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March 16-20, 2014
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Division of Medicinal Chemistry
247th ACS National Meeting, Dallas, TX, March 16-20, 2014

J. Macor, Program Chair

SUNDAY MORNING

Recent Advances in Treating Neuropathic Pain
C. Dzierba, Organizer; C. Dzierba, Presiding Papers 1-5

Small Molecules in Chemical Biology
G. Georg, Organizer; S. Malhotra, Organizer; G. Georg, Presiding; S. Malhotra, Presiding Papers 6-11

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Young Investigators in Medicinal Chemistry
T. Prisinzano, Organizer; T. Prisinzano, Presiding Papers 32-37

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Alfred Burger Award in Medicinal Chemistry: Symposium in Honor of John E. Macor
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WEDNESDAY EVENING

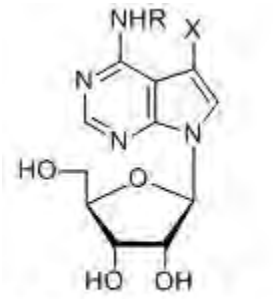
General Poster Session
J. Macor, Organizer Papers 236-350

MEDI

Synthesis of 6-N-substituted 7-deazapurine nucleoside antibiotics: Potential nucleoside transport inhibitors

Ramanjaneyulu Rayala[1]*, rraya002@fiu.edu, Patricia Theard*[1]*, Heysel Ortiz*[1]*, Stanislaw F. Wnuk*[1]*, Morris J. Robins*[2]*. (1) Department of Chemistry and Biochemistry, Florida International University, Miami, Florida 33174, United States (2) Department of Chemistry and Biochemistry, Brigham Young University, Provo, Utah 84602, United States*

The 6-*N*-(4-nitrobenzyl) derivatives of nucleoside antibiotics of type **1** (R = H, X = H, tubercidin; R = H, X = CN, toyocamycin; R = H, X = $CONH_2$, sangivamycin) were synthesized, since the corresponding 6-*N*-(4-nitrobenzyl) adenosine derivatives act as potent nucleoside transport inhibitors. First approach for the syntheses involved the treatment of 7-deazapurine nucleosides with 4-nitrobenzyl bromide to give 1-*N*-(4-nitrobenzyl) intermediates. The resulting intermediates were treated with dimethylamine to give access to 6-*N*-(4-nitrobenzyl) analogues *via* Dimroth Rearrangement. However, it was observed that 6-*N*-(4-nitrobenzyl) toyocamycin (R = 4-nitrobenzyl, X = CN) was formed *via* a direct alkylation on exocyclic amine group and not *via* Dimroth rearrangement pathway. In the second approach, treatment of 6-fluoro-2',3',5'-tri-*O*-acetyltubercidin with 4-nitrobenzylamine and deprotection gave 6-*N*-(4-nitrobenzyl)tubercidin. The 6-fluoro-2',3',5'-tri-*O*-acetyltubercidin precursor was prepared by diazotization-fluorodediazoniation of 2',3',5'-tri-*O*-acetyltubercidin with $NaNO_2$ and 55% HF/pyridine. The 6-*N*-(4-nitrobenzyl)-7-deazapurine derivatives are under evaluation for their inhibitory activity in nucleoside transport systems as well as in viral and cancer culture systems.



1; X = H, CN, $CONH_2$
R = H, benzyl, 4-nitrobenzyl

MEDI

Synthesis and cytostatic evaluation of 4-*N*-alkanoyl and 4-*N*-alkyl gemcitabine analogs

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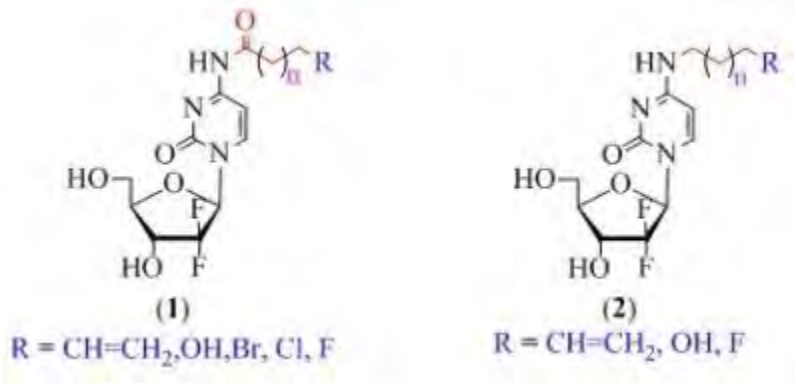
Gemcitabine (2',2'-difluoro-2'-deoxycytidine, dFdC) is a potent chemotherapeutic nucleoside analogue in the treatment of cancers and solid tumors. Coupling of gemcitabine (NMM/HOBt/EDCI) with varying carboxylic acids of different chain lengths (C9-C13) afforded the 4-*N*-alkanoylgemcitabine analogues (**1**) bearing a hydroxyl, fluoro, chloro or alkene functional group suitable for further chemical modification. Displacement of *p*-toluenesulfonylamido group in 4-*N*-tosylgemcitabine with 11-aminoundecanol or 10-undecenyl amine gave the 4-*N*-alkylgemcitabines (**2**). The analogues bearing a terminal hydroxyl group on the 4-*N*-alkanoyl or 4-*N*-alkyl chain were efficiently fluorinated either with DAST or under conditions that are compatible with synthetic protocols for ^{18}F labeling. The 4-*N*-alkanoylgemcitabines **1** displayed potent cytostatic activities against L1210, CEM, HeLa and MCF-7 tumor cell lines with IC_{50} values in the nM range, while activities for the 4-*N*-alkylgemcitabines **2** were in the μM range.



MEDI

Strain promoted click chemistry (SPAAC) of 8-azido purine and 5-azido pyrimidine nucleosides with cyclooctynes

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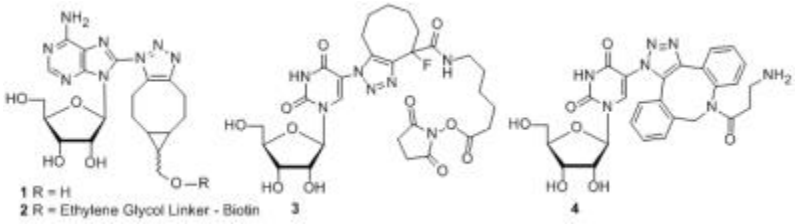
Click chemistry is an important tool for drug discovery, bioconjugation, and identification of cellular targets. Click chemistry between 8-azidoadenosine and a terminal alkyne usually requires a copper catalyst and microwave assistance or prolonged reaction times. These prerequisites are incompatible with biological applications. We have developed a protocol for the convenient strain promoted click chemistry (SPAAC) of 8-

azido purine nucleosides with various cyclooctynes in aqueous solution without a copper catalyst. A click reaction between 8-azidoadenosine and symmetrically fused cyclopropyl cyclooctynes occur rapidly (64% in 18 minutes, 92% in 2 hours) in ACN:H_2O (3:1) at rt to give triazole **1** or biotin modified adduct **2** . The click reaction of other 8-azido purine analogues including 8-azido-2'-deoxyadenosine and 8-azido-*arabino*adenosine with more complex cyclooctynes such as dibenzylcyclooctyne and monofluorocyclooctyne also proceeded smoothly to give triazole products. Furthermore, 5-azidouridine reacted efficiently with cyclooctynes to give products modified with an NHS ester **3** or a terminal amine **4** in less than 15 minutes. Bioorthogonal labeling of cellular targets using described click chemistry will also be discussed.



MEDI 1

Mechanisms and identification of targets for neuropathic pain

***Frank Porreca**, frankp@u.arizona.edu. Department of Pharmacology, University of Arizona, Tucson, ARizona 85724, United States*

Pain is produced by high intensity stimuli that can damage tissues. Following injury, healing processes are engaged and pain almost always resolves. In a minority of cases, however, tissues heal but pain persists chronically. In many cases, such pain may be related to damage to peripheral nerves resulting in a pathological state in which pain is no longer protective or useful to the patient. Patients with neuropathic pain experience pain persisting months to years following initial injury. Neuropathic pain is characterized by spontaneous pain (i.e. pain at rest). Some patients also experience pain in response to low intensity stimuli that were previously not painful. The phenomenon of pain from a previously non-noxious stimulus such as touch or cold is termed allodynia and reflects adaptive changes that occur both in peripheral nerves that convey sensations of pain as well as within the central nervous system. Multiple mechanisms have been suggested to underlie the clinical features of neuropathic pain. One prominent mechanism is that injured nerves become hyperexcitable and discharge spontaneously driving the pain pathways within the central nervous system. This idea is supported by many lines of evidence including the clinical efficacy of sodium channel blockers such as lidocaine and calcium channel modulators such as w-conotoxin (ziconotide). Additionally, rare mutations in some sodium channel subtypes (e.g., Nav1.7) can produce a complete insensitivity to pain (loss of function) or states of neuropathic pain (gain of function). Pain can be modulated by agonists acting at G-protein coupled receptors on the central

terminals of primary afferent fibers including opioid and a2 adrenergic receptors. Preclinical evaluation of ongoing pain has been challenging making the discovery of new therapies even more difficult. New approaches have been developed that appear to capture spontaneous pain and that may allow increased confidence in choice of targets for drug development.

MEDI 2

Applications of conotoxins for the treatment of pain

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Peptides derived from the venoms of marine cone snails have attracted recent attention as potential therapeutic agents for the treatment of chronic pain. These conotoxins are 12-40 amino acids in size and are typically stabilized by two or more disulfide bonds. Although conotoxins share the attractive features of peptides in general of having exquisite selectivity and potency for specific ion channels, membrane receptors or transporters, they also share the general disadvantages of peptides of short biological half-lives and poor oral bioavailability. Cyclization has been used in the past as a strategy in the pharmaceutical industry for stabilizing and locking the conformation of small peptides of 5-12 amino acids. This cyclization strategy can also be applied to conotoxins to produce additional stabilization, with the potential to dramatically increase the therapeutic potential of these molecules. In this study, we describe the development of a cyclic conotoxin analogue that has potent analgesic activity in the chronic constriction injury model of neuropathic pain in rats when administered orally. This result demonstrates the effectiveness of the cyclisation approach for the stabilization of peptide therapeutics. The cyclization approach has been successfully applied to a range of conotoxins, highlighting its broad potential in drug design.

MEDI 3

Ligands that target MOR-mGluR5 heteromer produce potent antinociception in mice with inflammatory or neuropathic pain

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Given the presence of opioid receptors (MOR) and metabotropic glutamate receptor-5 ($mGluR_5$} in glia and neurons, together with reports that suggest coexpressed MOR/$mGluR_5$ receptors in cultured cells associate as heteromer, the possibility that such a heteromer could be a target *in vivo* was addressed by the design and synthesis of a series of bivalent ligands that contain mu opioid agonist and $mGluR_5$ antagonist pharmacophores linked through spacers of varying length (10-24 atoms). The series

was evaluated for antinociception using mouse models for inflammatory or neuropathic pain. The bivalent ligand **MMG22** (22-atom spacer) was the most potent member of the series (i.t. ED_{50} ~9 fmol/mouse) in alleviating inflammatory pain and was devoid of tolerance.The exceptional potency of **MMG22** may be due to optimal bridging of protomers in upregulated, putative MOR-$mGluR_5$ heteromer. In the spared injury model of neuropathic pain, **MMG22** was active in the pmol range. Given its unprecedented i.t. potency, **MMG22** has potential as a spinally administered analgesic for intractable pain that is refractory to morphine or other analgesics. It is noteworthy that **MMG22** possesses a therapeutic index of greater than a million.

MEDI 4

Discovery and pharmacological characterization of the selective NaV1.7 inhibitor AZD3161

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Based on the recently discovered genetic linkage between mutations in gene coding for the NaV1.7 channel protein and inheritable pain states, selectively inhibiting this sodium channel subtype represents an attractive approach to provide analgesia in conditions associated with neuropathic pain. These genetic mutations in NaV1.7 have been identified in both inheritable painful states such as erythromelalgia, as well as congenital insensitivity to pain. NaV1.7 is expressed in the peripheral nervous system and is responsible for the conduction of painful stimuli from nerve endings and along the peripheral afferent fibers to the spinal cord and then to supraspinal sites. NaV1.7 expression is increased in painful neuromas in man, and is increased in pre-clinical models of peripheral neuropathy.

One of the main challenges to develop NaV1.7 inhibitors devoid of side effects has been to achieve sufficient selectivity vs. off-targets such as hERG or the other NaV channel subtypes 1.5 and 1.2, which are associated with cardiac and CNS side effects. This contribution will describe AstraZeneca's internal lead generation and lead optimization campaign to identify *selective* 1.7 inhibitors and the discovery and pharmacological characterization of lead AZD3161.

MEDI 5

Targeting calcium channel blockers for the treatment of pain

***Chih-Hung (Lance) Lee**, Lance.lee@abbvie.com. Neuroscience, AbbVie Inc., North Chicago, IL 60064, United States*

Multiple subtypes of voltage-gated calcium channels (VGCCs) including N-, P/Q- and T-types have been implicated in mediating the nociceptive behaviors observed in preclinical models of inflammatory and neuropathic pain using both selective peptide

blockers and genetic knockout animals. The implication of VGCCs in nociception is consistent with N- and P/Q-type calcium channels being localized in the spinal cord and having a critical role in regulating neurotransmitter release, whereas T-type calcium channels are expressed peripherally and have been demonstrated to modulate neuronal excitability. N-type calcium channels have also been clinically validated with the FDA approval of the selective peptide blocker Prialt® for the treatment of severe chronic pain. Recent efforts have focused on identifying orally bioavailable small molecule blockers that circumvent the need for intrathecal delivery that is required for peptide blockers. We now report the discovery of orally active and structurally novel mixed N-, P/Q- and T-type calcium channel blocker. Electrophysiology studies demonstrated that these molecules inhibits recombinant N- and T-type calcium channels in a state-dependent manner with a potency similar to that observed at native calcium currents in DRG neurons. These VGCC blockers also inhibited potassium-evoked release of the pro-nociceptive peptide CGRP from rat DRG neurons in a dose-dependent manner. Following oral administration, compound completely reversed grip force impairment in a rat model of osteoarthritic and neuropathic pain. This observation was associated with a concomitant reduction in osteoarthritic-induced MAPK activation in DRG neurons and the spinal cord of animals treated with VGCC blockers. Taken together, these studies demonstrate that VGCC blockers can effectively modulate the neurochemical signaling pathways associated with central sensitization in this preclinical model of osteoarthritis pain.

MEDI 6

Design and application of affinity labels to characterize antiviral drug targets

Nicholas A Meanwell*, Nicholas.Meanwell@bms.com. Department of Discovery Chemistry, Bristol-Myers Squibb Research and Development, Wallingford, CT 06410, United States*

We have employed phenotypic screening using cell-based assays that capture either a complete or partial virus replication cycle to identify mechanistically novel and selective antiviral agents including inhibitors of influenza and respiratory syncytial virus fusion, the influenza NP protein, HIV-1 attachment, hepatitis C virus entry and the HCV NS5A protein. The specificity of a hit for a viral target can be established by developing and sequencing resistant viruses, with a reverse genetics approach used to demonstrate that a particular mutation confers resistance to a molecule of interest. However, whilst this is a useful tool for target elucidation, with mutations frequently betraying the target protein, the mode of action of a compound can frequently be less than obvious. In this presentation, we will summarize our experiences with several antiviral programs where the careful design and application of affinity probes provided critical insights into mode of action and significantly affected program evolution.

MEDI 7

Organelle-specific small molecule delivery using mitochondria-penetrating peptides

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The mitochondria of human cells play a central role in the life and death of the cell due to the diverse processes and proteins, such as energy production and cell death regulators, that it houses. The role of mitochondria in cancer progression and tumorigenesis has been widely acknowledged. A major challenge to the study of mitochondrial processes and the development of mito-targeted therapies is presented by the impermeability of the innermost mitochondrial membrane and its highly negative membrane potential, which exclude most exogenous molecules from the organelle. We have developed a new class of peptide-based mitochondria-targeting vectors that can deliver various cargos to this previously impenetrable organelle. We have used these vectors to understand the chemical requirements for mitochondrial entry, to study oxidative stress in the organelle, and to deliver several different therapeutics. Insights into the unique chemical and biochemical features of this organelle gained from the use of these peptides will be presented.

MEDI 8

NCI's drug discovery and development programs, CBC and NExT

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The National Cancer Institute (NCI) has been charged with the discovery and development of new anti-cancer therapeutics since 1955. The structure of this program has changed numerous times over the past 63 years to meet the needs of the oncology field and most recently involved the creation of the Chemical Biology Consortium (CBC) as the discovery engine of the NCI's Experimental Therapeutics (NExT) Program. The CBC was created to increase the flow of early stage drug candidates into the development pipeline of NExT. The creation of the CBC enables the NCI to focus on unmet needs in oncology such as Natural Products, rare malignances and undruggable targets by establishing an integrated network of chemists, chemical biologists and molecular oncologists from government, academia and the drugindustry. This consortium is designed to significantly enhance the interactions among the participants leading to a more robust pipeline in the NExT Program from target validation through drug development and proof of concept (POC) Phase 0 and I clinical trials.

MEDI 9

Novel targeting ligands for imaging and therapy of human diseases

***Philip S. Low**, plow@purdue.edu. Department of Chemistry, Purdue University, West Lafayette, IN 47907, United States*

We are developing methods to target drugs specifically to pathologic cells, thereby avoiding collateral toxicity to healthy cells. In the case of cancer, we have exploited up-regulation of the folate receptor on cancers of the ovary, lung, kidney, endometrium and breast to target imaging and therapeutic agents to these cancers. Clinical trials of six folate-linked drugs demonstrate that the aforementioned strategy holds promise for increasing drug potency while reducing toxicity. Data on the design, synthesis, development and human testing of several of these drugs will be presented.

We have also developed a targeting ligand that selectively delivers attached drugs to PSMA on prostate cancer cells. Imaging and therapeutic studies suggest that this targeting ligand can not only improve diagnosis of the disease, but also enhance treatment of prostate cancer. Recent pre-clinical and clinical data on this targeting ligand will also be presented.

Additional cancer-specific ligands that target malignancies of the pancreas, stomach, brain, liver, colon, skin and esophagus will also be described. Moreover, use of these ligands to “light up” cancer tissues with tumor-targeted fluorescent dyes will be summarized.

Finally, targeted imaging and therapeutic agents for the diagnosis and treatment of autoimmune, inflammatory and infectious diseases will be briefly described.

MEDI 10

Targeting the VHL/PHD/HIF pathway therapeutically

***Amato J. Giaccia**, giaccia@stanford.edu. Division of Radiation and Cancer Biology, Department of Radiation Oncology, Stanford University School of Medicine, Stanford University, Stanford, CA 94305-5152, United States*

Targeted therapy for the treatment of cancer and metastasis can be divided into two main categories: monoclonal antibodies and small molecules. In this talk, we will discuss the approach of employing synthetic lethality to target cancer, specifically renal cell carcinoma. The concept of synthetic lethality is used to describe a genetic interaction of two non-allelic and non-lethal genes that when mutated simultaneously results in cell death. Recently, we screened for small molecules that function in a synthetic lethal manner to the loss of VHL, a mutation responsible for the vast majority of renal cell carcinoma. In this screen, we identified several small molecules, which demonstrated selective toxicity against cells that had lost VHL compared to isogenic matched cell lines with wild-type VHL both *in vitro* and *in vivo*. One ofthese small molecules kills VHL deficient cells by inducing autophagy and another kills by inhibiting

glucose uptake and retention. Both of these small molecules illustrate the power of using synthetic lethality in mammalian cells to develop new therapeutic strategies.

MEDI 11

X-aptamers: A novel small-molecule conjugated aptamer selected from split-pool bead libraries

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We have developed next-generation DNA oligonucleotide aptamers selected from large combinatorial libraries to target a number of proteins. We have developed both *in vitro* enzymaticcombinatorial selection and split-synthesis chemical combinatorial methods to identify phosphorothioate-modified oligonucleotide “thioaptamers” and next-gen “X”-aptamers to a number of different protein targets for both nanomedicine imaging, therapeutic and diagnostic methods. The X-aptamers also include a large range of chemical (X) modifications to the 5-X-dU position and thus represent a hybrid of aptamer backbone, protein amino acid-like sidechains, and small molecule leads in a self-folding scaffold that can be readily identified by oligonucleotide sequencing. Combinatorial libraries on beads can be readily prepared by click chemistry or amide coupling chemistry to modified-dU sequences. Compared to conventional aptamers, this approach dramatically expands the chemical diversity that can be incorporated to select X-aptamers with high affinity for diverse molecular biomarkers. Large bead-based combinatorial libraries of these aptamers can be rapidly selected. These X-aptamers are being used as antibody substitutes in biomarker identification to tumor cells and tumor vasculature and in various microfluidics and mass spec chips for nanomedicine, proteomics and diagnostics. Examples of application of the bead-based thioaptamer and X-aptamer selection are demonstrated for targeting cancer tissue and cells expressing CD44, E-Selectin and Annexin A2.

Disclosure: Financial interest in AM Biotechnologies, Inc.

MEDI 12

Towards glycosyltransferase inhibitors: Non ionic replacements for the diphosphate of sugar nucleotides

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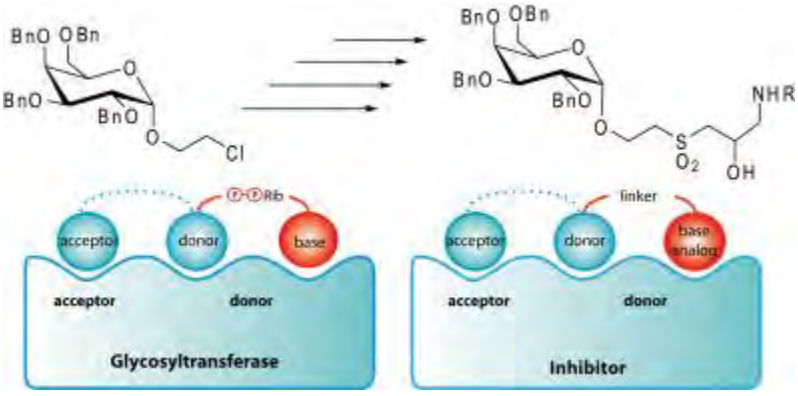
Glycans play diverse and important roles in biological systems. Glycosyltransferases are key enzymes in the biosynthesis of glycans catalyzing the specific transfer of carbohydrate moieties from an activated donor (nucleotide-diphosphate-sugar) to a

specific acceptor substrate. The human galactosyltransferase B (GTB) is responsible for the transfer of an α-D-galactose residue from UDP-galactose to the H-antigen yielding the blood group B antigen. Pancreatic tumors are much more prevalent in persons with blood group B than in those with other blood groups. Therefore GTB inhibitors promise to be important tools to regulate cancer progression. Rational design of an inhibitor was achieved using the crystal structure of GTB with bound donor and acceptor substrate.



Here, we report the design and synthesis of a modified linker replacing the pyrophosphate and the ribose residues to optimize the pharmacokinetic and pharmacodynamic properties of the inhibitor. *In silico* studies showed that a sulfone can act as a good binder to the manganese ion present in the enzyme. The synthesis of this donor substrate inhibitor will be discussed along with affinity data.

MEDI 13

***Cryptosporidium parvum* IMPDH inhibitors for the treatment of cryptosporidiosis: SAR, pharmacokinetic and pharmacodynamic studies**

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Cryptosporidium parvum (*Cp*) is a major cause of diarrhea and malnutrition in the developing world. This protozoan parasite is also a frequent cause of waterborne

disease in the developed world and presents a credible bioterrorism threat to potable water supplies. Although cryptosporidiosis is self-limiting in immune competent adults, it can be fatal in children and immune compromised adults. Current treatments are restricted to one FDA approved drug (e.g. nitazoxanide) that is not particularly effective. Therefore, new treatment options are urgently needed.

Inosine 5′-monophosphate dehydrogenase (IMPDH) is a metabolic enzyme that catalyzes the NAD^+-dependent oxidation of inosine 5'-monophosphate (IMP) to xanthosine monophosphate (XMP). Since *Cp*IMPDH is structurally distinct from its human counterpart and the organism is completely dependent on this enzyme for the production of guanine nucleotides, we have hypothesized that *Cp*IMPDH may represent a viable molecular target for the development of effective cryptosporidiosis chemotherapy with minimal host toxicity.

In this presentation, the structure-activity relationship (SAR) of several structurally distinct compound series of *Cp*IMPDH inhibitors will be highlighted, including descriptions of co-crystallization studies of inhibitors with *Cp*IMPDH and the generation of a pharmacophore model. In addition, mouse pharmacokinetic and pharmacodynamic studies of *Cp*IMPDH inhibitors will be presented. These studies will also feature the importance of tissue distribution required for efficacy. Finally, important questions concerning optimum physical-chemical properties and the impact of *Cp*IMPDH inhibitors on the host's gastrointestinal microbiota will also be discussed.

MEDI 14

Discovery of transcriptional modulators that target the vitamin D receptor

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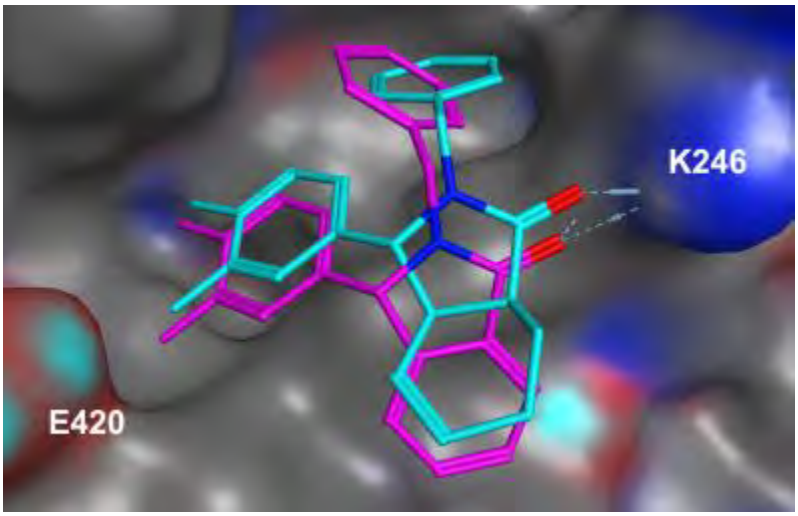
The vitamin D receptor (VDR) belongs to the superfamily of nuclear receptors and transcribes genes responsible for metabolism, calcium homeostasis, cell proliferation, and cell differentiation. The regulation of VDR is mediated by endogenous secosteroid ligands, including 1,25-dihydroxyvitamin D3, and coregulator proteins, which have been identified as master regulators of transcription. In order to identify molecular probes to determine the biological functions of coregulators in respect to VDR-mediated transcription a high throughput screen (HTS) was carried out in collaboration with the National Institute of Health. The HTS identified different classes of compounds that modulate the interaction between VDR and coregulators. Herein, we present the discovery and synthesis of VDR-coregulator inhibitors. Using parallel chemistry, small molecule libraries were synthesized and characterized with a battery of biochemical assays. In addition, early stage preclinical *in vitro* assays were used for their

characterization and to guide multiple rounds of computational design, synthesis and evaluation. A core isoindolone scaffold was identified with examples of highly selective low-micromolar inhibitors.



Our current effort is focused on the development of these compounds into anticancer drugs. In contrast to the very successful nuclear receptor ligands tamoxifen and flutamide targeting the estrogen and androgen receptor, these inhibitors target VDR-coregulator interactions and inhibit the proliferation and differentiation of cancer cells. We hope that crystallographic data will be available in time to disclose the exact binding modes of these novel molecules. Overall, we will present a chemical approach to identify the role of coregulators including that of coactivator AIB1 (amplified in breast cancer-1) in concert VDR in cancer.

MEDI 15

***N*-Myristoyltransferase inhibitors as anti-leishmanial agents**

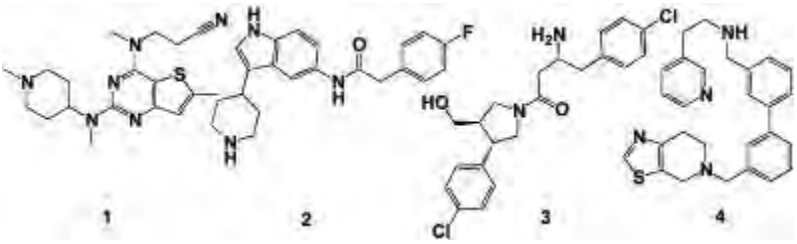
Andrew S Bell[1]*, a.bell11@imperial.ac.uk, Victor Goncalves*[1]*, Jennie A Hutton*[1]*, Thomas M Waugh*[1]*, Megan H Wright*[1]*, Zhiyong Yu*[1]*, James A Brannigan*[2]*, Daniel Paape*[3]*, Robin J Leatherbarrow*[1]*, Edward W Tate*[1]*, Anthony J Wilkinson*[2]*, Deborah F Smith*[3]*. (1) Department of Chemistry, Imperial College, London, United Kingdom (2) Department of Chemistry, University of York, York, Yorkshire YO10 5DD, United Kingdom (3) Department of Biology, University of York, York, Yorkshire YO10 5DD, United Kingdom*

The Leishmaniases, a spectrum of neglected tropical disease caused by the protozoan parasites of the genus *Leishmania*, are a major health problem in many developing countries. Cutaneous leishmaniasis, caused mainly by *L. major,* can lead to permanent scarring, whilst visceral leishmaniasis, caused by *L. donovani*, is often fatal due to

failure of the host immune system. Current therapies for leishmaniasis are highly toxic with severe side effects.

N-Myristoyltransferase (NMT) catalyses the transfer of myristate to the *N*-terminal glycine of specific target proteins. Up to 3% of the proteome is thought to be *N*-myristoylated, a vital modification for many regulatory processes within the cell. NMT is essential for many eukaryotes including *Leishmania*, therefore inhibition of this enzyme represents a good drug target for the treatment of this neglected disease.

We previously published the output of a high-throughput screening, which led to the identification of four series of inhibitors that selectively inhibit *Leishmania donovani* NMT over both human NMTs.



The initial hits were profiled in a novel cell-based anti-*Leishmania* assay and by high-throughput crystallography studies using *L. major* NMT. Structure-based SAR development in series 1-3 has lead to hybrid single-digit nM inhibitors, which has helped determine the appropriate physicochemical properties for good cellular activity. Activity in the cell-based screen has been correlated with effects on protein myristoylation through a non-radioactive, intracellular tagging assay.

MEDI 16

Anticancer agents targeted against cyclin-dependent kinase 2 (CDK2): Structure-based design of irreversible and reversible inhibitors

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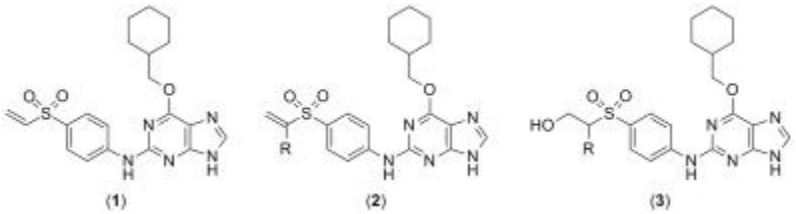
Inhibition of enzymes that participate in the cell cycle is a valuable approach to targeted cancer therapy. The cyclin-dependent kinase (CDK) family of enzymes plays a key role in the regulation of the mitotic cell cycle, and dysfunctional CDK activity has been observed in essentially all cancers. CDK2, which facilitates transition through two phases of the cell cycle, is overexpressed in a range of tumors including breast and

colorectal cancer, and melanoma. Modulation of CDK2 activity with small-molecule inhibitors has been demonstrated to elicit selective antitumor activity.

We have previously identified potent and selective CDK2 inhibitors, exemplified by the 6-alkoxypurine derivative NU6102. The vinylsulfone analogue NU6300 (**1**) was subsequently found to inhibit CDK2 in a time-dependent manner. The compound reacts covalently with CDK2, specifically by Michael addition of a lysine residue (Lys89), located in the 'hinge region' of the ATP-binding domain, to the vinylsulfone of **1** . However, the high reactivity of NU6300 was found to compromise selectivity and chemical stability, and further optimisation was required.

Using the crystal structure of the CDK2-NU6300 complex as a guide to inhibitor design, analogues (**2**) were synthesized bearing substituents (R) on the vinylsulfone group, predicted to attenuate reactivity whilst retaining the required potency against CDK2. The design, synthesis and biological activity of this new series of irreversible CDK2 inhibitors and their corresponding hydrolysis products (**3**), which are putative ATP-competitive inhibitors, will be discussed.



MEDI 17

Discovery of BMS-903452, an anti-diabetic clinical candidate targeting GPR119

Dean A. Wacker*, dean.wacker@bms.com, Ying Wang, Matthias Broekema, Karen Rossi, Steven O'Connor, Zhenqiu Hong, Ginger Wu, Sarah E. Malmstrom, Chen-Pin Hung, Linda LaMarre, Anjaneya Chimalakonda, Lisa Zhang, Li Xin, Cindy Cai, Cuixia Chu, Stephanie Boehm, Jacob Zalaznick, Larisa Serada, Song-Ping Han, Bradley Zinker, C. Kate Wu, Michelle Lee, Steven Griffen, John Krupinski, Keith J. Miller, Jeffrey A. Robl. Departments of Discovery Chemistry, Metabolic Diseases, Lead Evaluation, Computer-Assisted Drug Design, Exploratory Clinical and Translational Research, and Pharmaceutical Candidate Optimization, Bristol-Myers Squibb, Princeton, New Jersey 08543-5400, United States*

GPR119 is a G protein-coupled receptor expressed predominantly in pancreatic b-cells and in enteroendocrine cells in the gastrointestinal tract. GPR119 agonists have been shown to stimulate glucose-dependent insulin release by direct action in the pancreas and to promote secretion of the incretin GLP-1 by action in the GI tract. This dual mechanism of action has generated significant interest in the discovery of small molecule GPR119 agonists as a potential new treatment for type 2 diabetes. We have

identified a new series of GPR119 agonists based on a pyridone core that has entered human clinical trials. We, herein, describe the discovery, structure-activity relationships (SAR) and preclinical in vivo efficacy studies. We will also provide preliminary data from the single ascending dose clinical trial.

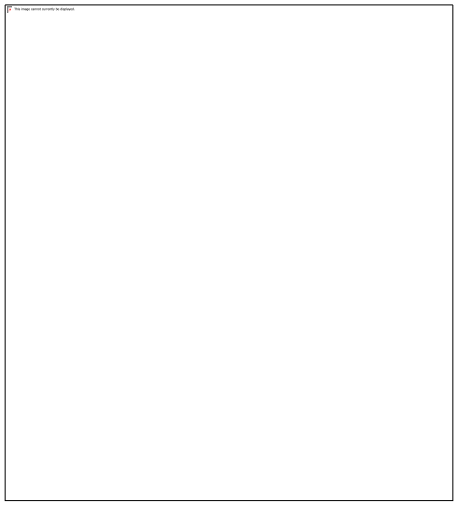
MEDI 18

Towards self-defending implants

***Cornelia G. Palivan**, Cornelia.Palivan@unibas.ch, Karolina Langowska, Wolfgang Meier. Department of Chemistry, University of Basel, Basel, Switzerland*

The number of medical and surgical procedures involving medical implant devices will continue to grow, especially due to aging of the population. It is expected an increase of device-associated infections, which are a consequence of bacterial adhesion and subsequent biofilm formation at the implantation site. Up to now, these device-associated infections are not eliminated by conventional antibiotic therapies, and very often lead to removal of the contaminated device.

Here we introduce a new antimicrobial strategy employing nanotechnology, which is more efficient and safer is highly desirable. Nanoreactors based on amphiphilic block copolymers are alternatives for controlled drug delivery and functionalization of biomaterials surfaces.[1] We have developed a polymeric nanoreactor[2], immobilized on a surface with an encapsulated biocatalyst that is responsible for the conversion of non-antibiotic substrates to a drug.



This system allows control of drug production at a specific rate for a specific period of time by adding predetermined amounts of substrate to the outer medium. In this way it is possible to both minimize the dosages and therefore systemic toxicity, and to limitat the immune response. The immobilization method, for medical applications and

biosensing, must fulfill certain criteria. We achieved this by an immobilization strategy based on Schiff base formation between the aldehyde groups presented on the outer surface of nanoreatcors and amino groups present on the solid support surface, followed by reductive amination. The resulting, immobilized, enzymatically active nanoreactors produced and released an antibiotic with sufficient efficiency to inhibit bacterial growth.

Refrences :

1. A. Najer et al; *Frontiers in Nanomedicine*, **2013** , *8*, 1.

2.K. Langowska et al; *Chem. Commun*., **2013** , *49*, 128.

MEDI 19

Development of small-molecular inhibitors of the oncogenic transcriptional co-repressor C-terminal binding protein (CtBP)

***Keith C. Ellis**, kcellis@vcu.edu. Department of Medicinal Chemistry, Virginia Commonwealth University, Richmond, Virginia 23119, United States*

C-terminal binding protein (CtBP) is an oncogenic transcriptional regulator of tumor suppressor genes that is overexpressed in several types of cancer including colon, breast, ovarian, lung, head and neck squamous cell, prostate, and melanoma. CtBP is overexpressed in clinical tumor samples and cancer cell lines but is expressed at lower levels in adjacent, healthy tissues and in the non-transformed cells.

The endogenous function of CtBP is to repress gene expression by interacting with DNA binding transcription factors and to recruit repressor complexes to targeted promoters. Tumor suppressor genes repressed by CtBP upregulation include *Bik*, *Brca1*, *PTEN*, *E-cadherin*, and many others. CtBP acts as a metabolic sensor and is activated to repress transcription of tumor suppressor genes under conditions of hypoxia or glycolysis when NADH levels are elevated, conditions often found in malignant tumors. The tumor-specific expression and activity of CtBP makes it a promising molecular target for a tumor-selective cancer therapy.

Inhibition of CtBP has been shown to restore the transcription and function of its tumor suppressor gene targets. On the basis of this data, we began a program to develop an inhibitor of CtBP's dehydrogenase activity as a tumor-selective therapy. Here we report the discovery of the first micromolar small-molecule inhibitor of CtBP as a lead molecule for further development. We will present data showing that the lead inhibits CtBP, is cytotoxic in only cancer cell lines, restores the transcription of the *Bik* tumor suppressor gene, and reduces tumor weight in a colon cancer mouse xenograft model.

MEDI 20

Discovery and optimization of a series of pyrimidine PDE10 inhibitors through fragment screening, structure-based design, and parallel synthesis

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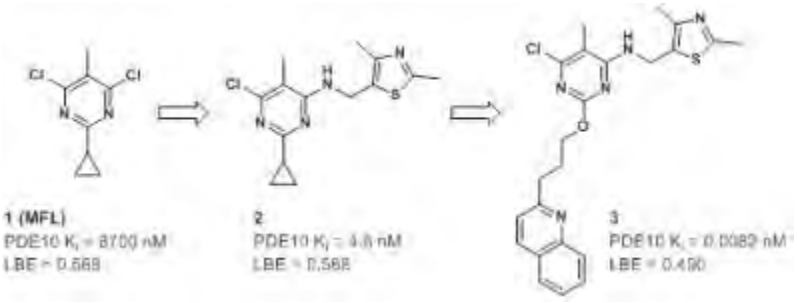
Large, drug-like lead compounds can require more time in the optimization phase to address problems inherent in the lead. Smaller leads have a higher chance of binding efficiently but can be harder to detect owing to lower affinity for the target. In recent years, several groups have demonstrated that evolution of low molecular weight, weakly-binding fragment hits is possible, representing a complementary approach to drug discovery that is especially attractive when co-crystal structure determination is used.

An effort at Merck recently identified a series of potent and selective phosphodiesterase 10 (PDE10) inhibitors employing the fragment approach. The Merck Fragment Library (MFL) is a collection of 1600 soluble, low molecular weight compounds designed to be screened at high concentration for weak but efficient binders. Screening the MFL for PDE10 inhibitors identified a low molecular weight pyrimidine (**1**) with a K_i of 8.7 μM. Initial optimization with similarity screening followed by parallel synthesis resulted in rapid potency improvements with minimal loss of binding efficiency.

An early lead identified in this process is **2** , a 4.8 nM inhibitor of the PDE10 enzyme with ~100x selectivity over other phosphodiesterases and excellent pharmacokinetics. Extensive use of inhibitor-bound X-ray crystal structures of PDE10 and in silico modeling suggested further modifications to the 2-position of the pyrimidine, resulting in the preparation of **3** , which has a K_i of 8.2 pM and selectivity of >5,000x over other PDEs.



MEDI 21

WITHDRAWN

MEDI 22

Synthesis of azapeptide CD36 modulators as potential treatments for age-related macular degeneration

William D Lubell[1]*, lubell@chimie.umontreal.ca, Yesica Garcia-Ramos*[1]*, Ngoc Duc Doan*[1]*, Mariam Traore*[1]*, Caroline Proulx*[1]*, Jinqiang Zhang*[1]*, Samy Omri*[2]*, Sylvain Chemtob*[2]*, Petra Pohankova*[3]*, Huy Ong*[3]*. (1) Département de Chimie, Université de Montréal, Montréal, Quebec H3C 3J7, Canada (2) Département d'Ophtalmologie, Université de Montréal, Montreal, Quebec H3C 3J7, Canada (3) Faculté de Pharmacie, Université de Montréal, Montreal, Quebec H3C 3J7, Canada*

The leading cause of adult blindness in North America, age-related macular degeneration (AMD) is characterized by two clinical forms of comparable incidence: an advanced 'dry' form (geographic atrophy) featuring accumulation of sub-retinal deposits and degeneration of photoreceptors and retinal pigment epithelium, and a vaso-proliferative 'wet' form associated with choroidal neovascularization. To develop novel treatments for both dry and wet AMD, we have targeted the cluster of differentiation 36 (CD36) receptor, because this multi-functional scavenger receptor is expressed on three main sub-retinal cell types and plays roles in the uptake of cytotoxic oxidized lipids and neovascularisation. Selective CD36 receptor ligands have been developed by introducing aza-amino acid residues into growth hormone-releasing peptide 6 (GHRP-6: His-D-Trp-Ala-Trp-D-Phe-Lys-NH2), such as [azaTyr4]-GHRP-6, which exhibited relatively tight affinity for the CD36 receptor in a surface plasmon resonance assay, and reduced neovascularization relative to control in a microvascular sprouting assay on mouse choroidal explants.[1] Our presentation will highlight advances in the synthetic methods and structure-activity studies of CD36 ligands towards effective prototypes to treat the pathology of AMD.

[1] Proulx, C.; Picard, E.; Boeglin, B.; Pohankova, P.; Chemtob, S.; Ong, H.; Lubell, W., D. J. Med. Chem. 2012, 55, 6502–6511.

MEDI 23

Discovery of *trans*-methyl N-(4-{2-[(1S)-1-{[4-(aminomethyl)cyclohexyl]formamido}-2-phenylethyl]pyridin-4-yl}phenyl)carbamate, a potent and selective factor XIa inhibitor

***James R. Corte**, james.corte@bms.com, Tianan Fang, Jon J. Hangeland, Todd J. Friends, Donald J. Pinto, Michael J. Orwat, Alan R. Rendina, Joseph M. Luettgen, Jeffrey M. Bozarth, Frank A. Barbera, Karen A. Rossi, Anzhi Wei, Paul E. Morin, Vidhyashankar Ramamurthy, Steven Sheriff, Dietmar Seiffert, Ruth R. Wexler, Mimi L. Quan. Department of Research and Development, Bristol-Myers Squibb, P.O. Box 5400, Princeton, New Jersey 08543-5400, United States*

Factor XIa (fXIa), a trypsin-like serine protease, functions early in the intrinsic pathway of the blood coagulation cascade. It is thought to play a key role in the amplification of thrombin production which leads to the growth and maturation of thrombi. Based on the preclinical and genetic evidence, fXIa is a potential target for anticoagulant therapy. FXIa inhibitors are efficacious in a variety of animal thrombosis models with minimal effects on bleeding time. Individuals deficient in fXI have a condition known as hemophilia C. In contrast to the severe bleeding observed with hemophilia A and B, individuals with hemophilia C exhibit a mild to moderate bleeding profile. Moreover, individuals with hemophilia C showed a reduced incidence of ischemic stroke. In addition, elevated levels of fXI are a risk factor for acute myocardial infarction and deep vein thrombosis. Work from our laboratories led to the discovery of an imidazole series, a compound from which is a picomolar fXIa inhibitor having potent in vivo antithrombotic efficacy in the rabbit AV-shunt model. Optimization of both the scaffold and P2 prime regions led to the discovery of potent and selective pyridine–based fXIa inhibitors.

MEDI 24

Discovery of GS-9973: A selective and orally efficacious inhibitor of spleen tyrosine kinase (Syk)

***Scott A Mitchell**, scotta.mitchell@gilead.com. Medicinal Chemistry, Gilead Sciences, Inc., Branford, CT 06405, United States*

Spleen tyrosine kinase (Syk) is an attractive drug target in autoimmune, inflammatory, and oncology disease indications. The most advanced Syk inhibitor, fostamatinb (prodrug of R406), has shown efficacy in multiple therapeutic indications but its clinical progress has been hampered by dose-limiting adverse effects that have been attributed, at least in part, to the off target activities of R406. It is expected that a more selective Syk inhibitor would provide a greater therapeutic window. Herein we report the discovery and optimization of a novel series of imidazo[1,2-a]pyrazine Syk inhibitors. This work culminated in the identification of GS-9973, a highly selective and orally efficacious Syk inhibitor which is currently undergoing clinical evaluation for autoimmune and oncology indications.

MEDI 25

Discovery of AMG 579, a novel phosphodiesterase 10 (PDE10A) inhibitor, as a potential treatment for schizophrenia

***Jennifer R Allen**, jeallen@amgen.com. Department of Medicinal Chemistry, Amgen, Thousand Oaks, CA 91320, United States*

Phosphodiesterase 10 (PDE10A) is an intracellular enzyme responsible for the breakdown of the two second messengers: cyclic AMP and cyclic GMP. Due to the localization of PDE10A in the medium spiny neurons of the striatum, inhibitors have the potential to address both dopamine hyperactivity and glutamate hypoactivity, two of the predominant signaling abnormalities thought to underpin schizophrenia symptoms. AMG 579 is a potent and highly selective small molecule inhibitor of PDE10A. In preclinical species, AMG 579 demonstrates promising pharmacokinetic (PK) properties with high oral bioavailability. AMG 579 also produces dose-dependent central nervous system (CNS) target occupancy of PDE10A, as measured using a specific PDE10A tracer. Additionally, in rodents, AMG 579 demonstrates efficacy in a pre-clinical model of psychosis similar to that observed with known antipsychotic drugs, however without affecting basal locomotor activity. These results are consistent with other published data on PDE10A inhibitors and we identified AMG 579 as a clinical candidate for the potential treatment for schizophrenia. Our supporting preclinical data, the chemical synthesis and structure activity relationships (SAR) leading to the discovery of AMG 579 and related analogs will be disclosed.

MEDI 26

Discovery of isonicotinamides as highly selective, brain penetrable, and orally active inhibitors of glycogen synthase kinase-3 (GSK-3) for the potential treatment of Alzheimer's disease

***Guanglin Luo**, guanglin.luo@bms.com, Ling Chen, Swanee Jacutin-Porte, Hong Xiao, Carol Krause, Nengyin Liu, Yang Cao, Catherine Burton, John E Macor, Gene M Dubowchik. Research and Development, Bristol-Myers Squibb Co., Wallingford, CT 06443, United States*

Glycogen Synthase Kinase-3 (GSK-3) has emerged as a potential target for treating a number of diseases from cancer to Alzheimer's Disease (AD). Hyperphosphorylation of tau, a GSK-3 substrate in the brain, leads to the destabilization of microtubules and aggregation to form intracellular neurofibrillary tangles (NFTs), a symptomatic sign of AD. Inhibitors of GSK-3 are thought to prevent hyperphosphorylation of tau, thus blocking the formation of NFTs. In this presentation, we will disclose a class of novel isonicotinamides as highly kinase selective, potent GSK-3 inhibitors, which are also brain penetrable and orally active in our pTau lowering *in vivo* model.

MEDI 27

Brain-penetrant, orally bioavailable microtubule-stabilizing small molecules as potential candidates for the treatment of Alzheimer's disease and related tauopathies

Carlo Ballatore[1,2]*, bcarlo@sas.upenn.edu, Kevin Lou*[1]*, Adam Hoye*[1]*, Bryant Gay*[1]*, Anne-Sophie Cornec*[1]*, Michael J. James*[2]*, Yuemang Yao*[2]*, Edward Hyde*[2]*, John Q. Trojanowski*[2]*, Virginia M.-Y. Lee*[2]*, Amos B. Smith*[1]*, Kurt R. Brunden*[2]*. (1) Department of Chemistry, University of Pennsylvania, Philadelphia, PA 19104, United States (2) Pathology & Laboratory Medicine, University of Pennsylvania, Philadelphia, PA 19104, United States*

In neurodegenerative tauopathies, of which the most prevalent example is Alzheimer's disease, the aggregation of the microtubule (MT)-associated protein tau is believed to have neuropathological consequences that result from toxic gains and/or losses of normal tau functions. MT-stabilizing drugs can compensate for the loss of MT-stabilizing tau function in tauopathy neurons and, therefore, these molecules have been suggested as potential candidates to treat tau-mediated neurodegeneration. Epothilone D has been the only brain-penetrant MT-stabilizer to be evaluated in tauopathy animal models, and this compound is presently undergoing testing in Phase 1b clinical trials. Epothilone D, however, exhibits potential deficiencies as a drug candidate, including an intravenous route of administration, the inhibition of the P-glycoprotein (Pgp) transporter, and a relatively complex/expensive synthesis. As a result, synthetically accessible, orally-active, brain-penetrant MT-stabilizing agents that do not interfere with Pgp function would be desirable. Towards this objective, our evaluation of different classes of known small-molecule MT-stabilizing agents led to the identification of selected triazolopyrimidines and phenylpyrimidines with these desired features. Pharmacodynamic studies confirmed that representative compounds produce MT-stabilization in the brain of mice, indicating that these synthetic small molecules may be promising leads to further develop as candidates for the treatment of tauopathies.

MEDI 28

From screening impurity to potent and selective ROMK inhibitors - the path to new mechanism diuretics

Haifeng Tang*, haifeng_tang@merck.com, Reynalda de Jesus, Shawn P Walsh, Aurash Shahripour, Yuping Zhu, Nardos Teumelsan, Lihu Yang, Emma Parmee, Sander G Mills, Birgit T Priest, John P Felix, Brande Thomas-Fowlkes, Andrew M Swensen, Gregory J Kaczorowski, Maria L Garcia, Adam Weinglass, Magdalena Alonso-Galacia, Xiaoyan Zhou, Lee-Yuh Pai, Caryn Hampton, Olga Price, Melba Hernandez, Charles Gill, Kashmira Shah, Joseph Metzger, Mike Forrest, James Ormes, Mengwei Hu, Karen Owens, Koppara Samuel, Randy R Miller, Vincent Tong, Sophie Roy, Kathleen Sullivan, Alexander Pasternak. Merck Research Labs, Kenilworth, New Jersey 07033, United States*

ROMK (Kir1.1) is a member of the inward rectifier family of potassium channels expressed in at least two regions of the kidney: the thick ascending loop of Henle (TALH) and the cortical collecting duct (CCD). At the TALH, ROMK participates in potassium recycling across the apical (urine-facing) membrane and is essential for function of the furosemide-sensitive Na+/K+/2Cl- (NKCC2) co-transporter, the rate-determining step for sodium reuptake in this part of the nephron. At the CCD, ROMK provides a pathway for potassium secretion that is tightly coupled to sodium uptake through the amiloride-sensitive epithelial sodium channel (ENaC). Human and rodent genetic data suggest that selective inhibition of ROMK will lead to natriuresis, diuresis and lowered blood pressure, with reduced loss of K+ in the urine. Thus, selective inhibitors of ROMK are expected to represent a new class of diuretics predicted to act at multiple sites in the nephron with a potential for greater efficacy and reduced urinary potassium excretion compared to standard-of-care loop and thiazide diuretics used in treating heart failure and hypertension. Unfortunately, investigation of the *in vitro* and *in vivo* pharmacology of ROMK has, to date, been hampered by the absence of potent and selective ROMK inhibitors with suitable characteristics to be used *in vivo* as tool compounds. The first small molecule ROMK inhibitors were reported by Denton at Vanderbilt University. More recently, we described the discovery of a new class of potent and selective small molecule ROMK inhibitors which, remarkably, originated from a minor impurity isolated from an otherwise inactive high throughput screening hit. This presentation will detail the discovery of our initial lead and subsequent program progress to improve ROMK potency, selectivity against the hERG channel, and poor initial PK properties. Several potent and selective benchmark ROMK inhibitors will be described in detail. Pharmacological assessment of highlighted inhibitors will be described, including pharmacodynamic characteristics in rat and dog diuresis models, and blood pressure effects in the spontaneous hypertensive rat model. These proof-of-biology studies establish for the first time that the human and rodent genetics accurately predict the *in vivo* pharmacology of ROMK inhibitors.

MEDI 29

Design of novel reagent sets to enhance drug discovery

***Frederick W Goldberg**, frederick.goldberg@astrazeneca.com. Department of Oncology, AstraZeneca, Macclesfield, Cheshire SK10 4TG, United Kingdom*

Numerous analyses have been published on the importance of high quality screening collections, focusing on factors such as lead-like properties, 3D shape, diversity, novelty and privileged substructures. One pragmatic way to achieve this is with improved access to novel, high quality building blocks, but the design principles that should govern strategic acquisitions and custom synthesis of such reagents has been rather overlooked in the literature. Reagents that can capture medicinal chemistry learning and are designed to impart favorable physical properties when used to synthesize project compounds can accelerate drug discovery programs and enhance the quality of compound collections. This is particularly true when attempting to incorporate saturation

and 3D shape, as the synthetic difficulties involved with the resulting chirality can be prohibitive.

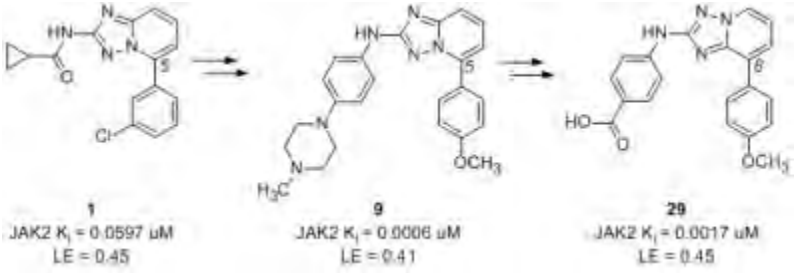
In this presentation we will present our strategy to address this with custom synthesis to harness the learning from our internal programs and external literature. >60k compounds in the AstraZeneca (AZ) compound collection have incorporated these reagents, resulting in numerous project impact examples and three candidate drugs. This presentation will discuss the methods we have used with examples, and the properties of the resulting reagent set *vs.* ACD. We will discuss what constitutes a "popular reagent" in medicinal chemistry, by analyzing the contribution of different reagent types to the AZ compound collection and the usage of reagents in the AZ electronic lab notebook. We shall also present the structures of real project examples to exemplify how these reagents have been used by our medicinal chemistry teams.

MEDI 30

2-Amino-[1,2,4]triazolo[1,5-a]pyridines as JAK2 inhibitors

Michael Siu[1]*, siu.michael@gene.com, Richard Pastor*[1]*, Wendy Liu*[1]*, Kathy Barrett*[1]*, Megan Berry*[1]*, Wade S Blair*[1]*, Christine Chang*[1]*, Jacob Z Chen*[1]*, Charles Eigenbrot*[1]*, Nico Ghilardi*[1]*, Paul Gibbons*[1]*, Haiying He*[2]*, Christopher A Hurley*[3]*, Jane R Kenny*[1]*, S Cyrus Khojasteh*[1]*, Hoa Le*[1]*, Leslie Lee*[1]*, Joseph P Lyssikatos*[1]*, Steve Magnuson*[1]*, Rebecca Pulk*[1]*, Vickie Tsui*[1]*, Mark Ultsch*[1]*, Yisong Xiao*[2]*, Bing-yan Zhu*[1]*, Deepak Sampath*[1]*. (1) Genentech, South San Francisco, CA 94080, United States (2) WuXi AppTec Co., Ltd, Shanghai, China (3) Argenta, Harlow, Essex CM19 5TR, United Kingdom*

The advancement of a series of ligand efficient 2-amino-[1,2,4]triazolo[1,5-a]pyridines, initially identified from high-throughput screening, to a JAK2 inhibitor with pharmacodynamic activity in a mouse xenograft model is disclosed



MEDI 31

Discovery of NVS-CRF38 a novel CRF-1 receptor antagonist

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Corticotropin-releasing factor (CRF) is a key mediator to adapt the body response to stress. CRF-1 receptor antagonists have been suggested as therapies for stress related disorders such as anxiety or irritable bowel syndrome. Since the disclosure of Pfizer's antalarmin (CP-154,526) the discovery of novel structural class CRF-1 receptor antagonists has been both characterized by and hampered by the high lipophilicity associated with the numerous chemical clasess disclosed to date. It can be assumed that the sub-optimal physicochemical properties are a major contributing factor for the poor advancement of this class of compounds through the clinic: this talk will centre on our drug discovery efforts and include the application of early toxicology studies which ultimately lead to the discovery of NVS-CRF38, a member of a novel structural class CRF-1 receptor antagonist with favourable biopharmaceutical properties.

MEDI 32

Traceless labeling of neuronal receptors using a modular, ligand based strategy

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Studies of dynamically trafficked neuronal proteins typically rely on the use of genetic engineering or antibodies. We have developed a simple, chemical biology-based method for observing these types of proteins. Our small, modular nanoprobe system uses a ligand to target fluorophore delivery to endogenous receptors. The fluorophore covalently binds the receptor, and the ligand can then be removed using light, returning the receptor to its non-liganded state. Virtually any tetherable ligand can be used for directing the probe to a target and, likewise, differently colored dyes can be employed as reporters. We have thus far designed different colored probes for NMDA and AMPA receptors. One of these probes employs a phenotype-specific small molecule that allows delivery of a fluorophore to endogenous calcium-permeable AMPA receptors (CP-AMPARs) on cultured neurons. Using this specific molecule, we observed an abundance of these receptors on dissociated hippocampal neurons, at synaptic spines and dendritic shafts. We also observed these labeled CP-AMPARs undergoing intracellular trafficking, sometimes moving into synaptic spines, and at other times

moving from spine bases to spine heads. The utility of these measurements could enable molecular-level visualization of processes such as synaptic potentiation.

MEDI 33

Discovery of 5''-chloro-N-[(5,6-dimethoxypyridin-2-yl)methyl]-2,2':5',3''-terpyridine-3'-carboxamide (MK-1064): A Potent, orally bioavailable, selective orexin 2 receptor antagonist that effectively promotes sleep in preclinical animal models

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The field of small molecule orexin antagonist research has evolved rapidly in the last fifteen years from the discovery of the orexin peptides to clinical proof-of-concept for the treatment of insomnia. Clinical programs have focused on the development of antagonists that reversibly block the action of peptides at both the orexin-1 and orexin-2 receptors (OX1R and OX2R), termed dual orexin receptor antagonists (DORAs). Full characterization of the pharmacology associated with antagonism of either OX1R or OX2R alone has been hampered by a dearth of suitable subtype selective, orally biovailable ligands. Herein we report the optimization of a selective orexin 2 antagonist (2-SORA) HTS screening lead to afford a potent, orally bioavailable 2-SORA ligand. During the course of these efforts several challenging medicinal chemistry issues were identified and overcome including reversible CYP inhibition, physical properties, P-glycoprotein susceptibility and bioactivation. This presentation will highlight the structural modifications the team utilized to drive compound design as well as in vivo characterization of 5''-chloro-*N*-[(5,6-dimethoxypyridin-2-yl)methyl]-2,2':5',3''-terpyridine-3'-carboxamide (MK-1064) in animal sleep models.

MEDI 34

Chemical biology approaches to investigate the Pup-proteasomal system in Mycobacterium tuberculosis

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The prokaryotic ubiquitin-like protein (Pup) was recently discovered in the pathogenic bacterium *Mycobacterium tuberculosis* (*Mtb*). Similar to the role of ubiquitin in humans, the covalent modification of mycobacterial proteins by Pup (pupylation) acts as a signal for their degradation by 20*S* proteasomes and is essential for persistent infection by *Mtb*. Therefore, enzymes associated with the Pup-proteasomal system are potentially susceptible targets in the face of rapidly escalating drug-resistance in *Mtb*. The deamidase of Pup (Dop) and proteasome accessory factor A (PafA) are enzymes involved in the C-terminal deamidation and subsequent conjugation of Pup, respectively, to lysine side-chains in target proteins. Understanding the mechanism of action of these enzymes, which are unique to actinobacteria, is an important step toward the rational design of novel enzyme inhibitors as TB therapeutics.

With this goal in mind, we developed new activity-based fluorescent probes of Dop and PafA activity. These probes permitted the identification of minimal amino acid sequences of Pup required for protein pupylation and depupylation, and for the identification of critical amino acids within these sequences. Surprisingly, we observed that the deamidating and depupylating activities of Dop have different sequence requirements in Pup, which is unusual for a single enzyme. Finally, the activity-based probes were also applied toward investigating the effect of S-nitrosylation of cysteine residues in PafA and Dop, biologically relevant modifications initiated by the host's immune response, on the rates of protein pupylation. Results from these studies and their implications in sustained infection by *Mtb* will be discussed.

MEDI 35

Chemistry, pre-clinical characterization, and clinical translation of BACE inhibitors

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Cerebral deposition of amyloid-β peptide (Aβ) is critical in Alzheimer's disease (AD) pathogenesis. Owing to its role in the generation of Aβ, the BACE1 enzyme continues to be prime target for designing drugs to prevent or treat AD; however, BACE1 has proven to be an exceedingly challenging target for drug discovery. In 2010, we demonstrated

for the first time that LY2811376, a small molecule BACE1 inhibitor discovered using a fragment-based approach, could produce profound Aβ-lowering effects in humans. We also advanced a more potent and selective BACE1 inhibitor, LY2886721, which ultimately progressed into Phase II clinical development. This presentation will describe key fragment starting points, complex synthetic chemistry, preclinical characterization of multiple molecules, and Phase I clinical PK/PD data for LY2886721. This presentation will also include the first disclosure of the chemical structure of LY2886721.

MEDI 36

Discovery of novel selective spleen tyrosine kinase inhibitors

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Selective inhibition of kinases remains an important challenge for medicinal chemists, particularly for the delivery of important therapeutics for non-oncology indications. The SYK (Spleen Tyrosine Kinase) inhibitor program for the treatment of immunological disorders provides an important case-study due to the hypothesis a selective SYK inhibition would retain the efficacy while dissociating the cardiovascular adverse events associated with the leading non-selective SYK inhibitor fostamatinib. This presentation will focus on the discovery, optimization and preclinical characterization of selective SYK inhibitors.

MEDI 37

Development of inhibitors against Venezuelan equine encephalitis virus (VEEV)

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Alphaviruses like VEEV are enveloped, RNA viruses that are geographically widely distributed and are known to cause rash, arthritis, encephalitis, and death in humans. Of the more than 30 alphavirus pathogens known, about a third contributes to human

disease, and currently there are no FDA approved treatments available for any of them. A renewed interest to find effective therapeutic leads for development has emerged due to the lack of effective countermeasures for these pathogens, the increased incidence of their prevalence with global climate changes, and the ease with which they can and have been weaponized as biological threats. A subset of scaffolds that inhibited a VEEV-induced cytopathic effect in the low micromolar range was identified using a high throughput-screen. Medicinal chemistry optimization of a selected chemotype revealed a unique chemical rearrangement that afforded a new scaffold and led to the development of ML336, a first-in-class probe that inhibited a VEEV-induced cytopathic effect in three strains of the virus in the low nanomolar range without showing cytotoxicity. Furthermore, ML336 dramatically reduced *in vitro* viral titer and featured a favorable *in vitro* pharmacokinetic profile which included moderate blood-brain barrier permeability. These combined characteristics have delivered an advanced lead which is suitable for further drug development.

MEDI 38

Use of metalloporphyrin catalyst(s) to evaluate biotransformations and potential drug-drug interactions

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Studying biotransformation is necessary in the early stages of drug development. Enzymes in the cytochrome P-450 superfamily are responsible for the biotransformation of over 90% of clinical drugs. In order to save time and money, *in vitro* methods have been developed to mimic this process to determine potentially toxic metabolites, as well as to examine potentially harmful drug-drug interactions. However, a number of problems are associated with current methods that use biological systems as *in vitro* models for drug metabolism. Here we present a new method utilizing metalloporphyrin catalyst(s) that mimics the oxidation of cytochrome P-450 in order to synthetically produce these metabolites. This technology introduces a new paradigm for drug discovery and mimicking drug-drug interactions for clinical diagnostics.

MEDI 39

Breaking the rule-of-5: Macrocycle leads for high-value drug targets

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Many high-value and structurally challenging drug targets have proven to be refractory to traditional small molecules. Using our proprietary DNA-Programmed Chemistry platform, Ensemble Therapeutics has created extensive libraries of macrocycles that are designed to modulate promising and challenging therapeutic targets such as protein-protein interactions. These libraries encompass a variety of non-peptidic scaffolds that can be rapidly screened using affinity-based selections. Ensemble has also developed a comprehensive drug discovery program blending library synthesis, chemistry, biology and modeling and leading all the way from initial hit discovery to clinical candidate. This process is not only providing insights into properties of non-traditional "beyond rule-of-5" drugs but has resulted in promising leads for previously undruggable proteins. We have recently demonstrated the utility of our macrocyclic platform by discovering and optimizing unprecedented leads for a high-value, clinically validated target that are both potent and highly selective over homologous proteins. This and other case studies provide promising glimpses into the potential of macrocycle drugs and validate Ensemble's approach to pursuing these novel chemical entities.

MEDI 40

Collective intelligence: A need for new drug discovery from medicinal plants, the African perspective

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The Chemistry, Manufacturing and Control (CMC) aspects of drug discovery is geared towards producing medicines suitable for their intended use with specified quality, safety and efficacy characteristics. This requires a thorough understanding of the drug product/drug substance performance, identification of drug product/substance critical characteristics (which is monitored on a batch-by-batch basis) and demonstration of drug safety and efficacy which ultimately leads to the review and/or approval of the drug product. To facilitate the management of the technical and regulatory CMC issues within core dossiers, companies have turned to a range of organizational structures and procedures to manage the input effectively. If drug discovery must happen in Africa the vertical and independent research approach will necessarily have to give way to the horizontal and collaborative research approach. Basic steps for drug discovery from medicinal plants (the Africans` perspective) have been highlighted with an unbiased emphasis on the Investigational New Drug (IND), a process which primarily ensures that a product does not expose human to unreasonable risk when used in early stage of clinical studies. Further highlights on IND requirements are clearly stated.

KEYWORDS: Chemistry, Control, Investigational, Manufacturing, Medicinal.

MEDI 41

Synthesis of resorufin derivatives as inhibitor indicators of cytochrome P450 enzymes

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Cytochrome P450 enzymes are hemoproteins found in all organisms, and responsible for the oxidation of many endogenous substances and xenobiotics as part of normal metabolism and detoxification processes. The P450-catalyzed monooxygenation reactions can however lead to the activation of certain environmental procarcinogens, particularly polyaromatic hydrocarbons (PAHs). In an attempt to understand the mechanisms of action of these enzymes, inhibitors have been developed to probe into their active site pockets. O-alkylresorufins fluoresce when dealkylated by P450 enzymes. By measuring the florescence intensity of the resorufin anion formed during the dealkylation reaction, enzyme activity and its inhibition by various inhibitors can be monitored. Our group has designed and synthesized a novel set of resorufin derivatives that have the potential to be used as inhibitor indicators in inhibition studies.

MEDI 42

Biotinylation of small molecules for target identification

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Biotinylation, the functional addition of a biotin molecule to a biologically active compound (small molecules) represents a common technique for identification of the intracellular binding partners that underlie the foundation of observed biological activity of the molecule. Many advances in the area of small molecule biotinylation have come about in recent years due to the explosion of advances in the fields of bioorthogonal chemistry and proteomics. Deciphering the mode of action and biological protein target of a drug candidate provides vital information on off-target effects, potential interactions, and other data valuable for bringing the compound to the clinic. However, the identification of such biological targets is extremely difficult in the area of chemical biology. One of the ways to overcome this problem is the use of biotinylated compounds for the identification of the target proteins.

In this poster we describe the methods for attaching the biotin moiety to some of our preclinical (ON 24160, ON 24860 and ON 1231360) and clinical (ON 01910, ON 03105 and ON 01210) compounds and we show the target identification using some of these biotinylated compounds.

MEDI 43

Evaluation of interactions of fullerene nanoparticles with proteins: An inverse docking study

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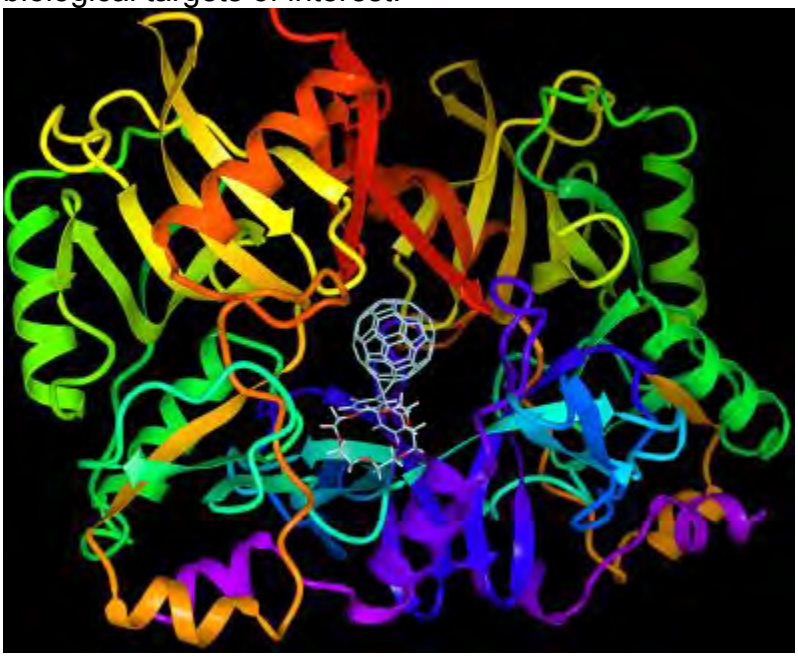
Identification of a potential drug for protein targets is one of the directions in drug discovery process. The aim of this study is to identify potential biological targets for fullerene derivatives and then score fullerene derivatives as potential drugs for particular targets. For this purpose a set of fullerene derivatives have been modeled and then docked against a series of proteins collected from potential drug target database (PDTD). The calculated binding affinities and calculated potential toxicity could be used to classify the pairs of fullerene derivative – biological target. The attained data also could support experimental studies for advancement of drug delivery agents to transport drug-like compounds to the target sites. Finally, a new set of rationally designed fullerene derivatives will be generated that may play as highly-potential drugs for biological targets of interest.



Keywords: Fullerene Nanoparticles, Inverse Docking, Drug Design

MEDI 44

WITHDRAWN

MEDI 45

Profound methyl effects in drug discovery and a call for new C-H methylation reactions

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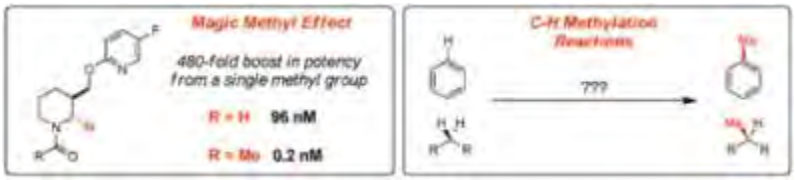
The methyl group is one of the most commonly occurring carbon fragments in small-molecule drugs. This simplest alkyl fragment appears in more than 67% of the top-selling drugs of 2011 and can modulate both the biological and physical properties of a molecule. This poster focuses on so-called magic methyl effects on binding potency, where the seemingly mundane change of C-H to C-Me improves the IC_{50}value of a drug candidate more than 100-fold. This discussion is followed by a survey of recent advances in synthetic chemistry that allow the direct methylation of $C(sp^2)$-H and $C(sp^3)$-H bonds. It is our hope that the relevance of the meager methyl group to drug discovery as presented here will inspire reports on new C-H methylation reactions.



MEDI 46

Structure-based design of novel SIRT3 inhibitors and activators

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Sirtuins are NAD^+-dependent enzymes that have been ascribed roles in numerous physiopathological conditions, including aging, metabolism, circadian clock regulation, nutritional behavior, but also cancer, inflammation, neurodegenerative disorders and cardiovascular diseases. Because of their broad involvement in the pathophysiology of highly prevalent diseases, sirtuins are appealing therapeutic targets for small-molecules. In particular, SIRT3 is a mitochondria-localized enzyme involved in the maintenance of mitochondrial integrity and metabolism during stress.

Several structures of human sirtuins are available in the Protein Data Bank, in unbound form or in complex with NAD^+, substrates, or different reaction intermediates. We carried out a structure-based molecular design approach devised to target SIRT3 with novel small-molecule. By means of SIRT3 direct fluorescent activity assay and western blot analysis for identification of the whole pattern of acetylated proteins and specific SIRT3 targets (i.e. SOD2) in isolated mitochondria, we report evidence of compounds able to inhibit and activate SIRT3. New families of compounds able to modulate the activity of SIRT3 hold promise to represent agents that could conceivably be used in a number of conditions like neurodegenerative diseases, aging-related pathologies, cancer and inflammation.

MEDI 47

Synthesis, ^{1}H- and ^{13}C- NMR spectroscopic studies of some novel emetine dithiocarbamate ester derivatives

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Emetine, an isoquinoline alkaloid from the ipecac species, has shown interesting medicinal properties including anti-cancer, anti-viral and anti-parasitic activities. In efforts to improve the pharmacological properties associated with emetine, we are exploring chemical modification of the N-2' position of emetine to compounds with improved efficacy but reduced host toxicity. A series of novel dithiocarbamate ester derivatives were synthesized in our research group for evaluation in some cancer cell lines. Using COSY, HETCOR and DEPT-NMR experiments, this paper presents detailed ^{1}H and ^{13}C NMR characterization of some dithiocarbamate ester derivatives of emetine.

MEDI 48

Encapsulation of (2E,6E)-2,6-bis(3-nitrobenzylidene)cyclohexanone in PLGA-based nanoparticles

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The therapeutic qualities of the Curcumin compound have been extendedly reported, showing anticancer, antioxidant and anti-inflammatory properties, among others. Nevertheless, dietary curcumin shows poor bioavailability due to its rapid metabolism. However, this obstacle can be overcome in many ways, one is to modify its molecular structure to create a more suitable compound that presents a longer availability within the organism in order to exert its therapeutic action. We have previously reported the cytotoxicity against prostatic and colon cancer cells, and normal peripheral blood mononuclear cells (PBMCs) of Curcumin analogues. Of these, compound (2E,6E)-2,6-bis(3-nitrobenzylidene)cyclohexanone was selected as a good candidate for future development due to its high cytotoxicity against cancer cell lines and relative low toxicity against PBMCs isolated from healthy volunteers, making this analog an excellent candidate for cancer treatment. The purpose of this new research project is to create a suitable drug delivery system to enhance the specific targeting and cytotoxicity of this drug towards cancer cells by achieving a prolonged release of the compound. To this extent, we decided to encapsulate this analog in Poly Lacto-co-Glycolic Acid (PLGA)-based nanoparticles (NPs). This kind of carrier has been reported to be easily absorbed by cells via endocytosis. PLGA-NPs have low toxicity provided by its biodegradable capabilities, degrading into lactic and glycolic acids in the organism, both metabolites of the Krebs' cycle. The formulation of these nanocarriers is achieved by a single emulsion method, and centrifuged to separate the NPs from the medium. The product and the supernatant will be analyzed to determine drug loading and entrapment, morphology and size of the NPs, zeta potential, drug release, and therapeutic potential. We expect that the cytotoxicity against prostatic and colon cancer cell lines of encapsulated drug to be higher than previous tests due to prolonged release of the compound in the cytosol.

MEDI 49

4-Alkyloxyimino derivatives of cytosine-5'-triphosphate: Distal modification of agonists and receptor docking at $P2Y_2$, $P2Y_4$, and $P2Y_6$ receptors

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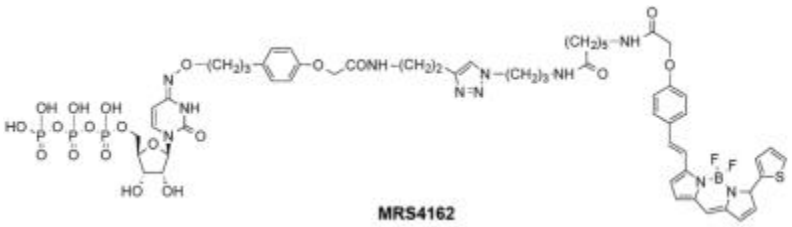
A series of N^4-(3-arylpropyl)oxy derivatives of cytidine-5¢-triphosphate were synthesized and found to display high potency as agonists of the G protein-coupled human $P2Y_4$ receptor ($P2Y_4R$). Stimulation of phospholipase C was measured in $P2Y_4R$-expressing 1321N1 astrocytoma cells and compared to other UTP ($P2Y_2R$) or UDP ($P2Y_6R$) receptors. The phenyl group was substituted with heterocyclic rings or a naphthyl ring with retention of affinity at P2YRs, indicating a broad tolerance for steric bulk in this distal region of the nucleotide. N^4-(3-(4-Methoxyphenyl)-propyl)oxy analogue displayed an EC_{50} at $P2Y_2R$ and $P2Y_4R$ of 47 nM and 23 nM, respectively. This potent ether was functionalized for chain extension using Click chemistry and functionalization with prosthetic groups, including fluorescent labels. MRS4162 containing the fluorophore BODIPY activated the $P2Y_2R$ and $P2Y_6R$ with EC_{50} values of 66 nM and 23 nM, respectively. Theoretical docking of MRS4162 and other 5¢-triphosphates to a homology model of the $P2Y_6R$ based on the X-ray structures of related GPCRs predicted a putative binding site and interactions of the chain tethering the fluorophore. Thus, an extended N^4-(3-arylpropyl)oxy group accessed a structurally permissive region on various Gq-coupled P2YRs and was modulated by distal structural changes to alter selectivity. This freedom of substitution can be utilized for the design of affinity probes of the uracil nucleotide-activated subset of P2Y receptors.



MRS4162

MEDI 50

Defining the role of reductase-incompetent ketoreductase in polyketide biosynthesis

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Modular polyketide synthases (PKSs) are giant multienzyme assembly lines that catalyze the biosynthesis of diverse polyketides, many of which are employed as antibacterials, antifungals, and anticancer agents. Polyketide intermediates are often epimerized at their α-carbon during polyketide synthesis, as in the first module of DEBS. Ketoreductases that lack the NADPH-binding motif but possess the tyrosine, serine, and lysine employed by reductase-competent KRs are referred to as C2-type Ketoreductase. Incubation of [2-^{2}H]-(2*R*,3*S*)-2-methyl-3-hydroxypentanoyl-SACP with

the non-epimerizing ketoreductase domain EryKR6 in the presence of a catalytic amount $NADP^+$ (0.05 equiv) and Ery KR3° resulted in time- and cofactor-dependent washout of deuterium from [2-^{2}H]-(2*R*,3*S*)-2-methyl-3-hydroxypentanoyl-SACP, as a result of equilibrium isotope exchange of transiently generated [2-^{2}H]-2-methyl-3-ketopentanoyl-ACP. The isotope exchange assay directly establishes that specific polyketide synthase ketoreductase domains also have an intrinsic epimerase activity, thus enabling mechanistic analysis of a key determinant of Polyketide stereocomplexity. The study not only establishes the fact that these silent ketoreductases have a specific function but also allow us to use the tool in understanding various other Polyketide formations.

MEDI 51

Simplifying the navigation through pharmacological data in the world-wide web

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Gathering publicly available pharmacological data from internet databases is annoying due to the large number of data sources, the different data formats and query mechanisms used. In 2011, the Open PHACTS project has been initiated to overcome these issues by integration of pharmacological data from a variety of information resources. Following an application-oriented approach, the project started with the definition of potential use cases in the form of prioritized research questions, most of which can only be answered by accessing multiple data sources in the web. The development of the platform as well as the services has been guided by these questions.

Here, we present the ChemBioNavigator (CBN), a web application allowing to navigate the Open PHACTS chem-bio space with a focus on small molecules and their targets. It allows interactive exploration of compound sets through sorting and subset selection as well as extending sets by substructure, similarity search or target specific assay data. Taking a step beyond the use-case driven development, the CBN is based on the analysis of several interviews with potential users from pharmaceutical industry. The interviews gave invaluable insights not only about the day to day use-cases, but also deficiencies as well as highly valued features of the existing tools. This leads to an agile and target-oriented development, which is able to quickly adapt to changing requirements from the scientist's daily work.

On our poster we present a couple of high-priority research questions and their answers found by the CBN.

MEDI 52

Biologically active extract of endophytic fungus from Wedelia texana

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The wedelia plant has been celebrated in traditional medicine as treatment for a diverse range of maladies, from liver problems to memory loss. Wedelia texana is a fast growing sunflower plant that is native to Central Texas. The leaves of the wedelia host symbiotic fungi that may confer medicinal activity through secretion of secondary metabolites.

The natural products of an endophytic fungus from Wedelia texana was harvested and tested for biological activity through a range of assays. The screens include anti-bacterial, anti-fungal, anti-oxidant, and allelopathic properties. There appears to be anti-oxidant and anti-bacterial activity of the crude extract and more results are to be pursued.

Screen	Activity
Anti-bacterial	- MIC of 0.005 mg of crude for Gram negative, also test for Gram negative
Anti-oxidant	- Anti-oxidant properties of separated compounds, DPPH and FTC assays
Anti-tumor	- Against potato tumors for potential agriculture applications
Allelopathy	- Used to coat lettuce seed as a potential protectant

MEDI 53

Extending matched molecular pairs analyses for novel chemical transformations

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Matched molecular pairs analysis (MMPA) is a method for analyzing SAR within a data set that considers pairs of molecules, and thus the relationship between compounds, rather than individual compounds themselves. MMPA is able to identify beneficial and deleterious transformations within a data set to apply or avoid in optimization efforts. Currently proposed MMPA methods require that a chemical transformation have already been observed. We present an extension of the matched pairs analysis that is adapted to allow for new or infrequently seen chemical transformations, and demonstrate its performance on several ChEMBL data sets with diverse endpoints including: antimalarial activity against Pf-3D7, Factor Xa inhibition, logP, and volume of distribution. In all cases, the protocol performs equally to, or better than, the standard matched pairs analysis, and more accurately than standard QSAR models. Notably, our methodology is able to return predicted activity changes for previously unseen chemical transformations, enabling a matched pairs analysis of a Vdss data set previously not analyzable by matched pairs due to insufficient numbers of pairs. The addition of more structural similarity will help identify and prioritize chemical transformations for the optimization of lead molecules.

MEDI 54

Synthesis and molecular docking thiazole-containing amino acids

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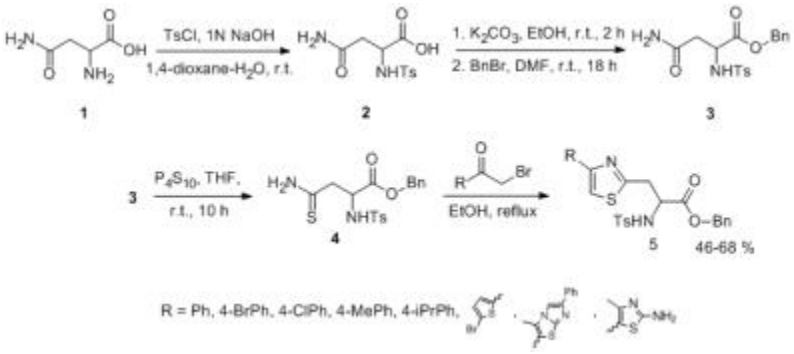
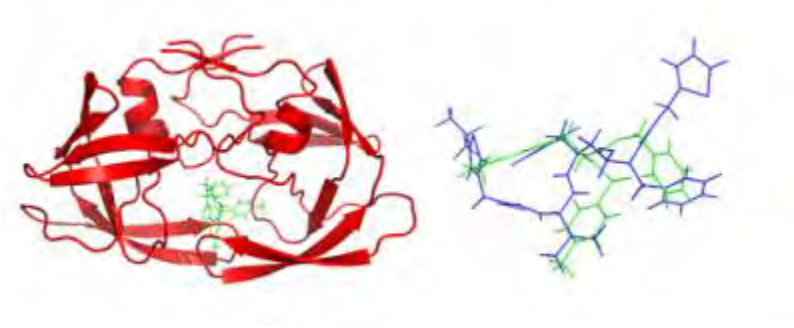
In the present study demonstrated the synthesis of a number of previously unknown thiazole-containing amino acids on the basis of asparagine 1.



We have done molecular docking synthesized thiazole-containing amino acids in the active site of binding of HIV-1 protease. For Series 5 amino acids studied were binding energy in the range 33 ÷ 44 kJ / mol (compared to ritonavir is 57 kJ / mol).



MEDI 55

Efficient virtual screening using ligand efficiency based approach

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Efficiency virtual high-throughput screening (vHTS) is an important manner to screen hits from massive database. We present the application of combining the ligand-based, structure-based and fragment-based screening for a novel VS protocol to screen in-house library of 125,000 compounds for aurora kinase A inhibitors. First, 20 known aurora kinase inhibitors were docked to aurora kinase A crystal structure (PDB ID: 2W1C) and the docked ligand conformations were used to create pharmacophore model (PH).The PH model was used to screen the database compounds, and rank (PH rank) them based on the predicted IC_{50} values. Next, a fragment-based ligand efficiency

(LE_Scale) function was derived from 294 known aurora kinase inhibitors. Using fit quality (FQ = LE/LE_Scale) score derived from LE_Scale function, the database compounds were reranked (PH_FQ rank) and the top 151 (0.12% of database) compounds were assessed for aurora kinase A inhibition biochemically. This VS protocol has led to the identification of 7 novel hits, with compound BPR0**5** showing aurora kinase A IC_{50} = 1.65 mM. Furthermore, synthesis and testing BPR0**5** against a panel of 31 kinase reveals that it is selective towards aurora kinase A & B, with < 50% inhibition for other kinases at 10 μM concentrations and is a suitable lead for further development. The LE based approach in the VS protocol not only helped in identifying a novel aurora kinase inhibitor BPR0**5** , but also increased the hit rate of VS protocol considerably by improving the Enrichment factor (EF) for Fit quality based screening (PH_FQ screening, EF = 828), compared to pharmacophore based screening (PH screening, EF = 237) alone, and the Goodness of fit score (GF) from 0.24 (PH screening) to 0.35. We suggest that this LE based application described here could be incorporated in the VS protocols for other targets to improve the hit rates.

MEDI 56

Can you keep all the SAR in your head? Using biological contexts to find the critical SAR regions

***Rae Lawrence**, rae@cresset-group.com, Tim Cheeseright, Mark D Mackey, Martin Slater. Cresset, Litlington, Cambridgeshire SG8 0SS, United Kingdom*

During the course of a lead optimization program hundreds or even thousands of compounds may be synthesized. These compounds hold a wealth of information on potency, selectivity and ADMET properties, but keeping all of this in mind, understanding it and using it to determine the optimal compound to make next is a challenging task.

One technique to extract the most important chemical transformations in a data set is to locate "activity cliffs": pairs of compounds where relatively small structural changes cause relatively large potency changes. The idea is that if a small structural change causes a large change in activity, then that structural change has high information content. A similar philosophy underlies the technique of matched molecular pair analysis.

However, the interpretation of activity cliffs can be difficult. A small structural change could cause a large change in potency due to many different reasons, such as steric clash with the protein, the loss of (or gain of) hydrogen bond donors/acceptors, or through forcing an alternative conformation of the ligand. We present a technique for locating activity cliffs in the context of the target active site. This clearly identifies the most important parts of the SAR, and visually shows the reason for the activity difference between each pair of compounds, assisting in a true understanding of the SAR landscape.

MEDI 57

Exploiting solvent effects in drug design and optimization

***Chris Williams**, cwilliams@chemcomp.com. Chemical Computing Group, Montreal, QC H3A 2R7, Canada*

Upon ligand binding, solvent molecules around the binding pocket and the ligand become displaced or rearranged. These desolvation energies can be a significant portion of the total binding energy, and thus represent opportunities for ligand design. Computing desolvation energetics typically requires lengthy simulations, but this talk presents a fast and easy-to-use method (3D-RISM) which computes desolvation energies in minutes, without using explicit simulations. Application to ligand optimization is demonstrated using case studies.

MEDI 58

Rationalization and visualization of non-bonded interactions using Extended Hckel Theory

***Alain Ajamian**, aajamian@chemcomp.com. Chemical Computing Group, Montreal, QC H3A 2R7, Canada*

Identification and rationalization of non-standard interactions presents many challenges during optimization of lead drug candidates. The typical approach of using SMARTS patterns to handle CH...O interactions is impractical as it requires a large set of patterns. An additional degree of difficulty is introduced when considering moities such as halogen bonds, proton-π interactions, sulfur-aromatic and the like. In order to address these challenges, Extended Hückel Theory is used to handle and account for the fundamentals of electron-withdrawal effects in relation to non-standard and hydrogen bond interactions.

MEDI 59

Computational approach for performing medicinal chemistry transformations within a 3D active site

***Nels Thorsteinson**, nthorsteinson@chemcomp.com, Elizabeth Sourial, Joe Loenard. Chemical Computing Group, Montreal, Quebec H3W3R7, Canada*

In this work, MedChem Transformations, a modeling application for performing medicinal chemistry transformations in the context of the 3D receptor and ranking the resulting molecules is presented. The methodology is outlined and a case study using a PDE5A-Sildenafil complex is performed. The results demonstrate that including pocket atoms and preserving key interactions help generate promising candidates that are

relevant to the PDE5A receptor as well as a known PDE5A ligand (Vardenafil) from the original sildenafil molecule.

MEDI 60

Carbamate co-drugs: Synthesis and inhibition of lipid peroxidation in skin

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The objective of this project was to synthesize and evaluate the antioxidant activity of new topical carbamate (α-TOC-CARB; δ-TOC-CARB) co-drugs and their metabolites (tocopherol (TOC) and lipoylamine (LAM) for protection of skin against oxidative damage. Carbamate co-drugs were synthesized from the isocyanate of lipoic acid, which was then coupled to α- or δ-TOC. The inhibitory activity of these antioxidants (AO) against lipid peroxidation (LP) using porcine skin as the lipid substrates in the thiobarbituric acid reactive substances (TBARS) assay was determined. IC_{50} values for α-TOC (32μM), δ-TOC (82μM), and LAM (527μM) were determined. The potencies of α-TOC and δ-TOC were consistent with values previously reported for these AO, however the potency of LAM was significantly greater than that observed for LA or LOH. Synergistic antioxidant activity was observed between α-TOC and LAM (IC_{50} =2.78μM) and δ-TOC with LAM (IC_{50}=210.3μM) (hydrolysis products of the co-drugs). The potency of the co-drugs was greater than expected for α-TOC-CARB and δ-TOC-CARB, suggesting the co-drugs hydrolyzed during the assay. α- and δ-TOC were potent inhibitors of LP. LAM was also found to inhibit LP, which has previously not been reported. Synergistic activity was observed for both α-TOC and δ-TOC with LAM. These data suggest that these carbamate co-drugs are excellent candidates for use as topical agents to protect the skin against oxidative damage.

MEDI 61

Interkingdom pharmacology of angiotensin-I converting enzyme inhibitor phosphonates produced by actinomycetes

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The K-26 family of bacterial secondary metabolites is a group of N-modified tripeptides, terminated by an unusual phosphonate analog of tyrosine. These natural products, produced via three different genera of the Streptosporangineae suborder of the Actinomycetales, are potent inhibitors of human angiotensin-I converting enzyme

(ACE). Herein we investigate the interkingdom pharmacology of the K-26 family by synthesizing these metabolites and assessing their potency as inhibitors of both the N-terminal and C-terminal domains of human ACE. In most cases, selectivity for the C-terminal domain of ACE is displayed. Co-crystallization of K-26 in both domains of human ACE reveal the structural basis of the potent inhibition and have shown an unprecedented binding motif that may guide future design of domain-selective inhibitors. Finally, the activity of K-26 is assayed against a cohort of microbially produced ACE relatives. In contrast to the synthetic ACE inhibitor captopril, which demonstrates broad interkingdom inhibition of ACE-like enzymes, K-26 selectively targets the eukaryotic family.

MEDI 62

Boron derivatives of DNA minor groove binders for the treatment of African Sleeping Sickness

Julius Green I*, jgreen25@student.gsu.edu, Shuo Wang, W David Wilson, Daivd W Boykin. Department of Chemistry, Georgia State University, Atlanta, GA 30312, United States*

Trypanosoma brucei, the parasite responsible for African Sleeping Sickness, kills approximately 10,000 people per year in Sub-Saharan Africa. This parasite has an organelle called a kinetoplast which contains thousands of circular DNA (kDNA) linked together like a chain mesh. kDNA has multiple phased AT regions and this organelle can be targeted for therapeutics. To this end, we have synthesized several compounds that use the lewis acidity of boron to increase DNA minor groove binding affinity. These fluorescent compounds show improved DNA binding and biological activity compared to the parent compounds.

MEDI 63

Ruthenium/phosphine complexes: Cytotoxicity and antiparasitic activities

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In this work we analyze the effects of ruthenium complexes containing lapachol(Lap) as ligand on the viability of breast cancer cells MDA-MB-231, prostate cancer cells DU-145, and fibroblasts HGF. The evaluation of antiparasitic activities of the complexes against *L. amazonensis* and *P. falciparum* demonstrated that the complexes are more potent than the free lapachol. The lapachol was choosen as ligand because it is a natural quinone isolated from brazilian *Tabebuia spp*. (Bignoniaceae family), which presents anticancer activity. Anticancer drug, cisplatin (CPt), and free Lapachol, were

tested for comparison. Cells viability was evaluated by the MTT method after 48 h of incubation with the complexes. All molecules affected tumor cells viability, as well as fibroblasts. The IC50 values (μM) for CPt, Lap, LapMe and LapMeO, respectively, are 2.437 ± 0.202, 38.912 ± 3.024, 0.478 ± 0.039, for MDA-MB-231; 2.001 ± 0.477, 9.204 ± 0.495, less than 0.19, for DU-145; and 1.422 ± 0.997, 9.298 ± 2.718, 2.386 ± 0.592, for HFG. RuLapMe was more cytotoxic for tumoral cell lines than the CPt and the free Lap, by about 5 and 80 times for MDA-MB-213, and about 10 and 48 times for DU-145. For fibroblasts, the effect of RuLapMe was almost the same as of CPt and about 4 times stronger than free Lap. Importantly, RuLapMe was more cytotoxic for tumoral cells, by about 5 and 12 times for breast and prostate cancer cells, respectively, while CPt and Lap were more effective for fibroblasts. Furthermore, 0,19 μM of RuLapMe increased the viability of fibroblasts, while the same concentration kills at least 50% of prostate tumor cells. The evaluation of antiparasitic activities of the complexes against *L. amazonensis* and *P. falciparum* demonstrated that the lapachol-ruthenium complexes are more potent than the free lapachol.

Financial support: CAPES, FAPESP, CNPq

MEDI 64

Synthesis of 4,6-diamino-1,2-dihydro-2,2-dimethyl-1-(3'-(3''-X-anilinomethyl)phenyl)-s-triazines as potential *Plasmodium falciparum* DHFR inhibitors

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As malarial drug resistance increases due to mutations in the DHFR domain, it is necessary to probe the enzyme with new, more flexible chemotypes in order to develop more effective antimalarial drugs. Novel compounds have been designed with a N-phenyl triazine moiety bridged with a CH_2NH linker to a hydrophobic X-phenyl group in order to overcome these mutations. A series of 4,6-diamino-1,2-dihydro-2,2-dimethyl-1-(3'-(3''-X-anilinomethyl)phenyl)-s-triazines have been synthesized and will be tested as potential inhibitors of the critical bifunctional enzyme, thymidylate synthase-dihydrofolate reductase that is essential for the biosynthesis of DNA, RNA, amino acids and proteins in the infectious plasmodium, *Plasmodium falciparum*.

MEDI 65

Study of quinoxaline-based new compounds against aminopeptidase N, and Plasmodium falciparum malarial parasite

***Anthony Addlagatta**[1], anthony@iict.res.in, Rajesh Gumpena[1], Anil Marapaka[1], Aparna Devi Allanki[1], Jung Ho Jun[3], Puran Singh Sijwali[2], **Sanjay V. Malhotra**[3], malhotrasa@mail.nih.gov. (1) Center for Chemical Biology, Indian Institute of Chemical Technology, Hyderabad, AP 500007, India (2) Center for Cellular and Molecular*

Biology, Hyderabad, AP 500007, India (3) Laboratory of Synthetic Chemistry, Leidos Biomedical Research, Inc, Frederick National Laboratory for Cancer Research, Frederick, Maryland 21702, United States

Malaria is oldest and the most important tropical diseases known to mankind, for which chloroquine (CQ) has been the primary therapy of choice for past several decades. However, CQ-resistant Plasmodium falciparum is now observed in majority of the malaria-endemic regions, leading to the most deadly form of malaria. This warrants urgent need to develop more effective drugs against this parasite. Quinoxaline-based compounds display diverse biological activities as antibacterial, antiviral, anticancer and antiparasitic agents. In the present study we synthesized a small chemical library of new compounds based on quinoxaline and investigated their effect on the growth of M1 alanyl-aminopeptidase (PfAPN), an enzyme involved in the terminal stages of hemoglobin digestion to generate an amino acid pool within the parasite. Also, to check if these molecules affect parasite growth, the most lethal human malaria parasite P. falciparum was grown in the presence of varying concentration of these compounds, and IC50 concentrations for each compound were determined. We found these compounds cause enzyme inhibition, and also parasite growth inhibition with IC50 values in the low micromoles. Details of the study will be presented.

MEDI 66

Synthesis and evaluation of 5-methyl-5-deaza folate analogs activity against *Staphylococcus aureus* type II topoisomerases

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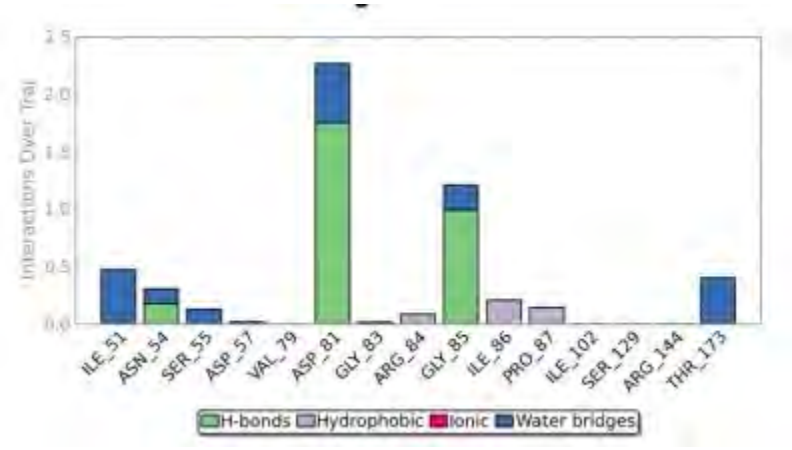
Methicillin-resistant *Staphylococcus aureus* (MRSA) infections have seen an increase and persistence in hospital and community settings due to mutations that render them resistant to commonly used antimicrobials. DNA gyrase and Topo IV are type II topoisomerases which regulate supercoiling of DNA driven by ATP hydrolysis. They are essential enzymes involved in bacteria replication. Using Shape Signatures screening, followed by receptor docking onto ATP binding site in crystallographic gyrase B, we were able to uncover a 5-methyl-5-deaza folate analog with high docking score, and a good interaction energy profile from molecular dynamics simulations. One of the analogs makes a hydrogen bond with ASP 81 throughout the course of the simulation. Interaction with this residue has been commonly found in potent Gyr inhibitors such as Novobiocin. ADME predictions suggest a good bioavailability and safety profile. Molecules in this class have previously shown antiplasmodium and antineoplastic activity as folate inhibitors. Following compound synthesis, the antimicrobial activity of the 5-methyl-5-deaza folate analogs are being determined through sensitivity assays by elucidating their minimal inhibitory concentrations against ISP 794 and RN4220 strains

of *S.aureus*. Relaxation assays will be performed to confirm inhibition of DNA gyrase and/or Topo IV activity. Evidence of ATPase activity will be evaluated through phosphate detection assays.



MEDI 67

Enzymatic synthesis of 3-deoxy-D-manno-octulosonic acid from in situ D-arabinose-5-arsenate as a substrate analog

Josh Halpern*, jrh11@geneseo.edu, Ronald W. Woodard, Andrew Pratt. Medicinal Chemistry, University of Michigan, Ann Arbor, Michigan 48104, United StatesDepartment of Chemistry, SUNY Geneseo, Geneseo, New York 14454, United States*

Kdo (3-deoxy-D-manno-octulosonic acid) is an acidic monosaccharide found in the inner core of lipopolysaccharide (LPS), the main component of the outer leaflet of the outer membrane critical for survival and growth of Gram-negative bacteria. These bacteria are human and plant pathogens that are responsible for many bacterial infections and understanding the biological roles Kdo can help us create novel antibiotics. Kdo serves as a linker between the core oligosaccharides and lipid A of LPS. Absence of Kdo in WT *E. coli* results in stagnant bacterial growth and increased sensitivity to antibiotics. The synthesis of Kdo using *in situ* D-arabinose-5-arsenate as a substrate mimic in place of the endogenous substrate, D-arabinose-5-phosphate, is a more facile, direct, and inexpensive *in vitro* synthesis. This synthesis requires the enzyme, KdsA (3-deoxy-D-manno-octulosonate 8-phosphate synthase) and lacks the need for the phosphatase activity of KdsB due to water mediated hydrolysis of the arsenate linkage. Large quantities of Kdo are needed in order to prepare analogues that will provide a deeper understanding of the chemical and biophysical properties of the enzymes involved in Kdo biosynthesis and incorporation into LPS with potential to discover novel inhibitors. KdsA was overexpressed and purified. The *in vitro* synthesis of Kdo tested KdsA from three species: *Escherichia coli, Arabidopsis thaliana,* and

Acinetobacter baumannii. It was found that KdsA from *A. baumanii* provided the maximal amount of Kdo. The concentrations of starting materials, D-arabinose, phosphoenolpyruvate, sodium arsenate, and protein concentrations were optimized to provide us with the highest yield of Kdo. Further optimization occurred by varying the reaction time and volumes. Kdo produced in these reactions was quantified via the Aminoff assay. Various purification methods of the Kdo produced via this method are currently being explored.

MEDI 68

Inhibitors of plasmodium falciparum methionine aminopeptidase 2 with antimalarial activities

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Plasmodium falciparum a is protozoan parasite that causes deathly cases of malaria in humans. There is a pressing need for the discovery of new antimalarial agents due to the rapid spread of multidrug-resistant strains of this organism. Methionine aminopeptidase (MetAP) carries out an important cotranslational N-terminal methionine excision of nascent proteins and was shown to be essential for the survival of many bacteria. Methionine aminopeptidase 2 (*Pf*MetAP2) is essential for *P. falciparum* making it an attractive antimalarial drug target. This poster focuses on the synthesis of potential inhibitors of PfMetAP2, identification of lead compounds from the synthesized inhibitors and compounds from Malaria Box collection, and design of novel *Pf*MetAP2 inhibitors using structural biology and molecular modeling. Up to date, the analogues of nitroxoline were identified as the most potent inhibitors against *Pf*MetAP2 with IC_{50} ranging from 60 to 100 μM and growth inhibition IC_{50} between 0.99 to 3.74 μM respectively.

MEDI 69

New *N-4* piperazinyl derivatives of norfloxacin: Correlation of lipophilicity, polarizability and topology parameters with antibacterial activity

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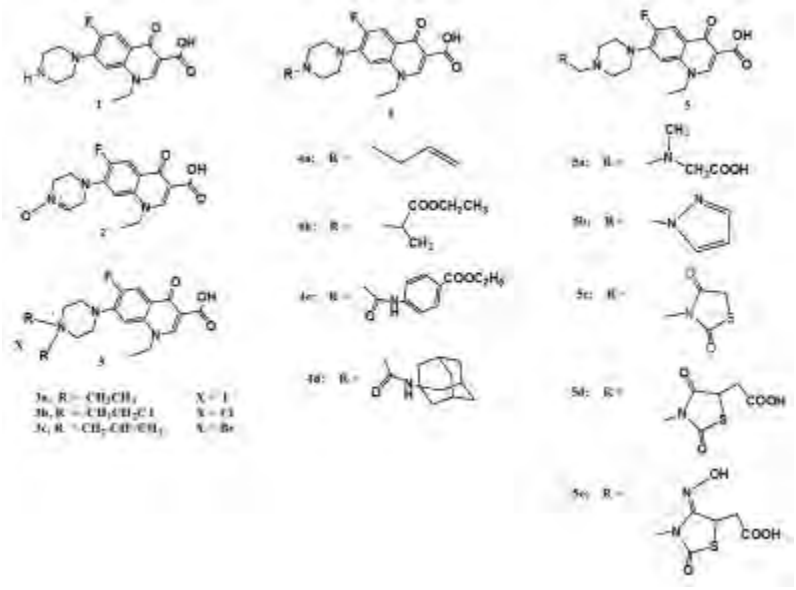
A group of *N*4-piperazinyl derivatives of norfloxacin **1** was synthesized and characterized by different spectroscopic techniques. The *N*4-piperazinyl substituents in the new compounds **2** , **3a-c, 4a-d** and **5a-e** were designed to alter the electronic, steric and physicochemical properties of norfloxacin structure.

The antibacterial properties of the newly synthesized compounds were evaluated against *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* strains using norfloxacin as a reference. Most of the tested compounds exhibited improved activity against *E. coli* and *K. pneumonia*e if compared to norfloxacin and five of them were more active against *P. aeruginosa*. None of the new compounds was more active against *S. aureus*than norfloxacin. The minimum inhibitory concentrations of the new compounds were shown to be correlated with lipophilicity, polarizability and topology parameters of the tested compounds.



MEDI 70

Design and synthesis of a dual inhibitor of falcipain-2 and dihydrofolate reductase: A prodrug approach

Jasper Werby, *jasperwerby@gmail.com, Cynthia Selassie. Chemistry, Pomona College, Claremont, California 91711, United States*

Malaria annually kills around one million people, mainly in the developing world. The rapid life cycle of the malaria parasite and the subsequent development of resistance have rendered most currently used drugs as ineffective. This project focuses on the development and synthesis of a new bifunctional, antimalarial drug that targets multiple inhibitory sites at the same time. Falcipain 2 and Dihydrofolate reductase (DHFR) are

enzymes that are critical to the growth of the plasmodium. A critical analysis of the current literature has led to the design and synthesis of a molecule that incorporates structural features necessary for binding to Falcipain 2 and DHFR. The multistep synthesis has involved the synthesis of two separate pharmacophores that are eventually connected to each other through an amide linkage. As amide linkages are labile within the bloodstream, and plasmodium falciparum is a blood borne disease, this method of creating a dual inhibitor shows the potential for a slow release prodrug targeting blood borne diseases. Further work will be done to test the kinetics of this separation as well as to test activity in both components.

MEDI 71

Discovery of next generation A_3 adenosine receptor selective agonists for treatment of chronic neuropathic pain

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The A_3 adenosine receptor (A_3AR) is a promising therapeutic target for a variety of chronic diseases including psoriasis, glaucoma, rheumatoid arthritis, and cancer. (N)-Methanocarba adenosine 5'-methyluronamides containing known A_3AR-enhancing modifications, i.e., 2-(arylethynyl)adenine and N^6-methyl or N^6-(3-substituted-benzyl) groups, were nanomolar full agonists of human and mouse A_3AR and highly selective (>3000-fold vs. other ARs). Combined 2-arylethynyl-N^6-3-chlorobenzyl substitutions preserved A_3AR affinity/selectivity in the (N)-methanocarba series better than that for ribosides. Receptor docking identified a large, mainly hydrophobic binding region and the vicinity of receptor-bound C2 groups was probed by homology modeling based on recent X-ray structure of an agonist-bound A_{2A}AR, with a predicted helical rearrangement requiring an agonist-specific outward displacement of TM2 resembling opsin. 2-(3,4-Difluorophenyl)ethynyl N^6-(3-chlorobenzyl) derivative MRS5698 was identified as a lead compound in a SAR study of receptor binding and in vivo. Chronic neuropathic pain is poorly managed and represents a huge unmet medical need. In models of chronic constriction injury (CCI) and chemotherapy-induced neuropathic pain (CIPN), we demonstrate the analgesic effects of the selective and orally available A_3AR agonist MRS5698. In mice and rats with CCI, systemic MRS5698 reversed mechano-allodynia in a dose-dependent manner. These effects were blocked by a selective A_3AR antagonist, lost in A_3AR-/- mice and had both peripheral and spinal sites of action. Our findings provide the pharmacological rationale towards the clinical development of A_3AR agonists for chronic pain.

Figure 1

MEDI 72

Discovery, optimization, and structure-activity relationship of a novel series of indole-3-carboxylamide $S1P_3$ selective antagonists

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Sphingosine 1-phosphate (S1P) mediates a variety of biological functions through five G-protein coupled receptors $S1P_1$-$S1P_5$. The development of FTY720 (fingolimod), a pan-antagonist at $S1P_1$, $S1P_3$, $S1P_4$, and $S1P_5$, as immunosuppressant has stimulated extensive interest in searching for S1P receptor modulators, particularly receptor sub-type selective agents for a broad range of therapeutic applications. Like $S1P_1$ and $S1P_2$, $S1P_3$ is widely expressed, although its genetic deletion has no obvious phenotypes. Studies have suggested that $S1P_3$ agonism is responsible for the transient bradycardia of FTY720 in humans and rodents. In addition, there is increasing evidence that implicates the role of $S1P_3$ in vascular permeability, inflammation, myofibroblast differentiation, and vasorelaxation. We herein describe the synthesis and structure-activity relationship of a novel series of indole-3-carboxylamides as $S1P_3$ selective antagonists with nanomolar potency, which are useful in further understanding the pharmacology of $S1P_3$ and its potential as a therapeutic target.

MEDI 73

Exploring a 2-naphthoic acid template for the design of $P2Y_{14}$ receptor antagonist molecular probes

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The $P2Y_{14}$ receptor ($P2Y_{14}R$), one of eight members of the P2Y family of purinergic G protein-coupled receptors (GPCRs), is activated by UDP and UDP-glucose. $P2Y_{14}R$ is broadly expressed in immune and epithelial cells and is involved in inflammation and hypoxic processes, thus making it an attractive target for further biological and pharmacological evaluation. Additionally, recent studies with $P2Y_{14}R$ knockout mice

have demonstrated its value as a potential drug target for diabetes treatment. This work in progress is directed toward the development of molecular probes for the $P2Y_{14}R$ structurally derived from a newly reported potent and highly selective antagonist (PPTN (4-((piperidin-4-yl)-phenyl)-(7-(4-(trifluoromethyl)-phenyl)-2-naphthoic acid). Our goal is introducing reliable $P2Y_{14}R$ affinity probes to provide methods for fluorescent characterization of the $P2Y_{14}R$ both in membranes and whole cells. In the absence of an atomic resolution structure of the $P2Y_{14}R$, we have constructed homology models based on recently reported GPCR X-ray structures and used them for virtual docking of the antagonists. The modeling results identify the piperidine ring as a likely site for chain derivatization with preservation of affinity, leading to a series of analogues that has been synthesized to probe the effect of distal changes on antagonist potency. The PPTN derivatives have been furnished with alkynyl or amino groups, suitable for attachment of fluorescent moieties by Click chemistry or amide coupling, respectively. All synthetic analogues are routinely evaluated functionally in CHO Chinese hamster ovary cells, stably expressing human $P2Y_{14}R$. More promising analogues can be studied in human neutrophils, where the $P2Y_{14}R$ promotes chemotaxis, or in LAD2 mast cells, where the receptor mediates release of inflammatory mediators. Results suggest that $P2Y_{14}R$ potency can be preserved by chain functionalization leading to selective fluorescent molecular probes for the receptor.

MEDI 74

Hit-to-lead optimization and kinase selectivity of imidazo[1,2-a]quinoxalin-4-amine derived JNK1 inhibitors

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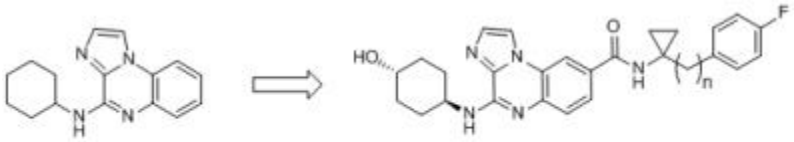
As the result of a rhJNK1 HTS, the imidazo[1,2-a]quinoxaline 1 was identified as a 1.6 μM rhJNK1 inhibitor. Optimization of this compound lead to AX13587 (rhJNK1 IC_{50} = 160 nM) which was co-crystallized with JNK1 to identify key molecular interactions. Kinase profiling against 125+ kinases revealed AX13587 was an inhibitor of JNK, MAST3, and MAST4 whereas its methylene homolog AX14373 (native JNK1 IC_{50} = 47 nM) was a highly specific JNK inhibitor.



1 **AX13587** (n = 0) **AX14373** (n = 1)

MEDI 75

PEG - 400 mediated microwave assisted synthesis of ethyl 2-substituted-4-(2-thienyl)thiazole-5-acetates and their pharmacological activities

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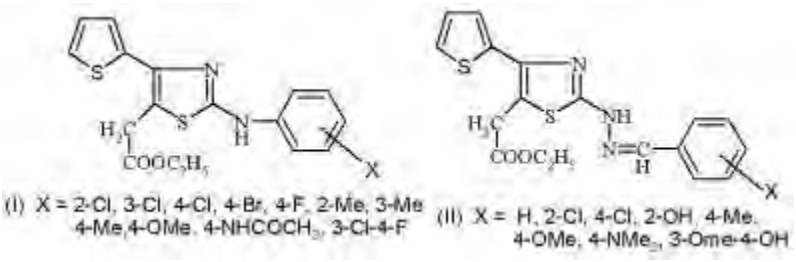
The compounds bearing thiazoles on verity of pharmacodynamic nuclei were found to possess potent anti-inflammatory, analgesic and antioxidant activities. Based on the activity data of a series of substituted thiazole compounds, Franklin et al. postulated that the minimum structural features necessary for the activity was 2-substituted amino thiazolyl moiety. Hence, a series of ethyl 2-arylamino-4-(2-thienyl)thiazole acetates (I) and ethyl 2-(arylhydrazino)-4-(2-thienyl)thiazole acetates(II) were synthesized by reacting substituted phenylthioureas (2-Cl, 3-Cl, 4-Cl, 4-Br, 4-F, 2-Me, 3-Me, 4-Me, 4-OMe, 4-NHCOMe, 3-Cl-4-F, 2,4-Cl_2) and araldehydethiosemicarbazones (H, 2-Cl, 4-Cl, 2-OH, 4-Me, 4-OMe, 4-NMe_2,3-Ome-4-OH) separately with ethyl 3-bromo-3-(2-theinoyl)propionate in PEG-400 under microwave irradiation.



The structure of newly synthesized thiazoles was confirmed by recording the IR, 1H NMR, 13C NMR and mass spectra of compounds. Considering the structural similarity to tolmetin zomepirac and fenclozic acid, the newly synthesized thiazoles were evaluated for anti-inflammatory activity by carraginan induced rat paw edema method and analgesic activity by acetic acid induced writhing method. The results of *invivo* tests showed that some of the investigated compounds exhibited good anti-inflammatory and analgesic activities similar to that of ibuprofen and aspirin respectively. A ethyl 2-(arylhydrazino)-4-(2-thienyl)thiazole acetates (II) showed good anti-oxidant activity (DDPH method) than compounds ethyl 2-arylamino-4-(2-thienyl)thiazole acetates (I).

MEDI 76

Synthesis of novel A_{2A} adenosine receptor selective 8-(*p*-substituted-phenyl/benzyl)xanthines with bronchospasmolytic activity

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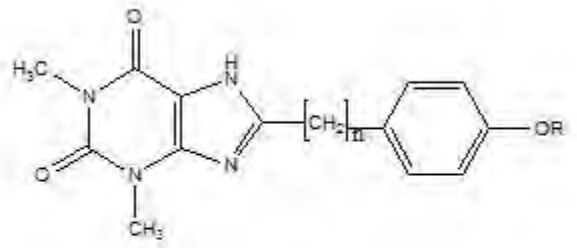
Pharmakologie und Toxikologie, Universität Würzburg, Germany, Wurzburg, Wurzburg, Germany

A new series of 8-(*p*-substituted-phenyl/benzyl)xanthines has been synthesized and evaluated *in vitro* for adenosine receptor binding affinity and *in vivo* for bronchospasmolytic effects. It was observed that the nature of substituent at *para*-position of 8-phenyl/benzyl group on the xanthine scaffold greatly affects the binding affinity and selectivity for various adenosine receptor subtypes. In order to examine specific structural features, a methylene spacer between the aromatic unit and the $C_8$of the xanthine nucleus was also introduced to study the resulting effects on biological activity.



In general, 8-phenylsubstituted xanthines displayed high binding affinity for all the adenosine receptor subtypes with maximum affinity and selectivity for the A_{2A} subtype. The imidazolyl substituted xanthine derivative was the most potent compound of the series with K_i = 42 nM for A_{2A} receptors. These compounds also produced potent bronchospasmolytic effects, however replacement of phenyl ring with benzyl moiety resulted in notable reduction in adenosine affinity and bronchospasmolytic effects. The effect of varying subtituents on the 8-phenyl ring on biological properties in case of both *in vitro* and *in vivo* assays is clearly visible. Suitable introduction of 8-phenyl/benzyl substituents on the xanthine scaffold results in potent and selective binding for A_{2A} adenosine receptors along with potent bronchospasmolytic effects. These dual action xanthine derivatives may act as useful candidates for asthma therapy.

MEDI 77

Identification of immunoproteasome selective inhibitors with structure-based molecular design approaches

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Immunoproteasome is an emerging biological target that constitutes a key element in antigen presentation, T cell and cytokine regulation as well as cellular homeostasis. Its inducible expression and different sensitivity as respect to standard proteasome renders it an appealing therapeutic target for central nervous system diseases, aging-related pathologies, tumours and autoimmune diseases. The three-dimensional structure of the immunoproteasome has been recently elucidated with X-ray crystallography, opening the way to use structure-based molecular design techniques aimed to identify novel and selective immunoproteasome inhibitors.

We report a virtual screening application on large database of commercial chemicals that allowed us to select new potential immunoproteasome selective inhibitors. These compounds have been than tested *in vitro* by short fluorogenic peptide assay on 20S standard proteasome and immunoproteasome purified from T2 and lymphoblastoid cell lines, respectively. We show that several compounds with non-peptide scaffold inhibit immunoproteasome, in particular its caspase-like activity, in the low micromolar range of concentration and have a considerable selectivity against the standard 20S proteasome.

MEDI 78

Identification of novel proteasome inhibitors from an enaminone library

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A library of structurally distinct enaminones was synthesized using sonication to accelerate the coupling of primary, secondary and tertiary thioamides with alpha halocarbonyls. Some of the precursors and resulting compounds shared structural similarities with amino acid functional groups, suggesting they might interfere with the ability of proteases to catalyze peptide bond cleavage. All 46 compounds were screened as potential inhibitors of the proteasome, which contains three distinct proteases (trypsin-like, chymotrypsin-like, or caspase-like) that degrade critical cellular proteins. Eight compounds of interest were identified in luciferase-based assays utilizing

a protease specific peptide. Two compounds turned out to be luciferase inhibitors. Of the remaining positives, three appeared to inhibit all three protease activities, while two only blocked caspase-like function. The final compound (Ethyl 2-bromo-3-oxo-3-phenylpropanoate) was specific for inhibition of the chymotrypsin-like protease activity and failed to inhibit purified chymotrypsin, suggesting it is specific for the proteasome. Compounds inhibiting the peptide-based proteasome assay were also able to inhibit proteasomal degradation of a model protein substrate in tissue culture cells. Because cytotoxicity often limits the efficacy of therapeutic agents, the library was evaluated for effects on mammalian cell viability. Of the six compounds of interest, five showed minimal cytotoxicity. Thus, the compounds identified in this study are good candidates for further development into novel proteasome inhibitors with potential therapeutic value and reduced cellular cytotoxicity.

MEDI 79

Discovery of novel spirocyclic diamine derivatives as highly potent sEH inhibitors for treating of chronic kidney diseases

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Soluble epoxide hydrolase (sEH) is an enzyme that catalyzes the conversion of epoxyeicosatrienoic acids (EETs) into dihydroxy eicosatrienoic acids (DHETs). EETs show physiologically beneficial effects such as vasodilatation, vasoprotection, and anti-inflammation. sEH inhibition exhibits the effects expected from an increase in EET level. We identified spirocyclic diamine derivatives as highly potent sEH inhibitors and orally active agents for treating chronic kidney diseases (CKD). Renal sEH expression is up-regulated in CKD rats. Oral administration of the derivatives at a dose of 30 mg/kg reduced serum creatinine levels in CKD rats. This result suggests that the derivatives are orally active drug candidates for the treatment of CKD. Along with the synthesis of these derivatives, their structure-activity relationships and pharmacological properties are described.

MEDI 80

Synthesis and structure-activity relationship of novel biarylcinnamamide derivatives as potent DGAT1 inhibitors

***Hiroyuki Ohno**, hir.oono@teijin.co.jp, Takaharu Tsutsumi, Katsushi Takahashi, Toshihiro Ishii, Kunio Minamizono, Masayuki Kurokawa, Hirotsugu Katoh, Akiko Suga. Teijin Institute for Bio-medical Research, Teijin Pharma Limited, 4-3-2 Asahigaoka, Hino, Tokyo 191-8512, Japan*

Diacylglycerol acyltransferase1 (DGAT1) catalyzes the final step in triglyceride biosynthesis and have been identified as potential therapeutic target for human obesity and type 2 diabetes. We identified a series of biarylcinnamamide derivatives as potent DGAT1 inhibitors. In this presentation, we will discuss synthetic method, the structure-activity relationship (SAR) of this series and our findings with DMPK profile and significant efficacy in obesity models.

MEDI 81

Highly potent aldose reductase inhibitors derived from botryllazine B

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Aldose reductase has been demonstrated to play important roles in the pathogenesis of diabetic complications. Aldose reductase inhibitors (ARIs), therefore, have attracted much attention of medicinal chemists as therapeutics for the diabetic complications. In the previous study (Saito *et al*. Tetrahedron, 2009, 3019–3026), we have evaluated *in vitro* inhibitory activities of botryllazine B, 6-(*p*-hydroxyphenyl)-2-(*p*-hydroxyphenyl)carbonylpyrazine, and its analogues of diverse substitution patterns against recombinant human aldose reductase (h-AR), and found that the replacing the *p*-hydroxy on the 6-phenyl group to *p*-amino group improved the inhibitory activity toward h-AR. This implies a kind of trends for developing higher ARIs, but the detailed structure-activity relationship study about the 6-position has not been performed. Thus, in the present work, we have synthesized botryllazine B analogues possessing various aryl groups at the 6-position and found that the derivatives modified with bicyclic heterocycles are potent ARIs exhibiting high inhibitory activities comparable to epalrestat, the sole drug available on the market for treating diabetic complications.

MEDI 82

Identification of adamantyl urea derivatives as novel and selective 11β-HSD1 inhibitors

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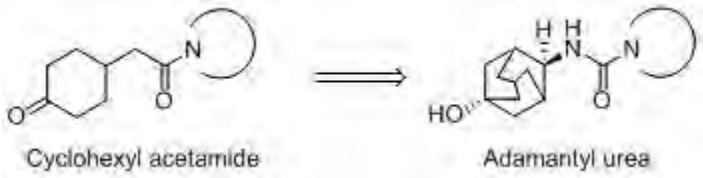
11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1) is a reductase converting inactive glucocorticoid hormone cortisone into active glucocorticoid hormone cortisol that regulates glucose and lipid homeostasis. Thus, 11β-HSD1 inhibitors have been explored as potential therapeutics for mainly type 2 diabetes, obesity and dyslipidemia by regulating the concentrations of cortisol. In our research project, we found a weak 11β-HSD1 inhibitory activity in a cyclohexyl acetamide compound. Exploration based on this compound led to a series of adamantyl urea derivatives possessing improved inhibitory potency. We will present the lead generation and further optimization toward our novel 11β-HSD1 inhibitors with nano molar inhibitory activities.



MEDI 83

Nitric oxide and nitroxyl releasing rosiglitazone derivatives: Design, synthesis and biological evaluation

Jingchao Liu[1,2]*, jcliuwj@email.arizona.edu, Lauren Elizabeth McClure*[2]*, Sixun Peng*[1]*, Katrina M Miranda*[2]*, Yihua Zhang*[1]*. (1) Center of Drug Discovery, China Pharmaceutical University, Nanjing, Jiangsu 210009, China (2) Department of Chemistry and Biochemistry, University of Arizona, Tucson, Arizona 85721, United States*

Diabetes typically leads to the accumulation of advanced glycosylation end products (AGEs), which are capable of increasing the probability of vascular endothelial dysfunction. This is a crucial factor in microvascular and macrovascular pathology resulting in diabetic complications. Among the drugs for treating type 2 diabetes mellitus (T2DM), rosiglitazone (trade name Avandia®, GlaxoSmithKline) is used clinically to treat insulin resistance. However, RECORD trial shows that long-term use of this drug will increase the incidance of cardiovascular side effects, which has led to restriction order issued by the FDA. Given the beneficial effects of nitric oxide (NO) on endothelial function and of nitroxyl (HNO) as an anti-hypertensive and contractile agent, we are motivated to synthesize hybrid compounds of rosiglitazone bearing nitrate- and diazeniumdiolate-based NO or HNO donating moieties. A goal of this project is to determine whether these adducts can synergistically exert hypoglycemic and cardioprotective activity. The synthetic methodology and biological evaluation will be reported.

MEDI 84

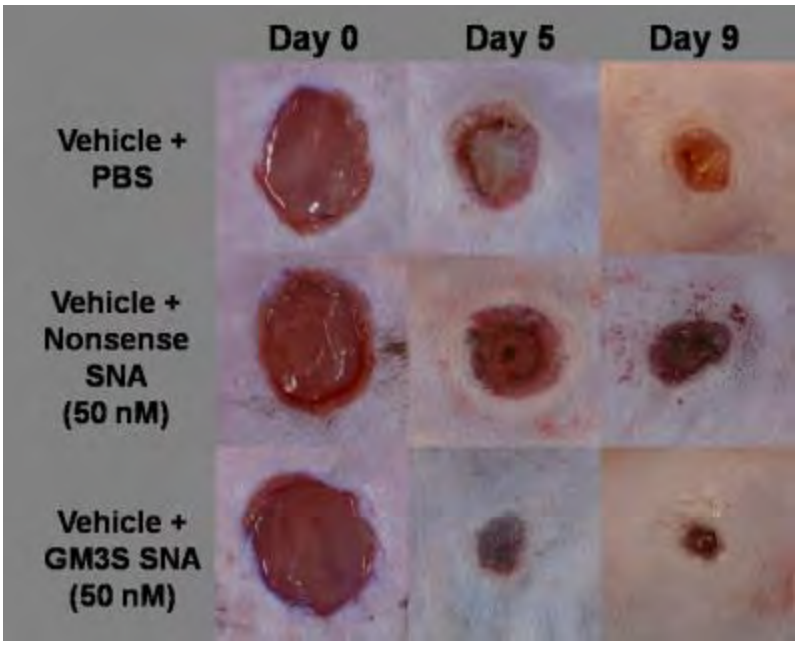
Topically applied spherical nucleic acids to increase the rate of wound healing in subjects with type 2 diabetes

Pratik S Randeria[1,2]*, prander2@u.northwestern.edu, Mark Seeger*[3]*, Desmond Shipp*[3]*, Xiaoqi Wang*[3]*, Amy Paller*[3]*, Chad A Mirkin*[2]*. (1) Department of Biomedical Engineering, Northwestern University, Evanston, IL 60201, United States (2) Department of Chemistry, Northwestern University, Evanston, IL 60201, United States (3) Feinberg School of Medicine, Northwestern University, Evanston, IL 60201, United States*

Normal wound healing is a process that requires keratinocyte (KC) proliferation and migration. In chronic subcutaneous wounds, KC function is impaired, largely due to insulin resistance. Studies show that a glycosylated sphingolipid, GM3, is increased in the skin of type 2 diabetic subjects, suggesting it as a critical mediator of insulin resistance.

Our lab has recently established spherical nucleic acids (SNAs), gold cores surrounded by a dense shell of highly oriented, covalently immobilized siRNA as a powerful tool for topically delivered gene suppression. Significantly, these structures can be delivered topically and do not require barrier disruption or transfection agents, exhibit remarkable stability towards nuclease degradation and decreased immunogenicity, and, to date, have caused no toxicity in cultured cells, mouse skin, or human skin equivalent models at concentrations required for effective gene knockdown.

Herein, we designed SNAs to suppress expression of GM3S in immortalized mouse KCs and in C57BL/6 mice. Upon treatment of KCs with the SNAs and appropriate controls, GM3S mRNA and protein levels were reduced by over 80% ($p<0.005$). SNA treated cells show significantly increased migration ($p<0.001$) and exhibit a four-fold increased rate of proliferation ($p<0.005$). To test *in vivo*, 6mm circular wounds were made on diabetic C57BL/6 mice. Preliminary animal results indicate significantly faster wound closure and reduced GM3S expression in harvested tissues with SNA in diabetic mice compared to untreated mice or mice treated with vehicle alone.



MEDI 85

Novel hydrogen-sulfide selective probes for the study of hydrogen sulfide-producing enzymes

Megan K Thorson[1]*, megan.thorson@gmail.com, Tomas Majtan*[2]*, Jan P Kraus*[2]*, Amy M Barrios*[1]*. (1) Department of Medicinal Chemistry, University of Utah, Salt Lake City, Utah 84112, United States (2) Department of Pediatrics, University of Colorado-Denver, Denver, Colorado 80202, United States*

While hydrogen sulfide (H_2S) is most often known for its distinctive odor and toxicity, this gaseous small molecule also plays important roles in human biology. Though the exact mechanisms are still being investigated, H_2S has a wide range of effects depending on its concentration, location, and target and appears to be especially important in the inflammatory, nervous, and cardiovascular systems. Hydrogen sulfide is produced *in vivo* through many different pathways with two predominant H_2S- producing enzymes being cystathionine β-synthase (CBS) and cystathionine γ-lyase (CGL). In particular CBS plays a key role in homocysteine homeostasis, catalyzing the first step in the conversion of toxic homocysteine to cysteine. Aberrant CBS activity has been implicated in a variety of diseases, including Down's Syndrome, hypertension, preeclampsia, and homocystinuria. Our overall goal in this project has been to discover new inhibitors and activators of these H_2S-producing enzymes that can be used both to elucidate the roles they play in human health and disease and also as potential therapeutics for those with a CBS-related disorder. To this end, we have developed fluorogenic H_2S selective probes and used these probes to screen for potent and selective inhibitors of CBS and CGL.

MEDI 86

Design, synthesis and structure–activity relationships of 5-alkylaminoquinolines: Potent, orally active corticotropin-releasing factor-1 receptor antagonists

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Corticotropin-releasing factor (CRF) is a 41-amino acid peptide that acts as the prime regulator of the hypothalamic-pituitary-adrenal (HPA) axis. CRF is a major modulator of the body's overall response to stressors and there is evidence supporting the hypothesis that over production of CRF may underlie the pathology of stress-related disorders, such as depression and anxiety. CRF_1 receptor is the most abundant subtype found in the pituitary and is involved in the regulation of adrenocorticotropic hormone (ACTH), a key mediator of the body's response to stress. Therefore a CRF_1 receptor antagonist is hypothesized to be a valuable target for the treatment of stress-related disorders.

To date, several CRF_1 receptor antagonists have been reported and prototypical antagonists have common structural features. Here we report the generation of 5-alkylaminoquinolines as novel CRF_1 receptor antagonists that have distinctive structural features compared to the currently known antagonists.

MEDI 87

Discovery of dihydropyrrolopyrazinone derivatives as metabotropic glutamate receptor 2 (mGluR2) positive allosteric modulators (PAMs)

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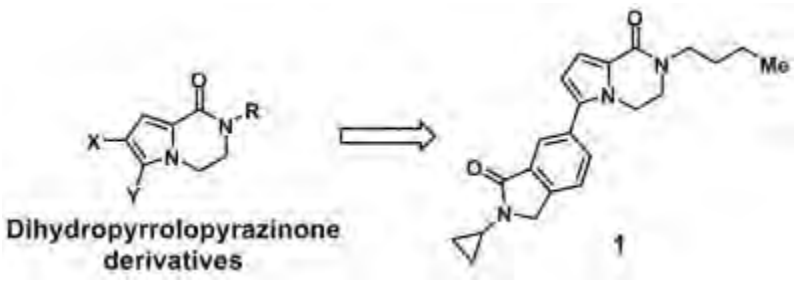
Glutamate is the major excitatory neurotransmitter in the central nervous system (CNS), and an imbalance in glutamatergic neurotransmission is believed to be linked to various neurological and psychiatric diseases. Metabotropic glutamate receptor 2 (mGluR2) belongs to the group II mGlu receptors that are widely expressed in the forebrain and localized presynaptically in the neurons, serving inhibitory regulation of related neurotransmission. Compounds that activate the group II mGlu receptors may offer anxiolytic and/or antipsychotic effects. Especially, it is expected that, unlike orthosteric

agonists, positive allosteric modulators (PAMs) would offer higher selectivity to mGluR2 in the mGluR family as well as the potential to avoid receptor desensitization. In addition, structural diversity and physicochemical properties suitable for passive brain penetration would likely be attained for PAMs, which is generally not easy for orthosteric agonists of mGluRs. While several structural classes of mGluR2 PAMs have been reported with this background, we identified dihydropyrrolopyrazinone derivatives as new chemotypes of mGluR2 PAMs with highly potent activity and wide substituent admissibility. The further lead optimization study led to discovery of novel mGluR2 PAM (**1**), which showed oral antipsychotic effects in rats. Herein, we will discuss the design, synthesis, structure-activity relationship (SAR), pharmacokinetic (PK) properties and biological activity of our novel mGluR2 PAMs.



MEDI 88

Design, synthesis, and pharmacological evaluation of novel positive allosteric modulators (PAMs) of 5-HT_{2C} receptor

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Allosteric modulators of the serotonin (5-HT) 5-HT_{2C} receptor (5-HT_{2C}R) present a unique drug design strategy to augment the response to endogenous 5-HT in a site- and event-specific manner with great potential as novel central nervous system probes and therapeutics. To date, there is only one reported 5-HT_{2C}R allosteric modulator, PNU-69176E. By using the scaffold of this lead compound and employing homology modeling via molecular docking techniques, a series of new small molecules such as CYD-1-79, CYD-6-10-2, and CYD-6-16-2 have been rationally designed and synthesized. Their pharmacological activities have been evaluated using a battery of *in vitro* functional and radioligand binding assays as well as *in vivo* behavioral studies to

assess allosteric modulation of the 5-HT_{2C}R. CYD-1-79 with a simplified polar head was found to enhance 5-HT_{2C}R-mediated Ca_i^{++} release and $ERK_{1/2}$ activation induced by the endogenous ligand 5-HT or the selective 5-HT_{2C}R agonist WAY 163909. It also exhibited a favorable overall PK profile such as oral bioavailability (F = 39.1%) and half-life ($t_{1/2}$ = ~6 hr). More importantly, the *in vivo* efficacy of the new 5-HT_{2C}R positive allosteric modulator CYD-1-79 was well demonstrated in our preliminary rodent behavior studies (e.g., spontaneous locomotor activity, self-grooming, impulsive action). Taken together, our target-based drug design and development efforts on 5-HT_{2C}R allosteric modulators open new avenues in probing the 5-HT_{2C}R function and discovering novel pharmacotherapeutics for CNS disorders including addictions and impulse control disorders.

MEDI 89

Synthesis of carbon-11-labeled 1,2,4-triazolo[4,3,*a*]quinoxalin-1-one derivatives for imaging of A_3 adenosine receptor

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Adenosine is a ubiquitous neuromodulator, and adenosine receptor A_3 subtype belongs to the G-protein-coupled receptor superfamily. A_3 receptor is involved in many important physiological effects and associated with a wide variety of brain, heart and cancer diseases. A_3 receptor has become a promising therapeutic target, and a series of 1,2,4-triazolo[4,3,*a*]quinoxalin-1-one derivatives has been recently developed as new potent and selective A_3 antagonists by Colotta et al. A_3 receptor is also an attractive imaging target. Here we report the synthesis of carbon-11-labeled 1,2,4-triazolo[4,3,*a*]quinoxalin-1-one derivatives for use in biomedical imaging technique positron emission tomography (PET) to image A_3 adenosine receptor. The reference standards 2-(4-methoxyphenyl)-[1,2,4]triazolo[4,3-*a*]quinoxaline-1,4(2*H*,5*H*)-dione, 2-(4-methoxyphenyl)-6-nitro-[1,2,4]triazolo[4,3-*a*]quinoxaline-1,4(2*H*,5*H*)-dione, 4-amino-2-(4-methoxyphenyl)-[1,2,4]triazolo[4,3-*a*]quinoxalin-1(2*H*)-one, 4-amino-2-(4-methoxyphenyl)-6-nitro-[1,2,4]triazolo[4,3-*a*]quinoxalin-1(2*H*)-one, *N*-(2-(4-methoxyphenyl)-1-oxo-1,2-dihydro-[1,2,4]triazolo[4,3-*a*]quinoxalin-4-yl)benzamide, *N*-(2-(4-methoxyphenyl)-1-oxo-1,2-dihydro-[1,2,4]triazolo[4,3-*a*]quinoxalin-4-yl)-2,2-diphenylacetamide, and *N*-(2-(4-methoxyphenyl)-6-nitro-1-oxo-1,2-dihydro-[1,2,4]triazolo[4,3-*a*]quinoxalin-4-yl)-2,2-diphenylacetamide were synthesized from 4-methoxyaniline, ethyl 2-chloro-acetoacetate and substituted benzene-1,2-diamines with 3, 5, and 6 steps in 67-75%, 38-47% and 26-35% overall chemical yield, respectively. The precursors 2-(4-hydroxyphenyl)-[1,2,4]triazolo[4,3-*a*]quinoxaline-1,4(2*H*,5*H*)-dione, 2-(4-hydroxyphenyl)-6-nitro-[1,2,4]triazolo[4,3-*a*]quinoxaline-1,4(2*H*,5*H*)-dione, 4-amino-2-(4-hydroxyphenyl)-[1,2,4]triazolo[4,3-*a*]quinoxalin-1(2*H*)-one, 4-amino-2-(4-hydroxyphenyl)-6-nitro-[1,2,4]triazolo[4,3-*a*]quinoxalin-1(2*H*)-one, *N*-(2-(4-hydroxyphenyl)-1-oxo-1,2-dihydro-[1,2,4]triazolo[4,3-*a*]quinoxalin-4-yl)benzamide, *N*-(2-(4-hydroxyphenyl)-1-oxo-1,2-dihydro-[1,2,4]triazolo[4,3-*a*]quinoxalin-4-yl)-2,2-

diphenylacetamide, and *N*-(2-(4-hydroxyphenyl)-6-nitro-1-oxo-1,2-dihydro-[1,2,4]triazolo[4,3-*a*]quinoxalin-4-yl)-2,2-diphenylacetamide were synthesized from 4-methoxyaniline, ethyl 2-chloro-acetoacetate and substituted benzene-1,2-diamines with 4, 6, and 7 steps in 54-63%, 30-40% and 22-32% overall chemical yield, respectively. The target tracers 2-(4-[^{11}C]methoxyphenyl)-[1,2,4]triazolo[4,3-*a*]quinoxaline-1,4(2*H*,5*H*)-dione, 2-(4-[^{11}C]methoxyphenyl)-6-nitro-[1,2,4]triazolo[4,3-*a*]quinoxaline-1,4(2*H*,5*H*)-dione, 4-amino-2-(4-[^{11}C]methoxyphenyl)-[1,2,4]triazolo[4,3-*a*]quinoxalin-1(2*H*)-one, 4-amino-2-(4-[^{11}C]methoxyphenyl)-6-nitro-[1,2,4]triazolo[4,3-*a*]quinoxalin-1(2*H*)-one, *N*-(2-(4-[^{11}C]methoxyphenyl)-1-oxo-1,2-dihydro-[1,2,4]triazolo[4,3-*a*]quinoxalin-4-yl)benzamide, *N*-(2-(4-[^{11}C]methoxyphenyl)-1-oxo-1,2-dihydro-[1,2,4]triazolo[4,3-*a*]quinoxalin-4-yl)-2,2-diphenylacetamide, and *N*-(2-(4-[^{11}C]methoxyphenyl)-6-nitro-1-oxo-1,2-dihydro-[1,2,4]triazolo[4,3-*a*]quinoxalin-4-yl)-2,2-diphenylacetamide were synthesized from their corresponding precursors with [^{11}C]CH_3OTf through *O*-[^{11}C]methylation and isolated by simplified solid-phase extraction (SPE, C18 Sep-Pak) in 30-60% radiochemical yields based on [^{11}C]CO_2 and decay corrected to end of bombardment (EOB), with 185-370 GBq/μmol specific activity at end of synthesis (EOS).

MEDI 90

Heterocycloalkyl-spiropiperidine analogs as histamine-3 receptor antagonists

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Histamine H_3 receptor antagonists have potential utility in addressing a variety of CNS disorders, including deficits in wakefulness, attention-deficit hyperactivity disorder (ADHD), Alzheimer's disease (AD), mild cognitive impairment, and schizophrenia. Modification of the 6-position on both the 1'-cyclobutylspiro[4H-1,3-benzodioxine-2,4'-piperidine] and 1'-cyclobutylspiro-[chromane-2,4'-piperidine] cores with various heterocycloalkyl groups produced 4-(1'-cyclobutylspiro[4H-1,3-benzodioxine-2,4'-piperidine]-6-yl)thiane-1,1-dioxide as a lead compound. The synthesis and structure-activity relationship of this novel series of heterocycloalkyl-spiropiperidine H_3R antagonists will be presented.

MEDI 91

Novel 6β-acylaminomorphinans with analgesic activity

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Opioid agonists comprise the backbone of analgesic therapy. There is an ever increasing need for the synthesis and pharmacological profiling of novel morphine-like substances that offer less side effects and superior analgesia over traditional opiates. Chemical transformations that alter the opioid receptor selectivity and efficacy may lead to useful drugs.

Aminomorphinans are a relatively young class of opioid drugs among which substances of high in vitro efficacy and favorable in vivo action are found. We report the synthesis and pharmacological evaluation of novel 6β-acylaminomorphinans. 6β-Morphinamine and 6β-codeinamine were stereoselectively synthesized by Mitsunobu reaction. The aminomorphinans were subsequently acylated with diversely substituted cinnamic acids. In vitro binding studies on cinnamoyl morphinamines showed moderate affinity for all opiate receptors with some selectivity for mu opioid receptors, while cinnamoyl codeinamines only showed affinity for mu opioid receptors. In vivo analgesia studies showed significant analgesic activity of 6β-cinnamoylmorphinamine mediated by mu and delta receptors. The lead compound was found to be roughly equipotent to morphine (ED_{50} 3.13 ± 1.09 mg/kg) but devoid of the dangerous side-effect respiratory depression, a major issue associated with traditional opioid therapy.

This work was supported by research grant from the National Institute on Drug Abuse (DA034106-01) to SM.

Results have been published in European Journal of Medicinal Chemistry (in press).

MEDI 92

Synthesis of bitopic muscarinic antagonists and functionally selective agonists for pharmaceutical applications

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The long-term research objective is to develop selective M_1/M_5 muscarinic agonists and M_2 antagonists for Alzheimer's, M_1/M_4 agonists for Schizophrenia, and M_1/M_4 antagonists for Parkinson's disease. In addition, selective M_2 and M_3 muscarinic antagonists are targets for chronic obstructive pulmonary disease (COPD), asthma and overactive bladder syndrome. We set to test the hypothesis that structural analogs of a newly synthesized bitopic antagonist and two potent and functional selective partial agonists would have improved receptor binding affinity and selectivity. The proposed hypothesis is partly based on a Shulman's model of drug-receptor interaction combined with chemical motifs known to achieve muscarinic receptor selectivity. This approach is quite innovative and has thus led to the discovery of the three muscarinic ligands mentioned above. The bitopic antagonist was shown to significantly slow down both the

dissociation of N-methyl scopolamine (NMS) and acetyl choline in kinetics experiments while other two partial muscarinic agonists were shown to be potent, efficacious and functionally selective on muscarinic cell lines. The project's hypothesis will be addressed in experiments of three specific aims. Specific aim 1 proposes to synthesize structural analogs of the bitopic antagonist by repositioning and varying the alkoxy group of the phenyl hydrophobic linker whereas the nitrogenous head group will be substituted with other five and six-membered heterocyclic moieties. Specific aim 2 proposes to synthesize structural analogs of the two partial agonists by repositioning and varying the substituent bonded to the thiophene ring while.the nitrogenous head group will be substituted with other five and six-membered heterocyclic moieties. And finally, specific aim 3 proposes to assay all synthesized compounds on cell lines stably transfected with the genes of human variants of muscarinic receptors. We anticipate that this study will lead to the discovery of both agonists and antagonists with much improved binding affinity and selectivity profiles.

MEDI 93

Discovery of ASP5736, a novel 5-HT_{5A} receptor antagonist as an antipsychotic drug

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The 5-HT_{5A} receptor is a member of the serotonin receptor subfamily. The 5-HT_{5A} receptor has been cloned from the human and rodents, and express predominantly in neural tissues such as hippocampus, thalamus, amygdala and cerebral cortex. 5-HT_{5A} KO mice were reported to show increased exploratory activity in response to novel environments. These observations suggested that the 5-HT_{5A} receptor is involved in regulation of cognition, exploratory behavior, anxiety, and circadian rhythm. Furthermore, schizophrenic populations were reported to exhibit an abnormality in 5-HT_{5A} coding sequences. Therefore, the 5-HT_{5A} receptor is considered as a potential clinical target for new treatment of schizophrenia.

As part of our research program directed at the development of new 5-HT_{5A} receptor antagonists, we have investigated a novel class of acylguanidine derivatives with heteroaromatic ring, and identified ASP5736 as a clinical candidate. ASP5736 is a potent and selective 5-HT_{5A} receptor antagonist with a good PK profile and no specific toxicity. Furthermore, ASP5736 (0.01–0.1 mg/kg, p.o.) ameliorated the PCP-induced deficit in prepulse inhibition in rats. In this presentation, we will show the synthesis, SAR of this novel series of 5-HT_{5A} receptor antagonists, and pharmacological profiles of ASP5736.

MEDI 94

Development of novel natural product hybrids as neuroprotectants

***Jeremy E Chojnacki**, Chojnackij@vcu.edu, Ahmed Salem, Kai Liu, Emily Wade, Shijun Zhang. Department of Medicinal Chemistry, Virginia Commonwealth University, Richmond, Virginia 23298, United States*

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by beta-amyloid (Aβ) aggregation/oligomerization,biometal dyshomeostasis, oxidative stress, and neuroinflammation. The multifaceted nature of AD may indicate the therapeutic potential of multifunctional ligands as effective AD-modifying agents. However, the rational design of lead compounds with polypharmacology is challenging. Curcumin, a natural product of the turmeric spice, has been shown to have anti-oxidative, anti-inflammatory, and neuroprotective activities. Melatonin, an important biomolecule in the control of circadian rhythm in mammals, has also recently been reported to exhibit similar protective properties, which could have possible beneficial effects in neurodegenerative disorders, including Parkinson's and Alzheimer's. Based on the structural features of these two natural products, we designed and developed chemical hybrids to investigate their potential neuroprotective effects, in particular whether they would retain their polypharmacological properties or display improved, synergistic effects. To this end a series of novel curcumin-melatonin hybrids were synthesized. Biological characterization of this series reveals two hybrid compounds AM-24 and AM-42 that exhibit marked neuroprotection of MC65 neuroblastoma cells with an EC_{50} of 28nM and 23nM, respectively. Further mechanistic studies demonstrated that they exhibited potent antioxidant effects in MC65 cells and suppressed the production of Aβ oligomers, as well. These results may suggest that the hybrid compounds exert their neuroprotective effects on MC65 cells via the blockage of Aβ oligomers, thus leading to the suppression of oxidative stress. In conclusion, our results suggest that the hybrid strategy is a viable approach to design novel compounds from known lead structures with polypharmacological activity and that these compounds are good candidates for optimization in the development of potential neuroprotective agents for Alzheimer's disease.

MEDI 95

Discovery of the first potent and orally available agonist of the orphan G protein-coupled receptor 52

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G protein-coupled receptor 52 (GPR52) is an orphan Gs-coupled G protein-coupled receptor. GPR52 inhibits dopamine D_2 receptor signaling and activates dopamine D_1/*N*-methyl-*D*-aspartate receptors via intracellular cAMP accumulation. GPR52 agonists are expected to have potential as a novel class of antipsychotics.

We successfully discovered 3-[2-(3-chloro-5-fluorobenzyl)-1-benzothiophen-7-yl]-*N*-(2-methoxyethyl)benzamide as the first GPR52 agonist. This compound showed potent GPR52 agonistic activity (EC_{50}= 35 nM) and good pharmacokinetic properties. Furthermore, the compound significantly suppressed methanphetamine-induced hyperactivity in mice after oral administration of 3 mg/kg and was devoid of disturbance of motor function in mice.

Design and synthesis of the first GPR52 agonist as well as biological evaluation results will be presented.

MEDI 96

Design and synthesis of 2-benzyl-1,3-thiazole derivatives as a novel class of G protein-coupled receptor 52 (GPR52) agonists

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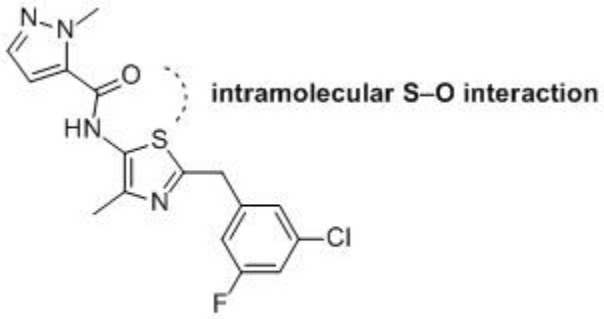
G protein-coupled receptor 52 (GPR52) is an orphan Gs-coupled G protein-coupled receptor. In this conference, our group reports the first GPR52 agonists, which is expected to be a novel class of antipsychotics. To acquire diverse lead compounds for drug discovery of GPR52 agonists, we continued to explore novel GPR52 agonists with different scaffolds.

As a result, we successfully discovered *N*-(2-(3-chloro-5-fluorobenzyl)-4-methyl-1,3-thiazol-5-yl)-1-methyl-*1H*-pyrazole-5-carboxamide (compound **1**) as a novel GPR52 agonist. This compound showed potent GPR52 agonistic activity (EC_{50}= 28 nM) and good pharmacokinetic profiles.

Design and synthesis of the novel GPR52 agonists will be presented. We would also like to discuss a role of an intramolecular S–O interaction for enhancement of GPR52 agonistic activity.



compound 1 (EC_{50} = 28 nM)

MEDI 97

Investigating sigma 2 receptor ligands for the targeted therapeutics of Alzheimer's disease by utilizing a novel *C. elegans* model of AD

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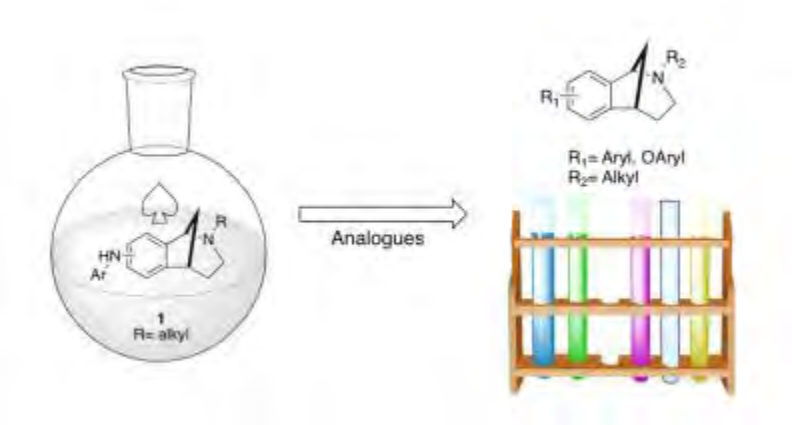
Alzheimer's disease (AD) is a neurodegenerative disease that currently affects over 5 million Americans. This common form of dementia does not have a cure, and the limited FDA approved drugs for AD merely treat the symptoms. Sigma receptors, a distinct class of chaperone proteins, have been found to be involved in many essential cellular processes, such as calcium signaling via sphinolipid products. The sigma 2 receptor (σ2R) subtype is a netrin receptor chaperone that could aid in regulation of the amyloid precursor protein (APP). By suppressing σ2R, we hope to reduce expression of competing netrin receptors in order to develop accessibility of netrin to improve APP regulation. In our current studies utilizing a recently developed *C. elegans* model of AD that expresses a single extra gene of APP, a novel heterocyclic compound, **1,** reduced neurodegeneration to non-AD control levels. Knock-out studies coupled with receptor binding assays has indicated this novel compound acts via the σ2R pathway. SAR studies will be conducted on hit compound **1** to generate analogues with enhanced potency and selectivity, improved physiochemical properties, and decreased lipophilicity. The overarching aim of this work is to validate σ2R as a biological target for AD that could be modulated in order to deliver an effective and metabolically sound pre-clinical candidate for the treatment of AD.



MEDI 98

Design, synthesis, and biological evaluation of alkaloids as potential antituberculosis drugs

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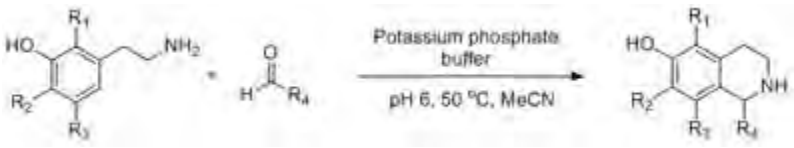
Tuberculosis (TB) is an infectious disease caused by the *Mycobacterium tuberculosis* pathogen. The increasing prevalence of multi-drug resistant strains of *M. tuberculosis*, means there is an urgent need to develop new anti-TB drugs with novel modes of action.

Aporphine alkaloid natural products, such as (*S*)-methoxynordomesticine, have demonstrated a specific anti-mycobacterial effect, as well as *M. tuberculosis* MurE inhibitory activity.[1] These aporphines contain a common tetrahydroisoquinoline skeleton, providing a unique template for the development of new anti-TB drugs.

Recently we have developed biomimetic reaction conditions for the Pictet-Spengler condensation of aldehydes and amines into tetahydroisoquinolines.[2] The reaction is mediated by phosphate and proceeds under mild reaction conditions, suitable for a variety of less stable aldehyde and amine substrates. Several tetrahydroisoquinolines synthesised using this method have been found active against *M. tuberculosis* $H_{37}Rv$.

We have been investigating the scope of the biomimetic Pictet-Spengler reaction, to access novel alkaloid structures, and identify new leads for mycobacterial growth inhibitors. Studies into asymmetric versions of the reaction using chiral phosphates, and extending the reaction for construction of larger ring sizes are also on-going. A number of novel tetrahydroisoquinoline derivatives have been synthesised and initial biological evaluation has allowed us to design a series of compounds for SAR studies, focussing on modification of substituents on the aromatic ring, and at the C-1 position.



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MEDI 99

Design, synthesis and target validation studies of aminothiazoles (ATs) as potent inhibitors of *Mycobacterium tuberculosis*

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Identification of the mode of action and cellular targets of known bactericidal agents is crucial for developing new drugs against *Mycobacterium tuberculosis* (*Mtb*). We have synthesized and evaluated a series of 2-aminothiazoles (ATs) for structure-activity relationship (SAR) detemination and identified a number of analogs that possess excellent activity against *Mtb* with rapid kill kinetics. However, the mode of action of this series is unknown. Furthermore, analogs of this series demonstrated poor pharmacokinetic (PK) properties. Thus, identifying the biological target for this series is vital for the strucutre-based design of novel AT compounds with enhanced PK properties. To this end, we have designed and synthesized AT-based molecular probes to elucidate the biological targets and cellular processes linked to bactericidal activity and mode of action. The synthesis and biological evaluation of AT analogs, as well as initial target identification studies of AT-based probes will be presented.

MEDI 100

Computational and structural biology approaches to the identification of potential binding sites of novel benzimidazole inhibitors with *Mtb*-FtsZ for antitubercular drug discovery

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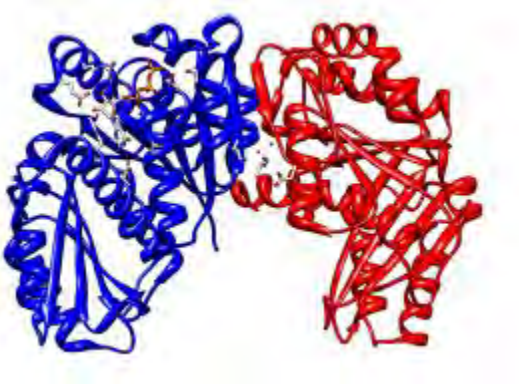
Filamentous temperature-sensitive protein Z (FtsZ), an essential bacterial cell-division protein, is a promising target for the development of new antitubercular agents. Series of novel trisubstituted benzimidazoles targeting FtsZ have been designed, synthesized and evaluated. Many of these novel FtsZ inhibitors exhibit excellent activities against drug-sensitive and drug-resistant tuberculosis strains. However, currently the binding site(s) of FtsZ with these highly potent antitubercular agents is still unclear. Thus, computational and structural biology approaches have been employed to investigate binding sites of these novel benzimidazole inhibitors with *Mtb*-FtsZ for antitubercular drug discovery and possible binding sites have been identified. The results on computational docking analysis, protein crystallography and photoaffinity labeling analysis will be discussed.



MEDI 101

Discovery and biological evaluation of Lusutrombopag (S-888711) as a novel nonpeptide drug candidate for thrombocytopenia

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As a drug candidate of thrombocytopenia, Lusutrombopag (S-888711) is in Phase III clinical trial stage right now. It is been proven that Lusutrombopag (S-888711) is excellent property in safety and efficacy by clinical trials. In this meeting, we will present in detail about the history of drug discovery of Lusutrombopag.Because Lusutrombopag (S-888711) acts specifically to human TPO receptor, we prepared TPOR-Ki/Shi mice expressing a mouse-human chimeric TPOR for evaluating the efficacy. This TPOR-Ki/Shi mice worked very well as an evaluation model of drug efficacy, so we were able to select Lusutrombopag from many candidate compounds. In this meeting, we will present the results of the efficacy in TPOR-Ki/Shi mice of Lusutrombopag and the similar drug (Eltrombopag).

MEDI 102

Design, synthesis, and biological evaluation of new triclosan analogs as potent inhibitors of the InhA enzyme in drug-sensitive and drug-resistant strains of *Mycobacterium tuberculosis*

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A series of new diaryl ethers was synthesized and evaluated for biological activity against the enoyl-acyl carrier protein reductase InhA in *Mycobacterium tuberculosis* (*Mtb*). Designed modifications to positions 5 and 4' of triclosan (TRC), a well-studied inhibitor of the mycobacterial InhA, afforded twenty-seven analogs. Biological testing revealed seven compounds possessing an improved potency against drug-susceptible and drug-resistant *Mtb* strains in comparison to the lead compound, TRC. Derivative **3** had an MIC value of 0.6 μg/mL (1.5 *μ*M) against wild type *Mtb* and it was identified to be the most active compound in this series. Additionally, enzymatic studies showed that compounds **3** and **14** (Figure 1) effectively inhibited the biosynthesis of mycolic acids. Furthermore, both analogs (**3** , **14**) had a substantial increase in the MIC value against $mc^2$4914, an *Mtb* strain overexpressing *inhA*, which further supports the notion that InhA is their likely molecular target. Molecular modeling of the most active TRC derivatives within the active site of InhA provided additional insights into the observed structure-activity relationships of the newly prepared diaryl ethers.

Figure 1. Modification of triclosan scaffold in search of improved inhibitors for mycobacterial InhA

MEDI 103

Discovery of a novel glucagon receptor antagonist for the treatment of type II diabetes

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Studies of type 2 diabetes (T2DM) have demonstrated a causal role for glucagon in promoting excessive hepatic glucose production (HGP), which is the predominant cause of fasting hyperglycemia and a major contributor to the postprandial hyperglycemia in T2DM. Glucagon receptor antagonists (GRAs) therefore have the potential to reduce HGP and be effective as anti-diabetic agents. Clinical studies of MK-0893 and LY-2409021 have demonstrated the superior efficacy of glucagon antagonists in lowering blood glucose and reducing hemoglobin A1c. In our effort to search for a backup to MK-0893, we have been interested in exploring structurally diverse b-alanine series, and a novel series of GRAs bearing a quinoline moiety was identified. The synthesis involved nucleophilic addition of a quinoline anion to the chiral ketone intermediate, followed by deoxgenation of the subsequent tertiary alcohol. Several synthetic strategies and methods, which were explored to overcome the challenges posted by the electron-

deficient nature of the quinoline ring, and the structure activity relationship that led to the discovery of a preclinical candidate, will be discussed.

MEDI 104

Anticancer activity of phenyl acrylamide based compounds

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Natural products from plants such as the *Gaillardia pulchella, Gochnatra rusbyana, Netoptergium incisum* and fruits of *Linum usitatissimum* have been reported to possess antimicrobial activity and antitumor activity against Sarcoma 180. These plants are known to contain derivatives of phenyl acrylamide and phenylacrylamide urea. The importance of phenyl acrylamides in therapeutic activity lead us to synthesize a library of molecules based on this pharmacophore and evaluate for anticancer activity. The compounds were screened against 60 human cancer cell lines at 10 µM concentration. Also, we examined the growth inhibitory effects of a representative (NSC-772451) from the most effective compounds, on breast cancer cells and elucidated the underlying molecular mechanisms. We first examined the effect of NSC772451 on clonogenic potential and anchorage-independent growth of breast cancer cells. NSC-772451 treatment resulted in dose-dependent and statistically significant inhibition of clonogenicity and soft-agar colony formation in MCF7, MDA-MB-231 and T47D breast cancer cells. NSC-772451 treatment resulted in elevated PARP cleavage. Analysis of upstream pathways showed that NSC-772451 activates ERK phosphorylation in breast cancer cells. Our recent studies have shown that elevated ERK signaling can mediate growth inhibition of breast cancer cells via up regulation of death receptor 5. We found that NSC-772451 treatment increases the expression of death-receptor 5. These data indicate a role of NSC-772451 as novel pro-apoptotic receptor agonists (PARAs) capable of inducing DR5 expression.

MEDI 105

Spherical nucleic acid nanoconjugates for identifying expression levels and intracellular localization of mRNA transcripts

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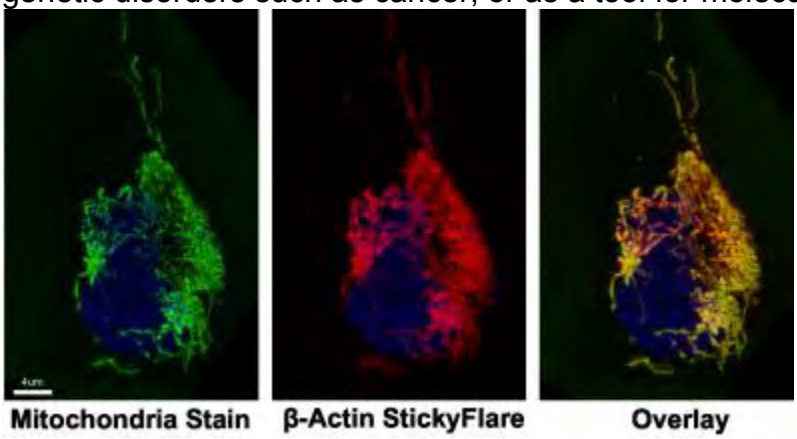
Careful observation of cellular processes has revealed exquisite examples of how the architecture of DNA and RNA can confer unique and complex functionality upon nucleic acid sequences. The realization that higher order nucleic acid architectures can afford unique functionality has ignited a new field of research that attempts to exploit such structures for a vast array of applications. Specifically, in the Mirkin lab we utilize a novel three-dimensional nucleic acid architecture, known as the spherical nucleic acid (SNA), which possesses a number of exceptional properties not observed with any other nucleic acid structural motif. These include the ability to enter live cells without the need for harmful transfection agents, minimal toxicity and immunogenicity, and a resistance to many nucleases. One SNA-based construct, known as the known NanoFlare, is capable of entering live cells and detecting the expression level of a targeted gene through a fluorescent output upon recognition. The gene recognition capability of the NanoFlare has been used to identify and separate specific cell types, such as cancerous cells, based entirely on their unique genetic profile. Further, we have recently developed this construct to be capable of tracking, in real time, the spatial distribution of mRNA transcripts within live cells. Together, these capabilities for a more complete understanding of mRNA function in living systems, whether it be for identifying genetic disorders such as cancer, or as a tool for molecular biology.



MEDI 106

Green approach to the synthesis of pyrrole C^5-nucleosides as potential antiviral agents

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Nucleoside analogs have a long history of use as anticancer or antiviral drugs. Since there is only one drug featuring a 5-membered nitrogen heterocyclic nucleoside currently in use, ribavirin, and none from the pyrrole class, then these nucleoside derivatives represent new and challenging targets for synthesis. The goal is to use green chemistry techniques such as an Ionic soluble support system to prepare such pyrrole nucleosides with a ribose group at the C-5 position, enhancing the yield and

decreasing the amount of solvents and chemical reagents used. The key benefit of the use of ionic liquids as a soluble support is the easy removal of excess reagents and by-products from the reaction. The pyrrole ring is constructed from a three-carbon synthon, a chloroenal, and an amino ketone under neutral conditions. This soluble support technique has shown an increase in yield of the pyrrole ring formation and isolation. This technique allowed for the quick isolation of the newly formed pyrrole by use of flash chromatography. The pyrrole-C^5-nulceoside is then prepared by treating the pyrrole with a Lewis acid ($TiCl_4$) followed by the addition of a protected ribose 1-acetate. Deprotection by sodium methoxide affords the pyrrole-C^5-ribonucleoside product and the detachment of the reusable soluble support ionic liquid.

MEDI 107

Discovery and structure-activity relationship (SAR) of novel pyridylureas as potent antibacterial agents

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The pyridylureas are a novel class of antibacterial agents that target DNA gyrase, an essential enzyme in bacteria. Inhibition of DNA gyrase prevents DNA synthesis and subsequently leads to cell death.

The pyridylureas were identified as a sub-series from known DNA gyrase inhibitors through virtual library enumeration utilizing x-ray co-crystal structures of the target protein. Focused libraries were designed and compounds were synthesized and tested for their biological activities. Enzymatic potency was measured as the IC_{50} against *Staphylococcus aureus* DNA gyrase ATPase activity, and cellular activity was measured as the minimum inhibitory concentration (MIC) against a panel of Gram-positive and Gram-negative bacteria. This effort led to the identification of a bis-pyridine ethyl urea compound containing a carboxylic acid as the initial lead with an IC_{50} = 0.85 μM against *S. aureus* DNA gyrase but with no MIC. Further optimization of the lead by modulating logD and replacing the carboxylic acid with an acidic heterocycle resulted in a very potent oxadiazolone-pyridyl compound, with an IC_{50} = 0.0005 μM and an MIC = 0.11 μM against *S. aureus*. The oxadiazolone compound also showed broad-spectrum antibacterial activity against other Gram-positive bacteria and fastidious Gram-negative bacteria such as *Haemophilus influenza* and *Moraxella catarrhalis.* Cross-resistance studies, radioactive precursor incorporation assays, and functional DNA supercoiling assays confirmed the mode of action. The oxadiazolone compound was efficacious in a *S. aureus* mouse thigh model for infection reducing bacterial load by 4 log units.

MEDI 108

Synthesis and antitumor activity of piperidinyl sulfamides

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The sulfamide (R2NSO2NR2) functionality is widely used in medicinal chemistry as potential targets for the development of novel small molecule therapeutic agents. In the present study we synthesized a set of piperidinyl-based sulfamide compounds through reductive amination and Mitsunobu reaction to generate sulfamides bearing chiral amino acid moiety. These compounds were screened for their anti-cancer activity against sixty human cancer cell lines, which represent a diverse histogolgies. Initial testing at 10 μM showed three of these compounds with high activity against several cell lines. While, most other compounds showed only low activities. The dose response studies and mechanism investigation suggest our representative compound inhibiting anchorage-independent growth. Also, it does not induce senescenece in A549 cells, but decreased cell density, thereby confirming inhibition of cell proliferation. Results of our studies will be presented.

MEDI 109

Synthesis and cytotoxic activity of novel conformationally restricted analogs of Combretastatin A4

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Vascular disrupting agents (VDAs), as Combretastatin A4 (CA-4), are potential anticancer agents. CA-4 targets the colchicine binding site of the β subunit of endothelial cell tubulin. The depolymerization of microtubules and disorganization of actin and tubulin that caused by CA-4 selectively damage the tumor vasculature and close the blood supply to solid tumors, causing massive tumor cell necrosis. Herein, we designed a group of novel conformationally restricted analogs of Combretastatin A4 to act as vascular disrupting agents (VDAs) with improved physical and biological characteristics. The newly synthesized compounds exhibited enhanced solubility in aqueous biological media, improved stability and longer shelf life than Combretastatin A4. The new compounds were evaluated for their cytotoxic activity as well as their tubulin polymerization inhibitory activity. Finally, the docking studies suggested an improved binding affinity of some of the new analogues to heterodimer tubulin.

MEDI 110

Structure-based design, synthesis and biochemical evaluation of reversible competitive inhibitors for Tissue Factor/Factor VII-a

***Cristina C Clement**, clement.cristina624@gmail.com, Manfred Philipp. Chemistry, City University of New York, Lehman College, Bronx, NY 10461, United States*

Activated factor VII (fVIIa) plays an important role in the initiation of blood coagulation. The fVIIa/TF complex hydrolyzes the zymogen coagulation factors (fX, fIX and fVII) to the corresponding active serine protease forms, culminating in the conversion of prothrombin to thrombin which will in turn transform fibrinogen into fibrin in the final stage of clotting. Different research groups demonstrated that specific inhibition of the TF/fVIIa complex results in antithrombotic effects without enhancing the bleeding propensity. These results suggested that fVIIa is a very promising target for a novel anticoagulant and stimulated substantial efforts in the pharmaceutical industry to discover new small molecules with anti-factor VIIa activity. Herein, we present a structure-based design approach to the discovery of peptidic and organic molecules reversible competitive inhibitors of TF/fVII-a. Screening of "*ZINCPharmer"* pharmacophore search software in combination with *"Vina Docking"* enabled the discovery of new drug like organic molecules potent competitive inhibitors of fVIIa using 4JZD.pdb as a target protein. The weakly basic or non-basic P1 groups were further selected for their biochemical evaluation of their inhibitory activity. In addition, "*SCULPT*" in combination with "*Vina Docking*" were used to generate *in silico* libraries of peptides with both D and L amino-acids, derived from the original irreversible peptide inhibitor D-Phe-Phe-Arg-chloromethylketone. The more hydrophobic, shallow binding site area bridging S2 and S4 pockets above the Trp215-Gly212 beta sheet region was explored as a binding site for rational design of new peptide sequences derived from D-Phe-Phe-Arg and extended at the amino-terminus with different variations of natural and non-natural amino acids. The original lead compound [Gly-Ser-Ala-D-Phe-Phe-Arg-CONH2] was synthesized and shown to inhibit *in vitro* the fVII-a/Tissue Factor (1:1 complex) with an IC_{50} of about 20 uM and the Ki (inhibitory constant) of 20 nM.

MEDI 111

Structure modifications of some non-steroidal anti-inflammatory drugs improved their safety and altered their selectivity

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In order to improve the safety and mask the gastrointestinal (GI) side effects of the known anti-inflammatory drugs Ibuprofen, Diclofenac, Mefenamic acid, and Ketoprofen, each drug has been coupled to Propyphenzone (**3** , **4** , **5** & **6**) to form esterase-sensitive mutual prodrug. The structures of the newly synthesized adducts were

confirmed by different spectroscopic techniques (^{1}HNMR and MS). Their analgesic and anti-inflammatory activities as well as their selectivity towards COX I and COX II enzymes were investigated. The results revealed that the newly synthesized propyphenazone adducts were able to exert *in-vivo* anti-inflammatory and analgesic activities with improved potency than the use of each drug individually. The *in-vitro* ability of each prodrug to inhibit COXI and/or COXII has been switched on upon incubation with bovine esterase suggesting the ability of plasma esterase to process these prodrugs. Interestingly, coupling of Propyphenazone to 4-Aminoantipyrine (**8**), to form a non-prodrug adduct, showed remarkably improved anti-inflammatory activity *in-vivo* and unique COXII selectivity in-vitro. The in-vitro COXII inhibitory activity of this adduct **(8)** was not affect by incubation was bovine esterase.

MEDI 112

Discovery and optimization of novel fatty acid transport protein 1 (FATP1) inhibitors

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Fatty acid transport protein 1 (FATP1) is known as a transmembrane protein with Acyl-CoA synthase activity that is highly expressed in a skeletal muscle as well as in adipose tissue. One of the important roles of FATP1 is thought to be the uptake and metabolism of a fatty acid by converting corresponding Acyl-CoA with an insulin signal. The previous report of a FATP1 deficient mouse showed the improvement of high fat-induced insulin resistance as well as the reduction of intramuscular accumulation of fatty acyl-CoA, suggesting that the inhibition of FATP1 is an attractive therapeutic target for insulin resistance. In this presentation, we report the first discovery and structure activity relationship of novel triazole and phenylpiperazine derivatives **1** -**6** as FATP1 inhibitors with improved blood or metabolic stability. We will also present preliminary *in vivo*results of these several compounds.

MEDI 113

Enhancement of drug activity by synthetic amphiphilic pore-formers

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A number of synthetic amphiphiles have been prepared and found to form pores in liposomal bilayers or in vital microbial and mammalian membranes. The formation of pores by these structures led to the hypothesis that their effect on the bilayer might permit other molecules to be carried along or otherwise co-transported. We have studied the ability of *tris*(crown) amphiphiles that we call hydraphiles and shown that they enhance the efficacy of such antibiotics as erythromycin, kanamycin, rifampicin, and tetracycline. The hydraphile family is large and evidence will be presented that only certain representatives of this group are effective. In addition, tetrameric amphiphiles called pyrogallol[4]arenes form pores in bilayers, the sizes of which are controlled at least by the cholesterol content. The pores formed by these molecules are large and could, in principle, assist in the passage of relatively large molecules through membranes. The results of studies involving a family of pyrogallolarenes will be presented.

MEDI 114

Discovery of new direct thrombin inhibitors (DTI) using a combination of pharmacophore searching with ZINCPharmer of the ZINC database and AutoDockVina molecular docking

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The screening of large compound libraries (>10 million compounds) is an alternative approach to other well established high-throughput screening (HTS) methodologies for the identification of leads for therapeutic targets. This research paper presents a new *in silico* HTS approach for screening the purchasable compounds of the ZINC database using the Pharmer-pharmacophore search technology provided by the ZINCPharmer (http://zincpharmer.csb.pitt.edu) online interface. The molecular therapeutic target of our interest, alpha human thrombin, is a serine protease, known to play a central role in the initiation and propagation of thrombotic events. Many parameters provided by the filtering screen in ZINCPharmer (such as selective hydrophobic, aromatic, acceptor/donor of hydrogen-bonds and MW functions) were rationally changed to generate new libraries containing ZINC compounds as potential DTI. The new discovered pharmacophore libraries were further imported as sdf files into the AutoDockVina and the molecular docking was performed using many reported alpha-thrombin X-Ray structures (such as 4AX9.pdb and 3U8O.pdb) in complex with peptidic or small organic DTI molecules. The compounds predicted to have free energies of interaction lower than “-9 kcal/mol” and lacking the strong basic functionality which could fit in the S1 pocket were further selected for the in vitro validation of their reversible competitive inhibition of alpha-thrombin. N-(4-fluoro-2-methoxy-phenyl)-3-(3-oxopiperazin-1-yl)sulfonyl-benzamide (ZINC23132663) and (N'1Z,N'3Z)-N'1,N'3-bis(2-

oxoindolin-3-ylidene)malonohydrazide (ZINC06062555) are some of the new classes of compounds found to be potential DTI and are predicted to have two or one digit nM Ki (inhibitory constants). Our new *in silico* HTS screening platform developed for the discovery of new DTI pharmacophores validated the ZINCPharmer as one the most reliable interactive searching tools for purchasing of new chemical space for drug discoveries.

MEDI 115

Structure-dependent inhibition of the ETS-family transcription factors by designed minor groove binders

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ETS transcription factors (TFs) mediate a wide array of cellular functions and are attractive targets for pharmacological control of gene regulation. Members of the ETS-family TFs, such as PU.1 and Ets-1, have winged-helix-turn-helix structures and bind sequence-specifically to 10-bp sites via a recognition helix bound in the major groove of a 5'-GGAA-3' consensus region, with additional contacts with the flanking minor groove. In this work, we have designed a panel of heterocyclic minor groove binders to interfere with ETS TFs binding, and systematically investigated the DNA-TF, DNA-ligand and DNA-TF-ligand interactions. We showed that the DNA binding activities of the TFs are specifically inhibited by the designed ligands bound in the minor groove of adjacent sequences of the consensus site. Moreover, the inhibition properties of the designed ligands are not only generally correlated with their DNA binding affinities but are also strongly dependent on ligand structures and their induced effects on DNA conformation. These results significantly broaden the application of minor groove binders, demonstrate that the reported ligands are powerful inhibitors of ETS TFs that act by targeting the minor groove of flanking sequences, and provide us better understanding for development of effective agents for specific modulation of DNA transcription. (Supported by NIH AI064200 to W.D.W, ACS IRG7700327 to G.M.K.P and NIH AI083803 to J.K.B)

MEDI 116

Design and synthesis of novel heterocyclic diamidines for specific DNA recognition: From AT-rich to mixed-base-pair DNA sequences

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To design compounds that can selectively target DNA and induce specific biological responses is a key goal of chemical biology. It offers many advantages such as specific control of cellular gene expression, development of new types of drugs, and agents for biotechnology. Most common minor groove binders from the natural products, netropsin and distamycin, to synthetic compounds, Hoechst 33258, pentamidine and related compounds, bind specifically to AT base pairs but weakly to GC sites. Based on extensive experience with minor groove agents for AT recognition, we have incorporated GC recognition motifs into compounds designed for mixed-base-pair recognition. A novel series of heterocyclic cations containing an azabenzimidazole ring has been synthesized in order to investigate both their sequence specificity and binding mode.The binding of the compounds was investigated by thermal melting, circular dichroism (CD), biosensor surface plasmon resonance (SPR) and molecular modeling studies. Compounds, bearing one or two azabenzimidazole-OCH_2-phenyl-amidine motifs, which could recognize a G base in a GC base pairs in an AT context, stand out as the most promising compounds. They bind strongly and selectively with the AAAGTTT and AAGAAATTGAA sequences, respectively. More importantly, this study opens new methods for the mixed-base-pair DNA sequences recognition. (supported by NIH grant AI064200)

MEDI 117

Novel heterocyclic minor groove binding compounds for targeting mixed-base-pair DNA sequences

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Sequence-specific recognition of DNA by organic small molecules offers a potentially general approach for the regulation of gene expression. It is necessary to develop a new class of compounds that can be rationally designed from the established modules which can bind strongly and selectively with specific DNA sequences. In this work we have designed a library of novel heterocyclic cations which can selectively bind with mixed-base-pair DNA sequences. Evidence of strong interaction of the compounds with a GC base pair (bp) adjacent to two AT rich sites has been obtained from thermal denaturation, circular dichroism, biosensor-surface plasmon resonance, isothermal calorimetric and mass spectrometry studies. Some of our new compounds bind to the target sequence with sub-nanomolar binding constants with very slow dissociation kinetics and very high selectivity over the related sequences without the GC base pair. By this approach we have introduced a new way to recognize more complex DNA sequences by synthetic organic molecules. The molecular modeling studies also provide the structure-sequence relationship between ligand-DNA complexes. (supported by NIH AI064200)

MEDI 118

WITHDRAWN

MEDI 119

Synthesis and structure-activity relationship of imidazoquinolone based inhibitors of glycogen synthase kinase 3β

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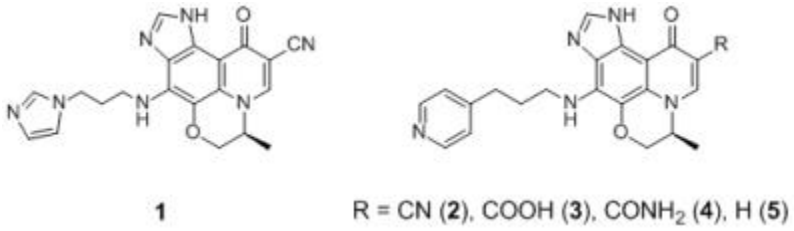
The synthesis and structure-activity relationship of imidazoquinolone GSK3β inhibitors are presented. Compounds **1** and **2** were identified as the most potent inhibitors in this study with GSK3β IC_{50} values of 700 pM and 800 pM, respectively. When profiled against 180+ kinases (KiNativ™) compounds **2** -**5** were identified as selective GSK3 inhibitors.



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R = CN (**2**), COOH (**3**), $CONH_2$ (**4**), H (**5**)

MEDI 120

(Z)-7-arylidene-2-(arylamino)-5H-pyrimido[4,5-b][1,4]thiazin-6(7H)-ones as specific inhibitors of PI3Kα and δ

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Phosphatidylinositol 3-kinase (PI3K) is a large duel lipid and protein kinase that catalyzes phosphorylation of the 3-hydroxyl position of phosphatidylinositides (PIs) and plays a crucial role in the cellular signaling network. Among the various subtypes of PI3K, class IA PI3Kα has gained increasing attention as a promising drug target for the treatment of cancer due to its frequent mutations and amplifications in various human cancers. Class I PI3Ks are heterodimers comprising a catalytic subunit (p110α, β, δ, or γ) and a regulatory subunit. The PI3K pathway has an important role in cell metabolism, growth, migration, survival and angiogenesis. Many receptor kinases implicated in

cancer development are upstream of the PI3K signaling pathway, and activation of this pathway is often responsible for promoting cancer. Because of the importance of this pathway to tumor cell growth and survival several small molecule PI3K inhibitors have been developed and have demonstrated anticancer efficacy in preclinical models. However initial clinical trials have shown weaker anticancer efficacy than expected. Hence there is a need to develop new small molecule PI3K inhibitors.

In our continuous search for potent small molecule kinase inhibitors we developed a series of (*Z*)-7-arylidene-2-(arylamino)-5*H*-pyrimido[4,5-*b*][1,4]thiazin-6(7*H*)-ones as specific PI3Kα and PI3Kδ isoform inhibitors. In this presentation, we will focus on the synthesis, structure activity relationship (SAR), cytotoxicity and kinase inhibitory profile of the lead compound ON 146040 on cancer cells.

MEDI 121

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

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A new class of DNA-alkylating agents, indolinobenzodiazepine dimers (IGNs), have been designed and synthesized. The lead compounds display potent DNA-binding affinity and sequence specificity. These compounds are highly cytotoxic *in vitro* towards cancer cell lines, with IC_{50} values in the picomolar range. Linkable versions of the lead IGN compounds were synthesized and conjugated to monoclonal antibodies directed against tumor-associated antigens. These antibody-IGN conjugates display high antigen-specific potency *in vitro* and anti-tumor activity *in vivo* at non-toxic doses. IGNs represent a promising new class of cytotoxic agents with a novel mechanism of action for use in the development of ADCs.

MEDI 122

Cytotoxic effects, DNA fragmentation and changes in gene expression of novel nitro and amino benzazolo[3,2-a] quinolinium (BQS)

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This study evaluates the toxic effects of two benzazolo[3,2-*a*]quinolinium salts (BQS) on tumor cells. The novel experimental drugs (ABQ48 and NBQ48) are cationic heterocyclic compounds that have demonstrated apoptosis induction. We here report biological activities including: Cell viability inhibition with one and 5 doses applying the NCI 60 cell line panel; mitochondrial membrane damage; DNA fragmentation and changes in cell cycle. Also preliminary *In-silico* gene expression analysis of ABQ48 (NSC 763307) has been performed by implementing the COMPARE algorithm program which reports a list of most important genes sorted by correlation coefficient R2. Cells were maintained in RMPI1640 media at 37ºC with 5% CO_2 and exposed to BQS for 48 hours to determine the concentration that inhibits 50% viability (IC50). The IC50 was then applied to measure the mode of action (MOA) using the Toledo lymphoma cell line. Viability inhibition was determined by trypan blue exclusion, changes to the mitochondrial membrane permeability applying the Nucleo Counter 3000 JC-1 imaging assay and for DNA fragmentation & Cell Cycle a DAPI assay was used. Results indicated that BQS presented various toxicities. The observed IC50s for ABQ48 and NBQ48 ranged from 10 µM to 50µM depending on the cell histology. Mode of action results indicated that BQS caused decreased in mitochondrial membrane potential, DNA fragmentation as well as cell cycle arrest in different stages. The presented preliminary results with the COMPARE analysis supports the BQS apoptosis induction hypothesis given the correlation in gene expression of cells treated with ABQ48 (R2=0.47) and an increase in the expression of gene NR2F2 (Nuclear receptor subfamily 2, group F, member 2) which is involved in the regulation of a variety of cellular processes, including cell differentiation; cell proliferation; and apoptosis.

MEDI 123

Discovery of ASP3026 as a potent and selective anaplastic lymphoma kinase (ALK) inhibitor for the treatment of non-small cell lung cancer

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Anaplastic lymphoma kinase (ALK) is a promising therapeutic target for the treatment of cancers, including echinoderm microtubule-associated protein-like 4 (EML4)-ALK positive non-small cell lung cancer (NSCLC). We synthesized a series of 1,3,5-triazine derivatives as novel ALK inhibitors. ASP3026 potently and selectively inhibited ALK, and demonstrated dose-dependent antitumor activity in mice bearing NCI-H2228 tumor xenografts. ASP3026 also showed inhibitory activity against the L1196M gatekeeper mutant of ALK that confers resistance to crizotinib. Synthesis and structure-activity relationships of ASP3026 and its derivatives will be presented.

MEDI 124

Novel STAT3 inhibitors: Synthesis, biological evaluation, SAR and modeling studies

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Signal Transducer and Activator of Transcription 3 (STAT3) is a transcription factor that plays a key role in regulating gene expression leading to cellular processes such as cell proliferation, differentiation and apoptosis. Constitutive activation of STAT3 has been detected at a high frequency in diverse human cancers. Inhibiting STAT3 results in decreased tumor growth and improvement in animal survival. Hence, STAT3 is considered a potential drug development target for novel anticancer therapeutics. STAT3 has been under intense investigation for many years, however no drug targeting it has been approved. In our search for novel potent STAT3 inhibitors, we have discovered a series of new compounds that interrupt STAT3-DNA binding at low micro molar concentrations. Subsequently, pharmacophore-based 3D QSAR studies were carried out to determine the role of functional groups and conformations required for activity. Docking and MM-GBSA-based refinement procedures suggested that these compounds bind to a DNA binding site of the STAT3 DNA binding domain, unlike many STAT3 inhibitors that are thought to bind to the SH2 domain. In the present study, we describe the biological testing, computational modeling, rational design and synthesis as well as SAR studies of the novel STAT3 inhibitors.

MEDI 125

Identification and mechanism of action studies of novel sumoylation inhibitors

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Sumoylation is a dynamic covalent modification of protein by Small Ubiquitin-like Modifier (SUMO) involved in various cellular processes, including protein stability, protein translocation, DNA repair, and transcription. In order to identify new sumoylation inhibitors we developed novel electrophoretic mobility shifty assay using a fluorescent peptide probe which contains a SUMO consensus sequence, and it was validated using in-gel fluorescence imaging and western blot. The assay monitors sumoylation events in real time and is tolerant to pH (7-10), temperature (RT-40 deg), and DMSO (0-5%). We screened small molecular libraries including 500 flavonoids and chalcones using electrophoretic mobility shifty assay and identified a flavonoid derivative 2-D08 as the most active sumoylation inhibitor in vitro. Mechanism of action studies revealed that 2-D08 inhibits SUMO transfer from Ubc9 thioester complex to the target protein. Cell-based assay showed that 2-D08 inhibits sumoylation of topoisomerase-I in response to

camptothecin treatment in ZR-75-1 and BT-474 breast cancer cells, while isomeric analogs are inactive. Importantly, the compound inhibits sumoylation pathway while not affecting ubiquitylation pathway in cancer cells. 2-D08 is a distinct sumoylation inhibitor compared to known sumoylation inhibitors which block SUMO-E1 thioester formation. We will describe the development of novel sumoylation assay and the discovery of sumoylation inhibitors and their structure-activity relationships and mechanism of action.

MEDI 126

Lipophilic isosteres of a π-π stacking interaction: New inhibitors of Bcl-2-Bak interactions

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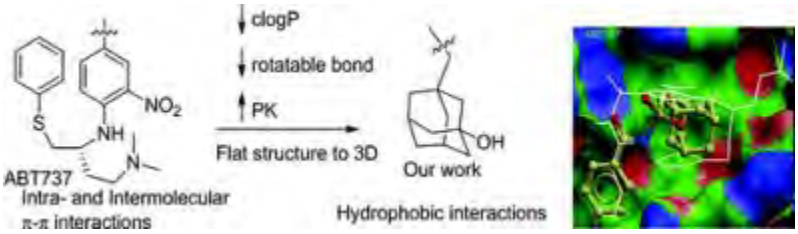
Overexpression of the antiapoptotic members of the Bcl-2 family of proteins is commonly associated with cancer cell survival and resistance to chemotherapeutics. The discovery of new Bcl-2 protein–protein interaction antagonists is described. We replaced the northern fragment of ABT737 (π–π stacking interactions) with structurally simplified hydrophobic cage structures with much reduced conformational flexibility and rotational freedom. The binding mode of the compounds was elucidated by X-ray crystallography, and the compounds showed excellent oral bioavailability and clearance in rat PK studies.



MEDI 127

Inhibitors of urokinase type plasminogen activator and cytostatic activity from crude plants extracts

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In view of the clear evidence that urokinase type plasminogen activator (uPA) plays an important role in the processes of tumor cell metastasis, aortic aneurysm, and multiple sclerosis, it has become a target of choice for pharmacological intervention. The goal of this study thus, was to determine the presence of inhibitors of uPA in plants known traditionally for their anti-tumor properties. Crude methanol extracts were prepared from the leaves of plants (14) collected from the Subtropical Dry Forest *(Guanica)* in Puerto Rico, and tested for the presence of inhibitors of uPA using Fibrin Plate Assay. The extracts that tested positive (6) were then partitioned with petroleum ether , chloroform , ethyl acetate and n-butanol , in a serial manner. The resulting partitions were then tested using again the fibrin plate assay. Extract partitions from leaves of *Croton lucidus* (*C. lucidus*) showed the presence of a strong uPA inhibitory activity. Serial dilutions of these *C. lucidus* partitions were performed to determine the uPA inhibition IC_{50} values. The chloroform partition showed the lowest IC_{50} value (3.52 µg/mL) and hence the most potent uPA inhibitor. Further investigations revealed that the crude methanol extract and its chloroform and n-butanol partitions did not inhibit significantly the closely related proteases such as the tissue type plasminogen activator (tPA) and plasmin, indicating their selectivity for uPA, and hence superior potential for medicinal use with fewer side effects. In a further evaluation of their therapeutic potential for prevention of cancer metastasis, the *C. lucidus*extracts displayed cytostatic activity over human pancreatic carcinoma (PaCa-2) cells, as determined through MTS assay. The cytostatic activities recorded for each of the partitions correlated with their relative uPA inhibitory activities. There are no existing reports of uPA inhibitors being present in any of the plants reported in this study.

MEDI 128

Synthesis and anticancer activity evaluation of piperidine and piperazine-linked chalcone derivatives

*Vineet Kumar[3], **Brajendra Kumar Singh**[1], singhbk@chemistry.du.ac.in, Vijay Bahadur[1], Christian E. Velez Gerena[2], Prasant Kumar[1], Beatriz Zayas[2], Sanjay V. Malhotra[3]. (1) Department of Chemistry, University of Delhi, Delhi, United States (2) School of Environmental Affairs, Universidad Metropolitana, San Juan, Puerto Rico 00928, United States (3) Laboratory of Synthetic Chemistry, Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Frederick, MD 21702, United States*

Chalcones (trans-1,3-diphenyl-2-propene-1-ones) are α,β-unsaturated carbonyl compounds with two aromatic rings. They are intermediates in the biosynthesis of flavonoids & isoflavonoids, and abundantly present in plants. These compounds show

anti-oxidant, anti-inflammatory, antiviral, antibacterial, antimalarial and anticancer activities. In the present study, we synthesized a series of piperidine- and piperazine-linked novel chalcones. A subset of our library was screened for their anticancer activities on the National Cancer Institute's 60 human cancer cell lines which cover diverse histologies. The screening showed some of the compounds to be highly active against different cancer types, causing growth inhibition at low micro molar dose. Further study through the Annexin V, DNA Fragmentation, Cell Cycle Effects and Mitocondrial Membrane Permeabilizationtest provided further insight into the mechanism. Details of these screening results, structure activity relationship and mechanism of action studies will be presented.

MEDI 129

Improved synthesis of furano analogs of Duocarmycin C1 and C2: Seco-isocyclopropylfurano[e]indoline-trimethoxyindole and seco-cyclopropylfurano[f]quinoline-trimethoxyindole

*Pravin Patil, **Moses Lee**, lee@hope.edu. Department of Chemistry, Hope College, Holland, MI 49422, United States*

CC-1065 and the duocarmycins are highly potent anticancer agents. Our group has recently developed a class of furan containing analogs, *seco*-iso-cyclopropylfurano[e]indoline-trimethoxyindole and *seco*- cyclopropylfurano[f]quinoline-trimethoxyindolle that are also highly cytotoxic against the growth of cancer cells. Previous synthesis of these compounds was achieved by separating mixtures of the respective isomeric indoline and quinoline intermediates formed from a radical cyclization reaction of allylic bromo starting material. In this presentation we will describe an improved and efficient synthesis of these compounds whereby either the indoline or quinoline intermediate could be synthesized selectively. Furthermore, the conditions of the stobbe condensation, selective bromination, 5-*exo-trig* radical cyclization, and debenzylation were modified and optimized. As a result the reaction times were shortened, the products were easier to isolate in greater than 99 percent purity (HPLC), and the overall yields were improved.

MEDI 130

Synthesis and cytotoxic properties of fused heterocyclic derivatives of curcuminoids: Bis- benzylidenyl-4-phenylcyclohexanone and -*N*-methyl-4-piperidone

*Pravin Patil[1], Kimberly A. Brien[1], Vijay Satam[1], Megan Lee[1], Mia Savagian[1], Samuel Tzou[1], Sarah Geoghan[1], Nicholas F. Dybdal-Hargreaves[2], Susan L. Mooberry[2], Matthias Zeller[3], **Moses Lee**[1], lee@hope.edu. (1) Department of Chemistry, Hope College, Holland, MI 49422, United States (2) Department of Pharmacology, University of Texas Health Science, San Antonio, TX 78229, United States (3) Department of Chemistry, Youngstown State University, Youngstown, OH 44555, United States*

A series of fused heterocyclic (pyrimidine-2-thione, dihydropyran, pyrazoline, and valerolactam) derivatives of bis-benzylidenyl-4-phenylcyclohexanone and bis-benzylidenyl-*N*-methyl-4-piperidone were synthesized. The structures of the products were ascertained by spectroscopic studies and one was unequivocally ascertained by X-ray single crystallography. The target compounds showed modest cytotoxicity against murine L1210 lymphoma and B16 melanoma cells, with the most active derivative giving IC_{50} values of 7 and 15 μM for the two cell lines, respectively. The compounds did not cause microtubule depolymerization in cells, but several induced apoptosis in HeLa cells.

MEDI 131

Validation studies with small-molecule modulators of the MDM2/MDMX-p53 binding interaction

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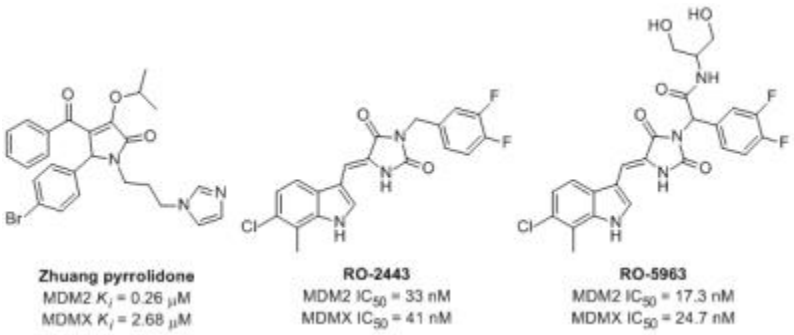
The tumour suppressor p53 is a transcription factor activating a number of genes responsible for cell growth arrest, senescence, and apoptosis in cells. Tumour cells evade apoptosis and proliferate by subverting the p53 pathway, either by mutation of the *TP53* gene, resulting in the expression of inactive p53, or through amplification/overexpression of proteins responsible for p53 regulation, most notably MDM2 and MDMX. Hence, modulators of the interaction of MDM2 and MDMX with p53 are of potential interest as antitumor agents (*Nat. Rev. Cancer* **2009,** *9*, 862).

As part of an ongoing programme to develop small-molecule modulators of the MDM2-p53 and MDMX-p53 binding interactions, we have established *in vitro* assays to examine compound potency. To validate these assays, and to enable a comparison with inhibitors reported in the literature, several compounds reported as MDM2/MDMX modulators were synthesised and evaluated.



Surprisingly, the *in vitro* results obtained with the Zhuang pyrrolidone (*J. Med. Chem.* **2012,** *55*, 9630) were in disagreement with the literature: the compound showed no activity against either target in an ELISA assay, and was inactive at 10 μM in p53 wild type MDM2/MDMX overexpressing cells. Contrastingly, whilst the potencies of RO-2443 and RO-5963 (*PNAS* **2012,** *109*, 11788) were 1000-fold lower than expected in a biochemical assay, the cellular data were in line with published results. Alternative assay formats are currently being established to explore correlations with compound potency in cells.

MEDI 132

New quinones as HER2 inhibitors for the treatment of trastuzumab resistant breast cancer

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HER2 overexpression is associated with aggressive breast cancer with high recurrence rate and poor patient prognosis. Treatment of HER2 overexpressing patients with the HER2 targeting therapy trastuzumab results in acquired resistance within a year. The HER2/EGFR dual kinase inhibitor lapatinib was shown to inhibit some trastuzumab resistant breast cancer cell lines and is currently in clinical trials. Our group has found new quinone compounds that show excellent inhibition of breast tumor cells expressing HER2 or the trastuzumab resistant HER2 oncogenic isoform, HER2Δ16. Compound **4** ((1R,2S,3S)-1,2,3,5,8-pentahydroxy-1,2,3,4-tetrahydroanthracene-9,10-dione) and compound **5** (5,8-dihydroxy-2,3-bis(hydroxymethyl)naphthalene-1,4-dione) showed sub-micromolar inhibition potency against these cell lines. These compounds also inhibit auto-phosphorylation of the Y1248 and Y1068 residues of HER2 and EGFR, respectively. The synthesis of new quinone derivatives aimed at enhancing the inhibition potency are presented.

MEDI 133

Effect of selenium: Comparative in vitro phototoxicity response of irradiated tumorigenic vs. non-tumorigenic prostate cancer cells

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Photodynamic therapy (PDT) is a slightly invasive form of treatment used for various types of cancer, including prostate cancer. PDT consists of two steps for treatment: introducing a photosensitizer (PS), exposing the PS with radiant energy of the appropriate wavelength in order for the PS to produce singlet oxygen, and then destroying the targeted cells. Limiting the effect of PDT to malignant tissues is a major challenge since this anticancer treatment contains side effects which include necrosis due to nonselective tissue targeting of the photosensitizers. Selenium compounds have been proven to act as antioxidants and prevent cell death on non-tumorigenic cells. The ultimate goal of this research is to study the mechanism of cell death pathway such as apoptosis and necrosis during photoirradiation, and to see if adding selenium compounds, namely, selenomethionine (SeM) or methylselenocysteine (SeMSC) will decrease non-tumorigenic cell death. A known water-soluble photosensitizer pyropheophorbide *a* (PyPPa) was incubated in non-tumorigenic (*PNT1A*) and malignant (*DU-145*) prostate cancer cells to compare its photosensitizing ability in the presence of selenium compounds. MTT assay was performed to determine the extent of cell survival upon irradiation with 650 nm light (fluence rate of 0.72 J/cm^2). The number of viable cells which is directly proportional to the absorbance was determined using a BioRad 550 Microplate Reader. Cell survival assay indicated that cell destruction increased with PS concentration and with increasing light dosage for the photosensitizer used for both normal prostate and tumorigenic prostate cancer cells. Selenium compounds protected both non- and tumorigenic cells at low light dosages from oxidative damage caused by singlet oxygen. Cell morphological changes associated with apoptosis or necrosis will be determined by fluorescence microscopy technique.

MEDI 134

Structural optimization and biological screening of a steroidal scaffold possessing cucurbitacin- like functionalities as BRAF inhibitors

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Cucurbitacins are highly oxygenated tetracyclic triterpene natural products. They are known for their broad spectrum of biological activities such as anti-inflammatory, hepatoprotective, and anti-carcinogenic activity against various cancer cell lines. Recently, we reported *in-silico* modeling coupled with *in-vitro* biological evaluations against mutant B-RAF melanoma cell lines as an initial investigation into the promising ability of cucurbitacins to target the Mitogen Activated Protein Kinase (MAPK) pathway. However, cucurbitacins are known for their multi-faceted biological effect by targeting

multiple molecular targets, sometimes undesirably. The potential utility of cucurbitacins as potential drug candidates drew attention towards optimizing the selectivity, by analyzing important chemical functionalities responsible for binding to B-RAF via molecular modeling. This approach resulted in the identification of a side chain at C-17, possessing an α,β-unsaturated ketone at ring D, of a steroidal skeleton using a structure-based drug design approach. Three main sites were studied synthetically; 1) Assembling different functionalities at C-25 via aldol condensation reaction, 2) Thiophenol and azide conjugate addition reaction at C-24, 3) *Trans*, *pseudo*, and *cis* configurations of the B/C ring juncture of the steroidal skeleton. Twelve synthetic steps were carried out resulting in 20 novel intermediates and final analogs. These analogs were biologically screened using cytotoxicity and in-cell based ELISA assays, to show the potential of analogs possessing *pseudo-cis* configuration and the actual side chain of cucurbitacins to inhibit cell growth of A-375 cell lines with IC_{50} ranging from 12.20 - 19.90 μM. The synthesized analogs also exhibited significant inhibition of phosphorylated ERK, induced by epidermal growth factor. These results offer a novel scaffold for targeting the MAPK pathway as a treatment for melanoma.

MEDI 135

Design and synthetic studies of a metabolically-stabilized zampanolide analog

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(-)-Zampanolide is a 20-membered macrolide that exhibits nanomolar cytotoxicity against both drug-sensitive and multidrug-resistant cancer cell lines. It exerts a mechanism of action similar to that of the clinically proven drug paclitaxel (Taxol®) by binding to the taxane pocket of β-tubulin. It uses its side chain to induce structuring of the M-loop into a short helix, which establishes lateral tubulin contacts in microtubules. As part of our ongoing program to identify drug-like zampanolide analogs with improved stability in vivo and the potential to treat multi-drug resistant cancer, a novel zampanolide analog has been designed by replacing the lactone moiety of zampanolide with pyrazole as its bioisostere. Two advanced intermediates have been successfully synthesized using more than 25 steps of reactions. Construction of the macrocyclic core structure from these two advanced intermediates, with Grubbs metathesis reaction as key reaction, is under study. The design, retrosynthetic analysis, and synthetic studies of this novel zampanolide analog will be presented.

MEDI 136

Exploration of novel scaffolds for MCT1 inhibition: New antitumor agents

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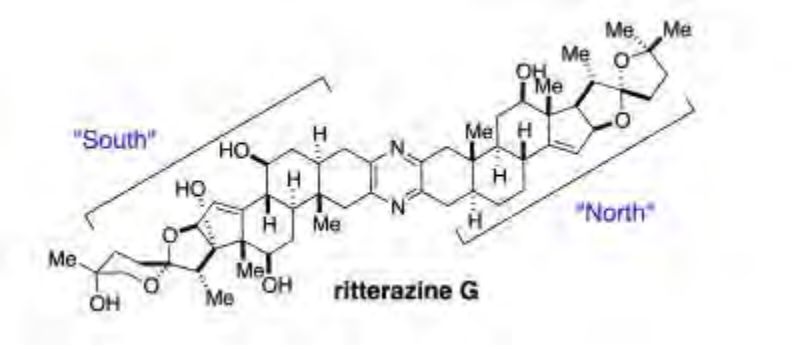
It is well-known that cancer cells gain energy through a high rate of glycolysis, even under conditions of sufficient oxygen. Lactate, the end product of glycolysis, must be effluxed by monocarboxylate transporters (MCTs) to maintain intracellular pH levels. Four such transporters, MCT1-4, are known, and the isoforms MCT1 and MCT4 are predominantly responsible for lactate transportation in tumor cells. Thus inhibitors of MCT1 and/or MCT4 are potential therapeutic agents for treatment of cancers. We herein report our efforts toward the development of MCT1 inhibitors in three novel scaffolds: pteridines, xanthines, and flavonoids. We will discuss their synthesis and SAR studies to optimize antitumor effects in cell-based assays.

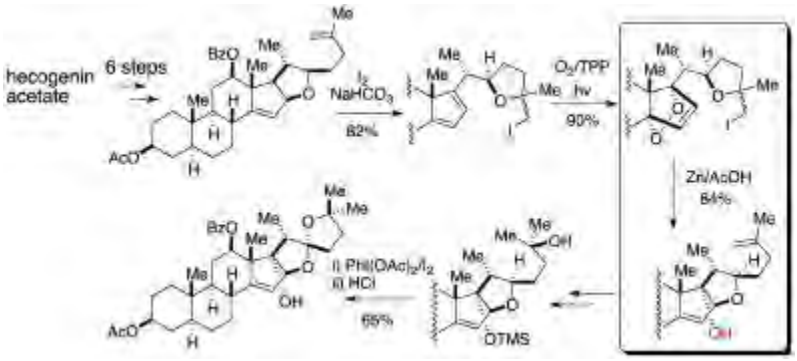
MEDI 137

Synthesis of C17-OH-north unit of ritterazine G via "Red-Ox" modifications of hecogenin acetate

Young K Cheun*, ykcheun@utexas.edu, Yi Kou, Begoña Stevenson, Hee-Kwon Kim, Myong C Koag, Seongmin Lee. Division of Medicinal Chemistry, College of Pharmacy, University of Texas at Austin, 2409 University Ave. Stop A1900, Austin, Texas 78712, United States*

Cephalostatin/Ritterazine family is a group of 45 tridecacyclic bissteroidal pyrazine marine natural products, which possess extreme antiproliferative activities. The unusual cytotoxic mechanism of cephalostatin/ritterazine is being studied at a slow pace due to the scarcity and the structural complexity of the molecules. Ritterazine G is an excellent study model for cephalostatin/ritterazine family because of its relatively simple structure and the decent cytotoxicity. C17-OH of ritterazine G is not only a critical site for its activity, but also a major challenge for the total synthesis. Utilizing a highly efficient and stereoselective introduction of the C17-OH via E-ring cleavage/F-ring formation, D-ring oxidation, and F-ring cleavage/E-ring formation, the C17-OH-north unit of ritterazine G can be prepared in 13 steps from hecogenin acetate. Since C17-OH moiety is a component of the common pharmacophore of extremely potent members of the cephalostatin/ritterazine family, our results may find applications in the synthesis of cephalostatin/ritterazine-related anticancer drugs.





MEDI 138

Primary study of vitro antitumor effect of DYC-279 on human hepatocellular carcinoma HepG-2 cells

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Recent research of 1,2,3-triazole's pharmacological effects became much more appealing and promising for anticancer agents design. 1,2,3-Triazoles conjugated with a wide range of moieties were reported to exhibit potent anticancer activity. DYC-279 is one of newly synthesized compound by our group which is one of a series of 1,2,3-triazoleedithiocarbamate hybrids with general structure by our research group(as shown Figure1 A). In the present study, we revealed that DYC-279 can inhibit the proliferation of HepG-2 cells in a dose- and time-dependent manner using the MTT test. No cross-resistance was detected. FACS-based cell cycle analysis revealed a significant increase in the G2/M phase and depletion in the G0/G1 phase populations. A concomitant down-regulation of cyclin D1 and up-regulation of CDC2 and Cyclin B1 in hepatocellular carcinoma HepG-2 cell was detected by Western blot. It means that DYC-279 could increase the expression of CyclinB1 and the phosphorylation of Cdc2 in concentration - dependent manners with decreasing the dephosphorylation of Cdc2 and in hepatocellular carcinoma HepG-2 cell in vitro. Cyclin D1 is the key protein in G1 phase and it has been shown to interact with tumor suppressor protein Rb and the expression of this gene is regulated positively by Rb. Mutations, amplification and overexpression of these genes, which alters cell cycle progression, is observed frequently in a variety of tumors and may contribute to tumorigenesis. Western blot analysis revealed that DYC-279 induced the activation of caspase-9 and the release of cytochrome C (Cyto C) from the mitochondria to the cytosol. Furthermore, the pro-apoptotic factor, Bax, was upregulated and the anti-apoptotic factor, Bcl-2, was downregulated, eventually leading to a reduction in the ratio of Bcl-2/Bax proteins. The results demonstrated that DYC-279 inhibits proliferation and induces apoptosis of HepG-2 cells human cells in vitro. The induction of apoptosis appears to occur through the upregulation of Bax, the

downregulation of Bcl-2, the release of Cyto C from the mitochondria to the cytosol and the activation of caspase-9, which subsequently trigger major apoptotic cascades. DYC-279 has potent antitumor activity in HepG-2 cells human cells and may be used as a novel effective reagent in the treatment of colon cancer.

MEDI 139

Nonpeptide macrocyclic histone deacetylase inhibitors: Targeting cancer and inflammation

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Histone deacetylase (HDAC) inhibition is a promising therapeutic strategy for cancer treatment because HDAC inhibitors have shown the ability to arrest proliferation of transformed cell lines by reactivating silenced tumor-suppressor-genes by modulating the condensation status of DNA. Recently, we have reported a new class of hydroxamic acid based HDAC inhibitors containing 14- or 15-membered non-peptide macrolide antibiotic skeletons as surface recognition group. In these HDAC inhibitors, the modified macrolide antibiotic is linked to the zinc chelating hydroxamic acid moiety via 5- or 6-methylene chain linker. Most of the compounds showed low nano-molar HDAC inhibitory activities and many of them inhibited proliferation of human lung, prostate, and breast cancer cell lines. Moreover, azithromycin-hydroxamic acid, with six methylene linker, selectively accumulates in vivo in the lung tissues of Bald/C mice, allowing for tissue targeted anti-cancer therapy. To further understand the depth of the SAR of this class of compounds on HDAC inhibitory activities, we investigated the effects of: (i) varying linker length; and (ii) macrolide modification. We observed that in most of the instances these modifications were well tolerated and the compounds retained their HDAC inhibitory and anti-proliferative activities and in some cases therapeutic profile is even better than previously reported compounds. We also tested all the potent HDAC inhibitors for their anti-inflammatory activities and interestingly some of the potent compounds inhibited bacterium NTHI induced NfkB-dependent inflammatory response in vitro in human lung epithelial cells.

MEDI 140

Fluorescent dye-labeled novel anticancer drug candidates: Cellular uptake and distribution studies

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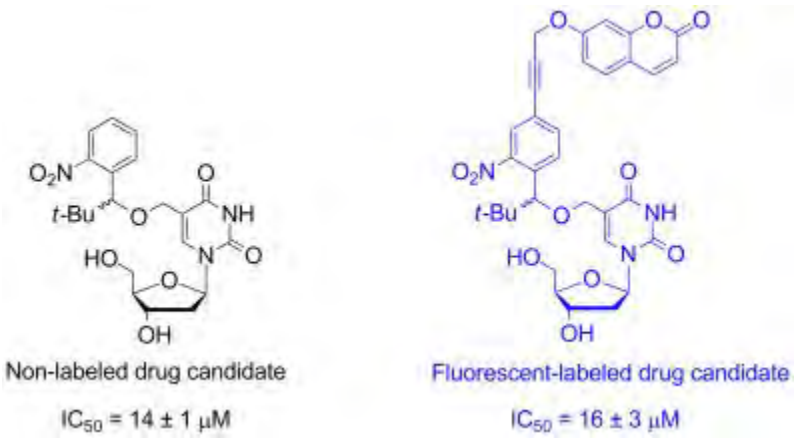
Interference with DNA replication affects rapidly proliferating cancer cells preferentially, which has prompted us to design and synthesize potential anti-cancer agents capable of halting DNA synthesis. The hypothetical mechanism of action of our base-modified nucleoside drug candidates is their intracellular metabolism into 5'-triphosphates whose subsequent incorporation into DNA replication fork obstructs further addition of nucleotides. In order to support the hypothesis, we have synthesized fluorescent dye labeled drug candidates, compared their activity to that of the unlabeled analog, and examined their cellular uptake and distribution using confocal microscopy.



MEDI 141

DNA binding, DNA methylation and cell toxicity of estradiol conjugated DNA methylating compounds

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Estradiol conjugated compounds that can target estrogen receptor positive cells and produce lethal N3-methyladenine adducts in these cells have been synthesized and investigated. Prior studies with these DNA methylating compounds had shown that the ability of these molecules to produce N3-methyladenine adducts was crucially dependent on the composition of the linker unit connecting the DNA methylating moiety to the estradiol unit. The DNA binding properties of this class of molecules was

investigated using stable non-methylating analogs, and the variation in the DNA binding ability of the compounds was compared to the corresponding DNA methylating ability. Cell toxicity studies were conducted using MCF-7 breast cancer cells in order to determine any correlation between toxicity and DNA methylation and estrogen receptor binding. The results of these studies will be presented.

MEDI 142

Targeting cancer-related kinases with metal-functionalized inhibitors

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Mutationally activated kinases are a major cause of aberrant cancer cell growth. Several kinases are currently being pursued as drug targets for cancer chemotherapy, including the ErbB family of enzymes, VEGFR, and FGFR, among others. Despite their clinical success, small molecule kinase inhibitors often lose their effectiveness in tumors over time as a consequence of secondary mutations that significantly decrease the drug-enzyme binding affinity. To overcome this type of acquired tumor resistance, irreversible inhibitors that covalently attach to the ATP binding pocket have been introduced. Using this strategy, we have designed kinase inhibitors containing cysteine-binding metal-based electrophiles. The compounds feature organic ATP-mimicking scaffolds that bind to the target kinase active site with high affinity and selectivity while the electrophilic metal induces a permanent coordinative bond with the amino acid residue to achieve irreversible inhibition. Here, we present the structure-guided design and synthesis of this class of molecules. Several of the target molecules showed binding constants (K_d) in the low-nanomolar range in competition binding assays and high selectivity for the targeted kinase domain in a panel of 145 wild-type and clinically relevant mutated kinases (KinomeScan, DiscoverX, Fremont, CA). The ability of the electrophilic agents to induce irreversible adducts was studied by LC ESI-MS/MS in pepsin digests of kinase incubated with inhibitor. Finally, the in-vitro cytotoxicity of selected derivatives was studied in solid tumor cell lines. The data generated in this study suggest that the new pharmacophores may have applications as therapeutics capable of overcoming acquired tumor resistance by irreversibly inhibiting deregulated kinases.

MEDI 143

Design and synthesis of norendoxifen analogs with dual aromatase inhibitory and estrogen receptor modulatory activities

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Both selective estrogen receptor modulators and aromatase inhibitors are widely used for the treatment of breast cancer. Compounds with dual aromatase inhibitory and estrogen receptor modulatory activities would be beneficial and have special advantages for treatment of breast cancer. Our previous efforts led to the discovery of norendoxifen as the first compound with dual aromatase inhibitory and estrogen receptor modulatory activities. To optimize its efficacy, CYP inhibition selectivity and ADME profiles, a series of structurally related analogues were designed based on molecular modeling and a structure-based drug design approach. These analogues were synthesized and biologically tested. Most of them displayed dual aromatase inhibitory activity and estrogen receptor binding affinity. The most potent compound (2) showed elevated potency against both aromatase and ER when compared to norendoxifen. The CYP inhibition selectivity profiles for compound 2 were also superior to norendoxifen. These results suggested compound 2 to be an interesting candidate for further evaluation in treatment of breast cancer.

Compound	Aromatase (IC_{50}, nM)	Aromatase (K_i, nM)	ER-α (EC_{50}, nM)	ER-β (EC_{50}, nM)
Norendoxifen	102 ± 33	77.0 ± 9.5	27.0 ± 4.8	35.2 ± 16.8
2	47.5 ± 1.5	17.3 ± 2.8	15.0 ± 3.3	9.5 ± 0.2

MEDI 144

Synthesis of 1 H-indazol-3(2H)-one derivatives as potential CDK inhibitors

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Cyclin-dependent kinases (CDKs) are serine/threonine kinases that play major roles in the cell cycle regulation process. Over expression of CDKs may cause cancer, proliferative renal diseases, and neurodegenerative disorders. The objective of this work is to inhibit the over expression of CDKs with 1 H-indazol-3(2H)-one and its derivatives. Three compounds- 1H-indazol-3-ol, 5-methyl-1H-indazol-3-ol, and 5,7-dibromo-1H-indazol-3-ol were synthesized. The identities were confirmed by H^1NMR. 1H-indazol-3-ol and 5,7-dibromo-1H-indazol-3-ol were tested for their efficacy for inhibition of the protein kinases CDK1 and CDK2. It showed that 1H-indazol-3-ol inhibited CDK1 and CDK2with an IC_{50} value of 2.95μM and 2.82μM. 5,7-dibromo-1H-indazol-3-ol inhibited CDK1 and CDK2with an IC_{50} value of 2.65μM and 3.07μM. Future work will include the synthesis of more derivatives and testing their inhibition of CDKs.

MEDI 145

Elucidating the binding site of epothilones on β-tubulin with epothilone photoaffinity probes

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Epothilones are tubulin-binding cytotoxic polyketide-derived macrolides. Although the binding sites for epothilones and taxanes on β-tubulin overlap, epothilones show efficacy against paclitaxel-resistant cancer cell lines, suggesting a significantly different binding mode. Two epothilone binding models have been proposed based on NMR and electron-crystallography data. In order to differentiate the proposed binding modes, we designed and prepared epothilone A photoaffinity analogues. We hypothesized that the protein region labeled by the probes is dependent on the epothilone conformation at the binding site. For one of the analogues we identified the probe-labeled peptide fragment 'TARGSQQY' in the β-tubulin isoform TBB3 (residues 274 to 281) by MS analysis. Our experimental results confirmed the consensus of both the models that Thr 274 and Arg 276 are necessary for binding of epothilones to β-tubulin. The synthesis of the photoaffinity analogues, their in vitro toxicity, the photolabeling experiments, and the mass spectrometric studies will be reported.

MEDI 146

Bioreductively activatable prodrug conjugates (BAPCs) of combretastatin A-1 (CA1) as anticancer agents targeted towards tumor hypoxia

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A significant number of solid tumor cancers are characterized by pronounced regions of hypoxia, which provide an opportunity for targeted therapies. One such therapeutic strategy utilizes highly cytotoxic small-molecule anticancer agents or vascular disrupting agents (VDAs), which are synthesized as their corresponding bioreductively activatable prodrug conjugates (BAPCs). Ideally, the BAPC remains structurally intact and non-cytotoxic until it reaches areas of tumor hypoxia in which reductase enzymes such as NADPH cytochrome P450 oxidoreductase cleave the prodrug portion of the conjugate thus releasing the cytotoxic anticancer agent or VDA site-specific to the tumor or tumor microenvironment. The natural products combretastatin A-4 (CA4) and combretastatin A-1 (CA1), originally discovered by Professor George R. Pettit (Arizona State University), have shown significant promise in the clinical trials as VDAs. A gem-

dimethylnitrothienyl BAPC of CA4 (previously prepared by Peter Davis and co-workers) demonstrated the ability to release CA4 from A549 cells under hypoxic conditions. Inspired by this work, a series of nitrothiophene-based BAPCs of CA1 were designed for chemical synthesis. The efficacy of these BAPCs will initially be evaluated by determining their differential cytotoxicity under normoxia versus hypoxia in selected human cancer cell lines. The most active BAPCs are candidates for future *in vivo* evaluation in mice bearing tumors with profound regions of hypoxia.

MEDI 147

Mitochondria-targeted nanoparticle assisted activation of dendritic cells for immune boosting treatment of breast cancer

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Photodynamic therapy (PDT) is an anti-cancer treatment that involves the administration of a photosensitizer (PS) followed by the irradiation by long-wavelength light. This leads to tumor cell destruction and induction of the host cell immune through the generation of reactive oxygen species. However, much optimization is needed in order to reach PDT's full potential to boost the host immune system. We speculated that by delivering the PS preferentially to its target organelle, the mitochondria, the result would be more efficient cancer cell apoptosis and immune system boosting. Recently, we developed a mitochondria-targeted polymeric nanoparticle (NP) platform that has the ability to deliver a wide array of payloads to the mitochondria.[1] Here, we report the potential of MCF-7 breast cancer cell antigens derived from mitochondria-targeted delivery of zinc phthalocyanine (ZnPc) using our NP system followed by light activation with a long wavelength laser of these cells in activating dendritic cells (DCs).[2] The tumor antigens generated were found to induce DC maturation and production of interferon-gamma.[2]

References:

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2) Marrache, S.; Tundup, S.; Harn, D.A.; Dhar, S. *Ex Vivo* Programming of Dendritic Cells by Mitochondria-Targeted Nanoparticles to Produce Interferon-Gamma for Cancer Immunotherapy. *ACS Nano*, **2013** , 7392–7402.

MEDI 148

Design, synthesis, and screening of novel dUTPase inhibitors for the treatment of resistant cancers

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A large number of cancer patients do not respond to 5-fluorouracil (5-FU) and other thymidylate synthase (TS) inhibitors, which have long being used as the standard of care for various cancers. This type of resistance can be attributed to the over-expression of the enzyme dUTPase, which allows the cells to regulate mis-incorporation of uracil through the conversion of deoxyuridine triphosphate (dUTP) to deoxyuridine monophosphate (dUMP). The development of selective dUTPase inhibitors, therefore, is of great interest to cancer therapy. Through the use of *in silico* modeling and virtual high-throughput screening, a new class of dUTPase inhibitors was designed, synthesized, and later optimized by SAR. Subsequent phenotypic screening revealed that these optimized molecules exhibit potent anticancer effects and enhance the cytotoxicity of TS inhibitors in various cancer cell lines. These new heterocyclic small molecules represent a potentially new class of therapeutics for the treatment of resistant cancers.

MEDI 149

Therapeutics resources for TB R&D: U.S. National Institute of Allergy and Infectious Diseases

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The Division of Microbiology and Infectious Diseases (DMID) of the National Institutes of Allergy and Infectious Disease (NIAID) of the National Institutes of Health (NIH) is committed to finding new ways to better understand, diagnose, treat, and prevent tuberculosis (TB). The high burden of TB disease in many countries of the world, and the emergence of multi-drug resistant (MDR) and extensively drug resistant (XDR) TB has intensified the need for new interventions. DMID supports a comprehensive research and product development grants portfolio and offers TB specific preclinical product development services, research reagents and bioinformatics resources to facilitate the discovery and development of new drugs.

This presentation focuses on TB drug candidates in world-wide development as well as on services and funding opportunities for biomedical research in TB that are issued through NIAID's Biodefense and Emerging Infectious Diseases Program. These services include *in vitro* evaluation of new chemical entities, *in vivo* animal model testing, access to mycobacterial research reagents, genome sequencing, bioinformatics support, formulation, and chemical synthesis including GMP manufacturing. Clinical resources include Phase 1 evaluations of candidate interventions.

Services are available without charge to U.S. and international investigators in academia, not-for-profit organizations, industry and government through an application process. The approval process is transparent and begins with an informal exploration of the request with the DMID Program Officer followed by institutional review using standardized criteria. Services are contingent upon availability of required preliminary data, and legal agreements ensure confidentiality and protection of intellectual property rights.

For more information see: http://www.niaid.nih.gov/labsandresources/resources/

MEDI 150

Discovery and development of bedaquiline: A rocky road

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Bedaquiline (TMC207) is the first drug with a new mechanism of action against M. tuberculosis in decades. Microbiologically this diarylquinoline is very potent *in vitro* and *in vivo*, but its physicochemical properties are far from ideal. Its development path was paved with ups and downs. FDA granted accelerated approval for Sirturo as part of combination therapy to treat adults with pulmonary Multi-Drug Resistant Tuberculosis

MEDI 151

Spectinamides: A new class of antituberculosis agents that overcome native drug efflux

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Spectinomycin is a potent bacterial protein synthesis inhibitor yet has poor antimycobacterial activity. Spectinomycin was modified using structure based drug design to generate a novel series of 3'-substituted spectinamides. From the 140 spectinamides synthesized, a clear structure activity relationship has been established with respect to antitubercular activity and protein synthesis inhibition. Analogs with 2-heteroaryl acetic acid substitution displayed the best activities. From this panel, the most potent compounds showed M. tuberculosis MICs that are far superior to the MIC of Spectinomycin and comparable to Streptomycin. Lead spectinamides are not cross resistant to any current tuberculosis therapeutics. Key to their potent antitubercular properties was their structural modification to evade the Rv1258c efflux pump. In murine

infection models, these spectinamides were well tolerated, significantly reduced lung tuberculosis burden alone and in combination with other agents.

MEDI 152

Bayesian models to accelerate tuberculosis drug discovery

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The identification of novel leads constitutes a significant hurdle in the resource-limited setting of drug discovery. This challenge is magnified in neglected diseases such as tuberculosis, characterized by ~2 million deaths annually and a need for shorter therapeutic regimens addressing drug resistance. We have leveraged high-throughput screening data, a multi-year and multi-million dollar investment by public and private institutions, to empirically validate single- and dual-event Bayesian models. We virtually screened a commercial library and experimentally confirmed actives with hit rates exceeding typical rates by 1–2 orders of magnitude. The first dual-event Bayesian model identified compounds with antitubercular whole-cell activity and low mammalian cell cytotoxicity from a literature set of antimalarial small molecules. The most potent hit exhibits the *in vitro* activity and *in vitro/in vivo* safety profile of a drug lead. These machine learning models offer significant economies in time and cost while being broadly applicable to drug discovery in the workflow ranging from hit to lead to clinical candidate.

MEDI 153

WITHDRAWN

MEDI 154

WITHDRAWN

MEDI 155

Molecular modeling and in silico characterization of mutations associated with multiple drug resistant tuberculosis (MDR-TB) and extensive drug resistant tuberculosis (XDR-TB)

***Sravanthi Joginipelli**, SXJOGINIPELL@UALR.EDU, Jerry A Darsey. Bioinformatics, University of Arkansas at Little Rock, University of Arkansas for Medical Sciences, Little Rock, AR 72204, United States*

Increasing emergence of multiple and extensive drug resistant tuberculosis poses a significant threat to the effective control of tuberculosis (TB) globally. Drug resistance in Mycobacterium tuberculosis arises from inadequate or inappropriate drug taking or drug prescribing. Drug resistance can be either primary drug resistance or secondary drug resistance (acquired drug resistance). Resistance of mycobacterium tuberculosis to most effective antibiotics is due to spontaneous mutations in its gene sequence, mutations causing resistance to drugs and posing new challenges to the treatment of disease. Here in this research, mutations associated with isoniazid, Pyrazinamide and flouroquinolone drug resistance were analyzed to get a better understanding of the disease. Highly frequent mutations in katG, inhA, pncA, gyrA, and gyrB genes causing resistance to isoniazid, pyrazinamide and flouroquinolones were chosen to predict three-dimensional structure of the modified protein due to mutations using comparative modeling techniques. The modified protein structures from mutated genes were taken for analysis using molecular modeling techniques and QSAR studies (Quantum Structure Activity and Relation) to understand the interactions with specific drug targets. Docking studies and QSAR studies were performed to predict binding affinities and ADME properties (Absorption, Distribution, Metabolism and Excretion). Structural properties of mutated genes were calculated with electronic structure modeling program and the properties were chosen to predict potential drug candidates using Artificial Neural Networks (ANN). Interactome of protein interactions is generated to understand the functions of mutated proteins. Insights into interactions with drug targets, binding affinities and ADME properties of mutated proteins would enable the development of new and rapid diagnostic techniques and will further help in development of new drugs for the treatment of TB, that can shorten the TB therapy.

MEDI 156

Studies directed toward the design of benzoxazinorifamycins less susceptible to emerging resistance

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Rifampin (RMP), a semisynthetic rifamycin, is the cornerstone of current tuberculosis treatment. Among many semisynthetic rifamycins, benzoxazinorifamycins (bxRIFs) have great potential for tuberculosis (TB) treatment due to their superior affinity for wild-type and rifampin-resistant *Mycobacterium tuberculosis* RNA polymerases and their reduced hepatic Cyp450 induction activity. We have determined crystal structures of

Escherichia coli RNA polymerase complexed with two newer generation bxRIFs, which show the C3′-tail of the bxRIF located in a gap between the β subunit fork loop 2 (green) and σ finger (orange). Predominant Rif-resistant mutations are D516, H526, and S531. **[figure 1]** Arg540 of the fork loop 2 is proximal to the ansa-naphthalene core of RMP/bxRIF. There are no reports of any mutation of this residue in the TB Drug Resistance Mutation Database (*PLoS Med* **2009** , *6*, e1000002). The fork loop 2 plays an important role in the DNA unwinding during transcription. Hence mutations of Arg540 may cause a larger defect in RNAP "fitness" that the organism cannot tolerate. This presentation will discuss the generation and characterization of Arg540 mutants of *E. coli* RNAP toward exploring fork loop 2 as a potential new target, as well as more recent structural biology studies that should aid in the development of rifamycin derivatives less susceptible to emerging resistance.

MEDI 157

Lessons learned in TB drug discovery

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Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb),is an airborne infectious disease that infects almost one-third of the world's human population. Effective chemotherapy for TB has existed since the 1940s, however, an important factor contributing to the resurgence of the disease is the emergence of multi-drug resistant tuberculosis (MDR TB). MDR TB is caused by Mtb that is resistant at least to isoniazid and rifampicin, the two most potent anti-TB drugs. Current treatment for MDR TB consists of lengthy regimens that are less effective, more expensive and less well tolerated. Therefore there remains an urgent need for new TB drugs that are able not only to shorten the long treatment regimen but also to control drug resistant forms of TB and that can be used along with the current AIDS/HIV retroviral treatments.

To identify a new starting point in the development of new TB drugs, the Novartis internal small molecule chemical library was screened for activity against *Mycobacterium bovis* BCG as a surrogate of Mtb by measuring ATP levels using the BacTiter-Glo assay.Subsequent hit confirmation with Mtb H37Rv led to the identification of a handfull novel chemical entities, which are partially described in this presentation. The key issues, from hit quality, hit to lead to lead optimization to identify preclinical candidates are captured as lessons learned.

MEDI 158

Design and development of quadruplex-targeted agents in human cancer

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Quadruplex nucleic acids can be formed in telomeric regions of chromosomal DNA as well as in promoter and other regulatory sequences of a number of genes involved in human cancer. Quadruplexes can be stabilised by a wide range of small molecules and the resulting complexes can act as impediments to effective telomere maintenance, to transcription or translation, depending on their genomic location. In practice, polyquadruplex targeting may be both an effective and feasible way of exploiting quadruplex genomics information since it is straightforward to devise compounds with selectivity for quadruplex over duplex DNA, but far more challenging to create compounds that effectively discriminate between different quadruplexes in favour of a single one.

It is now sixteen years since the initial publication of the concept that a disubstituted amidoanthraquinone can stabilise a human telomeric quadruplex sequence and inhibit the action of the telomerase enzyme (Sun et al, J Med Chem, 1977). A very large number of these polyheterocyclic compounds, usually with attached cationic side-chains compounds have subsequently been reported, although rather few have been evaluated in human cancer cell lines. Some such as the naphthalene diimides, are exceptionally potent, with an ability to inhibit cell growth at the nM level. In vivo and mode of action data will be presented for a lead compound in this class, optimised by structure-based methods, and which is currently undergoing development for the treatment of pancreatic cancer. Evidence will be presented that a primary mode of action involves binding to telomeric DNA single-stranded ends and induction of quadruplex formation, although other genomic quadruplex targets may also be implicated.

MEDI 159

Macrocyclic polyoxazoles as highly selective G-quadruplex ligands

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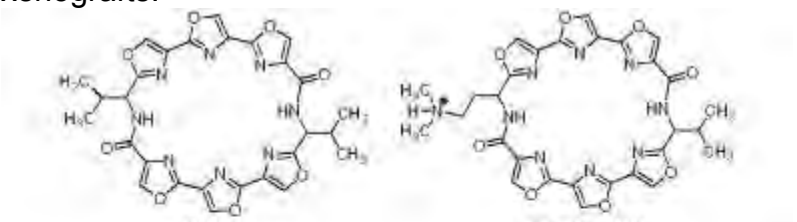
Oxazole-containing macrocycles represent a promising class of anticancer agents that target the G-quadruplex structural form. This presentation will emphasize the quadruplex-targeting, cytotoxicity, and *in vivo*antitumor properties of a series of synthetic oxazole-containing macrocyclic compounds. These properties include the following:

1. Highly selective binding to the G-quadruplex form of both DNA and RNA, with little or no affinity for the duplex or triplex form of either nucleic acid.
2. A nonintercalative, “terminal capping” mode of binding to G-quadruplex structures.

3. Potent anti-proliferative activity versus ***both*** telomerase-positive and telomerase-negative human tumor cells, suggesting that the anti-proliferative activity of the compounds is independent of telomerase status.
4. Induction of rapid apoptotic cell death and cell cycle arrest at the prometaphase stage of mitosis.
5. *In vivo* antitumor efficacy in athymic nude mice containing human breast tumor xenografts.



MEDI 160

Shape-dependent, multiple binding modes of hetero-polycyclic cations with DNA quadruplexes

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DNA quadruplex structures are important for the regulation of genomic function in disease cells from cancer to parasitic eukaryotic microorganisms. Selective targeting of quadruplex structures is thus an area of significant promise for discovery of novel drug candidates. Although the G-quadruplex has various potential recognition sites for small molecules, the primary interaction site of most compounds discovered to date is the terminal quadruplex tetrads. Similar to duplex-DNA groove recognition, however, quadruplex groove recognition by designed compounds offers the potential for additional selectivity in recognition among different quadruplexes. Analysis of quadruplex structures suggested the following initial discovery criteria for groove complexes: positive charges to complement the negative charges on quadruplexes as well as help with solution properties, polycyclic heterocycles with hydrogen bond donors

and acceptors, a variety of linking methods for the heterocyclic units, and a variety of molecular shapes to find an appropriate complement for different quadruplex grooves and to minimize any match for duplex grooves. For initial evaluation of binding mode we have relied on induced changes in compound circular dichroism, although this method has limitations. We have supplemented the CD binding mode studies with NMR analysis of several quadruplex complexes. As with the vast majority of compounds demonstrated to bind strongly to quadruplexes to date, most of the systems that we prepared also preferred an end-stacking binding mode, although with a significance preference over DNA duplex binding. With some compounds small induced CD signals were observed at low ratios of compound to quadruplex but strong induced CD at higher ratios. This indicates at least two binding modes with an initial end-stacking interaction. Although limited, evidence suggests that end stacking is followed by a groove-binding mode for some compounds. Both the induced CD and NMR results have an interesting dependence on compound and quadruplex structure. (Support NIH–NIAID Grant AI064200)

MEDI 161

Dissection of individual G-quadruplex structures with optical scalpels

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Enzymes involved in many fundamental cellular processes, such as DNA replication, RNA transcription, and protein translation, are motor proteins that apply a load force during catalytic reactions. Interaction of these motor proteins with secondary structures in nucleic acid templates can therefore be understood from a mechanical perspective. To serve as an effective mechanical blocker to motor proteins, the mechanical stability of these secondary structures, which include G-quadruplex and i-motifs, must be strong enough to withstand the load force of motor proteins. Optical tweezers provide a convenient tool to evaluate the mechanic stability of nucleic acid structures. Since 2009, we have evaluated mechanical stabilities of hairpins, G-quadruplexes, and i-motifs in DNA, and G-quadruplexes in RNA. Under physiologically relevant conditions, such as in double stranded DNA templates or in crowded solutions, mechanical stabilities of G-quadruplexes are higher than stall forces of polymerases. Mechanical stability of a structure is dependent on unfolding angles. Due to the limitation in current molecular biology strategies that process nucleic acid templates along the 5'-3' direction, most of mechanical unfolding has been confined into the same direction, which may not necessarily be a physiologically relevant angle. Assisted with click chemistry modifications, we expanded the unfolding into other geometries by introducing two unfolding handles at specific residues inside a G-quadruplex forming sequence. Statistical analyses allowed retrieving kinetic, thermodynamic, and mechanical information of nucleic acid structures along desired trajectories. In the presence of G-quadruplex specific ligands, such information demonstrates significant difference, suggesting that the interaction between small molecules and G-quadruplexes not only

changes the kinetic and thermodynamic properties, but also varies the mechanical stability of G-quadruplexes.

MEDI 162

Transcriptional complex between the BCL2 i-motif and hnRNP LL is a molecular switch to control gene expression that can be modulated by small molecules

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In this presentation I will demonstrate that the C-rich strand of the cis-regulatory element in the *BCL2* promoter element is highly dynamic in nature and can form either an i-motif or a flexible hairpin. Under physiological conditions these two secondary DNA structures are found in an equilibrium mixture, which can be shifted by the addition of small molecules that trap out either the i-motif (IMC-48) or the flexible hairpin (IMC-76). In cellular experiments we demonstrate that the addition of these molecules has opposite effects on *BCL2* gene expression and that these are antagonistic. Furthermore, we have identified a transcriptional factor that recognizes and binds to the *BCL2* i-motif to activate transcription. The molecular basis for the recognition of the i-motif by hnRNP LL is determined, and we demonstrate that the protein unfolds the i-motif structure to form a stable single-stranded complex. In subsequent experiments we show that IMC-48 and IMC-76 have opposite, antagonistic effects on the formation of the hnRNP LL–i-motif complex as well as on the transcription factor occupancy at the *BCL2* promoter. For the first time we propose that the i-motif acts as a molecular switch that controls gene expression and that small molecules that target the dynamic equilibrium of the i-motif and the flexible hairpin can differentially modulate gene expression.

MEDI 163

Dengue virus: A major public health problem worldwide

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Dengue virus (DENV) is a significant cause of morbidity and mortality in tropical and subtropical regions, causing hundreds of millions of infections each year. Infections range from asymptomatic to a self-limited febrile illness, dengue fever (DF), to the life-threatening dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS). The expanding of the habitat of DENV-transmitting mosquitoes has resulted in dramatic increases in the number of cases over the past 50 years, and recent outbreaks have occurred in the United States. Developing DENV-specific vaccines and antivirals is a global health priority. DENV vaccine and antiviral development is challenging due to the existence of four serotypes of DENV (DENV1-4), which a vaccine or an antiviral must protect against. Additionally, the adaptive immune response to DENV may be both

protective and pathogenic upon subsequent infection, and the precise features of protective versus pathogenic host response to DENV are unknown, complicating vaccine and antiviral development.

MEDI 164

Identification and optimization of novel dengue virus inhibitors

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The World Health Organization estimates dengue viruses (DENV) infect 50-100 million people worldwide every year, of which 500,000 develop severe life-threatening disease. It causes approximately 25,000 deaths annually. Despite extensive research, there are not yet any approved vaccines or therapeutics commercially available to prevent or treat DENV infection. SIGA screened a 200,000 small molecule library using a whole virus assay and identified four series of compounds. Each series was evaluated for its chemical tractability, potency and selectivity. Preliminary structure-activity relationship (SAR) studies resulted in two lead series. Compounds in both series have shown efficacy in a sub-lethal murine model of DENV infection with the ability to significantly reduce viremia and viral load in target organs compared to vehicle-treated controls. Further optimizations considerably improved the potency against all four dengue serotypes and ADME properties. Compound resistances for ST-148 and ST-610 were mapped to the DENV capsid (C) gene and the NS3 helicase domain respectively. Ultimately a lead candidate was selected for preclinical development.

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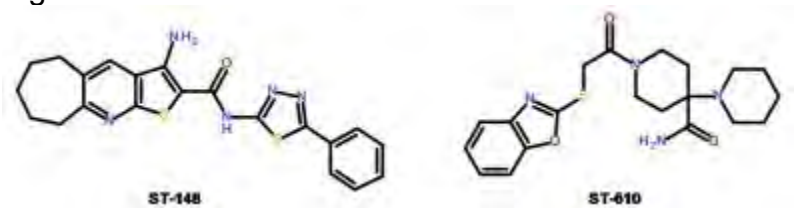

MEDI 165

Dengue drug discovery

***Pei Yong Shi**, pei_yong.shi@novartis.com. Department of Disease Biology, Novartis Institute for Tropical Diseases Pte Ltd, Singapre Country, Singapore*

Dengue virus (DENV) is the most prevalent mosquito-borne viral pathogen for humans. DENV annually causes about 390 million human infections, leading to 96 million cases with manifest symptoms. No clinically approved vaccine or antiviral is currently available

for DENV. In this presentation, I will review the current status of dengue drug discovery; in addition, I will present progresses made at Novartis Institute for Tropical Diseases (NITD) towards the development of dengue antivirals. Four strategies have been pursued to identify inhibitors of DENV through targeting both viral and host proteins. (i) HTS (high-throughput screening) using virus replication assays; (ii) HTS using viral enzyme assays; (iii) Structure-based *in silico* docking and rationale design; (iv) Repurposing hepatitis C virus inhibitors for DENV. Along the developmental process from hit finding to clinical candidate, many inhibitors did not advance beyond the stage of hit-to-lead optimization, due to poor selectivity, physiochemical properties, or pharmacokinetic properties. Only a few compounds showed efficacy in the AG129 mouse model. Two nucleoside analogs, NITD-008 and Balapiravir, entered preclinical animal safety study and clinic trial, but both were terminated due to toxicity and lack of potency, respectively. Celgosivir, a host alpha-glucosidase inhibitor, is currently under clinical trial; its clinical efficacy remains to be determined. The knowledge accumulated during the past decade has provided better rationale for the ongoing dengue drug discovery. Though challenging, I am optimistic that the continuous, concerted effort will lead to an effective dengue therapy.

MEDI 166

Fighting against malaria: Strategies and latest advances in the discovery of new effective therapies

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Nowadays, Malaria is still one of the major global major health problems. Plasmodium has been able to adapt to the different treatment developed by human along history however there has not been such a wide knowledge as currently. This fact joined to the urgent need for novel antimalarial drugs that can replace ACTs and the awareness of governments/health system/funding agencies makes of these times a unique opportunity to change the course of this disease and achieve the control and finally the eradication.

We at the Tres Cantos, Medicines Development Campus of GlaxoSmithKline (GSK) have been working align through different public-private-partnerships (PPP) in developing efficacious tools (chemicals and biologicals) to help in stop this burden. The machinery to find drugs with a profile suitable to control and block the transmission of the disease is running and starting to deliver.

This communication will focus on the progress in developing the necessary technology and chemical series that can deliver assets able to cure and eradicate malaria.

MEDI 167

Development of antimalarial compounds with new mechanisms of action using next-generation synthesis

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Malaria is one of the most threatening diseases worldwide with approximately one third of the world's population at risk and mortality estimated at 780,000 deaths per year. Since an effective vaccination is not yet available, small-molecule-based medicines are currently the best options for treating patients suffering from malaria. However, given the emergence of resistance against existing medicines, there is a pressing need for the discovery of new classes of compounds not related to existing pharmacophores, but with unique cores that are more likely to have novel mechanisms of action. We have reported the preparation of a screening collection of 100,000 diverse small molecules using a diversity oriented synthesis (DOS) strategy. The DOS compounds combine the complexity of natural products and the efficiency of high-throughput synthesis. This library of compounds was screened in a blood stage phenotypic assay against the multi-drug resistant *Dd2 Plasmodium falciparum* strain. Screening and triaging strategies to maximize the potential of identifying anti-malarials with novel mechanisms of action as well as medicinal chemistry optimization efforts will be discussed.

MEDI 168

Discovery, optimization, and mode of action of spiroindolone antimalarials

***Bryan KS Yeung**, bryan.yeung@novartis.com. Novartis Institute for Tropical Diseases, Singapore*

The antimalarial drug arsenal has historically benefitted from the phenotypic screening approach to identify lead molecules in the search for new drugs; with artemisinin being one of the most notable examples. As part of an effort to discover new antimalarial drugs we screened the Novartis natural product library to identify a spiroindolone hit which was the focus of a lead optimization effort resulting in the discovery and development of KAE609 (NITD609). In a parallel effort, we applied a full genome analysis of spiroindolone-resistant parasites to conclude that the spiroindolones work though a unique mode of action, namely the inhibition of a P-type *Plasmodium falciparum* ATPase4 (a Na^+ efflux pump). Disruption of Na^+ homeostasis by the spiroindolones was shown to rapidly lead to parasite death. KAE609 represents the first antimalarial chemotype working though a novel mechanism of action to enter Phase II clinical trials in over 20 years.

MEDI 169

Discovery of the Nedd8 activating enzyme inhibitor MLN4924

***Steven P Langston**, steve.langston@mpi.com. Department of oncology chemistry, Takeda Pharmaceuticals International Co., cambridge, MA 02139, United States*

The ubiquitin-proteasome system (UPS) and ubiquitin-like protein (Ubl) conjugation pathways are integral to cellular protein homeostasis. NEDD8 conjugation is essential for the enzymatic function of cullin-RING ligases (CRLs) a family of ubiquitin ligases (E3s) that target protein substrates for degradation by the proteasome, including several substrates with important roles in cancer growth and survival. To test the therapeutic potential of NEDD8 pathway inhibition, we identified an investigational small molecule inhibitor of the NEDD8 Activating Enzyme (NAE) known as MLN4924. NAE catalyses the formation of a NEDD8-inhibitor adduct with MLN4924 by a mechanism referred to as 'substrate assisted inhibition'. MLN4924 is currently being evaluated in clinical trials for solid and hematological malignancies. The discovery of MLN4924, a first in class inhibitor of NAE, and the mechanism of enzyme inhibition will be presented.

MEDI 170

Novel targets in oncology: Discovery of novel allosteric inhibitors of IRE1α

***Karen A Evans**, karen.a.evans@gsk.com. Department of Oncology R&D, GlaxoSmithKline, Collegeville, PA 19426, United States*

Inositol-requiring enzyme 1α (IRE1α) is being studied as a potential cancer target due to its role in the unfolded protein response (UPR) signaling pathway. Multiple myeloma (MM) cells are particularly dependent on the UPR for survival. Use of high-throughput screening of the GSK proprietary compound collection identified a novel class of allosteric inhibitors of IRE1α. During the hit-to-lead optimization phase, key structure-activity relationships (SAR) were rapidly identified, and a crystal structure of hIRE1α with a small molecule inhibitor bound was obtained. Details of the early exploratory efforts using these small molecule inhibitors, including SAR, synthesis, and structure-based drug design, will be presented.

MEDI 171

Drug discovery efforts towards the identification of potent and selective Tankyrase inhibitors

***Michael Visser**, michael.visser@novartis.com, Michael Schultz, Atwood Cheung, B. Barry Toure, Brant Firestone, Christina Kirby, Donovan Chin, Ty Gould, Jianmei Fan, Lucian DiPietro, Mark Palermo, Troy Smith, Ronald Tomlinson, Dyuti Majumdar, Etienne Ochala, Nigel Waters, Pascal Fortin, Zhoulinang Chen, Wenlin Shao. Novartis Institutes for Biomedical Research, Cambridge, MA 02139, United States*

Tankyrase 1 and 2 are members of the poly(ADP-ribose) polymerase (PARP) family of enzymes that have been shown to modulate the Wnt pathway of signaling. Deregulation of the Wnt pathway has been implicated in many cancers, making it an attractive target

for anticancer therapies. A structure based drug design driven approach to optimization of multiple chemical series has lead to an understanding of a common pharmacophore that is necessary to demonstrate in vitro and in vivo functional activity. Herein we describe our drug discovery effort in the identification and optimization of potent selective inhibitors which are well suited for further in vivo validation studies.

MEDI 172

Allosteric inhibition of the oncogenic serine/threonine phosphatase Wip1

Dirk A. Heerding*, dirk.a.heerding@gsk.com. Oncology R&D, GlaxoSmithKline, Collegeville, PA 19426, United States*

The Wild-type p53-Induced Phosphatase (Wip1, PPM1D) is an oncogenic type 2C serine/threonine phosphatase that negatively regulates key regulatory proteins in the DNA damage response pathway. Amplification of the PPM1D gene locus on 17q23 has also been reported in various cancers and Wip1 overexpression is believed to promote tumorigenesis by inactivating the tumor suppressor function of multiple substrates. These attributes provide evidence for the therapeutic potential of inhibiting Wip1 as a strategy to treat certain cancers. Here we report the discovery of a series of selective allosteric small molecule inhibitors of Wip1 phosphatase. The detailed characterization of the mechanism of action and key structure-activity relationships for this series will be presented.

MEDI 173

Discovery of poly(ADP-ribose) polymerase-1 (PARP-1) inhibitor clinical candidate CEP-9722

Robert L. Hudkins*, robert.hudkins@tevapharm.com, Allison L. Zulli, James L. Diebold, Greg Wells, Lisa D. Aimone, Jean Husten, Sheila Miknyoczki, Bruce A. Ruggeri, Mark A. Ator. Discovery and Product Development, Teva Pharmaceutical Global R&D, West Chester, PA 19380, United States*

Poly (ADP-ribose) polymerase 1 (PARP-1) is a nuclear enzyme that catalyses the synthesis of poly (ADP-ribose) chains from NAD^+ as part of the single-strand DNA repair process. PARP-1 inhibitors have therapeutic utility in oncology through potentiation of the anti-tumor activity of radiation or DNA damaging chemotherapeutic agents, and 'targeted' therapy in BRCA-mutation associated breast cancers. We previously reported a potent pyrrolocarbazole PARP-1 HTS hit (IC_{50} value of 36 nM) identified from our internal library. Lead optimization on this novel chemotype to improve cellular potency, physical properties and selectivity produced lead CEP-8983. A novel Mannich base prodrug strategy led to clinical candidate CEP-9722. The synthesis, SAR and preclinical development leading to the identification of CEP-9722 will be presented.

MEDI 174

Discovery of potent and highly selelctive inhibitors of CYP17 lyase

Aaron Balog[1]*, aaron.balog@bms.com, Audris Huang*[1]*, Upender Velaparthi*[1]*, David B Frennesson*[1]*, Mark G Saulnier*[1]*, Chetan Darne*[1]*, Peiying Liu*[1]*, Lata Jayaraman*[2]*, Thomas E Spires*[2]*, Venessa Rodriguez*[2]*, Cheryl A Rizzo*[2]*, Mary T Obermeier*[3]*, Aberra Fura*[3]*, Paul A Elzinga*[3]*, Gordon L Todderud*[5]*, Yi Fan*[5]*, John Newitt*[6]*, Sophie M Beyer*[4]*, Marco M Gottardis*[2]*, Geroge L Trainor*[1]*, Dinesh Vyas*[1]*, Gregory D Vite*[1]*. (1) Department of Oncology Chemistry, Bristol-Myers Squibb, Princeton, NJ 08543, United States (2) Department of Oncology, Bristol-Myers Squibb, Princeton, NJ 08543, United States (3) Department of Preclinical Candidate Optimization, Bristol-Myers Squibb, Princeton, NJ 08543, United States (4) Department of Veterinary Sciences, Bristol-Myers Squibb, Princeton, NJ 08543, United States (5) Department of Lead Evaluation, Bristol-Myers Squibb, Princeton, NJ 08543, United States (6) Department of Protein Science and Structure, Bristol-Myers Squibb, Princeton, NJ 08543, United States*

Prostate cancer (CaP) is one of the leading causes of death in men in the U.S. and Europe. Endogenous androgens promote the development and progression of CaP via the androgen receptor. While surgical or chemical castration can reduce circulating androgen levels by ~90% and are effective therapies, CaP patients eventually progress to castration-resistant prostate cancer (CRPC) where tumor growth can be driven by very low levels of circulating androgens. The dual function cytochrome P450 enzyme CYP17 produces androgen precursors such as dehydroepiandrosterone (DHEA) and androstenedione (AdT) in both the adrenal glands and the testes. These steroids are then converted peripherally to the androgens testosterone (T) and dihydrotestosterone (DHT). Inhibition of CYP17 function can therefore reduce androgen levels beyond castration which eliminates gonadal androgen production only. Clinical proof of concept has been achieved for this approach with abiraterone acetate, which has been shown to significantly reduce circulating androgens in castrate patients, leading to significant clinical benefit. However, abiraterone acetate requires co-administration of steroids, such as prednisone, in the clinic to combat mineralocorticoid excess, presumably due to non-selective inhibition of CYP17 lyase. Our program objective was to find a highly selective non-steroidal inhibitor of CYP17 that has the potential to avoid mineralocorticoid excess in the clinic. We utilized iterative design and molecular modeling to discover novel inhibitors of CYP17 lyase. Compounds with the desired *in vitro* profile were dosed in both gonadally-intact and chemically-castrated cynomolgus monkeys. In these studies, levels of T, progesterone, hydroxy-progesterone and cortisol were monitored to gauge the pharmacodynamic (PD) effect and level of selectivity associated with lead compounds. These findings will be presented along with the chemical synthesis of the lead compounds.

MEDI 175

TRP channels as targets for drug discovery

Magdalene M Moran*, mmoran@hydrabio.com. Biology, Hydra Biosciences, Cambridge, MA 02138, United States*

The 28 mammalian TRP channels comprise a structurally and functionally diverse superfamily. Though TRPV1 remains the best-characterized member of the family, significant progress has been made elucidating the biological roles of several family members in areas ranging from pain and neurodegeneration to cardiovascular and pulmonary. Modulators of at least four TRP channels are in clinical trials in these indications. Somewhat surprisingly, the products that have gained clinical approval so far have been agonists rather than antagonists. The goal of this talk will be to discuss the current status of the field and provide an introduction to these complex and fascinating channels.

MEDI 176

TRPM8 blockade and its role in cold pain signalling

David C Pryde[1]*, David.Pryde@pfizer.com, Robert M Owen*[1]*, Alan D Brown*[1]*, Wendy J Winchester*[2]*, Mark Andrews*[3]*, Graham Lunn*[3]*. (1) Worldwide Medicinal Chemistry, Pfizer Neusentis, Cambridge, Cambridgeshire CB21 6GS, United Kingdom (2) Department of Biology, Pfizer Neusentis, Cambridge, Cambridgeshire CB21 6GS, United Kingdom (3) Department of Chemistry, Peakdale Molecular, Sandwich, Kent CT13 9NJ, United Kingdom*

The transient receptor potential (TRP) family of ion channels is made up of non-selective cation channels that respond to a wide range of chemical and thermal stimuli.

TRPM8, which is a member of the melastatin subfamily, is activated by cold temperatures (<25 °C) and antagonists of this channel have the potential to treat cold induced allodynia (pain caused by normally innocuous stimuli) and hyperalgesia (a prolonged or more intense pain response). However, TRPM8 has also been implicated in mammalian thermoregulation and antagonists have the potential to induce hypothermia in patients. In order to better understand this potential risk, we initiated a drug discovery program focused on delivery of a clinical tool compound.

This talk will cover the identification and optimization of a series of TRPM8 antagonists which ultimately led to the discovery of a clinical tool compound PF-05105679. The screening campaign that was initiated, hit selection and hit to lead studies will be described, through to the SAR that led to the identification of the clinical tool. In addition, the clinical results for this compound will be discussed, including both clinical efficacy and its ability to affect thermoregulation processes in humans.

MEDI 177

Identification of a selective TRPA1 antagonist that demonstrates potent *in vivo* inhibition

Laurie Schenkel*, schenkel@amgen.com. Department of Medicinal Chemistry, Amgen, Inc., Cambridge, MA 02142, United States*

TRPA1 is a nonspecific cation channel and member of the TRP super-family of ion channels. A single point, gain-of-function mutation in TRPA1 was identified in a Colombian family, and carriers of this mutation experience severe pain resulting from a range of stimuli. Activation of TRPA1 in animal models also caused neurogenic inflammation and pain. Conversely, knockout of TRPA1 and pharmacological antagonism of the channel in rodents reduced mechanical hypersensitivity in *in vivo* pain models. While the reported *in vivo* pharmacological data are encouraging, it has been challenging to confirm that efficacy is on-target because the inhibitors reported to date exhibit micromolar potencies and/or suffer from poor pharmacokinetic properties. Here we report the discovery of a series of potent and selective TRPA1 inhibitors. Optimization of a high-throughput screening hit led to a compound that demonstrated nanomolar potency in *in vitro* assays, and after oral dosing in rats provided unbound plasma concentrations >100-fold over the *in vitro* IC_{50} and strong inhibition of AITC-induced flinching. Data from *in vivo* pain efficacy studies as well as a comparison between effect of genetic ablation and pharmacological blockade of TRPA1 in *ex vivo* skin-nerve studies will also be presented.

MEDI 178

TRP channels in *C. elegans* sensory physiology

***Shawn Xu**, shawnxu@umich.edu. Life Sciences Institute, University of Michigan, Ann Arbor, MI 48109, United States*

The nematode *C. elegans* has emerged as a popular model for the study of various phenomena in neurobiology because of its simple and well characterized nervous system and amenability to genetic manipulation. The *C. elegans* genome encodes 17 TRP channel genes that fall within all of the seven TRP subfamilies and are highly homologous to their vertebrate counterparts. Genetic analyses in *C. elegans* have implicated TRP channels in a wide spectrum of behavioral and physiological processes, ranging from sensory transduction to drug dependence, fertilization, organelle biogenesis, apoptosis, gene expression, and neurotransmitter/hormone release. Many *C. elegans* TRP channels share similar activation and regulatory mechanisms with their vertebrate counterparts. Studies in *C. elegans* have also revealed some previously unrecognized functions and regulatory mechanisms of TRP channels. *C. elegans* represents an excellent genetic model organism for the study of function and regulation of TRP channels *in vivo*. Here we will discuss our recent work on the role of TRP channels in sensory physiology. Using a combination of genetic, behavioral and electrophysiological assays, we have characterized the *in vivo* function and regulation of a number of TRP channels, particularly TRPC, TRPV, TRPA, and TRPN channels. Our data show that these TRP channels are involved in regulating sensory physiology, including mechanosensation, thermosensation, and chemosensation.

MEDI 179

Discovery of GSK2193874: An orally active, potent and selective blocker of the transient receptor potential vanilloid 4

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TRPV4 is a member of the Transient Receptor Potential (TRP) superfamily of cation channels which is expressed in the lung and regulates the integrity of the alveolar septal barrier. Increased pulmonary vascular pressure evokes TRPV4-dependent pulmonary edema; therefore, inhibition of TRPV4 represents a novel approach for the treatment of pulmonary edema associated with congestive heart failure. 3-(1,4'-Bipiperidin-1'-ylmethyl)-7-bromo-N-(1-phenylcyclopropyl)-2-[3-(trifluoromethyl)phenyl]-4-quinolinecarboxamide (GSK2193874) is an orally active, potent and selective TRPV4 blocker. Our approaches leading to the discovery of GSK2193874 and its pharmacokinetic properties and biological profiles will be discussed.

MEDI 180

Diverse TRPA1 antagonists: Unexpected mechanisms of action, binding properties, and functional utility

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TRPA1 is a member of the transient receptor potential family of ion channels with genetic linkage to pain in man. These non-selective cation channels are activated by a variety of chemical mediators produced during tissue damage and inflammation. Most notably, covalent modifiers such as 4-hydroxynonenol and 15d-PGJ(2), which are generated during inflammation and modify N-terminal cysteines, activate TRPA1 channels expressed in nociceptive trigeminal and dorsal root neurons. Pharmacological inhibition of TRPA1 channel activation is therefore an attractive therapeutic strategy for the treatment of pain associated with tissue damage and inflammation.

This talk will cover the identification and characterization of TRPA1 antagonist chemotypes that are structurally unique from literature compounds. *In vitro* studies investigating the mechanism of action of these chemotypes will be discussed along with findings from *in vivo* behavioural studies. The results demonstrate that these identified compounds differentiate from known literature compounds in possessing unique target pharmacology and potentially a preferred mechanism of action.

MEDI 181

Metabolomics as a unique biochemical approach for understanding disease pathogenesis

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Metabolomics, the quantitative global analysis of endogenous metabolites from cells, tissues, fluids and whole organisms, is becoming an integral part of functional genomics efforts as well as a tool for understanding fundamental biochemistry. While the genome and proteome represent upstream biochemical events, metabolites correlate with the most downstream biochemistry and therefore most closely represent the phenotype. This has been proven by the broad success of metabolite analysis in clinical diagnostics. The experimental aim in our studies is to obtain a comprehensive quantitative view of the metabolome to expand our understanding of what pathways are altered in specific diseases. We have developed a novel mass spectrometry platform for metabolomics including XCMS Online data analysis combined with METLIN, a comprehensive MS/MS metabolite database. As well as nanostructure-initiator mass spectrometry (NIMS) imaging. These technologies will be presented in the context of their application to discovering new disease therapies/pathways for chronic pain and cancer.

MEDI 182

Metabotyping: Metabolic phenotypes for characterising rodent models of disease

***Ian D Wilson**, i.wilson@imperial.ac.uk. Surgery and Cancer, Imperial College, London, England SW7 2AZ, United Kingdom*

The application of modern MS and LC techniques (e.g., UHPLC-MS), has greatly increased the potential of this technology for “global” metabolic profiling (metabotyping) as used in metabonomics/metabolomics in the search for biomarkers. The ability to provide broad metabolite profiles of biological fluids and tissue extracts can be used to advantage in characterising disease rodent models of disease, and in monitoring response to therapy (including potential toxicity). Such methods offer opportunities in drug discovery and development, and also perhaps in translation to patients. A range of applications of different types of metabolic profiling will be used to illustrate some of the benefits of this “global” approach to metabolic profiling and also highlight the difficulties. One particular problem is not the detection of metabolites that *might* be biomarkers of a particular/disease conditions, but identifying them, and *then* understanding what they are actually biomarkers of. At its simplest the question that these metabolites pose is, are they really a set of useful mechanistic biomarkers delivering real insight to the biology being studied?, or just a more general, non-specific, response?, or worse an artefact of the experimental design!.

The potential of some emerging developments in analytical technology will be considered, together the approaches that can be taken once you have found your biomarker such as systems biology modelling and the construction of a combined systems biology/PBPK model that provides testable predictions.

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MEDI 183

Metabolic profiling of rodents and children with acetaminophen overdose

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Drug-induced liver injury (DILI) is a reason many drugs fail in preclinical and early phase clinical testing. Even marketed drugs have such liabilities. The commonly used, OTC drug, acetaminophen (APAP) is a major cause of DILI. APAP-induced toxicity causes oxidative stress, ROS production, glutathione depletion, mitochondria dysfunction, disruption of energy metabolism, and altered immune response. Biofluid and tissue samples from preclinical APAP toxicity studies were analyzed by genomics, proteomics and metabolomics technologies to discover omics biomarkers of liver injury and recovery. Some of the metabolomics biomarkers were found in the preclinical studies are being evaluated in blood and urine samples from children that were accidentally overdosed with APAP. Omics evaluations of samples from a preclinical study of APAP toxicity identified changes related to fatty acid beta-oxidation metabolism and bile acids transportation. Evaluation of these biomarkers in clinical samples showed elevations of bile acid metabolites occurred at the early stages of liver injury in association with elevations of APAP-protein adducts. Importantly, bile acid metabolites and APAP-protein adducts were increased prior to the elevation of ALT, a standard clinical test for liver injury. The data suggest that bile acid metabolites and APAP-protein adducts are very sensitive indicators of liver toxicity due to APAP overdose. These data can be used to stratify patients for potential clinical intervention, thus allowing a personalized medicine approach. To explore the universality of these biomarkers, efforts are underway to evaluate other drugs in animals and in humans including those of an idiosyncratic nature.

MEDI 184

Metabolomics in pharmaceutical research and development

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Metabolomics (also known as metabonomics or metabolic profiling) has emerged as a powerful tool for various applications within pharmaceutical research and development. Within the industry the technology has broad application from phenotyping transgenic models in early discovery through assessing bioreactor media in the manufacture of protein products. In particular, metabolomics is returning to its early discovery roots as a screening approach revealing unexpected off-target effects and unanticipated pharmacology of novel targets. Furthermore, metabolomics has proven invaluable in evaluating preclinical models and in reverse-translation approaches where clinical metabolomic data from target populations can be used to assess how well preclinical models mimic clinical physiology and pathology providing novel insights as to why drugs that are promising in preclinical evaluations fail for lack of efficacy in the clinic. Clinical applications including patient stratification, efficacy and safety biomarker discovery are currently emerging as the new frontier for the technology. Technologically, annotated metabolite lists have largely replaced the ubiquitous PCA plots of early efforts. It is not uncommon to measure several hundred metabolites across urine and plasma, even more if tissue extracts are assessed. Applications to be discussed include metabolomics in early discovery screening for identification of unexpected off-target effects and unanticipated pharmacology of novel targets. Other examples will demonstrate how metabolomics has proven invaluable in evaluating preclinical models and in reverse-translation approaches. The technology also has proven powerful for providing fresh insights into nettlesome toxicities, revealing unanticipated pathophysiology and the potential for novel biomarkers.

MEDI 185

Atropisomer axial chirality as a valuable tool in drug discovery

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The optimization of ligand binding to a receptor requires an appropriate 3D complementarity. Currently, medicinal chemists employ atom-centered chirality as a valuable molecular engineering tool to design ligand substituents as vectors to optimize this complementarity. This presentation focuses on a largely overlooked alternative source of drug chirality – atropisomerism,which has the distinct feature of creating molecular chirality as a result of hindered rotation about a bond axis. Practical tools and strategies are discussed to reveal and exploit this time-dependent property. A variety of chemotypes will be presented as an aid for recognizing atropisomeric compounds, along with computational and experiment tools for predicting and monitoring rotational characteristics. This should provide medicinal chemists with multiple options for designing compounds that rotate faster or slower, with the goal of optimizing 3D complementarity and specificity for the desired receptor. Furthermore, a categorization

scheme is proposed as a guide to help bridge the efforts of chemists at the early drug discovery stages with later efforts of development scientists. Overall, this presentation emphasizes the view that ligand atropisomerism can be successfully employed as a valuable tool in drug discovery.

MEDI 186

Design of non-planar HIV integrase inhibitors

Emile J Velthuisen*, emile.j.velthuisen@gsk.com. GlaxoSmithKline Research & Development, Infectious Diseases Therapy Area Unit, GlaxoSmithKline, Research Triangle Park, NC 27709, United States*

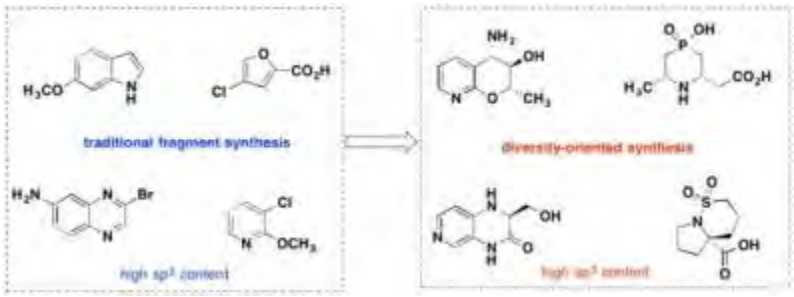
Dolutegravir (DTG) and GSK1265744A are second generation integrase inhibitors that have superior resistance profiles against clinically relevant integrase mutations. Evidence suggests that the stereochemical 3-dimensional conformation of the bicyclic N-acyl hemiaminal ring system contributes both to the exceptional potency and DMPK of this series. Our objective was to incorporate non-planar structural elements into other scaffolds to serve as a potential backup for these clinical assets. This presentation will focus on the 8-hydroxyquinoline tricyclic lactam (HQT) scaffold and describe a novel strategy for their construction, early chemistry problems encountered, and their virological profiles.

MEDI 187

Diversity-oriented synthesis for fragment-based discovery

Damian W Young*, dyoung@broadinstitute.org. Pharmacology, Baylor College of Medicine, Houston, TX 77030, United States*

Fragment-based screening (FBS) has become a popular and complementary strategy to the high-throughput (HTS) screening of large libraries of drug-like molecules. However, the diversity in fragment screening collections is lower than that of larger drug-like molecules. Fragment chemical space has traditionally relied heavily on the use of highly sp^2-carbon atom enriched aromatic compounds. Accordingly, it is rational to consider that a limited subset of biological space can be targeted with such compounds. We have utilized diversity-oriented synthesis as a guiding algorithm to produce fragments having greater sp^3/sp^2 carbon ratios toward the goal of engaging a wider spectrum of biological targets using FBS. In addition to enabling the discovery phase, DOS fragment pathways through their highly modular synthesis anticipate the chemical optimization of fragments. The talk will focus on the synthetic design and execution of DOS pathways to generate fragment libraries. It will further illustrate the utility of DOS derived fragments in screening campaigns.



MEDI 188

Use of the bicyclo[1.1.1]pentane motif as a nonclassical phenyl bioisostere with increased 3-dimensionality

***Antonia F Stepan**, Antonia.Stepan@pfizer.com. Department of Neuroscience Medicinal Chemistry, Pfizer, Cambridge, MA 02139, United States*

Replacement of the para-substituted fluorophenyl ring in the γ-secretase inhibitor BMS-708,163 with the bicyclo[1.1.1]pentane motif led to the discovery of an equipotent enzyme inhibitor with significant improvements in passive permeability, metabolic stability and aqueous solubility. These favorable *in vitro* properties translated into a significantly improved oral absorption and can be attributed to the increased 3-dimensionality relative to the parent biaryl compound. This work enhances the scope of the [1.1.1]-bicycle beyond that of a mere spacer unit and presents a case for its broader application as a phenyl group replacement in scenarios in which the aromatic ring count negatively impacts biopharmaceutical properties and overall drug-likeness.

The presentation outlines the rational design that led to this finding and demonstrates how the unusual [1.1.1]-moiety enabled fast project advancement and access to novel IP and design space. A matched molecular pair analysis of Pfizer's compound collection will be included to understand whether the observed changes in *in vitro* parameters are a general phenomenon or limited to this specific series of γ-secretase inhibitors.

MEDI 189

3D molecular descriptors important for clinical success

***David C Kombo**, david.kombo@proteostasis.com, Kartik Tallapragada, Rachit jain, Joseph Chewning, Anatoly Mazurov, Jason D Speake, Steve Toler. Department of Discovery Chemistry, Targacept, Inc., Winston-Salem, NC 27101, United States*

The pharmacokinetic and safety profiles of clinical drug candidates are greatly influenced by their requisite physicochemical properties. In particular, it has been shown that 2D molecular descriptors such as fraction of Sp3 carbon atoms (Fsp3) and number of stereo centers correlate with clinical success. Using the proteomic off-target hit rate of nicotinic ligands, we found that shape-based 3D descriptors such as the radius of gyration and shadow indices discriminate off-target promiscuity better than do Fsp3 and the number of stereo centers. We have deduced the relevant descriptor values required for a ligand to be non promiscuous. Investigating the MDL Drug Data Report (MDDR) database as compounds move from the preclinical stage toward the market, we have found that these shape-based 3D descriptors predict clinical success of compounds at preclinical and phase1 stages *vs.* compounds withdrawn from the market better than do Fsp3 and LogD. Further, these computed 3D molecular descriptors correlate well with experimentally observed solubility, which is among well-known physicochemical properties that drive clinical success. We also found that about 84% of launched drugs satisfy either Shadow index or Fsp3 criteria, whereas withdrawn and discontinued compounds fail to meet the same criteria. Our studies suggest that spherical compounds (rather than their elongated counterparts) with a minimal number of aromatic rings may exhibit a high propensity to advance from clinical trials to market.

MEDI 190

Design of chiral DPPIV inhibitors

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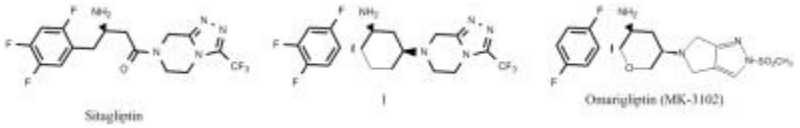
Sitagliptin phosphate (JANUVIA™) is the first DPP-4 inhibitor approved by the US FDA for the treatment of patients with Type 2 diabetes. The objective of the 2nd generation DPP-4 program is to identify a long acting DPP-4 inhibitor for a once weekly dosing. Based on X-ray crystallography of sitagliptin bound to DPP-4, we proposed that a

cyclohexylamine group could be an appropriate replacement for the central β-amino butanoyl portions of sitagliptin, providing a ring constrained analog such as **1** . Compound **1** which has three chiral centers proved to be a potent, selective DPP-4 inhibitor with excellent pharmacokinetic profile in preclinical species. Further modification of **1** and continued effort in this area provided Omarigliptin (**MK-3102)** as a clinical candidate, that is currently in Phase 3 clinical studies. In this presentation, the discovery of **MK-3102** and its biological profile including efficacy in reducing glucose will be discussed.



MEDI 191

Carfilzomib/Kyprolis™: The next generation proteasome inhibitor inspired by Nature

***Craig M Crews**, craig.crews@yale.edu. Department of Molecular, Cellular, and Developmental Biology; Chemistry; Pharmacology, Yale University, New Haven, CT 06520-8103, United States*

Each year, many promising natural products are identified as being biologically active in cell culture assays. Despite their proven *in vitro* efficacies, development of these 'drug candidates' into clinically useful therapeutic agents is an arduous procedure. Initially discovered by Bristol-Myers Squibb, the microbial anti-tumor natural product epoxomicin was not initially developed as a drug candidate due to 1) its peptide structure, 2) potentially labile epoxyketone pharmacophore, and 3) lack of a known mode of action. After developing the first total synthesis of epoxomicin, the Crews lab discovered it be a potent and selective proteasome inhibitor with a unique pharmacophore. Further optimization by the Crews lab yielded the synthetic tetrapeptide epoxyketone YU-101, which served as the parent compound for Carfilzomib (Kyprolis™), the newly approved therapeutic agent for multiple myeloma.

MEDI 192

Discovery and development of cabozantinib for the treatment of metastatic medullary thyroid carcinoma

***Alison H Joly**, ajoly@exelixis.com. Department of Program Management, Exelixis, South San Francisco, California 94083, United States*

Hepatocyte growth factor (HGF) and its tyrosine kinase receptor MET are overexpressed in a wide variety of tumor types, promoting tumor growth, invasion and

metastasis. Upregulation of MET occurs in response to multiple therapies including VEGF-pathway inhibition, and promotes resistance. Additionally, HGF and VEGF cooperate to promote endothelial cell proliferation, tubule formation, and growth of new vessels in vivo. Cabozantinib was derived through optimization of a lead compound selected for potent MET and VEGFR inhibition. In preclinical in vivo tumor models cabozantinib has potent anti-tumor and anti-angiogenic activity. Cabozantinib also inhibits additional receptor tyrosine kinases implicated in oncogenesis, including RET. Activating translocations involving RET occur in subsets of papillary thyroid and lung cancers, and activating RET point mutations, in particular M918T, occur in medullary thyroid cancer (MTC) and may be associated with a poor prognosis.

In a phase 1 study in patients with advanced solid tumors, 85 patients (37 with MTC) were enrolled to evaluate safety, pharmacokinetics, and to determine the maximum-tolerated dose. Ten of 37 MTC patients achieved a confirmed partial response.

A phase 3 study of cabozantinib vs placebo in patients with progressive, metastatic MTC was conducted. The primary endpoint was progression-free survival (PFS). Secondary endpoints included objective response rate (ORR) and overall survival (OS). 330 patients were randomized 2:1 to cabozantinib or placebo. Median PFS for cabozantinib was 11.2 months vs 4.0 months for placebo (HR0.28, 95% CI 0.19-0.40, $p<0.0001$). PFS results favored the cabozantinib group across pre-defined subset analyses. ORR was 28% for cabozantinib vs 0% for placebo ($p<0.0001$). An interim analysis of OS (44% of the 217 required events) did not show a difference between treatment arms. The most frequent grade ≥3 adverse events were diarrhea, palmar-plantar erythrodysesthesia, fatigue, hypocalcemia, and hypertension. Additional trials of cabozantinib in multiple tumor types are in progress.

MEDI 193

Development of Kynamro (mipomersen), the first second generation antisense inhibitor to be approved

***Rosanne M Crooke**, rcrooke@isisph.com. Department of Cardiovascular Disease Antisense Drug Discovery, Isis Pharmaceuticals, Inc., Carlsbad, California 92010, United States*

Antisense drugs are designed to bind to specific RNAs, via Watson-Crick base pairing, leading to downstream modulation of mRNA translation, and ultimately, suppression of dysregulated proteins involved in various diseases. Over the last 25 years, the advances in all aspects of antisense technology, as well as a detailed understanding of the mechanism of action of antisense drugs, has facilitated their use as therapeutic agents. These advancements culminated in the January 2013 FDA approval of Kynamro (mipomersen), a second-generation 2′MOE gapmer targeting human apoB-100 mRNA. Kynamro received orphan drug status from the FDA for the treatment of homozygous familial hypercholesterolemia (HoFH), and entered clinical trials in December 2003. It is being co-developed by Genzyme and Isis Pharmaceuticals as an

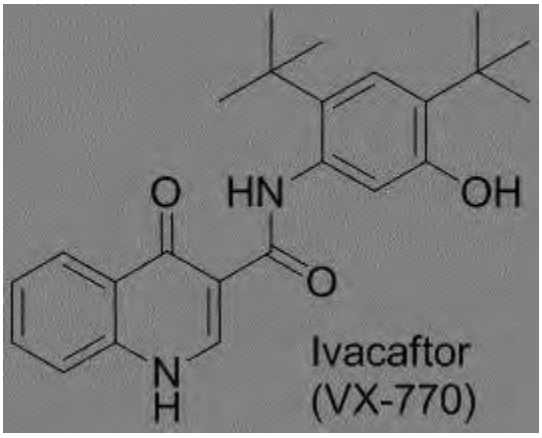
add-on therapeutic agent for patients with severely elevated cholesterol levels at high risk for coronary artery disease (CAD) and unable to reach their target LDL-C levels on maximally tolerated lipid-lowering drugs. Kynamro has been evaluated in multiple clinical trials and most recently, successfully completed four Phase 3 studies in which all the primary, secondary and tertiary efficacy endpoints were achieved. A brief history of antisense technology and highlights of the progression of Kynamro from early preclinical studies to multiple Phase 3 registration trials will be presented.

MEDI 194

Discovery of VX-770 (Ivacaftor), a CFTR potentiator for the treatment of cystic fibrosis in G551D patients

Peter DJ Grootenhuis*, peter_grootenhuis@vrtx.com. Drug Innovation, Vertex Pharmaceuticals, San Diego, CA 92121, United States*

Cystic Fibrosis (CF) is an autosomal recessive disorder affecting ~70,000 patients worldwide. CF is caused by defects in the cystic fibrosis conductance regulator (CFTR) protein that result from mutations in the *CFTR* gene. Defects in the CFTR protein lead to reduced chloride transport resulting in thick, sticky mucus that causes abnormalities in multiple organs. In the lungs, this excess mucus can lead to progressive loss of lung function and premature death. Currently, over 1900 CFTR mutations are known. The *G551D* mutation is a gating mutation that is present in 4-5% of CF patients. High throughput screening was used to identify pharmacological agents with the ability to modulate CFTR protein activity for improved chloride transport. One of the scaffolds identified in the screen was characterized as appropriate for further exploration; extensive medicinal chemistry and formulation efforts resulted in the identification and development of ivacaftor (VX-770) for clinical evaluation. Clinical studies in patients aged 6 years and older with CF and the *G551D* mutation demonstrated that treatment with ivacaftor led to significant improvements in lung function and other clinical endpoints. Ivacaftor is now approved for treatment of CF patients aged 6 years and older who have the *G551D*mutation.



MEDI 195

Precision cancer therapy: The transition of cancer to orphan diseases

***J. Jean Cui**, jean.cui@tptherapeutics.com. TP Therapeutics Inc, San Diego, CA 92121, United States*

Cancer results from an accumulation of a wide range of genomic alterations in normal cells. Advances in DNA sequencing technology have accelerated the large-scale complete sequencing of cancer genomes to reveal the evolutionary processes that underlie tumor origin, progression and metastasis. This comprehensive cancer genome data provides new insights for the treatment of cancers. Thousands of somatically acquired mutations are often associated with solid tumors. However, only some of the mutations, termed as driver mutations, play an important role in driving cancer biology. Targeting oncogenic driver mutations has been proven to produce effective therapeutic interventions to control the tumor growth and progression. Although a cancer is traditionally classified according to the tissue from which the cancerous cells are generated, it could be more completely characterized by its key genomic abnormalities. For example, lung cancer is a general term for a number of genetically distinct diseases, each with its own unique potential genetic targets for drug therapy. The oncogenic driver *EML4-ALK* is presented in ~5% of non-small cell lung cancer (NSCLC) and *CD74-ROS* in ~1% of NSCLC. Crizotinib, a multi-targeted kinase inhibitor for MET/ALK/ROS, has demonstrated marked efficacy in patients with abnormal *ALK* or *ROS* genes. The success of crizotinib has shifted the traditional one-pill-for-all chemotherapy to a new paradigm of molecularly targeted therapy, and represents the true transition of cancer to orphan diseases.

MEDI 196

Vignettes in kinase inhibitor drug discovery: Evolution of a drug target class

***Joel C Barrish**, joel.barrish@bms.com. Discovery Chemistry, Bristol-Myers Squibb, Princeton, New Jersey 08543, United States*

Protein kinases are critical players in intracellular signal transduction pathways and the etiology of many human diseases. Over the last three decades, our understanding of this biological target class has grown such that there are now over 20 kinase inhibitors on the market for the treatment of cancer and now inflammatory disorders. This presentation will describe the evolution of kinase drug discovery and development using three clinical candidate case histories to highlight key past milestones and future challenges.

MEDI 197

Natural products as leads for CNS drug discovery

***Thomas E Prisinzano**, prisinza@ku.edu. Department of Medicinal Chemistry, University of Kansas School of Pharmacy, Lawrence, Kansas 66045, United States*

Natural products (NPs) play an important role in drug discovery, serving as either a source or inspiration for approximately half of all approved small-molecule drugs. Although a large number of these drugs are naturally occurring substances, derivatives of NPs are often necessary to improve their pharmacokinetic properties. Perhaps the best examples of NP derivatives that have been developed into drugs are the many morphine-derived opioids prescribed for the treatment of pain. Opioid receptors, the site of action of morphine and related compounds, have also been implicated in other CNS disorders such as Parkinson's disease, depression and drug abuse. This talk will outline our recent progress in developing novel treatments for CNS disorders by targeting opioid receptors. In particular, our efforts to develop drug abuse medications through a better understanding of the chemistry and pharmacology of salvinorin A will be described.

MEDI 198

From natural product synthesis to methods for medicinal chemists

***Phil S Baran**, pbaran@scripps.edu. Department of Chemistry, The Scripps Research Institute, La Jolla, California 92037, United States*

This talk will focus on how studies in something as fundamental as natural product synthesis can have a tangible impact in the day to day lives of medicinal chemists.

MEDI 199

Award Address (Alfred Burger Award in Medicinal Chemistry sponsored by GlaxoSmithKline). Confessions of a medicinal chemistry fanatic

***John E Macor**, john.macor@bms.com. Department of Discovery Chemistry, Bristol-Myers Squibb Research and Development, Wallingford, CT 06492, United States*

This presentation will cover stories related to the discovery of a number of molecules which made their way into clinical trials. Lessons learned and the science and people behind the compounds will be discussed.

MEDI 200

Design of dermal and transdermal actives

***Jonathan Hadgraft**, Jonathan.hadgraft@btinternet.com. Pharmaceutics, UCL School of Pharmacy, London, United Kingdom*

The skin is an effective barrier to most xenobiotics. Its main function is to prevent excessive water loss from the body and to minimise the ingress of potentially toxic materials into the systemic circulation. The outermost layer, the stratumcorneum, is the main barrier and its structure can be likened to a brick wall. The major route of penetration is through the 'mortar', the intercellular spaces. These are filled with a complex mixture of lipids which are structured into bilayer arrays. A diffusing molecule therefore has to cross sequentially from a hydrophilic to a lipophilic environment. It is not surprising that molecules that permeate best have reasonable solubility in water and oil, are small, have a logPbetween 1 and 3 and are low melting point. Because of the good barrier function they should also be very potent. Actives delivered transdermally which fulfil these criteria include nitroglycerin, nicotine and rivastigmine. In conventional drug delivery often the salt will be administered. For dermal and transdermal delivery the free acid or free base is often far more appropriate (e.g. fentanyl, rivastigmine)

Molecules that lay outside this rather narrow range can also permeate to some extent but, importantly, their permeation can be significantly improved when formulated with components which modify the barrier properties of thestratum corneum. The excipients can alter the solubility properties of the skin thus facilitating the ingress of the active. They may also disrupt the lipids in the skin thereby improving the diffusion process.

This presentation will review the ideal properties of a transdermal or dermal drug and provide examples in which excipients can be shown to have a major effect on the penetration process. This will be a function of the physicochemical properties of both the active and the formulation components.

MEDI 201

Luminally restricted therapeutics: Low permeability molecules for gastrointestinal targets

***Steven M. Sparks**, steven.m.sparks@gsk.com. Enteroendocrine DPU, GlaxoSmithKline, Research Triangle Park, NC 27502, United States*

Identifying novel therapies with increased safety margins and robust efficacy for chronic conditions like diabetes and obesity remains a formidable task for drug discovery. To achieve this goal our group has focused on the restriction of therapeutic molecules to the gastrointestinal tract, referred to as luminal restriction, as a method to increase the safety margin for new anti-diabetic and anti-obesity therapies. This talk will describe the design parameters and assays used to identify luminally restricted compounds as well as examples from our ileal bile acid transport inhibitor (iBATi) program and additional early discovery programs.

MEDI 202

Relationship between drug properties and oral-mucosoal route of drug delivery

***Tarun Goswami**, tarungos@gmail.com. Department of Transdermal Formulations, Amneal Pharmaceuticals, Piscataway, NJ 08854, United States*

The oral-mucosal route is one of the early routes of administration for non-invasive systemic drug delivery. This route avoids first-pass metabolism and provides quick drug entry into the systemic circulation. Oral mucosal route includes buccal, sublingual and ginigival routes of drug delivery.

Different in-silico models were developed to study the relationship between oral mucosal permeability and physico-chemical and molecular properties of drugs. Drugs with diverse physicochemical and molecular properties such as, molecular volume, molecular weight, partition coefficient, distribution coefficient, pKa, total polar surface area, hydrogen bond acceptors and donors, number of rotatable bonds, solubility and melting point were used to develop the models. It was shown that owing to differences in barrier properties, the permeability of drugs can vary between buccal and sublingual mucosa. Oral-mucosal permeability was found to be dependent on molecular volume, distribution coefficient, number of hydrogen bond donors, and number of rotatable bonds. Smaller molecular size, high lipophilicity, lower hydrogen bond capability and greater flexibility were important for oral-mucosal permeability.

Due to the relatively hydrophilic nature of the oral-mucosa, in addition to the lipophilic drugs, oral-mucosal delivery can be an attractive alternative for the hydrophilic or charged drugs which otherwise have poor permeability across more lipophilic biological barriers. Nimesulide was used as a model drug to study the effect of the degree of ionization on sublingual permeation of ionized and unionized drug species. It was shown that ionized drug species can permeate across the sublingual mucosa and contribute significantly to the total drug flux.

The analysis of the contributions of drug properties to the permeability provided mechanistic insights in to drug permeation across oral mucosa from molecular structure perspective. This data can serve as an initial evaluation tool to screen prospective drug candidates for sublingual delivery.

MEDI 203

All metrics are wrong, some might be useful

***W. Patrick Walters**, pat_walters@vrtx.com. Vertex Pharmaceuticals, Cambridge, Massachusetts 02139, United States*

Publications by Lipinski and others during the 1990s led to a proliferation of metrics for compound “quality” in drug discovery programs. Over the last 15 years, these metrics have become part of medicinal chemistry dogma in many organizations, and molecules that violate these “rules” are often frowned upon. More recently, there appears to be a backlash against many of these metrics. In a number of recent publications, authors have complained that these metrics may inhibit creativity, and may not be appropriate

for the targets currently being pursued by the pharmaceutical industry. This presentation will provide an overview of the current state of the art in compound metrics for drug discovery, and examine areas where these metrics can provide benefits as well as liabilities.

MEDI 204

Medicinal chemistry design for inhaled medicines: Application to long-acting bronchodilators

***John R Jacobsen**, jjacobsen@theravance.com. Department of Medicinal Chemistry, Theravance, Inc., South San Francisco, CA 94080, United States*

Bronchodilators and anti-inflammatory drugs have been effectively used in the treatment of respiratory disease for decades. Delivered orally, members of these classes generally have dose-limiting effects mediated systemically. Consequently, many respiratory drugs have been developed for topical delivery to the lung by oral inhalation in order to achieve efficacious concentrations at the desired site of action while limiting drug levels in the systemic circulation. An inhaled medicine must be efficiently delivered to the lung, reach its pharmacological target, maintain activity over the dosing interval and be safely and rapidly cleared from the body. Understanding of how physicochemical properties and the in vitro biological profiles of compounds can impact these parameters is still at an early stage but is growing rapidly. Examples of successful inhalation medicines highlight some very different strategies that have addressed the challenges posed by different chemical spaces as well as different target classes. Our discovery efforts in the field of bronchodilators have demonstrated the potential for very different properties to provide compounds with the potential for potent once-daily dosing. The β_2-adrenoceptor agonist milveterol (TD-3327) was selected for development based on functional potency, suitability for inhalation formulation and duration of action in a guinea pig model of bronchoprotection. In seeking a second development candidate, we identified a chemically differentiated backup (TD-5471) with markedly different physicochemical properties and profile in vitro. This may reflect different mechanisms driving potency and duration of action in vivo and supports the potential for multiple and quite different solutions to challenges in inhaled drug discovery.

MEDI 205

Role of physicochemical and biochemical properties influencing topical ocular drug delivery

***Nagendra Venkata Chemuturi**, chemuturi@gmail.com, Andrew Rusinko. Alcon Labs, a Division of Novartis, Fort Worth, Texas 76134, United States*

The eye, responsible for the sense of vision, and being in contact with the environment, is susceptible to physicochemical and microbial assaults, which manifest themselves as various diseases of the eye. Being a complex organ, any disruption of the internal

homeostasis leads to various diseases like glaucoma, AMD, etc. The predominant route to treat ocular diseases has been the topical ocular route. However, dilution by the tears, rapid clearance by the movement of the eyelids, binding to melanin in the eye, etc., limit the bioavailability of the molecules in the target ocular tissues. While there is better understanding of drug delivery to the front of the eye, little is understood about the physicochemical properties of molecules and biochemical properties of the ocular barriers affecting the delivery to retina and choroid in the eye. The current presentation will review the correlation of physicochemical properties as they relate to ocular drug delivery, both for the front and the posterior segments of the eye.

MEDI 206

Targeting CNS availability for oncology: Role of P-gp, BCRP, and CNS MPO desirability in CNS drug discovery

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A key strategic imperative of research and medicinal chemistry is designing molecules that survive to achieve positive proof of mechanisms. Several *in vitro* cell-based assays to assess blood brain barrier (BBB) permeability exist. Useful high-throughput assays have been developed employing cell lines such as MDCK stably transfected with the human multidrug resistance 1 (MDR1) p-glycoprotein (P-gp). Based on the prominent of P-gp in efflux mechanisms at the BBB, these assays have been instrumental in lead development for optimizing brain permeability. One strategy to avoid P-gp and optimize brain availability of drug candidates is to understand how physicochemical properties impact P-gp liability and passive permeability. Advances in prospective design include the development of a novel approaches to assess drug-likeness. A new multi-parameter design tool (CNS MPO Desirability) focuses on a holistic approach to drug discovery and aims to align drug-like attributes such as low P-gp liability, low metabolic clearance, high passive permeability and safety in one molecule. Examination of the *desirability* approach to CNS Oncology drug discovery will be presented, including: case studies of past oncology and CNS drugs.

*Average values	CNS MPO Desirability	MW	clogP	tPSA	clogD	HBD
CNS Drugs*	4.61	298.7	2.72	47.6	1.7	1
Kinases Drugs*	3.56	461.2	4.24	85.6	2.63	2

MEDI 207

Clinical and preclinical considerations for neuro-oncology drug development

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Brain tumors are amongst the deadliest of all cancer subtypes. Despite vast advancements in our understanding of the basic biology of brain tumors, there has been little improvement in patient outcomes. Glioblastoma (GBM), the most common primary brain tumor, remains an essentially incurable disease. Despite multimodality treatment with surgery, chemotherapy and radiotherapy, median survival is only 12-15 months. There is a vast unmet need for improved therapies for GBM, but also many impediments to neuro–oncology drug development. For example, emerging evidence suggests that the subpopulation of cancer initiating cells is intrinsically chemo- and radio-resistant. Moreover, the blood-brain-barrier is an impediment to therapeutic drug delivery. This presentation will review some of the pre-clinical and clinical considerations for novel therapeutic development for brain tumors. I will discuss studies focused on increasing GBM apoptosis with the goal of overcoming resistance to therapy. I will also discuss an emerging strategy of inhibiting nuclear export.

MEDI 208

Drug delivery to brain tumors and the problem of the blood-brain barrier

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INTRODUCTION: The role of the blood-brain barrier in therapeutic failure of brain metastases has remained unanswered for many years. Blood vessels within the brain are uniquely characterized by low passive permeability and active efflux transport which limits brain penetration for many agents. In this presentation, we address this issue using novel imaging methods to simultaneously assess brain metastasis vascular permeability, chemotherapeutic drug uptake, and drug efficacy in preclinical models of breast cancer.

METHODS: Brain-seeking breast carcinoma cells were injected into the left cardiac ventricle of immune-compromised mice. Tumors were allowed to seed and grow within the central nervous system for 2-6 weeks. Mice were then anesthetized and administered radiolabeled chemotherapeutic drug (0.5 – 8 hr) as well as a vascular permeability marker. At the end of the circulation period, brain was removed from the skull, snap frozen, and cut into sections for fluorescence/phosphorescence permeability, drug distribution, and immunohistochemical analyses.

RESULTS: Barrier passive permeability was elevated 2-30 fold in most brain metastases relative to surrounding brain. Chemotherapeutic drug (e.g. paclitaxel, doxorubicin, vinorelbine, lapatinib, capecitabine, vorinostat) uptake was also heterogeneously enhanced in most (85%) brain metastases. However, drug concentrations in brain metastases were, on average, only 2-15% of those in metastases in other tissues (e.g., liver, kidney) that lack a tight vascular barrier. Apoptosis was observed only in the subset (~10%) of the most highly permeable brain metastases where drug concentration approached that of systemic lesions. Enhanced brain metastasis uptake for paclitaxel and doxorubicin correlated with elevated barrier

passive permeability but not with altered active efflux transport. Uptake could be enhanced up to 100-fold through inhibition of active efflux transport. The results demonstrate that the brain metastasis barrier, though partially compromised, still markedly limits drug delivery for many chemotherapeutic agents.

MEDI 209

Designing brain-penetrant small molecule inhibitors of PI3K

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The well documented deregulation of the PI3K/AKT/mTOR pathway in numerous tumor types has established a significant desire for PI3K inhibitors. To that end, numerous PI3K and PI3K/mTOR inhibitors are currently under evaluation in clinical trials. Among the cancer types that should benefit from PI3K inhibition is glioblastoma in which, conservatively, PI3K pathway activation is observed in >70% of all cases. This unmet medical need represents a significant opportunity for the use of PI3K inhibitors. However, targeting glioblastoma requires PI3K inhibitors capable of crossing the blood-brain barrier

We established a program to identify brain penetrant PI3K inhibitors for the treatment of glioblastoma. We began by demonstrating that both Pgp and bcrp mediated efflux were responsible for the low brain exposure observed with our earlier clinical PI3K inhibitors. This led us to evaluate how we might reduce efflux mediated by both Pgp and bcrp. A thorough analysis of physicochemical property correlations with efflux revealed striking trends which enabled the establishment of clear guidelines for new compounds.

We also identified trends in physicochemical properties that correlated with metabolic stability. Our analyses enabled us to make brain penetrant PI3K inhibitors that had good pharmacokinetics in a very short period of time.

The talk will discuss the analyses we performed and how they impacted the medicinal chemistry program. We will also identify multiple brain penetrant PI3K inhibitors with attractive comprehensive profiles that were studied in *in vivo* glioblastoma models. Data will be presented to show that brain penetrant molecules demonstrate efficacy in relevant models where clinical inhibitors of PI3K do not.

MEDI 210

Identification and preclinical evaluation of NT113, a novel pan-ErbB kinase inhibitor for the treatment of glioblastoma (GBM)

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Glioblastoma (GBM) is the most common and most aggressive malignant primary brain tumor in adults, accounting for about 50% of all functional tissue brain tumor cases and 20% of all intracranial tumors. Deregulated ErbB signaling is associated with the development of GBM, of which approximately 45% have EGFR amplification. A tumor specific and constitutively activated mutant, EGFRvIII is often co-expressed with amplified EGFR. Unfortunately, small molecule ErbB inhibitors used to date have poor CNS penetration, and thus failed clinically against GBM.

Our strategy to design blood-brain-barrier (BBB) penetrating ErbB inhibitors involved physicochemical property calculation, activity screening, and rapid pharmacokinetic (PK) profiling. This effort led to the discovery of NT113, a potent, irreversible pan-ErbB inhibitor that has excellent CNS exposure in rats and mice. NT113 completely suppressed tumor growth for a two-week period of NT113 administration and significantly prolonged the lifespan of treated mice in an EGFRvIII mutant GBM xenograft model. In a second intracranial xenograft experiment involving GBM cells with amplification of wild-type EGFR, NT113 significantly outperformed both erlotinib and lapatinib, with each therapeutic administered at its daily MTD for athymic mice. In addition, NT113 also exhibited potent anti-cancer activity in subcutaneous xenograft models with either HER2 over-expression or T790M/L858R EGFR mutation. Therefore, it may also find applications in treating cancer patients with brain metastases from extra-cranial primary tumors.

MEDI 211

Is CNS availability for oncology a no-brainer? Discovery of PF-06463922, a novel small molecule inhibitor of ALK/ROS1 with pre-clinical brain availability and broad spectrum potency against ALK-resistant mutations

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The 1st generation ALK inhibitor crizotinib demonstrated impressive clinical benefit in ALK-fusion positive lung cancers and was approved by the FDA for the treatment of ALK-fusion positive NSCLC in 2011. However patients treated with crizotinib eventually develop resistance to therapy. Acquired ALK kinase domain mutations and disease progression in the central nervous system (CNS) are reported as main contributors to patient relapse after ALK inhibitor therapy. A drug discovery program was initiated

aimed to develop a next generation ALK inhibitor that is more potent than existing ALK inhibitors, capable of inhibiting the resistant ALK mutants and penetrating the blood-brain-barrier. These objectives present a considerable challenge in kinase inhibitor chemical space.

Here we report that PF-06463922, a novel small molecule ATP-competitive inhibitor of ALK/ROS1, showed exquisite potencies against non-mutant ALK (Ki <0.2 nM; cell IC_{50} ~2 nM) and ROS1 kinase (Ki <0.005 nM; cell IC_{50} ~0.2 nM), and demonstrated low nanomolar inhibitory activity against a panel of ALK kinase domain mutants representing all of the patient crizotinib resistant mutations reported to date. The more commonly reported L1196M gatekeeper mutant shows significant sensitivity to PF-06463922 (Ki 0.7 nM; cell IC_{50} 21 nM). PF-06463922 is also very selective, and showed >100 fold kinase selectivity against 95% of the kinases tested in a 207 recombinant kinase panel.

Specific design considerations were developed leading to novel ATP-competitive kinase inhibitors with desired low efflux in cell lines over-expressing p-glycoprotein and breast cancer resistance protein, providing excellent blood-brain-barrier and cell penetration properties. Efforts to optimize ligand efficiency and lipophilic efficiency leveraging structure based drug design techniques led to ligands with overlapping broad spectrum potency and low efflux. Single and repeat dose preclinical rat *in vivo* studies of PF-06463922 demonstrated excellent oral bioavailability and CNS availability with free brain exposure approximately 30% of free plasma levels.

MEDI 212

Three peptide-drug conjugates for treatment of brain tumors: From concept to clinic

Gaoqiang Yang, Christian Che, Marie-Claude Lacoste, Jean-Christophe Currie, Michel Demeule, Anthony Regina, Jean-Paul Castaigne, ***Jean Lachowicz****, jlachowicz@angiochem.com. Angiochem, Inc, Montreal, Quebec H2X 3Y7, Canada*

While drugs from numerous classes of anti-cancer therapies have been approved, very few are positioned to treat tumors of the brain, due to the obstacle of the blood-brain barrier (BBB). Both the structure of the barrier, with unfenestrated capillaries connected by tight junctions, and the function, with efflux pumps such as the MDR gene product, P-glycoprotein, serve to prevent the majority of drugs from achieving therapeutic concentrations within brain parenchyma. By contrast, endogenous agents enter from plasma to brain via transporters and receptors with narrow and broad specificities. In the latter category is LRP-1 (low-density lipoprotein receptor-related protein-1). Using peptide libraries based on a consensus sequence from LRP-1 ligands, peptides were identified and optimized to utilize LRP-1 receptor-mediated transcytosis to cross the BBB. Peptide-drug conjugates constructed with the brain-penetrant peptide Angiopep-2 are also recognized by LRP-1 to gain the property of BBB penetrability. This technology has been demonstrated with three anti-tumor drugs of different classes: a taxane

spindle inhibitor (ANG1005), an anthracycline DNA intercalator (ANG1007), and a podophyllotoxin derivative topoisomerase II inhibitor (ANG1009). Unlike their parent molecules, these new chemical entities are brain penetrant, achieving therapeutic concentrations in brain. The most advanced, ANG1005, has been tested in over 200 patients with primary and secondary brain cancers, with anti-tumor activity observed. These studies further validate the utility of Angiopep technology for multiple classes of anti-tumor agents and demonstrate the potential for ANG1005 to be a first-in-class brain-penetrant taxane therapy.<ins cite="mailto:Jean%20E.%20Lachowicz" datetime="2013-10-16T13:06">

MEDI 213

Addressing cardiovascular issues of SSTR3 antagonists in MK-4256 structural class

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Type 2 diabetes mellitus (T2DM) has become a worldwide epidemic, causing significant morbidity and mortality. Despite the availability of a range of agents, effective treatments for T2DM continue to be a huge, unmet medical need. We have focused on the development of novel agents that promote glucose-dependent insulin secretion (GDIS) from pancreatic β-cells, thus minimizing the potential risks associated with hypoglycemia. Somatostatin (also known as growth hormone-inhibition hormone or somatotropin release-inhibiting factor, SRIF) derived its names from its action to inhibit the release of growth hormone from the anterior pituitary. Somatostatin also suppresses the production of the pancreatic hormones (e.g., insulin and glucagon), has a role in central nervous system as a neurotransmitter, is involved in the regulation of gastric secretion, and may regulate cell proliferation. The functions of somatostatin are mediated through five G-protein coupled receptors (SSTR1 to SSTR5). We had found that antagonism SSTR3 has the potential to be a novel GDIS mechanism for the treatment of T2DM. Our initial SAR work led to the discovery of MK-4256, a potent and subtype selective SSTR3 antagonist, which demonstrated superior efficacy in a mouse oGTT model. In this talk, we will present additional profiling of MK-4256, especially

relevant to unexpected cardiovascular effects and will discuss our efforts to resolve these issues.

MEDI 214

Small molecule disruptors of the GK-GKRP interaction as potential antidiabetics

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Glucokinase (GK) is an enzyme that converts glucose to glucose-6-phosphate (G6P) and is predominately expressed in pancreatic β-cells and liver hepatocytes. It's endogenous inhibitor, glucokinase regulatory protein (GKRP), binds to and sequesters GK in the nucleus, preventing glucose phosphorylation at fasting. Thus the GK↔GK-GKRP equilibrium plays an important role in regulating glucose uptake and glycogen synthesis. As a result, modulating blood glucose via the GK pathway presents a promising treatment of type II diabetes mellitus (T2DM). The prevailing approach has focused on compounds that directly hyperactivate GK via allosteric binding (GK activators – GKAs). One potential liability associated with this class of compounds is the development of hypoglycemia upon alteration of the intrinsic enzyme kinetics of GK. To mitigate this risk, we explored an alternative mechanism that increases GK-mediated glucose phosphorylation by disrupting the binding of GKRP to GK. In this presentation, we will describe the identification of a screening hit that led to the discovery of the initial tool compound (AMG-1694) with a suboptimal PK profile. Subsequent metabolic profiling along with structural-based optimization resulted in the discovery of a novel and stable small-molecule GK-GKRP disruptor (AMG-3969). This compound potently induced the dissociation of the GK-GKRP complex as well as promoted GK translocation in both *in vitro* and *in vivo* assays. Furthermore, AMG-3969 reduced blood glucose levels in rodent models of diabetes while showing no effect in euglycemic animals. These results represent the first successful discovery of a small molecule that targets the GK-GKRP complex as a novel pathway for managing blood glucose levels with reduced hypoglycemic risk.

MEDI 215

Identification of novel imidazo [1,5-*a*]pyrazine-6-carboxamide-containing mitogen-activated protein kinase kinase (MEK) inhibitors which exhibit a monodentate interaction with Ser212

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Starting from a novel 6,5-heterocyclic indazole- **G-894** which was capable of interacting with Ser212 in a bidentate manner to give exquisite potency, further structural diversity and improved properties were obtained by a core change to the imidazo[1,5-*a*]pyridine **GDC-0623,** a second novel 6,5-heterocyclic MEKi. Imidazo [1,5-*a*]pyridine **GDC-0623** taught us that a 6,5 heterocycle could engage Ser212 in a monodentate interaction and retain cell proliferation potency. However, that slight loss in potency was offset by gains in solubility and PK versus **G-894.** The 6,5-imidazo [1,5-*a*]-pyridine scaffold was further optimized by incorporating a nitrogen at the 7 position to give the 6,5-heterocyclic imidazo [1,5-*a*]pyrazine. The introduction of the C7 nitrogen was driven by blocking the 7 and C8 positions from metabolism for improved metabolic stability (and PK). It was found that introduction of a nitrogen at C7 was not sufficient to result in an imidazo[1,5-*a*]pyrazine analog that improved upon **GDC-0623** . However, increasing the polarity by combining a C7 nitrogen with a diol hydroxamate gave imidazo[1,5-*a*]pyrazine **G-479** , which was improved over **GDC-0623** in many aspects .

MEDI 216

Design and optimization of coagulation factor XIa inhibitors: Discovery of *trans*-N-((*S*)-1-(4-(3-amino-1H-indazol-6-yl)-5-chloro-1H-imidazol-2-yl)-2-phenylethyl)-4-(aminomethyl)cyclohexane-carboxamide, a potent factor XIa inhibitor with in vivo antithrombotic activity

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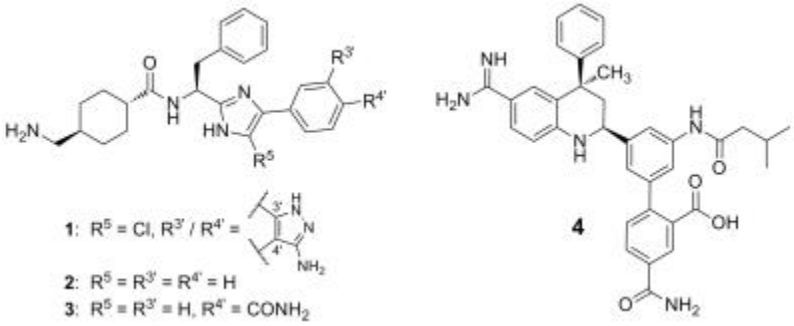
FXIa plays a key role in the formation of a stable thrombus, however, it is not critical for normal hemostasis. Individuals deficient in FXI (hemophilia C) experience bleeding associated with surgical procedures, but rarely spontaneous bleeding. In contrast, deficiencies in FIX, or its cofactor FVIII, are associated with a severe bleeding diathesis (hemophilia B and A, respectively). In addition, reduced incidence of ischemic stroke in patients with severe FXI deficiency is observed, whereas elevated FXI levels are associated with venous thrombosis, myocardial infarction, increased odds ratio for cerebrovascular events, and coronary artery disease. Moreover, preclinical studies have shown that FXI-deficient mice were protected against ferric chloride induced carotid artery thrombosis and venous thrombosis, while maintaining normal tail bleeding times as compared to wild-type litter mates. Novel inhibitors of FXIa containing an (*S*)-2-

phenyl-1-(4-phenyl-1H-imidazol-2-yl)ethanamine core have been designed and optimized to provide compound **1** , a potent inhibitor of FXIa (K_i = 0.2 nM) having *in vivo* antithrombotic efficacy in the rabbit AV-shunt thrombosis model (IC_{50} = 0.8 mM). Initial analog selection was informed by molecular modeling using compounds **2** and **3** overlaid onto the x-ray crystal structure of tetrahydroquinoline **4** (FXIa K_i = 0.2 nM) complexed to FXIa. Further optimization was achieved by specific modifications derived from careful analysis of the x-ray crystal structure of the FXIa/**3** complex.



MEDI 217

Discovery of a cardiac late sodium current inhibitor (Na_v 1.5) GS-458967, a triazolopyridine, structurally distinct from ranolazine with improved intrinsic efficacy and potency

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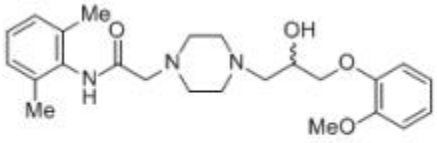
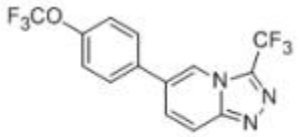
Ischemic heart disease (IHD) is associated with an increase in reactive oxygen species (ROS), which can modify $Na_v1.5$ and result in an enhanced late Na^+ current (Late I_{Na}, I_{NaL}). In IHD enhanced I_{NaL} leads to calcium overload, which in turn activates CAMKII and further increases I_{NaL}, creating a vicious cycle. Within its therapeutic range, (2 – 8 μM) ranolazine (Ran, **1**) selectively inhibits late I_{Na} compared to peak I_{Na}, but also inhibits other cardiac ionic currents, including I_{Kr}. Herein, we report the discovery of a potent and selective Late I_{Na} inhibitor, triazolopyridine GS-458967 (**2**), which has suitable PK profile consistent with QD dosing. Enhanced late I_{Na} generated by *Anemonia sulcata* toxin II (ATX-II) was inhibited by Ran (**1**) and GS-458967 (**2**) with IC_{50}'s of 6.9 and 0.25 μM, respectively. GS-458967 (**2**) was >50 times more potent than Ran (**1**) in reducing ischemia-induced contracture in an isolated heart model (rat) of global no-flow ischemia. GS-458967 represents a new class of potent late I_{Na} inhibitors

that will be useful in further delineating the role of inhibitors of this current in the treatment of IHD.



1 ranolazine



2 *GS-458967*

MEDI 218

Discovery of MK-4698 as a candidate for broad-spectrum beta-lactamase inhibitor

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Beta-lactamase, the enzyme that catalyzes the chemical degradation of beta-lactam antibiotics, is a leading cause of the fading efficacy of beta-lactam antibiotics, particularly in problematic Gram-negative organisms. Co-administration of beta-lactam antibiotics with beta-lactamase inhibitors (BLIs) has been a time honored strategy to restore clinical efficacy. Beta-lactamases are categorized into four classes: Classes A, C and D are serine-based hydrolases; Class B comprises zinc–metallo hydrolases. Unfortunately, the currently marketed beta-lactamase inhibitors are only effective on a subset of beta-lactamases. Thus there is an important medical need to develop new beta-lactamase inhibitors to combat the growing threat of antibiotic resistance. To this end, MK-4698 was discovered as a potent extended-spectrum BLI clinical candidate with excellent preclinical efficacy. Compared with marketed inhibitors, it offers much greater coverage for beta-lactamase panels of resistant bacterial strains. In animal models of resistant infection, it potentiated existing beta-lactam antibiotic therapies effectively.

MEDI 219

From lead to candidate drug selection of AZD6642, a 5-lipoxygenase activating protein (FLAP) inhibitor

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The candidate drug AZD6642, a potent small molecule inhibitor of the 5-lipoxygenase activating protein (FLAP) in the 5-LO pathway, will be disclosed together with learnings

and high-lights from the medicinal chemistry strategies used in this drug discovery program from lead to candidate drug selection.

Inhibitors of 5-lipoxygenase activating protein (FLAP) reduce the production of the bioactive leukotrienes (LTs) $LTB_4$and cysteinyl leukotrienes (cysLTs), lipid mediators derived from arachidonic acid that have inflammatory and vasoactive actions. Reduction of these lipid mediators could be beneficial in the treatment of a variety of inflammatory diseases including asthma and atherosclerosis.

A fast-follower approach was taken and properties optimized to significantly improve parameters such as Ligand Lipophilic Efficiency (LLE), free potency, reactive metabolite and metabolic stability. Critical structure-activity relationship, pharmacokinetic data, metabolism and predicted human dose will be discussed together with synthesis and overall properties of the candidate drug AZD6642.

MEDI 220

Towards novel anti-cancer therapeutics: Pharmacologic inhibitors of c-Myc–Max dimerization

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c-Myc is a proto-oncogenic transcription factor that is dysregulated in most, perhaps all, human cancers, and contributes to both the development and progression of the disease. Until recently, considerable skepticism was held towards the inhibition of c-Myc as an anti-tumour strategy given the significant role of c-Myc in various key processes, such as proliferation, differentiation and apoptosis, in normal cells. However, the apparent narrow therapeutic window of c-Myc has been widened thanks to the revelation that c-Myc does not turn on and off the transcription of target genes, as was previously thought. Rather, c-Myc acts as an “amplifier”, elevating or depressing transcription of genes that have already become transcriptionally activated. Intrinsically disordered in the monomeric state, c-Myc, a member of the basic helix-loop-helix-leucine zipper (bHLH-ZIP) family, provides little opportunity for targeted drug design, which is likely one reason why no “c-Myc drug” has entered the clinic. Nevertheless, a chemically diverse group of small-molecules has been identified that inhibit the obligatory dimerization of c-Myc with the bHLH-ZIP family member Max. Beginning with c-Myc inhibitor 10074-G5 (*N*-([1,1'-biphenyl]-2-yl)-7-nitrobenzo[*c*][1,2,5]oxadiazol-4-amine), a structure-activity relationship study led to the discovery of JY-3-094 (4-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)benzoic acid), which exhibits almost a five-fold improvement in the inhibition of c-Myc–Max dimerization (IC_{50} = 33 μM vs. 146 μM). Current efforts towards the further optimization of JY-3-094 include the introduction of bioisosteric replacements of the nitro group and the carboxylic acid function, both of

which will improve metabolic stability, promote cell penetration and enhance c-Myc–Max inhibitory activity.

MEDI 221

Discovery of new acylaminopyridines as GSK-3 inhibitors by a structure guided in-depth exploration of chemical space around a pyrrolopyridinone core

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Glycogen synthase kinase-3 (GSK-3) has been proposed to play a crucial role in the pathogenesis of many diseases including cancer, stroke, bipolar disorders, diabetes and neurodegenerative diseases. GSK-3 inhibition has been a major area of pharmaceutical interest over the last two decades. A plethora of reports appeared recently on selective inhibitors and their co-crystal structures in GSK-3β. We identified several series of promising new GSK-3β inhibitors from a coherent design around a pyrrolopyridinone core structure. A systematic exploration of the chemical space around the central spacer led to potent single digit and sub-nanomolar GSK-3β inhibitors. An exemplary compound from one series showed significant pTau lowering in a transgenic AD mouse model. X-ray crystallographygreatly aided in validating the binding hypotheses. The most salient features of our work including the design hypothesis, modeling studies that helped in achieving improved off-target kinase selectivity, key SAR, *in vitro* and *in vivo*, and X-ray crystallography data on select compounds will be presented.

MEDI 222

Identification of AQW051, an alpha7 nicotinic acetylcholine receptor partial agonist for the treatment of cognitive impairment associated with schizophrenia

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The α7 nicotinic acetylcholine receptor (nAChR α7) has been genetically linked with schizophrenia, its expression is reduced in the hippocampi of schizophrenia patients. Agents targeting nAChR α7 mediated signaling have shown broad efficacy in animal cognition models.

AQW051, an orally active nAChR α7 partial agonist, is in Phase II clinical evaluation for the treatment of cognitive impairment associated with schizophrenia (CIAS). Hit finding, structure-activity relationship and medicinal chemistry optimization of a series of nAChR α7 agonists will be presented, and the chemical structure of AQW051 will be disclosed for the first time.

AQW051 demonstrates high affinity for nAChR α7 (K_i=27±0.9nM in an [^{125}I]α-bungarotoxin binding assay) and acts as a potent agonist at the receptor (pEC_{50}=7.41±0.09 in a calcium flux assay). No stimulation of α4β2, α3β4 and α1β1γδ nicotinic subtypes and the 5-HT3 receptor was seen up to a concentration of 100 μM. AQW051 rapidly crosses the blood-brain barrier (brain/plasma ratios of 10 to 80 at 0.08 to 7 h in mice) and significantly improves memory function in rodent cognition paradigms, e. g. in the mouse object recognition test. Thus, AQW051 demonstrates strong preclinical potential as a therapeutic agent for cognition indications. Clinical studies show that AQW051 is safe and well tolerated in young and elderly healthy volunteers. Its pharmacokinetic properties make it suitable for once daily oral administration.

MEDI 223

Discovery and early development of MK-1064: A potent, orally bioavailable, selective orexin 2 receptor antagonist (2-SORA) for the treatment of insomnia

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The field of small molecule orexin antagonist research has moved swiftly from the discovery of the orexin peptides in 1998 to clinical proof-of-concept for the treatment of insomnia in 2007 to the first NDA filing with the FDA in 2012. Clinical programs have focused on the development of DORAs, or dual orexin receptor antagonists, that block the action of endogenous, wake-promoting peptides at the orexin 1 (OX_1R) and orexin 2 (OX_2R) receptors. This presentation will highlight drug development efforts focused on selective orexin 2 receptor antagonists (2-SORAs). These efforts resulted in the discovery of MK-1064 [5''-chloro-*N*-[(5,6-dimethoxypyridin-2-yl)methyl]-2,2':5',3''-terpyridine-3'-carboxamide], a clinical candidate for the treatment of insomnia. Selected data from early clinical development will also be disclosed.

MEDI 224

Antiviral drug discovery and development: Progress of the HCV inhibitors asunaprevir and BMS-791325 and the HIV-1 attachment inhibitor BMS-663068

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In this presentation, we will provide an update on the current status of three antiviral drug candidates for which the preclinical profile and structures were released at *First Time Disclosures of Clinical Candidates* sessions held at recent ACS National Meetings. The updates will focus on the HCV NS3 protease inhibitor asunaprevir (BMS-650032), disclosed at the Fall 2009 meeting, and the non-nucleoside HCV NS5B RNA dependent, RNA polymerase inhibitor BMS-791325, disclosed at the Spring 2012 meeting, which are currently in clinical trials in combination with the NS5A inhibitor daclatasvir. In addition, further development of the HIV-1 attachment inhibitor BMS-663068, a prodrug of BMS-626529 that was presented at the Spring 2011 ACS meeting, will be summarized.

MEDI 225

Development and biology of antitumor effect of novel inhibitor of VDR and its co-activator interactions

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The vitamin D receptor (VDR) belongs to the family of nuclear receptors and is known to transcriptionally regulate calcium balance, cell proliferation and cell differentiation. The design of inhibitors that selectively target the interactions between VDR and

transcriptional coregulators, in the presence of endogenous ligand calcitriol, is a novel approach to inhibit cancer cell growth without modulating calcium homeostasis. We identified an indole-based inhibitor using high throughput screening that selectively disrupted the interaction between VDR and coregulator peptide SRC2-3. Lead optimization resulted in compound PS121912, a VDR-coregulator inhibitor with nanomolar activity in cells. The global anti-cancer potential of PS121912 was determine with the NCI-60 cancer cell screen. Detailed proliferation studies with DU145, Caco2, HL-60, SKOV3 and OVCAR8 cells confirmed the tissue-specific inhibition of cancer cell growth induced by PS121912. Importantly, the anti-proliferative action of very low concentrations of PS121912 was mediated by liganded VDR as shown in the presence of calcitriol. At higher concentrations, a calcitriol independent activation of caspase 3/7 induced apoptosis at different concentrations for all cancer cells. Further genomic and proteomic changes were determined by rt-PCR and antibody arrays for various apoptosis markers. These studies revealed that among other proteins various death receptors and their ligands such as FAS, FASL, TNFR2, CD40 and CD40L were up-regulated. The time-dependent and dose-dependent responses of translation were confirmed by western blot. We hope that *in vivo* data will be available in time to disclose the anti-tumor activity of PS121912. Overall, we developed the first non-secosteroidal modulator of VDR-mediated transcription with tissue-specific inhibition of cancer cell proliferation.

MEDI 226

Targeting TrkC expressing tumors in vitro and in vivo using a TrkC ligand conjugated to a BODIPY-based photodynamic therapy (PDT) agent

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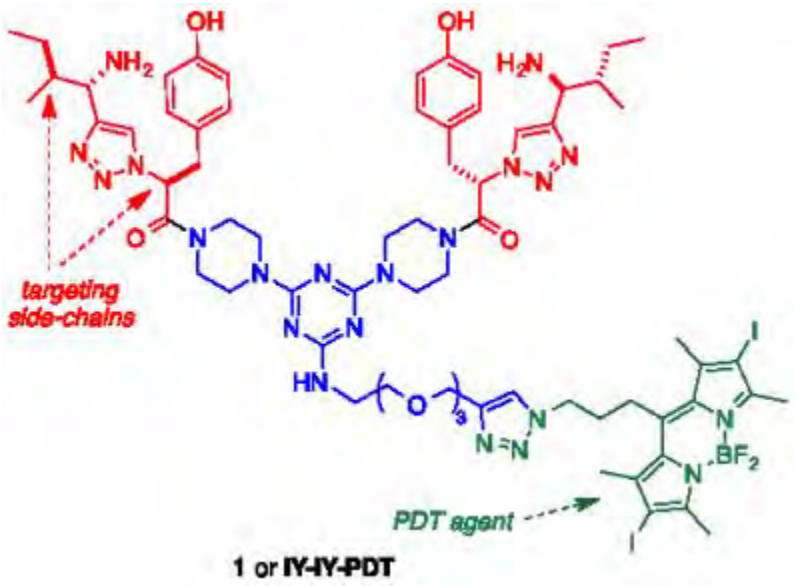
Our group has designed a small molecule, non-peptidic, ligand that binds the cell surface receptor TrkC. This receptor is overexpressed on various types of tumors. A molecule **1** (IY-IY-PDT)was designed to contain a fragment (IY-IY) that targets the TrkC receptor, and a photosensitizer that acts as an agent for photodynamic therapy (PDT). Molecule **1** has sub-micromolar photocytotoxicities to cells that were either engineered to stably express TrkC (NIH3T3-TrkC) or that naturally express high levels of TrkC (SY5Y neuroblastoma, 4T1 murine breast cancer, and Hs578t human breast cancer lines). Control experiments showed **1** is not cytotoxic in the dark, and has significantly less photocytotoxicity towards cells that do not express TrkC. Another control featuring a scrambled agent **2** (YI-YI-PDT), isomeric with **1** , showed it is considerably less photocytotoxic than **1** on TrkC expressing cells. Our data illustrates the beneficial effects of targeting in cellular assays, including internalization of cargo agents and selective cell killing *in vitro* and *in vivo*.



MEDI 227

Stimulus-responsive polymer nanoreactors for efficient photodynamic therapy

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Theranostic is a modern approach in medicine, which profits from its dual-functionality – combining diagnostic with treatment. Polymeric compartments encapsulating active molecules, which serve both to detect and treat a pathologic condition act as efficient multifunctional nanoreactors. The exchange of desired molecules through the compartment membrane, a feature essential for an *in situ* reaction of nanoreactors, is obtained by a selective permeable membrane. A smart combination of stimulus-responsive polymer compartments, and encapsulated active molecules supports a nanoreactor activity 'on demand', when the stimulus is present in their environment.

We present here stimulus-responsive nanoreactors based on encapsulated photosensitive conjugates in polymer vesicles with sizes in the nanometer domain. Upon irradiation with a specific wavelength, the photosensitive conjugates produce *in situ* reactive oxygen species (ROS) serving for photodynamic therapy [1]. Encapsulation of rose bengal conjugated to bovine serum albumin inside the cavity of polymer vesicles served to: i. improve the local concentration of photosensitizer, ii. protect the photosensitizer from degradation, iii. decrease its intrinsic toxicity, and iii. support the detection via the fluorescence signal of rose bengal. We selected as polymer compartments polymethyloxazoline-b-polydimethylsiloxane-b-polymethyloxazoline

(PDMS-PMOXA) because these polymer vesicles were up-taken by various cell lines without being toxic, and possess a ROS permeable membrane [2]. ROS production was turned on/off by irradiation with a wavelength specific to the photosensitizer. ROS amount in HeLa cells increased significantly due to the activity of the nanoreactor, and induced cell death in the region where the nanoreactors were irradiated. Our nanoreactor represents an efficient candidate for theranosic approaches in photodynamic therapy because of its dual-function: generation of ROS, and easy detection by the fluorescent signal of the photosensitizer.

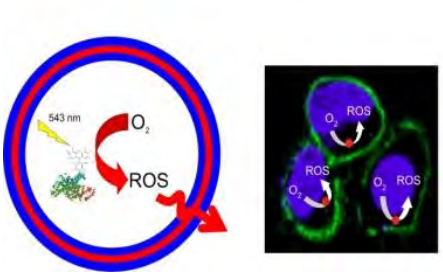

Figure 1. Schematic representation of a stimulus responsive polymer nanoreactor serving as a source of ROS “on demand” inside HeLa cells.

Refrences :

1. P. Baumann et al; *Nanoscale.* **2013** , *5*, 217.

2. F. Axthelm et al.,*J. Phys. Chem. B*, **2008** , *112*, 8211.

MEDI 228

Targeting specific interactions to improve binding properties of EGFR-kinase ligands

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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 antiproliferative activity against certain tumour types in the clinic. Resistance to EGFR inhibitors is mediated by mutation in the ATP site and often through activation of the MAPK pathways by other receptor tyrosine kinases. This inspired the investigation of 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 EGFR inhibitor. A structure-based drug design modeling program, combined with PDB data-mining, protein structural fingerprints and pharmacophore searches was used to help identify and characterize linkers for connecting EGFR-binding moieties to DNA and Src targeting functionalities. The resulting compounds showed EGFR inhibitory potency in the low micromolar to nM range and retained significant activity against their divergent targets.

MEDI 229

Prediction of activity spectra of substances (PASS): Twenty years of development

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Among the numerous ligand-based drug discovery tools PASS occupies a special place because its development has been started over 20 years ago (Poroikov et al. Automatic Documentation and Mathematical Linguistics, 1993, 27: 40-43). During the past years PASS is improved and extended permanently (Filimonov and Poroikov, In: Chemoinformatics Approaches to Virtual Screening, RSC Publishing, 2008, 182-216). Current PASS version predicts 6,400 biological activities with a mean accuracy of about 95% based on structure-activity relationships elucidated from the training set consisted of 330,000 biologically active compounds. Freely available online resource (http://www.way2drug.com/passonline) is used by ~9,000 researchers from ~90 countries. Over 300,000 predictions were performed; over 50 independent published studies confirm PASS predictions by subsequent synthesis and biological testing.

Using PASS predictions, novel pharmaceutical agents have been discovered with anxiolytic, anti-inflammatory, antihypertensive and other actions. By PASS application to the antihypertensive drugs Perindopril, Quinapril and Monopril we identified a nootropic action, which is likely not related to their antihypertensive effect. To find new anticancer agents, we have analyzed dozens of millions of structures from ChemNavigator database and selected a few dozen compounds for biological testing. Two out of eleven tested compounds were found to be potent anticancer NCEs, with synergistic action to the known p53 reactivator RITA. PASS application significantly increases the chances for discovery of new more safety and potent pharmaceutical agents, and predicts biological activity profiles of drug-like substances in chemical biology.

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MEDI 230

Discovery of novel and selective pan-Trk inhibitors for chronic pain

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The NGF/TrkA pathway plays a central role in the biology of chronic pain. The Trk A receptor kinase is a member of a family of Trk receptor kinases that includes Trk B and Trk C. Activation of the receptor tyrosine kinase TrkA by NGF triggers intracellular signaling cascades and protein expression that increases the sensitivity of nociceptors leading to chronic sensitization and pain. Inhibition of the NGF/TrkA pathway has been validated clinically using NGF-neutralizing monoclonal antibodies that is currently in Phase II-III trials for OA pain. Using a variety of biophysical techniques, unique HTS hits were validated and establish as competent lead series with novel binding paradigms. These leads were further optimized for potency and selectivity resulting in several small molecule pan-Trk inhibitors series that exhibit high selectivity for TrkA/B/C versus a diverse panel of kinases. We have also demonstrated efficacy in both inflammatory and neuropathic pain models upon oral dosing. This talk describes the identification process, hit-to-lead progression, and binding profiles of these selective pan-Trk kinase inhibitors.

MEDI 231

Can peptidomimetic antibiotics overcome bacterial resistance? Elucidating molecular level binding and specificity details of DD-peptidases and β-lactamases

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β-lactam antibiotics constitute the most diverse and extensively used group of antimicrobials in the world. This antibiotic class inhibits bacterial cell wall biosynthesis by mimicking the D-alanyl-D-alanine subunit of peptides that are a precursor to the cell wall structure. The acylation reaction forms a long lasting acyl-enzyme intermediate between the β-lactam and a catalytic serine residue. Upon forming this intermediate, the biosynthesis process is severely inhibited and can ultimately lead to bacterial cell death. Though the identity of the main catalytic residue is known (i.e. serine), acylation mechanistic details including the general base responsible for deprotonating the serine and the general acid that protonates the nitrogen of the β-lactam ring remain unknown. Some details of this have been gleaned from studies of the deacylation step, however characterization of the acylation mechanism has proven more difficult (largely due to rapid turnover) and many questions remain unanswered. Recently, my research group has elucidated details that will greatly contribute to unraveling many of the outstanding questions; for example 1. protonation state assignment of key active site residues (i.e. Lys65 and His298) and determination of their likely roles preceding antibiotic deactivation in DD-peptidases; 2. chemical level details of the acylation enzyme reaction mechanism in DD-peptidases; 3. identification of antibiotic - enzyme intermolecular interactions that govern binding and specificity in DD-peptidases and β-lactamases.

MEDI 232

Identification of novel ligands and insight into the structural switch between agonist and antagonist activity from an optimized model of the human aryl hydrocarbon receptor ligand binding domain

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The Aryl Hydrocarbon Receptor (AHR) is a ligand-activated transcription factor that regulates the expression of a diverse group of genes. Interestingly, the PAS-B ligand binding domain of AHR binds ligands with a variety of chemical scaffolds, including the potent carcinogenic 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), which to date is the most potent AHR agonist. In the absence of an experimentally-determined structure of this domain, previous AHR homology models in the *apo* conformation were successfully used for virtual ligand screening (VLS) to predict new AHR ligands that were confirmed with testing in cells. In this study, we sought to refine this technique by optimizing the protein model with the agonist TCDD docked within its binding pocket. The result was an “agonist-optimized” homology model which increased the volume of the binding

pocket and had reduced energy strained compared to the previous *apo* model. We found that the agonist-optimized model was able to discriminate known AHR agonists including TCDD from inactive compounds, indicating that it was an improved version with the potential to identify novel AHR agonists. To search for new agonists, we performed VLS against our agonist-optimized model and identified sixteen candidate compounds. In cells, four of these compounds were able to significantly activate the AHR gene CYP1A1. In addition, we performed molecular dynamics simulations of an agonist-bound, and antagonist-bound version of this improved model in order to gain insight on the mechanics of the AHR PAS-B domain. In the context of these simulations we discovered that a specific portion of the protein comprised a flexible segment with helices that coiled or uncoiled as needed to accommodate the different ligand shapes. Based on these findings, we hypothesize that this segment is a key adaptable component that might disrupt HSP90 binding and highly influences how AHR interacts with either an agonist or an antagonist.

MEDI 233

Targeting tumors by their addiction to amino acids: Glutamine–containing anticancer agents

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Tumor cells require a constant supply of essential and nonessential amino acids to support their growth. To achieve this they trap amino acids by expressing high levels of Asct2 and LAT1 transporters. Cleveland group has shown that tumor cells deal with lactate dilemma by markedly upregulating the expression of transporters that direct lactate efflux, specifically the monocarboxylate transporters Mct1 and/or Mct4. These are 12-membrane pass bidirectional exchange transporters and little has been known regarding control of their expression. Since very few compounds having high affinity for these transporters are known, every little effort has been done to study how disruptions in lactate transport may impede tumor cell viability.

The Asct2 Gln transporter is highly expressed in Myc-driven lymphomas implying that agents that block Asct2 may be effective therapeutics, though none are known yet. An inhibitor for Asct2 may be exploited to be a tumor cell delivery device; i.e., a glutamine micic could be selectively concentrated near or within tumor cell. The glutamine like compound could then deliver the anti-Mct1-specific agent. Since tumor type that is chosen for therapy would over express Mct1 as well as Asct2, this double amplification scenario should result in high efficacy with an acceptable therapeutic index.

AstraZeneca has reported few of its novel compounds which are active in inhibiting tumors. Based on the structural model, we have been using the art and skill of organic synthesis to synthesize new analogs with different alkyl and aryl groups, linkers and

terminal attachment with glutamine. The compounds synthesized are purified, analyzed and then submitted to cancer biology, where their activities are tested. We have synthesized more than 35 compounds with a variety of heterocycles and found that these compounds are active in transporting amino acids as well as inhibiting tumors. The synthesis and SAR of all the compounds studied will be discussed in this presentation.

MEDI 234

Emerging strategy for controlling drug release using visible/near IR light

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Spatio-temporal controlled release of therapeutic agents (drug) is critical to achieve local expression of pharmacological action of drug. One strategy of controlled release that has gained recent attention is the use of light preferably NIR light.

We first propose 'Click and Photo-unclick Chemistry' of aminoacrylate (β- enamino eater) which can be built readily and cleaved fast by amine-yne and dioxetane reactions after 690 diode laser irradiation.

We designed and synthesized a prodrug of combretastatin A4. Combretastatin A4 was conjugated to a photosensitizer through a photo-cleavable aminoacrylate linker. PS-L-CA4, PS = photosensitizer) and L = aminoacrylate linker.

Two pseudo-prodrugs (PS-NCL-CA4 and PS-L-Rh) also were prepared: PS-NCL-CA4 could not release free CA4 even after the irradiation (NCL = non-cleavable linker) and PS-L-Rh as a special fluorescence probe that emits bright rhodamine fluorescence only after cleavage of the linker to releases fluorescent rhodamine after the irradiation.

In vitro study using MCF-7 cells, the prodrug conjugate PS-L-CA4 was 5 fold less toxic than parent drug CA4 without NIR laser irradiation IC_{50D} = 164 nM → IC_{50P} = 28 nM which is presumably due to the release of CA4. The dark toxicity and photo toxicity of PS-NL-CA4 was quite similar IC_{50D}: 1802 nM → IC_{50P}= 1063 nM (PS-NCL-CA4) since the release of CA4 is not possible. Most exciting result was that PS-L-CA4 showed better antitumor effects than PS-NCL-CA4 upon irradiation.

This concept of release mediated by singlet oxygen cleavable linker provide control in term of the quantity, location and time of release of drug. The easy and high yield reaction and the photo-unclick chemistry of of aminoacrylate linker can find many applications, not limited to anticancer drugs and prodrugs, for spatio-temporally controlled release of active compounds but delivery vehicles liposomes, polymers, quantum dots, gold nanoparticle, carbon-nanotube etc.

MEDI 235

Development of robust tumor-targeted drug delivery system (TTDDS) platform and its applications to novel taxane-based drug conjugates with biotin as the tumor-targeting module

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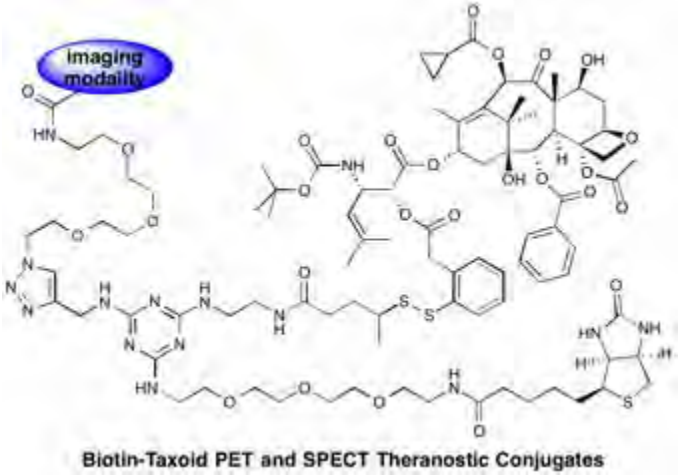
Novel tumor-targeting drug conjugates (TTDCs) bearing a second-generation taxoid, SB-T-1214, as the warhead and biotin as the tumor-targeting module (TTM) were designed and synthesized. Biotin receptors are overexpressed in various human cancers and serve as promising tumor-specific biomarkers. Thus, biotin was empolyed as the primary TTM in those TTDCs. Biotin-linker-taxoid conjugates demonstrated highly promising activity in mice bearing MX-1 tumor xenografts. Also, a novel dual-warhead conjugate bearing SB-T-1214 and camptothecin with a self-immolative disulfide linkers via a 1,3,5-triazine splitter demonstrated excellent target-specificity. This modular TTDDS platform can be applied to various cytotoxic agents in combination. Building upon this versatile platform, theranostic conjugates have been synthesized through incorporation of various imaging modules for PET and SPECT. In addition, a novel TTDDS bearing dual-TTM modules, bearing biotin and folic acid as TTMs, was constructed. Furthermore, three novel PET tracers were designed to assess the biodistribution, pharmacokinetics, and tumor-specificity of a biotin-probe, biotin-linker-taxoid conjugate, and SB-T-1214 in mice. The syntheses and biological evaluations of these TTDCs and tracers will be presented.



Biotin-Taxoid PET and SPECT Theranostic Conjugates

MEDI 236

Mass-directed flash purification of peptides

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Synthetic peptides represent an increasingly important class of therapeutic agents. Peptide purification represents a major bottleneck in peptide discovery and development. Advances in peptide synthesis have resulted in significantly improved crude purities of even complex peptides. As a result, the goal of peptide purification is often to remove non-peptide impurities and does not require the time, resolution and effort required for semi-preparative scale HPLC.

Here we report expedited purification methods for a variety of peptides over a broad range of scales utilizing mass-directed reversed-phase flash chromatography. Results demonstrate how transitioning from traditional HPLC purification methods to flash chromatography for peptide purification following synthesis can significantly impact peptide throughput and ease peptide discovery and development.

MEDI 237

MOE in education: Problem-based learning in medicinal chemistry

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The Molecular Operating Environment (MOE) is a comprehensive, user-friendly molecular modeling and cheminformatics software package. Here we demonstrate how MOE is used as an integral tool for teaching fundamental and applied aspects of the drug discovery process to Medicinal Chemists using a problem-based approach. More specifically, we present examples that show how MOE's medicinal chemistry workflows can be applied towards understanding fundamental principles in drug discovery, and from this, provide a means of visualizing, analyzing and correlating data from ligand-receptor interactions. We conclude with an example of how students can use this knowledge to rationally design novel compounds using problem-based learning.

MEDI 238

Microwave-assisted granuated-copper metal-catalyzed azide-alkyne “Click” synthesis of dendritic antioxidants

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Preparation of antioxidant dendrimers with metal chelating core and radical scavenging moieties using traditional “click chemistry” methods resulted in chelation of the copper catalyst, thus negating the positive effects of the molecules for their intended use. Herein we report syntheses of four unique antioxidant dendrimers containing surfaces rich in radical quenching phenolic hydroxyl groups as well as metal chelating cores without apparent copper contamination. They were prepared through microwave reaction in the presence of granulated Cu(0) metal as the catalyst, replacing the Cu(I)catalyst in the traditional Click synthesis.

MEDI 239

On comparison of parabolic and hyperbolic diffusion models for use in design of boronate affinity high performance liquid chromotography

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Trinity Biotech, Dublin, Ireland has obtained FDA approval for Hb9210 in 2011. This is a method using boronate affinity high performance liquid chromotography to for use in precise HbA1c analysis in less time. Each unit costs ~ $25,000. Hyperbolic diffusion models have the capability to predict the sigmoidal shape of the break through curves. Parabolic Fick diffusion and hyperbolic damped wave diffusion models are used to predict the elution from the chromotographic column. Glycation specific binding of boronate affinity is used in the integrated HPLC system for detection of all of teh glycalated Hb species present. The diabetes testing time of 66 s is less compared with the tests that need prolonged periods of fasting. The analytic column used is aminophenylboronic acid bound to porous polymer support. The glycalation reaction is also taken into account in the model. Different regimes of diffusion and reaction are identified. Based on the models the cost of the unit can be reduced and test optimized.

MEDI 240

Quantitative analysis of acetaminophen, acetylsalicylic acid, and cetirizine dihydrochloride by q-NMR (quantitative nuclear magnetic resonance) technique

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The pharmaceutical industry mainly uses chromatographic techniques such as high performance liquid chromatography (HPLC) and gas chromatography (GC), to determine the quantity of the active ingredients and other materials in the drugs. Quantitative proton-nuclear magnetic resonance technique (q-NMR) provides an alternative method for quantification of these drugs and a wide variety of other organic compounds. In this study quantitative analysis of three representative drugs — two

analgesic drugs, acetaminophen and acetylsalicylic acid and one antihistamine, cetirizine dihydrochloride — were accomplished by q-NMR technique using simple organic compounds as internal standards in 300 MHz NMR instrument. Those internal standards were chosen whose proton-NMR spectrum did not interfere with the spectrum of the analyte. Two sets of concentrations of acetaminophen in deuterated dimethyl sulfoxide – ranging from 6.12 mM to 102 mM and 0.84 mM to 6.75 mM – were analyzed using ethanol as an internal standard in quintuplicate to show the robustness of the method. The peak area of three sets of protons in acetaminophen – methyl group peak and two sets of aromatic ring peaks – were quantified with respect to the methyl group peak area of the internal standard, ethanol. The calibration curves of peak area vs. concentration for both sets of concentrations and three different sets of protons showed linear relationship with regression values ranging from 0.9979 to 0.9998. More than half of the data showed percent standard deviation values under 1%, indicating that the data had high precision. The percent standard deviation values over 4% were seen for aromatic peaks of the three lowest concentrations, suggesting that methyl peak areas of acetaminophen should be used for analysis of low concentration solutions. Two sets of similar concentration of cetirizine dihydrochloride, (ethyl alcohol as internal standard in deuterated water), acetaminophen and salicylic acid (ethyl ether in deuterated methanol) were also analyzed with similar results.

MEDI 241

Effective one-step microwave extraction of PCB congeners from soil followed by GC-MS analysis

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The subject of our study is to set time and coast effective extraction method for Polychlorinated biphenyls found in soils by the use of microwave extraction system Mars-X-traction (CEM) which is followed by GC-MS analysis. According to the literature Soxhlet extraction of mentioned compounds is widespread applying method; our study is presented an easy and simple method of sample preparation of 10 PCB (PCB congener numbers: 18, 28, 31, 101, 118, 138, 149, 153, 180 and 194) compounds from soil. Microwave assisted extraction procedure is applied by; weight of 0.5g soil (CRM) in PTFE capped reaction vessel (4mL) ; spike at concentration of 1000µg/kg with PCB congeners; adding ~1g Sodium sulphate anhydrous and 2mL of solvent mixture hexan:acetone (1:1), then subjected the sample to the microwave system at 50^{o}C within 15min. extraction time. The supernatant of centrifugated solution is transferred to the Kuderna-Danish evaporator and final volume is adjusted to the 500µL. GC-MS analysis is performed by injection of 1µL volume of sample solution. Experiments were carried out in 3 parallels and each one solution is injected 3 times. Recoveries of 99% - 105% were obtained for target compounds while RSD% varied between %2 and %5. Results showed that method is fast and reproducible for analysis of PCB congeners in soil samples

MEDI 242

In silico-investigation of the multitarget therapeutic concept to evaluate synergy between phytotherapy and medical therapy using modeling studies and bioinformatics approaches

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Over the past two decades, there has been a tremendous increase in the use of medicinal plants to treat diseases; however, there is still a significant lack of research data in this field. The World Health Organization (WHO) estimates that 80 percent of the world's population relies principally on traditional medicines, in which phytotherapy plays a central role. There is a need to integrate and to address the gap between phytotherpy (using chemical constituents from medicinal plants) and medical therapy. This may lead to the cheaper, faster and more effective drugs to majority of people either living in rural areas or for people relying upon the medicinal plants for their pharmaceutical requirements. A list of all medicinal plants from India with anti-ailment property against the ailments of our interest was prepared. The validated protein targets for all the ailments under investigation were retrieved from PDB. Docking studies were performed between the chemical constituents of all medicinal plants against their validated protein targets. The analysis of the data generated through modeling studies is done using the methods of statistical analysis. A relational database is prepared with all possible docking interactions for all phytochemical compounds and the protein targets. The scoring functions from the docking results test for ADME properties, ranked, and segregated. This will provide some important information about the possibility of lead optimization and new drug discovery. A phylogenetic tree representing all the plant species for which the data is available is constructed. This will provide the necessary relevant information about the relatedness between those medicinal plants curing an ailment. This will lead to the phylogenetic cross comparisons that can focus screening efforts on a subset of traditionally used plants, richer in bioactive compounds and could revitalize the use of traditional medicinal plants in the process of new drug discovery.

MEDI 243

Multiple labeling of peptides via orthogonal chemistries

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Labeling peptides have been widely practiced in numerous applications in chemistry and biology. For example, fluorescent dyes make peptides useful probes for *in vitro* and *in vivo* imaging and substrates for enzyme activity assays; biotinylation has vast applications including affinity-based purification of proteins, FRET-based flow cytometry

and receptor localization; PEGylation enhances plasma half-life of peptides and proteins and stabilizes them against metabolic degradation; radiolabeled peptides are versatile probes for *in vivo* imaging and targeted radionuclide therapy and so on. Similarly, peptides bearing different labels serve as a powerful research tool since multiple labels bestow a variety of new functions. To the end, a number of orthogonal chemistries have been reported, such as click chemistry, thiol-maleimide chemistry, Staudinger ligation, and oxime/hydrazone formation. Despite the versatility and broad applications in traditional organic reactions, Suzuki-Miyaura coupling has been rarely used in peptide chemistry. In addition, while these chemistries have been efficiently used alone in peptide conjugation and labeling, combination of orthogonal chemistries for multiple labeling of peptides is not so common. We have developed an efficient synthetic method of carrying out Suzuki-Miyaura cross-coupling with peptides that gives in high yield and purity. Using it along with other orthogonal chemistries such as thiol-maleimide chemistry and click chemistry, we have developed chemistry and strategy to make multiple labeling of peptides. The feasibility of these orthogonal chemistries was demonstrated by labeling a peptide with a fluorescent dye, biotin, and PEG which can have many potential applications in biomedical research.

MEDI 244

Novel antioxidant bioassay, qsar and docking studies of 1-phenyl, 3-(nitro furan) prop-2-ene-1-one derivatives

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A novel *in vitro* antioxidant spectrophotometric method is visualized and implemented from the charge transfer complex. A simple, rapid and sensitive antioxidant bioassay study of chalcones was monitored at 370nm. The antioxidant property of chalcones is influenced to a great extent by two aryl structures and their substitution pattern. The hydroxyl substituent is the key group to enhance the efficacy of antioxidant activity for easy conversion of phenoxy radicals through the hydrogen transfer mechanism. Quantum chemical methods, AM1 and PM3 were used to estimate different physicochemical parameters and different QSAR models were generated. Docking studies were also performed with the active site of cyclo- oxygenase-2 to identify hydrogen bonding, hydrophobic and ionic interactions. GOLD, Auto dock and Argus lab docking results revealed that the active site residue, TYR355 plays a key role in the formation of hydrogen bonding with chalcones. Mainly, the compound-2 has shown highest inhibitory activity against cyclo-oxygenase-2 with the formation of strong hydrogen bond interactions with the residues of active site. This chemical environment may serve as a starting point for synthesis of cyclo-oxygenase-2 inhibitors with improved efficacy

MEDI 245

High-yield synthesis of P-glycoprotein radioligands [^{11}C]*N*-desmethyl-loperamide and [^{11}C]loperamide

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P-Glycoprotein (P-gp) overexpression has been observed in several cancer types and neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, and traumatic brain injury. P-gp has become an attractive target for molecular imaging of cancer and brain diseases using biomedical imaging technique positron emission tomography (PET). Several PET radioligands have been investigated for their feasibilities to visualize P-gp at the blood-brain barrier (BBB). [^{11}C]*N*-desmethyl-loperamide ([^{11}C]dLop) is one of the promising candidates progressing to human PET studies, which is derived from the parent radiotracer [^{11}C]loperamide and originally developed and characterized at the National Institute of Mental Health. Here we present a high-yield synthesis of [^{11}C]dLop and [^{11}C]loperamide. *N*-Desmethyl-loperamide and loperamide were synthesized from α,α-diphenyl-γ-butyrolactone and 4-(4-chlorophenyl)-4-hydroxypiperidine in 5 and 4 steps with 8% and 16% overall yield, respectively. The amide precursor was synthesized from 4-bromo-2,2-diphenylbutyronitrile and 4-(4-chlorophenyl)-4-hydroxypiperidine in 2 steps with 21-57% overall yield. [^{11}C]dLop and [^{11}C]loperamide were prepared from their corresponding amide precursor and *N*-desmethyl-loperamide with [^{11}C]CH_3OTf through *N*-[^{11}C]methylation and isolated by HPLC combined with solid-phase extraction (SPE) in 20-30% and 10-15% radiochemical yields, respectively, based on [^{11}C]CO_2 and decay corrected to end of bombardment (EOB), with 370-740 GBq/μmol specific activity at EOB.

MEDI 246

Design of argyrin analogs as proteasome inhibitors with subunit specificity

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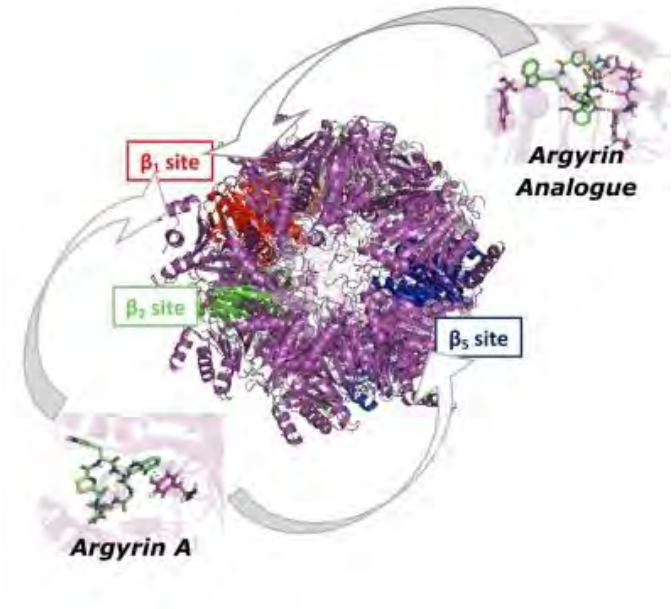
Due ti its involvement in many cellular pathways, the human proteasome has been a popular target for drug discovery. Inhibitors of the proteasome have to be highly specific and inhibit only one of proteasome's activities in order to minimize toxic effects and maximize therapeutic potential. In this study, we developed a computational methodology to guide the design of proteasome inhibitors with subunit specificity. The naturally occuring proteasome inhibitor, argyrin A, that targets active sites β1, β2 and β5, served as the starting point upon which the new analogues were designed. Using the crystal structure of the yeast proteasome we developed humanized models and analyzed the binding conformations of argyrin A to the β1, β2 and β5 sites by molecular docking simulations. The subunit selectivity was determined by analysis of the binding

conformations and site interactions of argyrin A and analogues. Predictions for selective binding distributions for β1, β2, and β5, were derived based on the relative binding affinity of each analogue towards argyrin A. Two new analogues were identified as promising β1 and one as β5 selective inhibitors. The computational protocol that was developed in this study may have a significant impact on the field of drug design when subunit selectivity of inhibitors is desirable.



MEDI 247

Structural probing of off-target receptor activities within a series of adenosine/adenine congeners

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The potential liabilities and advantages of drug promiscuity are a growing concern in drug development, and modulation of polypharmacology can be useful in drug optimization. Here, we have studied patterns of off-target receptor interactions, mostly at G protein-coupled receptors (GPCRs) in the μM range, of nucleoside derivatives that are highly engineered for nM interaction with adenosine receptors (ARs). Because of the considerable interest of using AR ligands for treating diseases of the CNS, we have utilized the Psychoactive Drug Screening Program (PDSP) for probing promiscuity of these adenosine/adenine congeners at over 50 diverse receptors, channels and transporters. The step-wise truncation of rigidified, trisubstituted (at N^6, C2, and 5' positions) nucleosides revealed unanticipated interactions with numerous biogenic

amine receptors, ion channels and transporters, with affinities up to the nM range. The unmasking of consistent sets of structure activity relationship (SAR) at novel sites suggested similarities between receptor families in molecular recognition. Extensive molecular modeling of the GPCRs affected suggested binding modes of the ligands that supported the patterns of SAR at individual receptors. The recognition patterns for different GPCRs were clustered according to which substituent groups were tolerated and which residues were involved in ligand recognition. Thus, some likely off-target interactions can be predicted for analogues of this set of substructures, and similar analyses could be performed for unrelated structural families for other GPCRs.

MEDI 248

Mass-directed flash purification of triglycerides

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Triglycerides are a class of natural products found in plant and animal sources. These fatty acids are comprised of a long chain of hydrocarbons saturated, mono- unsaturated or, poly unsaturated. These compounds have several uses including the manufacture of surfactants and soaps while some are thought to have medicinal and/or nutritional value.

Purification of these compounds can be challenging using liquid chromatography with UV detection as most of these compounds are UV transparent. The use of non-spectroscopic detectors such as ELSD can also be problematic as compound detection is non-specific and all compounds in the sample will be detected.

In this poster we will show the benefits of mass-directed flash purification for the isolation of several fatty acid methyl esters.

MEDI 249

Recent developments in automated flash chromatography improve purity and productivity

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Delivering large quantities of high purity compounds in the shortest possible time is the goal of a purification chemist. Two of the most popular purification techniques are Automated Flash Column Chromatography (AFCC) and Preparative HPLC.

Traditionally, AFCC is characterized by the ability to load large amounts of material and short purification times, while Prep HPLC is valued for high resolution separations resulting in very pure products. As a result, AFCC is typically used as a complementary technique whereby the crude sample is enhanced to a higher level of purity before final purification using Prep HPLC.

With new developments in AFCC instruments and cartridge media, the gap between 'high speed' flash purification and 'high efficiency' Preparative HPLC purification is rapidly shrinking. In many cases AFCC can deliver large quantities of product comparable in purity to Prep HPLC, with significantly less time and expense.

In this study, we demonstrate the performance improvements of AFCC, based on recent developments in instrumentation and cartridge media, in terms of increased resolution, increased flexibility, and time, solvent and cost savings.

MEDI 250

Determinations of targeted metabolites using capillary ion chromatography high resolution accurate mass spectrometry

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Ion chromatography (IC) has been used extensively to separate and determine ionic and charged small molecules. When coupled with high resolution accurate mass (HRAM) spectrometry, the combined techniques provide confirmatory identification, structural interpretation, and higher sensitivity in complex matrices. With its unique selectivity and high retention and peak area stability, IC has been successfully applied to the identification and quantification of targeted charged metabolites in biological samples. Capillary IC-HRAM (Cap IC-HRAM) at µL/min flow rates furthers the capability of IC with respect to metabolite identification and quantification by improving the system sensitivity and stability as well as reducing the amount of sample required.

Here we demonstrate the targeted analysis of metabolites. The analytes were separated on a capillary (0.4 mm i.d.) anion-exchange packed-resin column optimized for organic acid separations using an electrolytically generated hydroxide gradient by a Reagent-Free IC (RFIC) instrument. The IC-MS interface was optimized by continuous online desalting of the mobile phase and the introduction of an acetic acid-methanol desolvation solution. The analytes were detected by HRAM in full scan negative mode for selective and sensitive quantification. Using this configuration, targeted metabolites can be quantified at fmol levels, requiring only µLs of sample, and linearity can be maintained over three to four orders of magnitude.

MEDI 251

Small molecule-based strategy for predictable enhancement of reactive oxygen species (ROS) in bacteria

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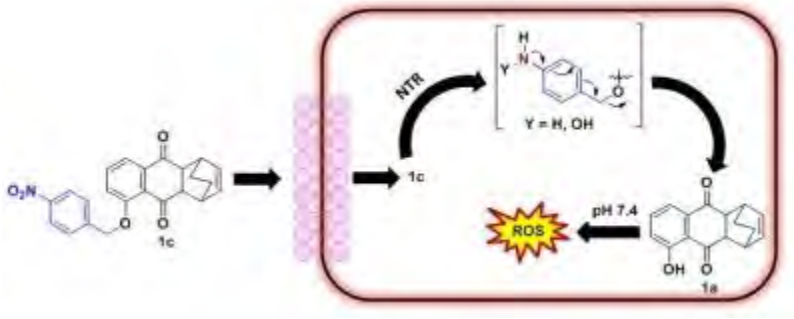
The precise role of reactive oxygen species (ROS), if any, in bacterial lethality by clinically used antibiotics remains unclear. Here, we report 5-((4-nitrobenzyl)oxy)-1,4,4a,9a-tetrahydro-1,4-ethanoanthracene-9,10-dione (**1c**),that is useful for predictably enhancing intracellular ROS in bacteria. A bacterial nitroreductase (NTR) enzyme was used to trigger **1c** for the generation of ROS intracellularly. Compound 5-hydroxy-1,4,4a,9a-tetrahydro-1,4-ethanoanthracene-9,10-dione (**1a)** was reacted with a NTR substrate, 4-nitrobenzyl bromide in the presence of silver oxide to produce **1c** in 66% yield. Compound **1c** generated ROS such as $O_2^{-\bullet}$ and H_2O_2 in pH 7.4 buffer only in the presence of NTR. Subsequently, treatment of *E. coli,* a model bacterium, with **1c** resulted in increased intracellular ROS. At elevated concentrations (250 μM) of **1c** no significant inhibition of bacterial growth is observed. Furthermore, no evidence for **1c** sensitizing *E. coli* to major classes of antibiotics was found. Thus, using this novel tool, we find that enhancing intracellular ROS was well tolerated by *E. coli*. This finding is consistent with several recent reports that indicate that ROS does not play a role in lethality induced by all major classes of antibiotics.



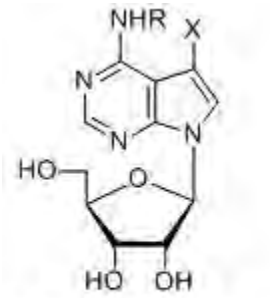
Scheme 1. Nitroreductase (NTR) triggered intracellular ROS generation by **1c**

MEDI 252

Synthesis of 6-N-substituted 7-deazapurine nucleoside antibiotics: Potential nucleoside transport inhibitors

Ramanjaneyulu Rayala[1]*, rraya002@fiu.edu, Patricia Theard*[1]*, Heysel Ortiz*[1]*, Stanislaw F. Wnuk*[1]*, Morris J. Robins*[2]*. (1) Department of Chemistry and Biochemistry, Florida International University, Miami, Florida 33174, United States (2) Department of Chemistry and Biochemistry, Brigham Young University, Provo, Utah 84602, United States*

The 6-*N*-(4-nitrobenzyl) derivatives of nucleoside antibiotics of type **1** (R = H, X = H, tubercidin; R = H, X = CN, toyocamycin; R = H, X = $CONH_2$, sangivamycin) were synthesized, since the corresponding 6-*N*-(4-nitrobenzyl) adenosine derivatives act as potent nucleoside transport inhibitors. First approach for the syntheses involved the treatment of 7-deazapurine nucleosides with 4-nitrobenzyl bromide to give 1-*N*-(4-nitrobenzyl) intermediates. The resulting intermediates were treated with dimethylamine to give access to 6-*N*-(4-nitrobenzyl) analogues *via* Dimroth Rearrangement. However, it was observed that 6-*N*-(4-nitrobenzyl) toyocamycin (R = 4-nitrobenzyl, X = CN) was formed *via* a direct alkylation on exocyclic amine group and not *via* Dimroth rearrangement pathway. In the second approach, treatment of 6-fluoro-2',3',5'-tri-*O*-acetyltubercidin with 4-nitrobenzylamine and deprotection gave 6-*N*-(4-nitrobenzyl)tubercidin. The 6-fluoro-2',3',5'-tri-*O*-acetyltubercidin precursor was prepared by diazotization-fluorodediazoniation of 2',3',5'-tri-*O*-acetyltubercidin with $NaNO_2$ and 55% HF/pyridine. The 6-*N*-(4-nitrobenzyl)-7-deazapurine derivatives are under evaluation for their inhibitory activity in nucleoside transport systems as well as in viral and cancer culture systems.



1; X = H, CN, $CONH_2$
R = H, benzyl, 4-nitrobenzyl

MEDI 253

Propargyl linked antifolates are dual inhibitors of Candida albicans and Candida glabrata

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Candidiasis is a systemic opportunistic fungal infection, most commonly caused by *Candida albicans*. However, a relatively non-pathogenic *Candida glabrata* has emerged as a source of blood stream infections in immune compromised patients. *Glabrata* species also exhibit resistance towards clinically used therapeutics, necessitating the need for new antimycotic agents and targets. Dihydrofolate reductase (DHFR) has been a validated drug target for antibiotics and anticancer research. In our study of propargyl-

linked antifolates, though we were able to develop potent compounds for the *glabrata* species, the enzyme inhibition did not translate to antifungal activity against *Candida albicans*. Screenings from our abandoned compound library gave insight into the optimal shape and polarity of molecules. Crystal structures of screened hits enabled further optimization to develop leads that potently inhibited the growth of both fungal species with MIC values less than 1µg/mL.

MEDI 254

Novel nitro(triazole/imidazole)-based heteroarylamides/sulfonamides as potential antitrypanosomal drugs

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We have previously shown that 3-nitro-1*H*-1,2,4-triazole-based arylamides and arylsulfonamides demonstrate significant activity *in vitro* against *T. cruzi,* the causative parasite of Chagas disease. Most importantly, several analogs demonstrated significant antichagasic activity *in vivo*, superior to that of benznidazole, currently used in the clinic. In the present work we have further expanded our research by synthesizing a series of novel nitro(triazole/imidazole)-based heteroarylamides/sulfonamides and by screening *in vitro*their trypanocidal activity to establish additional SARs. 3-Nitro-triazole-, 2-nitro-imidazole- and 4-nitroimidazole-based compounds were included.

All nitrotriazole-based compounds were active or moderately active against *T. cruzi* and all but three (two furanocarboxamides and one pyridinecarboxamide) were not toxic to the host cells. The 2-nitroimidazole-based derivatives were moderately active against *T. cruzi* but toxic to the host cells, whereas the 4-nitroimidazole-based derivatives were mostly inactive and toxic. In addition, only derivatives from the 3-nitrotriazole-based series showed activity against *T.b. rhodesiense* and selectivity up to 1410. No activity was observed by any derivative against *L. donovani*. The most active antichagasic 3-nitrotriazoles were chlorinated thiophene/benzothiophenesulfonamides (up to 14-fold more potent than the reference compound benznidazole). Detailed SARs will be discussed. Two nitrotriazole-based sulfonamides were superior than benznidazole in *in vivo* studies, by using a fast luminescence assay.

MEDI 255

Antimicrobial 4-hydroxy-2-octadec-(11*Z*)-enoylcyclohexane-1,3-dione and other secondary metabolites from various Jamaican *Peperomia* species

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In 2009 a new species of *Peperomia* (Piperaceae) was discovered on the western outskirts of the biodiversity rich Cockpit Country in central Jamaica. *Peperomia* sp. nov. yielded one novel (**1**) and two known lignans (**2** , **3**) and two known 2-acylcyclohexane-1,3-dione derivatives (**4** , **5**). Of these, the most abundant (2.63% of dried plant material) is 4-hydroxy-2-octadec-(11*Z*)-enoylcyclohexane-1,3-dione (**4**) . Compound **4** showed significant antibacterial activity against *Staphylococcus aureus* with an IC_{50} of <0.8µg/mL compared to Ciprofloxacin at 0.1 µg/mL. Also, two novel compounds (**6** , **7**) were isolated from *Peperomia hernandiifolia* and two prenylated secondary metabolites (**8** , **9**) from *Peperomia amplexicaulis*. These compounds were characterized mainly by 2D- NMR. NMR spectral parameters of compounds **1** , **4** , **6** and **7** along with biological activity data of compound **4** will be presented.

MEDI 256

Bacteria-triggered release of antimicrobial agents

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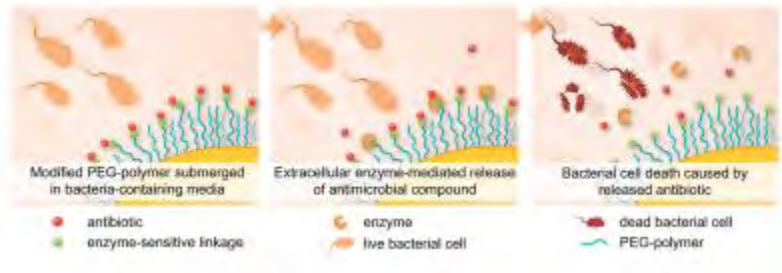
Medical devices employed in healthcare practice are often susceptible to microbial contamination. Pathogenic bacteria may attach themselves to device surfaces of catheters or implants by formation of chemically complex biofilms, which may be the direct cause of device failure. Treatment of device-related infections is often difficult as pathogens exhibit a high degree of antibiotic resistance in the biofilm mode-of-life. Extracellular bacterial lipases are particularly abundant at sites of infection, and we therefore envisioned that active or proactive compounds attached to polymeric surfaces via lipase-sensitive linkages, such as fatty acid esters or anhydrides, could be released in response to infection. Here, we communicate our investigations on such bacteria-responsive materials and report a novel lipase-triggered release system for the control of bacterial populations and modulation of quorum sensing.



The active compound precursor can be synthesized directly on the surface via solid-phase synthesis techniques, and many compounds with a diverse range of biological activities may in principle be released. This approach may address some of the key challenges for the treatment of bacterial infectious disease, including both the mode of delivery, dosage of antibiotics at the site of infection, and development of antibiotic resistance. The self-regulating system provides the basis for the development of device-relevant polymeric materials, which only release antibiotics in dependency of the titer of bacteria surrounding the medical device.

MEDI 257

Approaches to investigate HMP kinase with small molecule probes

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Drug resistance of pathogens requires identification of novel targets for antibiotics. Thiamin is an essential cofactor for all organisms in its active form, but bacteria synthesize thiamin, while humans obtain if from their diet. The chemical space of HMP kinase, an unexplored and essential enzyme within the Thiamin biosynthetic pathway, was investigated by modulation with small molecules. HMP kinase is responsible for catalyzing two sequential phosphorylations of HMP to HMP mono- and di-phosphate within the same catalytic domain. The preparation of various classes of HMP analogues is discussed, revealing convenient and divergent preparations of highly substituted pyrimidines. Luminescent kinase assays were utilized to investigate an initial substrate scope and inhibitor pharmacophore with synthetic HMP analogues. Phosphorylated compounds were analyzed by mass spectroscopy to evaluate phosphorylated end products. The initial studies and future discovery strategy will be discussed.

MEDI 258

Benzopyrano-4(1*H*)-ones as *Cryptosporidium Parvum* inosine 5′-monophosphate fehydrogenase (*Cp*IMPDH) inhibitors

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Cryptosporidium are protozoan parasites causing diarrheal disease in both humans and animals. Although infection is limiting in healthy adults, it can be life-threating in children and immune compromised individuals. Also since the organisms can easily be introduced into the water supply, they present credible bioterrorism threats. Currently, effective therapy is lacking. Inspection of *Cryptosporidium* genomes revealed the enzyme inosine 5′-monophosphate dehydrogenase (IMPDH) catalyzes the rate-determining step in the only pathway to guanine nucleotides in both *C. parvum* and *C. hominis*. Moreover, the *Cryptosporidium* IMPDH gene was likely obtained by lateral gene transfer from bacteria. Hence, *Cp*IMPDH is highly divergent from its host counterparts making it a potentially viable molecular target for treating cryptosporidiosis. Compounds based on a benzopyrano-4(1*H*)-one derivative were identified as selective inhibitors of *C. parvum* IMPDH in a previous study. Herein we report the initial structure-activity relationship (SAR) for this compound series, which will guide additional optimization in order to obtain candidate compounds for evaluation in cell infectivity models and potentially in an *in vivo* disease model.

MEDI 259

Inhibition studies of β-cyclodextrin derivatives against Staphylococcus aureus

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One of the widely spread bacterium found in the human respiratory tract and on the skin is Staphylococcus aureus. This bacterium produces α-hemolysin (α-HL), which is considered as a major virulence factor playing an important role in staphylococcal infection. In this work, we aimed to design and synthesize novel compounds which have similar structure, size and affinity to the target pore. As a model compound we selected β-cyclodextrin, which has similar hexagonal geometry to the target pore caused by α-HL. In order to increase the chemical binding between the pore and compound we tried to alter the size, pH and charge of β-cyclodextrin derivatives by changing the periphery groups. We identified several amino- and azido- derivatives of β-cyclodextrin which showed inhibiting activity of α-HL *in vitro* at relatively low concentrations. Further *in vivo* studies are underway on skin model of rat. We assume that these research findings can

serve as the basis for structure/property related studies against different types of pathogens which utilize pore-forming proteins.

MEDI 260

Understanding SARs of the 6-alkylidenepenicillin sulfones, potent inhibitors of Class A, C, and D □lactamases

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A library of substituted 6-pyridylmethylidenepenicillin sulfones was prepared and evaluated as inhibitors of representative class A, C, and D ß-lactamases, including class A and D carbapenemases, and also for synergy with imipenem against resistant strains of Pseudomonas aeruginosa, Klebsiella pneumoniae, and Acinetobacter baumannii. The compounds were potent inhibitors of the representative serine ß-lactamases, and, with appropriate substitution patterns, could display synergy with imipenem against resistant Gram-negative pathogens. The synthesis, as well as the biochemical and microbiological activity will be presented, together with a synopsis of the SAR trends.

MEDI 261

Fragment-based design, synthesis, and evaluation of a new class of effective non-β-lactam inhibitors of high molecular mass penicillin-binding proteins

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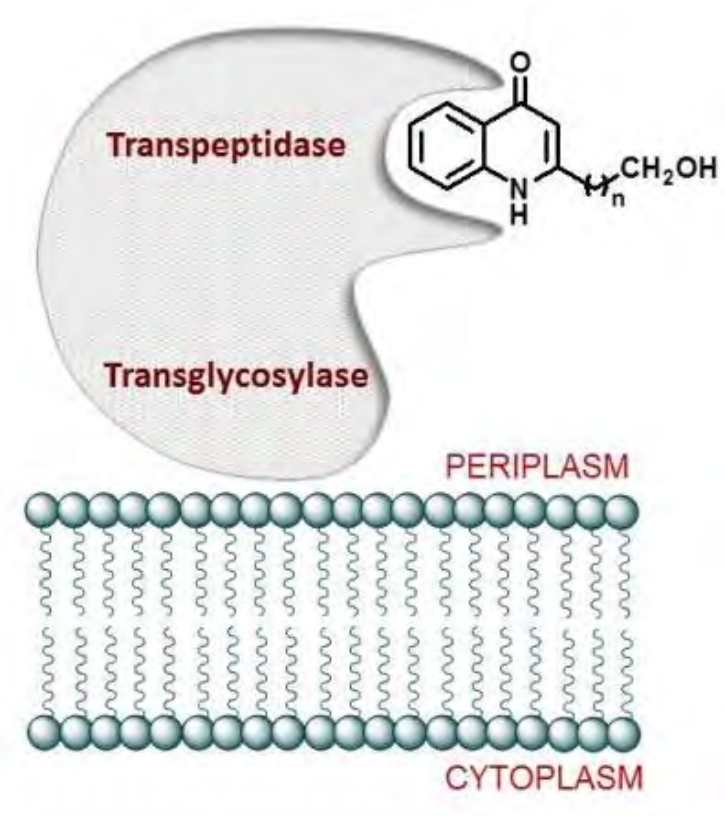
The increasing bacterial resistance to currently available antibiotics has created an urgent need for discovery of novel effective antibacterial agents. Among the other strategies, a promising approach is the discovery of new non-β-lactam antibiotics. The targets of these antibiotics are the penicillin-binding proteins (PBPs) or DD-peptidases. Penicillin-binding proteins are important bacterial enzymes, which catalyze the final peptide cross-linking step in peptidoglycan synthesis.



Directed by a computational fragment-based docking procedure, carried out on *Escherichia coli* PBP5, we have designed and synthesized a series of 4-quinolones derivatives as potential inhibitors of high molecular mass PBPs. We describe their binding to the PBPs by measuring dissociation constants (K_i) of these molecules from their PBP complexes in a competition with the irreversible reaction of the fluorescent penicillin Bocillin FL with membrane-bound PBPs of *E. coli* and *B. subtilis.* This research has shown that small suitably functionalized 2-hydroxyalkyl-4-quinolones are quite striking inhibitors of the high molecular mass PBPs of *E. coli* and *B. subtilis*, *e.g.* for *E. coli* PBP4, K_i values are ranging from 3 to 50 mM.

MEDI 262

Identification of proton-pump inhibiting drugs as inhibitors of *Trichomonas vaginalis* uridine nucleoside ribohydrolase using an ^{19}F NMR-based activity assay

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Trichomoniasis is the most prevalent non-viral sexually transmitted disease, with increasing resistance to existing therapies underscoring the need for novel antitrichomonal agents. The causative agent is the parasitic protozoan *Trichomonas*

vaginalis, an obligate parasite that must scavenge host cell nucleosides to obtain its nucleobases via salvage pathway enzymes. One such enzyme is uridine nucleoside ribohydrolase (UNH), a fundamental constituent in the uridine salvage pathway. After the K_m value for 5-fluorouridine was determined to be 15 μM, an ^{19}F NMR-based activity assay was developed to monitor the UNH-catalyzed conversion of 5-fluorouridine to 5-fluorouracil. The NIH Clinical Collection and NIH Clinical Collection 2 were then screened in a compressed format. A total of 23 out of 573 compounds tested exhibited significant inhibition at 50 μM including the proton-pump inhibiting drugs omeprazole, pantoprazole, and rabeprazole. Dose-response curves using fresh solutions made from commercially-sourced solid compounds confirmed NMR IC_{50} values less than 10 μM.

MEDI 263

Design and synthesis of novel antimicrobials for the treatment of drug resistance bacterial infections including M. tuberculosis

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The alarming increase in bacterial resistance over the last decade along with a dramatic decrease in new treatments for infections has led to problems in the healthcare industry. Tuberculosis (TB) is caused mainly by *Mycobacterium tuberculosis* which is responsible for 1.4 million deaths per year. A world-wide threat with HIV co-infected with multi and extensively drug-resistant strains of TB has emerged. In this regard, herein, novel acrylic acid ethyl ester derivatives were synthesized in simple, efficient routes and evaluated as potential agents against several *Mycobacterium* species. These were synthesized via a stereospecific process for structure activity relationship (SAR) studies. Minimum inhibitory concentration (MIC) assays indicated that esters **12** , **13** , and **20** exhibited greater *in vitro* activity against *Mycobacterium smegmatis* than rifampin, one of the current, first-line anti-mycobacterial chemotherapeutic agents. Based on these studies the acrylic ester **20** has been developed as a potential lead compound which was found to have an MIC value of 0.4 μg/mL against *Mycobacterium tuberculosis*. The SAR and biological activity of this series is presented; a Michael – acceptor mechanism appears to be important for potent activity of this series of analogs.

MEDI 264

De novo design antimicrobial peptides using self-organizing maps

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Antimicrobial peptides (AMPs) represent a class of bioactive agents that can be used to target multidrug resistant bacteria. Their mode of action is diverse and not fully understood, and innovative peptide design strategies are needed to generate novel AMPs with improved properties. Here we present the computer-assisted *de novo* design of AMPs. We selected two potent AMPs, protonectin and decoralin, as seed peptides and synthesized randomly scrambled derivatives based on these two templates. Membrane disruption was tested using large unilamellar vesicles (LUVs) fluorescence assay. Some of the scrambled peptides exhibited significant discriminatory activity for zwitterionic vesicles or anionic vesicles and presented a broader spectrum of antibacterial activity. For *de novo* design, we encoded the above tested peptides and 10000 pseudo-random peptides composed by the same amino acid distribution as the known actives by the PEPCATS descriptor and projected the distribution on a self-organizing map (SOM, Kohonen network). New peptides that fell together with tested active peptides in the same cluster were selected for synthesis and testing. Three synthesized peptides showed significant activity in the LUVs assays and two of them even exhibited stronger membrane lytic activities than the seeds. Inducible α-helix structure in circular dichroism emerged as a critical feature for those membrane active peptides. Surface plasmon resonance and atomic force microscopy were also used to explore peptide-membrane interaction. Isothermal titration calorimetry suggested an entropy-driven mechanism for peptide-membrane interaction. The study demonstrates the sustained potential of advanced computer-assisted methods for designing peptides with desired properties and activity.

MEDI 265

Strategies for the extension of plasma half life of the complement inhibitor compstatin

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Inappropriate or uncontrolled activation of the complement system contributes to the damage of host cells in many immune and inflammatory disorders. Consequently, there is an emerging need to develop specific complement inhibitors for the treatment of complement-mediated diseases. Complement component C3, the most abundant protein of the complement system, plays a central role in the complement activation cascade and presents a valuable target for therapeutic intervention. The Compstatin family of cyclic, synthetic peptides are highly potent and selective C3 inhibitors that regulate complement activation and have demonstrated strong therapeutic potential in various disease models. Considering long-term treatment in chronic diseases, new generations of compstatin derivatives were developed in order to improve the

pharmacokinetic profile of these peptidic C3 inhibitors. Conjugates between compstatin and albumin-binding molecules were designed and synthesized in an effort to prolong the plasma residence time of compstatin by taking advantage of the large serum albumin pool in circulation. In addition, site-specific PEGylation of compstatin analogs was investigated as an alternative measure for extending the plasma half life, since PEG polymers have the ability to delay the elimination of biomolecules from the circulation by decreasing renal clearance, proteolysis and immunogenicity. These new generations of long-acting compstatin derivatives were evaluated both in vitro and in vivo, and showed promising characteristics for a use in chronic complement-related disorders.

MEDI 266

Design and synthesis of novel small molecule stilbenes with activity against gram-positive bacteria and mycobacterium

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Novel small molecule stilbenes with activity against of number of bacterial strains have been synthesized via a transition metal Heck reaction. These compounds exhibit potent activity against Methicillin-resistant *Staphylococcus aureus* (MRSA) with MIC's as low as 1 µg/mL. Many of these molecules also exhibit activity against other gram-positive bacteria as well as mycobacterium. These small molecules may also represent a new class of antibiotics and perhaps involve a unique mechanism of action that in the future may help curb the spread of multi-drug resistant bacteria. Design and synthesis of new novel small molecules via the optimized Heck reaction are ongoing and mechanism-of-action studies are currently being initiated by our collaborators.

MEDI 267

Targeting vitamin pathways for development of novel antibacterials

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Current R&D has been facing a significant innovation deficit as it struggles to manage with new and always increasing economic pressures. On the other hand, bacterial chemotherapy exhibits a reduction in efficacy due to an overwhelming rise in bacterial resistance, leading to millions of deaths worldwide and a futile health care system. Vitamin pathways offer an unexplored and unique source for novel anti-infectives, resulting in the crucial investigation of these major enzymes. HMP kinase, a vital

enzyme in the thiamine metabolism, stands out as a promising target for drug discovery. Efforts towards the synthesis of HMP analogs and their respective evaluation via high throughput assays were completed to aid in the exploration of chemical space within the enzyme's active site. Incorporation of other analytical tools will enhance our understanding of the catalytic process of HMP kinase; ultimately, leading to the synthesis of a better pharmacophore.

MEDI 268

Antimicrobial cyclic lipo-α-AApeptides

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The emergent resistance of bacteria against the conventional antibiotics has motivated the search for novel antimicrobial agents. Nature abounds with a number of host defense peptides (HDPs). HDPs are present in virtually all life forms, acting as the first line of defence against microbial infection. While they are broad-spectrum in their activity and show less drug-resistance induction, their intrinsic metabolic stability limits their potential therapeutic applications. We have previously reported a group of unnatural HDP mimics, α-AApeptides (N-**a** cylated-N-**a** minoethyl peptides), as effective antimicrobial agents against various bacterial strains. Herein, we report the development of modified α-AApeptides, through simultaneous lipidation and cyclization. These novel cyclic lipo-α-AApeptides demonstrate significantly improved potency and broad-spectrum activity against a range of clinically relevant Gram-positive and Gram-negative bacteria. Furthermore, the lead cyclic lipo-α-AApeptide is the first of its kind shown to mimic some HDPs by antagonizing TLR4-induced nuclear factor kappa-light-chain-enhancer of activated B cells (NF-*κ*B) and nitric oxide (NO) signaling responses, as well as inhibiting production of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α). The linear analogue of the lead cyclic lipo-α-AApeptide shows weaker antimicrobial and inferior anti-inflammatory activity, indicating that both lipidation and cyclization are critical for the dual functions. Our results suggest that these cyclic lipo-α-AApeptides may emerge as a new class of antibiotic agents with both direct bacterium killing and immunomodulation ability.

MEDI 269

Design and synthesis of piperidinyl sulfamides against malarial aminopeptidase N, with micromolar efficacy against the parasite

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Malaria claims about a million deaths worldwide annually. The widespread resistance to current drug therapies is of major concern and necessitates the need for identification of new drug targets and new drugs. Malaria parasite needs to degrade the host cell hemoglobin to obtain amino acids for its growth during its development in erythrocytes. M1 alanyl-aminopeptidase (PfAPN) is an enzyme involved in the terminal stages of hemoglobin digestion to generate an amino acid pool within the parasite, and this enzyme has been validated as a potential drug target. To develop selective and effective inhibitors against PfAPN, we designed and synthesized a host of Piperidinyl Sulfamides basedcompounds. These molecules displayed inhibition of the recombinant PfAPN at low micromolar concentrations. To assess if these compounds also affect parasite growth, the most lethal human malaria parasite P. falciparum was grown in the presence of varying concentration of these compounds, and IC50 concentrations for each compound were determined. Similar to the enzyme inhibition, these molecules showed parasite growth inhibition with IC50 values in the low micromoles. Structural studies are in progress to understand their selectivity for PfPAN and improve their potency.

MEDI 270

Role of S*uperoxide-dismutases* and A*lkyl-hydroperoxidases* in pathogenesis of *Pseudomonas syringae*

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Plants, such as *Arabidosis thaliana*, release reactive oxygen species (ROSs) such as the superoxide anion, hydroxyl radical, and hydroxyl ion as a defense mechanism when they are infected with microorganisms. The production of ROS results in restriction of the growth of pathogen like *Pseudomonas syringae*. In order to be a successful pathogen, *P. syringae* must suppress these responses when it infects plants. *P. syringae* has evolved redox-related enzymes to detoxify ROS. In *P. syringae,* there are genes encoding such enzymes including superoxide dismutases (SODs), peroxidases, and catalases. *P. syringae* is able to relieve oxidative stresses by detoxifying ROS into less reactive molecules using these redox-related enzymes during infection. Superoxide SODs and catalases catalyze coupled reactions to reduce ROS converting O_2^- into H2O2 which is further degraded by catalases to H2O. Peroxidases detoxify organic peroxides. Catalases collectively contribute to *P. syringae* pathogenesis. However, it remains unclear whether SODs and peroxidases also play a role in pathogenesis.

In this project, we set out to1) Clone and express sodA, sodB and sodC genes in *E. coli*, 2) Perform pathogenicity assay with the *sodC* mutant, and 3) Generate deletion mutants lacking both *AhpC* and *AhpF*. We successfully cloned the sodA, sodB and sodC genes using plasmid pLN615, and verified their authenticity by immunoblot of the expression extract. Pathogenicity assays revealed that the *sodC* mutant did not show significant differences in virulence from the wild type strain DC3000 in *Arabidopsis* plants. This suggests that the *sodC* gene may be redundant with the s*odA* and *sodB* in DC3000. We were also successful in generating *P. syringae* mutant lacking both *AhpC* and *AhpF* using an unmarked mutagenesis approach.

MEDI 271

Synthesis of liposomal MUC1 glycopeptide-based three component vaccine and evaluation of the effect of L-rhamnose on the immune response

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$CD8^+$ response to extracellular antigens, an important aspect of anti-tumor vaccines, is generated by presentation of the antigen by antigen presenting cells (APC) and cross presentation to $CD8^+$ T cells via MHC class I molecules. Cross presentation is augmented by efficient antigen uptake followed by immune-complex-mediated maturation of the APCs. We have used liposomes with an L-Rhamnose epitope displayed on its surface as a ligand for anti-Rhamnose antibodies. Anti-Rhamnose antibodies are among the most abundant naturally occurring antibodies in humans. The formation of an anti-Rha-liposome immune complex should mediate an antibody-dependent antigen uptake mechanism for better antigen uptake and cross presentation. We synthesized a 20 amino acid MUC1-Tn peptide carrying a $CD8^+$ T cell epitope. A Toll-like receptor ligand (TLRL) was attached to it by Cu(I) assisted click chemistry. The TLRL-MUC1-Tn vaccine was incorporated on liposome by extrusion method. The liposomes were constructed using TEG-cholesterol or rhamnose-TEG-cholesterol, and 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine. The vaccine was tested on groups of C57BL/6 mice. Naïve mice were prepared with endogenous anti-rhamnose antibodies by immunization with rhamnose-ficoll prior to vaccination with liposomal TLRL-MUC1-Tn. The $CD8^+$ T cell response was evaluated by measuring $CD8^+$ T cell proliferation, cytotoxicity and IFNγ production. The anti-Rha antibodies producing mice showed higher $CD8^+$ T cell response compared to the control groups. The results suggest that the antigen was better taken by the APCs and that the MUC1 $CD8^+$ T cell epitope was cross presented to the MHC class I molecule more efficiently as a result of anti-rhamnose antigen uptake mechanism.

MEDI 272

Targeting cell wall biosynthesis in TB bacteria: Decaprenyl diphosphate synthase inhibitor design

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Decaprenyl diphosphate synthase (DPPS) catalyzes the formation of decaprenyl diphosphate, an essential component in *Mycobacterium tuberculosis* cell wall biosynthesis and thus, DPPS is a drug target for disrupting cell wall formation in *M. tuberculosis*. We carried out high through-put screening with a library of 700 compounds using a colorimetric assay for diphosphate release and found 39 potent inhibitors, which were then confirmed with an assay using ^{3}H-labeled substrates. Quantitative structure-activity relationship models were built with the 39 hits and five types of interactions were identified: steric, electrostatic, hydrophobic, possible hydrogen bond donor and possible hydrogen bond acceptor. More importantly, one inhibitor with a bisamidine structure was potent *M. tuberculosis* cell growth inhibitor, with an MIC of 0.31-1.25 μg/ml. We also obtained the first inhibitor bound crystal structure of DPPS, finding that DPPS adopts the ζ-fold and the inhibitor binds to the substrate site, consistent with the fact that this compound acts as a competitive inhibitor. We also performed molecular dynamics (MD) simulations on DPPS in its apo form and in complex with various ligands to investigate its dynamic behavior. Binding pocket volume calculations from the MD trajectories reveal the structural plasticity of the DPPS active site, opening up future possibilities of virtual screening with open DPPS conformations. Taken together, these results provide new opportunities for structure-based inhibitor design targeting *M. tuberculosis* DPPS.

MEDI 273

Design and synthesis of a series of small molecule CCR2 receptor antagonists to probe the structure-kinetic relationship

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The chemokine CCR2 receptor has been considered as a potential target for the treatment of several immune-based inflammatory diseases. Inhibiting the CCR2 receptor with small molecule antagonists has been of great pharmaceutical interest in the development of potential therapeutics. Due to lack of efficacy, high affinity CCR2 inhibitors have failed in clinical trials for the treatment of inflammatory diseases. Our

goal is to develop new CCR2 small molecule antagonists with long residence times (RT) which could potentially be linked to duration of the *in vivo* efficacy. The work presented here describes the design and synthesis of a series of compounds through a parallel synthesis based approach with a known orthosteric tetrahydroisoquinoline CCR2 antagonist scaffold. Compounds were tested for their affinity as well as for their ligand-receptor residence time (RT) using a novel kinetic dual-point competition association assay. Chiral synthesis of the optically pure compounds resulted in single enantiomers with long residence time (RT). From this study, it was clear that the structure-affinity relationship (SAR) and structure-kinetic relationship (SKR) do not correlate: the compounds synthesized showed difference in SAR and SKR which demonstrates that the additional parameter residence time (RT) along with the affinity can help in compound optimization in later stages of drug discovery process which cannot be achieved by SAR alone.

MEDI 274

Design, synthesis, and biological evaluation of tetrazole analogs as protein arginine deiminase inhibitors

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Protein arginine deiminases (PADs), members of the amidinotransferase superfamily of enzymes, are responsible for an important posttranslational modification of arginine called protein citrullination or deimination. This once obscure modification is involved in the onset and progression of many autoimmune diseases such as Rheumatoid Arthritis (RA), Multiple Sclerosis (MS), Lupus, Ulcerative Colitis (UC), and in some forms of cancer. Among the five human PADs (PAD1, 2, 3, 4 and PAD6), it is unclear which of the PAD isozymes contributes to disease pathogenesis. Towards the identification of potent, selective, and bioavailable PAD inhibitors as useful chemical probes to elucidate the specific roles of each isozyme, we describe tetrazole based analogs of Cl-amidine as PAD inhibitors: The tetrazole serves as suitable amide bioisostere for the parent Cl-amidine.

These compounds are highly potent and some of the analogs show excellent

selectivity towards particular isozymes. These compounds also possess enhanced

pharmacokinetic properties. Importantly, one of the compounds, biphenyl tetrazole

tert butyl Cl-amidine exhibits significant cell killing in the PAD4 expressing, osteosarcoma

bone marrow (U2OS) cell line. These tetrazole based analogs represent an important step in

our efforts to develop stable, bioavailable and highly selective inhibitors for the PADs.

MEDI 275

IL-8, NGAL & KIM-1 as early biomarkers for acute kidney injury diagnosis

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Acute kidney injury (AKI) is a common cause of morbidity (5-7%) in hospitalized patients with over 50% mortality. AKI is particularly common in patients in intensive care units. Currently, AKI is diagnosed based on elevations in the level of serum creatinine and blood urea nitrogen; however, they lack required sensitivity for early detection of AKI. Identification of biomarkers for the early detection of AKI is a high priority for patient management and survival. We examined three urine biomarkers proposed to help in early detection of AKI: interleukin 8 (IL-8), neutrophil gelatinase-associated lipocalin (NGAL), and kidney injury molecule 1 (KIM-1). Findings indicate that measurement of urine IL-8 and NGAL may identify patients with early AKI on admission to the ICU. Elevated levels of KIM1 in the urine on admission may identify patients at risk for subsequent AKI.

MEDI 276

Synthesis and biological activity of novel synthetic benzothiophene SERMs and use of the phytoestrogen glycinol as a guide: The quest for an ideal SERM

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An ideal selective estrogen receptor (ER) modulator (SERM) has actions contrary or antagonistic with estrogen in the breast and actions similar to or agonistic in bones and the brain. The benzothiophene SERM, raloxifene, has been used safely in postmenopausal osteoporosis and breast cancer chemoprevention for many years. Botanicals containing phytoestrogens are also reported to be protective against diseases such as menstrual irregularities, osteoporosis, cardiovascular diseases and various types of cancer. However, in general, these phytoestrogens are agonists in breast epithelial cells and often lack the potency of synthetic SERMs. In developing novel ER ligands based upon the benzothiophene (BT) scaffold with mixed, agonist, antagonist, and partial agonist actions at ER isoforms, the incorporation of a hybrid scaffold derived from the phytoestrogen glycinol, presented an interesting target. The synthesis of novel ER ligands based upon these scaffolds has revealed that small modifications can strongly influence isoform selectivity and both pharmacological efficacy and potency.

MEDI 277

Novel small molecules for treating kidney disease

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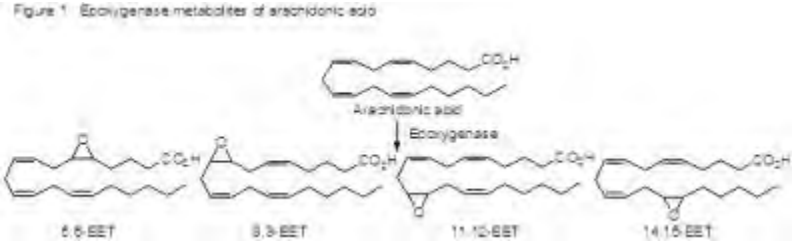
Loss of kidney function may develop immediately (acute) or gradually (chronic). Acute kidney injury (AKI) typically occurs after exposure to drugs, certain medical procedures, or exogenous toxins. Building upon our original observations that arachidonic acid metabolite



8,9-epoxyeicosatrienoic acid (EET) maintains the glomerular filtration barrier. Our recent novel results show that 8,9-EET analogs mimic the protective effect of the labile natural 8,9-EET and our recent studies led to a discovery of two lead analogs CRO-94 and CRO-95 which preserved the glomerular filtration barrier and protected the kidney from puromycin and cisplatin-induced.

MEDI 278

WITHDRAWN

MEDI 279

Optimization of a series of an N-arylsulfonamido-N'-aryl-piperazine GK-GKRP modulators: Exploration of a novel binding pocket in GKRP

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Glucokinase (GK) is an enzyme in the hexokinase family that plays a pivotal role in blood glucose homeostasis. Allosteric activation of GK with small molecules has been shown to lower blood glucose levels in rodents as well as in human clinical trials. Although this class of therapeutics have demonstrated robust efficacy, a potentially serious side effect is hypoglycemia as a result of over activation of GK. To address this liability, our team has initiated a program designed to target GK pathway by inhibiting the interaction of GK with its liver-specific glucokinase regulatory protein (GKRP). In this poster we report the results of our expanded SAR investigation of the *N*-arylsulfonyl-*N*-2-pyridinyl-piperazine series that focused on the 6-position of the *N*-pyridine ring. These studies resulted in the discovery of 2-(2-(4-((6-aminopyridin-3-yl)sulfonyl)piperazin-1-yl)-[3,3'-bipyridin]-5-yl)-1,1,1,3,3,3-hexafluoropropan-2-ol. The X-ray co-crystal data showed that the 3-pyridine ring of this compound bound in a novel area of the GKRP protein. This compound was potent in the biochemical and cellular assays for GK-GKRP and exhibited favorable PK properties for in vivo evaluation. When administered orally to db/db mice this compound demonstrated a robust pharmacodynamic response, and led to a significant reduction in plasma glucose levels.

MEDI 280

Treatment of metabolic syndromes: Synthesis and pharmacological evaluation of newer pparδ agonists

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Metabolic syndrome (MS) is defined by a cluster of interconnected factors that directly increase the risk of coronary heart disease (CHD), other forms of cardiovascular atherosclerotic diseases (CVD) and Type 2 diabetes mellitus (T2DM). Several studies have suggested important role of PPARδ in regulating lipid metabolism and energy homeostasis in muscle and fat. The present research work was undertaken to design and develop novel moieties derived from anthranilic acid scaffold as PPARδ agonists that can be developed further for the management of metabolic disorders. The molecules were designed using receptor based drug design approach, by utilizing the X-ray crystallographic information of PPARδ from PDB database. Based on the pharmacophoric requirements for PPARδ binding, the anthranilic acid nucleus was chosen for the design of newer analogs by substitution of amide linker and introduction of lipophilic groups on aromatic system by using sulfonamide group as a linker.

Amongst the several synthesized anthranilic acid derivatives, 5-Chloro-2-[3-(4-nitro-phenylsulfamoyl)-benzoyl amino]-benzoic acid showed highest antidiabetic activity. The experimental results were found to be in concordance with that of the *in silico* results. Overall, this research work revealed the potential of novel anthranilic acid based PPARδ agonists in the management of MS. Further, these molecules can serve as the starting point for the development of more potent lead molecules for MS.

MEDI 281

Fragment-based GPCR drug discovery using a stabilised receptor (StaR): Identification of an mGlu5 negative allosteric modulator (NAM) pre-clinical candidate

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An mGlu5 NAM pre-clinical candidate molecule has been identified through fragment-based drug discovery using a stabilised receptor (StaR). The candidate has more than ten-fold better affinity and ED_{50} in an *ex vivo* receptor occupancy model than mavoglurant, the most advanced clinical mGlu5 NAM, and has a significantly superior *in vivo*PK profile in rat and dog. Excellent selectivity within the mGlu receptor sub-family is matched by a clean profile against an extensive panel of CNS targets. Details of the data package associated with the candidate molecule will be disclosed.

The G protein-coupled receptor (GPCR) superfamily is critically involved in many diseases and has been a drug-discovery focus for decades, with approximately 30% of current drugs modulating these important targets. Despite this, many receptors with good pre-clinical or clinical validation have been difficult to prosecute using traditional strategies. Instability of GPCRs when removed from their membrane environment has severely limited the application of fragment-based techniques, an issue overcome at Heptares by generation of stabilised receptors (StaR proteins) which facilitate biophysical and biochemical fragment screening and allow X-ray structural information to be obtained.

In the first application of our approach to a family C target, an mGlu5 StaR has been created, using a NAM ligand to introduce a conformational bias towards the desired pharmacology for subsequent drug discovery. Enhanced stability of the mGlu5 StaR, coupled with dramatically increased expression and significantly higher DMSO tolerance, allowed us to conduct a robust fragment screen. The screen, in high-concentration radioligand binding format, yielded high-quality hits from a family C focused set and the general Heptares fragment collection. The most favourable series was rapidly advanced in a hit to lead to candidate process lasting approximately one year with less than 100 compounds synthesized before candidate identification.

MEDI 282

Chiral subtype selective imidazobenzodiazepines important as potential agents to treat schizophrenia

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Schizophrenia is a mental disorder which causes the breakdown of thought processes and affects almost 1% of the world's population. The primary treatments prescribed are antipsychotics that focus on suppressing dopamine receptor activity. These antipsychotics can fail to be effective, especially with cognitive dysfunction and the negative symptoms, and can even worsen some of the positive symptoms of schizophrenia. Recently we have developed a chiral subtype selective α5-GABAergic positive allosteric modulator, SH-053-2'F-R-CH_3 (**1**), which has very little to no efficacy at the α1, α2, and α3 BzR/GABAergic subtypes. In the MAM model of schizophrenia in rats, **1** reduced the number of spontaneously active dopamine neurons. The enantiomer of **1** , SH-053-2'F-S-CH_3 (**2**), is an α2/α3/α5 ligand that has shown to reduce the startle response in the prepulse inhibition model in rats. Because of the low efficacy at α1, there will be a very low abuse potential for these ligands. Both **1** and **2** have shown that they do not cause catalepsy, unlike some antipsychotics prescribed that act as dopamine inverse agonists. These ligands and other related compounds are being developed to treat schizophrenia, especially with regard to the cognitive dysfunction and negative symptoms of schizophrenia.

MEDI 283

Synthesis and TRPV1 antagonist activities of quinolinoxypyrimidine derivatives

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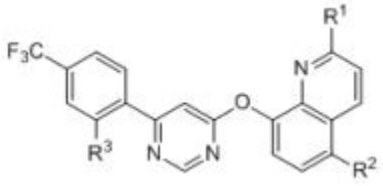
Quinolinoxypyrimidine derivatives **1** and **2** are potent TRPV1 antagonists with pK_b values 7.91 and 7.54, respectively. A series of ten quinolinoxypyrimidine analogues with modifications centered around positions 2 and 5 of the quinoline ring and position 2 of the trifluoromethylphenyl ring was designed and synthesized in an effort to gain a better understanding of the SAR of this series of TRPV1 antagonists. Substitution of bromoacetamide group at position 2 of the quinoline ring led to the most potent member of this series (**3**) with a pK_b value of 7.2.



1 $R^1 = NH_2, R^2 = H, R^3 = H$
2 $R^1 = H, R^2 = NH_2, R^3 = H$
3 $R^1 = NHCOCH_2Br, R^2 = H, R^3 = H$

MEDI 284

Design, synthesis, and biological evaluation of coumarin analogs as acetylcholinesterase inhibitors

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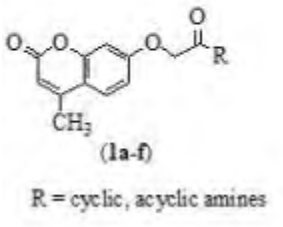
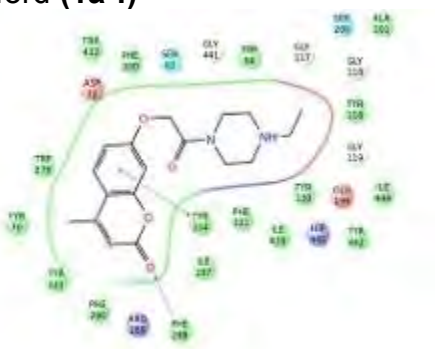
Alzheimer's disease (AD) is a complex neurodegenerative disorder characterized by the central cholinergic depletion and amyloid-*β* (A*β)* plaques. Acetylcholinesterase (AChE) inhibitors not only suppress the normal break down of acetylcholine (ACh) from the synaptic cleft, but also prevent the proaggregating activity of AChE toward A*β*. Therefore, dual binding AChE inhibitors can alleviate cognitive deficits by binding to both catalytic and peripheral site of the enzyme. A number of coumarin analogues have been reported as potential cognitive enhancers. It was thus envisaged to exploit this versatile pharmacophore. Hence, a series of substituted coumarin derivatives was synthesized by refluxing the 7-hydroxy-4-methyl-coumarin with methylchloroacetate to afford the ester derivative which was subsequently refluxed with cyclic and acyclic amines respectively to afford **(1a-f)**



(1a-f)

R = cyclic, acyclic amines



The structures of the synthesized compounds were confirmed by spectral analysis. The compounds were evaluated for anti-amnesic activity and AChE inhibition using Morris water maze and *Ellman* method. Docking study of the compounds was performed using Glide program and crystal structure of *Tc*AChE. Compound **1f** was found to be the most potent heterodimer (IC_{50} = 3.42 µM). Escape latency of the **1f** was comparable to standard drug rivastigmine. Docking studies also revealed that **1f** could bind to both the

catalytic and peripheral sites. Thus, coumarin analogues may act as disease modifying agents in AD.

MEDI 285

On nanorobot drug delivery of Curcuma longa for treatment of Alzheimer's disease

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There is a swarm of therapeutics arriving that used nanostructuring principles. Nanocoatings can be used in design and application of nanorobots for drug delivey. Nanoelevator was built at UCLA and is molecular scale device that is 2.5 nm high and can move up and down by 1 nm. Drugs are packed with nanoagents and are used to knock-out cancer cells. In 1995, Doxil was approved for use in the United States. The chemotherapy doxorubicin is encapsulated into a fat bubble/liposome and another coating of hair like strands made of rubbery material is applied. The agent is 100 nm in diameter. Doxil liposomes are small and are able to circulate for long periods of time. They posess the capability of penetration of vasculature or arrangement of blood vessels or tumors. Upon arrival at the tumor tissue the drug is slowly released. It can be used for treatment of patients with ovarian cancer.

Principles from photodynamic therapy, fullerene chemistry, nanostructuring, x-rays, computers,

pharmacokinetics and robotics are applied in developing a strategy for treatment of Alzheimer's disease using nanorobots. The curcuma longa that has shown curative effects in rats' brain with Alzheimers is coated on fullerenes. The drug is inactive when in caged or coating form. It is infused intrathecally into the cerebrospinal system. Irradiation of the hypothalamous

and other areas of the brain where Alzheimer's disease is prevalent lead to breakage of

fullerenes and availability of the drug with the diseased cells. Due to better mass transfer

better cure is effected. The other plausible reactions such as addition polymerization of

fullerene, polycurcumin formation and other hydrolysis reactions are modeled along with

the drug action under the Denbigh scheme of reactions. The fractional yield of drug curcumin

interaction is a function of intensity of radiation, frequency of radiation, patient demographics, age, gender, other disorders etc. Chromophore in curcumin is used as a sensor and computer imaging and feedback control design can result in more bioavailability for curcumin therapeutic action to cure Alzheimer's disease. The principles used in the design, the strategy of the design of the nanorobot drug delivery system with a specific target and pharamacokinetic formulation of the associated competing parallel reactions are discussed.

MEDI 286

Indole-based inhibitors of the mitochondrial human Lon protease

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The human Lon protease (hLon) plays an important role to clear oxidized proteins and to maintain mitochondrial homeostasis in relation to the high level of reactive oxygen species (ROS) produced. The basic processes by which hLon works are still mostly unknown but its role has been linked to several conditions including aging, neurodegeneration, and cancer. Central to fully characterize these functional roles is the design of novel compounds that are able to modulate the activity of the human Lon protease. Besides unselective molecules like tripterpenoids and coumarin-based ligands, no specific and mitochondrial-permeable inhibitors of the hLon are available yet.

In our efforts to discover new hLon modulating agents, we have used structure-based molecular design techniques to screen *in silico* compounds bearing novel chemical scaffolds. Here we present the biological characterization of new indole-based compounds that inhibit protease, peptidase and ATPase activities of the hLon in the low micromolar concentration. The mitochondrial activity of these compounds was tested on HeLa cells and compared to HeLa cells with hLon depleted, demonstrating their permeability to mitochondrial membranes. The use of these compounds will help to understand the role of hLon in the maintenance of the mitochondrial proteome and genome, to study biological effects on processes like mitochondrial functionality, apoptosis, cell proliferation and senescence and, finally, as new lead compound for pharmaceutical development.

MEDI 287

Synthesis and biological evaluation of oxazole and thiazole based enkephalin peptidomimetics

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The role of opioid peptides as endogenous analgesics suggests a possible use as pharmacological tools for pain relief, devoid of undesired secondary effects such as induced tolerance and dependence. Due to the rapid degradation by enzymes and the poor hydrophilic character of natural Leu-enkephalin peptide, there is a need to design and synthesize Leu-enkephalin peptidomimetics. This study presents the synthesis of oxazole and thiazole based heterocyclic aminoacids for the preparation of Leu-enkephalin peptidomimetic analogs and their biological evaluation. Oxazole aminoacids were obtained from the corresponding aminoacid and Serine ester, whereas thiazole aminoacids were prepared via Hantzsch cyclization starting from the corresponding thioamide. It is known that the incorporation of oxazole and thiazole heterocycles in the peptide back bone imparts conformational restriction which can enhance binding and hence the therapeutic potential. The current approach involves the combinatorial solid phase synthesis of oxazole and thiazole aminoacids containing tri- and tetra-peptides. All the compounds are tested for their affinity for μ- (MOR), d- (DOR), and κ- (KOR) opioid receptors. Good activity was obtained for the compounds with a general sequence Leu(Oxz)-Gln(Thz)-XXa-Tyr(Y) [Leucine-Oxazole, Glutamine-Thiazole and other Tyrosine aminoacids]. The design and the synthesis of the peptidomimetics and their biological activity profiles will be presented.

MEDI 288

Cyclopropenes as warheads for inhibitors of cysteine proteases

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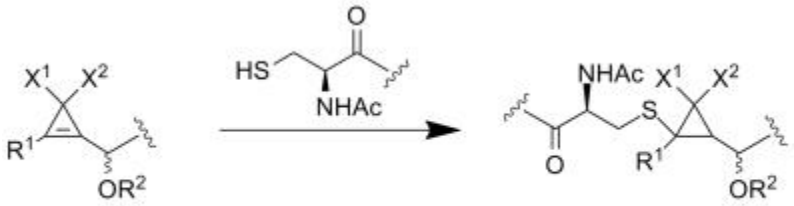
Cysteine proteases are protein processing and protein degrading enzymes whose overexpression in human body results in serious pathological changes, such as neurodegenerative diseases (*e.g.*Alzheimer's, multiple sclerosis, ischemic stroke, myocardial infarcts, cataract formation, *etc.*), destruction of cartilage tissue, and bone atrophy. Other cysteine proteases play an essential role in life cycles of some viruses (*e.g.* coronavirus) and parasites (*e.g.* malaria). The affinity labeling agents used as inhibitors of cysteine proteases normally bear an electrophilic “warhead”, a reactive group that covalently binds the active site cysteine residue thereby inactivating the enzyme. Examples include Michael acceptors, 1,2-diketones, and three-membered ring heterocycles (*e.g.* aziridines, epoxides), the latter generally having greater potency, but lower selectivity, often affecting aspartate and serine proteases as well. Among

carbocyclic unsaturated three-membered ring compounds, only BDA-410, a cyclopropenone derivative, has been used as a cysteine protease inhibitor, but its binding appears to be reversible, which suggests the addition of the thiol group to the 3-carbonyl, rather than to the cyclopropene double bond. Several derivatives of cyclopropenes have been synthesized and evaluated as potential cysteine-binding "warheads". Their stability and reactivity toward cysteine and other amino acid chemical probes was examined. The 1,2-cyclopropene moiety *irreversibly* binds the thiol group of cysteine, leaving other amino acid residues unaffected, which has the advantage for targeting enzymes expressed in foreign organisms or promoting carcinoma progression (*e.g.* cathepsins L, B, H, and S).



R^1 = H, Ph; R^2 = H, Ac, X^1, X^2 = H, F, COOMe, CN

MEDI 289

Small-molecule anticonvulsant agents with potent in vitro neuroprotection and favorable drug-like properties

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Severe seizure activity is associated with reoccurring cycles of excitotoxicity and oxidative stress that result in progressive neuronal damage and death. Intervention with these pathological processes is a compelling disease-modifying strategy for the treatment of seizure disorders. We have optimized a series of small molecules for neuroprotective and anticonvulsant activity as well as altered their physical properties to address potential metabolic liabilities, to improve CNS penetration and to prolong the duration of action in vivo. Utilizing phenotypic screening of hippocampal cultures with nutrient medium depleted of antioxidants as a disease model, cell death and decreased neuronal viability produced by acute treatment with glutamate or hydrogen peroxide were prevented. Modifications to our previously reported proof of concept compounds have resulted in a lead which has full neuroprotective action at < 1 nM and antiseizure activity across six animal models, including the kindled rat, and displays excellent pharmacokinetics including high exposure to the brain. These modifications have also eliminated the requirement for a chiral molecule, removing the possibility of racemization and making large scale synthesis more easily accessible. These studies

strengthen our earlier findings which indicate that potent, multifunctional neuroprotective anticonvulsants are feasible within a single molecular entity which also possesses favorable CNS-active drug properties in vitro and in vivo.

MEDI 290

Dendron-functionalized polymeric nanoparticles for cocktail therapy of advanced prostate cancer

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Prostate cancer (PCa) is one of the lethal forms of cancer that initially grows in the prostate gland.[1] Androgen ablation/castration therapy has been used extensively as anticancer modality for the initial stage of this disease. PCa that progresses even in the presence of androgen ablation is defined as Castration-Resistant Prostate Cancer (CRPC).[2] At this stage PCa tends to spreads from its original place to another part of the body, such as bones, and lymph nodes and
this stage of PCa further defined as metastatic PCa and it affects more than 80% of CRPC patients.[3] Cancer-associated inflammation functionally plays an important role in the formation of metastasis. Treatment of CRPC primarily relies on the use of cytotoxic chemotherapy following hormonal manipulation. However, chemotherapeutic agents do not help to relieve many of the symptoms related with CRPC, such as chronic inflammation and bone metastases.
Recently anti-inflammatory drugs have been used as adjuvant for chemotherapy for the treatment of metastasis PCa.[4] However the choice of correct drug combination and its synergistic ratio are ill defined. By combining controlled release polymer technology and targeted drug delivery approaches, we aim to differentially deliver chemotherapeutic drugs to PCa cells along with synergistic anti-inflammatory agents and inhibitors of bone resorption in a temporally regulated manner resulting in safer and more effective management of deadly CRPC. Polymeric NPs of dual drug functionalized polylactide (PLA) and poly(lactide-*co*-glycolide)-*b*-polyethyleneglycol (PLGA-*b*-PEG) block copolymers demonstrate significant better cytotoxic profile as compared to that of individual drugs.

References

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MEDI 291

Rational design, synthesis and biological studies of two series of novel indenoquinolone derivatives targeting wild-type and R364H mutant of topoisomerase I

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DNA topoisomerase I (Top1) is a well-validated antitumor target of camptothecin (CPT) derivatives, including irinotecan and topotecan. However, CPTs are suffered several limitations, e.g. low solubility, poor stability, high toxicity, and the occurrence of resistance. Several non-CPT Top1 inhibitors demonstrated promising antitumor activities and are now under clinical investigations. Crystallographic studies demonstrated that a key hydrogen bond was observed between all the three major classes of Top1 inhibitors (including camptothecin, indolocarbazole and indenoisoquinoline) and Arg364 of Top1. Therefore, R364H mutation, firstly observed in CPT-resistant tumor cell line, will probably render resistance to all the three classes of Top1 inhibitors in clinical trials. Thus, it is necessary to discover novel Top1 inhibitors targeting wild-type and R364H mutant of Top1. Using rational design and medicinal chemistry approaches, we have discovered two series of novel indenoquinolone derivatives that have shown potent activities against both wild-type and R364H mutant of Top1. This study also provided an elegant example that targeting drug-resistant mutant could be achieved by QM-guided scaffold hopping.

MEDI 292

NMR-based methods for screening inactivating molecular interactions of boron-based drugs

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The introduction of Bortezomib (Velcade), a proteasome inhibitor approved for the treatment of multiple myeloma, a growing number of additional boron-based drugs are under investigation. Considering that the boron moiety in these agents typically retains an electrophilic character, it is possible to interfere with other molecules that are taken at the same time either as part of the diet or for medicinal purposes. Indeed, our earlier studies revealed that green tea polyphenols such as epigallocatechin (EGCG) suppress the cytotoxicity of bortezomib. In order to further understand the mechanism behind such inactivating interactions we employed several NMR-based methods, including 1H-NMR, 13C-NMR, 11B-NMR, and 19F-NMR for the purpose of characterizing the

adducts of bortezomib EGCG and related polyphenols. By monitoring and quantifying a single peak we were able to evaluate the likely inactivation of the boron group. Using a 11B-NMR-based SAR study, it was determined that bortezomib has the ability to form a boronate adduct with EGCG as well as other polyphenols of variable structures. These results were further validated with cell-based assays that produced analogous results. Overall, this approach may offer a simple and novel method for screening food-drug and drug-drug interactions of boron-containing drugs.

MEDI 293

Design, synthesis and biological evaluation of novel and selective SIRT6 inhibitors

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SIRT6 is a member of the sirtuin family and has been recently shown to be crucial for a number of processes including telomere maintenance, DNA repair and genome stability, secretion of inflammatory cytokines, regulation of glycemia and epigenetic control of acetylated histones.

Based on the crystal structure of SIRT6 and by means of structure-based molecular design techniques, we identified several scaffolds as good binders of SIRT6. Drug-like analogs of these scaffolds were revealed to inhibit SIRT6 with a considerable selectivity (more than 20-fold) over SIRT1 and SIRT2. We will discuss the synthesis and biological characterization of these inhibitors as well as the molecular determinants for the design of SIRT6-selective inhibitors.

These inhibitors may have properties to sensitize cancer cells to chemotherapeutics, treat aging-related pathologies, act as anti-inflammatory compounds or as glucose-lowering agents with potential clinical values.

MEDI 294

Postsynthetic modifications of DNA with boronic acid

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DNA molecules, known as important materials for genomic and structural purposes, are also useful in other applications such as the development of new diagnostics and materials. Boronic acid is well-known by its strong interactions with diols, alcohols, and nucleophiles. Therefore incorporation of the boronic acid moiety into DNA could lead the discovery of new scaffolds for diagnostic and therapeutic applications. Herein, We describe a series of our work in synthesis and incorporation of modified nucleotide (thymidine) with a “click handle” for incorporation of boronic acid into DNA.

MEDI 295

Tuning the subcellular distribution and target selectivity of cytotoxic platinum-intercalator conjugates

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The formation and stabilization of G-quadruplex nucleic acid structures has potential applications in the development of more selective cancer chemotherapeutics with decreased systemic toxicity. These non-classical nucleic acid structures are found in many regions of the human genome and transcriptome, including sequences that have been linked to aberrant cancer cell proliferation, such as the telomeres and ribosomal RNA genes (rDNA). G-quadruplexes in guanine-rich nucleolar rDNA have recently attracted attention as targets of new chemotherapeutic agents aiming to exploit the unique morphological differences between normal and malignant cells. Toward this goal we are pursuing novel platinum-intercalator conjugates capable of inducing irreversible damage in form of monofunctional adducts with nucleobases in the loop regions of the G-quadruplexes. This form of damage has the ability to elicit a rapid damage signal in treated cells potentially leading to apoptotic or autophagic cell death. Novel hybrid agents have been rationally designed and synthesized exhibiting unique geometry and electronic properties. The goal of the design was to minimize the dose-limiting genotoxicity of the parent compounds by minimizing their reactivity with dsDNA and by hijacking them into cellular structures harboring targetable G-quadruplexes. To identify potential “leads”, a small library of analogues was tested for biological activity in solid tumor cell lines using a modular combinatorial approach. Selected derivatives were re-synthesized and studied for uptake and subcellular localization using confocal fluorescence microscopy and inductively-coupled plasma mass spectrometry (ICP-MS).

In addition, their binding properties with telomeric and nucleolar G-quadruplex sequences have been studied using CD spectroscopy, ESI-HRMS, and various biochemical assays. Potential structure-activity and structure-target selectivity relationships will be discussed.

MEDI 296

Synthesis of the common intermediate to stabilized zampanolide analogs

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(-)-Zampanolide was first isolated from marine sponge in 1996, representing a novel macrolide with potent cytotoxicity (IC_{50} = 1-5 ng/mL) against both drug-sensitive and multidrug- resistant cancer cell lines. Zampanolide binds to β-tubulin through a unique covalent interaction with amino acid residues within the paclitaxel-binding site. So far, only a few zampanolide analogs and structure-activity relationships have been reported. Our long-standing program on zampanolide focuses on the identification of its new drug-like analogs with improved physicochemical and pharmaceutical properties. We embarked on the design of zampanolide analogs with improved stability (both in vitro and in vivo) and the potential to treat multi-drug resistant cancer. We mainly engineer the lactone moiety and side chain of zampanolide. Fragment C9-C20 is the common intermediate to these target analogs according to our retrosynthetic analysis. We will present the synthesis of the common intermediate---Fragment C9-C20, as well as the design and retrosynthetic analysis of our target zampanolide analogs.

MEDI 297

Cytotoxic effect of novel dehydroepiandrosterone derivatives on different cancer cell lines

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This work describes the synthesis and pharmacological evaluation of three series of dehydroepiandrosterone derivatives having a pyrazole or triazol ring at C-17 of the dehydroepiandrosterone skeleton and an aromatic ester having a variety of electron donating and electron attracting groups. In another series, the aromatic ester function was replaced with esters having a ring size of 3-5 carbon atoms at C-3 position. The results from this study indicated that the steroidal compounds having a triazole ring at C-17 showed a much higher cytotoxic activity in PC-3, MCF-7 and SKLU-1 cell lines (human prostate, human breast cancer and human lung cancer respectively) as compared to the compounds having a pyrazole function at the same position as well as

an ester function at C-3. On the other hand, the compounds having the aromatic ester at C-3 position (triazole ring at C-17), showed a much higher cytotoxic activity as compared to their aliphatic counterparts (cycloalkyl esters) having the same azole ring at C-17. It is important to emphasize that the aromatic esters having an electronegative group in the *para* position of the phenyl group (triazole ring at C-17) showed a higher cytotoxic activity as compared to those containing an electropositive group in the same position. On the basis of these results, the active compounds could be subjected to further pharmacological studies in order to select the best candidate for the treatment of the above mentioned cancers.

MEDI 298

Synthesis of novel c5-curcumin-fatty acid conjugates and evaluation of their anticancer properties

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The importance of this project is underscored by the problem of the current chemotherapy treatments that are either highly inefficient or toxic, which weakens the immune system allowing opportunistic bacteria to infect the cancer patients. One way to address this problem is to design and synthesize more effective and less toxic compounds with both antibacterial and anticancer properties. Among the compounds that are being evaluated as anticancer agents, curcumin has demonstrated to have this property. Although preclinical and clinical studies have shown that curcumin is not toxic against normal human cells, several pharmacokinetics disadvantages, such as poor bioavailability, fast metabolism and requiring of repetitive oral doses, have been reported, which limits its pharmacological applications. However, curcumin is still an excellent compound for drug design and development based on the basis of its explicit bioactivities, non-toxicity, and easy synthesis. In order to find novel molecules with the potential to be used as anticancer drugs, we performed the synthesis of novel C5-curcumin-fatty acid conjugates in order to study their anticancer properties. We are particularly interested in synthesizing C5-curcumin-palmitic acid (C5-curc-PA) because our preliminary results support the hypothesis that the chemical linking of C5-curcumin and palmitic acid will increase its anticancer properties. Once the C5-curc-PA and other conjugates were synthesized, their anticancer properties were evaluated by using standard *in vitro* bioassays. Finally, the inhibitory properties of C5-curc-FA conjugate toward human DNA topoisomerase I was addressed because this enzyme is a known molecular target of anticancer drugs.

MEDI 299

Structure-based design, synthesis, and biological activity study of Hsp90 inhibitors

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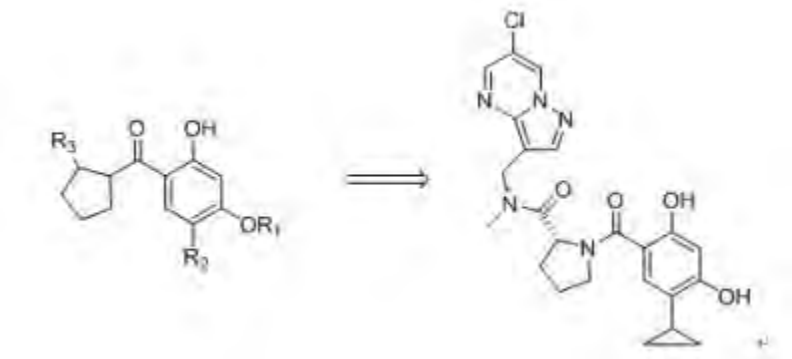
90 kDa heat shock proteins belong to a family of chaperones that regulate intracellular functions and are required for the refolding of denatured proteins following heat shock, as well as the conformational maturation of a large number of key proteins involved in cellular processes. Inhibitors of Hsp90 disrupt the protein folding process, resulting client protein degradation via the ubiquitin proteasome pathway. Hsp90 multi-protein complex in tumor cells exhibits a higher affinity for N-terminal ligands and malignant cell and highly dependent upon the Hsp90 protein folding machinery for cell survival. Inhibition of Hsp90 could result in combination of disruption of multiple signaling pathways in which is important for the tumor cell survival and bring a potential method for carcer treatment.

In the past several years, based on Hsp90 crystal structure, we found a series of new Radicicol derived compounds (Fig 1) which show good potency in Hsp90 binding and inhibitory effect on human cancer cell growth in vitro, indicating further application in anti-tumor drug development.



Recently, several Phase II-IIIclinical trial candidates showed some side effect on inducing glaucoma problems. Related with our Radicicol derived compounds, we found some synthesized compounds with lass Hsp90 inhibition effect ion could kick out this concerning based on rabbit model. The work is supported by NSFC 30973639,21310005081323and2012G0021840

MEDI 300

Novel organometallic anticancer agents

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Novel organometallic ruthenium-carbohydrate anticancer agents were synthesized and characterized by spectroscopic methods and single crystal X-ray crystallography. In vitro anticancer activity of these new compounds were evaluated against several types of human cancer cell lines and compared against clinically used drug cisplatin. Anticancer activity is many times more potent than cisplatin.

MEDI 301

Strain promoted click chemistry (SPAAC) of 8-azido purine and 5-azido pyrimidine nucleosides with cyclooctynes

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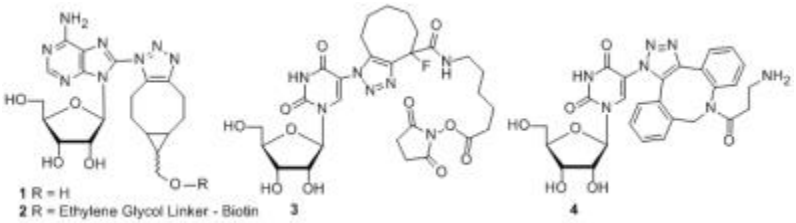
Click chemistry is an important tool for drug discovery, bioconjugation, and identification of cellular targets. Click chemistry between 8-azidoadenosine and a terminal alkyne usually requires a copper catalyst and microwave assistance or prolonged reaction times. These prerequisites are incompatible with biological applications. We have developed a protocol for the convenient strain promoted click chemistry (SPAAC) of 8-azido purine nucleosides with various cyclooctynes in aqueous solution without a copper catalyst. A click reaction between 8-azidoadenosine and symmetrically fused cyclopropyl cyclooctynes occur rapidly (64% in 18 minutes, 92% in 2 hours) in ACN:H_2O (3:1) at rt to give triazole **1** or biotin modified adduct **2** . The click reaction of other 8-azido purine analogues including 8-azido-2'-deoxyadenosine and 8-azido-*arabino*adenosine with more complex cyclooctynes such as dibenzylcyclooctyne and monofluorocyclooctyne also proceeded smoothly to give triazole products. Furthermore, 5-azidouridine reacted efficiently with cyclooctynes to give products modified with an NHS ester **3** or a terminal amine **4** in less than 15 minutes. Bioorthogonal labeling of cellular targets using described click chemistry will also be discussed.



MEDI 302

Tris-benzamide analogs for inhibiting Bcl-2 proteins in prostate cancer

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Apoptosis is an important cellular mechanism for tissue homeostasis and aberration in the process is well documented in cancers including castration-resistant prostate cancer (CRPC). Since it is regulated by heterodimerization of Bcl-2 family proteins, disrupting such protein complexes is attractive for therapeutic intervention. Structural studies revealed that the helical BH3 domain of pro-apoptosis proteins including Bak binds to a hydrophobic pocket in anti-apoptotic proteins like Bcl-xL. To mimic helical BH3 domain, we have reported the synthesis and biological activity of tris-benzamides which can place three functional groups corresponding to the side chains found at the i, i+4, and i+7 positions in a α-helix. To improve the activity of tris-benzamides, we have modified them by introducing additional side chain group at either end of molecules. This library of tris-benzamide analogs as BH3 mimetic was then examined for their binding affinity to anti-apoptotic Bcl-xL protein, inhibition of cell proliferation of various prostate cancer cell lines, mechanism of action, and in vivo efficacy. We have identified several leading compounds that showed strong binding affinity as well as high metabolic stability and cell permeation. These compounds were found to be effective in inhibiting cell growth of several prostate cancer cell lines and provided evidences of inducing apoptosis. These results show a high potential of BH3 mimetics in treating prostate cancer.

MEDI 303

Synthesis and cytostatic evaluation of 4-*N*-alkanoyl and 4-*N*-alkyl gemcitabine analogs

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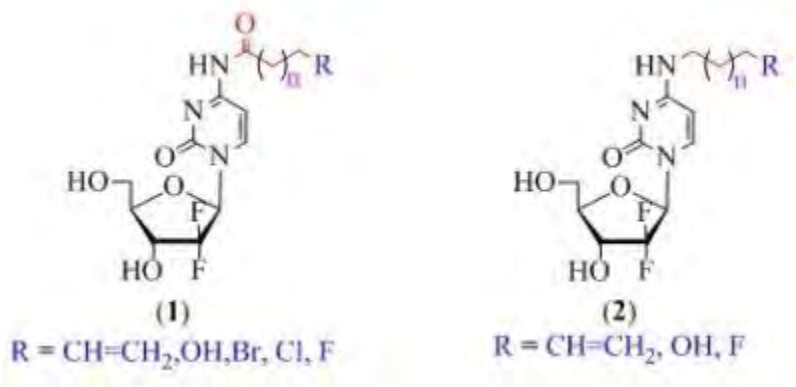
Gemcitabine (2',2'-difluoro-2'-deoxycytidine, dFdC) is a potent chemotherapeutic nucleoside analogue in the treatment of cancers and solid tumors. Coupling of gemcitabine (NMM/HOBt/EDCI) with varying carboxylic acids of different chain lengths (C9-C13) afforded the 4-*N*-alkanoylgemcitabine analogues (**1**) bearing a hydroxyl, fluoro, chloro or alkene functional group suitable for further chemical modification. Displacement of *p*-toluenesulfonylamido group in 4-*N*-tosylgemcitabine with 11-aminoundecanol or 10-undecenyl amine gave the 4-*N*-alkylgemcitabines (**2**). The analogues bearing a terminal hydroxyl group on the 4-*N*-alkanoyl or 4-*N*-alkyl chain

were efficiently fluorinated either with DAST or under conditions that are compatible with synthetic protocols for ^{18}F labeling. The 4-*N*-alkanoylgemcitabines **1** displayed potent cytostatic activities against L1210, CEM, HeLa and MCF-7 tumor cell lines with IC_{50} values in the nM range, while activities for the 4-*N*-alkylgemcitabines **2** were in the μM range.



MEDI 304

Biological activity of N-Hydroxyethyl-4-aza-didehyropodophyllotoxin derivatives upon colorectal adenocarcinoma cells

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Podophyllotoxin is a natural product and precursor for several antitumor drugs. Recently, we reported the synthesis of a library of aza-podophyllotoxin derivatives, close structural analogues of podophyllotoxin that displays promising competitive biological activity (Kumar, et.al.). Several compounds from this library of aza-podophyllotoxin derivatives were submitted to NCI for antitumor activity on 60 types of human cancer lines. The majority of the aza-podophyllotoxin derivatives submitted for testing were found very active at single dose (stage I) and five (5) concentrations dose studies (stage II) level, most of them entered into stage III (repetition of stage II) followed by stage IV (Hollow fiber studies on human carcinoma in mice model) protocols of NCI to evaluate anticancer activity of these new compounds. Based on these exciting anti-tumor activity results of aza-podophyllotoxin derivatives on the 60 cell line panel from NCI, it is worthwhile to investigate this new scaffold extensively for further studies.

Selected aza-podophyllotoxin derivatives from this compound library were further investigated to understand their mode of action in order to establish a structure activity relationship. Two compounds (4-(2-Hydroxyethyl)-10-(3,4,5-trimethoxyphenyl)-3,4,6,7,8,10-hexahydro-1H-cyclopenta[g]furo[3,4-b]quinolin-1-one) (**8a**) and 6-(2-Hydroxyethyl)-10-(3,4,5-trimethoxyphenyl)-2,3,7,10-tetrahydro-[1,4]dioxino[2,3-g]furo[3,4-b]quinolin-9(6H)-one **(9a)** were found to be very active against COLO 205

type of human colon cancer cells at NCI. The aza-podophyllotoxin derivatives **8a** and **9a** have been studied extensively in order to determine the mechanism by which compounds induces cell death i.e. apoptosis or necrosis; IC_{50} determination of **8a** and **9a** against COLO 205 cells, activation of caspase 3 and 7 assay, and mitochondria permeability assay.

Ref: Kumar A, Kumar V, Alegria A, Malhotra S. (2011). N-hydroxyethyl-4-aza-didehydropodophyllotoxin derivatives as potential antitumor agents. Eur J Pharm Sci. Sep 18;44(1-2):21-6. doi:10.1016/j.ejps.2011.04.013. Epub 2011 May 13. PubMed PMID: 21601635; PubMedCentral PMCID: PMC3278235.

MEDI 305

Ferrocenyl thiourea derivatives as potential anticancer agents: Synthesis, DNA binding, and micelle interaction study

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A general protocol for the synthesis of ferrocene based thiourea and its derivatives have been reported in this presentation. Single crystal X-ray diffraction analysis and multi-nuclear NMR spectroscopy (^{1}H & ^{13}C) have been used to establish their identities in the solid state and also in solution. DNA binding studies based on UV-vis spectrophotometric titration shows the potential of these organometallic compounds as an anti-tumor drug. Membrane penetration studies have been carried out for some compounds with micelle membrane interfaces prepared from CTAB, TTAB and SDS surfactants using $^{1}HNMR$ and UV-Vis spectroscopic techniques. Results show the presence of these molecules in the interfacial regions of the self assembled systems.

MEDI 306

Base-modified nucleotide terminators of DNA synthesis as novel anticancer agents

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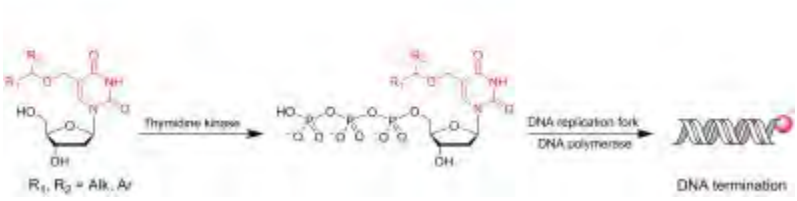
Interfering with DNA synthesis represents a plausible approach to cancer chemotherapy. The most potent FDA-approved nucleoside-based anti-cancer drugs elicit severe side effects that are sometimes lethal. Therefore, there is a critical need to explore the therapeutic potential of alternative agents that have wider therapeutic window. One of the venues to fill this gap is to develop novel antimetabolites with the

mechanism of action exclusively focused on targeting replicating DNA directly. Recently, we discovered base-modified 2'-deoxynucleotides that undergo template-driven incorporation into DNA molecule by natural polymerases *more efficiently* than their natural counterparts. Once the incorporation occurs, further addition of nucleotides is obstructed due to the presence of a terminating moiety attached to the nucleobase. Since nucleosides readily undergo cellular uptake followed by intracellular metabolism into 5'-triphosphates, it was hypothesized that treatment of cancer cells with appropriate base-modified nucleosides will lead to obstruction of their DNA replication process, so the cell division will not pass the restriction checkpoint, thus triggering apoptosis. A library of novel base-modified thymidines was synthesized followed by cytotoxicity study (MTT assay). The SAR has revealed a lead compound.



MEDI 307

Discovery of SYL933: A 2-aminopropane-1, 3-diol derivative as potent and selective $S1P_1$ receptor agonist (prodrug)

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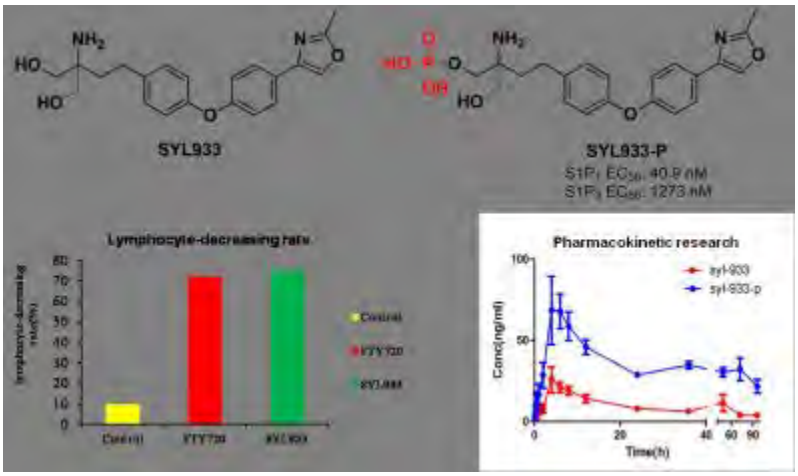
The $S1P_1$ receptor agonist FTY720 (fingolimod), as the immunomodulator, has been approved by the FDA for treating multiple sclerosis, but its $S1P_3$ agonism may be relative to a number of side effects such as bradycardia. In our group, a series of 2-aminopropane-1, 3-diol derivatives were designed and synthesized. Extensive structure–activity relationship studies led to the discovery of SYL933, a selective $S1P_1$ receptor agonist (prodrug). Upon phosphorylation, the compound (SYL933-P) showed nanomole $S1P_1$ agonist activity (EC_{50}=40.9 nM) with >30-fold selectivity over $S1P_3$ (EC_{50}=1273 nM). Dosed orally at 1 mg/kg in SD rats, SYL933 significantly reduced blood lymphocyte counts, which was equivalent to FTY720, but showed much less

effects on heart rate than FTY720. Impressively, SYL933 showed good *in vivo*activitiesin rat adjuvant induced arthritis model, rat type IIcollagen induced arthritis model, mouse EAE model and mouse psoriasis model. The PK/PD study reveals good pharmacokinetic properties of SYL933 (bioavailability: 48.6%, cumulative excretion: 37.3%, plasma protein binding: 95.09%). It was found that SYL933 was relatively stable in human/monkey/rat liver microsomes and had no obvious inhibitory effect on human CYP450s. Moreover, low Cardiac hERG toxicity of SYL933 was discovered (IC_{50}=8.07 μM). On the basis of the favorable *in vitro*, *in vivo* activities, good PK/PD profiles as well as broad toxicology evaluations, SYL933 was considered as a promising candidate in preclinical trial.



MEDI 308

Structure-activity relationships within the aryl carbinol region of the N-arylsulfonamido-N'-aryl-piperazine series of GK-GKRP disruptors

Kevin C Yang, *chaoy@amgen.com, Nobuko Nishimura, Mark H Norman, Longbin Liu, Kate S Ashton, Michael D Bartberger, Samer Chmait, Jie Chen, Rod Cupples, Christopher Fotsch, Joan Helmering, Roxanne K Kunz, Steve F Poon, Aaron Siegmund, Glenn Sivits, David J Lloyd, Clarence Hale, David J St. Jean, Jr. Department of Therapeutic Discovery, AMGEN, Thousand Oaks, CA 91320, United States*

As an alternative approach to glucokinase activators (GKAs) for the management of type 2 diabetes, we focused on compounds that promote the release of glucokinase by disrupting the glucokinase-glucokinase regulatory protein (GK-GKRP) interaction. These initial investigations led to the development of a series of *N*-arylsulfonamido-*N'*-aryl-piperazines and culminated in the identification of AMG-3969, a compound that effectively enhanced GK translocation and reduced blood glucose levels in diabetic animals. Herein we report the results of our investigations that focus on modifications at the aryl carbinol group of this series. Guided by structure-based design utilizing an X-ray co-crystal structure of AMG-3969 bound to hGKRP, we identified several potent GK-

GKRP disruptors bearing a diverse set of functionalities in the aryl carbinol region such as sulfoximine and pyridinyl derivatives. The structure-activity relationships, pharmacokinetic data as well as the in vivo pharmacodynamic and efficacy data for representative compounds will be presented.

MEDI 309

Imidazopyridine PDE10 inhibitors

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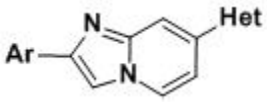

A series of [1,2-a]imidazopyridines were prepared and found to be potent and selective PDE10A inhibitors. Efficacy in both a conditioned avoidance response model (CAR) and novel object recognition model for cognition in rodents demonstrate the promise of this series as a potential therapy for schizophrenia.

MEDI 310

Combination of tamoxifen and retinoic acids, all trans, 9-cis, 13-cis, on spindle breast cancer cells: Monolayer films, spectral studies, and electron microscopy

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Retinoids inhibit growth of breast cancer cell lines in culture and inhibit breast tumor growth in animals. Retinoid signals are mediated through the retinoic acid receptors (RARs) and the retinoid X receptors (RXRs), with each family represented by three distinct receptor genes designated alpha, beta, and gamma. All-trans retinoic acid (atRA) preferentially binds RARs but not RXRs; however, atRA can be converted intracellularly to 9-cis-retinoic acid (9-cis-RA), an RXR ligand. 9-cis-RA and 13-cis-RA bind both RARs and RXRs. Following stimulation by retinoids, RAR-RXR heterodimers and RXR-RXR homodimers can form. The receptor dimers bind to retinoic acid response elements or retinoid X response elements in the promoter sequences of target genes, and they modulate gene transcription. Effects of retinoids on signaling by ER and HERS have been reported. Inhibition of breast tumor cell growth by retinoids is greater for ER-positive cells than ER-negative cells, which may be partly related to alterations in retinoid metabolism. Some studies have found that RA increased the

amount of ER in MCF7 breast cancer cells, although others reported that RA downregulated ER in this cell line. Regardless, activated RARs have been observed to exert anti-estrogenic effects by directly or indirectly impairing binding of ER to estrogen response elements (EREs). Conversely, the N-terminal region of ER-alpha modulates the transcriptional activity of RAR. Both RAR-alpha and RAR-beta have been implicated in the anti-proliferative effects of retinoids against breast cancer. RAR-alpha expression is correlated positively with ER and with RA sensitivity, and is inducible by estrogen. RAR-beta has been ascribed tumor suppressor=type activity and is often down regulated in breast cancer; it is inducible by atRA, and inducibility correlates with atRA sensitivity. In both ER-positive T47D cells and ER-negative SKBR3 cells, some evidence suggests that RAR-alpha is the receptor solely sufficient for the growth inhibition, cell cycle arrest, apoptosis, and modulation of RAR levels. Inhibition of breast cancer cell growth by atRA and 4-HPR has been associated with downregulation of HER2; while atRA induced morphologic changes consistent with differentiation, 4-HPR induced those of apoptosis. atRA and 9-cis-RA caused decreases in HER2 and HER3, and inhibited SKBR3 cell growth with cell cycle arrest and induction of apoptosis,, and the retinoids downregulated HER4 in T47D cells; atRA also downregulated HER2 and HER3 in MCF cells.Retinoids have been found to delay the onset of mammary tumors in HER2. Our lab wants to examine the effects of various retinoids (atRA, 9-cis-RA, and 13-cis-RA), tamoxifen, or combinations of these drugs on proliferation, cell cycle, differentiation, and apoptosis in BT474 and SKBR3 cells. Since retinoids and tamoxifen are individually agents that possess anti-tumor activity toward breast cancer, combinations of these drugs may translate into improved therapy for breast cancer

MEDI 311

Functionalized benzofulvenes: Coupling synthesis and computational chemistry to develop small molecule inhibitors at a primarily undergraduate institution

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Human thioredoxin reductase 1 (TrxR1) is an enzyme of therapeutic interest in cancer treatment, due to its involvement in regulating the redox-signaling enzyme thioredoxin 1. Interestingly, cancerous cells can attribute many of their negative clinical features as a result of thioredoxin 1's interaction with the cellular redox cycle. Reduced (activated) thioredoxin is involved in a host of intra and extra cellular signaling pathways that can lead to increased cellular proliferation and decreased apoptosis. Predictably, thioredoxin has also been shown to be overly expressed in cancerous cells, particularly the most malignant (e.g., pacreatic, liver and breast). We are currently working towards a small molecule inhibitor developed to target TrxR1, the only known enzyme that reduces and activates thioredoxin. Current research suggests that inhibition of TrxR1 leads to decreased cancer cell fitness through interaction with the previously discussed mechanisms, (i.e., cellular growth control). Encouragingly, computational modeling has demonstrated the possible effectiveness of highly functionalized benzofulvene moieties.

We have determined quite high *in-silico* binding affinities (i.e. >10 kcal/mole) to the TrxR1 active site.

From readily available indanone a pharmaceutically relevant synthetic route for the benzofulvene scaffold has been developed. We can access highly functionalized benzofulvenes through a four step/two pot route that utilizes reduced toxicity solvents, avoids the use of heavy metals, and eliminates cryogenic conditions. Currently, we have synthesized twelve novel benzofulvenes with various functional properties. We will apply this route to synthesizing *in-silico* directed target molecules that could possibly inhibit TrxR1.

MEDI 312

Synthesis and anticancer activity of flavone derivatives against estrogen dependent cancers by rational approach

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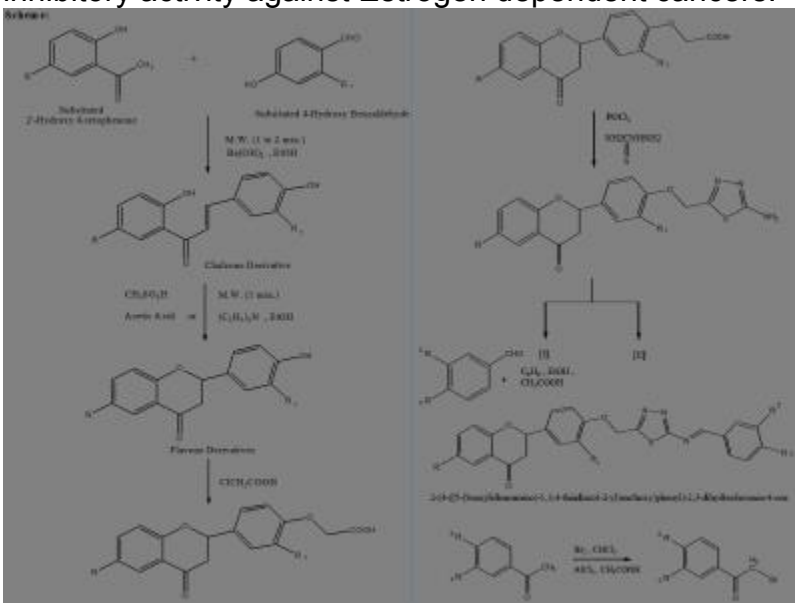
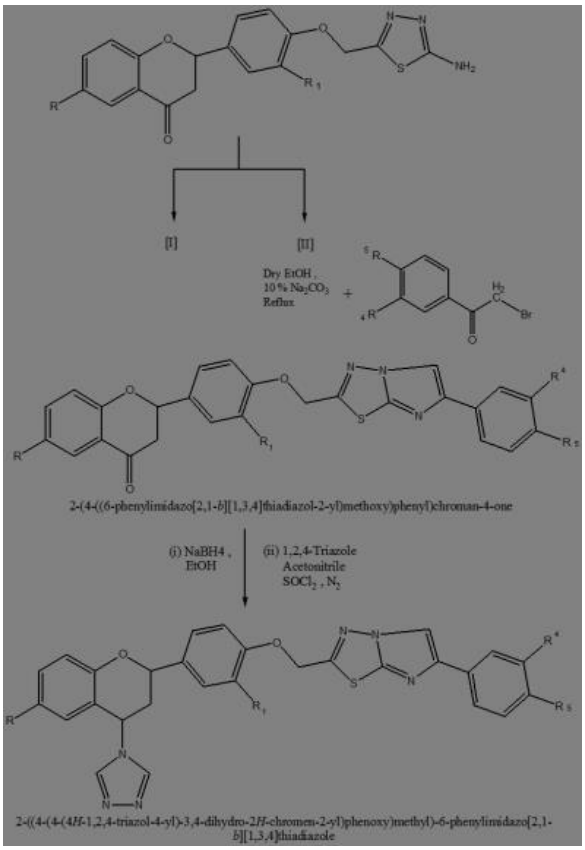
Aromatase and 17-ßHSD inhibitors are main target of pharmacological interest for the treatment of estrogen dependent cancers. Coumarins, Flavones, Isoflavones have been reported for such inhibition and are used for treatment of brest tumers. So in this topic, Flavone derivatives containing Imidathiadiazole, Thiadiazole, Triazole and benzimidazole hetrocycles were synthesised by using simple laboratory reagents like 2-Hydroxy Acetophenone and 4-Hydroxy Benzaldehyde to convert chalcone leads to formation of Flavones by cyclazation using Microwave and followed by attachment of different Hetrocycles and charactrarized by IR, ^{1}H NMR, ^{13}c NMR spectroscopy and elemantal analysis. This Flavone derivatives were found to exhibit moderate to high inhibitory activity against Estrogen dependent cancers.





MEDI 313

Treatment of colon cancer using small molecule activators of 15-PGDH

***Melissa Moss**, melissa.desilets@utsouthwestern.edu. Department of Biochemistry, UT Southwestern Medical Center at Dallas, Dallas, Texas 75204, United States*

Colonic cyclooxygenase 2 (COX-2) is a potent oncogene and is elevated in 85% of colon cancers. It is critical for the conversion of arachadonic acid to the tumor promoting molecule PGE2. Likewise, 15-PGDH is a negative regulator of COX-2 and its expression is substantially reduced in 90% of colon cancers. It functions to reduce PGE2 levels through oxidation to 15-keto-PGE2. Thus, small molecules that enhance expression or activity of PGDH would represent preventative and therapeutic agents for colon cancers. Such small molecules would provide a novel strategy for treating a deadly and common cancer. Through the use of high throughput screening a small molecule has been identified and a medicinal campaign initiated to find a viable compound that will activate 15-PGDH.

MEDI 314

Design and synthesis of novel indazole derivatives as Rho kinase inhibitors

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Rho-associated coiled-coil forming protein kinase (ROCK), members of the serine/threonine kinase family, plays a key role in cellular phenomena such as contraction of smooth muscles, morphological changes, etc. Therefore, inhibition of ROCK has been expected to provide effective anti-glaucoma activity.

Our investigations of structure-activity relationship for ROCK inhibitors started from Y-27632 and Y-39983, and resulted in the identification of novel trisubstituted olefin derivatives, 6-substituted isoquinoline derivatives and 5-substituted indazole derivatives. Especially, 4-cyclopropyl, 5-pyridino indazole derivatives were significantly soluble in water, and showed the potent inhibition at in vitro studies and the strong reduction of intraocular pressure in cynomolgus monkey.

MEDI 315

DBPR112, a potent and selective EGFR kinase inhibitor in preclinical study

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Lung cancer is the major cause of cancer death in the world while non small cell lung cancer (NSCLC) accounts approximately 85% of all lung cancer diagnosis. EGFR mutations, found in 10–30% of patients with NSCLC characterize a subpopulation with exquisite sensitivity to EGFR tyrosine kinase inhibitors (EGFR-TKIs). However, the clinical benefits of first-generation TKIs (like gefitinib or erlotinib) can be further improved because of the development of drug-acquired resistance within 10–14 months in patients who initially respond to the treatment. Therefore, there is a need to discover next generation medicines as EGFR-TKIs for NSCLC patients.

Our EGFR program was first started to screen 20,000 in-house compounds for $EGFR^{WT}$ activity in EGFR-transfected 32D cells and further performed knowledge-based design. We had identified DBPR112 as a potent EGFR-TKI with oral *in vivo* activity in a mouse model for lung adenocarcinoma.

DBPR112 showed IC_{50} of 2 nM in HCC827 cells and potent $EGFR^{WT}$ (IC_{50}: 10 nM) and $EGFR^{L858R/T790M}$ (IC_{50}: 50 nM) kinase inhibition which are better than gefitinib. DBPR112 was orally (F = 49%) administered against the growth of human lung HCC827 tumors subcutaneously xenografted in nude mice. A dramatic reduction of the tumor size was

noted in the tumors treated with DBPR112 without significant loss of body weights in the nude mice.

Considering the fact that EGFR kinase plays an important role in lung adenocarcinoma, our goal is to develop novel and potent EGFR kinase inhibitors for the treatment of lung cancer, either as a single agent or in combination with existing cancer treatment. DBPR112 is a highly potent small-molecule against various forms of EGFR kinase. The non-clinical profile of DBPR112 showed promising *in vivo* efficacy. We, therefore, propose to design a preclinical program to assess the developability of DBPR112 and conduct comprehensive preclinical studies. We hope to make DBPR112 an investigational new drug for the treatment of lung cancer in the near future.

MEDI 316

Design and synthesis of dual FLT3-Aurora kinase inhibitors for the treatment of acute myeloid leukemia

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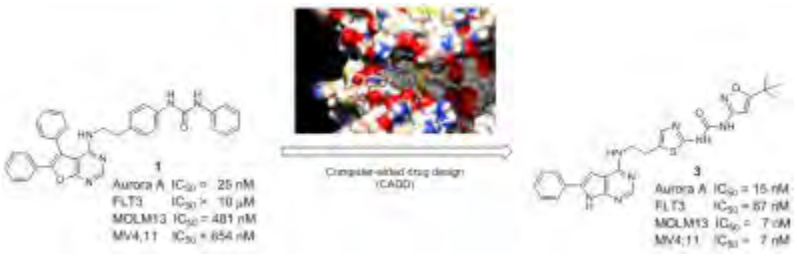
FMS-like receptor tyrosine kinase-3 (FLT3) is a tyrosine kinase protein, encoded by the FLT3 gene, and highly related to acute myeloid leukemia (AML). FLT3 inhibitory was proven as a critical factor to destroy the leukemia cells. However, limited responds in the clinical study were usually observed after patients with AML treated selective FLT3 inhibitors. Notably, some reports demonstrated that dual FLT3-Aurora inhibition not only benefits patients with mutated-FLT3 AML, but also is effective against selective FLT3 inhibitor resistance, due to the ability of extra Aurora inhibitory. Hence, dual FLT3-Aurora inhibitory may raise the opportunity for being the novel therapeutic strategy for the treatment of AML.

Recently, our group reported a novel Aurora A kinase inhibitor by using high-throughput parallel synthesis and structure-based drug design (SBDD). Based on this, we wanted to modify the lead (**1**) to maintain its Aurora A inhibition as well as improve its FLT3 activity to develop dual FLT3-Aurora kinase inhibitors. We envisaged replacing the pharmacophore of **1** by use of in-house amine compounds of our institute. After the compounds with the great diversity of pharmacophores were scored by virtual screening in FLT3 homology model, 26 compounds with high docking scores were selected to synthesize. Among these 26 compounds, compound **2** bearing the pharmacophores of the thiazole moiety revealed Aurora A activity with IC_{50} of 50 nM and FLT3 activity with IC_{50} of 322 nM. Furthermore, we sought a better dual kinase inhibitor **3** , compared with compound **2** , utilizing SBDD and binding energy calculation. Inhibitor **3** possessed Aurora A activity with IC_{50} of 15 nM and FLT3 activity with IC_{50} of 67 nM as well as inhibited the proliferation against MOLM13 and MV4;11 with IC_{50} of 7 nM, which was a promising drug candidate to as a dual FLT3-Aurora kinase inhibitor.



MEDI 317

Discovery of potent, selective and non-ATP competitive c-Met inhibitors: Daphnetin C-3 dimer derivatives

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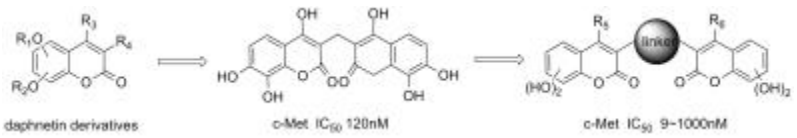
c-Met is a receptor tyrosine kinase that plays a important role in embryonic development and organ regeneration; aberrant c-Met signaling also has been identified in a wide range of human malignancies. Consequently, c-Met receptor tyrosine kinase is an attractive oncology target for therapeutic intervention. Daphnetin, a derivative of coumarin, was reported to be a protein kinase inhibitor which inhibits tyrosine-specific protein kinase, EGFR (IC_{50}=7.67 μM), and serine/threonine-specific protein kinases, including PKA (IC_{50}=9.33 μM) and PKC (IC_{50}=25.01 μM). However, no c-Met inhibitory activity of this compound has been reported.

In the present work, we synthesized a series of daphnetin C-3 dimer derivatives with different linkers and found that these compounds show potent c-Met inhibitory activity with IC_{50} ranging from 9nM to 1μM, as well as a non-ATP-competitive mode of action. The corresponding monomer had poor inhibitory activity against c-Met kinase, with IC_{50} at the micromolar level.

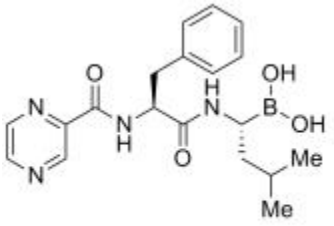


MEDI 318

Design and synthesis of putative small-molecule inhibitors targeting the SCF^{SKP2} E3 ligase complex

Andrew E. Shouksmith[1], *a.e.shouksmith@ncl.ac.uk, Laura E. Evans*[2], *Roger J. Griffin*[1,2], *Bernard T. Golding*[1,2], *Herbie Newell*[1,2], *Duncan C. Miller*[1], *Martin E. M. Noble*[1,2], *Jane A. Endicott*[1,2], *Deborah Tweddle*[2]. *(1) Department of Chemistry, Newcastle University, Newcastle-Upon-Tyne, United Kingdom (2) Department of Cancer Research, Newcastle Cancer Centre, Newcastle-Upon-Tyne, United Kingdom*

The SKP1-Cullin-F-box (SCF) E3 ligases are the largest family of E3 ubiquitin ligases and promote the ubiquitination of regulatory proteins, targeting them for degradation or otherwise affecting their function/activity.[1] Several subunits of the SCF complex have demonstrated oncogenic activity, including the F-box protein S-phase Kinase-associated Protein 2 (SKP2). The SCF^{SKP2} E3 ligase targets several cell cycle negative regulators, e.g. p21 and p27, and oncogenic SKP2 increases ubiquitination of these proteins enabling replicative immortality.[2] Reduced levels of p27 are common in many human cancers and are associated with an aggressive phenotype.[3-5] Currently, the only marketed drug that targets the ubiquitin-proteasome system is Bortezomib (Velcade; Millennium Pharmaceuticals) (**1**), which is used to treat multiple myeloma.[3,6]



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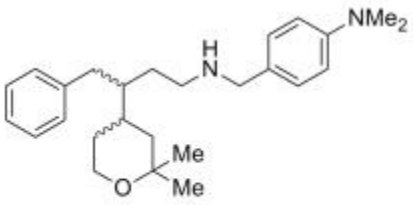

2

Targeting the F-box protein of an SCF E3 ligase is an attractive approach because the F-box protein defines E3 ligase selectivity and each E3 ligase has fewer target proteins than the 26S proteasome. To date, no small molecule targeting an F-box protein has entered clinical trials, but a recently reported compound, **2** , has shown promising therapeutic potential with evidence to suggest it inhibits the SCF^{SKP2} pathway.[3] The synthesis of **2** (diastereomer mixture) has now been completed at Newcastle University, confirming reported cytotoxic activity in HeLa cells, and SAR studies around the dimethylaniline ring, amine linker and the pyran ring are discussed. The first enantioselective synthesis of this chemotype, utilising Evan's auxiliary, is reported herein.

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(2)Wang, D., *et al.*; *PNAS*,**2012** , *109*, 9493-9498

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(5)Esposito, V., Baldi, A., De Luca, A., *et al*.; *Cancer Res*., **1997** , *57*, 3381-3385

(6)Paramore, A., Frantz, S.; *Nature Rev. Drug Disc*., **2003** , *2*, 611-612

MEDI 319

Design, synthesis, and biological evaluation of ring-opened bengamide analogs as anticancer agents

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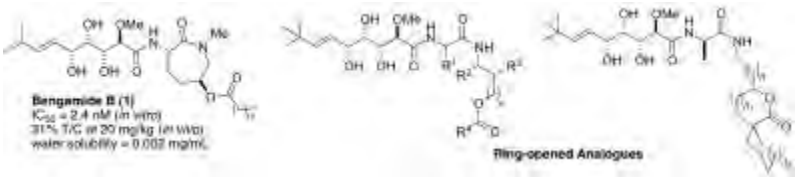
The bengamides are marine natural products, isolated from Jaspidae sponges, which display a wide range of biological activities. Their antitumor properties in particular have prompted intense research efforts, including the development of LAF389 by Novartis as a clinical candidate. The study of LAF389 was halted, however, by poor pharmacokinetic properties and unclear side effects during the phase I clinical trial.

In an effort to improve the pharmacokinetic and antitumor properties of bengamide-related compounds, we have designed a novel series of analogues in which the caprolactam ring is replaced with a linear alkyl chain. Among these caprolactam ring-opened analogues, several show potent antitumor activity against MDA-MB-435 human breast cancer cells (IC_{50}= 4-20 nM) and good water solubility. These analogues are synthetically more accessible than the parent natural products and provide possibilities for further SAR studies to afford novel candidates for development.



MEDI 320

Synthesis and anticancer evaluation of pyrrolyl-1,3,4-oxadiazole conjugates characterized by Schiff base and amide linkages

Bereket Mochona[1], Elizabeth Mazzo[2], ***DeCoria McCauley****[1], decoria.mccolium@famu.edu, Kinfe K. Redda[2]. (1) Department of Chemistry, Florida A&M University, Tallahassee, Florida 32307, United States (2) College of Pharmacy &Pharmaceutical Sciences, Florida A&M University, Tallahassee, Florida 32307, United States*

1,3,4-oxadiazole and pyrrole structural motifs were connected using a Schiff base and /or amide groups to investigate the combination of two distinct structural motifs on cancer cell lines. Friedel-Crafts acylation of 2-pyrrolyl carboxylates followed by hydrolysis afforded 4-substituted 2-pyrrole carboxylic acids. Subsequent coupling with 2-amino-1,3,4-oxadiazole derivatives formed the targeted amides. The Schiff bases were formed by the reaction of 2-amino-1,3,4-oxadiazoles with appropriately substituted aldehydes. Preliminary screening of the synthesized compounds for their anticancer potential against a panel of three breast cancer cell lines (MDA MB231, Ishikawa and BT 474cells) at six concentration levels is reported.

MEDI 321

Design, synthesis, biological evaluation, and structure-activity relationship study of hydroxylated 2-phenyl-4-aryl indenopyridines as selective topoisomerase IIα inhibitor

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Topoisomerase (topo) II plays crucial role in many nuclear processes that generate topological problems.In addition, topo II is the only enzyme available to disentangle the topological problems in chromosomes, which must occur during the completion of replication, and to decantenate the replicated chromosomes by transiently breaking of double strands of DNA. Therefore, many researches are attracted to develop selective topo II inhibitors as antitumor agents. Our previous studies showed that conformationally constrained rigid analogs of 2,4,6-trisubstituted pyridine containing 5,6-dihydrobenzo[*h*]quinoline moiety is important for topo inhibitory activities, and cytotoxicity against several human cancer cell lines.Various compounds possessing indenopyridine moiety were reported to have anticancer activity.In this study, twenty-one2-phenyl-4-aryl indenopyridines containing hydroxyl group at *ortho, meta, or para* position of phenyl ring were systematically designed and synthesized. They were evaluated for their topo I and II inhibitory activities, and cytotoxicity against several human cancer cell lines. Compounds **8** , **11** , **14** , and **17** showed significant selective topo II inhibitory activityat both 100 μM and 20 μM. Compounds **17** showed the most significant cytotoxicity against T47D cell line whereas weaker cytotoxicity against HCT15, DU145 and HEK293 cell lines as compared to positive controls. Structure-activity relationship (SAR) study revealed that hydroxyl group at *meta* or *para* position of 2-phenyl ring of indenopyridinehas an important role in displaying selective topo IIα inhibitory activity.

Keywords: Antitumor agents,Cytotoxicity, Hydroxylated 2-phenyl-4-aryl indenopyridine, Terpyridine, Topoisomerase IIαinhibitor

MEDI 322

Targeting cancer metabolism with biguanide-based Hexokinase-II inhibitors

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One of the primary metabolic change observed in malignant transformation is an increased catabolic glucose metabolism characterized by high rates of anaerobic glycolysis regardless of oxygen concentration (Warburg effects). Hexokinase-2 (HK2) regulates the cellular entrapment of glucose by catalyzing it to glucose-6-phosphate (G6P). In cancer cells the glucose affinity and mitochondrial localization of HK2 are highly advantageous for tumor survival and growth and, like other enzymes of the glycolytic pathway, HK2 may constitute potential target for cancer therapy.

In this work we show that metformin, a widely use antidiabetic drug, and related biguanide-scaffold analogs are able to allosterically inhibit HK2 by blocking the synthesis of G6P. These results provide new leads for the development of biguanides-based selective inhibitors of HK2, which, once extended to other cancer models, may be a valuable strategy to devise new compounds that impact on the bioenergetic metabolism of cancer cells.

MEDI 323

Phosphatidylserine-targeted bimodal liposomal nanoparticles for in vivo imaging of breast cancer in mice

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Phosphatidylserine -Targeted Bimodal Liposomal Nanoparticles for *In Vivo* Imaging of Breast Cancer in Mice

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Phosphatidylserine (PS) that is normally constrained to the inner plasma membrane becomes exposed on the surface of endothelial cells (ECs) in tumor vasculature due to the harsh tumor microenvironment. In the present study, we report the development of a novel tumor vasculature-targeted liposomal nanoprobe by conjugating a human monoclonal antibody, PGN635 that specifically targets PS to polyethylene glycol-coated liposomes. Superparamagnetic iron oxide nanoparticles (SPIO) were packed into the core of liposomes to allow for MR contrast, while near-infrared dye, DiR was incorporated into the lipophilic bilayer. The liposomal nanoprobe PGN-L-IO/DiR (111 nm hydrodynamic diameters) was fully characterized, and its binding specificity and subsequent internalization into PS-exposed vascular ECs was confirmed by *in vitro* MRI and histological staining. *In vivo* longitudinal MRI and optical imaging were performed after *i.v.* injection of PGN-L-IO/DiR into mice bearing breast MDA-MB231 tumors. At 9.4T, T_2-weighted MRI detected drastic reduction on signal intensity and T_2 values of tumors at 24h (mean tumor/normal ratio (TNR) decrease = 15 ± 3%). Ionizing radiation significantly increased PS exposure on tumor vascular ECs, resulting in a further MRI signal loss of tumors (mean TNR decrease = 47 ± 6%; $p < 0.01$). Concurrent with MRI, optical imaging revealed a clear tumor contrast at 24h (TNR = 3.8 ± 0.6). Intriguingly, PGN-L-IO/DiR exhibited distinct pharmacokinetics and biodistribution with significantly reduced accumulations in liver or spleen. Localization of PGN-L-IO/DiR is antigen specific, since a control antibody probe of irrelevant specificity, showed minimal accumulation in the tumors. Our studies indicate that PS-targeted liposomes may provide a useful platform for tumor-targeted delivery of imaging contrast agents or potentially anti-cancer drugs for cancer theranostics.

Acknowledgments: We thank Peregrine Pharmaceuticals Inc., Tustin, CA, for the provision of PGN635 antibody.This work was supported in part by DOD W81XWH-12-1-0317. Imaging was conducted by DOE grant #DE-FG02-05CH11280 and NIH BTRP # P41-RR02584.

MEDI 324

Novel small molecule MCT inhibitors as anticancer agents

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All advanced stage tumors adopt vigorous glycolysis and the production of lactic acid to produce energy and other essential metabolites for proliferation. Export of this lactic

acid out of the cell is essential to prevent a decrease in intracellular pH and avoid apoptosis. Lactic acid influx and efflux from cells is facilitated by members of the mono-carboxylate transporter family known as MCT1 and MCT4. Thus, our hypothesis is that target inhibition of MCT1 and MCT4 with specific, novel, small molecule inhibitors will lead to vulnerability and death of cancer cells within both hypoxic and aerobic regions of neoplastic tissue. Over the past three years we have created novel small molecule MCT inhibitors and synthesized and evaluated several new compounds. From these studies, we have discovered numerous potent MCT inhibitors that are active at very low nM concentrations. Anticancer efficacy studies in nude mice using a colon cancer model and preliminary toxicology studies in healthy mice have proven that these new molecules efficiently reduced the tumor growth without causing any systemic toxicity. MCT inhibitors have the potential to act as single agent anti-cancer agents as well as in combination with standard chemo/radiotherapy to realize their synergistic potential.

MEDI 325

Design and synthesis of cyclopropylamide analogs of combretastatin-A4 as novel microtubule-stabilizing agents

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A series of novel cyclopropylamide analogues of combretastatin-A4 (CA-4) were designed and synthesized. Most of them had significant in vitro antiproliferative activities, particularly for compounds 7i4, 7c4, 8a4, and 8c4. Moreover, compound 8c4 was also equally potent against paclitaxel resistant cancer cells. Interestingly, the novel cyclopropylamide analogues had different binding mechanisms from CA-4. Instead of inhibiting tubulin polymerization, these CA-4 derivatives were able to stimulate tubulin polymerization. Flow cytometry revealed that compound 8c4 arrested A549 cancer cells in the G2/M phase and resulted in cellular apoptosis. Further immunofluorescence assays revealed that compound 8c4 induced mitotic arrest in A549 cells through disrupting microtubule dynamics. In addition, compound 8c4 also effectively inhibited the tumor growth in the A549 xenograft model without causing significant loss of body weight. Compound 8c4 represents a novel class of microtubule-stabilizing agent and can be used as a promising lead for the development of new antitumor agents.

MEDI 326

Towards novel anti-cancer agents: Tetrahydroquinoline-based small-molecules as selective Mcl-1 inhibitors

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The Mcl-1 (myeloid cell leukemia-1) protein is an anti-apoptotic member of the Bcl-2 family. Structurally, its BH1 (Bcl-2 homology), BH2 and BH3 domains form a hydrophobic groove that can accommodate the BH3 "death" domain of pro-apoptotic Bcl-2 proteins, thereby neutralizing their cell-killing effects. Mcl-1 up-regulation is implicated in the initiation, progression and chemoresistance of many human malignancies. Accordingly, Mcl-1 is an attractive target for the development of novel, stand-alone anti-cancer agents, and/or synergistic therapies with conventional anti-cancer drugs, such as Taxol and cisplatin. Using molecular modeling as a guide, we have developed potent inhibitors of Mcl-1 based on a tetrahydroquinoline (THQ) scaffold. Significantly, our THQ derivatives are selective for Mcl-1 over the Bcl-2 family member Bcl-x_L. Furthermore, the THQ scaffold offers several opportunities for optimization, and our progress in this regard will be described.

MEDI 327

N-Substituted, (*Z*)-5-arylidene-thiazolidine-2,4-diones as BH3 α-helix mimetics to inhibit the anti-apoptotic proteins Bcl-x_L and Mcl-1

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The anti-apoptotic proteins Bcl-x_L and Mcl-1 of the Bcl-2 family are over-expressed in a variety of cancers and contribute to chemoresistance through “neutralizing” the BH3 α-helical “death domain” of pro-apoptotic Bcl-2 proteins, which include Bak and Bim. Employing structure-based drug design, we have rationally designed synthetic BH3 α-helix mimetics based on a thiazolidine-2,4-dione (TZD) core, which are readily accessible in just three steps. Construction of the TZD scaffold was accomplished in a one-pot reaction with carbonyl diimidazole, thioglycolic acid and various amino acids in which the amino group forms part of the ring, whilst the carboxylic acid and side-chain are intended as mimetics of Asp67 and Phe69, respectively, of the Bim-BH3 α-helix. Functionalization of the 5-position of the TZD scaffold using Knoevenagel chemistry introduced moieties to mimic Leu65 and Leu62 of the Bim-BH3 α-helix. Our TZD-based α-helix mimetics are currently undergoing biological evaluation in a fluorescence polarization competition assay.

MEDI 328

2,6,9-Tri-substituted purines as amphipathic α-helix mimetics to inhibit the anti-apoptotic Bcl-2 proteins

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Over-expression of the anti-apoptotic Bcl-2 proteins, such as Mcl-1 and Bcl-x_L, is associated with the development and progression of a variety of cancers. Through the "neutralization" of the amphipathic, α-helical BH3 "death" domains of pro-apoptotic Bcl-2 proteins, such as Bak and Bim, the anti-apoptotic Bcl-2 proteins inhibit cell death. Towards the dual inhibition of Mcl-1 and Bcl-x_L, we have rationally designed amphipathic α-helix mimetics of the BH3 α-helix based on a 2,6,9-tri-substituted purine scaffold. Starting from N^2-Boc-2-amino-6-chloropurine, two sequential Mitsunobu reactions at the N9 and N2 positions installed mimetics of the side-chains of Phe69 and Ile65, respectively, of the Bim-BH3 helix, which, along with the N^2-Boc group as a mimetic of Leu62, completed construction of the hydrophobic face. Finally, displacement of the 6-chloro group employing S_NAr chemistry introduced mimetics of Asp67 on the hydrophilic face of the purine-based α-helix mimetic. Preliminary studies indicate our compounds function as pan-Bcl-2 inhibitors.

MEDI 329

Design, synthesis, and biological evaluation of 1-hydroxy-2-carboxy-substituted arenes as selective inhibitors of the Mcl-1 oncoprotein

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Mcl-1 is an anti-apoptotic protein of the Bcl-2 family that plays crucial roles in the regulation of apoptosis in homeostatic cells. As the over-expression of this protein has been associated with a myriad of lethal chemotherapeutically resistant cancers, Mcl-1 is a critical target for the design of small-molecule inhibitors. Currently, no potent Mcl-1 selective inhibitors have reached clinical trials, emphasizing the urgent need for such clinically-effective and specific chemotherapeutics. The oncogenic activity of Mcl-1 is mediated through protein–protein interactions with the pro-apoptotic members of the Bcl-2 family. Specifically, a hydrophobic binding groove on Mcl-1 binds the BH3 α-helices of pro-apoptotic Bcl-2 proteins that include Bak and Bim, resulting in the regulation of intrinsic apoptosis. In a structure-based drug design strategy, we have rationally engineered and synthesized small-molecules based on 1-hydroxy-2-carboxy-

substituted arene motifs as specific inhibitors of Mcl-1. Biological evaluation of our novel compounds is currently underway.

MEDI 330

Manganese-doped zinc oxide nanoparticles for the treatment of B-chronic lymphocytic leukemia by photodynamic therapy

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Zinc (Zn) is a well known important element involved in normal human physiology. Also, metal Zn oxide nanoparticles (ZnO NPs) could be made in order to induce a wide range of biological responses in cell systems. Doping ZnO NPs with transition metals like manganese (Mn) increases their surface defects. Therefore, this modification affects the optical and electronic properties of these NPs and could improve photo-oxidation reactions in photodynamic therapy (PDT). In this way, Mn-doped ZnO NPs could be designed and used to treat B-CLL, especially when combined with PDT. If these NPs are excited by light emission from a green laser with a wavelength of around 532nm, intracellular generation of cytotoxic reactive oxygen species, especially singlet oxygen occurs leading to a possible therapeutic apoptotic effect that could kill B-CLL cells even those with resistant phenotypes. Normal lymphocytes as well as B-CLL cells were penetrated by Mn-doped ZnO NPs when cultured at 37^{o} C for two hours and observed under TEM. A significant production of intracellular singlet oxygen was detected in both normal and B-CLL cells after being penetrated by NPs and laser irradiated.

0.5% Mn-doped ZnO NPs produce a high level of apoptosis on un-mutated B-CLL cells compared to normal lymphocytes, especially when they are laser irradiated. This significant difference in the apoptotic level between normal lymphocytes and leukemic cells could be related to the difference in the redox state usually higher in the malignant B-CLL cells. Resistant to traditional therapies un-mutated B-CLL cells responded strongly to the cytotoxic effect of Mn-doped ZnO NPs after PDT, suggesting that this innovative therapy with modifications could be used in the near future as an alternative effective treatment specially under the occurrence of B-CLL difficult to treat cell phenotypes.

MEDI 331

Synthesis of triazole-vanillin molecular hybrids and their cytotoxic studies

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Triazole drugs are widely used in cancer patients for treatment of life-threatening invasive fungal infections. A recent report also suggested 1,2,3-triazole as a useful scaffold for the inhibition of tyrosine kinases, which stimulated our curiosity to design new molecules based on this moiety. We synthesized a host of new compounds containing 1,2,3-triazole moiety tethered with substituted vanillin or isovanillin. In our synthesis first vanillin and isovanillin were converted into cyclopentyl/cyclohexyl ethers, and subsequently treated with MeMgI to give carbinols. These carbinols on reaction with $TMSN_3$ and $ZrCl_4$ as Lewis acid gave the desired azides. Click chemistry on azides with diverse acetylenes furnished the final triazoles. Products were screened for potential anticancer activity on 60 human cancer cell lines at a 10 μM dose. With exception of two compounds, most others had weak or no activity. The two active compounds showed strong inhibitory effect against different cell lines, with highest inhibition against Breast cancer panel. To elucidate the underlying molecular mechanisms we examined the clonogenic potential and anchorage-independent growth of estrogen receptor positive (MCF7 and T47D) and estrogen receptor negative (MDA-MB-231 and MDA-MB-468) breast cancer cells and investigated induction of apoptotic pathways. Results of our investigation will be presented.

MEDI 332

Labeling of tubastatin A in the hydroxamic acid functional group with carbon-11

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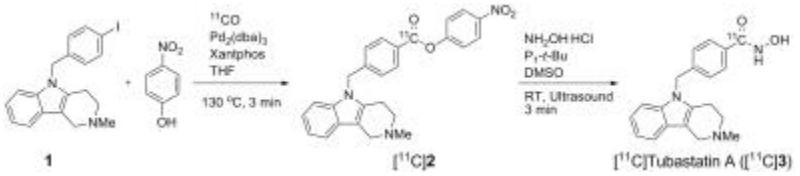
The hydroxamic acid group features prominently in many histone deacetylase (HDAC) inhibitors, including tubastatin A (**3**), a potent and selective HDAC6 inhibitor (IC_{50} = 15 nM) because of strong interaction of this functional group with zinc(II) at the enzyme active site. A generic method for labeling hydroxamic acids with carbon-11 ($t_{1/2}$ = 20.4 min) could be useful for developing PET radioligands for HDAC, or other zinc(II)-dependent enzymes of molecular imaging interest, such as the matrix

metalloproteinases. In this work the labeling of [^{11}C]tubastatin A ([^{11}C]**3**) was achieved (RCY = 16.1 ± 5.6%, n = 4) with a two-step process from [^{11}C]carbon monoxide via Pd-mediated synthesis of a [^{11}C]*p*-nitrophenyl ester ([^{11}C]**2**), followed by an ultrasound-assisted aminolysis with excess NH_2OH in the presence of phosphazene base P_1-t-Bu in DMSO. This approach is expected to have broad applicability to labeling the hydroxamic acid group with carbon-11 in future candidate PET radiotracer development.



MEDI 333

Synthesis and biological evaluation of new carbohydrate-substituted indenoisoquinoline Topoisomerase I inhibitors and improved syntheses of the experimental anticancer agents indotecan (LMP400) and indimitecan (LMP776)

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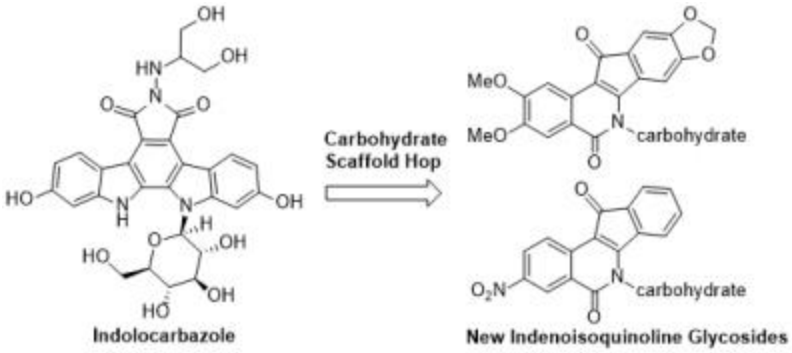
Inhibition of Topoisomerase I (Top1) is an established mechanism of action for the treatment of cancer. Two camptothecin-derivative Top1 inhibitors, topotecan and irinotecan, are FDA-approved cancer chemotherapeutic drugs. Synthetic indenoisoquinolines represent an alternative source of Top1 inhibitors that overcome many of the drawbacks associated with the camptothecins. Our laboratory has engaged in structure-based drug design focused on the optimization of an indenoisoquinoline lead compound. In the present work, carbohydrate moieties were strategically transported from the indolocarbazole Top1 inhibitor class to the indenoisoquinoline system in search of structurally novel and potent Top1 inhibitors. The synthesis and biological evaluation of 20 new indenoisoquinolines glycosylated with linear and cyclic sugar moieties are reported. Aromatic ring substitution with 2,3-dimethoxy-8,9-methylenedioxy or 3-nitro groups exerted strong effects on antiproliferative and Top1 inhibitory activities. While the length of the carbohydrate side chain clearly correlated with antiproliferative activity, the relationship between stereochemistry and biological activity was less clearly defined. Twelve of the new indenoisoquinolines exhibit Top1 inhibitory activity equal to or better than that of camptothecin. An advanced synthetic intermediate from this study was also used to efficiently prepare indotecan (LMP400) and indimitecan (LMP776), two anticancer agents currently under investigation in a Phase I clinical trial at the National Institutes of Health.



MEDI 334

Synthesis, SAR studies and in vitro evaluation of eEF-2K inhibitors

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Eukaryotic elongation factor-2 kinase (eEF-2K), an atypical protein kinase and also known as calcium/calmodulin dependent protein kinase-III (CaM kinase-III), has been reported to be upregulated in various malignant cell lines such as MCF7 and identified as a potential therapeutic target for breast cancer, gliomas and depression. eEF-2K phosphorylates eEF2 at T56 and T58, and inactivates eEF2, and thus regulate the protein synthesis during elongation step of translation.

To probe the role of eEF-2K in regulation of protein synthesis and develop a potent and selective eEF-2K inhibitor, few small molecules including NH125, and Rottlerin were identified. Recently, a high-throughput screen of Abbott compound library for eEF-2K inhibitors revealed A-484954 (IC_{50} = 280 nM *in vitro*)

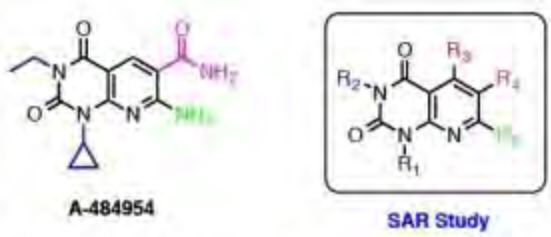

figure1. Structure and SAR study of A-484954.

, an ATP-competitive and cell-permeable pyrido-pyrimidinedione derivative, inhibitor of eEF-2K. A homology model

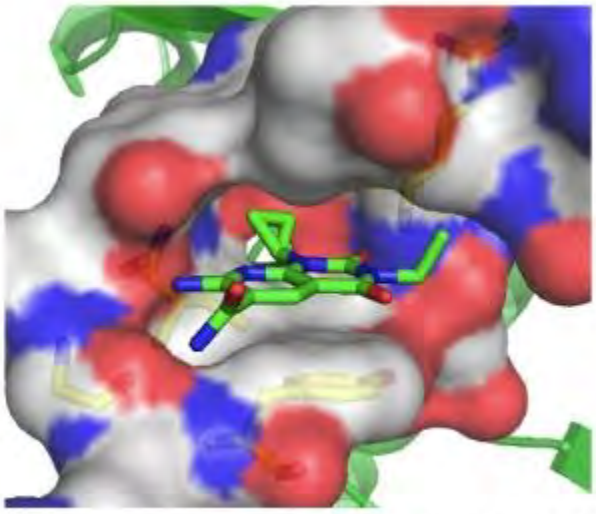

figure2. Homology model of eEF-2K with A-484954.

has been created and a series of A-484954 analogs by modifying the pyrido-pyrimidinedione core structure have been synthesized and screened for inhibitory activity against eEF-2K. Synthesis, structure-activity relationship (SAR) studies and *In vitro* evaluation of new eEF-2K inhibitors will be presented.

MEDI 335

Co-crystal structures of GSK3β and the 4-quinolone inhibitor AX9839: Specific and potent binding facilitated by a fluorine H-bond acceptor, an arginine stacking interaction, and a water bridge

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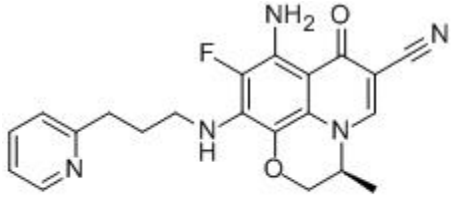
The GSK3β inhibitor **AX9839** is both highly potent and selective (KiNativ™). Due to a variety of molecular interactions detailed herein, **AX9839** is a slow, tight-binding inhibitor of GSK3. The co-crystal structure also explains aspects of structure-activity relationships seen during the development of these inhibitors.



AX9839

GSK3β IC_{50} = 0.6 nM

MEDI 336

Discovery of an APOBEC3G inhibitor from a decomposed high-throughput screening hit

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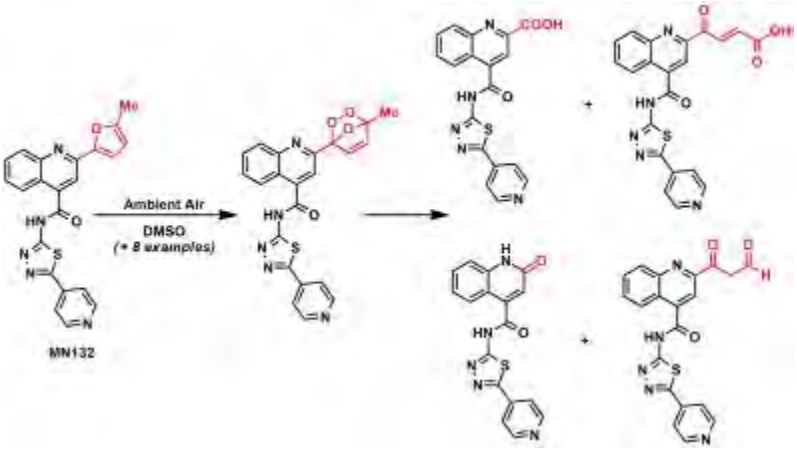
A putative inhibitor of APOBEC3 (A3) DNA cytosine deaminases A3A and A3G was identified by small molecule high-throughput screening (HTS). Confirmatory dose-response assays with synthesized MN132, however, failed to recapitulate the results observed in HTS. Curiously, repeated screening of MN132 DMSO stocks revealed increasing A3G inhibitory activity with time, suggesting that the parent scaffold is susceptible to decomposition. LC/MS analyses of MN132 stocks aged over one month demonstrated that the furan of MN132 reacts with molecular oxygen in a [4+2] cycloaddition, followed by Baeyer-Villiger rearrangement to yield four decomposition products. Control aging experiments with [^{18}O]-O_2resulted in transfer of the heavy

oxygen to the byproducts, whereas aging experiments under a nitrogen atmosphere failed to yield any measurable decomposition. A study of 8 structurally analogous scaffolds found in common screening libraries revealed identical patterns of oxidative decomposition, generally implicating the liability of the 2-furylquinoline substructure. This poster reports the oxidative liabilities of 2-furylquinolines, as well as reports a new chemotype of APOBEC3 inhibitor.



MEDI 337

DUPA conjugation of cytotoxic indenoisoquinoline topoisomerase I inhibitors as a method for selectively targeting prostate cancer cells

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Prostate cancer is the second-leading cause of cancer death of men in the United States. Current treatments are compromised by adverse side effects. Fortunately, most prostate cancer cells overexpress the prostate-specific membrane antigen (PSMA), whereas the receptor is present at low or undetectable levels in normal cells. This difference could be taken advantage of in order to selectively deliver non-specific cytotoxic drugs to these pathogenic cells while sparing normal cells that lack PSMA, thus improving potencies and reducing toxicities. PSMA is a very attractive therapeutic target that has high affinity for the ligand 2-[3-(1,3-Dicarboxypropyl)-Ureido]Pentanedioic Acid (DUPA) (K_i = 8 nM). After binding to a DUPA-drug conjugate, PSMA internalizes, unloads the conjugate, and returns to the surface. A release mechanism that would facilitate the intracellular cleavage of indenoisoquinoline topoisomerase I inhibitors from their DUPA conjugates was investigated in the present study. A suitable peptide linker was added as a spacer between the drug and the DUPA ligand in order to ensure the binding of PSMA and its ligand. In order to provide

preliminary support for this methodology, two indenoisoquinoline topoisomerase I inhibitors and their DUPA conjugates were synthesized and tested in both LNCaP and 22RV1 cell cultures, both of which overexpress PSMA. All of the compounds exhibited GI_{50} values in the low nanomolar range. The efficacy in an animal model was determined by administering one of the two DUPA-drug conjugates to 22RV1 tumor xenograft-bearing mice, and the results showed a complete cessation of tumor growth and no toxicity (loss of body weight or death of mice) during the treatment period. The results demonstrate that it is possible to selectively target cytotoxic indenoisoquinoline topoisomerase I inhibitors to prostate cancer cells while reducing adverse side effects to normal tissues.

MEDI 338

Design, synthesis, and biological evaluation of berberine and its analogs as novel drugs for breast cancer

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Breast cancer the most common cancer among women in the United States and is the second leading cause of cancer-related death in women. The majority of diagnosed breast cancers are estrogen receptor (ER) positive providing a favorable target for endocrine therapy. Currently tamoxifen (TAM) is administered as first-line endocrine therapy and its development represents a major advancement in the treatment of ER positive breast carcinoma (1, 3-4). Despite the advances in chemotherapeutic agents for breast and prostate cancer, an acquired resistance to endocrine therapy still remains a major obstacle in the treatment of breast and prostate cancer. Studies have shown that phytochemical, Berberine, a benzyl-tetra isoquinoline alkaloid extracted from plants of the Berberidaceae family, has been extensively used for many centuries, in the traditional Chinese and Native American medicine. Several evidences suggest that berberine possesses therapeutic uses, including anti-tumoral, antidiabetic and anticancer activity. We hypothesizethat structural modifications of berberine may be lead to effective anticancer agents used to overcome endocrine resistance in both breast and prostate cancer. About 20 analogues have been synthesized and the preliminary data shows that berberine analogs could effectively inhibit the growth of breast cancer cell lines to about 50% at a micromolar range.

MEDI 339

WITHDRAWN

MEDI 340

Structure-activity relationship study of betulinic acid as TGR5 agonist

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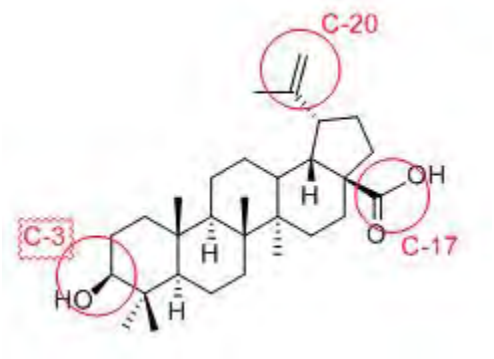
TGR5 is a member of the rhodopsin-like superfamily of G-protein coupled receptors (Class A), which is activated by bile acids (BAs). Pharmacological targeting of TGR5 may be a promising strategy for the treatment of diabetes and associated metabolic disorders.

Previously reported TGR5 agonists include natural products, such as steroidal ligands and triterpenoids, and synthetic small molecules. The most noteworthy compound is 6α-ethyl-23(S)-methyl cholic acid (S-EMCA, INT-777), which has shown good results in both EC50 (0.82 μM) and in vivo experiments, and is undergoing clinical trials.

In an effort to discover more potent TGR5 agonists, we chose betulinic acid, the most potent natural triterpenoid, as a starting point for a SAR studies. Modifications were made to the betulinic acid scaffold, focusing on structural modifications of the C-3 alcohol, the C-17 carboxylic acid, and the C-20 alkene. Structural variations gave rise to major improvements in potency, yielding compounds more potent than INT-777. Our new approach to modifying betulinic acid, especially at the C-20 alkene, opens a broader space for derivatization of the triterpenoids and provides useful evidence for the design of the next generation of TGR5 agonists.



MEDI 341

Synthesis of inositol-(1,4,5,6)-tetrakisphosphate—based probes for HDAC3 function

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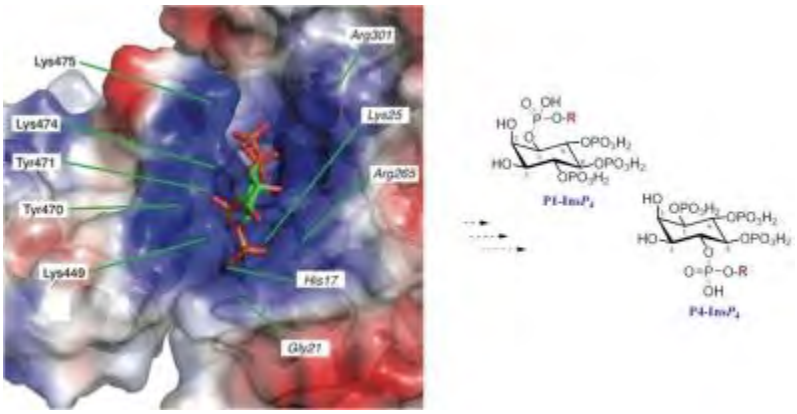
Histone deacetylase enzymes (HDACs) are emerging as important therapeutic targets in cancer. These enzymes regulate gene expression by modulating chromatin lysine actylation state. Removal of an acetyl group from lysine results in chromatin condensation and a concomitant reduction in gene transcription. Full enzymatic activity of most class I HDACs requires recruitment into multi-subunit co-repressor complexes, which are subsequently recruited to chromatin by repressive transcription factors. Recently the structure of a complex between human HDAC3 and the deacetylase activation domain (DAD) from the human SMRT co-repressor has been reported.[1]



This structure reveals two remarkable features: firstly, the SMRT-DAD undergoes a large structural rearrangement on forming the complex; secondly, D-*myo*-inositol-(1,4,5,6)-tetrakisphosphate (Ins(1,4,5,6)P_4) mediates that protein-protein interaction, acting as an 'intermolecular glue' between the two proteins. As assembly of the complex is dependent on Ins(1,4,5,6)P_4, it seems likely that this small molecule has a regulatory role in HDAC3 function—potentially explaining the link between inositol phosphates kinases and transcriptional regulators. This mechanism for the activation of HDAC3 appears to be conserved in class I HDACs from yeast to humans, and presents novel therapeutic opportunities. To understand and establish the role played D-*myo*-inositol-(1,4,5,6)-tetrakisphosphate in mediating protein-protein interactions in the regulation of HDAC1 and 3 activity, the synthesis of P1- and P4-modified Ins(1,4,5,6)P_4 derivatives acting as potential selective HDAC1 and 3 inhibitors will be presented.

[1] Watson P. J. and all. *Nature*, **2012** , *481*, 335–340

MEDI 342

Triphenylmethanol derivatives of triptorelin: Design and antiproliferative evaluation

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Triptorelin (TRP) a well-known decapeptide, is a gonadotropin-releasing hormone agonist (GnRH agonist), widely used anticancer agent for the treatment of a wide variety of hormone-responsive cancers such as prostate and breast cancer. The major limitations of cancer chemotherapy treatment are low cellular uptake, high efflux rate and the development of resistance to a certain dose of anticancer drugs, such as TRP. During the treatment of prostate cancer, TRP does cause a surge of testosterone known as a flare effect, therefore Improving of cellular uptake and duration of action of TRP is very demanding. The biological activity of TRP can potentially be enhanced by increasing and optimizing its hydrophobicity using appropriate hydrophobic linker attachment to the TRP using triphenylmethanol (TPM) which led to higher cellular uptake. In this regard, TPM-TRP prodrugs were designed to deliver and release TRP into cells. The attachment of TPM analogs along with fatty linker significantly enhanced the lipophilicity and thus the cellular uptake of these multifunctional conjugates. In this study, a number of TPM derivatives of TRP were synthesized by the reaction of 2-substituted methoxy benzenes and 1,3,5-trioxane in glacial acetic acid in 62-77% yield. The reaction of TPM derivatives with fatty linkers containing up to twelve carbons continued with conjugation to TRP afforded eight TPM-TRP conjugates with different hydrophobicity in 38-53% overall yield. Comparative antiproliferative assays between TPM-TRP conjugates and the corresponding noncovalent physical mixtures of the TPM derivatives and TRP were performed. TPM-TRP conjugates inhibited the cell proliferation of breast carcinoma cells by 55-97% at a concentration of 5 μM after 96-120 h of incubation. These data suggest that TPM-TRP derivatives can be used as a potential prodrug for improving the cellular delivery and retention of TRP.

MEDI 343

WITHDRAWN

MEDI 344

Toward the synthesis of novel boronates as potential HIV-1 protease inhibitors

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Drug discovery for HIV/AIDS has resulted in many life-saving therapies, making a great impact on modern medicine. Even though new therapies are available many drugs are highly susceptible to resistance development, have poor bioavailability, and cause several side effects. Therefore, there is an urgent need for the development of new types of inhibitory compounds, with better resistance profiles, better bioavailability, higher affinity, and lower toxicity. We are synthesizing novel boronates that were designed as compounds with potential dual-mode, both competitive and associative, of

HIV-1 protease inhibitory action. A library of straight chain and cyclic boronates are being synthesized with the hope that those compounds will demonstrate a greater inhibitory activity than their non-boronated analogs and currently used drugs.

MEDI 345

Development of small molecule inhibitors against neurotropic alphavirus replication

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Neurotropic alphaviruses are arboviruses that infect the central nervous system (CNS). These viruses may cause a wide range of ailments from mild fevers to potentially fatal encephalitis. Alphaviruses include the western equine encephalitis virus (WEEV), Fort Morgan virus (FMV), and neuroadapted Sindbis virus (NSV). Previously, we reported the preliminary development of indole-2-carboxamides as inhibitors of alphaviral replication. One of these analogs offered protection from NSV infection in mice. Further development of this series resulted in 10-fold improvement in potency in a WEEV replicon assay and significant increases in half-lives in mouse liver microsomes. We utilized a rhodamine123 uptake assay in MDR1-MDCKII cells to assess recognition of new analogs by P-glycoprotein (Pgp), the key efflux transporter at the blood brain barrier. Preliminary PK study in mice demonstrated that two new analogs could achieve higher and/or longer plasma drug exposures than our previous lead, and that one compound achieved measurable drug levels in the brain.

MEDI 346

Novel quinoline-based P2-P4 macrocyclic derivatives as pan-genotypic HCV NS3/4a protease inhibitors

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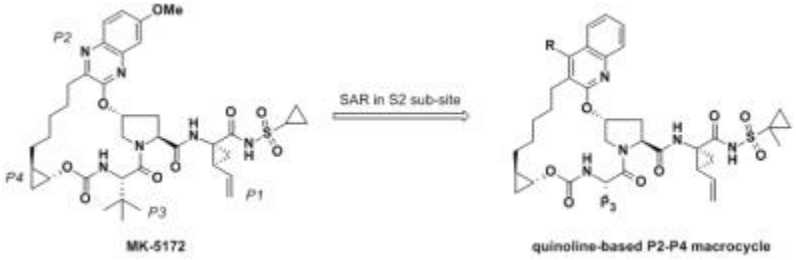
With the introduction of inhibitors of the HCV serine protease NS3/4a such as boceprevir and telaprevir, given in combination with interferon and ribavirin, the sustained virological response rate has improved to up to 75% for naïve HCV gentotype 1 patients. Additional NS3/4a protease inhibitors with broad genotype activity are currently being investigated as interferon sparing all-oral regimens. We have previously reported the discovery of our P2-P4 macrocyclic HCV NS3/4a protease inhibitor MK-5172, which is currently undergoing clinical trials. One of the areas for follow-up investigation involved replacement of the quinoxaline moiety in MK-5172 with a quinoline, and studying the effect of substitution at 4-position of the quinoline. The rationale for this effort was based on molecular modeling, which indicated that such modifications would improve interactions with the S2 sub-site, in particular with D79. We wish to report herein the discovery of highly potent inhibitors with pan-genotypic activity and an improved profile over MK-5172, especially against gt-3a and gt-1b A156 mutants.



MEDI 347

Development of novel small molecule antivirals for Western equine encephalitis virus

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Mosquito-borne viruses (arboviruses) represent an emergent public health threat largely due to the widespread prevalence of mosquitoes. Among these viruses, the equine encephalitis viruses are of particular interest to our lab because they are debilitating and potentially weaponizable infectors of the central nervous system (CNS). This is particularly troublesome because of the absence of effective human vaccines and therapeutics for these viruses. We are therefore interested in the development and

optimization of novel small-molecule antivirals, using both a Western equine encephalitis virus (WEEV) replicon assay and live virus. We explored structural modifications of the previously reported indole-based CCG-205432 (WEEV replicon IC_{50} = 0.53 ± 0.1 μM) in an effort to improve physicochemical properties predictive of successful CNS drugs, as well as to establish essential pharmacophore features and reduce P-glycoprotein (Pgp) interaction. Our investigations revealed the importance of the secondary amide to antiviral activity and demonstrated a dependence of Pgp interaction on both molecular weight and conformation.

MEDI 348

Design, synthesis, and testing of novel substituted pyridones as human concentrative nucleoside transporter 1 (SLC28a1) inhibitors

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Concentrative nucleoside transporters belong to a class of transmembrane proteins which work to transfer their substrates into or out of the cell and have been shown to be efficacious drug targets. Two highly attractive areas in which they are involved are anti-cancer and anti-viral therapeutics, but there is currently a lack of selective, potent, small molecule inhibitors.

A human concentrative nucleoside transporter (hCNT1) pharmacophore was designed using biological data from a testing of commercially available flavones and related compounds (Wang et al. Biochem. Pharmacol. 79:307-20, 2010). The resulting pharmacophore hypothesis was used to search a proprietary database of more than 360,000 compounds and ninety-six compounds were selected for testing. A whole-cell based assay employing [^{3}H]- uridine substrate was used to identify active compounds with novel CNT1 inhibitory activity. Two hit molecules, 58B and 82B of a pyridone scaffold showed moderate *in vitro* activity with IC_{50}s of 12.9 μM and 16.9 μM, respectively. This is tenfold more potent than the standard non-selective inhibitor, Phlorizin, with a reported IC_{50} of 210 μM. The hits were selected for optimization. Analogues were synthesized for testing to explore the structure-activity relationship of compounds with the pyridone scaffold.

MEDI 349

***In vitro* and *in vivo* anti-hepatitis B virus activities of novel 2-pyridone derivatives**

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We designed and synthesized a series of novel 2-pyridone derivatives and evaluated their anti-hepatitis B virus activities and cytotoxicities both *in vitro* and *in vivo*. Moderate to good activities against HBV DNA replication were observed in the target compounds. Among them, the most potent ones are compounds 11d and 11g, with profound inhibitory activity against HBV DNA replication (IC50 = 0.61 and 0.11 mM, respectively) and remarkably high selectivity (selectivity index 483.3 and = 1600.0, respectively). The *in vivo* study conducted on Pekin ducklings showed that compound 11d significantly reduced plasma and hepatic DHBV DNA levels in a dose-dependent manner with low toxicity. Compound 11d hence represents a promising drug candidate for the cure of HBV infections.

MEDI 350

Picomolar inhibitors of HIV reverse transcriptase featuring bicyclic replacement of a cyanovinylphenyl group

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Members of the catechol diether class are highly potent non-nucleoside inhibitors of HIV-1 reverse transcriptase (NNRTIs). The most active compounds yield EC_{50} values below 0.5 nM in assays using human T-cells infected by wild-type HIV-1. However, these compounds like rilpivirine, the most recently FDA-approved NNRTI, bear a cyanovinylphenyl (CVP) group. This is an uncommon substructure in drugs that gives reactivity concerns. In the present work, computer simulations were used to design bicyclic replacements for the CVP group. The predicted viability of a 2-cyanoindolizinyl alternative was confirmed experimentally and provided compounds with 0.4-nM activity against the wild-type virus. The compounds also performed well with EC_{50} values of 10 nM against the challenging HIV-1 variant that contains the Lys103Asn/Tyr181Cys double mutation in the RT enzyme. Indolyl and benzofuranyl analogs were also investigated; the most potent compounds in these cases have EC_{50} values towards wild-type HIV-1 near 10 nM and high-nM activities towards the double-variant. The structural expectations from the modeling were much enhanced by obtaining an X-ray crystal structure at 2.88-Å resolution for the complex of the parent 2-cyanoindolizine **10b** and HIV-1 RT. The aqueous solubilities of the most potent indolizine analogs were also measured to be ca. 40 µg/ml, which is similar to that for the approved drug efavirenz and ca. 1000-fold greater than for rilpivirine.