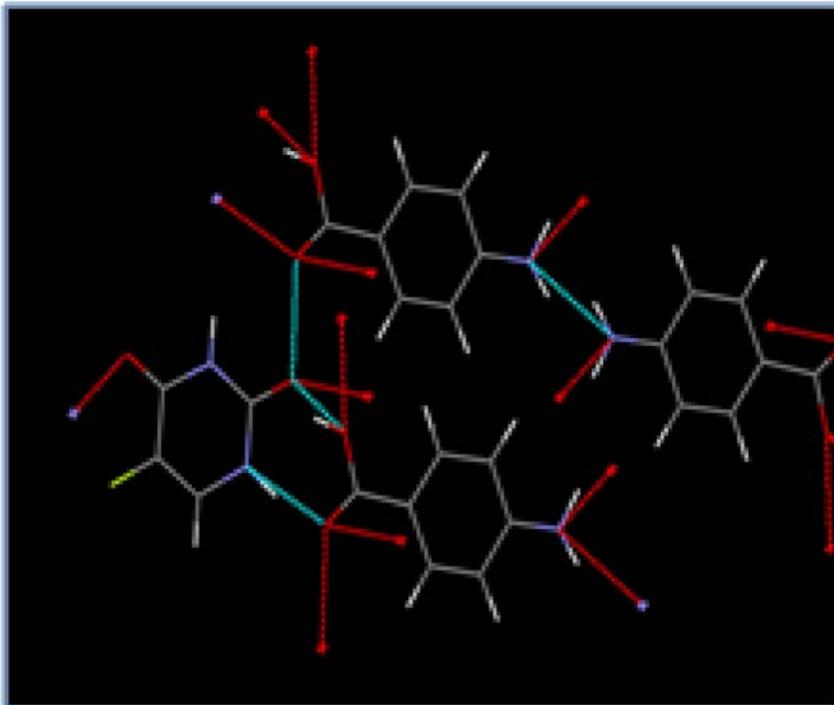
Cocrystal of 5-fluorouracil with nicotinamide to improve its biopharmaceutical attributes using crystal engineering approach

Manoj K. Gautam, *manojgautamup@gmail.com*, Renu Chadha. *Pharmaceutical Chemistry, University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh, Chandigarh, Chandigarh, India*

Pharmaceutical cocrystallization is proposed as a novel approach to improve biopharmaceutical attributes of BCS class III drug such as membrane permeability and solubility. In this regard, 5-fluorouracil (5-FU) has been taken as a model drug to prepare its cocrystal using nicotinamide (NT) as a cofomer. The aim of the present study is focused on the preparation, characterization and biopharmaceutical evaluation of cocrystal (5-FUNT).

Characterization of the prepared cocrystal was done by using various analytical tools such as differential scanning calorimetry (DSC), fourier transform infrared spectroscopy (FT-IR), powder X-ray diffraction (PXRD). DSC scan of 5-FUNT shows the appearance of single endothermic transition at 166°C which is different from the melting peaks of drug (284°C) and cofomer (128°C) that indicate the formation of new phase. FT-IR study indicated amide–amide interaction between the participating molecules and appearance of new peaks in PXRD pattern confirms the formation of new cocrystal (5-FUNT). Crystal structure of 5-FUNT was determined using material studio software (Biovia) from PXRD data and revealed triclinic crystal system, P-1space. Silicon membrane, equilibrium solubility and disk intrinsic dissolution rate of 5-FUNT showed improvement in permeability and solubility as compared with pure drug.

Pharmacokinetic studies were revealed 2 fold improvement in relative bioavailability than 5-FU. Thus cocrystallization approach has potential in ameliorating the dissolution limited bioavailability and permeability of BCS class III drug.



MEDI 509

Solving challenging structural motifs in natural products using concerted DFT modeling and 2-D INADEQUATE NMR

Jacob R. Powell², **Tyler M. McCullough**¹, tymac1029@gmail.com, Robbie Iulicci¹, James K. Harper². (1) Chemistry, Washington & Jefferson College, Washington, Pennsylvania, United States (2) Chemistry, University of Central Florida, Orlando, Florida, United States

Natural products and their derivatives continue to provide a novel source for medicinal compounds used to combat illnesses including cancer, malaria, and Alzheimer's. In modern applications, the pharmaceutical industry commonly utilizes the structure of natural products as models on which combinatorial chemistry and molecular biology can be applied. NMR measurement of $^1J_{CC}$ coupling by 2-D INADEQUATE allows for efficient structure elucidation in hydrogen poor compounds and can be measured in a single experiment at natural ^{13}C abundance. Additionally, modern DFT computations of model compounds are functionally accurate with B3LYP/EPR-III $^1J_{CC}$ calculations correlating with experimental data. By combining theoretical predictions with experimental INADEQUATE $^1J_{CC}$ coupling values, challenging structural problems can be solved. 5-methylmellein, a DNA Polymerase I inhibitor, hydroheptelidic acid, which displays antitumor and antimalarial activity, and austrocortinin, an anthraquininoid pigment, are three natural products extracted from endophytic fungi found in the Central Florida area with potential medical applications. Structural elucidation of these compounds was performed in order to evaluate the accuracy of this concerted model; particularly, by aiming to solve difficult structural motifs including strong intramolecular

hydrogen bonding, verifying heteroatom identity, assigning double bond configuration, and assigning the correct tautomeric form. In all cases, the structural ambiguities were resolved and single best-fit structures were uniquely identified; verifying that such motifs can be accurately modeled.