



Division of Medicinal Chemistry  
Scientific Abstracts  
for the  
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**American Chemical Society  
Division of Medicinal Chemistry  
254th ACS National Meeting, Washington, DC, August 20-24, 2017 Fall  
Meeting**

**A. Stamford, Program Chair**

**SUNDAY MORNING**

**Treatment of Chronic Neuropathic Pain**

K. A. Jacobson, Organizer; D. Salvemini, Organizer; K. A. Jacobson, Presiding; D. Salvemini, Presiding Papers 1-6

**General Orals**

A. W. Stamford, Organizer; J. R. Allen, Presiding Papers 7-17

**SUNDAY AFTERNOON**

**General Orals**

A. W. Stamford, Organizer; A. W. Stamford, Presiding Papers 18-26

**Biophysical Methods in Drug Discovery**

M. J. Blanco, Organizer; N. A. Meanwell, Organizer; P. M. Scola, Organizer; K. Yeung, Organizer; N. A. Meanwell, Presiding; P. M. Scola, Presiding; K. Yeung, Presiding Papers 27-31

**SUNDAY EVENING**

**General Posters**

A. W. Stamford, Organizer; Papers 32-204

**MONDAY MORNING**

**Insights on Medicinal Chemistry from Hardcore Practitioners**

J. Barrow, Organizer; J. Barrow, Presiding Papers 205-209

**Addiction: The Unmet Medical Need of the 21st Century**

M. J. Blanco, Organizer; J. V. Aldrich, Organizer; M. J. Blanco, Presiding Papers 210-214

**MONDAY AFTERNOON**

**Encoded Technologies for Lead Generation, Successes & Challenges**

H. Deng, Organizer; K. Leftheris, Organizer; J. Messer, Organizer; N. V. Prabhu, Organizer; J. Messer, Presiding; K. Leftheris, Presiding Papers 215-220

**Off Targets No More: CYP450 Enzymes as Drug Discovery Targets**

S. B. Hoyt, Organizer; S. Hoyt, Presiding Papers 221-225

**MONDAY EVENING**

**Sci-Mix**

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**TUESDAY MORNING**

**Award Symposium**

A. W. Stamford, Organizer; W. B. Young, Presiding Papers 226-232

**Recent Advances in the Treatment of HIV-1 Infection & Approaches to a Cure**

N. A. Meanwell, Organizer; B. N. Naidu, Organizer; S. Runyon, Organizer; N. A. Meanwell, Presiding; B. N. Naidu, Presiding; S. Runyon, Presiding; E. Velthuisen, Presiding Papers 233-238

**TUESDAY AFTERNOON**

**Recent Advancements & Therapeutic Opportunities in Muscarinic Receptors**

R. Mazzola, Organizer; M. P. Bourbeau, Organizer; R. Mazzola, Presiding; M. P.

Bourbeau, Presiding Papers 239-244

**General Orals**

A. W. Stamford, Organizer; J. Ramanjulu, Presiding Papers 245-253

**WEDNESDAY MORNING**

**First Time Disclosure of Clinical Candidates**

J. B. Schwarz, Organizer; J. B. Schwarz, Presiding Papers 254-258

**Unusual Protein-Ligand Interactions in the Design of Novel Pharmaceuticals**

D. F. Ortwin, Organizer; H. E. Purkey, Organizer; H. E. Purkey, Presiding Papers 259-264

**WEDNESDAY AFTERNOON**

**First Time Disclosure of Clinical Candidates**

J. B. Schwarz, Organizer; J. B. Schwarz, Presiding Papers 265-269

**General Orals**

A. W. Stamford, Organizer; A. Ali, Presiding Papers 270-280

**WEDNESDAY EVENING**

**General Posters**

A. W. Stamford, Organizer; Papers 281-366

## MEDI 1

### Purine receptors as drug targets in pain

**Kenneth A. Jacobson<sup>3</sup>, kajacobs@helix.nih.gov, Dilip K. Tosh<sup>1</sup>, Antonella Ciancetta<sup>1</sup>, Daniela Salvemini<sup>2</sup>.** (1) *Lab of Bioorganic Chem Msc-0810, NIDDK, NIH, Bethesda, Maryland, United States* (2) *Department of Pharmacological and Physiological Science, St. Louis University, St. Louis, Missouri, United States* (3) *Laboratory of Bioorganic Chemistry, NIDDK, National Institutes of Health, Bethesda, Maryland, United States*

Extracellular nucleosides and nucleotides have widespread functions in controlling physiological homeostasis. The “purinome” encompasses four G protein-coupled receptors (GPCRs) for adenosine, eight GPCRs activated by nucleotides (P2YRs), seven ATP-gated P2X ion channels, and the associated enzymes and transporters that regulate the levels of the native agonists. Modulators of purinergic signaling, such as receptor agonists and antagonists, have potential for treating chronic pain. Signaling molecules acting at P2X<sub>3</sub>, P2X<sub>4</sub>, P2X<sub>7</sub> and P2Y<sub>12</sub> receptors play critical roles in maladaptive pain neuroplasticity. Recent efforts have provided structurally novel ligands for these nucleotide receptors. Adenosine and its analogues potently suppress nociception in preclinical models by activating A<sub>1</sub> and/or A<sub>3</sub> adenosine receptors (ARs), but safely harnessing this endogenous pathway to clinically treat pain has not been achieved. However, we discovered that this can be achieved through selective targeting of A<sub>3</sub>AR (1,2) spromting exploration of the structure activity relationship of nucleoside derivatives at this subtype using a computational structure-based approach. Novel A<sub>3</sub>AR agonists for pain control that contained a bicyclic ring system (bicyclo[3.1.0]hexane) in place of ribose were designed and screened using an in vivo phenotypic model (3), which reflected both pharmacokinetic and pharmacodynamic parameters. High specificity (>10,000-fold selective for the A<sub>3</sub>AR) was achieved with the aid of receptor homology models based on related GPCR structures. These A<sub>3</sub>AR agonists were well tolerated in vivo and highly efficacious in models of chronic neuropathic pain.

## MEDI 2

### A<sub>3</sub> adenosine receptor subtype agonists as novel non-narcotic analgesics for neuropathic pain

**Daniela Salvemini<sup>2</sup>, salvemd@slu.edu, Kenneth A. Jacobson<sup>1</sup>, Dilip K. Tosh<sup>3</sup>, Gary Bennett<sup>4</sup>.** (1) *Lab of Bioorganic Chem Msc-0810, NIDDK, NIH,*

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Chronic neuropathic pain is a huge unmet medical need affecting millions of individuals throughout the world. Neuropathic pain is poorly managed and novel targeted-based therapeutics are needed. Adenosine has long been known to exert potent analgesia in animal models of neuropathic pain and there are proof-of-concept clinical trials in patients with neuropathic pain. The beneficial effects exerted by adenosine were thought to be driven mainly by the A<sub>1</sub>AR and perhaps by A<sub>2A</sub>AR; however strategies targeting these receptors subtypes failed due to cardiovascular side effects. We have discovered that adenosine can also exert its beneficial effects through the A<sub>3</sub>AR and that selective A<sub>3</sub>AR agonists block and reverse neuropathic pain in several models of neuropathic pain without the development of analgesic tolerance and without discernable side effects. The beneficial effects are independent of the endogenous opioid and endocannabinoid systems. A<sub>3</sub>AR agonists are more potent than currently used analgesics including opioids; moreover when given in combination they enhance the efficacy and potency of opioids, gabapentinoids, and NE/5HT re-uptake blockers. Noteworthy, A<sub>3</sub>AR agonists block the development of opioid-induced tolerance and opioid-induced hyperalgesia, block the acquisition of morphine-induced Conditional Place Preference (an indicator of abuse potential), and greatly reduce the signs of addiction (naloxone-evoked withdrawal). Beneficial effects are driven by inhibition of astrocyte-driven neuroinflammation via an increase in spinal levels of IL-10 and also by inhibition of mitochondrial dysfunction. A<sub>3</sub>AR agonists for clinical development have been identified. We anticipate that these finding will have a major impact on the treatment of neuropathic pain.

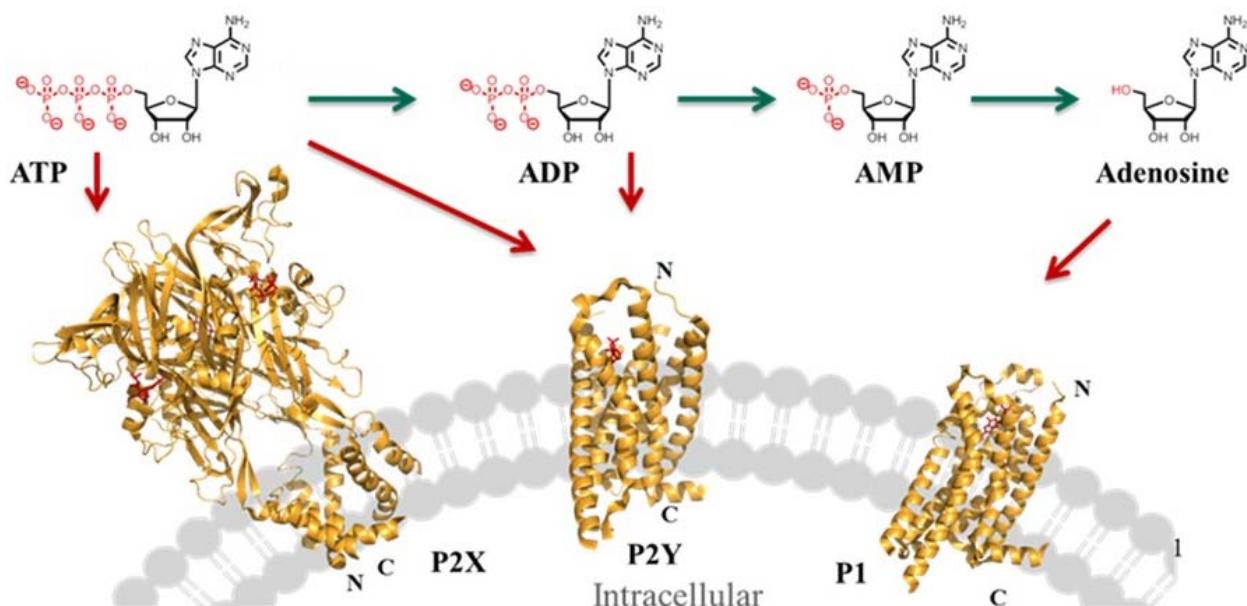
### **MEDI 3**

#### **Design of new antagonists of P2X and P2Y receptors**

**Christa E. Mueller**<sup>1,2</sup>, christa.mueller@uni-bonn.de. (1) Pharmaceutical Chemistry, University of Bonn, Bonn, Germany (2) PharmaCenter Bonn, Bonn, Germany

Nucleotides such as ATP, ADP, UTP and UDP are important extracellular signalling molecules, that activate two classes of cell membrane receptors: G protein-coupled P2Y receptors (subtypes: P2Y<sub>1,2,4,6,11,12,13,14</sub>) and ligand-gated ion channels termed P2X receptors (subtypes: P2X<sub>1-7</sub>). These receptors are

involved in inflammatory processes including neuroinflammation and pain sensation. The P2X4 receptor subtype has been proposed as a novel drug target for neuropathic and inflammatory pain. We have developed allosteric modulators for P2X receptor subtypes with a focus on P2X4 antagonists. Moreover we identified and characterized allosteric binding sites on the P2X4 receptor using a combination of mutagenesis studies and molecular modeling based on published X-ray structures. Recently, we succeeded in developing the first potent and selective antagonist for the UTP-activated P2Y<sub>4</sub> receptor. The new tool compounds will allow target validation studies and may serve as lead compounds for drug development.



Purinergic receptors: P2X, P2Y and adenosine receptors

#### MEDI 4

#### **Endocannabinoid system as a target for neuropathic pain treatment**

**Alexandros Makriyannis**, [a.makriyannis@northeastern.edu](mailto:a.makriyannis@northeastern.edu). Center for Drug Discovery, Northeastern University, Boston, Massachusetts, United States

The CB2 cannabinoid receptor is recognized as a validated therapeutic target. We have shown that activation of the CB2 cannabinoid receptor can have a therapeutic effect in the treatment of neuropathic pain. To this effect, we have developed novel cannabinergic ligands possessing excellent binding and functional properties for CB2 over the CB1 receptor. This has been the basis for the development of high-quality drug leads that lack the undesirable CNS

effects of CB1.

Our novel proprietary compounds have shown very robust efficacy in rodent models of chemotherapy-induced neuropathy. Gabapentin, the compound currently used to treat conditions involving neuropathy, exhibits a delayed analgesic effect in rodents. By contrast, our CB2 ligands, including AM1710, act immediately and do not have this therapeutic limitation. When AM1706 injected through a mini-pump, its effects continuously persist even after the treatment is discontinued. I shall discuss the experiments that confirm the CB2 effects in appropriate preclinical models.

## MEDI 5

### **Benzo[c][2,7]naphthyridin-5(6H)-one and 5H-chromeno[3,4-c]pyridine as potent inhibitors of a novel serine/threonine kinase for the potential treatment of neuropathic pain**

**Carolyn D. Dzierba**, carolyn.dzierba@bms.com. Bristol Myers Squibb Co, Wallingford, Connecticut, United States

Effective treatment of chronic pain, in particular neuropathic pain, is a significant unmet medical need. Current standard of care compounds have limited efficacy and are often associated with dose limiting side-effects, such as dizziness, drowsiness, and fatigue. We recently identified adaptor associated kinase 1 (AAK1), also known as AP2-associated protein kinase 1, as a potential novel therapeutic target for neuropathic pain. AAK1 is a member of the Ark1/Prk1 family of serine/threonine kinases and plays a role in modulating receptor endocytosis. AAK1 had not previously been associated with neuropathic pain. It was found that AAK1 knock-out mice exhibit reduced pain behavior in the formalin assay for persistent pain and a reduced neuropathic pain response (mechanical allodynia) in the Chung model for neuropathic pain without producing motor side effects. This presentation will cover the discovery, synthesis, and *in vivo* evaluation of AAK1 inhibitors in the benzo[c][2,7]naphthyridin-5(6H)-one and 5H-chromeno[3,4-c]pyridine class.

## MEDI 6

### **Biasing opioid receptor signaling away from opiate side effects**

**Laura M. Bohn<sup>1</sup>**, lbohn@scripps.edu, Thomas D. Bannister<sup>2</sup>. (1) Molecular Medicine & Neuroscience, The Scripps Research Institute, Jupiter, Florida, United States (2) #3A1, The Scripps Research Institute-Florida, Jupiter, Florida, United States

GPCRs can signal via multiple pathways and in the endogenous environment, these pathways may vary depending on where the receptor is expressed. The key challenge is to determine how receptors function *in vivo* to mediate diverse physiologies, depending on where they are located, and how they signal. Early genetic knockout studies support a hypothesis that an agonist that promotes mu opioid receptor (MOR) coupling to G protein pathways without beta-arrestin2 recruitment might be a way to promote antinociception while limiting the side effects associated with opiate narcotic analgesics. Therefore, we have developed several selective MOR biased agonists designed to activate G protein signaling pathways over beta-arrestin2 pathways and have investigated whether divergent signaling *in vitro* can predict divergent physiologies in mouse models of opioid responsiveness. Our studies show that for such compounds, G protein signaling is preserved *in vivo* as well as the antinociceptive properties in mice. However, respiratory suppression is greatly attenuated. A correlation between calculated bias factors, used for ranking compound selection, and the widening of the therapeutic window *in vivo* will be discussed.

## MEDI 7

### **6-((2-Oxo-1-substituted-1,2-dihydropyridin-3-yl)amino)imidazo[1,2-b]pyridazine derivatives as potent, selective, and orally active Tyk2 JH2 inhibitors**

**Chunjian Liu**, chunjian.liu@bms.com, James Lin, Ryan Moslin, John S. Tokarski, Jodi Muckelbauer, Hyunsoo Park, Peng Li, Dauh-Rurng Wu, Joann Strnad, Adriana Zupa-Fernandez, Lihong Cheng, Charu Chaudhry, Christine Huang, Jing Chen, Cliff Chen, Huadong Sun, Paul Elzinga, Celia D'arienzo, Kathleen Gillooly, Tracy L. Taylor, Kim W. McIntyre, Luisa M. Salter-Cid, Louis Lombardo, Percy H. Carter, Nelly Aranibar, James R. Burke, David S. Weinstein. Bristol-Myers Squibb, Princeton, New Jersey, United States

Tyrosine kinase 2 (Tyk2), a member of the Janus kinase (JAK) family of non-receptor tyrosine kinases, regulates the signaling of pro-inflammatory cytokines IL-12, IL-23 and type 1 IFN, and is therefore believed to be a promising small molecule target for developing orally active therapeutic agents for the treatment of autoimmune and inflammatory diseases such as psoriasis and lupus. We are particularly interested in Tyk2 JH2 (pseudokinase domain) due to its unique structural feature in the binding pocket, allowing us to target the Tyk2 dependent signaling pathway with excellent selectivity. In this presentation, discovery of 6-((2-oxo-1-substituted-1,2-dihydropyridin-3-

yl)amino)imidazo[1,2-b]pyridazine derivatives as potent, selective, and orally active Tyk2 JH2 inhibitors will be described.

## MEDI 8

### **Discovery of small molecule protease-activated receptor 2 (PAR2) antagonists and agonists using DNA-encoded library (DEL) screening technologies**

**Dean G. Brown<sup>1</sup>, dean.brown@astrazeneca.com, Andrew Ferguson<sup>1</sup>, Hongming Chen<sup>1</sup>, Linda Sundstrom<sup>1</sup>, Stefan Geschwinder<sup>1</sup>, Arjan Snijder<sup>1</sup>, Maria Saxin<sup>1</sup>, Jing Zhang<sup>1</sup>, Ye Wu<sup>1</sup>, Holly Souter<sup>2</sup>, Dawn M. Troast<sup>2</sup>, Christoph Dumelin<sup>3</sup>, Giles A. Brown<sup>3</sup>, Robert K. Cheng<sup>3</sup>, Cedric Fiez-Vandal<sup>3</sup>, Robert Cooke<sup>3</sup>, Rudi Prihandoko<sup>3</sup>, Benjamin Tehan<sup>3</sup>, Giselle Wiggin<sup>3</sup>, Andrei Zhukov<sup>3</sup>, Miles S. Congreves<sup>3</sup>, Barry Teobald<sup>3</sup>, Oliver Schlenker<sup>3</sup>, Qingqi Liu<sup>5</sup>, Wenzhen Yang<sup>5</sup>, Rongfeng Chen<sup>5</sup>, Shawn Johnstone<sup>4</sup>, Roland Burli<sup>1</sup>, Niek Dekker<sup>1</sup>.** (1) AstraZeneca Pharmaceuticals, Waltham, Massachusetts, United States (2) X-Chem Inc., Waltham, Massachusetts, United States (3) Heptares Therapeutics Ltd, Welwyn Garden City, Hertfordshire, United Kingdom (4) IntelliSyn Pharma, Saint-Laurent, Quebec, Canada (5) Pharmaron Beijing Co., Beijing, China

PAR2 is a G-protein coupled receptor (GPCR) known to mediate inflammatory pathways and is implicated in several diseases such as pain, airway inflammation, and skin disorders. We report a novel series of antagonists discovered by DEL screening of purified and stabilized PAR2. Optimization of this series resulted in potent and selective compounds which demonstrated antagonism across a range of PAR2 cellular models. The crystal structure of the stabilized receptor in complex with a member of this series revealed an allosteric binding mode at the surface of the receptor within the hydrophobic membrane spanning region. In addition, another series was identified by DEL screening of an unknown binding mode. Chemical expansion of this series resulted in potent and selective agonists of PAR2. Thus, DEL screening in combination with GPCR stabilization technology and crystallography, has led to novel antagonists and agonists of PAR2. These compounds have proven to be useful tools in helping to understand the complex pharmacology of this receptor.

## MEDI 9

### **Creating the ideal vaccine formulation: Attenuating inflammation while maintaining the adaptive response**

**Brittany Moser**, [bamoser@uci.edu](mailto:bamoser@uci.edu), **Rachel C. Steinhardt**, **Aaron P. Esser-Kahn**. Chemistry, University of California, Irvine, Irvine, California, United States

The design of successful vaccines depends on eliciting a strong innate immune response to potentiate the adaptive immune system. Designing safe and effective adjuvants requires a thorough understanding of the signaling pathways that drive an innate immune response. Although many Toll-like receptor agonists have demonstrated great promise in eliciting protective responses, few have made it through clinical trials due the systemic inflammatory response they induce. The ideal adjuvant would activate the immune response and elicit an adaptive response, but not cause a systemic inflammatory response. The innate immune response is controlled, in part, by the transcription factor nuclear factor k-B (NF-kB), which primes the transcription of proinflammatory genes, cell surface makers or antiviral responses. NF-kB is composed of two subunits—a DNA binding domain and a transactivation domain. There are three possible DNA binding domains RelA, RelB and RelC and three possible transactivation domains p65, p50 and p52. By blocking one or more of these subunits from entering the nucleus, we have shown that it is possible to shift the immune response to decrease inflammation while maintaining or increasing cell surface receptors necessary for communication with adaptive immune cells. We have demonstrated success of this method using common Toll-like receptor agonists in immortalized and primary mouse cells as well as demonstrated a variety of protective responses *in vivo*. The results of these experiments will guide the design of next-generation vaccine formulations.

## MEDI 10

### High confidence protein-ligand complex modeling by NMR-guided docking enables early hit optimization

**Andreas Lingel<sup>1,2</sup>**, [andreas.lingel@novartis.com](mailto:andreas.lingel@novartis.com), **Dirksen Bussiere<sup>1</sup>**, **Andrew Proudfoot<sup>1</sup>**. (1) Global Discovery Chemistry, Novartis Institutes for Biomedical Research, Emeryville, California, United States (2) Global Discovery Chemistry, Novartis Institutes for Biomedical Research, Basel, Switzerland

Structure-guided optimization of ligands engaging with a specific molecular target is a key approach in drug discovery today. As a prerequisite, detailed knowledge of the types and quality of interactions, ligand and protein geometry, as well as available nearby space and interaction opportunities is necessary. Currently, X-ray crystallography is by far the most applied

technique to elucidate protein-ligand co-structures; however, the success rate is often variable, in particular when working with dynamic proteins or weakly binding ligands. As a result, structural information is not routinely obtained in these situations and ligand optimization is often challenging or not pursued at all, representing a substantial limitation in the diversity of chemical scaffolds explored for a given target.

To overcome this impediment, we have developed a robust NMR restraint-guided docking protocol to generate high quality models of protein-small molecule complexes. A comprehensive, experimentally determined and easily accessible set of protein-ligand intermolecular distances is used to drive the docking process and enables selection of the correct ligand conformation in the bound state. Using the antibacterial target CoaD as a model system, the method was first benchmarked with several compounds for which X-ray co-structures were available. Afterward, and for the first time according to our knowledge, the utility and performance of such a method was fully demonstrated by applying it to molecules with completely unknown binding mode. Data on two independent compound series is presented showing that the generated docking models could be employed for the successful, prospective optimization of crystallographically non-tractable hits into more potent binders. The improved molecules are shown to interact with the target as predicted and can serve as novel chemistry starting points.

## MEDI 11

### **Identification of potent, selective, and cellularly-active KDM2B inhibitors by utilizing structure- and property-based design**

***Jun Liang, liang.jun@gene.com. Genentech, Los Altos Hills, California, United States***

Lysine demethylase KDM2B (also referred to as JHDM1B/FBXL10) is a 2-oxoglutarate (2-OG)-dependent hydroxylase that catalyzes the removal of methyl group(s) from histone H3K36Me3. Recent biological studies showed that KDM2B plays a critical role in transforming hematopoietic progenitor cells, suggesting that its inhibition might be an effective strategy to treat myeloid leukemia.

Attracted by this therapeutic potential, we set out to identify potent and selective KDM2B inhibitors. A high-throughput screen (HTS) of our compound collection was undertaken, in hope of uncovering chemical matters against this target. Two structurally-distinct scaffolds, which we named “ketone” and “sulfonamide”, respectively, emerged as attractive hits after a successful HTS campaign. Their binding to KDM2B protein was further confirmed

independently through both SPR and NMR. Furthermore, even though their potency against KDM2B was still moderate ( $IC_{50}$  0.3-1.2  $\mu M$ ), they were found to be quite selective against other KDMs ( $IC_{50} > 80 \mu M$ ).

In order to de-risk any potential off-target activities associated with each scaffold, we decided to pursue SAR optimization of both scaffolds in parallel. Our medchem effort was greatly accelerated when co-crystal structures were obtained for multiple analogs in each scaffold. Guided by structure- and property-based design principles, we were able to quickly identify potent KDM2B inhibitors from both scaffolds with KDM2B  $IC_{50} = 2$  and 16 nM, respectively, while maintaining selectivity against other KDMs ( $IC_{50} > 25 \mu M$ ). Additionally, the lead molecules displayed good solubility and showed excellent cell permeability in MDCK cells. Therefore, they qualified as suitable chemical probes for in vitro target validation of KDM2B biology.

## MEDI 12

### Selectively targeting *MYC* expression with nucleic acid binding small molecules

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The *MYC* gene encodes for the transcription factor MYC, which is responsible for the regulation of 15-90% of the human genome and has been linked to proliferation, differentiation, apoptosis, and oncogenesis. Importantly, *MYC* is overexpressed in 20% of all cancers. However, it has proven difficult to develop direct small molecule inhibitors of MYC. An attractive alternative route is the prevention of *MYC* expression via small molecule-mediated stabilization of the G-quadruplex (G4) present within its promoter region. However, a major barrier in developing biologically active small molecules that bind to nucleic acids has been the identification of selective interactions. Previous approaches have generally yielded pan-quadruplex binding molecules, and strategies to generate selective ligands are lacking. Here, we report an approach for the identification of G4-binding small molecules using small molecule microarrays (SMMs). We use the SMM screening platform to identify a novel G4-binding small molecule (D089). Biophysical techniques, such as surface plasmon resonance (SPR), thermal melt assays, WaterLOGSY NMR,

and fluorescence titrations, demonstrate that D089 binds reversibly to the *MYC* G4 with single digit micromolar affinity, with weaker or no measurable binding to several other G4s. Additionally, D089 inhibits *MYC* expression in cell models, with minimal impact on the expression of other G4-associated genes, while *MYC* target genes are downregulated. Development of a synthetic route to D089 has enabled the discovery of an improved analog (DC-34) that has superior binding affinity and efficacy in silencing *MYC* expression. Furthermore, from a preliminary NMR structure of DC-34 and the *MYC* G4, we can observe that the *MYC* G4 undergoes a conformational change to accommodate ligand binding in a way that appears unique for this molecular scaffold. Biochemical and cell-based assays demonstrate that DC-34 effectively silences *MYC* transcription and translation via a G4-dependent mechanism of action. We demonstrate that the SMM approach can reveal a selective G4 binder for oncogene inhibition. Efforts toward applying SMMs to other G4-associated oncogenes are being pursued to discover new selective binding scaffolds.

## MEDI 13

### **Different modes of activation of the four regulatory pyruvate dehydrogenase kinases by the E2 and E3 binding protein components of the human pyruvate dehydrogenase complex**

**Elena L. Guevara, elg6110@gmail.com, Luying Yang, Natalia S. Nemeria, Jieyu Zhou, Frank Jordan. Chemistry, Rutgers University, Newark, New Jersey, United States**

The human pyruvate dehydrogenase complex (PDC) comprises four multidomain components, E1, E3, E2 and an E3-binding protein (E3BP), the latter two forming the core as E2-E3BP sub-complex. Pyruvate flux through PDC is regulated via phosphorylation (inactivation) at E1 by four PDC kinases (PDKs), and reactivation by two PDC phosphatases. Up-regulation of PDK isoform gene expression is reported in several forms of cancer, while PDKs may be further activated by PDC by binding to the E2-E3BP core. Hence, the PDK:E2-E3BP interaction provides new therapeutic targets. Inactivation of E1 via phosphorylation by PDK1-4 was initially studied to determine PDK interaction and therefore activation by E2-E3BP or its C-terminally truncated proteins. The studies demonstrated significant differences in activation of PDK isoforms by binding to the E2-E3BP core. Recently, the Jordan group defined the interaction loci between PDKs and the E2-E3BP core by two complementary methods, HD-exchange MS (HDX-MS) and NMR. Interrogation of the sites on E2-E3BP identified to interact with PDKs was

accomplished by site-directed mutagenesis studies in order to validate the identified ‘hot spots’. We have successfully shown that the variants on the L1L2S region of E2 effect inactivation of E1 via phosphorylation by the PDKs. To date, drug design to inhibit these overactive kinases were based on limited data on the activation of these kinases leading to non-specific, and ultimately ineffective, drugs. Therefore, it is crucial to paint a much more detailed picture of the interaction and resulting activation of these kinases with the E2-E3BP core in order to formulate a novel and impactful drug design treatment for many diseases.

## MEDI 14

### **Discovery of a selective androgen receptor degrader (SARD) for treatment of castration-resistant prostate cancer**

*Zhong-Ke Yao<sup>3</sup>, Suzanne E. Wardell<sup>4</sup>, Ivan Spasojevic<sup>4</sup>, John D. Norris<sup>4</sup>, John A. Katzenellenbogen<sup>1</sup>, Donald P. McDonnell<sup>4</sup>, Jatinder S. Josan<sup>2</sup>, jsjosan@vt.edu. (1) 461 RAL, Box 37-5, University of Illinois, Urbana, Illinois, United States (3) Chemistry, Virginia Tech, Blacksburg, Virginia, United States (4) Duke University School of Medicine, Durham, North Carolina, United States*

Current treatments for prostate cancer are centered on blocking androgen-signaling axis, which is the target of several clinical AR antagonists, including enzalutamide. Recent studies have shown that a single AR mutation (F876L) converts enzalutamide from an antagonist to an agonist – a fate shared with earlier antagonists such as bicalutamide and flutamide. Thus, while hormonal therapy is often initially successful, prostate tumors inevitably become resistant. Remarkably though, several mechanistic studies suggest that AR remains a viable target in these hormone refractory cancers. Thus, novel therapeutics that target and degrade AR could provide unique druggable opportunities for complete and sustained AR inhibition in prostate cancers. Based on an AR-gelsolin interaction screen of an in-house library of 170,000 compounds, and further follow-up of top hits with AR-driven gene expression, inhibition of VCaP cell proliferation, transcriptional and conformational profiling, and AR degradation studies, we have identified a Selective Androgen Receptor Degrader (SARD) that shows efficacy against both WT and AR mutants. Further structure-activity relationship (SAR) revealed analogs with IC<sub>50</sub> equivalent or superior to enzalutamide. In vivo studies are currently underway to evaluate efficacy in mouse tumor xenografts. These preclinical findings highlight the utility of SARDs as effective therapeutic

prostate tumor strategy in the context of AR overexpression and mutations that confer resistance to second-generation AR antagonists.

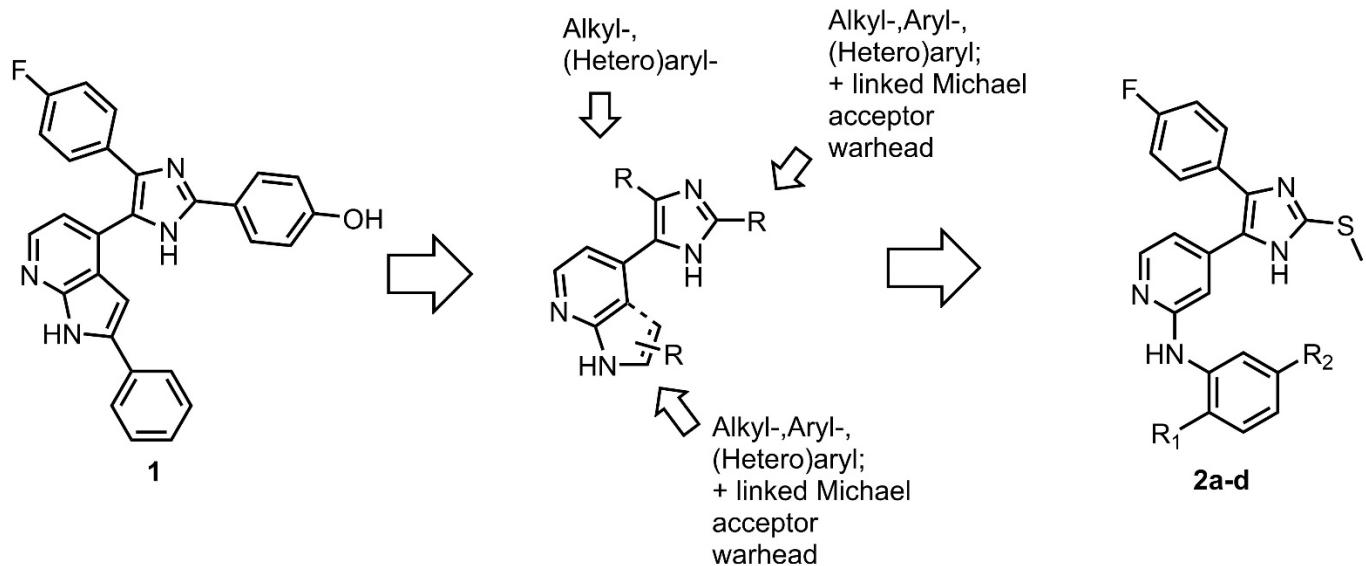
## MEDI 15

### **EGFR triple mutant: Recent set-backs and new hopes in fighting mutant non-small cell lung cancer**

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The discovery of EGFR L858R and del19 activating mutations in non-small cell lung cancer patients intensified the change of thinking towards personalized tumor therapy. “Oncogene addiction” to the EGFR signalling pathway paved the way for the development of the small molecules erlotinib and gefitinib as mutant selective, first generation tyrosine kinase inhibitors. Initial good results were overshadowed by imminent resistance development mainly via the gatekeeper point mutation T790M. Rational efforts in drug design finally led to irreversible third generation, mutant selective EGFR inhibitors with promising results in patient with acquired T790M mutations that became resistant to first generation TKIs. Recently, a third point mutation C797S was discovered in the cancer tissue of patients. This particular mutation renders irreversible bond formation with the cysteine impossible. Thus this acquired mutation leads to resistance to the actual gold standard osimertinib (FDA-approved 2015). Beside the development of potent allosteric inhibitors, a target hopping approach from pyridinylimidazole-based p38 MAPK inhibitors to EGFR inhibitors led to trisubstituted imidazoles as structural novel class of EGFR-inhibitors. The approach yielded very potent reversible and irreversible inhibitors of the EGFR L858R, L858R/T790M and L858R/T790M/C797S mutants with submicromolar IC<sub>50</sub>s. These compounds show apart from a covalent binding mode to the double mutant additional noncovalent binding properties at the triple mutant. Furthermore, high cellular as well as wild type sparing activity (comparable to osimertinib) in L858R/T790M mutant cancer cell lines, good kinase selectivity profile and metabolic stability could be achieved. Example compound shows IC<sub>50</sub> (EGFR-L858R/T790M) = < 0.5 nM and EGFR- L858R/T790M/C797S down to 6 nM. Cellular EC<sub>50</sub> value reaches down to 14 nM in a double mutant L858R/T790M cell line.

In sum, this new class of EGFR inhibitors together with this rational approach to inhibit EGFR L858R/T790M/C797S may stimulate the development of either improved trisubstituted imidazoles as candidates or probes. In addition the design approach might be transferred to other structural classes of EGFR inhibitors.



## MEDI 16

### Development and optimization of a selective MYST histone acetyltransferase inhibitor that induces cellular senescence

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Is the histone acetyltransferase (HAT) family druggable? This question has been frequently asked in the field of epigenetics. We have demonstrated through a HTS screen and medicinal chemistry optimization that one member of the MYST family (MOZ; KAT6A) can be selectively inhibited in the nanomolar range. KAT6A is an oncogene and is involved in critical roles by

promoting cell proliferation *via* regulation of the tumor suppressor locus *Cdkn2a*. We have discovered a selective high affinity inhibitor (WM-8014) of KAT6A, which is a reversible acetyl-CoA competitor with binding affinity of 8 nM. WM-8014 induces cellular senescence, without DNA damage, in an INK4A/ARF dependent fashion, without general cell toxicity either in culture or vertebrate model systems. We anticipate that this class of inhibitors will be useful in accelerating the development of therapeutics targeting gene transcription mediated by histone acetylation.

## MEDI 17

### **Mnk1/2 and Abl inhibitions for the treatment of blast crisis chronic myelogenous leukemia**

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MAP kinase-interacting serine/threonine-protein kinases 1 and 2 (MNK1/2) as targets for the development of oncologic, metabolic and CNS therapies have recently gain a tremendous amount of interest. This, in conjunction with the increasing knowledge on the impact of mRNA translation reprogramming and control in tumorigenesis.

MNK1/2 are serine and threonine kinases that phosphorylate eukaryotic translation initiation factor 4E (eIF4E) on Ser209. eIF4E and especially, phosphorylated eIF4E play an important role in oncogenic mRNA translation. Overexpression of phosphorylated eIF4E drive oncogenesis, tumor progression with often a poor prognosis as seen with patients in Blast crisis chronic myeloid leukemia (BC-CML).

Imatinib (Gleevec<sup>TM</sup>), a marketed BCR-ABL tyrosine kinase inhibitor (TKI), has not only galvanized targeted therapy but has specifically revolutionized the treatment of early stage or chronic phase (CP) chronic myeloid leukemia (CML). Unfortunately, a proportion of CP-CML patients do not respond to Imatinib treatment and will progress to BC-CML. A consistent feature in these

patient samples is an elevated level of phosphorylated eIF4E. Consequently, our strategy to treat to BC-CML is to develop MNK inhibitors that will moderate the level of phosphorylated eIF4E.

Here, we report our hits finding schemes, as well as our hit to lead optimization process. Structure activity relationships, pharmacokinetics properties, and efficacies studies will be presented.

Our data demonstrate that drug-like molecules can be developed to potently and specifically inhibit the MNK kinases. We will also show that simultaneous inhibition of MNK and BCR-ABL is effective at preventing BCR-ABL-driven growth and proliferation, as well as inhibiting the MNK-eIF4E-dependent self-renewal function of BC leukemic stem cells. We have identified a selective Mnk1/2 inhibitor ETC-206 currently in clinical trial.

## MEDI 18

### **Integration of x-ray crystallography, computational modelling and NMR conformational analysis data in fragment-based drug design**

***Emiliano Tamanini, emiliano.tamanini@astx.com. Chemistry, Astex Pharmaceuticals, Cambridge, United Kingdom***

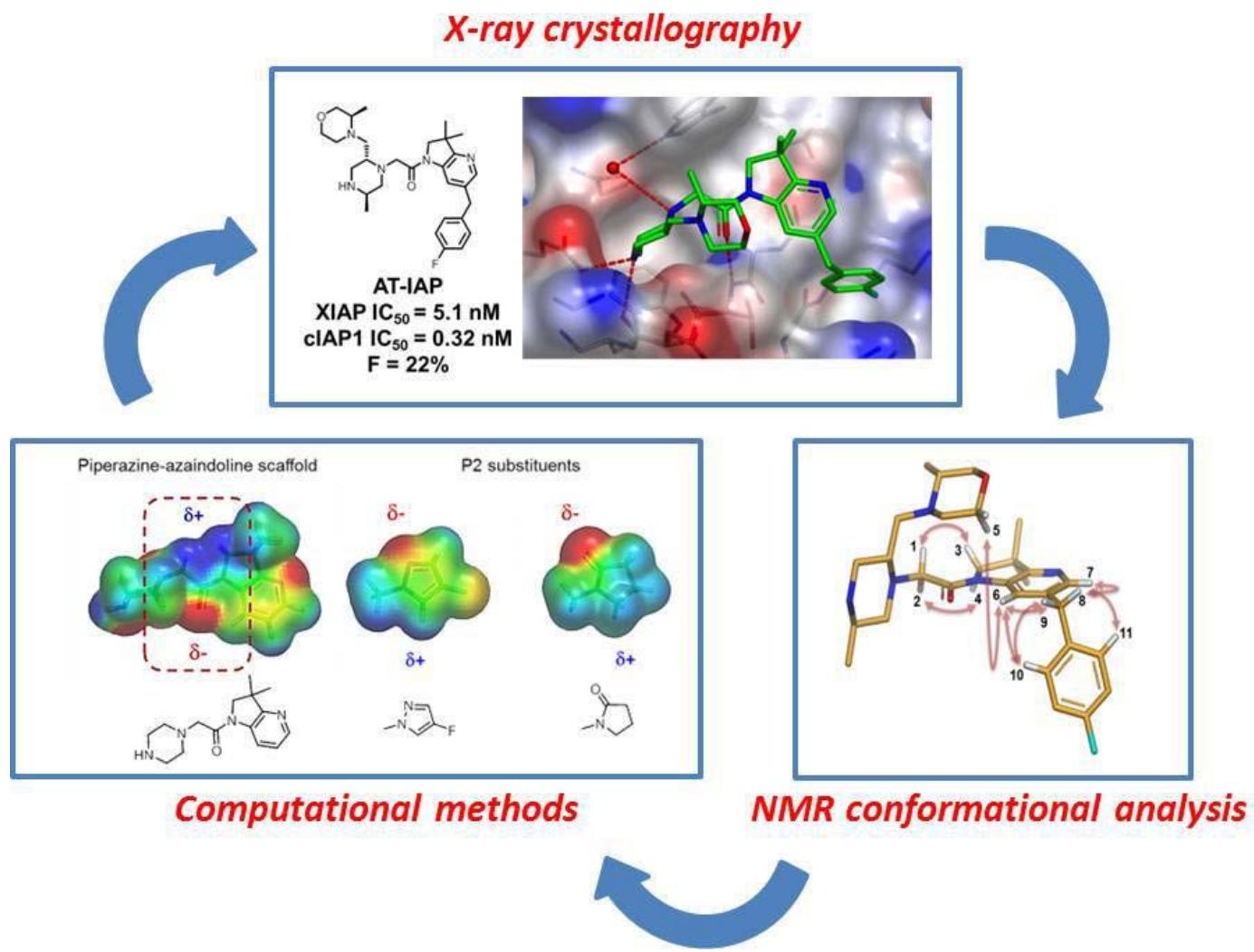
A deep structural understanding of ligand-protein binding is important for successful conduct of fragments-based drug design (FBDD) projects. X-ray crystallography plays a crucial role, but computational approaches also play an important part in optimizing key contacts between protein and ligand. Also important is an appreciation of the preferred solution conformation of the ligand. The integration of these three key areas is illustrated for the optimization of a series of dual cIAP1 and XIAP inhibitors.

The Inhibitor of Apoptosis Proteins (IAPs) are key regulators of anti-apoptotic and pro-survival signalling pathways. Overexpression of IAPs occurs in various cancers and has been associated with tumour progression and resistance to treatment.

Astex has successfully applied FBDD to develop a non-peptidic, potent dual cIAP1 and XIAP antagonist with a different pharmacological profile compared to previously reported, alanine-based, peptidomimetic antagonists.

This presentation will describe the key steps of the fragment evolution to generate the lead series starting from fragment identification using our screening approach Pyramid™. X-ray crystallography enabled us to identify non-alanine fragments, which bind with mM affinity ( $LE << 0.3$ ) to both XIAP and cIAP1 which were optimised to a potent, dual XIAP and cIAP1 antagonist, which we have designated **AT-IAP**. The presentation highlights all the different aspects of the design process, from fragment to lead, that enabled us

to quickly reach low nM level of potency (LE > 0.3) and to generate an orally bioavailable antagonist which showed *in vivo* efficacy in a number of mouse tumour xenograft models.



## MEDI 19

**NMR conformational signatures guide the design of macrocycle drug cell activity and permeability: AstraZeneca case studies**

**Amber Y. Balazs**, [ybalazs@hotmail.com](mailto:ybalazs@hotmail.com), **Rodrigo Carbajo**, **Nichola Davies**, **Elisabetta Chiaparin**. IMED Oncology, AstraZeneca Pharmaceuticals LP, Waltham, Massachusetts, United States

In recent years, synthetic macrocycles are increasingly considered as a source of drugs. Macrocycle drugs present several attractive features. For example, they can impart exquisite potency and selectivity, especially with challenging Protein-Protein Interactions (PPIs) targets. Additionally, macrocycles often retain optimal physico-chemical properties, such as good solubility and permeability, despite high molecular weight or lipophilicity. A distinctive feature of macrocycles is greater conformational restriction due to fewer rotatable bonds. As a consequence, the ability to accurately and robustly predict free as well as bioactive conformations are key steps in structure-based macrocycle design. Unfortunately, computational conformational analyses often fail to predict global minimum energy states of free ligands. Despite the availability of bioactive conformations from protein-ligand x-ray crystallography, optimization of macrocycles potency can still be an empirical process. Moreover, conventional metrics to design oral bioavailability for small molecule drugs, such as of Lipinski's 'Rule of Five', are of little use in macrocycle physical chemistry optimization. We will show, with examples from AstraZeneca oncology discovery projects, how measurement by NMR spectroscopy of the conformational landscape of free macrocycles in solution can aid and focus drug design hypotheses, leading to accurate predictions of affinity and physical chemistry properties.

## MEDI 20

### **Discovery of CC-671: A TTK/CLK2 inhibitor for the treatment of triple negative breast cancer**

**Jennifer R. Riggs**, *jennifer.riggs@yahoo.com. Medicinal Chemistry, Celgene, San Diego, California, United States*

Triple negative breast cancer (TNBC) remains a serious unmet medical need in oncology. Although the standard of care (Taxotere) treatment produces a high initial response rate, relapses are significant with no clear treatment options remaining for this patient population. High TTK (Mps1) expression in TNBC patients is linked to poor prognosis with target validation data supporting tumor survival dependency. A unique dual inhibition profile targeting TTK (mitotic transit) and CLK2 (mRNA splicing) was discovered from a phenotypic screen targeting TNBC. TNBC tumor cell driven SAR created potent and selective compounds that demonstrate favorable PK properties leading to the development candidate CC-671. SAR, compound profile, and significant single agent in vivo efficacy in multiple TNBC xenograft models will be presented.

## MEDI 21

### Optimization of macrocyclic ring containing Mcl-1 inhibitors through SAR and rational design

**Todd Kohn**, tkohn@amgen.com. Amgen, South San Francisco, California, United States

Mcl-1 is a member of the Bcl-2 family of proteins which act via protein-protein interactions between pro- and anti-apoptotic factors to regulate the process of programmed cell death. Identification of the anti-apoptotic Mcl-1 protein as a critical survival factor in a broad range of human cancers that leads to tumor progression along with the target's resistance to chemotherapy has made it a highly desirable drug target. Compounds containing a macrocyclic ring were designed based on NMR studies between the Mcl-1 protein and early stage ligands. This observation was followed by an iterative process using crystal structure, SAR and rational design to identify a macrocyclic compound that is now in clinical development for the treatment of multiple myeloma. This presentation will focus on how different classes of macrocycles were exploited to enhance potency, cellular shift and pharmacokinetics.

## MEDI 22

### Discovery of GDC-0077: A highly selective inhibitor of PI3K-alpha that induces degradation of mutant-p110 alpha protein

**Marie-Gabrielle Braun<sup>1</sup>**, braun.mariegabrielle@gene.com, Connie Chan<sup>1</sup>, Saundra Clausen<sup>1</sup>, Kyle Edgar<sup>1</sup>, Charles Eigenbrot<sup>1</sup>, Richard Elliott<sup>2</sup>, Nick Endres<sup>1</sup>, Lori Friedman<sup>1</sup>, Keira Gerland<sup>1</sup>, Xiao-Hu Gu<sup>3</sup>, Pat Hamilton<sup>1</sup>, Chong Han<sup>1</sup>, Emily J. Hanan<sup>1</sup>, Rebecca Hong<sup>1</sup>, Philip Jackson<sup>2</sup>, Sean Kelly<sup>1</sup>, Jamie Knight<sup>2</sup>, Man-Ling Lee<sup>1</sup>, Aijun Lu<sup>3</sup>, Calum MacLeod<sup>2</sup>, Aija McKenzie<sup>1</sup>, Michelle Nannini<sup>1</sup>, Raman Narukulla<sup>2</sup>, Amanda Nguyen<sup>1</sup>, Jodie Pang<sup>1</sup>, Hans E. Purkey<sup>1</sup>, Laurent Salphati<sup>1</sup>, Deepak Sampath<sup>1</sup>, Stephen Schmidt<sup>1</sup>, Leah Schutt<sup>1</sup>, Robert Heald<sup>2</sup>, Kyung Song<sup>1</sup>, Mark Ultsch<sup>1</sup>, Jianfeng Xin<sup>3</sup>, Kuen Yeap<sup>2</sup>, Amy Young<sup>1</sup>, Zoe Zhong<sup>1</sup>, Steven T. Staben<sup>1</sup>. (1) Genentech Inc, South San Francisco, California, United States (2) Charles River, Harlow, United Kingdom (3) Pharmaron, Beijing, China

The phosphatidylinositol 3 kinase (PI3K) signaling pathway plays a critical role in regulating tumor cell growth, proliferation and survival. Hotspot mutations in *PIK3CA*, the gene that encodes for the p110-alpha catalytic subunit of phosphatidylinositol-3-kinase, are highly prevalent in cancer and thus PI3K-

alpha is a promising target for the treatment of cancer. However, exposure of PI3K inhibitors in the clinic can be limited by PI3K-isoform driven toxicities. We hypothesized that improving selectivity for PI3K-alpha relative to the other isoforms has the potential for increased tolerability in the clinical setting. Structure-based design was utilized to enhance isoform-specific interactions with Gln 859, leading to potent inhibitors of PI3K-alpha with greater than 300-fold selectivity over the other Class I PI3K isoforms. Optimization of the physico-chemical properties lead to improved permeability and decreased clearance, and overall increased bioavailability, resulting in the identification of GDC-0077 which demonstrates strong efficacy in PIK3CA mutant tumor models. In addition, GDC-0077 induces the selective degradation of the mutant p110-alpha protein in a proteasome dependent fashion. *In vitro* characterization and pharmacokinetic parameters modeled from preclinical species predicted low plasma clearance in human, supporting the selection of GDC-0077 as a development candidate for the treatment of patients with *PIK3CA*-mutant cancer. This compound is now in Phase I clinical trials.

## MEDI 23

### Discovery of the JAK1 selective kinase inhibitor AZD4205

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JAK1 together with JAK2, JAK3, and TYK2 belong to the JAK (Janus-associated kinase) family of cytoplasmic tyrosine kinases that play important roles in cytokine and growth factor mediated signal transduction. Activated by cytokine or growth factor engagement, JAKs are autophosphorylated to stimulate the recruitment and phosphorylation of the signal transducers and activators of transcription (STAT), leading to the dimerization and translocation of phosphorylated STATs into the nucleus and increasing cellular proliferation and resistance to apoptosis. Up to 70% of human cancers are linked to persistent elevated STAT3 activity that is associated with resistance or poorer response to chemotherapy agents and treatment with inhibitors of targeting oncogenic signalling pathways such as EGFR, MAPK and AKT. JAK1 is believed to be a primary driver of STAT3 phosphorylation and signalling. JAK2 is essential for the signal transduction downstream of erythropoietin, thrombopoietin and related receptors that control erythrocyte

and megakaryocyte expansion. Therefore, JAK1 selective inhibition may enable improved target coverage of JAK1-STAT3 signalling by sparing toxicities such as thrombocytopenia and anaemia due to JAK2 inhibition. We will present our detailed medicinal chemistry optimization from a non-kinome selective screening hit to the candidate drug AZD4205, a highly selective novel JAK1 kinase inhibitor. AZD4205 displayed high levels of JAK1 biochemical and cellular potency with favourable physical properties and excellent preclinical in vivo pharmacokinetics. AZD4205, in a combination with low dose osimertinib, demonstrated a greater antitumor activity in an H1975 (T790M) NSCLC xenograft model. Furthermore, AZD4205 enhanced osimertinib-induced tumor regression in an LG1049 (T790M) NSCLC PDX model. These findings warrant the further clinical investigation of AZD4205 to fully understand its potential as an anti-cancer therapeutic.

## MEDI 24

### **Discovery of LY3200882: A highly specific and potent TGF $\beta$ RI small molecule inhibitor**

**Saravanan Parthasarathy**, *parthasarathy\_saravanan@lilly.com. Eli Lilly and Company, Indianapolis, Indiana, United States*

The multifunctional transforming growth factor  $\beta$  (TGF $\beta$ ) is a member of a large family of cytokines involved in the regulation of critical biological processes such as cell proliferation, cell migration, invasion, extracellular matrix production, and immune suppression. TGF $\beta$  signaling deregulation is frequent in tumors and has crucial roles in tumor initiation, development, and metastasis. Thus it is an attractive target for the development of novel inhibitors for the treatment of cancer. However, developing inhibitors with high specificity and desirable potency has posed a significant challenge due to high homology in the adenosine triphosphate (ATP) binding pocket among the other kinase family members. Reported herein is the discovery of LY3200882, a small molecule ATP competitive inhibitor of TGF $\beta$ RI currently in Phase I clinical trials. LY3200882 demonstrates desirable potency and high specificity for TGF $\beta$ RI over other kinases.

## MEDI 25

### **Discovery of BMS-135: An orally active imidazo[2,1-f][1,2,4]triazine pan-CK2 inhibitor for the treatment of cancer**

**Ashok V. Purandare**, [ashok.purandare@bms.com](mailto:ashok.purandare@bms.com), Kurt Zimmermann, Walter Johnson, Honghe Wan, Amy C. Hart, Christine M. Tarby, Liqi He, Brian E. Fink, Ashvinikumar V. Gavai, Gregory Vite, Yufen Zhao, Wayne Vaccaro, Tram Huynh, Harold Mastalerz, Jennifer A. Inghrim, John S. Tokarski, Xiaopeng Sang, Brent Rupnow, Chiang Yu, Joseph Farnoli, Benjamin Henley, Francis Lee, Aberra Fura, Mary Oberneier, Paul A. Elzinga, William Foster, Bogdan Slezcska, PN Arunachalam, Anuradha Gupta, Muthalagu Vetrichelvan, Nirmala Raghavan, Zheng Yang, Arvind Mathur, Richard Rampulla, Dauh-Rurng Wu, Peng Li, Herbert Klei, Gerry Everlof, Shu Zhong, Greg Locke, John T. Hunt, Jodi Muckelbauer, Wei Yong, Tai Wong. Bristol Myers Squibb, Princeton, New Jersey, United States

CK2A1/A2 are highly conserved, and constitutively active serine/threonine kinases. High levels of CK2A1 correlate with disease aggressiveness and is associated with poor prognosis in HNSCC, SqNSCLC, prostate cancer and AML. Deregulation of the enzymes has been shown to promote and maintain a malignant phenotype through mechanisms that affect anti-apoptotic and pro-proliferative signaling

pathways. CK2 has been reported to phosphorylate and modulate the activity of several oncogenic transcription factors, including CREB, Myc, Max, Jun, Fos and Myb. In addition, CK2 inhibition or knockdown with RNAi results in growth suppression and/or apoptosis of both solid and hematologic cancer cell lines.

This presentation will focus on the SAR studies leading to the discovery of a highly potent, ATP competitive pan-CK2 inhibitor, BMS-135 with a commensurate level of cellular potency. Upon oral dosing, BMS-135 demonstrated strong PK/PD correlation and robust, anti-tumor efficacy in CK2-driven xenograft models. The presentation will also discuss further optimization to identify an orally bioavailable prodrug BMS-159 with improved pharmaceutical properties suitable for further development.

## MEDI 26

### Discovery of CC-90003: A covalent ERK1/2 inhibitor

**Li-Xin Qiao**, [lqiao@celgene.com](mailto:lqiao@celgene.com). Celgene Corp., Cambridge, Massachusetts, United States

MAPK (RAF/MEK/ ERK) kinase pathway is a validated pathway for cancer therapeutic intervention (e.g. melanoma). Blockade of this pathway is expected to shut down an mTORC1 escape mechanism involving activation of MEK pathway signaling. Though some BRAF and MEK inhibitors have been

FDA-approved or are at late stage of clinical trials, the resistance to BRAFi and MEKi presents an emerging unmet medical need. ERK is a major signaling convergence point in human cancers. Tumor biology data suggests that targeting ERK hold high potential to overcome or prevent resistance from BRAFi and MEKi. Using structure-based drug design, we discovered CC-90003, which forms covalent bond with the Cys in the ATP binding sites of ERK1/2. The enhanced pharmacodynamimcs effect was demonstrated by prolonged inhibition of p-RSK and extended ERK occupancy recovery after compound washout. CC-90003 inhibited the cell growth against not only a broad spectrum of BRAF- and KRAS-mutant cell lines, but also against vemurafenib- and trametinib-resistant A375R clones and trametinib-resistant HCT116R polyclonal cells. *In vivo*, tumor growth inhibition results in BRAF<sup>V600E</sup> (A375, LOX IMVI melanoma) and KRas<sup>G13D</sup> (HCT116 colon) xenograft models correlated with ERK occupancy in tumor tissues, as presented in QD and QOD dose regimen.

## MEDI 27

### **Two photon fluorescence polarization microscopy for imaging and quantifying drug target binding *in vitro* and *in vivo***

**Claudio Vinegoni<sup>1</sup>, cvinegoni@mgh.harvard.edu, Ralph Weissleder<sup>2</sup>.** (1) Center for Systems Biology, MGH - Harvard University, Boston, Massachusetts, United States (2) Massachusetts General Hospital, Boston, Massachusetts, United States

For drugs to be therapeutically successful, they must reach their intended cellular targets, modulate specific protein function while avoiding off-target effects. This process of delivery and effector function can be difficult to decipher, especially *in vivo*. Fluorescence polarization microscopy is commonly used in screening assays during drug development, and has recently been shown to offer unique possibilities for imaging drug target engagement when used in combination with fluorescently labeled companion drugs.

Building upon intravital microscopy imaging modalities developed in our group to image drug pharmacokinetics and cellular heterogeneity, we have recently exploited fluorescence polarization to study target engagement in several model systems. While traditional polarization assays are somewhat limited to artificial system such as biochemical assays or cell lysates, microscopy based methods offer quantitative pharmacological binding information at the single cell level.

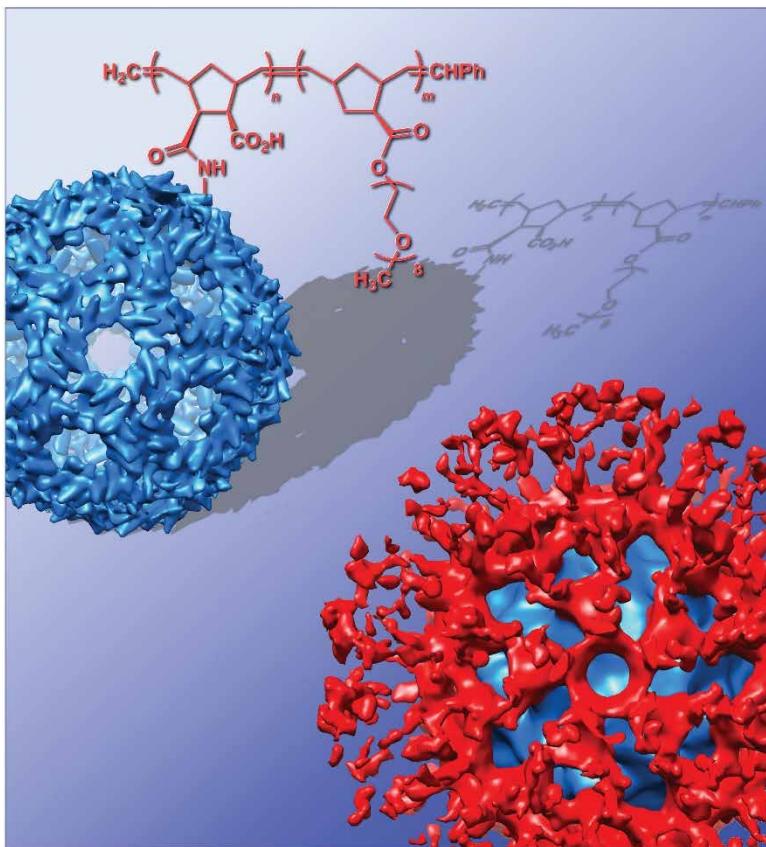
Here we report our recent results in the development of an imaging platform for two photon fluorescence anisotropy microscopy.

## MEDI 28

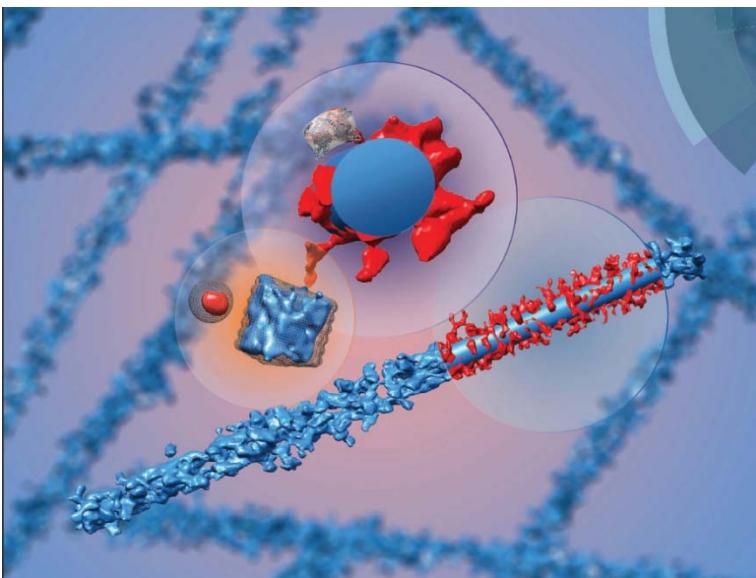
### Cryo-EM applications from viruses to nanoparticles

**Phoebe L. Stewart, [pls47@case.edu](mailto:pls47@case.edu).** Pharmacology, Case Western Reserve University, Cleveland, Ohio, United States

Cryo-electron microscopy (cryo-EM) can be used to study the structure of a wide variety of biological assemblies from icosahedral viruses and membrane proteins to engineered nanoparticles. Recent technical advances are enabling atomic resolution structure determination for favorable samples. The advantages of cryo-EM include the facts that a crystal is not required and the sample is imaged in a near-native state. If the sample has a homogeneous structure, then thousands of 2D cryo-EM images of particles oriented randomly on the grid can be averaged to generate a 3D structure. If the sample is heterogeneous or includes flexible linkers, another approach is cryo-electron tomography where a 3D structure is generated from a tilt-series of 2D images collected from the same specimen area. CryoEM structures of viruses and engineered viral nanoparticles will be presented.



Polymers provide antibody shielding for virus-like particles, such as those derived from the bacteriophage Q $\beta$ . Cryo-EM structures are shown for Q $\beta$  (blue) and Q $\beta$  shielded with polynorbornene (red).



Tobacco mosaic virus (TMV) has been engineered as a drug delivery vehicle and as an MRI contrast agent. Coating TMV with human serum albumin provides camouflaging from immune recognition and clearance. The serum albumin coating

(red) on TMV (blue) can be visualized and quantified by cryo-electron tomography.

## MEDI 29

### Discovering drug leads by practical NMR strategies

**Steven Laplante<sup>1,2</sup>, stevenlaplante@gmail.com.** (1) University Quebec (INRS-IAF), NMX, Laval, Quebec, Canada (2) NMX Research and Solutions, Laval, Quebec, Canada

Small molecule drugs continue to be crucial for combating diseases. This presentation will convey the critical role NMR has been playing for discovering the seeds for new drugs starting from substrate peptides, high-throughput screens and fragment-based screens. Central to all the examples was the need to better understand the properties of small molecules when free in solution and to decipher the various types of binding to macromolecules. To do so, appropriate NMR methods/strategies were developed to prioritize quality ligands for downfield medicinal chemistry purposes. Examples will be shown where NMR strategies revealed compound solution behavior (solubility, aggregation, atropisomer chirality), exposed target protein features (folding and changes), and determined stoichiometric binding attributes.

## MEDI 30

### Applications of SPR to drug discovery: Understanding LXRB agonist binding profile to two key serum proteins

**Mark R. Witmer<sup>2</sup>, mark.witmer@bms.com, Kamelia Behnia<sup>3</sup>, Susan Johngahr<sup>3</sup>, Qi Wang<sup>3</sup>, James Smalley<sup>3</sup>, Deepa Calambur<sup>2</sup>, Punit Marathe<sup>3</sup>, David Rodrigues<sup>3</sup>, Ellen K. Kick<sup>1</sup>.** (1) Hw 13-1.10, Bristol-Myers Squibb, Princeton, New Jersey, United States (2) Molecular Discovery Technologies, Bristol-Myers Squibb, Pennington, New Jersey, United States (3) Pharmaceutical Candidate Optimization, Bristol-Myers Squibb, Princeton, New Jersey, United States

Surface plasmon resonance or SPR, is a biophysical method that provides kinetic information on biomolecular interactions, through measuring association and dissociation kinetics in real time. The applications of SPR to drug discovery are widespread, and this talk will focus on the interactions of drug molecules with serum proteins, rather than the more common study of candidates with the target protein of interest. The Phase I clinical PK profile of

BMS-779788, an agonist for the LXR $\beta$  receptor, showed an unexpectedly high level of plasma exposure, in contrast to relatively normal values seen in preclinical models in rat and dog. Specifically, low volume of distribution (V<sub>d</sub>) and low clearance (CL) values were seen, consistent with tight binding in the plasma. A hypothesis was formulated that the clinical observation was due to tight binding of BMS-779788 to a major serum protein, either human serum albumin or human  $\alpha$ 1-acid glycoprotein (HAGP). Experimental methods including equilibrium binding and SPR were used to probe the hypothesis. The binding kinetics for BMS-779788, a second generation molecule BMS-852927, and various control compounds were analyzed by SPR using purified human serum albumin, and  $\alpha$ 1-acid glycoprotein from human, dog and rat. The data from equilibrium binding and SPR were consistent with high affinity binding of BMS-779788 to HAGP, but not to human serum albumin. SPR revealed the interaction was driven by a relatively slow rate of dissociation from human AGP. The compound showed relatively fast dissociation rates from rat and dog AGPs, as well as from human serum albumin. The kinetics of BMS-852927 dissociation from AGPs, in contrast, were relatively rapid, especially from HAGP, predicting preclinical PK to be predictive in a Phase I study, which was subsequently demonstrated.

## MEDI 31

### **Not all sites are equal: Using biophysics to probe the biological relevance of fragment binding sites**

**Susanne Saalau**, susanne.saalau@astx.com. Molecular Science, Astex Pharmaceuticals, Cambridge, Cambridgeshire, United Kingdom

Fragment-Based Drug Discovery (FBDD) is now well established as an approach for identifying lead molecules with low molecular weight, good ligand efficiency and low overall complexity. Our fragment library (average MW ~176Da) generally displays low target-binding affinities, typically in the uM to mM range, which necessitates the use of sensitive biophysical techniques to detect specific binding events. Astex has successfully prosecuted >50 high-throughput crystallographic and biophysical fragment screens (Pyramid<sup>TM</sup>) against a wide variety of targets, and has consistently discovered alternative binding sites, some of which have been shown to regulate function via allosteric mechanisms. This presentation will use specific case studies to illustrate how the use of orthogonal biophysical techniques enabled: 1. The elucidation of the mode of action of ligands and biological relevance of the targeted allosteric sites, and 2. The interaction of compounds with different conformational protein species present in solution. Finally, we

would like to discuss best practice approaches implemented for the different methods and the importance of experimental rigour to avoid pitfalls.

## MEDI 32

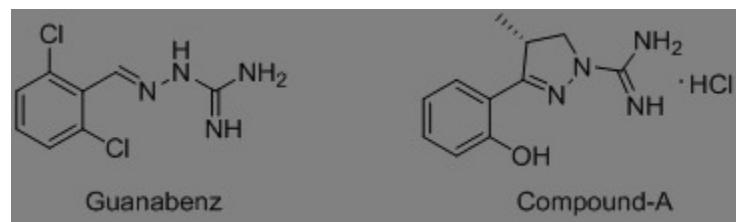
### New selective 5-HT<sub>2B</sub> receptor antagonists for the treatment of fibrosis

**Lars Pettersson**, *larspettersson59@live.se*. AnaMar AB, Lund, Sweden

Serotonin (5-HT) is known to be associated with fibrosis and recent studies support that 5-HT<sub>2B</sub> receptors have an important role in fibrotic disease by regulating production of pro-fibrotic mediators and modifying cell differentiation and activation.

Most 5-HT<sub>2B</sub> receptor antagonist developed so far are associated with certain liabilities, such as poor selectivity and inadequate pharmacokinetic properties. Starting from benzylidene-aminoguanidine derivatives, e.g. Guanabenz, an unselective α<sub>2</sub>-adrenergic agonist with moderate 5-HT<sub>2B</sub> receptor binding, we have developed new, highly potent and selective antagonists of the 5-HT<sub>2B</sub> receptor. The lead compound (Compound-A) was prepared in two synthetic steps, a Mannich reaction and a ring-forming condensation reaction with aminoguanidine, followed by chiral separation. Compound-A is a potent and selective 5-HT<sub>2B</sub> receptor antagonist ( $IC_{50}$  2.4 nM, Mw 218) with no agonistic effect at 10 μM and with high aqueous solubility (>10 mg/mL), and has been shown effective both in *in vitro* and *in vivo* models of fibrosis.

Here we report on the synthetic preparation and the very robust SAR of the 5-HT<sub>2B</sub> receptor binding for this compound class. Variations of the aromatic substituents, the pyrazoline regio- and stereochemistry, and the guanidine moiety all have major and distinct impact on the receptor binding. Virtual docking to the 5-HT<sub>2B</sub> receptor was used to guide the compound development providing high affinity ligands ( $IC_{50}$  0.1–1 nM).



## MEDI 33

### Novel pirfenidone derivatives: Potent antifibrotic agents

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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 vivo* is reported. In this study, **8d** effectively inhibits TGF- $\beta$ 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- $\beta$ 1, p38 MAPK and  $\alpha$ -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- $\beta$ 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 34**

### **Discovery of novel benzo[*b*]thiophene tetrazoles as non-carboxylate GPR40 agonists**

**Mark R. Player**<sup>1</sup>, *mplayer@its.jnj.com*, **Hui Huang**<sup>1</sup>, **Michael P. Winters**<sup>1</sup>, **Sanath K. Meegalla**<sup>1</sup>, **Seunghun P. Lee**<sup>1</sup>, **Tonya Martin**<sup>1</sup>, **Jianying Liu**<sup>1</sup>, **Meghan Towers**<sup>1</sup>, **Fran Xu**<sup>2</sup>, **Heng-Keang Lim**<sup>2</sup>, **Jose Silva**<sup>2</sup>, **Monicah Otieno**<sup>2</sup>, **Eric Arnoult**<sup>2</sup>, **Alessandro Pocai**<sup>1</sup>. (1) *Cardiovascular & Metabolism TA, Janssen Research and Development, LLC, Spring House, Pennsylvania, United States* (2) *Janssen, Spring House, Pennsylvania, United States*

GPR40 agonism is a promising new mechanism for the treatment of type 2 diabetes mellitus due to its ability to mediate glucose-stimulated insulin secretion (GSIS) from pancreatic β-cells. Tak-875 (fasiglifam, Takeda), the most clinically advanced GPR40 agonist, demonstrated clinical efficacy comparable to sulfonylureas without propensity to cause hypoglycemia and weight gain. However, fasiglifam was terminated in phase III trials due to liver toxicity. Most GPR40 agonists in the literature (including fasiglifam) have a carboxylic acid group, which may form an acyl glucuronide (AG), a reactive metabolite that may pose a risk for idiosyncratic drug toxicity. One way to mitigate this potential toxicity is to replace the carboxylic acid with a bioisostere. A novel series of GPR40 agonists containing a tetrazole as a carboxylic acid surrogate was identified. This series of compounds features a benzo[*b*]thiophene as the center ring, which is prone to oxidation during phase 1 metabolism. Following SAR optimization targeting GPR40 agonist activity and intrinsic clearance in microsomes (human and rat), potent and metabolically stable compounds were selected for in vivo evaluation. The compounds are efficacious at lowering blood glucose dose-dependently in a SD rat oGTT model. This class of non-carboxylate GPR40 agonists have the potential to be next generation glucose lowering agents without the threat of forming potentially reactive AG metabolites.

## **MEDI 35**

### **GPR40 full agonists for the treatment of type 2 diabetes**

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The G-protein-coupled receptor 40 (GPR40) mediates fatty acid-induced insulin secretion from pancreatic  $\beta$ -cells. Though GPR40 contains 3 binding sites, GPR40 partial agonists (GPR40PA) are thought to bind to site 1 through the cell membrane and GPR40 full agonists bind to site 3 with extracellular entry. Among the many GPR40 agonists that have been investigated, the most advanced, fasiglifam, is a site 1 binding GPR40PA that stimulates IPone and improves glycemic control in diabetic patients via glucose-dependent insulin secretion ( $\sim 1\% \downarrow$  HbA1c). Recently, a novel GPR40 agonist (JNJ-4307) was discovered that binds to site 3, stimulates IPone and cAMP and results in both insulin and glucagon-like peptide-1 (GLP-1) secretion.

In an ipGTT study in C57 mice, JNJ-4307 improves glucose handling and demonstrates GLP-1 secretion in wt but not GPR40 KO mice. Oral administration of JNJ-4307 (3-30 mg/kg) in ZDF rats dose-dependently controlled glucose excursion with a minimum efficacious dose of 3 mg/kg with increased GSIS. JNJ-4307 also dose dependently increases insulin secretion in ex vivo T2D human islets. The observed pharmacology of JNJ-4307 suggests that GPR40 full agonists represent a novel class of oral anti-diabetic anti-hyperglycemic medication.

## MEDI 36

### **Discovery of clinical candidate MR1704: A novel isothiazole based GPR40 agonist for diabetes**

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GPR40 is one of GPCRs predominantly expressed in pancreatic  $\beta$ -cells. Free fatty acids activate GPR40 as natural ligands and insulin secretion is promoted depending on the plasma glucose level. GPR40 agonist is expected as an effective anti-diabetic drug with the low risk of hypoglycemia. To date, several potent GPR40 agonists have been reported. Most of them have phenyl propionic acid moiety. The methylene protons of the phenyl propionic acid are known as metabolic soft spot, and many of GPR40 agonists have a substituent on the benzyl position to improve oral bioavailability. Although some acid surrogates for GPR40 agonists have been reported, no effective agonist having an acid surrogate directly bonding with the phenyl ring is known. As the results of our exploration of acid surrogates, we found the isothiazole S-oxide worked as a good acid group for orally available GPR40

agonists. Further optimization of isothiazole derivatives led to the discovery of MR1704 as a clinical candidate. In this presentation, we will describe the exploration of acid surrogates, lead identification & optimization, SAR of isothiazole-based GPR40 agonists and the profiles of MR1704.

## MEDI 37

### **Discovery of a novel series of heterocycles as potent EP3 antagonists for the treatment of type 2 diabetes**

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The Prostaglandin EP3 receptor is a 7-transmembrane G-protein coupled receptor found in a variety of human tissues including the kidney, uterus, bladder, stomach, brain, and pancreas. Prostaglandin E2 (PGE2), a primary product of arachidonic acid metabolism by the cyclooxygenase pathway, is the natural ligand which activates EP3 as well as other EP receptor subtypes. Recent studies have provided strong evidence underscoring the role of increased levels of PGE2 contributing to defective insulin secretion in diabetic patients. Animal models have demonstrated that PGE2 suppression of glucose-stimulated insulin secretion (GSIS) operates through the EP3 receptor. It is hypothesized that increased PGE2 signaling through the EP3 receptor might also help drive the development of diabetes and contribute to β-cell dysfunction. Therefore, EP3 receptor antagonists may be an effective treatment for type 2 diabetes by relieving the inhibitory action of PGE2 to partially restore defective GSIS in diabetic patients.

Herein we report our discovery of a novel series of potent heterocycles EP3 antagonists. We describe an extensive medicinal chemistry campaign to identify a lead compound with potent hEP3 binding affinity and functional activity, good physical properties, an excellent PK profile, and promising *in vivo* efficacy as a pre-clinical candidate for the treatment of type 2 diabetes.

## MEDI 38

### **Synthesis of 5-(3-(2-[<sup>18</sup>F]fluoroethoxy)phenyl)-1,3-dihydro-2*H*-benzofuro[3,2-e][1,4]diazepin-2-one as a new potential PET radioligand for P2X4 receptor**

**Min Wang**, wang1@iupui.edu, **Mingzhang Gao**, **Jill Meyer**, **Jonathan Peters**,  
**Hamideh Zarrinmayeh**, **Paul Territo**, **Gary Hutchins**, **Qi-Huang**

**Zheng**. Department of Radiology and Imaging Sciences, Indiana University School of Medicine, Indianapolis, Indiana, United States

The purinergic P2X receptors are a family of cation-permeable ligand gated ion channels that open in response to the binding of extracellular adenosine 5'-triphosphate (ATP). This ionotropic receptor family contains seven subunits P2X1-7. P2X receptors are involved in various physiological processes and associated with a variety of diseases including cancer, cardiovascular and neurological diseases. P2X4 is a predominant subtype highly expressed in various central nervous system (CNS) areas, on immune cells and peripheral macrophages. The over expression of P2X4 is linked to neuroinflammation, which is an essential step in the progression of brain diseases. P2X4 has become a novel molecular target for treatment and PET (positron emission tomography) imaging of neuroinflammation and associated brain diseases such as Alzheimer's disease. 5-BDBD (5-(3-bromophenyl)-1,3-dihydro-2H-benzofuro[3,2-e][1,4]diazepin-2-one) is a specific P2X4 antagonist. We are interested in the development of PET P2X4 radioligands. In our previous work, we have developed carbon-11-labeled 5-BDBD derivatives as new potential PET probes for imaging of P2X4 receptor. In this ongoing study, we report the synthesis of a fluorine-18-labeled 5-BDBD derivative 5-(3-(2-[<sup>18</sup>F]fluoroethoxy)phenyl)-1,3-dihydro-2H-benzofuro[3,2-e][1,4]diazepin-2-one ([<sup>18</sup>F]FE-5-BDBD) as a new potential PET radioligand for P2X4 receptor. The reference standard 5-(3-(2-fluoroethoxy)phenyl)-1,3-dihydro-2H-benzofuro[3,2-e][1,4]diazepin-2-one (FE-5-BDBD) and its corresponding tosylated precursor 2-(3-(2-oxo-2,3-dihydro-1H-benzofuro[3,2-e][1,4]diazepin-5-yl)phenoxy)ethyl 4-methylbenzenesulfonate (TsOE-5-BDBD) were synthesized from commercially available starting materials 2-hydroxybenzonitrile with 2-bromo-1-(3-methoxyphenyl)ethan-1-one in 5 steps with 22%, and 5 steps with 34% overall chemical yield, respectively. The target tracer [<sup>18</sup>F]FE-5-BDBD was prepared by nucleophilic substitution of the tosylated precursor TsOE-5-BDBD with K[<sup>18</sup>F]F/Kryptofix 2.2.2 and isolated by HPLC combined with solid-phase extraction (SPE) in a home-built automated multi-purpose <sup>18</sup>F-radiosynthesis module. The decay corrected radiochemical yield from K[<sup>18</sup>F]F of [<sup>18</sup>F]FE-5-BDBD was 15-25%, and specific activity at end of bombardment (EOB) was 111-740 GBq/mmol.

## MEDI 39

### Novel and widely-applicable method to uncover pharmacologically active metabolites using metabolic biotransformation, affinity selection-mass spectrometry, and 2D NMR technique

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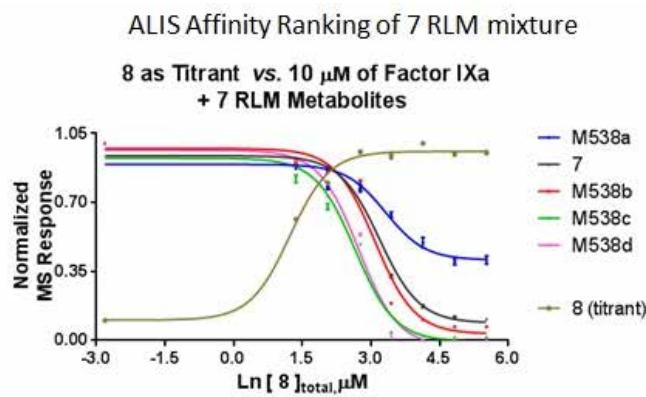
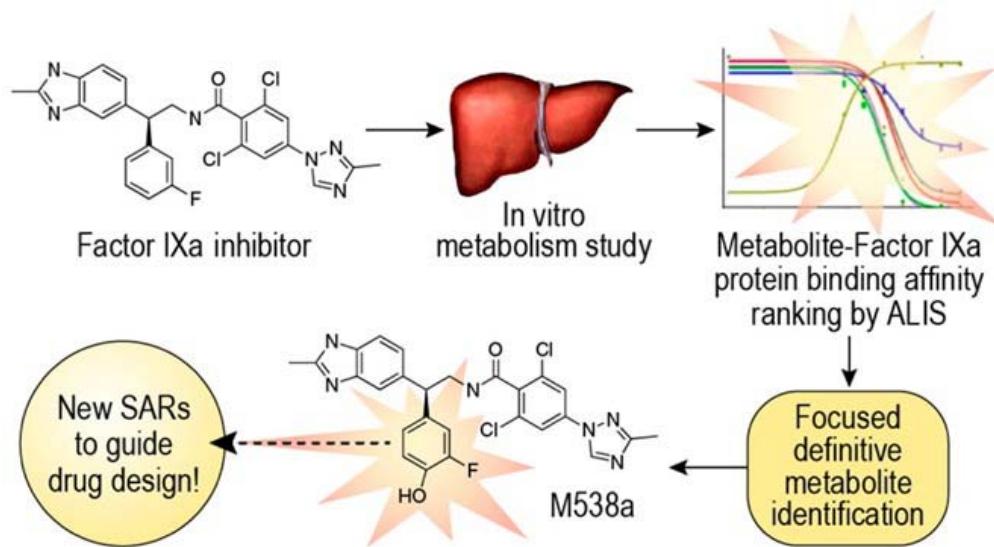
#### Introduction:

Metabolism of marketed drugs and lead compounds in the drug discovery pipeline can generate a complex mixture of metabolites that can be equally or even more pharmacologically active compared to parent compound at the therapeutically-targeted protein. Active metabolites of marketed drugs have been successfully developed as second generation drugs like desloratadine (Claritin), which is a metabolite of loratadine (Claritin). Metabolism studies providing full structural elucidation of metabolites is a challenge to perform routinely during drug discovery. A new platform that could identify active metabolites, establish their relative potency to parent, and fully determine metabolites' structures is needed.

#### Methods:

We present a novel platform which employs metabolic biotransformation methods, affinity-selection/mass spectrometric method (Automated Ligand Identification System, ALIS), and a MicroCryoProbe heteronuclear 2D NMR method. Metabolite mixtures were generated from traditional, liver preparations to turnover test drugs. ALIS can accurately rank-order the binding affinity, as surrogate for the potency, to therapeutic target of parent drug and any drug-derived metabolites. This can be done without the need for either a priori definitive structural elucidation or purification with scale-up of metabolites; only active metabolites are isolated and purified from mixtures by semi prep-

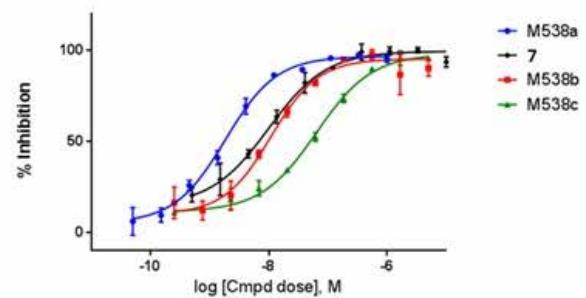
HPLC method. The chemical structures of the active metabolites in microgram quantities are characterized using MicroCryoProbe heteronuclear 2D NMR method. The potency of active metabolites is tested in functional assays.



ALIS binding affinity ranking  
M538a > 7 > M538b > M538c, M538d

FIXa enzymatic assay titration of 7 and its metabolites

2 nM Human Factor IXa- Titration of Metabolites



Sample ID	$IC_{50} (\text{nM})^*$
M538a	1.86
7	9.99
M538b	11.0
M538c	63.9

## MEDI 40

### Structural optimization of atropisomeric pyrrolopyrimidine RET kinase inhibitors

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Aberrant kinase activity is involved in many different diseases, focusing research efforts towards the development of small molecule kinase inhibitors. Although each kinase plays a specific role in these pathways, the active sites

of kinases are highly conserved throughout the kinase, making it difficult to selectively inhibit a specific kinase.

82% of FDA approved kinase inhibitors contain at least one rotational axis between two sp<sup>2</sup> carbons. This leads to an extended form of chirality called atropisomerism, where the two different rotational conformers can either exist as a rapidly racemizing mixture or isolable enantiomers. Most bioactives, as designed, exist as a rapidly interconverting atropisomeric mixture, however, when they bind to target active site, they tend to do so in an atropisomeric fashion. The presence of the non-relevant atropisomer via interconverting atropisomerism or a stable racemic mixture can result in off-target inhibition and unwanted side effects.

In efforts to solve this problem, our lab exploited atropisomerism as a selectivity filter to represent a strategy to increase kinase selectivity. In our report, we rigidified a biaryl axis by adding steric bulk adjacent to the axis and found the (R)-conformer to be 5x more selective towards RET kinase than the (S)-conformer after subjecting the conformers to a partial kinase screen. One drawback to this work was a loss in potency from the parent compound (no steric bulk). To fully exploit the strategy of spatial preorganizing an inhibitor as a selectivity filter, the analogs need to be optimized for both potency and selectivity. To accomplish this, we first identified potential analogs by screening various substituent combinations of the (R)-configuration against RET using MOE, a molecular modeling software. Using SVL code to measure poses' dihedral angles (determinant of R or S), the docking results were ranked by both binding score (potency) and ΔR/S (atropisomer preference) to establish priority molecules to synthesize. The optimized molecules were synthesized and their R and S conformers (separated via HPLC) were screened *in vitro* against RET using ADP-Glo kinase inhibition assay. Preliminary *in vitro* data (consistent with *in silico* studies from MOE) demonstrated that 1) any loss of potency from the parent compound may be regained while maintaining RET's atropisomer preference and 2) MOE can confidently be used as a tool to design spatially preorganized inhibitors.

## MEDI 41

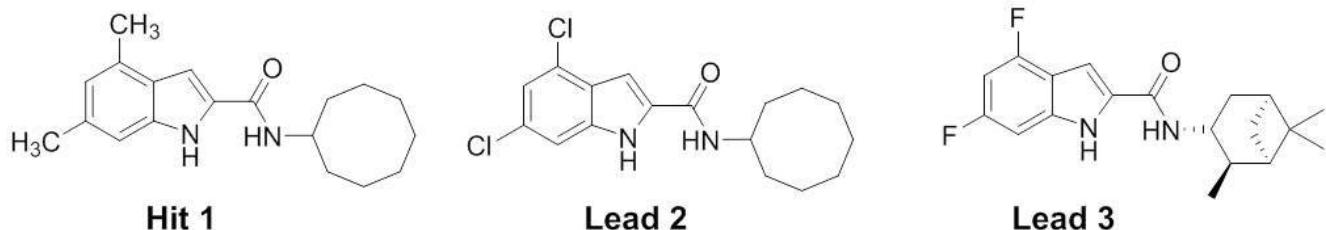
### Molecular docking of potent MmpL3 inhibitors based on the indole-2-carboxamide scaffold

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The Mycobacterial membrane protein Large 3 (MmpL3) is a transmembrane transport protein that belongs to the resistance, nodulation, and cell division (RND) family of membrane transporters. The RND family transporters are important for the survival and pathogenesis of *Mycobacterium tuberculosis* (*Mtb*). MmpL3 was characterized to be responsible for the export of mycolic acids in the form of trehalose monomycolates (TMM) from the cytoplasm to the periplasmic space. Mycolic acids are the essential component of the outer layer of the thick and waxy cell envelope of *Mtb*. Accordingly, MmpL3 has emerged as a feasible molecular target for the discovery and development of new and effective medications to combat tuberculosis. Previously, we identified and reported a novel synthetic molecule **1** based on the indole-2-carboxamide scaffold (Figure 1). Further medicinal chemistry optimization of hit **1** led to the development of better and more effective derivatives **2** and **3** (Figure 1) that showed not only superior *in vitro* and *in vivo* activity but also desirable absorption, distribution, metabolism, excretion, and toxicity (ADME/Tox) profile.

We used molecular docking techniques to investigate the possible mode of binding of the active indole-2-carboxamides at the MmpL3. A putative homology model for the MmpL3 transporter was built using the MexB transporter as a template. While considering the limitations typical for the homology models, this computational study provided possible modes of binding and thus further insights into the structure-activity relationship of the indolecarboxamides.



**Figure 1.** Indole-2-carboxamides show high inhibition of *Mtb*'s growth

## MEDI 42

### Longitudinal murine biodistribution and MRI study of a gavage-administered gadolinium pegylated metallofullerene nanoparticle

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There is increasing concern regarding the administration of gadolinium-based MRI contrast agents because of recent reports of gadolinium deposition in brain and bone tissue after clinical administration of commercial contrast agents. While the clinical significance of these findings and potential toxicity issues remain to be determined, it is clearly prudent to explore other promising MRI contrast modalities besides the currently available commercial linear and macrocyclic gadolinium contrast agents. PEGylated trimetallic nitride metallofullerenes ( $\text{Gd}_3\text{N}@\text{C}_{80}[\text{DiPEG(OH)}_x]$ ) nanoparticles have the potential to be an effective MRI contrast agent because of the robust nature of the fullerene cage which isolates the metal cluster from the bioenvironment. Although most animal studies to date have involved intravenous delivery of metallofullerene nanoparticles, oral administration has not been extensively explored. Furthermore, the presumed increase in chemical stability could potentially allow metallofullerene nanoparticles as an oral administered MRI GI tract contrast agent. In the present study, we report the oral bioavailability and biodistribution of  $\text{Gd}_3\text{N}@\text{C}_{80}[\text{DiPEG(OH)}_x]$  in mice up to 28 days after feeding of the nanoparticles. Furthermore, the *in vitro* MRI characterization of  $\text{Gd}_3\text{N}@\text{C}_{80}[\text{DiPEG(OH)}_x]$  will be described, and MRI studies of  $\text{Gd}_3\text{N}@\text{C}_{80}[\text{DiPEG(OH)}_x]$  within the lumen of mice GI tract will be presented.

## MEDI 43

### Chemistry, anti-coxsackievirus B study on tricyclic matrinane derivatives

**Yinghong Li**, *yinghongli523@aliyun.com*, **Sheng Tang**, **Dan-Qing Song**. Institute of Medicinal Biotechnology, Chinese Academy of Medical Science & Peking Union Medical College, Beijing, Beijing, China

Group B Coxsackieviruses tend to infect a variety of organs, such as heart, pleura, pancreas, and liver and cause pleurodynia, myocarditis, pericarditis, and hepatitis. However, there is no well-accepted treatment for the Coxsackie B group of viruses by now. In this investigation, taking 12-N-*m*-trifluoromethylbenzenesulfonyl matrinic butanol (**1**) as the lead, totally 50 tricyclic matrinane derivatives were designed, synthesized and evaluated for their anti-CVB3 activity to obtain ideal candidates by pharmacology, safety and pharmacokinetics evaluations. SAR revealed that matrinic acid core was the optimal core structure, and the variations on the 11- and 12- side chain were also emphasized in the report. Among all the compounds, 12N-*m*-cyanobenzenesulfonyl matrinic butane (**24d**) which demonstrated high sensitivities to all CVB strains and pleconaril-resistance CVB3 strain was screened out, the further druglike study indicated an excellent PK and a good safety profile of it. Mechanism study indicated that it targeted on the viral transcription and translation stage. Thus, we considered that **24d** is a promising anti-coxsackievirus B candidate.

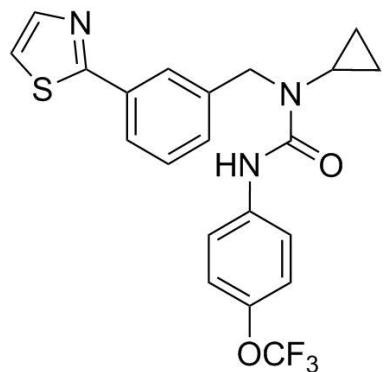
## MEDI 44

### Urea TrkA kinase inhibitors: How the hinge helped open the door to improved potency

**Kristen Jones**, *kristen\_jones@merck.com*. Merck & Co., Inc., West Point, Pennsylvania, United States

Tropomyosin receptor kinases (Trks) are a family of cell surface receptor kinases including TrkA, TrkB and TrkC, that play an important role in cell signaling. When a neurotrophic partner binds with the extracellular domain of each of these kinases, activation of the receptor occurs. Activation of TrkA by nerve growth factor (NGF) results in an increase in sensitivity of nociceptors leading to chronic sensitization and pain. Direct inhibition of TrkA kinase is one possible approach to inhibit NGF/TrkA signaling. While inhibition of the NGF/TrkA pathway has been clinically validated with NGF-neutralizing

monocloidal antibodies (tanezumab, Phase III), our interest was in small molecule inhibitors. From HTS screening, two small molecule pan-Trk inhibitor leads were discovered that bind to the TrkA enzyme in the DFG-out mode, spanning both the kinase active site and the nearby allosteric pocket. Optimization of these lead compounds was explored. Azaindazole hinge binders improved potency 10-30-fold in an open urea series, with selective modulation of P-gp susceptibility possible. Gem-dimethyl cyclic urea analogs improved potency 280-fold by water expulsion, while the combination results in cell-active analogs with moderate plasma clearance in rat.



## MEDI 45

### Repurposing of a conformationally locked nucleoside scaffold: Enhanced activity at the dopamine and norepinephrine sodium symporters

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We have repurposed (N)-methanocarba adenosine derivatives (A<sub>3</sub> adenosine receptor (AR) agonists) to enhance radioligand binding allosterically at the human dopamine (DA) transporter (DAT) and inhibit DA uptake. We extended the structure-activity relationship of this series with small N<sup>6</sup>-alkyl substitution, 5'-esters, deaza modifications of adenine, and ribose restored in place of

methanocarba. C2-(5-halothien-2-yl)-ethynyl 5'-methyl (MRS7292) and 5'-ethyl (MRS7232) esters enhanced binding at DAT ( $EC_{50} \sim 35$  nM) and at norepinephrine transporter (NET). Both compounds were selective for DAT compared to A<sub>3</sub>AR in the mouse, but not human. At DAT, binding of two structurally dissimilar radioligands was enhanced; NET binding of only one radioligand was enhanced; SERT radioligand binding was minimally affected. MRS7232 was more potent than cocaine at inhibiting DA uptake ( $IC_{50} = 107$  nM). Ribose analogues were weaker in DAT interaction than corresponding bicyclics. Thus, we enhanced the neurotransmitter transporters activity of rigid nucleosides while reducing A<sub>3</sub>AR affinity.

## MEDI 46

### **Structure-based fragment growing and serendipity: First discovery of S1 benzylamine-derived potent and selective reversible inhibitors binding to an ‘unlocked’ conformation of the serine protease Complement Factor D**

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Dysregulation of the complement alternative pathway (AP) and its activation against the host has been associated to various prevalent and rare diseases such as age-related macular degeneration (AMD), atypical hemolytic uremic syndrome (aHUS) and paroxysmal nocturnal hemoglobinuria (PNH). The trypsin-like S1 protease Factor D (FD) plays a central function in AP amplification and is highly substrate specific due to a unique active site architecture that requires induced-fit conformational transition for proteolytic activity. Selective inhibition of FD has been considered recently as an attractive mode of action to reduce AP overactivity at the first and rate-limiting step of the complement pathway.

Previously, we have reported the first potent and orally efficacious (S)-proline-based reversible FD inhibitors which emerged from a FD-tailored library design based on target enzyme family hopping in combination with *in silico* docking of a focused fragment collection and hit evaluation by ligand/protein-observed NMR. In a complementary design approach starting

from the X-ray crystal structure of a 2-indole-carboxamide fragment (NMR  $K_D = 1,600$  mM) bound to the S1 pocket of FD in a closed conformation, we investigated several strategies for fragment growing by targeting the prime site and the flexible acidic 60-loop of the enzyme. Initial SAR identified a weak-affinity analog (NMR  $K_D = 440$  mM) bearing a tethered benzylamine structural motif at the C4-position. Intriguingly, crystal structure determination revealed a flipped binding pose with the basic benzylamine directly interacting with the carboxylate of Asp<sub>189</sub>, accompanied by displacement of the Asp<sub>189</sub>-Arg<sub>218</sub> ionic interaction at the bottom of the S1 pocket and major conformational movements of the self-inhibitory loop. Further SAR-by-archive provided a S1 benzylamine derivative extending into the S1beta sub-pocket of FD. Subsequent structure-based efforts by fragment morphing and ultimately merging with a privileged proline-benzylamide S1'/S2' motif culminated in the design of cyclopentyl-(1*R*,2*R*)-dicarboxamides as novel FD inhibitors with low nano-molar potencies and selectivity against related serine proteases.

## MEDI 47

### Organizing 3D project data for structure-based drug design

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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 48

### Targeting specific interactions to improve EGFR-ligand binding

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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 49

### MOEsaic: Application of matched molecular pairs to interactive SAR exploration

*Alain Ajamian, aajamian@chemcomp.com. Chemical Computing Group, Montreal, Quebec, Canada*

SAR analysis can be huge challenge in a medicinal chemistry program. Often multiple chemical series are pursued in parallel. The number of assays involved in a screening cascade (selectivity, physico-chemical and ADME assays) can lead to the generation of hundreds to thousands of data points for each chemical series. The difficulty in managing the data means the analysis of historical results is seldom done or left to expert users. Other typical workflows are shown below.

- Review what has been made / not made
- Explore effects of structural change at a certain position
- Investigate if a trend is general or scaffold dependent
- Rationalise trends based on calculated or measured properties
- Determine if different series share the same SAR
- Is the SAR additive and/or transferable

These workflows are very difficult to perform with the existing analysis tools available.

## SAR by spreadsheet is not sufficient

1. Ensure smooth navigation & focus on key information

2. Interpretable visualization of SAR data and properties

### MEDI 50

#### Exploiting solvent effects in drug design and optimization

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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 51

#### Design, synthesis, and evaluation of potent and selective inhibitors of mono-(ADP-ribosyl)transferases, PARP10 and PARP14

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Poly (ADP-ribose) polymerases (PARPs) are a family of proteins that are responsible for post-translational modifications via the transfer or polymerization of ADP-ribose units. Many members of this family, both poly-(ADP-ribose)polymerases and mono-(ADP-ribosyl)transferases, are implicated in DNA damage repair and genomic integrity. The best known member, PARP1, has been a promising drug discovery target for the last three decades resulting in the development of several clinical candidates and two recently approved drugs for cancer therapy. Some of these PARP1

inhibitors have demonstrated broad, non-specific inhibition of other PARP family members, including many of the mono-(ADP-ribosyl)transferases. This pan-PARP inhibition and its pharmacological effects have not been well studied. Thus, specific, potent inhibitors must be designed in order to delineate the effects of this inhibition. Herein, we describe a series of compounds that selectively inhibit PARP10 (a.k.a. ARTD10) and PARP14 (a.k.a. ARTD8), both mono-(ADP-ribosyl)transferases. PARP14 is upregulated in multiple myeloma while PARP10 has been shown to play a role in chromatin remodeling and DNA repair, making both enzymes attractive drug discovery targets. In this poster, we present the synthesis and evaluation of selective inhibitors of PARP10 and PARP14. Several of the compounds presented exhibit unique binding modes, sub-micromolar potency against both PARP10 and PARP14 and most importantly selectivity over many other members of the PARP superfamily.

## MEDI 52

### **Development of azole antifungal analogues to treat cancers dependent on Hedgehog signaling**

**Kelly A. Teske**, kelly.teske@gmail.com, **Jennifer R. Pace**, **Albert M. DeBerardinis**, **Matthew K. Hadden**. *Pharmaceutical Sciences, University of Connecticut, Storrs, Connecticut, United States*

For the treatment of different cancers there remains a need for the design of targeted therapies that, unlike standard chemotherapies, block tumor growth at precise molecular targets without causing cytotoxic effects to healthy tissue. Although known for its role in regulating cell proliferation and differentiation during embryonic development, inappropriate activation of the Hedgehog (Hh) signaling pathway has been implicated in many cancers such as basal cell carcinoma and medulloblastoma. As a result, the Hh signaling pathway has emerged as a promising target for drug intervention. Itraconazole and posaconazole are azole antifungals that have previously been identified as Hh inhibitors with the ability to decrease tumor growth in models of Hh-dependent basal cell carcinoma and medulloblastoma. Using the azole antifungal scaffold, we report specific structural modifications to develop improved analogues with enhanced activity against Hh-dependent cancers.

## MEDI 53

### **Development of novel NK3 receptor antagonists with reduced environmental impact**

**Koki Yamamoto**, *yamamoto.koki.74s@st.kyoto-u.ac.jp*, **Hiroaki Ohno**,  
**Nobutaka Fujii**, **Shinya Oishi**. Kyoto Univ Gra Sch Pharm SCI, Kyoto, Japan

Neurokinin B (NKB)-neurokinin-3 receptor (NK3R) signaling plays an important role in controlling mammalian reproduction via regulation of the hypothalamo-pituitary-gonadal (HPG) axis. NKB positively regulates the reproductive hormone cascade via activation of the gonadotropin-releasing hormone (GnRH) neuron in the hypothalamus, leading to the pulsatile secretion of luteinizing hormone (LH) from the pituitary gland. NK3R-selective antagonists could suppress the reproductive functions of mammals. However, the NK3 modulators and the bioactive metabolites excreted from treated humans or animals may cause environmental and health risks including reproductive disturbances of non-target species via water pollution and/or soil contamination. To minimize the possibility of these adverse effects on non-target species, the bioactive ingredients need be converted into the inactive substances by spontaneous degradation in the environment soon after excretion. We investigated the structure–activity relationship study a quinolone-based NK3R antagonist for development of novel antagonists with reduced environmental toxicity.

Talnetant derivatives with hydroxy- or mercapto-group modifications exhibited comparable NK3R binding inhibition to that of the parent compound talnetant. Air-oxidation of 3-mercaptopquinoline derivative afforded the disulfide or isothiazolone form with no binding affinity to NK3R. This novel NK3R antagonist would be applicable to the treatment of sex-hormone disorders with decreased environmental impact.

#### MEDI 54

**Synthesis of [<sup>11</sup>C]methyl 3-((2,2-difluoro-5*H*-[1,3]dioxolo[4',5':4,5]benzo[1,2-*d*]imidazol-6-yl)carbamoyl)benzoate as a new potential PET agent for imaging of casein kinase 1**

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The human casein kinase 1 (CK1) family of protein kinases are serine/threonine-specific enzymes, consisting of at least 6 different isoforms ( $\alpha$ ,  $\gamma_1$ ,  $\gamma_2$ ,  $\gamma_3$ ,  $\delta$ , and  $\epsilon$ ). Deregulation of CK1 activity is linked to several pathological disorders and diseases like cancer, neurodegenerative diseases, and inflammatory disorders. CK1 has become an interesting molecular target for the treatment and PET (positron emission tomography) imaging of CK1

associated diseases such as cancer cell proliferation. Recently difluoro-dioxolo-benzoimidazol-benzamides have been developed as potent and selective CK1 $\delta$  and CK1 $\epsilon$  inhibitors with nanomolar inhibitory activity. We are interested in the development of PET CK1 imaging agents. Here we report the synthesis of a carbon-11-labeled difluoro-dioxolo-benzoimidazol-benzamide [ $^{11}\text{C}$ ]methyl 3-((2,2-difluoro-5H-[1,3]dioxolo[4',5':4,5]benzo[1,2- $d$ ]imidazol-6-yl)carbamoyl)benzoate. The reference standard methyl 3-((2,2-difluoro-5H-[1,3]dioxolo[4',5':4,5]benzo[1,2- $d$ ]imidazol-6-yl)carbamoyl)benzoate and its corresponding desmethylated precursor 3-((2,2-difluoro-5H-[1,3]dioxolo[4',5':4,5]benzo[1,2- $d$ ]imidazol-6-yl)carbamoyl)benzoic acid were synthesized from commercially available starting materials 2,2-difluorobenzo[ $d$ ][1,3]dioxol-5-amine and 3-(methoxycarbonyl)benzoic acid in 6 steps with 45%, and 7 steps with 41% overall chemical yield, respectively. The target tracer [ $^{11}\text{C}$ ]methyl 3-((2,2-difluoro-5H-[1,3]dioxolo[4',5':4,5]benzo[1,2- $d$ ]imidazol-6-yl)carbamoyl)benzoate was prepared from the acid precursor with [ $^{11}\text{C}$ ]CH<sub>3</sub>OTf under basic condition (2 N NaOH) through O-[ $^{11}\text{C}$ ]methylation and isolated by HPLC combined with solid-phase extraction (SPE) in 30-40% radiochemical yield based on [ $^{11}\text{C}$ ]CO<sub>2</sub> and decay corrected to EOB. The radiosynthesis was performed in a home-built automated  $^{11}\text{C}$ -radiosynthesis module. The radiochemical purity was >99%, and specific activity at EOB was 370-1110 GBq/ $\mu\text{mol}$ .

## MEDI 55

### Strategies for improving flash chromatography efficiency

**John R. Bickler**, *bob.bickler@biotage.com, Elizabeth Denton. Biotage, LLC, Charlotte, North Carolina, United States*

For most medicinal, organic, and natural product chemists flash chromatography is a necessary part of their research. As such, many chemists need quick isolation of at least one desired component from a crude mixture in relatively high yield and purity. This need for speed, purity, and yield pits these desires against each other as you can typically optimize on only two of the three goals.

In this poster, we will describe some techniques that help chemists optimize flash purification and maximize speed, yield, and purity.

## MEDI 56

### **Mass-directed flash purification – a new tool for isolating natural products**

**John R. Bickler**, *bob.bickler@biotage.com*, *Elizabeth Denton. Biotage, LLC, Charlotte, North Carolina, United States*

Natural product chemists are like hunters – they try to find interesting molecules in a sea of other compounds. By employing various extraction and purification techniques these chemists can often isolate compound classes and then even individual species using preparative chromatographic techniques. However, these chemists are often working “blind” as they do not know what they are collecting.

In this poster, we will show how an in-line mass detector on a flash chromatograph can help identify and isolate compounds based on molecular mass.

## MEDI 57

### **Synthesis and Structure–Activity Relationship (SAR) of tetra-substituted cyclohexyl diol inhibitors of pan-PIM kinases**

**Wooseok Han**, *wooseok.han@novartis.com. Mail Stop 4.5, Novartis Institutes for Biomedical Research, Emeryville, California, United States*

Overexpression of Proviral Insertion of Moloney virus (PIM) 1, 2 and 3 kinases is frequently observed in many human malignancies, including multiple myeloma, non-Hodgkins lymphoma, and myeloid leukemias. Pan-PIM 1, 2 and 3 kinase inhibitors have recently begun to be tested in humans to assess whether pan PIM kinase inhibition may provide benefit to cancer patients. We discovered a potent and selective pan-PIM kinase inhibitor, PIM447, which is currently being assessed in phase IB studies of patients with hematological malignancies. A key structural motif of PIM447 is the 1,3-cis-disubstituted cyclohexyl amine, where the amino group participates in H-bond interactions with the PIM protein residues which are crucial for potency. In the early stage of the discovery of PIM447, we were interested in replacing the amino group with isosteres such as a hydroxyl group as an alternative H-bond acceptor and donor. In this presentation, the cellular potency and metabolic stability of cyclohexyl diol inhibitors of pan-PIM kinases will be reported. In addition, the synthesis of synthetically challenging tetra-substituted cyclohexyl diol intermediates will be described.

## MEDI 58

### Morphing of antimicrobial peptides towards selective antibiotic agents

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There is a great need for novel antibiotics, which antimicrobial peptides (AMPs) could potentially satisfy. AMPs are part of the host-defense mechanisms of most multicellular organisms. Certain AMPs directly act on bacterial cell membranes without the need for interaction with target proteins prone to potential mutation. This raises the question of which molecular features equip AMPs with their direct membranolytic antimicrobial activity. We have tried to pin down these features by synthesizing and testing a range of linear cationic peptides with a fixed  $\alpha$ -helical amphipathic secondary structure but varying amino acid sequence. Novel peptides were derived from a gradual transformation of the AMPs Aurein2.2d2 (GLFDIVKKVVGALG-NH<sub>2</sub>) to Klk14 (KLLKLLKKLLK-NH<sub>2</sub>) and vice versa. Of the 18 synthesized peptides, 11 peptides inhibited the growth of *S.aureus* at concentrations of below 50 $\mu$ M. Hemolysis and HDME cell selectivity assays revealed that six peptides exhibit therapeutic indices greater than three. Circular dichroism and nuclear magnetic resonance spectroscopy confirmed an overall helical secondary structure of all molecules. Molecular dynamics simulations in membrane mimicking environment additionally supported these findings. In conclusion, the presented rectilinear morphing strategy is a simple method to explore peptide sequence space, while retaining overall structural features. By help of this new approach, we have discovered novel synthetic AMPs that are potent and selective towards Gram-positive bacteria.

## MEDI 59

### Problem-based learning in drug discovery with MOE

**Audrey Bonin**, [abonin@chemcomp.com](mailto:abonin@chemcomp.com). Chemical Computing Group, Montreal, Quebec, Canada

Problem-Based Learning (PBL) is an 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 includes: 1. A guided learning process with challenging open-ended problems where there are multiple solutions and 2. 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. Problem-Based Learning (PBL) 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: 1. A guided learning process with challenging open-ended problems where there are multiple solutions and 2. 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 60

### **Identification and characterization of small molecule scaffolds as inhibitors of the translesion synthesis pathway**

**Zuleyha Ozen**, ozen49@gmail.com, Matthew K. Hadden. *Pharmaceutical Sciences, University of Connecticut, Storrs Mansfield, Connecticut, United States*

Resistance to standard anti-cancer treatments is a persistent concern in the field of oncology. In general, there is an initial overall response to first line chemotherapeutics in patients; however, overtime they acquire resistance and experience relapse, leading to an increase in dose or a change in drug regimen. The translesion synthesis (TLS) pathway is a DNA damage tolerance mechanism, which allows proliferating cells to bypass DNA lesions. Previous studies examining the suppression of TLS signaling have increased sensitivity to first-line chemotherapeutics, marking the pathway as an emerging therapeutic target. We have identified small molecules as inhibitors of key protein-protein interactions in the TLS pathway and have evaluated their ability to increase the efficacy of cisplatin in preliminary cellular assays. Herein, we report the identification of these compounds as well as describe their anti-TLS activity.

## MEDI 61

### Development of affinity probes for identification of the molecular target for a novel series of Rho/MRTF/SRF-mediated gene transcription inhibitors

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Novel biological targets for fibrotic diseases are of great interest since existing therapies do not address the underlying pathophysiology. A major hallmark of fibrosis is the fibroblast-to-myofibroblast transition. Myofibroblasts overproduce extracellular matrix components and impart contractile forces that cause architectural distortion of the surrounding tissue. This myofibroblast transition is driven by extracellular signals including transforming growth factor  $\beta$  (TGF $\beta$ ), lysophosphatidic acid (LPA) and others, which can activate Rho family GTPases and their downstream kinase, Rho-associated coiled-coil containing protein kinase. This leads to activation of myocardin-related transcription factor (MRTF)/serum response factor (SRF)-mediated gene transcription, ultimately inducing myofibroblast differentiation. A series of potent oxadiazole-thioether inhibitors that block Rho/MRTF/SRF-mediated gene expression was identified from a phenotypic high-throughput screen. The high level of potency observed for this series is hypothesized to result from a unique irreversible covalent mechanism with the biological target(s). Structure-activity relation studies yielded novel inhibitors that show promising results in multiple *in vitro* and *in vivo* models of fibrosis. Due to the phenotypic nature of the assay, the molecular target(s) for this series remain(s) unknown. In hopes of identifying potentially new molecular targets that block mechanisms involved in the progression and maintenance of pathological fibrosis, we designed and synthesized both an active resin-immobilized affinity probe and an inactive control resin. Importantly, these probes were designed taking into consideration the hypothesized irreversible covalent mechanism of the series with the biological target(s). Following incubation with HEK 293T cell lysate and trypsin digestion of the resin-immobilized proteins, proteomics analysis of the protein fragments selectively immobilized on active resin generated a list of putative targets. These proteins are currently undergoing validation as potential biological target(s) for our potent oxadiazole-thioether inhibitors.

## MEDI 62

### Asymmetric synthesis of novel antimalarial agents with fluorene core

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Malaria is a neglected tropical disease that remains a leading cause of morbidity and mortality among the world's poorest populations. More than 100 tropical and sub-tropical countries are endemic for this infectious disease. Pregnant women and children are the most sensitive to this infection and, in 2015, 429 000 people died. Among the five species of *Plasmodium* responsible for human malaria, *P. falciparum* is the parasite which causes the most serious form of the disease. More recent efforts focused on the development of antimalarial vaccines and since 2006, World Health Organization (WHO) recommends artemisinin-based combination therapies (ACTs). In drug resistance areas, several antimalarial drugs, such as aminoalcohol-aryl (mefloquine (MQ), lumefantrine (LM)), are currently used in combination with artemisinin derivatives. However, the emergence of multi-drug-resistant parasites decreases efficacy of ACTs. Thus, the design of new active compounds on *Plasmodium*-resistant strains is urgently. We have previously developed an asymmetric synthesis to prepare 4-aminoalcohol-quinoline enantiomers (AQ) as MQ analogs. They were active on nanomolar range against 3D7 (chloroquine-sensitive) and W2 (chloroquine-resistant) *P. falciparum* strains. Interestingly, (S)-enantiomers displayed an activity increased by 2 to 15-fold as compared to their (R)-counterparts. During the *Plasmodium* intra-erythrocytic asexual stages, hemozoin formation and the oxidative and glutathione-dependent degradation of heme are inhibited by these aminoalcohol-aryls (MQ, LM). Currently their mechanisms of actions are not totally clear and remain to be explored. In continuation of our work, we are interested now to study the change of heterocycle (fluorene vs quinoline) on the antimalarial activity. We focus on the design and the preparation of novel asymmetric 2,4,7-trisubstituted fluorenes, new aminoalcohol-fluorene derivatives (ALF) as LM analogs. The evaluation of their antiplasmoidal activity against *P. falciparum* and their corresponding cytotoxicity is under progress.

## MEDI 63

### Small molecule and peptidic ligands as PCSK9-LDLR inhibitors

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Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a genetically validated target for hypercholesterolemia. PCSK9 binds to the low-density lipoprotein receptor (LDLR) and facilitates its lysosomal degradation reducing cell surface LDLR levels thereby increasing circulating levels of low-density lipoprotein cholesterol (LDL-C). There are two approved human monoclonal antibodies that bind to PCSK9 and inhibit interaction with the LDLR. Both of these antibodies bind to the epitope of PCSK9 that is adjacent to the region required for LDLR interaction. The current presentation will describe both peptidic and fragment-derived small molecule approaches that are designed to inhibit the interaction of PCSK9 with LDLR.

## MEDI 64

### **Novel Wnt/β-catenin inhibitors for the treatment of colorectal cancer**

**Yong Ai**, yai@rx.umaryland.edu, **Wei Yang**, Yingjun Li, Yan Shu, Fengtian Xue. *Department of Pharmaceutical Sciences, University of Maryland School of Pharmacy, Baltimore, Maryland, United States*

Colorectal cancer (CRC) is one of the leading causes of cancer-related deaths in the United States. The Wnt/β-Catenin signaling pathway plays a vital role in CRC initiation and progression. Constitutive activation of the Wnt/β-catenin signaling pathway is found in CRCs, and thus, it is an attractive target for anti-CRC therapy. A series of pyrazole-based pyrvinium analogs were designed and synthesized. Of which, **YW2065** inhibits Wnt/β-catenin signaling activity with an IC<sub>50</sub> value of 74 nM. This new inhibitor displays potent antiproliferative activity against SW480 cells (IC<sub>50</sub> = 2.5 μM), is not toxic and shows significantly improved pharmacokinetic profile. In addition, **YW2065** represses the growth of implanted CRC in mice. Together, **YW2065** acts a potential Wnt/β-Catenin inhibitor and may be a promising candidate for intervention of CRC.

## MEDI 65

### Selective inhibition of Hedgehog (Hh) signaling by analogues of vitamin D3 and calcitriol

*Chad Maschinot, maschinotc1@gmail.com, Matthew K. Hadden. Pharmaceutical Sciences, University of Connecticut, Storrs Mansfield, Connecticut, United States*

The Hedgehog (Hh) signaling pathway is a developmental pathway that has emerged as a target for anti-cancer drug development, where aberrant signaling has been linked to the development of a variety of cancers, specifically basal cell carcinoma (BCC) and medulloblastoma (MB). The first Hh inhibitor, GDC-0449 (Vismodegib), was approved in 2012 followed by LDE225 (Sonidegib) in 2015 for advanced BCC by targeting the trans-membrane receptor Smoothened (Smo). Since their approval, Smo mutations have led to resistance to both compounds. In recent years, the endogenous seco-steroids, vitamin D3 (VD3, cholecalciferol) and calcitriol (1,25-dihydroxyvitamin d3) were identified as inhibitors of Hh signaling. Due to the innate activation of canonical vitamin D signaling through the Vitamin D Receptor (VDR) by calcitriol, VD3 was utilized as our lead scaffold. Initial SAR studies indicated coupling of a phenolic A-ring motif with Grundmann's alcohol increased both the potency and selective for Hh signaling and provided a new lead. The focus of this project is to synthesize analogues of VD3 and calcitriol to further explore the SAR of VD3 to identify the requirements for anti-Hh activity and understand its mechanism of action. Progress towards these studies will be described herein.

## MEDI 66

### Studies towards the identification of small molecule regulators of SWI/SNF chromatin remodeling

*Angela Zaino, angela.zaino@uconn.edu, Matthew K. Hadden. Pharmaceutical Sciences, University of Connecticut, Storrs Mansfield, Connecticut, United States*

Epigenetics is the regulation of gene expression through the reversible modification of DNA and proteins that bundle DNA, such as histones. Chromatin remodeling is an epigenetic process that regulates gene expression by modifying chromatin structure to allow transcriptional proteins access to DNA. The SWI/SNF family of chromatin remodelers consist of

multiprotein complexes that recognize specific histone modification and utilize an ATP-dependent process to modify nucleosome structure and regulate gene expression. Remodeling by SWI/SNF complexes occurs in an ordered way during normal differentiation and development but aberrant remodeling can contribute to many disease states, including cancer, inflammation, metabolic disease, and neuropsychiatric disorders. To date, small molecule regulators of SWI/SNF remodeling function have not been disclosed, but they are needed to more clearly understand the role of these proteins in both normal and disease states. Our progress towards the identification of selective inhibitors of SWI/SNF remodeling activity will be presented herein.

## MEDI 67

### **Imine-based dynamic combinatorial chemistry for discovery of multivalent RNA-binding ligands**

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Non-protein coding RNA transcripts have been increasingly recognized as potential drug targets owing to their important roles in cellular processes. Since peptide-based and RNA-based therapeutics often exhibit poor *in vivo* delivery and pharmacokinetics, small molecules offer an excellent alternative tool for targeting RNA. However, due to its unique chemical and structural properties compared to protein targets, RNA has been difficult to target with drug-like small molecules. For example, despite the proven promise of targeting RNA with multivalent ligands, progress in this area has been hampered by current limitations in three-dimensional structure characterization of large RNAs. There is thus a need to develop techniques that do not rely on structure-based design. We are developing an imine-based dynamic combinatorial chemistry (DCC) technique for multivalent ligand discovery for large RNAs. In DCC, a target biomolecule is incubated with a thermodynamically-controlled dynamic library of small molecules, allowing it to select its highest affinity binders. DCC is thus superior to structure-based design since all thermodynamically stable conformations of the target participate in the ligand discovery process. To date, we have identified favorable conditions for imine formation in aqueous media, conducted comparative studies of amine reactivity towards imine formation, and begun validation studies on a known RNA binding scaffold. For validation studies, an aldehyde scaffold will be incubated with a diverse library of primary amines to

discover ligands for the HIV-1 Transactivation Response (TAR) RNA. Upon validating the DCC method, we will set out to identify first multivalent ligands for a number of disease-relevant RNA targets. Ultimately, this work will provide a much-needed platform for multivalent ligand discovery for large RNAs, particularly those that have yet to be structurally characterized.

## MEDI 68

### Diversification of nitrogen containing fused heterocycles for selective recognition and binding to RNA

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Despite the strong evidence showing significant involvement of RNA in a variety of diseases, development of small molecule-based strategies to selectively target RNAs remains a significant challenge. A major hurdle is the weak understanding of guiding principles for selective recognition and binding of small molecules to RNA structure fragments. In an attempt to address this challenge, our lab has developed a cheminformatics analysis based small molecule design approach for infusing bias towards binding RNA, which builds on the chemical and spatial properties that are unique to the known bioactive RNA-binding small molecule ligands. In this presentation, we will discuss our efforts to diversify various nitrogen-based heterocyclic scaffolds, which are biased towards binding RNA in biological assays, for selective RNA recognition and binding. Stepwise diversification strategies have yielded a library of over a 30 small molecules featuring an array of heterocyclic rings that contain easily accessible modifications that in turn lead to significant scaffold diversity. The binding of these scaffolds is then studied against a variety of RNAs featuring different structural elements. Pattern recognition protocols such as principal components analyses (PCA) can be performed using this data in order to understand the relationship between the small molecule structural features and their binding potency as well as selectivity towards RNA structure motifs. These results will provide crucial insights into small molecule:RNA recognition, which will strongly contribute towards developing and validating the hitherto unavailable guiding principles for rational design of small molecule scaffolds for selectively targeting RNA.

## MEDI 69

### Targeting the EWS-FLI1 pre-mRNA in Ewing sarcoma through small molecule microarray screening

**Robert Boer<sup>1</sup>, reboer@loyola.edu, Carla Neckles<sup>2</sup>, David Calabrese<sup>1</sup>, Guillermo Rangel-Rivera<sup>2</sup>, Suntae Kim<sup>2</sup>, Natasha J. Caplen<sup>2</sup>, John Schneekloth<sup>1</sup>.** (1) Chemical Biology Laboratory, National Cancer Institute, Frederick, Maryland, United States (2) Genetics Branch, National Cancer Institute, Bethesda, Maryland, United States

The main oncogenic event in approximately 85% of Ewing Sarcoma (ES) tumors is a chromosomal translocation that produces a fusion gene containing the 5' end of the *EWSR1* gene and the 3' end of the *FLI1* gene. In an estimated 30% of tumors, translocations that retain exon 8 of the *EWSR1* gene generate an out-of-frame transcript unless the exon is removed by alternative splicing. Previous work has demonstrated ES cells with a genomic breakpoint that retains exon 8 of *EWSR1* require the heterogeneous nuclear ribonucleoprotein H1 (HNRNPH1) to express an in-frame *EWS-FLI1* mature mRNA. Thus, the ability to find a small molecule that inhibits alternative splicing by HNRNPH1 would provide an opportunity to uncover new therapies for ES. In this work, we demonstrated that the G-rich regions within exon 8 of *EWSR1* pre-mRNA fold into G-quadruplexes *in vitro*. Additionally, we employed a Small Molecule Microarray (SMM) approach to identify compounds that selectively bind to the *EWSR1* G-quadruplex over other DNA and RNA G-quadruplexes. We developed a short and flexible synthetic sequence to access a lead compound as well as a number of analogues to provide a structure-activity relationship for this series. Compounds identified from this approach have shown good affinity for the RNA. Biochemical and cell-based experiments have demonstrated that the lead compound identified from our screen prevents HNRNPH1 binding. Therefore, the SMM approach has allowed for the rapid identification of a selective RNA-binding small molecule that can be developed into a novel therapy for ES.

## MEDI 70

### Exploiting amino acid differences: Design, synthesis and biological evaluation of substituted pyrido[3,2-*d*]pyrimidines as potent and selective dihydrofolate reductase inhibitors for pneumocystis pneumonia infection

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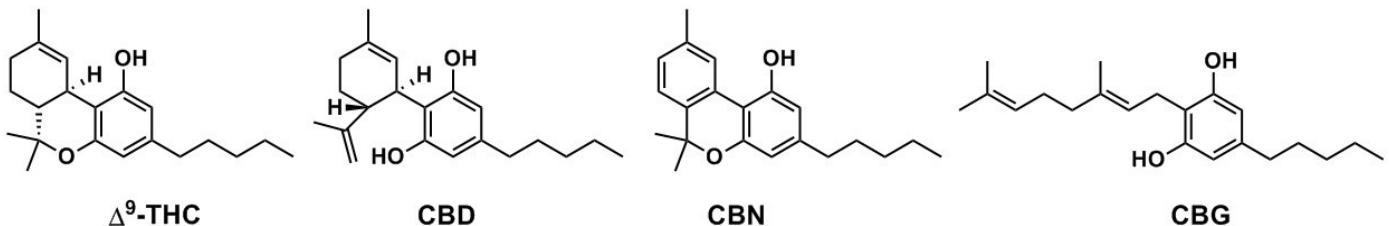
Pneumocystis jirovecii (*Pj*) causes Pneumocystis pneumonia (PCP), which can be fatal for patients with HIV/AIDS, those undergoing chemotherapy for cancer and organ transplantation. Both treatment and prophylaxis for PCP involves combination of trimethoprim (TMP) with sulfamethoxazole (SMX). Though low potency of TMP towards the *Pj* dihydrofolate reductase (*Pj*DHFR) is augmented by SMX, it can lead to discontinuation due to clinically resistant strains of DHFR, dihydropteroate synthase (DHPS) or allergies to SMX. Piritrexim and trimetrexate are potent DHFR inhibitors, but are non-selective for *Pj*DHFR with dose-limiting toxicities. For patients, irresponsive or resistant to the first line treatment, new drugs are critically needed. To predict binding of proposed compounds in absence of a *Pj*DHFR crystal structure, we developed a *Pj*DHFR homology model. Comparison of active sites of *Pj*DHFR and human DHFR (hDHFR) revealed amino acid differences which could be exploited to gain potent and selective *Pj*DHFR inhibitors. For instance, M33/F31 in *Pj*DHFR/hDHFR can affect binding due to their distinct steric and electronic effects. Gangjee *et al.* reported 6-substituted pyrido[3,2-*d*]pyrimidines with nitrogen linkers which displayed nanomolar potency and 28-fold selectivity for *Pj*DHFR. To further potentiate activity, isosteric replacement of nitrogen with sulfur at the 6-position was performed. Compared to the lead, sulfur linked analogs could change bond angle, distance and electronics of the side chain aryl group and cause a clash with F31 in hDHFR, whereas they appropriately fit with M33 in *Pj*DHFR. The 7-substituted analogs of these compounds were also synthesized. As predicted by modeling, enzyme assays of these compounds showed picomolar potency and about 100-fold selectivity for *Pj*DHFR. Further evaluation in *P. carinii* cell assay and *P. murina* animal model showed significant reduction in infection burden, compared to TMP alone and efficacy similar to TMP/SMX. The synthesis, molecular modeling, *in vitro* and *in vivo* evaluation of these compounds will be presented

## MEDI 71

### Chemistry of Canadian medical cannabis

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Cannabis was approved in Canada in 2000 for medical use for the treatment of various clinical ailments. Several chemical constituents in this plant exhibit anti-inflammatory, antibacterial, antiviral, anticancer activities; they have shown efficacy in neuropsychological disorders as well. To date, 564 compounds were characterized in *Cannabis sativa L.*, including the unique pharmacologically-active cannabinoids, which act primarily through the CB1 and CB2 cannabinoid receptors. In their natural state within the cannabis plant, the cannabinoids carry a carboxylic acid moiety, and are physiologically almost inactive. In their decarboxylated forms, these phytocannabinoids such as Δ<sup>9</sup>-THC and CBD, either alone or in combination with other phytocannabinoids, exhibit a variety of physiological activities. We were interested in characterizing different commercially available strains of Canadian medical cannabis in order to understand their chemical composition, hence, potentially their therapeutic benefits. Cannabis resin was extracted from a commercial sample of medical cannabis and was subsequently subjected to decarboxylation. We employed UPLC-MS to analyze the extracted chemicals prior to and post-decarboxylation. Within the pre-decarboxylated extract, 63 compounds including the characteristic phytocannabinoids, sesquiterpenes and plant fatty acids were observed. Changes in the chemical composition of the post-decarboxylated cannabis extract were revealed through the disappearance of 26 compounds and the emergence of 22 new ones found, including the neutral cannabinoids. Subsequently, we extracted and activated two additional strains of medical cannabis, and performed similar analysis. The decarboxylated cannabis extracts, possessing the neutral cannabinoids and other plant components may serve as a starting point for development of potential lead compounds. This serves as a stepping stone to implement the analyses of commercial medical cannabis samples available in the country, and to understand their chemical composition, hence, potential pharmacological profiles.



Major cannabinoids

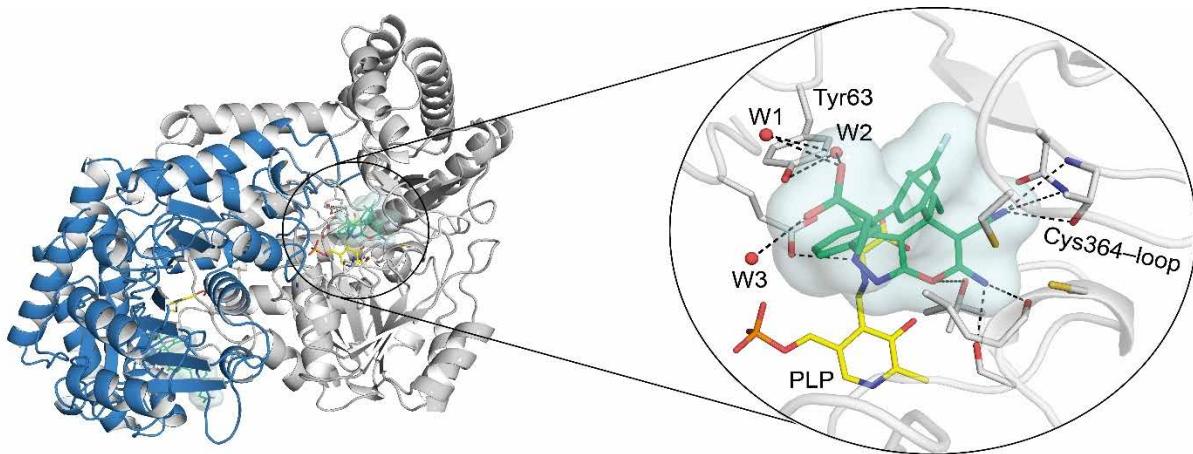
## MEDI 72

### Identification of a potent *in vivo* candidate inhibiting SHMT, an underexploited antimalarial target

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Malaria is mainly caused by the parasite *Plasmodium falciparum* (*Pf*). Despite the numerous treatments available, emerging drug-resistant strains render the discovery of novel active substances urgent. In this respect, the folate cycle with its several enzymes was validated as an antimalarial target. Until now, few antimalarials address this pathway. However, inhibition of serine hydroxymethyltransferase (SHMT), a key enzyme of the folate cycle, has not been investigated so far. *Arabidopsis thaliana* (*At*) SHMT inhibitors, based on a pyrazolopyran core, from an herbicide optimization program at BASF-SE demonstrated promising antimalarial activity on *P. falciparum* and *P. vivax* (*Pv*) SHMT. Our pioneering work on the inhibition of SHMT shed light on this novel antimalarial target. Several new cocrystal structures with *Pv*SHMT allowed to understand the binding mode of this class of molecules in detail. In this work, the optimization of pyrazolopyran-based inhibitors guided by computational modeling was focused on improving liver microsomal stability while keeping high *in vitro* potency. Subtle alteration of the core, derivatization of the peripheral moiety occupying the *para*-aminobenzoate binding site, and resolution of the single enantiomers led to the identification of a promising candidate with microsomal stability exceeding 4 h and *in vitro* potency on the *Pf*NF54 strain in the two-digit nM range. The optimized ligand is currently investigated *in vivo* in a mouse model. Finally, several new X-ray crystal

structures of *PvSHMT*-ligand complexes were solved, showing for the first time a small water cluster bridging a ligand with Tyr63. Also, the conformational flexibility of the Cys364-loop was analyzed thanks to the 7 ligand-protein complexes obtained so far in this optimization program.



## MEDI 73

### Macrocyclic triazolopyridines as potent inhibitors of myeloperoxidase

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Myeloperoxidase (MPO), a heme-containing peroxidase, releases hypochlorous acid as an important component of the body's defense against invading microbes. However, high plasma MPO levels and MPO-specific oxidation products have been associated with chronic inflammatory diseases including atherosclerosis, so inhibitors of MPO have been proposed to provide therapeutic potential. The crystal structure overlay of two potent MPO inhibitors inspired the design and synthesis of macrocyclic MPO inhibitors. The macrocycles were designed with the goal to improve potency and physical properties. A variety of macrocyclization synthesis strategies were evaluated for rapid SAR study, including ring-closing Mitsunobu, Ullmann, and palladium-catalyzed cross-coupling reactions. An intramolecular Suzuki-Miyaura reaction provided the best general access to several macrocycles with varying ring size and structural complexity, resulting in MPO inhibitors with nanomolar potency.

## MEDI 74

### **Design, synthesis, and anti-neoplastic evaluation of dimeric amino-naphthoquinones against acute myeloid leukemia (AML) cells**

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The current therapeutic strategies against acute myeloid leukemia (AML) including conventional antimetabolites and DNA damaging agents, and hematopoietic stem cell transplantation cure only a small fraction of patients, underscoring the need for the design and synthesis of agents with novel mechanisms of action. We have investigated dimeric naphthoquinones as novel agents against solid and hematologic neoplasms. Here, we present incorporation of amine-based functional groups into dimeric naphthoquinone cores, which culminated in improvement of potency, stability and solubility of these new analogs against AML cell lines. Preliminary mechanistic studies have suggested dual mechanism of action involving perturbation of cellular oxidative state and immunomodulatory effect.

## MEDI 75

### **Discovery and characterization of 1*H*-pyrazol-5-yl-2-phenylacetamides as novel, non-urea containing GIRK1/2 potassium channel activators**

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The G protein regulated inwardly rectifying potassium channels (GIRK, Kir3) are a family of inward-rectifying potassium channels and are key effectors in

signaling pathways. Four GIRK channel subunits are expressed, either as homo- or heterodimers in mammals: GIRK1, GIRK2, GIRK3 and GIRK4 and are expressed in both the CNS and the periphery. There is significant evidence supporting the roles of GIRK in a number of physiological processes and potential target for numerous indications, such as pain, epilepsy, reward/addiction and anxiety. Previously, our laboratory has identified a series of urea containing molecules as GIRK1/2 preferring activators. Unfortunately, the urea series suffers from significant PK liabilities (solubility, brain penetration) and due to these short-comings, we have identified a novel series of 1*H*-pyrazolo-5-yl-2-phenylacetamides in an effort to improve upon the PK properties. This poster will detail our efforts in the identification, structure-activity relationship (SAR) and the *in vitro* and *in vivo* pharmacokinetic profiling of this new series of compounds.

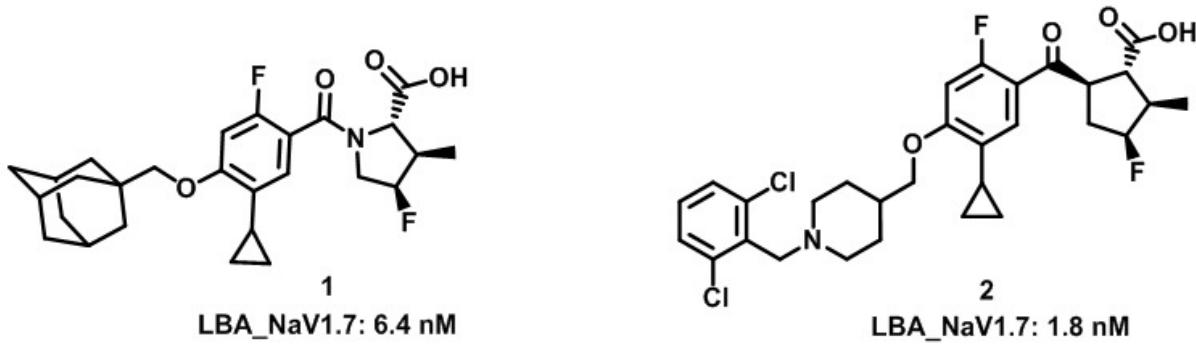
## MEDI 76

### Design and development of new potent and selective inhibitors of NaV1.7

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Voltage-gated sodium channel NaV1.7 is a central player in human pain. Loss of NaV1.7 function in humans causes profound insensitivity to pain.

Reproducing this effect with small molecule inhibitors is an attractive potential way to treat pain. Using structure and properties based design, we developed a series of novel, potent and isoform selective inhibitors of human NaV1.7 that bind to the fourth voltage-sensor domain (VSD4). Derived from the initial proline series (compound **1**), cyclopentane carboxylic acid **2** demonstrated robust PK/PD in our IEM mouse assay.



## MEDI 77

### Novel indole pharmacophore series of irreversible MPO inhibitors

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Myeloperoxidase (MPO) activity and subsequent generation of hypochlorous acid has been associated with the killing of host-invading microorganisms (e.g., bacteria, viruses, and fungi). However, during oxidative stress high plasma activity of secreted MPO can cause host tissue damage. As such, MPO levels and activity have been linked to several chronic inflammatory diseases. Herein we describe the discovery of a novel indole pharmacophore series, originating from an HTS hit. The indoles are mechanism-based irreversible inhibitors, and have been optimized for potency, *in vitro* safety selectivity, and oral exposure.

## MEDI 78

### Novel inhibitors of the NLRP3 inflammasome

**Jacob Fulp, fulpjw@vcu.edu, Liu He, Yuqi Jiang, Shijun Zhang. Medicinal Chemistry, Virginia Commonwealth University, Richmond, Virginia, United States**

Inflammasomes play a vital role in our innate immune system by recognizing molecular danger signals associated with pathogenic invasion and

endogenous host molecules correlated with cellular damage and stress. The NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome, is the most well elucidated inflammasome. Activation of the NLRP3 inflammasome stimulates the secretion of both interleukin IL-1 $\beta$  and IL-18. These two proinflammatory cytokines drive inflammation and promote a type of cell-death known as pyroptosis. Because studies suggest that the dysregulation of the NLRP3 inflammasome and IL-1 $\beta$  plays an important role in the pathogenesis of a variety of diseases, including Alzheimer's disease and multiple sclerosis, developing novel NLRP3 inflammasome inhibitors capable of combatting these illnesses is of significant importance. However, NLRP3 inhibitors are currently lacking. Although glyburide, an anti-diabetic drug which stimulates the release of insulin from pancreatic b-cells, has been shown to inhibit the NLRP3 inflammasome in myeloid cells *in vitro*, it is not a realistic NLRP3 inhibitor *in vivo* because this drug would induce lethal hypoglycemia at concentrations required for NLRP3 inhibition. We hypothesized that it would be possible to alter the structure of glyburide to retain its NLRP3 inhibitory effect while eliminating this molecule's insulin releasing activity. Currently, we have synthesized novel molecules, based off the structure of glyburide, that inhibit the production of IL-1 $\beta$  without affecting glucose levels. The initial results of our small molecule glyburide analogues are quite promising. So far, we have developed compounds that inhibit the production of IL-1 $\beta$  in J774.A1 macrophage cells, with IC<sub>50</sub> values in the low nanomolar range.

## MEDI 79

### Synthesis of novel tanshinones for probing the inflammatory response in zebrafish

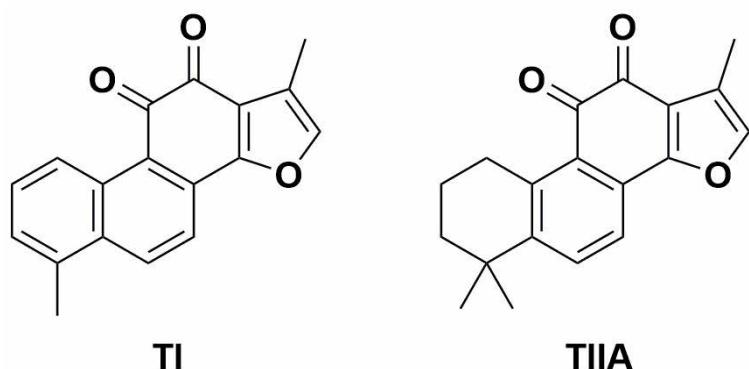
**Matthew J. Foulkes**<sup>1,2</sup>, *mjfoulkes@hotmail.co.uk*, Simon Jones<sup>1</sup>, Stephen A. Renshaw<sup>2</sup>. (1) Department of Chemistry, The University of Sheffield, Sheffield, United Kingdom (2) Department of Infection, Immunity and Cardiovascular Disease (IICD), The University of Sheffield, Sheffield, United Kingdom

Inflammation is the natural biological response to injury or invading pathogens, in which neutrophils play a vital role. However, neutrophil clearance from the site of injury or invasion, known as resolution of inflammation, can sometimes fail. This particularly applies to diseases such as chronic obstructive pulmonary disease (COPD) and arthritis. Current treatments are largely ineffective, non-specific and exhibit side-effects, so there is an unmet need for novel, effective clinical treatments.

A transgenic zebrafish model of inflammation can be used to identify compounds which accelerate inflammation resolution *in vivo*, and also those which suppress initial recruitment of neutrophils. Counting the number of neutrophils at various time points after injury enables quantification of any effects exhibited by compounds in solution.

Tanshinones are a group of naturally-occurring molecules which are found in the Chinese red sage *Salvia miltiorrhiza*, and include tanshinone I (TI) and tanshinone IIA (TIIA) (**Figure 1**). In a compound screen using the zebrafish inflammation model, TIIA was found to exhibit significant pro-resolution activity, whilst TI significantly decreased initial neutrophil recruitment.

A range of novel TI analogues and their isomers have been synthesised in an optimised six-step route, and evaluated *in vivo*. This has included detailed optimisation studies on key Minisci and intramolecular Heck reaction steps, involving some use of design of experiments software. Furthermore, TIIA analogues have been investigated in a similar manner.



**Figure 1.** Structures of TI and TIIA.

## MEDI 80

### Small molecule activators of the leukotriene A<sub>4</sub> hydrolase enzyme for pulmonary inflammation

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*Walter Reed Army Institute of Research, Silver Spring, Maryland, United States*

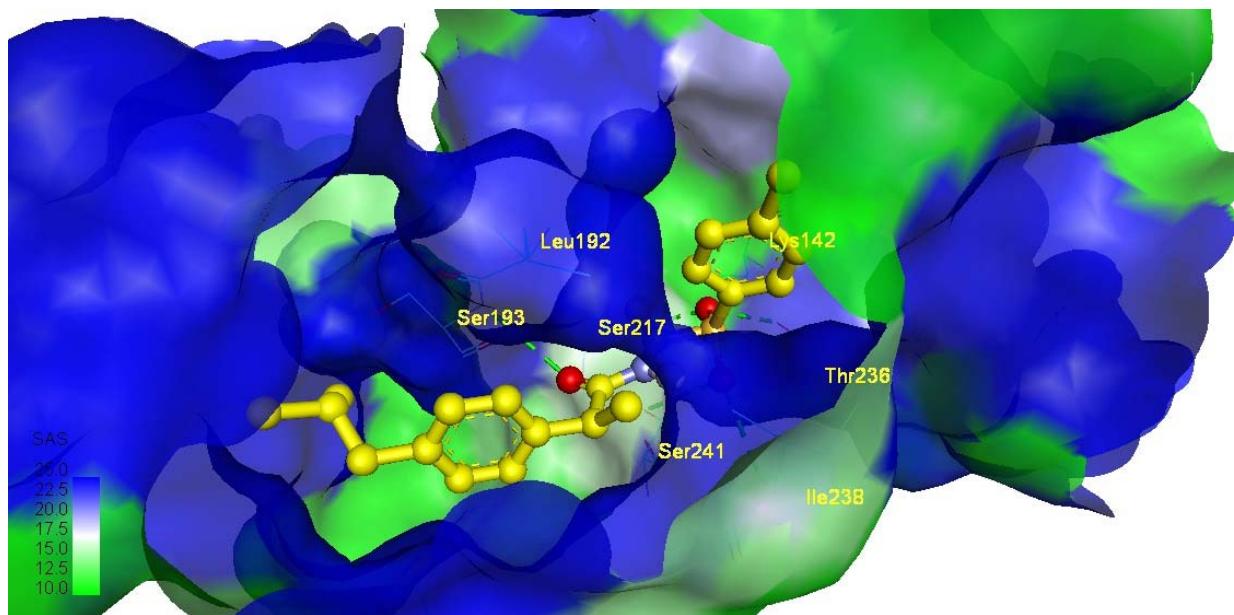
Previously, we have shown that the leukotriene A<sub>4</sub> hydrolase enzyme participates in both pro-inflammatory and anti-inflammatory pathways. The aminopeptidase activity of the enzyme was shown to promote resolution of pulmonary inflammation. Herein, we screen a series of small molecules for enzyme activation, determine enzyme activation mechanism for the endogenous substrate Pro-Gly-Pro, and present a computational model using the X-ray crystal structure of the leukotriene A<sub>4</sub> hydrolase protein.

## **MEDI 81**

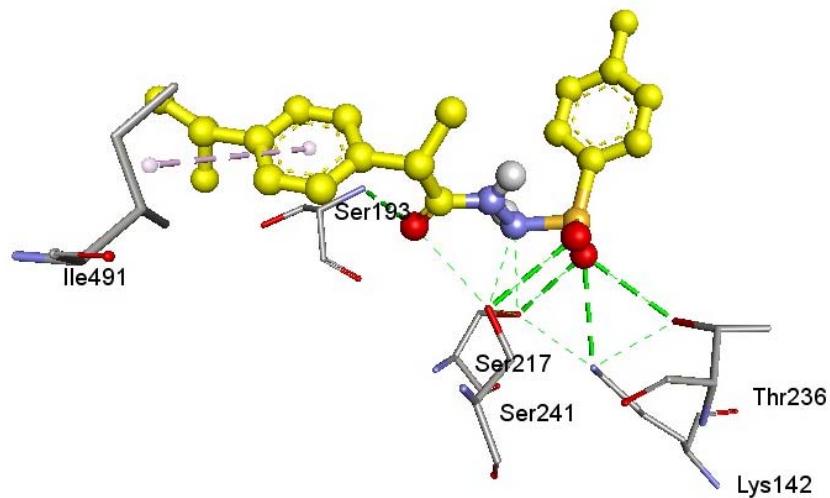
### **Synthesis, docking and biological evaluation of certain class of nonsteroidal anti-inflammatory drugs as fatty acid amide hydrolase inhibitors**

**Leena S. Saad<sup>1,2</sup>, leena99m@hotmail.com, Fatmah A. Alasmary<sup>1</sup>, Moustafa E. EL-Araby<sup>3</sup>.** (1) Chemistry, King Saud University, Riyadh, Saudi Arabia (2) Chemistry, King Faisal University, Alahsa, Saudi Arabia (3) Medicinal Chemistry, King Abdulaziz University, Jeddah, Saudi Arabia

Fatty acid amide hydrolase (FAAH) degrades fatty acid amides like oleamide to terminate the signaling in the central nervous system. When FAAH breaks down the two important amides AEA and 2-AG, this will lead to less stimulus of the endocannabinoid receptor CB1 and CB2. Since there has been some research confirming the involvement of the CB1 and CB2 on the anti- cancer affects, then inhibiting the FAAH could maintain the anticancer affects to last longer in the body by increasing the residence time of AEA and 2-AG at their receptors. In present study, we report the design, synthesis and biological screening studies of a series of substituted ibuprofen sulfonohydrazide derivatives (**LSQ1-LSQ20**) from the corresponding ibuprofen hydrazide. Molecular docking studies of all compounds (**LSQ1-LSQ20**) were performed to figure out the binding interactions with the active sites of FAAH and other amino acid residues of the enzyme. Compounds (**LSQ1**), (**LSQ2**) and (**LSQ4**) fit well at the catalytic triad and are stabilized by H-bonds and hydrophobic interactions. The synthesis starting from Ibuprofen led to preparation of 18 novel compounds of the virtually active compounds (top scorers in the docking study). Work is still undergoing to complete the biological screening.



3D representation of docked LSQ2



The interactions of (LSQ2) on active site of FAAH

## MEDI 82

### Anti-proliferative and anti-inflammatory estrogen receptor modulators

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Breast cancer is the most commonly diagnosed cancer among U.S. women. Approximately 70% of these cases express estrogen receptor alpha (ER $\alpha$ ) extensively and can be targeted with anti-estrogens. However, many patients relapse while maintaining a functional ER $\alpha$  (wild type or mutant). Like the glucocorticoid receptor, activated ER inhibits inflammatory gene expression via protein-protein interactions that block NF- $\kappa$ B transcriptional activity. Importantly, NF- $\kappa$ B is a primary mediator of treatment resistance in many cancers, including breast cancer; so, suppression of NF- $\kappa$ B signaling should be of benefit in breast cancer therapies. All the current endocrine treatments in breast cancer, however, block both the ER proliferative and the anti-inflammatory signaling pathways. Using a novel x-ray crystallographic screening approach, we have developed ER ligands that strongly inhibit both proliferation and NF- $\kappa$ B activity, thereby embodying the desirable *antiproliferative effects of antiestrogens while retaining the anti-inflammatory effects of estrogens*. We present here a high-affinity ER ligand series, named OBHS for its prototypical member (oxabicycloheptenesulfonate phenyl ester), several analogs of which show marked anti-proliferative and anti-inflammatory activity. We associate this ER-mediated NF- $\kappa$ B inhibition to a unique binding conformation of ER to OBHS utilizing a part of the pocket that faces the dimer interface and that has not been exploited by other known anti-estrogens.

## MEDI 83

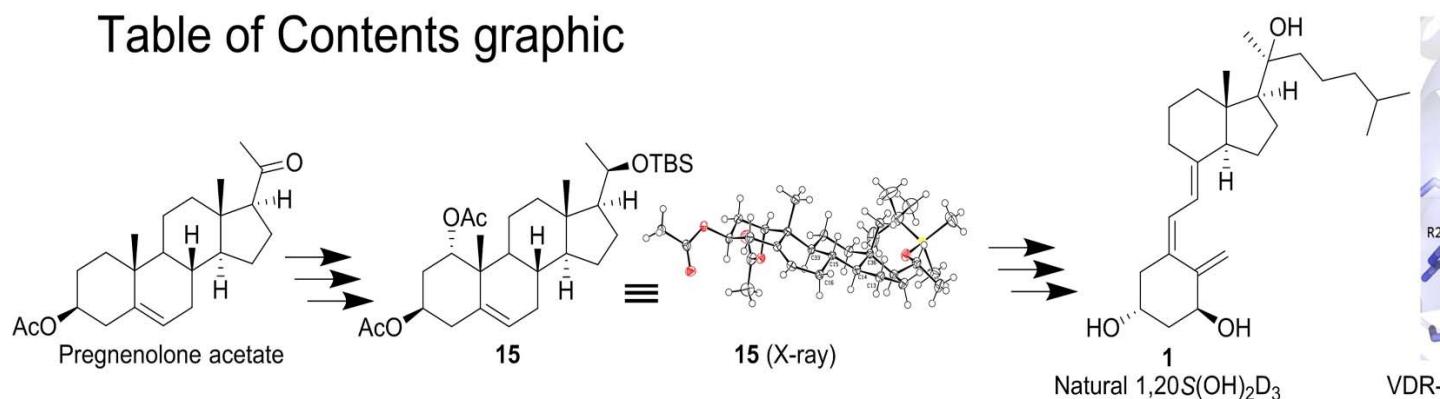
### **Synthesis of natural 1 $\alpha$ ,20S-dihydroxyvitamin D3 as a potent vitamin D receptor agonist and anti-inflammatory agent**

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1 $\alpha$ ,20S-Dihydroxyvitamin D3 [1,20S(OH)2D3], a natural and bioactive vitamin D3 metabolite, was chemically synthesized for the first time. A semi-reduced

intermediate (14a) of the Birch reduction during  $1\alpha$ -OH formation was obtained, and was used to propose the reaction mechanism. X-ray crystallography analysis of intermediate 15 confirmed its  $1\alpha$ -OH configuration.  $1,20S(OH)2D3$  binds efficiently to vitamin D receptor (VDR), with similar affinity with its native ligand  $1\alpha,25$ -dihydroxyvitamin D3 [ $1,25(OH)2D3$ ]. However, their co-crystal structures revealed differential molecular interactions of the  $20S$ -OH moiety and the  $25$ -OH moiety to the VDR, which may help explain their biological activities. In addition,  $1,20S(OH)2D3$  functions as a VDR agonist with comparable activities to  $1,25(OH)2D3$  with respect to of VDR stimulation, VDR translocation, regulation of VDR downstream genes (VDR, CYP24A1, TRPV6 and CYP27B1), and inhibition of inflammatory markers (IFNy and IL1 $\alpha$ ). This study offers a convenient synthetic route using a novel intermediate  $1\alpha,3\beta$ -diacetoxy pregn-5-en-20-one (3), and provides a molecular and biological basis for the development of  $1,20S(OH)2D3$  and its analogs as potential therapeutic agents.

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## MEDI 84

### Phospholipase A2: A pharmaceutical target to diminish inflammation

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Phospholipase A<sub>2</sub> (PLA<sub>2</sub>) enzymes are the upstream regulators of the eicosanoid pathway liberating free arachidonic acid (AA) and other polyunsaturated fatty acids (PUFA). The liberation of AA by PLA<sub>2</sub> enzymes sets off a cascade of molecular events that involves downstream regulators such as cyclooxygenase (COX) and lipoxygenase (LOX) metabolites leading to inflammation. Nonsteroidal anti-inflammatory drugs (NSAIDs) work by

inhibiting COX and LOX, but both rely on PLA<sub>2</sub> enzymes to provide them with AA. That means PLA<sub>2</sub> enzymes can potentially also be targeted to diminish inflammation at an earlier point in the process. Using molecular dynamics (MD) simulations guided by hydrogen/deuterium exchange mass spectrometric (DXMS) experimental data, we have recently elucidated the catalytic cycle of two cellular enzymes, the Group IVA cytosolic (GIVA cPLA<sub>2</sub>) and Group VIA calcium-independent (GVIA iPLA<sub>2</sub>) which are the main AA providers in the eicosanoid pathway.<span style="font-size:10.8333px"></span> Our data showed the channels to the active sites of these PLA<sub>2</sub>s are opened upon allosteric interaction of the enzyme surface with the membrane to facilitate entry of the substrate phospholipid. This constitutes the first detailed study describing the binding and the interaction mechanism of intracellular PLA<sub>2</sub>s with the membrane bilayer as well as how they bind a single phospholipid molecule in the catalytic site. Similar methods were also employed to understand the binding of various inhibitors to generate structure-activity relationships that facilitate the development of new potent and selective inhibitors for these two enzymes. In fact, we have developed thioether fluoroketone compounds that selectively inhibit GIVA cPLA<sub>2</sub> and GVIA iPLA<sub>2</sub>. These enzymes are implicated in many diseases, and the development of potent and selective inhibitors will aid in the discovery of new therapeutics.

## MEDI 85

### Design and synthesis of curcumin conjugates as potential anti-inflammatory agents

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Phytochemical products constitute one of the basic resources for many human needs including food and medicines. Many clinically used drugs are either derived from medicinal plants or inspired by chemical scaffold of biologically active agents of plant origin. Curcumin, a component of turmeric (*Curcuma longa*), is a compound that is beginning to gain significant notoriety for its many medicinal uses. While curcumin has been used as a remedy to treat a wide variety of ailments for centuries, a considerable amount of research is currently being conducted to determine its various biological abilities. Inflammation is associated with many pathological diseases such as rheumatoid arthritis, lupus, periodontitis, diabetes, chronic hepatitis, myocardial infarction (cardiovascular diseases), brain ischemic injury such as

stroke, cancer, pulmonary diseases or inflammatory bowel disease. Although many non-steroidal anti-inflammatory drugs (NSAIDs) (e.g. ibuprofen, naproxen, indomethacin ..... etc) are in practice long ago, gastrointestinal ulceration, bleeding, nephrotoxicity side effects including heart and kidney failure limit their long-term utilization. Although curcumin exhibit qualities that show promise for effectively treating certain life-threatening diseases, they do come with some drawbacks.

This research is concerned with synthesizing potential curcumin based drug candidates that combat inflammation and overcome the limitations. By synthesizing curcumin conjugates with amino acids, we hoped to develop potent conjugates and diminished side effects. In doing so, an efficient methodology for synthesizing these conjugates was developed. The synthesized conjugates proved as potent anti-inflammatory agents in compared to current standard commercially available drugs. The details of chemistry and pharmacological studies will be discussed in the conference.

## MEDI 86

### Selective JAK1 inhibitors for treatment of inflammatory diseases: Design and synthesis

**Mihir D. Parikh<sup>2</sup>, mihparikh@gmail.com, Ralph P. Robinson<sup>1</sup>.** (1) MS 220-3419, Pfizer Inc, Groton, Connecticut, United States (2) MS 220-3446, Pfizer Inc, Groton, Connecticut, United States

Janus Kinases (JAKs) are intracellular tyrosine kinases which mediate the signaling of cytokines and growth factors involved in the regulation of immunity, inflammation and hematopoiesis. Interest remains high in the identification and development of anti-inflammatory JAK inhibitors that are specific to a single Janus kinase isoform. Non-selective JAK inhibitors that inhibit JAK2 (which mediates EPO and TPO signaling) have the potential to induce anemia and/or thrombocytopenia. A JAK1-selective inhibitor is expected to retain the inhibition of key cytokines (e.g. Type 1 interferons, interferon gamma, IL-4, -5, -13,-31 and -21, while minimizing potential for JAK2-mediated hematopoietic effects.

Using biochemical and cellular assays for screening, we identified a series of JAK1-selective inhibitors from Pfizer's compound file. Crystallographic data, structure-based drug design and lead-transformation tools were used to optimize the series. Favorable interactions with the P-loop of JAK1 improved JAK1 potency and increased selectivity over JAK2. Several compounds were selected for potential advancement based on JAK1 selectivity, preclinical *in*

vivo efficacy and favorable pharmacokinetics. PF-04965842 is being evaluated in clinical trials for autoimmune disease. Synthetic routes that enabled the delivery of lead preclinical compounds and innovations in chemical synthesis that assured rapid progress of the program through discovery will be highlighted.

## MEDI 87

### **Design and synthesis of *N*-alkylated tubulysin analogs and their folate conjugates**

*Iontcho R. Vlahov, Fei You, xingzheyou@gmail.com, Kevin Y. Wang, Hari K. Santhapuram, Hanna F. Klein, Marilynn Vetzel, Joseph Reddy, Christopher P. Leamon. Endocyte Inc, West Lafayette, Indiana, United States*

Tubulysins are natural products isolated from myxobacterial species. They are potent mitotic poisons as they inhibit the polymerization of tubulin into microtubules. Majority of natural isolated tubulysins possess an acid-, base-, and enzyme-sensitive *N*-acyloxymethyl substituent, as well as an enzyme-labile acetate group. Both of these functional groups are essential for their potent cytotoxicity. Herein, we present the design and synthesis of more stable tubulysin analogs based on our reported synthesis of tubulysin B. These synthetic tubulysin analogs are less prone to degradation under acidic/basic conditions and enzymatic hydrolysis. Folate conjugates of these highly potent tubulysin analogs were also synthesized.

## MEDI 88

### **Pro-Pyrrolobenzodiazepine (pro-PBD) bioconjugates, part 1: Design and synthesis of pro-PBD conjugates containing a cleavable disulfide linker**

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Pyrrolobenzodiazepines (PBDs) and their dimers (bis-PBDs) have emerged as some of the most potent chemotherapeutic compounds, and are widely used as payloads in antibody drug conjugates. However, when used as stand-alone therapeutics or as the warhead for small molecule drug conjugates (SMDCs), the reactive imine functionality has the potential to cause off-target toxicities. As an elegant solution to this inherent problem, we designed

conjugated prodrugs lacking the imine moiety. Once the prodrug (pro-PBD) conjugate enters a targeted cell, cleavage of the linker system triggers the generation of a reactive intermediate possessing an aldehyde and aromatic amine. An intramolecular reaction subsequently takes place as the aromatic amine adds to the aldehyde with the loss of water to give the imine, and as a result, the diazepine ring. In our pro-PBDs, we mask the aldehyde as a hydrolytically sensitive oxazolidine moiety as part of a reduction-cleavable disulfide linker system for conjugation. We designed and synthesized several novel warheads: pro-PBD dimers and hybrids of pro-PBD with DNA minor groove binders.

## MEDI 89

### **Targeted folate-aminopterin anti-inflammatory conjugates: Synthesis and activity of an enzymatically labile lysine-linked conjugate and its pegylated analogs**

**Paul J. Kleindl, pkleindl@endocyte.com, Fei You, Hari K. Santhapuram, Hanna F. Klein, Spencer J. Hahn, June Lu, Satish Rao, Michael Pugh, Vickey Cross, Christopher P. Leamon, Iontcho R. Vlahov. Endocyte Inc, West Lafayette, Indiana, United States**

Macrophages are a class of white blood cells tasked with the elimination of diseased or dying cells and foreign substances, and as a result, play an important role in maintaining the health of the biological systems in which they are employed. Unrestrained, they have also been shown to cause tissue damage in a number of auto-immune diseases, including: rheumatoid arthritis, psoriasis, Crohn's disease, lupus, and atherosclerosis. In addition, activated macrophages have also been shown to release pro-inflammatory cytokines such as TNF- $\alpha$ , IL-6, and IL-1, promoting and intensifying the disease. Activated macrophages are known to over-express folate receptor (FR) isoform  $\beta$ , a cell-surface glycoprotein which binds with high affinity to the vitamin folic acid (FA). Our group has previously shown that activated macrophages and hence, sites of inflammation can be visualized by FA conjugates of the radioimaging agent  $^{99m}\text{Tc}$ . Treatment of Lewis rats with a conjugate of the anti-folate aminopterin (AMT) hydrazide, a more active but also more toxic structural relative of the ubiquitous anti-inflammatory methotrexate (MTX), to FA through a reducible disulfide linker system resulted in the reduction of symptoms seen in adjuvant induced arthritis (AIA), autoimmune uveitis (EAU), and autoimmune encephalomyelitis (EAE). Herein, the synthesis of a series of FA-AMT conjugates which link AMT to our FA-spacer via lysine is reported. Due to the slower release of AMT from this

non-reducible linker system, a series of analogs incorporating structural modifications on the spacer portion of the conjugate, including the addition/removal of sacchro-peptidic moieties and polyethylene glycols (PEGs) of various chain lengths, were synthesized to determine how these changes might affect the pharmacodynamic (PD)/pharmacokinetic (PK) profile. Fmoc-based solid phase peptide synthesis (SPPS) plays a prominent role in the conjugate/spacer synthesis.

## MEDI 90

### **Targeted folate-aminopterin anti-inflammatory conjugates: Optimization of a reductively/enzymatically labile cysteine-derived linker system**

**Paul J. Kleindl, pkleindl@endocyte.com, Fei You, Hari K. Santhapuram, Jeremy F. Vaughn, Hanna F. Klein, June Lu, Satish Rao, Michael Pugh, Vickey Cross, Christopher P. Leamon, Iontcho R. Vlahov. Endocyte Inc, West Lafayette, Indiana, United States**

Macrophages are a class of white blood cells tasked with the elimination of diseased or dying cells and foreign substances, and as a result, play an important role in maintaining the health of the biological systems in which they are employed. Unrestrained, they have also been shown to cause tissue damage in a number of auto-immune diseases, including: rheumatoid arthritis, psoriasis, Crohn's disease, lupus, and atherosclerosis. In addition, activated macrophages have also been shown to release pro-inflammatory cytokines such as TNF- $\alpha$ , IL-6, and IL-1, promoting and intensifying the disease. Activated macrophages are known to over-express folate receptor (FR) isoform  $\beta$ , a cell-surface glycoprotein which binds with high affinity to the vitamin folic acid (FA). Our group has previously shown that activated macrophages and hence, sites of inflammation, can be visualized by FA conjugates of the radioimaging agent  $^{99m}\text{Tc}$ . Treatment of Lewis rats with a conjugate of the anti-folate aminopterin (AMT) hydrazide, a more active but also more toxic structural relative of the ubiquitous anti-inflammatory methotrexate (MTX), to FA through a reducible disulfide linker system resulted in the reduction of symptoms seen in adjuvant induced arthritis (AIA), autoimmune uveitis (EAU), and autoimmune encephalomyelitis (EAE). Herein, the synthesis of a series of FA-AMT conjugates which link AMT to various FA-peptidic/sacchro-peptidic spacers via various cysteine/penicillamine/cysteinamine derived linker systems is described. As designed, the linker allows for two distinct sites of release: 1) a reductively labile disulfide bond, the stability of which can be increased by increasing the adjacent stearic bulk, and 2) an enzymatically-cleavable ( $\Gamma$ -glutamyl

hydrolase) amide bond, the stability of which is effected by the substituents on the  $\alpha$ -carbon as well as the steric bulk at the  $\beta$ -position relative to the amide. Testing in AIA models showed L-cysteine methyl ester with dimethyl cysteine (penicillamine) providing additional steric bulk on the spacer side of the disulfide bond was optimal.

## MEDI 91

### **Pro-Pyrrolobenzodiazepine (pro-PBD) bioconjugates, part 2: Design and synthesis of pro-PBD conjugates containing an enzyme-responsive linker**

*Iontcho R. Vlahov, Ning Zou, zoun2000@hotmail.com, Albert Felten, Kevin Y. Wang, Spencer J. Hahn, Christopher P. Leamon. Endocyte Inc, West Lafayette, Indiana, United States*

Folic acid (FA) is a high affinity natural ligand that binds to the folate receptor which is over-expressed on the cell surface in a variety of cancers. The pyrrolo[2,1-c][1,4]benzodiazepines (PBDs) are a group of antitumor antibiotics which exert their biological activity by sequence-selective DNA minor-groove binding that form a covalent aminal bond between their C11-position and the C2-NH<sub>2</sub> groups of guanine bases. The PBD dimers which contain two alkylating imines could form nondistorting interstrand DNA cross-links. They exhibited potent cytotoxicity, antitumor activity, and antibacterial activity. Unfortunately, all PBDs and their conjugates cause undesired off-target toxicity. In an attempt to improve the therapeutic index of PBD conjugates, we designed a novel class of prodrugs referred to as pro-PBDs. In our approach, the imine moiety in the PBD molecular framework was converted to its two parental integral parts: an aromatic amine and an aldehyde. Next, we integrated the aldehyde into the oxazolidine ring system. This pro-PBD motif was further connected to FA through an enzymatically-cleavable peptide-based linker system and a spacer unit. When such a conjugate enters diseased cells upon receptor targeting and receptor-mediated endocytosis, an active PBD will be formed as a result of enzymatic fragmentation of the linker system.

## MEDI 92

### **Discovery of potent transglutaminase 2 inhibitors for the treatment of renal cell carcinoma (RCC)**

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Renal cell carcinoma (RCC) is the most common malignancy of the human kidney. Also, TGase 2 expression is consistently increased in all RCC cell lines compared to other NCI 60 cancer cell lines, including lung, ovarian, CNS, blood, colon, melanoma, breast, and prostate cancer cell lines.

Transglutaminase 2 (TGase 2, E.C. 2.1.2.13) is an enzyme that catalyzes an isopeptide bond between protein glutamine and lysine residues, resulting in a covalent cross-link. TGase 2 expression is much more pronounced in RCC cell lines. Therefore, the development of a new inhibitor of TGase 2 could be a novel and effective strategy to treat RCC.

With this respect, we have designed a new scaffold of small molecule inhibitors of TGase 2. Over 200 compounds were synthesized and their biological activities were tested by in vitro enzyme assay and cell-based assay. Among the tested compounds, several of them are identified as highly potent TGase 2 inhibitors. These results were possible by modifying several substituents base on our SAR analysis. Series of compounds were selected for ADME/T test and xenograft experiments. As a result, we have found that our TGase 2 inhibitors of novel scaffold abrogates RCC growth in xenograft tumor models, suggesting the possibility of a new therapeutic approach to RCC. Herein, detailed experimental data including xenograft results are presented.

## MEDI 93

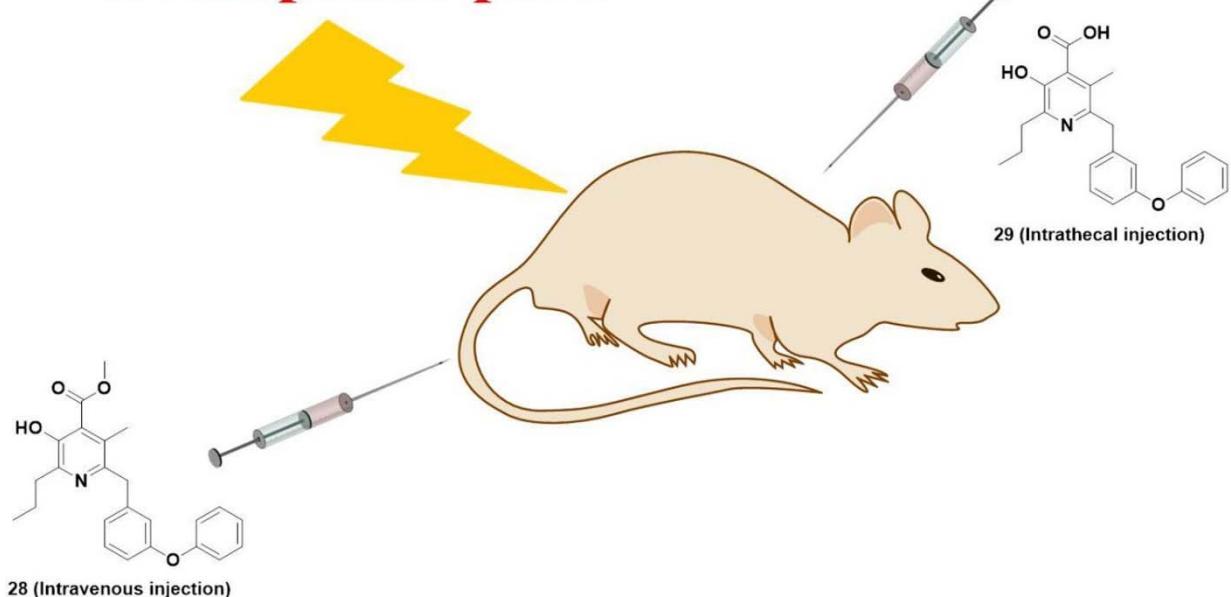
### Discovery of potent antialloodynic agents for neuropathic pain targeting P2X3 receptors

**Young-Hwan Jung**<sup>1</sup>, jyh0401@gmail.com, **Yeo Ok Kim**<sup>2</sup>, **Hai Lin**<sup>2</sup>, **Joong-Heui Cho**<sup>3</sup>, **Jin-Hee Park**<sup>3</sup>, **So-Deok Lee**<sup>1</sup>, **Jinsu Bae**<sup>4</sup>, **Koon Mook Kang**<sup>1</sup>, **Yoon-Gyoong Kim**<sup>5</sup>, **Ae Nim Pae**<sup>6</sup>, **Hyojin Ko**<sup>1</sup>, **Chul-Seung Park**<sup>1</sup>, **Myung Ha Yoon**<sup>2</sup>, **Yong-Chul Kim**<sup>1</sup>. (1) School of Life Sciences, Gwangju Institute of Science & Technology, Gwangju, Korea (the Republic of) (2) Department of Anesthesiology and Pain Medicine, Chonnam National University, Gwangju, Korea (the Republic of) (3) New Drug Development Center, Daegu-Gyeongbuk Medical Innovation Foundation , Daegu, Korea (the Republic of) (4) Department of Biomedical Science and Engineering, Gwangju Institute of

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Antagonism of the P2X3 receptor is one of the potential therapeutic strategies for the management of neuropathic pain because P2X3 receptors are predominantly localized on small to medium diameter C- and A $\delta$ -fiber primary afferent neurons, which are related to the pain-sensing system. In this study, 5-hydroxy pyridine derivatives were designed, synthesized, and evaluated for their in vitro biological activities by two-electrode voltage clamp assay at hP2X3 receptors. Among the novel hP2X3 receptor antagonists, intrathecal treatment of compound **29** showed parallel efficacy with pregabalin (calcium channel modulator) and higher efficacy than AF353 (P2X3 receptor antagonist) in the evaluation of its antiallodynic effects in spinal nerve ligation rats. However, because compound **29** was inactive by intraperitoneal administration in neuropathic pain animal models due to low cell permeability, the corresponding methyl ester analog, **28**, which could be converted to compound **29** in vivo, was investigated as a prodrug concept. Intravenous injection of compound **28** resulted in potent antiallodynic effects, with ED<sub>50</sub> values of 2.62 mg/kg and 2.93 mg/kg in spinal nerve ligation and chemotherapy-induced peripheral neuropathy rats, respectively, indicating that new drug development targeting the P2X3 receptor could be promising for neuropathic pain, a disease with high unmet medical needs.

# Neuropathic pain



## MEDI 94

### Pyrrolo-triazine derivatives as atypical antipsychotics for the treatment of schizophrenia

**Mohammed Rasheed**, rasheed@suven.com, Anil K. Shinde, Meghana Dasoju, Shankar Reddy Gagginapally, Vanaja Middekkadi, Ramkumar Subramanian, Gopinadh Bhyrapuneni, Pradeep Jayarajan, Venkata Satya Ramakrishna Nirogi. Discovery Research, Suven Life Sciences Ltd, Hyderabad, Telangana, India

Schizophrenia is a chronic and serious mental disorder affecting 1-2% of the global population. It becomes difficult for the people diagnosed with this serious condition to differentiate between reality and imagined experiences and behave normally. People who suffer with schizophrenia often spend their lives in monitored isolation due to psychological barriers that prevent them from mingling with the people. Antipsychotic medications are the most common form of treatment. The current antipsychotic (schizophrenia) medications predominantly improve positive symptoms, agitation and aggression but have limited efficacy for negative and cognitive symptoms, which not only affect functional outcome but also the patient's quality of life. Therefore, development of new therapeutic agents which treat both negative and cognitive symptoms of schizophrenia patients is the current un-met medical need. The atypical antipsychotics, possesses a higher antagonist

affinity at the serotonin 5-HT<sub>2A</sub> as compared with the dopamine D<sub>2</sub> receptor. The atypical antipsychotic agents, offer improved treatment of schizophrenia by combining efficacy with less propensity to cause extra pyramidal side effects (EPS). A series of pyrrolo-triazine derivatives were designed, synthesized and evaluated for their in-vitro potencies toward receptors such as 5-HT<sub>1A</sub>, 5-HT<sub>2A</sub>, SERT, D<sub>2</sub>, adrenergic α<sub>1b</sub> and H<sub>1</sub>. The potent affinity towards 5-HT<sub>1A</sub>, 5-HT<sub>2A</sub>, SERT and D<sub>2</sub> and no or minimal potency towards adrenergic α<sub>1b</sub> and H<sub>1</sub> is the desired feature to show the efficacy in treating both negative and cognitive symptoms associated with schizophrenia. Details of design, chemistry, structure activity relationship and *in vitro* potencies of pyrrolo-triazine derivatives will be disclosed in this poster presentation.

## MEDI 95

### **Preclinical characterization of indole carboxamide derivatives: Novel, potent and selective muscarinic M1 positive allosteric modulators**

**Anil K. Shinde**, *anilshinde@suven.com*, **Mohammed Rasheed**, **Rajesh K. Badange**, **veena reballi**, **Kumar Boja**, **Santhi Sree Kommineni**, **Sravanthi Manchineela**, **Vinod Kumar Goyal**, **Santosh Kumar Pandey**, **Vijay Benade**, **Pradeep Jayarajan**, **Venkata Satya Ramakrishna Nirogi**. *Discovery Research, Suven Life Sciences Ltd, Hyderabad, Telangana, India*

Muscarinic acetylcholine subtype 1 (M<sub>1</sub>) receptor agonists though showed cognitive improvement in human clinical trial, their clinical utility was limited by insufficient muscarinic subtype selectivity mediated side effects. The conservative nature of the orthosteric site makes it difficult to identify selective M<sub>1</sub> receptor agonists. A novel series of indole-3-carboxamide compounds as positive allosteric modulators (PAM) have been designed, synthesized and their *in vitro* potencies established. The series in general had good *in vitro* affinity and functional selectivity over M<sub>2</sub> to M<sub>5</sub> muscarinic receptor subtypes. The lead compound has adequate water solubility, orally bioavailability with adequate brain penetration and high free fractions. It has reversed memory deficit in object recognition task and promotes non-amyloidogenic APP processing in rats. Details of design, chemistry, structure activity relationship and *in vitro* potencies will be disclosed in this poster presentation.

## MEDI 96

### Towards the development of a peptide-PROTAC conjugate targeting a viral protein: Rational design and optimization of a stapled alpha-helical peptide that binds HPV16 E2 protein

**Stacie L. Richardson**, *stacie.richardson@gmail.com*, **Matthew C. Hartman**. Chemistry, Virginia Commonwealth University, Richmond, Virginia, United States

Human papillomaviruses (HPV) are involved in a variety of cancers, including anogenital, head, and neck cancers. Nearly 100% of cervical cancer cases (approximately 530,000 new cases each year) can be attributed to HPV infections, with over 50% associated with high-risk type HPV16. As HPV infections are often subclinical, there is a very high risk of transmission. Thus, there is an urgent need for therapeutics that can prevent its progression and transmission. Targeting host-viral protein-protein interactions (PPI) is a strategy that can overcome problems with resistance, since host-viral PPI are much less likely to develop mutations. However, inhibiting only one PPI may not be sufficient to fully suppress viral activity. A recent strategy, called PROteolysis TArgeting Chimeras (PROTAC), utilizes small molecules to recruit the ubiquitin-proteasome pathway, which degrades the target protein. To this end, we are developing a heterobivalent ligand composed of a small molecule PROTAC conjugated to a peptide with affinity for an HPV16 protein. In HPV16, the E2 protein is a critical regulatory factor, which binds to the chromatin-associated protein BRD4 to control viral DNA replication. It has been shown that the binding interaction of BRD4 to E2 depends upon an alpha helical motif in the C-terminus of BRD4. However, we found that this peptide has very little affinity for E2, likely due to its inability to maintain an alpha helical structure. Starting with this peptide sequence, we designed a small library exploring peptide length and staple positions and have identified a stapled peptide with low micromolar affinity for HPV16 E2. We have performed alanine scanning to determine which positions are important for affinity. We have replaced residues showing minimal impact on affinity with positively charged residues to enhance cell permeability. Progress toward attachment of the PROTAC molecule to this peptide and evaluation of its activity will be discussed.

## MEDI 97

### Synthesis and biological evaluation of phosphoantigens for gamma-delta T cell stimulation

**Michael M. Poe**, [michael.poe@uconn.edu](mailto:michael.poe@uconn.edu), **Chia-Hung Christine Hsiao**,  
**Andrew J. Wiemer**. *Pharmaceutical Sciences, University of Connecticut, Storrs, Connecticut, United States*

Immunotherapy can promote the ability of the immune system to detect and clear cancerous cells. Our approach in this field targets the activation of gamma-delta ( $\gamma\delta$ ) T cells through the use of phosphoantigens. These specialized V $\gamma$ 9V $\delta$ 2 T cells may function as “early responders” in fighting malignancies and are stimulated by the natural phosphoantigens *E*-4-hydroxy-3-methyl-but-2-enyl diphosphate (HMBPP) and isopentenyl diphosphate (IPP). The success of synthetic analogs is limited by stability of the diphosphates *in vivo* and internalization of the ligand to reach the cytoplasmic binding site on the butyrophilin protein BTN3A1. Instead, our approach substitutes the diphosphate structure with a monophosphonate which results in superior stability, in addition to the utilization of prodrug forms of the ligands to allow for rapid permeation of the cell membrane. These protecting groups are then cleaved intracellularly resulting in the active drug form. The combined alterations have led to the pivaloyloxymethyl-protected phosphonate POM<sub>2</sub>-C-HMBP, which has been shown to strongly stimulate the proliferation of  $\gamma\delta$  T cells. Herein, we present the synthesis of novel phosphoantigens and determine their ability to stimulate  $\gamma\delta$  T cell proliferation and bind to the target BTN3A1, as we investigate their effectiveness as a potential cancer immunotherapy.

## MEDI 98

### Synthesis and evaluation of vitamin D3-based probes for cellular target(s) verification

**Jiachen Wen**, [wjc4ever@hotmail.com](mailto:wjc4ever@hotmail.com), **Matthew K. Hadden**. *Pharmaceutical Sciences, University of Connecticut, Storrs, Connecticut, United States*

The seco-steroid vitamin D3 (VD3) has been recognized as a hedgehog (Hh) signaling inhibitor in recent years. Initial studies in this area suggested that VD3 inhibited Hh signal transmission at the level of smoothened, a 7-transmembrane GPCR-like protein. Follow-up studies in our laboratory suggest that VD3 does not bind to the conventional small molecule binding site on the transmembrane domain of smoothened. In order to more clearly define the direct binding target(s) and to fully understand the relationship between VD3 and Hh inhibition, we have synthesized a series of VD3-based probe molecules. Herein, we report their synthesis and preliminary activity against the Hh pathway.

## MEDI 99

### Design and synthesis of siderophore-antibiotic conjugates

Jean-Pierre Jourdan<sup>1,2</sup>, Alexandra Dassonville-Klimpt<sup>1,2</sup>, Catherine Mullié<sup>1,2</sup>, Jean-Paul Becker<sup>1,2</sup>, Jean-Paul.Beauger@u-picardie.fr, Pascal Sonnet<sup>1,2</sup>. (1) UFR de Pharmacie, Université de Picardie, Jules Verne, Amiens, France (2) LG2A CNRS UMR 7378, Amiens, France

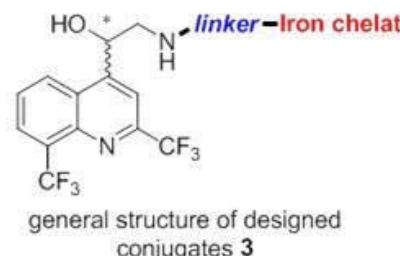
The dissemination of multidrug-resistant bacteria has reduced the therapeutic efficacy of antibiotic drugs, and is now part of the major human healthcare emergencies. Among the bacteria ESKAPE, Gram negative pathogens such as *Pseudomonas aeruginosa* or *Acinetobacter baumannii* are very virulent. Bacteria require iron for many vital functions. However, in the oxidative atmosphere iron exists as insoluble salts what makes its bacterial assimilation hard. To overcome iron-deprivation, pathogens use small iron chelators named siderophores which transfert iron within bacteria by specific receptors. Siderophores are classed in four main families: hydroxamates, catecholates, carboxylates and phenolates. Previous studies have shown that the motif hydroxypyridinone is recognized by the same kind of transporters than catechol group. This chelating agent has recently been combined to a monosulfactam to lead to conjugate BAL30072 which is active on multidrugresistant strains of *P. aeruginosa* and *A. baumannii*.

In previous researches, we have showed that aminoquinolinemethanols (AQMs), as mefloquine analogues, possess an antibacterial activity only against Gram positive bacteria.

Inspired by the Trojan horse strategy, we have grafted hydroxypyridinone group to these AQMs with different linkers to obtain conjugates **1** and **2**.

Conjugate **1** is not active against *P. aeruginosa* DSM1117 while conjugate **2** possesses an interesting activity (MIC 128µg/mL).

In order to better understand this difference of activity and to establish new structure activity relationships, we are now designing novel conjugates **3** with various linkers and iron chelators.



First series of iron chelator-AQM conjugates

## MEDI 100

### **Design and validation of a peptidomimetic ligand as a translesion synthesis inhibitor**

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Alkylating agents are standard first-line chemotherapeutic drugs for several tumor types; however, the development of acquired resistance and dose-dependent side effects can limit their long-term efficacy. Recent studies suggest that DNA translesion synthesis (TLS) is an important pathway that influences the cytotoxic properties of alkylating agents and can introduce acquired resistance against standard anti-cancer regimens. Developing new small molecules to target TLS activity has recently emerged as a promising strategy for enhancing the efficacy of alkylating agents. Using a structure-based computational approach, we have rationally designed a small molecule scaffold that mimics a key protein-protein interaction in TLS signaling. Follow-up molecular dynamics and MM/PBSA aided in optimization of the scaffold. Validation of the initial scaffold as well as the synthesis and evaluation of a series of analogues has been completed and will be presented herein. The rationally designed molecules present new scaffolds with potential to inhibit TLS and enhance the efficacy of first-line alkylating agents.

## MEDI 101

### **Strategies for the modulation of protease-activated receptors (PARs)**

**Disha Gandhi**, *gandhi\_disham@yahoo.co.in*, Mark Majeswski, Ricardo Rosas, Trevor J. Foster, Kaitlin Kentala, Allison Stephans, Khia Kurtenbach, Ryan Engel, Kristen Lucknow, Chris Dockendorff. *Chemistry, Marquette University, Milwaukee, Wisconsin, United States*

Recent advances in the understanding of G-protein coupled receptor (GPCR) pharmacology have demonstrated that certain ligands can selectively engage different GPCR-mediated signaling pathways. This phenomenon, called biased signaling, has been demonstrated with protease-activated receptors (PARs), a unique subset of GPCRs with profound effects on diverse phenomena such as platelet activation, inflammation, and cancer cell metastasis. We previously discovered a class of small molecules, called parmodulins, that act to selectively inhibit platelet granule secretion via PAR1-

activated Gq. Presented here is a selection of our efforts to 1) identify analogs with improved properties using medicinal chemistry; 2) characterize the promising cytoprotective effects of these compounds; 3) synthesize first-in-class bivalent ligands.

## MEDI 102

### Discovery of novel frizzled-7 inhibitors through structure-based virtual screening

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Frizzled (Fzd) proteins are seven transmembrane receptors that belong to a novel and separated family of G-protein-coupled receptors (GPCRs). The Fzd receptors can respond to Wnt proteins to activate the canonical β-catenin pathway which is important for both initiation and progression of cancers. Disruption of the Wnt/β-catenin signal thus represents an opportunity for rational cancer prevention and therapy. Of the 10 members of the Fzd family, Fzd7 is the most important member involved in cancer development and progression. In the present studies, we applied structure-based virtual screening targeting the transmembrane domain (TMD) of Fzd7 to select compounds that could potentially bind to the Fzd7-TMD and block the Wnt/Fzd7 signaling and further evaluated them in biological assays. Six small molecule compounds were confirmed as Fzd7 inhibitors. The best hit, SRI37892, significantly blocked the Wnt/Fzd7 signaling with IC<sub>50</sub> values in the sub-micromolar range and inhibited cancer cell proliferation with IC<sub>50</sub> values around 2 μM. Our results provide the first proof of concept of targeting Fzd-TMD for the development of Wnt/Fzd modulators. The identified small molecular Fzd7 inhibitors can serve as a useful tool for studying the regulation mechanism(s) of Wnt/Fzd7 signaling as well as a starting point for development of cancer therapeutic agents.

## MEDI 103

### Discovery of novel class of alpha selective PI3K inhibitors

**Keira Garland**<sup>1</sup>, garland.keira@gene.com, **Emily J. Hanan**<sup>1</sup>, **Steven T. Staben**<sup>1</sup>, **Marie-Gabrielle Braun**<sup>1</sup>, **Kyle Edgar**<sup>1</sup>, **Nick Endres**<sup>1</sup>, **Lori Friedman**<sup>1</sup>, **Amanda Nguyen**<sup>1</sup>, **Jodie Pang**<sup>1</sup>, **Hans E. Purkey**<sup>1</sup>, **Laurent Salphati**<sup>1</sup>, **Stephen Schmidt**<sup>1</sup>, **Kyung Song**<sup>1</sup>, **Mark Ultsch**<sup>1</sup>, **Allan Jaochico**<sup>1</sup>, **Connie Chan**<sup>1</sup>,

*Charles Eigenbrot<sup>1</sup>, Calum MacLeod<sup>2</sup>, Philip Jackson<sup>2</sup>, Raman Narukulla<sup>2</sup>, Jamie Knight<sup>2</sup>, Kuen Yeap<sup>2</sup>, Kristen Messick<sup>1</sup>, Nicole Valle<sup>1</sup>, Robert Heald<sup>2</sup>, Michelle Nannini<sup>1</sup>, Pat Hamilton<sup>1</sup>, Saundra Clausen<sup>1</sup>, Amy Young<sup>1</sup>, Deepak Sampath<sup>1</sup>, Rebecca Hong<sup>1</sup>, Man-Ling Lee<sup>1</sup>, Toby Blench<sup>2</sup>, Richard Elliott<sup>2</sup>, Aijun Lu<sup>3</sup>, Xiao-Hu Gu<sup>3</sup>, Jianfeng Xin<sup>3</sup>. (1) Genentech, South San Francisco, California, United States (2) Charles River Laboratories, Harlow, United Kingdom (3) Pharmaron, Beijing, China*

Phosphoinositide 3-kinase (PI3K) is an important member of the PI3K/Akt/mTOR signaling pathway, playing a role in cell growth, proliferation, and survival. PIK3CA, the gene that encodes the catalytic subunit of PI3K-alpha protein, is a commonly mutated oncogene. It is implicated in a wide range of cancers, particularly in breast cancer. Herein we report a scaffold hopping approach that led to the discovery of a novel, highly potent chemical series. These compounds demonstrate highly selective inhibition of PI3K-alpha over the other Class I PI3K isoforms as well as the broader kinase. This series presented challenges such as poor solubility and permeability, and low oral bioavailability. The structure activity relationships indicated specific areas of polarity and the presence of hydrogen bond donors were essential for maintaining activity. These challenges were addressed through the use of structure based design, modulation of physicochemical properties, and in-silico ADME property modelling. Optimized inhibitors with good oral bioavailability show strong efficacy in PIK3CA tumor xenograft models. Overall, the high potency and selectivity make this series of great interest, as selective inhibitors have the potential to allow for treatment of PI3K-alpha-driven cancers with a greater therapeutic index.

#### **MEDI 104**

#### **Discovery of pan-active and isoform selective inhibitors of class I phosphoinositide-3-kinases (PI3Ks) utilizing a DNA-encoded discovery platform**

*Christopher D. Hupp, chupp@x-chemrx.com, Daniel I. Resnicow, Diana Gikunju, Matthew A. Clark, Ying Zhang, Anthony D. Keefe, John W. Cuozzo, Eric A. Sigel, Paolo A. Centrella, Marie-Aude A. Guie, Sevan Habeshian, Kaitlyn M. Kennedy. X-Chem Pharmaceuticals, Waltham, Massachusetts, United States*

The phosphoinositide-3-kinase (PI3K) pathway has been linked to a variety of diseases, such as cancer, diabetes, cardiovascular disease and even Alzheimer's. As a result, these enzymes are a compelling class of targets for

the identification of small molecule inhibitors. Utilizing our DNA-encoded discovery platform (DEX™), we were able to identify potent pan-active and nanomolar isoform selective ( $\delta$  and  $\gamma$ ) inhibitors for this intriguing class of enzymes. The two pan-active series were discovered from two separate heterocyclic core based DNA encoded libraries, while a  $\delta$ -selective and  $\gamma$ -selective series was identified from the same linear based DNA encoded library.

## MEDI 105

### Potent and selective PI3K $\delta$ inhibitors: Structure-activity relationships of 8-alkoxy-2-(benzimidazol-1-yl)-6-morpholinopurines

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Cell signaling through phosphoinositide-3-kinase delta (PI3K $\delta$ ) has been shown to be involved in lymphocyte-mediated inflammatory diseases and B-cell malignancy. Morpholinopyrimidine and related scaffolds have been extensively explored for pan and isoform-selective PI3K inhibitors. In our effort to discover selective PI3K $\delta$  inhibitors, we explored SAR in 8-alkoxy-2-(benzimidazol-1-yl)-6-morpholinopurines. Modifications were examined in the 8-alkoxy region with steric and polar factors that could affect potency, selectivity, and pharmacokinetics. Additional examinations were made in the 2-(benzimidazol-1-yl) substituents. These studies resulted in molecules with nanomolar potency, high delta isoform selectivity, and negative *in vitro* genotoxicity.

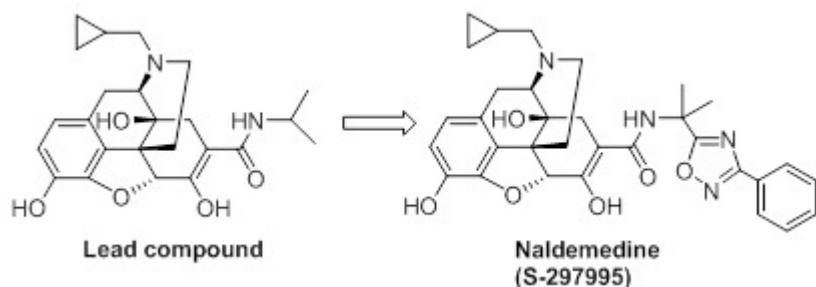
## MEDI 106

### Discovery of naldemedine (S-297995): A potent and orally available opioid receptor antagonist for treatment of opioid-induced adverse effects

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Opioids are widely used for pain management in moderate-to-severe pain, but some adverse effects such as constipation and emesis/vomiting frequently

appear. Opioid receptor antagonists are reported to be effective on these opioid-induced adverse effects. To reduce these undesired effects, we performed synthesis and structure-activity relationship of morphinan derivatives, and obtained promising novel chemotype and lead compound with strong inhibitory effects on opioid receptors. Further improvement of potencies and PK profiles of the lead compound finally led to discover Naldemedine (S-297995), which demonstrated outstandingly strong anti-constipation effect on small intestinal transit model in rat and anti-emetic effect on ferret model.



## MEDI 107

### Synthesis and biological evaluation of matrix metalloproteinase 9 inhibitors for cancer therapeutics

**Xiaodong Ren<sup>2</sup>, rxdcpu@hotmail.com, Vincent Alford<sup>1</sup>, Qianwen Gan<sup>2</sup>, Monaf Awwa<sup>2</sup>, Iwao Ojima<sup>2</sup>.** (1) Stony Brook University, Stony Brook, New York, United States (2) Chem Dept/ICBDD, Stony Brook University, Stony Brook, New York, United States

Emerging evidence indicates an important role of matrix metalloproteinases (MMP) in the early stage of cancer dissemination. Detailed structural information of MMPs has revealed that targeting the non-catalytic domain of proteases can overcome the lack of specificity and selectivity. Non-catalytic sites like, hemopexin (PEX) domain, which is indirectly crucial for the catalytic activity of MMPs and not highly conserved as compared to catalytic sites, can be an alternative site to target MMPs. Previously, Dufour *et al* utilized an *in silico* docking approach to screen a library of compounds against the MMP-9 PEX domain and identified several compounds with high docking scores. One of the compounds, *N*-(4-difluoromethoxyphenyl)-2-(6-oxo-4-propyl-1,6-dihydropyrimidin-2-ylthio)acetamide (1403-A) exhibited micromolar affinity to MMP-9 with very good selectivity. This hit compound was also found to inhibit cell migration, proliferation, invasion, tumor growth and metastasis induced by MMP-9. In this work, 14 new analogs were designed based on computational

modeling and synthesized. Biological evaluation of these new analogs showed that one of the compound **Bcy** exerts a substantially better effect on inhibiting proMMP-9 mediated migration than parental compound 1403-A as shown by a 2-D dot migration assay, which monitors proMMP-9 mediated cell migration. **Bcy** inhibits proMMP-9 mediated migration in a dose-dependent manner and may even have therapeutic effects in the nanomolar scale as shown by our EC<sub>50</sub> curve. **Bcy** exerts a selective inhibition of proMMP-9 mediated migration over other homologous and non-homologous MMPs as evidenced by our 2-D dot migration assay. Further studies suggest that chronic exposure of cancer cells to **Bcy** has minimal cytotoxic effects in cells treated for multiple days. It was also found that **Bcy** inhibits cellular migration and has no observed off-target effects on the catalytic activity of MMPs as observed by our FITClabeled gelatin substrate degradation assay. The design, synthesis and biological evaluations of **Bcy** and its analogs will be presented.

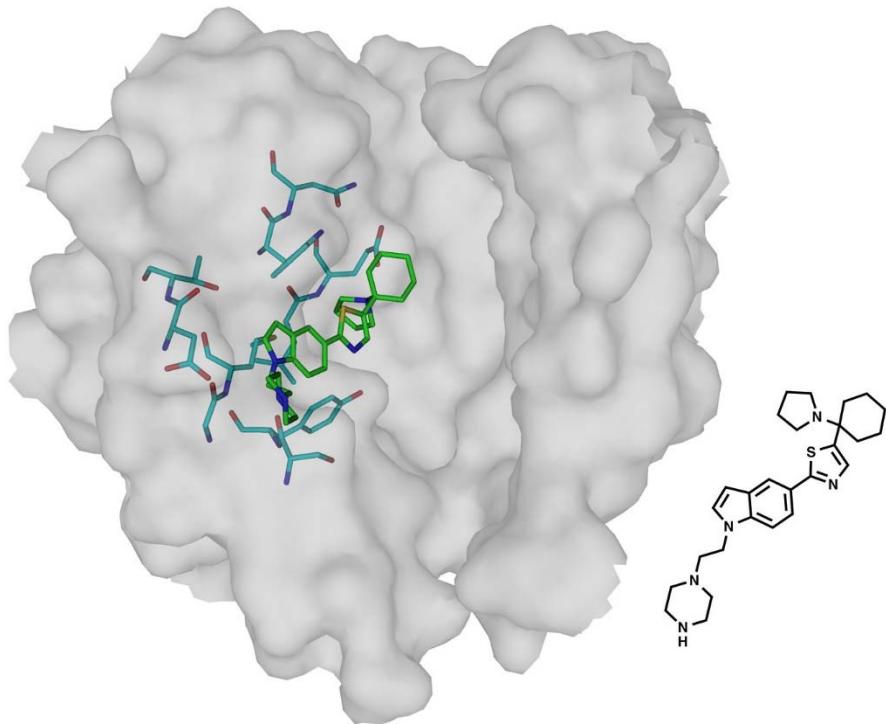
## MEDI 108

### Addressing a large active site: Inhibition of trypanothione reductase with cyclohexylpyrrolidine-based ligands

**Raoul E. De Gasparo**<sup>1</sup>, *raoul.degasparo@org.chem.ethz.ch*, **Elke Persch**<sup>1</sup>, **Steve Bryson**<sup>2</sup>, **Marcel Kaiser**<sup>3</sup>, **Emil F. Pai**<sup>2</sup>, **R. Luise Krauth-Siegel**<sup>4</sup>, **Francois N. Diederich**<sup>1</sup>. (1) *Laboratory of Organic Chemistry, ETH Zurich, 8093 Zurich, Switzerland* (2) *Departments of Biochemistry, Medical Biophysics & Molecular Genetics, University of Toronto, Toronto, Ontario, Canada* (3) *Swiss Tropical and Public Health Institute, 4002 Basel, Switzerland* (4) *Biochemistry Center, Heidelberg University, 69120 Heidelberg, Germany*

Inhibition of the enzyme trypanothione reductase (TR) has proved to be effective against the parasite *Trypanosoma brucei*, which causes the african sleeping sickness. Causative agents of chagas disease and leishmaniasis, namely *Trypanosoma cruzi* and *Leishmania donovani*, also possess this enzyme and, therefore, represent potential targets of TR inhibitors. In 2014, we reported the biological evaluation and the first co-crystal structures showing the binding mode of small TR inhibitors derived from BTCP (1-[1-(benzo[b]thien-2-yl)cyclohexyl]piperidine), previously reported to be a low-molecular-weight inhibitor of TR by Fairlamb and co-workers. The information about the binding mode of these inhibitors laid the foundation for the rational structure-based design of small molecules to be bound in the large solvent exposed active site of TR. Some of the reported analogues exhibited poor water solubility, which severely impeded their biological evaluation and co-

crystallization. We have now developed other, promising derivatives with better water solubility and a 3-fold increase in binding affinity ( $K_{ic} = 3.9 \pm 0.3 \mu\text{M}$ ) for *Trypanosoma brucei* TR. Newly obtained co-crystal structures confirmed our previously reported binding mode for these derivatives in the large active site of TR.



## MEDI 109

### Stereoselective synthesis of rhodotorulic acid analogues with potential siderophore properties

*Timothée Garnerin<sup>1,2</sup>, Alexandra Dassonville-Klimpt<sup>1,2</sup>, Jean-Paul Becker<sup>1,2</sup>, Jean-Paul.Becker@u-picardie.fr, Pascal Sonnet<sup>1,2</sup>. (1) UFR de Pharmacie, Université de Picardie, Jules Verne, Amiens, France (2) LG2A CNRS UMR 7378, Amiens, France*

Antibiotic resistance is an emerging disease and a real problem of health. Resistance of Gram negative bacteria such as *Acinetobacter baumannii* and *Escherichia coli* to conventional antibiotics lead to therapeutic failure and require new antibiotherapies. The use of the iron transport systems is one of the most promising strategies to overcome this resistance phenomenon. These specific routes of entry, essential for the survival of the

microorganisms, allow ferric siderophore complexes to carry iron within the bacteria.

These systems allow the introduction of antibacterial agents (conjugates antibiotic-siderophore) or toxic complexes (gallium complexes) into the bacteria to kill them. Rhodotorulic acid (RA) is a siderophore transported by TonBox dependant Fhu receptors. These kinds of receptors are expressed by *Acinetobacter baumannii* and *Escherichia coli*. RA is dioxopiperazine iron chelator with hydroxamate as iron ligands and two asymmetric centers (S,S configuration). This spatial orientation is essential for the Fhu receptor recognition.

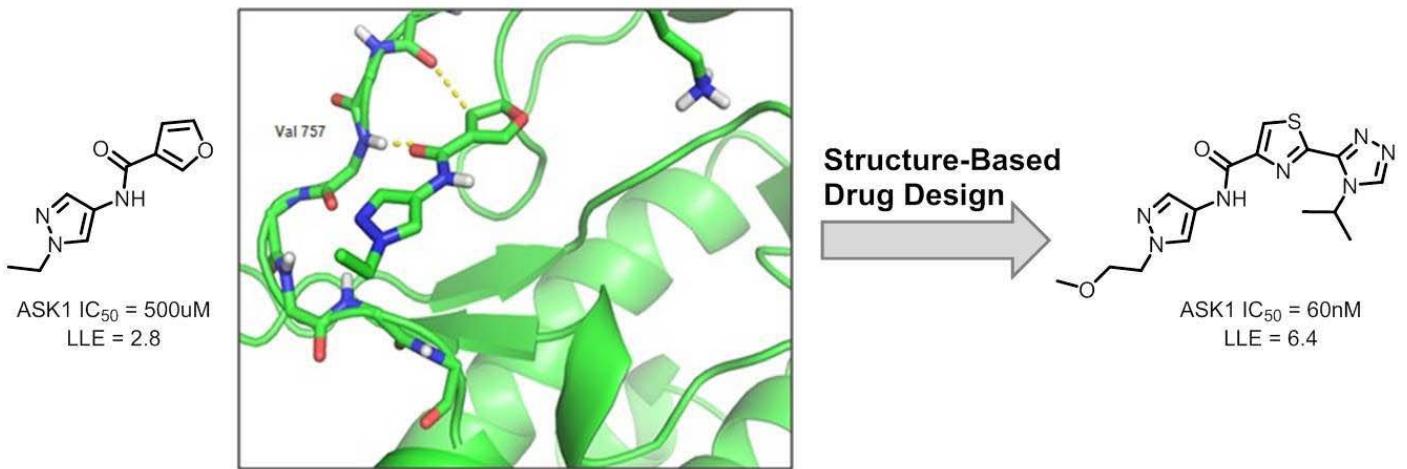
We have previously reported the asymmetric synthesis of 3-substituted 2-oxopiperazines. Herein, we present an original and a convergent strategy to synthesize RA and corresponding 3,6-disubstituted analogues. Their iron complexation capacities such as their possible intake by the bacteria will be carried out.

## MEDI 110

### Structure-based drug design of novel ASK1 inhibitors using an integrated lead optimization strategy

**Tony S. Gibson<sup>1</sup>, tony.gibson@takeda.com, Benjamin Johnson<sup>1</sup>, Andrea Fanjul<sup>2</sup>, Petro Halkowycz<sup>2</sup>, Douglas R. Dougan<sup>3</sup>, Derek C. Cole<sup>1</sup>, Steve Swann<sup>1</sup>.** (1) Medicinal Chemistry, Takeda California, Inc., San Diego, California, United States (2) Discovery Biology, Takeda California, Inc., San Diego, California, United States (3) Structural Biology and Biophysics, Takeda California, Inc., San Diego, California, United States

Structure-based drug design is an iterative process that is an established means to accelerate lead optimization, and is most powerful when integrated with information from different sources. Herein is described the use of such methods in conjunction with deconstruction and re-optimization of a diverse series of ASK1 chemotypes along with high-throughput screening that lead to the identification of a novel series of efficient ASK1 inhibitors displaying robust MAP3K pathway inhibition.



## MEDI 111

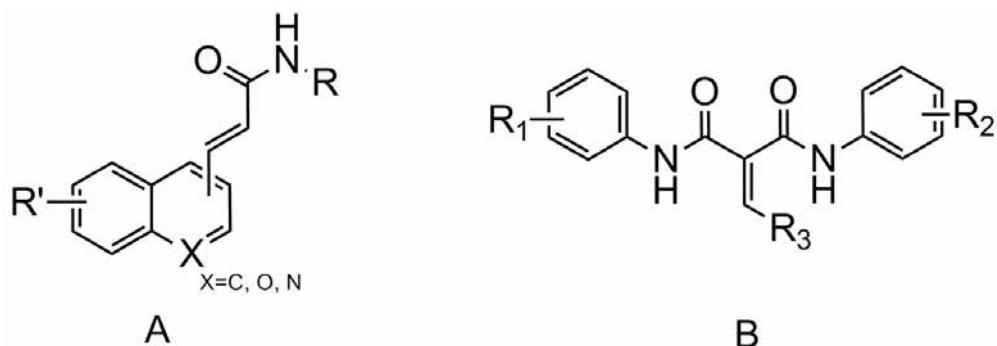
### Lead identification of activators of the Nrf2 pathway via targeting repression of Bach1

**Hong Nie<sup>1</sup>, hong.nie@gsk.com, Alicia Davis<sup>2</sup>, James F. Callahan<sup>2</sup>, Rabin Carr<sup>2</sup>, Jeffrey K. Kerns<sup>2</sup>, Ami Lakdawala-Shah<sup>2</sup>, Tindy Li<sup>2</sup>, Brent McCleland<sup>2</sup>, Jen-Pyng Kou<sup>2</sup>, Ruth Osborn<sup>2</sup>, William Rumsey<sup>2</sup>, Yolanda Sanchez<sup>2</sup>, Thomas Sweitzer<sup>2</sup>, Lawrence Wolfe<sup>2</sup>, John Yonchuk<sup>2</sup>, Hongxing Yan<sup>2</sup>. (1) GSK, King of Prussia, Pennsylvania, United States (2) GlaxoSmithKline, King of Prussia, Pennsylvania, United States**

The nuclear factor-E2 related factor 2 (Nrf2) pathway plays a pivotal role in orchestrating the body's defenses against oxidative stress by regulating the expression of a large set of Phase II enzymes, e.g., heme oxygenase-1 (HO-1) and NADPH quinone oxidoreductase-1 (NQO1). As one of the key transcription factors responding to cellular stress, the activation of Nrf2 is tightly regulated. Cytoplasmic Nrf2 binds to Kelch-like ECH associating protein 1 (KEAP1) which targets Nrf2 for ubiquitin (Cul3) mediated degradation. Under oxidative stress conditions, the Keap1/Cul3-dependent ubiquitination of Nrf2 is disrupted and Nrf2 accumulates in the cytoplasm of the cell, and translocates to the nucleus where it complexes with other proteins (such as MafK), binds Antioxidant Response Elements (ARE) of DNA and ultimately leads to induction of various Phase II genes. As a further control of Nrf2 pathway activation, the BTB and CNC homology 1 protein (Bach1) also complexes with MafK, functioning as a repressor of the Nrf2 pathway by competing with Nrf2 and blocking its binding to many of the same ARE sequences. Oxidative stress, especially stress that increases nuclear levels of heme, disrupts Bach1/MafK/ARE binding, allowing Nrf2 to complex with MafK,

bind to DNA and thus increase the transcription of Phase II genes, including HO-1.

In our efforts to identify inhibitors of Bach1 repression of the Nrf2 pathway, we utilized diversity screening of the GSK compound collection using both a biochemical assay that measured the inhibition of Bach1/MafK binding to an ARE double standard oligo and also a cellular reporter assay that measured the induction of HO-1. Two main hit series were identified from HTS: Quinoline Propenamides (A) and Malonamides (B). The team then initiated a hit to lead chemistry campaign to improve the target potency and improve the physiochemical properties of the initial hits. This poster will describe our hit-to-lead chemistry efforts to optimize these two hit series and identify viable lead series that targeted disruption of Bach1 repression of the Nrf2 pathway.



## MEDI 112

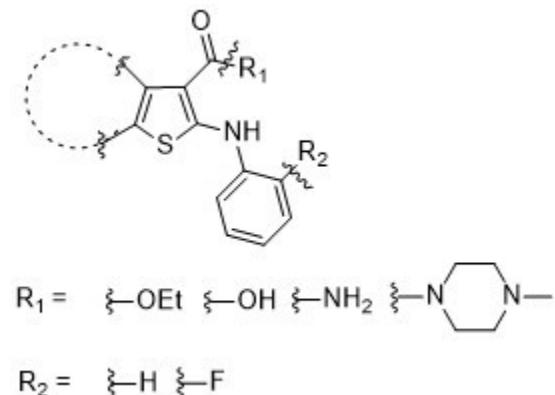
### Novel thiophene analogs as potential MEK5/ERK5 inhibitor

**Mohit Gupta**<sup>1</sup>, guptam@duq.edu, Patrick T. Flaherty<sup>1</sup>, Akshita Bhatt<sup>2</sup>, Thomas Wright<sup>2</sup>, Jane Cavanaugh<sup>2</sup>. (1) Department of Medicinal Chemistry, Duquesne University, Pittsburgh, Pennsylvania, United States (2) Department of Pharmacology, Duquesne University, Pittsburgh, Pennsylvania, United States

Inhibition of mitogen-activated protein kinase (MAPK) pathways is an established anti-cancer therapeutic strategy. The MAPK pathway proceeds by sequential protein phosphorylation. For example, MEK5 phosphorylates and activates ERK5. Activated ERK5 induces transcription to increase cell proliferation, cell growth, cell differentiation, and angiogenesis. The MEK5/ERK5 pathway is significantly upregulated in breast cancer, triple-negative breast cancer (TNBC), prostate cancer, colon cancer, leukemia, hepatocellular carcinoma, osteosarcoma, pancreatic cancer and lung cancers.

Despite correlation of increased activity of the MEK5/ERK5 pathway to negative outcomes in breast cancer, selective inhibition of MEK5/ERK5 pathway is understudied.

The Gewald reaction produces 2-amino-3-carboxythiophenes. This core has found application in several drug design projects including MEK1/2 inhibitors. *N*-Arylation with an Ullman coupling followed by functionalization of the 3-acyl substituent provided compounds that were deemed potent and selective by *in silico* screening as MEK5 inhibitors. The design, synthesis, and biological testing of 4/5 substituted *N*-aryl-3-carboxy thiophenes will be presented.



## MEDI 113

### Design and synthesis of phenylthiourea emetine analogs for studies in prostate cancer

**Nabil Idris**<sup>1</sup>, nidris@outlook.com, Emmanuel S. Akinboye<sup>2</sup>, Oladapo Bakare<sup>1</sup>. (1) Chemistry, Howard University, Silver Spring, Maryland, United States (2) Johns Hopkins University, The Sidney Kimmel Comprehensive Cancer Center, Baltimore, Maryland, United States

Emetine could be a suitable cytotoxic agent in cancer therapy and can be used to overcome multidrug resistance or to take advantage of synergistic effects in order to minimize side-effects due to the high dosage of other cytotoxic agents. However, severe side-effects such as cardiac damage and muscle weakness greatly discouraged its use as a chemotherapeutic agent. In an attempt to explore the usefulness of emetine as an anticancer agent and eliminate or drastically reduce the undesirable toxic side effects, our research group recently derivatized the N-2' position of emetine to prepare the thiourea, urea, sulfonamide, dithiocarbamate, carbamate and pH responsive hydrolyzable amide analogs and prodrugs of emetine. These compounds

show significant anticancer activities when screened against some prostate cancer cell lines (PC3 and LNCap). In addition, the compounds did not show sign of toxicity in mice when compared with emetine at the same dosage. Encouraged by these results, we have continued to synthesize and study the anticancer activities of emetine analogs and prodrugs in our prostate cancer drug development program. In this study, we report the synthesis and anticancer studies of a library of phenylthiourea analogs of emetine in the androgen receptor negative and aggressive human prostate cancer cell line (PC3).

## MEDI 114

### **Improving solubility, permeability and bioavailability of imatinib using crystal engineering approach with nicotinamide and glutamic acid**

**Manoj Kumar Gautam<sup>1</sup>, manojgautam08@gmail.com, Mousumi Besan<sup>2</sup>, Renu Chadha<sup>3</sup>.** (1) *Pharmaceutical Sciences, UIPS, PU, CHD, Chandigarh, India* (2) *Department of Pharmaceutics (IITBHU), Indian Institute of Technology, Varanasi, Uttar Pradesh, India* (3) *Pharmaceutical Sciences, UIPS, PU, CHD, Chandigarh, India*

Crystal engineering comprises rational design and modified fabrication of crystal structures offers diverse prospects to selectively enhance the physicochemical properties of drugs via cocrystallization processes. The present study is focused on the preparation, characterization and evaluation of imatinib co-crystals with conformers nicotinamide (ND) and glutamic acid (GA) using crystal engineering approach and their biological evaluation was done in breast adenocarcinoma (MCF7) and Colon Cancer (COLO205) cell lines. Characterization of the prepared co-crystal was done by using analytical tools such as differential scanning calorimetry (DSC), fourier transform infrared spectroscopy (FT-IR), powder X-ray diffraction (PXRD). DSC thermographs of IMND and IMGA cocrystals showed single sharp melting endotherm at 208°C and 149°C respectively, which were in between the melting of drug and both coformer. FT-IR study indicated anime–carbonyl interaction between the participating molecules and appearance of new peaks in PXRD pattern confirms the formation of new co-crystal. Crystal structure of both cocrystals was determined using material studio software (Biovia) from PXRD. In-vitro permeation studies of IM and its cocrystals were determined using Franz-type diffusion cells using silicon membrane as well as in caco-2 cell line also which showed improvement in permeability. Equilibrium solubility and disk intrinsic dissolution rate of cocrystals were found more as compared with pure drug. Pharmacokinetic studies were revealed 3 fold improvement in

relative bioavailability than free imatinib. All the results indicate that imatinib cocrystals possess better antitumor efficacy than free drug. Thus cocrystallization approach has been found to be viable technique for improving the solubility, permeability, dissolution and bioavailability of poorly aqueous soluble drug.

## MEDI 115

### Identification of novel 5,6-dimethoxy indan-1-one derivative as potent antiviral agent

*Siddappa A. Patil<sup>2</sup>, Vikrant Patil<sup>2</sup>, Renukadevi Patil<sup>1</sup>, Kenneth Beaman<sup>3</sup>, Shivaputra Patil<sup>1</sup>, shivaputrap@yahoo.com. (1) Pharmaceutical Sciences, Rosalind Franklin University, North Chicago, Illinois, United States (2) Centre for Nano and Material Sciences, Jain University, Bangalore, Karnataka, India (3) Microbiology and Immunology, Rosalind Franklin University, North Chicago, Illinois, United States*

The 5,6-dimethoxy indan-1-one nucleus is of considerable interest as this ring is the key constituent in a range of bioactive compounds, both naturally occurring and synthetic, and often of considerable complexity. In an effort to identify the broad-spectrum of antiviral agents, we screened focused set of 5,6-dimethoxyindan-1-one analogs (6-8) along with a thiopnene derivative (9). These molecules demonstrated considerable antiviral activity towards variety of viruses. Compound 7 showed very high potency towards vaccinia virus (EC50: <0.05 µM) and it is nearly 232 times more potent than the standard drug Cidofovir (EC50: 11.59 µM) tested. Compound 7 has been further selected for secondary screening for the vaccinia virus at National Institute of Allergy and Infectious Diseases (NIAID) antiviral screening program.

## MEDI 116

### Phosphatase-stable peptidomimetic ligands of the polo-like kinase 1 polo-box domain

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Polo-like kinase 1 (Plk1) is a serine/threonine protein kinase that plays critical roles in cell cycle regulation. Because aberrant expression of this enzyme has been observed in various cancers, Plk1 has emerged as a clinically relevant

target for the development of new anti-cancer agents. Aside from a canonical ATP-dependent kinase domain, Plk1 also contains a C-terminal polo-box domain (PBD), which is responsible for allosteric regulation of the kinase domain through protein-protein interactions with phosphoserine (pSer) or phosphothreonine (pThr)-containing peptide sequences. Our group has developed peptide mimetics containing “Ser-pThr” motifs that display low-nanomolar PBD-binding affinities. These compounds were designed to cause selective cytotoxicity in cancer cells that overexpress Plk1 by inhibiting proper localization of the enzyme and in so doing, result in disordered mitotic function. However, the constructs show decreased potency in cell-based assays, mainly due to poor membrane permeability and inactivation by cellular phosphatases. To address hydrolytic lability of the phosphoryl ester group, we have devised an efficient synthetic route to the phosphonate-based pThr mimetic, (2S,3R) 2-amino-3-methyl-4-phosphonobutanoic acid (Pmab), which also provides access to analogs having various substituents at the C3 position. When incorporated into our peptidomimetic scaffold, several of the C3 analogs exhibit several-fold enhancements in binding affinity, while maintaining excellent binding selectivity for Plk1 versus the PBDs of Plks 2 and 3. This work significantly advances the state of our current PBD-binding peptidomimetics, and it could be more broadly applicable for the investigation of structure-activity relationships of peptidomimetics targeted to other pThr-binding sites.

## MEDI 117

### **Exploration of intramolecular protein-protein interaction inhibitors of polo-like kinase 1**

**Kohei Tsuji**, kohei.tsuji@nih.gov, **David Hymel**, **Terrence R. Burke**. CBL, NCI, NIH, Frederick, Maryland, United States

The polo-like kinase 1 (Plk1) is a serine/threonine kinase that plays crucial roles in mitosis. Plk1 is overexpressed in many cancers and inhibition of Plk1 function causes mitotic arrest and subsequent apoptosis. Therefore, this kinase is a clinically-relevant target for anti-cancer therapeutic development. Although a number of highly potent ATP-competitive inhibitors that target the N-terminal kinase domain (KD) have reached clinical trials, cytotoxicity potentially arising from off-target activity has occurred. This may reflect the fact that ATP binding sites are highly conserved throughout the kinome, making it challenging to develop selective kinase inhibitors. However, the Plk family is distinguished by the presence of unique C-terminal polo-box domains (PBDs), which recognize phosphoserine and phosphothreonine-containing

sequences, thereby serving to localize the enzyme to specific interaction sites. The PBD also modulates the kinase activity of Plk1 via intramolecular interactions with its KD. It has been reported that over-expression of the Plk1 PBD prevents the formation of normal bipolar spindles, resulting in mitotic arrest. Therefore, inhibitors targeting the PBD could provide a means of achieving highly selective inhibition of Plk1 function. Inhibitors designed to disrupt PBD-mediated protein-protein interactions typically derive much of their affinity from replicating aspects of phosphopeptide recognition within the PBD. As reported herein, we have developed PBD-binding peptides that take advantage of additional interactions outside the canonical PBD. This has resulted in the discovery of PBD-binding inhibitors, which can show up to two-orders of magnitude enhancement in their abilities to disrupt PBD-ligand interactions relative to our previously disclosed peptidomimetics.

## MEDI 118

### **Application of oxime-diversification to optimize ligand interactions within a cryptic pocket of the polo-like kinase 1 polo-box domain**

**Xue Zhi Zhao**, *xuezhizhao@hotmail.com*, **David Hymel**, **Terrence R. Burke**. NIH, Frederick, Maryland, United States

Members of the polo-like kinase (Plk) family of serine/threonine protein kinases play crucial roles in cell cycle regulation and proliferation. Of five Plks (Plk1 – 5), Plk1 is recognized as an anticancer drug target. Plk1 contains multiple structural components that are important for its proper biological function. These include an N-terminal catalytic domain and a C-terminal non-catalytic polo-box domain (PBD). The PBD binds to phosphothreonine (pThr) and phosphoserine (pSer)-containing sequences. Blocking PBD-dependent interactions offers a potential means of down-regulating Plk1 function that is distinct from targeting its ATP-binding site. Oxime-based post-solid phase diversification is a form of directed fragment screening, which can be highly effective in optimizing protein-ligand interactions. As one example, starting from the known PBD-binding peptide “PLHSpT,” we have previously used this approach to identify a hydrophobic cryptic binding pocket on the surface of the PBD, whose access can enhance peptide-binding affinity by approximately 1000-fold. As reported herein, we have employed this technology to further extend and optimize PBD-ligand interactions. By a process involving initial screening of a set of 87 aldehydes using an oxime ligation-based strategy, we were able to achieve a several-fold affinity enhancement over one of the most potent previously known Plk1 PBD-binding inhibitors. This improved binding may result from accessing a newly identified auxiliary region proximal to a key

hydrophobic cryptic pocket on the surface of the protein. We have also shown that selectivity for the PIk1 PBD relative to the PBDs of PIk2 and PIk3 can be significantly enhanced by modulating interactions within this region. Our findings could have general applicability to the design of PIk1 PBD-binding antagonists.

## MEDI 119

### **Novel 5-substituted pyrrolo[2,3-d]pyrimidines with pyridine glutamate side chain as selective folate receptors and proton-coupled folate transporter substrates: Potential targeted chemotherapeutic agents**

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Pemetrexed (PMX, Alimta: total annual sale of 2.28 billion dollars in 2016) is the current standard for treating malignant pleural mesothelioma and non-small cell lung cancer. Despite being a blockbuster drug, PMX suffers from neutropenia as a dose-limiting toxicity in part due to its non-selective transport by the reduced folate carrier (RFC). RFC is expressed ubiquitously in normal tissues and tumor cells. Unlike RFC, folate receptors (FR $\alpha$  and FR $\beta$ ) and the proton coupled folate transporter (PCFT) show limited expression in normal tissues. In normal tissues, FR $\alpha$  (eg. renal tubule) is inaccessible to circulating chemotherapeutic agents and FR $\beta$  (eg. thymus) is nonfunctional. On the other hand, FRs are overexpressed in malignancies such as ovarian cancer (FR $\alpha$ ) and in myeloid leukemia (FR $\beta$ ), where they are accessible to circulation.

PCFT functions optimally between pH range 5.5 – 6.9 which mimics the acidic microenvironment of solid tumors and becomes non-functional at physiological pH (7.2 - 7.4). Hence agents targeting FRs and/or PCFT selectively over RFC are expected to have superior safety profile as novel cytotoxic targeted cancer therapeutics. PMX is a 5-substituted pyrrolo[2,3-d]pyrimidine linked to a phenyl glutamate side chain via a two carbon bridge. Our previously reported 5-substituted pyrrolo[2,3-d]pyrimidines with phenyl side chains and three (**AGF126**) or four carbon (**AGF127**) linkers, when compared with PMX showed potent KB tumor cell inhibition with very little or no selectivity for FR $\alpha$ , FR $\beta$  or PCFT over RFC. To improve selectivity, we designed and synthesized 5-substituted pyrrolo[2,3-d]pyrimidines with a pyridine side chain and two-(**AGF324**), three- (**AGF315**) and four- (**AGF317**) carbon bridges. **AGF315** and **AGF317** showed outstanding selectivity for FR $\alpha$

(32- and 22- fold, respectively), FR $\beta$  (226- and 55- fold, respectively) and moderate selectivity for PCFT (6- and 3- fold, respectively) over RFC with potent KB tumor cell inhibition ( $IC_{50}$  = 15.9 nM and 2.51 nM, respectively). To our knowledge, this is the first example of 5-substituted pyrrolo[2,3-d]pyrimidine analogs with remarkable (> 10 fold) selectivity for both FR $\alpha$  and FR $\beta$  over RFC in *in vitro* studies compared to PMX. The design, synthesis, and biological evaluation of these pyridine analogs will be presented.

## MEDI 120

### Design of alkylarylsubstituted targeted thieno[2,3-d]pyrimidines as cancer chemotherapeutic agents with fluorine insertion on aryl the side chain

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The use of currently marketed anticancer agents such as methotrexate (MTX) and pemetrexed (PMX) is limited by dose-limiting toxicity due to non-selective transport by the ubiquitously expressed reduced folate carrier (RFC). As a result, there is an urgent need to design targeted cancer chemotherapeutic agents to avoid the dose-limiting toxicities and chemotherapy drug resistance. In addition to the ubiquitously expressed RFC, two other transporters are responsible for the uptake of antitumor agents. These include folate receptor (FR)  $\alpha$  and  $\beta$ , and the proton-coupled folate transporter (PCFT). Both FRs and PCFT exhibit narrower patterns of tissue expression and are likely to serve more specialized physiologic roles. Our group is systematically developing novel folate analogs with selective membrane transport via FRs and PCFT over the ubiquitously expressed RFC. We previously published the 6-substituted thieno[2,3-d]pyrimidine analogs which showed complete selectivity for RFC and inhibited *de novo* purine biosynthetic pathway at the steps catalyzed by glycynamide ribonucleotide formyl transferase (GARFTase) and 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase (AICARFTase). In this report, fluorines were strategically placed on the thiophene side chain in order to improve potency and selectivity through induced conformational restriction caused by a plausible intramolecular fluorine-hydrogen bonding interactions. The fluorinated analogs designated as **AGF298** and **AGF302**, had  $IC_{50}$ s in the single digit nanomolar ( $IC_{50}$  = 6.84

nM and 2.6 nM, respectively) for KB tumor cells with selective uptake by FRs and PCFT over RFC. The molecular modeling, synthesis, *in vitro* evaluation and SAR of these compounds as substrates for folate transporters FR and PCFT over RFC and as potent inhibitors of human KB tumor cells ( $IC_{50}$ ) due to inhibition of GARFTase and AICARFTase, will be presented.

## MEDI 121

### Optimizing the cystargolide scaffold for the selective treatment of cancer by proteasome inhibition

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Proteasome inhibitors are an intriguing class of compounds that can be used for the selective treatment of cancer. Even though there are currently three clinically approved proteasome inhibitors for the treatment of multiple myeloma, the development of a potent inhibitor with minimal side effects associated with non-specificity remains a challenge. The development of new proteasome inhibitors with enhanced selectivity is paramount and may enable the use of these drug leads for the treatment of other types of cancer. In order to achieve optimal selectivity the structure activity relationship for drug internalization and enzyme binding must be defined. This research explores the structural modification of the benzyl ester analog of the  $\beta$ -lactone proteasome inhibitor, Cystargolide B, which has been shown in previous studies by this group to selectively target breast cancer cell lines MCF7 and MDA-MB-231, leaving the normal breast tissue cell line, MCF10A, unaffected up to a drug concentration of 100  $\mu$ M. Structural modification focuses on three aspects of the inhibitor's structure:  $\beta$ -lactone substitution, dipeptide variation, and C-terminal substitution. Changing the isopropyl group side-chain at the  $\beta$ -lactone to a sec-butyl group present in Belactosin led to an improvement of cytotoxicity by almost ten times, completely reorienting the molecule within the active site of the  $\beta$ 5 subunit of the proteasome, as observed by x-ray crystallography. Exploring the ester substitution, the best lead molecule was determined to be the allylic ester, exhibiting nanomolar anticancer  $IC_{50}$  values: 365 nM in MCF7, 350 nM in MDA-MB-231, and 94 nM in RPMI 8226 while

maintaining >100 µM toxicity in MCF10A. Changing the valine residues of the dipeptide unit to either phenylalanine or glycine led to a loss of specificity and efficacy, underlining the importance of the dipeptide functionality for enzyme affinity and cytotoxicity. Additional modification of the β-lactone to alicyclic substituents caused significant decrease in efficacy due to poor membrane internalization. Further efforts are being made to explore structural characteristics that control drug internalization, stability of the scaffold *in vitro*, proteasome-drug affinity, and proteasome-drug selectivity.

## MEDI 122

### Coupled enzyme assay for screening of effector molecules of nicotinamide mononucleotide adenylyltransferase (NMNAT)

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NMN<sup>+</sup> is converted to NAD<sup>+</sup> by reaction with ATP catalyzed by nicotinamide mononucleotide adenylyltransferase (NMNAT). NMNAT is important in NAD<sup>+</sup> homeostasis and a potential drug target for various types of cancer and neurodegenerative diseases. We have developed a luciferase-coupled assay for NMNAT, which is suitable for high-throughput formats and rapid MMOA experiments. This method was used to screen 912 compounds from the NCI mechanistic oncology set and approved oncology set against NMNAT isoform 1 (NMNAT1), and five organic molecules were identified to be time-dependent inhibitors. This is the first report of a bioluminescent assay for NMNAT suitable for screening.

## MEDI 123

### Identification and characterization of a new series of calcium/calmodulin-dependent protein kinase kinase-2 (CAMKK2) inhibitors

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CAMKK2 is a member of the serine/threonine-specific protein kinase family. It is essential in calcium signaling cascades and may be an attractive therapeutic target in range of diseases, including hepatic cancer, prostate cancer, and ovarian cancer. Despite the importance of this kinase in calcium signaling, there are few reports on structure activity relationships of CAMKK2 inhibitory series. We have identified potent inhibitors of CAMKK2 based on a furo[2,3-b]pyridine scaffold. Here we describe preliminary structure activity relationships, initial kinase selectivity data, and x-ray crystallography elucidation of the binding mode of these new CAMKK2 inhibitors.

## MEDI 124

### **Palladacycle-facilitated ligand-free Suzuki coupling of hindered aryl bromides yields potent and selective COX-2 inhibitors**

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Inflammation is an important pathological component of many diseases. It has been treated for many years with steroids and non-steroidal anti-inflammatory drugs (NSAIDs). NSAIDs non-selectively inhibit cyclooxygenases 1 and 2 (COX-1 and 2), the key enzymes responsible for the synthesis of prostaglandins (PGs). Although these drugs have good activity, they still suffer from many side effects which, in many cases, are not tolerable. The side effects are mainly due to their inhibition of COX-1 enzyme, which has an important physiological role in many tissues. Selective COX-2 inhibitors have been introduced to the US market as safer anti-inflammatory agents with lower side effects than associated with conventional NSAIDs. Unfortunately, two of these selective inhibitors were withdrawn from the market due to clinically reported side effects. Accordingly, there is a need for safer compounds from this class to fill the gap. During our optimization of Suzuki-type coupling reaction for the synthesis of some biologically active compounds, we discovered a novel class of COX-2 inhibitors that is based on the benzothiazole ring system. Similarity search for one of our reaction substrates suggested that our compounds could be selective COX-2 inhibitors. This new class can be synthesized in two synthetic steps from commercially available aromatic carboxylic acids or aldehydes by applying the newly developed reaction in our lab which uses palladacycles to facilitate Suzuki-type coupling. The optimized reaction proceeds in very good yield (75-99%) with wide substrate scope and functional group tolerability. Six compounds showed promising activity and selectivity against COX-2 and one of them has activity comparable to celecoxib in the biochemical assay. The

encouraging activities of these new compounds and their rapid accessibility via the optimized coupling reaction make positive contributions in the areas of palladium coupling chemistry and COX-2 selective inhibitors.

## MEDI 125

### **Design, synthesis and evaluation of 8-(methylamino)-2-oxo-1,2-dihydroquinoline derivatives as novel DNA gyrase and topoisomerase IV inhibitors**

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Bacterial type II topoisomerases, DNA gyrase and topoisomerase IV, are essential enzymes for DNA replication and well-established antibacterial targets. Fluoroquinolones are known as these inhibitors. However fluoroquinolone-resistant bacteria are increasing recently and cause serious clinical problems. Therefore, there is a clinical need to develop a novel chemical class of antibacterial agent. We identified 8-(methylamino)-2-oxo-1,2-dihydroquinoline scaffold as a potent and novel series of GyrB/ParE inhibitor. A representative compound showed antimicrobial activity against both Gram-positive and Gram-negative pathogens, especially *Neisseria gonorrhoeae* including antibiotic-resistant strains. It also showed *in vivo* efficacy in a mouse model of *N. gonorrhoeae* infection. Details of design, synthesis, structure activity relationship and biological activity of these compounds will be disclosed.

## MEDI 126

### **Evaluation of a FLT3 inhibitor as an anti-leukemic agent for acute myeloid leukemia**

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FMS-like receptor tyrosine kinase-3 (FLT3) belongs to the family of receptor tyrosine kinase (RTK), FLT3 mutation is observed in 1/3 of acute myeloid leukemia (AML) patients. We have identified potent FLT3 inhibitor containing indirubin skeleton, compound **4**. Potent inhibitory activity of the compound **4** against FLT3 was shown in *in vitro* kinase assay ( $IC_{50} = 3$  nM). The compound **4** selectively inhibited the growth of MV4;11 cells ( $GI_{50} = 1$  nM) and induced apoptotic cell death. The compound **4** caused cell cycle arrest at G<sub>2</sub>/M phase and increased cell population at sub-G<sub>1</sub> phase. Phosphorylation of STAT5, which is downstream signaling of FLT3, was significantly reduced by compound **4** dose-dependently. Pharmacokinetic properties of compound **4** in mice were investigated. And *in vivo* anti-tumor effect was carried out using MV4;11 xenograft. With 5 mg/kg and 10 mg/kg of intravenous administration to nu/nu mice, tumor volume and weight was significantly reduced compared to control.

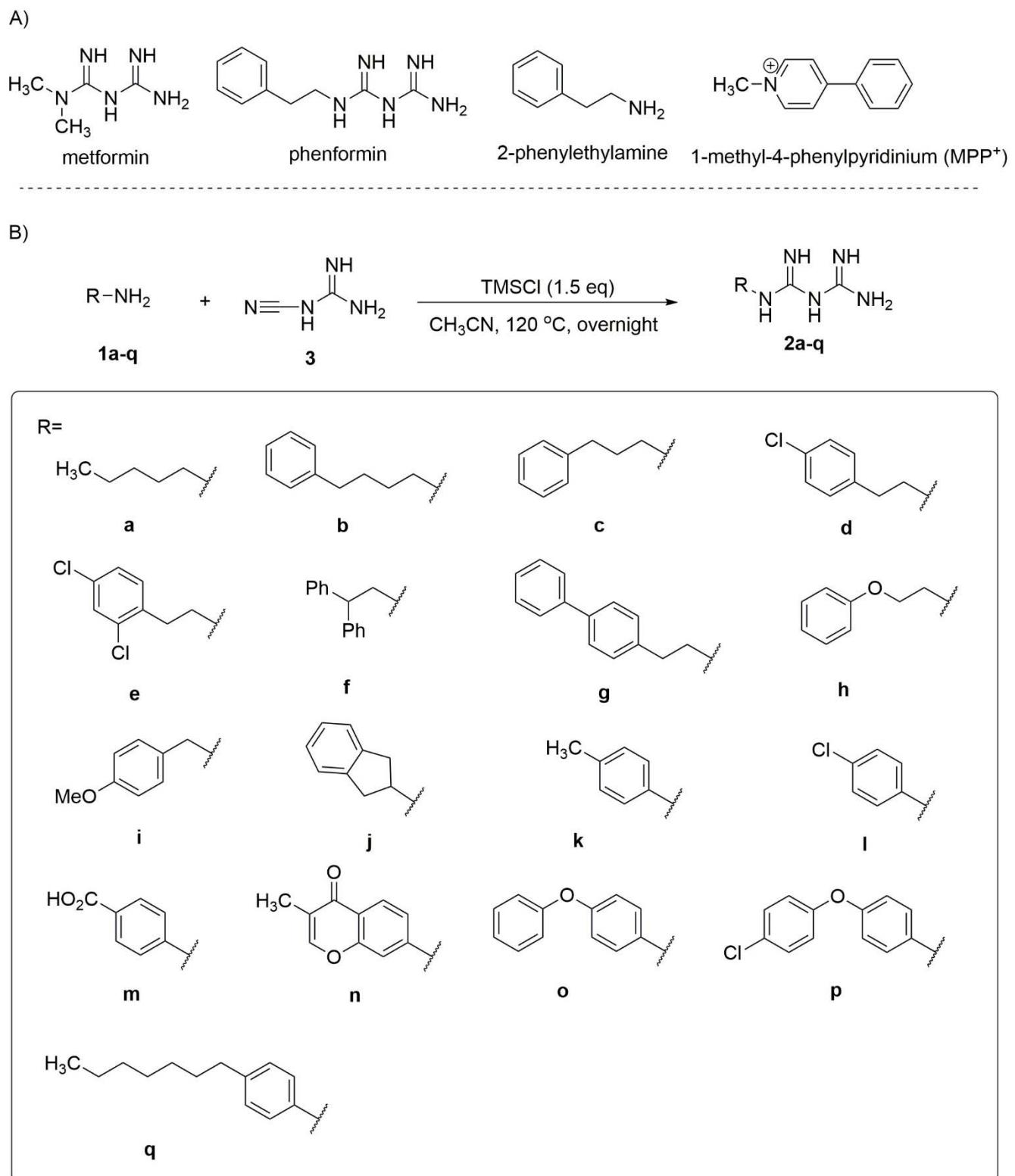
## MEDI 127

### **Incorporation of a biguanide scaffold enhances uptake by organic cation transporters (OCT) 1 and 2**

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Membrane transporters play a significant role in the transport of many endogenous and exogenous compounds. Here we demonstrate that inclusion of a biguanide functionality can improve the compound uptake mediated by organic cation transporters 1 and 2 (OCT1 and OCT2). We synthesized 18 pairs of potential OCT substrates with either an amino compound or a biguanide group; and assessed their uptake in cells overexpressing human OCT1 or OCT2. Our results showed that the presence of the biguanide functionality significantly improved OCT1 and OCT2-mediated update. The biguanides also inhibited the uptake of prototypical substrates of both transporters, 1-methyl-4-phenylpyridinium (MPP<sup>+</sup>) and metformin. In addition, we found that molecular weight, molecular volume, LogD (pH 7.4) and

accessible surface area were key determinants of OCT2 substrates, but none of these parameters was a significant factor for OCT1. Moreover, the inhibition of MPP<sup>+</sup> uptake correlated linearly with that of metformin uptake for the tested biguanides in both cell lines. OCT1-mediated MPP<sup>+</sup> and metformin inhibition was negatively correlated with biguanide molecular weight, molecular volume, and accessible surface area.



**Figure 1. Chemical synthetic scheme and compounds assayed.** a) Structure of metformin, phenformin, phenformin parent amine (2-phenylethylamine), and

MPP<sup>+</sup>. **b)** General scheme for synthesis of biguanides. TMSCl – Trimethylsilyl chloride; CH<sub>3</sub>Cl – acetonitrile.

## MEDI 128

### P38 MAPK kinase inhibitor for steroid insensitive asthma

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While the normal mild and moderate asthma symptoms can be well controlled with inhale corticosteroid, there are still approximately 10% patients require high dose steroid and second control therapies. Steroid insensitive severe asthma represents a major unmet medical need with disproportionate health care cost in total asthma treatment. P38 mitogen-activated protein kinase (MAPK) signaling pathway regulates pro-inflammatory transcription factors such as NK-kB and has been proposed for the treatment of many inflammatory diseases. Increased P38 MAPK activation was reported in severe asthma patients, and the mechanism may involve the nuclear translocation of glucocorticoid receptors (GR). By inhibiting the phosphorylation of GR serine 226, p38 MAPK kinase inhibitor may restore the corticosteroid sensitivity of severe asthma patients. We have identified a novel chemistry series with potent P38 MAPK inhibition activities. These compounds were evaluated in ex vivo and in vivo models and showed promising activity for further investigation, detailed ADME/PK, in vitro, in vivo results will be presented.

## MEDI 129

### Design, synthesis and biological evaluation of heteroaryl amine derivatives as potential anticancer agents

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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 - thiadiazole 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<sub>50</sub> 35 μM against MCF-7 (breast cancer), HCT116 (Colon) and U251 (Glioma) cells.

## MEDI 130

### **Method for the analysis and quantification of 3-methylene furanone: A biomarker of oxidative damage to DNA**

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Oxidative stress is defined as a disturbance in the balance between the production of reactive oxygen species (free radicals) and antioxidant defenses. Reactive oxygen species are harmful to the body because they have the potential to cause damage lipids, proteins, RNA, and DNA. Thus, the inability of the body to metabolize these species could be the cause of numerous diseases such as, atherosclerosis, diabetic, cancer, rheumatoid arthritis, cardio vascular diseases, and chronic inflammation. In the study of the C3'-thymidyl radical, several damage lesions have been identified. Among these are 3'-oxothymidine and its associated elimination products. 3'-oxothymidine was postulated to form as a result of the generation of the C3'- radical under anaerobic conditions; however, we have also observed its formation in the presence of oxygen as well. One of the resulting elimination product is 2-Methylene-3(2H) furanone, a characteristic biomarker of oxidative DNA damage occurring at the C-3' and C-2' of DNA. The high reactivity of the lesion has made its isolation, identification and quantification difficult. Taking advantage of known synthetic routes we report the synthesis and

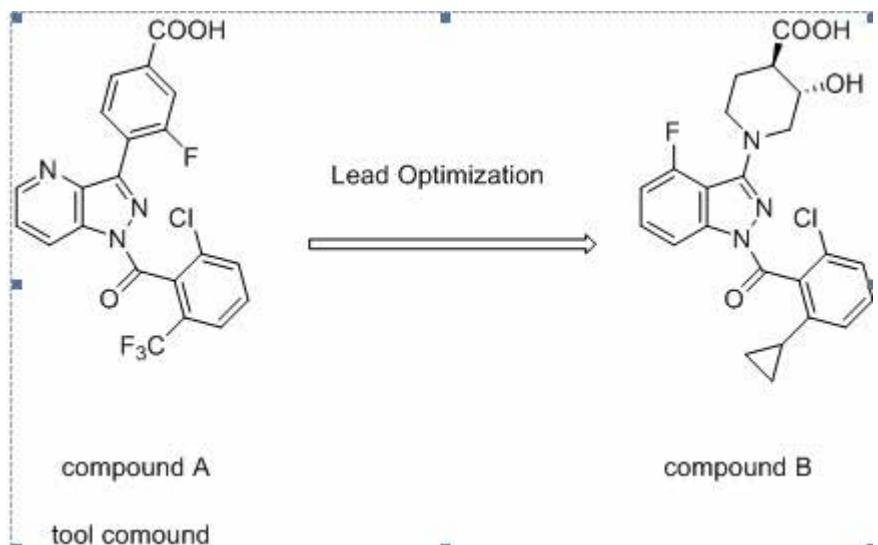
quantification of thiol analogs of 2-methylene 3(2H) furanone to facilitate its isolation. LC-MS analysis was then conducted to establish a standard concentration curve that will enable us to routinely quantify 2-methylene-3 (2H) furanone release from DNA damage.

## MEDI 131

### **Discovery of (3S,4R)-1-(1-(2-chloro-6-cyclopropylbenzoyl)-4-fluoro-1H-indazol-3-yl)-3-hydroxypiperidine-4-carboxylic acid as potent and selective allosteric inhibitors of ROR $\gamma$ t for the treatment of autoimmune diseases**

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Targeting the Th17 pathway has increasingly become an attractive therapeutic approach for the treatment of autoimmune diseases. Recent clinical success and FDA approval of anti-IL-17 antibody Secukinumab further validates the feasibility of this mechanism. Nuclear hormone receptor ROR $\gamma$ t is a master regulator of the differentiation and development of Th17 cells. Such regulation by ROR $\gamma$ t affects the production of not only IL-17, but also a host of other pro-inflammatory cytokines. Inhibition of ROR $\gamma$ t may provide an alternative or even better option than those antibodies targeted for neutralizing a single cytokine. In the past few years, a number of structurally diverse ROR $\gamma$ t inverse agonists as well as several cocrystal structures of these ligands have been reported. The majority of such inhibitors bind similarly in the classical orthosteric binding pocket. Recently, we disclosed a new class of inhibitors, which bind to a non-classical and novel allosteric binding pocket. This unprecedented binding mode was established unequivocally by the cocrystal structures. In addition, we also showed that one tool molecule (compound A) demonstrated robust efficacy in a dose-dependent manner in a thymocyte PD model. In this poster, we will describe our optimization effort which led to the discovery of (3S,4R)-1-(1-(2-chloro-6-cyclopropylbenzoyl)-4-fluoro-1H-indazol-3-yl)-3-hydroxypiperidine-4-carboxylic acid (compound B) as a potent, selective ROR $\gamma$ T allosteric inhibitor with improved pharmacokinetics and physiochemical properties.



MEDI 132

# Design, synthesis, and biological evaluation of flexible acyclic nucleoside analogues against human coronaviruses and filoviruses

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To date, there are no FDA approved treatments or vaccines for diseases caused by coronaviruses (CoVs) or filoviruses. Over the past decade, two deadly human coronaviruses, Severe Acute Respiratory Syndrome CoV (SARS) and Middle East Respiratory Syndrome CoV (MERS), have emerged as lethal pathogens with high mortality rates. Filoviruses, such as the Ebola (EBOV), Sudan (SUDV), and Marburg (MARV) viruses, also represent a severe health threat with mortality rates reaching 90%. With the potential of global reemergence of SARS, as well as the recent outbreaks of MERS, EBOV, and SUDV, it is imperative that a viable and efficient treatment is developed in order to increase survival rates of these lethal diseases.

Nucleoside analogues have long served as the cornerstone for antiviral therapeutics due to their ability to inhibit viral DNA or RNA replication; however, one major issue is the moderately high genetic mutation rate associated with these viruses, which alters the enzymatic binding site and renders the antiviral agents ineffective. One way to potentially overcome drug resistance is to create a more flexible nucleobase scaffold in order to increase adaptability of the drug once bound within the target enzyme. The Seley-

Radtke lab has developed various types of flexible nucleoside analogues, called “fleximers”, that have demonstrated the ability to overcome point mutations within the binding site of biologically significant enzymes, as well as to increase interactions in the binding pocket that were unattainable by the parent nucleoside. Preliminary results have shown that several acyclic Flex-analogues of the FDA-approved drug Acyclovir have shown activity against both SARS and MERS *in vitro*. These findings are groundbreaking since these compounds represent the first nucleosides to exhibit potent activity against SARS and MERS. More recently, studies have uncovered activity against various filoviruses including EBOV, SUDV, and MARV. The results of these studies are reported herein.

## MEDI 133

### Dentification of novel inhibitors of glucose transporter 3 (GLUT3) through structure-based virtual screening

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Glioblastomas (GBMs) are one of the most aggressive, deadly cancers. Microenvironments of nutrient deprivation often occur in GBMs and contribute to treatment failure. We found that increased glucose uptake was a mechanism for the survival advantage of brain tumor initiating cells (BTICs) which are subsets of highly tumorigenic cells implicated in tumor maintenance and therapeutic resistance, and the elevated glucose uptake of BTICs was due to significantly higher levels of the glucose transporter GLUT3. We also found that higher levels of GLUT3 correlated with poor patient outcomes in many other tumor types including those of the breast, lung, and colon. These data indicate the potential importance of GLUT3 as a therapeutic target.

However, no GLUT3 specific inhibitors are currently available. In the present study, we applied structure-based virtual screening (SBVS) to identify GLUT3 inhibitors and evaluated their potential therapeutic effects using GBM xenografts. Two of the SBVS selected hits were found to significantly diminish GBM growth but with limited toxicity agonist astrocytes and neurons.

Mechanism studies further confirmed that the two identified inhibitors blocked glucose uptake in GLUT3 over-expressed cells. Our results strongly support the concept of targeting GLUT3 for the treatment of GBMs. The identified small molecule GLUT3 inhibitors can serve as a useful tool for studying the

mechanism(s) of glucose uptake as well as potential leads for the development of cancer therapeutic agents.

## MEDI 134

### **Discovery and development of novel diazeniumdiolate derivatives as nitric oxide donors**

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Nitric oxide (NO) is widely known to be involved in cellular signaling that results in vasodilation in the cardiovascular system, contributing to decreased blood pressure. Organic nitrates such as glyceryl trinitrate have been developed as NO donors, but they can be limited by short half-lives, tolerance, and adverse side-effects. Diazeniumdiolates offer a novel method of systemic introduction of NO with a distinct release mechanism through CYP3A4 metabolism. This novel mechanism allows for control over the kinetics of NO release and gives compounds with improved peak-to-trough ratios as demonstrated with *in vivo* telemetry studies. Here we will discuss the initial lead diazeniumdiolate compounds as well as the evolution of SAR based on *in vivo*, DMPK, and metabolism identification studies.

## MEDI 135

### **Glutathione as an herbal molecule with potential for zinc chelation therapy**

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Despite the essentiality of the element zinc to all organisms, its excess is toxic. A chelating agent used to treat zinc poisoning is CaEDTA. However, EDTA (ethylenediaminetetraacetic acid) has severe tissue irritating properties. Therefore, it is desirable to replace CaEDTA with a less hazardous, yet efficient, chelating agent.

A previously developed QSPR (Quantitative Structure-Property Relationship) model for predicting zinc(II) binding by organic ligands was used to explore a

natural chelate found in fruits and/or vegetables that is capable of binding zinc(II) nearly as strongly as EDTA. Glutathione, with its 3 amine groups, 2 carboxylic acid groups, and 1 sulfhydryl group, binds zinc(II), at pH 7.0, with a predicted conditional binding constant of  $\log K_f = 15.20$  for zinc(II)-glutathione 1:1 complex. This means that glutathione at pH 7.0 binds zinc(II) ~19 times more strongly than EDTA, as the experimental conditional binding constant for zinc(II)-EDTA 1:1 complex is  $\log K_f = 13.92$ . Since glutathione is abundantly available in all vegetables, it is predicted to be a great replacement for EDTA for zinc chelation therapy.

## MEDI 136

### Non-psychoactive cannabinoid CBD modulates the orphan receptor GPR3

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Cannabidiol (CBD) is one of the major components of Cannabis sativa. This non-psychoactive molecule has shown neuroprotective, antiepileptic, anxiolytic, antipsychotic, and anti-inflammatory properties among others. However, its complex pharmacology it is not yet fully understood.

GPR3 is an orphan receptor that belongs to the Class A family of G-Protein Coupled Receptors. It shares high sequence similarity with GPR6, GPR12, the lysophospholipid receptors S1P1 and LPA1, and the cannabinoid receptors, CB1 and CB2. GPR3 has been shown to be a possible target for the treatment of pathological conditions such as Alzheimer's disease (AD), oocyte maturation or neuropathic pain. Nevertheless, the lack of potent and selective GPR3 modulators is delaying the exploitation of this promising therapeutic target. Interestingly, we have recently discovered that CBD acts as a GPR3 inverse agonist of the G-protein Independent  $\beta$ -arrestin (GprotIndepBarr) signaling pathway, but has no effect on GPR3 G-protein Dependent (GprotDep) signaling. Such GPR3 GprotIndepBarr signaling biased inverse agonists have great therapeutic potential in AD.

In this context, we have developed a GPR3 homology model that may help us to elucidate the structural determinants governing key ligand-receptor interactions. Our GPR3 model is based on the crystallized S1P1 receptor structure. Sequence divergences in transmembrane helices 1, 4, 6 and 7 have been explored using the Monte Carlo/simulated annealing program,

Conformational Memories. The extracellular and intracellular loop geometries were calculated using Modeller v9.1. The GPR3 inactive state model has helped us to elucidate the molecular interactions of CBD with this orphan receptor rationalizing the structural basis of its selective GprotIndepBarr vs. GprotDep signaling. This homology model will enable the design of more potent and selective GprotDep and GprotIndepBarr-biased GPR3 ligands to further understand GPR3's biological role and its possible relation with the endocannabinoid system.

## MEDI 137

### Reduced synthesis time of an acidic α-diimine ligand using flow chemistry

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Metal complexes of 1,4-diazabutadiene ligands have received attention due to their utility as homogeneous polymerization catalysts. One example, 4-[3-(4-carboxyphenyl)iminobutan-2-ylideneamino]benzoic acid, is synthesized via flow chemistry. Flow chemistry reduces the reaction time to 20 minutes instead of the 24 hour reaction time needed for traditional techniques. This example uses a single pump and a constant temperature bath. The total time required for both the reaction and purification steps is less than a day resulting in a significant improvement in productivity.

## MEDI 138

### Panamanian cyanobacterial metabolite with antitrypanosomal activity

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American trypanosomiasis or Chagas disease, caused by the protozoan *Trypanosoma cruzi*, is a vector-borne neglected tropical disease. The causative parasite is endemic in 21 Latin American countries and as of now infects about 6 to 7 million people in that geographic region and imposes a large economic burden on people of these countries with widespread

poverty. The parasite also imposes a global threat because of population migration, which brings it to the USA, Canada, Europe, and Western Pacific countries. Unfortunately, the effectiveness of available therapeutics for the Chagas disease is limited by long-term therapy, serious side effects, and inadequacy in the chronic phase of the disease, thus demands development of new therapeutic agents. Recently, an *N*-methylated peptide, naranjamide, was discovered by bioactivity-guided fractionation from a Panamanian cyanobacterium, which showed an inhibition of 81.51% of *T. cruzi*. The structure of the compound was elucidated by using NMR and MS/MS. We completed the total synthesis of naranjamide with commercially available building blocks. The reaction scheme utilized four types of reactions including peptide coupling, ester hydrolysis, Boc-deprotection, and *N*-methylation of amides. The major peptide coupling reaction employed was *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide (EDC) coupling with 1-hydroxybenzotriazole (HOBr) as an additive. HATU mediated coupling reaction was also performed in the final step of the synthesis. Base (NaH) promoted methylation with methyl iodide (MeI) yielded the *N*-methyl version of the corresponding amide. To obtain a better insight of the structure-activity relationship of the lead compound, several series of naranjamide analogs have been designed. Here we also report the synthesis of a series of naranjamide analogs that constitutes both methylated and non-methylated peptides with variation in their chain length, and positions of amino acids. Analogs synthesis applied the similar set of reactions used for naranjamide synthesis. The anti-parasitic activity of these analogs will be presented. Other analogs, designed with modification in the head and tail part of the peptide, will be reported in future.

## MEDI 139

### Investigating the impact of pore size and chain length when purifying peptides

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Peptide synthesis and purification continues to increase in importance. Peptides are used as active site models in drug discovery and are increasingly being used as Active Pharmaceutical Ingredients (APIs). Earlier chromatographic work focused on the analytical chemistry of peptides, but there have been few studies that looked at the relationship of chain length and pore size and their corresponding impact on larger scale purification of

peptides where resolution and loading are both important. This study compares the purification of several different sized peptides where reverse phase stationary phase chain length, and are varied to determine their effect on the resolution and loading capacity of peptides.

## MEDI 140

### Optimal light conditions and nitrogen treatments for growth and for accumulation of phytochemical groups in *Calendula officinalis*

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*Calendula officinalis* is an ornamental plant whose extracts have been known to possess various classes of compounds including terpenoids, flavonoids, coumarins, quinones and volatile oils that exhibit anti-inflammatory, anti-HIV, spasmogenic and antiseptic properties among others. The goal of this study was to determine optimal light intensity and nitrogen-based treatment for growth and for high expression of beneficial phytochemical compounds in *Calendula*. Levels of total polyphenolic content (TPC) and total antioxidant capacity (TAC) were quantitatively analyzed using visible spectrphotometry. Four plants were cultivated under each of the following eight light conditions and ammonium nitrate treatments: 100% light and distilled water (control), 100% light and 0.01 M, 100% light and 0.05 M, 100% light and 0.1 M, 50% light and distilled water (control), 50% light and 0.01 M, 50% light and 0.05 M, and 50% light and 0.1 M. Each plant received weekly treatments of 100 mL of ammonium nitrate solutions at the mentioned concentrations for 7 weeks. Biweekly observations for plant height, leaf length and leaf count indicated that *Calendula* growth was optimal at 50% light intensity and 0.01 M ammonium nitrate. Maximum TPC accumulation and highest TAC levels also occurred at 50% light intensity and lower concentrations of nitrogen in samples of leaves collected on the first sampling date (S1). A downward trend in TPC was observed from S1 to S3, but maximum TPC still accumulated under the same conditions (50% light and low nitrogen) in S3 samples. TAC levels decreased drastically for samples at 50% light intensity from S1 to S3. Results from this study indicate that 50% light intensity and low concentrations of nitrogen-based treatment are ideal for *Calendula* growth and for high yield of therapeutic compounds.

## MEDI 141

### Identification and optimization of 4-anilinoquinolines as selective inhibitors of cyclin G associated kinase

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4-Anilinoquinolines were identified as potent and narrow spectrum inhibitors of the cyclin G Associated Kinase (GAK), an important regulator of viral and bacterial entry into host cells. Optimization of the 4-anilino group and the 6,7-quinoline substituents produced GAK inhibitors with nanomolar activity and high selectivity relative to other members of the Numb-Associated Kinase (NAK) sub-family. These compounds could be useful tools to explore the therapeutic potential of GAK in prevention of a broad range of infectious diseases. Our results demonstrate that quinoline-based ATP-competitive kinase inhibitors can be designed with exquisite selectivity within the NAK sub-family. Iterative medicinal chemistry led to a member of the 4-anilinoquinoline series with over 50,000-fold selectivity for GAK over other members of the NAK sub-family. Analogs within 4-anilinoquinolines series have the potential to yield high quality chemical probes for use in the elucidation of GAK function in cells and *in vivo*.

## MEDI 142

### Targeted antitumor agents for the inhibition of one-carbon metabolism associated with purine biosynthesis: Altering sterics, electronics and conformation for tumor selectivity and potency

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Cancer chemotherapy is in urgent need of agents devoid of dose-limiting toxicities (DLTs) and drug resistance. Targeted chemotherapy utilizes selective uptake by transporters expressed in tumor cells to circumvent DLTs. Multi-enzyme inhibition can evade tumor resistance by attacking two or more targets in tumor cells. The folate receptors (FRs) are functionally overexpressed in several tumor cells including ovarian cancers and the proton-coupled folate transporter (PCFT) is the principal folate transporter in the acidic micro-environment of solid tumors. The reduced folate carrier (RFC) is ubiquitously expressed major tissue folate transporter, and is non-functional in acidic environment. Substrates of folate transporters inhibit one or more of the following intracellular enzymes that catalyze one-carbon transfer during purine/pyrimidine biosynthesis: glycinamide ribonucleotide formyl transferase (GARFTase), aminoimidazole carboxamide ribonucleotide formyl transferase (AICARFTase), thymidylate synthase (TS) and dihydrofolate reductase (DHFR). DLTs due to non-selective RFC mediated transport, and tumor resistance are the drawbacks of currently used drugs such as methotrexate (MTX) and pemetrexed (PMX). Our previous reports demonstrated that scaffold hopping and 2C bridge extension to 3C and 4C in 6-5 fused aroyl glutamates that are structurally and mechanistically distinct from MTX and PMX, improve antitumor potency and selectivity (selectively inhibit FR/PCFT over RFC over-expressing KB and IGROV1 tumor cells at sub-nM to nM IC<sub>50</sub> values due to GARFTase and/or AICARFTase inhibition). We recently reported that fluorine substitution on the phenyl side chain, *ortho*(o-) to the glutamate improves potency and selectivity of our pyrrolo[2,3-*d*]pyrimidine analogs. Fluorine substitution can lower binding energies and/or favor the bound conformation by a multitude of effects such as intramolecular F-H bonding, halogen bonding, dipole interactions and steric effects. In the current report we extend the rational design of 6-substituted pyrrolo[2,3-*d*]pyrimidine analogs to explore the effects on the biological activity by replacing the *o*-fluorophenyl side chain with *o*-methylphenyl (steric), *o*-trifluoromethylphenyl (steric and electronic) and pyrimidyl (electronic) side chains. Novel compounds in this series have a remarkable picomolar inhibition of tumor cells. The molecular modeling, synthesis, NMR studies for F-H bonding and *in vitro* evaluation will be presented.

## MEDI 143

### **Discovery of N-substituted 2-phenylcyclopropylmethylamines as functionally selective serotonin 2C (5-HT<sub>2C</sub>) receptor agonists for potential use as antipsychotic medications**

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The serotonin 2C (5-HT<sub>2C</sub>) receptor has been identified as a promising drug target for obesity and central nervous system (CNS) disorders, such as schizophrenia and drug addiction. In particular, 5-HT<sub>2C</sub> agonists with high subtype selectivity against 5-HT<sub>2B</sub> and 5-HT<sub>2A</sub>, and functional selectivity (namely ligand bias, *i.e.*, G<sub>q</sub>-linked calcium flux vs. β-arrestin recruitment) are advantageous to reduce side effects (especially lethal cardiac valvulopathy from activation of 5-HT<sub>2B</sub> receptor) and enhance therapeutic efficacy for clinical studies. In our prior work, we have developed a series of 2-phenylcyclopropylmethylamine (2-PCPMA) derivates as potent, and subtype-selective 5-HT<sub>2C</sub> agonists. Herein, in order to improve brain penetrance and functional selectivity, while maintaining subtype selectivity, a new series of N-substituted 2-PCPMAs have been designed, synthesized and pharmacologically evaluated. Some of these compounds exhibit highly selective 5-HT<sub>2C</sub> vs. 5-HT<sub>2B</sub> receptor agonism with excellent functional bias for G<sub>q</sub>-linked calcium flux vs. β-arrestin recruitment signaling. The N-methyl compound (+)-**3a**, which displayed an EC<sub>50</sub> of 12 nM at 5-HT<sub>2C</sub> with no β-arrestin recruitment activity, is the first potent and at the same time fully G<sub>q</sub>-biased 5-HT<sub>2C</sub> agonist reported to date, while the N-benzyl compound (+)-**9a** with an EC<sub>50</sub> of 24 nM at 5-HT<sub>2C</sub> is fully selective over 5-HT<sub>2B</sub>. Preliminary studies in an amphetamine-induced hyperactivity model *in vivo* indicate that (+)-**9a** shows potential antipsychotic action.

## MEDI 144

### **Design and synthesis of 1,4-benzodioxane-6-carboxylic acid derivatives for studies in prostate cancer drug development**

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Our research group continues to explore the chemical modification of natural products to identify new bioactive agents that could be useful in the development of new chemotherapeutic drugs for the treatment of castration resistant prostate cancer. In our attempt to study some newly designed 1,4-benzodioxane derivatives in castration-resistant prostate cancer, we found the antioxidant natural product, gallic acid, an attractive scaffold for chemical transformation to unique 1,4-benzodioxane-6-carboxylic acid derivatives. In this study, a new library of 6,8-disubstituted 1,4-benzodioxanes, including 1,4-benzodioxane-emetine hybrids, were synthesized for studies in our prostate cancer drug development program.

#### **MEDI 145**

#### **Development of thiol specific fluorogenic agents for cell surface thiol imaging in live cells**

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Thiols play a significant role in cell structure and function. Thiols have been found intracellularly, in extracellular fluids, and on cell surface. Thiol density on cell surface is easily affected by extracellular oxidant insults. Therefore, an agent that can map thiol distribution on cell surface in live cells will be a valuable tool in understanding how cells respond to oxidative stress stimuli. We would like to present the design and synthesis of thiol specific fluorogenic agents for imaging thiols on cell surface in live cells through fluorescence microscopy. These agents are designed based on a benzofurazan sulfide structure previously reported by us to be thiol specific and fluorogenic. Imaging of an analyte in live cells through fluorescence microscopy enables us to visualize the analyte in its native environment without breaking the cell and reveals information that cannot be obtained from broken cells.

#### **MEDI 146**

#### **Efforts towards the development of new ERR $\gamma$ modulators via structure-based drug design**

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The estrogen-related receptors (ERRs) were the first orphan nuclear receptors to be identified and as their name suggests are similar to the estrogen receptors (ER $\alpha$  and ER $\beta$ ) but do not bind endogenous ER ligands. However, the synthetic ER ligand 4-hydroxytamoxifen has been shown to bind to ERR $\gamma$  as an inverse agonist. Additionally, a non-selective ERR $\beta/\gamma$  agonist GSK4716 has also been reported. With crystal structures of both ligands in ERR $\gamma$  available, we have taken a structure-based drug design approach to develop new chemical templates that act as selective modulators of ERR $\gamma$ . This poster will describe the synthesis and basic structure-activity relationships of these novel thiophene and pyrazole based templates.

#### MEDI 147

#### Targeting inhibitor of apoptosis proteins: Identification of potent dimeric antagonists of IAPs

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Inhibition of apoptosis, or programmed cell death, has been associated with cancer cell survival and the development of resistance to chemotherapeutic agents. The inhibitor of apoptosis proteins (IAPs) block cell death in response to a variety of stimuli and are overexpressed in many human malignancies. Consequently, IAPs are attractive targets for the treatment of cancer. We describe the discovery of a series of heterodimeric IAP antagonists which demonstrate potent *in vitro* antiproliferative activity in a range of human cancer cell lines. Several compounds have further shown curative efficacy in human cancer xenograft models.

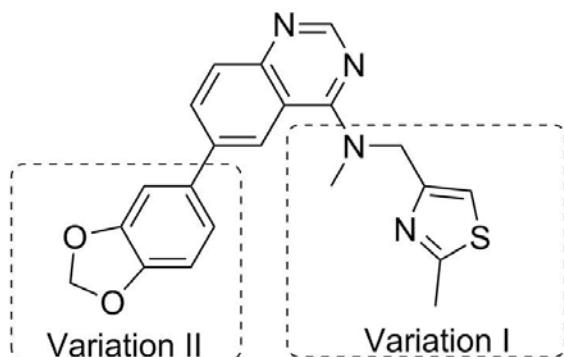
#### MEDI 148

#### Optimization of quinazoline derivatives as selective MEK5 inhibitors

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The Mitogen Activated Protein Kinase (MAPK) pathway relays extracellular signals by sequential phosphorylation of proteins. In this pathway the serine/threonine kinase mitogen-activated protein kinase kinase 5 (MAP2K5 or MEK5) phosphorylates and activates extracellular signal-regulated kinase 5 (ERK5). Up regulation of the MEK5/ERK5 pathway is reported in various types of cancers especially in triple negative breast cancer. Elevated activity of the MEK5/ERK5 pathway reduces disease-free survival time in breast-cancer patients. The development of small molecule selective inhibitors of MEK5 may have relevant therapeutic application. Compound **1** prepared originally by Craig Thomas was developed as a cdc2-like kinase inhibitor and identified to inhibit MEK5 in a counter-screen. Compound **1** was selected as a MEK5 scaffold and examined for optimization of MEK5 activity. An *in-silico* binding analysis of **1** to MEK5 suggested variation on the two side-chains for further optimization (variations I and II). These aim to exploit unique P-loop residues, the putative Thr gatekeeper residue, and MEK5-specific C-terminal domain (CTD) residues.



**1**

## MEDI 149

**Potent and selective inhibitors of receptor-interacting protein kinase 1 that lack an aromatic back pocket group**

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Receptor-interacting protein kinase 1 (RIP1K), a key component of the cellular necroptosis pathway, has gained recognition as an exciting therapeutic target.

Pharmacologic inhibition or genetic modulation of RIP1K has shown promise in models of disease ranging from acute ischemic conditions, chronic inflammation, and neurodegeneration. Accordingly, there has been a surge of interest in developing small molecule inhibitors of RIP1K. We present here a class of RIP1K inhibitors that is distinguished by a lack of a lipophilic aromatic group present in most literature inhibitors that typically occupies a hydrophobic back pocket of the protein active site. Despite not having this ubiquitous feature of many RIP1K inhibitors, we were able to obtain compounds with good potency, selectivity, and pharmacokinetics.

## MEDI 150

### **Novel 6-substituted pyrrolo[2, 3-d] pyrimidines with substituted nitrogen bridges and fluorinated benzoyl regioisomers as selective folate receptor substrates and antitumor agents**

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Folates are involved in one-carbon transfer reactions, required for DNA synthesis. In mammals, there are three major folate transport systems, the reduced folate carrier (RFC), folate receptors (FRs), and proton-coupled folate transporter (PCFT). Currently used antifolates, such as methotrexate (MTX), pemetrexed (PMX), and pralatrexate (PDX), are significantly transported by the ubiquitously expressed RFC and result in dose-limiting toxicity, due to non-selective drug uptake into normal tissues. FRs and PCFT have limited expression in human tissue and over-expressed in tumor cells. Antifolates that are selectively taken up by FR and/or PCFT into tumors would circumvent the dose-limiting toxicities of currently used clinical antifolates. We previously reported 6-substituted pyrrolo[2,3-d]pyrimidines with three-atom bridged N-substituted analogs that have superior FR and good PCFT activity. However, the selectivity for FR and/or PCFT over RFC is not optimal. In this study, fluorinated benzoyl regioisomers with 6-substituted three-atom bridged N-substituted pyrrolo[2,3-d]pyrimidine analogs were designed and synthesized to evaluate the effects of fluorinated benzoyl regioisomers on transport by RFC, FR and/or PCFT and as inhibitors of human tumor cells targeting

enzymes involved in the purine biosynthetic pathway. Some of these analogs were single digit nanomolar and picomolar inhibitors (IC<sub>50</sub>) of tumor cells in cultures. This report will discuss the molecular modeling, design, synthesis and potent biological results of these compounds.

## MEDI 151

### Design, synthesis and *in combo* antidiabetic bioevaluation of multitarget phenylpropanoic acids

**Gabriel Navarrete Vazquez**<sup>1</sup>, gabriel\_navarrete@uaem.mx, Blanca Colin-Lozano<sup>1</sup>, Samuel Estrada-Soto<sup>1</sup>, Julio Cesar Almanza-Pérez<sup>2</sup>, Xin Xie<sup>3</sup>, Umberto Mura<sup>4</sup>. (1) Facultad de Farmacia, Universidad Autónoma del Estado de Morelos, Cuernavaca, Morelos, Mexico (2) Laboratorio de Farmacología, Depto. Ciencias de la Salud, Universidad Autónoma Metropolitana-Iztapalapa, Mexico City, Mexico (3) CAS Key Laboratory of Receptor Research, the National Center for Drug Screening, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shangai, China (4) Dipartimento di Biologia, Unità di Biochimica, University of Pisa, Pisa, Italy

We synthesized a small series of five 3-[4-arylmethoxy]phenyl]propanoic acids using an easy and short step synthetic route. All compounds were tested *in vitro* against a set of four protein targets identified as key elements in diabetes: Aldose reductase, GPR40, PPAR $\gamma$  and GLUT-4. Compound **1** was an aldose reductase inhibitor, showing IC<sub>50</sub> = 7.4  $\mu$ M. This compound also displayed an EC<sub>50</sub> value of 0.075  $\mu$ M against GPR40.

Compounds **2** and **3** behave as aldose reductase inhibitors, GPR40 agonists and showed an increase of 4-times in the mRNA expression of PPAR $\gamma$ , as well as the GLUT-4 levels. Docking studies were conducted in order to explain the polypharmacological mechanism of action and the interaction binding mode of the most active compounds on these three targets. Compounds **1**-**3** were tested *in vivo* at 100 mg/kg dose, but only compounds **2** and **3** were orally actives, reducing glucose levels in a non insulin-dependent diabetes mellitus mice model. These compounds showed robust *in vitro* and *in vivo* efficacy, and could be considered as promising multitarget antidiabetic drug candidates.

## MEDI 152

### Phytochemical approach for therapeutic efficacy enhancement of FeNP: As biomedicine

**Anamika Mubayi**, [anamika.mubayi@gmail.com](mailto:anamika.mubayi@gmail.com), Geeta Watal. University of Allahabad, Allahabad, India

Exploitation of phytochemicals as reducing agents for synthesizing nanoparticles is an economical, quick and ecofriendly approach. The present study deals with the synthesis of iron nanoparticles (FeNP) using ferric chloride solution in *Moringa oleifera* extract and the synthesized FeNP were characterized by different analytical techniques viz. UV-Vis, XRD, SEM, FTIR, LIBS. It has been observed that the therapeutic efficacy of these particles helps in raising the haemoglobin (Hb) level from 13.1 to 14.9 mg/dl within a week in normal cases and hence manages iron deficiency in general as well as in patients associated with diabetes. Iron deficiency is mostly supplemented with oral medication available in the market, which could not be easily assimilated in the system due to their low absorption rate. In addition to it they also have some side effects. Enhanced Hb level is due to the higher absorption rate, because of the increased surface area of FeNP.

## **MEDI 153**

### **PTX-NPs encapsulated by metal-polyphenol: Synthesis and cytotoxicity**

**Michelle Hung<sup>3</sup>**, [michellemwhung@gmail.com](mailto:michellemwhung@gmail.com), **Ping Li<sup>4</sup>**, **Wei Liu<sup>2</sup>**, **Yanlian Yang<sup>1</sup>**. (1) National Center for Nanoscience Technology, Beijing, China (2) University of Chinese Academy of Sciences, Beijing, China (3) St. Mark's School, Southborough, Massachusetts, United States (4) National Center for Nanoscience and Technology, Beijing, China

Nano-particulate drugs hold great promise of improving drug efficacy because of their enhanced solubility, prolonged retention time, and higher bioavailability with tissues or cells. Generally, these nanoparticles are encapsulated to keep them well dispersed with specific particle size in the manufacturing process. The additional advantage of encapsulation is that a rationally selected coating agent may tailor the pharmacokinetics and control the release of therapeutic nanoparticles in the targeted tissue, simultaneously reducing the toxicity and side effects of drugs. Here we report paclitaxel nanoparticles (PTX-NPs) encapsulated by metal-polyphenol layer, produced by one-step aerosol spray method. PTX-NPs act as a template, which enables in-situ formation of a membrane of coordination complexes of polyphenol tannic acid and Fe(III) in the solution. The produced PTX-NPs with metal-polyphenol encapsulation (PTX-C) are stable in water and have pH responsiveness for releasing. To investigate the safety and effectiveness of the PTX-C, an in vitro drug release and cytotoxicity assay is performed on

human breast cancer cell line (MCF-7). The IC<sub>50</sub> of PTX-C is lower than that of PTX-NPs and ABI-007. Cellular uptake and distribution of Rhodamine B labelled PTX-C in MCF-7 cells indicates that PTX-C could be endocytosed by cells and localized in lysosomes. A control experiment proved that TA/Fe(III) complex is nearly non-cytotoxic, evidencing the potential of PTX-C for cancer therapies.

## MEDI 154

### Synthesis of $\alpha,\beta$ -unsaturated phosphonate esters as DXR inhibitors

**Kenneth Heidel<sup>1</sup>, kennyheidel@gwu.edu, Robert C. Brothers<sup>1</sup>, Rachel Edwards<sup>3</sup>, Amanda Haymond<sup>4</sup>, Helena I. Boshoff<sup>5</sup>, Marvin J. Meyers<sup>2</sup>, Stacy Arnett<sup>2</sup>, Ana Rodriguez<sup>6</sup>, Audrey R. Odom<sup>3</sup>, Cynthia S. Dowd<sup>1</sup>.** (1) Department of Chemistry, George Washington University, Washington, District of Columbia, United States (2) Center for World Health & Medicine, Saint Louis University, Saint Louis, Missouri, United States (3) School of Medicine, Washington University, St. Louis, Missouri, United States (4) Department of Chemistry and Biochemistry, George Mason University, Manassas, Virginia, United States (5) Tuberculosis Research Section, NIAID, NIH, Bethesda, Maryland, United States (6) School of Medicine, Department of Microbiology, New York University, New York, New York, United States

*Plasmodium falciparum* (*Pf*) and *Mycobacterium tuberculosis* (*Mtb*) are infectious microorganisms of particular interest, as these organisms infect millions worldwide. Unfortunately, both *Pf* and *Mtb* continually develop resistance to current drug therapies. Thus, resistance presents an imperative need to develop next-generation drugs to combat these infectious agents. One such approach is by inhibition of the non-mevalonate pathway (NMP), a pathway not found in humans. The NMP is responsible for the biosynthesis of five-carbon building blocks, called isoprenes. By inhibiting the NMP, isoprene biosynthesis in microorganisms is halted, and the growth and spread of the infectious agents are impeded. We aim to disrupt the NMP through inhibition of the enzyme 1-deoxy-D-xylulose-5-phosphate reductoisomerase (Dxr), which is the first committed step of the pathway. In our prior work, an  $\alpha,\beta$ -unsaturated lipophilic phosphonate prodrug, RCB-185, was shown to have potent activity against *Pf*. New analogs of RCB-185 are being synthesized in effort to understand the relationships between structure and *in vitro/in vivo* activities.

## MEDI 155

### Synthesis of enantiopure 10-nornaltrexone as potential TLR-4 antagonist and opioid receptor ligand

**Christine A. Herdman**<sup>1,2</sup>, christine.herdman@nih.gov, **Arthur E. Jacobson**<sup>1,2</sup>, **Kenner C. Rice**<sup>1,2</sup>. (1) National Institute on Drug Abuse, National Institutes of Health, Bethesda, Maryland, United States (2) National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, Maryland, United States

The use of opiates for pain relief has been a long established practice, allowing for the treatment of both short and long term pain. Opiates interact at three opioid receptors known as the m, k, and d receptors. While opiate agonists that bind to these receptors provide pain relief, many that bind to the m-receptor also elicit unwanted physiological responses such as respiratory depression, constipation, vomiting, nausea, and hyperalgesia. Recently it has been shown that some opiates activate the Toll-like 4 receptors (TLR-4), which stimulates an immune response decreasing tolerance and hyperalgesia. Two known TLR-4 antagonist opiates, (+)-naloxone and (+)-naltrexone, are inactive at the opioid receptors and do not antagonize the beneficial effects of opiates.

In order to better understand the structure activity relationship of TLR-4 antagonists, a simpler more flexible analogue has been synthesized that contains a more ideal geometric shape for TLR-4 antagonism, utilizing the 10-nornaltrexone structure. This simpler analogue may also be useful in the treatment of opioid dependence. Previous synthetic work in our group has been utilized to produce the *trans* 10-nornaltrexone analogue, while the synthesis of the *cis* analogue will be presented in this poster.

## MEDI 156

### Targeted BET protein degradation for the treatment of acute myeloid leukemia (AML) and acute lymphoma leukemia (ALL)

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*University of Michigan, Ann Arbor, Michigan, United States (4) Pharmaceutical Sciences, University of Michigan, Ann Arbor, Michigan, United States*

The bromodomain and extraterminal (BET) family proteins consisting of BRD2, BRD3, BRD4 and BRDT, are the most extensively studied epigenetic “reader” proteins among bromodomain-containing proteins. Recently, several inhibitors of BET family proteins have been advanced into clinical trials for various human diseases, especially for human cancers. Although BET protein inhibitors activity against cancer cells arises from mechanisms such as c-MYC suppression, BET protein degradation based on the Proteolysis Targeted Chimeras (PROTAC) technology has been demonstrated to induce additional cellular effects and more significant cell apoptosis. As our continuing interest for targeting BET protein for cancer drug discovery, herein, we report our discovery of a small-molecule pan-BET degrader. Compared with known BET inhibitors and degraders, the new degrader demonstrates dramatically improved cellular growth inhibition activities against RS4;11, MOLM13 and MV4;11 cell lines. Moreover, the compound results in complete and rapid tumor growth regression in RS4;11 and MV4;11 mouse xenograft models. These data establish that our newly developed degrader is a promising candidate for further evaluation.

## MEDI 157

### **Design, synthesis and evaluation of potent DNA-alkylating agents for use in antibody-drug conjugates (ADCs)**

*Emily E. Reid, Katie E. Archer, Chen Bai, Nicholas C. Yoder, Dilrukshi Vitharana, Leanne Lanieri, Megan Bogalhas, Rui Wu, Qifeng Qu, Erin K. Maloney, Olga Ab, Jose F. Ponte, Ravi V. Chari, Michael L. Miller, michael.miller@immunogen.com. 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. Here we present preclinical results from a head-to-head comparison of ADCs of the monoimine containing **DGN549** with its corresponding DNA cross-linking diimine version, **IGN-P1 diimine**. These IGNs were conjugated using a peptidase-labile linker to monoclonal antibodies directed against tumor-associated antigens. The resulting ADCs were highly cytotoxic *in vitro* towards cancer cell lines, with IC<sub>50</sub> values in the picomolar range. Studies leading to the selection of the

monoimine **DGN549** ADC for clinical advancement, will be described, along with methods to improve its overall synthesis.

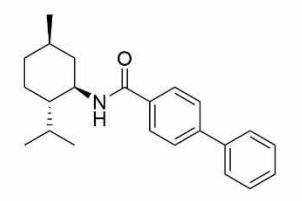
## MEDI 158

### Towards a structure-based pharmacophore for the transient potential melastatin 8 (TRPM8) ion channel: Ligand recognition at the menthol receptor

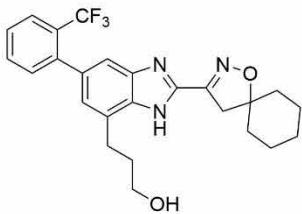
*V B. Journigan, journigan@marshall.edu, Colleen E. Heffner. Department of Pharmaceutical Sciences and Research, Marshall University, Huntington, West Virginia, United States*

The transient potential melastatin 8 (TRPM8) ion channel is a target of emerging interest with regards to its role in nicotine addiction, in addition to its more traditionally explored roles in neuropathic pain and prostate cancer. While the majority of TRPM8 ligands were discovered from high throughput screening efforts, no structure-based pharmacophore exists for the rational design of chemical probes or therapeutic small molecules. We hypothesized that insights into structure-based ligand recognition at TRPM8 could be revealed by flexible docking of several structurally similar TRPM8 antagonists in a published homology model of the closed state. Selective compounds **1-2, AMTB** and **AMG2850**, with TRPM8 IC<sub>50</sub> values ranging from 0.2-156 nM, were docked into the active site of a single monomer using Schrodinger's induced-fit docking protocol (version 11.0). Tyr745, located in the S<sub>2</sub> helix, was selected as the centroid of the active site based on mutagenesis studies implicating its role in endogenous ligand (menthol) binding. Residues within 4.4 Å of the ligand poses were refined. The docked poses of **1-2, AMTB** and **AMG2850** suggest binding in a pocket consisting of both hydrophobic and polar residues in the S<sub>1-2</sub> and S<sub>4-5</sub> helices, as well as domains between these helices. The pharmacophoric features of each ligand resulting from interactions in the active site were detected using Schrodinger's e-pharmacophore. The individual pharmacophores were then merged to reveal three overlapping hydrophobic and aromatic features, three non-overlapping hydrophobic and aromatic features within 2.3-3.3 Å, and two H-bond acceptor groups. Overlapping pharmacophoric regions were validated with the published SAR of nine known active and inactive TRPM8 ligands, and able to discriminate between the majority of actives and decoys. These results suggest an initial pharmacophore based on the overlapping regions, as well as additional pharmacophoric regions that could be harnessed to yield a more comprehensive understanding of TRPM8 ligand recognition.

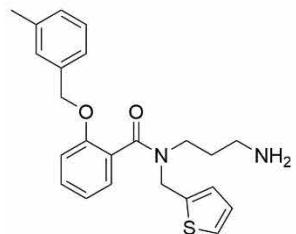
**TRPM8 Antagonists**



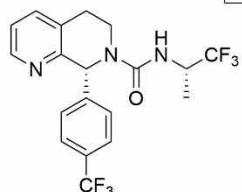
**1**  
TRPM8 IC<sub>50</sub> (menthol): 20 nM



**2, Janssen**  
TRPM8 IC<sub>50</sub> (icilin): 0.2 nM

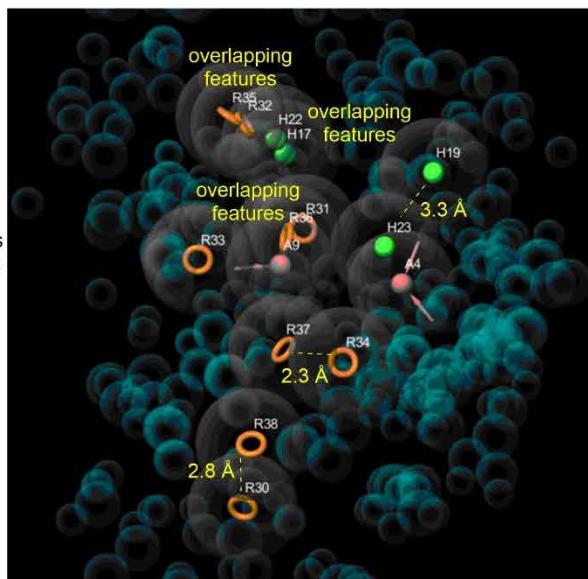


**AMTB, Bayer**  
TRPM8 IC<sub>50</sub> (menthol): 8 nM



**AMG2850, Amgen**  
TRPM8 IC<sub>50</sub> (menthol): 156 ± 110 nM

Structure-based  
pharmacophoric features  
from induced-fit docking



## MEDI 159

### Development of bis{N,N'-rhodamine-7,7'-aminosulfonyl(benzo[c][1,2,5]oxadiazol-4-yl)}sulfane (BiROS) as a thiol specific fluorogenic agent for mitochondrial thiol imaging in live cells

**Shenggang Wang**<sup>2</sup>, shenggang.wang@sdstate.edu, **Huihui Yin**<sup>3</sup>, **Yinghong Li**<sup>1</sup>, **Xiangming Guan**<sup>2</sup>. (1) Institute of Medicinal Biotechnology, Chinese Academy of Medical Science & Peking Union Medical College, Beijing, Beijing, China (2) Pharmaceutical Sciences, South Dakota State University, Brookings, South Dakota, United States (3) Guangxi Veterinary Research Institute, Nanning, Guangxi, China

Thiols play significant roles in various cellular functions. A change in thiol concentration has been linked with various disease states. Numerous reagents have been developed to detect thiols in biological samples. However, few are able to detect and quantify thiols in live cells, especially in mitochondria of live cells. Live cell thiol detection has the advantage of revealing information that cannot be revealed from thiol quantification of homogenized samples. BiROS was designed and synthesized as a thiol specific and mitochondria selective fluorogenic agent. BiROS effectively imaged and quantified thiols in mitochondria in live NCI-H226 cells, and effectively detected thiol change, created by a thiol-modulating agent NEM, in mitochondria at a concentration that was nontoxic to the cells. The

mitochondrial thiol imaging was confirmed through a co-localization experiment with DIOC<sub>6</sub>(3) - a mitochondria imaging agent. BiROS will be a valuable tool for the study of thiols' function in mitochondria.

## MEDI 160

### **Design, synthesis, and evaluation of glutathione-cholesterol sulfide and its derivatives as brain-targeting agents**

***Yue Huang, Yue.Huang@sdstate.edu, Shenggang Wang, Asim Najmi, Xiangming Guan. Pharmaceutical Sciences, South Dakota State University, Brookings, South Dakota, United States***

The blood-brain barrier (BBB) serves as a protection barrier for the brain. The barrier also present a challenge for therapeutic agents to reach the brain for the treatment of various brain diseases such as Parkinson's disease, Alzheimer's disease, and brain cancers. Extensive research efforts have been made to improve the delivery of therapeutic agents to the brain.

The BBB is featured with various receptors and transporters. Ligands of some of these receptors and transporters are exploited for brain-targeting.

Glutathione (GSH) is an endogenous three amino acid peptide. GSH enters the brain through GSH transporter that is enriched on the BBB. GSH has been successfully used for brain-targeting. In this poster, the design, synthesis, and evaluation of glutathione-cholesterol sulfide as a brain-targeting agent and its derivatives will be presented.

## MEDI 161

### **Defining the pharmacokinetic and pharmacodynamic parameters of potent and selective heteroaryl sulfonamide Nav1.7 inhibitors with robust *in vivo* analgesic activity**

***Benjamin Milgram, bmilgram@amgen.com. Amgen, Cambridge, Massachusetts, United States***

The voltage-gated sodium channel Nav1.7 serves as a primary driver of action potential firing and neuronal excitability in the pain processing pathway. Genetic evidence supports the role of Nav1.7 in a range of inherited pain syndromes. Consequently, there has been considerable interest in the development of a subtype selective Nav1.7 inhibitor for the management of chronic neuropathic pain. Herein, we provide an in depth analysis of the *in vitro* and *in vivo* pharmacokinetic and pharmacodynamic parameters for

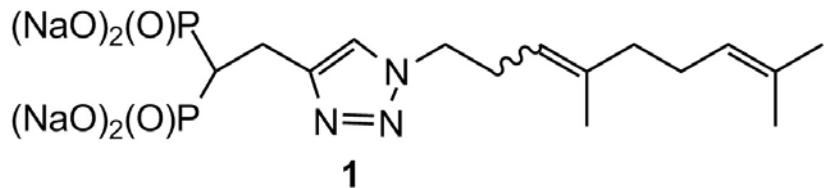
several sub-series of heteroaryl sulfonamide Nav1.7 inhibitors. Lessons learned led to the discovery of sulfonamides with favorable rat, dog, and cyno pharmacokinetic profiles that demonstrate robust efficacy in several mouse pain models, including UVB and capsaicin-induced nociception.

## MEDI 162

### Novel isoprenoid triazole bisphosphonates as potential GGDPS inhibitors

**Robert A. Mattheissen<sup>1</sup>, robert-matthiesen@uiowa.edu, Michelle L. Varney<sup>2</sup>, Sarah A. Holstein<sup>2</sup>, David F. Wiemer<sup>1</sup>.** (1) Department of Chemistry, Univ of Iowa, Iowa City, Iowa, United States (2) Internal Medicine, Univ of Nebraska Medical Center, Omaha, Nebraska, United States

Bisphosphonates that inhibit the enzyme farnesyl diphosphate synthase (FDPS) are used for treatment of patients with osteoporosis and multiple myeloma. However, there is evidence that these drugs express their pharmacological effects through depletion of the downstream isoprenoid geranylgeranyl diphosphate (GGPP). Therefore inhibition of geranylgeranyl diphosphate synthase (GGDPS), the enzyme that mediates formation of GGPP from FPP and IPP, may offer a more direct way to achieve the biological effects desired from FDPS inhibition. In an effort to inhibit GGDPS several isoprenoid bisphosphonates have been made and tested, and some show IC<sub>50</sub> values as low as 45 nM (**1**). Previous efforts focused on the formation of individual olefin isomers and comparison of their activity. However, the mixture of olefin isomers showed more potent biological activity than either of the individual isomers. Based on those findings, current work is focused on design and synthesis of analogues with increased biological activity. The details of the synthesis of novel bisphosphonates similar to compound **1**, and their activity as inhibitors of GGDPS, will be presented.



## Synthesis of Pan-CMP mimics to inhibit CoaBC

**Hailey Butman<sup>1</sup>, haileybutman@gmail.com, Xu Wang<sup>1</sup>, Robert C. Brothers<sup>1</sup>, Joanna C. Evans<sup>2</sup>, Valerie Mizrahi<sup>2</sup>, Erick Strauss<sup>3</sup>, Cynthia S. Dowd<sup>1</sup>.** (1) *Chemistry, The George Washington University, Washington, District of Columbia, United States* (2) *MRC/NHLS/UCT Molecular Mycobacteriology Research Unit & DST/NRF Centre of Excellence for Biomedical TB Research, Institute of Infectious Disease and Molecular Medicine and Department of Pathology, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa* (3) *Dept of Biochem Stellenbosch U, Matieland, South Africa*

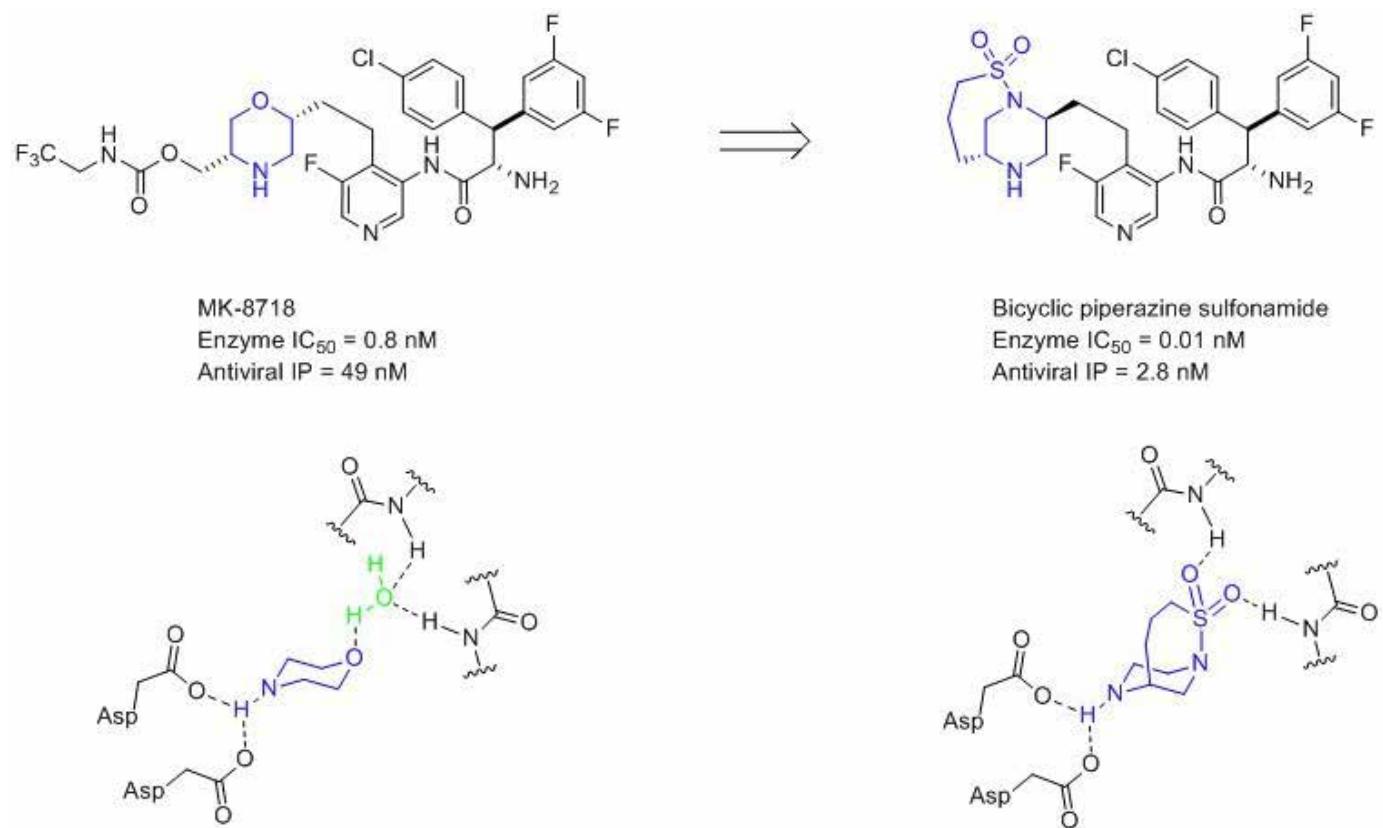
The increase in multidrug-resistant pathogens due to the overuse of antibiotics, as well as the lack of development of novel therapeutics, has presented an urgent need for the discovery of next-generation antibacterial agents. In particular, the spread of multi-drug resistant tuberculosis (MDR-TB) and extensively-drug resistant tuberculosis (XDR-TB) has been a growing societal concern. The enzyme cofactor CoA plays an essential role in the biosynthesis of fatty acids and the generation of energy. The significant differences between microbial and mammalian CoA biosynthesis pathways make it an attractive target for drug development. In *Mycobacterium tuberculosis* (*Mtb*), CoA precursor pantothenate (Pan) is synthesized by PanB, PanC, PanD, and PanE. In the second stage of biosynthesis, Pan is converted to CoA in five steps that are catalyzed by PanK, CoaBC, CoaD, and CoaE enzymes. It was recently shown that, of all the enzymes in the pathway, depletion of only CoaBC resulted in bactericidal activity. Depletion of other enzymes was only bacteriostatic. The importance of CoaBC in prokaryotic metabolism leads to the hypothesis that inhibitors of CoaBC will disrupt CoA synthesis and kill bacterial cells. Bacterial CoaBC is bifunctional and contains both phosphopantothenoylcysteine synthetase (PPCS) and phosphopantothenoylcysteine decarboxylase (PPCDC) activities. Together, these activities catalyze the transformation of 4'-phospho-pantothenic acid (P-Pan) into 4'-phospho-pantetheine (P-PantSH). This reaction proceeds through formation of the reactive 4'-phospho-pantothenoyl-CMP (Pan-CMP) intermediate. Mimics of Pan-CMP have been synthesized as inhibitors of CoaBC. This family of compounds has the potential to further validate CoaBC as a new antitubercular drug target.

## MEDI 164

### Design and synthesis of bicyclic piperazine sulfonamides leading to highly potent HIV protease inhibitors

**Christopher J. Bungard**, christopher\_bungard@merck.com. WPP14-2, Merck and Co, West Point, Pennsylvania, United States

Using the morpholine core of **MK-8718** as inspiration, a novel aspartate binding bicyclic piperazine sulfonamide core was designed and synthesized. The resulting HIV protease inhibitor containing this core showed an 80-fold increase in enzyme binding affinity and a 20-fold increase in antiviral activity relative to **MK-8718**.



## MEDI 165

### Identification of potent 17 $\beta$ -hydroxysteroid dehydrogenase type 3 (17 $\beta$ -HSD3) inhibitors by systematic structural modifications of the lead compound RM-532-105

**Francisco Cortés-Benítez**<sup>1,3</sup>, franciscoqfb@comunidad.unam.mx, **Jenny Roy**<sup>1,2</sup>, **Martin Perrault**<sup>1,2</sup>, **Rene Maltais**<sup>1,2</sup>, **Donald Poirier**<sup>1,2</sup>. (1) CHU de Québec – Research Center, Quebec, Quebec, Canada (2) Université Laval, Quebec, Quebec, Canada (3) National Autonomous University of Mexico, Mexico City, Mexico

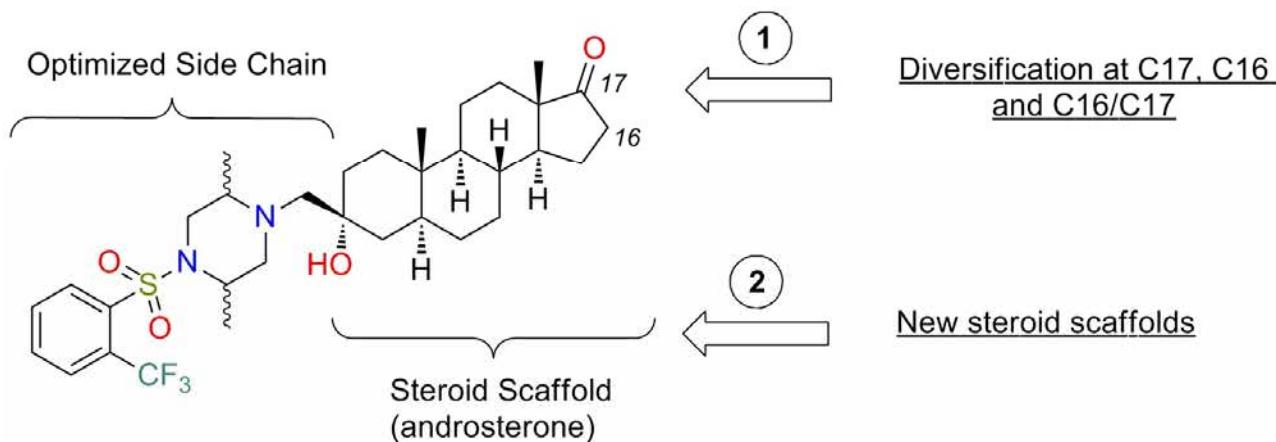
Prostate Cancer (PCa) has been a major public health concern in North America since it is the second most common cancer in men. Although there are currently several hormonal therapies to improve health or prolong life expectancy, PCa often evolves into a resistant form. The androgen receptor (AR) signaling pathway plays a central role in PCa development, as the initial growth of prostate carcinomas are androgen-sensitive. Thus, maximum blocking of androgen production is crucial to counteract the proliferative effect on prostate cancer cells. Since 17 $\beta$ -hydroxysteroid dehydrogenase type 3 (17 $\beta$ -HSD3) is an overexpressed enzyme in PCa which plays an important role in the biosynthesis of Testosterone (T), inhibition of this target is an attractive strategy to treat PCa.

Herein, we report the synthesis by systematic structural modifications of the RM-532-105 compound, which is a substituted 3 $\beta$ -androstane derivative. To obtain a more potent and metabolically stable 17 $\beta$ -HSD3 inhibitor with null androgenicity, we carried out several reactions modifying the C16 and C17 positions. Moreover, we explored some other steroid scaffolds. Structure activity-relationships for 17 $\beta$ -HSD3 inhibition on transfected LNCaP [17 $\beta$ -HSD3] cells, androgenicity on the LAPC-4 cell line, as well as metabolic stability on human hepatocytes will be presented. From the lead RM-532-105, a novel class of estrone derivatives was discovered as potent and metabolically stable 17 $\beta$ -HSD3 inhibitors with null androgenic effect.

## 17 $\beta$ -HSD3 Inhibitor

(lead compound: RM-532-105)

### SAR Studies



## MEDI 166

### Targeting cancer cell metabolism using sugar-based small molecules

**Fidelis Ndombera**, *fidelis@chem.wayne.edu*. Chemistry, Wayne State University, Detroit, Michigan, United States

Metabolic reprogramming occurs in cancer cells leading to an altered metabolism. Small molecules that block this altered metabolism in cancer or that increase the production of reactive oxygen species (ROS) are emerging as potential anti-cancer agents. This is because increased generation of ROS observed in most cancer cells relative to normal cells suggest that this biochemical property provide a therapeutic window for selective killing of cancer cells using ROS-modulating small molecules. ROS-modulating small molecules such as phenethyl isothiocyanate, piperlongumine and 2-deoxy-D-glucose exploit cancer cell vulnerability to reach lethal ROS levels above the antioxidants protective threshold.

To explore the generality of these observations, we hypothesized that carbohydrate based ROS-modulating molecules would more selectively enhance ROS levels in cancer cells relative to normal cells. Furthermore, cancer cells overexpress glucose (GLUT-1) transporters in order to facilitate enhanced sugar entry necessary to fuel high cancer-cell metabolism. Previous studies have demonstrated that GLUT-1 tolerates substitution at positions 1, 2 and 6 of a glucose molecule. Consequently, we ensured our sugar-conjugated small molecules maintained vital structure-activity relationships with GLUT-1. Considering that various carbohydrates can be used for cellular energetics or protein N-glycosylation of which interruption can lead to cellular stress, we

synthesized and evaluated a library of N-aryl glycosides for induction of ROS and cytotoxicity in H1299 lung cancer cell line. Two N-aryl glycosides (K8 and H8) were identified that induce about 2-fold ROS levels and cytotoxicity in H1299 cells. K8A was recently evaluated in 60 cell lines by the National Cancer Institute and found to inhibit growth in UO-31, NCI H522 and CCRF-CEM. We further showed that the acetylated form of K8 (K8A) activates AMPK, and stabilizes p53 and induce a higher cytotoxicity than 2-deoxy-D-glucose in H1299 cell line. In addition, K8A induces ER stress indicated by upregulation of glucose-regulated protein-78 (GRP-78). Interestingly, initial mechanistic studies using click chemistry suggest that K8A blocks global protein glycosylation in H1299 cells.

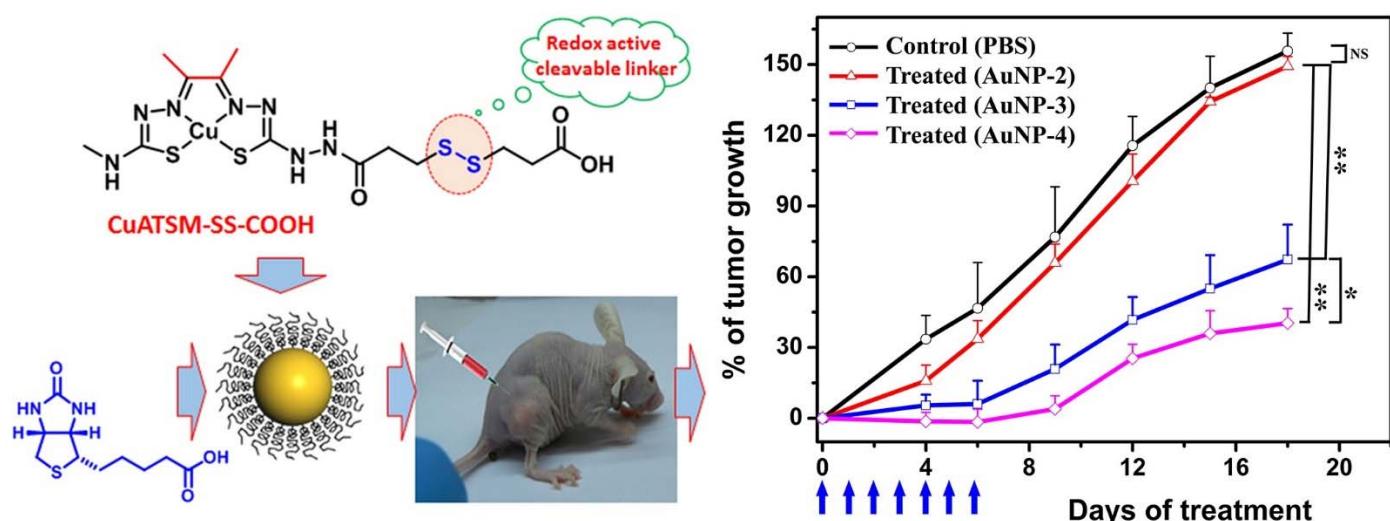
## MEDI 167

### **Smart and targeted delivery of an anticancer active copper complex: *In vitro* and *in vivo* studies**

**Anup Kumar Pramanik<sup>1</sup>, Kumaravel Somasundaram<sup>2</sup>, Ashoka G. Samuelson<sup>1</sup>, ashoka@ipc.iisc.ernet.in.** (1) *Inorganic and Physical Chemistry, Indian Institute of Science, Bengaluru, Karnataka, India* (2) *Molecular and Cell Biology, Indian Institute of Science, Bangalore, Karnataka, India*

Targeted anticancer activity is an eagerly sought after goal in the global fight against cancer. While cytotoxic agents of copper(II) diacetyl-bis(N4-methylthiosemicarbazone) complexes are known, targeted delivery of copper(II) thiosemicarbazone complexes is not known. We present the design and synthesis of a copper(II) complex connected to a carboxylic acid group through a cleavable disulfide link to enable the smart delivery of a cytotoxic agent. The carboxylic acid group attached to the complex is tethered to highly water soluble 20 nm gold nanoparticles (AuNPs). The gold nanoparticles are stabilized by amine terminated lipoic acid-polyethylene glycol (PEG) and some of them further decorated with biotin to achieve targeted action. Results of testing the copper complex and the gold nano conjugates with and without biotin, against HeLa and HaCaT cells are presented. They show very good anticancer activity against HeLa cells, a cell line derived from cervical cancer and are less active against HaCaT cells. *In vitro* modeling of the slow and sustained release of the complex from conjugates is demonstrated through cleavage of disulphide linker in the presence of glutathione (GSH). As GSH is a reducing agent intrinsically present in higher concentrations within cancer cells compared to normal cells, a second level of targeting is achieved. Surprisingly, *in vitro* MTT assays suggest that biotin appended conjugates do not show greater activity than conjugates without biotin against HeLa cells.

This is consistent with drug uptake studies which suggests similar uptake profiles for both conjugates *in vitro*. However, *in vivo* studies using a HeLa cell xenograft tumor model shows 3.8 fold reduction in tumor volume for the biotin conjugated nanoparticle compared to the control whereas the conjugate without biotin shows only 2.3 fold reduction in the tumor volume suggesting significant targeting.



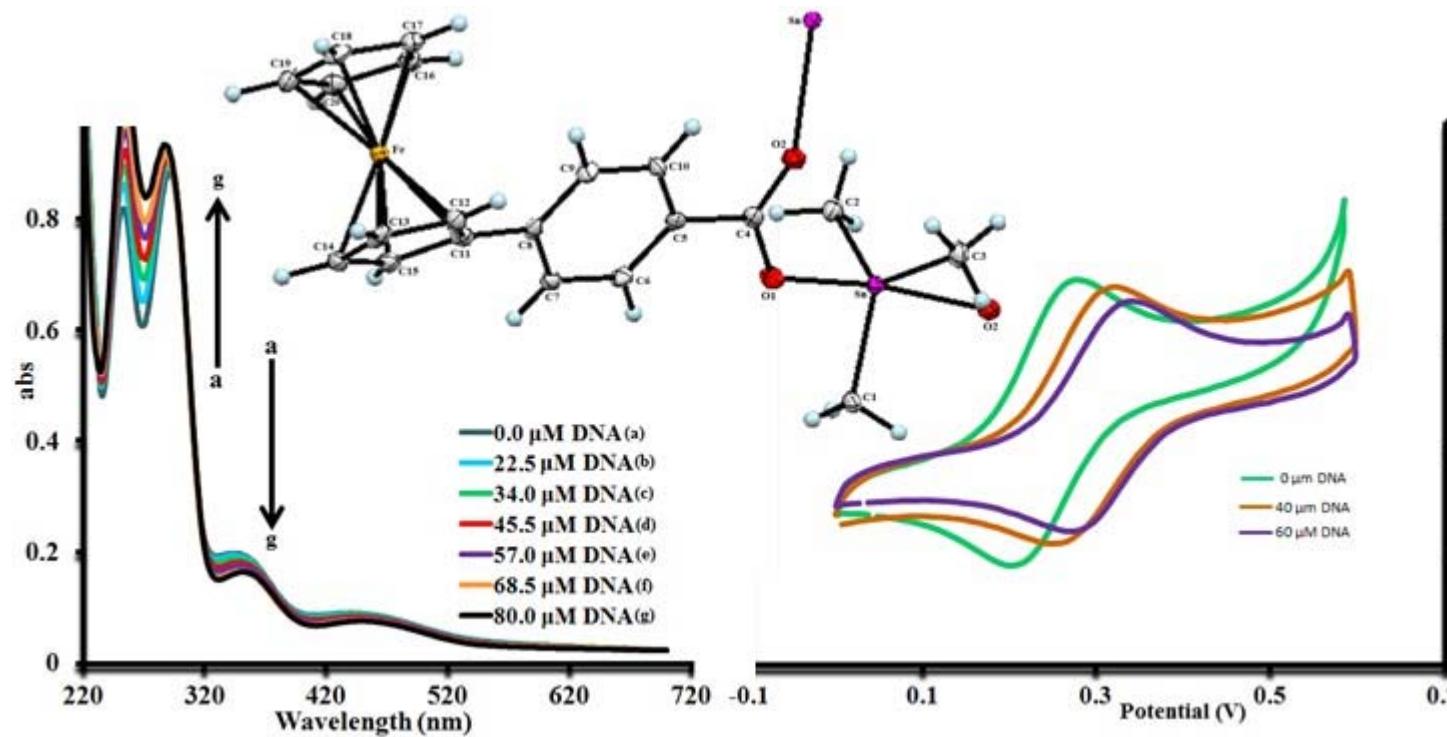
## MEDI 168

### Ferrocene based Fe-Sn heterobimetallics: Synthesis and DNA binding potentials

**Ataf Ali Altaf**<sup>2</sup>, [atafali\\_altaf@yahoo.com](mailto:atafali_altaf@yahoo.com), Nasir Khan<sup>1</sup>, **Amin Badshah**<sup>1</sup>, [aminbadshah@qau.edu.pk](mailto:aminbadshah@qau.edu.pk), Bhajan Lal<sup>3</sup>. (1) Quaid-i-Azam University, Islamabad, Pakistan (2) Department of Chemistry, University of Gujrat, Gujrat, Punjab, Pakistan (3) IBA Sukkur, Sukkur, Pakistan

Reports on the medicinal utility of heterobimetallics are too limited in literature. We have designed the ferrocene based Fe-Sn bimetallics. Multistep synthesis was utilized to make bimetallic organometals. The synthetic compounds were characterized by multinuclear NMR and single crystal X-rays difraction analysis. Reports on the medicinal utility of heterobimetallic are too limited in literature. We have designed the ferrocene based Fe-Sn bimetallic. Multistep synthesis was utilized to make bimetallic organometals. The synthetic compounds were characterized by multinuclear NMR and single crystal X-rays diffracton analysis. The X-ray structure of one example compound is given in graphical abstract. The synthesized compounds were evaluated for the interaction with DNA using physicochemical techniques like UV-visible

spectroscopy, cyclic voltammetry, dynamic light scattering and viscometry. The interaction studies indicate that some of these the synthesized molecules intercalate between the DNA bases, while the other compounds interact in the minor grooves of the DNA helical structure. Further investigations are on the way and will be shared in the meeting.



Graphical Abstract: Crystal Structure selected compound and its DNA interaction probing with UV-visible spectroscopy and Cyclic voltammetry.

## MEDI 169

### Design and synthesis of novel pH-responsive multifunctional lipid-like carriers for siRNA delivery

**Zhanhu Sun**, sunzhanhu@hotmail.com, **Hongfa Jiang**, **Jingcan Qin**, **Da Sun**, **Zheng-Rong Lu**. Department of Biomedical Engineering, Case Western Reserve University, Cleveland, Ohio, United States

Gene therapy has returned to center stage in precision medicine. It has attracted substantial interests from academia and industry. Since the 2006 Nobel Prize in Physiology and Medicine was awarded to Andrew Fire and Craig C. Mello, RNA interference (RNAi) has been found broad therapeutic applications in treatment of various human diseases. Recent clinical trials

have demonstrated the promise as novel therapeutics in precise treatment. However, broad clinical application of RNAi is still limited by the lack of safe and efficient delivery systems for siRNA to different organs and tissues. Our group has been working the design and development of pH-responsive multifunctional lipids to efficiently delivery siRNA for RNAi. Herein, we reported the design, synthesis, and characterization of new optimized lipid-like carriers and their application in RNAi. The carriers encompass three major moieties, ethylenediamine moiety for protonation and interacting with negative-charge siRNA via electrostatic interactions, cysteine or cysteinyl-containing dipeptide moiety acting a glue for polymerization via disulfide bonding to form nanoparticles and Glutathione-tuned siRNA release, and oleyl moiety for tuning amphiphilicity and facilitating trans-membrane delivery. The synthetic procedure of the new lipids was significantly simplified. The effect of amino acid sequence and number of protonatable amino moieties were explored in the design of the new lipid carriers. Gene silencing efficiency of the lipids was assessed in U87-luc cells and MDA-MB-231-Luc cells to explore the effects of N/P ratios and number of amino acids. The new lipids medicated up to 90% silencing of luciferase expression in both cell lines. The lipid/siRNA nanoparticles exhibited minimal cytotoxicity. The results suggest that these new pH-sensitive multifunctional lipids have the potential to act as safe and efficient vehicles for RNAi therapeutics.

## MEDI 170

### **3D imaging detection method of HER2: Application of conjugated affibody-quantum dots probes and ratiometric analysis**

**Perla I. Pérez Treviño<sup>1</sup>, a006173282@itesm.mx, Hector Hernández de la Cerdá<sup>1</sup>, Noemí García<sup>1,2</sup>, Julio Altamirano<sup>1,2</sup>. (1) Escuela de Medicina, Tecnológico de Monterrey, Monterrey, Nuevo León, Mexico (2) Basic and Translational Research Center, Hospital Zambrano-Hellion, San Pedro, Garza García, Nuevo León, Mexico**

HER2 overexpression is associated with Breast Cancer (BC) poor prognosis, due to increased metastasis and angiogenesis, and decreased apoptosis. HER2 expression status is commonly assessed by immunohistochemistry, however, this technique requires tedious sample manipulation, since antibodies have poor penetration, therefore thin fixed samples (3-5 mm) are used, and labelling interpretation is based on subjective algorithms. Consequently, lacks accuracy and reproducibility, and could lead to misdiagnosis. Therefore, we aimed to develop a 3D confocal imaging detection method of HER2 using the small recognition Affibody (Aff)

molecules conjugated with Quantum Dots (QD), and ratiometric analysis (RMA). Aff anti-HER2 and Aff negative control were conjugated by the maleimide reaction with QD605 and QD545, respectively (where numbers refer to emission wavelength, and both were exited at 488 nm). Fixed BC spheroids of HCC1954 (HER2+) and MCF-7 (HER2-) cell lines were incubated with a mixture (1:1) of both Aff-QD probes, and confocal image stacks were recorded in the z-axis. Images were processed by RMA (AffantiHER2-QD605/Affneg-QD545 fluorescence), and (%) of HER2+ cells, as well as HER2 density were assessed in the stack. We found in HCC1954 spheroids, that Aff-QDs could be optimally resolved up to 50 mm depth, and the AffantiHER2-QD605 signal was significantly higher than that of Affneg-QD545 (due to unspecific accumulation). After unspecific signal removal by RMA, the HER2+ expression status was objectively quantified. In MCF-7 spheroids, Aff-QDs could be resolved up 80 mm, however, no significant differences between AffantiHER2-QD605 and Affneg-QD545 signals were found. Therefore, after RMA almost all unspecific AffantiHER2-QD605 fluorescence was removed, and no false HER2+ cells (%) or cellular HER2 labeling were found. The results demonstrate that conjugated Aff-QDs could efficiently penetrate in spheroids, as 3D BC models, with minimal sample manipulation. Importantly, RMA, efficiently removed unspecific fluorescence, allowing objective HER2 quantification in relatively thick samples.

## MEDI 171

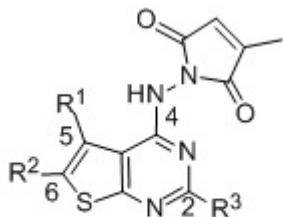
### **Improving solubility of thieno[2,3-d]pyrimidine based FLT3 inhibitor via structural modifications at the C<sub>2</sub> and C<sub>6</sub> position**

***Changmok Oh, hiuliul123@naver.com, Hyuntae Kim, Gyoonehee Han. #605, 2nd Engeering Hall II, Yonsei University, Seoul, Korea (the Republic of***

Acute myeloid leukemia (AML) is highly aggressive cancer that affects the blood and bone marrow. Many studies had revealed that FLT3 plays a key role in the development of AML and poor prognosis. FLT3 is aberrantly expressed in most patients with AML, and about one third of patients have a mutation in FLT3. The two major types of FLT3 mutations are internal tandem duplications (FLT3/ITDs) and tyrosine kinase domain point mutations (FLT3/TKDs). These mutations give a constitutive activation in FLT3 signaling, which is associated with a poor prognosis in AML.

We had previously reported that thieno[2,3-d]pyrimidine derivatives showed inhibitory activity against FLT3 and mutant FLT3. Among them, compound **1** exhibited best antiproliferative activity against human leukemia cell lines which harbor FLT3 or mutant FLT3 than **AC220**, which is a well-

known FLT3 inhibitor, and has good microsomal stability. However, compound **1** had poor solubility (2.96 uM in 100 mM pH 7.4 phosphate buffered saline; PBS) due to a bulky moiety at the C<sub>6</sub> position. Therefore we performed structural modification at the C<sub>2</sub> and C<sub>6</sub> position utilizing compound **1** as a starting point. As a result of optimization, we developed compound **13b** with a thiazole moiety at the C<sub>2</sub> position. It exhibited much better growth inhibition activity against human leukemia cell lines than compound **1** and showed desirable drug-like properties including moderate microsomal stability and increased solubility. Therefore, we concluded that compound **13b** is suitable for being a structural basis for further modification and as well as a promising potential FLT3 inhibitor for AML treatment.



Thieno[2,3-d]pyrimidine derivatives

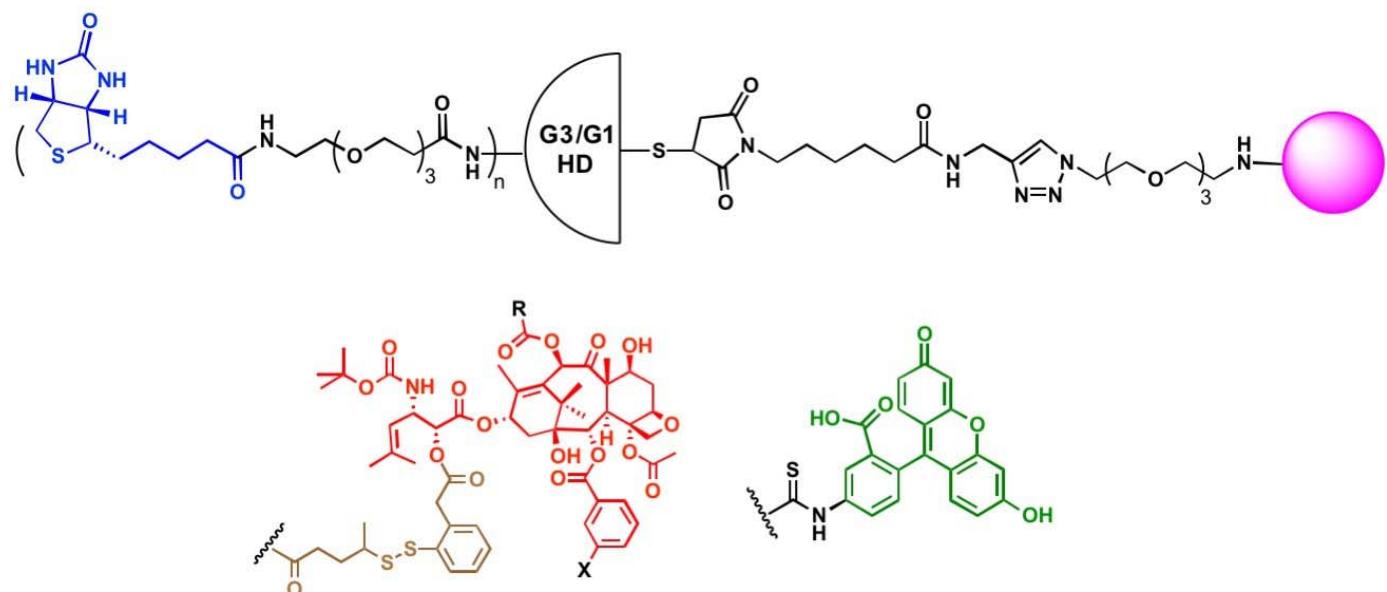
## MEDI 172

### Dendrimer-based multifunctional conjugates of new-generation taxoids for tumor-targeted drug delivery

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Cancer remains the second leading cause of death in the United States. In spite of tremendous efforts, there is still no common cure for cancer. Over the past decades, significant achievements have been made in the development of tumor-targeted drug delivery systems (TTDDS) to distinguish cancer cells from normal cells. Vitamin receptors are overexpressed on the surface of some cancer cell lines to maintain rapid cancer cell growth. Dendrimers are well-defined three-dimensional macromolecules, which can be used to increase the payload of the drugs, targeting efficacy of the conjugate, and other biological and physiological properties. Based on these advantageous features, PAMAM dendrimer-based multifunctional conjugates were designed and synthesized by connecting a biotin-PEGlated G3/G1 PAMAM half-dendron with a new-generation taxoid or a fluorescent probe.

Biological evaluations (MTT, CFM and flow-cytometry analyses) of these conjugates against various cancer cell lines, overexpressing biotin receptors (BRs), indicate that these dendrimer-based tumor-targeting drug conjugates exhibited excellent BR-specific cytotoxicity, and substantially enhanced receptor-mediated endocytosis (RME).



G3/G1PAMAM Half-Dendron-Based Tumor-Targeted Delivery System

## MEDI 173

### Synthesis of flexible, purine analogue inhibitors of NCp7

**Therese Ku**, chaku1@umbc.edu, Katherine L. Seley-Radtke, Yafet Arefeayne. Univ of Maryland Baltimore Co, Baltimore, Maryland, United States

Anti-HIV-1 drug design has been notably challenging due to the virus' ability to mutate and develop immunity against commercially available drugs. This project aims to discover a new series of nucleobase analogues that not only possess inherent flexibility that could withstand active site mutations, but also target a non-canonical, more conserved target, NCp7. Interestingly, these compounds are not predicted to work by zinc ejection, which would endow them with significant advantages over currently reported zinc-ejectors, which are toxic. We have synthesized several series of these fleximer base analogues using palladium-catalyzed coupling techniques and tested them against NCp7 specifically, and HIV-1 in general. The results are presented herein.

## MEDI 174

### **Discovery of novel series of LasR quorum sensing inhibitors in *Pseudomonas aeruginosa***

**Pathi Suman, Lark J. Perez, Subash C. Jonnalagadda,**  
*jonnalagadda@rowan.edu. Chemistry and Biochemistry, Rowan University,  
Glassboro, New Jersey, United States*

Bacteria communicate with each other by producing and detecting small molecules in a process known as quorum sensing (QS). QS enables the bacteria to adjust gene expression to coordinate their behavior in a cell-density dependent manner. Biofilms are associated with majority of bacterial infections and the inhibition of QS can lead to the inhibition of biofilm formation thereby reducing infection or enhancing the effect of traditional antibiotics. We envisioned the development of functionalized cinnamates as QS inhibiting antimicrobial agents. Application of the Baylis-Hillman reaction provides allylic alcohols and amines in a one-step transformation and facilitates the generation of a large library of structurally diverse scaffolds. Employing this strategy, we prepared a series of α-piperazinylmethylcinnamates. Biological evaluation of these compounds enabled the identification of a new structure class of drug-like reversible inhibitors of LasR quorum sensing in *Pseudomonas aeruginosa*. This presentation will provide insights into the synthesis and biological evaluation of these small molecule inhibitors.

## MEDI 175

### **Cefiderocol (S-649266): A new siderophore cephalosporin exhibiting potent activities against *Pseudomonas aeruginosa* and other gram negative-pathogens including multi-drug resistant bacteria: Structure activity relationship**

**Toshiaki Aoki, toshiaki.aoki@shionogi.co.jp, Hidenori Yoshizawa, Kenji Yamawaki, Katsuki Yokoo, Jun Sato, Shinya Hisakawa, Yasushi Hasegawa, Hiroki Kusano, Masayuki Sano, Hideki Sugimoto, Yasuhiro Nishitani, Yoshinori Yamano, Takafumi Sato, Masakatsu Tsuji, Rio Nakamura, Toru Nishikawa. Shionogi & Co., LTD., Osaka, Japan**

#### **Background**

In recent years, the threat of multi drug resistant Gram negative pathogens e.g. *P.aeruginosa* (MDRP) and *A.baumanii* is expanding all over the world.

One strategy to discover a new cephalosporin capable of eradicating multi drug resistant Gram negative pathogens is to utilize a bacterial iron acquisition system by employing siderophore. Here we report the structure activity relationship of the new siderophore conjugated cephalospolins including Cefiderocol, which has a potent *in vitro* and *in vivo* correlated *in vivo* microbial activity against several Gram negative pathogens including MDRP and *A.baumanii*.

### **Method**

Cefiderocol and the analogues modified the side chains at the C-3 and the C-7 were prepared in Shionogi & Co., Ltd.. MICs of those compounds against drug resistance Gram negative pathogens including *P.aeruginosa*, *A.baumanii* and *K.pneumoniae*, were determined by cation adjusted mueller hinton broth (CAMHB) supplemented with 20 $\mu$ M human apo-transferriin (apo-T) to mimic the iron deficiency condition on the basis of broth microdilution method. The *in vivo* potency was determined using systemic mouse infection model. The 50% effective dose (ED<sub>50</sub>) was calculated from survival rate on the 7<sup>th</sup> day after infection.

### **Result**

Cefiderocol showed excellent *in vitro* activities against various Gram negative pathogens including IMP-1 producing multi drug resistant *P. aeruginosa* (MIC against SR27001; 1 $\mu$ g/mL), *A. baumanii* (MIC against SR27323; 1 $\mu$ g/mL), KPC producing *K. pmeumoniae* (MIC against ATCC BAA-1705; 0.063 $\mu$ g/mL) and *S.maltophilia* (MIC against SR21970; 0.5 $\mu$ g/mL). Depending on the structures of the side chains at the C-3 and the C-7 or a combination in them, the activity against the target pathogens changed and we identified that cefiderocol was one of the best molecules among the prepared compounds at the point of antibacterial activity and efficacy. Some analogues didn't show *in vivo* potency in spite of having *in vitro* activity against *P.aeruginosa* SR27001. However, Cefiderocol showed a potent *in vivo* efficacy against *P.aeruginosa* SR27001 (ED<sub>50</sub> = 5.48 mg/kg) compared to the known antimicrobial agents such as CFPM (ED<sub>50</sub> > 100 mg/kg).

### **Conclusion**

We have demonstrated the structure activity relationship of the new catechol conjugated analogues with excellent *in vitro* and *in vivo* efficacy. These results show that Cefiderocol is a highly promising parental cephalosporin targeted for multidrug-resistant Gram-negative infection.

## **MEDI 176**

### **Inhibiting effect of essential oils and methylglyoxal with carrier oils on the growth of *Pseudomonas aeruginosa***

**Aashna Patel<sup>1</sup>, s0980228@monmouth.edu, James P. Mack<sup>1</sup>, Albert Rojtman<sup>2</sup>. (1) Department of Biology, Monmouth University, West Long Branch, New Jersey, United States (2) Department of Pathology, Jersey Shore University Medical Center, Neptune, New Jersey, United States**

Due to global overuse of antibiotics, some bacteria have evolved to become resistant to drugs normally used to treat their respective bacterial infections. Since traditional antibiotics are ineffective to treat infections caused by antibiotic-resistant bacteria, alternative methods are sought to combat the emergence of these bacteria. One such method is the use of natural products derived from plants to effectively inhibit the growth of multidrug-resistant bacteria. Essential oils from plants are known to be highly potent and have natural antibacterial properties that may be useful to treat infections due to drug resistant bacteria. In this study, two highly potent essential oils, cassia and cinnamon bark, and the aldehyde methylglyoxal (the main active antibacterial ingredient in Manuka Honey) were used in conjunction with three carrier oils (olive oil, jojoba oil, and lanolin) to determine their efficacy in inhibiting the growth of *Pseudomonas aeruginosa*, a multidrug-resistant bacterium. *Pseudomonas aeruginosa* is a gram-negative, aerobic, and coccobacillus bacterium that infects open airways and wounds. *Pseudomonas aeruginosa* infections have become a serious problem for patients who have weakened immune systems. The Kirby-Bauer disk diffusion method was used to test the efficacy of the essential oil and carrier oil mixtures. The essential oils used were diluted to lower concentrations with carrier oils to determine their minimal inhibitory concentration (MIC) as essential oils can be irritating if used independently. The results were compared to colistin, which is an antibiotic normally used to treat *Pseudomonas aeruginosa* infections. The essential oils and methylglyoxal were diluted and tested at 100%, 75%, 50%, 25%, and 12.5% concentrations in carrier oils. The results were compared to the colistin for relative effectiveness. It was determined that at a 50% concentration, the essential oils and methylglyoxal were more effective than colistin in inhibiting the growth of *Pseudomonas aeruginosa* in the Petri dish experiments. The results show a potential topical treatment that can be used in health care facilities to effectively treat infections caused by this bacterium.

## **MEDI 177**

### **Inhibition of the *Pseudomonas aeruginosa* heme oxygenase**

**Elizabeth Robinson<sup>2</sup>, erobinson@umaryland.edu, Dongdong Liang<sup>3</sup>, Kellie Hom<sup>4</sup>, Angela Wilks<sup>1</sup>, Fengtian Xue<sup>2</sup>. (1) Pharmaceutical Sciences, University**

*of Maryland, Baltimore, Maryland, United States (2) School of Pharmacy, University of Maryland, Baltimore, Baltimore, Maryland, United States*

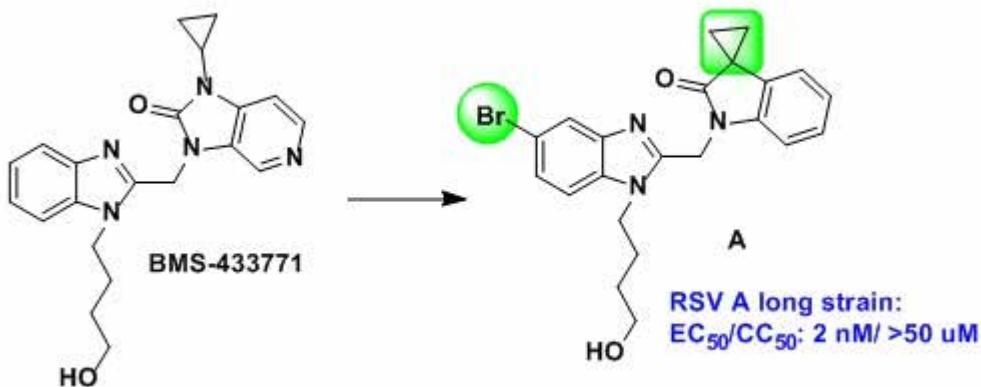
Iron is an essential nutrient for growth and virulence for *Pseudomonas aeruginosa*, a multidrug resistant and nosocomial infection causing bacteria. Therefore, targeting the iron-regulated heme oxygenase (HemO) of *Pseudomonas aeruginosa* serves as a novel idea to overcome multidrug resistant infections. Here we present the synthesis and binding activity of a series of rhodanine-based inhibitors of HemO. This class of inhibitors acts by binding in the heme binding pocket site which is further supported by saturation transfer difference (STD) NMR as well as by heteronuclear single quantum coherence (HSQC) spectroscopy. An in vitro binding assay using fluorescence quenching was optimized to be automated for high throughput screening where one inhibitor, FX3028, showed a binding affinity of 3.3  $\mu\text{M}$ . Further assays to test in cell activity as well as toxicity on this novel rhodanine family are currently being developed and optimized to be automated for high throughput screening to gather more supporting data.

## MEDI 178

### **Discovery of 1H-benzo[d]imidazol-2-yl-methyl-spiro [cyclopropane-1,3'-indolin]-2'-one derivatives as fusion inhibitors for treatment of respiratory syncytial virus infection**

**Haiying He, he\_haiying@wuxiapptec.com. DDSU, WuXi AppTec, Shanghai, China**

RSV is the most common viral cause of severe acute lower respiratory infections (ALRI, mainly bronchiolitis and pneumonia) in infants, young children, immunocompromised adults and the elderly. So far, no specific anti-RSV therapeutics or effective anti-RSV vaccines have been reported. RSV envelope glycoprotein F plays an important role in RSV fusion with, and entry into, the host cell and, consequently, serves as an attractive target for developing RSV entry inhibitors. A new series of spiro[cyclopropane-1,3'-indolin]-2'-one derivatives were synthesized and evaluated against RSV. Their extensive SAR study led to compound **A**, with excellent in vitro potency ( $\text{EC}_{50} = 2 \text{ nM}$ ), and preferable drug exposure in lung. RSV infection mouse model demonstrated it possessed superior efficacy against RSV with 96% reduction of viral load in lung at 50 mg/kg via oral gavage, which proves it as a promising lead for RSV treatment.



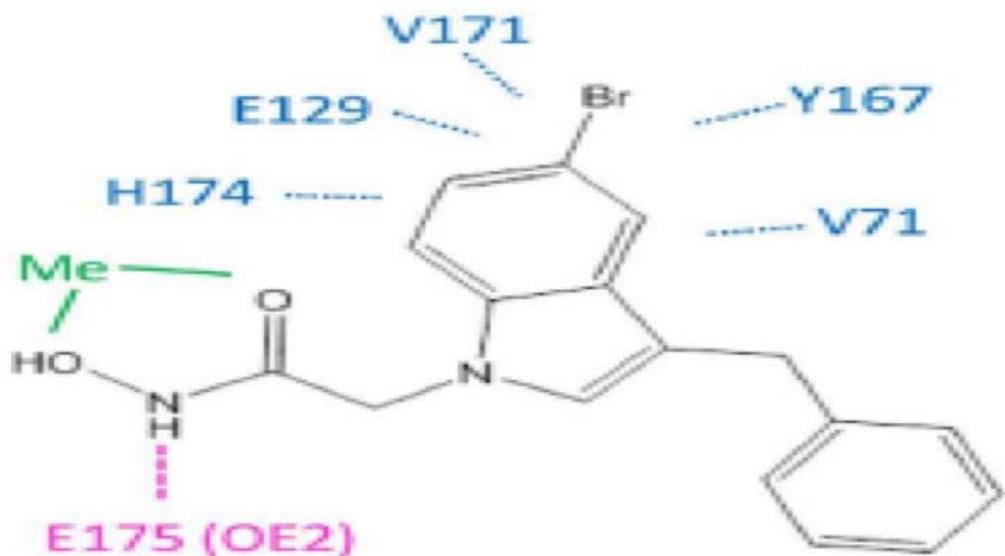
## MEDI 179

### Molecular-based design, synthesis and docking studies of new benzimidazole derivatives as potential bacterial peptide deformylase inhibitors

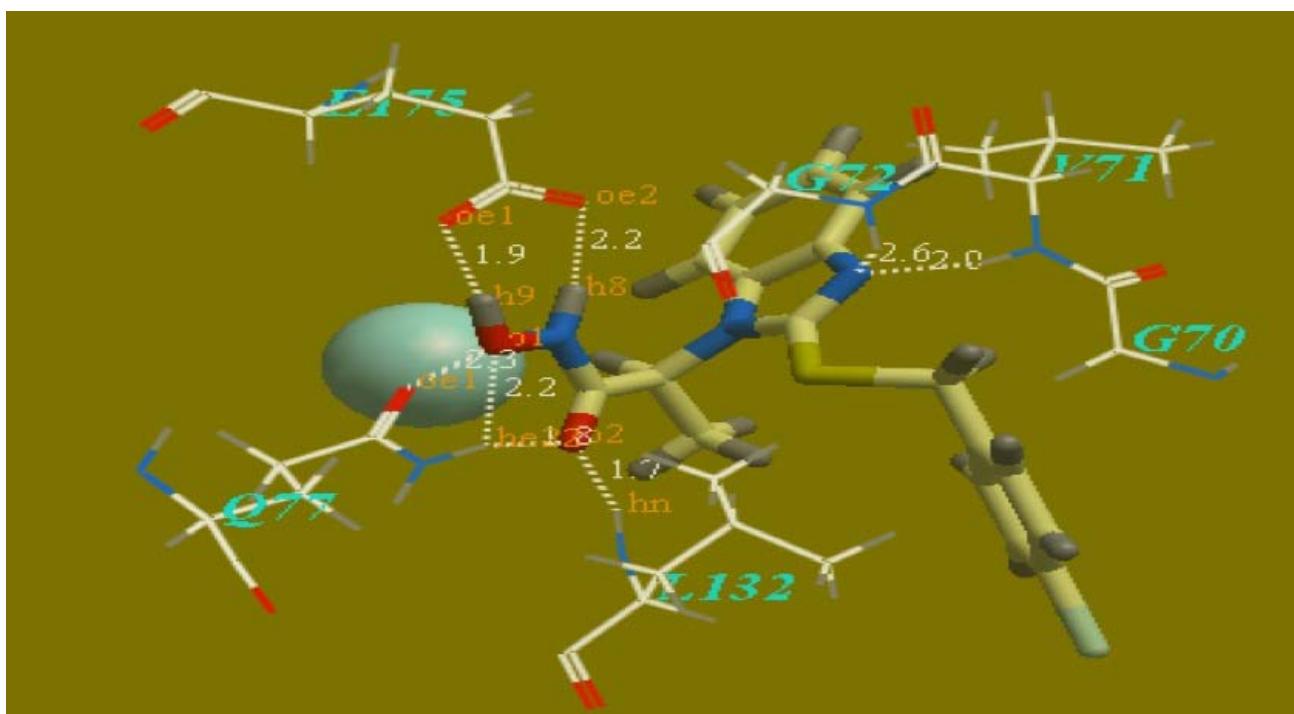
**Shaymaa E. Kassab**, shaymaa.kassab@yahoo.com. Pharmacy, Damanhour University, Cairo, Egypt

New N-hydroxyprpanamide and butanamide derivatives of benzimidazole have been designed on molecular basis to perform potential inhibitory activity against bacterial peptide deformylase (PDF) selectively. The design of the new derivatives is based on benzimidazole as large aromatic core to occupy the large S1 pocket of bacterial PDF active binding site that is essential for selectively against PDF since S1 pocket of human PDF (HsPDF) couldn't accommodate large volume of aromaticity. A hydrocarbon tail ended by hydroxamic acid substituted the N1-heteroatom of benzimidazole to stabilize the Metal of the metalloenzyme via formation coordinate bond. Presence of N1 and 2-thiol in the benzimidazole offered the opportunity to explore diverse derivatives in which we can shift the substitution of hydroxamic tail of hydrocarbon from N1 to SH and vice versa. Simple synthetic routes and lab friendly reaction conditions were adopted to generate the new candidates in quantitative yields. The new candidates were docked into the active site of PDF taking the potent inhibitor 5-bromo-3-benzyl-1-N-hydroxyacetamide as a ligand inhibitor. 2-(2-((4-bromobenzyl)thio)-1H-benzo[d]imidazol-1-yl)-N-hydroxypropanamide was one of the most derivative that showed high binding affinity, exhibited proper interaction pattern, and high possibility of the hydroxamic acid end to stabilize the metal. The benzimidazole core occupied the S1 pocket and formed hydrogen bond with amino acids comparable to that formed with the original ligand inhibitor. The new candidates will be

biologically evaluated against PDF and HsPDF to test the antibacterial potential and cytotoxicity as well.



Binding mode of the ligand inhibitor with PDF



Binding mode and orientation of 2-((4-bromobenzyl)thio)-1H-benzo[d]imidazol-1-yl)-N-hydroxypropanamide into the active binding pocket of PDF

## MEDI 180

### **Discovery of small molecules that inhibit the LRS-RagD interaction and their potential use as anti-cancer drugs**

**Kilsoo Jung**, *Kilsoo@umich.edu, Chulho Lee, Gyoонhee Han. Yonsei University, Seoul, Korea (the Republic of)*

The mTORC1 pathway regulates protein synthesis, autophagy and cell growth in mammals. This pathway is activated by leucine and this leucine dependent mTORC1 activation is mediated by leucyl-tRNA synthetase (LRS) which serves as a GTPase-activating protein for RagD3. LRS takes on a unique role in regards to the control of leucine-dependent Rag GTPases via RagD. RagGTPases can be switched “On” by LRS via RagD. Synthetic compounds, specifically BC-LI-YS-0186, that bind to the RagD-interaction region of LRS were identified and found to efficiently inhibit the interaction between LRS and RagD resulting in mTORC1 suppression as well as suppression of cell growth without affecting the catalytic activity of LRS for protein synthesis. BC-LI-YS-0186 was discovered through HTS, synthesis of derivatives using SAR analysis, computer aided pharmacophore search and evaluation of mTORC inhibition through biological assay. BC-LI-YS-0186’s ability to suppress the mTORC1 pathway through inhibiting the interaction between LRS and RagD give it promise as a potential anti-cancer small molecule.

## MEDI 181

### **First insight into structure-activity relationships of selective Meprin $\beta$ inhibitors**

**Daniel Ramsbeck**, *daniel.ramsbeck@izi.fraunhofer.de, Antje Hamann, Dagmar Schlenzig, Stephan Schilling, Mirko Buchholz, Hans U. Demuth. Department of Drug Design and Target Validation, Fraunhofer IZI, Halle/Saale, Germany*

Together with BMP-1/tolloid-like enzymes and ovastacin, Meprins (Meprin  $\alpha$  and  $\beta$ ) are members of the astacin family of metalloproteinases. Meprin  $\alpha$  is secreted, meprin  $\beta$  represents a membrane-bound isoform. Both enzymes exhibit slightly different substrate specificities, e.g. meprin  $\beta$  shows a unique preference for acidic amino acids neighbouring the scissile bond. Meprin  $\alpha$  and  $\beta$  represent procollagenases and are linked to disorders such as fibrosis, pulmonary hypertension or keloids. Due to its ability to release N-truncated A $\beta$  peptides from the amyloid precursor protein, meprin  $\beta$  might be also

associated with Alzheimers disease, thus acting as an alternative beta secretase. Hence, meprins are interesting drug targets for treatment of various diseases. However, only few examples of inhibitors have been presented to date.

On our quest to develop selective inhibitors of meprin  $\beta$ , we utilized a combined structure and ligand-based approach. A short overview of the structure-activity relationship of the inhibitors will be given, including an initial selectivity profile.

## MEDI 182

### Evaluating p97 inhibitor analogues for potency against different p97-p97 cofactor complexes

**Tsui-Fen Chou**, *tsuifenchou@ucla.edu. Department of Pediatrics, Harbor-UCLA and LABioMed, Torrance, California, United States*

We will present studies to evaluate different p97 inhibitors against two ATPase domains of p97 and a few p97-p97 complexes. Our studies identify several potential starting points for future SAR analyses, to improve the potencies of D1-specific inhibitors and selective inhibitors of the p97–p47 complex. It also highlights the potential importance of context-based inhibition of p97 ATPase activity. The important implications of this study are: 1) To improve SAR studies of p97 inhibitors *in vitro*, one needs to use D1 and D2 mutants to determine the extent to which compounds inhibit the D1 and D2 sites. 2) To correlate *in vitro*, cellular, and *in vivo* potencies of p97 inhibitors, one needs to consider the presence of different p97–cofactor complexes. 3) Further work is needed to develop specific cell-based assays for specific p97–cofactor complexes, to facilitate the development of complex-specific inhibitors.

## MEDI 183

### Examining the activity of HIV protease inhibitors against human endogenous retrovirus-K: A potential treatment for amyotrophic lateral sclerosis

**Rachel Abrams<sup>1</sup>**, *rachel.abrams87@gmail.com*, **Richa Tyagi<sup>1</sup>**, **Wenxue Li<sup>1</sup>**, **Mario Blanchet<sup>2</sup>**, **Avindra Nath<sup>1</sup>**. (1) *Section of Infections of the Nervous System, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland, United States* (2) *Department of*

*Neurology, Johns Hopkins School of Medicine, Baltimore, Maryland, United States*

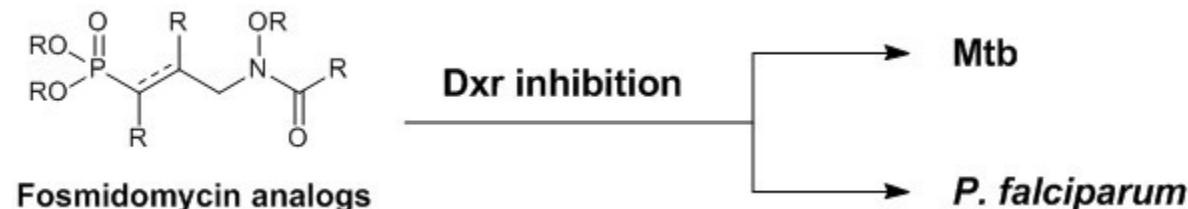
Retroviruses evolved to infect animal hosts at least 450 million years ago. Over the course of evolution, retroviruses have infected cells of the germ line, allowing the genome of the virus to be passed down from parent to offspring. In humans, these endogenous retroviruses (HERVs) make up about 8% of the genome, but in most cases, multiple mutations have made them inactive. One of the most recently incorporated (HERV-K), however, has been implicated in the development of amyotrophic lateral sclerosis (ALS). Like HIV, HERV-K utilizes an aspartic acid protease to process the viral polyprotein into its active components. HIV protease inhibitors were tested in an in vitro HERV-K infection model and demonstrated a moderate protective effect, though they were less effective than against HIV infection. To further investigate HERV-K inhibition, a detailed comparison of the HIV and HERV-K proteases was conducted. Since there is no crystal structure of the HERV-K protease available, comparative modeling was performed using the sequence alignment with HIV protease. A homology model was generated and refined using the Prime program in the Schrodinger Suites Software package. Overall, the model of the HERV-K protease produced a similar structure to that of HIV protease; however, the differences caused by a few amino acids in the active site could explain the reduction in antiviral activity. To experimentally correlate the level of antiviral activity to protease inhibition, a functional assay measuring the activity of HERV-K protease is currently being established. The first stage of assay development involved the optimization of HERV-K protease expression in Escherichia coli. Progress towards the identification of appropriate assay conditions employing a cleavable fluorescent peptide substrate will be discussed.

## MEDI 184

### **Evaluating fosmidomycin analogs as antimicrobial agents through 1-Deoxy-D-xylulose-5-phosphate reductoisomerase (Dxr) inhibition**

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Tuberculosis (TB) and malaria are severe, life-threatening infectious diseases that torture millions of people every year. Both diseases are caused by microorganisms: *Mycobacterium tuberculosis* (Mtb) causes TB and *Plasmodium falciparum* causes malaria. Due to the unavailability of new antibiotics and the increasing emergence of drug-resistant strains of these organisms, there is an urgent demand for novel drug therapies. We try to find new drug candidates that would effectively and efficiently kill Mtb and *Plasmodium falciparum*. The isoprene unit, made of 5-carbons, is used and made by all living cells. Halting isoprene production leads to cell death in Mtb and *P. falciparum*. 1-Deoxy-D-xylulose-5-phosphate reductoisomerase (Dxr) is a crucial enzyme in the nonmevalonate pathway to make isoprenes. This pathway is found in many pathogenic organisms including Mtb and *P. falciparum*, but not humans. Thus, Dxr inhibitors may be promising therapeutic candidates with low human toxicity. It has long been known that fosmidomycin is a potent inhibitor of Dxr. Unfortunately, fosmidomycin is not effective against Mtb and has failed in clinical trials against malaria. We synthesized and evaluated fosmidomycin analogs as improved Dxr inhibitors that act as potent antimicrobial agents, which shed light on its SAR and the potential of Dxr inhibitors becoming antimicrobial drug candidates.



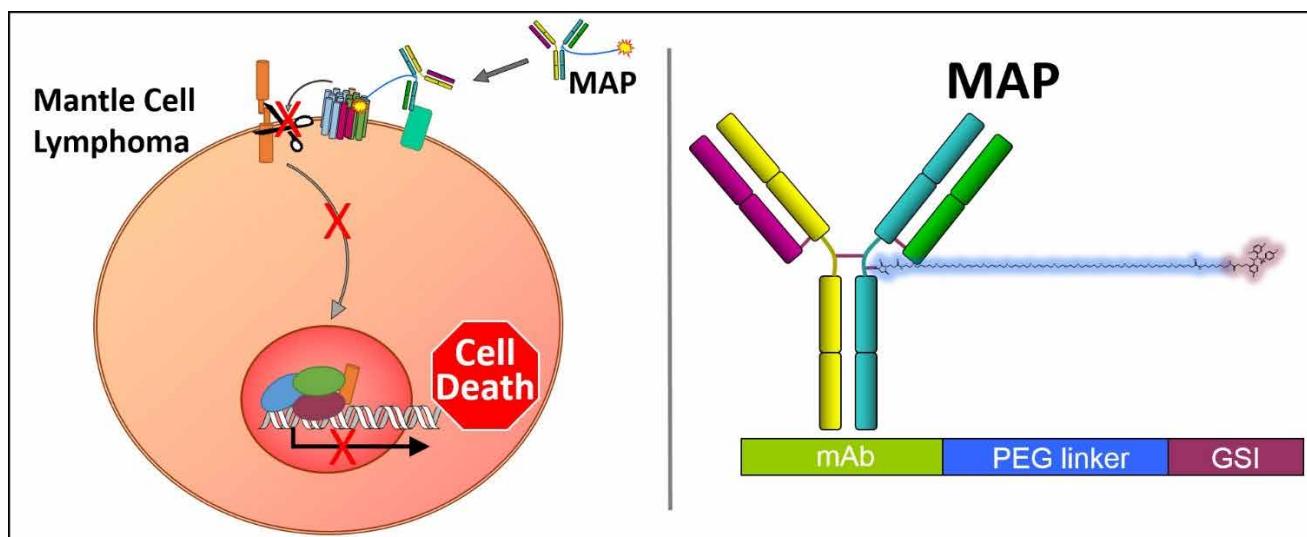
## MEDI 185

### Targeting the notch pathway in mantle cell lymphoma using $\gamma$ -secretase inhibitor-antibody conjugates

**Benjamin Buer**, [ben.buer@wolfelabs.com](mailto:ben.buer@wolfelabs.com), Michael S. Wolfe, Janet L. Wolfe. Wolfe Laboratories Inc, Woburn, Massachusetts, United States

Dysregulation of the Notch signaling pathway has been implicated in numerous malignancies, including mantle cell lymphoma (MCL), making this pathway a particularly attractive therapeutic target. Small molecule therapeutics targeting the Notch pathway, including  $\gamma$ -secretase inhibitors (GSIs), have seen limited clinical success because of numerous unwanted side effects that stem from the ubiquitous nature of Notch in human cells and the numerous cellular context-dependent roles Notch plays. To limit the

amount of off-target effects in blocking the Notch pathway, we have developed a series of antibody-based conjugates we refer to as Matched Antibody-Payloads (MAPs). These MAPs are composed of a targeting antibody, a long, flexible linker and a GSI. In this study, we demonstrate the utility of MAPs by specifically blocking the Notch pathway through  $\gamma$ -secretase inhibition in MCL, resulting in reduced cell viability.



## MEDI 186

### N6-benzyladenosine derivatives inhibit replication of RNA viruses from flavivirus and enterovirus geni

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Nucleoside analogs play an important role in antiviral drug design, comprising the majority of approved therapeutics. Numerous nucleoside analogs were tested against well-studied viruses, such as HIV-1, Hepatitis C virus, Herpes simplex virus 1, allowing to establish structure-activity relationships. However, approximately 200 pathogenic viruses, including Ebola, dengue, and Zika virus, are studied less thoroughly, and data on antiviral activity of nucleosides against them are scattered. Systematic studies of antiviral chemical space are thus required for successful drug discovery targeting emerging pathogens.

In this study antiviral activity of 57 nucleoside analogs, mostly derivatives of N<sup>6</sup>-benzyladenosine, was assessed against tick-borne encephalitis virus (TBEV), belonging to genus *Flavivirus*, and against a panel of enteroviruses (enterovirus A71, poliovirus, coxsackieviruses A16 and B1). TBEV is an enveloped virus transmitted by ticks, commonly causing deadly encephalitis in northern Eurasia. Enteroviruses are small widespread non-enveloped picornaviruses, causing poliomyelitis and hand, foot and mouth disease. Anti-TBEV activity was observed for molecules bearing large aromatic moieties in the N<sup>6</sup> position of adenosine or bulky hydrophobic moieties in 5'-O position. One of the most important targets of anti-flaviviral nucleosides is non-structural protein 5, the enzyme mediating virus replication, composed of two domains: methyltransferase (MTase) and RNA-dependent RNA polymerase (RdRp). 5'-O-Tritylated uridine analogs were earlier shown to inhibit dengue virus replication through RdRp. On the other hand, 5'-O-silylated compounds inhibited dengue MTase activity. To reveal TBEV life cycle stage affected by nucleosides, we conducted a series of time-of-addition experiments. Possible binding modes of the active compounds we predicted by docking studies to the sites in MTase and RdRp. These findings lay the foundation for further search of new nucleoside inhibitors of TBEV reproduction.

Replication of enteroviruses was inhibited most efficiently by N<sup>6</sup>-halobenzyladenosines. Viruses belonging to Enterovirus A species were consistently susceptible to these compounds, whereas for Enterovirus B and C only fluorination of the benzyl led to acceptable activity. Time-of-addition studies revealed inhibition of the viral replication stage, and possible modes interaction with viral proteins were analyzed by docking of the most potent compounds.

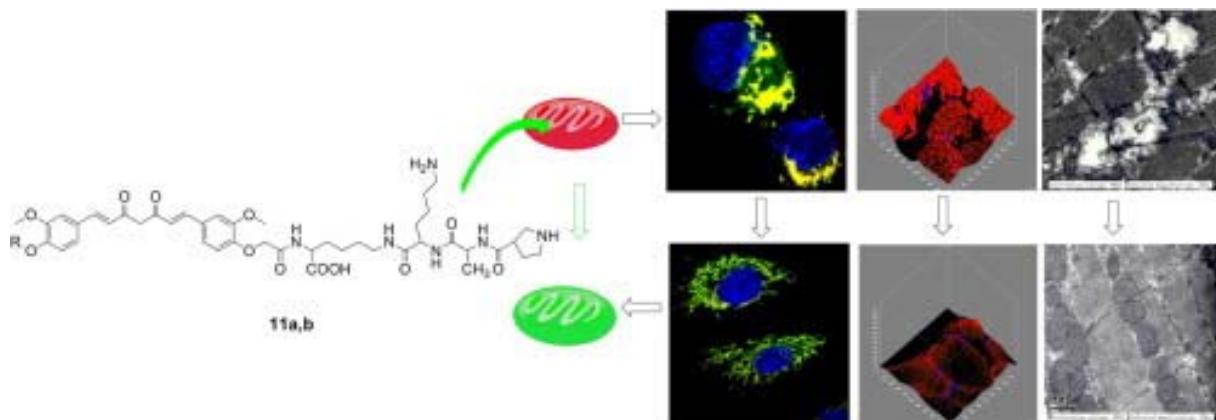
## MEDI 187

### **Pharmacological protection of mitochondrial function mitigates acute limb ischemia/reperfusion injury**

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We describe several novel curcumin analogues that possess both anti-inflammatory antioxidant properties and thrombolytic activities. The therapeutic efficacy of these curcumin analogues was verified in a mouse ear edema model, a rat arterial thrombosis assay, a free radical scavenging assay performed in PC12 cells, and in both in vitro and in vivo ischemia/reperfusion

models. Our findings suggest that their protective effects partially reside in maintenance of optimal mitochondrial function.



## MEDI 188

### Catch and release strategy to treat bacterial infections

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A new bio-orthogonal strategy, termed ‘catch and release’, to battle bacterial infections will be presented. The approach is based on recent developments of the inverse electron demand Diels-Alder (IEDDA) reaction that allows release of therapeutic payloads attached to the *trans*-cyclooctene group after the initial cycloaddition step. The strategy starts with the injection of the biocompatible hydrogel modified with tetrazine near the site of the infection. An antibiotic with attenuated activity containing a releasable *trans*-cyclooctene moiety (pro-drug) is injected intravenously and travels through the circulatory system. When the pro-drug and the hydrogel come near, the bio-orthogonal agents react with each other through IEDDA reaction localizing the therapeutic payload. The multivalency of the hydrogel’s surface provides a large number of tetrazine groups capable of ‘catching’ the systemically administered antibiotic pro-drug. Finally, the resulting intermediate isomerizes spontaneously releasing the active antibiotic from the hydrogel to perform its therapeutic function locally. Evaluation of the ‘catch and release’ strategy in standard laboratory strains, *Staphylococcus aureus* (*Staph.aureus*) *methicillin resistant* (MRSA) and *Staphylococcus aureus methicillin sensitive* (MSSA) will be described.

A. Local Hydrogel Injection	B. Pro-Drug Dose	C. Concentration	D. Activation
<p>Hydrogel Modified with Tetrazines (HMT)</p>	<p>TCO Modified Antibiotic (Pro-Drug)</p>	<p>Hydrogel + N<sub>2</sub> → NH<sub>2</sub> + CO<sub>2</sub></p>	<p>Antibiotic</p>

## MEDI 189

### Multi-target molecular profiling using MOE: A CYP450 isoform selectivity case study

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Abductive reasoning applied to modeling chemical biology can only be made possible if both quality datasets and versatile cheminformatics and molecular modeling methods are available. Identifying compounds that are susceptible to metabolic degradation via CYP450 pathways, and identifying which major isoform is responsible is an ongoing problem. Here we demonstrate using a combination of 2D and 3D cheminformatics and modeling tools built into MOE (Molecular Operating Environment) how a rational workflow can be developed to predict CYP450 isoform specificity and even reactivity.

Using a combination of carefully curated *in vitro* Cytochrome P450 data (from the NCBI, the FDA, Flockhart's tables and the SuperCyp database) and a variety of modeling techniques implemented in MOE (binary classification

trees, 2D-molecular fingerprints, binary QSAR, pharmacophores and molecular docking models) we developed efficient computational approaches to identify putative CYP450 interacting ligands using very simple models that required no more than 1-3 descriptors with accuracies > 75%. In addition, reactivity models that use fingerprints derived from reactive functional groups (both MACCS-key and SmartCYP) were used to differentiate substrates from inhibitors, or more generally the propensity for CYP450 ligand reactivity.

The simple ligand-based and structure-based models can be used to develop tiered molecular triage workflows in KNIME using the MOE extensions for KNIME to interrogate large datasets of compounds for potential CYP450 binders with sub-type profiling capabilities, and propensity for biotransformation. We add that these multi-target approaches can be directly generalized to other protein families in which isoform-specificity and poly-pharmacological interactions are of interest. These studies place emphasis on the need for accurate curated data in addition to versatile and inter-operable modeling platforms, such as MOE, that enable multi-dimensional modeling approaches for interrogating chemical biology for large scale molecular profiling.

## MEDI 190

### **Phytoestrogens: New ligands targeting the estrogen receptor domains**

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Estrogen receptor, aromatase and 17- $\beta$ 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 IC<sub>50</sub> 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 191

### **Structure-based drug design of new indole and benzpyrazole analogs with expected activity**

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Hypoxia Inducible Factor (HIF-1) has been proved to be an important cancer target. Inhibition of HIF-1 activity has significant effect on tumour growth. There are certain ways to target HIF-1 protein like, inhibiting i) mTOR signaling pathway ii) topoisomerase-I iii) dimerization of HIF-1 iv) HIF m-RNA translation and destabilizing HIF-1 $\alpha$  protein. Eventually, in this course, we have approached to target HIF-1 $\alpha$  protein. Thus, the PDB ID: 1YCI binding site is potentially a good target for new anti-tumour drugs which will either allosterically or directly target Hypoxia Response Element (HRE). Docking studies using crystal structure of 1YCI binding domain suggested that tetrahydro indole and benzpyrazole derivatives could be good candidates. The pharmacophore model and SAR was develop for 1-(1-((3s,5s,7s)-adamantan-1-yl)-4,5,6,7-tetrahydro-1H-indol-3-yl)ethanone and 1-(1-((3s,5s,7s)-adamantan-1-yl)-4,5,6,7-tetrahydro-1H-indazol-3-yl)ethanone scaffolds. The binding energies derived from Insilco molecular docking studies shows ADMTH 3 and ADMTH78 ligands could be considered for their efficacy as anticancer therapeutics.

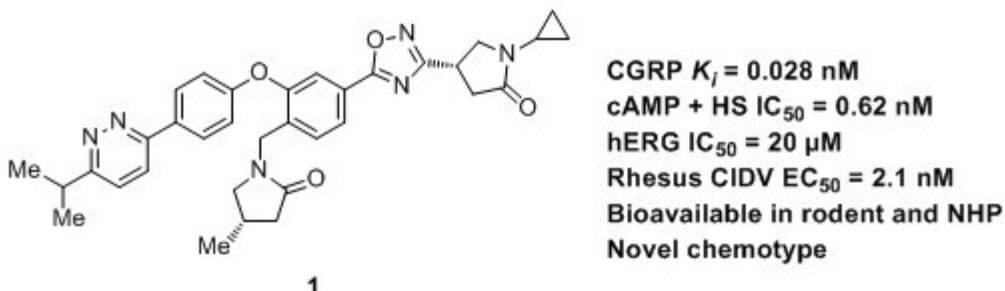
## MEDI 192

### **Rapid identification and optimization of a novel CGRP receptor antagonist chemotype**

**Brendan M. Crowley**<sup>1</sup>, brendan.crowley@gmail.com, **Craig M. Potteiger**<sup>1</sup>, **Diem N. Nguyen**<sup>1</sup>, **John Lim**<sup>1</sup>, **Cheng Wang**<sup>1</sup>, **Helen Mitchell**<sup>1</sup>, **Kathy Schirripa**<sup>1</sup>, **Melody McWherter**<sup>1</sup>, **Robert Gilfillan**<sup>1</sup>, **Mehul Patel**<sup>1</sup>, **Ken L. Arrington**<sup>1</sup>, **Eric L. Moore**<sup>2</sup>, **Joseph G. Bruno**<sup>3</sup>, **Amanda Kemmerer**<sup>3</sup>, **Avni Soni**<sup>3</sup>, **Rebecca B.**

*White<sup>4</sup>, Dan Cui<sup>4</sup>, Andrew Danziger<sup>5</sup>, Scott T. Harrison<sup>6</sup>, John C. Culberson<sup>6</sup>, Hua-Poo Su<sup>7</sup>, Gopal Parthasarathy<sup>7</sup>, Ian M. Bell<sup>1</sup>, Mark E. Fraley<sup>1</sup>, Scott D. Mosser<sup>3</sup>, Christine Fandozzi<sup>4</sup>, Christopher A. Salvatore<sup>2</sup>, Christopher S. Burgey<sup>1</sup>. (1) Discovery Chemistry, Merck & Co., Inc., West Point, Pennsylvania, United States (2) Pain & Migraine, Merck & Co., Inc., West Point, Pennsylvania, United States (3) In Vitro Pharmacology, Merck & Co., Inc., West Point, Pennsylvania, United States (4) Pharmacokinetics Pharmacodynamics & Drug Metabolism, Merck & Co., Inc., West Point, Pennsylvania, United States (5) In Vivo Pharmacology, Merck & Co., Inc., West Point, Pennsylvania, United States (6) Chemistry Modeling and Informatics, Merck & Co., Inc., West Point, Pennsylvania, United States (7) Structural Chemistry, Merck & Co., Inc., West Point, Pennsylvania, United States*

Calcitonin gene-related peptide (CGRP) is a 37 amino acid neuropeptide widely expressed in the peripheral and central nervous system that has been implicated in the pathogenesis of migraine headache. Antagonism of the CGRP receptor has been clinically validated to provide effective migraine relief comparable to the triptans with an improved adverse event profile. In our efforts to develop structurally novel, low-dose successors to telcagepant, our first-generation clinical candidate, we investigated a series of trisubstituted benzene compounds that represent a novel chemotype for CGRP receptor antagonists. This series makes interactions with the CLR portion of the heterodimer not observed for other chemotypes by inducing a new pocket to form in this part of the receptor (determined by receptor mutagenesis, X-ray crystallography, and NMR). Prior to these efforts, notably, only a small number of structural classes have been disclosed against this target despite nearly two decades of searching by the community. Initial hit to lead efforts led to the identification of compounds that possess good potency (CGRP cAMP IC<sub>50</sub> < 10 nM) and selectivity (AM<sub>1</sub> and AMY<sub>3</sub> cAMP IC<sub>50</sub> > 20 μM) but suboptimal pharmacokinetic properties (rat F = 0%). Optimization of this series resulted in compounds with further improved potency and selectivity and substantially improved rodent and NHP PK, ultimately leading to the identification of a compound, **1**, with high affinity for the receptor (*K<sub>i</sub>* = 0.028 nM), potent *in vivo* activity (rhesus CIDV EC<sub>50</sub> = 2.1 nM), a low potential human dose, and good off-target selectivity (e.g. hERG IC<sub>50</sub> = 20 μM).



## MEDI 193

### **Discovery of (*E*)-(4-(3-methylbut-2-en-1-yl)-3-(3-phenylpropanamido)cinnamic acid as highly potent and selective inhibitor of AKR1C3 for the treatment of castration-resistant prostate cancer (CRPC) and acute myeloid leukemia (AML)**

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Aldo–keto reductase 1C3 (AKR1C3), also known as type 5 17 $\beta$ -hydroxysteroid dehydrogenase, is required for the synthesis of testosterone and 5 $\alpha$ -dihydrotestosterone in prostate cancer. Upregulation of AKR1C3 occurs during the emergence of castration-resistant prostate cancer (CRPC) the fatal form of prostate cancer and contributes to androgen deprivation drug resistance. The prostaglandin F synthase activity of AKR1C3 also leads to an increase in proliferation of myeloid precursor cells in the bone marrow that contributes to acute myeloid leukemia (AML) pathogenesis. Moreover, AKR1C3 confers chemotherapeutic resistance to the anthracyclines and enzalutamide: first-line agents for the treatment of CRPC and AML. AKR1C3 shares close homology to its isoforms: AKR1C1 and AKR1C2 which are implicated in normal steroid metabolism. Hence, the synthesis of potent and isoform-selective AKR1C3 inhibitors is a considerable drug discovery challenge.

Baccharin, isolated from Brazilian green propolis was identified as a hit compound exhibiting potent AKR1C3 inhibition activity (IC<sub>50</sub> = 100 nM) and selectivity for the AKR1C3 isoform. In order to improve the AKR1C3 inhibitory

activity and selectivity and to address the metabolic concerns within the baccharin structural scaffold, we conducted an in-depth structure-activity relationship study. We report the identification of novel AKR1C3 inhibitors that exert up to two-fold enhancement in enzyme inhibitory potency than baccharin and demonstrate exquisite isoform selectivity. Our lead compound, exhibiting >2800-fold selectivity towards AKR1C3 inhibition is the most selective AKR1C3 inhibitor discovered to date. Amide bearing derivatives maintained a greater hydrolytic stability ( $t_{1/2} = 240$  min) as compared to baccharin and ester-bearing derivatives ( $t_{1/2} < 1$  min) when incubated with human hepatic S9 fractions. Biological evaluation of lead compounds demonstrated a very high degree of synergistic drug action when combined with enzalutamide, etoposide, and anthracyclines in *in vitro* cell models of CRPC and AML: indicative of repressing chemotherapeutic resistance.

AKR1C3 inhibition has been proven to be a valid approach for the treatment of CRPC and AML. Based on the preliminary biological screen, the lead compounds represent a promising approach to obtain clinical drug candidates to overcome therapeutic resistance, reduce severe toxicity associated with the use of chemotherapy drugs and improve patient survival.

## MEDI 194

### Synthesis of $\beta$ -monoadducts using oligonucleotides

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The drug Mitomycin C (MC) is currently used to treat stomach, anal, or lung cancer. Its cytotoxicity is due to the formation of DNA N 2 -dG adducts, in particular interstrand crosslinks known as  $\alpha$ -ICLs. A close analog of MC, 10-decarbamoyl mitomycin C (DMC), generates the same  $\alpha$ -ICL as MC in the presence of DNA as well as a second stereoisomeric crosslink known as the  $\beta$ -ICL. When human cancer cells are treated with DMC and MC, DMC appears more toxic than MC and is able to initiate a p53 independent type of cell death. We believe that the different biochemical responses exhibited by the two drugs are due to the opposite stereochemistry of the  $\alpha$  and  $\beta$  ICLs. Our aim is to develop an efficient method of producing the  $\beta$ -ICL and to investigate if, and how, the difference in the ICL structures determine the cell signaling outcome. In particular, we are interested in the cell death pathway triggered by the 2 isomeric ICLs. We describe here a new method to generate the DMC  $\beta$  monoadduct, a precursor of the  $\beta$ -ICL. This DMC DNA-adduct was

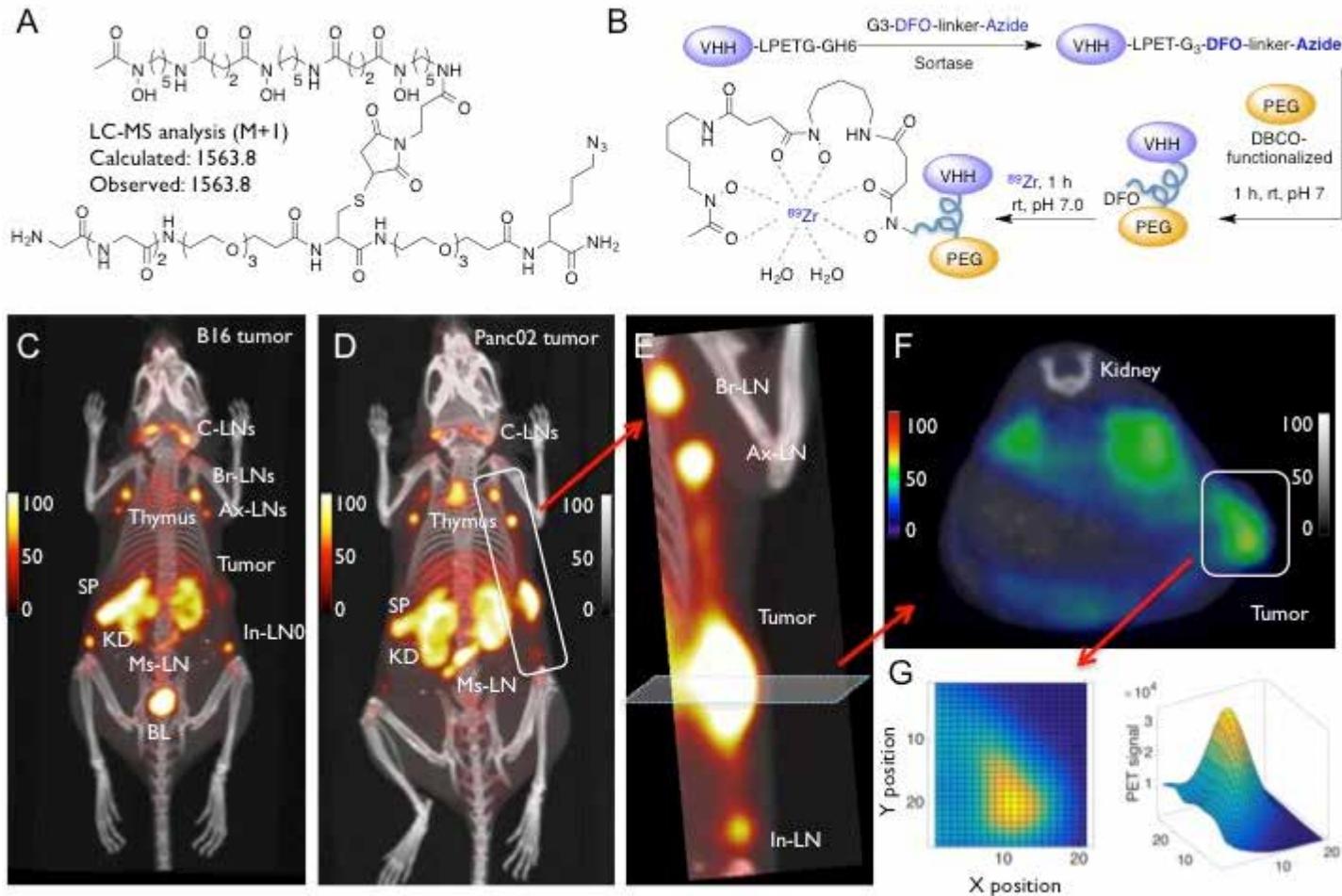
obtained from the bifunctional activation of DMC in the presence of duplex DNA. The alkylation reactions have been performed with oligonucleotides in different sequence context in order to identify the sequence preference for the formation DMC  $\beta$  monoadducts.

## MEDI 195

### Profiling CD8 T cells in tumor microenvironment using PEGylated single domain antibodies and immunoPET

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Immunotherapy using checkpoint-blocking antibodies can cure cancer in a subset of patients. The presence of CD8 T cells in the tumor correlates with improved survival. We show that immuno-positron emission tomography (immuno-PET) can visualize tumors by detecting infiltrating lymphocytes. We developed a method using  $^{89}\text{Zr}$ -labeled PEGylated single-domain antibody fragments (VHHs) specific for CD8 to track the presence of intratumoral CD8 $^+$  T cells in the immunotherapy-susceptible B16 melanoma model in response to checkpoint blockade. We can distinguish responding tumors from those that do not respond to therapy. Animals that responded to CTLA-4 therapy showed a homogeneous distribution of the anti-CD8 PET signal throughout the tumor, whereas more heterogeneous infiltration of CD8 T cells correlated with faster tumor growth and worse responses. It may thus be possible to use immuno-PET and monitor antitumor immune responses as a prognostic tool to predict patient responses to checkpoint therapies.



A) Structure of the bi-orthogonal sortase substrate. The azide functionality allows installation of PEG groups, and the DFO chelator is used to install  $^{89}\text{Zr}$  for PET imaging. B) Schematic representation of preparing PEGylated  $^{89}\text{Zr}$ -labeled VHHs for PET imaging. C-G) Anti-CD8  $^{89}\text{Zr}$ -labeled PEG20-VHH detects lymphoid organs and tumor-infiltrating CD8 $^{+}$  lymphocytes. C&D) PET-CT images of tumor-bearing mice (C: B16 tumor and D: Panc02 tumor) injected with  $^{89}\text{Zr}$ -PEGylated VHH ( $n=3$  for each experiment). E) Enlarged view of the tumor and draining lymph nodes. F) A cross-section of the tumor shows the intratumoral distribution of infiltrated CD8 $^{+}$  T cells. G) Enlarged view 2&3D-representation of the cross section in (F) shows CD8 $^{+}$  T cells deep inside the tumor.

## MEDI 196

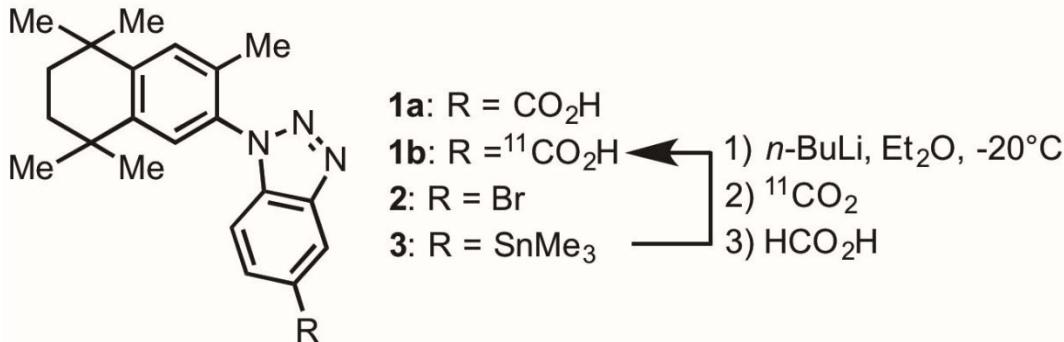
**Synthesis of  $^{11}\text{C}$  labeled RXR partial agonist CBt-PMN by [ $^{11}\text{C}$ ] carbon dioxide fixation via organolithiation of trialkyltin precursor and PET imaging thereof**

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Hiroyuki Hirano<sup>3</sup>, Hiroki Kakuta<sup>1</sup>.** (1) Graduate School of Medicine, Dentistry,  
and Pharmaceutical Sciences, Okayama University, Okayama, Japan (2)  
Collaborative Research Center for Okayama Medical Innovation Center  
(OMIC), Okayama University Graduate School of Medicine, Dentistry and  
Pharmaceutical Sciences, Okayama, Japan (3) SHI Accelerator Service Ltd.,  
Okayama, Japan

**[Objective]** We reported RXR partial agonist CBt-PMN (**1a**,  $E_{max} = 75\%$ ,  $EC_{50} = 143 \text{ nM}$ ) exerts potent glucose-lowering effect without the serious adverse effects caused by RXR full agonist bexarotene (targretin<sup>®</sup>), which has applications for treatment of cutaneous T cell lymphoma (CTCL) in US and Japan. Since bexarotene is reported to show curative effect for central nervous system disease (CNS disease) such as Alzheimer's or Parkinson's diseases, **1a** is also expected to show similar therapeutic effect. In this study, we determined brain uptake and biodistribution of carbon-11 labeled CBt-PMN (**1b**) by performing PET imaging study.

**[Results]** It is thought that induction of carbon-11 to carboxylic moiety is more easily than to a methyl group at a benzyl position because of the steric hindrance by the triazole ring.  $^{11}\text{C}$ -labeled carboxylation is performed by using  $[^{11}\text{C}]CO$  or  $[^{11}\text{C}]CO_2$ .  $[^{11}\text{C}]CO_2$  fixation can be performed directly by using organolithium or Grignard precursors or by the reaction with boronic ester precursors in the presence of copper(I)-catalysts. Thus,  $[^{11}\text{C}]CO_2$  fixation was selected and applied to **1b**. Lithiation of bromo derivative **2** was failed because of the low tolerance of the triazole ring. Though using turbo-Grignard, which is applicable to highly functionalized precursors, enabled to produce target molecule from **2**, the low yield and the existence of byproduct hardly separated were problems. Cu-mediated  $[^{11}\text{C}]CO_2$  fixation applied to  $[^{11}\text{C}]bexarotene$  faced production of Cu-triazole complex. Finally, tin–lithium exchange reported by Staubitz *et al*, was applied and succeeded in synthesis of **1b** from **3** with >99% radiochemical purity within 30 min. Positron emission tomography-computed tomography(PET-CT) using **1b** revealed brain uptake and enterohepatic circulation in mice.

**[Conclusion]** This work is the first  $[^{11}\text{C}]$ carboxylic acid radiotracer synthesized from  $[^{11}\text{C}]CO_2$  using tin–lithium exchange, and this methodology may be useful in PET tracer syntheses with compounds chelating to Cu ion. And the results show **1a** has potential to be a curative candidate for CNS disease such as Alzheimer's disease, and Parkinson's disease.



## MEDI 197

### Predicting ADME and PK properties of antivirals for Ebola

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The West African Ebola outbreak of 2013-2016 resulted in over 11,000 deaths. To date there is no FDA approved antiviral or vaccine for the Ebola virus. We have previously detailed the creation of *in silico* Bayesian models using pseudotype viral entry assay and Ebola virus replication assay data for over 800 compounds. These resulting models were then validated both internally and externally and used to score a library of drugs available from MicroSource. Quinacrine, pyronaridine and tilorone, three of the highest scoring molecules that were not in either of the model training sets, were tested *in vitro* and had EC<sub>50</sub> values of 350, 420 and 230 nM, respectively. All three compounds have been moved forward into *in vivo* efficacy testing in the infected mouse model.

In preparation for the murine *in vivo* testing, absorption, distribution, metabolism, excretion (ADME) and pharmacokinetic (PK) experimental data for pyronaridine and tilorone was obtained. The values pertaining to mouse liver microsomal stability, plasma protein binding, Caco-2 permeability and CYP inhibition (against CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4) were determined. We then used this *in vitro* data as a “real life” comparison to the computational predictions for microsomal stability, Caco-2 permeability

and CYP inhibition using our previously described Bayesian models for these properties.

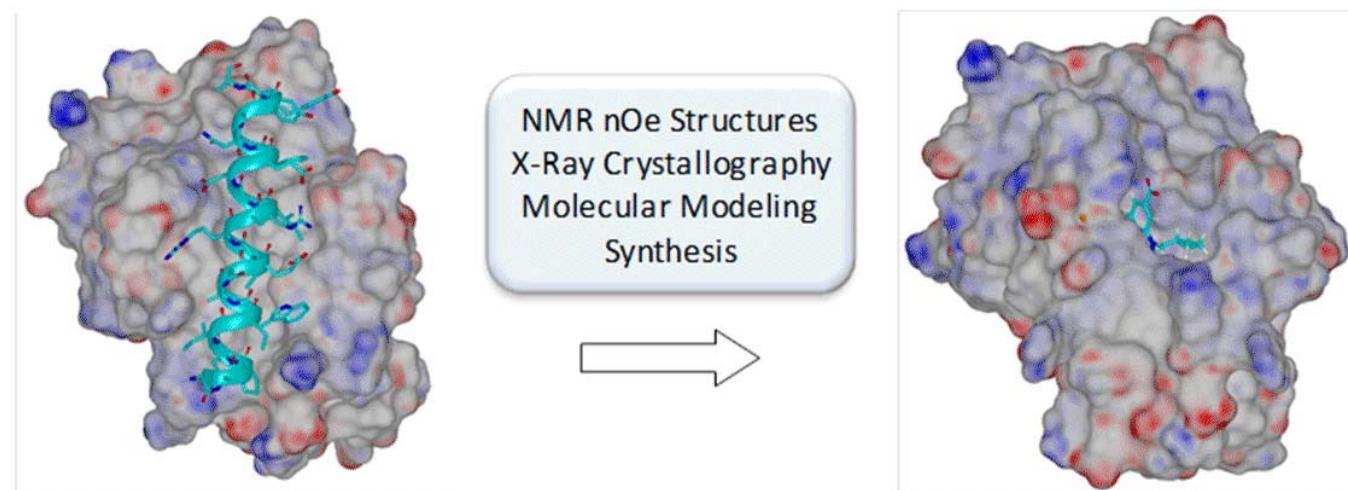
Pyronaridine and tilorone had good ADME and PK properties (demonstrating adequate exposure) as would be expected for potential clinical candidates, and therefore further justified *in vivo* efficacy testing. Our combined *in vitro* and *in silico* results suggest a strategy that could be used for identifying additional antivirals.

## MEDI 198

### Interdiction at a protein—protein interface: Structure-based design and optimization of spirocyclic Mcl-1 inhibitors

**Kexue Li, kexuel@yahoo.com, Sean P. Brown. Medicinal Chemistry, Amgen, Inc., Thousand Oaks, 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. Overexpression of Mcl-1 is observed in many human cancers and is associated with tumor progression and resistance to chemotherapy. Converging structural information and conformational analysis has culminated in the design and synthesis of potent conformationally constrained Mcl-1 inhibitors featuring the spirocyclic scaffold. X-Ray crystallographic data has revealed that this series of inhibitors binds in a deep hydrophobic pocket that is not observed in the presence of native ligands. This presentation will describe the design and optimization of spirocyclic Mcl-1 inhibitors.



## MEDI 199

### **Indole-TEMPO conjugates alleviate ischemia-reperfusion injury via attenuation of oxidative stress and preservation of mitochondrial function**

**Shanshan Hou<sup>3</sup>, 414762160@qq.com, xin yan<sup>2</sup>, Lanrong Bi<sup>1</sup>.** (1) Michigan Tech Univ, Houghton, Michigan, United States (2) Michigan Technological University, Houghton, Michigan, United States (3) chemistry, Michigan Technology University, Houghton, Michigan, United States

Mitochondrial oxidative damage contributes to a wide range of pathologies including ischemia/reperfusion injury. Accordingly, protecting mitochondria from oxidative damage should possess therapeutic relevance. In the present study, we have designed and synthesized a series of novel indole-TEMPO conjugates that manifested good anti-inflammatory properties in a murine model of xylene-induced ear edema. We have demonstrated that these compounds can protect cells from simulated ischemia/reperfusion (s-I/R)-induced reactive oxygen species (ROS) overproduction and mitochondrial dysfunction. Furthermore, we have demonstrated that indole-TEMPO conjugates can attenuate organ damage induced in rodents via intestinal I/R injury. We therefore propose that the pharmacological profile and mechanism of action of these indole-TEMPO conjugates involve convergent roles, including the ability to decrease free radical production via lipid peroxidation which couples to an associated decrease in ROS-mediated activation of the inflammatory process. We further hypothesize that the protective effects of indole-TEMPO conjugates partially reside in maintaining optimal mitochondrial function.

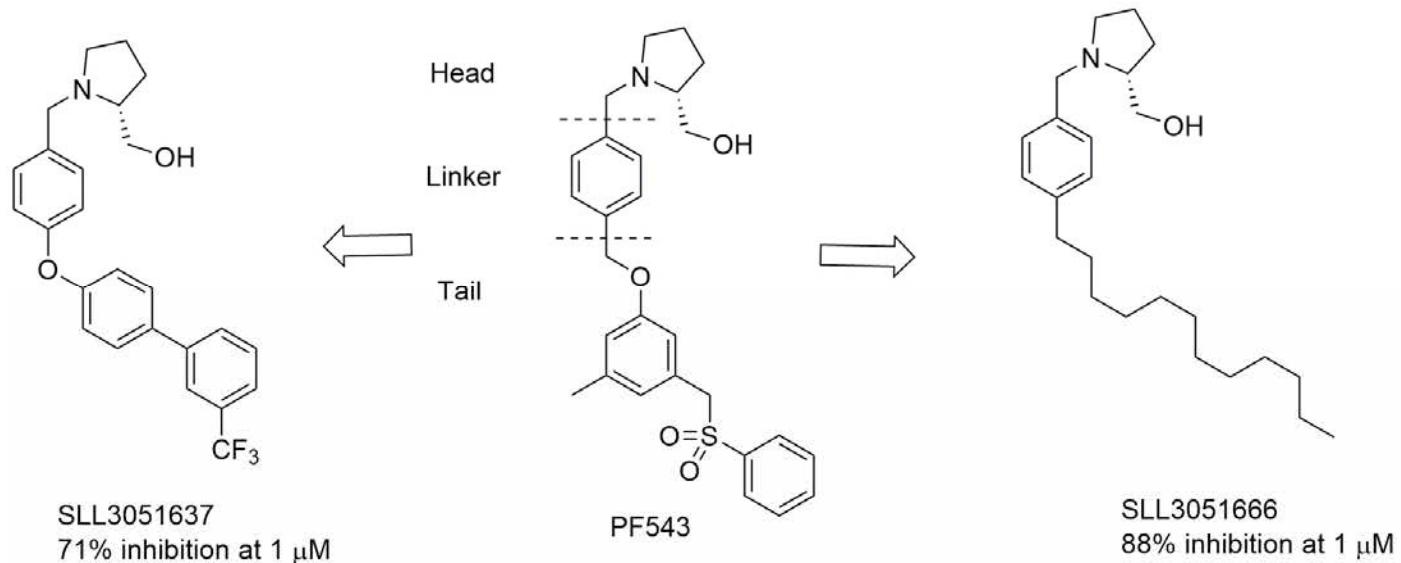
## MEDI 200

### **Development of prolinol based derivatives targeting sphingosine kinase-1**

**Hao Li<sup>1</sup>, lhao1@vt.edu, Yugesh Kharel<sup>3</sup>, Kevin Lynch<sup>3</sup>, Webster L. Santos<sup>2</sup>.** (1) Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, United States (2) Chem Dept, Virginia Tech, Blacksburg, Virginia, United States (3) Department of Pharmacology, University of Virginia, Charlottesville, Virginia, United States

Sphingosine kinase 1 (SphK1) is the key enzyme catalyzing the formation of sphingosine-1-phosphate (S1P), which is an important signaling molecule

regulating multiple biological process including inflammatory responses. Elevated SphK1 activity as well as upregulated S1P level is linked to various diseases, such as cancer, fibrosis and sickle cell disease. Therefore, there is a growing interest in studying on SphK1 as a potential target for the treatment of aforementioned diseases. Through high throughput screening, various SphK1 inhibitors have been discovered, among which PF 543 is the most potent inhibitor reported to date ( $K_i=3.6$  nM). Previous research indicated that SphK1 inhibitor PF543 was effective in reducing S1P levels and slowing down the development process of sickle cell disease *in vivo*. However, the lack of *in vivo* stability of PF543 still makes it necessary to develop inhibitors with improved pharmacokinetic profile. In this study, PF543 was employed as the lead compound, and the influence of different tails groups upon binding affinity and *in vivo* stability were investigated. In brief, 28 compounds with different tail groups including alkyl, alkoxy, phenoxy and phenethyl groups were synthesized, and their inhibition potency were tested by broken-cell assay, with the hit compound being further evaluated on mice model for *in vivo* effect. Our preliminary results indicated that compounds SLL051666 and SLL05637 were the best two hits discovered so far, with the SphK1 inhibitions of 71% and 88%, respectively at 1  $\mu$ M. Future study will focus on head group modification to improve *in vivo* stability.



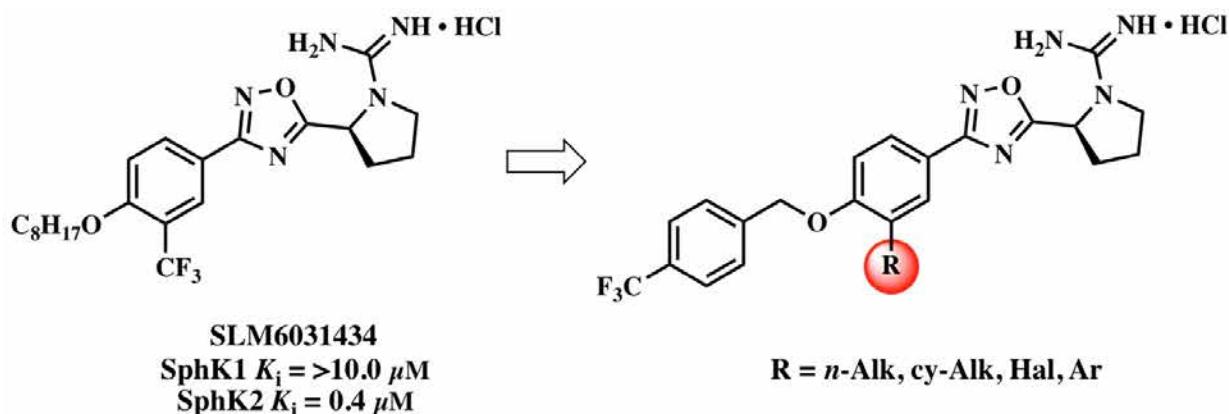
## MEDI 201

### Aryl ring modifications of sphingosine kinase 2 selective inhibitors

**Christopher Sibley<sup>1</sup>, sibleycd@vt.edu, Yugesh Kharel<sup>2</sup>, Kevin R. Lynch<sup>2</sup>, Webster Santos<sup>1</sup>.** (1) Chemistry, Virginia Tech, Blacksburg, Virginia, United

*States (2) Pharmacology, University of Virginia, Charlottesville, Virginia, United States*

Sphingosine-1-phosphate (S1P) is a ubiquitous signaling molecule synthesized by its generative enzymes sphingosine kinase 1 and 2 (SphK1 and SphK2). The S1P signaling pathway has been implicated in various disease states such as cancer, sickle cell disease and renal fibrosis. Inhibition of SphK1 and 2 to attenuate levels of S1P have exhibited therapeutic efficacy in battling the symptoms of these disorders. Recently, work done with **SLM6031434** demonstrated that introduction of a trifluoromethyl group on the internal phenyl ring increased potency toward inhibiting SphK2. Herein, we disclose the synthesis and characterization of compounds with varying substitutions on the internal phenyl ring (Fig. 1). Our studies suggest that a small pocket on this region is present and the current investigations probe this site.



## MEDI 202

### **Investigation of the oprin protein from North American opossum (*Didelphis virginiana*) as a potential inhibitor of Western diamondback rattlesnake (*C. atrox*) venom metalloproteinases**

**Robert M. Werner, mwerner@lssu.edu. Chemistry, Lake Superior State University, Sault Ste. Marie, Michigan, United States**

Numerous mammalian species, including the mongoose, hedgehog, and opossum appear to be resistant to a variety of snake venoms. It is currently accepted that this resistance is conferred through two mechanisms: 1) the resistant animal displays a mutation in the receptor targeted by the snake's toxin, and/or 2) the resistant animal contains serum proteins, referred to as snake venom metalloproteinase inhibitors (SVMPIs), that form non-covalent

complexes, thus neutralizing the toxin. One such protein, oprin, has previously been identified in the N. American opossum (*Didelphis virginiana*). This work will present our efforts to clone the oprin gene from opossum tissue via polymerase chain reaction (PCR), insert it into an expression vector, and to over-express and purify this protein. In addition, we will discuss our efforts to develop protein and peptide based inhibitors of Western diamondback rattlesnake (*C. atrox*) venom components using both a gelatinase and fluorescent peptide assay.

#### MEDI 203

#### Synthesis and cytotoxicity of Baylis-Hillman reaction derived betulinic acid analogs

*Pathi Suman<sup>1</sup>, Amardeep Patel<sup>1</sup>, Lucas Solano<sup>2</sup>, Anupama Indukuri<sup>1</sup>, Sai K. Kommineni<sup>1</sup>, Ryan M. Rutkoski<sup>1</sup>, Michael Collins<sup>1</sup>, Subash C. Jonnalagadda<sup>1</sup>, jonnalagadda@rowan.edu. (1) Chemistry and Biochemistry, Rowan University, Glassboro, New Jersey, United States (2) Chemistry, University of Minnesota, Duluth, Minnesota, United States*

Betulin and betulinic acid are natural products readily isolated from the bark of birch trees. Betulinic acid has been found to possess selective toxicity towards cancer cells while being relatively non-toxic towards healthy cells. The ready availability and favorable therapeutic index makes betulinic acid an attractive candidate for drug development. Previously, we had reported the synthesis of betulinic acid analogs using reactions such as aldol condensation, Passerini reaction, and reductive amination. Recently, we also reported the preparation of triazole conjugates of betulinic acid employing click chemistry and identified two series of these conjugates for further development as potential anti-cancer agents. This poster will detail our recent efforts on the detailed SAR of these analogs.

#### MEDI 204

#### Design of $\alpha$ -(benzoboroxolyl) and $\alpha$ -(benzoboroxolylmethyl) acrylamides as potential anti-cancer agents

*Pathi Suman, Md. Ashiq Ur Rahman, Md. Reazul Islam, Phillip M. Mastoridis, Reuben D'Souza, Subash C. Jonnalagadda, jonnalagadda@rowan.edu. Chemistry and Biochemistry, Rowan University, Glassboro, New Jersey, United States*

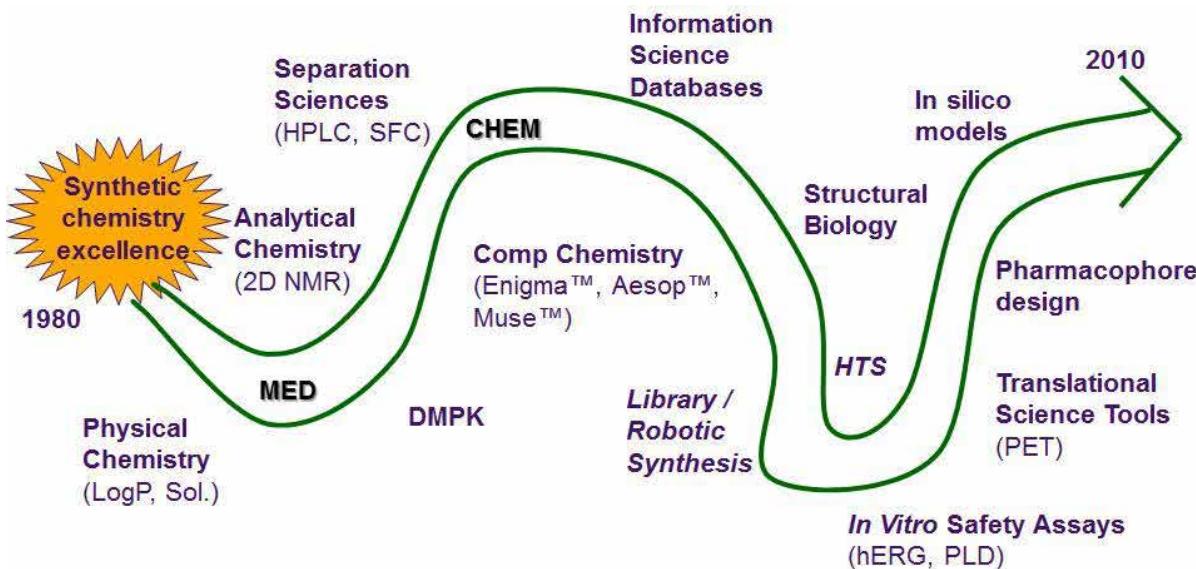
Benzoboroxoles are cyclic boronic acids that have gained prominence owing to the recent FDA approval of tavaborole (Kerydin®) for treatment of onychomycosis. We have reported several methods for the formation of functionalized benzoboroxoles employing reactions such as Baylis-Hillman, Passerini, aldol condensation, and reductive amination. In continuation of this project, we undertook the synthesis of the title compounds for their development as potential *anti*-cancer agents. Reaction of  $\alpha$ -formylphenylboronic acid with methyl acrylate under BH conditions yielded  $\alpha$ -(benzoboroxolyl)acrylate, while the analogous reaction with methyl  $\alpha$ -bromomethylacrylate under Barbier allylation conditions furnished  $\alpha$ -(benzoboroxolylmethyl)acrylate. The final compounds were obtained upon alkaline hydrolysis followed by amide coupling. Detailed *in vitro* evaluation was carried out for all the synthesized compounds and our efforts in this project will be described.

## MEDI 205

### Roles of chemists and chemical technology in a changing drug discovery environment

**Peter R. Bernstein**<sup>1,2</sup>, bernsteinpr@gmail.com. (1) PharmaB LLC, Rose Valley, Pennsylvania, United States (2) Department of Chemistry and Biochemistry, University of Delaware, Newark, Delaware, United States

Over the last several decades the roles filled by chemists and the chemical technologies used in small molecule drug discovery have changed dramatically. The functions that chemists fill in R&D efforts have become more diverse, complex and specialized, all at the same time. However, at the end of the day an effective small molecule drug is a chemical with a very specialized set of properties. Those imbue it with the dual ability to be effective as a treatment and safe, when administered to people. Using examples from 30+ years of Drug Discovery this talk will illustrate how from a starting point at which time synthetic organic chemistry was the “core” chemical science it is now but one part of a complex amalgam. Currently, the medicinal chemist needs to be conversant in multiple technologies and is more of a conductor/composer who designs and develops a drug via the integration of input from synthetic, analytical, computational, structural biological, physical, and informatics chemists.



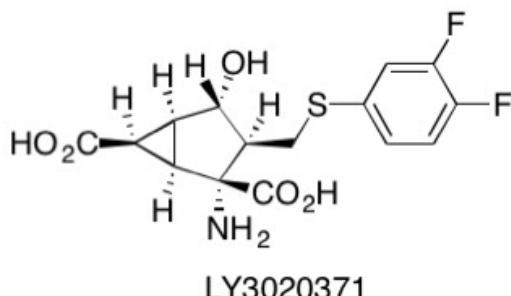
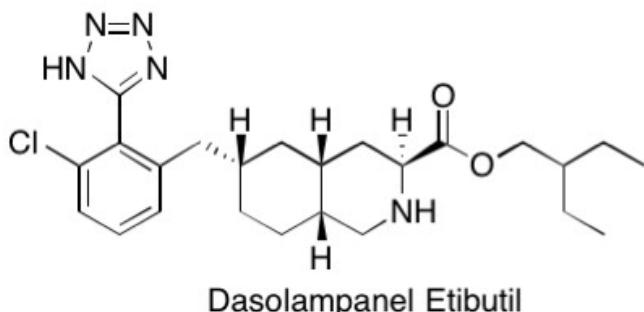
Navigating the wild river "Chemistry in Drug Discovery"

## MEDI 206

### Adventures in the discovery of excitatory amino acid antagonist therapeutics: The value of perseverance

**Paul L. Ornstein, apolloddc@gmail.com. School of Pharmacy, Medical College of Wisconsin, Milwaukee, Wisconsin, United States**

Glutamate is the major excitatory neurotransmitter in the CNS, and its role in modulation of fast and slow excitatory neurotransmission opens many opportunities for the discovery and development of therapeutics for neurological and psychiatric diseases. In my 28 years at Eli Lilly and Company and beyond, I have been privileged to work with exceptional chemistry, pharmacology, medical, ADME and toxicology partners, and our combined efforts led to the discovery of many development candidates for CNS disorders. Key to our success was the ability to persevere on our discovery efforts through multiple project iterations, allowing us to evolve compounds that incorporated the best aspects of the science as it matured over more than 20 years. I will discuss two stories that evolved clinical candidates in that time frame, dasolampanel etibilil and LY3020371.



## MEDI 207

### Role of tacit knowledge in medicinal chemistry

**Robert L. Dow**, [rldgrotton@gmail.com](mailto:rldgrotton@gmail.com). *Medicine Design, Pfizer Inc, Cambridge, Massachusetts, United States*

The discipline of medicinal chemistry requires the development of a wide scope of hard knowledge, though this does not in and of itself necessarily spell success for the practitioner. Creativity in the scientific endeavor requires not only our ability to filter a large influx of data, but also the ability to utilize intuition or tacit knowledge. Interestingly it was a chemist who pioneered the study of tacit knowledge, providing a framework for pushing back against scientific reductionism. While capturing the essence of tacit knowledge is difficult at best, this talk will attempt to articulate key features of this skill set and how we might consider employing it in the medicinal chemistry setting.

## MEDI 208

### Find out what you don't know: A recurring lesson from years of lead generation research

**Michael R. Wiley**, [wileymr@lilly.com](mailto:wileymr@lilly.com). *Discovery Chemistry Research and Technologies, Eli Lilly and Company, Indianapolis, Indiana, United States*

In the pursuit of drug discovery efforts, it can be tempting to try to accelerate access to chemical starting points using predictive methods to minimize the size of screening sets based on structural homology with natural substrates or other previously reported ligands. However, staged investments in exploratory screening strategies can often reveal that targets can be successfully engaged with drug-like starting points bearing little resemblance to their native biological ligands. In addition to the advantages of evaluating structural diversity, atypical mechanisms of drug action (eg, non-competitive or un-

competitive binding) can also be discovered that can help accelerate the resolution of challenges such as translation to in vivo efficacy or selectivity vs target family homologs. Thus, a thorough experimental evaluation of the intersection between the available chemistry space and the biology of interest can pay significant dividends by enabling teams to initiate research efforts from starting points that are much closer to the finish line. Selected vignettes from our research efforts will be presented to illustrate the advantages of “finding out what you don’t know”.

## MEDI 209

### **Tales from the hood: Three vignettes focused on optimization of human dose**

***Harold B. Wood, blair\_wood@merck.com. Discovery Chemistry, Merck & Co. Inc., Kenilworth, New Jersey, United States***

Why target compounds with low human doses? There are several desirable aspects to having a low predicted human dose including: reductions of APIs in the environment, lower cost to the patient and minimizing the risks of adverse events. This talk will describe three vignettes focused on approaches to consider when optimizing human dose predictions. Part 1 discusses the impact of non-specific binding on in vitro intrinsic activity. Part two describes the use of definitive met ID from microsomal incubations to better understand metabolism. Part three discusses progress towards an in silico human dose prediction to guide compound design.

## MEDI 210

### **Addictive diseases: Molecular neurobiology, behavior, human genetics, and treatments**

***Mary Jeanne Kreek, kreek@rockefeller.edu. Laboratory of the Biology of Addictive Diseases, The Rockefeller University, New York, New York, United States***

Over the past 17 years, misuse of prescription opiates (primarily oxycodone) and overdose due to opioids, including heroin, have soared, now reaching three deaths per hour in the United States. Central to the neurobiology underlying the development of addictive diseases is the endogenous opioid system, both the mu opioid receptor system involved in “reward” and the kappa opioid receptor system involved in countermodulation of “reward.” Our

Laboratory has undertaken studies of the effects of self-administration of oxycodone in adolescent and adult mice. Many genes whose mRNA levels are changed significantly by oxycodone self-administration in mice also have SNPs that have been shown to be significantly associated with opiate addiction in humans. We developed methadone maintenance treatment for heroin addiction over 50 years ago, and numerous studies have shown that 60-80% of unselected opioid addicted persons respond to this long-term treatment. Currently, over 1.3 million persons worldwide are in methadone maintenance treatment. Buprenorphine-naloxone, developed in the late 1980s, has also been shown to be an effective treatment for opiate addiction. Since we have shown that dynorphin acting at kappa receptors significantly lowers dopaminergic tone in rodents, we are now conducting translational work focused on the kappa opioid receptor as a possible biological target for novel pharmacotherapies for cocaine addiction, for which there is no pharmacotherapy, and alcoholism, for which naltrexone and naloxone are the only available treatments and are effective in only around 30% of unselected alcoholics. Our ongoing human molecular genetics work has identified over 100 SNPs associated with opiate addiction, many of which have been replicated by other groups. A large number of these SNPs are in genes of the opioid, stress-responsive, and dopaminergic systems, which we have demonstrated to be of central importance for the development of specific addictive diseases.

## MEDI 211

### Discovery of selective orexin-1 receptor antagonists

**Brock T. Shireman**, bshirema@its.jnj.com, **Cathy Preville**, **Jeannie M. Ziff**, **Curt A. Dvorak**, **Heather Coate**, **Christine Gelin**, **Terry Lebold**, **Pascal Bonaventure**, **Christine Dugovic**, **Tatiana Koudriakova**, **Brian Lord**, **Diane Nepomuceno**, **Jonathan Shelton**, **Timothy Lovenberg**, **Nicholas I. Carruthers**. Janssen Pharmaceutical Research & Development, San Diego, California, United States

The neuropeptides orexin-A and orexin-B derives from a common precursor peptide exclusively produced by perifornical and lateral hypothalamic neurons orexin neuropeptides hypocretin-1 (OX1) and hypocretin-2 (OX2) originate in the hypothalamus. Orexin-producing neurons project widely to key areas of the brain and are predominantly involved in the control of wakefulness and also in the regulation of food intake, reward, addictive behaviors and stress. The OX neuropeptides mediate their effect by stimulating two distinct G-protein coupled receptors, orexin-1 (OX1R) and orexin-2 (OX2R) that are co-

located or selectively located in specific brain areas suggesting differentiated roles from a common precursor and have been shown to exert their mode of action on the Orexin-1 (OX1) or Orexin-2 receptors (OX2). These receptors are either co-expressed or selectively expressed in key areas of the central nervous system that mediate sleep-wake, addiction, reward, mood, panic, and anxiety. The selective expression of these receptors suggest differentiated roles within the CNS. For example, the OX2Rs are exclusively expressed in the tuberomammillary nuclei which play a critical role in wake promotion. In agreement with this, the ability of OX2R antagonists to promote sleep by inhibiting wake is now well established. This has led to a marketed dual OX1/OX2 receptor antagonist (DORA), Belsomera® (suvorexant). In line with pre-clinical studies indicating that antagonism of the OX2 receptor is required to induce sleep, but not the OX1 receptor, Janssen and others have reported that selective OX2R antagonists, are effective at promoting sleep in healthy human volunteers. In contrast to the OX2Rs, OX1Rs are selectively expressed in the bed nucleus of the stria terminalis, amygdala, cingulate cortex and locus coeruleus which play a role in panic and anxiety. In addition to the ventral tegmental area which is thought to mediate addictive behaviors. As researchers in academia and industry look to more clearly delineate the role and selectively target the OX1R in more complex emotional behavior (addiction, panic, anxiety) improved tool compounds are needed.

Presented here will be the discovery, synthetic methods and SAR associated with novel selective OX1R antagonists and their evaluation in selected preclinical models of panic, anxiety and addiction. In addition, we will highlight our first candidate for preclinical development.

## MEDI 212

### **Targeting the dopamine D<sub>3</sub> receptor for treatment of opioid and cannabis use disorders**

**Amy H. Newman**, [anewman@intra.nida.nih.gov](mailto:anewman@intra.nida.nih.gov). NIDA IRP, Baltimore, Maryland, United States

The dopamine D<sub>3</sub> receptor (D<sub>3</sub>R) is a target for development of medications to treat substance use disorders. D<sub>3</sub>R-selective compounds with high affinity and varying efficacies have been discovered, providing critical research tools for cell-based studies that have been translated to *in vivo* models of drug abuse. D<sub>3</sub>R antagonists and partial agonists have shown especially promising results in rodent models of relapse-like behavior. However, to date, advancement to human studies has been limited. The high resolution D<sub>3</sub>R crystal structure has

provided clues for structure-based drug design in combination with small molecule structure activity relationships (SAR). Using this hybrid approach, highly potent and selective dopamine D<sub>3</sub>R antagonists and partial agonists have been discovered. For example, VK4-116 is a metabolically stable D<sub>3</sub>R antagonist that demonstrates high D<sub>3</sub>R binding affinity, (Ki=7 nM) and ~1700-fold selectivity over D<sub>2</sub> receptors. VK4-116 attenuates self-administration of the prescription opioid oxycodone, in rats (FR1, 15-25 mg/kg), significantly attenuates the acquisition of oxycodone self-administration when pretreated with VK4-116 and blocks oxycodone-induced reinstatement to drug seeking. VK4-116 also significantly attenuates naloxone-precipitated conditioned place aversion in chronic oxycodone treated rats, but does not affect oxycodone-induced analgesia, in the hot plate test. These data suggest that D<sub>3</sub>R antagonists may be suitable alternatives or adjunctive to opioid-based medications currently used to treat opioid addiction. As VK4-116 proved successful in this model, we then explored its potential for treatment of cannabis dependence. VK4-116 dose-dependently blocked THC self-administration in squirrel monkeys self-administering THC (FR10, 1-10 mg/kg) without affecting food self-administration. In addition, VK4-116, in the same dose range, blocked THC- or cue-induced reinstatement of drug seeking behavior. This is the first demonstration that D<sub>3</sub>R is a medication target for cannabis use disorders. We have also discovered the highly selective and metabolically stable D<sub>3</sub>R partial agonist, VK 4-40 (D<sub>3</sub>R Ki=0.36 nM and 400-fold selectivity over D<sub>2</sub> receptors.) Separation and evaluation of the enantiomers of both VK4-116 and VK4-40 have revealed enantioselective profiles of these agents and provide new lead molecules for medication development.

## MEDI 213

### **Substance use disorders: Vaccination as a therapeutic strategy**

**Kim D. Janda**, *kdjanda@scripps.edu. Scripps Rsrch Inst, La Jolla, California, United States*

Substance use disorders (SUDs) are a global public health concern with less than optimal treatment outcomes. For example while there are medications approved by regulatory agencies to treat nicotine and opioid use disorders. There are no approved medications for cocaine, methamphetamine, and cannabis use disorders, despite over 25 years of research and a plethora of medications evaluated. Moreover many patients receiving treatment relapse; therefore there is an urgent need to discover effective medications to treat SUDs. Traditional, small-molecule approaches have only been marginally

successful in treating SUDs; as such we have sought alternative treatments to conventional SUD pharmacotherapies. Protein based therapeutics offer an alternative to customary pharmacodynamic approaches to treating addiction. Specifically we will discuss how vaccination can alter the pharmacokinetic properties of an abused drug without burdening the recipient with untoward CNS side effects. Moreover the lecture will detail the chemistry, immunology and behavioral findings from our most recent vaccines as a means for treating SUDs including heroin, cocaine, and methamphetamine.

## MEDI 214

### **Development of M<sub>5</sub> muscarinic acetylcholine receptor negative allosteric modulators for the treatment of opioid use disorder**

**Carrie K. Jones**, carrie.jones@vanderbilt.edu. Vanderbilt Center for Neuroscience Drug Discovery, Vanderbilt University, Nashville, Tennessee, United States

Opioid use disorder (OUD) represents a debilitating psychiatric disorder that has reached epidemic proportions across the United States. Accumulating evidence suggests that selective inhibition of the M<sub>5</sub> muscarinic acetylcholine receptor (mAChR) subtype may provide a novel target for the treatment of OUD. M<sub>5</sub> is the only mAChR subtype expressed on midbrain dopamine (DA) cells and selective activation of M<sub>5</sub> regulates mesolimbic DA reward circuitry. M<sub>5</sub> knockout mice exhibit reduced morphine place preference with no effect on morphine-induced analgesia. Recently, we reported the successful optimization of the selective M<sub>5</sub> negative allosteric modulator (NAM) ML375 that acts at a less highly conserved allosteric site on the receptor than the ACh binding site. In previous studies, we demonstrated that ML375 can attenuate cocaine self-administration in rodents. Here, we investigated the ability of ML375 to attenuate opioid self-administration without blocking opioid-induced analgesia in rats. Male Sprague-Dawley rats were trained to lever press for a 3 ug/kg/injection of remifentanil under a 10-response, fixed ratio schedule of i.v. drug injection during daily 1-h sessions. Following stable rates of remifentanil self-administration, a complete remifentanil dose-response curve was completed and the ability of ML375 to attenuate the reinforcing effects of remifentanil at each unit dose was then determined. Next, the effects of ML375 on reducing the reinforcing strength of remifentanil were assessed in rats responding under a progressive ratio (PR) schedule of reinforcement. Finally, the potential analgesic effects of ML375 alone and in combination with morphine were measured using a hot plate apparatus. Selective inhibition of M<sub>5</sub> by ML375 produced a dose-related attenuation of

remifentanil self-administration and reduction in the reinforcing strength of remifentanil under a PR schedule. ML375 also had no effect on morphine-induced analgesia within the dose range tested. These findings suggest that M<sub>5</sub> NAMs may represent an exciting novel pharmacotherapy for the treatment of OUD.

## MEDI 215

### **ALIS affinity selection in pharmaceutical discovery**

**Peter Dandliker**, *peter.dandliker@merck.com. Pharmacology, Merck & Co, Inc., Boston, Massachusetts, United States*

Affinity selection mass spectrometry (ASMS) is a general, high-throughput method to select and identify small molecule ligands from complex compound mixtures. Merck has advanced a specific ASMS approach termed ALIS (Automated Ligand Identification System), a two-dimensional LC/MS system in line with high-resolution mass spectrometry, to routinely assess one million compound / target encounters per day. This high throughput capability, while traditionally employed for small molecule hit identification, has recently been adapted to deconvolute molecular targets of phenotypically active compounds of unknown mechanism, in an approach termed Protein Array ALIS (PA-ALIS), and to quantitatively rank order the binding affinity of medicinal chemistry analogs in complex mixtures (Protein Titration or PT-ALIS). The PT-ALIS method, when combined with nanoscale parallel or mixture synthesis permits identification of analogs most likely to exhibit potent functional activity starting from very small quantities of material and without need for compound purification prior to biological assay. An introduction to ALIS and the novel application to medicinal chemistry and target identification will be presented.

## MEDI 216

### **Synthesis strategies to DNA-encoded small molecule libraries – of a chemoresistant sequence, and micellar nanoreactors**

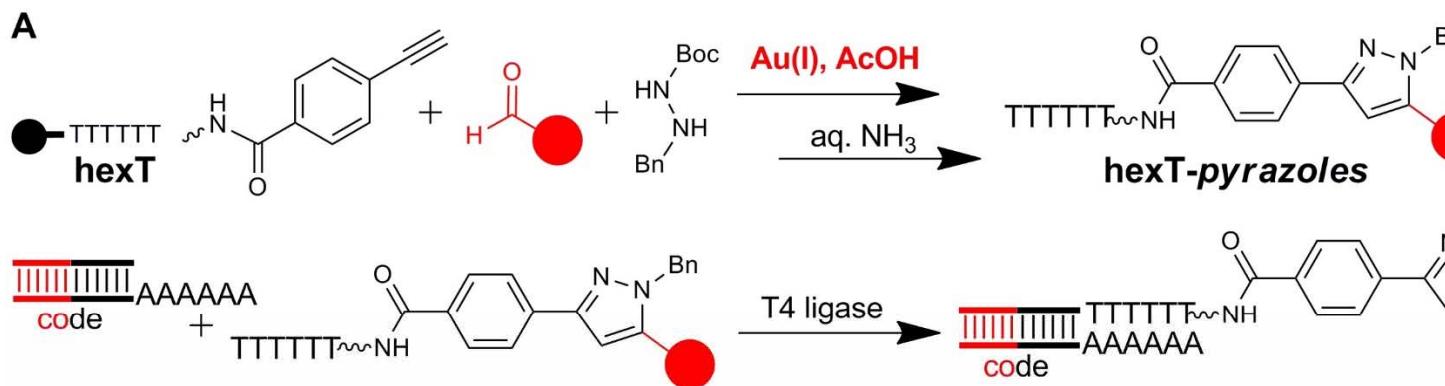
**Andreas Brunschweiger**, *andreas.brunschweiger@tu-dortmund.de, Mateja Klika Skopic, Hazem Salamon. Department of Chemistry and Chemical Biology, TU Dortmund, Dortmund, Germany*

DNA-encoded small molecule libraries (DELs) have found widespread use in drug research as screening technology. Tagging compounds with genetic information allows for pooling them to large collections, and efficient

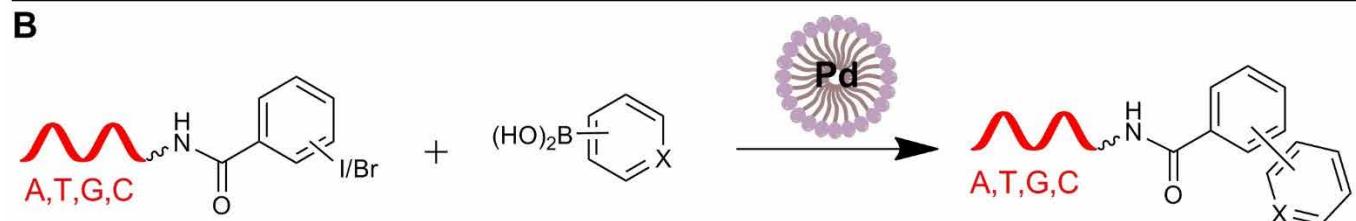
identification of bioactive compounds from these pools by selection and DNA-sequencing. Heterocycles are essential structures in the chemical space of bioactive compounds. Currently, only few heterocycle-forming reactions are available for DEL synthesis. Transition metal catalysts, and acid organocatalysts enable access to diverse drug-like heterocycles from simple starting materials, but interact or even react with purine bases eventually causing depurination of the DNA tag. To circumvent this impediment, we utilize a hexathymidine sequence “hexT” as an adapter oligonucleotide in the initial step of DEL synthesis. Testimony of the stability of the hexT sequence to reaction conditions and catalysts was the synthesis of hexT-pyrazole conjugates through a Au(I)-mediated annulation reaction in glacial acetic acid (**Figure 1A**). The hexT-heterocycle conjugates were readily ligated to coding DNA sequences.

A conceptually different strategy for DEL synthesis rests on the steep solubility gradient of DNA-small molecule conjugates. This gradient is mirrored by oil-in-water (o/w) micelles formed by detergents. Such micelles serve as nanoreactors, separating the water-soluble DNA from the lipophilic core that takes up the conjugated small molecule, catalyst, and reactants. Micellar Suzuki reaction led to the synthesis of DNA-biaryl conjugates from DNA-aryl halide educts and diverse boronates under mild conditions (**Figure 1B**).

**A**



**B**



**Figure 1:** Synthesis strategies to DNA-encoded libraries. A) The solid phase-bound hexathymidine sequence “hexT” tolerated harsh reaction conditions, among them a Au(I)-mediated annulation reaction in glacial acetic acid; B) micellar Pd-catalyzed

synthesis of DNA-biaryl conjugates. Wavy bond: polyethylene glycol linkage; black filled circle: controlled pore glass solid support.

## MEDI 217

### **DNA-encoded library technology (ELT): Challenges and advances in chemistry and library development**

***Yun Ding***, *yun.x.ding@gsk.com. GlaxoSmithKline, Waltham, Massachusetts, United States*

DNA-encoded libraries (DELs) represent an up-and-coming strategy for significantly enhancing compound collections used for screening and ligand identification against therapeutic targets of interest. GlaxoSmithKline (GSK) has established DNA-Encoded Library Technology (ELT) as a platform that utilizes this strategy to identify small molecules for both target validation and medicinal chemistry progression. A primary factor for the success of the technique is the chemical diversity of the libraries. This presentation will review the evolution of the platform over the past 12 years within GSK and the technical advances in library chemistry which enable the expansion of diversity and novel chemical space of the DEL collection.

## MEDI 218

### ***In vitro selection assays: New approaches and applications***

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The *in vitro* selection of synthetic molecule libraries allows a collective querying of function for many molecules simultaneously. As an assay, this approach has several advantages over assays employed in traditional screening campaigns, such as improved throughput and lower cost. We present a critical evaluation of *in vitro* selection assays with regard to their application to discovery from DNA-encoded libraries and also to selection-based sensing, a new assay approach using DNA-linked activity probes. These selections include commonly employed affinity purifications, as well as enzyme substrate selections and selections enabled by covalent crosslinking

of DNA-linked ligands to target proteins. We present quantitative assay parameters of these specific approaches and their application with DNA-encoded libraries of peptidomimetics against several proteins. In addition, we explore the application of selection-based sensing for enzyme activity detection in complex proteomic samples and in the screening of conventional small molecule libraries for enzyme inhibition by DNA sequence analysis.

## MEDI 219

### **Revolution will be compartmentalized: Technology for next-generation small molecule discovery**

**Brian Paegel**, *briandna@gmail.com. Chemistry, The Scripps Research Institute, Jupiter, Florida, United States*

The NIH Molecular Libraries Program (MLP) was founded to translate the discoveries of the Human Genome Project into therapeutics through a network of high-throughput screening (HTS) centers. A decade of discovery produced hundreds of probes — highly selective small molecules that modulate cellular function — but centralized compound screening bears the same cost and infrastructure burdens of millennial DNA sequencing centers, which has limited access to the technology and, more significantly, the rate of small molecule discovery. We are building a distributable drug discovery platform analogous to next-generation DNA sequencing. New DNA-encoded solid-phase synthesis strategies produce ultra-miniaturized compound libraries of microscopic bead each displaying many copies of a small molecule library member and a corresponding amplifiable DNA encoding its structure. Microfluidic instrumentation engineering for miniaturizing automated screening has now yielded integrated circuits that load individual compound library beads into picoliter-scale droplets of assay reagent, photochemically cleave the compound from the bead into the droplet in a UV dose-dependent fashion (0.01–10  $\mu\text{M}$  compound), incubate the dosed droplets, detect activity using laser-induced confocal fluorescence detection, and sort hit-containing droplets for PCR amplification and high-throughput sequencing. To demonstrate the feasibility of the platform, we synthesized a modest (~50k compounds) DNA-encoded combinatorial protease inhibitor library and developed droplet-scale biochemical assays of HIV-1 protease, ZIKV NS2B-NS3 protease, and cathepsin D. Not only are the molecular libraries and screening technology deployable in any laboratory setting, but dose-response screening promises whole-library structure activity relationship profiles. The unprecedented molecular detail of these data will yield portfolios of new leads and replenish

the pipeline of therapeutics, especially those targeting rapidly-evolving bacterial and viral pathogens.



## MEDI 220

### Application of DNA-encoded technology to lead generation of challenging targets

***Ying Zhang, yzhang@x-chemrx.com. X-Chem, Inc., Waltham, Massachusetts, United States***

The DNA encoded platform at XChem allows for the synthesis of large, highly diverse libraries of lead-like small molecules. Identification of unique clusters of library members that interact with therapeutic targets proceeds by performing affinity-mediated selection for target-binding followed by sequencing of the associated oligonucleotide tags. The biological activities for discovery targets are confirmed by resynthesizing exemplary binders without DNA tags, and assaying them in relevant biochemical and/or cell based assays. An overview of the technology along with case studies of successful programs, including GPCR's and protein-protein interaction (PPI) programs at X-Chem, will be presented.

## Steroidogenic cytochrome P450 enzymes as drug targets

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**Lina Yin**<sup>1,2</sup>, **Amjad Ali**<sup>3</sup>, **Scott Hoyt**<sup>3</sup>, **Qingzhong Hu**<sup>1,2</sup>, **Chris van Koppen**<sup>2,4</sup>. (1)  
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Steroid hormones are not only important for vital physiological processes, they are also associated with severe diseases (like cancer, cardiovascular and metabolic diseases, and chronic wounds). For their treatment, inhibition of the biosynthesis of the corresponding hormones has turned out to be an effective strategy. Six Cytochrome P450 (CYP) enzymes are involved in steroidal hormone biosynthesis pathways, four of which are well established drug targets or at a target discovery stage. This talk gives an overview of these targets with a focus on the work from our lab. Research in this field started with attempts to inhibit aromatase (CYP19A1) more than three decades ago. The third generation aromatase inhibitors are used today as first line therapeutics for the treatment of hormone-dependent breast cancer. Ten years later research started on CYP17A1 (17 $\alpha$ -hydroxylase-C17,20-lyase) to block androgen biosynthesis. But only recently, an inhibitor of this enzyme was approved for the treatment of castration-refractory prostate cancer. Selective inhibitors of mineralo- and glucocorticoid biosynthesis were not in the focus of research efforts until a couple of years ago. This was due to the fact that the homology between aldosterone synthase (CYP11B2) and cortisol synthase (CYP11B1) is as high as 95 %, and it was considered very difficult to obtain selective inhibitors of one enzyme versus the other. Nevertheless, we started research in this field and succeeded in obtaining highly active compounds with selectivity factors exceeding 1000 over CYP11B1. As such compounds could be candidates for the treatment of hypertension, congestive heart failure, and myocardial fibrosis, there is presently a high degree of interest in this field. Furthermore, we recently developed potent inhibitors of CYP11B1. Such compounds are highly interesting for treating Cushing's Syndrome (CS). They also represent a new concept for promoting chronic wound healing. We recently developed metabolically stable, orally bioavailable inhibitors for the systemic treatment of CS, and, for the topical treatment of chronic wounds, metabolically unstable compounds which are stable in wound fluid. In the research efforts until now, a major focus was on

the selectivity of compounds: only one target enzyme should be inhibited. We will present examples of dual inhibitors that may be more advantageous over selective inhibitors in the treatment of certain patient cohorts ("personalized medicine")

## MEDI 222

### LFF269: A cortisol-sparing CYP11B2 inhibitor that lowers aldosterone in human subjects

**Julien P. Papillon**, *julien.papillon@novartis.com. Global Discovery Chemistry, Novartis, Somerville, Massachusetts, United States*

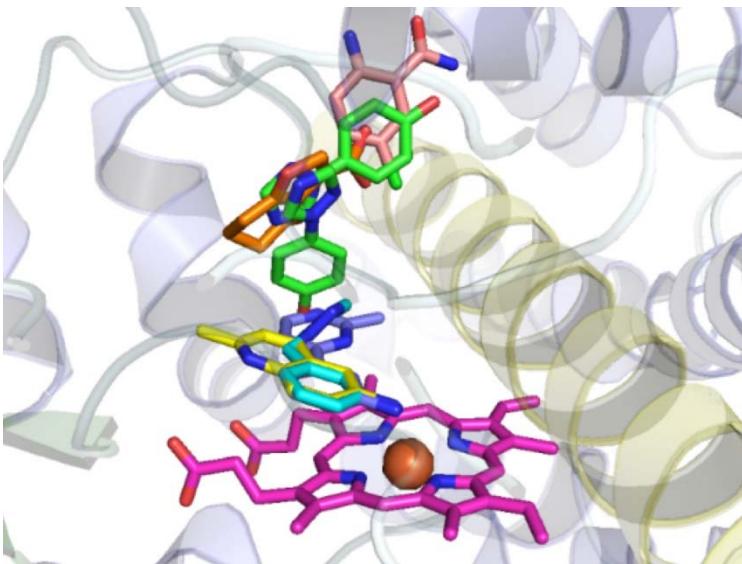
CYP11B2, the aldosterone synthase, and CYP11B1, the cortisol synthase, are two homologous enzymes implicated in a range of cardiovascular and metabolic diseases. We have previously reported the discovery of LCI699, a dual CYP11B2 and CYP11B1 inhibitor which has provided clinical validation for the lowering of plasma aldosterone as a viable approach to modulating blood pressure in humans, as well as normalizing urinary cortisol in Cushing's disease patients. This lecture will describe how despite the 93% identity between the two enzymes, highly selective inhibitors could be identified, with pharmaceutical properties suitable for clinical development. Clinical data demonstrating that *in vitro* selectivity translated into *in vivo* selectivity in humans will be presented.

## MEDI 223

### Using fragment-based approaches to probe the *Mycobacterium tuberculosis* CYPome

**Christopher Abell**, *ca26@cam.ac.uk. Department of Chemistry, University of Cambridge, Cambridge, United Kingdom*

In our laboratory we have pioneered the use of fragment-based approaches in chemical biology and drug discovery. We have a specific interest in developing novel inhibitors of enzymes from *M. tuberculosis* that may lead to the development of novel therapeutics. *M. tuberculosis* has an unusually high number of cytochrome P450 enzymes, some of which are essential for survival. We have used our fragment-based methodology in a number of creative ways to profile fragment-binding across several enzymes, to try to identify substrates, and to develop inhibitors of specific CYPS.



## MEDI 224

### CYP51 inhibitors for Chagas disease

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Chagas disease (American trypanosomiasis) is a deadly insect vector-borne infection caused by a variety of naturally occurring strains of the unicellular eukaryotic parasite *Trypanosoma cruzi*. The disease is endemic in South and Central America and is becoming a serious global health problem due to human migration. In the USA, the situation is particularly alarming because of the broadening of the vector distribution area. Kissing bug bites have been reported in 44 states, and an estimate suggests that there are >2 million infected here. The only two drugs available in South America (benznidazole and nifurtimox) are highly toxic, have not been approved by FDA and are not prescribed in the USA. New, safer and more efficient therapies are urgently needed.

Similar to fungi, the life of *T. cruzi* depends on the production of endogenous sterols. Ergosterol derivatives serve as essential membrane components and regulate the parasite multiplication and morphological transformation. The attempts to repurpose antifungal agents for Chagas disease have been undertaken. However, the drug candidate ravuconazole exhibited bioavailability problems, and the 20% success rate of the drug posaconazole has been declared as a failure. Both these compounds inhibit fungal sterol

14 $\alpha$ -demethylase (CYP51), the cytochrome P450 enzyme that has about 25% sequence identity to the *T. cruzi* ortholog.

We have solved the X-ray structure of *T. cruzi* CYP51 in complex with 16 inhibitors, including posaconazole, built the structure-based 3D-inhibitory pharmacophore, identified a novel, highly potent inhibitor (VNI) that cures the acute and chronic Chagas disease in mice infected with Tulahuen strain of *T. cruzi*, and used the structural information for further VNI scaffold development aimed at broadening its antiprotozoan spectrum of activity (VFV). VFV completely suppresses parasitemia in mice infected with Tulahuen, Y, and Colombiana *T. cruzi* and is more potent than VNI against mice infection with *Leishmania donovani*. Non-toxic, non-mutagenic, having favorable pharmacokinetics, high oral bioavailability (maximal plasma concentration 25  $\mu$ M vs. ~6  $\mu$ M for posaconazole and ~1  $\mu$ M for raruconazole) and broad tissue distribution, VFV is a promising drug candidate suitable for entering clinical trials for Chagas disease.

## MEDI 225

### Discovery of selective CYP11B2 inhibitors as potential treatments for resistant hypertension

**Scott B. Hoyt**<sup>5</sup>, scott.hoyt@nih.gov, Whitney Petrilli<sup>5</sup>, Min K. Park<sup>5</sup>, Jerry A. Taylor<sup>5</sup>, Clare London<sup>5</sup>, Andrew Cooke<sup>5</sup>, Jiaqiang Cai<sup>5</sup>, Emma Carswell<sup>15</sup>, John Robinson<sup>5</sup>, John Maclean<sup>7</sup>, Lindsay Brown<sup>5</sup>, Simone Belshaw<sup>5</sup>, Tom Clarkson<sup>5</sup>, David J. Bennett<sup>5</sup>, Kun Liu<sup>3</sup>, Gui-Bai Liang<sup>9</sup>, Feroze Ujjainwalla<sup>4</sup>, Jim Tata<sup>5</sup>, Qingzhong Hu<sup>8</sup>, Lina Yin<sup>8</sup>, Chris van Koppen<sup>8</sup>, Rolf W. Hartmann<sup>8</sup>, Bheemashankar Kulkarni<sup>9</sup>, Swapna K. Samanta<sup>9</sup>, Rohit Saxena<sup>9</sup>, Mary Struthers<sup>2</sup>, Doris Cully<sup>2</sup>, Tom Wisniewski<sup>2</sup>, Ning Ren<sup>2</sup>, Charlene Bopp<sup>2</sup>, Andrea Sok<sup>2</sup>, Tian-Quan Cai<sup>2</sup>, Sloan Stribling<sup>2</sup>, Lee-Yuh Pai<sup>2</sup>, Xiuying Ma<sup>2</sup>, Joseph Metzger<sup>2</sup>, Andreas Verras<sup>2</sup>, Daniel McMasters<sup>2</sup>, Qing Chen<sup>2</sup>, Elaine Tung<sup>1</sup>, Wei Tang<sup>2</sup>, Gino Salituro<sup>2</sup>, Nicole Buist<sup>2</sup>, Joe Clemas<sup>2</sup>, Gaochao Zhou<sup>2</sup>, Mark Rosenbach<sup>2</sup>, Yusheng Xiong<sup>6</sup>, Amjad Ali<sup>5</sup>. (1) Caridome Pharma Corp, Vancouver, British Columbia, Canada (2) Merck, West Point, Pennsylvania, United States (3) BMB 3-132, Merck Co., Inc., Boston, Massachusetts, United States (4) K15-MN2, Merck Co., Inc., Kenilworth, New Jersey, United States (5) Discovery Chemistry, Merck & Co., Hoboken, New Jersey, United States (6) K15-MW110, Merck Co Inc, Kenilworth, New Jersey, United States (7) Computational Chemistry, RedX Pharma, Macclesfield, United Kingdom (8) Saarland University, Saarbruecken, Germany (9) Medicinal Chemistry, WuXi AppTec, Scotch Plains, New Jersey, United States

Aldosterone is a steroid hormone that promotes increased blood pressure, inflammation and fibrosis. The final three steps of its biosynthesis are catalyzed by CYP11B2 (aldosterone synthase). A closely related enzyme, CYP11B1, catalyzes the biosynthesis of cortisol, an important regulator of glucose metabolism. Small molecule inhibitors of CYP11B2 such as LCI699 have recently been shown to lower aldosterone levels and blood pressure in the clinic, thus validating this mechanism as a treatment for hypertension. LCI699, which inhibits CYP11B2 with only modest selectivity vs. CYP11B1, also produces an undesired impairment of cortisol response, presumably as result of CYP11B1 inhibition. More selective inhibitors of CYP11B2 are thus desired as treatments for hypertension.

This talk will highlight the discovery and optimization of several structurally distinct series of CYP11B2 inhibitors. These efforts culminated in the identification of key compounds that display potent CYP11B2 inhibition, high selectivity versus related CYPs, good pharmacokinetic properties in rat and rhesus, and good physical properties. In a rhesus pharmacodynamic model, key compounds display dose dependent aldosterone lowering efficacy, with significant reductions in plasma aldosterone, and no apparent effect on cortisol levels.

## MEDI 226

### Synthesis and evaluation of itraconazole analogues for the treatment of medulloblastoma

**Jennifer R. Pace**, *jennifer.pace@uconn.edu*, **Matthew K. Hadden**. *Pharmaceutical Sciences, University of Connecticut, Storrs, Connecticut, United States*

Itraconazole (ITZ) is a clinically efficacious antifungal agent that has recently been determined to inhibit the hedgehog (Hh) pathway ( $IC_{50} = 690$  nM). The Hh pathway is an embryonic developmental signaling pathway that plays a role in cell differentiation and tissue growth. Dysregulation of the Hh pathway may lead to cell proliferation and tumor growth most notably seen in basal cell carcinoma (BCC) and medulloblastoma (MB). MB is the most common pediatric malignant brain tumor accounting for ~25% of childhood brain cancer. Current treatment for MB consists of surgery followed by high dose chemotherapy and radiation. While survival rates are generally high, long-term side effects are evident from this harsh treatment regimen. ITZ is currently administered to and tolerated by children for the treatment of various fungal infections and serves as a promising scaffold for pediatric cancer treatment. Our ongoing structure-activity relationship (SAR) studies for the ITZ scaffold

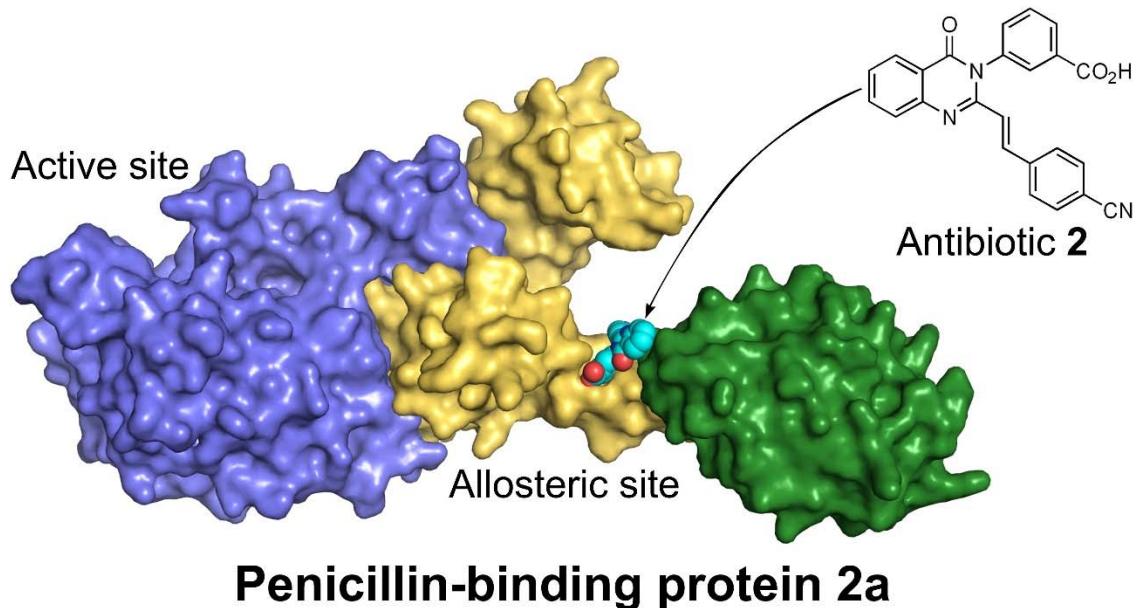
aim to determine the structural requirements for anti-Hh activity. In addition, analogues will be focused on improving general “drug-like” properties necessary to penetrate the blood-brain barrier (BBB) and treat MB. The design, synthesis, and preliminary evaluation of these analogues will be reported herein.

## MEDI 227

### **Discovery of new quinazolinone antibiotics for the treatment of methicillin-resistant *Staphylococcus aureus***

**Renee Bouley<sup>3,5</sup>, bouleyr@gmail.com, Mark Suckow<sup>1</sup>, Juan Hermoso<sup>4</sup>, Mayland F. Chang<sup>2</sup>, Shahriar Mobashery<sup>1</sup>.** (1) Univ Notre Dame, Notre Dame, Indiana, United States (2) Dept of Chem, Univ of Notre Dame, Notre Dame, Indiana, United States (3) Department of Pharmacology, University of Michigan, Ypsilanti, Michigan, United States (4) Consejo Superior de Investigaciones Científicas, Madrid, Spain (5) University of Notre Dame, Notre Dame, Indiana, United States

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a leading cause of hospital-acquired infections and is the cause of approximately 11,000 deaths a year in the United States alone. This organism displays broad resistance to the β-lactam class of antibiotics by expressing an additional penicillin-binding protein (PBP), called PBP2a, which displays low affinity to the β-lactams. This protein is able to evade inhibition by the β-lactams through a closed active site that is regulated allosterically. The quinazolinones were discovered through *in silico* screening of a 1.2 million compound library against the PBP2a active site. The quinazolinones affect peptidoglycan synthesis through inhibition of PBP2a and PBP1 of *S. aureus*. Elucidation of the structure of PBP2a in complex with the lead quinazolinone showed binding to the allosteric site, which produced conformational changes at the active site. An initial lead quinazolinone was identified that demonstrated excellent solubility and good pharmacokinetics in mice. This compound was the result of over 60 structural variations of the initial hit compound, which allowed us to deduce a structure-activity relationship for the quinazolinones. Ten compounds were selected based on antibacterial activity to be tested for *in vivo* efficacy, pharmacokinetics, and *in vitro* cytotoxicity. Three compounds from this library, all containing carboxylic acid functionalities, demonstrated good *in vivo* efficacy as well as pharmacokinetics. From these variations a new lead compound was identified that shows a higher volume of distribution, more potent *in vitro* antibacterial activity, and efficacy *in vivo*.



**Penicillin-binding protein 2a**

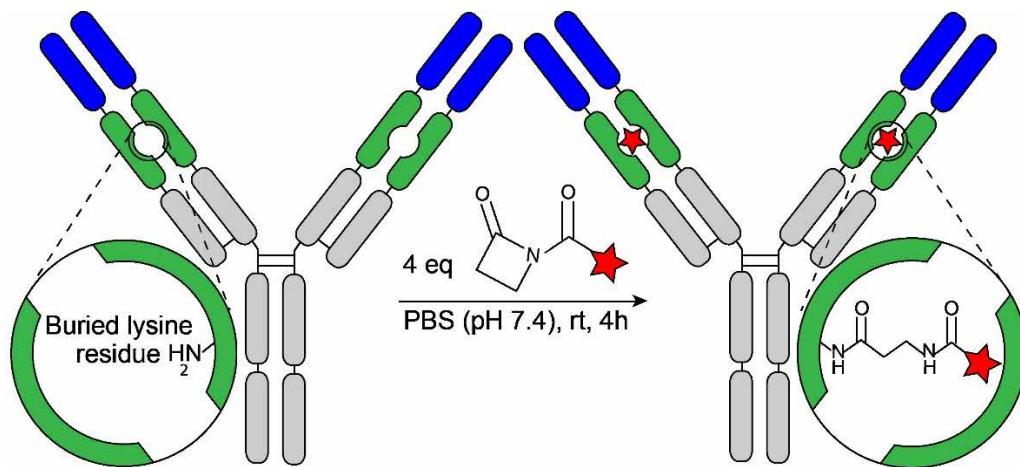
## MEDI 228

### Harnessing a catalytic lysine residue for the rapid, one-step preparation of homogeneous antibody-drug conjugates

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Antibody drug conjugates (ADCs) are emerging as a promising class of cancer therapeutics. The strategy of conjugating potent cytotoxic compounds to highly specific antibodies has already been proven effective with two FDA approved ADCs and >60 in clinical trials. Although ADC development has progressed rapidly, a major challenge has been the production of homogeneous, site-specific ADCs that have defined drug loading. Site-specific ADCs are expected to have well defined pharmacokinetic and pharmacodynamic properties and should translate more quickly from preclinical to clinical investigations. Even though there are several strategies to produce site-specific ADCs, they all rely on mutations or inefficient conjugation chemistries. Here we present a novel strategy to produce site-

specific ADCs using a highly reactive natural buried lysine embedded in a dual variable domain (DVD) format. This approach is mutation free and drug conjugation proceeds rapidly at neutral pH in a single step without removing any charges. The conjugation chemistry is highly robust, enabling the use of crude DVD for ADC preparation. In addition, our strategy affords to precisely monitor the efficiency of drug conjugation with a catalytic assay. ADCs targeting HER2 were prepared and demonstrated to be highly potent and specific *in vitro* and *in vivo*. Furthermore, the modular DVD platform was used to prepare potent and specific ADCs targeting CD138 and CD79B, two clinically established targets overexpressed in multiple myeloma and Non-Hodgkin lymphoma, respectively.



## MEDI 229

### Dual inhibition of the oncoproteins MCL-1 and BCL-2 by rationally designed polypharmacology

**Brandon Drennen<sup>2</sup>, bdrennen\_92@umaryland.edu, Samuel J. Hughes<sup>3</sup>, Steven Fletcher<sup>1</sup>.** (1) Dept of Pharmaceutical Sciences, University of Maryland School of Pharmacy, Baltimore, Maryland, United States (2) Pharmaceutical Sciences, University of Maryland School of Pharmacy, Baltimore, Maryland, United States (3) Cardiff University, Cardiff, United Kingdom

Apoptosis, or programmed cell death, is controlled by the B-cell lymphoma 2 (BCL-2) family of cellular proteins, which contains both pro-apoptotic (e.g. BAK, BAX, BIM and NOXA) and anti-apoptotic (e.g. BCL-2, BCL-XL and MCL-1) members. The pro-apoptotic members can be further divided into the BCL-2 effectors (BAK, BAX), multi-BH domain proteins located within the mitochondria membrane, and the BH3-only activators (BID, NOXA), proteins

that solely express the  $\alpha$ -helical BH3 domain of the effector proteins. Under homeostatic conditions, the effector proteins'  $\alpha$ -helical BH3 domain interacts with the hydrophobic binding groove on the surface of the anti-apoptotic proteins, capturing the effector proteins and blocking apoptosis. Once a cell is exposed to apoptotic stress, it expresses the BH3-only activator proteins which release the effector proteins from sequestration to initiate apoptosis. Various human cancers exploit this pathway by upregulating the expression of the anti-apoptotic proteins, resulting in the capture of the BH3-only proteins before they can release the effector proteins, thus inhibiting apoptosis. A current strategy deployed to combat this oncogenic transformation is BH3 mimicry, that is the development of small molecules that can mimic the  $\alpha$ -helical BH3 domain and thereby free up the native BH3-only proteins to initiate apoptosis. BH3 mimetics have shown promising activity in clinical studies. Indeed, ABT-199, a selective BH3 mimetic for BCL-2, has recently been approved by the FDA for chronic lymphocytic leukemia. Unfortunately, resistance has been observed in cancer cells exposed to ABT-199, manifested by the upregulation of MCL-1 as a compensatory mechanism to counter BCL-2 inhibition. Hence, to overcome the resistance mechanism associated with ABT-199 and to avoid thrombocytopenia, which is a known consequence of BCL-XL inhibition in platelets, we will report our progress towards the development of dual MCL-1/BCL-2 inhibitors via a hybridization strategy between fragments of potent MCL-1 and BCL-2 inhibitors.

## MEDI 230

### Novel HIV-1 protease inhibitors: Design, synthesis, and biological evaluation

***Heather L. Osswald, hosswald@purdue.edu. Purdue University, West Lafayette, Indiana, United States***

HIV-1 protease inhibitors are vital members of highly-active antiretroviral therapy (HAART). Upon the introduction of HAART, the mortality and morbidity rate for HIV/AIDS patients receiving HAART therapy has substantially decreased. Still, the emergence of multi-drug resistant (MDR) strains of HIV often renders current therapies inadequate. The design of novel, highly potent HIV-1 protease inhibitors is necessary to address the MDR viral issue. The design, synthesis, and biological evaluation of novel classes of HIV-1 protease inhibitors will be discussed. 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. Design strategies are focused on the optimization of the non-prime

side of the active site. Hydrogen bonding interactions can be optimized, particularly in the S2 subsite of the active site. Modulation of the P1 hydrophobic ligand indicates an expanded hydrophobic space in the S1 subsite. Furthermore, the optimization of van der Waals interactions in the S1-S2 subsites resulted in exceedingly potent inhibitors. Inhibitors of all classes proved to be potent against HIV in enzymatic and antiviral assays. Selected inhibitors were tested further against multi-drug resistant HIV. X-ray crystallographic elucidation of an inhibitor-enzyme co-crystal provided structural information into the binding mode of each inhibitor class.

## MEDI 231

### **From endocrine regulation to bacterial quorum sensing (QS): Design and optimization of compounds for the treatment of endocrine disorders and infectious diseases**

**Rolf W. Hartmann**<sup>1,2</sup>, rolf.hartmann@helmholtz-hzi.de, **Qingzhong Hu**<sup>1,2</sup>,  
**Chris van Koppen**<sup>2,3</sup>, **Sandrine Marchais-Oberwinkler**<sup>2</sup>, **Christine Maurer**<sup>1</sup>,  
**Martin Empting**<sup>1</sup>. (1) Department of Drug Design and Optimization, Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Saarbrücken, Germany (2) Pharmaceutical and Medicinal Chemistry, Saarland University, Saarbrücken, Germany (3) Elexopharm GmbH, Saarbrücken, Germany

Steroid hormones are essential for many physiological processes. Acting similarly, QS signal molecules regulate the cell-to-cell (c2c) communication in bacteria and thereby control important pathogenic processes like virulence and biofilm formation. As steroid hormones are also associated with severe diseases, inhibiting the biosynthesis of the corresponding hormone has turned out to be an effective therapeutic strategy. In bacteria, on the other hand, interference with the c2c communication has recently emerged as an alternative anti-infective treatment. In this talk examples of our recent achievements in both fields will be presented.

Selective inhibitors of mineralo- and glucocorticoid biosynthesis have only recently come into the focus of research efforts. This was due to the fact that the homology between aldosterone synthase (CYP11B2) and cortisol synthase (CYP11B1) is very high and obtaining selective inhibitors was considered impossible. Nevertheless, we could develop highly active and selective CYP11B2 inhibitors as candidates for the treatment of several cardiovascular diseases as well as potent inhibitors of CYP11B1 for treating Cushing's syndrome and promoting chronic wound healing. Research efforts for the treatment of hormone-dependent diseases are not only focused on steroid biosynthesis in the endocrine glands. A more targeted approach

addresses the activation or deactivation of the steroid in the target cell. For the modulation of estrogen activity, 17 $\beta$ -hydroxy-steroid dehydrogenases are responsible. As one example of our work in this field, the design and optimization of highly active and selective 17 $\beta$ HSD2 inhibitors for treating osteoporosis and bone fracture healing will be described.

The blockade of bacterial pathogenicity without affecting cell viability is a new paradigm for the treatment of bacterial infections. In contrast to conventional antibiotics such compounds should not instigate selection pressure and treated bacteria should thus be less prone to resistance development. We developed the first compounds which interfere with the PQS c2c communication of *P. aeruginosa*. Inhibitors of PqsD, an enzyme involved in the biosynthesis of the QS molecule PQS, and antagonists of the PQS receptor PqsR were designed, synthesized and shown to block biofilm and virulence factor formation. Importantly, they were active in an in vivo infection model.

## MEDI 232

### **Activity-based proteomics: Protein and ligand discovery on a global scale**

**Ben F. Cravatt**, 30404955@acs.org. *The Scripps Research Institute, La Jolla, California, United States*

Genome sequencing projects have revealed that eukaryotic and prokaryotic organisms universally possess a huge number of uncharacterized proteins. The functional annotation of these proteins should enrich our knowledge of the biochemical pathways that support human physiology and disease, as well as lead to the discovery of new therapeutic targets. To address these problems, we have introduced chemical proteomic technologies that globally profile the functional state of proteins in native biological systems. Prominent among these methods is activity-based protein profiling (ABPP), which utilizes chemical probes to map the activity state of large numbers of proteins in parallel. In this lecture, I will describe the application of ABPP to discover and functionally annotate proteins in mammalian physiology and disease. I will also discuss the generation and implementation of advanced ABPP platforms for proteome-wide ligand discovery.

## MEDI 233

### **Curing HIV infection: Going beyond N = 1**

**Robert F. Siliciano**<sup>1,2</sup>, rsiliciano@jhmi.edu. (1) Department of Medicine, Howard Hughes Medical Institute, Chevy Chase, Maryland, United States. (2) Molecular Biology and Genetics, Pharmacology and Molecular Sciences, and Biology, Johns Hopkins University, Baltimore, Maryland, United States

Only a single patient has been cured of HIV infection despite the availability of extremely effective antiretroviral drugs. This talk will discuss the unexpected finding that some antiviral drugs have highly cooperative dose response curves that allow near complete inhibition of viral replication. The cure of hepatitis C infection will be discussed in this context. The reason that HIV infection cannot be similarly cured is a small reservoir of resting memory CD4+ T cells harboring a latent, non-replicating form of the virus. The challenges involved in the ongoing search for small molecules that target this latent reservoir will be described.

## MEDI 234

### **Exploring epigenetic regulatory proteins and their inhibition for HIV latency disruption**

**Lindsey I. James**, ingerman@email.unc.edu. Center for Integrative Chemical Biology and Drug Discovery, UNC Chapel Hill, Chapel Hill, North Carolina, United States

Current antiviral treatments are primarily limited to drugs targeting viral enzymes that are present during an active, productive infection, while latent virus persists under these regimens. Manipulation of viral latency via forced reactivation has emerged as a promising strategy for viral eradication in chronically infected patients. Chemical strategies that promote viral reactivation by disrupting repressive epigenetic processes represent an exciting step toward a cure for HIV. Proof of concept studies in reactivating HIV transcription with histone deacetylase (HDAC) and bromodomain inhibitors have yielded promising results; however, many of these compounds exhibit only modest latency reversing activity and only a limited number of chemical tools are currently available. A better understanding of the epigenetic pathways that influence proviral latency and novel chemical tools are clearly needed to develop fully effective latency-reversing agents (LRAs) or combinations of LRAs. Our current efforts are aimed at 1) defining new epigenetic regulators involved in maintaining the latent HIV population, 2) discovering first-in-class chemical probes of epigenetic regulators hypothesized to repress viral transcription, and 3) characterizing novel combinations of LRAs by screening combinations of kinase signaling inhibitors

and chromatin targeted agents. We are particularly interested in the components of and reagents that target repressive complexes that are involved in chromatin regulation, such as the Polycomb Repressive Complexes (PRC1 and PRC2) and the Human Silencing Hub (HUSH) complex. Taken together, we hope that these studies will function as a stepping stone to uncover new regulators of HIV and LRAs, or combinations of LRAs, which have the potential to pave the way for future therapeutic interventions to treat this disease.

## MEDI 235

### **Long acting HIV antiretroviral agents: Moving beyond one pill once a day**

**Brian A. Johns**, *brian.a.johns@gsk.com*, **Emile Velthuisen**. *GlaxoSmithKline, Efland, North Carolina, United States*

The discovery and development of antiretroviral agents for the treatment of HIV/AIDS has changed the prognosis of infection from a death sentence to a manageable chronic disease, and represents one of the most miraculous accomplishments in science and medicine over the past three decades. There are now over thirty drugs and drug combinations available for physicians to build an appropriate regimen to battle the virus in the context of needing life-long therapy. All of these drugs need to be dosed daily or more frequently and with one exception all are oral formulations. In the context of chronic daily therapy, patients are challenged with needing to be highly adherent to taking their medicines as well as burdened with a daily reminder of their underlying disease. Our group has been thinking beyond the one pill once a day mantra that has driven the current standard of care options and setting a new objective of designing treatment options that require infrequent dosing in the realm of once a month or less. This presentation will cover the discovery and preclinical development of new antiretroviral agents from our laboratories as well as the evolution of this new area of HIV research.

## MEDI 236

### **Second generation HIV-1 maturation inhibitors: The discovery of BMS-955176**

**Alicia Regueiro-Ren**, *alicia.regueiroren@bms.com*. *Medicinal Chemistry, Bristol Myers Squibb, Middletown, Connecticut, United States*

Clinical validation of virus maturation inhibition as an approach to the control of HIV-1 infection was first achieved by bevirimat (BVM), a first generation maturation Inhibitor (MI). Poor polymorphic coverage as well as challenges with drug formulation prevented BVM from being further developed. BMS-955176 is a second generation HIV-1 maturation inhibitor that combines a broader spectrum of antiviral activity with good oral exposure in preclinical species. Both BVM and BMS-955176 are derivatives of the triterpenoid belutinic acid; however, key structural differences between the two compounds provided the targeted improvement in virological and pharmacokinetic profiles displayed by BMS-955176. The structure-activity studies leading to the design, synthesis, and preclinical characterization of BMS-955176 will be discussed.

## MEDI 237

### **Phosphonamide prodrugs GS-7340 (tenofovir alafenamide) and GS-9131 for the treatment of HIV**

**Richard L. Mackman, rmackman@gilead.com. Medicinal Chemistry, Gilead Sciences, Foster City, California, United States**

Since the discovery of nucleoside reverse transcriptase inhibitors (NRTIs) for the treatment of HIV, efforts to improve the potency, resistance profile, and long term safety have continued. GS-7340 (tenofovir alafenamide, TAF) is a phosphonamide prodrug of the acyclic NRTI tenofovir (TFV) that was recently approved for the treatment of HIV and HBV. Compared to the prodrug tenofovir disoproxil fumarate (TDF), GS-7340 preferentially delivers TFV to immune cells and tissues resulting in a lower oral dose and improved long term safety profile. There is also a need for new NRTIs with potent activity against HIV viral strains with NRTI resistance. GS-9131 is a phosphonamide prodrug of a novel cyclic nucleoside phosphonate, GS-9148 (2'Fd4AP). GS-9131 demonstrates a superior resistance profile compared to all approved NRTIs and importantly a low potential for mitochondrial toxicity that supports its clinical development for treatment experienced patients with NRTI resistance mutations. The preclinical discovery and profiling of these prodrugs, along with clinical data supporting the approval of GS-7340 and the potential of GS-9131, will be presented. Additionally, there is an interest in application of nucleoside phosphonate antivirals toward RNA viruses. Aided by structural studies in HIV reverse transcriptase, the challenges associated with the extension of the class of nucleoside phosphonate inhibitors to RNA viruses will be discussed.

## **MEDI 238**

### **Allosteric inhibitors of HIV-1 integrase**

**Mamuka Kvaratskhelia**, *kvaratskhelia.1@osu.edu. College of Pharmacy, The Ohio State University, Columbus, Ohio, United States*

Evolution of HIV-1 strains resistant to current therapies is a major clinical problem in the fight against AIDS. Therefore, new inhibitors with alternative mechanisms of action are needed. Allosteric HIV-1 integrase (IN) inhibitors (ALLINIs) have recently emerged as a promising class of antiretroviral agents and are currently in clinical trials. Our research has focused on dissecting the mode of action of these inhibitors. ALLINIs target the clinically unexploited dimer interface of HIV-1 IN and exhibit dual function *in vitro*: impairing IN binding to its cellular cofactor LEDGF/p75 and promoting IN aggregation. Unexpectedly, in infected cells ALLINIs were significantly more potent during virion maturation rather than integration. These inhibitors induced hypermultimerization of IN in virions and yielded eccentric, non-infectious virus particles, where the ribonucleoprotein complexes (RNPs) were mislocalized outside the protective capsid core. While the essential catalytic role of IN during HIV-1 integration into the human genome is well established, our findings have prompted us to investigate the non-catalytic function of IN during virion maturation. We have found that IN binds the viral RNA genome and ensures the correct localization of RNPs within protective capsid core. ALLINIs inhibited IN binding to the viral RNA genome in virions of wild type but not the escape mutant virus. Collectively, these findings have uncovered a non-catalytic function of IN during virion maturation and elucidated the mode of action of ALLINIs. Our current efforts are focused on developing improved ALLINIs with novel scaffolds using crystallographic screening of fragments which bind to the catalytic core domain of IN followed by a fragment expansion approach to obtain potent inhibitors. The ultimate goal of these studies is to develop clinically useful ALLINIs.

## **MEDI 239**

### **Mutant muscarinic receptors as novel chemogenetic tools to identify new therapeutic targets**

**Jürgen Wess**, *jwess@helix.nih.gov. Mol. Signaling Section, LBC, NIH-NIDDK, Bethesda, Maryland, United States*

Mutant muscarinic receptors referred to as DREADDs (designer receptors exclusively activated by designer drug) represent powerful novel chemogenetic tools to study the physiological relevance of signaling pathways activated by different functional classes of G protein-coupled receptors (GPCRs). Structurally, DREADDs are mutant muscarinic receptors that can be activated by clozapine-N-oxide (CNO), an otherwise pharmacologically inert agent, with high potency and efficacy. Importantly, these new designer receptors cannot be activated by acetylcholine, the endogenous muscarinic receptor agonist. At present, muscarinic receptor-based DREADDs that selectively activate  $G_{q/11}$ ,  $G_{i/o}$ , or  $G_s$  are available. Moreover, we developed an  $M_3$  muscarinic receptor-based DREADD that is unable to active  $G_{q/11}$  but retains the ability to recruit b-arrestins and initiate beta-arrestin-dependent signaling in response to CNO treatment. More recently, we also generated a  $G_{q/11}$ -biased,  $M_3$  muscarinic receptor-derived DREADD that lacks the ability to interact with beta-arrestins. During the past few years, we expressed DREADDs with different coupling properties in a cell type-specific fashion in mice, with primary focus on metabolically relevant cell types including hepatocytes, pancreatic beta-cells, and certain neuronal subpopulations of the hypothalamus. CNO treatment of the DREADD-expressing animals leads to the selective stimulation of distinct GPCR signaling pathways only in the DREADD-expressing cells. This approach makes it possible to assess the *in vivo* consequences of activating distinct GPCR signaling pathways in specific cell types. Clearly, such studies are difficult to perform with ligands targeting native GPCRs which are typically expressed in multiple tissues and cell types. These studies clearly demonstrated that DREADDs represent highly useful tools to delineate GPCR-dependent signaling pathways that can be targeted for the treatment of various pathophysiological conditions including type 2 diabetes and obesity.

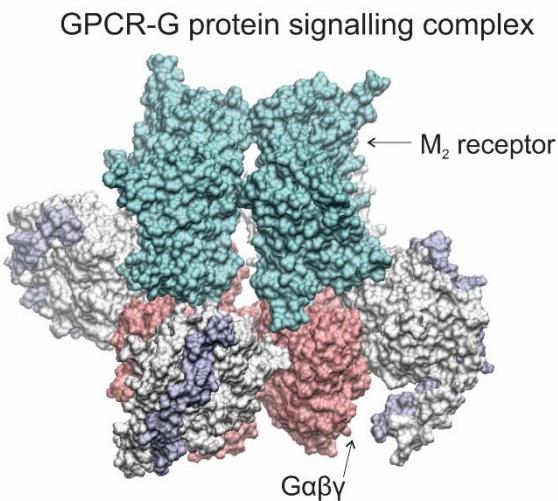
## **MEDI 240**

### **Allosteric regulation and oligomerization of muscarinic cholinergic receptors**

**Rabindra V. Shivnaraine**, [rvshivnaraine@gmail.com](mailto:rvshivnaraine@gmail.com). Molecular Cellular Physiology, Stanford University, Stanford, California, United States

Muscarinic cholinergic receptors have served as prototypical models for understanding signaling and allostery in GPCRs. However, aspects of signaling related to the role of oligomers on the interaction between the receptor and G proteins and of the receptor and allosteric ligands were largely

unexplored. Monomers and oligomers of the M<sub>2</sub> muscarinic receptor therefore have been compared to identify properties that are gained in oligomers. Allosteric interactions were monitored by means of a FRET-based sensor of conformation at the allosteric site and in pharmacological assays involving mutants engineered to preclude intramolecular effects. Allosteric effects in monomers were exclusively negative and derived primarily from intramolecular electrostatic repulsion between the allosteric and orthosteric ligands. Allosteric effects in oligomers could be positive or negative, depending upon the allosteric-orthosteric pair, and they arose from interactions within and between the constituent protomers. The size of the oligomer was determined by single-particle photobleaching of immobilized complexes of eGFP-tagged M<sub>2</sub> muscarinic receptor complexes. The method was calibrated using multiplexed controls comprising 1–4 copies of fused eGFPs. The patterns indicated that the receptor was a tetramer and the structural feasibility of a tetrameric complex was demonstrated in molecular dynamics (MD) simulations. Such MD simulations were also used to examine electrostatic, steric, and conformational determinants of allostery at the atomic level. The complex pharmacological behavior observed for oligomers and various engineered oligomers is characteristic of muscarinic receptors measured in binding assays from myocardial preparations from porcine tissue.



## MEDI 241

### Convulsion and cholinergic toxicity of subtype selective M<sub>1</sub> positive allosteric modulators (PAMs)

**Jennifer E. Davoren**, *jennifer.e.davoren@pfizer.com. Pfizer, Cambridge, Massachusetts, United States*

It was hypothesized that selective muscarinic M<sub>1</sub> activation could be a strategy to provide cognitive benefits to schizophrenia and Alzheimer's disease patients while minimizing the cholinergic side effects observed with nonselective muscarinic orthosteric agonists. Recent data disputes this hypothesis by demonstrating that activation of the M<sub>1</sub> receptor by subtype-selective positive allosteric modulators (PAMs) contributes to the gastrointestinal (GI) and cardiovascular (CV) cholinergic adverse events (AEs) previously attributed to M<sub>2</sub> and M<sub>3</sub> activation. These studies were completed using PAMs that also exhibited allosteric agonist activity leaving the possibility that direct agonism, rather than allosteric modulation, could be responsible. To test this hypothesis we compared the safety of three structurally distinct M<sub>1</sub> PAMs with varying degrees of allosteric agonism. The potency of each compound was measured in mouse striatum using an ex-vivo inositol monophosphate (IP<sub>1</sub>) accumulation assay. Safety TI's were calculated using the unbound compound concentration in the brain corresponding to a 2-fold increase of IP<sub>1</sub> as the denominator. Holistically we found that the safety risks presented by all three ligands are similar, which suggests that convulsion and GI AE's are not compound or chemotype specific.

## **MEDI 242**

### **Targeting positive allosteric modulators of the M<sub>1</sub> muscarinic receptor: Identification of MK-7622**

**Douglas C. Beshore**, *douglas\_beshore@merck.com. Medicinal Chemistry, Merck & Co., Inc., Lower Gwynedd, Pennsylvania, United States*

Addressing the cognitive symptoms of patients afflicted with Alzheimer's disease represents a critical unmet medical need. Restoring cholinergic signaling, specifically via excitation of the M<sub>1</sub> signaling pathway, has been targeted for several decades. Identification of truly selective muscarinic agonists has been hampered due to high sequence homology for these receptors at the orthosteric site, resulting in non-selective agonists like xanomeline. In recent years, targeting allosteric activation has proved more fruitful, resulting in the identification of highly selective, positive allosteric modulators. Herein, we detail and disclose our efforts that led to the discovery of MK-7622, a positive allosteric modulator of the M<sub>1</sub> receptor.

## MEDI 243

### Discovery, development, mechanistic insights and therapeutic potential of M<sub>4</sub> PAMs

**Craig W. Lindsley**, *craig.lindsley@vanderbilt.edu. Dept of Pharmacology, Vanderbilt University, Nashville, Tennessee, United States*

The seminal finding that the M<sub>1</sub>/M<sub>4</sub>-preferring muscarinic acetylcholine receptor (mAChR) agonist xanomeline demonstrated robust antipsychotic efficacy in schizophrenia patients generated a major interest in developing selective M<sub>1</sub> and M<sub>4</sub> agonists and understanding the roles of these receptors. Unfortunately, all known mAChR agonists are non-selective and activate peripheral M<sub>2</sub> and M<sub>3</sub> receptors, leading to adverse effects. By targeting allosteric sites on mAChRs, we and others have succeeded in identifying highly subtype-selective positive allosteric modulators (PAMs) of individual mAChR subtypes that avoid activation of peripheral mAChRs. Interestingly, highly selective M<sub>4</sub> PAMs have robust antipsychotic-like effects in multiple rodent models as well as cognition. However, M<sub>4</sub> PAMs have progressed more slowly for many reasons, including species differences in M<sub>4</sub> PAM potency (i.e., affinity and cooperativity), challenges with respect to M<sub>2</sub> selectivity, and P-gp efflux as well as limited chemical diversity. Clearly, these are significant roadblocks en route to an M<sub>4</sub> PAM preclinical candidate. In this talk, I will detail our navigation of these issues within the VU0467154 series of M<sub>4</sub> PAMs, leading to the discovery of a potent, selective, and orally bioavailable M<sub>4</sub> PAM VU0467485/AZ13713945 with robust efficacy in behavioral models that was evaluated as a preclinical candidate. While the mechanisms by which M<sub>4</sub> PAMs exert their behavioral effects are not entirely clear, these compounds reverse multiple *in vivo* effects of psychomotor stimulants that induce increases in extracellular dopamine. These studies raise the possibility that M<sub>4</sub> PAMs may act by inhibiting DA release from midbrain DA neurons. However, the hypothesis that selective M<sub>4</sub> PAMs inhibit DA release has not been directly tested. Surprisingly, both the sustained inhibition of DA release and antipsychotic-like behavioral effects induced by an M<sub>4</sub> PAM were found to require intact endocannabinoid (eCB)-mediated CB<sub>2</sub> cannabinoid receptor signaling. Taken together, these studies identify a novel signaling pathway through which activation of M<sub>4</sub> receptors on SPNs dampens dopaminergic signaling and highlights the importance of this pathway to the antipsychotic-like efficacy observed with M<sub>4</sub> PAMs.

## MEDI 244

### Discovery and clinical progression of highly selective M<sub>1</sub> agonists utilizing structure-based drug design

**Giles A. Brown**, *giles.brown@heptares.com. Heptares Therapeutics Ltd, Cambridge, United Kingdom*

G protein-coupled receptors (GPCRs) are an important and long-standing family of drug targets. Despite many historical success stories, today there are still a significant number of GPCRs with compelling pre-clinical validation that remain highly challenging for drug discovery. Over the last 8 years there has been significant progress in the structural biology of GPCRs facilitating Structure-Based Drug Design (SBDD) approaches. Heptares uses its proprietary StaR® technology to thermostabilise GPCRs by mutagenesis into a precisely defined biologically-relevant conformation. StaR® proteins are amenable to techniques that cannot be readily used with wild-type GPCRs, including fragment screening, biophysical kinetic profiling and X-ray crystallography.

There is currently a major unmet medical need in the treatment of Alzheimer's disease, where current agents typically offer only modest and transient cognitive benefit. Muscarinic M<sub>1</sub> receptor agonism has shown clinically efficacy in the treatment of both Alzheimer's dementia and cognitive impairment associated with schizophrenia, yet previous compounds lacked the necessary selectivity against receptor sub-types and resulted in undesired cholinergic side effects.

Utilizing the proprietary StaR® technology the structures of both muscarinic M<sub>1</sub> and M<sub>4</sub> receptors in their agonist conformations have been solved, with multiple agonist ligands, and will be disclosed. These structural insights have been instrumental in the design of highly selective M<sub>1</sub> agonists with good ligand efficiency and drug-like properties. The chemical structure, SAR development and in vivo pharmacological data of a series of highly selective M<sub>1</sub> agonists will be disclosed. This work has led to the first highly selective M<sub>1</sub>agonist candidate (HTL9936) to entered clinical trials.

## MEDI 245

### Design of liver-targeting, glucose-responsive insulin

**Dmitri A. Pissarnitski, dmitri111@hotmail.com, Songnian Lin, Lin Yan, Zhiqiang Zhao, Ahmet Kekec, Yuping Zhu, David N. Hunter, Pei Huo, Danqing Feng, Christopher Moyes, Brenda Pipik, Joseph L. Duffy, Erin Guidry, James Mu, Margaret Van Heek, Peter Zafian, Terri Kelly, Ester Carballo-Jane, Ravi P. Nargund. Merck Research Laboratories, Kenilworth, New Jersey, United States**

Insulin therapy is required in all patients with type 1 diabetes, and is being increasingly used in type 2 diabetes. Therapeutic insulin is typically administered via SC injections. By contrast, insulin in healthy individuals is secreted by pancreas into the hepatic portal vein, delivering up to 60% of total insulin into the liver. With SC insulin delivery, liver is relatively under-insulinized while the periphery is over-insulinized, compared to distribution of endogenous insulin. Furthermore, the dose has to be carefully chosen for individual patients to avoid overdose leading to potentially life-threatening hypoglycemia, and injections need to be properly timed to compensate for post-prandial glucose excursions.

We have studied neo-glycoconjugates of recombinant human insulin (RHI) with carbohydrate ligands for the endogenous mannose receptor (MR). After in vivo dosing, the drug targets both insulin receptor (IR) and MR. The biodistribution of the drug between IR and MR is dependent on the concentration of glucose in plasma. At the state of hyperglycemia, high levels of glucose reduce the ability of MR to bind the drug, and thus a larger proportion of the drug is being channeled to IR, providing “insulin on demand”. As MR is largely expressed in the liver, these glycoconjugated insulins predominantly function in this organ.

## **MEDI 246**

### **Identification of potent and selective covalent monoacylglycerol lipase (MAGL) inhibitors for treatment of neuroinflammation**

**Laura A. McAllister<sup>1</sup>, laura.mcallister@pfizer.com, Elizabeth M. Beck<sup>1</sup>, Michael A. Brodney<sup>1</sup>, Christopher Butler<sup>1</sup>, Adam M. Gilbert<sup>2</sup>, Anthony R. Harris<sup>2</sup>, Christopher J. Helal<sup>2</sup>, Douglas S. Johnson<sup>1</sup>, Scot Mente<sup>1</sup>, Justin I. Montgomery<sup>2</sup>, Steven V. O'Neil<sup>2</sup>, Justin R. Piro<sup>1</sup>, Bruce N. Rogers<sup>1</sup>, Tarek Samad<sup>1</sup>, Damien Webb<sup>1</sup>. (1) Pfizer Inc., Cambridge, Massachusetts, United States (2) Pfizer Inc., Groton, Connecticut, United States**

Monoacylglycerol Lipase (MAGL) is the key enzyme controlling brain levels of the endocannabinoid 2-arachidonylglycerol (2-AG). MAGL catalyzes 2-AG

degradation to arachidonic acid (AA), which is a precursor to pro-inflammatory eicosanoids such as prostaglandins. Inhibiting MAGL has been demonstrated to reduce inflammation in a bi-directional manner. By reducing levels of arachidonic acid, levels of downstream inflammatory mediators are reduced. Additionally, the increased 2-AG levels activate the cannabinoid pathway through CB1 receptor agonism, which has been reported to be beneficial in reducing neuroinflammation. Centrally active MAGL inhibitors with a suitable profile are therefore targeted for the treatment of conditions where neuroinflammation is a significant feature.

We will report the discovery of a novel series of carbamate based irreversible MAGL inhibitors, which feature a heterocyclic core and a trifluoromethyl glycol leaving group. Compounds from this class are in improved physicochemical property space relative to literature tool inhibitors, allowing for improved oral drug properties. SAR efforts to identify an optimum leaving group which balances MAGL potency with serine hydrolase selectivity will be described. A key aspect of leaving group optimization is balancing inherent chemical reactivity, which is a particularly important feature in the design of any irreversible inhibitors to mitigate the safety risks of non-selective covalent binding. Lead molecules from the series with suitable ADME properties such as permeability and brain penetration achieved sufficient PK exposure for in vivo evaluation in models of neuroinflammation.

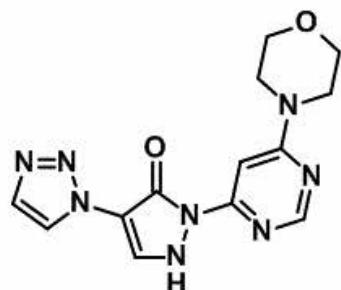
## MEDI 247

### **Discovery of molidustat (BAY 85-3934): A small-molecule oral HIF-prolyl hydroxylase (HIF-PH) inhibitor for the treatment of renal anemia**

**Hartmut Beck**, *hartmut.beck@bayer.com. Pharmaceuticals, Bayer AG, Wuppertal, Germany*

Hypoxia inducible transcription factor (HIF) is the key activator of erythropoietin (EPO) gene expression in response to hypoxia. Inhibition of HIF-prolyl hydroxylases (HIF-PHs) leading to increased EPO expression and subsequently increased erythropoiesis may therefore be suited as a novel therapeutic principle for the treatment of various forms of anemia without the increased risk of adverse cardiovascular effects seen for patients treated with rhEPO. Herein we describe – for the first time – the discovery, SAR, synthesis and proposed binding mode of novel 2,4-diheteroaryl-1,2-dihydro-3*H*-pyrazol-3-ones as orally active HIF-PH inhibitors for the treatment of anemia. From high-throughput screening hit BAY-908 – derived from our corporate compound library – lead optimization led to the identification of BAY 85-3934

(molidustat), a novel HIF PH inhibitor, currently in clinical development for the therapy of anemia in patients with chronic kidney disease (CKD).



**molidustat**

**(BAY 85-3934)**

## MEDI 248

### Discovery of potent and orally bioavailable macrocyclic FXIa inhibitors

**Wu Yang, [pz\\_1998@yahoo.com](mailto:pz_1998@yahoo.com). Discovery chemistry, Bristol-Myers Squibb, Princeton Junction, New Jersey, United States**

The novel oral anticoagulants have largely addressed many limitations of vitamin K antagonists, the previous standard of care. However, there still remains a medical need for novel antithrombotic agents with an improved therapeutic index. Human genetic and epidemiologic data suggest that FXIa inhibition may provide a new antithrombotic therapy with an improved therapeutic benefit relative to bleeding risk profile. We and others have reported preclinical models with FXIa inhibitors which show an improved therapeutic index as compared to other mechanisms. Clinical support for this concept was demonstrated with an antisense oligonucleotide which reduced FXI expression and provided efficacy superior to enoxaparin without increasing bleeding risk in a knee arthroplasty Phase II study. We have previously reported the discovery of potent 12- and 13-membered macrocyclic FXIa inhibitors and the subsequent optimization of the substitutions on the macrocycle linkers through structure-based drug design. Our initial disclosure showed that exquisite potency could be achieved using a chlorophenyltetrazole P1 group to interact with the S1 of FXIa. However, these analogs generally had poor PK properties due to their high PSA (polar surface areas). In this presentation, we will describe our effort to improve oral bioavailability by removing an H-bond donor from the P1 group via cyclization to form conformation-constrained analogs and by reducing overall PSA via removal of the tetrazole group. Further SAR studies of P1 groups as well as

heterocyclic core modifications led to the discovery of sub-nanomolar inhibitors with good oral bioavailability and selectivity against most relevant serine proteases. A key compound will be presented that showed superior PK properties (excellent oral bioavailability and low clearance) in multiple preclinical species (cyno, dog, and rat). In the rabbit models, this compound achieved robust antithrombotic efficacy at doses which did not prolong cuticle bleeding time.

## MEDI 249

### Cleavable photoprobes enable binding site identification of a gamma secretase inhibitor

**Christopher am Ende<sup>1</sup>, Christopher.amEnde@pfizer.com, Natalya Gertsik<sup>4</sup>, Kieran F. Geoghegan<sup>2</sup>, Chuong Nguyen<sup>1</sup>, Paramita Mukherjee<sup>5</sup>, Scot Mente<sup>5</sup>, Uthpala I. Seneviratne<sup>1</sup>, Douglas S. Johnson<sup>3</sup>, Yueming Li<sup>4</sup>. (1) Pfizer Inc., Mystic, Connecticut, United States (2) MS 8220-3263, Pfizer Inc., Groton, Connecticut, United States (3) Pfizer Worldwide Research Development, Cambridge, Massachusetts, United States (4) Sloan Kettering, New York, New York, United States (5) Pfizer, Groton, Connecticut, United States**

Gamma secretase cleaves the amyloid precursor protein, which results in the formation of neurotoxic A $\beta$ 42 peptides that have been implicated in the pathogenesis of Alzheimer's disease. Gamma secretase inhibitors (GSIs) have been unsuccessful in clinical trials, likely due to toxicity related to the inhibition of the NOTCH signaling pathway. Therefore, gamma secretase modulators (GSMs) have been developed to reduce the production of the A $\beta$ 42 peptides without inhibiting the processing of gamma secretase substrates. Utilizing clickable photoprobes, we have shown that GSIs and GSMs have distinct binding sites on gamma secretase, although the precise binding sites have remained elusive. In this presentation, we demonstrate a cleavable photoprobe of BMS-708163 labels the intracellular inhibitory loop of presenilin-1 within the gamma secretase complex, revealing the binding site of this class of GSIs. Furthermore, modeling of gamma secretase and the photoprobe inhibitor using molecular dynamic simulations corroborates photolabeling experiments.

## MEDI 250

### Identification of LYS228: A Novel monobactam with activity against extended spectrum $\beta$ -lactamase expressing and carbapenem-resistant enterobacteriaceae

**Anthony Casarez**<sup>1</sup>, diggshouse@yahoo.com, **Alun Birmingham**<sup>1</sup>, **Johanne Blais**<sup>3</sup>, **Vladimir Capka**<sup>3</sup>, **Richard Colvin**<sup>3</sup>, **Charles Dean**<sup>3</sup>, **Alexander Fekete**<sup>3</sup>, **Wanben Gong**<sup>4</sup>, **Ellena Growcott**<sup>3</sup>, **Hongqui Guo**<sup>3</sup>, **Xiaodong Lin**<sup>1</sup>, **Mika Lindvall**<sup>1</sup>, **Sara Lopez**<sup>3</sup>, **David McKenney**<sup>1</sup>, **Heinz Moser**<sup>1</sup>, **Dita Rasper**<sup>1</sup>, **Vijay Sethuraman**<sup>1</sup>, **Xiaoyu Shen**<sup>3</sup>, **Robert Simmons**<sup>1</sup>, **Dazhi Tang**<sup>1</sup>, **Meiliana Tjandra**<sup>2</sup>, **Nancy Turner**<sup>3</sup>, **Tsuyoshi Uehara**<sup>3</sup>, **Charles Vitt**<sup>3</sup>, **Steven Whitebread**<sup>3</sup>, **Aregahegn Yifru**<sup>1</sup>, **Xu Zang**<sup>5</sup>, **Qingming Zhu**<sup>1</sup>, **Folkert Reck**<sup>1</sup>. (1) *Global Discovery Chemistry, Novartis Institutes for BioMedical Research, Emeryville, California, United States* (2) *Aduro Biotech, Berkeley, California, United States* (3) *Novartis Institutes for BioMedical Research, Emeryville, California, United States* (4) *Suzhou Novartis Pharma Technology, Suzhou, China* (5) *Genentech, South San Francisco, California, United States*

Gram-negative infections due to multi-drug resistant bacteria have been on the rise for some time and have the potential to lead to a public health crisis. Within the  $\beta$ -lactam class of antibiotics, resistance in Gram-negative organisms is primarily mediated by expression of  $\beta$ -lactamases. Particularly concerning is the emergence of metallo- $\beta$ -lactamases (MBLs, e.g. New Delhi MBL, NDM-1), which have the power to inactivate all  $\beta$ -lactams aside from the monocyclic variant aztreonam. Unfortunately, aztreonam is unstable to many class A and C serine  $\beta$ -lactamases (SBLs), limiting its clinical use. One strategy to expand the utility of aztreonam is by co-administration with the serine  $\beta$ -lactamase inhibitor avibactam, currently under clinical investigation. We chose to address SBL stability directly through structural modification of the monobactam ring itself. Stability of analogs towards relevant  $\beta$ -lactamases was monitored by activity against an *E. coli* isogenic strain panel, each expressing a unique  $\beta$ -lactamase of the Ambler classes A, B, C and D. New monobactams demonstrated potent activity against a broad range of cephalosporin and carbapenem-resistant Enterobacteriaceae (CRE), with significantly improved stability against SBLs compared to aztreonam, while retaining stability towards MBLs. Most optimized analogs had limited activity against Gram-negative non-fermenters like *Pseudomonas aeruginosa*. Since induction of seizures through inhibition of the GABA<sub>A</sub> receptor is a class effect of  $\beta$ -lactam antibiotics, we monitored this in vitro using a GABA<sub>A</sub> yellow fluorescent protein-based assay and in vivo by intraventricular injection (IVC). The culmination of our efforts led to the identification of LYS228, a single agent monobactam with excellent activity against CRE and a low potential for CNS effects. Our data support the development of LYS228 for treatment of infections caused by CRE and extended spectrum  $\beta$ -lactamase (ESBL)-expressing Enterobacteriaceae. This agent is currently in phase I clinical trials.

## MEDI 251

### Chemoinformatic-driven design and synthesis of an RNA-targeted small molecule library

**Brittany Morgan**, *brittany.s.morgan@duke.edu*, **Jordan Forte**, **Bilva Sanaba**, **Yuqi Zhang**, **Diane Karloff**, **David Bertan**, **Amanda E. Hargrove**. *Chemistry, Duke University, Durham, North Carolina, United States*

Despite the discovery of many therapeutically relevant RNAs, there are no FDA-approved drugs that target RNA, excluding selected antimicrobials, which recognize the most abundant RNA—the ribosome. Currently, most RNA-based, small molecule screens utilize commercially available libraries, which are proposed to be biased to protein binding, leading to low hit rates for RNA and the identification of promiscuous ligands with limited efficacy *in vivo*. Therefore, the goal of this work is to identify RNA-privileged physicochemical and spatial properties, design an RNA-biased library, and synthesize RNA-targeted small molecules. To begin, a database of bioactive, RNA-binding ligands (RVD) was compiled from the literature with activity in cell culture and/or mouse models. Chemoinformatic parameters and principal moments of inertia vectors were calculated and compared to FDA-approved drugs, general RNA-binding ligands, and screening libraries to distinguish key two-dimensional properties and the molecular shape of the ligands, respectively. Three key guiding principals emerged for bioactive RNA ligands: i) compliance to medicinal chemistry rules; ii) an increase in nitrogen atom count and ligand rigidity; and iii) a statistically significant shift in rod-like character. To incorporate these properties into a synthetic combinatorial library, RETrosynthetic Combinatorial Analysis Procedure was used to fragment the RVD into building blocks, which inspired the selection and purchase of amine-based subunits. Using these subunits, a theoretical library of 2500 small molecules was generated based on a designed oxazolidinone scaffold, a RNA-privileged core with known biological activity. The small molecules that reflected the identified guiding principals were selected for synthesis. The novel scaffold was synthesized from 3-butene-1,2-diol, where the primary alcohol is first protected and the secondary alcohol is then converted to a trichloroacetyl carbamate. A copper-assisted palladium cyclization yields the oxazolidinone-based scaffold, where both protecting groups are removed under mild conditions. Utilizing the subunits, orthogonal substitution is achieved at two positions, as well as oxidation followed by amide coupling for synthesis of the selected library. The methodology developed in this work can be applied to additional RNA-privileged scaffolds to synthesize diverse RNA-

targeted libraries for the discovery of novel RNA-based chemical probes and therapeutics.

## MEDI 252

### Discovery and optimization of a novel class of selective NaV1.7 antagonists

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Nav1.7 is a voltage gated sodium channel that has been implicated in nociception and pain signaling in humans. While several non-selective sodium channel pore blockers can demonstrate efficacy, their lack of selectivity is believed to be responsible for dose-dependent side effects that limit their clinical utility. Compounds selective for NaV1.7 have recently been reported that contain a sulfonamide pharmacophore. We recently reported the Xray crystal structure of a NaV1.7-selective arylsulfonamide complexed with

voltage sensor domain 4 (VSD4). This structure shows that the anionic sulfonamide warhead forms an ion pair with Arg residues in S4 of VSD4 (Ahuga et al, 2015). We have used this structural information to design alternative anionic warheads in order to mitigate scaffold risk. Our presentation details the discovery and optimization of the first non-sulfonamide based selective Nav1.7 inhibitors that bind to VSD4 of hNaV1.7. GX-725 is a highly potent and selective Nav1.7 inhibitor that demonstrated analgesic activity in a transgenic mouse that expresses hNaV1.7 with an inherited erythromelalgia mutation is highlighted.

## MEDI 253

### **Discovery of clinical candidate GDC-0276: A selective NaV1.7 inhibitor for the treatment of pain**

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The voltage gated sodium channel Nav1.7 has been shown to be an essential component for the transmission of pain. The generation of small molecule modulators to this protein that have the appropriate potency, selectivity, and drug like properties to adequately effect pain in animals, and ultimately humans, has been a significant challenge due to multiple factors. We describe the discovery of GDC-0276, a selective and orally available inhibitor that binds to the voltage-sensing domain 4 (VSD4) of Nav1.7. This compound was discovered using a series of tailored in vitro and in vivo assays that were

designed to address some of the significant challenges with this class of inhibitors. The SAR leading to the identification of GDC-0276 through these and other assays will be highlighted.

## MEDI 254

### **Discovery and initial clinical evaluation of trigriluzole: A tripeptide prodrug of riluzole for the treatment of glutamate-associated disorders such as ataxia**

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Trigriluzole (BHV-4157, FC-4157) is a tripeptide conjugated prodrug of riluzole, the only FDA-approved treatment for amyotrophic lateral sclerosis (ALS). Riluzole increases the expression and activity of the excitatory amino acid transporters responsible for the clearance of synaptic glutamate. Additionally, riluzole modulates a broad range of ion channels to inhibit presynaptic glutamate release. Prodrugs of riluzole were developed to address variable drug metabolism and pharmacokinetics for riluzole mediated by variable Cyp1A2 levels, a negative food effect requiring fasting and twice daily (BID) dosing. Riluzole also has a 60% oral bioavailability and with its known dose-dependent liver effects, a prodrug could increase bioavailability and permit lower drug loads to the liver. A family of three generations of riluzole prodrugs comprising ~300 analogs were prepared by appending the exocyclic amine of riluzole to a functionality that would be released in the blood, a Type II prodrug approach. Trigriluzole shows excellent stability in the GI tract, is absorbed via the PepT1 transporter, and rapidly converts to riluzole upon in vivo administration. Mechanistic studies indicate the terminal amino acid is cleaved enzymatically, followed potentially, by dipeptide cyclization or further enzymatic cleavage. The preclinical safety profile of trigriluzole is similar to that of riluzole. The original synthesis of trigriluzole has been adapted to >30 kg GMP scale, with high yields and >99% purity. A Phase I clinical trial with trigriluzole showed ~30% greater bioavailability and a longer

Tmax than for riluzole itself. Trigriluzole is currently in Phase II/III clinical trials for the treatment of familial ataxia, and in Phase I trials in combination with anti-PD1 antibodies for the treatment of metastatic melanoma.

## MEDI 255

### **Allosteric antagonists of sigma-2/PGRMC1 complex: Brain penetrant orally active amyloid oligomer-displacing agents for the treatment and prevention of mild cognitive impairment and Alzheimer's disease**

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Accumulation of soluble amyloid 1-42 (Abeta) oligomers is proposed to underlie cognitive decline in mild cognitive impairment (MCI) and Alzheimer's disease (AD). An unbiased phenotypic assay in mature cultures of rat brain cells was utilized to identify small molecules that prevent the binding and synaptotoxic effects of soluble Abeta oligomers. These studies demonstrated that synthetic and human-derived soluble Abeta oligomers act as pharmacologically-behaved ligands and exhibit saturable binding at neuronal receptors. Soluble Abeta oligomers exert functional synaptotoxic effects related to their binding to neuronal surfaces. The displacement Abeta oligomers by small molecule antagonists block these effects. The small molecule amyloid oligomer-displacing agents identified in our phenotypic neuronal assay were screened against a broad panel of CNS receptors and found to be potent and specific antagonists of the sigma-2/PGRMC1 receptor complex. The Abeta oligomer-displacing drug candidates reported here were demonstrated to be orally absorbed and brain permeable in animal PK studies. Select agents restored cognitive function in transgenic hAPP Swe/Ldn mice and normalized memory in multiple AD models. These novel Abeta oligomer-displacing compounds are first-in-class small molecule drug candidates and represent a novel mechanism of action for disease-modifying MCI and AD therapy. Our superior candidate molecule CT1812 has been advanced to clinical study.

## MEDI 256

### **Discovery of RG7314: A vasopressin 1a receptor antagonist for the treatment of social communication deficits in autism spectrum disorders**

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The structurally and evolutionarily closely related neuropeptides vasopressin and oxytocin are known to play an important role in the regulation of social behavior in animals and humans. Intranasal administration of vasopressin was shown to modulate the social brain, increasing threat perception in healthy volunteers and impairing emotion recognition in men. Furthermore, elevated vasopressin levels in plasma as well as genetic variation in the vasopressin receptor 1a gene (AVPR1A) have been associated with autism. Altogether, this suggests that a vasopressin 1a (V1a) receptor antagonist has the potential to treat disorders with social emotional dysfunction, including autism spectrum disorder (ASD).

Currently no treatments are available for the ASD core social communication deficits. Evaluation of V1a receptor blockade in animal models with the potential for translational validity has so far been hampered by the lack of selective and robustly brain penetrant small molecule antagonists. In a move towards the validation of V1a receptor antagonism for the treatment of social communication deficits of autism directly in humans we had conducted a proof-of-mechanism study with RG7713, a tool compound which has a PK-PD profile suitable for humans but not rodents. Here we report the discovery of RG7314, the first V1a receptor antagonist developed for the treatment of social communication deficits in ASD. While a phase 2 study in adults has already been completed, evaluation in children and adolescents is ongoing.

## MEDI 257

### **Discovery of TAK-041: Potent and selective GPR139 agonist for treatment of negative symptoms associated with schizophrenia**

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Schizophrenia involves diminished or altered motivation, deficits in social behavior, and difficulties with complex cognitive tasks. Patients often manage their psychoses to some degree with prescription antipsychotics, but there are no effective therapies for the negative and cognitive symptoms, which remain

significant unmet medical needs.

The habenula is a small nucleus that gates information flow from higher brain centers to the monoaminergic nuclei of the midbrain and brainstem, and is essential for assigning negative value to unrewarding situations. Lesions of the habenula cause deficits in social behavior and cognitive ability, and in schizophrenics, the habenula fails to activate when the patient is challenged with a negative reward. We identified the orphan G-protein coupled receptor GPR139 as a novel excitatory Gq-coupled receptor enriched in the dorsal medial habenula, a small subregion in the habenula that has not been studied extensively. Thus, agonists of GPR139 have the potential to be first-in-class therapies for the treatment of psychiatric diseases with debilitating deficits in social domains such as negative symptoms of schizophrenia.

High-throughput screening of GPR139 yielded promising starting points for medicinal chemistry, which rapidly led to the development of compounds with sufficient potency, selectivity, and brain-penetration to be useful as *in vivo* tool molecules. *In vivo* target validation as well as strategies towards the confirmation of *in vivo* target engagement and PK/PD will be presented in the talk. Last but not least, the first-in-class Takeda GPR139 agonist and FIH molecule will be revealed for the first time.

## MEDI 258

### **Discovery of a ketohexokinase inhibitor for the treatment of NAFLD/NASH: Fragment-to-candidate via structure-based drug design and parallel chemistry**

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Inhibition of ketohexokinase (KHK, fructokinase) may ameliorate non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) by decreasing fructose conversion to fructose-1-phosphate. Initial low-molecular weight hits were identified by fragment screening; subsequent file-mining provided multiple starting points for hit-to-lead chemistry. A combination of

parallel synthesis and structure-based drug design yielded an in vivo tool compound that recapitulated the efficacy reported in a KHK-null rodent model on a high-fructose diet. Further optimization provided the clinical candidate, currently in clinical trials. This fragment-to-candidate story will present the fragment-based screen triage, compound optimization via structure-based drug design and parallel chemistry, in vivo target validation, clinical candidate selection and initial clinical data.

## MEDI 259

### 40 Years of structure-based design: What have we learned?

**Francois N. Diederich**, *diederich@org.chem.ethz.ch. Laboratory of Organic Chemistry, ETH Zürich, Zurich, Switzerland*

With more than 125 000 protein crystal structures in the Protein Data Bank (PDB) and many more unpublished in-house structures, abundant structural information has become available to successfully pursue structure-based design. Besides this essential information, another pillar of structure-based ligand design is conformational analysis, aided by more than 875 000 small molecule structures in the Cambridge Structural Database (CSD). The third pillar is the in-depth understanding of weak intermolecular interactions, including the role of solvent. An emerging fourth pillar is the simultaneous combination of structural design work with physico-chemical and ADME properties, provided by big-data mining. All these topics are addressed in today's symposium.

We pursue since the 1980ies a multi-dimensional, highly collaborative approach towards deciphering and quantifying weak intermolecular interactions in chemical and biological systems. Experimental study in this research involves the investigation of protein-ligand interactions, synthetic host-guest complexation, and dynamic processes in designed unimolecular model systems, such as molecular torsion balances. It is complemented by computational analysis and data base mining in the CSD and the PDB. Examples of intermolecular interactions quantified by this approach are orthogonal dipolar interactions, organofluorine interactions, stacking on peptide bonds, cation- $\pi$  interactions, halogen bonding, and chalcogen bonding. Enantioselective complexation based solely on shape complementarity and dispersion interactions is investigated with new chiral cage compounds, validating the 55% rule for optimal cavity filling established by Mecozzi and Rebek. Extensive protein and small-molecule crystallographic work is essential to all study. We also explore the energetics of the

replacement of conserved water molecules in protein co-crystal structures by ligand parts. This work established that drug designers can learn a lot from host-guest model systems, as Martin Stahl stated in a recent F1000 recommendation. In fact, many of the weak interactions of current interest could only be quantified in model systems. The multi-dimensional approach is illustrated in examples taken from our structure-based drug design projects. Lessons learned are directly applicable to ligand design and optimization in drug discovery and crop protection research, but equally to the assembly of synthetic supramolecular systems.

## MEDI 260

### **Binding pockets make the difference: Morphing banal water–ligand interactions into determining ones**

**Stefan G. Krimmer<sup>1</sup>, krimmer@uni-marburg.de, Jonathan Cramer<sup>1</sup>, Michael Betz<sup>1</sup>, Veronica Fridh<sup>2</sup>, Robert Karlsson<sup>2</sup>, Andreas Heine<sup>1</sup>, Gerhard Klebe<sup>1</sup>.** (1) *Pharmaceutical Chemistry, University of Marburg, Marburg, Germany* (2) *GE Healthcare Bio-Sciences AB, Uppsala, Sweden*

In any biological system, the binding reaction between an inhibitor and its target protein takes place in water. Thus, water molecules need to be considered as third player in the protein–ligand recognition process. In due course of ligand binding, both binding partners have to shed part of their solvation shell, which covers the binding interface prior to complex formation. Subsequently, water molecules re-arrange around the newly-formed, solvent-exposed surface of the protein–ligand complex. With the aim to study the thermodynamic influence of this rearrangement of water molecules, we analyzed a series of nine congeneric thermolysin inhibitors exhibiting hydrophobic side-chains of increasing size (from a methyl to a phenylethyl group) — that interact with a flat, apolar and well-solvated binding pocket of thermolysin — by high-resolution crystallography and isothermal titration calorimetry. Across the nine complexes, the crystal structures revealed water networks of different degrees of completeness adjacent to the protein-bound ligands. The observed structural differences correlated remarkably well with the differences observed between the thermodynamic binding signatures. The establishment of a well-ordered, pronounced water network resulted in an increase in enthalpy, whereas the disruption of a water network resulted in an increase in entropy. The inhibitor with the highest affinity exhibited a medium-sized hydrophobic side-chain stabilizing a pronounced water network, resulting in a highly favorable enthalpy overcompensating losses in entropy. Based on these observations, we designed new inhibitors with the aim to

further improve the water network stabilization and thereby boost affinity. Prior to ligand synthesis, we validated the newly designed ligands by predicting the putative water networks by MD simulations. As a result, one of the new ligands showed the most pronounced water network, and, consequently, the highest affinity with an overall 50-fold improvement. By facing matching pairs of polar and apolar substituents it was shown that the attachment of a charged ammonium group to the parent ligand scaffold reduces the affinity by a factor of up to 180 in  $K_d$ . Even when the polar moiety remains partly solvated or engages in strong interactions to the protein, an enthalpic penalty for the partial desolvation of the functional group is observed. The charged group does not induce a pronounced ordering of the surrounding hydration shell.

## MEDI 261

### Tales from the trenches: Case histories of exploiting surprising interactions in drug discovery

**Neysa Nevins**, neysa.2.nevins@gsk.com. UP1210, GlaxoSmithKline, Collegeville, Pennsylvania, United States

This talk will share unusual protein-ligand interactions encountered in structure-based design program work at GlaxoSmithKline. Examples include an electrostatic potential difference for a scaffold that provided insight into a four log unit potency (pIC<sub>50</sub>) difference and an electrophile that was predicted to weakly react with a cysteine (covalently) but led to a non-covalent series. Of note is that complicated, time-consuming calculations may not be needed for project impact, especially when a particular component (e.g. electrostatic) dominates a given interaction.

## MEDI 262

### Quantum mechanical approaches to structurally informed design

**Alexander Heifetz**, heifetz.alexander@gmial.com. Computational Chemistry, Evotec, Abingdon, United Kingdom

The understanding of binding interactions between any protein and a small molecule is a cornerstone of any efficient structure-based drug design (SBDD) process. X-ray crystallography and homology modelling are the main source of structural information required for rational SBDD. However, even with the crystal structure in hand, “visual inspection” and force field-based molecular mechanics calculations often used for the rationalization of ligand-protein

potency cannot always explain the full complexity of the molecular interactions. Quantum mechanical (QM) approach was always considered as promising direction to achieve this goal however, traditional QM are not feasible for large biological systems, due to their high computational cost. FMO method offers a considerable computational speed-up over traditional QM methods. One of the key features of the FMO approach is that it can provide a list of the interactions formed between the ligand and the receptor and a chemically intuitive breakdown of these interactions. Such information is essential for medicinal chemists to be able to rationally approach modification of lead compounds in order to increase favourable interactions. We will demonstrate the prospective application of FMO method in drug-discovery programs.

Recently, we have demonstrated that FMO can be even faster (secs instead of hours) without compromising the accuracy by combining it with density-functional tight-binding (DFTB) method. We will exemplified the use of FMO-DFTB in three GPCR-ligand systems.

## MEDI 263

### **Noncovalent sulfur interactions in drug design: Conformational control and intermolecular association**

*Michael D. Bartberger, michael.d.bartberger@gmail.com. Molecular Engineering, Therapeutic Discovery, Amgen, Inc., Thousand Oaks, California, United States*

As underscored by other presentations in this Symposium, rational conformational control of drug-like molecules is a key factor in the overall strategy of maximizing ligand-target binding affinity and selectivity. The ability to recognize and successfully exploit the potential of nonbonding interactions in their various forms should be an integral part of the drug discovery thought process and toolkit.

While intramolecular hydrogen bonding, lone pair-lone pair repulsion, and other more traditional nonbonded interactions are often readily recognized during SAR interpretation and utilized as a means for conformational modulation, other, more atypical interactions have gained interest in recent years. This presentation will focus on the use of noncovalent interactions involving sulfur atoms as a rational design element. Descriptions of such interactions—origins, potential applications, and limitations—on the basis of electronic structure calculations will be presented, and key successes in the published literature will be discussed.

## MEDI 264

### How significant are unusual intermolecular interactions?

**Bernd Kuhn**, bernd.kuhn@roche.com, Oliver Korb. F. Hoffmann-La Roche, Basel, Switzerland

In recent years a large number of novel interaction types have been postulated to have a stabilizing effect on protein-ligand complex formation. However, the significance for some of these “unusual” interactions has yet to be validated with experimental and theoretical studies of model systems as well as statistical analyses of crystallographic databases. We have pursued the latter approach and extended the recently published line-of-sight analysis by Taylor to protein-ligand complexes from the Protein Data Bank. With this method confounding secondary interactions are pruned out and statistically significant interaction propensities for different functional groups can be derived. In addition this approach provides insights into the geometric preferences of intermolecular contacts.

As a result of our studies we will present crystal structure based statistical analyses of different interaction types and highlight preferred protein environments of selected functional groups of relevance for medicinal chemistry. This will be complemented by illustrative examples from drug discovery projects.

## MEDI 265

### S-033188: A novel, first-in-class, orally bioavailable inhibitor of influenza virus cap-dependent endonuclease

**Makoto Kawai<sup>2</sup>**, makoto.kawai@shionogi.co.jp, **Masayoshi Miyagawa<sup>1</sup>**, **Toshiyuki Akiyama<sup>1</sup>**, **Yoshiyuki Taoda<sup>1</sup>**, **Kenji Takaya<sup>1</sup>**, **Takao Shishido<sup>1</sup>**, **Ryu Yoshida<sup>1</sup>**. (1) Shionogi Co Ltd, Toyonaka Osaka, Japan (2) Shionogi & Co., Ltd., Toyonaka, Japan

Influenza is an acute respiratory infectious disease caused by influenza virus. Neuraminidase inhibitors suppress budding and release of influenza viruses from host cells. However, there are still unmet needs for new anti-influenza drugs that have better efficacy and safety profile, and activity against resistant strains and highly pathogenic strains.

Cap-dependent endonuclease (CEN) is an enzyme residing in the PA subunit of influenza virus polymerase. CEN mediates the “cap-snatching” process during viral mRNA biosynthesis and is essential for viral replication. Therefore,

CEN is an attractive target for anti-influenza drugs.

S-033447, an active form of orally available prodrug S-033188, is a novel small molecule inhibitor of CEN, and exhibited broad and potent antiviral activity against clinically isolated influenza virus A and B strains that were collected from hospitals in Japan between 2006 and 2014 ( $EC_{50}$  in plaque reduction assay ranged from 0.20 to 0.99 nM for type A and from 4.01 to 11.26 nM for type B, data from poster# 645 of ID week in 2017). In clinical studies, S-033188 has demonstrated a favorable PK profile and evidence of anti-viral activity.

In this presentation, we will describe how we successfully discovered a novel and orally active CEN inhibitor, S-033188 and the early clinical profile.

## MEDI 266

### First time disclosure of BAY 1128688: A novel AKR1C3 inhibitor for the treatment of endometriosis

**Ulrich Bothe<sup>1</sup>, ulrich.bothe@bayer.com, Matthias Busemann<sup>1</sup>, Andreas Steinmeyer<sup>1</sup>, Peter Droscher<sup>1</sup>, Oliver-Martin Fischer<sup>1</sup>, Michael Peters<sup>1</sup>, Thomas Zollner<sup>1</sup>, Florian Sohler<sup>1</sup>, Andrea Rotgeri<sup>1</sup>, Karsten Denner<sup>1</sup>, Naomi Barak<sup>1</sup>, Margrit Hillmann<sup>2</sup>, Pascal Savy<sup>3</sup>, Nick Ray<sup>3</sup>. (1) Bayer AG, Drug Discovery, Pharmaceuticals, Berlin, Germany (2) Bayer Intellectual Property GmbH, Schoenefeld, Germany (3) Medicinal Chemistry, Charles River Laboratories, Harlow, United Kingdom**

Endometriosis is a chronic, mainly estrogen-dependent disease characterized by the presence of endometrial tissue outside the uterus in the peritoneal cavity. Aldo-keto reductase 1C3 (AKR1C3/ 17 $\beta$ -hydroxysteroid dehydrogenase type 5) is an enzyme, combining 17 $\beta$ -hydroxysteroid dehydrogenase and prostaglandin (PG) F synthase activity. AKR1C3 has been described to play a crucial role in intra-tissue steroid and prostaglandin metabolism. Expression of the enzyme in lesions is expected to lead to increased local concentrations of steroid hormones (especially estradiol) and of PGF2 $\alpha$ . This pro-estrogenic environment with increased PGF2 $\alpha$  concentration is thought to stimulate proliferation and growth of estrogen-sensitive endometriotic lesions. To demonstrate the role of AKR1C3 in endometriosis, the novel and selective AKR1C3 inhibitor BAY 1128688 was identified and tested in a marmoset monkey endometriosis model. The identification of BAY 1128688, which is currently in clinical trials, will be presented. Moreover, SAR aspects and research data related to the new compound class will be reported.

## MEDI 267

### Discovery and evaluation of clinical candidate IDH305: A brain penetrant mutant IDH1 inhibitor

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Isocitrate dehydrogenase 1 (IDH1) is a metabolic enzyme that catalyzes the oxidative decarboxylation of isocitrate to produce alpha-ketoglutarate (a-KG). Heterozygous mutations in IDH1 at Arg<sup>132</sup> cause a neomorphic catalytic activity resulting in the processing of a-KG to R-2-hydroxyglutarate (2-HG), an oncometabolite. 2-HG accumulation in tumors likely interferes with a variety of a-KG dependent enzymes that regulate epigenetic state and cellular differentiation. Therefore, inhibition of 2-HG production with small molecule inhibitors has emerged as a treatment option for patients with IDH1 mutant malignancies. Herein, we describe our efforts to identify potent and selective inhibitors of mutant IDH1. These efforts led to the discovery of **IDH305**, which demonstrated excellent physicochemical and pharmacological properties suitable for clinical evaluation. **IDH305** exhibited robust inhibition of 2-HG production and efficacy in IDH1<sup>mut</sup> xenograft models. Furthermore, brain exposure of **IDH305** was observed in multiple animal models suggesting potential utility in gliomas, which harbor a high frequency of IDH1 mutations. These data support the clinical evaluation of **IDH305** across multiple IDH1<sup>mut</sup> cancers, including AML, solid tumors, and central nervous system malignancies.

## MEDI 268

### Discovery of M2951: A selective, covalent inhibitor of BTK for the treatment of autoimmune diseases

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Bruton's Tyrosine Kinase (BTK) is a member of the TEC kinase family that is expressed in cells of hematopoietic lineage (e.g., in B cells, macrophages, monocytes, and mast cells). Btk is critical for the activation of these cells and its impairment has been shown to be beneficial in rodent models of lupus, rheumatoid arthritis, multiple sclerosis, and type 1 diabetes. Therefore, Btk inhibitors are expected to be efficacious in various autoimmune diseases, besides their already established utility in B cell malignancies. In autoimmune indications the requirements for better tolerability than in oncology dictate a higher level of kinase selectivity. Using rational drug design, and starting from scaffolds identified by a kinase platform approach, we discovered M2951, an irreversible, covalent, and selective inhibitor of BTK that exhibits an oral pharmacokinetic profile suitable for once daily dosing in man. M2951 is efficacious in different mouse and rat models of human autoimmune disease. In the mouse CIA model of auto-reactive arthritis, the ED<sub>50</sub> was 0.75 mg/kg, PO, QD. In a mouse model of SLE in monotherapy, the ED<sub>50</sub> was 1.36 mg/kg, PO, QD. Currently, M2951 is in Phase II clinical trials for various autoimmune indications, including Rheumatoid Arthritis, Systemic Lupus Erythematosus, and Multiple Sclerosis.

## MEDI 269

### Discovery of a macrocyclic peptide inhibitor of programmed death-ligand 1 (PD-L1)

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Structurally distinct classes of macrocyclic peptides were identified as inhibitors of PD-L1 through mRNA display, an *in vitro* selection technique. These classes of macrocycles demonstrated modest *in vitro* activity in PD-L1 binding assays, while they proved inactive in functional assays. Co-crystal structures of selected analogues with PD-L1 illustrated a distinct binding mode for each structural class and provided insight into the nonbonding interactions between these macrocyclic peptides and the PD-L1 protein. The structure-based insights gleaned enabled the rapid optimization of these macrocycles with respect to PD-L1 inhibitory activity and the mitigation of off-target liabilities identified in early leads. This rational drug design approach led to the discovery of a macrocyclic peptide with activity in binding and functional assays comparable to a PD-L1 antibody. Additional structural modifications led to the discovery of a PD-L1 imaging agent. Details of these discoveries will be discussed.

## MEDI 270

### Bayesian models for Chagas disease

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Over 7 million people in Latin America are infected with *Trypanosoma cruzi* (*T. cruzi*), the eukaryotic parasite that gives rise to Chagas disease. Chagas disease has also begun to gain a foothold as an expanding infection in the United States where an estimated 300,000 people may be infected. The cost of treatment in the United States alone is estimated to be over \$900 million annually showing that Chagas has the potential to have serious economic impact on the United States and the world. We previously used data from public whole-cell, phenotypic high throughput screens completed by the Broad

Institute for *T. cruzi* and generated Bayesian models that resulted in the selection of 97 compounds for *in vitro* testing. Eleven of these were found to have EC<sub>50</sub> values less than 10 μM against intracellular parasites in cell-based assay, and five compounds advanced to an *in vivo* mouse efficacy model of Chagas disease. In particular, the antimalarial pyronaridine presented an 85.2% reduction in parasite burden after 4 days of treatment, and is ready for assessment in a chronic Chagas disease model. Additional literature *T. cruzi* datasets have also been produced and collated since the completion of the *in vivo* testing. We now present examples of combining public datasets with curated data from comparable assays using an in-house software, Assay Central, and the resulting validated Bayesian machine learning models. We also describe these seven machine learning models and evaluate how our original models perform using new data as test sets. This novel approach will ultimately be used to streamline identification of additional potential candidate drugs for the treatment of not only Chagas, but other diseases.

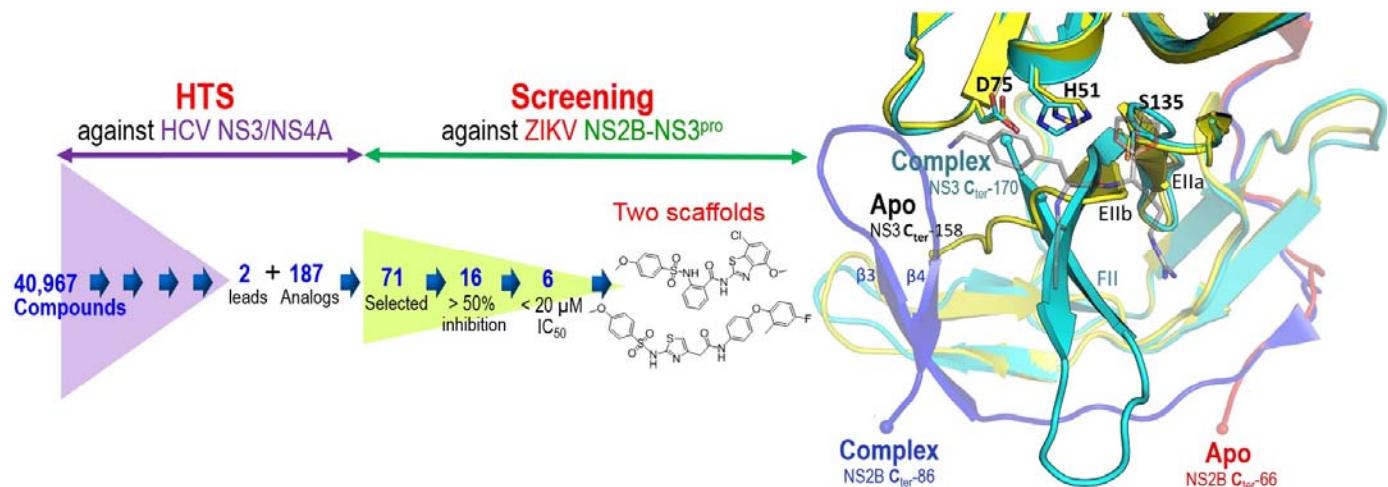
## MEDI 271

### Identification of novel small molecule inhibitors against NS2B/NS3 serine protease from Zika virus

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Zika flavivirus infection during pregnancy appears to produce higher risk of microcephaly, and also causes multiple neurological problems such as Guillain–Barré syndrome. The Zika virus is now widespread in Central and South America, and is anticipated to become an increasing risk in the southern United States. With continuing global travel and the spread of the mosquito vector, the exposure is expected to accelerate, but there are no currently approved treatments against the Zika virus. The Zika NS2B/NS3 protease is an attractive drug target due to its essential role in viral replication. Our studies have identified several compounds with inhibitory activity (IC<sub>50</sub>) and binding affinity (K<sub>D</sub>) of ~5-10 μM against the Zika NS2B-NS3 protease from testing 71 HCV NS3/NS4A inhibitors that were initially discovered by

high-throughput screening of 40,967 compounds. Competition surface plasmon resonance studies and mechanism of inhibition analyses by enzyme kinetics subsequently determined the best compound to be a competitive inhibitor with a  $K_i$  value of 9.5  $\mu\text{M}$ . We also determined the X-ray structure of the Zika NS2B-NS3 protease in a “pre-open conformation”, a conformation never observed before for any flavivirus proteases. This provides the foundation for new structure-based inhibitor design.



## MEDI 272

### Bacterial natural products as a renewed source of novel antibiotics: Isolation, characterization, and evaluation of antibacterial agents produced by soil bacteria

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The increased emergence of bacterial resistance over the past two decades has greatly reduced the effectiveness of nearly all clinical antibiotics, bringing infectious disease to the forefront as a dire threat to global health. To combat these infections, new antibiotics need to be rapidly discovered, and bacterial natural products have reemerged as an abundant source of novel bioactive molecules. Herein, the isolation and evaluation of over 400 bacteria from bulk and rhizosphere soil native to western North Carolina and the southwestern U.S. in a novel and robust liquid-based high-throughput antagonism assay against *Staphylococcus aureus* and *Escherichia coli* is presented.

Approximately 23% of bacteria screened have been found to produce an antibiotic capable of inhibiting cell growth in at least one of the pathogenic target strains. Of these more than 10 bacteria have been subjected to large

scale culture and extraction techniques to isolate the produced antibiotic. One of those, a *Pseudomonas* sp., was found to produce the natural product pseudopyronine B, and we have further improved the antibiotic activity of this natural product through SAR evaluation of the alkyl side chains.

## MEDI 273

### Targeting the influenza RNA-dependent RNA polymerase

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Influenza is an infectious disease responsible for up to 500,000 deaths worldwide annually despite the availability of vaccines and antiviral drugs. Vaccines target the most common strains of the virus, leaving those exposed to other strains at risk for infection. Most small molecule antiviral drugs currently on the market act as neuraminidase inhibitors (zanamivir, oseltamivir, peramivir) or target the M2-ion channel (amantadine, rimantadine); however, due to the lack of viral proof-reading enzymes, these targets are prone to rapid mutations that often confer antiviral resistance. In contrast, the viral RNA-dependent RNA polymerase (RdRp) is an attractive drug target because it is relatively slow to develop drug resistance, conserved across genotypes, and essential to viral replication. With no eukaryotic homologue, the potential for toxicity due to off-target effects is low for RdRp targeting compounds. Our research focuses on targeting the endonuclease domain of the RdRp, located on the PA N-terminal domain, which has a two metal binding active site. We have developed a series of 2-substituted dihydroxypyrimidine carboxamides which bind to the endonuclease active site and disrupt its activity in vitro. The activity of these compounds has been validated by fluorescent polarization binding assays and plaque inhibition assays. The most potent inhibitors have been co-crystallized with PAN to determine the structure-activity relationships, allowing us to improve their efficacy. We will discuss the structure-activity relationship of our analogs and several interesting protein-small molecule X-ray crystal structures, as well as our progress on the development of therapeutic lead compounds targeting influenza endonuclease.

## MEDI 274

### Inhibitors of the DNA repair enzyme AAG as leads for potential new chemoprotectives and stroke treatments

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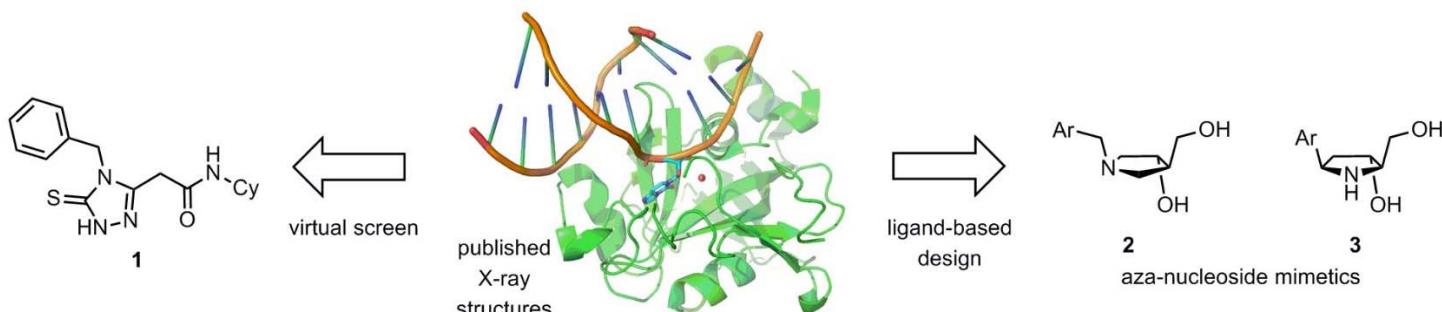
Alkyladenine glycosylase (AAG) is a DNA repair enzyme which initiates the Base Excision Repair pathway by hydrolysis of a range of alkylated, oxidised and deaminated bases from the DNA backbone. This leaves abasic sites which are further repaired by downstream enzymes. However, overactivity of AAG in mice has been associated with necrosis of certain cell types.

Specifically, in wild type mice treated with DNA alkylating agents, retina, spleen, thymus and cerebellum cell death is observed which is absent in AAG-knockout mice. Furthermore, in a brain ischemic reperfusion model, which mimics a stroke, AAG-knockout mice suffer reduced tissue necrosis compared to wild type. Both phenomena are hypothesised to result from the action of AAG on extensive DNA damage leading to the accumulation of toxic abasic sites in the DNA with which the downstream enzymes cannot keep up. Stable, membrane permeable inhibitors of AAG are required as tools to further investigate these mechanisms and to form leads to potential chemoprotectives for patients on alkylative chemotherapy and as potential rapid treatments to minimise tissue damage from stroke. We are addressing this in a two-pronged approach:

1) a virtual screen identified triazole-thione **1** as a hit inhibitor. Synthetic chemistry was optimised around this structure to provide access to analogues at the amide, linker and core.

2) small molecule aza-nucleoside mimetics **2** and **3** were designed, based on known ethenocytidine- and abasic pyrrolidine-containing DNA oligomer inhibitors. The position of the (protonated) N-atom in these may be critical for optimum hydrogen bonding with the active site nucleophilic water molecule. Asymmetric synthesis was adapted from published routes to include final, new methods to install a variety of aromatics.

Finally, a new LCMS-based biochemical assay was developed to generate IC<sub>50</sub> values of the new inhibitors.



Two-pronged approach to the discovery of inhibitors of AAG

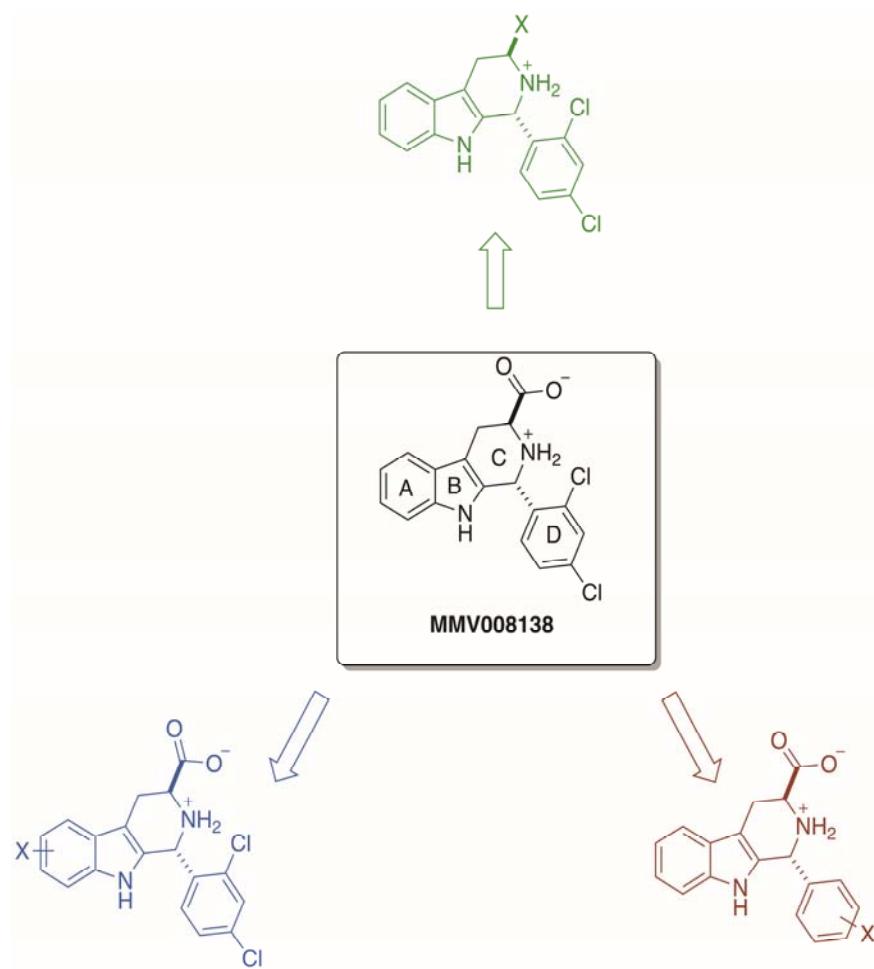
## MEDI 275

### Exploration of A, C, and D-ring SAR of the IspD-targeting antimalarial agent MMV008138

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Malaria is caused by an infection from a protozoan parasite of the genus *Plasmodium*. In 2014, it resulted in an estimated 438,000 deaths among 214 million cases. Continued emergence of drug resistance is a constant threat, therefore identification and development of new scaffolds for the development of antimalarial drugs bearing different mechanisms of action has attracted close attention. In *Plasmodium* parasites, the methylerythritol phosphate (MEP) pathway is used to synthesize isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP), which are essential for parasite growth. This pathway takes place in the apicoplast of the parasite and is absent in humans. We rely on the mevalonate pathway for IPP biosynthesis instead, thus MEP pathway inhibitors are potentially safe and effective therapeutic candidates. Among a phenotype-based screen of the 400 compounds present in the Malaria box, MMV008138 was the only compound that inhibited the MEP pathway. MMV008138 did not show a delayed death phenotype, and inhibition of apicoplast elongation was reversed by IPP supplementation. These observations in addition to the high efficacy of MMV008138 against FOS-resistant parasites suggested that its target within the MEP pathway is not IspC, and other researchers determined that the

target was a cytidylyltransferase (IspD) in the MEP pathway. We have synthesized numerous analogs of MMV008138 to study the structure-activity relationship within this scaffold on the IspD enzyme inhibition and *P. falciparum* growth inhibition. It is worth mentioning that no effect on the *E. coli* at 500  $\mu$ M was observed for MMV008138 and its potent analogs (*P. falciparum* IC<sub>50</sub> = 190-500 nM). Various A-, C- and D-ring substituted analogs in addition to carboxylic acid bioisosteric replacements are studied. Possible binding modes to *P. falciparum* IspD will be evaluated based on the enzyme inhibition data.



## MEDI 276

**Synthesis of ADMDP-typed iminosugars to develop pharmacological chaperones for the treatment of Fabry disease and potential enhancers to increase enzyme replacement therapy efficiency**

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A unique molecular library consisting of all sixteen synthetic ADMDP (1-aminodeoxy-DMDP) stereoisomers has been prepared and evaluated for inhibitory activity against  $\alpha$ -Gal A, and ability to impart thermal stabilization of this enzyme. The results of this testing led us to develop a novel pharmacological chaperone for the treatment of Fabry disease. 3-Epimer ADMDP was found to be an effective pharmacological chaperone, able to rescue  $\alpha$ -Gal A activity in the lymphoblast of the N215S Fabry patient-derived cell line, without impairment of cellular  $\beta$ -galactosidase activity. When 3-epimer ADMDP was administered with rh- $\alpha$ -Gal A (enzyme replacement therapy) for the treatment of Fabry patient-derived cell lines, improvements in the efficacy of rh- $\alpha$ -Gal A was observed, which suggests this small molecule can also provide clinical benefit of enzyme replacement therapy in Fabry disease. Besides, two polyhydroxylated pyrrolidines with the (3R,4S,5R) configuration pattern underwent rapid substituent diversity by conjugating the primary aminomethyl moiety of each with a variety of carboxylic acids to generate two libraries (2 x 60 members). Our bioevaluation results showed one member with the (2R,3R,4S,5R) configuration pattern and bearing a 5-cyclohexylpentanoyl group as a substituent moiety possessed sufficient chaperoning capability to rescue  $\alpha$ -Gal A activity in the lymphocyte of the N215S Fabry patient-derived cell line and other  $\alpha$ -Gal A mutants in COS7 cells.

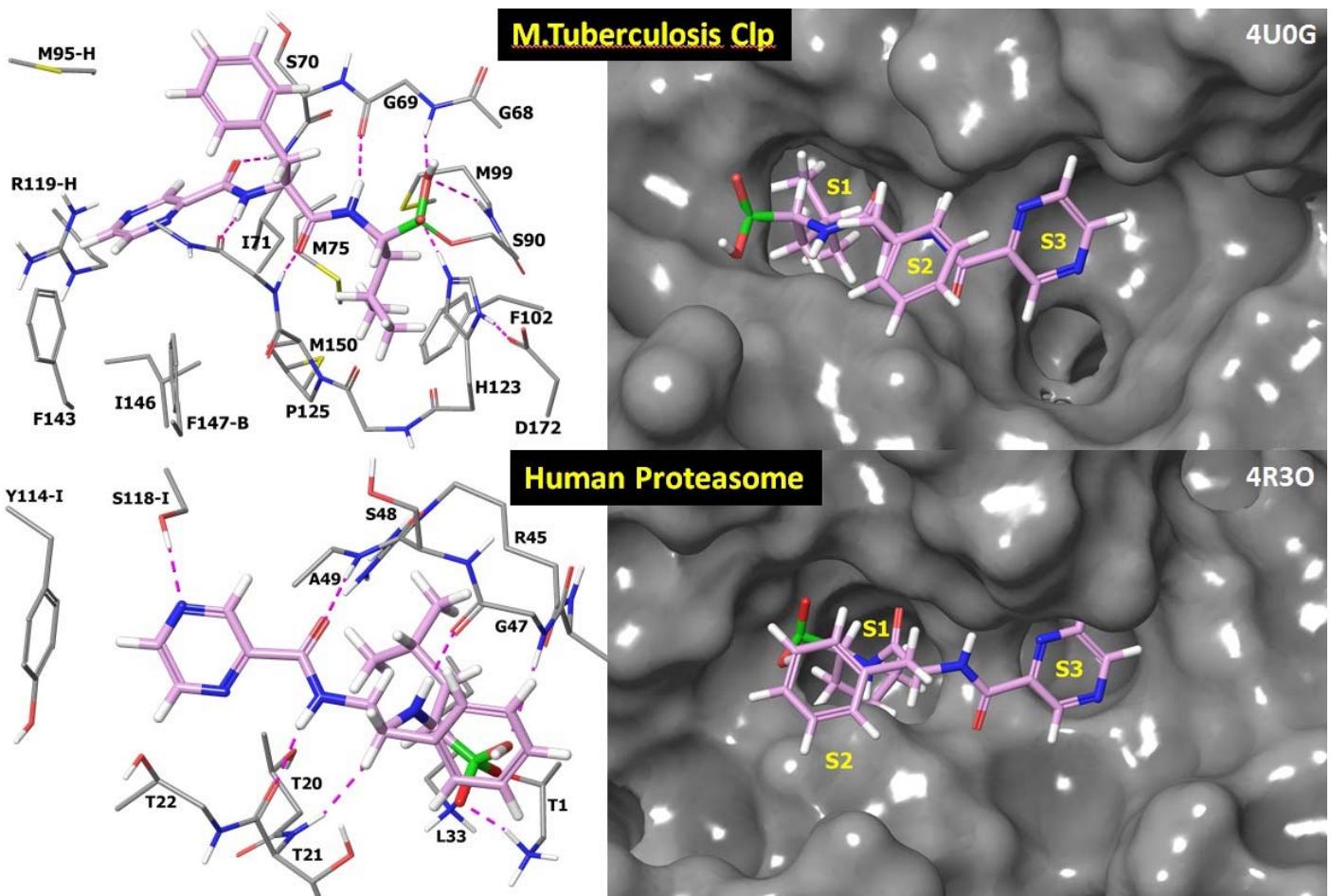
## MEDI 277

### Dipeptidyl boronates as ClpP1P2 inhibitors: A novel approach to anti tuberculosis therapy

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Mycobacterium tuberculosis is and has throughout history been one of the most lethal bacterial pathogens. We have established the mycobacterial

caseinolytic proteases P1 and P2 (ClpP1P2) as a novel promising antituberculosis target. In a phenotypical screen for inhibitors of ClpP1P2 we identified bortezomib (Velcade) as a hit with bactericidal activity. However, bortezomib is approved for cancer therapy and exhibits significant toxicity due to on target inhibition of the human-proteasome. We started a hit to lead optimization project aiming for a compound with selective inhibition of the bacterial protease over the human proteasome while maintaining or improving the antibacterial activity. We made use of substrate preference and protein structural data for inhibitor design. The human proteasome has a preference for hydrophobic P2 sidechains while mycobacterial ClpP1P2 does not. The site where the P1 sidechain binds may accommodate more bulky residues in ClpP1P2 than the human proteasome. The boronic acid warhead and peptidic backbone was not changed as it is deemed essential for binding. Our lead compound has 100-fold reduced proteasome inhibition while the antibacterial activity has been slightly improved. The lead has been extensively profiled and found to have good IV PK, low toxicity towards liver cells and high selectivity against a panel of 62 human proteases. Our lead has properties that are especially desirable for an antibiotic like moderate plasma binding, moderate clearance (in vivo half life of 4 hr) and no cytochrome inhibition. The project recently entered the lead optimization phase. Our current efforts are mainly centered on improving oral bioavailability and antibacterial activity.



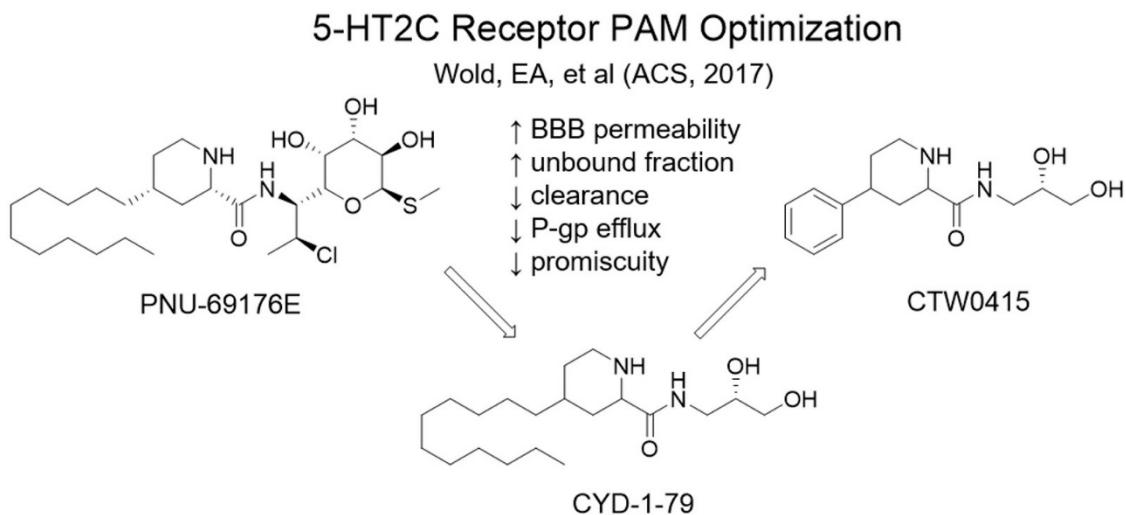
## MEDI 278

### Discovery and synthesis of 4-phenylpiperidine-2-carboxamides as selective 5-HT<sub>2C</sub> receptor positive allosteric modulators

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Evidence supports that the serotonin 2C (5-HT<sub>2C</sub>) receptor is a regulatory component of neurobehavioral processes, and aberrant signaling at this receptor may contribute to several CNS disorders such as depression, schizophrenia, impulsivity disorders and drug addiction. To augment pathologically-decreased signaling, positive allosteric modulators (PAMs) of the 5-HT<sub>2C</sub> receptor represent a favorable strategy to selectively enhance the

functional response to endogenous 5-HT while preserving spacial and temporal signaling features. Therefore, to achieve a 5-HT<sub>2C</sub> receptor PAM lead compound, we introduced chemical modifications to simplify and optimize PNU-69176E, the only reported synthetic 5-HT<sub>2C</sub> receptor PAM. Our initial chemistry and SAR resulted in CYD-1-79 as a novel, selective 5-HT<sub>2C</sub> receptor PAM with improved drug-likeness. To achieve CYD-1-79, an optically pure 1,2-propanediol was introduced to the piperidine 4-carboxamide. We then continued to a second series of analogues, in which we challenged the assumption that the piperidine 2-position undecyl moiety was a privileged fragment. Excitingly, this recent series has yielded CTW0415, which retained 1,2-propanediol and introduced a phenyl to the piperidine 4-position, replacing the undecyl moiety. This chemical modification resulted in a “lead-like” 5-HT<sub>2C</sub> receptor PAM that displays markedly improved distribution, metabolism and pharmacokinetic (DMPK) properties while retaining selectivity and efficacy at the 5-HT<sub>2C</sub> receptor. In conclusion, these efforts have yielded a selective and efficacious 5-HT<sub>2C</sub> receptor PAM lead compound that exhibits promising DMPK properties and modulates 5-HT<sub>2C</sub> receptor-associated behaviors, *in vivo*, in a dose-dependent manner. CTW0415 can perform as a unique tool to elucidate neuropathological serotonergic function, and further optimization will yield promising therapeutic candidates with a potential for a first-in-class neurotherapeutic.

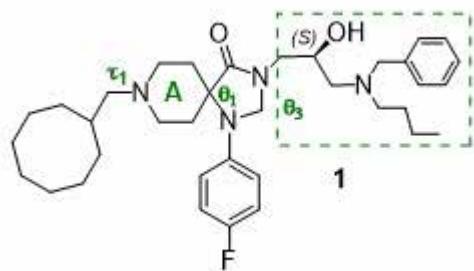


## MEDI 279

**Selective small molecule Nociceptin (NOP) agonist for the treatment of anxiety related disorders**

**Tina M. Ross<sup>1</sup>, tinamross2@gmail.com, Gilles Bignan<sup>2</sup>, Peter J. Connolly<sup>2</sup>, John Moyer<sup>2</sup>.** (1) Tindey Technologies LLC, West Chester, Pennsylvania, United States (2) Clinical, Johnson & Johnson PRD LLC, Spring House, Pennsylvania, United States

Research will be presented on Nociceptin (NOP) agonists for the treatment of anxiety related disorders. Nociceptin is a peptide ligand that has homology to other opioid receptors, however it is uniquely different. The synthesis of partial agonist (**1**) involved using a molecular diversity approach, to rapidly advance a library of compounds for biological testing. Through design and structure activity results, we were able to progress a full NOP agonist to clinical for exploration. A lead selective full agonist JNJ-19385899 (100-fold NOP/Mu) progressed to clinical studies. The synthetic approach and biological data for the related chemical series will be presented.



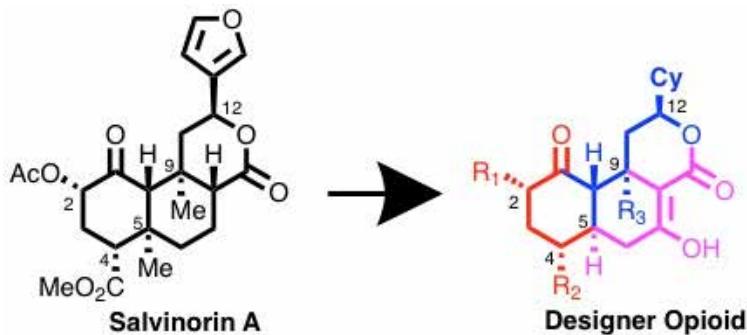
## MEDI 280

### Modular total synthesis approach towards salvinorin A inspired designer opioids

**Alexander M. Sherwood, ams1@ku.edu, Samuel Williamson, Rachel M. Saylor, Thomas E. Prisinzano.** Medicinal Chemistry, University of Kansas, Lawrence, Kansas, United States

An adaptable total synthesis approach of designer opioids based upon the salvinorin A scaffold is valuable for the development of analgesics with reduced abuse liability and drug abuse pharmacotherapies. The natural product salvinorin A is the prototypical non-nitrogenous opioid receptor ligand and has atypical pharmacology compared to classical morphine-derived opioids. Drugs inspired by and built upon this natural product scaffold yield valuable probes for understanding opioids and are potentially capable of circumventing some of the known abuse liabilities associated classical alkaloid-derived opioids. Our total synthesis approach permits functionality to

be introduced deliberately within the molecules with the goal of systematically exploring their activity by *in vitro* studies at opioid receptors and ultimately in animal models of pain and addiction. We have designed molecules able overcome potential shortcomings in salvinorin A, such as rapid metabolism, so that they may be useful for clinical pharmacotherapies. The desired chemical scaffolds have been accessed by a straightforward approach to bisenone 14-membered macrolides that are capable of undergoing a transannular Michael reaction cascade to assemble the tricyclic neoclerodane core representative of salvinorin A. The compounds produced provided access to otherwise unattainable molecular features on salvinorin A by semisynthesis on plant-derived material. The tricyclic neoclerodane core has been synthesized with manipulations targeting key features that are required for activity and an array of salvinorin A inspired structures was accessed. The compounds produced are being evaluated for activity at opioid receptors and in animal models with the goal of developing clinically relevant analgesics with reduced abuse liability and drug abuse pharmacotherapies.



## MEDI 281

### Essential oil content of the seeds of wonderful kola, African walnut and guinea plum and their potentials on hyperlipidemic male Wistar rats

**Eucharia O. Nwaichi**, nodullm@yahoo.com, **Justice O. Osuoha**, **Michael O. Monanu**. Biochemistry, University of Port Harcourt, Port Harcourt, Nigeria

Essential oil contents of seeds of Wonderful kola, African walnut and Guinea plum and their potentials on hyperlipidemic Wistar rats were investigated. Diet-induced type II A hyperlipidemia in rat model was achieved by oral administration of egg yolk and groundnut oil formulation for two weeks. Rats fed normal diet constituted the negative control for comparison. The chromatogram obtained using GC/MS for Wonderful kola revealed the presence of fifteen compounds. The major compound in the harvested essential oil was 1, 2- benzenedicarboxylic acid mono (2-ethylhexyl) ester

(74.88%). Those for African walnut seeds revealed the presence of ten compounds which amounted to 86.2 % of the total oil composition. The major abundant compounds in the essential oil was Diethyl Phthalate (76.94%) and results from the essential oil composition of Guinea plum showed the presence of fifteen compounds which amounted to 100% of the total oil composition. The compounds in the essential oil were 1,2-benzenedicarboxylic acid, diisooctyl ester (34.65%), diethyl phthalate (14.95%), cyclopentaneacetic acid, ethenyl ester (10.1 %), fumaric acid, 3-methylbut-3-enyl tridecyl ester, (7.79 %), Phytol (6.7%) 2,4- and Nonadienal, (E, E) (4.33%). In comparison to control, administration of tried samples markedly lowered weight gain by animals, total cholesterol, triglycerides, AST, ALT ALP, plasma contents of LDL and atherogenic indices in a dose dependent fashion. Their administration also produced significantly higher ( $p<0.05$ ) plasma HDL cholesterol levels indicating recovery. These results showed the nutraceutical potentials of these three seeds and suggest their use in management of hyperlipidemia in the order. Similarly, abundance of 1,2-benzenedicarboxylic acid mono (2-ethylhexyl) ester in harvested essential oil from Wonderful kola suggests their possible use in the management of cancer given their established potentials. However, heavy presence of phthalate esters in harvested essential oil from African Walnut suggest possible varied toxicity on consumption and may portend danger.

## MEDI 282

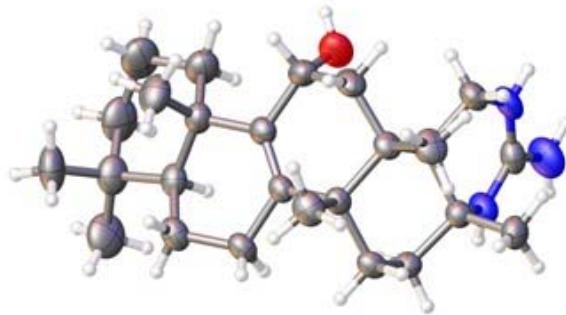
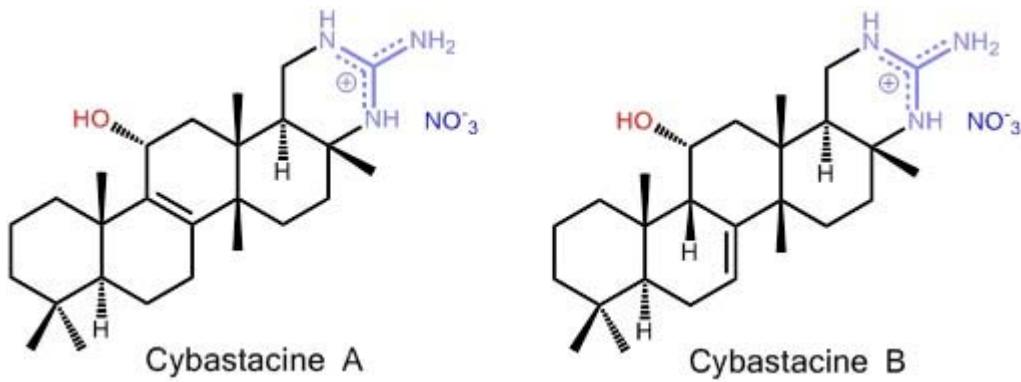
### Promising antibacterial sesterterpenes: Cybastacine A and B from blue-algae cyanobacteria *Nostoc sp.*

**Víctor Tena Pérez<sup>1</sup>, victor.tena@estudiante.uam.es, Alfredo Hernández Cabanillas<sup>1</sup>, Diego F. Rosero Valencia<sup>2</sup>, Santiago Maderuelo Corral<sup>2</sup>, Montserrat Ortega Doménech<sup>2</sup>, Ángel Rumbero Sánchez<sup>1</sup>.** (1) Organic chemistry, Universidad Autónoma de Madrid, Madrid, Madrid, Spain (2) Biotechnology, Valoralia I más D, Tres Cantos, Madrid, Spain

Multi-drug resistance of microorganisms is considered a serious problem from a clinical and epidemiological point of view. Infections caused by these resistant microorganisms fail to respond to standard treatments, resulting in prolonged illness with increased risk of death and high economic costs. Considering this situation, new chemical entities presenting innovative mechanisms of action and generating more effective clinical treatments are in huge demand. For this purpose, cyanobacteria compounds hold a bright and promising future in scientific research and provide a great opportunity for new drug discovery.

Two novel pentacyclic sesterterpenoid-alkaloid (Cybastacine A and B) were isolated from cyanobacteria *Nostoc sp.* collected in the Canary Islands. Their structures were elucidated by a combination of spectroscopic analyses such as 1D, 2D NMR and HRMS measurements. The relative configuration of A was determined by single-crystal X-ray diffraction using Mo K $\alpha$  radiation.

The *in vitro* antibiotic activity of Cybastacine A and B was tested against clinically important bacteria strains, and expressed in terms of the minimum inhibitory concentration (MIC). Cybastacine A exhibited limited antibiotic activity, with MICs ( $\leq$ 18 to 32  $\mu$ g/ml) that were not comparable to reference antimicrobials MICs. Relevant results were found with Cybastacine B which exhibited strong activity against clinical isolates of *Enterococcus spp.*, *Staphylococcus spp.*, *Mycobacterium abscessus* and *Nocardia spp.*, with MICs of  $\leq$ 4  $\mu$ g/ml, and *Tsukamurella pulmonis* with a surprising MIC of  $\leq$ 2  $\mu$ g/ml. These results are comparable or lower to those of other reference antimicrobial agents.

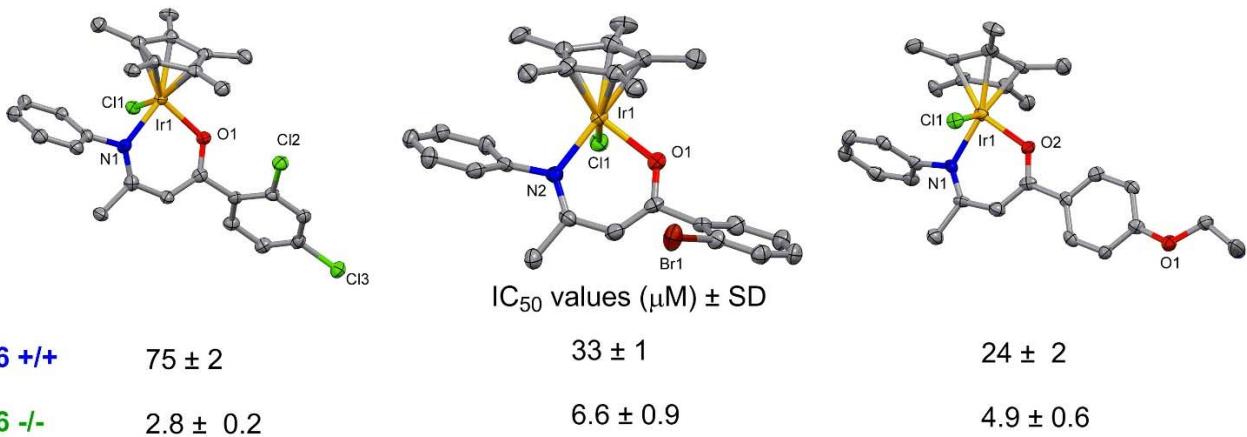


## MEDI 283

**Organometallic iridium compounds: Cytotoxic potential against p53wt and p53-/ human colon cancer HCT116**

**Rianne M. Lord<sup>1</sup>, r.lord@brad.ac.uk, Imogen Henderson<sup>2</sup>, Patrick McGowan<sup>2</sup>.** (1) School of Chemistry and Biosciences, University of Bradford, Bradford, United Kingdom (2) School of Chemistry, University of Leeds, Leeds, United Kingdom

The tumour suppressor p53 (TP53) is crucial in multicellular organisms as it prevents the formation of cancer. Studies have shown links to the variation in p53 polymorphism and cancer susceptibility. Many cancer types show a high occurrence of TP53 mutations, which lead to the expression of mutated p53 alleles. Growing evidence shows that the mutant genes which have lost the wild-type TP53 activity will gain functions which lead to tumour progression. Therefore, TP53 has become the most appealing target for mechanism-driven anticancer drug discovery. The changes in p53 conformation from mutant to wild-type p53 (p53 reactivation), the death of cancer cells with mutant p53 or death of cancer cells via a p53-independent pathway are less understood for inorganic compounds. This work presents the first iridium compounds designed to target p53 -/- and induce tumour cell apoptosis via a p53-independent pathway. The data presented will discuss the chemosensitivity studies against human colon cancers, p53wt (HCT116 +/+) and p53 -/- (HCT116 -/- null), alongside cell viability assays against healthy cells, apoptosis, cell cycle progression and DNA strand breaking via the Comet assay. These iridium compounds are active against the null p53 -/- cells, which do not express a p53 function, concluding our compounds are active via a p53-independent pathway.



## MEDI 284

**Novel ensemble approach to providing small molecule support for validation of cellular targets confirms that glycolysis is a viable antiproliferative strategy in leukemic cells**

**Adam Zweifach**, adam.zweifach@uconn.edu. Molecular and Cell Biology, University of Connecticut, Storrs, Connecticut, United States

Cancer drug discovery programs fail too often. One cause may be weak small molecule support at the target validation stage, since the tool compounds available at that point are not optimized and often have multiple effects. Aerobic glycolysis- the Warburg effect- has recently reemerged as a potential cancer target, attracting considerable interest, but small molecule support that blocking glycolysis is an effective antiproliferative strategy is not strong. We developed a new assay based on expression of an intramolecular FRET sensor for ATP in K562 cells, and used it to screen a National Cancer Institute compound collection for metabolically-active small molecules. We identified 10 compounds that inhibit glycolysis and 7 that inhibit ATP produced via oxidative phosphorylation in the absence of glucose and glutamine. The oxidative phosphorylation inhibitors were no more effective at blocking proliferation than the overall compound set, while all of the glycolysis inhibitors were in the top third of most effective antiproliferative compounds. Knowing the growth-inhibiting properties of the library, the odds of this occurring by chance can be estimated and are vanishingly small. Our results point to an approach to providing small molecule support for targets that could be applied prospectively prior to large scale screening and which could help reduce the failure rate of drug development efforts.

## MEDI 285

### Generation of natural products-based screening libraries for drug discovery

**Folake A. Egbewande**, folake.egbewande@griffithuni.edu.au, Mark J. Coster, Rohan A. Davies. Griffith University, Brisbane, Queensland, Australia

Natural products (NPs) represent a unique source of chemical and structural diversity that exhibit a wide variety of biological activities. Exploring and designing diverse compound collections using unique and under-utilized NP scaffolds increases the chances of discovering chemical probes and/or future leads that could be developed into new drugs.

This project is significant as it combines knowledge from synthetic and combinatorial chemistry, and uses novel NP-templates to create smaller and smarter libraries. This study will allow for the exploration of unique chemical space and provide distinctive screening libraries containing potential drug, lead or probe molecules.

To date, three unique scaffolds, valerenic acid, gibberellic acid, and

thiaplakortone A have been modified to generate libraries of analogues via parallel solution-phase synthesis. The library based on the valerenic acid scaffold has recently been screened for anti-inflammatory activity at LEO Pharma's Open Innovation platform. This library was tested in two separate anti-inflammatory *in vitro* assays based on IL-8 and TNF- $\alpha$  inhibition. Six analogues showed moderate inhibitory activity in the IL-8 assay with IC<sub>50</sub> values of 2.8–8.3  $\mu$ M, while none of the tested compounds showed any significant effect on inhibiting TNF- $\alpha$  release. The gibberellic acid library is undergoing high-throughput screening for anti-cancer activity against several promising targets in prostate cancer that are linked to oxidative stress, redox homeostasis, lipid metabolism and mitochondrial function; all these processes are critical for prostate cancer growth and survival. The small library based on thiaplakortone A is currently being synthesized and will be evaluated for anti-malarial activity.

## MEDI 286

### **Stabilization of quadruplex DNAs by tetraurea macrocycles: Synthesis, DNA binding and beyond**

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G-quadruplexes are distinct secondary structures formed by DNA or RNA strands rich in guanine bases. These unique structures, often found in chromosomal extremities (telomeres) and intra-chromosomal region (oncogenes promoters), have been proven to play an important role in oncogene expression regulation and cell proliferation. Telomerase is a reverse transcriptase ribonucleoprotein found in rapidly proliferating cells but inactive in normal somatic cells and results in the lengthening of the telomeres. This lengthening has major consequences on the cell life cycle since it leads to immortalization.

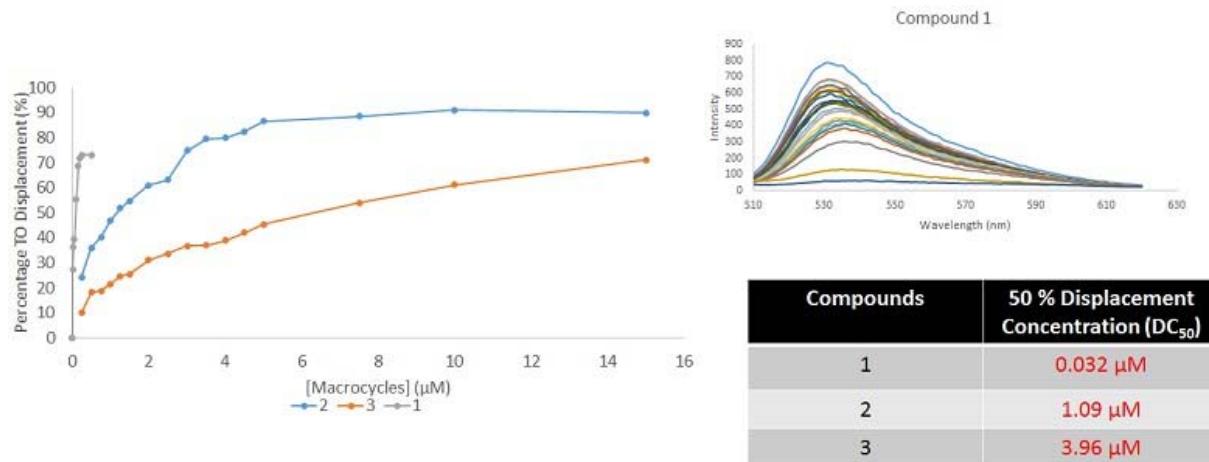
Efforts have been made to inhibit the activity of telomerase. The discovery of the natural product telomestatin, which was proven to bind and induce G-quadruplexes stabilization and result in subsequent indirect telomerase inhibition, has led to the synthesis of small molecular analogues that would mimic the potency of telomestatin toward telomerase through indirect inhibition.

In this work, we have synthesized a series of aromatic tetraurea macrocycles which have shown through DNA melting temperature, isothermal calorimetry and fluorescence displacement assays to bind and induce the stabilization of

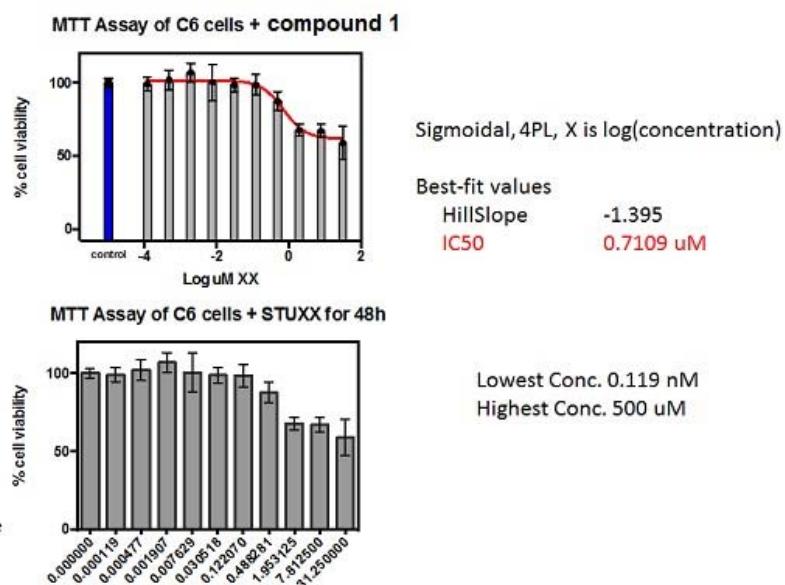
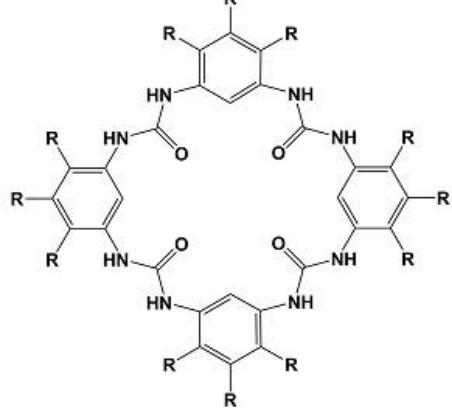
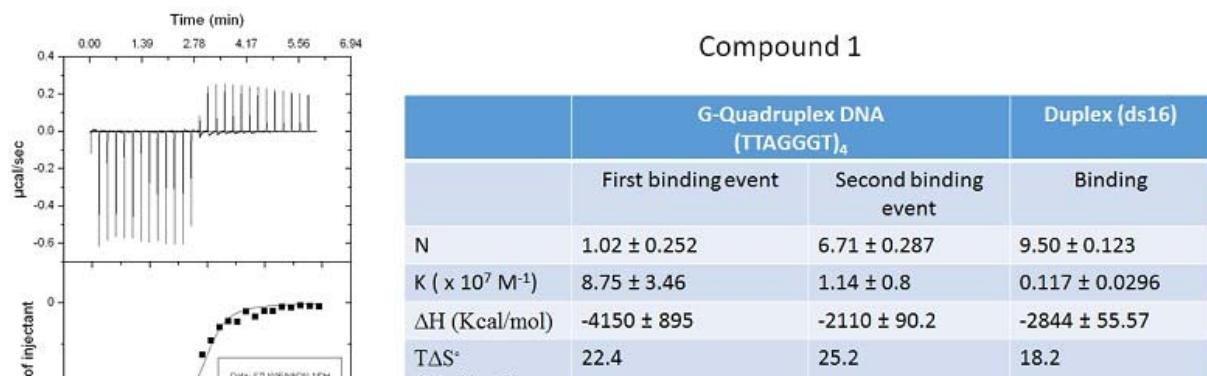
human telomeric G-quadruplex. We have also performed MTT cell viability assays in C6 glioma cell lines where one of the macrocycles yielded an IC<sub>50</sub> of 710 nM after 48 hours.

The selectivity of these aromatic macrocycles was also investigated by isothermal calorimetry. The results show a 10-fold binding preference for quadruplex DNA over duplex DNA.

# Florescence Intercalator Displacement Assay (G4-FID) using Thiazole Orange Dye



## Comparing Isothermal Calorimetry Data G-Quadruplex vs Duplex



## MEDI 287

### Production of the antidote of cyanide poison (sodium and hydrogen cyanide) known as sodasulphanecobalamin

**Salako N. Olatunji**, salakoolatunji9@gmail.com. Federal Institute of Industrial Research Oshodi, FIIRO, Oshodi, Lagos, Nigeria

**Abstract:** SodaSulphanecobalamin ( $\text{Na}_4\text{S}_5\text{CoC}_{69}\text{N}_{15}\text{H}_{89}\text{O}_{26}$ ) is an antidote for Cyanide poison, mainly high concentration of Cyanides (Sodium and hydrogen Cyanide) which displaces the Cyanides to a free toxic compound, thiocyanocobalamin .It also added the amide group of protein when used. However, recent studies shows that this antidote can serve as a replacement for the antidote of Orange agent ( 2,3,4,7-tetra chlorobenzodioxin) which displaced millions of Vietnam Citizens during the world war II. Though Mercury (I) Oxalate is been used for this antidote for the orange agent, but we all know that Mercury is highly toxic and poisonous to the human. It is produce by using 100g of Sodium nitrite ( $\text{NaNO}_2$ ) when heated with a burner in its Combustion Furnace at a temperature of  $340^{\circ}\text{C}$  it produces 44.93g of Sodium oxide ( $\text{Na}_2\text{O}$ ) in a crystalline form, while 21.74g and 33.33g of Nitric Oxide ( $\text{NO}$ ) and Nitrogen (IV) oxide will be liberated as gas respectively, thereafter the 4130g of Sodium thioSulphate ( $\text{Na}_2\text{S}_2\text{O}_3$ ) decomposes on heating at  $330^{\circ}\text{C}$  to give 2783.83g of sodium sulfate ( $\text{Na}_2\text{SO}_4$ ) and 1346.17g of  $\text{Na}_2\text{S}_5$ . Sodium polySulphide ( $\text{Na}_2\text{S}_5$ ) which is a dark-red liquid separated itself due to its characteristics of its separating agent, dissolves in the distillated water to gives a solution of these component, furthermore 1,500g of Hydroxocobalamin red solids is dissolved by distilled water ( $\text{H}_2\text{O}$ ) of volume of 1.115dm<sub>3</sub> (1liter and 115 ml) to give a red solution of Hydroxocobalamin [ $\text{C}_{62}\text{H}_{89}\text{CoN}_{13}\text{O}_{15}\text{P}$ ] of 1molar concentration. The resulting components now mixed together in a reaction to produces TERTSodium1,2-diithiosulphite-3,4diintrosoco- $\alpha$ ( $\alpha$ -5,6diimethylbenzylmizazonly)co- $\beta$ -hydroxocobalamin( $\text{Na}_4\text{S}_5\text{CoC}_{69}\text{N}_{15}\text{H}_{89}\text{O}_{26}$ )  
 $\text{NO} + \text{Hocbl} + 2\text{NaOH} + \text{NO}_2 + 3\text{Na}_2\text{SO}_4 + \text{Na}_2\text{S}_5 + 2\text{Na}_2\text{S}_2\text{O}_3 + 2\text{NaNO}_2 + 4\text{NaOH} + \text{HOSCb1} + \text{SO}_{2(g)} + \text{Na}_4(\text{S}_2\text{O}_3)_2 + (\text{NO}_2)_2 + \text{C}_{62}\text{H}_{87}\text{SCoN}_{13}\text{O}_{16}\text{P}$

Hydroxocobalamin with the decomposition of Sodium nitrite and Sodium thiosulfate will led to a faster return to baseline mean arterial pressure compared with sodium nitrite with sodium thiosulfate; however, there was no difference between the antidotes combinations in mortality, serum acidosis, or serum lactate.

**The most efficient and reliable way to treat cyanide poison is by using SodaSulphaneCobalamin. It is non-carcinogenic, non-mutagenic and**

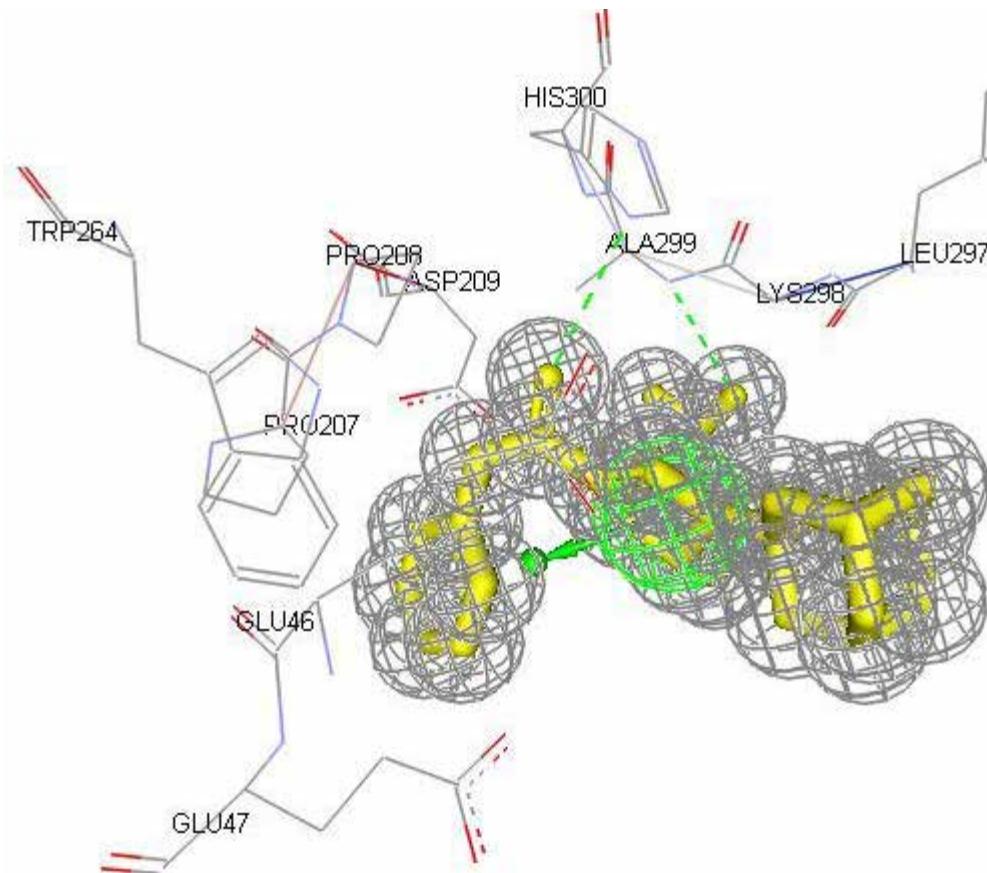
**non-teratogenic compound which is composition doesn't has any toxicity and health effect when administered**

## **MEDI 288**

### **Design and structural modification of adamantane analogs for their anti-cancer activity**

**Vanrajsinh Thakor, vanrajsinh.thakor@outlook.com, Arzoo Shaikh, arzshaikh@gmail.com. Department of Pharmaceutical Chemistry, Shree Dhanvantary Pharmacy College, Kim, Surat, Gujarat, India**

This research activity led to the development of hydrophobic ligands which are the mimics that can act as Bcl-2 molecular switchers. Several peptides and drugs are reported which are able to reproduce the pro-apoptotic effects of paclitaxel. These ligands predominantly target Bcl-2 pathway. The function of Bcl-2 from anti-to pro-apoptotic pull of either by interacting with either Bcl-2 protein or  $\beta$ -tubulin. So, in this course, The QSAR and structure based drug design approaches were considered to screen the ligands. With interaction energies and IC<sub>50</sub> values, we have achieved significant results. The QSAR and SBDD data of designed ligands confirms their effective binding affinity towards Bcl-2 proteins.



pharmacophore development for adamantine analogs

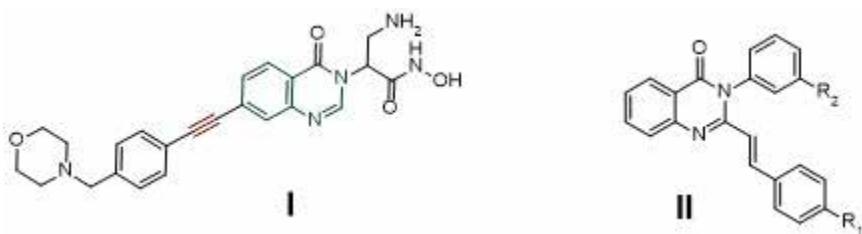
## MEDI 289

### Design, synthesis and biological evaluation of new quinazolinone derivatives as potent antimicrobial agents

**Srinivas Nanduri<sup>1</sup>, nandurisrini92@gmail.com, Srikanth Gatadi<sup>2</sup>, Madhavi V. Yeddanapudi<sup>1</sup>, Sidharth Chopra<sup>3</sup>.** (1) Process Chemistry, National Institute of Pharmaceutical Education & Research, Hyderabad, Telangana, India (2) Medicinal Chemistry, National Institute of Pharmaceutical Education & Research, Hyderabad, Telangana, India (3) Department of Microbiology, Central Drug Research Institute, Lucknow, Uttarpradesh, India

Quinazolinone class of compounds is known to be an important chemical class with varied biological activities of pharmaceutical importance. They possess a variety of biological effects including antihypertensive, antimicrobial, anti-hyperlipidemic, antioxidant, anti-inflammatory, anticonvulsant, and anticancer activities<sup>1</sup>. Interest in quinazolinones has further increased since the report of compounds I and II as potent anti-

bacterial agents. While compound **I** is found to be a potent Lpxc inhibitor<sup>2</sup>, compound **II** has been reported to be an inhibitor of penicillin binding protein PBP2a<sup>3</sup>. Inhibition of Lpxc leads to bactericidal activity against gram negative bacteria. Compound **II** and its related derivatives have been found to be inhibitors of methicillin-resistant S.aureus (MRSA). A new library of quinazolinone derivatives have been synthesized utilizing the structural features from the above two series of compounds and are evaluated against gram negative, gram positive bacteria and mycobacterium. The structure Activity Relationships will be discussed.



## MEDI 290

### Alteration in mode of cell death of NSCLC cultures after treatment with various imidazolium salts

**Marie Southerland**, mariezie914@yahoo.com, **Michael DeBord**, **Patrick O. Wagers**, **Kerri L. Shelton**, **Sailaja M. Paruchuri**, **Claire Tessier**, **Matthew Panzner**, **Wiley J. Youngs**. Department of Chemistry, The University of Akron, Akron, Ohio, United States

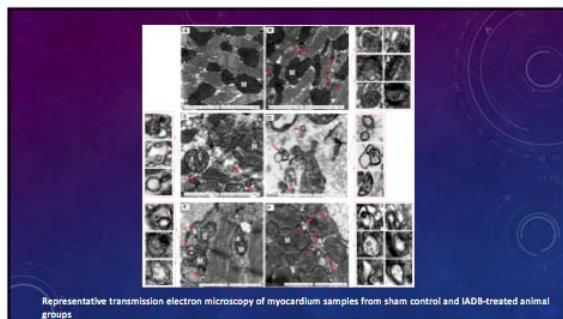
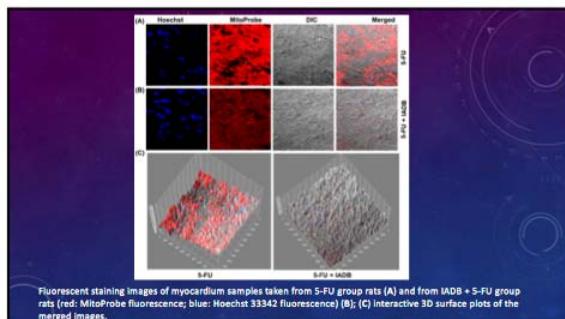
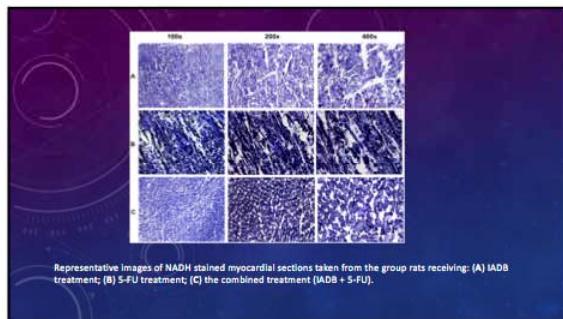
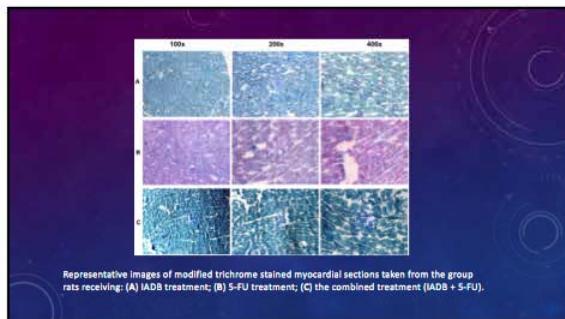
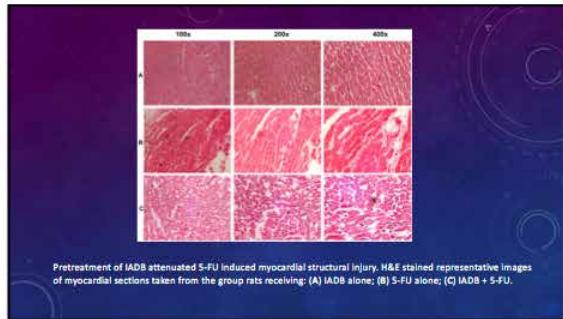
One focus of current cancer research is the development of new chemotherapeutic agents that minimize negative side effects seen with current drugs without minimizing potency. A class of compounds known as imidazolium salts has shown promising anti-cancer activity against several non-small cell lung cancer (NSCLC) cell lines by MTT assay with IC<sub>50</sub> values in the low μM range. Various functional groups used to modify the general imidazolium core have demonstrated a structure activity relationship between anti-cancer activity and the chemical properties of the functional group. In order to understand the activity of these compounds even further, a study to differentiate between necrosis and apoptosis in cell death has been initiated. The same functional groups that give rise to various MTT assay values also modify the time frame of cell death and the mode of cell death, which has been visualized through Annexin V assays using fluorescence microscopy.

## MEDI 291

### **Discovery of a novel dual functional compound (IADB) as chemo-sensitizing and cardio-protective agent**

**Lanrong Bi**, *lanrong@mtu.edu. Michigan Tech Univ, Houghton, Michigan, United States*

Chemotherapy-induced cardiotoxicity is a serious complication that limits the clinical use of chemotherapeutic agents. Currently, there is no clinically proven treatment available for chemotherapy-induced cardiotoxicity. Recently, we discovered an indole alkaloid derivative B (IADB) as a novel dual-functional agent (antioxidant and autophagy-modulating activity). We have demonstrated that IADB exhibiting potent free radical scavenging activities in the acetylcholine (Ach)-induced relaxation of the rat thoracic aorta assay. Moreover, we observed that treatment with IADB induced the appearance of autophagy-associated structures in the cytoplasm in both cancer and normal cell lines. Interestingly, the cytotoxicity of IADB was much lower in normal cell lines (i.e. H9C2 rat cardiomyocytes) than cancer cell lines (i.e. MDA-MB-231, HT-29, HeLa), suggesting that IADB was more potent in cancer cells. We further demonstrated the treatment of colon cancer HT-29 cells with the combination of IADB with 5-FU resulted in potentiation of the inhibitory effect on the proliferative activity and also an increase in the percentage of apoptotic cells. On the other hand, the histological results showed that of 5-FU alone treatment induced a degree of cardiotoxicity. The severity of the histological change was notably alleviated in sections from animals pretreated with IADB. At the subcellular level, 5-FU driven mitochondrial ROS correlated with the mitochondria dysfunction. However, a partial rescue of mitochondrial oxidative stress upon treatments with IADB + 5-FU was observed. The presently observed reversal of a number of the signs of toxicity associated with 5-FU by IADB are likely through its anti-oxidative properties and also maybe mediated in part via modulation of autophagy, and further exploratory *in vivo* studies is warranted.



## MEDI 292

### Design and synthesis of PC-PLC selective self quenching near-infrared fluorescing probes

**Benjamin K. Liebov**, benjamin.liebov@gmail.com, E. J. Delikatny, Anatoliy V. Popov. Anat Chem Bldg 317, Univ of Pa Dept Radiology, Philadelphia, Pennsylvania, United States

The design and synthesis of PC-PLC targeted fluorescent probes could lead to a new and improved technique for the diagnosis and treatment of breast cancer. PC-PLC is strongly up-regulated in epithelial ovarian and breast carcinoma cell lines compared with non-tumor counterparts. In contrast to normal cells, PC-PLC accumulates primarily on the outer leaflet of the cancer cell plasma membrane where the enzyme reveals abnormally high activity. Amongst a series of breast cancer cell lines the highest level of PC-PLC activity was found in the triple negative highly-metastatic breast cancer cell line MDA-MB-231. These data suggest that PC-PLC may be an effective target for breast cancer therapy, particularly for tumors with high metastatic potential. In order to design a PC-PLC selective probe, a phospholipid is synthesized that contains a bulky substituent hindering the *sn*2-position to prevent attack by PLA<sub>2</sub>, a ubiquitous phospholipase. For this purpose, the phospholipid phosphatidylethanolamine (PtdEtn) is chosen and the bulky moiety at its *sn*-2 position can be a porphyrin e.g., pyropheophorbide a (Pyro) or bacteriochlorin e6 (Bchl), which can play three roles: a bulky substituent, NIR dye, and a photosensitizing agent. For PC-PLC-activatable probes, a quencher BHQ-3 or QSY21 is attached to the nitrogen containing head group of PtdEtn. Non-quenched probes based on phosphatidylcholine (PtdCho) with a porphyrin moiety (Pyro or Bchl) attached at the *sn*-2 position are also being studied. The probes have been tested *in vitro* for chemical stability serum stability, enzymatic cleavage and optical imaging of breast cancer cells.

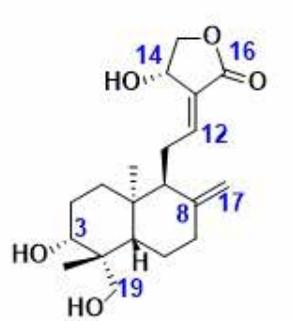
## MEDI 293

### **Andrographolide: A versatile natural product for the generation of structurally diverse bioactive diterpenes**

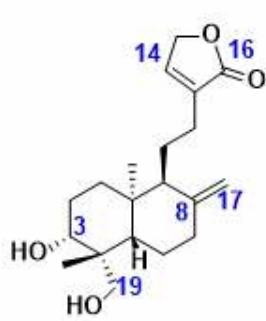
**Srinivas Nanduri<sup>1</sup>**, nandurisrini92@gmail.com, **Sai Giridhar Sarma S. Kandanur<sup>2</sup>**, Nageswara Rao Golakoti<sup>2</sup>. (1) Process Chemistry, National Institute of Pharmaceutical Education & Research, Hyderabad, Telangana, India (2) Department of Chemistry, Sri Sathya Sai Institute of Higher Learning, Prasanthinilayam, Andhra Pradesh, India

From times immemorial, natural products have played an important role in the management of human health. *Andrographis paniculata*, belonging to the family Acanthaceae and its constituents are reported to possess a wide range of biological properties such as anti-cancer, anti-diabetic, anti-inflammatory,

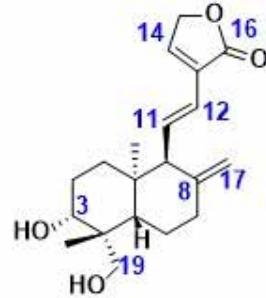
anti-bacterial, anti-malarial, anti-hepatitis, anti-HIV, anti-atherosclerosis, hepatoprotective, FXR antagonist, and  $\alpha$ -glucosidase inhibitory activities. Andrographolide (**1**), 14-deoxy andrographolide (**2**), 14-deoxy 11,12-didehydro andrographolide (**3**) and other related labdane diterpenes are identified as the bioactive constituents of the plant. Promising biological properties of the major constituent Andrographolide (**1**) along with structural amenability for facile semi-synthetic modifications have led to the generation of structurally diverse bioactive labdane diterpenes. Structure Activity Relationship (SAR) studies on the new analogues synthesized by our group by modifying the 3,14,19- hydroxyl groups, double bond across C-8,17 and the lactone moiety of Andrographolide will be discussed.



**Andrographolide (I)**



**14-deoxy andrographolide (II)**



**14-deoxy 11,12-didehydro andrographolide (III)**

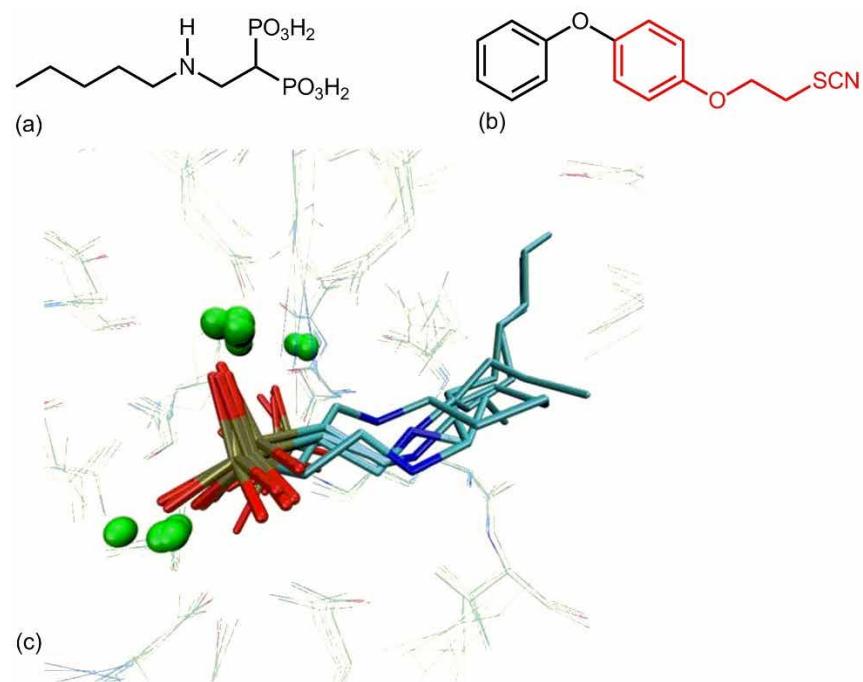
## MEDI 294

### Isoprenoid pathway as a valid target to control parasitic diseases

**Juan B. Rodriguez**, *jbr@qo.fcen.uba.ar*, **Sergio H. Szajnman**, **Maria N. Chao**. Quimica Organica, Universidad de Buenos Aires, Buenos Aires, Argentina

American trypanosomiasis is a chronic parasitosis caused by *Trypanosoma cruzi*, which is the largest parasitic disease burden of the Americas. The only drugs to treat *T. cruzi* infection are nifurtimox and benznidazole. Neither of these two compounds are FDA-approved drugs, and in the United States they are available only from CDC under investigational protocols. In addition, the etiologic agent for toxoplasmosis, *Toxoplasma gondii* is an opportunistic protozoan parasite that is able to infect humans and warm-blooded animals. This illness is one of the most prevalent parasitic diseases affecting close to one billion people worldwide. The current chemotherapy for toxoplasmosis is also still deficient.

Isoprenoid biosynthesis has been selected as a target for many parasitic diseases caused by trypanosomatids and Apicomplexan parasites. In this sense, **WC-9** and 2-alkylaminoethyl-1,1-bisphosphonates, developed in our laboratory, proved to be effective inhibitors of the enzymatic activity of two key enzymes of the isoprenoid biosynthesis, squalene synthase (SQS) and farnesyl diphosphate synthase (FPPS). The availability of the crystal structure of several complexes of 2-alkylaminoethyl-1,1-bisphosphonates with *T. cruzi* FPPS facilitated a rational approach to obtain new bisphosphonate inhibitors. Besides, FPPS of *T. gondii* is a bifunctional enzyme that catalyzes the condensation of isopentenyl diphosphate with three allylic substrates: dimethylallyl diphosphate, geranyl diphosphate, and farnesyl diphosphate. On the other hand, aimed at searching new SQS inhibitors structurally related to **WC-9**, several optimized analogues have been envisioned. Therefore, the recent progresses made in our laboratory on new bisphosphonate derivatives targeting FPPS as well as **WC-9** analogues targeting SQS will be presented.



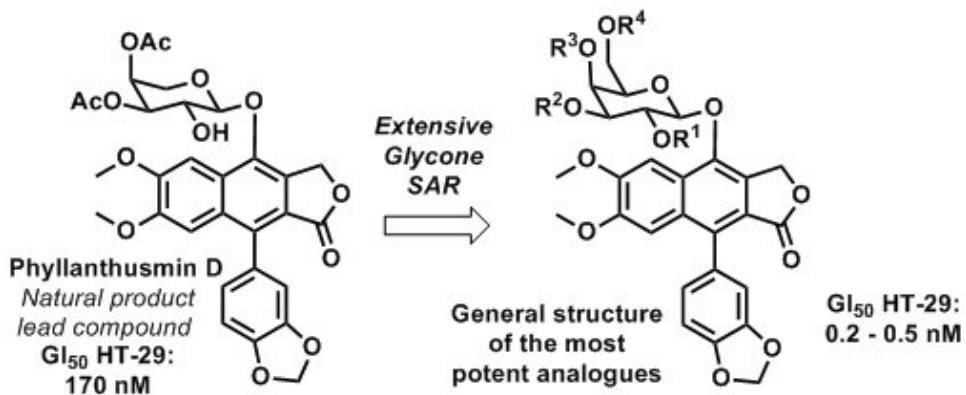
(a) representative lineal 1,1-bisphosphonate; (b) chemical structure of **WC-9**; (c) conformations at the binding site of *TcFPPS* of different 2-(*n*-alkyl)aminoethyl 1,1-bisphosphonates.

## MEDI 295

### Lead optimization and drug development of antiproliferative constituents from *Phyllanthus poilanei*

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Historically, natural products have been an important starting point for developing new cancer therapeutics. In 2014, bioactivity-guided fractionation furnished several novel arylnaphthalene lignan glycosides from extracts of *Phyllanthus poilanei* possessing potent in vitro activity against the HT-29 cancer cell line. In an effort to improve both potency and drug properties, our research group has carried out structure-activity relationship (SAR) studies on this class of compounds. The synthesis of more than 30 novel analogues designed to explore the effects of functionalization of the glyccone has revealed structural motifs key to the significant improvements in activity (between 100- and 1000-fold) observed for these analogues as compared to the natural product phyllanthusmin D. One of these potent compounds has been shown to possess single digit to sub-nanomolar activity in vitro in three different cancer cell lines and proven effective in two different in vivo murine models, a hollow fiber assay and a xenograft study. The biological target for this compound, however, is as of yet unknown, making further structural optimization more challenging and hindering preclinical development. Therefore, mechanistic probes have also been designed and synthesized in an effort to identify the key protein interactions responsible for their activity. Furthermore, additional modifications have been investigated for the purpose of reducing metabolic vulnerability and increasing oral bioavailability. The SAR studies, biological results, and current efforts towards further drug development will be reported.



## MEDI 296

### Sensing bacterial growth and measuring antibiotic susceptibility via laser diffraction

**Nick K. Kotoulas**, nicholas.kotoulas@mail.utoronto.ca, M. Cynthia Goh. Chemistry, University of Toronto, Toronto, Ontario, Canada

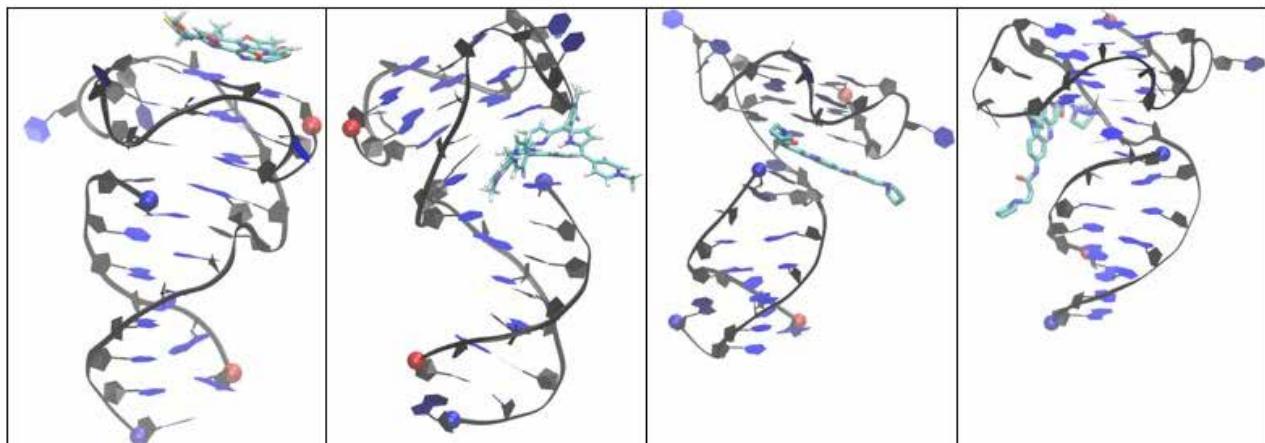
The rise of antibiotic resistance is a critical problem impacting healthcare networks worldwide. A common bottleneck in the assessment and treatment of antibiotic resistant infections is the length of time required to both identify the bacteria causing infection and to measure their antibiotic susceptibility. The latter, which is critical in ensuring an effective treatment for patients with antibacterial resistant infections, can take between 24-72 hours to complete. This work aims to reduce the negative impact of antibiotic resistant infections by utilizing laser diffraction to monitor the growth of bacteria of interest in the presence of selected antibiotics to screen for resistance more rapidly. Bacterial-surface interactions were investigated to optimize the immobilization of bacteria on micro-printed polymer patterns in order to obtain diffraction signals. Diffraction spot intensities obtained from immobilized *Escherichia coli* growing in liquid culture were monitored and compared with experiments exposing the bacteria to an antibiotic (ampicillin). Significant differences in these signal intensities were detected within a two-hour period, highlighting the potential for diffraction sensing as a means to dramatically reduce the time required to measure antibiotic susceptibility. With further study, this method may contribute to an effective solution to the exponentially rising risk and impact of antimicrobial resistance.

## MEDI 297

### Binding at the telomeric G-quadruplex-duplex interface: A computational study

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A promising anticancer therapeutic strategy is the stabilization of telomeric G-quadruplexes using quadruplex-binding small molecules. Though various motifs of quadruplex-specific ligands have been explored, many lack sufficient potency and selectivity to G-quadruplexes. With that, efforts have now begun to focus on targeting the junction formed between a G-quadruplex and duplex DNA as a solution to these issues. Recently, a crystal structure of a telomeric 3' quadruplex-duplex formed from putative parallel G-quadruplex and duplex DNA was reported in attempt to characterize this complex interface, but no structural data on ligand binding was able to be determined. This study uses computational methods to further investigate efficient binding poses and mechanisms of quadruplex-binding ligands to the quadruplex-duplex 3' interface. Molecular dynamics binding simulations with a free ligand were used to study binding poses and dynamics of four well-known, diverse ligands: telomestatin, TMPyP4, BSU6037 and BRACO19. Our data showed that BRACO10, BSU6037, and TMPyP4 were able to bind to the interface while telomestatin remained to only bind at the quadruplex and duplex ends. The binding modes and the binding pathways were characterized and the binding energy was calculated and compared.



## MEDI 298

### **Triggering a peptidomimetic's oxidative activity to reduce survival of intracellular pathogens**

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Copper (Cu) ions play a critical role in controlling bacterial infections. For example, several studies have determined that different pathways contribute to Cu resistance in *Mycobacterium tuberculosis* (Mtb). We report, as proof of concept, that a novel Cu hypersensitivity phenotype can be induced in intracellular pathogens, including Mtb, through the peptidomimetic DAB-10 that is able to form Reactive Oxygen Species (ROS) following Cu-binding. We found that DAB-10 induces oxidative stress at growth inhibitory concentrations in intracellular bacteria in a Cu-dependent manner. DAB-10 penetrates murine macrophages and microscopy studies establish the co-localization of the peptidomimetic with intracellular bacteria. Overall, our studies show that DAB-10 exploits the pool of Cu ions in the host-bacteria interface to augment the macrophage response to infection.

## MEDI 299

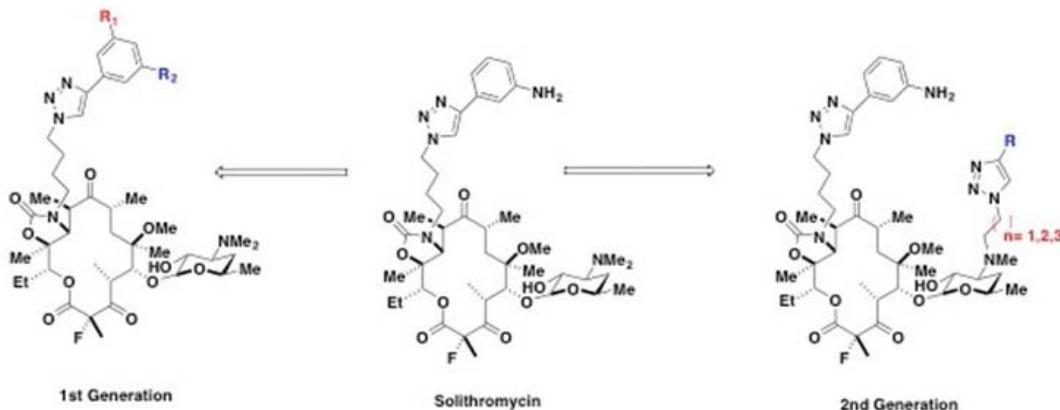
### **Addressing antibiotic-resistance targeting ketolide drugs by developing novel analogs generated via click & *in situ* click chemistry**

**Samer Daher**, *tuf24815@temple.edu*. *Chemistry, Temple University, Philadelphia, Pennsylvania, United States*

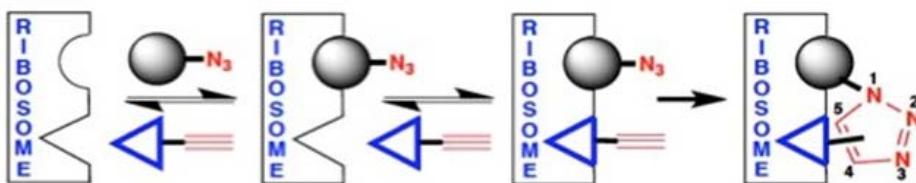
The field of antibiotics and drug development is susceptible to onset of antibiotic resistance, and increasingly common problem, especially with the widespread use of antibiotics in healthcare. The demand for new classes of drugs that can overcome bacterial resistance threats is high and can be accomplished by synthesizing novel targets via structure-activity relationship (SAR), or developing enormous methodologies capable of screening larger scope of antibiotics, amongst others. Our lab is interested in exploring macrolides such as Solithromycin, developed in 2005 by Optimer Pharmaceuticals, via employing Cu (I) catalyzed combinatorial click chemistry. Currently, Solithromycin is in phase 3 clinical trials and has proven to be most efficacious amongst Ketolides developed to date. Macrolides' mechanism of action is by binding to the 50S subunit and inhibiting protein synthesis, which motivated us to investigate non-covalent interactions comprised of hydrogen

bonding and Pi- stacking interactions. Accordingly, a series of analogs fulfilling the latter characteristics have been designed based on Cu click chemistry. Alternatively, inspired by target-guided *in situ click chemistry*, a library consisting of 5-15-membered alkynes has been developed in which E.coli 50S/70S ribosomes substituted the Cu in templating the macrolide azide with various alkyne fragments. An advantage of this powerful drug discovery platform is that it eliminates the arduous task of independent synthesis. All analogs have been evaluated by minimum inhibitory concentration (MIC) assays. Our initial data showed promising MIC levels compared to Solithromycin. In conclusion, the promising MICs values dedicate a positive impact for discovery of potential drug candidates that would have an enormous benefit on healthcare given the ever-expanding use of antibiotics.

#### First Approach: Synthesis of analogs via click chemistry



#### Second Approach: Generation of analogs via *in situ click chemistry*



## MEDI 300

### Synthesis, design and computational studies of anticancer agents

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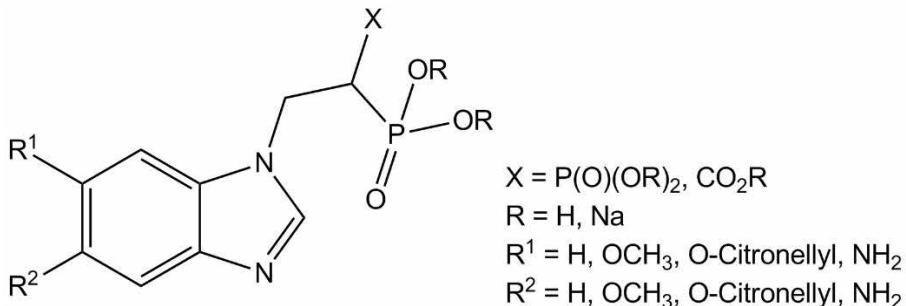
Anticancer agents are effective in the treatment of malignant, or cancerous, disease. There are several major classes of anticancer drugs; these include alkylating agents, antimetabolites, natural products, and hormones. Novel 3,5-bis(benzylidene)-1-[3-(2-hydroxyethylthio) propanoyl] piperidin-4-ones and 1-[3-(2-hydroxyethylsulfanyl)propanoyl]-3,5-bis(benzylidene)-4-piperidones display potent cytotoxicity and a preferential lethality toward various neoplasms compared to some normal cells have been reported by our research group. In order to facilitate the drug discovery process, the QSAR paradigm is a promising technology that may enable correlations to be found between the structural features of a compound with its respective biological activities. In this study we focused on the development of a QSAR model for the prediction of cytotoxicity data of a series of piperidones and similar compounds.

## MEDI 301

### New motif for targeting isoprenoid biosynthetic pathway enzymes

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Protein prenylation is a post-translational modification which is necessary for the activity of many key members of the Ras GTPase superfamily. The isoprenoid moieties used in the prenylation reactions are derived from the isoprenoid biosynthetic pathway (IBP). Both IBP and prenyltransferase inhibitors have been of interest from a therapeutic perspective for the treatment of diverse diseases including cancer and bone disorders. Many of the reported IBP and prenyltransferase inhibitors incorporate nitrogenous bisphosphonate or carboxy phosphonate moieties. A small set of novel bisphosphonates and carboxy phosphonates now has been synthesized based on the heterocycle benzimidazole. The synthesis of these analogues was accomplished by a set of transformations which includes formation of the substituted benzimidazoles, conjugate addition with a vinyl bisphosphonate or carboxy phosphonate, and a final ester hydrolysis. The synthesized analogues were tested for their ability to inhibit IBP enzymes in *in vitro* enzyme assays. The synthesis of these compounds, together with their effects on protein geranylgeranylation and monoclonal protein secretion in human myeloma cells, will be presented.



## MEDI 302

### Design, synthesis, and biological evaluation of small molecule drug conjugates targeting carbonic anhydrase IX positive cancers

**Isaac Marks**, *imarks@purdue.edu*. Chemistry, Purdue University, West Lafayette, Indiana, United States

Carbonic anhydrase IX (CAIX) is a zinc membrane metalloenzyme that catalyzes the hydration of carbon dioxide in response to a hypoxic and acidic extracellular environment. It is prevalent in many human cancers including renal cell carcinomas, colorectal, and lung cancer. Its overexpression on the membrane of malignant cells and low expression levels on normal tissues make CAIX a particularly attractive target for small molecule inhibitors, antibody drug conjugates, and small molecule drug conjugates. In this study, small molecule inhibitors were used as targeting moieties and converted to small molecule drug conjugates (SMDCs) through the conjugation of various imaging and therapeutic cargos. Fluorescent SMDCs were used to characterize the *in vitro* binding affinity and kinetics in several human cancer cell lines that constitutively express CAIX. Near infrared and SPECT/CT SMACs were used in *in vivo* xenograft models of CAIX to demonstrate the tumor-specific uptake and tissue biodistribution patterns. Finally, the *in vitro* and *in vivo* evaluation of CAIX targeted therapeutic tubulysin B SMDCs show the rapid and permanent elimination of tumors in mouse xenograft models.

## MEDI 303

### Design, synthesis, and evaluation of derivatives of glutathione linked to cholesterol via a link for brain-targeting drug delivery

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**Xiangming Guan**. *Pharmaceutical Science, South Dakota State University, Brookings, South Dakota, United States*

Treatment of brain diseases has been hampered by the blood brain barrier (BBB) - a barrier protects the brain from exogenous toxins. The high lipophilic nature of the BBB prevents hydrophilic molecules from penetrating through the barrier, and the existence of various drug efflux pumps reduces hydrophobic drugs' absorption into the brain. Extensive research efforts have been made to help therapeutic agents penetrate the BBB.

There are a number of receptors/transporters on the BBB. One of the approaches to improve brain drug delivery is through the use of a brain-targeting agent that is a ligand for a receptor or transporter on the BBB. Glutathione (GSH) enters the brain through the GSH transporter that is distributed on the BBB. GSH has been found to be a good brain-targeting ligand. In this poster, the synthesis and evaluation as a brain-targeting agent of glutathione coupled to cholesterol through various links will be presented.

#### **MEDI 304**

#### **Synthesis and evaluation of 1, 3, 5 (10) estratriene aminoalkyloxy, 16-formyl derivatives of estrone as potential anti-breast cancer agents**

**Christopher Sullen**, *cgsullen@aol.com*. *College of Pharmacy and Pharmaceutical Sciences, Florida A&M University, Tallahassee, Florida, 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<sup>+</sup>), MDA-MB-231 (ER<sup>-</sup>) and Ishikawa Cell (Human endometrial cancer). With an IC<sub>50</sub> ( $\mu$ M) 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 of 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 305

### Novel computer-assisted drug design (CADD) AKT pathway inhibitors

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The AKT kinase signaling pathway regulates growth and survival of cells, and it is linked to the progression of numerous types of human cancers. Several studies have shown that deactivation of Akt can promote inhibition of cancer cell growth. The objective of this study was to test the efficacy of novel AKT pathway inhibitors in terms of their ability to deactivate AKT and inhibit human tumor cell growth. These compounds were identified using computer-assisted drug design (CADD) methods., Pharmacophore methods were used to screen hundreds of small molecule compounds as potential AKT pathway inhibitors. Five commercially available putative AKT pathway inhibitors were identified that had the necessary pharmacophoric features. Cultured tumorigenic ras-transformed cells (WBras1) and human lung carcinoma cells H2009 were treated for 4, 18, 24, or 48 hours with one of the test compounds at varying non-cytotoxic concentrations, or with the vehicle (DMSO). Total protein was extracted from the cells and Western blot analysis was used to monitor phosphorylation changes in the AKT protein at the Ser473 and Thr308 activation sites. The results indicated down-regulation of AKT phosphorylation in tumorigenic cells at micro-molar concentrations in 2 of 5 compounds. Compound B was the most effective of the 5 compounds in inhibiting AKT phosphorylation. Compound B was also the most effective in inhibiting H2009 cell growth. These results highlight the utility of CADD in identifying promising anti-cancer agents.

## MEDI 306

### **Discovery of selective low molecular weight VAV1 guanine nucleotide exchange factor inhibitors**

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Guanine nucleotide exchange factors (GEFs) are a class of multi-domain proteins involved in the activation of small GTPases. Although GEFs are viewed as potential drug targets relevant for human diseases, they are highly dynamic in nature which makes it challenging to modulate their function with low molecular weight (LMW) compounds. We will report on the discovery and mode of action characterization of a series of soluble and permeable small molecular weight compounds that have been discovered as potent and selective inhibitors of the VAV1-GEF dependent activation of the Rho family GTP binding protein RAC1. We could demonstrate that these compounds show iso-form selective VAV1-inhibition in biochemical assays, and significant isoform-selective VAV1-mediated inhibitory effects in cellular assays. From analysis of inhibitor-VAV1 co-crystal structures we could conclude that compound binding induces conformational changes preventing VAV1 binding to RAC1 through an allosteric mechanism, revealing a previously unrecognized functional compound binding site between the C1- and DH-domains of VAV1.

## MEDI 307

### **Synthesis and preliminary biological evaluation of [<sup>11</sup>C]methyl (2-amino-5-(benzylthio)thiazolo[4,5-d]pyrimidin-7-yl)-D-leucinate as a new potential PET radioligand for the fractalkine receptor (CX3CR1)**

***Mingzhang Gao, migao@iupui.edu, Min Wang, Jill Meyer, Jonathan Peters, Hamideh Zarrinmayeh, Paul Territo, Gary Hutchins, Qi-Huang Zheng. Department of Radiology and Imaging Sciences, Indiana University School of Medicine, Indianapolis, Indiana, United States***

CX3C chemokine receptor 1 (CX3CR1), also known as fractalkine receptor or G-protein coupled receptor 13 (GPR13), is a protein in humans. CX3CR1 binds the chemokine CX3CL1, also called fractalkine ligand or nurotactin. CX3CR1 is expressed in the brain, spleen, and in subpopulations of leukocytes, cells of monocytic lineage, and neutrophils but also in lymphocytes, and associated with various neurological, cancer and cardiovascular diseases. CX3CR1 has become a novel molecular target for treatment and PET (positron emission tomography) imaging of CX3CR1 associated diseases such as Alzheimer's disease. Methyl (2-amino-5-(benzylthio)thiazolo[4,5-*d*]pyrimidin-7-yl)-*D*-leucinate recently developed by AstraZeneca is a potent and selective CX3CR1 antagonist with *K*<sub>i</sub> 8.3 and 1940 nM for CX3CR1 and CXCR2, respectively, and selectivity index (SI) 230 (Karlström S, et al. J Med Chem. 2013;56:3177-90). Here we report the synthesis and preliminary biological evaluation of [<sup>11</sup>C]methyl (2-amino-5-(benzylthio)thiazolo[4,5-*d*]pyrimidin-7-yl)-*D*-leucinate as a new candidate PET radioligand for imaging of CX3CR1. The reference standard methyl (2-amino-5-(benzylthio)thiazolo[4,5-*d*]pyrimidin-7-yl)-*D*-leucinate and its desmethylated precursor 2-amino-5-(benzylthio)thiazolo[4,5-*d*]pyrimidin-7-yl)-*D*-leucine were synthesized from 6-amino-2-mercaptopurimidin-4-ol and BnBr with overall chemical yield 7% in five steps and 4% in six steps, respectively. The target tracer [<sup>11</sup>C]methyl (2-amino-5-(benzylthio)thiazolo[4,5-*d*]pyrimidin-7-yl)-*D*-leucinate was prepared from the acid precursor with [<sup>11</sup>C]CH<sub>3</sub>OTf through O-[<sup>11</sup>C]methylation and isolated by HPLC combined with solid-phase extraction (SPE) in 40-50% radiochemical yield, based on [<sup>11</sup>C]CO<sub>2</sub> and decay corrected to end of bombardment (EOB). The radiosynthesis was performed in a home-built automated <sup>11</sup>C-radiosynthesis module. The radiochemical purity of the radiotracer was >99%, and the specific activity (SA) at EOB was 370-1110 GBq/ $\mu$ mol with a total synthesis time of ~40-minutes from EOB. The preliminary biological evaluation of the radiotracer via radioligand depletion experiment indicated high non-specific binding to CX3CR1.

## MEDI 308

### Macrocyclic factor Xla inhibitors containing phenyl azole carboxamide P1 groups

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*Watson<sup>1</sup>, Earl Crain<sup>1</sup>, Joseph M. Luettgen<sup>1</sup>, Dietmar A. Seiffert<sup>1</sup>, Patrick Y. Lam<sup>1</sup>, Ruth R. Wexler<sup>1</sup>, William R. Ewing<sup>1</sup>. (1) Bristol-Myers Squibb, Princeton, New Jersey, United States (2) Bristol-Myers Squibb Research Center, Syngene International Pvt. Ltd., Bangalore, India*

Factor Xla (FXIa), a trypsin-like serine protease, is a key enzyme in the intrinsic pathway of the blood coagulation cascade. Inhibitors of FXIa are promising novel anticoagulants since they have shown robust efficacy in a variety of preclinical thrombosis models with minimal effects on bleeding. We recently demonstrated that modifying the P1 region in our novel macrocyclic FXIa inhibitors was a successful strategy for improving the oral bioavailability for the series, however these changes resulted in a loss in potency. Herein, we describe the optimization of the P1 group which resulted in the discovery of the phenyl azole carboxamide P1 groups. Macrocycles containing the phenyl azole-linked carboxamide P1 groups exhibited improved potency and metabolic stability, excellent selectivity against the relevant blood coagulation enzymes, and displayed potent antithrombotic efficacy in a rabbit thrombosis model.

## **MEDI 309**

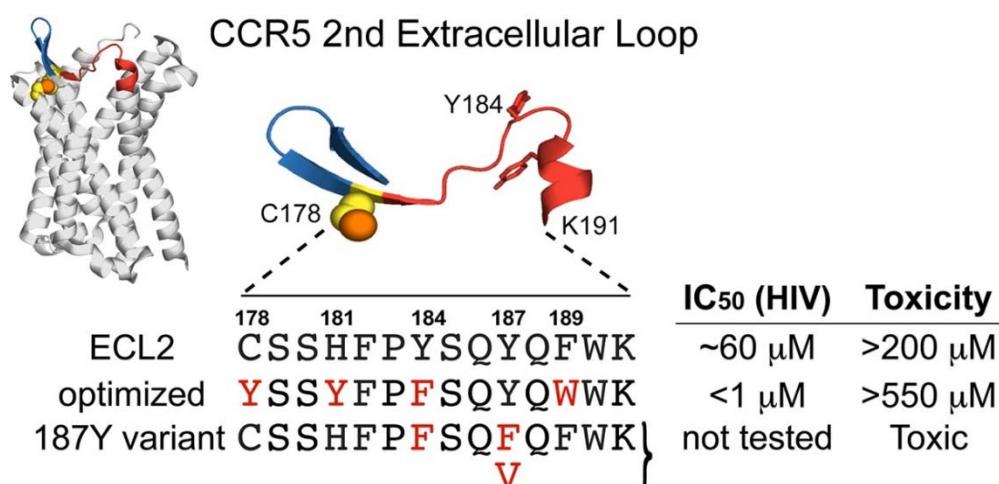
### **Design of HIV co-receptor derived peptides that inhibit viral entry at submicromolar concentrations**

*Sivakoteswara Rao Mandadapu<sup>2</sup>, siva.mandadapu@nih.gov, Kostyantyn Bobyk<sup>3</sup>, Katheryn Lohith<sup>4</sup>, Carole A. Bewley<sup>1</sup>. (1) Bldg 8, Room 1A-02, NIH, Bethesda, Maryland, United States (2) National Institutes of Health, Gaithersburg, Maryland, United States (3) National Institutes of Health, Rockville, Maryland, United States*

HIV/AIDS continues to pose an enormous burden on global health. Current HIV therapeutics include inhibitors that target the enzymes HIV protease, reverse transcriptase and integrase, along with viral entry inhibitors that block the initial steps of HIV infection by preventing membrane fusion or virus-co-receptor interactions. With regard to the latter, peptides derived from the HIV co-receptor CCR5 were previously shown to modestly inhibit entry of CCR5-tropic HIV strains, with a peptide containing residues 178-191 of the second extracellular loop (peptide 2C) showing the strongest inhibition. Here we use an iterative approach of amino acid scanning at positions shown to be important for binding the HIV envelope, and recombining favorable substitutions to greatly improve the potency of 2C. The most potent candidate peptides gain neutralization breadth and inhibit CXCR4 and CXCR4/CCR5-

using viruses, rather than CCR5-tropic strains only. We found that gains in potency in the absence of toxicity were highly dependent on amino acid position and residue type.

Using virion capture assays we show that 2C and the new peptides inhibit capture of CD4-bound HIV-1 particles by antibodies whose epitopes are located in or around variable loop 3 (V3) on gp120. Analysis of antibody binding data indicates that interactions between CCR5 ECL2-derived peptides and gp120 are localized around the base and stem of V3 more than the tip. In the absence of a high-resolution structure of gp120 bound to co-receptor CCR5 these findings may facilitate structural studies of CCR5 surrogates, design of peptidomimetics with increased potency, or use as functional probes for further study of HIV-1 gp120-coreceptor interactions.



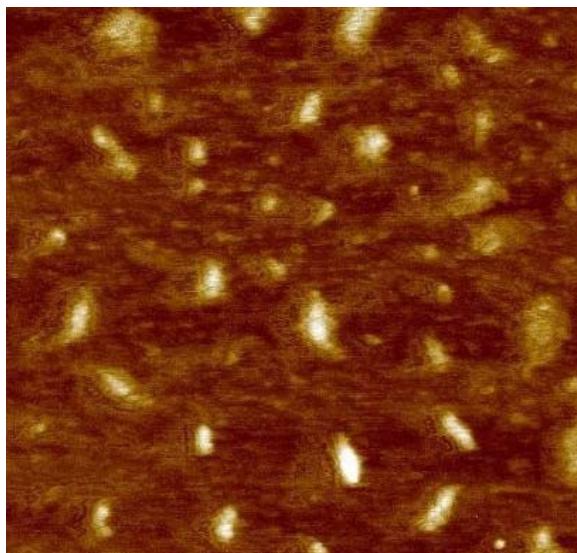
## MEDI 310

### Study co-aggregations of nucleic acid nanostructures with tetracycline molecules and their potential applications in smart drug delivery

**Nouf Alzahrani<sup>1</sup>, dr-noufalzahrani@hotmail.com, Jinglin Fu<sup>1</sup>, Dong Yang<sup>1</sup>, Zhicheng Wang<sup>2</sup>.** (1) Chemistry Department, Rutgers University, Camden, New Jersey, United States (2) School of Biomedical Engineering, Science and Health Systems, Drexel University, Philadelphia, Pennsylvania, United States

Recently, self-assembled DNA structures have been broadly used for various research areas, including drug delivery, biocatalysis and nanomaterials. DNA can bind to small molecules, such as antibiotics due to its highly programmable structure and high efficiency. Several reported studies have demonstrated that DNA could bind to small molecules to enhance their

release. In this project, we are exploring DNA nanoscaffolds for their ability to bind with a neuroprotective minocycline (MO), and to control its release kinetics. We discovered that magnesium played a critical role to bridge DNA with minocycline through electrostatic charge. Further,  $\pi$ - $\pi$  stacking is suggested to form between the nitrogen base and MO's rings. We have demonstrated that, the encapsulation yield of Minocycline with DNA depended on length, shape of DNA. Moreover, pH level of buffer play important role on the aggregations of DNA with MO, where the pH=7 or 6 are compatible with body physiological conditions. The assembled DNA-MO complex was visualized by using Atomic Force Microscopy(AFM). Figure shows the aggregations between DNA and minocycline in the presence of  $Mg^{2+}$ , there are particles formed. The study will have important impact on drug delivery for spinal cord therapy.



## MEDI 311

### Investigation of a new DMC-DNA monoadduct

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The drug Mitomycin C (MC) is currently used to treat stomach, anal, or lung cancer. In the presence of DNA, it is known to form one stereoisomeric interstrand crosslink (ICL) known as alpha-ICL. Another drug, 10-decarbamoyl mitomycin C (DMC) has been found to form the same crosslink as MC in the presence as DNA as well as a second stereoisomeric crosslink known as

beta-ICL. DMC has been found to induce apoptosis more efficiently than MC and we believe that the different biochemical responses exhibited by the two drugs are due to the opposite stereochemistry of the alpha and beta ICLs. Two new compounds were identified from the analyses of DMC reactions with synthetic oligonucleotides under bifunctional activation. The structure of these adducts was determined by UV-vis spectroscopy, mass spectroscopy and co-elution with authentic standards obtained via direct reactions between DMC and deoxyadenosine. The new compounds are isomeric N6-deoxyadenosine adducts of decarbamoyl Mitomycin C i.e. DMC-dA monoadducts. Circular dichroism spectroscopy was used to assign the stereochemistry of these new minor adducts and provide further proof of their structure. These are the first deoxyadenosine adducts identified from the reaction of DMC and DNA fragments. Knowledge of how these compounds are generated will further our understanding of DMC binding selectivity to DNA and provide insight for developing efficacious cancer targeting drugs.

## MEDI 312

### **Re-engineering the natural product, emetine, towards achieving a therapeutically useful drug**

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Emetine is a natural product alkaloid with protein synthesis inhibitory activity. It is known to have a number of medicinal properties including anti-parasitic and anticancer activities. However, extreme toxicity including cardiotoxicity rendered it clinically unsafe as a drug. Our research group has been involved with the re-engineering of emetine to obtain a therapeutically useful anticancer agent that eliminates or significantly reduce the undesirable toxic side effects associated with emetine. To this end we have pursued the design, synthesis and biological evaluation of several analogs and prodrugs of emetine and studied their anticancer properties in prostate and breast cancer cell lines. An overview of our strategy, studies and results to date will be presented.

## MEDI 313

### Novel selective dopamine D3 receptor modulators for the treatment of cocaine addiction

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Cocaine, also known as benzoylmethylecgonine, a stimulant found in the leaves of the *Erythroxylon coca* plant, was first characterized by Friedrich Gaedcke in 1855. Although it has found some use in the medical community, it is most commonly used as a recreational drug. Cocaine addiction has brought about significant societal and health problems. The National Survey on Drug Use and Health estimated in 2008 that there were 1.9 million cocaine users in the U.S. These individuals suffer from numerous adverse changes in physiology, neurophysiology, and behavior.

Several treatment approaches have been developed based on the principles of detoxification and drug withdrawal. Among these approaches, abstinence can be highly effective, but difficult to accomplish by the patient alone. Recent studies have implicated the D<sub>2</sub> and D<sub>3</sub> dopamine receptors in cocaine reinforcement, reinstatement, and drug seeking behaviors. It has been demonstrated that D<sub>3</sub> receptor expression is increased in rodents upon exposure to cocaine. In addition, human post-mortem studies of cocaine overdose fatalities found elevated D<sub>3</sub> receptor expression in the limbic areas of the brain. The D<sub>2</sub> receptor also plays a role in cocaine addiction, but it is known that long-term treatment with typical antipsychotics possessing D<sub>2</sub> antagonist properties can increase the risk of extrapyramidal side-effects. Our efforts are focused on the design and development of selective D<sub>3</sub> receptor modulators that may provide an effective treatment for cocaine addiction, while minimizing the risk of D<sub>2</sub> related side effects. The synthesis and study of a novel series of phenylpiperazine analogs as potential D<sub>3</sub> receptor modulators will be discussed.

## MEDI 314

### **Structure based discovery of host-targeted antiviral (HTA) small molecules: Ribosomal protein RACK1 as a potential broad antiviral target**

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Because of the small number of molecular targets in viruses and the rapid evolution of viral genes, it is very challenging to develop specific antiviral drugs. Viruses require host factors to translate their transcripts and targeting the host factor(s) offers a unique opportunity to develop antiviral drugs. It is well documented that some RNA viruses utilize an Internal Ribosome Entry Site (IRES). To accomplish this, viruses utilize a host's protein, Receptor for Activated C Kinase 1 (RACK1), which is found to play a role in IRES-mediated viral translation. It is reported that RACK1 is essential for hepatitis C virus (HCV), HIV1, CrPV and HSV1 translation and more than 80 different viruses are known to use IRES based translation. Therefore host RACK1 protein can be an attractive target for developing broad anti-viral drug. Depletion of host's RACK1 will potentially inhibit virus replication. This background study has led us to the investigation for a possible development of novel antiviral therapeutics such RACK1 inhibitors or activators. By utilizing our lab developed crystal structure of the RACK1A protein from model plant *Arabidopsis*, and using a structure based drug design method, we discovered dozens of small compounds that can potentially inhibit the RACK1 functional tyrosine residue (Y248) phosphorylation. SD-29 is identified as the most potent binder to the RACK1A Y248 phosphorylation pocket. We found in-vitro studies that SD-29 can potentially repress viral (HCV and HIV1) IRES based luciferase reporter gene translation in HSB-1 and Hela cells. Current investigation is focused on the use of the compounds to inhibit virus proliferation.

## MEDI 315

### **Targeting challenging protein-protein interactions with DNA-encoded libraries**

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DNA-encoded libraries (DELs) are becoming increasingly utilized to identify chemical probes and hit molecules of pharmaceutical interest. DELs allow for library sizes in vast excess of those achieved in traditional high-throughput screening campaigns by combining the encoding properties of DNA and the molecular diversity achieved through combinatorial synthesis. Using DNA-programmed combinatorial chemistry (DPCC), we present two peptidomimetic libraries specifically designed for targeting protein-protein interactions. These libraries include natural amino acids, unnatural amino acids, peptoids (*N*-substituted glycines), and extended peptoid submonomers. The first library is approximately 500,000 small molecule peptidomimetics and allows for further derivatization to append known pharmacophores or crosslinking groups. The second library of 250 million members occupies the middle space of ligands, larger in molecular weight than traditional small molecules but smaller than biologics, including both linear and macrocyclic peptidomimetics. We present selection data from these two libraries against several target proteins using both traditional affinity selections, as well as alternative selection approaches for the enrichment of low affinity ligands.

## MEDI 316

### **Formulating a toothpaste that intraorally delivers vitamin D using penetration enhancers**

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The predominant source of vitamin D in humans is exposure to sunlight. Hence, the fact that people living in the sunny region of the world still suffer from vitamin D deficiency remains a mystery. Various vitamin supplementary products are sold dramatically. However, everyone admits that it is very difficult to maintain their vitamin intake on a daily basis. On the contrary, most people brush their teeth once a day at least. Combining the fact that most people brush their teeth at least once a day with the idea of easy vitamin D delivery deemed to be an effective method. In this study, a vitamin D-deliverable toothpaste was formulated with an emulsion base that was formulated with distilled water, olive oil and emulsifying wax. On top of that, traditional essential ingredients of toothpaste (TSPP, Sorbitol, Xanthan Gum, Sodium Bicarbonate, Silica) were carefully mixed with Vitamin D and

penetration enhancers Sodium Dodecyl Sulfate (SDS) and Polysorbate 80. After it was formulated, various properties of the formulation, such as the presence of abrasive particles, abrasiveness, spreadability, pH, foaming ability and cleaning ability were tested through recommended methods and were compared with commercial toothpastes, Colgate and Crest. Our formulations' toxicity was tested by using Daphnia through serial dilution. Transepithelial electric resistance (TEER) Values of earthworm skin were monitored and its weight reduction capability of earthworm were evaluated. As a result, the vitamin D toothpaste that formulated in this study had more favorable properties in terms of its spreadability, pH and cleaning ability, but its abrasiveness and foaming ability were inferior to the two other commercial toothpastes. The daphnia study, TEER Values and L. terrestris weight reduction capability test demonstrated that this formulation intraorally increased the vitamin D delivery. It was expected that the SDS and polysorbate 80 might facilitate vitamin D to deliver across the skin which was the epithelial tissue like intraoral area.

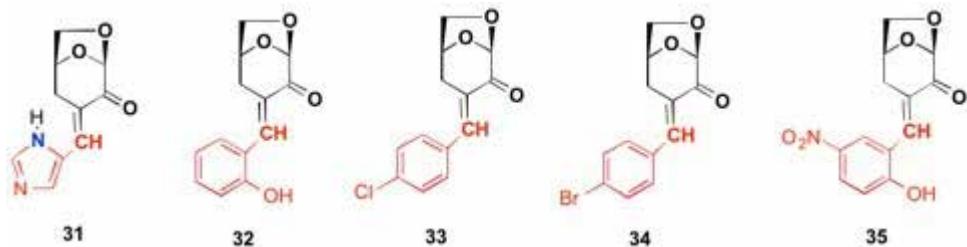
## MEDI 317

### Synthesis and biological evaluation of novel thiophene, pyrrole and aromatic exo-cyclic carbohydrate enone derivatives. Part II

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In search of new heterocyclic anticancer agents a series of newly conceptual 1,6- anhydrosugar derivatives **31-35** functionalized at C-3 position with thienyl, pyrrole and furyl moieties have been designed, synthesized and screened for their cytotoxic activity against human cancer cell lines such as human ovarian carcinoma (A2780) and variant of ovarian carcinoma resistant to cisplatin (A2780cis), human colon cancer LoVo and human brain astrocytoma U87 cells. Cancer cells were incubated in the presence of increased concentrations of tested compounds for 24h. Their cytotoxic properties were evaluated using colorimetric cell viability assay CCK-8. The most promising of the three screened compounds was compound **33**. The most sensitive cell line was LoVo ( $IC_{50} = 200\mu M$ ). We noticed also cytotoxic effect on A2780 cells ( $IC_{50} = 500\mu M$ ) and multidrug-resistant (MDR) derivatives (A2780cis). Morphological changes suggest the induction of apoptosis. The collected data with other previously results suggests that the most anticancer potential has a

1,6-anhydrosugar derivatives with heterocyclic moieties with sulfur as heteroatom within the chlorine atom at position C-4 in aromatic ring. They would be used as templates to screen new analogs linked with thiosugar at C-3 position. Additionally, the new analogs will merit further development as preclinical drug candidates for treating cancer, including MDR phenotype. We also plan to evaluate mechanism of anticancer properties such modified compounds.



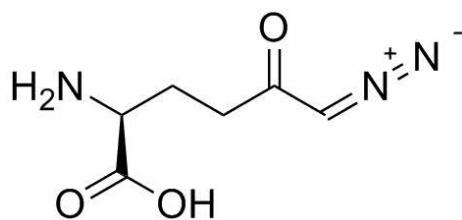
## MEDI 318

### Novel cell directed glutaminase inhibitors as chemotherapeutic agents for hematological malignancies

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The glutamine antagonist 6-diazo-5-oxo-L-norleucine (DON) broadly blocks glutamine utilizing reactions critical for the synthesis of nucleic acids, proteins and the generation of alpha-ketoglutarate for energy metabolism. DON has shown robust efficacy in both lymphoma animal models and exploratory clinical studies, but its development was halted due to marked dose-limiting toxicities, many of which were gastrointestinal (GI)-related, as the GI system is highly dependent on glutamine utilization. We hypothesized that a novel cell-directed prodrug of DON which could deliver the drug selectively to the lymphoid cells would permit significant dose reduction, greatly alleviating the adverse events. Our confidence in this approach is supported by the recent success of Gilead's lymphoid cell-targeted prodrug of the antiviral agent tenofovir, called tenofovir alafenamide (TAF), which in Ph 3 clinical trials provided similar efficacy with a 30-fold dose reduction and less toxicity. By exploiting a similar concept yet *taking a unique molecular design strategy*, we have identified DON prodrugs, which preferentially delivered 30-fold more DON to peripheral blood mononuclear cells (PBMCs) versus human plasma in

vitro. Furthermore, a tissue distribution/tolerability study in swine confirmed the PBMC targeting of the prodrug. In a preliminary toxicity study, versus equimolar DON, the DON prodrug showed enhanced DON delivery to PBMCs, reduced delivery to GI tissues, with less GI pathology and clinical symptoms. Thus through these studies, we have identified several novel analogs that can provide safe and efficacious levels with better tolerability than DON, for cancer treatment.



### **6-diazo-5-oxo-L-norleucine (DON)**

**MEDI 319**

### **Structure-activity relationships for rigid amphipathic fusion inhibitors suppressing tick-borne encephalitis virus reproduction**

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More than twenty dozens viral pathogens of humans are known nowadays, but less than twenty are manageable with prophylactic or therapeutic treatment. Numerous viral infections caused by Ebola virus, West Nile virus, Zika virus etc. have no approved therapeutics and only symptomatic treatment is in use. Given large number of pathogenic viral species, broad-spectrum antivirals should be prioritized. A specific class of compounds, RAFIs (Rigid Amphipathic Fusion Inhibitors), potently inhibits reproduction of enveloped viruses. RAFIs are usually composed of two parts: polycyclic aromatic moiety and polar hydrophilic head, connected by a rigid linker. The hydrophobic nature of RAFIs allows them to interact with the membranes of the virions and

cells and inhibit the viral fusion process.

In this study we investigated the activity of new RAFI series against tick-borne encephalitis virus (TBEV), a small enveloped (+)ssRNA virus from Flavivirus genus. There is no approved specific treatment for TBEV-caused infections, and only vaccination is used as a preventative measure. However, the vaccination coverage is considered to be insufficient. Five RAFI series (Fig. 1) were assessed for anti-TBEV activity in a cell-based assay. All studied compounds inhibited TBEV reproduction at low micromolar concentrations without any signs of cytotoxicity in the studied concentration range. Perylene moiety and rigid linker were found to be crucial for activity. As compared to a typical RAFI, 5-(perylene-3-yl)ethynyl-arabino-uridine (TBEV EC<sub>50</sub> = 0.018 μM), representatives of all series showed similar or superior activity. The most active series of studied RAFIs is (perylene-3-yl)ethynyl-phenols. Thus, the presence of large hydrophilic head appears to be not necessary for RAFI activity.

We conducted the time of addition experiments to reveal the role of RAFI binding to virions. Pre-incubation of the compounds with the virus for 1 h before mixing with the cells does not influence the activity significantly. On the contrary, pre-incubation with the cells for 1 h before the virus addition decreased the EC<sub>50</sub> values by two orders of magnitude. This observation supports the assumption that RAFI anti-TBEV activity is realized through the inhibition of viral fusion stage. The exact mechanism of action as well as the exact role of the perylene moiety remains to be investigated further.

## MEDI 320

### **Design and synthesis of selective histone deacetylase 6 inhibitors based on nexturastat A and evidence of efficacy in melanoma xenograft models**

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Nexturastat A (Nex A) is a potent and highly selective histone deacetylase 6 (HDAC6) inhibitor well known as a potential candidate to treat melanoma. Further efforts were directed towards structure-activity relationship (SAR) studies in a series of compounds based on its phenylhydroxamate scaffold,

with a urea moiety as linker. Fourteen new derivatives with different caps, linkers, and ZBGs were designed, synthesized, and initially evaluated in class I and class IIb HDACs. Several hydroxamate-based analogs exhibited improved potency against HDAC6 compared to Nex A while maintaining acceptable selectivity over HDAC1 and 8. The selectivity of **6c**, **6d**, and **6e** was further verified in the melanoma cells in terms of increasing levels of acetylated tubulin rather than levels of acetylated histone. Moreover, the analogs **6c** and **6d** exhibited low *in vitro* anti-proliferation effects in various melanoma cells and human lymphoma cells, but significantly down-regulated the production of immunosuppressive cytokine IL-10 in macrophages. To evaluate the *in vivo* efficacy of the metabolically stable HDAC6 inhibitor **6d**, C57BL/6 mice bearing B16-F10-luc melanoma tumors were treated with **6d**, resulted in 100% survival rates compared to the control group (60% survival) and more reduced tumor volumes in comparison with the group treated with Tubastatin A. In contrast, no significant effect on the growth of melanoma tumors was observed in the immunodeficient (SCID) mice after the treatment of compound **6d** for 20 days. Taken together, the initial finding suggests that the developed Nex A analog **6d** displays non-cytotoxic property and improved capability to impair tumor growth in melanoma models, which is involved in the regulation of inflammatory and immune response.

## MEDI 321

### **Design, synthesis, and biological evaluation of novel histone deacetylase inhibitors as anti-cancer agents**

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Despite major advances in cancer treatment strategies in recent years, significant limitations still remain. Selectively targeting cancer cells without affecting normal cells is a challenging task. Histone deacetylase (HDAC) enzymes, which are overexpressed in many cancer tissues, provide a potential target for cancer chemotherapy. Therefore, HDAC inhibitors (HDACis) are currently being widely investigated as anticancer agents. Most of the current HDACis are not selective and have undesirable side effects. Selective inhibition of specific HDAC isoforms to preferentially suppress the proliferation of cancer cells is a goal yet to be achieved. Most of current clinically used HDAC inhibitors have hydroximic acid as the zinc-binding group. We used molecular modeling studies to design a new class of HDAC inhibitors with a novel zinc-binding group. Some of these

compounds showed higher cell growth inhibition than the FDA approved drug vorinostat (SAHA) and showed mitotic arrest in the cell inhibition assay. The design, synthesis, and biological activity of these compounds will be presented.

## MEDI 322

### Surfing the kinetic and thermodynamic map in a hit to lead process

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Drug Discovery is a multi-parameter process. One of the key parts of drug design is the understanding and optimization of molecular interactions between compound and protein target. It has been recognized that in addition to the binding affinity, both kinetics and thermodynamics of binding can potentially give further insights into compound binding and help to prioritize chemical series. Here we describe a case study wherein a set of 100 compounds were profiled by both ITC (Isothermal Calorimetry) and SPR (Surface Plasmon Resonance) binding studies for a drug target. As expected, screening hits displayed weak affinity with no measurable binding kinetics. As compounds become more potent, cellular activity of compounds correlated with slower dissociation rate constants but not with association rate constants. We also sought to relate the thermodynamics of compound binding to potency. A significant challenge is that thermodynamics parameters are quite sensitive to purity and chirality of compounds however, potency in compounds were driven more by favorable change in enthalpy compared to entropy. This case study shows how kinetics and thermodynamics of compound binding may give deeper insights into compound-protein interactions. We also describe some of the challenges associated with obtaining these data and considerations related to how to efficiently apply them to a hit-to-lead-campaign.

## MEDI 323

### PROTAC design of Mdm2 degraders: A novel efficient approach for cancer therapy

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Human murine double minute 2 (Mdm2) protein is a primary, endogenous cellular inhibitor of the tumor suppressor p53 through their direct protein-protein interaction, which makes it an attractive cancer therapeutic target. Although highly potent and specific small-molecule inhibitors of Mdm2-p53 protein-protein interaction were discovered in the past decades with several of them in clinical development for cancer treatment, activation of p53 by Mdm2 inhibitors leads to dramatically upregulation of Mdm2 mRNA and accumulation of Mdm2 protein since Mdm2 is a direct transcriptional target of p53. Dramatic upregulation of Mdm2 protein by Mdm2 inhibitors not only limit their potential clinical efficacy but also may have unwanted deleterious effects due to the oncogenic activity of Mdm2 protein.

In present study, we have designed and synthesized several proteolysis-targeting chimera (PROTAC) small-molecule molecules to induce the degradation of Mdm2 protein and investigated their therapeutic potential and mechanism of action *in vitro* and *in vivo*. Our best Mdm2 degrader (**LE-004**) could effectively induce Mdm2 degradation in leukemia cells at as low as 1 nM within 1 hour. Degradation of Mdm2 induced by **LE-004** could effectively induce accumulation of wild-type p53 protein and activate of p53 transcriptional activity in leukemia cells without accumulation of Mdm2 protein. **LE-004** could potently inhibit cell growth and induce apoptosis in leukemia cells in low nano-molar concentrations, which is 10-100 times more effective than the Mdm2 inhibitors. The PD and efficacy studies have shown that **LE-004** could strongly activate wild-type p53 in xenograft AML tumor tissues in mice and achieve complete tumor regression in mice at well-tolerated dose-schedules in mice. In conclusion, PROTAC designed Mdm2 degraders could successfully induce Mdm2 degradation and corresponding cancer cell growth inhibition, which might be developed as a novel efficient strategy for cancer therapy.

## MEDI 324

### **Synthesis of FR900098 analogs as inhibitors of *Plasmodium Falciparum* and *Mycobacterium tuberculosis* 1-deoxy-D-Xylulose-5-Phosphate Reductoisomerase (Dxr)**

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*Plasmodium falciparum* (Pf) and *Mycobacterium tuberculosis* (Mtb) are responsible for millions of infections and deaths each year. Although chloroquine and artemisinin combination therapy have been effective treatments for malaria, Pf resistance has been observed in many parts of the world including southeast Asia. Similarly, multi-drug and extensively-drug resistant strains of Mtb have resulted in diseases that are very difficult to treat. New therapies are needed to fight these infectious diseases. The non-mevalonate pathway of isoprenoid synthesis (NMP) is a promising drug target due to its importance in the life cycles of Pf and Mtb and its absence in humans. 1-deoxy-D-xylulose-5-phosphate reductoisomerase (Dxr) is the first committed step in the NMP, and FR900098 is a natural product and potent inhibitor of Dxr activity. The polar nature of FR900098, however, limits its efficacy against many bacterial species, including Mtb. We have focused on the design, synthesis, and evaluation of new analogs of FR900098 with improved activity against Dxr and enhanced efficacy against whole cells. These modifications further validate this enzyme as a novel drug target, worth pursuit in our efforts toward new antimicrobial compounds.

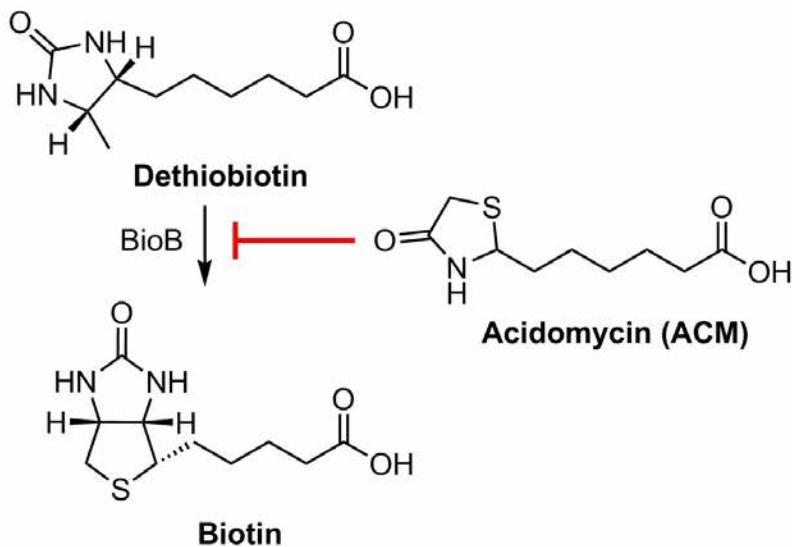
## **MEDI 325**

### **Revitalizing an old molecule: Investigating acidomycin as an inhibitor of *Mycobacterium Tuberculosis* biotin synthase**

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The antibiotic Acidomycin (ACM) discovered in 1952 was shown to possess selective activity against *Mycobacterium tuberculosis* (Mtb). Based on its structural similarity to biotin it was hypothesized to be an antimetabolite and early experiments demonstrated addition of exogenous biotin could rescue Mtb from ACM. However, the specific molecular mechanism of action of ACM remains unresolved. Given the paucity of new drugs for tuberculosis,

the continued increase of drug-resistant *Mtb* strains, coupled with the simple chemical structure and selective antitubercular activity, we initiated a program to investigate the detailed mechanism of action, discern the structure-activity relationships, and use contemporary medicinal approaches to improve upon the drug disposition properties to enhance oral bioavailability and tissue distribution. Using classic feeding experiments of biotin pathway intermediates, we isolated the penultimate step performed by BioB, a radical S-adenosylmethionine (SAM) enzyme that catalyzes the C-H activation and insertion of sulfur in dethiobiotin (DTB) to afford biotin as the likely target based on the ability of biotin to rescue activity in ACM-treated *Mtb* and failure of DTB and all upstream metabolites to restore growth. *Mtb* strains that allow differential expression of BioB showed a monotonic relationship between BioB protein levels and ACM susceptibility further supporting this molecular target. To biochemically validate these findings, we established an in vitro enzyme assay for this notoriously challenging radical SAM enzyme and confirmed ACM is a potent enzyme inhibitor of BioB. Structure-activity relationships have confirmed the activity resides in the (–)-antipode and demonstrated the C5 alkyl chain is optimal and thiazolidinone heterocycle is essential for activity. These results provide a foundation for future studies aimed to revitalize this old antibiotic that acts by a novel mechanism of action.



## MEDI 326

### Antimycobacterial 2-aminoquinazolinones: Synthesis and biological evaluation

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Tuberculosis (TB) is a life-threatening infectious disease caused by *Mycobacterium tuberculosis* (*Mtb*). Globally, TB is a major public health burden with an estimated 10.4 million new cases and 1.8 million deaths reported in 2015. Although TB is curable, the treatment options currently available are beset by numerous shortcomings such as lengthy and complex treatment regimens, drug-drug interactions, drug toxicities, as well as emergence of widespread multi-drug resistance. Therefore, there is an urgent and compelling need to develop new, more effective, safer drugs with novel mechanisms of action, and which are capable of shortening treatment duration. 2-aminoquinazolinones with low  $\mu\text{M}$  antimycobacterial activity were identified through phenotypic whole-cell *in vitro* screening. In this study, about 90 analogues were synthesized in an effort to deliver an optimized lead compound. When evaluated against H<sub>37</sub>Rv *Mtb* strain grown on GASTE-Fe media, several compounds exhibited potent activity ( $\leq 10 \mu\text{M}$ ) and clear SAR trends. In addition, potent compounds had low cytotoxicity ( $> 25 \mu\text{M}$ ) and high microsomal metabolic stability. Candidates subjected to *in vivo* pharmacokinetics studies in mice exhibited good plasma exposures, favorable half-lives and were well tolerated. However, the representative compound evaluated for *in vivo* efficacy studies in an acute mouse model lacked activity. Further studies, including use of different growth media, showed that 2-aminoquinazolinones killed *Mtb* *in vitro* via a glycerol-dependent mechanism of action. These findings correlated well with the results from resistant mutant generation and whole genome sequencing studies, which revealed that all strains resistant to the investigational compound had SNPs in glycerol metabolism genes: *glpD2* and *glpK*.

## MEDI 327

### **Synthesis and microbiological evaluation of 2-amino-4,5,6,7-tetrahydrothieno[2,3-c]pyridines against sensitive and drug resistant *Mycobacterium tuberculosis***

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Tuberculosis (TB) is an infectious disease caused by the bacterium *Mycobacterium tuberculosis* (*Mtb*). One-third of the world's population is thought to be infected with TB. New infections occur in about 1% of the population each year. In 2014, there were 9.6 million cases of active TB which resulted in 1.5 million deaths. The World Health Organization declared TB a "global health emergency". For all of this a lot of studies are developed to stop tuberculosis that aimed to save 14 million lives. We synthesized a group of 2-aminothiophene carboxamides which have been reported to inhibit Pks13, a validated anti-TB drug target. Pks13 catalyzes the condensation of key lipids to produce  $\alpha$ -alkyl  $\beta$ -ketoacids. The  $\alpha$ -alkyl  $\beta$ -ketoacids, with the help of the thioesterase (TE) domain of Pks13, are transferred onto trehalose. This step is followed by reduction of a keto moiety by corynebacterineae mycolate reductase A (CmrA) to produce trehalose monomycolate (TMM). TMM is utilized by the Ag85 complex for the biosynthesis of trehalose dimycolate (TDM, Cord Factor) and mycolylarabinogalactan (mAG). TDM and mAG are constituents of *Mtb* cell wall. We prepared the compounds by using *N*-ethylpiperidine-4-one, cyanoacetamide, sulfur and morpholine in first step followed by *N*, *N*-(dimethylamino) pyridine, triethylamine and 2,3,4,5-pentafluorobenzoyl chloride in the second step. Several of the compounds showed potent anti-*Mycobacterium tuberculosis* activity. Further, the compounds were characterized for protein binding, CACO-2 permeability, cytochrome P450 inhibition, stability in human liver microsomes and in cell based toxicity assays. Based on the results some compounds showed dramatically increased microsomal stability and showed significantly reduced toxicity. Most of the compounds retained their antimicrobial activity.

## MEDI 328

### New carbapenem antibiotics with activity against mycobacterium tuberculosis and mycobacterium abscessus

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A series of atypically substituted carbapenem antibiotics was synthesized and evaluated against replicating and dormant Mtb as well as Mabs. Some of these new carbapenems have activity superior to meropenem. Structure-activity relationships will be discussed.

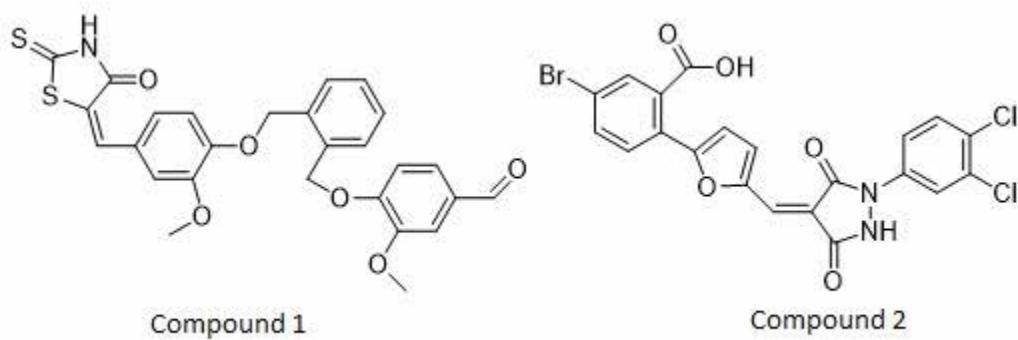
## MEDI 329

### Imparting intrinsic fluorescence as an approach towards rapid inhibitor screening and mechanistic evaluation of tuberculosis shikimate kinase

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The fate of the shikimate pathway is the synthesis of aromatic and some cellular metabolites. Shikimate kinase (SK) alongside other enzymes of the pathway are essential for viability of pathogens like *Mycobacterium tuberculosis* (*M. tb*). Being absent in mammalian metabolism makes its enzymes attractive targets for development of new antitubercular agents. Our aim is to develop a rapid identification tool for potential SK inhibitors and characterize their mechanisms of inhibition. Interestingly, *M. tb* is devoid of tryptophan (Trp). Sequence alignments and structural studies were used to guide trp substitution on key components of the enzyme. Variants generated

were expressed and purified: N151W (nucleotide-binding domain), E54W (shikimate-binding domain) and V116W (Lid domain). Kinetic parameters (ATP- and Shikimate-dependent) were similar across all variant and wild-type. The three variants showed characteristic and distinct trp emission spectra. Hyperbolic decreases in fluorescence emission were observed with ATP titration for all variants, with  $K_{D5}$ s ranging from 0.2-0.4 Mm, similar to  $K_{Mapp}$  (ATP). In contrast, titration with shikimate produced no change in fluorescence emission by either E54W or N151W *MtSK*, but there was a 30% decrease in V116W emission intensity in the presence of shikimate. V116 is part of the conformationally dynamic lid domain. This observation may point toward shikimate-induced conformational changes in *MtSK*. We also evaluated two inhibitors (see below). Both compounds produced a hyperbolic decrease in fluorescence intensity.  $K_{D5}$ s for Compound 1 ranged from 16 to 33 mM depending on the variant evaluated. For each variant,  $K_{D5}$ s determined for compound 2 were about two fold lower than those of compound 1. Strikingly, emission spectra for the variants were differentially affected by inhibitor binding. ESI-LC-MS data suggest these inhibitors form no covalent adducts with the enzyme and dilution experiments also suggest a slow reversible mechanism in play. Our data suggest that these variants will serve as valuable mechanistic probes of *MtSK* catalysis and inhibition.



## MEDI 330

### Novel pyrimidine antituberculars discovered through machine-learning Bayesian method

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Despite millions of people being infected by *M. tuberculosis* annually, the pipeline of tuberculosis drug discovery still suffers from a lack of novel chemical entities that inhibit validated biological targets and lack cross-resistance to current frontline therapies. Our efforts with machine learning models to discover novel chemotypes with promising growth inhibitory activity versus *in vitro* cultured *M. tuberculosis* have led to the discovery of diaminoaryl-triazine nitrofurylhydrazones. Optimization efforts of the triazine series have led to a focused set of pyrimidines. Compounds were evaluated for *in vitro* activity and cytotoxicity, physiochemical and absorption-distribution-metabolism-excretion (ADME) properties to select candidates for pharmacokinetic profiling in mice. The findings in this program will be disclosed and suggest a potential of these pyrimidine antituberculars for further optimization to develop novel antitubercular agents.

## **MEDI 331**

### **Discovery of 2-aminobenzimidazoles that sensitize *M. smegmatis* and *M. tuberculosis* to β-lactam antibiotics in a pattern distinct from β-lactamase inhibitors**

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A library of 2-aminobenzimidazole derivatives was screened for the ability to suppress β-lactam resistance in *Mycobacterium smegmatis*. Several non-bactericidal compounds were identified that reversed intrinsic resistance to β-lactam antibiotics in a manner distinct from β-lactamase inhibitors. Activity also translates to *M. tuberculosis*, with a lead compound from this study potently suppressing carbenicillin resistance in multiple *M. tuberculosis* strains (including multi-drug resistant strains). Preliminary mechanistic studies revealed that the lead compounds act via a mechanism distinct from traditional β-lactamase inhibitors.

## MEDI 332

### Rational design, synthesis and preliminary biological evaluation of novel C8-linked pyrrolobenzodiazepine-5'-O-[N-(salicyl)sulfamoyl]adenosine conjugates (PBD-Sal-AMS) as anti-tubercular probes with dual mode of action

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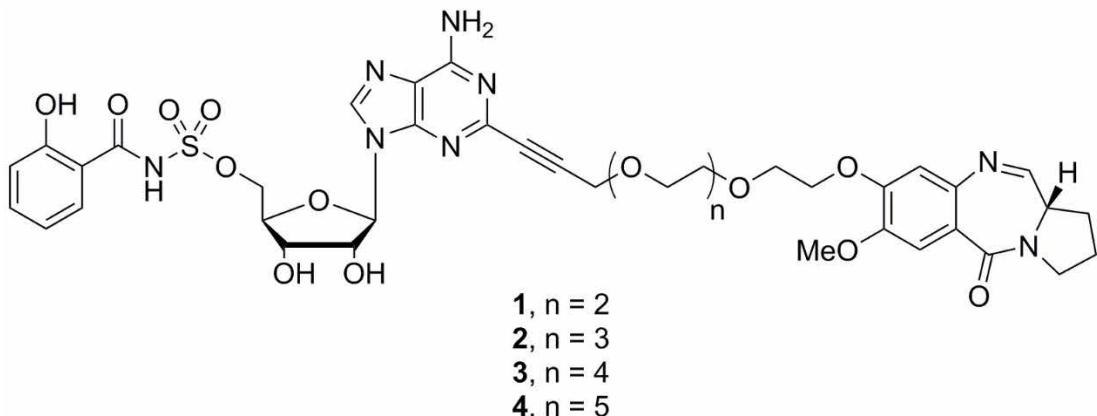
Tuberculosis (TB), a disease caused by *Mycobacterium tuberculosis*, affects an estimated 10 million people worldwide, causing around 2 million human deaths yearly. The emergence of multi-drug resistant strains and lack of new drugs significantly contribute to the tuberculosis pandemic, and new chemotherapeutic agents with novel mechanisms of action are urgently required to fight this global health threat.

The aim of this study was to synthesise and evaluate for biological activity a small library of C8-linked pyrrolo[2,1-c][1,4]benzodiazepine(PBD)-5'-O-[N-(salicyl)sulfamoyl]adenosine(Sal-AMS) conjugates (**1-4**) comprised of a PBD unit tethered to a Sal-AMS moiety through small-chain polyethylene glycol (PEG) linkers. The rationale for the design of **1-4** was provided by our recent findings that PBD-C8-polyamides, which are DNA sequence-selective binding agents, exhibited significant growth inhibitory activity against *M. tuberculosis* (H37Rv) and *M. bovis* (BCG), albeit with some degrees of mammalian cell toxicity.

Therefore, we have postulated that linking the PBD unit to Sal-AMS, a potent anti-tuberculosis agent with an excellent therapeutic index, would provide highly-effective, safer anti-tubercular agents with a two-fold mechanism of action. Upon selective uptake by *M. tuberculosis*, Sal-AMS exerts its activity by inhibiting MbtA, a newly emerging TB enzyme-target involved in the biosynthesis of mycobacterial iron-chelators essential for virulence and growth. Multipharmacophore-containing antibiotics might have the clear advantages of slowing-down drug resistance development, broadening the antimycobacterial spectrum and potentially reducing drug cytotoxicity.

Herein, the synthesis of the four novel PBD-Sal-AMS conjugates **1-4** is reported through a convergent approach. The PBD and Sal-AMS units were independently synthesised and joined through four short-chain PEG<sub>(4-6)</sub> spacers, each containing distal-end reactive groups for attaching the two therapeutic payloads. Notably, a novel oxidation method was used to access the PBD 1,4-diazepine ring.

Conjugates **1-4** are currently undergoing DNA- and MbtA-binding activity evaluation and will be tested *in vitro* for *M. tuberculosis* growth inhibitory activity and human cell cytotoxicity.



## MEDI 333

### Synthesis, optimization, and biological evaluation of novel analogs of DG85 as antitubercular agents

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Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, is an infectious disease that killed ca. 1.4 million people in 2015. It is believed that one-third of the world population is infected with TB and ~9 million new infections are reported every year. The current therapy for drug-sensitive infection consists of isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA), and ethambutol (EMB). However, multi drug resistant (MDR) and extended-drug resistant (XDR) *M. tuberculosis* strains have emerged to the current therapy. Therefore, new antitubercular drugs, with a novel mechanism of action are needed to target drug-resistant as well as dormant/persistent strains. We report the optimization and biological evaluation of novel analogs of a diamide screening hit (MIC = 0.39  $\mu$ M towards *M. tuberculosis* H37Rv). The novel analogs, exhibited significant improvements in their *in vitro* efficacy, physiochemical, ADMET, and pharmacokinetic profiles, enabling their further study as chemical tools and drug discovery entities.

## MEDI 334

### Evaluation of 5-substituted 1,10-phenanthroline and nickel complexes as G4 ligands and telomerase inhibitors

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DNA G-quadruplex (G4) structures have attracted much attention due to their ability to regulate gene expression and the potential role of cancer intervention. The formation of DNA G-quadruplexes requires monovalent cations ( $\text{Na}^+$  and  $\text{K}^+$ ), which can be facilitated by small molecules known as G4 ligands. Phenanthroline is a type of G4 ligand scaffold that forms metal complexes with a large aromatic surface suitable for stacking with G-quartets. In the present work, we evaluated a series of 5-substituted 1,10-phenanthroline-based nickel complexes binding to telomeric G-quadruplex DNA. Results from several biophysical methods including ESI-MS, CD thermal denaturation, CD titration, and FID assay suggested that the side chains at the 5 position of 1,10-phenanthroline were crucial for G-quadruplex recognition. Arylsulfanyl groups were the best side chains regarding binding affinity and selectivity towards G-quadruplex DNA. Several G-quadruplex binding Phen-Ni complexes inhibited telomerase activity *in vitro* and exhibited cytotoxicity against three cancer cell lines. Our results here provide a guidance of utilizing 5- substituted phenanthroline derivatives to design novel G4 ligands.

## MEDI 335

### Discovery of potent BET inhibitors as potential treatments for cancer: Optimization of pharmacokinetic and pharmaceutics properties

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Despite recent advances in cancer treatment, significant unmet need persists. Small molecule approaches remain attractive methods to address many oncologic targets, including the bromodomain and extra-terminal (BET) proteins. We previously disclosed a carboline-containing clinical candidate (BMS-986158) that demonstrated BET-mediated tumor inhibition in preclinical models. Herein, we describe efforts towards a second-generation inhibitor. Deuteration and fluorination strategies were pursued to reduce clearance. Concurrent with these studies, heterocyclic phenyl replacements were employed to drive increases in free fraction and aqueous solubility. This work culminated in the identification of potent BET inhibitors with increased exposure and robust efficacy in a mouse solid-tumor model.

## MEDI 336

### **Discovery of highly potent BET protein degraders based on novel inhibitors inducing complete and durable tumor regression in human acute leukemia xenografts**

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Bromodomains containing protein interacts with acetylated lysine residues on histone tails and functions as epigenetic “readers”. Among them, the bromodomain and extra-terminal (BET) family proteins, including BRD2, BRD3, BRD4 and BRDT, have emerged as ideal ‘druggable’ targets for a number of human diseases, including cancer, inflammation, HIV infection, CNS disorders, and cardiovascular diseases. Here, we report the design, synthesis, and optimization of series of degraders based on our unique inhibitors. Coupling of BET inhibitors with thalidomide/Lenalidomide generated CRBN-based BET degraders. Through optimization of the linker region and evaluation of pharmaceutical properties, the best degrader was identified and was capable of achieving complete and tumor regression at very low dose in RS4;11 mouse xenografts. Our data demonstrate that it is a highly promising BET degrader not only for extensive preclinical investigation but also as a potential clinical development candidate.

## MEDI 337

### N7-substituted pyrrolo[3,2-d]pyrimidine analogues - new small molecule anticancer agents

**Brian Cawrse**, *bcawrse1@umbc.edu. Chemistry and Biochemistry, University of Maryland Baltimore County, Baltimore, Maryland, United States*

Pyrrolo[3,2-d]pyrimidines, commonly called 9-deazapurines, are a medicinally important group of compounds that have shown activity as bactericides and protozoocides, and act as anti-tumor agents through inhibition of a variety of enzymes including tubulin, NEDD8-activating enzyme (NAE), and several kinases. These compounds closely resemble natural purine nucleobases and have been investigated by our laboratory for their use as potential therapeutics against triple negative breast cancer (TNBC), leukemia, non-small cell lung cancer, and pancreatic cancer. A structure-activity relationship (SAR) study showed that the compounds are potent inhibitors of the TNBC MDA-MB-231 cell line, and caused an accumulation of cells in the G<sub>2</sub>/M stage with little apoptosis. This was consistent with earlier reports of 9-deazapurines used as antiproliferative agents. The introduction of a halogen at C9 resulted in increased activity, with the IC<sub>50</sub> against HeLa cells decreasing from 19±3 µM to 0.92±0.04 µM. The halogen also altered the apparent mechanism of action, with the majority of the cells now undergoing apoptosis, although the mechanism for this is still being elucidated. These finding led us to further investigate the effect of substituents on the pyrrole moiety of these compounds with the aim to retain or increase activity while decreasing toxicity. The resulting compounds were synthesized and evaluated for activity against a multiple cancer cell lines. The most promising lead compounds were chosen for toxicity and pharmacokinetic studies to determine therapeutic index as well as to identify the metabolic pathway.

## MEDI 338

### Late-stage modification of tigloyl moiety to ipomoeassin F to enable SAR studies of the natural product

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The resin glycoside, Ipomoeassin F has been shown to be extremely potent again multiple cancer lines with single-digit IC<sub>50</sub> in the nanomolar range. However, the mechanism of the action of the potent natural product is still not fully understood. Some SAR studies have confirmed the pharmacofore importance some of the moieties of Ipomoeassin F. A previous study showed the α,β- unsaturated ketone was vital to overall cytotoxicity of Ipomoeassin F. Nevertheless, none of these studies focused on the importance of the C-3 tigloyl moiety, individually. To study the pharmacofore importance of the tiglic ester, an efficient, scalable, and flexible synthesis route was designed. The 18-linear step synthesis uses several regioselective and chemoselective reactions, while not affecting the highly functionalized disaccharide. This synthesis route modifies the C-3 position in the penultimate step, making the preparation of multiple analogs easily obtainable. The obtained analogs of Ipomoeassin F will help investigate the scientific values of an underexplored natural product with very promising therapeutic potential.

## MEDI 339

### Highly-active influenza endonuclease inhibitors developed from a designer metal-binding pharmacophore library screen

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Annual influenza epidemics are responsible for substantial morbidity and mortality, as well as significant financial burden worldwide. While vaccinations are a reasonable prophylactic for healthy adults, they are less effective for individuals with compromised immunity. The efficacy of these vaccines is also heavily dependent on correctly predicting the predominant infectious strains for any one year, and incorrect predictions can render vaccination less than 30% effective. Existing drugs, such as Relenza (zanamivir; GSK) and Tamiflu (oseltamivir; Roche) which target viral neuraminidase can be useful in treating influenza infections, but must be administered within 1-2 days of infection to be effective and have many undesirable side-effects. Considering this, there is an urgent need for the development of new drugs to prevent and treat influenza infection.

One attractive target for viral inhibition is the cap-dependent endonuclease domain of the viral RNA-dependent RNA polymerase, which mediates 5' cap-snatching. This target is a dinuclear Mg<sup>2+</sup> or Mn<sup>2+</sup> metalloenzyme that lacks a human counterpart. To identify novel inhibitor scaffolds a targeted fragment-

based screen was conducted employing a designer metal-binding pharmacophore (MBP) library. Using a FRET-labeled DNA oligonucleotide substrate, we measured the endonuclease activity of the PA subunit of H1N1 influenza A polymerase in the presence of MBP molecules and identified two MBP families that exhibited strong inhibition: 3,4-hydroxypyridinone-2-carboxylates and α-hydroxytropolones. Several fragments in these families were found to have IC<sub>50</sub> values of <100 nM and were identified as leads for further elaboration. Structure-activity relationships found in the library screen were expanded upon, including a screen of carboxylic acid metal-binding isosteres (MBIs) of the 3,4-hydroxypyridinone-2-carboxylic acid lead. Guided by these findings and previously established SAR, derivatives of both scaffolds were optimized for inhibitory activity. This has resulted in the development of several lead molecules with sub-nanomolar in vitro inhibitory activity (IC<sub>50</sub> <1 nM) against viral PA endonuclease in protein based assays.

## MEDI 340

### **Cholestosome™ mediated delivery of nucleic acids into MCF7 cells**

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This laboratory has developed a neutral lipid based vesicle (the Cholestosome™), that uses naturally occurring lipids to encapsulate and deliver a wide variety of substances, including fluorescein isothiocyanate (FITC) and other small molecules, vancomycin and other antibiotics, insulin and other peptides, IgG antibodies and other proteins as well as plasmid DNA and other nucleic acids. Previous work has shown Cholestosome-mediated delivery of FITC-labelled peptides into various mouse tissues (including brain) after oral administration. Cholestosomes can therefore potentially be used to orally deliver compounds for which intravenous administration is the only effective dosing route. Especially exciting is the potential to orally deliver nucleic acid therapeutics. The present study reports preliminary work on the encapsulation and delivery of plasmid DNA encoding Green Fluorescent Protein (GFP), a molecule widely used as a co-transfection marker and to study protein interaction and localization. Successful transfection of this plasmid results in a cell that displays bright green fluorescence when

visualized under ultraviolet light. Encapsulated GFP was placed on MCF7 cells and examined for presence of green signal indicating successful delivery and conversion of plasmid to protein. Future potential applications for the Cholestosome™ include the delivery of a variety of different molecules used in the treatment and diagnosis of cancer, muscular dystrophy, and Alzheimer's.

## MEDI 341

### **Thiohydroxypyridinones as a scaffold for the development of potent New Delhi metallo- $\beta$ -lactamase-1 inhibitors**

**Rebecca Adamek**<sup>2</sup>, *radamek@ucsd.edu*, **Cy V. Credille**<sup>2</sup>, **Pei Thomas**<sup>4</sup>, **Walter Fast**<sup>3</sup>, **Seth Cohen**<sup>1</sup>. (1) *Chemistry and Biochemistry, U.C. San Diego, La Jolla, California, United States* (2) *Chemistry and Biochemistry, UC San Diego, San Diego, California, United States* (3) *Phar Med Chem, University of Texas at Austin, Austin, Texas, United States* (4) *University of Texas, Austin, Texas, United States*

Since their discovery in 1928 by Alexander Fleming,  $\beta$ -lactam drugs have been the most successful and commonly prescribed class of antibiotics. Unfortunately, the widespread use of this powerful drug class has created a natural selection pressure for bacteria to adapt and develop resistance mechanisms to these therapeutics; most notably the emergence of  $\beta$ -lactamases enzymes.  $\beta$ -lactamases confer bacterial resistance through hydrolysis of the  $\beta$ -lactam bond that is crucial for the activity of these drugs. New Delhi Metallo- $\beta$ -lactamase (NDM-1) is a particularly worrisome metallo- $\beta$ -lactamase that utilizes two active site Zn<sup>2+</sup> ions to achieve this hydrolytic activity. NDM-1 has been shown to be capable of hydrolyzing all clinically relevant  $\beta$ -lactams, including the carbapenems – which are considered a last resort drug against resistant infections. Furthermore, NDM-1 is easily communicable between bacteria populations as it is encoded in *bla*<sub>NDM-1</sub>, a plasmid capable of horizontal transfer, and has already been detected in multiple strains of enterobacteriaceae. Indeed, since its discovery in India in 2008, NDM-1 has rapidly spread among the general population worldwide and has not been limited to hospital-acquired infections. In addition to this ease of transmission, there are currently no clinically approved drugs available against NDM-1, implicating that NDM-1 has a strong potential to lead to untreatable “superbug” type infections.

The ultimate goal of this research is to develop potent inhibitors of NDM-1 to restore  $\beta$ -lactam activity against resistant bacteria. In order to meet this goal,

we have synthesized and screened metal binding pharmacophore (MBP) libraries against NDM-1 to identify MBP fragments that selectively bind to the dinuclear Zn<sup>2+</sup> NDM-1 active site. This screen has revealed thiohydroxypyridinone-based compounds, 3-carboxy-1,2-HOPTO and 2-hydroxyisoquinoline-1-thione, as novel scaffolds for the development of NDM-1 inhibitors. The use of a fragment growth strategy to derivatize these compounds has resulted in the discovery of a new, thiohydroxypyridinone-based NDM-1 inhibitor with a potent IC<sub>50</sub> of 240 ± 10 nM.

## MEDI 342

### **Therapeutic effects of novel benzylguanidine derivative on neuroblastoma tumor cells**

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Neuroblastoma (NB) is a type of cancer that forms in certain types of nerve tissue. Metaiodobenzylguanidine (MIBG) is a compound that can be combined with radioactive iodine (I-131) in diagnostic imaging and therapy of neuroblastoma cancer cells. The purpose of this study is to synthesize and characterize novel benzyl guanidine (BG) derivative, which has similar active group like MIBG, and its polymer conjugated analogue (PLGA-PEG-BG) to compare their effects on neuroblastoma cancer cells with MIBG. We used click reaction method to conjugate the BG to polymer (PLGA-PEG). Dialysis method was used to purify the polymer and the synthesized structures were characterized by <sup>1</sup>H-NMR spectroscopy. Furthermore, we tested the effects of the synthesized compounds on in vivo neuroblastoma cell growing in nude mice. We found that the new compounds significantly decreased tumor growing compared to control group and also, they showed more effective growth inhibition than that of MIBG.

## MEDI 343

### **Discovery of potent and selective Axl/Mer dual inhibitors**

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*Mark A. Wolf<sup>2</sup>, Vijay D. Pawar<sup>3</sup>, Santhosh K. Chittimalla<sup>3</sup>, Chennakesavulu Bandi<sup>3</sup>, Anjan Chakrabarti<sup>3</sup>, Jun Takeuchi<sup>1</sup>. (1) Ono Pharmaceutical Co Ltd., Mishima Gun Osaka, Japan (2) Albany Molecular Research Inc., Albany, New York, United States (3) Albany Molecular Research Singapore Research Centre, Pte. Ltd., Singapore, Singapore*

Axl and Mer are members of the TAM (Tyro3, Axl, Mer) receptor tyrosine kinase family. Axl and Mer play an important role in regulating cell proliferation, survival, migration and cytokine production. Axl/Mer are known to be over-expressed in various types of haematological and solid tumour cancers and have been reported as poor prognostic factors. Recent studies indicate that Axl also appears to play a key role in epithelial-to-mesenchymal transitions (EMTs), which is involved in metastases and drug resistance in solid tumour such as NSCLC and breast cancer, and TAM receptor signaling increases innate immune regulation.

Hit series of pyrrolopyrimidine derivatives were obtained through high throughput screening, and then modifying of them resulted in the identification of a highly potent and selective Axl/Mer dual inhibitor, N-[4-(4-amino-7-methyl-7H-pyrrolo[2,3-d]pyrimidin-5-yl)phenyl]-5-cyclopropyl-2-oxo-1-phenyl-1,2-dihydro-3-pyridinecarboxamide (ONO-6990554, Axl IC<sub>50</sub>: 1.8 nM, Mer IC<sub>50</sub>: 1.2 nM, KDR IC<sub>50</sub>: 1300 nM, IGF1R IC<sub>50</sub>: 1200 nM, BaF3 Axl IC<sub>50</sub>: 6.5 nM). This presentation will describe the drug design which includes initial SAR exploration and efforts for improvement of selectivity, pharmacokinetic properties and *in vivo* efficacy. In addition, we will present the discovery of covalent inhibitor targeting specific cysteine residue at allosteric pocket by analysis of X-ray crystal structure and modeling.

#### **MEDI 344**

#### **Design, synthesis and biological evaluation of 6-aminopenicillanic acid and 7-aminocephalosporanic acid derivatives of emetine**

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6-Aminopenicillanic acid (6-APA) and 7-aminocephalosporanic (7-ACA) have been used in conjugation with anticancer agents to induce dramatic change in solubility, toxicity and chemotherapeutic sensitivity against certain types of cancer. In an ongoing study to explore the anticancer properties of the natural product, emetine, and develop it into a clinically useful anticancer agents, we

have designed emetine hybrids with other anticancer and antibiotic agents. The present study is focused on the design and synthesis of 6-APA and 7-ACA derivatives of emetine towards the development of new anticancer antibiotics.

## MEDI 345

### Synthesis of azotochelin analogues as antibiotic leads

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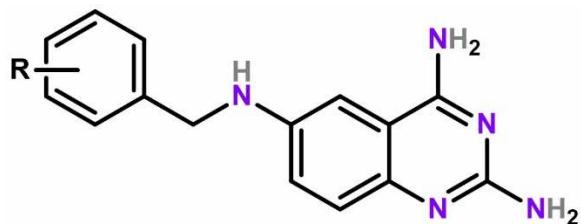
Iron is used in various biological pathways in bacterial cells. Various types of bacteria, including pathogenic organisms produce siderophores to acquire the iron from the environment for growth and reproduction. Siderophores are biosynthesized via organized enzymatic machinery, and they chelate iron, and bacteria uptake chelated form of siderophores via dedicated membrane channels. As antibiotic resistant is a growing problem, inhibiting siderophore production or transport may provide a unique way to kill pathogenic bacteria. Brominated analogues of siderophores like enterobactin have been found to be active against certain bacteria. These analogues inhibit an enzyme that biosynthesizes an enterobactin-like siderophore. The other siderophores which have catechols in them, like azotochelin, can be a promising lead as an antibiotic. We have synthesized natural azotochelin and its brominated analogues by electrophilic aromatic halogenation. We will be synthesizing second generation analogues of azotochelin using Pd-catalyzed cross coupling technique using brominated azotochelin as a precursor. Our future goal is to perform antibacterial testing for these analogues against Gram-negative and Gram-positive bacteria.

## MEDI 346

### Design, synthesis and *in vitro* antiproliferative evaluation of quinazoline 2,4,6-triamine and 6-aminoquinazoline-4-(3H)-one derivatives in ovarian cancer skov-3 cell line

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Ovarian cancer is any cancerous grow that occurs in the ovary. Furthermore, represents one of deadliest tumor of the female genital tract and it is one of the main causes of the death in women. Among one of the characteristics to be highlighted in this type of cancer is the overexpression of Epidermal Growth Factor Receptor (EGFR), which belong to the family of tyrosine kinase receptor protein and this is related with a poor prognosis in people presenting it. Although several drugs have been developed as egfr inhibitors, such as gefitinib, erlotinib, vandetanib, among others, all of them are characterized by present side effects. Therefore, the need to develop new molecules that provide a new alternative to society in the treatment of such ailment. In that sense, we have designed and synthetized a series of eighteen quinazolines analogues to the gefitinib, which have different substituents in the phenyl ring that is in the position six of the quinazoline, and then we evaluated the antiproliferative activity in a cell line of ovarian cancer (SKOV-3). Among quinazoline derivatives evaluated, the compound with substituent trifluoromethoxy presented the best antiproliferative activity with a IC<sub>50</sub> of 10.58 uM to 24 hours of exposition.



## MEDI 347

### Efforts towards the structure diversification of amorfrutins to investigate the structure activity relationship

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PPAR $\gamma$  (peroxisome proliferator-activated receptor gamma) is a nuclear receptor and has emerged as a druggable target for metabolic diseases and inflammation associated health conditions. Amorfrutins, a unique class of prenylated 2,4-dihydroxybenzoic acid containing natural products have been identified as potent modulators of PPAR $\gamma$ . As they have been proven to be partial agonist at low  $\mu$ M concentration, there is a high interest in developing structural analogs with improved pharmacological properties, and low toxicity. Our research lab has a long-standing interest in developing novel chemical

methods to modify natural products of biological interest. Hence, we have initiated a research program to investigate a direct and highly efficient synthetic approach to generate structurally diverse analogs of amorfrutins. We have converted the commercially available 4-methoxysalicylic acid into an advance precursor by an esterification, and subsequent silyl protection of 2-OH group. Using a selective halogenation strategy, we have synthesized both brominated and iodinated derivatives. These halogenated compounds serve as the precursor for structure diversification via metal catalyzed cross-coupling reactions. Current effort is focused on optimizing the cross-coupling conditions to install various alkyl and aryl motifs at C-3, C-5 and C-6 positions. This method enables us to readily functionalize the aromatic ring with various substituents to generate a library of analogs for structure activity relationship (SAR) evaluation. We plan to evaluate the anti-inflammatory effects and anticancer activity of these compounds in suitable *in vitro* systems. Since there have been no reports on extensive SAR of amorfrutins, the first generation analogs we synthesize will shed light on the SAR. As toxicity associated with PPAR $\gamma$ modulators has been a major concern, we plan to test the hepatotoxicity profile of the analogs as well.

## MEDI 348

### Closing the loop between synthesis and design: Balancing optimisation of potency with selectivity

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Drug discovery is a multi-parameter optimisation (MPO) process, in which the goal is to simultaneously optimise target potency, selectivity and a broad range of Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) properties, prioritising those compounds most likely to succeed against a project's objectives. However, the ultimate goal is not simply to select from those compounds already available, but to design new compounds with an improved balance of properties.

*De novo* design approaches typically result in more *in silico* compound ideas than can reasonably be synthesised and tested. Assessment of these virtual compounds therefore requires development and use of *in silico* models which predict potency, or other properties, based upon information derived from the known structure-activity relationships (SAR). These predictive models can be used in an MPO assessment of selectivity, optimising for high potency at one

receptor and low potency at others.

We present a truly MPO approach to *de novo* design, using Probabilistic Scoring and quantitative structure-activity relationship (QSAR) models to generate and prioritise high quality compounds ideas. This approach enables simultaneous optimisation of the virtual compounds for high potency with selectivity over multiple receptors, whilst also considering a balanced ADMET profile. It is exemplified with optimisation of selective dipeptidyl peptidase (DPP) inhibitors.

## MEDI 349

### Structure-based drug design (SBDD) and SAR of tetrapeptides competitive inhibitors of Y-49 β-lactamase

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Pathogen resistance to β-lactam antibiotics is spreading. One of the most effective resistance mechanisms involves the production of β-lactamases that hydrolyze β-lactam antibiotics. More than 840 β-lactamases are known to date. One of the most effective approaches to overcome the resistance to β-lactam antibiotics involves the discovery of new *non β-lactam* scaffolds, inhibitors of β-lactamases. Herein, we report a structure-based design approach for the discovery of potential tetrapeptides inhibitors of Y-49 enzyme, a class A beta-lactamase, from *Mycobacterium tuberculosis*. The tetrapeptide scaffold was derived from the original sequence RRGHYY which was found to inhibit class A *Bacillus anthracis* Bla1, ( $K_i = 42 \mu\text{M}$ ) and class A TEM-1 β-lactamase, ( $K_i = 136 \mu\text{M}$ ) (Huang W et al., *Protein Eng Des Sel* 16:853-860). *In silico* docking experiments were performed with Autodock Vina coupled with “SeeSAR” module from Optibrium and the beta-lactamase 3M6B.pdb as target protein leading to the discovery of novel tetrapeptides 2HN-R-X-H-Y-CONH<sub>2</sub>, potential competitive inhibitors of beta-lactamase. X was varied with all 20 natural L- and -D-amino acids. Our initial structure-activity relationship (SAR) studies established that acidic and basic amino acids (such as Asp, Lys and Arg) and small neutral like Gly occupying the X-position (P2) would increase the inhibitory activity ( $K_i$ ). Moreover, we are showing that cyclic peptides derived from cyclo(2HN-dR-X-H-Y-CONH<sub>2</sub>) have

at least fivefold better Ki than the linear analogous tetrapeptide. The new tetrapeptide dRGH<sub>Y</sub> is a lead competitive inhibitor of Y-49 beta lactamase with Ki of 2.2  $\mu$ M. The SBDD and SAR lead to the discovery of a new tetrapeptide pharmacophore which could be used for further designing of linear and cyclic peptides with D- and unnatural amino acids with improved anti- $\beta$  lactamase activity.

## **MEDI 350**

### **Design and synthesis of novel uridine analogue with possible anti-HCV activity**

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According to the World Health Organization (WHO), hepatitis C affects chronically 130-150 million people worldwide. Seven hundred thousand patients die each year from hepatitis C-related liver diseases. The risk of death from liver cancer and cirrhosis can be reduced by antiviral medications, but the access to medications is limited due mainly to the high cost of treatment [\$31,452 to \$410,548 per Quality-Adjusted-Life-Year (QALY) gained owing to the individual patient characteristics such as fibrosis stage, comorbidities, estimated life expectancy, and HCV genotype]. The recent publication of the crystal structure of the HCV RNA-dependent RNA-polymerase (RdRp) NS5B with an inhibitor in the active site has enabled elegant development of new agents targeting the NS5B enzyme. Uridine analogues modified at the 2' position have been proven to be effective against the HCV RdRp NS5B as a chain terminator. We present here the design and synthesis of new uridine analogue starting from uridine in six steps. The synthesis will be extended to the synthesis of phosphoramidate prodrugs. This modified uridine will be tested against HCV using the Replicon assay.

## **MEDI 351**

### **Synthesis of 2'-C-methyl pseudouridines for the inhibition of HCV RNA-polymerase**

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Studies of the structure and function of Hepatitis C Virus (HCV) RNA-dependent RNA polymerase (RdRp) have broadened our understanding of HCV viral RNA replication and the mechanism of action of this RNA polymerase. These findings have encouraged the development of inhibitors of this target for antiviral therapy. Anti-HCV activity has been shown in-vivo with C-nucleosides containing a 2'-C-methyl (Me) substituent. The presence of the 2'-C-Me group prevents the chain elongation catalyzed by the RdRp NS5B. To further investigate this phenomenon, the synthesis of modified pseudouridines was performed using earlier developed strategies for the unmodified nucleoside. By coupling of the protected pyrimidine to a likewise protected 2'-C-methyl-D-ribono-lactone the C-nucleoside was formed. Subsequent reduction and ring closure generated alpha and/or beta- 2'-C-Me pseudouridines. Through the work presented here, this modified pseudouridine synthesis was optimized and will be utilized in the synthesis of other modifications of this naturally occurring nucleoside and evaluated for their antiviral activity. This will include conversion to substrates suitable for the monophosphate prodrug strategy.

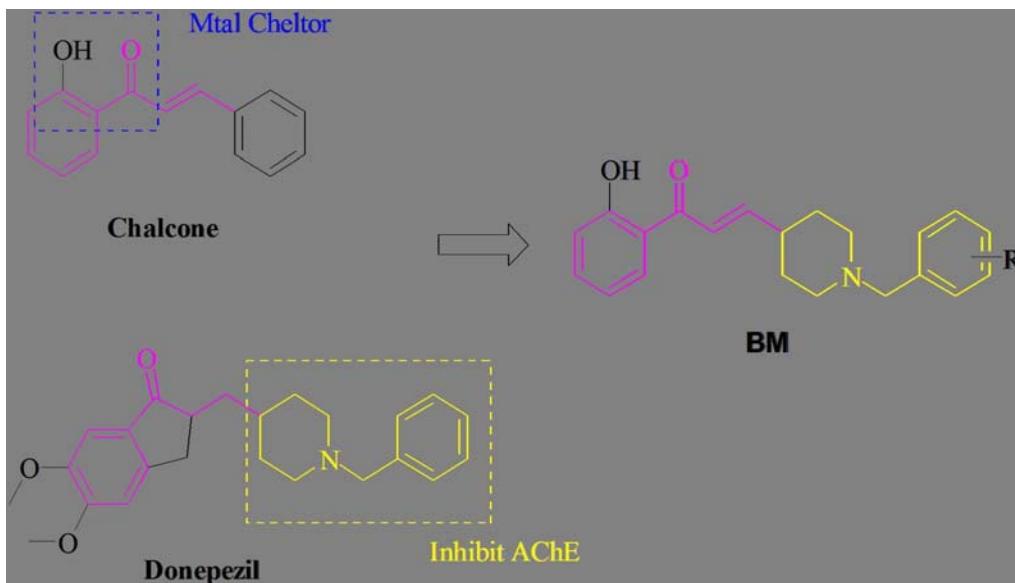
## **MEDI 352**

### **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- $\beta$  (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.



## MEDI 353

### Design and development of pramipexole-donepezil hybrids as potential therapeutics for Alzheimer's disease

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Alzheimer's disease (AD) is considered as one of the leading causes of deaths among the various neurodegenerative diseases in the old age peoples. AD is characterized by excessive deposition of extracellular  $\beta$ -

amyloid plaques and intracellular neurofibrillary tangles. Although, number of hypotheses regarding the etiology of AD have been reported in the past but the exact cause of AD still remains unknown. Hyperphosphorylation of tau protein is considered as one of the most accepted reasons by the scientists and researchers throughout the world. According to the latest WHO report, there are 47.5 millions of people worldwide sufferings from dementia and this disease will devastate 75.6 million people by 2030 and this figure will multiply thrice by 2050.

Keeping in the mind urgent need for the development of novel potential therapeutics for the treatment of AD, in this presentation we report the design, synthesis and biological evaluation of a series of novel multi-targeted ligands. The synthesis of multi-targeted ligands is achieved through combining benzylpiperidine moiety of donepezil with pramipexole scaffold.

Benzylpiperidine moiety is a key pharmacophore for the AChE inhibition whereas Pramipexole has neuroprotective role in AD through its antioxidant effect. The designed prototypes are synthesized and evaluated for their anti-Alzheimer potential using preliminary cholinesterase inhibition (AChE and BuChE) assay. Among the synthesized derivatives, compound **6** was found to be the most potent with an  $IC_{50}$  value of 0.127  $\mu M$  against AChE in comparison to donepezil ( $IC_{50} = 0.01 \mu M$ ) while compound **5** showed activity against BuChE with an  $IC_{50}$  value of 3.068  $\mu M$  (Donepezil  $IC_{50} = 1.26 \mu M$ ). Further biological screening of the synthesized compounds is in pipeline.

## MEDI 354

### **SUVN-502, A novel, potent and pure 5-HT<sub>6</sub> receptor antagonist - proof-of-concept study design in moderate Alzheimer's disease patients**

**Venkata Satya Ramakrishna Nirogi**, nvsrk@suven.com, Kambhampati R. Sastry, Anil K. Shinde, Mohammed Rasheed, Rajesh K. Badange, Thrinath Bandyala, Venugopalrao Bhatta, veena reballi, Pramod Kumar Achanta, Kiran Kumar kandukuri, Kumar Bojja, Sangram Keshari Saraf, Koteshwara Mudigonda, Pradeep Jayarajan, Gopinadh Bhyrapuneni, Vinod Kumar Goyal, Venkat Jasti. Discovery Research, Suven Life Sciences Ltd, Hyderabad, Telangana, India

Optimization of a novel series of 3-(piperazinylmethyl)indoles as 5-hydroxytryptamine-6 receptor (5-HT<sub>6</sub>R) antagonists resulted in identification of 1-[(2-bromophenyl)sulfonyl]-5-methoxy-3-[(4-methyl-1-piperazinyl)methyl]-1H-indole dimesylate monohydrate (SUVN-502) as a clinical candidate for potential use in the symptomatic treatment of Alzheimer's disease. SUVN-502 is a pure 5-HT<sub>6</sub>R antagonist with >100 fold selectivity against other closely

related serotonin subtypes. It exhibited excellent ADME properties and robust preclinical efficacy. Co-treatment of SUVN-502 with standard of care memantine and donepezil (triple combination) produced synergistic effects in animal models. SUVN-502 showed excellent margin of safety in preclinical long-term safety studies. The clinical portions of the single and multiple ascending dose studies evaluating safety, tolerability and pharmacokinetics have been successfully completed allowing the initiation of a Phase-2 proof of concept study in USA. The efficacy and safety of SUVN-502 is currently being evaluated in moderate AD patients aged between 50 to 85 years currently treated with donepezil and memantine. Details of the preclinical data and Phase-2 study design will be presented.

## MEDI 355

### **Pyrimidine carboxamide derivatives as muscarinic acetylcholine subtype 1 positive allosteric modulators (M<sub>1</sub> PAM) for the treatment of cognitive deficits in Alzheimer's disease**

**Venkata Satya Ramakrishna Nirogi, nvsrk@suven.com, Mohammed Rasheed, Anil K. Shinde, Parijatha Kalukuri, Durga Malleshwari Kancharla, Narsimha Bogaraju, Ramkumar Subramanian, Nageswara Rao Muddana. Discovery Research, Suven Life Sciences Ltd, Hyderabad, Telangana, India**

Positive allosteric modulation (PAM) of the muscarinic acetylcholine receptor subtype 1 (M<sub>1</sub>) has drawn the attention of the researchers across the world as novel therapeutic approach for the treatment of cognitive deficits associated with Alzheimer's disease (AD). Moreover, selective M<sub>1</sub> PAMs also showed disease modifying potential, in addition to symptomatic cognition enhancing properties. A series of pyrimidine carboxamide derivatives were designed synthesized and evaluated for their in-vitro potencies towards muscarinic receptors. Most of the compounds showed potent in vitro potencies towards M<sub>1</sub> receptor and found to be selective against other sub types M<sub>2</sub> to M<sub>5</sub>. The selected compounds were further evaluated in pharmacokinetic studies to assess their exposures in plasma and brain. Details of design, chemistry, structure activity relationship, *in vitro* potencies and pharmacokinetic studies of pyrimidine carboxamide derivatives will be disclosed in this poster presentation.

## MEDI 356

### **Design and synthesis of novel [F18]-labeled histone deacetylase inhibitors as potential molecular imaging agents for Alzheimer's disease**

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Due to the lack of tools for early diagnosis of Alzheimer's disease (AD), the patients were usually diagnosed with AD after exhibiting significant clinical symptoms and it is extremely difficult to develop new drugs for AD. Histone deacetylase inhibitors (HDACIs) are potential agents to enhance the memory, learning ability, and cognitive function of patients with neurodegenerative diseases, such as AD. Hence, molecular imaging agents for noninvasive real-time *in vivo* visualization and quantification of the contents and activity of HDAC in human brains would be valuable for the determination of the roles HDAC plays in AD. In this study, a series of fluoroalkyl-substituted benzamide derivatives was designed and synthesized as novel HDACIs with potential blood-brain barrier (BBB) permeability. In the series, most ligands demonstrated potent inhibition against HDAC1 and HDAC2, whereas these compounds had negligible ability to inhibit other subtypes of HDAC. Several compounds exhibited high potency and selectivity for HDAC1 ( $IC_{50} = 50\text{-}100$  nM), and other ligands showed similar potency for both HDAC1 and HDAC2 ( $IC_{50} = 60\text{-}200$  nM). In PAMPA assay, several probes demonstrated high potential to penetrate through BBB. A fluoropropyl-substituted derivative was radiolabeled and this  $^{18}\text{F}$ -labeled HDACI was evaluated *in vivo* as a potential positron emission tomography (PET) imaging agent for study of AD and other CNS applications.

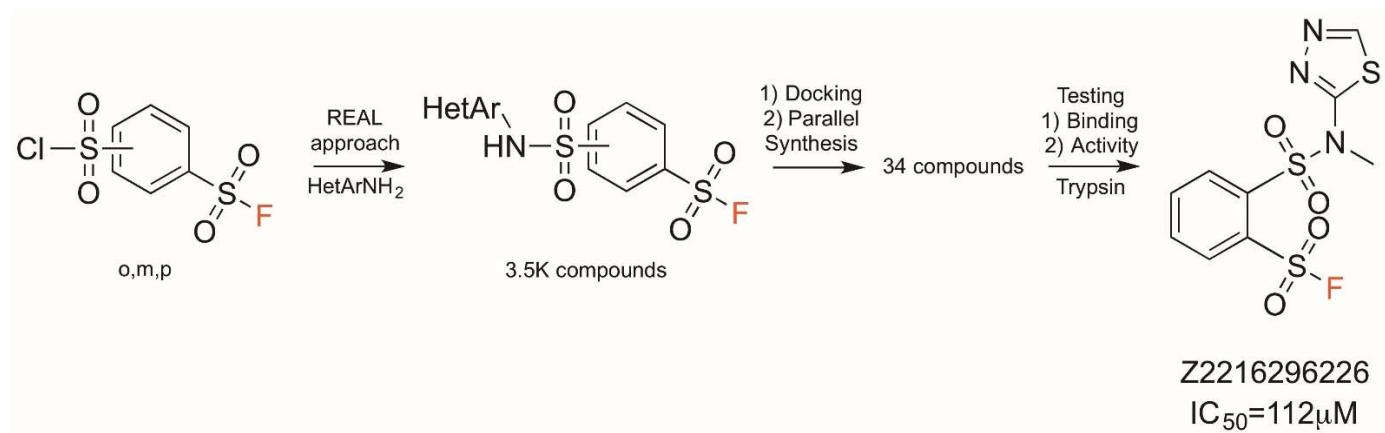
## MEDI 357

### **REAL fragment-like covalent modifiers: N-arylsulfamoylbenzenesulfonyl fluorides as potent protease inhibitors**

*Oleksii Gavrylenko<sup>1</sup>, oleksii.gavr@gmail.com, Alexander Chupryna<sup>2</sup>, Oleksandr Vasylchenko<sup>1</sup>, Maxim Platonov<sup>2</sup>, Petro Borysko<sup>2</sup>, Yurii Moroz<sup>1</sup>, ysmoroz@gmail.com. (1) ChemBioCenter, National Taras Shevchenko University of Kyiv, Kyiv, Ukraine (2) Enamine Ltd, Kyiv, Ukraine*

More than 40 approved drugs possess covalent mechanism of action, the interest for discovery of novel entities has recently increased. High efficiency, selective binding to rare and specific targets, and longer duration of action are main advantages of covalent modifiers. To be efficient for early stage drug discovery, covalent modifiers have to integrate novel scaffolds and easy, potentially enumerated setups. Exploring a 3,000,000 fragment space of REAL arrays (REAL - REadily AccessibLe), we have established sets of fragment-like covalent modifiers containing commonly employed binding motifs of boronic acid, acrylamide, and sulfonyl fluoride.

We have shown applicability of the sulfonyl fluoride set to identify protease inhibitors. Docking screen against a model protease, trypsin, parallel synthesis of selected virtual hits followed by *in vitro* assays identified two potent hit compounds.



## MEDI 358

### Synthesis and SAR studies of positive allosteric modulators of mGluR2 for treatment of neurological and psychiatric diseases

**Zhaoxing Meng<sup>1</sup>, joshuameng1989@yahoo.com, Ronald J. Mattson<sup>2</sup>, Michael Parker<sup>1</sup>, Leatte Gurenon<sup>1</sup>, Amy Easton<sup>5</sup>, Walter Kostich<sup>5</sup>, Matthew Seager<sup>5</sup>, Clotilde Bourin<sup>5</sup>, Linda Bristow<sup>5</sup>, Kim Johnson<sup>5</sup>, Regina Miller<sup>5</sup>, John Hogan<sup>5</sup>, Valerie Whiterock<sup>5</sup>, Micheal Gulianello<sup>5</sup>, Meredith Ferrante<sup>5</sup>, Yanling Huang<sup>5</sup>, Adam Hendricson<sup>5</sup>, Andrew Alt<sup>5</sup>, John Macor<sup>3</sup>, Joanne J. Bronson<sup>4</sup>, joanne.bronson@bms.com.** (1) Chemistry, Bristol-Myers Squibb, Middletown, Connecticut, United States (2) Retired, Meriden, Connecticut, United States (3) Sanofi, Waltham, Massachusetts, United States (4) Chemistry, Bristol-Myers Squibb, Wallingford, Connecticut, United States (5) Bristol-Myers Squibb, Wallingford, Connecticut, United States

Positive allosteric modulators(PAMs) of metabotropic glutamate 2 receptor (mGluR2), which bind at an alternative site to the orthosteric endogenous agonist, may have advantages such as increasing mGluR2 signaling with greater selectivity, maintaining activity based on transient and dynamic release of glutamate without inducing over-activation or desensitization. Herein we report a series of novel substituted phenylcyclopropylmethyl triazolopyridinamines. The syntheses and the SAR of these compounds will be discussed. Some of the compounds reversed PCP stimulated locomotor activity and were also active in Y-maze test. Those compounds that showed *in vivo* activity had also good metabolic stabilities and brain exposures.

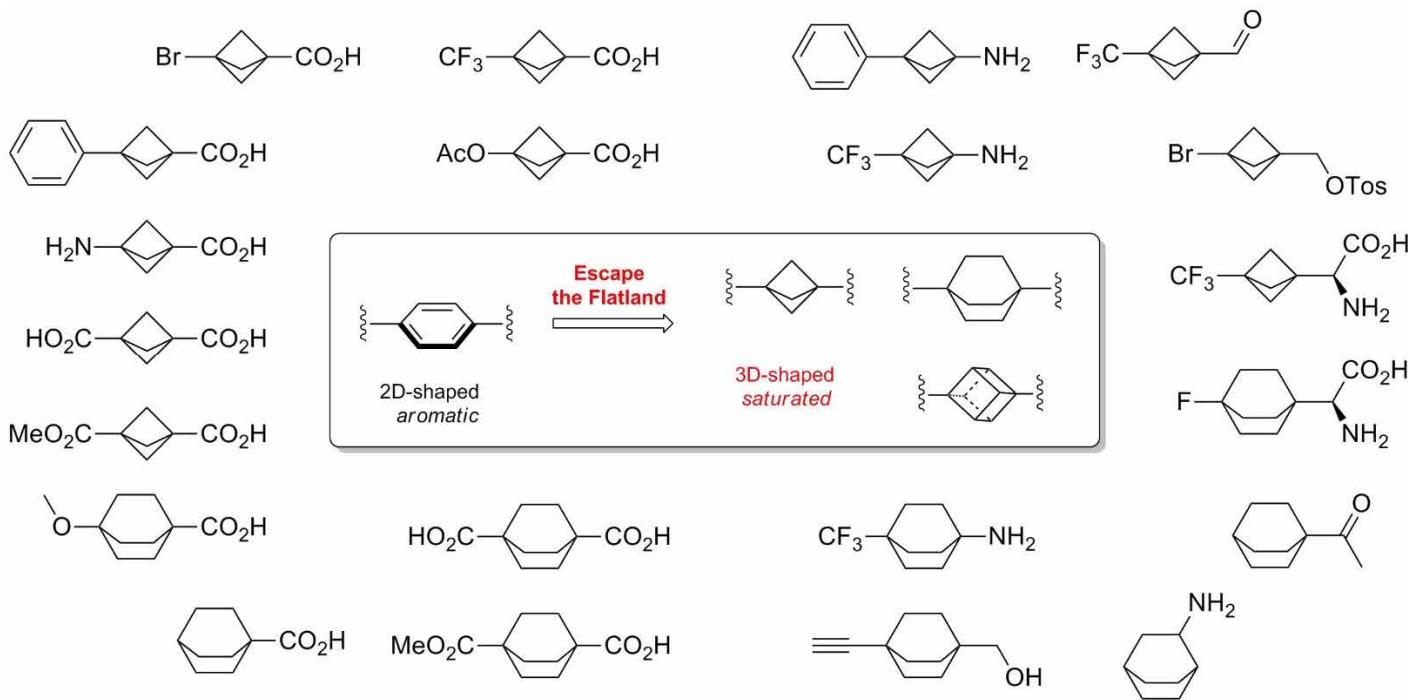
### **MEDI 359**

### **Design, synthesis and application of novel building blocks to Escape the Flatland**

**Pavel Mykhailiuk, Pavel.Mykhailiuk@gmail.com. Chemistry, Enamine Ltd, Kiev, Ukraine**

Given the modern trend in medicinal chemistry – “Escape the Flatland” – saturated 3D-shaped building blocks do play an important role.<span style="font-size:10.8333px"> </span>Compared to their aromatic 2D-shaped counterparts, the saturated analogues usually possess higher water solubility, higher activity and lower toxicity.

In this work, therefore, we have rationally designed and synthesized a library of novel saturated bioisosters of benzene. Details of the synthesis and application of the obtained compounds will be discussed.



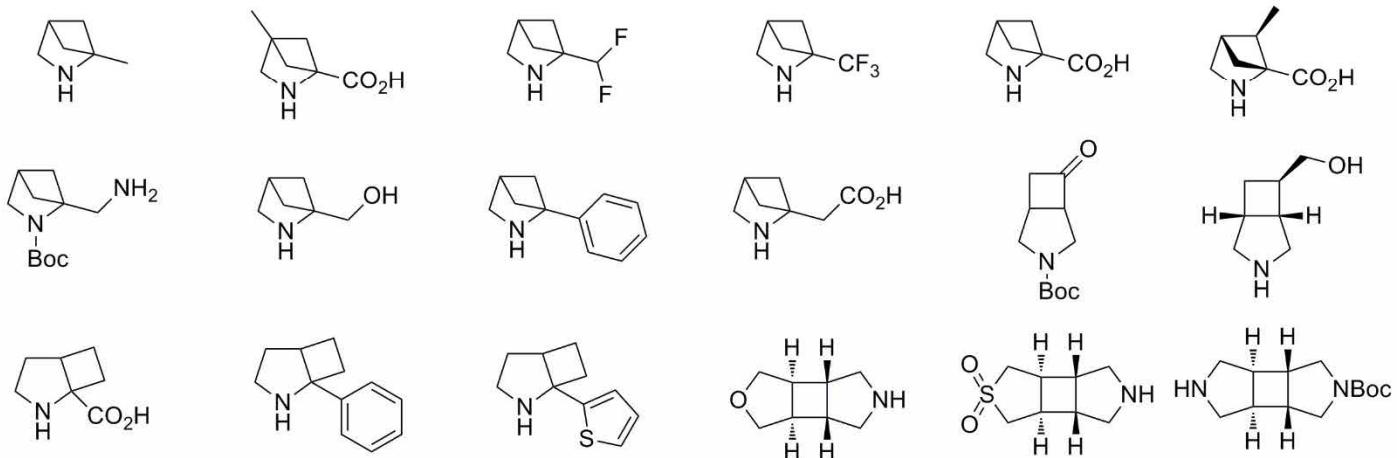
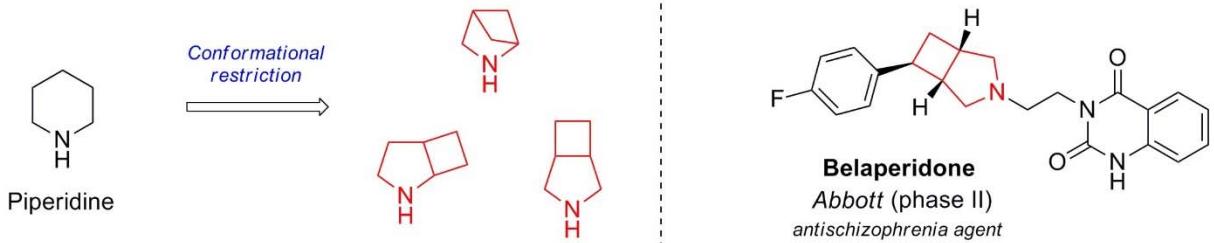
## MEDI 360

### [2+2]-photochemical synthesis and application of bicyclic amines: Advanced building blocks for medicinal chemistry

**Pavel Mykhailiuk**, Pavel.Mykhailiuk@gmail.com. Chemistry, Enamine Ltd,  
Kiev, Ukraine

“Conformational restriction” concept has already gained a considerable attention in medicinal chemistry. Scientists are looking more and more now on 3D-shaped saturated building blocks. In this context, intrinsically conformationally rigid bicyclic amines seem to be promising for drug discovery. For example, *Belaperidone* - a drug candidate of Abbott - bearing a residue of a bicyclic amine, reached phase II of clinical trials.

In this work, we have rationally designed, synthesized and applied a library of novel/Previously scarcely available diverse bicyclic amines in medicinal chemistry. The key synthesis step was photochemical [2+2]-cyclization. Details of the synthesis and application of the obtained compounds will be discussed.



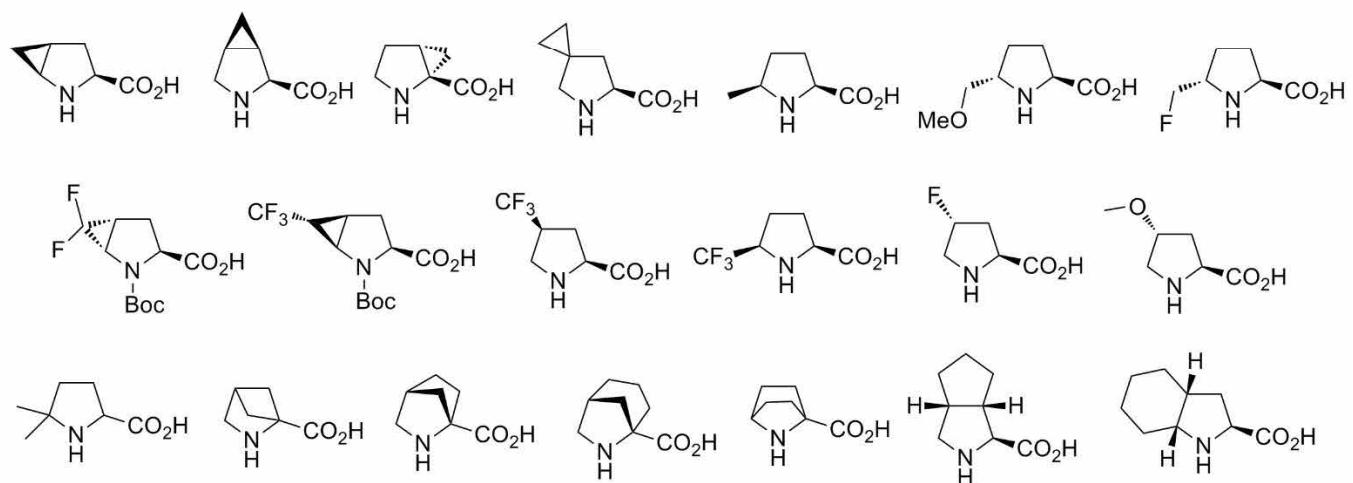
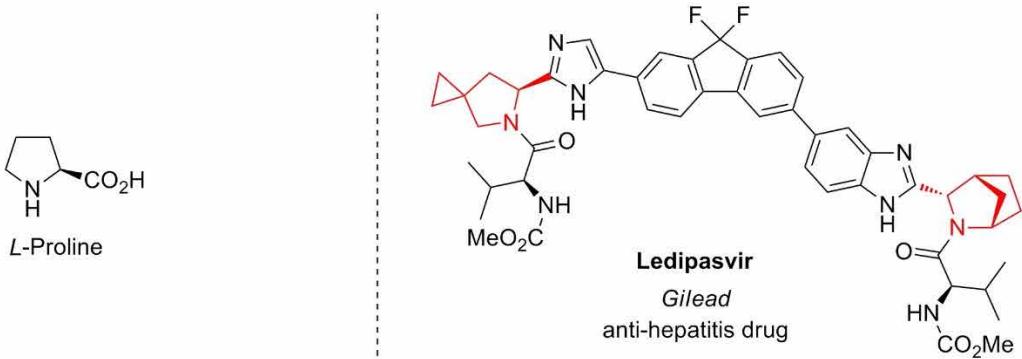
## MEDI 361

### Synthesis and application of unnatural Proline analogues: Advanced building blocks for medicinal chemistry

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*L*-Proline is a natural amino acid playing an important role in drug discovery as a cheap chiral bifunctional building block. In this context, over the past decade unnatural analogues of Proline also became extremely popular. For example, in 2010 Gilead launched *Ledipasvir* – a drug bearing the residues of two unnatural analogues of *L*-Proline.

In this work, we have rationally designed, synthesized and applied a library of novel/previously scarcely available analogues of Proline in medicinal chemistry. Details of the synthesis and application of the obtained compounds will be discussed.



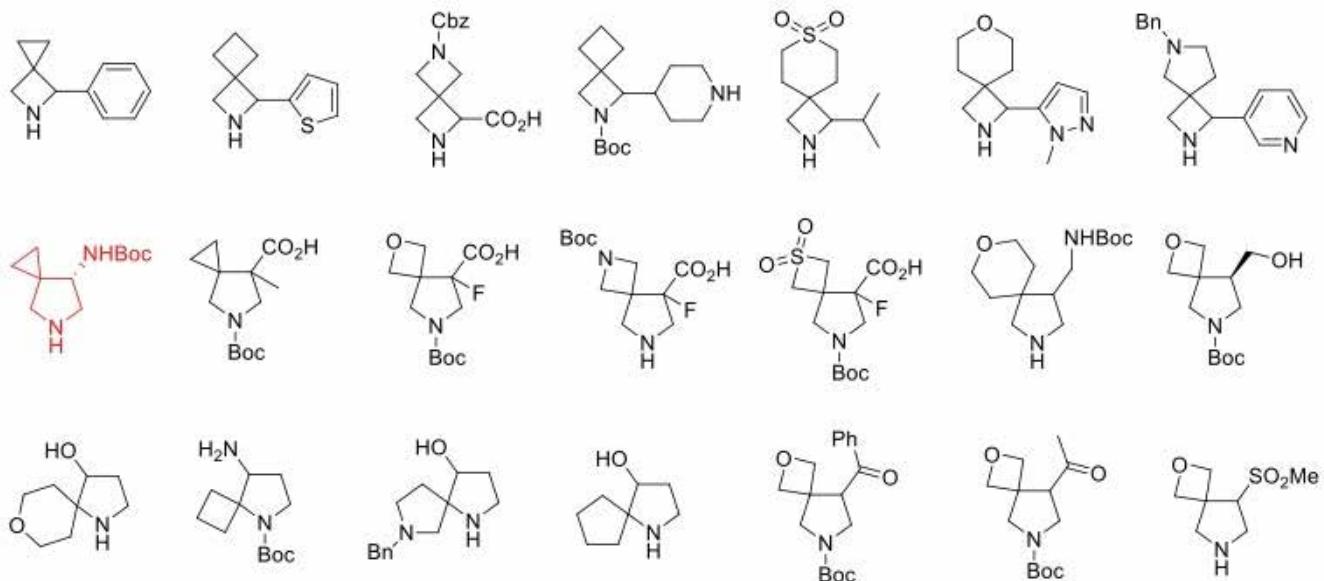
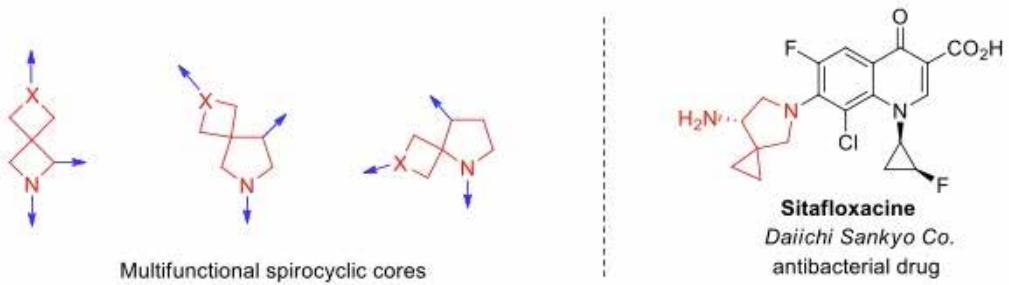
## MEDI 362

### Rapid access to novel multifunctional spirocyclic cores for drug discovery

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Trends in drug discovery are changing rapidly. During the past decade, terms “Scaffold hopping,” “Escape the Flatland” and “Conformational restriction” have been introduced, and have already found huge practical application. Spiro compounds are especially interesting, because they are intrinsically both - 3D-shaped and conformationally restricted.

In this work, we have rationally designed, synthesized and applied a library of novel multifunctional spirocyclic cores for drug discovery. Details of the synthesis and application of the obtained compounds will be discussed.



## MEDI 363

### Synthesis of triazole as GABA analogues

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**Introduction.** GABA is the major inhibitory neurotransmitter in the CNS. Due to its low degree of lipophilicity, it cannot cross the blood brain barrier and is not considered an efficient drug therapy. In this context, the synthesis of the GABA analogues with increased lipophilicity and promising therapeutic potential has been the subject of intensive research.

### Results and discussion.

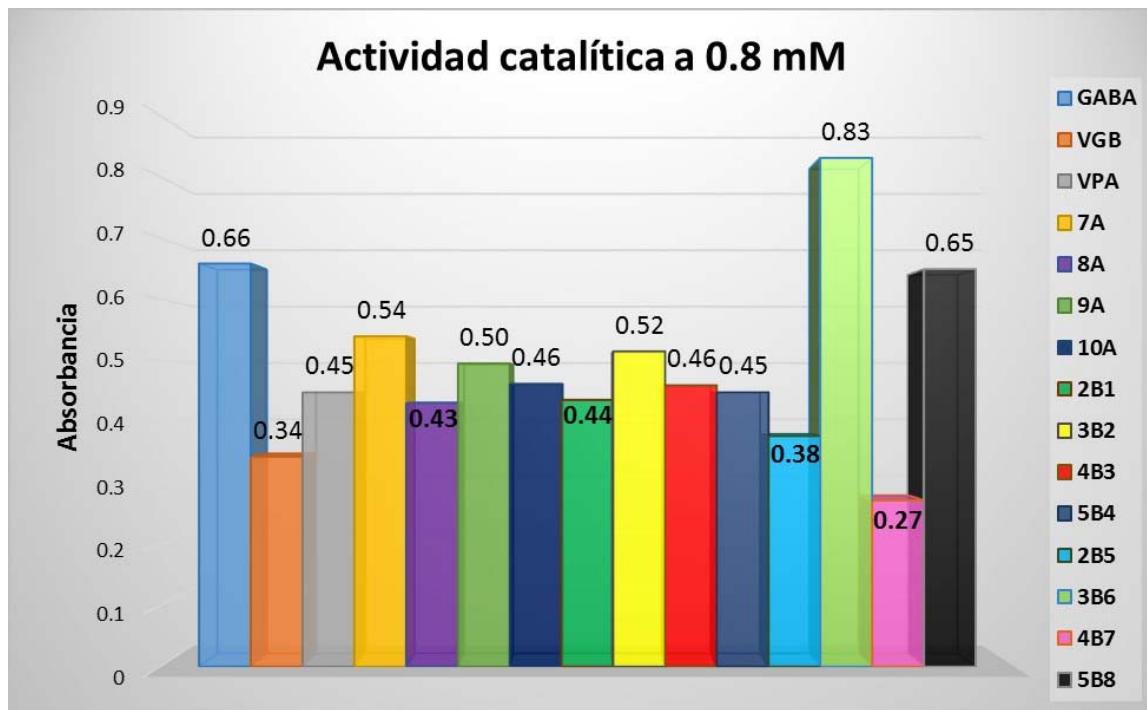
**Chemistry.** In this work, we present the synthesis of some structural

analogues of GABA where the nitrogen atom is embedded in a triazole heterocyclic ring system designed as GABA-aminotransferase (GABA-AT) inhibitors. The synthesis of analogues **7A-10A** was accomplished via a copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC) followed by basic hydrolysis. For the synthesis of analogues **2B1-5B4** and **2B5-5B8** the same methodology. In a subsequent step, the conjugate addition of *i*-butyl and *p*ClC<sub>6</sub>H<sub>4</sub> cuprates to **2Bi-5Bi** and **2Bp-5Bp** followed by basic hydrolysis of the corresponding esters afforded the analogues final.

**Preliminary tests of enzyme inhibition on GABA-AT *Pseudomonas fluorescens*.** Preliminary tests were performed to determine the inhibitory potential of **7A-10A**, **2B1-5B4** and **2B5-5B8** analogues compared to the positive controls Vigabatrin (VGB) and valproic acid at a final concentration of 0.8 mM.

According to the Figure, the absorbance value of GABA represents 100% of catalytic activity, therefore the absorbance of VGB represents a percentage activity of 52% and 48% inhibition, for valproic acid, 68% activity and 32% inhibition. According to these data analogues **8A** and **2B1** show a **35%** and **34%** degree of inhibition compared with the positive control valproic acid (32%), **2B5** and **4B7** show a **43%** and **59%** degree of inhibition compared with the positive control VGB (48%).

**Conclusions.** We have developed an optimal procedure for the synthesis of the triazole-derived analogues **7A-10A**, **2B1-5B4** and **2B5-5B8**. Our preliminary inhibitory potential results found that the compounds **8A**, **2B1**, **2B5** and **4B7** are the most promising GABA-AT inhibitors to further its development as new drugs.



## MEDI 364

### Novel deuterated GABAAR- $\alpha$ 6 subtype selective ligands with improved metabolic stability and enhanced bioavailability: Targeting trigeminal orofacial pain, neuropsychiatric disorders, & depression

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GABA<sub>A</sub> receptors (GABA<sub>AR</sub>) are the major inhibitory neurotransmitter receptors in the mammalian brain and the target of many clinically important drugs which act at GABA<sub>AR</sub> binding sites. GABA<sub>AR</sub> containing the  $\alpha$ 6 subtype are primarily expressed in the granule cells of the cerebellum, as well as in the cochlear nucleus. Lower expression has recently been reported also for the hippocampus. Recent publications suggest that  $\alpha$ 6 receptors may play a role in trigeminal orofacial pain, neuropsychiatric disorders with sensori-motor gating deficits (such as tic disorders, certain symptoms of schizophrenia,

obsessive compulsive disorder and attention deficit disorders), and depression. However, the function of  $\alpha 6$ -containing receptors in brain physiology is still largely unclear. Consequently, designing compounds selective for  $\alpha 6$  receptors would greatly assist in the determination of which physiological processes they mediate. Recently, pyrazoloquinolinones such as compound 6 were reported as the first  $\alpha 6$  subtype selective ligands to date. However, the bioavailability and the half-life of these compounds is of concern due to their limited solubility in water. To increase the bioavailability of the unmetabolized active ligands, we utilized the primary deuterium kinetic isotope effect (DIE). DIE results from the difference in the zero-point energy of C-D and C-H bonds due to the lower vibrational frequency of C-D bonds. Consequently, C-D bond cleavage has a higher activation energy and slower rate than a C-H bond. Here we report the synthesis of the first deuterated GABA<sub>AR</sub>- $\alpha 6$  subtype selective ligands and demonstrate that OCD<sub>3</sub> substituted aryl-pyrazoloquinolinones exhibit improved metabolic stability and enhanced bioavailability over their respective OCH<sub>3</sub> counterparts.

## MEDI 365

### **Second-generation inhibitors of the hepatitis C virus NS3/4A protease: Discovery of BMS-986144 with pan-genotypic antiviral activity**

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Combinations of mechanistically orthogonal direct acting antiviral agents have achieved high cure rates in the treatment of genotype 1 hepatitis C virus infection. As part of the evolution of therapy, we sought pan-genotypic HCV NS3 inhibitors that could be used in combination therapy. In this presentation, we will describe the discovery of BMS-986144, a potent HCV NS3 protease inhibitor that demonstrates potent pan-genotypic antiviral activity *in vitro*. This molecule incorporates P3 and P1 into a macrocycle ring that is combined with

specific manipulation of substitution patterns of the tether to achieve high potency across genotypes and toward resistant variants. The pharmacokinetic profile of BMS-986144 in three preclinical species predicts the potential for once daily administration in combination therapy. The principles behind the design of BMS-986144 will be described in detail along with preclinical profiling data that characterize this compound as a 2<sup>nd</sup> generation protease inhibitor.

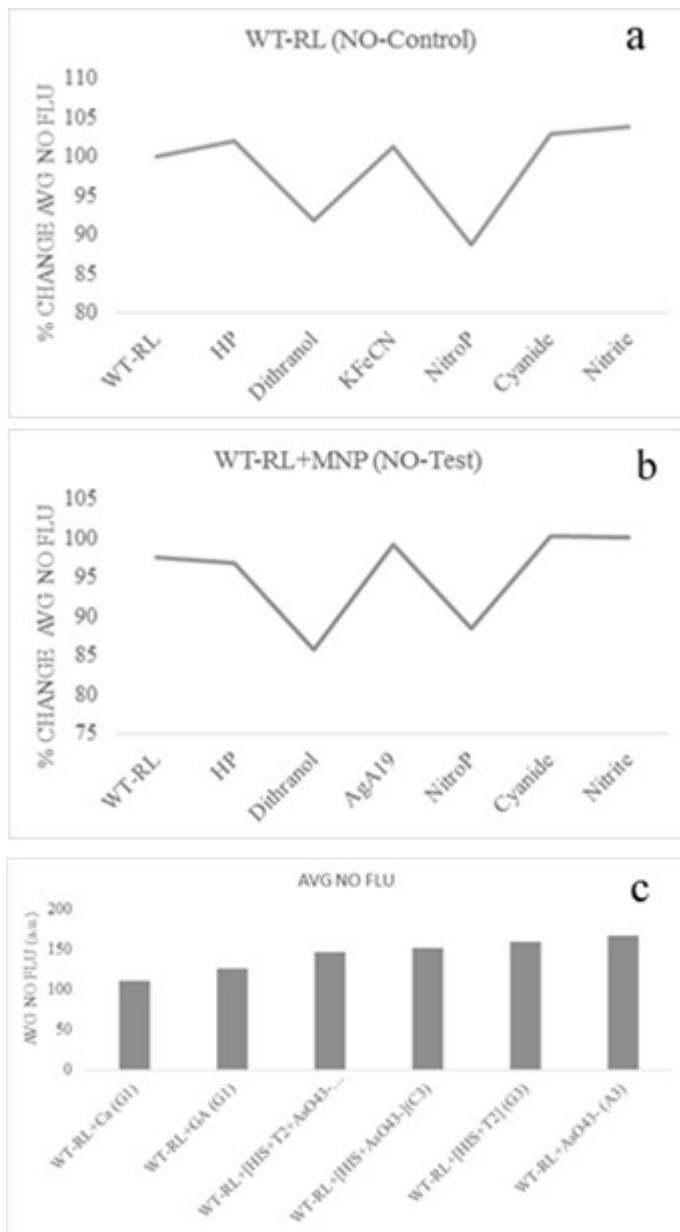
## MEDI 366

### Toxicological evaluation of magnetic nanoparticles

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Iron based magnetite nanoparticles (MNPs) were synthesized via co-precipitation method under cost-effective and environment-friendly conditions. The natural products served two purposes: to coat and improve particle solubility and to increase MNP zeta potential as a means of increasing particle stability. The coatings collectively serve to enhance the performance of heavy metal removal. The coated surface enhance the adsorption capability of the generated MNPs. The removal efficiency was improved by 10% compared to the porous Fe<sub>3</sub>O<sub>4</sub> particles. Chitosan showed enhanced adsorption relative to gum Arabic shielding due to the difference of amine group.

To ensure the eco-friendliness of the MNPs, we tested their toxicological behavior towards the retinal pigmented epithelial (RPE) wild-type cells. Data indicated that the Gum Arabic can facilitate the cell recovery upon being poisoned by heavy metal ions. Example (Fig. a) includes oxidizers such as hydrogen peroxide (HP), sodium cyanide (CN) and sodium nitrite (Nit). The addition of a reducing agent, such as dithranol (Dit) did result in a drop of nitric oxide (NO) by approximately 10%. Lastly, use of sodium cyanide and sodium nitrite which both affect electron respiratory proteins appears to be unaffected by T2, since the NO values between the T2-stress agent are the same as the stress agent alone (Fig. b). A kinetic analysis over 3 hours indicates that T2 reduced NO emissions (indicative of arsenate stress) by 12% at 330 ppm dose of arsenate and T2 at 1:1 ratio v/v (c).



- (a) The amount of nitric oxide (NO) in control cells was evaluated against known classes of stressors;
- (b) compared with gum Arabic shielded magnetite; and
- (c) The NO under arsenate stress is shown in the form of higher NO values, which are reduced with gum Arabic shielded MNPs (T2) incorporation by 12%.