



Division of Medicinal Chemistry
Scientific Abstracts
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Division of Medicinal Chemistry
252nd ACS National Meeting, Philadelphia, PA, August 21-25, 2016 Fall Meeting

W. Young, Program Chair

SUNDAY MORNING

Renaissance of Estrogen Receptor-Based Therapy

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General Orals

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General Orals

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Role of Water in Ligand Design & Optimization

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WEDNESDAY EVENING

General Posters

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MEDI 1

SERMs and SERDs as the cornerstone of endocrine therapy in ER α -positive breast cancer

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Until recently it was considered that resistance to tamoxifen and/or to an aromatase inhibitor signaled the end of the utility of estrogen receptor-alpha (ER α) as a therapeutic target in breast cancer. Based primarily on the results of studies performed *in vitro*, it was proposed that receptor mutations, the production of non-classical ER ligands, or activation of signaling pathways that converge upon and enhance ER-transcriptional activity enabled cells to bypass the inhibitory effects of endocrine therapies. However, it has now become apparent that even in heavily treated patients with late stage disease, ER α remains a viable therapeutic target. This contemporary view of resistance has driven the search for Selective Estrogen Receptor Degraders (SERDs), compounds that target the receptor for proteasomal degradation. The clinical activity of fulvestrant, the first drug in this class, confirmed the utility of SERDs in late stage disease; however the poor pharmaceutical properties of this drug have limited its use. Previously, we reported the discovery and early clinical development of the first orally bioavailable SERD (GW5638) and although its development was discontinued, the early positive signals from clinical studies of this drug provided others with the impetus to develop additional molecules that function using the same mechanism of action (GDC810 and AZ9694). These latter drugs, if approved, will be significant additions to the breast cancer armamentarium. Anticipating that acquired resistance is likely to be a response to SERD treatment, we have recently embarked on an effort to identify targets/pathways that are required for the maintenance of the receptor expression. It is anticipated that combined treatment with SERDs and agents that inhibit ER α expression would result in more durable clinical responses. These efforts have led to the discovery that the AGR2/LYPD3 signaling axis is required for ER α expression and that its inhibition achieves a quantitative downregulation of this receptor in tumor models. The impact of targeting ER α expression, directly and/or indirectly in breast cancer will be the focus of this presentation.

MEDI 2

Benzothiophene SERMs, SERDs, MERDs, SEMs, and ShERPAs in endocrine-independent ER+ breast cancer therapy

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Despite treatment options for estrogen receptor positive (ER+) breast cancer, more women die of ER+ than triple-negative breast cancer. Resistance to the standard-of-

care treatment, the selective estrogen receptor modulator (SERM) tamoxifen, occurs in up to 50% of patients. Orally bioavailable selective ER downregulators (SERDs) and paradoxically, agonists at ER α , selective human estrogen receptor partial agonists (ShERPAs), were successfully designed and developed to treat drug resistant, endocrine-independent, ER+ breast cancer. Chemistry: The 6-OH-benzothiophene (BT) SERM, raloxifene, has been used effectively in the clinic for almost 2 decades. We adapted the BT scaffold to design and synthesize novel derivatives to modulate the pharmacological response at ER α . Truncation of the "side chain" resulted in selective estrogen mimics (SEMs) and modifications designed to reposition in the ligand binding pocket yielded ShERPAs. Further modifications resulted in SERDs and mixed activity ligands, including mixed ER downregulators with ER α agonist activity (MERDs). Biology: Three endocrine-independent ER+ breast cancer cell lines in 2D and 3D cultures and mouse xenografts were provided assays, representing models of tamoxifen and aromatase inhibitor resistant cell lines: T47D:TAM1; T47D:PKC α ; and MCF-7:5C. Parent endocrine-dependent MCF-7 and T47D cell lines were used for comparison. Pharmacology: SERMs are ineffective or weakly inhibitory in endocrine-independent ER+ cell cultures and xenografts. In contrast, E2, SEMs and ShERPAs were observed to inhibit growth of endocrine-independent ER+ cell lines in 3D and/or 2D cultures and cause regression of xenografts. Extranuclear translocation of ER α was correlated with, in most cases, with SEM efficacy. ShERPAs, as classic pharmacologic partial agonists, did not support growth of an endocrine-dependent xenograft and did not increase uterine weight. The response of resistant breast cancer cell lines to SERDs was in most cases similar in direction and magnitude to the response of these cell cultures to SEMs, and the response to a combination was not additive. Transcriptional response, defined by luciferase reporter and gene products, was cell line dependent, and uncorrelated with binding affinity to isolated ER: however, all pharmacologic classes contained low- or sub-nanomolar potency ligands. Differential ligand affinity/stabilization of ER complexes at response elements likely explains pharmacologic response.

MEDI 3

GDC-0810: An orally bioavailable selective estrogen receptor degrader for breast cancer

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Breast cancer is the most studied malignancy in the world. It is the most common cancer among women and affects one out of every eight women in the U.S. It remains the second leading cause of cancer death in women. At least 70% of all breast cancer expresses estrogen receptor alpha (ER α), making it a prime target for the treatment of breast cancer. Despite effective endocrine therapies, many patients eventually relapse and become resistant to standard of care treatments. These endocrine resistant tumors often continue to depend on ER α for growth and survival, as evidenced by their sensitivity to the selective estrogen receptor degrader (SERD), fulvestrant. However,

fulvestrant may be limited by its pharmaceutical and pharmacokinetics properties and intramuscular route of administration to achieve sufficient target occupancy for maximal efficacy. Consequently, orally bioavailable SERDs were sought, allowing consistent and rapid achievement of therapeutic exposure. GDC-0810 is an oral SERD currently in Phase 2 clinical trials, which was discovered through a prospective lead optimization on ER α degradation. Structure activity relationship of ER α degradation efficiency, pharmacokinetics, PD, and *in vivo* efficacy in wildtype and ER mutant models will be discussed, as well as corresponding early clinical data.

MEDI 4

Tetrahydroisoquinolines as selective estrogen receptor degraders with good oral bioavailability in preclinical species

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Estrogen receptor (ER) α -positive breast cancers are an unmet medical need; while there are first line therapies available like tamoxifen, resistance often emerges and disease progression continues. Fulvestrant is the only clinically approved second-line therapy available for tamoxifen-resistant ER α -positive patients. Fulvestrant is an ER α antagonist as well as a selective estrogen receptor degrader (SERD); however, the poor oral bioavailability limits its effectiveness in the clinic. We sought to develop an orally bioavailable low molecular weight entity that would antagonize ER α as well as reduce receptor level concentrations through the degradation of ER α . Herein, we disclose the optimization and identification of a tetrahydroisoquinoline (THIQ) compound bearing a phenyl acrylic acid that offers the characteristics of an ER α antagonist, degrader, and is orally bioavailable in three preclinical species. The lead molecule affords efficacy on par to that of fulvestrant in a 90 day MCF7 xenograft mouse model.

MEDI 5

Fifty shades of SERD: Designing and characterizing selective estrogen receptor degraders towards clinical candidates

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Finding potent and orally bioavailable estrogen receptor (ER) downregulators has challenged the industry for over 20 years. Designing pure ER downregulator-antagonists offers the advantage of not only antagonising ER α -driven tumor cell growth but also degrading ER α via ubiquitylation and thus diminishing growth due to reduced ER α content. This lecture will describe the medicinal chemistry challenges associated with designing novel, orally bioavailable non-steroidal ER α antagonists and down-

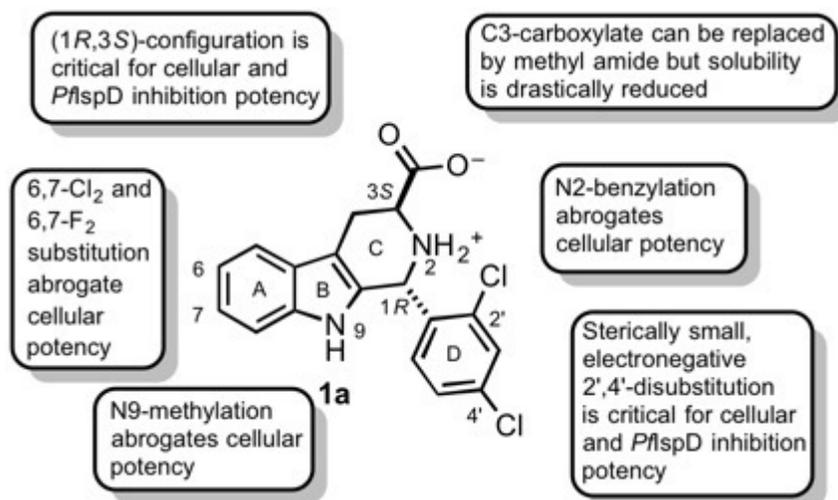
regulators including AZD9496 and other novel series. It will describe the process of how we design and characterize selective ER downregulators and some of the common pitfalls associated with working with known ER ligands and combining the right phenotype with the right oral bioavailability.

MEDI 6

Structure-activity studies of IspD-targeting antimalarials related to MMV008138

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Worldwide, malaria caused an estimated 438,000 deaths in 2014. Due to resistance to current antimalarial drugs such as chloroquine and artemisinin derivatives, there is a pressing need to discover and develop new chemotherapeutic agents that engage new targets in the pathogen. We and others have recently reported studies on the antimalarial compound MMV008138, which inhibits isoprenoid precursor biosynthesis in the apicoplast of the malaria parasite *Plasmodium falciparum*. Since the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway used by *Plasmodium sp.* for synthesis of isoprenoid precursors is absent in humans, MEP pathway inhibitors could offer effective malaria chemotherapy/chemoprotection with minimal dose-limiting toxicity. The target of MMV008138 is IspD, a cytidyltransferase that is the third enzyme in the MEP pathway. To date we have prepared 92 analogs of MMV008138: we will report the effects of structure modification on inhibition of both IspD and *P. falciparum* growth. MMV008138 and its potent analogs (*P. falciparum* growth IC₅₀ = 190 - 500 nM) do not effect *E. coli* at 100 μM, suggesting that these compounds would not affect the human gut microbiome. Ligand-based models for growth inhibition and *P. falciparum* IspD (*PfIspD*) inhibition will be presented, and possible binding modes to *PfIspD* will be evaluated based on the enzyme inhibition data. Finally, ADME-Tox data on MMV008138 and an analog will be reviewed.

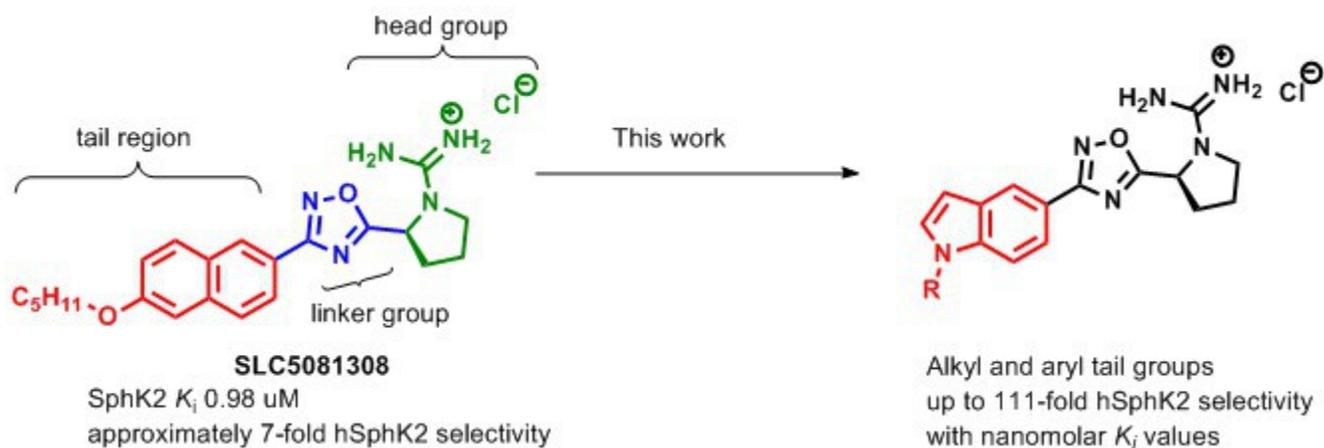


MEDI 7

Structure-activity relationship studies of the lipophilic tail region of indole derived sphingosine kinase 2 inhibitors

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A variety of diseases, including Alzheimer's, asthma, cancer, fibrosis, and sickle cell disease have been associated with elevated levels of sphingosine-1-phosphate (S1P). S1P is synthesized by the transfer of a phosphoryl group catalyzed by the two isoforms of sphingosine kinase (SphK1 and SphK2). While SphK1 is the more studied isoform, the functional role of SphK2 is still emerging. Therefore, selective, small molecule inhibitors of SphK2 are necessary to aid in determining its physiological role in vivo. Previously, our group reported a SphK2 selective inhibitor, **SLC5081308**, which displays approximately 7-fold selectivity for hSphK2 over hSphK1 and a hSphK2 K_i of 0.98 μ M. Herein, we report the design, synthesis, and biological evaluation of **SLC5081308** derivatives which substitute the 2,6-naphthalene moiety for a 1,5-indole. These compounds display excellent inhibition activity, good SphK2 selectivity, and nanomolar K_i values for hSphK2. Our study highlights key electrostatic and steric interactions in the binding pocket.



MEDI 8

Discovery of the first subfamily-selective inhibitor of FTO for novel treatment of obesity and related metabolic syndrome

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Obesity is currently one of the leading cause of premature death worldwide. Despite major therapeutic advances, there is currently no safe and effective drug for the long-term treatment of obesity. A series of recent, high-profile studies has identified *FTO* (fat mass and obesity-associated) gene to be strongly-linked with an increased risk of obesity in both children and adults, and in all major ethnic groups worldwide. Unlike other 'obesity genes' discovered previously, the *FTO* risk allele is highly prevalent across populations (14-52%). Importantly, inactivation of FTO protein was found to protect against diet-induced obesity in several animal studies. Hence, FTO inhibition may provide a novel, genetic-based approach to the treatment of obesity and related metabolic syndrome.

FTO belongs to the AlkB family of nucleic acid demethylases. These enzymes share a high level of sequence and structural homology (>60%), but have distinct physiological functions. In this presentation, we will describe the use of an innovative dynamic combinatorial chemistry (DCC)-based strategy, which led to the discovery of the first subfamily-selective and cell-active inhibitor of FTO **CA** (IC_{50} = 0.81 μ M). **CA** not only demonstrates considerable (30 to 100-fold) selectivity for FTO over other AlkB subfamily members, but also discriminates against several other structurally-related oxygenases, such as PHD2 and JMJD2A. Such selectivity is rarely achieved for any enzyme family. The mechanism of FTO inhibition was subsequently analysed using kinetic, crystallographic and cell-based studies (*Chemical Science*, 2015, 6, 112). Remarkably, treatment of human myotubes with **CA** dramatically reversed several of

the metabolic impairments associated with obesity and diabetes, leading to significant improvement in mitochondrial oxidative function, fatty acid oxidation and basal glucose uptake (unpublished results). Similar metabolic benefits were also achieved with human adipocytes. These preclinical findings suggest that **CA** is a highly promising FTO lead, and FTO inhibition could be a viable therapeutic strategy for obesity and related metabolic diseases.

MEDI 9

Discovery of a novel binder of Lp-PLA₂ and subsequent optimization through rational target design

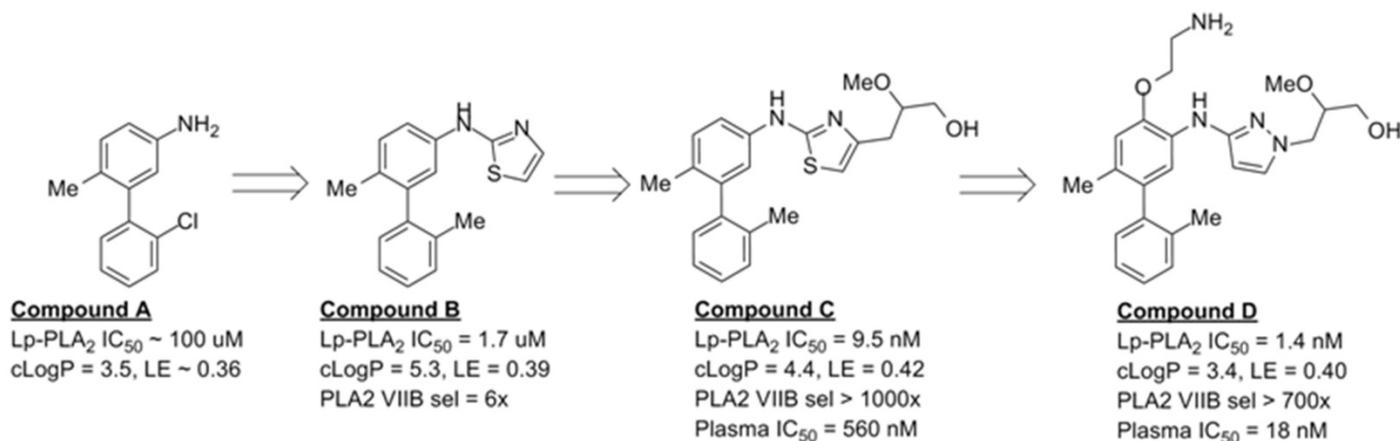
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Lipoprotein-associated phospholipase A2 (Lp-PLA₂) is a key driver in the hydrolysis of oxidized phospholipids in low-density lipoproteins (LDL's). Localized in the plasma, an elevated level of the enzyme has been correlated with inflammation as well as atherosclerosis and dementia. As such, Lp-PLA₂ inhibitors have the potential to lead to novel therapies to combat these disease states. To this end, collaboration between GSK and Astex Pharmaceuticals led to the discovery of Compound A, which was a novel lead structure originating from a fragment screen of approximately 1,000 compounds. Interestingly, Compound A exhibited a unique binding mode featuring an induced pocket formed through the rotation of the C_α-C_β bond of residue Phe357. Furthermore, this binding mode conferred potency without interacting with the catalytic triad of the enzyme. This piqued our interest with the hopes of unearthing novel pharmacology.

Target design guided by X-ray crystallography proved instrumental in the elaboration of Compound A. In a pivotal example, exploiting a growth vector off of the aniline moiety led to Compound B, which exhibited a 60-fold increase in potency. Strategically leveraging additional growth vectors in conjunction with functional group manipulations led to exemplars C and D. The latter, in particular, displayed low-nanomolar potency in a whole human plasma assay, which was most likely due to improved physicochemical properties (i.e. lower cLogP). Importantly, Compounds C and D featured exquisite selectivity over PLA2VII-B, which is a closely-related phospholipase sharing 42% sequence homology with Lp-PLA₂.

A discussion in regards to rationale target design, interpretation of structure-activity

relationships and chemical synthesis in addition to key learnings applicable to a wide audience will be presented in detail.

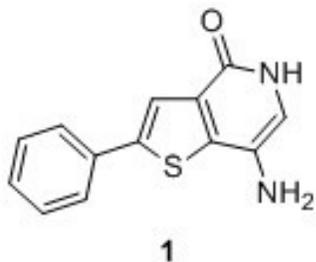


MEDI 10

Synthesis and biological characterization of a novel PTP4A3 inhibitor

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The protein tyrosine phosphatase 4A family (PTP4A1, PTP4A2, and PTP4A3) has recently garnered much interest as an anticancer target. PTP4A3 is overexpressed in many human cancers and this overexpression is correlated with increased tumor invasiveness and poor patient prognosis. Detailed understanding of the protein's exact role in tumor formation and progression remains incomplete. In an effort to expand the knowledge of the structural and mechanistic features of PTP4A3, we designed a concise synthesis of thienopyridone **1**, the most potent PTP4A3 inhibitor reported in the literature. Interestingly, **1** mimicked the phenotypic response of gene knockdown studies, where, in the absence of PTP4A3, colony formation and migration was significantly reduced in colorectal cancer cells. This result warranted the exploration of small molecule inhibitors of PTP4A3 as potential anticancer agents. During our pharmacological characterization of **1**, we discovered a structurally unique, more active analog **2**. Both compounds inhibited the activity of PTP4A3 at nanomolar concentrations (**1** IC₅₀ = 173 nM; **2** IC₅₀ = 49 nM), had potent growth inhibition of mouse colorectal and ovarian tumor cells, and severely hindered wound healing in a mouse colorectal tumor cell migration assay. Additionally, they showed inhibitory selectivity when compared to tyrosine phosphatases CDC25B and PTP1B, and serine/threonine phosphatase PP2A. Current efforts are focused on obtaining additional SAR information on **2** and more comprehensive biological profiling of its activity against PTP4A3.



MEDI 11

Discovery of potent, selective, CNS-penetrant potentiators of glycine receptors

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Glycine receptors (GlyRs) are members of the Cys-loop family of pentameric ion channels. GlyRa1 and GlyRa3 are expressed throughout the adult central nervous system (CNS) where they modulate inhibitory glycinergic neurotransmission. Previous studies with modestly potent pharmacological modulators of GlyRs have implicated these channels in the control of both inflammatory (GlyRa3) and neuropathic (GlyRa3, GlyRa1) pain processing. Thus far, the published small molecule tools interrogated in the context of preclinical pain models have lacked in both potency and pharmacokinetics. As such, new potent, CNS penetrant, positive modulators of GlyRs would serve as valuable proof-of-concept tools and may ultimately offer mechanistically differentiated therapies for the treatment of chronic inflammatory and neuropathic pain. Herein, we describe the identification of a novel class of allosteric GlyR potentiators, originating from a 3 mM hit derived from a functional cellular HTS assay. Property-guided optimization resulted in potent, selective, orally bioavailable, CNS penetrant tool molecules. These tools demonstrated (1) *in vitro* potentiation of Gly-evoked current in stably expressed cells, (2) *ex vivo* potentiation of Gly-evoked current in mouse spinal dorsal horn slices, and (3) *in vivo* proof-of-concept in a mouse model of neuropathic pain. Furthermore, a highly potent binder (K_i : 0.011 mM; EC_{50} : 0.066 mM) from this series was leveraged facilitated the solution of the first high resolution X-ray co-crystal structure of hGlyRa3 in complex with both glycine and an allosteric potentiator. The co-crystal structure reveals a novel binding pocket, which explains the high degree of selectivity over other Cys-loop family receptors and introduces opportunities for structure-based lead optimization and additional lead generation.

MEDI 12

Isoform selective AMPK activators

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5'-Adenosine monophosphate-activated protein kinase (AMPK) is a complex, heterotrimeric protein kinase involved in maintaining cellular energetics. AMPK is phosphorylated and activated under conditions of energetic stress and in turn phosphorylates a number of downstream substrates to modulate pathways involved in restoring energy homeostasis. We will describe the discovery and optimization of two lead series that bind to and activate AMPK from two unique allosteric binding pockets, the 'alpha/beta' site and a novel, inducible binding pocket on the gamma-1 subunit. The two series have distinct isoform selectivity profiles and data suggest they have different mechanisms for activation. Lead matter that binds to the alpha/beta site was optimized to a potent and selective beta-1 AMPK activator that was progressed to the clinic. A unique profile of gamma-1 selectivity was identified in a second series and this series was optimized to a tool compound. Co-crystal structures for both sites have been solved. We will summarize our hypothesis for activation from the 2 sites based on a combination of biophysical data and computational methods.

MEDI 13

Discovery of the potent and selective pyridine M₁ PAM PF-06767832: Evaluation of efficacy and cholinergic side effects

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It is hypothesized that selective muscarinic acetylcholine receptor (mAChR) M₁ subtype activation could be a strategy to provide cognitive benefits to schizophrenia and Alzheimer's disease (AD) patients while minimizing the cholinergic gastrointestinal (GI) and cardiovascular (CV) side effects observed with non-selective muscarinic orthosteric agonists. Achieving subtype selectivity has been challenging due to the high sequence homology of the orthosteric binding site across the five mAChRs. Selective activation of the M₁ mAChR receptor via a positive allosteric modulator (PAM), which binds to an allosteric pocket exhibiting less sequence homology, has emerged as a new approach to achieve selective M₁ activation. Described is a novel series of M₁-selective pyridone and pyridine amides and their key SAR for modulating potency, CNS penetration and clearance.

PF-06767832 is a potent M₁ PAM that has well-aligned physicochemical properties, good brain penetration and pharmacokinetic properties, and is active in multiple in vivo assays. Extensive safety profiling suggested that despite being devoid of mAChR M₂/M₃ subtype activity, compound PF-06767832 still carries GI and CV side effects. This data provides strong evidence that M₁ activation contributes to the cholinergic liabilities that were previously attributed to activation of the M₂ and M₃ receptors.

MEDI 14

Discovery of a novel series of aminopyrazine-based A2a antagonists for the treatment of Parkinson's disease: Integration of an intramolecular H-bonding strategy in the design of brain-penetrant scaffold

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Parkinson's disease (PD) is a chronic, progressive neurodegenerative disease characterized by loss of dopamine-producing cells in the substantia nigra, which results in a syndrome of movement disorders. Adenosine A2a receptors interact with the dopamine D2-receptors, and play an important role in modulating the effect of dopamine. Selective antagonism of the A2a receptors has been shown to restore the relative balance between dopamine and adenosine signaling, and thus represents a potential novel treatment of Parkinson's disease.

This presentation will describe our efforts towards the discovery of a novel class of selective A2a antagonists based on an aminopyrazine core. The integration of intramolecular H-bonding strategy in the design of the brain-penetrant scaffold and the use of definitive metabolic ID study in the optimization of this series of antagonists will be discussed.

MEDI 15

Design and synthesis of a potent, reversible covalent, Oxaborininol inhibitor of Lp-PLA₂

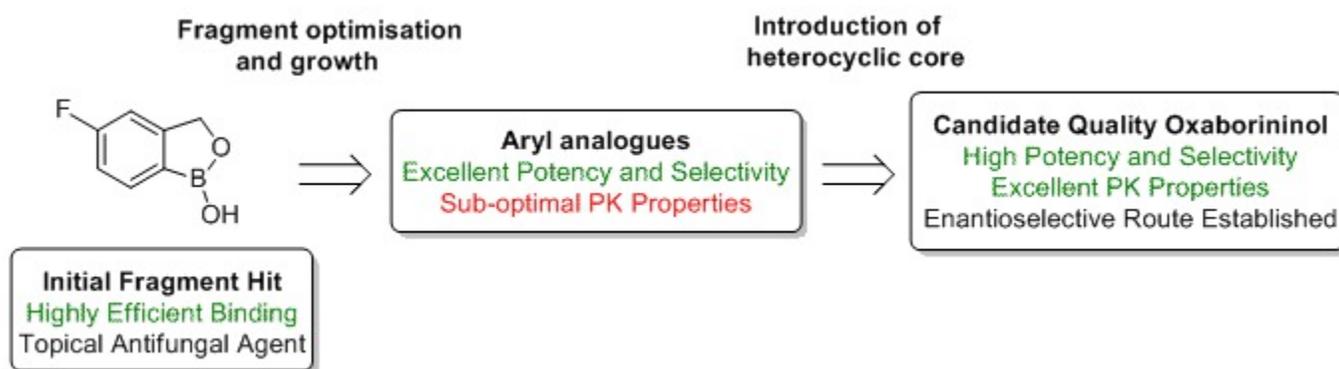
Storm Hart^{3,4}, *storm.j.hart@gsk.com*, Valerio Berdin⁵, Eric E. Boros⁶, Neil Curtis⁴, Phil Day⁵, Alain Daugan¹, Nicolas E. Faucher¹, Marie-Hélène Fouchet¹, Pascal Grondin¹, Istvan Kaldor⁶, William Kerr³, Eric S. Manas², Mark Mitchell⁶, Vipulkumar Patel⁴, Donald Somers⁴, Eric Talbot⁴, Matt Toczko⁶, Lionel Trotter¹, Gemma White⁴, Alison Woolford⁶. (1) GlaxoSmithKline, Les Ulis, France (2) Computational Chemistry US, GlaxoSmithKline, Lafayette Hill, Pennsylvania, United States (3) Chemistry, University of Strathclyde, Stevenage, United Kingdom (4) Flexible Discovery Unit, GlaxoSmithKline R&D, Stevenage, United Kingdom (5) Astex Pharmaceuticals, Cambridge, United Kingdom (6) R&D, GlaxoSmithKline, Chapel Hill, North Carolina, United States

Inhibition of lipoprotein-associated phospholipase A₂ (Lp-PLA₂) has been implicated in a variety of disease states, most notably heart disease, atherosclerosis, Alzheimer's disease, and diabetic macular oedema. The topical antifungal agent, tavaborole, was identified as a covalently bound hit in the PyramidTM fragment screen (ligand efficiency = 0.44). Herein, we disclose the identification of potent, selective, reversible covalent, cyclic boron based inhibitors of Lp-PLA₂, suitable for clinical development from this hit (ligand efficiency = 0.41).

The presentation discusses the modulation of pharmacokinetic properties of the boron based series through incorporation of heterocyclic core structures. Replacement of a

phenyl group with a pyridyl in the bicyclic core significantly reduced the lipophilicity, and further elaboration of this core was able to identify a molecule with excellent oral bioavailability and developability characteristics.

In vivo safety studies necessitated the synthesis of significant quantities of enantiopure material. The original medicinal chemistry routes to these compounds were protracted (11 linear steps), low yielding (approximately 1% to racemic material), and required chiral separation. The presentation will discuss the development of a more concise (6 linear steps), enantiospecific synthetic route to these compounds, which enabled both rapid analogue synthesis and safety studies to be conducted.



MEDI 16

Discovery of 4-undecylpiperidine-2-carboxamides as selective positive allosteric modulators of the serotonin (5-HT) 5-HT_{2C} receptor

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Signaling at the serotonin (5-HT) 5-HT_{2C} receptor (5-HT_{2C}R) is a regulatory component of neurobehavioral processes, and decreased signaling at this receptor may contribute to chronic health disorders such as impulsivity disorders, drug addiction, schizophrenia, and depression. To rescue the pathologically decreased signaling, positive allosteric modulators (PAMs) of the 5-HT_{2C}R present a novel and favorable strategy to fine-tune binding and/or signaling in response to endogenous 5-HT in a site- and event-specific manner. PNU-69176E, identified by means of a high-throughput screen, is the only reported selective PAM of the 5-HT_{2C}R. While biological characterization via intracellular calcium (Ca_i²⁺) release assay suggests efficacy and potency at 5-HT_{2C}R, PNU-69176E has sub-optimal drug-like properties and poor bioavailability. Therefore, optimization of this lead is of high importance to promote translational therapeutic

development. A series of novel analogues (e.g., CYD-1-79) have been achieved through the modification of the lipophilic long alkyl chain (undecyl) and polar moiety that flank the piperidine-2-carboxamide core scaffold. Excitingly, these efforts have yielded a selective and efficacious 5-HT_{2C}R PAM that exhibits promising pharmacokinetic properties and modulates 5-HT_{2C}R-associated behaviors *in vivo* in a dose-dependent manner. These novel 5-HT_{2C}R PAMs are capable of performing as unique tools to elucidate neuropathological serotonergic function and further medicinal chemistry optimization will yield promising therapeutic candidates with a potential for a first-in-class neurotherapeutic.

MEDI 17

Balancing selectivity and safety in a MAP4K4 kinase inhibitor: Advancing potent, selective, and orally bioavailable leads to preclinical toxicity

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MAP4K4 (mitogen-activated protein kinase kinase kinase kinase 4) is a serine/threonine protein kinase that has been implicated as a therapeutic target in a number of indications, e.g. oncology, type-II diabetes, neurodegenerative and more recently in heart failure. This presentation will describe the identification of an aminopyridine lead series from virtual screening and evolution of multiple aminopyridine leads that led to discovery of advanced potent, selective and orally bioavailable MAP4K4 inhibitors. Given the concerns of chronic safety for a kinase inhibitor in a non-oncology indication, this presentation will discuss strategies that were employed to advance two chemotypes (PF-06279789 and PF-06745013) with excellent kinase selectivity but orthogonal profiles to assess safety of the mechanism. Results from various preclinical toxicological assays will be disclosed.

MEDI 18

Discovery and structure-activity relationships of BMS-820132, a potent partial glucokinase activator

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Kristin Williams, Richard Sulsky, Bruce A. Ellsworth, Michael Wong, Jung-Hui Sun, Leslie Leith, Dawn Z. Sun, Dauh-Rung Wu, Anuradha Gupta, Richard Rampulla, Arvind Mathur, Bang-Chi Chen, Aiyang Wang, Helen Fuentes, Lori Kunselman, Michael Cap, Jacob Zalaznick, Xiaohui Ma, Heng Liu, Joseph Taylor, Rachel Zebo, Beverly Jones, Stephen Kalinowski, Joann Swartz, Ada Staal, Kevin Omalley, Lisa M. Kopcho, Jodi Muckelbauer, Chiehying Chang, Stanley R. Krystek, Steven Spronk, Jovita Marcinkeviciene, Gerry Everlof, xue-qing chen, Carrie Xu, Robert Langish, Yanou Yang, Qi Wang, Kamelia Behnia, Aberra Fura, Evan Janovitz, Nicola Pannacciulli, Steven Griffen, Bradley Zinker, John Krupinski, Mark Kirby, Jean Whaley, Robert Zahler, Joel C. Barrish, Jeffrey A. Robl, Peter T. Cheng. Bristol-Myers Squibb, Princeton, New Jersey, United States

Glucokinase is a key regulator of glucose homeostasis and small molecule activators of this enzyme have been actively investigated for the treatment of Type 2 diabetes. In the pancreas, GK functions as the glucose sensor that determines the threshold for β -cell glucose-stimulated insulin secretion. In the liver, GK removes glucose from the blood through its function as a high-capacity enzyme that catalyzes the first step in glucose metabolism. Activation of glucokinase in both liver and pancreas would be an effective strategy for lowering blood glucose by up regulating hepatic glucose utilization, down regulating hepatic glucose output and normalizing glucose stimulated insulin secretion. Several glucokinase activators have advanced to clinical studies and demonstrated promising efficacy, but also revealed hypoglycemia as a key risk. To mitigate this hypoglycemia risk while maintaining the promising efficacy of this mechanism, we have investigated a series of phosphonate containing aminoheteroaryl benzamides as “partial activators” of the glucokinase enzyme. Herein, we present the SAR of this series of GK activators, which culminated in the discovery of the potent “partial GK activator” BMS-820132. The synthesis, X-ray crystal structure and preclinical biological & ADME characterization of BMS-820132 will also be presented. Based on its promising in vivo efficacy and preclinical safety data, BMS-820132 was selected as a development candidate and advanced to Phase Ib clinical trials in diabetic patients.

MEDI 19

Discovery of ubrogepant (MK-1602): A potent, selective and orally bioavailable CGRP receptor antagonist for the acute treatment of migraine

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Calcitonin gene-related peptide (CGRP) receptor is a clinically validated target for the treatment of migraine headache. While a number of small molecule CGRP receptor antagonists have demonstrated efficacy in the clinic, none has reached regulatory filing. Concerns for liver safety factored into the discontinuation of Merck’s telcagepant in Phase III and MK-3207 in Phase II clinical studies. In our efforts to identify a development candidate with lower risk of liver injury, our strategy centered on the identification of a molecule with a differentiated structure and metabolism profile

compared to telcagepant and MK-3207, and a low human dose projection. These criteria were met with the discovery of ubrogepant. The presentation will describe the origin of a new structural series of lactam amides and optimization that led to the identification of ubrogepant. An X-ray crystal structure of ubrogepant bound to the extracellular domain of the CGRP receptor will be utilized to illustrate the key binding interactions. The preclinical profile of the compound will be discussed in detail and compared to telcagepant and MK-3207.

MEDI 20

Discovery of AZN001: A broad-spectrum capsid-binding human rhinovirus inhibitor

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Human rhinoviruses (HRVs) are non-enveloped, positive-sense single-stranded RNA viruses which are the most common cause of upper respiratory illness¹ including the common cold. While most HRV infections are mild and self-limiting, in susceptible populations they can lead to exacerbations in asthma and COPD which often require hospitalization. Currently there are no marketed antivirals against HRV although capsid-binding compounds pleconaril, pirodavir and vapendavir, which target the HRV structural protein VP1 have been in clinical development. Pleconaril, the most advanced HRV inhibitor, produced significant reductions in the severity and duration of exacerbations in clinical trials; however, it has unfavorable physicochemical properties and drug interaction concerns. We initiated a project to identify a novel broad-spectrum capsid-binding antirhinoviral with improved physicochemical properties, pharmacokinetics (PK) and CYP induction profile compared to pleconaril and other VP1

inhibitors reported in the literature.

The VP1 antiviral binding site is a largely hydrophobic tunnel and while the logD reported for most published inhibitors is >4 we determined that broad serotype coverage was best accomplished by generating compounds within a relatively narrow logD_(pH=7.4) range of 2.0 – 2.4. In general, these compounds also possessed improved solubility and higher free fraction. Additional exploration of functional group tolerance, the effect of sterics and incorporating chirality resulted in analogues having improved in vitro PK, metabolic stability and CYP induction profiles. Ultimately we identified a compound, AZN001, that had favorable physicochemical properties and an improved in vitro safety profile while demonstrating excellent potency against an expanded 46-serotype HRV panel. Consequently, AZN001 was progressed into preclinical in vivo safety and tolerability studies.

MEDI 21

Discovery of in vivo inhibitors of lactate dehydrogenase A (LDHA)

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Tumor cells often show signs of metabolic alteration when compared to normal cells. Instead of the mitochondrial tricarboxylic acid (TCA) cycle utilized by healthy cells, they often rely on glycolysis for energy generation, even in the presence of normal oxygen levels. Lactate dehydrogenase A (LDHA) is critical to this process by catalyzing the conversion of pyruvate to lactate in the final step of glycolysis. Overexpression of LDHA is found in many types of cancer cells, and shRNA mediated LDHA depletion results in the inhibition of tumor growth in glycolytically dependent cancer cell lines, xenografts, and genetically engineered murine models. Together, these facts have focused attention on the therapeutic potential of LDHA inhibition for the treatment of cancer. We have discovered series of trisubstituted hydroxylactams that are potent enzymatic and cellular inhibitors of LDHA. Utilizing structure based design and physical property optimization, multiple inhibitors were discovered with <10 µM lactate IC₅₀ in a MiaPaca2 cell line. Optimization of the series led to GNE-140, a potent cell active molecule (MiaPaca2 IC₅₀ = 0.67 µM), that also possesses good exposure and pathway activity modulation when dosed orally to mice.

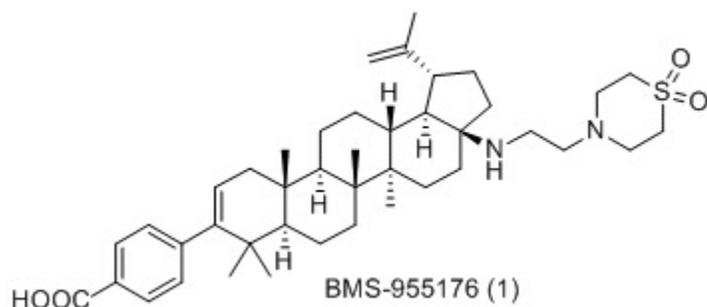
MEDI 22

SAR evolution of C-17 amines triterpenoids leading to the discovery of the second generation HIV maturation inhibitor BMS-955176

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BMS-955176 (1) is a second generation HIV maturation inhibitor (MI) with broad antiviral activity currently in Phase IIb studies. BMS-955176 is a derivative of the triterpenoid betulinic acid (BA). Four other modified triterpenoid MIs have been evaluated in humans but none of these compounds have advanced beyond Phase II clinical studies. The key structural modifications leading to the successful identification of BMS-955176 were performed at the C-3 and C-17 positions of BA. While the C-3 position incorporates a novel benzoic acid that maintains the virological profile of the dimethylsuccinic acid moiety previously deemed essential for antiviral activity, it is the C17 modification that enhances the virological profile and confers targeted exposure in vivo. In this presentation, we focus on modifications to the C-17 position, specifically C-17 amines and the careful SAR that led to the discovery of BMS-955176.



MEDI 23

Discovery of a first-in-class, potent, selective and orally bioavailable inhibitor of the p97 AAA ATPase (CB-5083)

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The p97 AAA-ATPase plays vital roles in mechanisms of protein homeostasis, including ubiquitin-proteasome system (UPS) mediated protein degradation, endoplasmic reticulum-associated degradation (ERAD) and autophagy. Herein we describe our lead optimization efforts focused on *in vitro* potency, ADME and pharmaceutical properties that led to the discovery of a potent, ATP-competitive, D2-selective and orally bioavailable p97 inhibitor, CB-5083. Treatment of tumor cells with CB-5083 leads to significant accumulation of markers associated with inhibition of UPS and ERAD functions which induces irresolvable proteotoxic stress and cell death. In tumor bearing

mice, oral administration of CB-5083 causes rapid accumulation of markers of the unfolded protein response (UPR) and subsequently induces apoptosis leading to sustained anti-tumor activity in *in vivo* xenograft models of both solid and hematological tumors. CB-5083 has been taken into phase 1 clinical trials in patients with multiple myeloma and solid tumors.

MEDI 24

Discovery of AZD2716: A novel, potent secreted phospholipase A2 (sPLA2) inhibitor for the treatment of coronary artery disease

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The structure-based optimisation of a fragment hit leading to the finding of AZD2716, a novel and potent secreted phospholipase A2 (sPLA2) inhibitor, will be presented. Key topics for the optimization were ligand efficiency, physicochemical properties and activity against sPLA2-IIa. By this rational AZD2716 could be identified in just a few design cycles. AZD2716, displaying an excellent preclinical pharmacokinetic properties across species, was selected for further PK-PD studies in cynomolgus monkeys where a clear *in vivo* efficacy was demonstrated. Based on these evidence and the pivotal role of sPLA2s in regulating lipoprotein function and inflammatory mechanisms, two crucial components of atherogenesis, AZD2716 was selected for further profiling as a potential treatment alternative of cardiovascular disease.

MEDI 25

Developing CDK8 inhibitors as tool compounds: A case study in lean decision making

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The cyclin-dependent kinase CDK8 is a critical component of the Mediator complex, which regulates the transcriptional activity of RNA polymerase II. By influencing

transcription, the complex plays a role in signalling in multiple signalling pathways involved in oncogenesis and proliferation, including the Wnt, Notch, p53 and TGF- β pathways. CDK8 has been reported to act as a colon cancer oncogene and also as a tumor suppressor in uterine leiomyomas. In order to begin to make sense of the complex biology of this kinase, we resolved to make a potent selective CDK8 kinase inhibitor.

Two separate approaches to inhibitors were taken. In the first, a known promiscuous inhibitor of CDK8, sorafenib, was optimized to improve its selectivity initially by modifying its interactions with the hinge region of the enzyme, and subsequently by using FastROCS to rescaffold the molecule to reduce the number of aromatic rings in the molecule and thereby increase its solubility. This had the additional benefit of improving the CDK8 selectivity of the molecules synthesized. Our optimized molecules were then co-crystallized with CDK8 and determined to share the DMG-out binding mode of Sorafenib.

Our second approach began with a search of our corporate database for compounds which serendipitously bound CDK8 in addition to their primary target. The most promising lead from this effort was a series of 6-azabenzothiophene inhibitors of COT. Following several rounds of SAR and structural based drug design, we obtained a series of potent, selective CDK8 inhibitors that bound the DMG-in conformation of the enzyme.

Both series of inhibitors were then profiled for their ability to inhibit the growth of colon cancer cell lines, and found to be substantially less active than anticipated. At the same time, our pharmacodynamic marker of activity, phosphorylation of the known CDK8 substrate STAT1, showed that our compounds were in fact inhibiting the kinase activity of the enzyme. We propose therefore that both the scaffolding function of CDK8 in the Mediator complex as well as its enzymatic activity may contribute to its role in signalling.

MEDI 26

Water, integral but often overlooked partner in protein-ligand binding

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Binding affinity is used as the target property for medicinal chemistry optimizations. It provides however, only limited insights as to whether the correct drug properties have been optimized with respect to a subsequent therapeutic application. Under equilibrium conditions affinity displays a Gibbs free energy and as such can be factorized into enthalpy and entropy, the latter two properties allow a more in-depth insight. The same holds for binding-kinetic k_{off} and k_{on} rates.

We have performed experimental and computational studies of congeneric ligand series and they show how water takes in manifold ways influence on the structure and energetics of protein-ligand complex formation. The impact of such effects is less apparent in a strong modulation of affinity but, owing to compensatory effects, it strongly shifts the enthalpy/entropy inventory and tunes binding kinetics. Upon ligand binding water molecules are displaced, rearranged or newly recruited to engage in contacts

between protein and ligand. In case of fragments, the binding signature is even more strongly determined by the contributions arising from the water molecules participating in the fragment's binding. Any newly formed complex will be coated by a rearranged water shell. All these processes take influence on the thermodynamic and binding-kinetic signature of the formed complex and they are decisive for the characteristics of the involved hydrophobic effect. In case binding occurs in empty or partly solvated but structurally stable pockets deviating characteristics are observed compared to transient pockets opened upon binding. The water network formed about exposed ligand functional groups in flat solvent-exposed pockets takes strong impact on the thermodynamic signature of the complex and seems to govern binding kinetics. As these parameters are determinant for the efficacy of drug binding, they must be optimized individually in tailored fashion. The combination of the results of high resolution X-ray and neutron diffraction, microcalorimetry, binding kinetics and computer simulations helps to characterize the determining influence of water on the efficacy of ligand binding.

MEDI 27

Interacting with visible or not visible water molecules to gain potency and selectivity

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Reaching high level of selectivity has been a challenging task in medicinal chemistry but remains a prerequisite for proof of concept in animal models and for limiting potential adverse effects. Interacting with specific/mutated residues in the target sites is the most common approach for achieving selectivity in target families (kinases, proteases, phosphatases...) or when several isoforms exist.

Investigating X-Ray structures of target sites usually reveals presence of water molecules which could be deeply embedded in the pocket. Development of hydrogen bonds in particular with those potentially trapped or stabilized water molecules with the ligand could result in gain of potency together with selectivity improvement as they can be very specifically localized into one protein. However it is essential to assess the thermodynamic properties of these water molecules as drug design strategies will differ depending on whether their free energy of binding is positive or negative.

In this presentation, we will first elaborate on two different case studies (PDE4, HSP90) where interactions with one identified water molecule or its displacement have resulted in spectacular affinity improvement. In the latter one (PI3K), WaterMap calculations have revealed the potential existence of unhappy water molecules in the vicinity of the ligand position and in the site entry. A rationale for the observed selectivity across the four PI3K isoforms with a series of closely related inhibitors has been established based on differential water destabilizations. Other methods, including crystal structure analysis, differential contact maps, and implicit solvent binding energy calculations were not able to explain the experimentally observed selectivity trends.

MEDI 28

Water, water, everywhere, nor any space left to link?

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In the course of historic projects, the analysis of water has played a key role. I will present results regarding the rationalization of largely different binding modes of very similar compounds as well as for the SAR exploration. However, crude scoring schemes such as MMPB/SA or simply the analysis of protein-ligand interactions are still powerful and should not be ignored.

MEDI 29

Applying CSD- and PDB-derived binding hotspot analysis to water molecules to aid in ligand design

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The Cambridge Structural Databases (CSD) contains over 820,000 small molecule organic and organo-metallic crystallographically determined atomic structures. Using this large dataset, along the protein-ligand complex structures from the Protein Data Bank (PDB), we have derived a knowledge-based method to predict and analyze fragment and lead compound binding hotspots. Using this method, we have the ability to analyze water molecule binding sites as well and have applied this technique to a number of targets of pharmaceutical interest. We will show this method as a relatively rapid tool that can aid ligand design.

MEDI 30

Water-centric methods in structure-based GPCR ligand design

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Water molecules play a crucial role in several complex aspects affecting G protein-coupled receptors (GPCRs) function. These include protein dynamics, conformational changes, receptor activation and druggability.

The increasing number of GPCR crystal structures allows us now to combine experimental ligand binding and functional activity data with high-resolution structural

analyses of GPCR-ligand interactions. This enables the estimation of the binding-site water molecules relative free energies and dynamics upon ligand binding to gain important insight to rationally optimize ligand affinity, kinetics and functional activity.

Results of molecular dynamics-based methods for predicting ligand kinetics will be presented. Examples of the critical role of waters in design will be discussed for a variety of GPCR targets including the multiple ligand structures from the StaR® (Stabilised Receptor) technology. The talk will focus in particular on the role of waters in the activity of allosteric GPCR ligands, covering also the presentation of the new glucagon receptor crystal structure in complex with MK-0893.

MEDI 31

Navigating the ocean: Importance of understanding active site waters in drug discovery

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Water plays a key role in the binding of small molecules to proteins and thus, for drug design, it is critical to have a clear understanding of active site water networks. Molecular dynamics simulations coupled with clustering and statistical thermodynamics allow for the prediction of the enthalpy, entropy and free energy of binding site waters. The energetics of these waters provide important insight into the druggability of the site and the locations of 'hot spots' which can be targeted in virtual screening and lead optimization. In addition, the enthalpic and entropic terms allow one to optimally navigate the water network by providing information about which waters to displace with a hydrophobic group, replace with a water mimetic, or avoid to improve potency. Once compounds are designed based on this information, free energy calculation methods are employed to prioritize compounds for synthesis. This talk will provide examples, both retrospective and prospective, of the successful use of binding site water energetics to evaluate druggability, identify 'hot spots', assess opportunities for bridging water interactions, and facilitate the design of compounds with improved potency and selectivity including details on the use of these approaches in Nimbus discovery programs.

MEDI 32

Transition of lipophilic imidazolium salts from *in vitro* to *in vivo* testing

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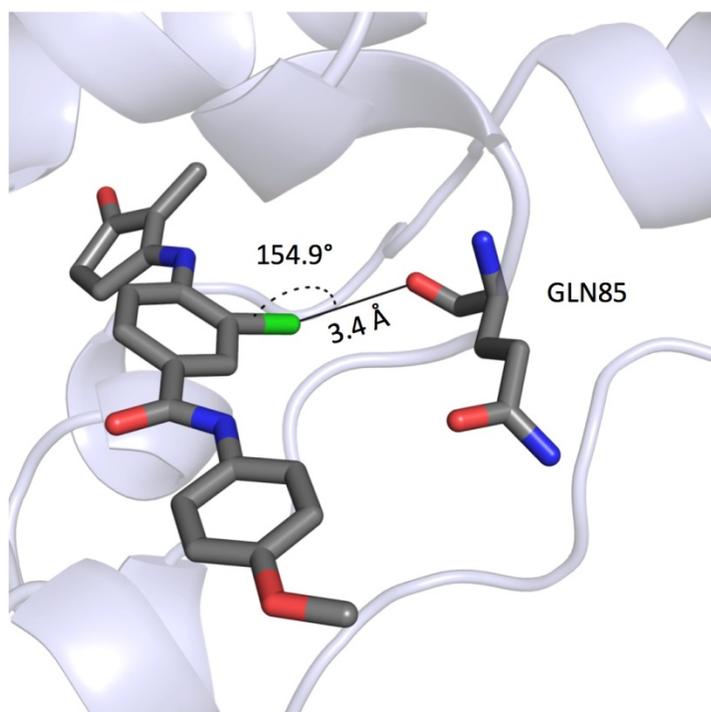
A new class of chemotherapeutic agents known as imidazolium salts has shown great potential against non-small cell lung cancer by MTT assay. Issues with aqueous solubility have limited the potential of these compounds to be transitioned from *in vitro* to *in vivo* studies. The introduction of excipients, such as 2-hydroxypropyl- β -cyclodextrin, greatly increases aqueous solubility of lipophilic compounds. Annexin V staining shows that the mode of cell death induced by imidazolium salts administered in either the presence or absence of cyclodextrin remains unchanged. However, the time frame for the compound to exert its effect is delayed when it is solubilized with cyclodextrin. Use of this excipient has also allowed for toxicity studies to be completed in C57BL/6 mice, finally permitting the transition of imidazolium salts into *in vivo* studies.

MEDI 33

New bromodomain inhibitors with halogen bonding interactions

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The BET proteins function through their tandem acetyl-lysine binding bromodomains (BrDs) as important transcriptional regulatory proteins to direct gene transcription in chromatin. As shown by numerous recent studies, the BET proteins, particularly, BRD4, are promising drug targets for cancer and inflammation. Growing evidence also shows that the two bromodomains of BRD4 have distinct functions in gene transcriptional regulation. We have used a structure-guided design approach to develop new small molecule inhibitors selective for the tandem BrDs of BD1 and BD2. In this study, we report a novel-binding mode for the rational drug design of BRD4 inhibitors. Ph with substitutions (Cl, OCH₃) and four amides are synthesized in sets (5×4), and tested against BRD4, BD1 and BD2. One set is identified with the strongest affinity to BD1 and selectivity between domains. From that set, one ligand is selected for X-ray crystal structure elucidation with BRD4-BD1. This is the first bromodomain inhibitor with a halogen bond seen to modulate affinity and selectivity. A series of analogs (F, Cl, Br, and I) are compared against the bromodomains of BRD4 and CBP. Two X-ray crystal structures with CBP are elucidated where halogen bonding effects are encountered.

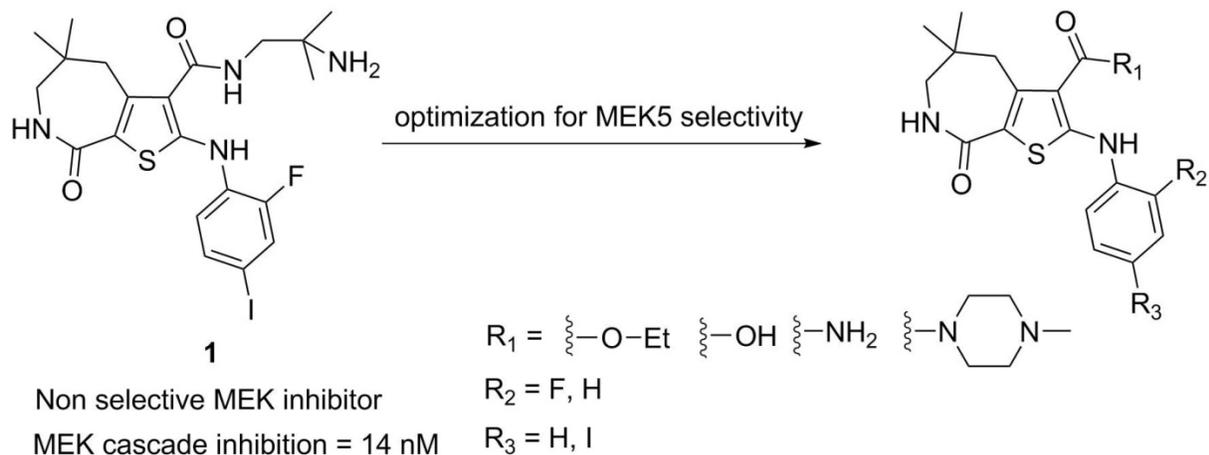


MEDI 34

***N*-arylated 2-amino fused thiophene analogs as potential MEK5/ERK5 pathway inhibitors**

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The MEK5/ERK5 signaling pathway belongs to the mitogen activated protein kinase (MAPK) family that mediates intracellular responses to extracellular events. The MEK5/ERK5 pathway is involved in cell survival, anti-apoptotic signaling, angiogenesis, and cell motility. It is significantly up-regulated in specific tumor types including breast and prostate cancers. There are currently no highly specific MEK5 inhibitors to explore the role of MEK5 in the MEK5/ERK5 pathway or related MAPK-associated responses. We have previously explored the SAR of several diphenylamine series in our lab for selective MEK5/ERK5 inhibition. In order to obtain more novel chemical scaffolds with selective inhibition of the MEK5, we began with a literature non-selective MEK/ERK inhibitor thiophene **1**, and incorporated SAR obtained from our previous diphenylamine series and used homology models for all MEK isoforms. Design, synthesis, and biological activity of selected molecules will be presented.



MEDI 35

Utility of monomethyl auristatin (MMA) analogs as payloads for targeted therapies of cancer: Design and synthesis of MMAE and MMAF folate conjugates

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The dolastatins are antimitotic and antineoplastic pseudopeptides isolated from the sea hare *Dolabella auricularia*. They bind to tubulin and inhibits tubulin polymerization. Monomethyl auristatin E (MMAE) and monomethyl auristatin F (MMAF) are synthetic analogs of dolastatins and are referred to as auristatins. They exhibit similar activity and are potent cytotoxins in preclinical models. The clinical application of these auristatins are limited due to high off-site toxicity. A monoclonal antibody conjugate of MMAE is approved for clinical use as a drug targeting CD30 expressed on cell surface in several cancers.

Selective targeting of receptors over-expressed on pathologic cells with small molecule drug conjugates (SMDC) provides an opportunity to reduce undesired toxicity experienced by normal cells. Herein we present the design and synthesis of MMAE and MMAF-folate conjugates targeting folate-receptor-positive tumor cells.

MEDI 36

Design and synthesis of *seco*-duocarmycin analogs as warheads in small molecule drug conjugates (SMDCs) for targeted cancer therapies

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Duocarmycins are naturally occurring antitumor agents originally isolated from *Streptomyces* bacteria. Being extremely cytotoxic with picomolar (pM) potency, duocarmycins are viable candidates for targeted cancer therapies. Mechanistically,

duocarmycins selectively alkylate into the minor groove of α -helical double stranded DNA via covalently binding to Adenine N-3. The result of this alkylation process is cell death. Mitigating off-target toxicity, prodrug *seco*-duocarmycin agents were designed and synthesized. Once delivered to the tumor site and upon entering the cell, the *seco*-compounds are converted to the active drug. Here, we explore the design and synthesis of small molecule drug conjugate (SMDC) platforms containing a folate binding ligand, a hydrophilic spacer, and a self-immolative linker system tethered to a *seco*-duocarmycin analog.

MEDI 37

Novel targets for small molecule drug conjugates: Design and synthesis of somatostatin analogs as targeting ligands and their conjugates with cytotoxic warheads

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Somatostatins are a family of cyclic peptides that mediate their hormone-releasing inhibitory effect through binding to cell surface G-protein coupled somatostatin receptors. These peptides can exhibit an anti-proliferative effect in cancerous cells expressing the SS2 receptor by inhibiting cell division or inducing apoptosis by initiating specific transduction pathways. Somatostatin analogues, octreotide and lanreotide, are designed for use in oncology based upon their potency and stability in comparison to their naturally occurring counterparts, somatostatin 14 and somatostatin 28. Here, we explore the dual role of somatostatin analogues as cytotoxic agents and as targeting ligands in Small Molecule Drug Conjugates (SMDCs). An efficient and regioselective method to synthesize the somatostatin analog-spacer unit is presented as part of the synthesis of the resulting SMDC.

MEDI 38

Design and synthesis of small molecule drug conjugates: Additional structural motifs

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EC1456 is an Endocyte proprietary Small Molecule Drug Conjugate (SMDC) containing a folate moiety linked to a potent cytotoxic agent, tubulysin B hydrazide (TubBH). Folic acid (FA) is a high affinity natural ligand that binds to folate receptor which are over-expressed on the cell surface in variety of cancers. TubBH is a member of the tubulysin class of anti-neoplastic agents that inhibits the polymerization of tubulin into microtubules, a critical component during cell division. EC1456 is currently in a Phase 1 clinical trial.

Herewith we present additional structural motifs, possessing enzymatically hydrolysable peptidic linker systems. Natural tubulysin B (and not TubBH) serves as warhead.

MEDI 39

Design, synthesis and early evaluation of hybrids of DNA minor groove binders and DNA-alkylating agents as warheads for small molecule drug conjugates (SMDCs) for targeted cancer therapies

Iontcho R. Vlahov, Longwu Qi, lqi@endocyte.com, Spencer Hahn, Kevin Y. Wang, Hari K. Santhapuram, Albert Felten, Jeremy F. Vaughn, Christopher P. Leamon. Endocyte Inc, West Lafayette, Indiana, United States

DNA minor groove binders (MGBs) are a class of compounds which selectively bind with high affinity to the DNA minor groove. Such compounds have been used as DNA-sequence-selective vehicles for the delivery of DNA-alkylating agents and/or DNA-cleaving agents. Combining an MGB and an alkylating agent in a single molecular entity frequently results in a hybrid compound with superior cytotoxicity in comparison to each of the constituent agents. In this presentation, we discuss the design and synthesis of structural hybrids of novel DNA-alkylating agents and MGBs, such as Distamycins and Hoechst 33258. The utility of these new hybrids as warheads in the design of small molecule drug conjugates (SMDC) for targeted cancer therapies is also discussed.

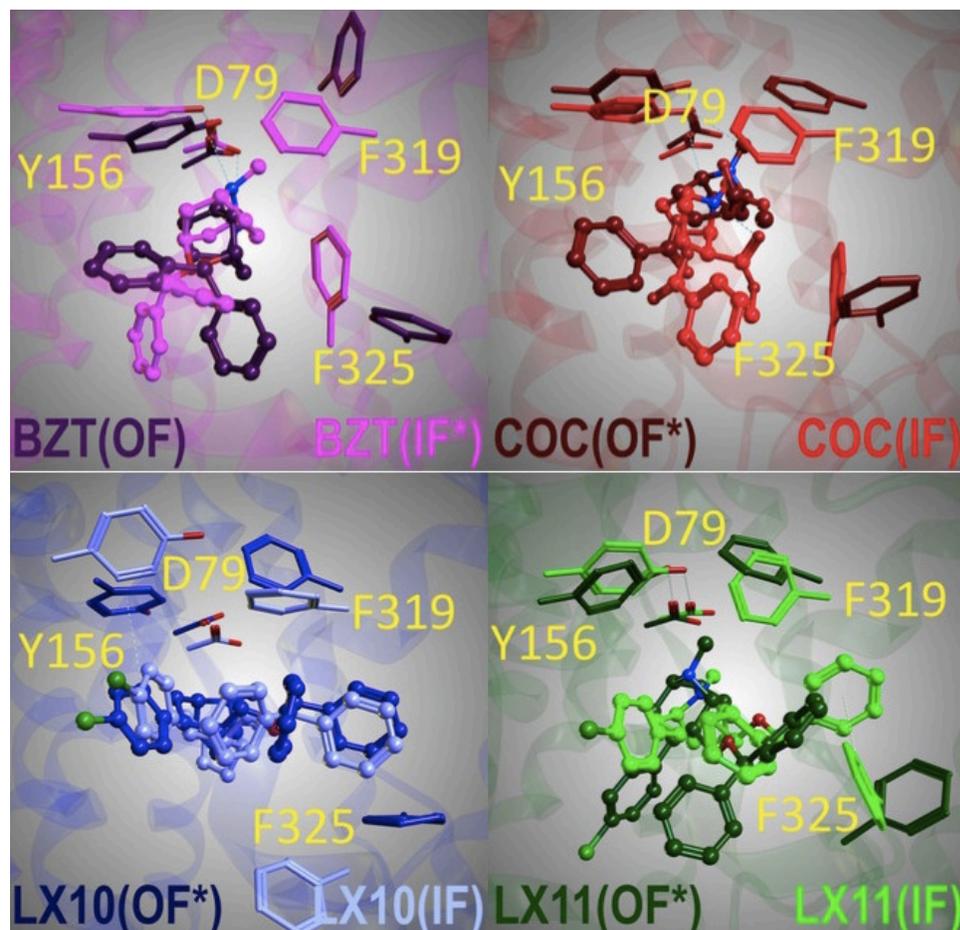
MEDI 40

Elucidating the binding mechanism of DAT inhibitors that result in abusable vs. non-abusable atypical DAT inhibitors

Bernardie Jean, jeanb@duq.edu, Jeffry D. Madura, Christopher K. Surratt. Duquesne Univ, Pittsburgh, Pennsylvania, United States

The nucleus accumbens, the brain's "pleasure center" is especially rich in dopamine neurons. Psychostimulants increase synaptic dopamine levels in this region, resulting in feelings of euphoria and the "high" associated with such drugs. It has been postulated that the abusable psychostimulants, such as cocaine (COC), stabilize the outward-facing (OF, extracellular-facing) conformation of the dopamine transporter (DAT), whereas high affinity but non-abusable DAT inhibitors, such as benztropine (BZT), stabilize the inward-facing (IF, intracellular-facing) conformation. However COC analogs, LX10 and LX11, which are 3 β -aryltropanes with respective 2 β - and 2 α -substituted phenyltropane substituents, bind to DAT with nanomolar affinity and similar mechanisms to that of COC while producing divergent behavioral effects. This work evaluates the molecular interactions between the IF and OF DAT conformation and the aforementioned DAT inhibitors. COC, BZT, and cocaine analogs, LX10 and LX11, were docked into the IF and OF DAT molecular models and simulated for 100 ns. Simulations of DAT binding COC, BZT, LX10, and LX11 reveal key interactions in the molecular mechanism of ligand-induced DAT conformations. Though LX10 and LX11 stabilize OF DAT,

interactions seen in the binding mechanism of COC differs from the binding of LX10 and LX11. The 2-substituted phenyltropane of LX10 and LX11 interact within hydrophobic regions of the binding site that is inaccessible to COC. Moreover, upon binding of LX10 and LX11, there is a loss of the salt bridge interaction between the intracellular gated residues, D435 and R60.



BZT, COC, LX10, and LX11 simulated for 100 ns in outward-facing (OF) and inward-facing (IF) DAT models superimposed respectively for comparison. The 2-substituted phenyltropane of LX10 and LX11 interact within hydrophobic regions of the binding site that is inaccessible to COC and BZT.

MEDI 41

Improved Grp94-selective inhibitors as therapies for glaucoma and metastasis

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The heat shock protein 90 kDa (Hsp90) family of proteins is responsible for the conformational maturation of over 200 client protein substrates. Many of these clients

are involved in signaling pathways that are commonly hijacked in cancer. In fact, client proteins of Hsp90 are represented in all ten hallmarks of cancer and provides an opportunity to simultaneously inhibit multiple oncogenic pathways similar to a combination therapy, but via a single agent. As a result, Hsp90 inhibitors have entered clinical trials, however, many have exhibited unanticipated liabilities suggesting a need for alternative approaches toward Hsp90 inhibition. One commonality between all clinical candidates is that they target all four Hsp90 isoforms with similar affinities. However, selective inhibition of one isoform would reduce the number of client proteins affected and reduce the potential liabilities associated with *pan*-Hsp90 inhibition. Unfortunately, the development of Hsp90 isoform-selective inhibitors is hindered by the high identity (>85%) shared amongst the four Hsp90 isoforms.

Glucose regulated protein 94 (Grp94) is the endoplasmic reticulum isoform of the Hsp90 family. Grp94 is responsible for the maturation and trafficking of proteins involved in cell adhesion and signaling. Clients of Grp94 include Toll-like receptors, integrins, IGF-I and -II, LRP6, and mutant myocilin. Grp94 possesses a 5 amino acid insertion in its primary sequence, which results in a unique secondary binding pocket within its N-terminal ATP-binding site. Previous work has demonstrated that incorporation of a *cis*-amide bioisostere into the radamide scaffold produces selective inhibition of Grp94 via interactions with this hydrophobic pocket. Structure-activity relationship studies have been performed on the aryl side chain, which interacts with this unique pocket and produced compounds that exhibit both increased affinity and selectivity for Grp94. These improved Grp94-selective inhibitors manifest activity in cellular models of metastasis and glaucoma.

MEDI 42

Discovery of anti-invasive tools and leads for the study and treatment of metastatic cancer: two case studies

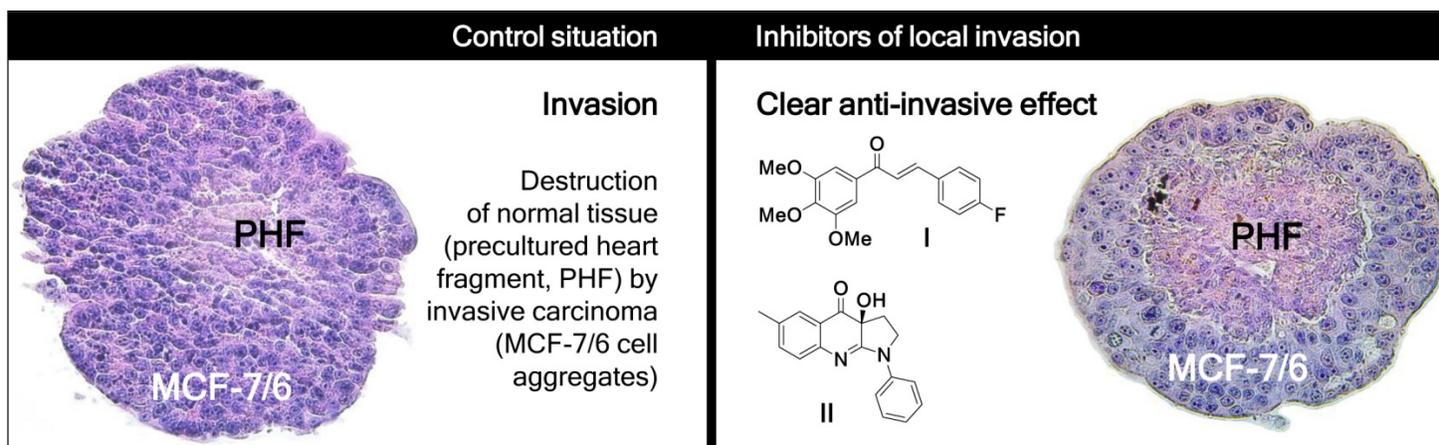
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The sequelae of invasion and metastasis are responsible for 90% of cancer-related mortality. Unfortunately, no effective inhibitors of these events are available in the clinic. In response to this unmet need, our laboratories have embarked on a quest for small-molecule tools and leads for the study and pharmacological manipulation of local invasion. An update on two of these projects will be presented. In both studies, the underlying therapeutic strategy aims at reducing mortality due to late metastasis in cancer patients.

In a first project, a screen of over 1,000 natural products using a highly relevant phenotypic invasion assay (3D confronting culture of precultured chick heart fragments (PHF) and aggregates of MCF-7/6 breast carcinoma cells) afforded micromolar

chalcone hits. A chemical optimization program yielded analogs with nanomolar potency. Their anti-invasive activity was confirmed against BLM (melanoma) and SK-OV-3 (ovarian carcinoma) cells. The compounds do not exert cytotoxicity up to a factor 1,000 above anti-invasive concentrations and do not exhibit promiscuous binding behaviour. No red flags emerged during extensive eADME, PK and MTD profiling. One compound was evaluated in vivo and increased survival time in an artificial metastasis model in nude mice. The molecular mechanism of action of the compounds remains unknown, but has been distinguished from that of known anti-invasive/antimetastatic molecules in development (e.g. integrin, chemokine, EGFR, TGF-beta, Hsp90 inhibitors). Results on the evaluation of our molecules in more elaborate in vivo models and on the identification of their biological target will be disclosed.

A second, target-based project concerns the discovery of improved inhibitors of non-muscle myosin II A/B. These molecular motor proteins are directly involved in malignant cell behavior. Today, blebbistatin is the standard chemical tool to probe myosin II. Unfortunately, this molecule is a less than ideal tool compound: it is photolabile, (photo)toxic, has limited water solubility and its fluorescence interferes with GFP visualization. Moreover, its potency in phenotypic assays is too low to be of clinical relevance. We will report on the discovery of novel blebbistatin analogs with improved physicochemical properties, potency and efficacy.



MEDI 43

3',4'-Dimethoxyflavonols: A new group of potential anti-prostate cancer agents

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Flavonoids are a class of polyphenolic compounds ubiquitously distributed in a variety of dietary plants with an array of biological activities. Flavonols are a sub-class of flavonoids featuring a hydroxyl group at C-3. Certain flavonols, such as quercetin and

fisetin, have been evidenced by *in vitro* cell-based and *in vivo* animal experiments as potential anti-prostate cancer agents. The Achilles' heel of flavonols as drug candidates is their poor bioavailability and moderate potency. The objective of this study is to explore the possibility of enhancing both bioavailability and anti-proliferative potency by chemical manipulations of 3-OH in flavonols. 3',4'-Dimethoxyflavonol, as our first model compound, and its fourteen derivatives have been synthesized through aldol condensation and Algar-Flynn-Oyamada(AFO) reaction. Their anti-proliferative activity towards three human prostate cancer cell lines has been assessed by WST-1 proliferation assay. Our findings indicate i) that 3-O-alkyl-3',4'-dimethoxyflavonols are more potent than the parent compound in suppressing prostate cancer cell proliferation and ii) that incorporation of a dibutylamine group to 3-OH through a three- to five-carbon linker leads to the optimal derivatives with over 200-fold enhanced potency when compared with the parent compound. The design, synthesis, anti-proliferative effect, and structure-activity relationships will be presented.

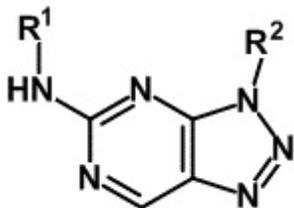
MEDI 44

Triazolopyrimidine derivatives as the first potent and selective inhibitors of the kinase GCN2

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GCN2 (general control nonderepressible 2 aka EIF2AK4) is a serine/threonine kinase that plays a key role in the integrated stress response pathways. During amino acid deprivation, GCN2 is activated by the accumulating deacylated tRNAs leading to an increased phosphorylation of the primary substrate protein eIF2a. This is a trigger for the specific expression of stress related target genes allowing the cell to adopt to nutrient deprivation conditions. The GCN2 pathway is believed to be an important regulator for the tumoral immune escape but also plays an active role in modulating tumor survival directly. Thus, GCN2 inhibitors may be beneficial agents for the treatment of cancers.

A high-throughput screening campaign for GCN2 inhibitors was conducted and a triazolo[4,5-d]pyrimidine derivative was found as hit with mediocre potency and kinase selectivity. We will present the optimization of this hit to compounds with double-digit nanomolar affinity on GCN2 and selectivity against a considerably large portion of the kinome.



MEDI 45

Synthesis and development of new, potent ROCK inhibitors for the treatment of glaucoma

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Inhibition of Rho Kinase (ROCK) in the trabecular meshwork of the anterior chamber of the eye is a new approach to lower intraocular pressure in patients with glaucoma. *Alpha*-aryl-*beta*-amino isoquinoline amide analogs were identified as potent ROCK inhibitors ($K_i = 0.2$ -10.3 nM). Their racemic syntheses involve a direct alkylation using *N*-(bromomethyl)phthalimide to incorporate the *beta*-amino methylene, followed by coupling the protected amino acid with 6-aminoisoquinoline. Studies towards the asymmetric synthesis demonstrate that chloroformates in DMF can be used to couple the weakly nucleophilic 6-aminoisoquinoline with *alpha*-aryl *beta* amino acids without partial racemization. 2,2,2-Trichloro,1,1-dimethylethyl chloroformate was identified as a new coupling agent to give *alpha*-aryl *beta*-amino isoquinolinyl amides in good yields and high enantiomeric excess.

MEDI 46

Synthetic strategies for the generation of aliphatic and aromatic bis-imidazoles as carbonic anhydrase activators

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The development of carbonic anhydrase (CA) activators was limited for a long time by the lack of powerful activators that will significantly enhance the activity of the various carbonic anhydrase isozymes and by the limited understanding of the mechanism of isozyme activation. Throughout a collaborative effort we were able to shed light into CA activation mechanism and to introduce substituted imidazoles as potent CA activators. Recently we showed that bis-imidazoles can act as powerful and selective CA activators, with nanomolar potency against several cytosolic and membrane-bound CA isozymes.

The purpose of this study was to explore different synthetic strategies for the efficient synthesis of aliphatic and aromatic bis-imidazoles, useful as carbonic anhydrase activators. We will present the results of our study in a comparative manner, together with selective activation data for the compounds generated via these procedures.

Synthesis and biological activity of triclosan based β -acetamido ketones

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Cancer has been emerged as a major health concern and it figure out main cause of mortality and morbidity worldwide. World cancer report estimates approximately 14 million new cases and 8.2 million cancer related deaths in 2012. With 1.6 million deaths in 2012, lung cancer is leading cause of cancer death. Inspired by recent reports on triclosan as a promising FASN inhibitor a series of novel triclosan based β -acetamido ketones have been synthesized by improved TFA (Trifluoro acetic acid)-catalyzed Dakin-West reaction. Structures of these synthesized compounds were characterized by IR, ¹H NMR, ¹³C NMR and LCMS. All the synthesized compounds were screened for their *in vitro* cytotoxicity against cancer cell lines including HepG2 (human liver carcinoma cells), A-549 (human lung adenocarcinoma cells), MCF-7 (human breast adenocarcinoma cells) and Vero (non-cancerous cell line) using MTT (3-[4, 5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) assay. Several of the synthesized compounds exhibited good anticancer activity especially compound 3d and 3j with IC₅₀: 5.63 μ g/mL and 9.86 μ g/mL respectively. Importantly, these compounds were found to possess higher anti-cancer potency at nontoxic concentration. Induction of antiproliferative activity of compound 3d and 3j was confirmed by AO/EB (acridine orange/ethidium bromide) nuclear staining method and also by DNA fragmentation study. Morphological analysis of 3d and 3j treated A-549 cells, clearly demonstrated the reduction of cell viability and induction of apoptosis. DNA fragmentation was observed as a characteristic of apoptosis in treated cells. Further cell cycle analysis was also performed by flow cytometry where compounds 3d and 3j significantly arrested the cell cycle at G0/G1 phase. The present study opens innovative platform for the development of triclosan (TCL) mimicking drugs for cancer chemotherapy.

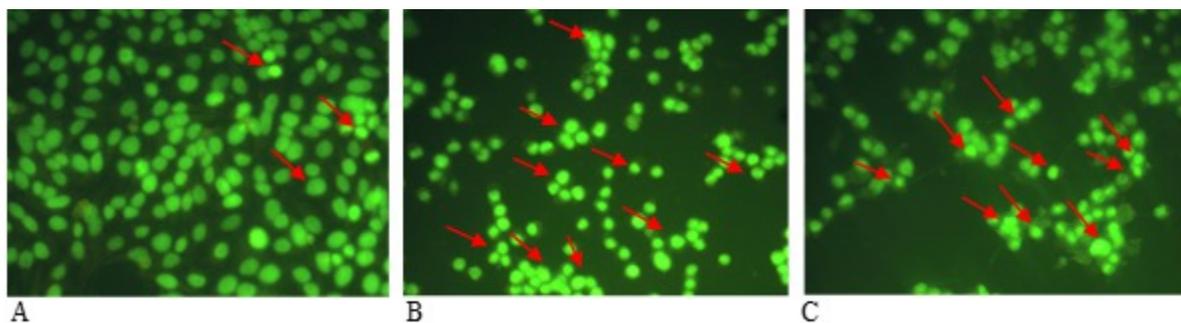


Figure 1. The representative images for induction of apoptosis by AO/EB stain in for 48 h in A-549 cells. A. Normal control, B. Compound **3d**, C. Compound **3j**.

MEDI 48

Discovery of BCL-3 inhibitor for the potential treatment of metastatic breast cancer

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In humans, the putative B-cell lymphoma 3 (Bcl-3) proto-oncogene is a Stat-3 responsive gene and an atypical member of the NFκB protein family, over-expressed in hematopoietic and solid tumours. Although it is involved in the regulation of cell death and proliferation, its exact role in endogenous tumors is still unknown. *In vivo* knockdown studies shown that Bcl-3 deficiency did not act on the primary tumour, but it reduced the occurrence of metastases by 80% without any effects on the normal mammary gland function. Bcl-3 modulates transcription of a different panel of genes involved in the metastatic progression of breast cancer by binding to p50/p52 proteins. Patients with this type of cancer have poor prognosis, they do not respond to the typical treatment or show resistance and side effects to the usual therapeutic options. Hence, there is an urgent need to find new candidate drugs.

A virtual screening targeted against an unreported Bcl-3 binding pocket has allowed the identification of an interesting hit molecule. This compound has been evaluated in several *in vitro* assays: cell viability assay; NFκB luciferase assay; migration assay; the Enzyme-Linked Immunosorbent Assay (ELISA) on both tumorigenic and non-tumorigenic breast cancer cell lines. Furthermore, the compound was also evaluated in an *in vivo* metastatic mouse model, producing a dramatic suppression of metastatic seeding compared to control.

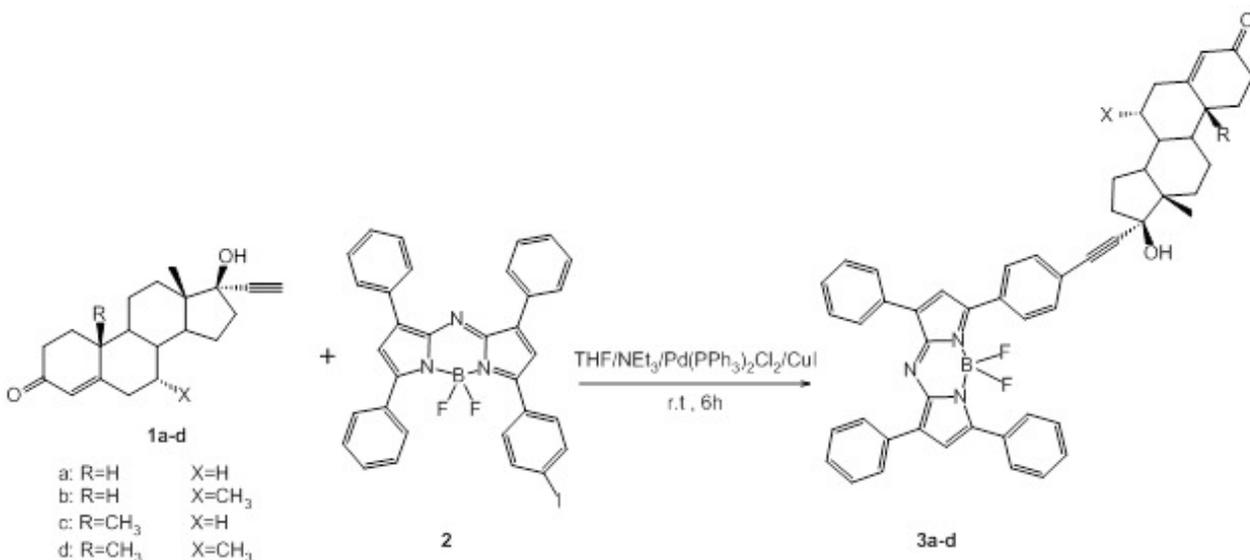
In conclusion, the compound proved to be non-toxic and it exhibits promising biological activity, both *in vitro* and *in vivo*. Further molecular modelling and Structure-Activity relationships studies have been performed and they represent the starting point of the development of a first-in-class Bcl-3 inhibitor with a unique therapeutic profile.

MEDI 49

Aza-bodipy-steroid conjugates for fluorescence imaging

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Advances in chemical synthesis techniques, such as cross-coupling and conjugation strategies, have enabled chemists to decorate a plethora of molecular species with targeting moieties, providing access to elaborate molecular architectures that can be tailored to occupy distinct binding sites within one or multiple biomacromolecules. Androgens are a class of steroid hormones that stimulates or controls the development and maintenance of male characteristics in vertebrates. Progesterone is also an endogenous steroid involved in the menstrual cycle and pregnancy. The effects of these hormones are mediated by their respective receptors. To date, there have been only limited examples evaluating the potential for targeting androgen and progesterone receptor (AR and PgR) with steroidal conjugates. They are important drug target for treatment of prostate and breast cancer respectively and have been the subject of research for several decades. There is also great interest in the development of multimodal imaging probes to monitor the mechanism of these biologically active components in living systems. The most common approach to facilitate their detection includes radiolabelling or coupling to a fluorophore, or a combination of both. To develop androgen and progesterone receptor-based ligands for fluorescence imaging we synthesized several steroid-AzaBODIPY conjugates for the first time. Aza-BODIPY (boron-azadipyrromethene) has recently attracted increasing attention as a fluorophore owing to high NIR extinction coefficients and moderate fluorescence quantum yields. We have prepared aza-BODIPY conjugates of C17 α -ethynyl derivatives of 19-norethindrone (**1a**), 7 α -methyl-19-nortestosterone (**1b**), testosterone (**1c**) and 7 α -methyl- testosterone (**1d**) using palladium catalyzed Sonogashira coupling method to furnish conjugates **3a-d**. All conjugates absorb at 675 nm, which is ideal for imaging purposes. Further studies to determine biological activities, including relative receptor binding affinity and fluorescence imaging, are being undertaken.



MEDI 50

Development of fused heterocyclic betulin conjugates as potential anti-cancer agents

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Betulin and betulinic acid are pentacyclic triterpene secondary metabolite natural products found ubiquitously in more than 200 different types of plants distributed across the plant kingdom. While betulin is abundantly available from the natural sources (eg. the bark of birch trees contain 15-20% by mass of betulin), the biologically more active betulinic acid is scarce in nature. However, betulinic acid could be conveniently synthesized from betulin via simple oxidation/reduction protocols. Betulinic acid was found to be non-toxic to normal cells however it showed significant *in vitro* cytotoxic effect against melanoma cell lines. Bevirimat is a dimethylsuccinic acid hemiester derivative of betulinic acid and it was found to exhibit potent anti-HIV activity with a new mechanism of action (maturation inhibition). However, one of the problems associated with further clinical development of betulin derivatives is the limited solubility of this molecule in the aqueous media such as the blood serum and the polar media that are used for bioassays.

Owing to the ready availability and the favorable biological efficacy (selective toxicity), we undertook the synthesis of novel betulin derivatives with increased water solubility and improved therapeutic profile. We have been able to synthesize several betulin conjugates employing reactions such as Passerini three-component coupling reaction, Fischer indole synthesis, reductive amination, Click cycloaddition chemistry, aldol reaction, etc. This presentation will outline with the synthetic details and the *in vitro* studies that were performed to determine their anti-cancer efficacy.

MEDI 51

Synthesis of novel benzothiadiazole derivatives and their biological evaluation against the oncogenic SHP2 phosphatase

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Protein tyrosine phosphatase SHP2 is of importance for the regulation of essential cellular processes that control growth, differentiation, metabolism, motility and apoptosis and has potential as drug target. The SHP2 specific small molecule agents to modulate cellular processes through direct interactions with their biological targets offer a number of attractive attributes to novel antitumor therapeutics. However, few compounds are identified as SHP2 inhibitor. Therefore, it is imperative to develop new SHP2 inhibitors. Based on our previous project to identify specific PTP1B inhibitors, we identified several compounds with benzothiadiazole scaffold showing SHP2 inhibitory activity. Therefore, we designed and synthesized a focused library with chemical diversity to study the structure-activity relationships to obtain potentially specific inhibitors for SHP2, and several compounds showed moderate SHP2 inhibitory activity and selectivity and can

be used as tool compounds to understand the detailed functions of SHP2 in biological condition.

MEDI 52

Design and synthesis of xanthone analogs based on α -mangostin analogs as new anti-cancer agents

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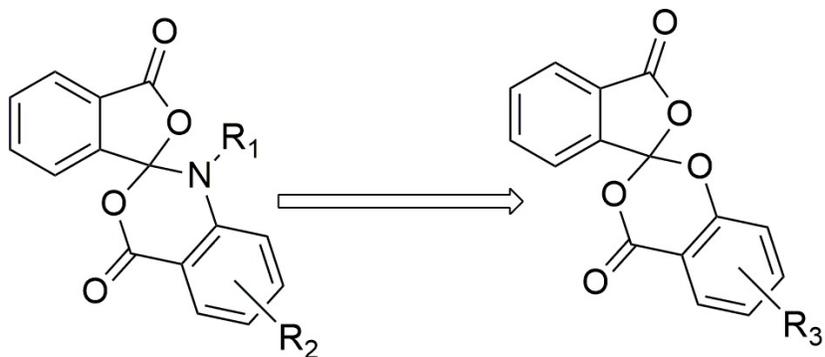
A xanthone-derived natural product, α -mangostin is isolated from various parts of the mangosteen, *Garcinia mangostana* L. (Clusiaceae), a well-known tropical fruit. Novel xanthone derivatives based on α -mangostin were synthesized and evaluated as anti-cancer agents by cytotoxicity activity screening using 5 human cancer cell lines. Some of these analogs had potent to moderate inhibitory activities. The structure–activity relationship studies revealed that phenol groups on C3 and C6 are critical to anti-proliferative activity and C4 modification is capable to improve both anti-cancer activity and drug-like properties. Our findings provide new possibilities for further explorations to improve potency.

MEDI 53

Design, synthesis and evaluation of spiro[benzo[d][1,3] dioxine-2,1'-isobenzofuran] -3',4(1H)-dione derivatives as potential anticancer agents

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Inspired by recent reports that anti-fungal agent griseofulvin inhibits cancer cell proliferation by interfering with microtubule function, a series of spiro-oxazine derivatives structurally related to griseofulvin was synthesized and evaluated against a panel of cancer cell lines which include KB-3-1, MCF-7, H460, and SW620. The IC₅₀ value of the most potent compound in this series was found to be $2.39 \pm 0.08 \mu\text{M}$ against the SW620 cell line. In an effort to further improve the potency of these compounds, a series of spiro[benzo[d][1,3] dioxine-2,1'-isobenzofuran] -3',4(1H)-dione derivatives was designed and synthesized. Cell proliferation assay using the cancer cell lines KB-3-1, MCF-7, H460, and SW620 showed that the dioxine analogues were, in general, more potent than the oxazine analogues. IC₅₀ value of the most potent compound in the dioxine series was found to be $4.51 \pm 0.03 \mu\text{M}$ against the KB-3-1 cell line.



MEDI 54

Design and synthesis of 4-anilinoquinazoline-acylamino derivatives as VEGFR-2 inhibitors

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The present report describes the design and discovery of 4-anilinoquinazoline-acylamino derivatives as VEGFR-2 inhibitors. VEGFR-2 is important target for cancer therapy, the VEGFR-2 signalling pathways represents a promising approach to the treatment of cancers with a synergistic effect. In this study, a series of novel 4-anilinoquinazoline-acylamino derivatives designed as VEGFR-2 inhibitors were synthesized and evaluated for biological activities. Most of them exhibited significant inhibitory potencies against VEGFR-2 as well as excellent antiproliferative activities. Compound 6 exhibited the most potent inhibitory activity against VEGFR-2 with IC_{50} of $1.81\mu\text{M}$, it also showed the highest antiproliferative activities against three cancer cell lines (HT-29, MCF-7 and H460) with IC_{50} of $7.65\mu\text{M}$, $8.611\mu\text{M}$ and $10.73\mu\text{M}$, respectively. Molecular docking established the interaction of compound 6 with the DFG-out conformation of VEGFR-2, suggesting that they might be type II kinase inhibitors.

MEDI 55

Design of inhibitors for the human papillomavirus E6 protein

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The Human papillomavirus (HPV) is a major cause of cancer worldwide, infecting close to 79 million people and claiming the lives of 300,000 annually. The situation is particularly grim in Sub-Saharan Africa, Eastern Europe, and Latin America, but even developed countries such as the United States contribute 14 million new infections every year. The only clinically approved products on the market, Gardasil and Cervarix,

while effective, are insufficient at treating patients post-infection *Therefore, there is a clear and urgent need for easy-to-administer, low-cost therapies, capable of preventing disease progression and treating both early and advanced HPV-associated cancers. The development of potent drug candidates, selective for the HPV-E6 protein required for disease progression, are a goal of this project.*

The efforts to date have provided several key insights into the chemical features of the binding pocket on HPV-16 E6 for the human E6AP/UE3A protein. While it is already known that the LxxLL α -helical motif is crucial for peptide recognition and binding, our work has shown that other factors contribute to the association with small molecule inhibitors. In particular, our data indicate potential roles for several arginine residues (R102, R129, R1031) in small-molecule binding, by hydrogen-bonding and pi-cation interactions with the ligands. Mutational analyses have shown that upon loss of one of these arginines, the binding pocket will rearrange to allow for compensatory interactions with alternative arginines and the ligand. Interactions with these residues offer additional potential for developing potent ligands. Further, computational studies have revealed the existence of three previously unknown sub-pockets, surrounding the main E6-E6AP binding groove. These sub-pockets are in close proximity to R102, R129, and R131, and have unique chemical features, which distinguish them from the E6AP binding groove and make them promising hot-spots for new ligand development.

MEDI 56

Synthesis and properties of curcumin conjugates as green cancer drug delivery system

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There is a pressing need for the development of green cancer drug delivery systems, which can selectively deliver the anticancer drug on the target cancer cells and reduces the toxicity of anticancer drugs. Curcumin, a natural polyphenol extracted from the rhizome of the perennial herb *Curcuma longa* is a good candidate for developing new green cancer drugs. However, its low bioavailability has restricted its development as an effective anti-cancer drug. The bioavailability of curcumin can be increased by conjugating it to proteins. We intend to synthesize well-defined water soluble Bovine Serum Albumin (BSA)-curcumin conjugate. Curcumin is attached to the BSA via a cleavable ester linkage which would be hydrolyzed by cytosolic esterases, releasing free drug inside the target cells. The conjugate is characterized via Gel electrophoresis

and Fast Protein Liquid Chromatography (FPLC). The drug loading is determined by MALDI-TOF. The anti-cervical cancer activity is evaluated by MTT assay on Hela cells *in vitro*.

MEDI 57

Breast cancer cell MDA viability of extracts from Taylor and Callahan counties of West-Central Texas plants: *Asclepias syriaca*, *Asclepias viridis*, *Solanum elaeagnifolium*, *Gaillardia pulchella*, and *Glandularia bipinnatifida*

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Due to the resistance of synthetic-cancer drugs and complexity of breast cancer disease our attention has been caught to investigate the extracts from natural resources as potentials against breast cancer. We screened some of plants in Taylor and Callahan counties of West-Central Texas Plants. Here we present the microtitration viability assay of between breast cancer cell MDA and each extract of *Asclepias syriaca*, *Asclepias viridis*, *Solanum elaeagnifolium*, *Gaillardia pulchella*, and *Glandularia bipinnatifida*.

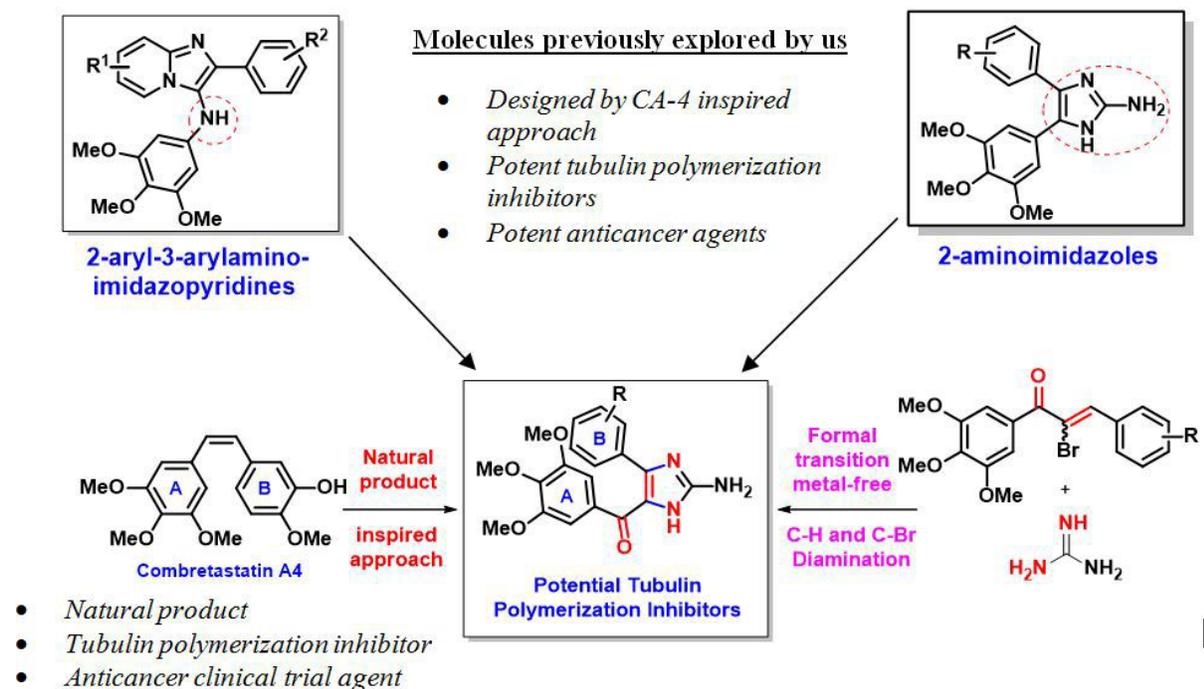
MEDI 58

Creating new from clinical agents: Discovery of combretastatin, a-4 inspired heterocycles as antitubulin anticancer agents

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“The most fruitful basis for the discovery of a new drug is to start with an old drug”- Sir James Black, Nobel Laureate in Physiology and Medicine said. Natural products have inspired the discovery of new drugs. Combretastatin A-4 (CA-4) is a natural product that has led to the discovery of several tubulin polymerization inhibitors. As our interest^{1,2} of medicinal chemistry research on anticancer drug discovery, in the present study, 2-aminoimidazole with carbonyl linker as key scaffold was considered as replacement of the double bond of CA-4. Other important pharmacophoric features like 3,4,5-trimethoxy substitution on the ring A, relevant substituted aryl as ring B, cis configuration between rings A and B and an additional linker like carbonyl group to provide the optimal dihedral angle for binding at the colchicine binding site were also considered in the design of novel 2-aminoimidazole-bridged analogs. A new and efficient approach for the synthesis of 4-aryl and 5-aryl substituted 2-aminoimidazoles via previously unknown formal diamination of guanidine to alkene of α -bromochoalcone has been developed. Utilizing this method, 2-aminoimidazole analogs with relevant substitutions, which were found to be important in other CA-4 analogs as potential tubulin polymerization inhibitors, were synthesized. Most of the compounds were found to exhibit pronounced antiproliferative activities in nanomolar concentrations. Compared to CA-4, they were also found to be

significantly potent in tubulin polymerization inhibition. Single crystal X-ray analysis confirmed the required geometry/orientation present in the structure. The detailed mode of tubulin-binding will also be included in my presentation.

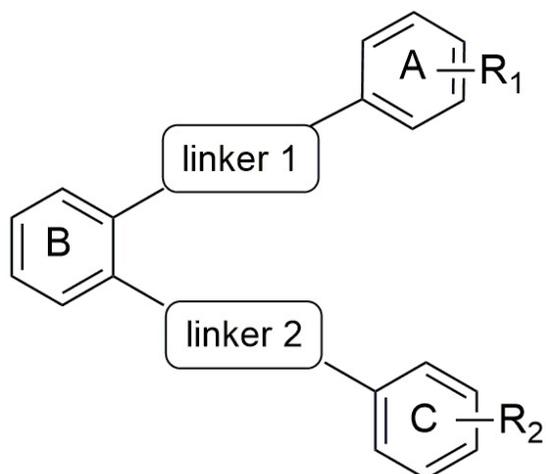


MEDI 59

Synthesis and evaluation of benzamide and phenyl tetrazole derivatives with amide and urea linkers as BCRP inhibitors

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A series of breast cancer resistance protein (BCRP/ABCG2) inhibitors derived from tariquidar was synthesized. Linkers such as amide or tetrazole between rings A and B and amide or urea linkers between rings B and C were explored along with various substitutions at R₁ and R₂ positions. The target compounds exhibited enhanced cytotoxicity of ABCG2 substrate anticancer drug, mitoxantrone, in drug resistant H460/MX20 cells overexpressing ABCG2. However, the target compounds do not have effect on the cytotoxicity of paclitaxel, a substrate of ABCB1/Pgp, in ABCB1 overexpressing KB-C2 cells. The results of these studies suggest that the new synthetic compounds are potent and selective modulators of ABCG2/BCRP.



MEDI 60

Cytotoxicity assay of *Combretum farinosum* extracts

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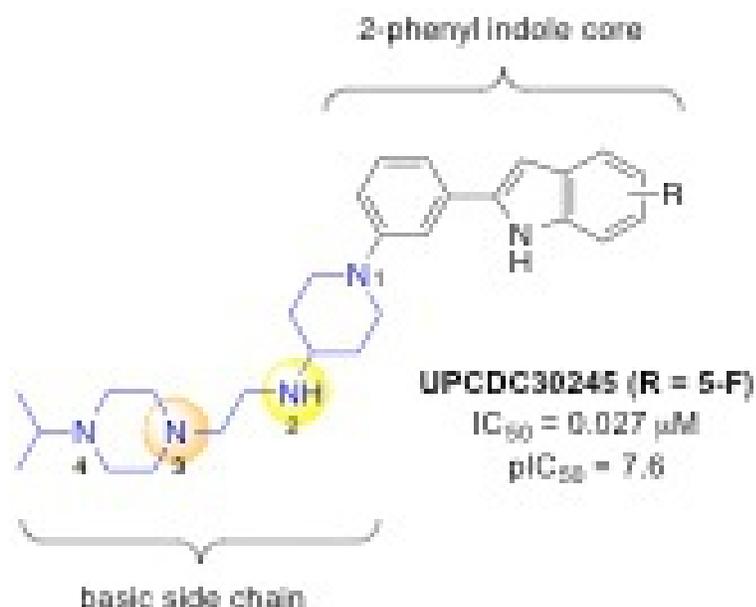
The use of plants from the Combretaceae family in medicine have been of much interest to scientists. Fossil evidence dates plant usage in healthcare as far back as 60,000 years. In fact, indigenous biomedicine in South Africa has an estimated economic value of over \$326 million. Researchers have concluded that approximately 200,000 healers deliver this form of healthcare to 27 million South Africans. Worldwide, plant usage in healthcare is estimated at 65%. A sequential Soxhlet extraction was performed on *Combretum farinosum* roots, fruits, leaves, and stems using petroleum ether, acetone, and ethanol solvents. These extracts then underwent a rotary evaporation and freezing drying process before being diluted in DMSO to make known concentrations. A three day Wallert and Provost Lab 96 well plate MTT cell proliferation assay was performed on lung fibroblast (from a cancer donor) LL 47 (MaDo) ATCC CCL-135, and normal foreskin BJ ATCC CRL-2522 cell lines. The MTT assay is a colorimetric assay which allows the quantification of percent inhibition of cell growth in response to treatment with test extracts through the detection and quantification of the cleaved MTT product. Preliminary results suggest that the ETOH stem extract inhibits growth of the lung cells while the fruit ETOH, stem ETOH, fruit AC, roots AC, fruit P. Ether, and fruit P.Ether extracts inhibited skin cell growth. The inhibition of cell growth is noteworthy as these extracts have potential use as anti-cancer drugs.

MEDI 61

Computational studies of 2-phenyl indole inhibitors of p97 chaperone

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The active form of the p97 AAA+ chaperone is a hexameric complex, and each monomer is composed of two ATPase domains, D1 and D2, that form two stacked rings and an N-terminal domain that binds numerous cofactor proteins. Due to the critical role of p97 in protein homeostasis, it is an emerging target for cancer drug development. As part of our effort to develop allosteric p97 inhibitors, our team developed a series of potent inhibitors represented by UPCDC30245. Based on data from > 200 analogs, we developed ligand- and structure-based pharmacophore models to elucidate essential scaffold substituents and conformational preferences, which were also in agreement with experimental data. Docking studies were used to rationalize the importance of the indole moiety and the preference for certain substituents at the indole 5-position, as well as the importance of basic side chains.



MEDI 62

Side chain optimizations of novel inhibitors of the AAA ATPase p97

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p97, a homo-hexameric complex, is a type II AAA ATPase that plays an essential role in protein homeostasis and is an appealing target for the development of chemotherapeutics. We recently reported 2-phenyl indoles containing a piperidine-linked piperazine side chain as potent inhibitors of p97. The key synthetic step in the preparation of these compounds was a titanium (IV)-mediated reductive amination of a 4-piperidone. Our structure-activity relationship (SAR) studies indicated that the piperidine-linked piperazine portion was readily modified without dramatic changes in biochemical potency. Our SAR results were consistent with a model placing the indole deep within a protein pocket with the flexible side chain extending into a surface cleft. A second generation series of compounds took advantage of this binding model and will be described in detail. This series included simple alkyl linkers between the piperidine and terminal piperazine, as well as several constrained analogs, including spirocyclic and fused ring systems. The synthetic efforts to be reported resulted in compounds with activities spanning a 100-fold range from micromolar to low nanomolar

MEDI 63

Design and synthesis of heterocyclic inhibitors of the AAA ATPase p97

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The AAA p97 influences cell cycle regulation, autophagy, endoplasmic reticulum associated degradation, ubiquitin fusion, and other cellular processes. It is an attractive target for anti-cancer therapy based on the correlation of increased p97 levels with poor disease outcomes. A competitive inhibitor of p97 is currently in early stage clinical trials. We identified a series of 2-phenyl indoles as allosteric inhibitors of p97. Furthermore, using molecular match pairing and isosteric heterocycle replacement strategies as guidance, we prepared 20 different heterocyclic replacements of the indole moiety. This presentation will also present the biochemical data that characterize the preferred 2-phenyl indole system.

MEDI 64

Design and characterization of a mercaptophile library for screening cysteine-containing target

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There is considerable interest in the design of drug candidates that covalently modify their targeted binding site. The majority of these small molecules utilize an electrophilic warhead to form either reversible or irreversible covalent bonds with proximal cysteine or serine residues at the binding site. Many of these candidates have been identified by a structure-based approach to design an electrophile as a “targeted covalent inhibitor (TCI)”. However, carefully designed small molecule libraries with reactive warheads have the potential to identify novel starting points for drug discovery projects. Employing this approach, we set out to design a diverse compound library of mercaptophiles with fragment-like characteristics for use in a mass spectroscopy-based tethering screen. Our target for this screen was ATG4b, a cysteine protease involved in multiple steps in autophagy. Utilizing a strategic cheminformatics-guided selection process, we examined our in-house library for thiol-capture moieties. The ~ 52,000 UPCMLD (Univ. Pittsburgh Center for Chemical Methodologies and Library Development) library compounds were initially clustered according to 21 different warhead groups. We defined a diverse compound set through physicochemical properties (MW and lipophilicity) followed by similarity analysis (Tanimoto coefficient <0.85). From this analysis we identified 300 compounds to create a focused “Mercaptophile Library” which was used to screen for inhibitors of ATG4b and generated confirmed hits that could be further elaborated. This focused library may prove useful for screening other proteases.

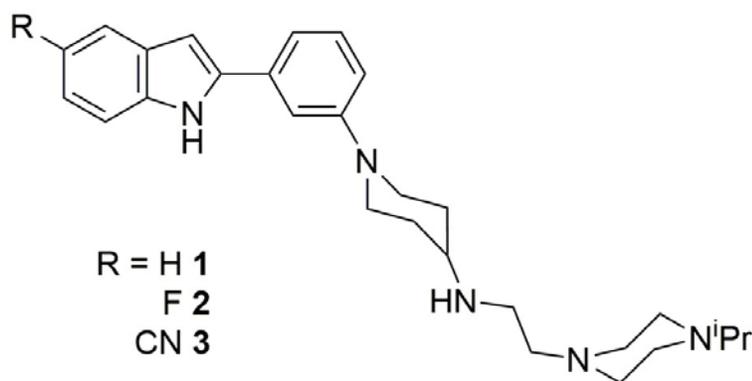
MEDI 65

2-Phenyl indole piperazine inhibitors of AAA ATPase p97

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Valosin-containing protein, also known as p97, plays a critical role in many crucial cellular pathways, including cell cycle regulation, endoplasmic reticulum-associated

degradation, transcription factor regulation and autophagy. Our initial optimization of lead compounds from the Chemical Biology Consortium (CBC) identified 2-phenyl indole **1**, which exhibited a low nanomolar IC_{50} in a biochemical assay of p97. The effect of substitutions on the indole portion of **1** will be discussed. For example, substitutions at indole N-1 and C-3 resulted in an almost complete loss of activity. Conversely, substitution at C-5 increased potency, with fluoro-2-phenyl indole **2** and cyano-2-phenyl indole **3** exhibiting IC_{50} values of 20-50 nM.



2-Phenyl indole piperazines **1-3**.

MEDI 66

PEG-conjugated aromatic and heterocyclic sulfonamides as potent carbonic anhydrase inhibitors with anti-tumor activity

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Over the last decade a connection was established between cancer and the expression of certain carbonic anhydrase isozymes. Recent studies have shown that membrane bound carbonic anhydrases CA IX and CA XII are over-expressed in many tumor cells. Pharmaceutical agents that can selectively inhibit CA IX and CA XII were shown to have therapeutic value for detection, imaging and treatment of a large variety of hypoxic tumors.

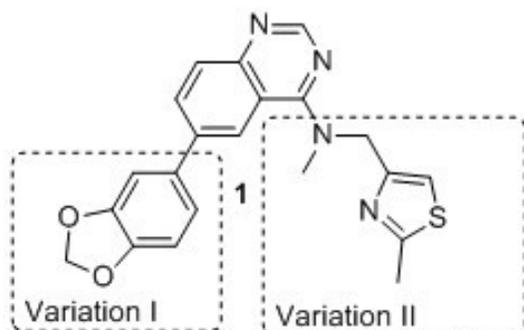
The purpose of this study was to generate membrane-impermeant inhibitors of CA IX and CA XII that may have the ability to inhibit the development of hypoxic tumors. In this context we are presenting our recent efforts towards generation of membrane-impermeant high molecular weight CA inhibitors obtained through conjugating PEG with known carbonic anhydrase pharmacophores. We are also presenting the in vitro biological properties of these compounds in 2D and 3D models of cancer.

MEDI 67

Exploration of quinazoline derivatives as MEK5 inhibitors

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The MAPK or Mitogen Activated Protein Kinase cascade is a sequence of stepwise phosphorylation events that both amplifies and modifies external cellular events such as a growth factor activation and stress to produce intercellular responses in both the cytosol and nucleus. There are seven human isoforms of MEK. MEK5 is upregulated in several cancer types including triple negative breast cancer cells recommending this pathway as a target for therapeutic intervention. Literature identification of a quinazoline derivative **1** that diverged from its intended activity as a Cdc2-like kinase (Clk) inhibitor and exhibited MEK5 inhibition provided the structural basis for further exploration of ATP-site MEK5 inhibitors. Use of a computational homology model permitted exploration of the proposed interaction at the ATP site. Design, synthesis, and cellular activity will be presented.



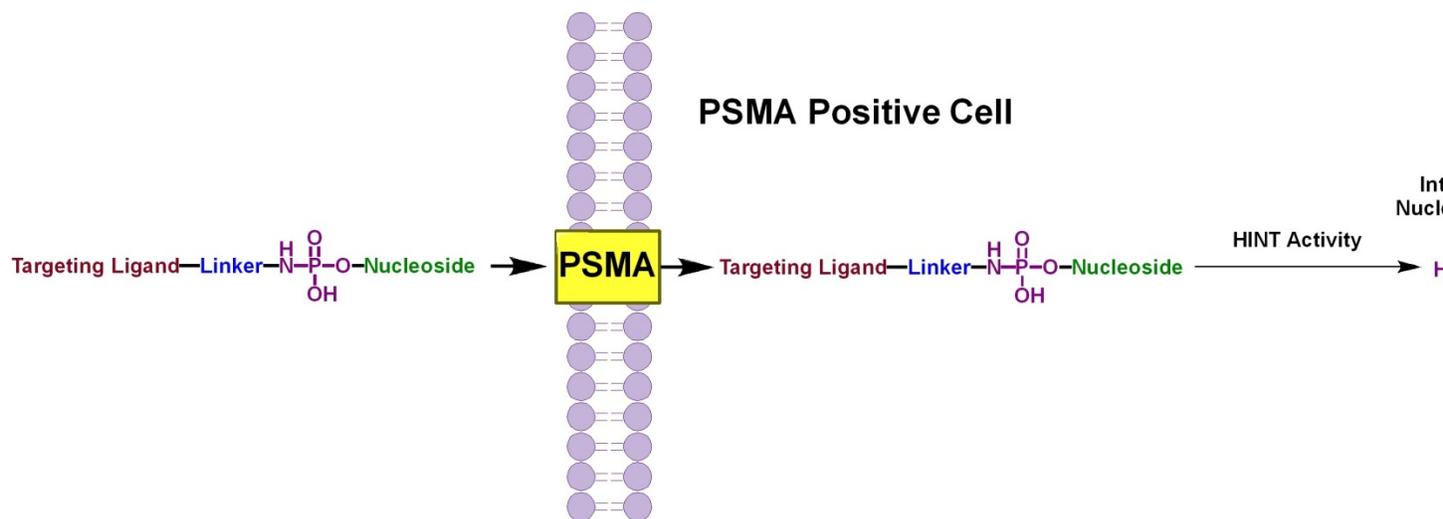
MEDI 68

Prostate-specific membrane antigen targeted phosphoramidate-pronucleotides

Harrison West², *westx359@umn.edu*, **Sidath C. Kumarapperuma**², **Alexander Hendricks**², **Carston R. Wagner**¹. (1) Weaver-Densford Hall, University Minnesota, Minneapolis, Minnesota, United States (2) Medicinal Chemistry, University of Minnesota, Minneapolis, Minnesota, United States

Prostate cancer (PCa) is the most prevalent malignancy and a major contributor to cancer related deaths in men, with an estimated 230,000 new cases and 28,000 deaths in 2015. Current chemotherapies for PCa are not selective for tumor tissue, but instead largely rely on the proliferative nature of dividing malignant cells over normal cells. This can preclude the use of potent anti-cancer compounds due to off-target toxicities. Prostate-specific membrane antigen is a cell-surface glycoprotein that has been

identified to be overexpressed in PCa compared to normal tissue, which exhibits little to no PSMA. The small molecule 2-[3-(1,3-dicarboxypropyl)ureido] pentanedioic acid (DUPA) selectively binds to PSMA and has been investigated as a targeting agent for tumor detection and therapeutic delivery. In an effort to develop a targeted pro-drug approach to deliver nucleoside antimetabolites for the treatment of PCa, we have synthesized phosphoramidate pronucleotides conjugated to a DUPA targeting moiety. The enzymatic activation and biological effects of these molecules will be assessed.



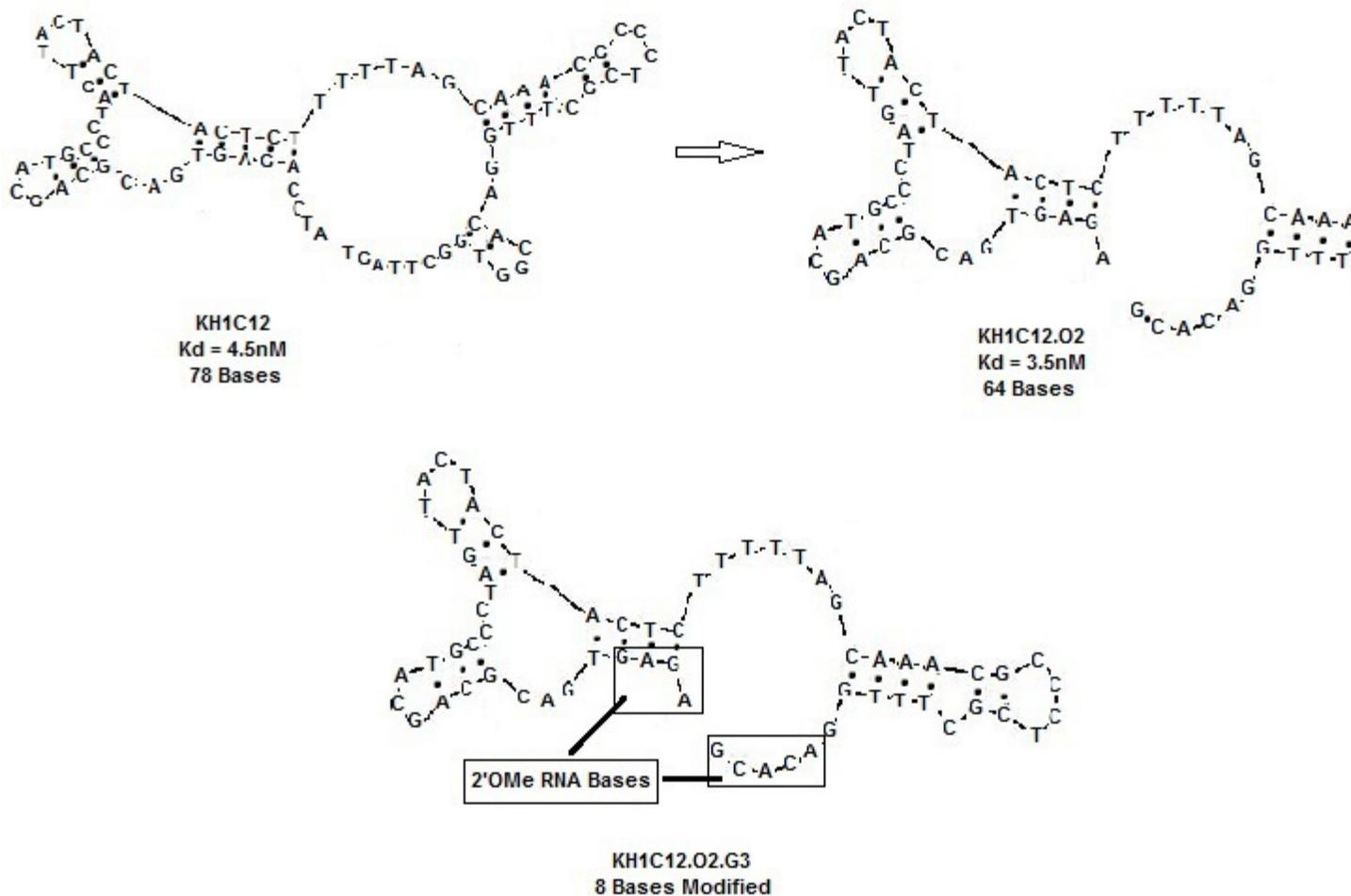
MEDI 69

Designing bispecific aptamers for increased stability in human serum

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Aptamers are designer synthetic DNA molecules with high specificity and affinity toward their target receptor on cells. Due to the nature of synthesis and ease of chemical manipulation, the application of DNA aptamers in designing bispecific aptamers are more desirable compared to antibodies. The aptamer KH1C12 has been identified against the myeloid leukemia cell line HL 60. It is an ideal candidate for the synthesis of a bispecific aptamer due to its already established high affinity to HL60 as well as its known structural stability at physiological temperatures. However, aptamer KH1C12 is 78 bases in length and composed of natural DNA bases. In order to develop a successful molecular tool based on aptamer KH1C12 against HL 60, it is necessary to develop a stable, truncated analogue. It has been shown that truncated aptamer sequences tend to have a higher affinity due to increment in homogenous secondary folds. Therefore, as a step in generating a stable aptamer, we have truncated aptamer KH1C12 by systematically removing bases from 3' and the 5' end. We have synthesized three analogues of KH1C12 and discovered that one of the analogues, KH1C12.O2,

shows an affinity constant of 3.5nM, slightly higher than the full-length aptamer but 14 bases shorter. In order to contract a nuclease stable KH1C12.O2 we have modified the KH1C12.O2 with 2'OMe RNA bases (KH1C12.O2.G_n) at the 5', 3', and both the 5' and 3' ends. The modification of 3' and 5' ends with nuclease resistant analogues will prevent exo-nuclease degradation. After exploring the effect of the modification of the aptamer by nuclease resistant modified nucleic acids, the KH1C12.O2.G3 modification showed no effect on affinity as well as an increased stability in human serum making it ideal for the synthesis of a bispecific molecule. Future work will include synthesis of a bispecific molecule comprised of anti-NKL aptamer CL0020 connected to KH1C12.O2.G3 via a polyethylene glycol linker and assess toxicity as a function of linker length.



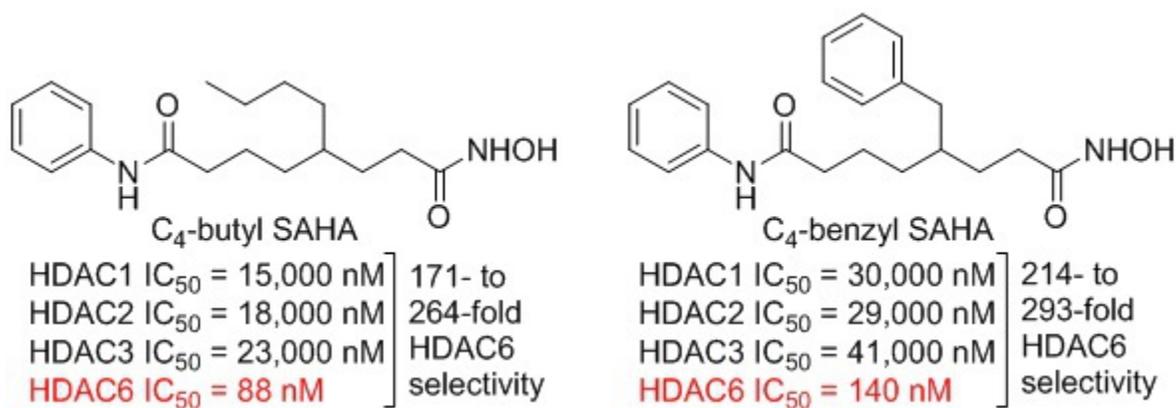
Aptamer KH1C12 Modification

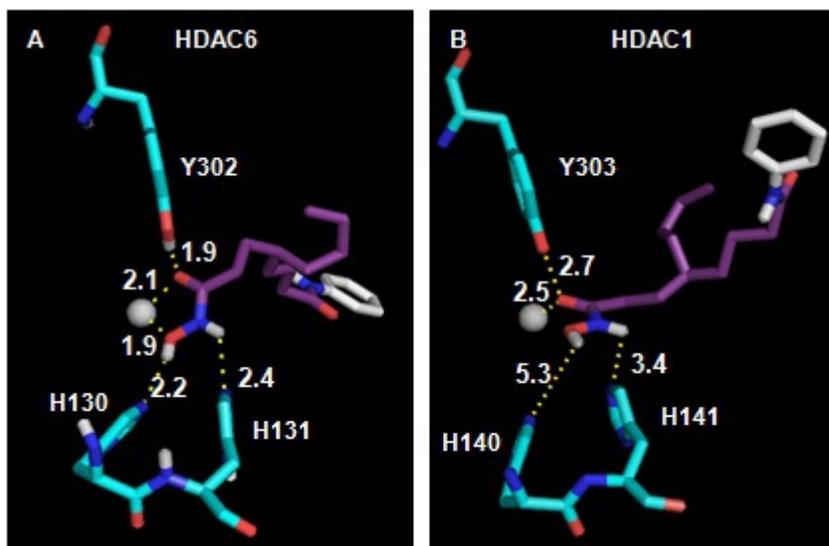
MEDI 70

Structural requirements of histone deacetylase inhibitors: Suberoylanilide hydroxamic acid analogs modified at the C4 position display HDAC6 selectivity

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Histone deacetylase (HDAC) proteins are epigenetic regulatory enzymes that deacetylate protein substrates, leading to subsequent changes in cell function. HDAC proteins are implicated in cancers and several HDAC inhibitors have been approved by the FDA as anti-cancer drugs, including SAHA (Vorinostat). Unfortunately, SAHA inhibits all eleven HDAC isoforms, which limit its use as a pharmacological tool and may lead to side effects in the clinic. In this work we modified the non-selective HDAC inhibitor SAHA by substituting the C4 position of the linker to explore the effect of this substitution on activity and/or selectivity. C4-SAHA analogs were synthesized and screened *in-vitro* and *in-cellulo* for HDAC isoform selectivity. C4-*n*-butyl SAHA and C4-benzyl SAHA displayed 88 and 140 nM potency with 171- to 293-fold selectivity for HDAC6 compared to HDAC1, 2, and 3. *In-cellulo* selectivity experiments showed the same selectivity pattern. Docking studies provided a structural rationale for selectivity. In summary, modifying the C4 position of the non-selective inhibitor SAHA led to substantial improvement in selectivity with a modest reduction in potency. HDAC6 selective C4-SAHA analogs will be useful biological tools to understand the role of HDAC6 in cancer and lead compounds to develop more effective anti-cancer drugs targeting HDAC6. More generally, these studies with SAHA analogs suggest that modifying current drugs can significantly improve their properties.





A-B) Docked pose of C4-*n*-butyl SAHA in the HDAC6 homology model (A) and HDAC1 crystal structure (B) using Autodock 4.2. Binding distances between the hydroxamic acid atoms and active site residues (numbered in figure) or the metal are displayed in Angstroms. Atom color-coding: C4-*n*-butyl SAHA (C=purple/white; O=red; N=blue; H=White); amino acids (C=deep teal; O=red, N=blue); Zn²⁺ metal ion (grey sphere).

MEDI 71

Design, synthesis, and biological screening of novel CUCS-inspired estrone analogues towards treatment of hepatocellular carcinoma

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Cucurbitacins (Cucs) are natural product with highly oxygenated tetracyclic triterpenes produced mostly by Cucurbitaceae. They are known for their therapeutic efficiency with different biological activities, such as anti-inflammatory, hepatoprotective and anti-cancer targeting different types of cancer. Hepatocellular carcinoma (HCC) is the third leading cause of death worldwide. Previous reports have shown the ability of cucs to inhibit the growth of HepG-2 (hepatocellular carcinoma) cell lines significantly. Structural activity relationship studies suggested the potential of the 23, 24 enone side chain of Cucs to bind to the Epidermal Growth Factor Receptor (EGFR). Due to the limited quantities of cucs upon isolation and the challenges of total synthesis of Cucs, therefore estrone skeleton was used as a starting scaffold to synthesize cucs like structures targeting HCC. Molecular docking of estrone analogs study was conducted using 1M17 (EGFR receptor) co-crystallized with Erolitinib (known EGFR inhibitor anti-cancer agent). Several analogs were identified for synthesis. Two novel steroidal analogs (Fig 1) were synthesized by installing the cucs side chain. The novel analogs showed a

comparable affinity to the receptor based on the docking study the synthesized estrone analogs showed hydrophobic filling the binding pocket of EGFR and hydrogen bonding interactions. This work will open novel frontiers towards investigating insertion of functional groups to be synthesized, followed by in-vitro biological screening.

MEDI 72

Anti-tumor studies of N,N'-naphthylmethyl-2-alkyl and N,N'-quinolylmethyl-2-alkyl substituted imidazolium salts

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Previously reported naphthylmethyl imidazolium salts have exhibited high anti-cancer activity using in vitro studies against non-small cell lung cancers comparable to the chemotherapeutic cisplatin. However, these compounds are limited by poor water solubility and therefore lack the potential ability to be systemically administered. Presented herein are a new class of naphthylmethyl and quinolylmethyl substituted imidazolium salts with lipophilic and hydrophilic functional groups at the C² position. The most active of these compounds has IC₅₀ values in the nanomolar range against the NCI-H460, NCI-H1975, and HCC827 non-small cell lung cancer lines. Unfortunately, these highly active imidazolium salts also have poor water solubility. However, these compounds were solubilized by the chemical excipient 2-hydroxypropyl-β-cyclodextrin, an FDA approved excipient. This is the first example of an imidazolium salt solubilized by a cyclodextrin for use as a chemotherapeutic. The synthesis, characterization, and in vitro anti-cancer activity of these compounds will be presented as well as results from an in vivo toxicity study.

MEDI 73

Discovery of novel leucyl adenylate analogues as leucyl tRNA synthetase (LRS)-mediated mTORC1 inhibitors

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Leucyl-tRNA synthetase (LRS) is a member of the class I aminoacyl-tRNA synthetase (ARSs) family, which catalyzes the ATP-dependent ligation of leucine to cognate transfer RNA (tRNA). Recently, it has been demonstrated that LRS plays a critical role in the activation of the mammalian target of rapamycin complex 1 (mTORC1), signaling pathway by sensing intracellular leucine concentration. In the presence of leucine, LRS translocates to the lysosome, where it directly binds to Rag GTPase, the mediator of

amino acid signaling to mTORC1, in an amino acid-dependent manner and functions as a GTPase-activating protein (GAP) for Rag GTPase to activate mTORC1. Since the hyperactivation of this mTORC1 pathway is implicated in the pathogenesis of many human diseases including cancer, the inhibition of LRS-mediated mTORC1 activation pathway has emerged as a potential target for drug development.

In this study, a series of leucyl adenylate analogues have been investigated as inhibitors of the LRS-mediated mTORC1 signaling pathway. Herein we identified the lead compound (**KSE-398**) as a potent inhibitor of mTORC1 signaling pathway by blocking leucine sensor and as a potential anticancer drug.

MEDI 74

Structure based design, synthesis, and study of potent and selective KDM5A/5B (JARID1A/1B) inhibitors

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Aberrant expression of histone-modifying enzymes resulting in epigenetic alterations have been implicated in tumorigenesis. The KDM5/JARID1 family of histone demethylases is comprised of 2-oxoglutarate and Fe(II)-dependent enzymes which remove a methyl group from the activating marks of H3K4me³ and H3K4me². Overexpression of KDM5A/5B (also known as JARID1A/1B) has been reported in a variety of malignant tumors. Gene knockdown studies demonstrate that suppression of KDM5A/5B can inhibit tumorigenesis, suggesting KDM5A/5B demethylase inhibitors may have efficacy in the treatment of cancer. Using structural information of known inhibitors and an in-house built homology model of KDM5B (based on KDM4A co-crystal structures), a potent series of pyrazolopyridines was designed. Co-crystal structure of in-house inhibitor (QC168) provided guidance in designing a novel benzyloxy pyrazolopyridine series. SAR exploration resulted in the identification of QC3611, an orally available, potent and selective inhibitor of KDM5A/5B. Cellular inhibition of KDM5A/5B was demonstrated with QC3611 using an immuno-blotting assay measuring tri-methylated H3K4 levels in the breast cancer cell line ZR-75-1.

MEDI 75

Regulation of neural stem cell proliferation and differentiation with molecules that stabilize nucleic acid secondary structure

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Most translational applications with neural stem cells require an expansion of cultures in order to generate a sufficient number of progenitors and the differentiation of

progenitors to specific phenotypes. Agents that facilitate either the proliferation or differentiation of neural stem cells will improve the efficacy in which therapeutically relevant cell types can be generated. In this study, we tested the ability small molecules that stabilize nucleic acid secondary structure to regulate the proliferation and differentiation of neurosphere cultures derived from adult mouse neural stem cells in the subventricular zone. These types of molecules have been extensively studied as anti-tumor agents in cancer systems, and our laboratory has recently showed that these compounds can also be used to manipulate neuronal gene expression levels. This study found that Quarfloxin, a fluoroquinolone derivative designed to target G-quadruplexes within ribosomal DNA and disrupt protein-DNA interactions, was a potent inhibitor of neural stem cell proliferation, but was also cytotoxic at low concentrations. TMPyP4, a cationic porphyrin that stabilizes multiple nucleic acid secondary structures, also blocked neural stem cell proliferation, but with minimal cytotoxicity. TMPyP2, a structural isomer of TMPyP4 that is unable to stabilize nucleic acid secondary structure, was also anti-proliferative without inducing cytotoxicity. Thus, Quarfloxin and TMPyP4 can effectively block neural stem cell proliferation, but their ability to stabilize nucleic acid secondary structure may not be necessary for this action. Under differentiation conditions, however, treatment with TMPyP4 reduced the expression of the astrocyte marker GFAP significantly more than with TMPyP2. This indicates that TMPyP4 can preferentially repress adoption of glial cell fate. Together, these findings indicate that molecules that stabilize nucleic acid secondary structure can regulate the proliferation and differentiation of adult neural stem cells.

MEDI 76

Synthesis and biological evaluation of novel 6-substituted pyrrolo[2,3-d]pyrimidines with substituted nitrogen bridges as targeted antifolates

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Reduced folates are essential cofactors for the biosynthesis of purines and pyrimidines. Since humans do not synthesize folate, these cofactors must be obtained from dietary sources. In mammals, three specialized systems exist that mediate membrane transport of folates and antifolates across biological membranes. These include the reduced folate carrier (RFC), the primary route for the uptake of folates and antifolates in mammalian cells, folate receptors (FRs) α and β , and the proton-coupled folate transporter (PCFT). Whereas RFC is ubiquitously expressed, FRs and PCFT show a narrower pattern of tissue expression. Toxicity of clinically used antifolates is attributed in part to their lack of selectivity for tumor cells over normal cells due to RFC transport. Antifolates with tumor-specific FR and/or PCFT drug uptake would circumvent major toxicities of currently used antifolates. Our three carbon atom chain analog **AGF17**

showed similar inhibitory activity towards Chinese hamster ovary (CHO) cells expressing FR α (RT16; IC₅₀ = 4.1 \pm 1.6 nM) and FR β (D4; IC₅₀ = 5.6 \pm 1.2 nM). **AGF17** was 100-fold more selective for FR transport (FR α and FR β) over RFC with excellent cell inhibitory activity against FR α -expressing KB human tumor cells (IC₅₀ = 1.8 nM). **AGF17** was identified as a potent inhibitor of cellular GARFTase. The natural substrate for GARFTase is N10-formyl tetrahydrofolate, which is a 6-substituted pteridine with a –CH₂-N- two-atom bridge. The N10 is substituted with a formyl (CHO) moiety which forms a hydroxylated, tetrahedral intermediate prior to transfer of the formyl group. Thus, substitution of the C10-benzylic CH₂ of the 6-substituted pyrrolo[2,3-*d*]pyrimidine **AGF17** with a N-COCH₃ (**AGF174**), N-CHO (**AGF219**) and N-COCF₃ (**AGF209**) affords mimics of the natural substrate. We have thus hypothesized that these 6-substituted three-atom bridged N-substituted analogs would function as potent inhibitors of human GARFTase rather than as substrates. **AGF174**, **AGF219** and **AGF209** showed low levels of inhibitory activity toward the growth of PC43-10 CHO cells expressing human RFC (IC_{50s} of 808, 642 and 783 nM, respectively). **AGF174**, **AGF219** and **AGF209** were significantly more selective towards D4 CHO cells expressing FR β than RT16 CHO cells expressing FR α . **AGF219**, **AGF174** and **AGF209** were also more potent inhibitors with isolated human GARFTase compared to **AGF17**. Thus, **AGF174**, **AGF219** and **AGF209** are potential analogs for further preclinical studies and analog design.

MEDI 77

Inhibitors of the mitochondrial citrate transport protein for targeting lung cancer

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An estimated 158,040 of people die each year from lung cancer. The mitochondrial citrate transporter (SLC25A1) is a potential target for lung cancer. Gene levels of *SLC25A1* are increased in human lung cancers and SLC25A1 is required for tumor proliferation. Pharmacological inhibition of SLC25A1 causes mitochondrial dysfunction and halts cancer cell proliferation. Herein, we present a series of sulfonamide-based inhibitors of the SLC25A1 protein as potential drug leads. Initial screening was performed by surface plasmon resonance spectroscopy to determine the ligand-protein binding constants. The effect of the drugs on lung cancer cells was determined using 3-dimensional cell cultures. Finally, an *in silico* model of our lead compound docked in a homology model of SLC25A1 is provided.

MEDI 78

Discovery and optimization of small molecule CSN5 inhibitors for the treatment of cancer

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Cullin-Ring E3 ligases (CRLs) target substrates for degradation in a complex and tightly regulated manner. One of the essential regulatory mechanisms to maintain ligase activity is a reversible post-translational modification with the ubiquitin-like protein NEDD8. Removal of NEDD8 from cullin proteins is mediated by the COP9 signalosome (CSN), a multi-protein complex comprising 8 subunits and CSN5 is the active metalloprotease subunit. CSN5 mediated deneddylation of cullin-Ring E3s accompanies recycling of E3 ligase components. Blocking deneddylation by specific inhibition of CSN5 with siRNA has been shown to affect growth of selected cancer cell lines, likely through stabilization of tumor suppressors. Thus specific CSN5 inhibition with a small molecule might offer a new therapeutic approach for the treatment of cancer. A high-throughput screening campaign using a biochemical assay with recombinant CSN and a NEDD8-modified CRL substrate led to the identification of a micromolar CSN5 inhibitor. The hit, which interestingly does not contain one of the well-known bidentate zinc-binding groups, was validated by various biophysical methods. Initial optimization aimed to rapidly navigate the structure-activity landscape of the hit and led to the identification of highly potent CSN5 inhibitors which enabled investigations of the mechanistic consequences of CSN5 inhibition on the protein level in HCT116 cells. Subsequently functional target validation by potent inhibition of cell proliferation completed the in vitro profiling. Moreover, a X-ray cocrystal structure elucidated the binding mode of these unusual metalloprotease inhibitors revealing them as active site directed monodentate Zn²⁺ ligands.

MEDI 79

Structure-activity and structure-toxicity relationships of 1,5-diarylpenta-1,4-dien-3-ones on prostate cell models

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The symmetric 1,5-diheteroaryl-1,4-pentadien-3-ones have recently been established by us as a promising class of curcumin-based anticancer agents. The present study aims to further explore the in-depth structure-activity relationships, structure-toxicity relationships, as well as the possible mechanism of action for this scaffold. Specifically, thirty-four novel asymmetric 1,5-diheteroarylpenta-1,4-dien-3-ones have been designed and successfully synthesized through two sequential Honor-Wadsworth-Emmons reactions. The WST-1 cell proliferation assay showed that this group of asymmetric 1,5-diheteroarylpenta-1,4-dien-3-ones possess very promising anti-proliferative activities towards three prostate cancer cell lines, demonstrating up to over 250-fold enhanced

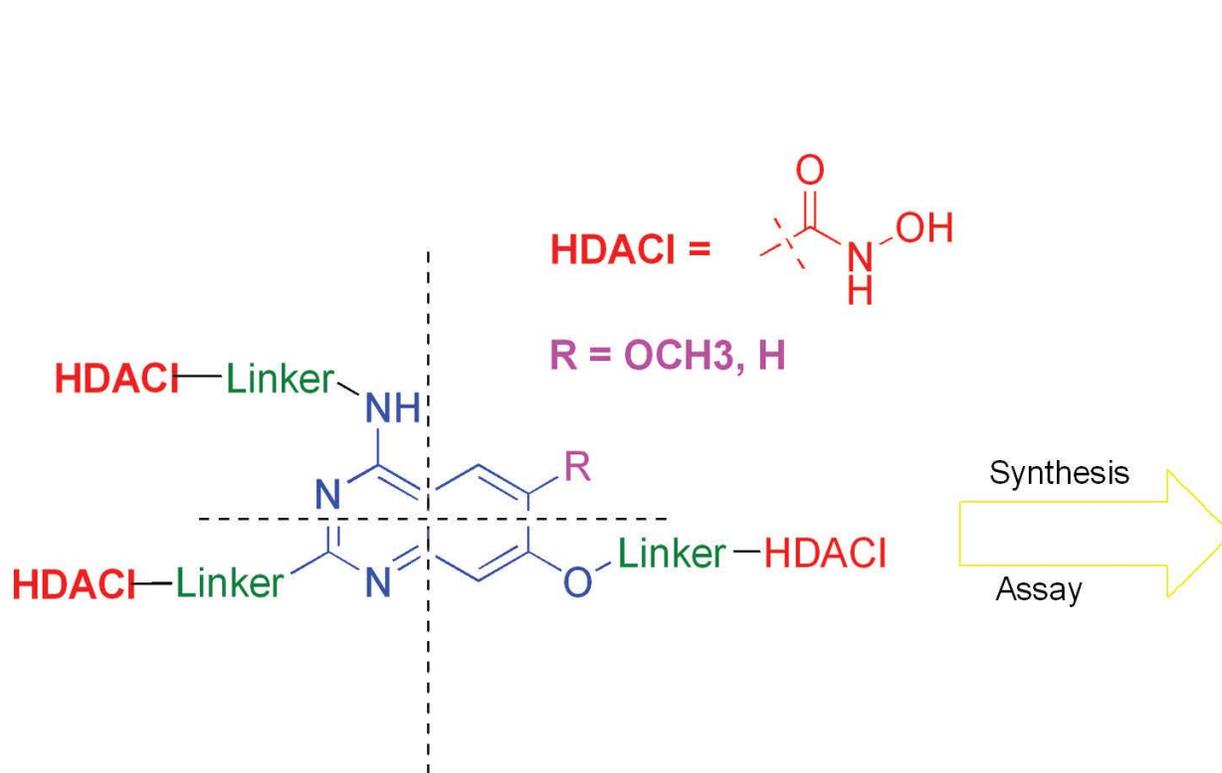
potency when compared to curcumin. Fifteen compounds were selected for toxicity evaluation against PWR-1E prostate epithelial cell. Two compounds were selected for assessment of their effect on PC-3 prostate cancer cell apoptosis and cell cycle regulation. Six 1,5-diheteroaryl-penta-3-ones were also synthesized to explore the contribution of diene moiety to antiproliferative effects in prostate cancer cell lines and to toxicity in the normal prostate cell line. The synthesis, structure-activity relationships, and structure-toxicity relationships of asymmetric 1,5-diheteroaryl-penta-1,4-dien-3-ones will be presented.

MEDI 80

Structure based design, synthesis and activity studies of small hybrid molecules as HDAC and G9a dual inhibitors

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Aberrant enzymatic activities or expression profiles of epigenetic regulations are a therapeutic target for cancers. Among these, histone 3 lysine 9 methylation (H3K9Me2) and global de-acetylation at histone proteins are associated with multiple cancer phenotypes including leukemia, prostate carcinoma, hepatocellular carcinoma and lung cancer. Cancer is a disease with difficult treatment options due to the multifactorial basis of initiation, and progression; a treatment targeting multiple components instead of a single component would therefore be of particular interest in cancer therapeutics. To meet this need, development of new or improved lead compounds targeting cancer during multiple points in its development is important in improving the therapeutic profile of the existing therapies. Developing new lead molecules which target the cancer from various stages of disease development from either known inhibitors or *de novo* is important for improving effectiveness and side effects/toxicity. Herein we report the discovery of the first small molecule capable of acting as a dual inhibitor targeting both G9a and HDAC. Our structure based design, synthesis and screening for the dual activity of the small molecules led to the discovery of compound **14** which displays promising inhibition of both G9a and HDAC in the low micro-molar range in cell based assays.



Compound	G9a IC ₅₀ (MDA-MB-231) μM	HDAC IC ₅₀ (K562) μM
14	7.136±1.62	5.735±1.23
SAHA	-----	2.056±0.59
BIX-01294	4.563±1.2	-----

Structure based design and synthesis of dual acting G9a and HDAC inhibitors

MEDI 81

***In silico* design and synthesis of novel thiourea and phenylsulfonyl-benzamide compounds as anti-prostate cancer agents**

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Prostate cancer is a major cause of male death worldwide and the identification of new and efficient treatment strategies is constantly required.

Among the available options, different non-steroidal androgen receptor antagonists are approved also in the case of castration-resistant forms. Most of these drugs show a

limited application due to the development of resistant mutants of their biological target. A homology model of the AR open antagonist conformation was built and a series of docking-based studies guided the design of new potential inhibitors, which might impede the receptor to adopt its closed agonist conformation even in the presence of adaptive mutations.

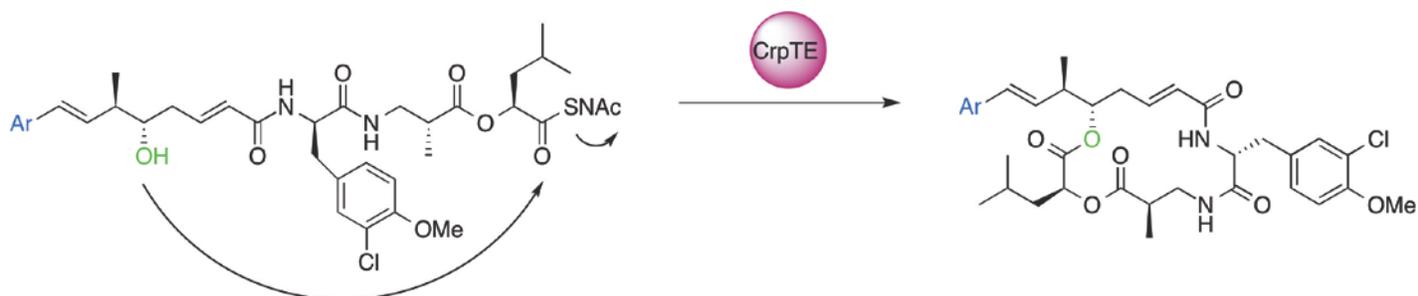
Two series of novel thiourea and phenylsulfonyl-benzamide compounds were synthesised. Among them, different new analogues displayed improved in vitro activity in comparison with standard bicalutamide and enzalutamide, with IC₅₀ values in the low micromolar range against four different prostate cancer cell lines (LNCaP, VCaP, DU-145, 22Rb1). These results represent a promising starting point for further development.

MEDI 82

Exploiting biocatalysis for the production of novel cryptophycin anticancer agents

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Macrocyclic natural products are an important class of molecules that display a wealth of pharmacological activities. These scaffolds are biosynthesized by megasynthases that terminate in a thioesterase (TE), which is responsible for effecting regio- and stereospecific macrocyclization. The cryptophycins are a family of macrolactone natural products that display not only some of the most potent anti-proliferative activity seen to date, but also exceptional activity against drug-resistant cancers. Difficulties in synthesizing this complex structure have hindered the medicinal chemistry efforts necessary to continue the clinical development of this promising scaffold leading us to investigate chemoenzymatic methodologies. The cryptophycin thioesterase (CrpTE) is a versatile enzyme that naturally fashions over twenty cryptophycin analogues, enabling us to envision a drug development paradigm that utilizes the CrpTE as a stand-alone biocatalyst for the production of a library of novel cryptophycins. To this end, we have synthesized a suite of unnatural linear intermediates as the N-acetyl cysteamine thioesters (NAC) and utilized the recombinant CrpTE cloned from the biosynthetic pathway to catalyze facile macrocyclization under mild conditions. The scope of substrates synthesized has not only given us fascinating insight into the CrpTE, but potent analogues for further clinical development.

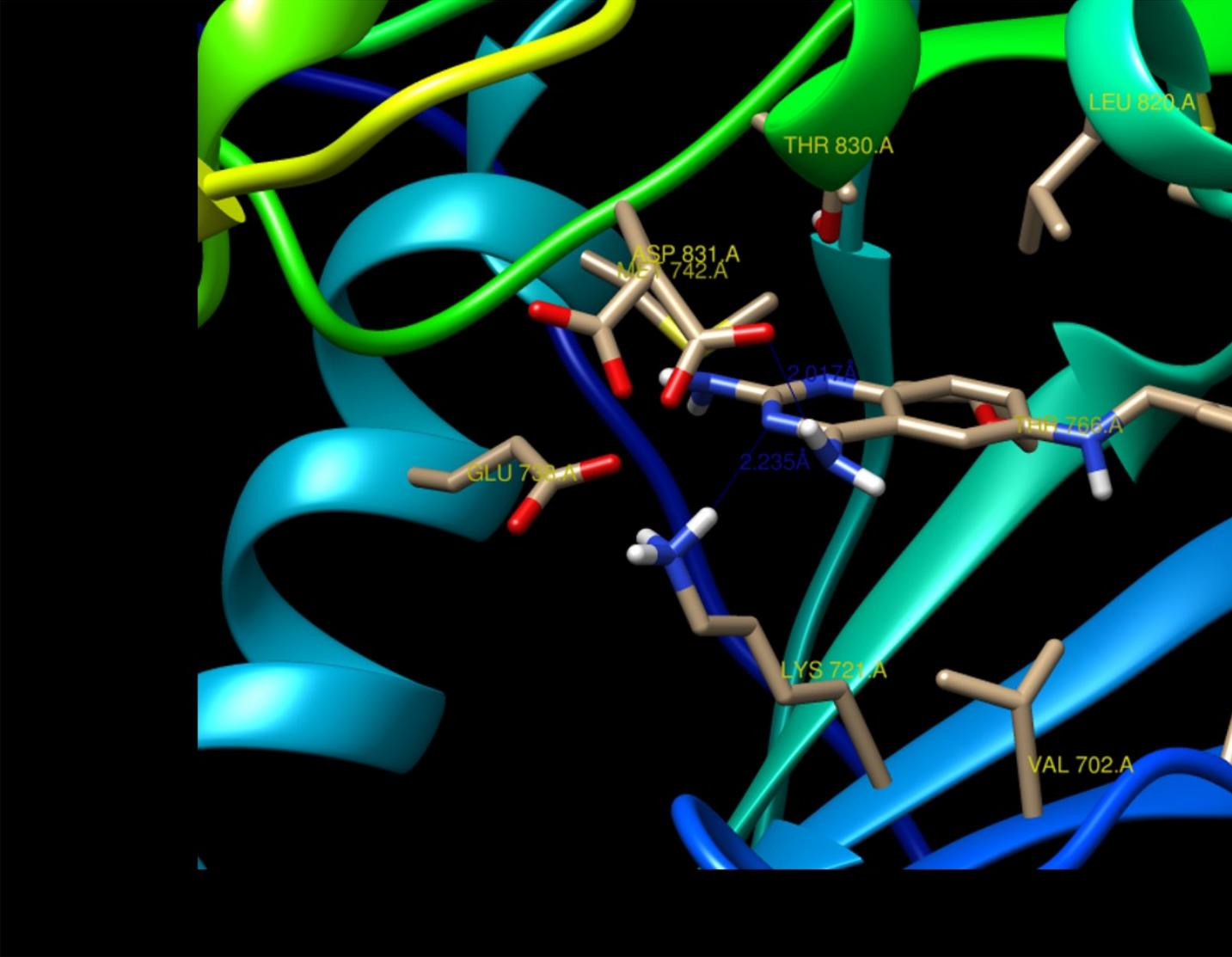


MEDI 83

Design, synthesis and evaluation antitumoral of quinazoline derivatives

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Epidermal Growth Factor Receptor (EGFR) belongs to the family of tyrosine kinase receptor protein. Which together with their main ligands (epidermal growth factor (EGF), epiregulin (EPR), betacellulin (BTC), among others) play an important role in the pathways that regulate biological processes like apoptosis, angiogenesis, cell proliferation and differentiation. Overexpression of EGFR is present in many types of tumors, such as ovarian cancer which is one of the most deadly tumors of the female genital tract. Although there are inhibitor drugs with tyrosine kinase activity that showing health benefits such as gefitinib, erlotinib and vandetanib, they have the disadvantage of causing side effects. In this regard, there is a need to design new molecules that may offer new ways to treat these disorders and furthermore have lower toxicity. Based on the above, we decided to design, synthesize and evaluate in an ovarian cancer cell line (SK-OV-3) and 2 cell line immortalized but non-cancerous, 10 triaminquinazolin-2,4,6-triamine derivatives. six compounds were shown to have antitumor activity on SK-OV-3; particularly, *N*⁶-[2-(trifluoromethyl)benzyl]quinazolin-2,4,6-triamine showed the highest rate of selectivity with respect to the other with an IC₅₀ = 2.76 μM and induced cell death by apoptosis. Molecular docking of this compound showed on a good affinity tyrosine kinase site (PDB ID: 1M17), and which could explain its possible inhibitory.



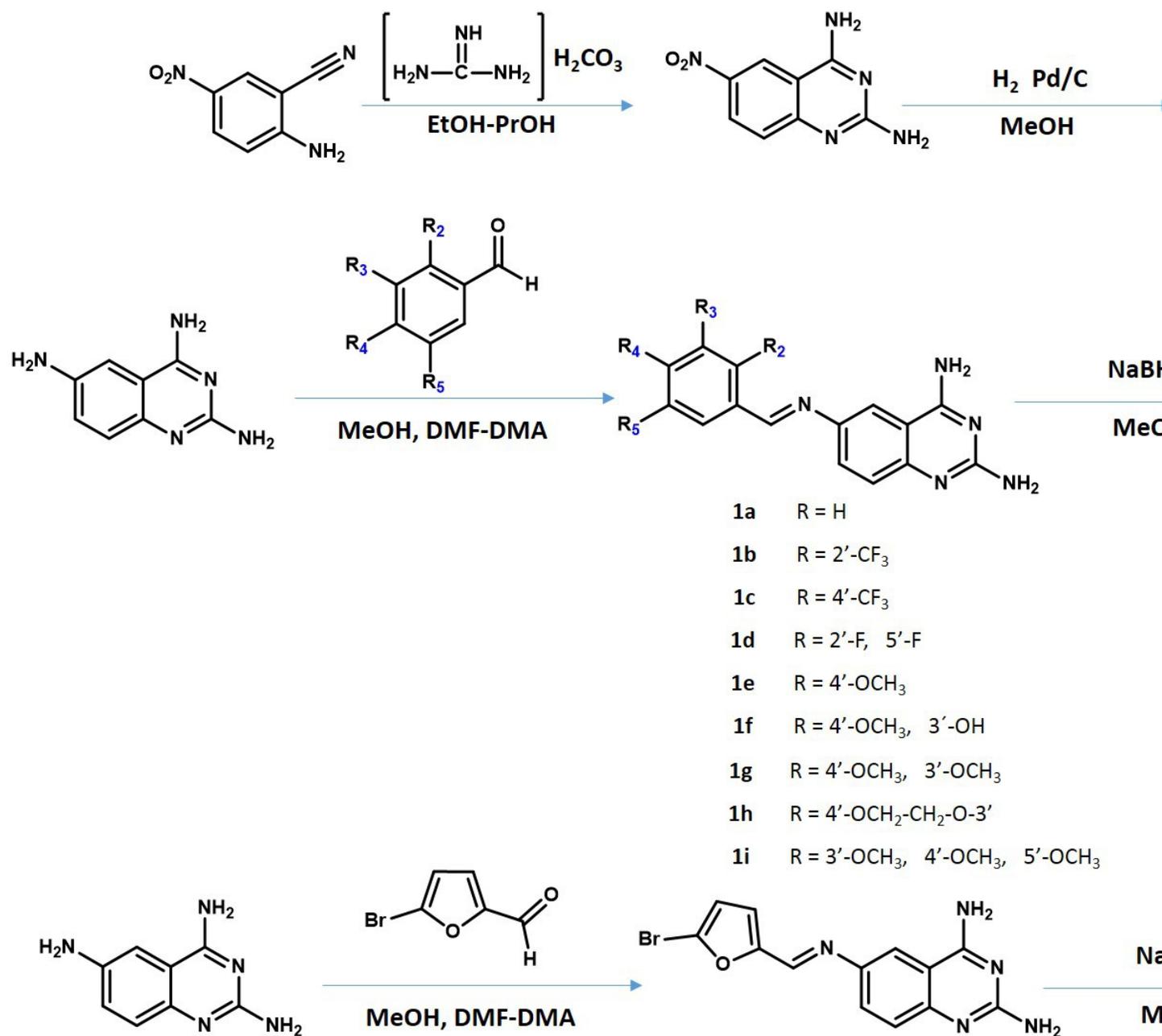


Fig. 1. Synthesis of the target compound

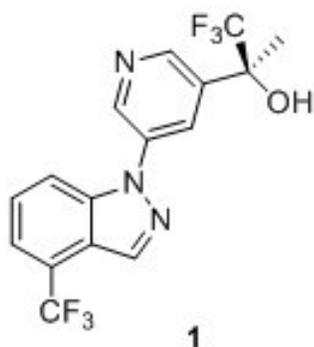
MEDI 84

Discovery of indazole aldosterone synthase inhibitors as potential treatments for resistant hypertension

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Aldosterone is a steroid hormone that promotes increased blood pressure, inflammation and fibrosis. The final three steps of its biosynthesis are catalyzed by aldosterone synthase (CYP11B2). A closely related enzyme, CYP11B1, catalyzes the biosynthesis of cortisol, an important regulator of glucose metabolism. Small molecule inhibitors of CYP11B2 such as LCI-699 have recently been shown to lower aldosterone levels and blood pressure in the clinic, thus validating this mechanism as a treatment for hypertension. LCI-699, which inhibits CYP11B2 with 4-fold selectivity vs. CYP11B1, also produces an undesired impairment of cortisol response, presumably as result of CYP11B1 inhibition. More selective inhibitors of CYP11B2 are thus desired as treatments for hypertension.

This presentation will outline the discovery and hit-to-lead optimization of a structurally novel indazole series of CYP11B2 inhibitors. These efforts culminated in the identification of compounds such as **1**, a potent CYP11B2 inhibitor that displays high selectivity vs. CYP11B1 and other related CYPs, high selectivity vs. other pharmacologically relevant targets, good pharmacokinetic properties, and good physical properties. On the basis of its favorable profile, the indazole lead series was selected for progression into lead optimization.



MEDI 85

Discovery and structural development of novel natriuretic peptide receptor A (NPR-A) agonists

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Atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) bind to natriuretic peptide receptor A (NPR-A) and exert diuresis, vasodilation and cardioprotective effects by elevating cGMP concentrations. Human atrial natriuretic peptide (hANP) has already been used in clinical for the treatment of acute heart failure in Japan. However, the clinical utility of hANP is limited to the acute setting because of its rapid clearance from the body, as is often the case with peptide drugs. The aim of this study was to identify the non-peptidic small molecule which can activate NPR-A, and cure chronic heart failure. We synthesized novel triazine compounds and evaluated their various biological activities. Among them, some triazine compounds showed highly potent NPR-A agonist activity *in vitro* and promoted diuresis *in vivo*. Thus, we discovered non-peptidic small molecules with novel triazine structure that showed NPR-A agonistic effect like ANP, and these compounds may open the possibility of new therapy for congestive heart failure.

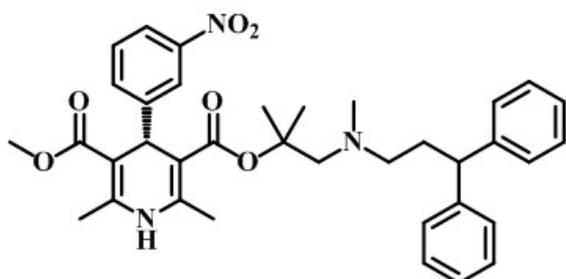
MEDI 86

Design & synthesis of novel dihydropyrimidine derivatives as potential L- and T-type calcium channels blockers

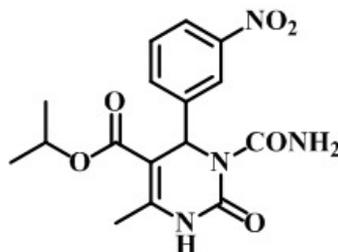
Mohamed T. Ismail^{1,2}, *mohamed.ismail@sdstate.edu*, **Fangxiong Zhang**³, **Ola H. Rizk**², **Ahmed M. Farghaly**², **Omaima M. AboulWafa**², **Gerald W. Zamponi**³, **Hesham T. Fahmy**¹. (1) Department of Pharmaceutical Sciences, College of Pharmacy, South Dakota State University, Brookings, South Dakota, United States (2) Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Alexandria University, Alexandria, Egypt (3) Department of Physiology & Pharmacology, Hotchkiss Brain Institute, University of Calgary, Calgary, NorthWest Territories, Canada

In 2011, The World Health Organization (WHO) published the global atlas for cardiovascular diseases (CVDs) prevention and control, confirming that CVDs is the biggest cause of deaths worldwide. Over 17 million people died from CVDs in 2008. Significantly, hypertension is the single most important contributing factor to CVDs. In recognition of the burden posed by hypertension, the European Society of Hypertension (ESH) and the European Society of Cardiology (ESC) published their '2013 Guidelines for Management of Arterial Hypertension'. Recently, the Eighth Joint National Committee on the Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 8) completed the '2014 Evidence-Based Guidelines for Management of High Blood Pressure in Adults'. The 2013 ESH/ESC guidelines in common with the 2014 evidence-based guidelines recommended calcium channel blockers (CCBs) as initial treatment. The most valuable CCBs are 1,4-dihydropyridines (1,4-DHPs). Later, interest has been focused on DHPs aza-analogs as dihydropyrimidines (DHPMs) which

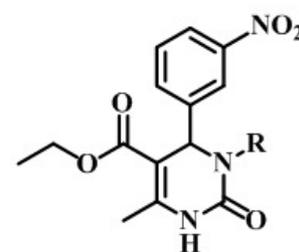
show very similar pharmacological profile to DHPs CCBs. Several lead compounds have been developed; of these, SQ 32926 is superior in potency and duration of activity to classical DHPs. The current study aims at developing novel DHPMs as aza-analogs of DHPs CCBs. A series of novel *N*3-substituted DHMPs were synthesized in high yield. Various moieties were introduced to the DHPM core via ester, amide and hydrazide linkages. The whole-cell patch clamp technique was applied to test L- and T-type calcium channels blockade. Preliminary screening suggested that introducing alkyl or aryl moieties to the DHPM core showed promising calcium channel blocking activities. Guided by primary data, structure optimization is concerned for improving calcium channel blocking activity. Within this scope, it will be possible to expand the existing SAR and to get further insight into molecular interactions at the receptor level.



Lercanidipine
"4th generation CCB"



SQ 32926
"Lead DHPM CCB in clinical trials"



Novel DHPMs
" Targeted DHPMs"

MEDI 87

ROCK kinase inhibitor prodrug to improve PK properties

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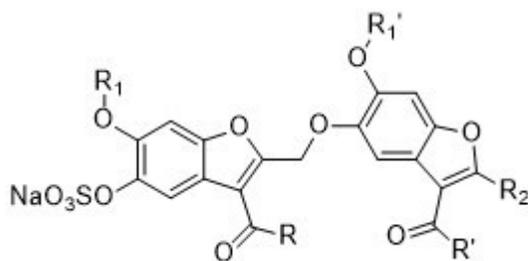
ROCK kinase is a promising target for the treatment of cardiovascular and autoimmune diseases. Fasudil is the only clinical approved drug for the treatment cerebral vasospasm due to subarachnoid hemorrhage. The active metabolite hydroxyfasudil was about four times higher than the parent drug in human. Hydroxyfasudil has been studied in vitro and in vivo to show similar activity as fasudil, and the action is more specific on Rho-kinase. Novel hydroxyfasudil prodrugs were designed, synthesized, and evaluated of the pharmacokinetic profiles. The new designed prodrugs showed improve exposure (C_{max} , AUC) and faster onset of action (T_{max}). Detailed synthesis and pharmacokinetic evaluation will be presented.

MEDI 88

Reducing bleeding risks of anticoagulants through a novel partial inhibition approach

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Thrombin, a key enzyme in the coagulation cascade, is considered to be an important target for anticoagulant development. Complete inhibition of thrombin leads to elimination of its proteolytic potential resulting in the enhanced risk of bleeding, a major side effect associated with all current thrombin-based anticoagulants. We have earlier demonstrated that sulfated benzofuran dimers (SBDs, see Figure) inhibit thrombin through an allosteric mechanism by binding in the region of Arg173. Additionally, some of these agents displayed only 75% inhibition efficacies of thrombin at saturation. Therefore, we reasoned that it should be possible to develop more potent thrombin inhibitors with inhibition efficacies in the range of 50%. We proposed that such partial inhibitors of thrombin will retain some proteolytic activity even at saturating conditions and thereby exhibit hemostatic potential but reduce or eliminate thrombotic potential. To develop such agents, we employed computational virtual screening on a library of SBD analogs. A select group of these analogs were synthesized in ~10 steps. Chromogenic substrate assay shows that a few of these analogs display inhibition efficacies of ~50-60% at saturation, whereas others exhibit efficacies of ≥80%. A specific agent shows similar partial inhibition phenomenon with fibrinogen, thrombin's in vivo substrate. Thus, exploiting allosteric regulatory properties of thrombin is likely to yield hemostatic inhibitor(s) that may resolve bleeding risk associated with thrombin-based anticoagulants.



General Structure of SBDs

MEDI 89

Sulfonylated benzothiazole based inhibitors of endothelial lipase

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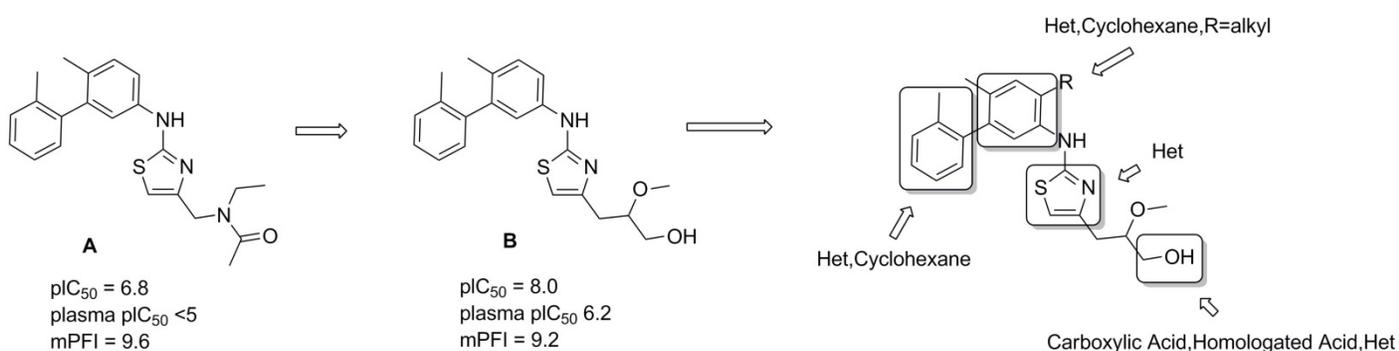
Serum concentrations of high density lipoprotein (HDL) are known to be inversely proportional to the incidence of coronary heart disease. Inhibition of endothelial lipase (EL) has been shown to increase HDL concentrations in preclinical animal models making EL a target for the potential treatment of atherosclerosis. A series of benzothiazole based EL inhibitors were synthesized with an alpha-sulfone moiety resulting in increased potency for EL. The optimization for selectivity against hepatic lipase as well as pharmacokinetic and pharmacodynamic properties will be described.

MEDI 90

Discovery and optimization of novel chemotype LpPLA₂ inhibitors featuring a unique binding mode

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Inhibition of lipoprotein-associated phospholipase A₂ (LpPLA₂), a member of the phospholipase A₂ family of enzymes believed to contribute to atherosclerotic plaque progression and instability, has become an attractive therapeutic target for atherosclerosis. Initial screening and follow-up effort through collaboration between GSK and Astex led to the identification of a series of biphenyl amino thiazoles exemplified by compound **A** and compound **B**, the latter of which demonstrated attractive *in vitro* potency but low plasma activity and an undesirable measure of property forecast index (PFI = chromLogD + number of aromatic rings). X-ray crystallography revealed that this chemotype adopts a unique binding mode in which the sidechain of phenylalanine 357 moves to form the binding pocket. Interestingly, representative examples of this “Phe-mover” series of LpPLA₂ inhibitors do not engage the catalytic Ser 273 residue known to be critical for the enzyme’s biological activity. As such, this structural class offered a tantalizing potential of unique pharmacology with related benefits of superior *in vivo* efficacy and/or safety margins. Balancing both enzyme and plasma potency with favorable physicochemical properties was critical for series progression. Towards this end, we exploited high-resolution structural data for the rational design of key compounds for program advancement. The design elements, chemical synthesis and biological data of these “Phe-movers” will be presented, in addition to key learnings that can be instructive to a broad audience of medicinal chemists.



MEDI 91

Strategies toward structurally constrained diamide inhibitors of FXIa

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Factor XIa (FXIa), a trypsin-like serine protease, is a key enzyme of the intrinsic phase of coagulation. Once activated, FXIa amplifies the production of thrombin, the final enzyme in the coagulation cascade, which ultimately leads to the formation of a stable fibrin clot. The therapeutic potential of inhibiting FXIa is supported by the observation that humans deficient in FXI (hemophilia C) exhibit a mild bleeding phenotype. In preclinical rabbit models of thrombosis, direct active-site inhibitors of FXIa show robust antithrombotic efficacy with no bleeding liability. This evidence suggests that FXIa inhibition could block pathologic thrombus formation while preserving normal hemostasis. Recently, we disclosed a novel series of phenylalanine derived diamide FXIa inhibitors. In this presentation, we describe a strategy to constrain the phenylalanine moiety in an effort to improve the overall profile for these analogs. This effort culminated in the discovery of tetrahydroisoquinolines (THIQs) as novel FXIa inhibitors with low nanomolar *in vitro* potency and improved selectivity against relevant serine proteases.

MEDI 92

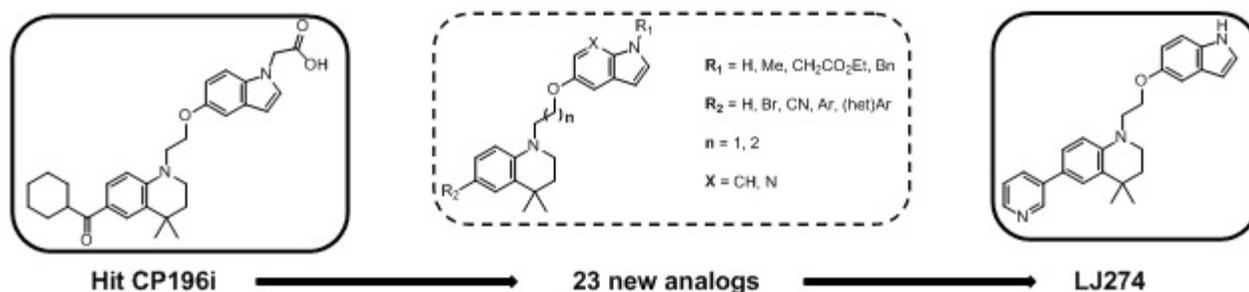
Stat5 and chronic myeloid leukemia: Synthesis and biological evaluation of novel inhibitors

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Chronic myelogenous leukemia (CML) is a myeloproliferative disease of the hematopoietic stem cell (HSC) due to t(9;22) genomic translocation-derived BCR-ABL fusion gene. BCR-ABL codes for a tyrosine kinase that activates multiple signaling pathways in CML cells including the transcription factors Stat5a and Stat5b (Signal Transducers and Activators of Transcription 5a/5b). Imatinib, an inhibitor of Bcr-Abl, is currently used as first-line treatment of CML. However most of the patients relapse after treatment interruption and about 15% experienced failed therapy.

Several experimental facts underlined the essential role of Stat5 proteins in the maintenance of CML. Inhibition of Stat5 would contribute besides to tackle the survival and self-renewing of leukemic stem cells, and also to reduce the resistance of CML cells to Bcr-Abl kinase inhibitors. In a first set of experiments, we tested a series of molecules from our chemical library to analyze their effects on CML cell proliferation and Stat5 activity. One molecule (CP-196i) was identified as able to inhibit Stat5 phosphorylation and leukemic cell growth.

Starting from this hit compound, 23 new analogs have been synthesized and evaluated by proliferation and viability studies carried out on CML cell lines KU812 and K562. Among them, LJ274 slowed proliferation and decreased viability of CML cell lines, with $EC_{50} = 6 \mu\text{M}$ on KU812 cells and $9 \mu\text{M}$ on K562 cells. Stat5 phosphorylation assays clearly showed that LJ274 inhibited Stat5 activation and expression. Apoptosis assays and evaluation of synergistic effects with Imatinib will be presented.



Scheme 1. Hit CP196i, new analogs and the lead LJ274

MEDI 93

Monitoring the progression of structure-activity relationship information during lead optimization

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Lead optimization (LO) aims to transform selected active compounds into clinical candidates through iterative analog design and is one of the most important tasks in the practice of medicinal chemistry. LO is largely driven by hypotheses and depends on

experience and intuition of medicinal chemists, focusing on the key question “which compound to make next?” It is essentially impossible to predict the ultimate outcome of an LO project and it is also very difficult to estimate when sufficient numbers of compounds have been evaluated before meaningful conclusions can be reached. Given the subjective nature of LO decisions and inherent optimism of project teams, very few attempts have been made to systematically evaluate LO progression. Herein we introduce a computational approach to monitor the evolution of structure-activity relationship (SAR) information during LO over a time course. The approach is based upon the use of SAR matrix data structures as a diagnostic tool and enables graphical analysis of SAR redundancy and project progression. This framework should merit further investigation in LO assessment.

MEDI 94

Design and synthesis of macrocyclic factor XIa inhibitor

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The trypsin-like serine protease Factor XIa (FXIa), the activated form of the zymogen Factor XI (FXI), is a key component in the intrinsic pathway of the blood coagulation cascade. A growing body of research indicates that FXIa plays a pivotal role in thrombosis (blood clots) but only a minor role in hemostasis. As a result, inhibition of FXIa could provide an effective anticoagulant therapy with a safer bleeding profile. We have previously described the discovery of potent, macrocyclic FXIa inhibitors that contained a key macrocyclic amide linker. Investigations of amide bioisosteres led to the discovery of amine-linked macrocyclic inhibitors described in this presentation, which are potent and selective FXIa inhibitors with improved physicochemical properties.

MEDI 95

Structure-based design of novel RORgamma inverse agonists

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Retinoic acid receptor-related orphan receptors (RORs) belong to the nuclear hormone receptor superfamily; three isoforms of RORs are known (RORalpha, RORbeta, and RORgamma). RORgamma has been implicated in the production and regulation of pro-

inflammatory cytokine interleukin-17 and has emerged as an important target for treating autoimmune diseases such as psoriasis, multiple sclerosis, and rheumatoid arthritis. A number of modulators of RORgamma are reported in the literature. Here we describe the discovery of a novel series of RORgamma modulators starting from a piperazine-containing screening hit. Interestingly, some of the enantiomeric pairs both bind tightly to RORgamma, but behave very differently in cell-based assays. X-ray crystal structures for one enantiomeric pair are also reported here.

MEDI 96

Protease inhibitors from derivatives of tranexamic acid

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Introduction:

Tranexamic acid (TA) is an antifibrinolytic drug that has proven effective for the treatment of heavy menstrual bleeding. TA has found application with fibrin sealants in surgical procedures in leak prevention. TA is known for its adverse side effects in high oral dosage. Its neurotoxicity prevents its use with fibrin sealants in neuro-surgical procedures. Therefore, an investigation was undertaken to make derivatives of TA at its acid and amine functional groups. The former entity prepared was the calcium salt of TA (Calcium Tranexmate, Ca-TA), and the latter, an amide derivative, was named MTA (modified TA). This study reports the preliminary efficacy data on Ca-TA and MTA in their respective protease inhibition activities in a proteolytic environment.

Experimental:

Ca-TA was synthesized as described under patent application WO 2014/124029A2. MTA, a novel molecule, was synthesized and characterized, though not disclosed at this time as its patent application is currently under preparation. These were both water soluble compounds. A gravimetric method was used for fibrin clot degradation profiles at pH 7.4 and 37 °C in a proteolytic medium containing plasmin. Longevity of clot under these conditions as compared to the control with no protease inhibitor was used as a measure of efficacy for protease inhibition of Ca-TA or MTA.

Results & Discussion:

Efficacy of Ca-TA was demonstrated and compared with that of TA. Ca-TA was efficacious at a concentration as low as 0.1 mE or below, comparable to that of TA at 0.1 mM

MTA at concentrations of 0.1 mM and 1 mM were evaluated in a proteolytic medium of plasmin for fibrin clot degradation over duration of 16 hours at pH 7.4 and 37 °C. While 0.1 mM MTA showed 100 % degradation of clot, 1 mM showed a degradation of 85.1+/- 0.6 %, indicating activity.

Conclusion:

Both Ca-TA and MTA were demonstrated to exhibit protease inhibition activity. Safety studies continue.

MEDI 97

New small organic ligands for the natural cytotoxicity receptor NKp30

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Natural killer (NK) cells are a type of cytotoxic lymphocyte critical to the innate immune system. NK cells provide rapid responses to viral-infected cells, and play a role in tumor immunosurveillance by directly inducing the death of tumor cells. Instead of acting via antigen-specific receptors, lysis of tumor cells by NK cells is mediated by alternative receptors, including NKG2D, NKp44, NKp46 and NKp30.

In recent developments, B7-H6, a surface protein present on a broad panel of tumor cells including lymphoma, melanoma, and carcinoma, was identified as a ligand for the NKp30 receptor. The structure of the NKp30-B7H6 complex has also been resolved. The comparison between the 3D structures of unbound and B7-H6-bound NKp30 demonstrated marked conformational changes that may be a key-factor for the NK-response activation role of B7-H6.

Our current work aims at designing a family of small organic molecules (SOMs) capable of mimicking the effect of B7-H6 on the NKp30 receptor. The main goal is to obtain an SOM capable of inducing an NK response, through binding to the NKp30 receptor, and structurally amenable to derivatization with tumor-targeting molecular units to produce a specific immune response against cancer cells.

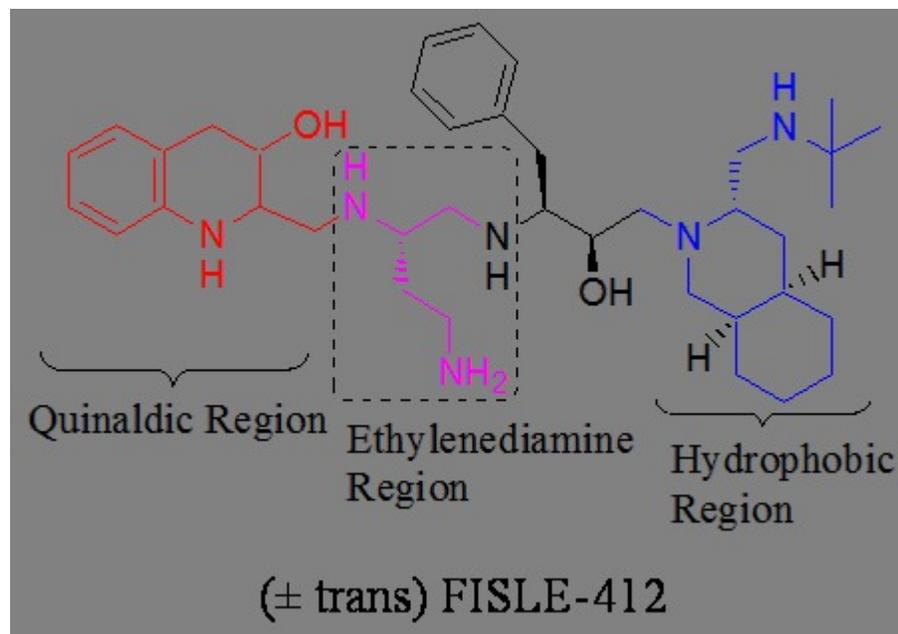
A combination of computational docking and molecular dynamics tools was extensively used to scan several ligand libraries, yielding core-structures as possible ligands for the receptor. These were further optimized to generate lead structures for chemical synthesis. Data from mass spectrometry-based screening of the initial leads as NKp30 ligands will be presented.

MEDI 98

Structural investigation of FISLE-412, a peptidomimetic compound derived from saquinavir that targets lupus autoantibodies

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FISLE-412 is the first reported small molecule peptidomimetic that neutralizes anti-dsDNA autoantibodies associated with systemic lupus erythematosus (SLE) pathogenesis. FISLE-412 is a complex molecule that involves a challenging synthesis scheme, but has attractive pharmacological activities as a potential small molecule therapeutic in lupus. Therefore, we initiated a structure-activity investigation of FISLE-412. We synthesized a small library of mimetopes around FISLE-412 and identified several analogues which could neutralize anti-DNA lupus antibodies *in vitro* and *in vivo*. Our strategies managed to reduce the structural complexity of FISLE-412 and provide important information that may guide potential autoantibody-targeted lupus therapeutics.



Structure of FISLE-412

MEDI 99

Anti-DNA antibodies as a drug target in systemic lupus erythematosus

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Current Systemic Lupus Erythematosus (SLE) treatment involves mainly non-specific immune system inhibition (corticosteroids) or targets features of the immune response

that are necessary for normal suppression of infection and endogenous immune function. In an attempt discover more specific SLE therapeutics, we began a program to develop small molecule decoy antigens which could bind exclusively to anti-dsDNA antibodies which are implicated in nephrotoxicity and neuropsychiatric features of the disease.

We have discovered several novel compounds which have the ability to neutralize anti-dsDNA antibodies *in vitro*, *in situ*, and *in vivo*. Of our top leads, pharmacokinetic and SLE model studies demonstrated that FISLE-412, a reduced saquinavir analogue, was well-tolerated, altered serum reactivity to DWEYS, reduced glomeruli IgG deposition, preserved kidney histology, and delayed SLE onset in NZB/W F1 mice.

MEDI 100

Discovery of potent and selective ROR γ t inverse agonists through scaffold hopping using CoreHopTM

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Retinoic acid receptor (RAR)-related orphan receptor (ROR γ t) plays an important role in autoimmune disorders such as psoriasis. We present here the application of scaffold hopping algorithm called CoreHopTM to identify novel ROR γ t agonists. CoreHop joins physically separated molecular fragments in the protein binding site with high resolution core fragments. Core fragment libraries were generated by recursive chopping of small molecules from high resolution X-ray structures. Further modification of one of the hits through molecular design led to potent and selective ROR γ t inverse agonists.

MEDI 101

Discovery of novel S1P₃-sparing S1P₁ & S1P₅ receptor agonists for treatment of multiple sclerosis

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Republic of) (6) Department of Integrated Biomedical and Life Science, Korea University, Seoul, Korea (the Republic of)

Multiple sclerosis (MS) is a chronic, progressive autoimmune disease in the CNS. The non-selective sphingosine-1-phosphate (S1P) receptor modulator, FTY720, binds to S1P_{1,3,4,5}. It could cause diverse side effects such as bradycardia. Our rationale of developing novel S1P₁ & S1P₅ agonist is to be lower the circulating lymphocytes more efficiently by internalization of S1P₁ on lymphocyte and to remyelinate by modulation of S1P₅ on oligodendrocytes. We attempted to design S1P₁ & S1P₅ dual receptor agonists *via in silico* study based on already disclosed S1P₁ & S1P₅ agonist such as ASP4058, BAF312, etc. Novel S1P₃-sparing S1P₁ & S1P₅ agonists were identified by Calcium Signaling assay, β -arrestin assay and Receptor Internalization assay. Furthermore, several compounds act as β -arrestin biased S1P₁ agonist. We are optimizing to improve pharmacokinetic properties remaining S1P₁ & S1P₅ affinity and selectivity against S1P₂ - S1P₄.

MEDI 102

Taurine prodrugs: a new class of amino acid anti-inflammatory drug devoid gastric toxicity

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It is estimated that exists more than 50 different non steroidal anti-inflammatory drugs (NSAIDs) in the market, but none of them are wholly without side effects such as gastro toxicity, even selective compounds to COX₂ (cyclooxygenase-2) receptors. For this reason none of NSAIDs is recommended for use in chronic inflammatory diseases. Taurine is a semi essential amino acid with high antioxidant activity, including intestinal anti-inflammatory activity. The purpose of this work was to synthesize mutual prodrug from classical NSAIDs (naproxen, diclofenac, salicylic acid, ibuprofen and indometacin) with taurine and evaluate the acute ant inflammatory effect and gastro toxicity compared to the physical association of taurine and NSAIDs. In addition, the prodrugs were submitted to colitis animal model. All NSAIDs tested have caused ulceration average number of 48 to 69, the extent of injury consistent with the highest lesion in grade 5. Taurine decrease the injury grade of diclofenac and naproxen to grade 1 and others NSAIDs to grade 3. All prodrugs showed no lesions with the maintenance of the anti-inflammatory effectiveness. All prodrugs showed to protect the bowel and recover the necrotic tissue to normal pattern observed in the histopathological analysis. No mutagenic effect was observed in micronucleus essay for all derivatives. This suggest that taurine can protect gastric toxicity effect from NSAID therapy. Also, its is highlight the taurine use as carrier of NSAID in prodrug strategy to obtain a new class of anti-inflammatory drug devoid gastro toxicity.

MEDI 103

Structure-based design of 3-(4-aryl-1*H*-1,2,3-triazol-1-yl)-biphenyl derivatives as P2Y₁₄ receptor antagonists

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UDP and UDP-glucose activate the P2Y₁₄ receptor (P2Y₁₄R) to modulate processes related to inflammation, diabetes, and asthma. A computational pipeline suggested alternatives to the naphthalene ring of a previously reported P2Y₁₄R antagonist (**3**, PPTN) using docking and molecular dynamics (MD) simulations on a human P2Y₁₄R model based by homology to hP2Y₁₂R X-ray structures. By reevaluating the binding of **3** to P2Y₁₄R using computational approaches, two non-naphthalene alternatives, *i.e.* alkynyl and triazolyl derivatives, were identified. A fluorescent antagonist **4** was prepared by an improved synthetic route to enable quantification of affinity using flow cytometry (FCM) of hP2Y₁₄R-expressing CHO cells. *p*-F₃C-phenyl-triazole **65** was more potent than a corresponding alkyne **11** and only 6-fold less potent than **3** in this FCM assay. Thus, additional triazolyl derivatives were prepared, as guided by docking simulations. Nonpolar aryl substituents, such as *p*-*n*-propyl-Ph, *p*-bromo-Ph, and 5-bromothien-2-yl, were favored. The simpler synthetic pathway presents ample opportunity for further structural optimization. Additionally, the higher P2Y₁₄R affinity observed with the triazoles was consistent with the predicted binding modes of alkynyl and triazole analogues. These triazoles, designed through a structure-based approach, can now be assessed in disease models.

MEDI 104

Non selective PDE inhibitors as promising anti-inflammatory medicine

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Pentoxifylline is a xanthine derivative approved for the treatment of chronic peripheral vascular occlusive disorders. In clinical, pentoxifylline has been off-label used for the treatment of various diseases, including alcoholic hepatitis and ischemic stroke. The mechanism of action may include: phosphodiesterase inhibition, increase cAMP/cGMP level and down regulate pro-inflammatory cytokine, like TNF α , IL1b, and IL6. There are clinical data support for pentoxifylline on anti inflammatory indications, though the efficacies were generally mediocre. After oral administration, Pentoxifylline was quickly metabolized and eliminated, a plasma half-life was less than a hour. Aimed to improve

efficacy and pharmacokinetic properties, an optimization effort that focused on xanthine 3,7-substitutions was initiated. Replacement of metabolic unstable ketone moiety with heterocyclic rings led to the discovery of a new chemical series with improved pharmacokinetic properties and better in vitro PDE and ex vivo TNF α inhibition. Detailed synthesis, biological activities and PK properties will be presented.

MEDI 105

Synthesis and development of novel compounds selectively targeting on sphingosine 1-phosphate receptor 1 (S1P₁) for treatment of multiple sclerosis

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The sphingosine-1-phosphate (S1P) receptors are the first lipid G protein-coupled receptors (GPCRs) that are divided into five subtypes; S1P₁, S1P₂, S1P₃, S1P₄, and S1P₅. In particular, targeting S1P₁ with the endogenous ligand, S1P, has shown substantial efficacy in treatment of multiple sclerosis (MS) since it promotes egress of lymphocytes from lymph nodes. The S1P receptor agonist has been shown to induce receptor down-regulation from the cell surface, suggesting that it acts as a functional antagonist of S1P₁ to prevent lymphocyte egress by internalization of the receptor. In this study, we synthesized a series of chemical library for a potent lead compound and evaluated their potency and selectivity using a set of complementary assays such as calcium flux, β -arrestin recruitment, and S1P₁ receptor internalization. Among the synthesized compounds, SKY-59 exhibited potent activities on β -arrestin recruitment and S1P₁ receptor internalization with an EC₅₀ of 180 nM and 10 nM, respectively, whereas it was slightly active (EC₅₀=1.72 mM) on Ca⁺⁺ assay. The selectivity profile of SKY-59 against S1P₃ receptor and in vivo studies are under investigation to be developed as a lead compound.

MEDI 106

2,5-Isomers of triazole-pyrrolopyrimidine act as selective inhibitors of Janus kinase 2 (JAK2) compared to JAK1 and JAK3

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Members of the Janus kinase (JAK) family are potential therapeutic targets. Abnormal signaling by mutant JAK2 is related to hematological malignancy, such as myeloproliferative neoplasms (MPNs), and tyrosine kinase inhibitor (TKI)-resistance in non-small cell lung cancer (NSCLC). We discovered a potent and highly selective inhibitor of JAK2 over JAK1 and -3 based on the structure of 4-(2,5-triazole)-pyrrolopyrimidine. Among all triazole compounds tested, 2,5-triazole regioisomers more effectively inhibited JAK2 kinase activity than isomers with substitutions of various alkyl groups at the R₂ position, except for methyl-substituted 1,5-triazole, which was more potent than the corresponding 1,4- and 2,5-triazoles. None of the synthesized 1,4-isomers inhibited all three JAK family members. Compounds with phenyl or tolyl group substituents at the R₁ position were completely inactive compared with the corresponding analogues with a methyl substituted at the R₁ position. As a result of this structure-activity relationship, **54**, which is substituted with a cyclopropylmethyl moiety, exhibited significant inhibitory activity and selectivity (IC₅₀ = 41.9 nM, fold selectivity JAK1/2 10.6 and JAK3/2 58.1). Compound **54** also exhibited an equivalent inhibition of wild type JAK2 and the V617F mutant. Moreover, **54** inhibited the proliferation of HEL 92.1.7 cells, which carry JAK2 V617F, and gefitinib-resistant HCC827 cells. Compound **54** also suppressed STAT3 phosphorylation at Y705.

MEDI 107

Targeting pulmonary inflammation by pharmacological augmentation of leukotriene A₄ hydrolase with 4-methoxydiphenylmethane

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The leukotriene A₄ hydrolase (LTA₄H) enzyme is a bifunctional zinc metalloenzyme that functions as an epoxide hydrolase and aminopeptidase. The epoxy hydrolase activity participates in a pro-inflammatory pathway by catalyzing the hydrolysis of leukotriene A₄ to leukotriene B₄. However, the aminopeptidase activity contributes to an anti-inflammatory pathway by catalyzing the hydrolysis of the tripeptide proline-glycine-proline. Herein, we present an X-ray crystal structure of LTA₄H in complex with 4-methoxydiphenylmethane (4MDM), a small molecule that augments the aminopeptidase activity of the LTA₄H enzyme. Furthermore, we present the effect of 4MDM in a murine model for adult respiratory distress syndrome.

MEDI 108

Design and characterization of peptide inhibitors of the interleukin-1 β receptor signaling complex

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Signal transduction by the heterotrimeric complex consisting of interleukin-1 β (IL-1 β), interleukin-1 receptor, type 1 (IL-1RI), and interleukin-1 receptor accessory protein (IL-1RAcP) is implicated in a number of inflammatory indications. Herein, we present the design of peptide inhibitors to block formation of the IL-1 β :IL-1RI:IL-1RAcP complex and a fluorescent-based assay for characterization. The peptides were further characterized by circular dichroism spectroscopy. Inhibition of complex formation was determined in a ligand pull-down assay for the complex, and by measuring inhibition of proinflammatory NF- κ B downstream signaling.

MEDI 109

Design and synthesis of small molecule inhibitors of interleukin-1 β for the treatment of osteoarthritis

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Osteoarthritis is the most common form of arthritis and is characterized by degenerative changes in articular cartilage, bone, and associated joint tissues. Post-traumatic arthritis (PTA) is one of the etiologic subtypes of osteoarthritis, which is derived from a physical injury that leads to a change in joint mechanics. Health care costs for PTA is \$7 billion annually. Therefore, there is an urgent need to develop novel therapeutic approaches for PTA post joint injury. Herein, we present our strategy for the design and synthesis of small molecule inhibitors of the heterotrimer complex formation of interleukin-1 β , interleukin-1 receptor type 1, and interleukin-1 receptor accessory protein as lead compounds for the treatment of osteoarthritis.

MEDI 110

Discovery of a novel allosteric thyroid hormone binding site on macrophage migration inhibitory factor (MIF)

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Macrophage migration inhibitory factor (MIF) is a pro-inflammatory cytokine and plays a critical role in the pathology of many inflammatory diseases. An enzymatic pocket in the

biologically-active MIF trimer is the prevailing site for drug development as its ligation has been shown to correlate with a reduction of MIF biological activities. Our previous study found that thyroxine (T4) was a potential endogenous ligand of MIF and that MIF activity could be modulated both in vitro and in vivo disease model by this prohormone, but not its metabolite triiodothyronine (T3). Structure activity relationship studies suggested that 5'- and 3'- iodination is critical for inhibition of MIF tautomerase activity, which conflicted with docking studies of thyroxine analogues on the MIF active site. Herein, we have conducted molecular dynamic simulation studies of thyroid hormone and its metabolites with the MIF protein. In contrast to previous studies, our data suggests that thyroxine may disrupt MIF enzymatic activities via a novel allosteric binding site.

MEDI 111

Design and synthesis of 1,3,4-thiadiazoles as S1P₁ and S1P₅ selective agonists for the treatment of autoimmune diseases

Brahmachary Enugurthi, benugurthi@celgene.com, Esther Martinborough, Adam R. Yeager, Liming Huang, Junko Tamiya, Manisha Moorjani, Marcus Boehm, Fiona Scott, Bryan Clemons, Jennifer Brooks, Rachel Powell, Harry Dedman, Hans Desale, Gregory Reinhart, Gregg Timony, Robert Peach. Receptos, a wholly owned subsidiary of Celgene Corp., San Diego, California, United States

Sphingosine 1 phosphate (S1P) is a lysophospholipid which is implicated in multiple cellular processes through its binding to five receptors, S1P₁ through S1P₅. Stimulation of S1P₁ receptor sequesters lymphocytes in peripheral lymphoid organs and prevents trafficking to inflamed tissue. Targeting S1P receptors to treat autoimmune disease such as relapsing multiple sclerosis (RMS) was clinically established by non-selective S1P receptor modulator, fingolimod (FTY720, GilenyaTM) and an S1P₁/S1P₅ selective agonist derived from a 1,2,4-oxadiazole core (Ozanimod) is currently in Phase 3 clinical trials to treat RMS and ulcerative colitis. In this presentation we will describe identification of structurally different molecules derived from a 1,3,4-thiadiazole core as S1P₁/S1P₅ selective agonists. Detailed SAR, synthesis, pharmacokinetics and pharmacodynamic data will be presented.

MEDI 112

Identification of novel arylpiperazinyl butyrolactones 5-HT₇ antagonists as potential inflammatory bowel disease (IBD) therapies

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McMaster University, Hamilton, Ontario, Canada (4) Praeventix, LLC, Exton, Pennsylvania, United States

Over 1.4 million Americans suffer from the life altering disease known as Inflammatory Bowel Disease (IBD). Severe, chronic inflammation of the gastrointestinal tract is the hallmark feature of this condition, which can be divided into two broad categories, Crohn's Disease and Ulcerative colitis. Crohn's Disease can affect any area of the GI tract, leading to patches of inflammation, while Ulcerative Colitis occurs only in the colon. Despite decades of research, the pathophysiology of IBD remains a mystery, and there is no cure. Current treatment protocols focus on symptom mitigation through the progressive application of anti-inflammatory amino-salicylates (5-ASA), corticosteroids (prednisone, prednisolone, budesonide), immunomodulator drugs (6-mercaptopurine, azathioprine, cyclosporin A, methotrexate) and anti-TNF- α antibodies (Remicade, Humira). Irrespective of treatment, however, ~33% of Ulcerative Colitis patients and ~70% of Crohn's Disease patients require surgery to remove the affected portion of the GI tract. Surgical intervention is curative but life changing in Ulcerative Colitis, as the standard protocol, an ileal pouch-anal anastomosis, removes the colon and rectum and the small intestine is attached to the anal area to allow stool passage. Initial surgery in Crohn's Disease patients is less severe, as only the afflicted region is removed, but inflammation of previously unaffected areas often leads to symptom resurgence. In the absence of novel therapies, patients will continue to suffer. Many will endure undesirable surgical interventions. There is a compelling need to identify novel IBD therapies. Recent literature has established a causal link between over activation of the 5-HT₇ receptor in GI dendritic cells and IBD. Specifically, the highly selective 5-HT₇ antagonist SB-269970 produces statistically significant improvement in the dextran sulfate sodium (DSS) induced mouse model of IBD. We recently identified a novel series of arylpiperazinyl butyrolactones that produce similar results in the DSS mouse model of IBD and have established collaborative efforts to advance our findings towards pre-clinical development. The synthesis, biological activity and *in vitro* ADME of this series will be discussed.

MEDI 113

Identification of Vps34 as a key-off target activity in the search for a suitable PI3K δ oral inhibitor for the treatment of respiratory diseases and its potential impact on toxicity

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Phosphoinositide 3-kinase δ (PI3K δ) is a lipid kinase expressed primarily in leukocytes. It plays a key role in immune cell signalling, and thus in inflammatory processes and is activated in response to multiple triggers of relevance to respiratory disease, making it

an attractive target for the treatment of asthma and chronic obstructive pulmonary disease (COPD).

This poster will describe the optimisation of a novel series of orally bioavailable PI3K δ inhibitors derived from our existing inhaled assets and how Vps34 off-target activity was identified and tackled to remove potential toxicity. Medicinal chemistry efforts initially focussed on improving selectivity and oral PK in the series, resulting in the discovery of a first potential candidate molecule. Additional profiling highlighted Vps34 as a key off-target activity for this compound and we believe this contributed to early toxicity findings which halted further development. Vps34 is a class III PI3K expressed in all eukaryotic cells which is involved in vesicle trafficking and autophagy. Further optimisation using structural knowledge resulted in removal of this liability and delivered a potent and selective PI3K δ inhibitor with an improved therapeutic index, suitable for oral administration.

MEDI 114

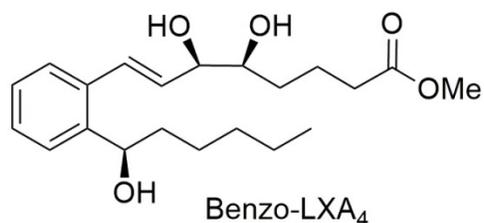
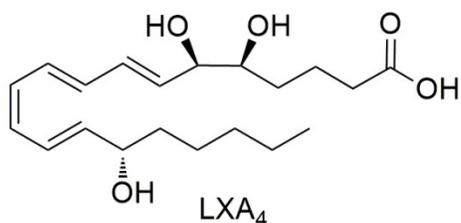
Asymmetric synthesis and preliminary biological evaluation of heteroaromatic lipoxin A₄ analogues

Andrea Zanetti¹, andrea.zanetti@ucdconnect.ie, **Eibhlin Butler**¹, **Christine Loscher**², **Patrick J. Guiry**¹. (1) Centre for Synthesis and Chemical Biology, University College Dublin, Dublin, Ireland (2) School of Biotechnology, Dublin City University, Dublin, Ireland

Lipoxins (LXs) are pro-resolving mediators which promote the resolution of inflammation. They are trihydroxytetraene-containing eicosanoids, derived from arachidonic acid and they are naturally produced by lipoxygenase enzymes at sites of inflammation.

LXs are labile molecules that are quickly metabolised *in vivo*. Recently, we have explored the synthesis of more stable LX analogues by substituting some motifs of the native molecule with more stable and resistant units.

One such analogue, Benzo-analogue *epimer* was previously synthesised and showed a 1000 time increase in potency compared to native LXA₄. A pyridine analogue has showed anti-inflammatory effects. Herein, the synthesis of lipoxin A₄ analogues containing a heteroaromatic motif is reported through a convergent approach. Two units are independently synthesised before a cross coupling reaction and an asymmetric reduction which are the key steps in the synthesis of the proposed novel analogues. A set of novel LXA₄ heteroaromatic analogues has been synthesised and is undergoing biological evaluation.



MEDI 115

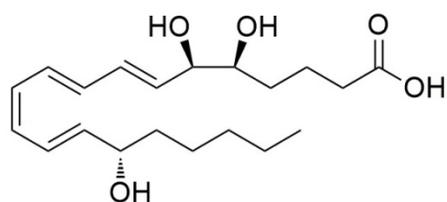
Asymmetric synthesis and biological evaluation of novel heteroaromatic lipoxin A₄ analogues for biological evaluation

Kevin Gahan¹, kevin.gahan@ucdconnect.ie, **Eibhlin Butler**³, **Monica de Gaetano**⁴, **Catherine Godson**⁵, **Patrick J. Guiry**². (1) University College Dublin, Dublin, Ireland (3) Centre for Synthesis and Chemical Biology, University College Dublin, Dublin, Ireland (4) Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Dublin, Ireland

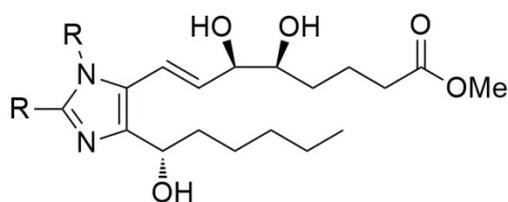
Since their initial discovery in 1984 by Samuelsson and Serhan, Lipoxins (LX's) have been shown to display both potent and selective anti-inflammatory activity. The two native Lipoxins; LXA₄ and LXB₄, both act by binding to a specific G-Protein Coupled Receptor named FPR2 on the cell membrane of polymorphonuclear leukocytes (PMNs) and monocytes, preventing the accumulation of neutrophils at the site of inflammation. However, LXs are seen to undergo rapid, irreversible metabolism *in vivo* forming biologically inactive metabolites, hence limiting their therapeutic potential. In LXA₄, one of the moieties most prone to metabolism is the triene core, which rapidly undergoes reduction.

In an effort to address the rapid metabolism of LXA₄ and LXB₄, it was proposed that replacement of the triene system with an aromatic or heteroaromatic unit may hinder metabolism as well as increase potency of the compound. Previous success within the group replacing the chemically-labile triene system with a benzene or pyridine ring resulted in the corresponding analogues displaying increased chemical stability as well as increased potency and bioactivity.

This work focuses on the synthesis and biological evaluation of novel imidazole-containing LXA₄ analogues as well as their initial biological evaluation. By carrying out a convergent synthesis utilising a palladium catalysed cross-coupling as the key step, the target analogues were produced and biologically tested for anti-inflammatory properties.



LXA₄



Target LXA₄ analogue



MEDI 116

Tryptamine derivatives, extracted from Syrian rue seeds, negatively affected *Leishmania tarentolae* in culture: A model study

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Leishmaniasis is a disease caused by the parasitic protozoan *Leishmania*. This disease affects people in over 100 countries worldwide with over one million new cases diagnosed every year. The CDC identifies leishmaniasis as one of the most neglected tropical diseases. The current treatment options are expensive and have harsh side effects, thus, new treatments are of interest. Tryptamine derivatives, such as dimethyltryptamine (DMT), and other β -Carbolines (harmine, harmaline and vascine) are commonly ingested, as a drink, during ritualistic ceremonies in some locations where leishmaniasis is endemic. Consequently, the effect of these compounds on *Leishmania* cell viability, motility, and secreted acid phosphatase activity is of interest. To investigate these effects, harmine or harmaline (purchased from Sigma) were added to *in vitro* cultures of *L. tarentolae*. Cell viability was measured using the dimethylthiazol-2,5-diphenyltetrazolium bromide (MTT) viability assay. Secreted acid phosphatase activity was investigated using the standard substrate *p*-nitrophenylphosphate. Relative to control cells, addition of harmine or harmaline reduced the detectable secreted acid phosphatase activity one day post addition by some 75-85%, however this enzyme activity appeared to return to control levels over the next 4 days of culture. The MTT viability was reduced by about 40% with harmine and 60% with harmaline on day 1 after addition, however, the viability over the next 4 days did not return to control cell values. Therefore, these compounds may have some potential *in vivo* negative effects on *Leishmania*. Future work will involve extraction of tryptamine derivatives from Syrian

Rue seeds, characterization of extracts using IR, NMR, and UV spectroscopy, and then addition of these compounds singly or as mixtures to cultured *Leishmania*.

MEDI 117

Synthesis and biological evaluation of 5,7-dihydroxyflavanone derivatives as antimicrobial agent

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In this study, seven 5,7-dihydroxyflavanone (FNN) derivatives were synthesized. Novel compounds are 4-F-FNN (2), 3,4-diCl-FNN (6), and 4-oMe-FNN (8) whereas 3,4-diF-FNN (3), 3,4,5-triF-FNN (4), 3F-4Cl-FNN (5), and 3,4,5-triOH-FNN (7) have been previously reported. However, this is first time screening for their antimicrobial efficacy on Gram-negative, Gram-positive bacteria and yeast. Among these compounds, 3,4-diCl-FNN showed the most potent antimicrobial activity against Gram-positive bacteria, the yeast *Saccharomyces cerevisiae*, and the Gram-negative bacterium *Vibrio cholera* (Table 1). Mammalian cytotoxicity was tested using MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt) cell viability assay on HepG2 cells. The 5,7-dihydroxyflavanone derivatives generally exhibited low cytotoxicity in HepG2 cells (Figure 1). The most antimicrobial compound, 3,4-diCl-FNN, showed the lowest cytotoxicity in HepG2 cells. This study suggested that halogenated flavanones may be the promising pharmacological candidates as effective antimicrobial agents with low mammalian cytotoxicity.

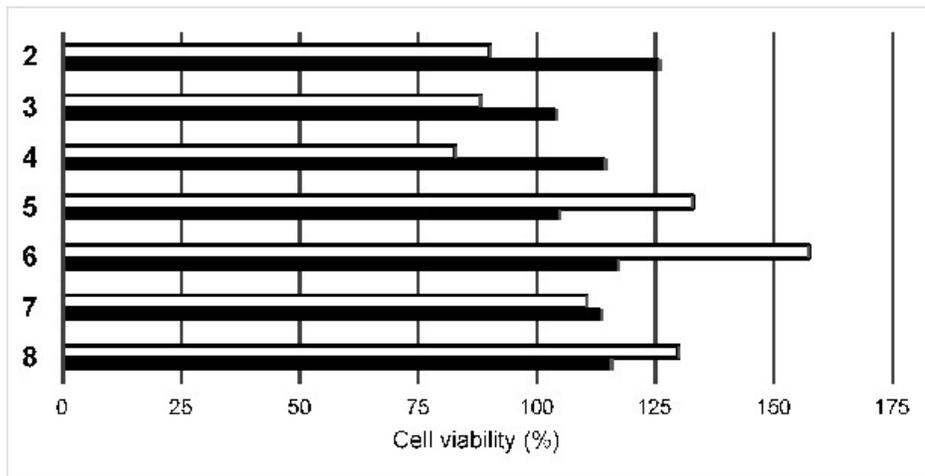


Figure 1. Cytotoxicity of 5,7 -Dihydroxyflavanone derivatives on HepG2 cells. Cell viability was measured after incubating HepG2 cells with 50 μM of flavanone derivative by MTS assay. Cell viability was normalized based on the negative control (100% growth medium) and positive control (100% DMSO). Flavanone **3** shows least toxicity out of the compounds that were tested. Open bars correspond to 24 h and closed bars correspond to 6 h.

Table 1. Non-natural flavanone MIC values ($\mu\text{g mL}^{-1}$)

Organism	Compound						
	2	3	4	5	6	7	8
<u>Gram-negative Bacteria</u>							
<i>Escherichia coli</i> BW25113	>140	>290	>310	>80	>40	>300	>290
<i>Pseudomonas aeruginosa</i> PA01	>140	>290	>310	>80	>40	>300	>290
<i>Vibrio cholerae</i> 0395N1	30	40	> 310	40	40	>300	> 290
<u>Gram-positive Bacteria</u>							
<i>Bacillus subtilis</i> 1012	30	40	> 310	40	20	> 300	> 290
<i>Bacillus anthracis</i> delta steme	30	20	>310	20	10	> 300	> 290
<i>Bacillus cereus</i> 10987	30	40	> 310	30	10	>300	> 290
<i>Stappyllococcus aureus</i> ATCC 33807	30	40	> 310	40	20	>300	> 290
<u>Eukaryote</u>							
<i>Saccharomyces cerevisiae</i> INVSc1	70	> 290	40	80	10	>300	> 290

MEDI 118

Inhibitors of LHR-1 as novel anti-parasitic drugs

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Greater than 1.4 billion people in the world are affected by Neglected Tropical Diseases (NTDs). The effectiveness of treatment of NTDs has been diminished due to the

following problems: 1) resistance spreads and develops quickly, 2) the parasites do not actively transport drugs into their bodies, 3) many drugs are only effective during specific developmental stages of the parasites, and 4) the drugs tend to target only a specific type of parasite. It is now known that free-living and parasitic nematodes are unable to synthesize heme, and without it they are unable to survive and reproduce.

Leishmania are heme auxotrophs that lack the necessary genes to biosynthesize heme, which plays an essential role in the replication and survival of the parasite. LHR-1 is a small 20 kDa protein, with four predicted transmembrane domains, that localizes to acidic intracellular compartments and the plasma membrane of promastigotes and amastigote stages of the parasite. It functions in the direct transport of heme from the environment into the cytoplasm and is essential for virulence. Importantly, LHR1 transcript levels increase when the parasites are grown under heme depleted conditions, is involved in the control of intracellular heme pools (in host macrophage), can rescue the growth of a yeast strain defective in heme biosynthesis, and can directly transport radioactive heme when expressed in a yeast system. Loss of a single LHR-1 allele results in severe growth defects, whereas a null mutant is not viable. These results along with the low sequence homology to the human homolog (hHRG-1) make LHR-1 an attractive drug target.

Several antagonists were identified from an initial high throughput screen that specifically inhibit the heme transport function of the Leishmania LHR1 but not the corresponding human homolog (hHRG1). Herein we detail the design, synthetic strategy, and structure-activity relationships of novel **low- μM** selective inhibitors of LHR-1.

MEDI 119

New vacuolar-ATPase inhibitors as antiviral therapies

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Filoviruses (Ebola virus and related viruses) are highly contagious, lethal pathogens that cause severe hemorrhagic fever in humans and primates. Currently, there are no approved therapeutic measures for the treatment of filovirus infections; which makes their development of paramount importance. A promising therapeutic target during filovirus infection is the infection of macrophages and dendritic cells early in the virus' lifecycle. By targeting viral inhibitors to the host lysosomes of macrophages, systemic and deadly infection by filoviruses could be averted. The cellular enzyme vacuolar-ATPase (v-ATPase) has been identified as integral to the entry of filoviruses into host cells. The discovery of the V-ATPase inhibitory property of the lignan diphyllin presents a new scaffold that is distinct from all previously known v-ATPase inhibitors. Diphyllin is

a natural, aryl-naphthalene lignan found in plants and has shown promising anti-tumor and anti-osteoclast activity, as well as strong anti-viral activity against Influenza. *Our hypothesis is that by inhibiting the cellular enzyme vacuolar-ATPase (v-ATPase) in macrophages, we will prevent the infection of these cells by Ebolavirus and reduce the spread of the virus throughout the body.* Using our lead compound, we will test the ability of liposomal nanocarriers and structural modifications of diphyllin to target our compounds to macrophage endosomes. We have synthesized several lactam analogues of diphyllin that show similar potency to diphyllin and improved physicochemical properties. Our diphyllin analogues exhibited potency of around 1 μ M against ebolavirus entry into primary human blood-derived macrophages. Since polyamines have shown preferential uptake in macrophages, we plan to incorporate polyamine chains into diphyllin as further lactam derivatives. We have also developed preliminary formulations of diphyllin and analogues for targeted delivery to macrophages and the analog liposomes have shown improved stability over diphyllin liposomes. The preferential uptake of our formulations and compounds into macrophages will be assessed in THP-1 macrophages.

MEDI 120

Synthesis and biological evaluation of polyalthic acid derivatives for the treatment of neglected diseases

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Leishmaniasis and trypanosomiasis are among the neglected tropical diseases with the highest mortality rate. Therapy for both diseases relies on drugs discovered more than 50 years ago and their efficacy is limited by several factors including drug resistance, cost and toxicity.

The copaiba oil is one of the most used natural medicines in the Amazon. The oil has been traditionally used by South American Indians for the treatment of several diseases including leishmaniasis. Polyalthic acid, a diterpene, is one of the major compounds found in copaiba oil. Based on the reported antileishmanial activity of copaiba oil, a series of amides and diols derivatives of polyalthic acid were synthesized and tested against *Leishmania donovani* and *Trypanosoma brucei*. Polyalthic acid was active in both assays with IC₅₀ ranging from 3.87 to 8.68 μ g/mL. The compound with best antileishmanial activity was

(1S,4aS,5R)-N-(cyclohexylmethyl)-5-(2-(furan-3-yl)ethyl)-1,4a-dimethyl-6-methylenedecahydronaphthalene-1-carboxamide (**2h**, IC₅₀ = 3.84 μ g/mL) and (1S,4aS,5R)-5-(2-(furan-3-yl)ethyl)-N-(2-methoxyethyl)-1,4a-dimethyl-6-methylenedecahydronaphthalene-1-carboxamide (**2c**) showed the best antitrypanosomal activity with an IC₅₀ of 2.54 μ g/mL

MEDI 121

Antibacterial activity of *Combretum farinosum* extracts

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About 20 genera and 600 species have been documented for the Combretaceae family. Reports evaluating the biological activities involving the genus *Combretum* have demonstrated to have properties against ailments such as malaria, dysentery, jaundice, influenza, different types of infections. This family also contains agents against inflammation, digestive problems and serves as a diuretic promoter. *C. farinosum* is an under-explored exemplar by the scientific community and thus, worthy for investigation on probable antimicrobial and antimycotic inhibition. This woody vine was partitioned into the following sections: roots, stems, leaves, and fruits. Each plant section underwent a 24 hour sequential Soxhlet extraction utilizing petroleum ether, acetone, and 90% ethanol in water. The resulting extracts were lyophilized and various concentrations (mg/mL) were prepared. The antimicrobial activity of the extracts was tested against *Staphylococcus aureus* (SA), *Methicillin Resistant Staphylococcus aureus* (MRSA), *Bacillus subtilis* (BS), *Enterococcus faecalis* (EF), *Salmonella enteritidis* (SE), *Pseudomonas aeruginosa* (PA), *Shigella flexneri* (SF), and *Escherichia coli B* (ECB). Whereas the petroleum ether extracts did not show any activity against any microbe, the acetone and ethanol mixture extracts exhibited potential activity.

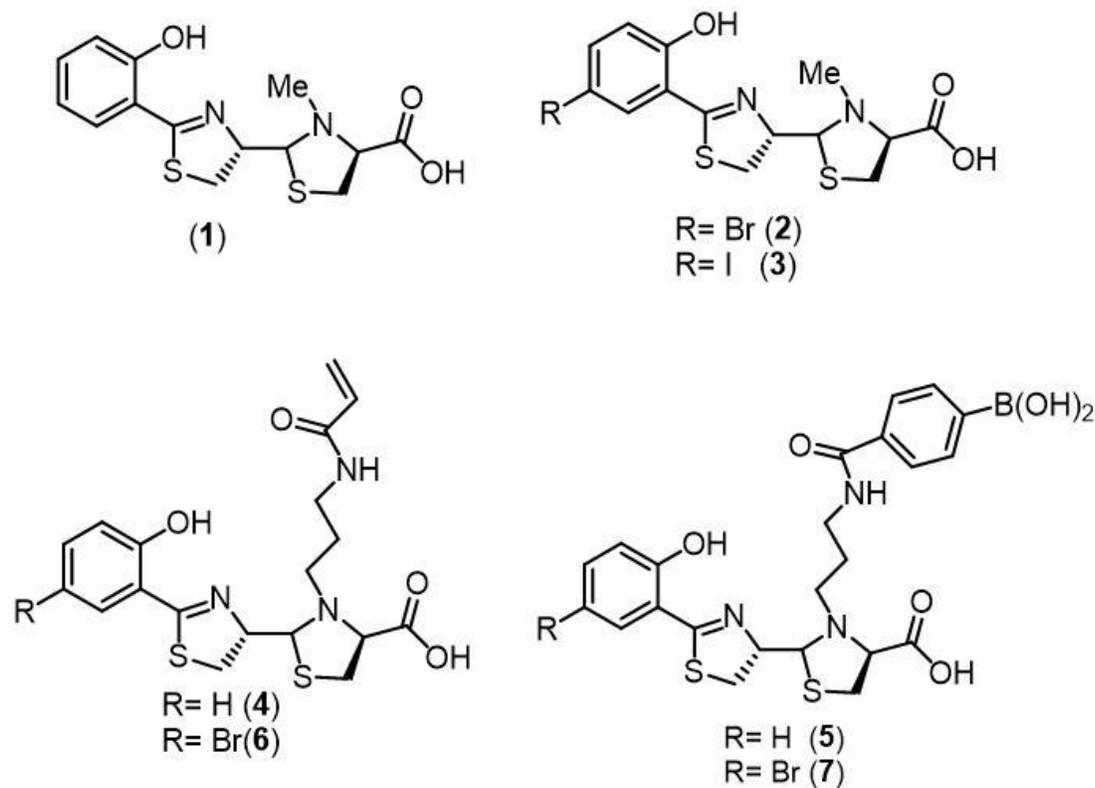
MEDI 122

Synthesis and biological evaluation of pyochelin analogs as potential antibacterial agents against pathogenic bacteria

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Pseudomonas aeruginosa is a typical opportunistic Gram-negative pathogen that is responsible for numerous infections, especially in immunocompromised patients. Even the most potent antibiotics, such as glycopeptides and lipopeptides have been shown to be ineffective against such pathogens due to drug resistance. Synthesis of siderophore analogs is one of the novel strategies to target these pathogens. Siderophores, like pyochelin (**1**) are biosynthesized via extensive non-ribosomal biosynthetic machinery by bacteria, and function as iron chelators to transport ferric ion via dedicated membrane transporters. So by inhibiting pyochelin synthesis or its transport, the growth and pathogenicity can be inhibited. Our research group is investigating the development of pyochelin analogs as potential covalent or non-covalent inhibitors of membrane transporters. A small library of pyochelin analogs (**2-6**) has been designed and synthesized. For non-covalent analogs, we attach halogen atoms to the aryl-ring of **1**, while covalent analogs have electrophilic motifs like Michael acceptors. The biological

activity of these synthetic analogs will be investigated against both Gram-positive and Gram-negative bacteria. In the future, we will design and synthesize second-generation analogs according to the initial bioactivity results. We also plan to synthesize pyochelin-antibiotic conjugates to evaluate the ability of pyochelin to transport antibiotics through the bacterial outer membrane. We hypothesize that this strategy is a valuable method to recover the clinical potential of some antibiotics currently being utilized solely for Gram-positive bacterial infections.



MEDI 123

Multidisciplinary approach to design New Delhi metallo- β -lactamase-1 (NDM-1) inhibitors

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New Delhi metallo- β -lactamase-1 (NDM-1) is a metal-containing β -lactamase that confers resistance to broad range of β -lactam antibiotics. Importantly, *Klebsiella pneumonia* and *Escherichia coli* that express NDM-1 are resistant to all β -lactam drugs, including the carbapenems – the antibiotic class designed to overcome antibiotic resistance. Currently, there is no NDM-1 inhibitor available as a therapeutic agent. As a result, the medical community is at present without any options to treat NDM-1-mediated antibiotic resistant infections. Our long term goal is to deliver clinically-viable NDM-1 inhibitors for evaluation in cells and *in vivo*. Previous research has shown that 4-

methyl-5-(trifluoromethyl)-4H-1,2,4-triazole-3-thiol is an inhibitor of imipenemase (IMP-1) and Verona integron-encoded (VIM-2) metallo- β -lactamases. However, the 1,2,4-triazole-3-thiol fragment has not been previously investigated for NDM-1 inhibition. To that end, we have synthesized ~40 analogs incorporating the 1,2,4-triazole-3-thiol pharmacophore for evaluation against NDM-1. This poster will present the synthesis and biochemical evaluation of 1,2,4-triazole-3-thiols against NDM-1.

MEDI 124

Antimicrobial and exfoliative properties of silver(I) N-heterocyclic carbenes

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We have previously reported that N,N-arylmethyl substituted imidazolium salts can be used to exfoliate bladder epithelial cells for potential use in urinary infections. Although these compounds exfoliate the epithelial layer, they have little inherent antimicrobial activity in the bladder. Therefore, silver(I) N-heterocyclic carbenes have been synthesized with various N,N-arylmethyl substituents with the intent to both exfoliate epithelial cells and expunge uropathogens. The synthesis, characterization, and in vitro studies of these compounds will be presented as well as in vivo studies evaluating the dual nature of these compounds.

MEDI 125

Potent influenza endonuclease inhibitors developed from metal-binding pharmacophore library screen

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The influenza virus is responsible for annual seasonal illness, resulting in between 250,000 and 500,000 deaths worldwide. The last century alone has seen the advent of four influenza pandemics, each resulting in over one million deaths. While vaccinations are a reasonable prophylactic for healthy adults, they are less effective for individuals with compromised immunity. The efficacy of these vaccines is also heavily dependent on correctly predicting the predominant infectious strains for any one year, and incorrect predictions can render vaccination less than 30% effective. Existing drugs, such as Relenza (zanamivir; GSK) and Tamiflu (oseltamivir; Roche) which target viral neuraminidase can be useful in treating influenza infections, but must be administered within 1-2 days of infection to be effective and have many undesirable side effects. Considering this, there is an urgent need for the development of new drugs to prevent and treat influenza infection.

One attractive target for viral inhibition is the cap-dependent endonuclease of the viral RNA-dependent RNA polymerase, which mediates 5' cap-snatching. This target is a dinuclear Mg^{2+} or Mn^{2+} metalloenzyme that lacks a human counterpart. Herein we report the development of novel inhibitors of influenza endonuclease derived from lead compounds identified in a targeted metal-binding pharmacophore (MBP) library screen. Using a FRET-labeled DNA oligonucleotide substrate, we measured the endonuclease activity of the PA subunit of H1N1 influenza A in the presence of MBP molecules, and identified more than 12 molecules with IC_{50} values of $<10 \mu M$ and 5 with IC_{50} values $<1 \mu M$ from a library of ~ 300 compounds. Computational and structural studies were performed to better understand the high potency of these compounds. Guided by these findings, a modest sublibrary of molecules was elaborated from the MBP hits and tested for activity. Structure-activity relationships were established, and favorable structural components were incorporated into previously disclosed inhibitor molecules to further increase potency. This has resulted in the development of several lead molecules with nanomolar in vitro inhibitory activity ($IC_{50} < 100 \text{ nM}$) against viral PA endonuclease in protein based assays, and low micromolar activity ($EC_{50} < 5 \mu M$) against virus particles in cell-rescue assays.

MEDI 126

Synthesis and antimicrobial studies of hydrophilic pyrazole derivatives as potent antibacterial agents

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Antibiotic resistance to infection has become a worldwide problem in recent years. According to the latest CDC report more than two million people are infected every year with antibiotic-resistant infections and at least 23,000 are dying as a result of these diseases in the US alone. One of the four guidelines recommended by CDC to combat antibiotic resistance is promoting the development of new antibiotics and developing new diagnostic tests for resistant bacteria. Pyrazoles (1,2-diazole) are among the privileged heterocycles in drug discovery. Many pyrazole derivatives have been approved as drugs to treat various kinds of diseases. In our efforts to get potent antimicrobial agents, we have synthesized several pyrazole derivatives to test against various bacteria. We have used efficient synthetic methods and inexpensive reagents to rapidly generate a library of new molecules. These new molecules have been tested against both Gram-negative and Gram-positive bacteria. Our synthetic and biological studies of more hydrophilic compounds will be presented.

MEDI 127

Design, synthesis, and evaluation of a carbapenem antibiotic with improved activity against carbapenemase-producing *Klebsiella pneumoniae*

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Carbapenem resistant enterobacteriaceae (CRE) represent a serious clinical challenge, with high mortality rates from *Klebsiella pneumoniae* infections in the US. While one approach is the administration of β -lactamase inhibitors together with existing β -lactam antibiotics, another potential strategy may be the design of carbapenem antibiotics less susceptible to prevalent carbapenemases. A series of atypically substituted (C6 and C5) carbapenems were prepared and evaluated against susceptible and carbapenemase producing Gram negative pathogens, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, and *Klebsiella pneumoniae*, using a meropenem comparator. One of the compounds, WR-3-15, demonstrated 2-fold improvement over meropenem against *K. pneumoniae* producing KPC-2 (MIC 8 mg/ml versus 16 mg/ml), 4-fold improvement over *K. pneumoniae* producing KPC-3 and CTX-M-14 (MIC 1 mg/ml versus 4 mg/ml), and better than 2-fold improvement (MIC 16 mg/ml versus >32 mg/ml) against *K. pneumoniae* producing NDM-1.

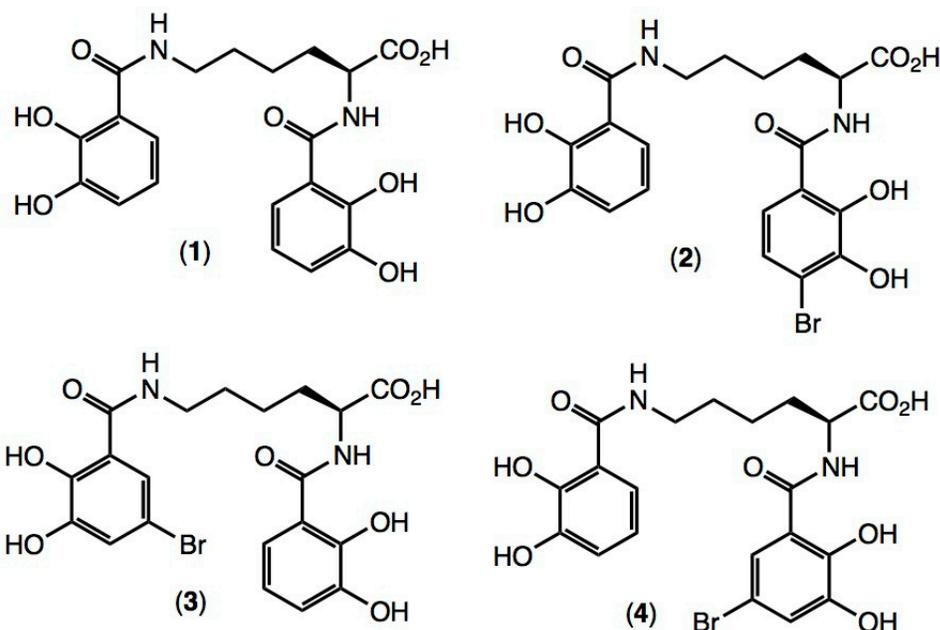
MEDI 128

Development of azotochelin analogues as potential antibacterial leads

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Siderophores (Greek: "iron carrier") are biosynthesized by bacteria and fungi for ferric ion uptake through chelation in iron deficient environment. These organisms require iron as an essential element in variety of cellular pathways. As current antibiotics are losing effectiveness against drug-resistant bacteria, it is of prime need to identify new antibiotic leads. It has been proved that the analogues of siderophores like brominated enterobactin show promising antibacterial activity through inhibition of enzymes involved in the biosynthesis of enterobactin-type siderophores. Siderophores like azotochelin which are also catechol-containing molecules are potential candidates as antibacterial agents. We have synthesized the natural azotochelin (**1**), and brominated analogues of azotochelin (**2** - **4**) using electrophilic aromatic halogenation approach. Since brominated azotochelin derivatives are synthetically useful precursors for further structure modification through Pd-catalyzed cross coupling reaction, we plan to synthesize several second generation analogs through this method. The future goal will

be to screening these analogs for their antibacterial activity against both Gram-positive and Gram-negative bacteria.



MEDI 129

Synthesis of 2'-C-methyl pseudouridines for the inhibition of HCV RNA polymerase

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Studies of the structure and function of Hepatitis C Virus (HCV) RNA-dependent RNA polymerase (RdRp) have broadened our understanding of HCV viral RNA replication and the mechanism of action of this RNA polymerase. These findings have encouraged the development of inhibitors of this target for antiviral therapy. Anti-HCV activity has been shown *in-vivo* with C-nucleosides containing a 2'-C-methyl (Me) substituent. The presence of the 2'-C-Me group prevents the chain elongation catalyzed by the RdRp NS5B. To further investigate this phenomenon, the synthesis of modified pseudouridines was performed using earlier developed strategies for the unmodified nucleoside. By coupling of the protected pyrimidine to a likewise protected 2'-C-methyl-D-ribo-lactone the C-nucleoside was formed. The impact of both the 5' and base protecting groups were also investigated to determine the effectiveness on the structure. Subsequent reduction and ring closure generated alpha and/or beta- 2'-C-Me pseudouridines. Through the work presented here, this modified pseudouridine synthesis was optimized and will be utilized in the synthesis of other modifications of this naturally occurring nucleoside and

evaluated for their antiviral activity. This will include conversion to substrates suitable for the monophosphate prodrug strategy.

MEDI 130

Boronic acid analogs of anti-HIV therapies: Synthesis and biological evaluation

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Boronic acids and their derivatives have recently emerged as biologically interesting moieties, with increased attention in their use as pharmaceutical agents. To date the FDA has approved two drugs containing boron atoms: Velcade, a peptidyl boronic acid treatment for multiple myeloma, and Kerydin, an oxaborole-containing antifungal. The purpose of this work is to provide further insights into boronic acids as a medically relevant functional group, specifically as phosphate isosteres. To this end, we created a library of boronic acid analogs of nucleoside monophosphates and phosphonates for examination as antiviral agents. These analogs were synthesized through substitution reactions between nucleobases and boron-containing alkyl halides, which were initially difficult to obtain. We therefore developed comparatively high yielding procedures (65-92%) of potassium haloalkyltrifluoroborate salt (TFBS) electrophiles through hydroboration of commercially available haloalkenes with dichloroborane, followed by treatment of the crude hydroboration products with potassium hydrogen difluoride. A hexaethylidisiloxane byproduct that hinders the isolation of boronic acids and esters was identified and easily removed in this procedure. Biological evaluation of these compounds as anti-HIV agents has revealed several leads that do not exhibit cytotoxicity.

MEDI 131

Synthesis of solithromycin analogues with acyclic desosamine surrogates

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Antibacterial resistance is becoming a severe widespread problem. One of the main mechanisms of resistance to macrolide antibiotics is based on methylation of A2058 by the methyltransferase encoded in *erm* genes. Methylation or dimethylation of A2058 leads to a steric clash with the macrolides and reduces the affinity of erythromycin for the ribosome.

Therefore, in order to reduce the effect caused by methylation, we need to reduce the

steric clash between desosamine and A2058. Based on this theory, a new class of macrolide antibiotics, ketolides was developed. Ketolides removed cladinose and replaced a ketone on the C3 position in order to leave some space for desosamine to rotate.

This idea is proved by solithromycin, a ketolide currently under phase III clinical trial. Solithromycin showed better anti-resistant potency. However, to get a better antibiotic, we need to apply more modifications.

One way to further release the steric clash is to remove the desosamine six member ring. However, this may result in less affinity since the restriction from six-member ring helped amine head to the right direction. To figure out whether desosamine is crucial to the interaction, we modified the desosamine.

Synthetically, we removed desosamine from solithromycin azide following Optimer's deglycosylation protocol: Swern oxidation of the 2'-OH followed by methanolysis. With free C5 hydroxyl product in hand, we applied the Pd-catalyzed allylation. MCPBA oxidation of terminal alkene formed only one diastereomeric due to the steric hindrance on the other side, and then treated with secondary amine immediately to give the regioselective ring-opening product. At last, the azide coupled with 3-ethynyl aniline to furnish desosamine analogues.

As a result, the Minimum inhibitory concentration of the analogues with dimethyl amine, morphine, piperidine, pyrrolidine are higher than solithromycin. This means the desosamine six-member ring played an important role supporting its amine binding to the A2058. Some other method should be used to reduce the steric clash.

MEDI 132

Structural characterization and inhibition of shikimate kinase from methicillin resistant *Staphylococcus aureus* through homology modeling and molecular docking simulations

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Staphylococcus aureus, the principal agent in nosocomial infections worldwide, is a particularly virulent microorganism that is resistant to antibiotics, the main impact is due to methicillin resistant strains of *S. aureus* (MRSA) commonly found in hospitals, creating an increased necessity to develop a new antibacterial therapy. In this context, shikimate kinase (SK), an essential enzyme in the shikimic acid pathway involved in the biosynthesis of aromatic compounds, has been considered as an excellent molecular target for antibacterial drug design. In this study, shikimate kinase from methicillin

resistant *S. aureus* (SaSK) was characterized *in silico* and a virtual screening protocol was applied to identify new potential inhibitors. After construction and validation of SaSK 3D model through homology modeling, using five different programs, structural analyses revealed the existence of typical three domains (the CORE, the LID, and the NMP-binding domain) presented in other bacterial SKs. On the other hand, a virtual screening strategy, using Glide software, was performed into the active site of the enzyme. The small molecules “Drug like” subset of the ZINC database was employed. Analyses of the three molecules with the highest binding energy showed that compound ZINC34616948 (-7.535 Kcal/mol) made hydrogen bonds with Asp117, Glu57, Arg138 and Arg61; while ZINC03162994 (-6.623 Kcal/mol) formed hydrogen bonds with Asp37 and Asp117, a salt bridge with Asp37, and a π - π stacking with Phe60. Compound ZINC70632388 (-6.257 Kcal/mol) made hydrogen bonds with Arg61 and Arg138, a salt bridge with Glu57, Arg61, and Arg138. Finally, drug likeness and toxicity scores indicated that according to their chemical structure, all of them have the potential to become drugs. Therefore, these molecules are possible inhibitors of SaSK and could serve as a guide in the search of a new chemotherapy against nosocomial infections caused by bacteria.

MEDI 133

Computer assisted drug design to find potential inhibitors of phosphoglycerate mutase 1 from *Plasmodium falciparum*

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Plasmodium falciparum, the causative agent of malaria, has developed resistance to current treatments, in this regard; it is necessary to develop new drugs that can solve this problem. In view of the parasite is entirely dependent on glycolysis as the sole source of energy, enzymes from this pathway, such as phosphoglycerate mutase (PGAM), are considered excellent targets to find new inhibitors in the search of new drugs against malaria. The aim of this work was to find new potential inhibitors of *P. falciparum* PGAM1 (PfPGAM) through computer assisted drug design. The search was performed applying a virtual screening strategy with Glide software (www.schrodinger.com), having the catalytic site as target. The crystal structure of PfPGAM (PDB ID: 1XQ9) and the library of small molecules from ZINC database were used. From the molecules docked, compounds ZINC15782377, ZINC64219552, and ZINC39095354 were the best ranked. According to an extra precision docking, the binding energy from these molecules was -7.303, -6.113, and -4.191 Kcal/mol, respectively. Hydrogen bond and cation- π interactions were formed between these molecules and residues from the catalytic site. A predicted drug likeness score of -0.76,

-0.84 and -0.70 for ZINC15782377, ZINC64219552, and ZINC39095354 was obtained, respectively. In conclusion, these molecules have the potential to inhibit PfPGAM, and could be considered as hits to obtain new antimalarial drugs.

MEDI 134

Structure-based drug design (SBDD), synthesis and evaluation of peptides inhibitors of Y-49 β -lactamase from *Mycobacterium tuberculosis*

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There is a persistent need to discover novel inhibitors to combat β -lactamase-mediated antibiotic resistance. Herein, we report a structure-based design approach for the discovery of potential tetrapeptides inhibitors of Y-49 enzyme, a class A beta-lactamase, from *Mycobacterium tuberculosis*. The tetrapeptide scaffold for the inhibitors was derived from the original sequence RRGHYY which was found to inhibit class A *Bacillus anthracis* Bla1, ($K_i = 42 \mu\text{M}$) and class A TEM-1 β -lactamase, ($K_i = 136 \mu\text{M}$) (Huang W et al., *Protein Eng Des Sel* 16:853-860). *In silico* docking experiments using *Autodock Vina* and *SCULPT* were performed using *M. tuberculosis* β -lactamase protein target 1YM1.pdb and different tetrapeptides 2HN-R-X-H-Y-CONH₂, where X was varied with all 20 natural L- and -D-amino acids. Our initial structure-activity relationship (SAR) studies established that acidic and basic amino acids (such as Asp, Lys and Arg) and small neutral like Gly occupying the X-position (P2) would favor a lower μM inhibitory constant (K_i). As such, tetrapeptide RRHY had K_i of 5.1 μM while the tetrapeptides RDHY and RGHY had K_i of 6.3 μM and 5.5 μM respectively. We propose a new tetrapeptide derived pharmacophore which could be used for further designing of linear and cyclic peptides with D- and unnatural amino acids with improved anti-beta lactamase activity.

MEDI 135

Development of inhibitors of the di-zinc metallo beta-lactamase NDM-1

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The need for new antibiotic treatments has increased as more bacteria acquire antibiotic resistance. One of the primary methods through which bacteria acquire antibiotic resistance is through uptake of foreign DNA containing genes coding for

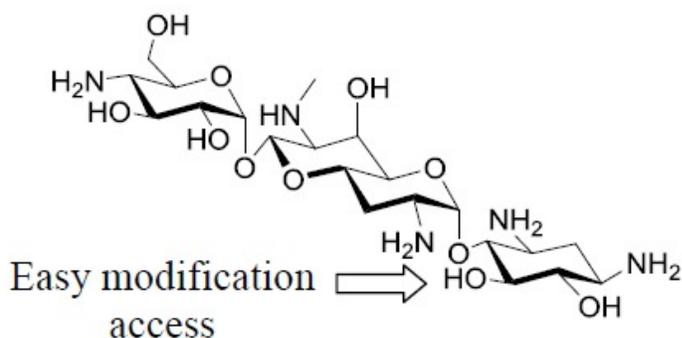
metalloenzymes which deactivate antibiotics. One such category of metalloenzymes is the beta-lactamases, which destroy beta-lactam antibiotics. Perhaps the most worrisome in this category is New Delhi metallo-beta-lactamase-1 (NDM-1), our chosen target. Our research focuses on the synthesis and optimization of inhibitors of NDM-1 that are designed to restore antibiotic efficacy. The divergent synthetic approach utilizes a 3-component copper-catalyzed coupling from a common indoline sulfonyl azide intermediate employing conditions described by Bae to prepare sulfonyl amidines, sulfonyl triazoles, and acyl sulfonamides (Bae et al. 2005). Using this chemistry, we have synthesized single-digit micromolar inhibitors of NDM-1. In addition to variation derived from the the sulfonyl azide coupling, we also report a synthetic strategy to vary substituents around the indoline scaffold. Thus, in the design of more potent NDM-1 inhibitors, we have the opportunity of developing a new therapeutic agent to be co-administered with beta-lactam antibiotics, restoring their efficacy.

MEDI 136

Design, synthesis, and evaluation of improved apramycin derivatives for the treatment of MDR infectious diseases

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Apramycin is a broad spectrum aminoglycoside antibiotic that is not susceptible to inactivation by most aminoglycoside modifying enzymes and which has minimal ototoxicity in animal models. With a view to further improving the profile of apramycin we have investigated derivatization at the 5-position. In this poster we describe an improved route for modification at the 5 position, and the synthesis and evaluation of various apramycin derivatives at that position including a number of glycosides and other derivatives. These derivatives give a better understanding of apramycin binding to the bacterial ribosomal decoding A-site useful in the design of more active and less ototoxic derivatives.



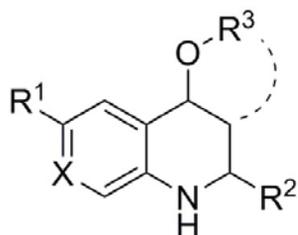
MEDI 137 – Withdrawn.

Fragment-based design, synthesis, and binding of non-peptide mimics of NS4A and their binding to HCV NS3/4A protease

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Since the approval of our project in 2012, the number of marketed Direct Acting Antivirals (DAAs) have grown dramatically. Their efficacies have been broadened to treat patients with other HCV genotypes than genotype-1. The advances brought a new era of Interferon-free HCV therapy (Second generation DAAs). Three new drugs and combinations have shown efficacy against HCV genotype-4, the prevalent viral strain in the Middle East. However, according to several reports in 2015, resistance is emerging against the new DAAs in a fast pace. In addition, adverse effects complicated the use of several new medicines especially the first generation DAAs.

In this research, we aim to validate the NS4A as a target for development of a new generation of NS3/4A inhibitors. The *de novo* computer-aided design tools were employed to rationalize the potentiality of peptidomimetic 1,2,3,4-tetrahydroquinoline and 1,2,3,4-tetrahydro-1,7-naphthyridine scaffolds as mimics of NS4A core moiety. The compounds were synthesized using accessible Povarov reaction. These allosteric fragments were tested for their binding to HCV-NS3 protease using label-free aggregation temperature change technique on Stargazer-2 instrument. Compared to NS3 control, Compound **1** (100 μ M) caused shift in the mid-point melting temperature (T_m) of the protein (5 μ M) from 42 to 45 °C while compound **2** caused no significant change indicating good binding of compound 1 with target protein (NS3).

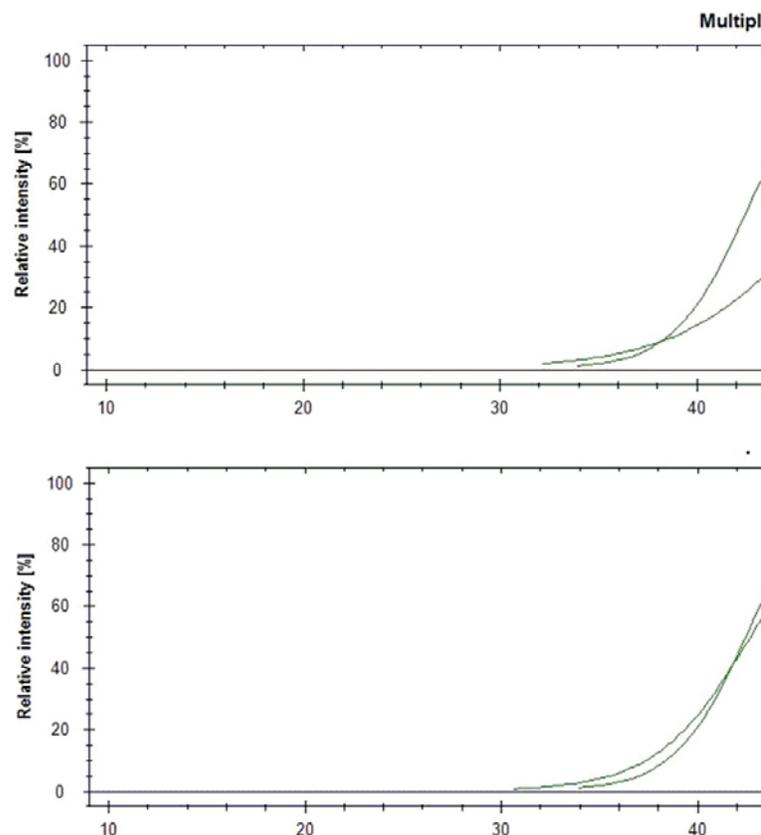


R¹ = H, NHBoc

R² = Ph, C₆H₅, n-Pr

R³ = Et, tetrahydrofuro, tetrahydropyrano

X = CH, N



MEDI 139

Synthesis and development of the endophenazines as new antibacterial drugs

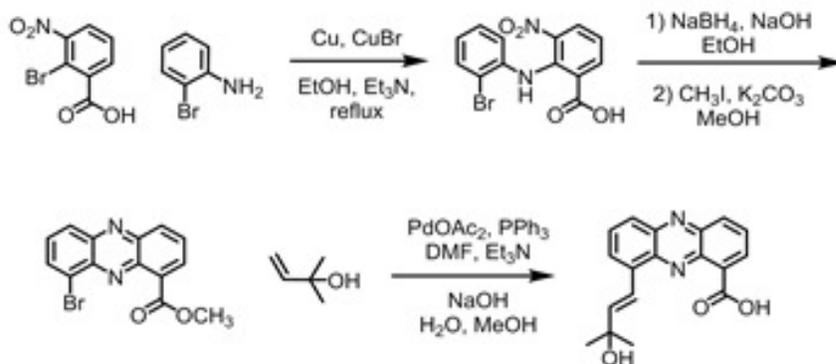
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The emergence of resistant bacterial strains is a real problem to human health. A problematic bacterial strain is MRSA (methicillin resistant staphylococcus aureus), a type of infection resistant to many current antibiotics.

The identification of new drugs that can prevent and inhibit MRSA growth is key to solve this health challenge. The endophenazines are heterocyclic antibiotics of natural origin that have shown promising results against various bacteria including MRSA.

This poster presents the design and a concise synthesis of the endophenazine natural products. In order to develop structure-activity relationships, we prepared a series of structural analogues. The biological evaluation and the physical properties of the synthesized compounds are presented. Some of the endophenazines present MIC in the low micromolar range and limited toxicity against mammalian cells. The obtained data indicates the potential of the endophenazines as potential new drugs against MRSA.

Scheme 1. Synthesis of the Endophenazine G



MEDI 140

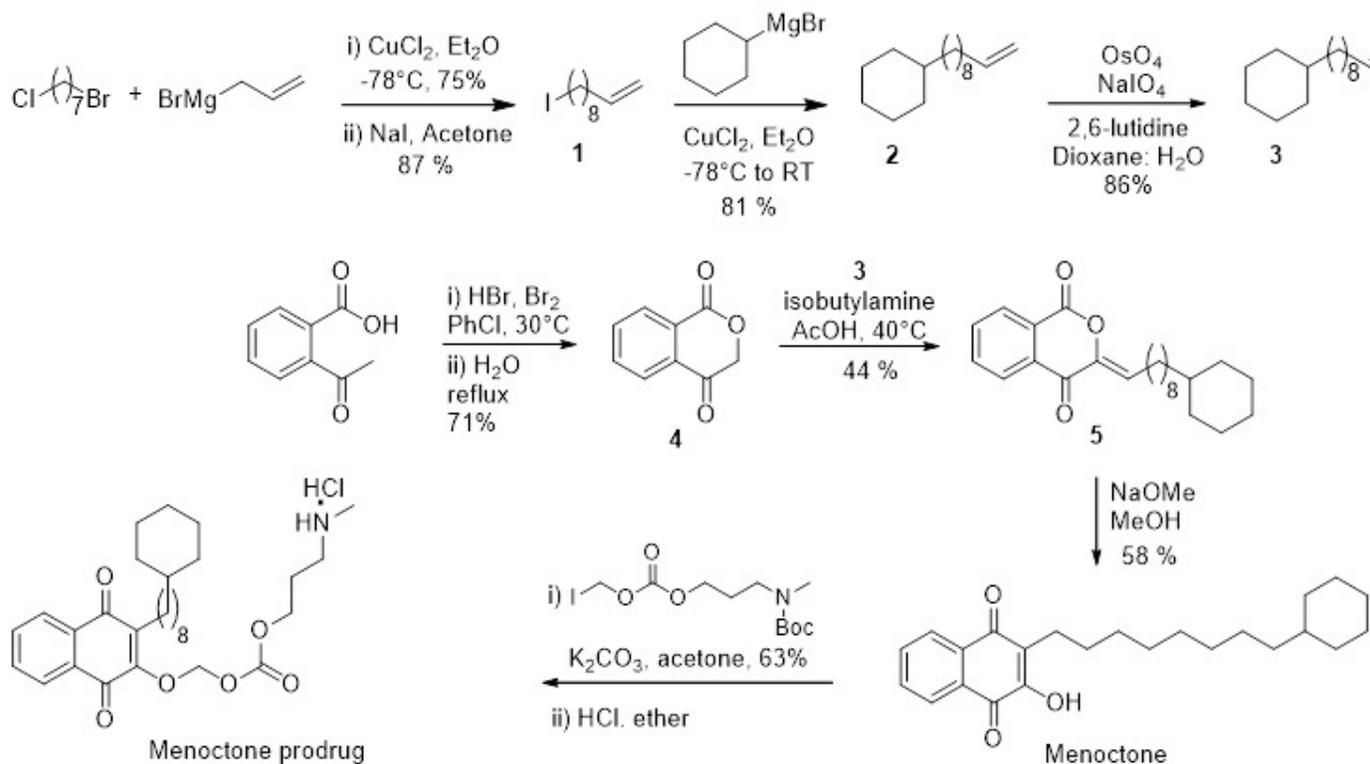
Characterization of menoctone efficacy against *Plasmodium berghei*

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Previously, menoctone demonstrated suppressive activity against blood-induced rodent malaria and was tested against sporozoite-induced *Plasmodium berghei* infections. Its development was abandoned due to its lack of aqueous solubility and poor bioavailability. In this study we report the synthesis and efficacy studies of menoctone and its prodrug.

The synthesis of menoctone involves successive copper catalyzed Kumada couplings followed by an oxidation to obtain the long chain aldehyde, which was subsequently condensed with isochromandione and rearranged to menoctone. The prodrug of menoctone was also synthesized to examine the enhancement in its solubility and oral bioavailability.

We used luciferase expressing *P. berghei* infections in HepG2 and found menoctone to be extremely potent (IC₅₀ = 0.41 nM). In these assays, menoctone was ~ 3 fold more potent than atovaquone. Previously, Peters and colleagues generated a menoctone resistant line of *P. berghei* (MEN). We first assessed the potential for cross-resistance between menoctone and atovaquone in the MEN line and found incomplete resistance. Currently, we are expanding these studies to characterize menoctone efficacy in *P. falciparum* and the potential mechanism(s) of resistance to menoctone in both *P. berghei* and *P. falciparum*.



Synthesis of menoctone and menoctone prodrug.

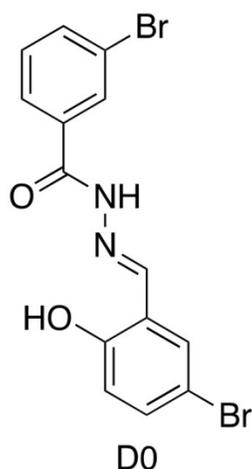
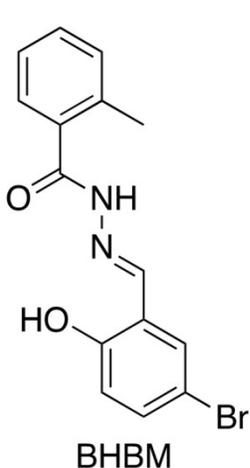
MEDI 141

SAR study of novel anti-fungal agents targeting the synthesis of fungal sphingolipids

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According to recent statistics, more than 300 million people are affected by serious fungal infections globally. Current drugs have a variety of drawbacks including drug-drug interactions, toxicity and narrow spectrum of activity. With the emergence of fungal infections resistant to current drugs, there is an urgent need for the development of new antifungal agents with novel mechanisms of action. In this context, fungal sphingolipids that are essential for their virulence is a potential target for the development of new

antifungal agents. Starting from the screening of a library of 49,120 compounds from ChemBridge against *C. neoformans*, followed by in vitro and in vivo assays, including the inhibition of the synthesis of the fungal but not of the mammalian sphingolipid glucosylceramide, two compounds, BHBM and D0 were identified first as hits with minimum fungicidal concentrations of 4 $\mu\text{g/mL}$ and 1.2 $\mu\text{g/mL}$ respectively. Further screening found three more hit compounds, which exhibited potent antifungal activity (Minimum Inhibitory Concentration (MIC_{80}) of 0.03 $\mu\text{g/mL}$). Based on these results, a new library of aromatic acylhydrazides has been designed and synthesized for SAR studies. Synthesis, biological activity, and SAR study of the new library of anti-fungal acylhydrazones will be presented.



MEDI 142

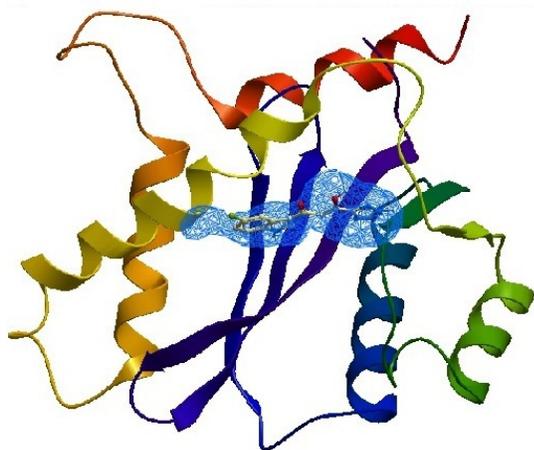
Estimated binding energies of molecules in the active site of HIV-1 integrase (1BIS.pdb): Results of drug-like and nondruglike molecules with consideration of mutations of 1BIS.pdb using ICM-Pro (Molsoft L.L.C.)

Julie B. Ealy, jbe10@psu.edu, Habib Yazgi, Noorhaan Abouomar, Justin Cogan. Chemistry, Penn State Lehigh Valley, Nesquehoning, Pennsylvania, United States

The Centers for Disease Control and Prevention (CDC) in Healthy People 2020 addressed 10-year national objectives to improve the health of Americans. Eighteen of the objectives focus on HIV and the acquired immunodeficiency syndrome (AIDS). The primary goal of Healthy People 2020 as it relates to HIV/AIDS is to prevent HIV infection and its related illness and death. Since there are approximately 56,000 new HIV infections each year in the United States interventions are needed such as the development of new drugs that can prevent transmission as well as disease progression. These interventions are of paramount public health importance, and targeting a critical viral enzyme is a proven approach to meet this need.

After examination of approximately 200 molecules identified as possible inhibitors of

HIV-1 integrase and consideration of their strand transfer values (≤ 87 nanomolar) as obtained from the literature, approximately 30 molecules were chosen to be docked into the active site of HIV-1 integrase (1BIS.pdb). Questions to be answered: Is there a difference in the estimated binding energies of the drug-like and nondrug-like molecules? Is there a relationship between the strand transfer value and estimated binding energy of the drug-like molecules, and separately, of the nondrug-like molecules. When the protein is mutated computationally is the estimated binding energy affected for the either the drug-like or nondrug-like molecules?



1BIS.pdb with 5citep Docked in the Active Site Pocket
Image from ICM-Pro (Molsoft L.L.C.)

MEDI 143

Dipicolinic acid derivatives as inhibitors of New Delhi metallo- β -lactamase-1 (NDM-1)

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β -lactams (penicillins, cephalosporins, and carbapenems) are the most widely prescribed antibiotics for combating a multitude of infections. However, the overuse of β -lactams has led pathogenic bacteria to generate various resistance mechanisms, including an ability to hydrolyze the β -lactam ring and render these drugs ineffective. While classes A, C, and D β -lactamases utilize serine as the key catalytic residue, class B, metallo- β -lactamases (MBLs), utilize one or more site Zn(II) ions at the active site. The class B strain of primary interest, New Delhi metallo- β -1 (NDM-1), is believed to be the most clinically concerning isoform, conferring resistance to all classes of β -lactams. NDM-1 is often carried on plasmids capable of horizontal gene transfer, escalating the

spread of NDM-1 between different strains of bacteria. There is currently no available treatment for this resistance mechanism. To date, there are only a small number of NDM-1 inhibitors; however, these compounds generally exhibit weak activity and inconsistent Structure-Activity Relationship (SAR) trends.

Herein, we describe a novel approach for the systematic design of a potent NDM-1 inhibitor. A metal-binding pharmacophore library (~300 compounds) was screened against NDM-1 and revealed dipicolinic acid (DPA) as a novel scaffold for inhibitor development. To identify key active site interactions, a fragment growth strategy was utilized where DPA was derivatized at various positions with various substituents. Utilizing several cycles of a fragment growth, computational modeling, and enzymatic screening, potent NDM-1 inhibitors have been identified. Ultimately, this fragment-based approach is expected to facilitate the identification of a potent NDM-1 inhibitor that can re-potentiate β -lactam drugs to multidrug resistant bacterial infections in animal models.

MEDI 144

Towards enhanced treatment of tuberculosis: Discovery and development of indole-2-carboxamide scaffold

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In our efforts to improve the current chemotherapy repertoire against tuberculosis (TB), we have identified and advanced the indole-2-carboxamide scaffold. The best compound in this series showed impressive activity against *Mycobacterium tuberculosis* (*Mtb*), including activity against both the multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB. The optimized lead compound acts as a likely inhibitor of the mycolic acid transporter MmpL3 in *Mtb* (Figure 1). The lead molecule showed desirable ADMET profile including good oral bioavailability, metabolic stability, lack of activation and inhibition of Cytochrome P450, and selectivity index over 16000. The lead is efficacious in reducing the count of colony-forming units (CFU) using the TB aerosol lung infection model in mice. Additionally, the lead showed favorable pharmacokinetics parameters in plasma and lungs. The findings that the best compound also works synergistically with rifampin may imply that treatment regimens including indole-2-carboxamide and lower rifampin doses could be efficacious. Homology model for the MmpL3 transporter has been generated and provides further insights into the structure-

activity relationships (SAR) of the indole-2-carboxamides.

Based on these findings, the best indole-2-carboxamide analog identified in this research represents a possible candidate to advance to the clinic with the aim to improve the current regimen of TB treatment.

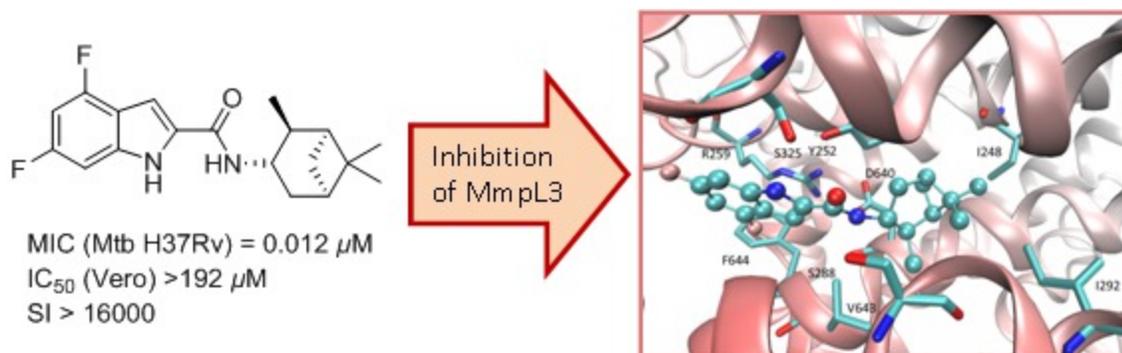


Figure 1. Optimization of indole-2-carboxamides as inhibitors of MmpL3

MEDI 145

8-Hydroxyquinoline as a scaffold for the development of New Delhi metallo- β -lactamase-1 Inhibitors

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β -lactam drugs have historically been the most successful and commonly prescribed class of antibiotics. However, the widespread use of these drugs has created a natural selection pressure for bacteria to adapt and develop resistance mechanisms to this class of therapeutics; most notably the emergence of β -lactamase enzymes. β -lactamases confer bacterial resistance through hydrolysis of the β -lactam bond that is critical for the activity of these drugs. New Delhi Metallo- β -lactamase (NDM-1) is a particularly worrisome Class B β -lactamase that utilizes two active site Zn²⁺ ions to achieve this hydrolytic activity. NDM-1 has been demonstrated to be capable of hydrolyzing all clinically relevant bicyclic β -lactams, including the carbapenems – which are often used as a last resort against drug resistant infections. Since its initial discovery in India in 2008, NDM-1 has spread among the general population and has not been limited to hospital-acquired infections. NDM-1 is easily communicable between bacteria populations because it is encoded in *bla*_{NDM-1}, a plasmid capable of horizontal transfer, and has already been detected in multiple strains of enterobacteriaceae. In addition to this ease of transmission, there are currently no clinically approved drugs available against NDM-1, implicating that NDM-1 has a strong potential to lead to “superbug” type infections.

The goal of this research is to develop potent inhibitors of NDM-1 that restore β -lactam

activity against resistant bacteria. In order to meet this goal, we have synthesized and screened metal binding pharmacophore (MBP) libraries against NDM-1. This screen revealed 8-hydroxyquinoline analogs, 5-chloro-8-hydroxyquinoline and 2-carboxyl-8-hydroxyquinoline, as novel scaffolds for the development of NDM-1 inhibitors. Based on these results from our MBP fragment screening, our research is focused on derivatizing 8-hydroxyquinoline in a fragment growth strategy in order to discover potent and effective NDM-1 inhibitors.

MEDI 146 – Withdrawn.

MEDI 147

Synthesis of novel 2-methoxylated fatty acids as effective inhibitors of clinical isolates of methicillin-resistant *Staphylococcus aureus* (CLMRSA)

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Previous research from our group has demonstrated that 2-methoxylated unsaturated fatty acids can be good candidates to display interesting antimicrobial, antibacterial, and anticancer properties. In this work we undertook the total synthesis of a series of racemic 2-methoxylated unsaturated fatty acids and tested them against methicillin-resistant *S. aureus* isolates. Among the series, the novel acid (\pm)-2-methoxy-6-octadecynoic acid (**1**) stood out as it displayed one of the best activities against MRSA at an $IC_{50} = 17 \mu\text{g/ml}$. The antibacterial activity of the novel 2-methoxylated acids correlated quite well with their critical micelle concentrations (CMC) where chain length was a critical factor. It was also found that, in general, 2-methoxylation tends to lower the CMC of fatty acids as **1** displayed a lower CMC (15-18 $\mu\text{g/ml}$) as compared to 6-octadecynoic acid (20-30 $\mu\text{g/ml}$). In addition, the combination of the 2-methoxy and acetylenic functionalities reduces the calculated hydrophobicity indexes (cLogP) of these compounds as compared to their olefinic analogs. These results, in combination with future directions in this field, will be presented.

MEDI 148

Orally bioavailable antimalarial 4(1H)-quinolone prodrugs with single-dose cures

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Malaria is estimated to have caused 438,000 deaths and 214 million cases of the disease globally in 2015. Four strains of *Plasmodium* parasite cause malaria in humans and the disease is transferred by *Anopheles* mosquitos. Though mortality rates are down 47% globally since 2000 and significant progress has been made in the quest for eradication, reported occurrences of resistance against current therapeutics threaten to reverse that progress. Longstanding treatment chloroquine has seen resistance since the 1950's, with resistance becoming widespread in the 70's and 80's. Artemisinin, the current main line of defense against malaria, is used in artemisinin combination therapies (ACTs) in order to curtail resistance, though at last count, artemisinin resistant parasites have been reported in 5 countries of the Greater Mekong sub region. In order to curb further resistance, it is essential that new antimalarial compounds be brought through the pipeline.

For approximately half a century, 4(1*H*)-quinolones such as endochin or ICI 56,780 were known to be causal prophylactic and potent erythrocytic stage agents in avian but not in mammalian malaria models. Hit-to-lead optimization of endochin lead to 4(1*H*)-quinolones ELQ-300 and P4Q-391, which target the liver, the blood as well as the transmitting stages of the parasite. Despite entering preclinical development, ELQ-300 did not enter phase I trials due to limited aqueous solubility and high crystallinity.

To overcome these limitations, we designed and developed a prodrug approach containing an amino group linked to the parent 4(1*H*)-quinolone by an acetal carbonate group. Different reaction conditions were found to attach the prodrug moiety selectively onto the oxygen or the nitrogen of the 4(1*H*)-quinolone scaffold. The resulting O-alkylated prodrugs P4Q-1290 and P4Q-1291 were profiled for physicochemical properties such as chemical stability and aqueous solubility. The prodrugs are stable at low pHs and start releasing the parent drug independently of any enzyme activity at a pH level of about 7. Furthermore, prodrugs P4Q-1290 and P4Q-1291 were highly efficacious in *in vivo* efficacy assays displaying single-dose cures at low doses.

The new discoveries are significant as mitochondrial inhibitors have the potential to advance the malaria elimination campaign by blocking parasite development in the blood and liver, as well as preventing transmission to mosquitoes.

MEDI 149

Synthesis and kinetic characterization of mechanism-based inhibitors of tubercular BioA

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Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (*Mtb*), is the leading cause of mortality among infectious diseases worldwide. The emergence of multidrug-resistant (MDR-TB) and extensively drug-resistant (XDR-TB) strains necessitates the

development of new antibacterial agents, ideally with novel mechanisms of action. Mechanism-based inhibitor **1** has been shown to kill *Mtb* in biotin-deprived conditions through inactivation of BioA, a PLP dependent enzyme in the biotin biosynthetic pathway. The rational optimization of mechanism-based inhibitors is challenging since standard equilibrium dissociation constants cannot be used, due to the irreversible nature of inhibition. Rather, one must optimize microscopic rate constants of the individual steps involved in ageing the enzyme. The inactivation of BioA by **1** is a four-step process, beginning with binding of **1** to the PLP and continuing with three more transformations before an irreversible PLP adduct is formed. Each step shows a unique absorbance signature, allowing for investigation of the kinetics using stopped-flow measurements. Preliminary analysis suggests the kinetic bottleneck occurs within the second step, suggesting that a new warhead should be designed that can be more easily deprotonated. Using this information, three new inhibitors that lower the pK_a of the alpha proton were designed. The synthesis of each of these will be described along with their comprehensive biochemical and biological characterization.

MEDI 150

Towards the synthesis of novel 1,3-azaborines as potential HIV-1 protease inhibitors

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Recent studies have shown that boron-modified inhibitors have a higher affinity for the protease than their corresponding non-boronated analogs and have inhibitory affinity towards an HIV-1 protease variant that is resistant to several HIV-1 protease inhibitors. The main goal is to synthesize a library of both straight chain and cyclic boronates that potentially have dual-mode, competitive and associative, inhibitory action against HIV-1 protease. The borinic acid target compounds are chiral 1,3-azaborines that will potentially have a greater affinity for the protease enzyme, better bioavailability, less toxicity, and fewer side effects. Both straight chain and cyclic boronates are being synthesized which will serve to expand molecular diversity, as well as organoboron chemistry in general. Due to the structural rigidity of cyclic boronates, they are expected to be better inhibitors than their straight chain analogs.

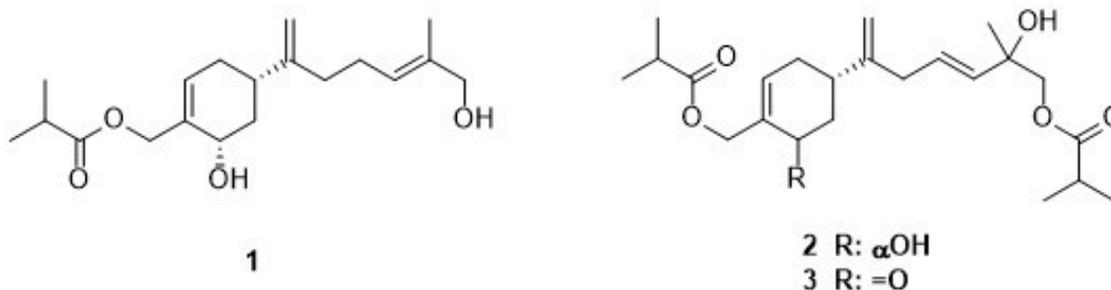
MEDI 151

New bisabolenes isolated from *Calea urticifolia*

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University, Mississippi, United States (3) Facultad de Química y Farmacia, University of El Salvador, San Salvador, El Salvador

Calea is a diverse genus in the Asteraceae family with close to 120 species distributed around the tropical and subtropical areas of North, Central and South America. Several species of *Calea* have been used in traditional medicine for hundreds of years for the treatment of diarrhea, fever, as a laxative and for its calming effects. As a part of our continuing search for novel, plant-derived bioactive agents, *Calea urticifolia* commonly identified in El Salvador as “Juanislama” was chemically studied. Three new sesquiterpenes with skeleton of bisabolanes, caleanolanes A-C [1-3] were isolated. The structures of compounds 1-3 were determined on the basis of HR-MS, and 1D- and 2D- NMR studies and its configuration was partly established by GIAO 13C-NMR, supported by DP4 probability analysis, and ECD calculations.



MEDI 152

Inhibition of phosphoglycerate mutase from *Entamoeba histolytica* by benzimidazole derivatives

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Entamoeba histolytica is the causative agent of amebiasis, it is estimated that 50 million people get sick each year worldwide. Because of adverse reactions and their treatment failure, it is necessary to develop new antiamebic drugs. The parasite uses glycolysis as their only source of energy, enzymes of this route, such as phosphoglycerate mutase (EhPGAM), are considered excellent targets to search new drugs. The aim of this work was to evaluate our in house library of benzimidazole derivatives as inhibitors of EhPGAM. Firstly, we cloned and overexpressed the *E. histolytica pgam* gen in *E. coli* strains, obtaining an outstanding band of approximately 64 kDa corresponding to the expected weight for EhPGAM. The enzyme was purified using immobilized metal-ion affinity chromatography and the protein was eluted with 90% purity. Around 300

benzimidazole derivatives were assessed at a concentration of 100 μM . The compounds designed as ARMD15e, CPM-2, and A-CBX-B4 were found that provoked an inhibition of approximately 70%. Docking studies using MOE software revealed that the three compounds formed hydrogen bonds with Arg169 and Arg205, and a cation- π interaction between the aromatic rings of the benzimidazole nucleus and the manganese ion in the enzyme. The inhibitors of EhPGAM also were probed in cultures of *E. histolytica* trophozoites, all the compounds had a CI_{50} in the nanomolar range, suggesting that they affected, into the parasite, something else besides EhPGAM. In conclusion, our findings indicated that these molecules could be used as hits to design more potent EhPGAM inhibitors and as a starting point to continue the process to obtain a new chemotherapy against amebiasis.

MEDI 153

Design, synthesis, and biological evaluation of pyrrolo[2,3-*d*]pyrimidines as potent and selective dihydrofolate reductase inhibitors and potential anti-opportunistic agents

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Pneumocystis pneumonia (PCP), caused by the fungus *Pneumocystis jirovecii* (*pj*), is one of the most common life-threatening opportunistic infections for HIV and non-HIV immunocompromised patients. *Pj* dihydrofolate reductase (*pj*DHFR) exhibits 38% amino acid sequence similarity to the DHFR enzyme in *P. carinii* (*pc*). Absence of a crystal structure of *pj*DHFR, difficulties in producing in-vitro cultures, and the lack of animal models have impeded drug discovery efforts to obtain selective *pj*DHFR inhibitors. First line therapy for PCP consists of trimethoprim (TMP), a weak *pj*DHFR inhibitor (co-administered with sulfonamides), but is limited due to severe side effects. Trimetrexate (TMQ) and piritrexim (PTX), which are potent, but non-selective *pj*DHFR inhibitors cause high rates of myelosuppression and require co-administration of leucovorin, increasing the cost of therapy and are not efficacious. Hence, it is highly desirable to develop single agent DHFR inhibitors that combine the potency of TMQ or PTX with the species selectivity of TMP. We previously reported a series of pyrrolo[2,3-*d*]pyrimidines that exhibited moderate potency (~ 200 nM) and selectivity (~ 10 fold) for *pj*DHFR versus human DHFR (*h*DHFR). Comparing the X-ray crystal structures of the lead pyrrolo[2,3-*d*]pyrimidine compounds in *h*DHFR and their docking in our *pj*DHFR homology model indicated a larger hydrophobic pocket in *pj*DHFR which could be exploited to increase potency and/or selectivity of pyrrolo[2,3-*d*]pyrimidine inhibitors of *pj*DHFR. A series of 7-substituted pyrrolo[2,3-*d*]pyrimidine analogs were designed and synthesized to explore this hydrophobic pocket. The best analogs displayed excellent potency (< 40 nM) and selectivity (~ 25 fold) for *pj*DHFR versus *h*DHFR. The design, synthesis, molecular docking and biological activity of these compounds will be presented.

MEDI 154

Synthesis and evaluation of boron-containing inhibitors of the non-mevalonate isoprenoid synthesis pathway

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With the increase in resistance of pathogens to common anti-infective agents, there is an urgent need to develop new classes of therapeutic molecules with novel mechanisms of action. The non-mevalonate isoprenoid synthesis pathway (MEP pathway) is essential to the survival of several pathogens including *Plasmodium falciparum* and *Mycobacterium tuberculosis*. This pathway has emerged as an attractive therapeutic target for drug design since it is not present in the human body.

Fosmidomycin, a phosphonate-containing natural product, is the most potent inhibitor of the MEP pathway to date. The molecular target of fosmidomycin is 1-deoxyxylulose-5-phosphate reductoisomerase (IspC), an enzyme which catalyzes the first committed step in this biosynthetic pathway. The phosphonate moiety mimics the phosphate of the natural substrate. However, the highly charged nature of this moiety hinders absorption and leads to poor pharmacokinetic properties. Therefore, fosmidomycin has found limited utility as a therapeutic agent. In an effort to overcome these issues, our work investigates the use of boronic acids and its analogs as neutral phosphate and phosphonate isosteres. Here, we report the synthesis of a small library of boron-containing analogs of fosmidomycin and their evaluation as inhibitors of IspC. Current work involves optimization of inhibitory assay conditions and docking studies to expand the compound library.

MEDI 155

Hydroxymethylnitrofurazone (NFOH) in chronic Chagas disease animal model

Caue B. Scarim¹, Rafael C. Consolin Chelucci¹, Isabel Martinez³, Elias Padilha⁴, João A. da Rosa³, Rosangela G. Peccinin⁴, Cleverton R. Andrade², Jean L. Santos¹, **Chung M. Chin**¹, *chungmanchin@gmail.com*. (1) Farmacos e Medicamentos, Faculdade de Ciências Farmacêuticas, Universidade Estadual Paulista "Júlio de Mesquita Filho, Araraquara, Sao Paulo, Brazil (2) Fisiologia e Patologia, Faculdade de Odontologia, Universidade Estadual Paulista Julio de Mesquita Filho, Araraquara, Sao Paulo, Brazil (3) Ciências Biológicas, Faculdade de Ciências Farmacêuticas, Universidade Estadual Paulista Julio de Mesquita Filho, Araraquara, Sao Paulo, Brazil (4) Produtos Naturais e Toxicologia, Faculdade de Ciências Farmacêuticas, Universidade Estadual Paulista Julio de Mesquita Filho, Araraquara, Sao Paulo, Brazil

There are two drugs for the treatment of Chagas` Disease worldwide: nifurtimox and benznidazole (BNZ), active only in the acute phase. Hidroximetilnitrofurazone (NFOH) is a prodrug of nitrofurazone (NF), effective against trypomastigotes and amastigotes forms

of *T. cruzi*, with low toxicity and increased biodistribution. The aim of this work was to evaluate NFOH in an *in vivo* chronic phase Chagas disease essay, using 10^2 trypomastigotes Y strains of *T. cruzi* inoculated in BALB/c mice. After no blood parasitemia comproved, the animals received NFOH (150 mg/kg) and BNZ (60 mg/kg) for 60 days followed by dexamethasone for 14 days. At the end, biochemical (ALT, AST, CK-MB, and GGT) and histopathological analysis of the organs were done. The results showed 55 % of reactivation in positive control and no parasitemia with NFOH and BZN. No biochemical alterations were observed in all all groups. Relative weights and macroscopic examination of the heart, gallbladder, spleen, and kidneys of NFOH group were similar to negative control while BZN group was similar to the positive control. The histopathological analysis showed no amastigotes in any organ/animal treated with NFOH. The treatment with BZN showed amastigoe nest in 50% heart/ (4/8) and in 12,5% liver (1 / 8) . The positive control showed several amastigotes nests in the heart (5/9) and also in liver (2/9). It was also observed the recovery of the intestinal plexus inflammation after treatment with NFOH. These data suggest that NFOH is a potential drug candidate for the treatment in chronic phase of Chagas Disease.

MEDI 156

Parmodulins: Biased ligands for protease-activated receptors (PARs)

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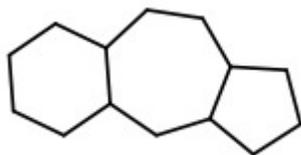
PARs are G-protein coupled receptors (GPCRs) that are activated by a variety of vascular proteases and mediate a range of both beneficial and potentially detrimental signaling pathways. Our progress in the study of the parmodylins, a class of PAR1 biased ligands with promising antithrombotic and cytoprotective activities, will be presented.

MEDI 157

Three-ring scaffold with rich biological activity but no commercial availability

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Many natural products contain the 6-7-5 ring scaffold. They come from a variety of sources: tropical fungi, marine algae and sea hares. Compounds with this ring system possess a broad range of biological activities: antitumor, antiangiogenic, antibacterial, antiviral, and antifoulant. Although compounds with the 6-7-5 ring scaffold are found in nature, they are not available commercially. We propose an explanation for the diverse biological activity of this class of natural products.



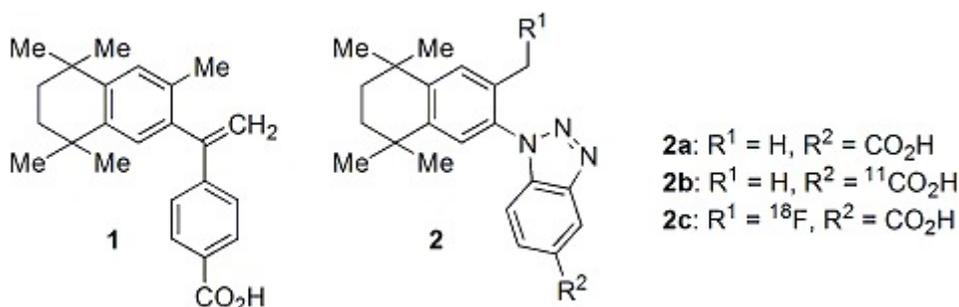
MEDI 158

Evaluation of brain migration and therapeutic effects of novel RXR partial agonist CBt-PMN on cognitive impairment in mice

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[Objective] Bexarotene (**1**), a retinoid x receptor (RXR) agonist, is used for the treatment of cutaneous T cell lymphoma (CTCL) and reported to show therapeutic effects against type 2 diabetes, Alzheimer's disease, and Parkinson's disease. However, **1** induces significant adverse effects such as blood triglyceride elevation and hepatomegaly in rodents. Since **1** is a RXR full agonist, which activates RXR completely, on the basis of our hypothesis that there is a threshold difference between the therapeutic effects and these adverse effects by RXR activation, we created RXR partial agonist CBt-PMN (**2a**, 1-[(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)amino] benzotriazole-5-carboxylic acid: $E_{max} = 75\%$, $EC_{50} = 143$ nM. CBt-PMN (**2a**) exerts the beneficial effects such as a potent glucose-lowering effect, improvements of insulin secretion, and glucose tolerance without serious side effects. In this study, we evaluated brain migration of **2a** using positron emission tomography (PET) imaging, and therapeutic effects on cognitive impairment in mice.

[Results] PET imaging of **2a** was performed by synthesizing [¹¹C]CBt-PMN (**2b**) and [¹⁸F]CBt-PMN (**2c**) as PET tracers. Intravenous administration of **2c** to mice showed migration to brain including hippocampus. At 1 hour after oral administration at 30 mg/kg of **2a** to mice, the brain concentration was 11.4 μ M whereas that of **1** was 5.6 μ M. Step-through test using 14-month-old SAMP8 dementia mice showed that **2a** induced more obvious improvement than **1**, and MRI revealed that **2a** improved hippocampal atrophy. LPS-induced nitric oxide production in RAW264.7 cells was inhibited by **2a** in accordance with its RXR activation. In addition, reporter gene assay using COS-1 cells showed that **2a** activated Nurr1/RXR heterodimers, similarly to **1**. These results indicate RXR partial agonist **2a** can be a new candidate of a therapeutic agent for cognitive impairment.



MEDI 159

Syntheses of 3-aminopiperidinone amides as CGRP receptor antagonists

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Calcitonin gene-related peptide (CGRP) is a potent vasodilator that has been implicated in the pathogenesis of migraine. Several clinical studies have validated the effectiveness of CGRP receptor antagonists in the acute treatment of migraine. Our CGRP receptor antagonist program focused on the development of a novel class of highly potent and orally bioavailable lactam amides. This presentation will describe a number of unique synthetic routes that were developed to prepare a series of 3-aminopiperidinone amides. Specifically, the talk will focus on novel approaches for the construction of highly substituted 3-aminopiperidinones and the introduction of methyl substitution at various sites on the lactam ring system, enabling the development of SAR studies. Highlighted will be asymmetric syntheses utilized in the preparation of leading molecules containing up to four chiral centers.

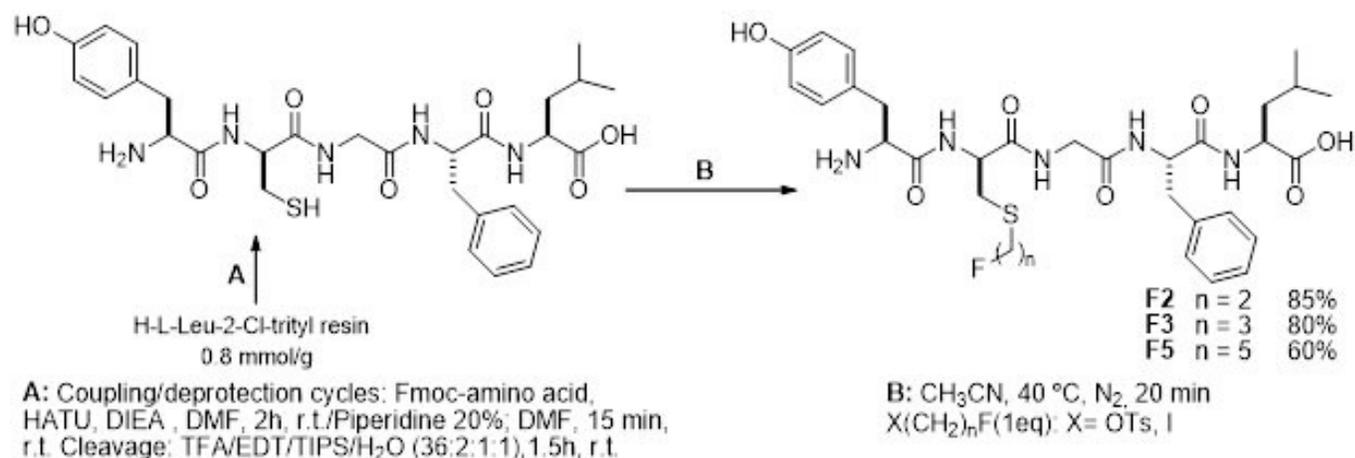
MEDI 160

Preparation and evaluation of a series of ${}^{19}F$ -enkephalin analogues: the first step in the design of potent and selective ${}^{18}F$ -labeled PET tracers for delta opioid receptor imaging

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Several studies, including ours, suggest that targeting the delta opioid receptor (DOPr) may provide a strategy to developing new therapies to alleviate chronic pain (and possibly mood disorders and other psychiatric diseases such as anxiety and

depression) without the usual adverse effects associated with narcotics. Although several PET-tracers have been used clinically for depiction and quantification of the opioid receptors, only one with pronounced selectivity for DOPr is currently available; the [^{11}C -Me]naltrindole. However, this tracer cannot be used for quantitative measurements. Thus, highly potent and selective PET tracers are needed to further progress in this field. Our approach consists in labeling enkephalin analogs with more hydrophobic [^{18}F]-prosthetic group to achieve high specific activity and facilitate/increase their stability and transport across the BBB. We have successfully prepared a series of Tyr-D-Cys(S(CH $_2$) $_n$ F)-Gly-Phe-Leu derivatives bearing ^{19}F -alkyl chains (n=2,3,5). The non-radioactive analogs showed excellent receptor binding affinity for DOP and their lipophilicities (LogD $_{7.4}$ values) increased with the chain length (from 0.04 to 0.95). Further *in vitro/in vivo* experiments with ^{18}F -labeled peptides as potential PET imaging agents are warranted.



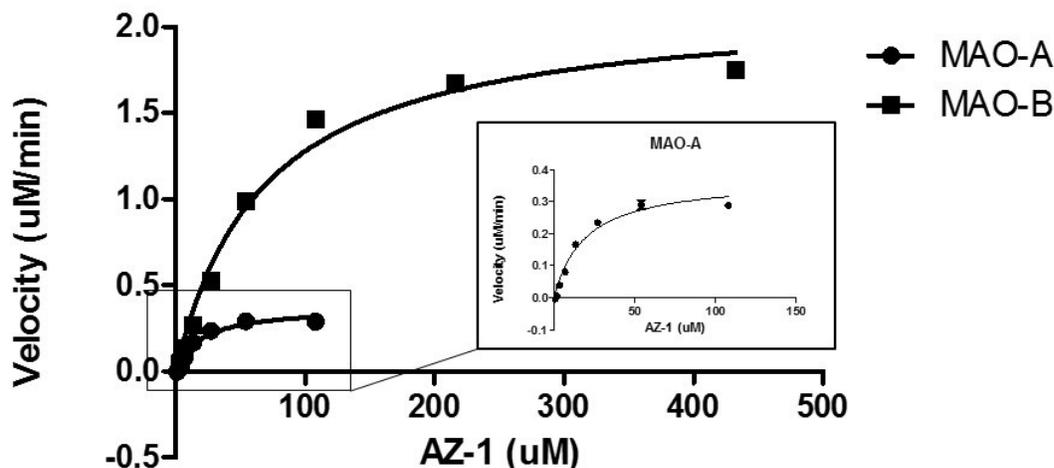
MEDI 161

Evaluation of 4-(2-fluoro-4-nitrophenoxy)-1-([^{11}C]methyl)-1,2,3,6-tetrahydropyridine as a MAO-A selective PET-MRI hybrid imaging probe

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Monoamine oxidase A (MAO-A) is a mitochondrial membrane bound flavoprotein that catalyzes the oxidative deamination of biologically important amines, including neurotransmitters. This enzyme is implicated in multiple neurological disorders and is a target for antidepressants. The complexity of these neurological disorders is an attractive application for the functional imaging modality of positron emission tomography (PET). As a potential hybrid PET-MRI imaging agent for monoamine oxidase-A (MAO-A) we combined our concept of carbon-11 labeled 1-methyl-4-aryloxy-1,2,3,6-tetrahydropyridines (Brooks AF, *et. al.* (2015) ACS Chem Neurosci,) to include a 2-fluoro-4-nitrophenoxy substituent, which has been reported useful as a chemical shift-switching ^{19}F MRI substituent in a MAO-A selective substrate (Yamaguchi K, *et. al.*

(2011) JACS). MAO-catalyzed oxidation and spontaneous hydrolysis of the proposed radiotracer would yield a non-toxic radiolabeled metabolite for PET imaging, and 2-fluoro-4-nitrophenol for possible chemical shift-switching MRI. *In vitro* ^{19}F -NMR studies showed a significant chemical shift difference between the substrate (-130.0 ppm) and product (-137.5 ppm). Syntheses of reference standard and precursor for radiolabeling were performed as described previously. *In vitro* kinetics were evaluated by MAO-A/B supersomes and monitored by appearance of absorbent product and by ^{19}F -NMR. *In vivo* pharmacokinetics were evaluated in rats and primates. Blocking experiments in primates with reversible inhibitors were used to determine selectivity. The *in vitro* studies showed a preference for MAO-A in binding (K_M), but no preference in overall catalytic efficiency (k_{cat}/K_M). The radiotracer displayed good initial brain uptake and trapping within rat and nonhuman primate brain. This report has been developed to prepare a MAO-A selective imaging agent with the potential to be utilized as a hybrid PET-MR imaging agent.



Kinetic curves shown for the standard compound in human MAO-A and MAO-B supersomes. (n=6)

MEDI 162

Pyrrolotriazines as potent inhibitors for a novel serine-threonine kinase for indication of neuropathic pain

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Jersey, United States (3) Lexicon Pharmaceuticals Inc, The Woodlands, Texas, United States

Effective treatment of chronic pain, in particular neuropathic pain, is a significant unmet medical need. Gabapentin and pregabalin are currently two of the most commonly prescribed drugs to treat neuropathic pain. However, both of these drugs have limited efficacy and are associated with dose limiting side-effects, such as dizziness, drowsiness, and fatigue. To look for potential novel therapeutic targets we utilized a mouse gene knock out (KO) approach combined with evaluation of pain behavior. We identified adaptor associated kinase 1 (AAK1), also known as AP2-associated protein kinase 1, as a potential novel therapeutic target for neuropathic pain. AAK1 is a member of the Ark1/Prk1 family of serine/threonine kinases and plays a role in modulating receptor endocytosis and had not previously been associated with neuropathic pain. It was found that AAK1 KO mice exhibit reduced pain behavior in the formalin model for persistent pain and a reduced neuropathic pain response (mechanical allodynia) in the Chung model for neuropathic pain without producing motor side effects. AAK1 is found in both Central Nervous System (CNS) and Peripheral Nervous System (PNS), although it was unknown if penetration into the CNS would be required for efficacy. Synthesis and structure-activity relationship (SAR) studies of a series of pyrrolotriazine AAK1 inhibitors led to the identification of BMS-832309, a brain penetrant, AAK1-selective compound and BMS-901715 a non-CNS penetrant, AAK1-selective compound. BMS-832309 was active in both the formalin model and the Chung model, whereas BMS-901715 showed activity in formalin model but not in the Chung model. BMS-832309 provided proof-of-concept demonstrating that CNS penetration is required for efficacy in neuropathic pain models. Recapitulation of the KO phenotype with a small molecule suggests that AAK1 may be a novel target for the treatment of neuropathic pain. The pyrrolotriazine-based chemotype SAR along with the in vitro profile for BMS-832309 and BMS-901715 will be presented.

MEDI 163

Design and synthesis of new acetylcholine analogues acting as full agonists for the nicotinic acetylcholine receptor subtype $\alpha 9\alpha 10$

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Nicotinic acetylcholine receptors (nAChRs) are transmembrane proteins that mediate rapid synaptic transmission and belong to the superfamily of ligand-gated ion channels. Seventeen nAChRs subunits have been identified in vertebrate species, the

last nAChR subunits that have been cloned are $\alpha 9$ and $\alpha 10$. These subunits assemble to form, *inter alia*, the $\alpha 9\alpha 10$ nAChR subtype which plays an important role in the central nervous system's modulation of sound detection and amplification. To the best of our knowledge, with the exception of acetylcholine (ACh), no effective full agonists, natural or synthetic, are known to exist for the $\alpha 9\alpha 10$ nAChR. Here we describe the design, synthesis and pharmacological properties of ACh analogs that are the first synthetic full agonists for the $\alpha 9\alpha 10$ nAChR subtype. A series of new ACh analogs were synthesized, characterized and assayed at 300 μM on *Xenopus* oocytes expressing the $\alpha 9\alpha 10$ nAChR subtype. Based on their electrophysiological responses, five compounds (**17a**, **17d**, **17e** and **17f**, Figure 1A) were selected for further study. Concentration-response curves for these five compounds were created (Figure 1B). The EC_{50} , the maximal evoked response and Hill coefficients derived from these concentration-response curves are summarized in Table 1.

In this study were discovered synthetic full agonists for the rat recombinant $\alpha 9\alpha 10$ receptor. Among these compounds, **17f**, bearing an octyl group on the ammonium nitrogen, was determined to be the most potent one. These results are a promising step toward the development of pharmaceutical agents targeting the $\alpha 9\alpha 10$ receptor.

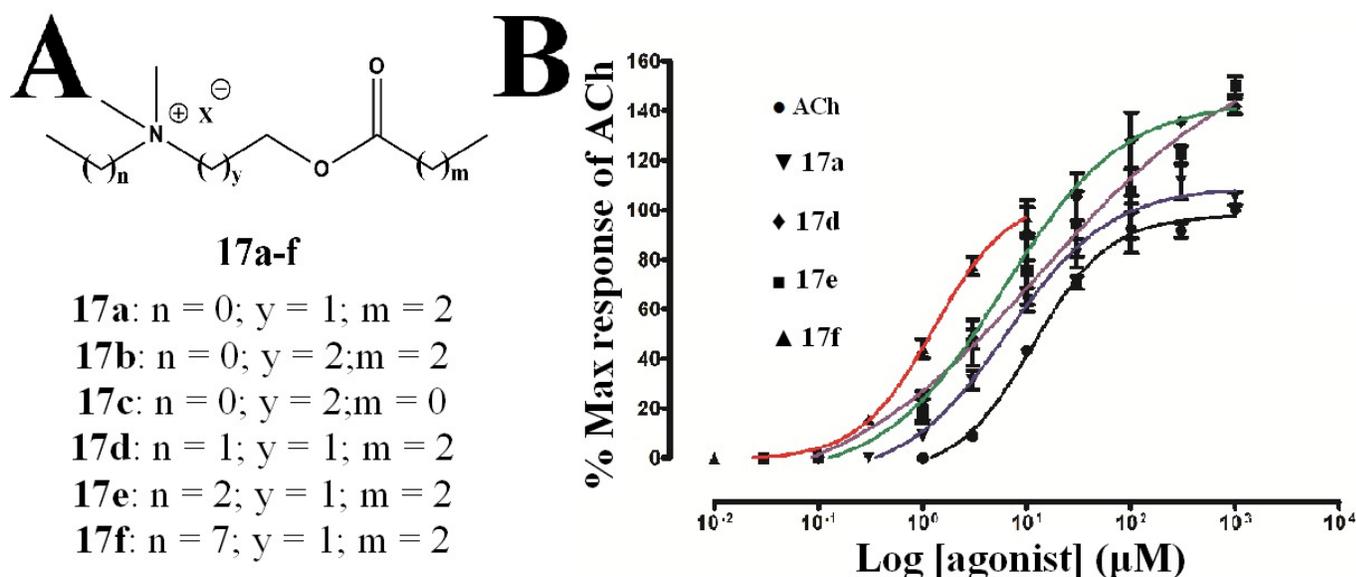


Figure 1. A: Chemical structure of new compounds. B: Concentration-response curves to ACh and compounds **17a**, **17d**, **17e** and **17f**.

Compound	$\text{EC}_{50}(\mu\text{M})$	Max resp (%)	n_H	n
ACh	13.21 \pm 1.05	100	1.58 \pm 0.18	7
17a	7.43 \pm 1,11	104,54 \pm 2,43	1.00 \pm 0.10	3
17d	6,93 \pm 1,14	141,71 \pm 3,17	0,80 \pm 0,06	4
17e	13.45 \pm 6.27	149.99 \pm 2.97	1.26 \pm 0.07	4
17f	0,91 \pm 0,11	97,17 \pm 5,25	1,58 \pm 0,18	5

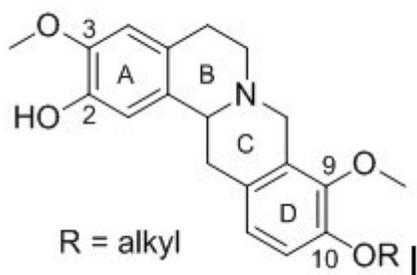
Table 1. Parameters derived from concentration-response curves in the presence of ACh, **17a**, **17d**, **17e** and **17f**.

MEDI 164

Synthesis and evaluation of C10 and flexible analogues of (\pm)-stepholidine at dopamine D₃ and σ ₂ receptors

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Novel C10 alkoxy homologues and de-rigidified analogues of the tetrahydroprotoberberine (THPB) alkaloid (\pm)-stepholidine were synthesized and evaluated at dopamine and σ receptors, in order to assess effects on D₃ and σ ₂ receptor affinity and selectivity. Our study revealed that small *n*-alkoxy groups are best tolerated at C10 for D₃ and σ ₂ receptor affinity and σ ₂ receptor selectivity. Introduction of flexibility to the rigid THPB scaffold of stepholidine, resulted in diminished affinity at all receptors evaluated. This indicates that the rigid tetracyclic framework is a critical requirement for the high affinity of THPBs to dopamine and σ receptors. Docking of the C10 alkoxy homologues at the D₃ receptor, revealed important ligand stabilizing interactions with the receptor *viz.*: i) an ionic interaction between the protonated nitrogen atom and Asp110, ii) a H-bond interaction between the C2 phenol and Ser192 and iii) hydrophobic interactions between ring A and Phe345 as well as ring D and Phe106.



MEDI 165

Discovery of C6-truncated purine (N)-methanocarba nucleoside derivatives as selective A₃ adenosine receptor agonists

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During the synthesis of carbocyclic adenosine derivatives, an unexpected Sonogashira coupling and subsequent amination and acid hydrolysis enabled the synthesis of purine (N)-methanocarpa-5'-N-alkyluronamidoribosides as A₃ adenosine receptor (A₃AR) agonists of atypical structure. The absence of an exocyclic amine represents a major adenosine modification, as it was previously considered important for nucleoside recognition at ARs (H-bond donation to a conserved Asn residue in transmembrane helix (TM) 6). Based on the unexpectedly high A₃AR affinity of initial C6-Me and C6-substituted styryl derivatives, other rigid nucleoside analogues lacking an exocyclic amine, including 6-H and 6-OMe derivatives, were prepared. C6-Me 2-phenylethynyl (MRS5919) and 2-(5-chlorothiénylethynyl) (MRS7195) analogues were particularly potent (human A₃AR K_i values of 6 and 45 nM, respectively), with other C6-Me analogues binding in the μM range. C2-(5-Chlorothiényl)-6-H analogue (MRS7220) was potent and selective at A₃AR (K_i 48 nM). An exocyclic amine was not required for full agonist efficacy in cAMP assays at mouse and human A₃ARs. In vivo, MRS7220 (3 μmol/kg, p.o.) completely reversed mechanoallodynia of the mouse sciatic nerve. The lack of a H-bond donor at the C6 position while maintaining A₃AR affinity and efficacy could be rationalized by homology modeling and docking of these hypermodified nucleosides, which have lower polar surface area. Thus, a suitable combination of stabilizing features can partially compensate for the lack of an exocyclic amine, an otherwise important contributor to recognition in the A₃AR binding site.

MEDI 166

Design, synthesis and in combo activity of selective σ-1 receptor ligands with robust antinociceptive effect

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A series of compounds were designed, prepared and the *in vitro* binding evaluation against σ₁ and σ₂ receptors was measured. Several compounds showed high σ₁ receptor affinity in the low nanomolar range, being more selective for σ₁ than σ₂ receptor. Also, it was performed a molecular docking of most active compounds into the ligand binding pocket homology model of σ₁ receptor, showing a salt bridge between the ionized morpholine ring and Asp126, as well as important short contacts with residues Tyr120, His154 and Trp164. Ligand efficiency indexes and predicted toxicity analysis revealed an excellent intrinsic quality of selected candidates. The

antinociceptive effect of compounds were determined using the formalin test, whereas the acetone test was employed for a rat model of neuropathic pain. The *in vivo* results indicated that compounds may be effective in treating inflammatory and neuropathic pain.

MEDI 167

2,4-Dioxo-3-aza-bicyclo[3.1.0]hexane-6-carboxamide derivatives as atypical antipsychotics for the treatment of schizophrenia

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Schizophrenia is a chronic, debilitating mental disorder affecting 1-2% of the global population. This brain disease makes it difficult for those diagnosed with it to differentiate between reality and imagined experiences, behave normally in social situations, and have normal emotional responses to everyday situations. People who suffer with schizophrenia often spend their lives in monitored isolation due to the disease's extreme psychological barriers that prevent them from having friends or a job. Antipsychotic medications are the most common form of treatment. The atypical antipsychotics possess a higher antagonist affinity at the serotonin 5-HT_{2A} as compared with the dopamine D₂ receptor. The atypical antipsychotic agents offer improved treatment of schizophrenia by combining efficacy with less propensity to cause harmful central nervous system (CNS) side effects. Our focused design and structure activity relationship efforts resulted in 2,4-dioxo-3-aza-bicyclo[3.1.0]hexane-6-carboxamide derivatives which have shown potent *in vitro* activity towards 5-HT_{2A}, D₂, 5-HT_{1A} and 5-HT₇ receptors and characteristic atypical antipsychotic signature. Details of design, chemistry, structure activity relationship and *in vitro* potencies will be disclosed in this presentation.

MEDI 168

Design, synthesis and pharmacological characterization of novel amides as 5-HT₄ receptor agonist

Anil K. Shinde, *anilshinde@suven.com*, Abdul Rasheed Mohammed, Sangram Keshri Saraf, Venugopala Bhatta, Kirankumar Kandukuri, Kambhampati R. Sastry, Ramkumar Subramanian, Venkatreddy Mekala, Gopinadh Bhyrapuneni, Vijay Benade, Pradeep Jayarajan, Ramakrishna Nirogi. Discovery Research, Suven Life Sciences Ltd, Hyderabad, Telangana, India

Alzheimer's disease (AD), which is characterized by gradual decline in cognitive function like thinking, remembering and reasoning skills represents the desperate unmet medical need of the 21st century. The 5-HT₄ receptor may represent a novel target for

the treatment of AD by providing both symptomatic relief of cognitive impairment as well as neuroprotection by increasing sAPP α secretion and reducing A β generation and toxicity. The novel series of amide compounds were designed, synthesized and characterized. The synthesized compounds were potent and selective 5-HT $_4$ agonists with adequate plasma exposures and brain penetration in Wistar rats. Design, synthesis and pharmacological profiling of novel amide derivatives in preclinical symptomatic and disease modifying cognition models will be presented.

MEDI 169

Conjugated amides: Potent and selective histamine H $_3$ receptor ligands

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The histamine-3 receptor (H $_3$ R) is presynaptically localized and functions both as an autoreceptor and a heteroreceptor by modulating the release of various neurotransmitters, including histamine, acetylcholine, dopamine, serotonin and norepinephrine. Activation of the H $_3$ R results in the inhibition of neurotransmitter release. In contrast, blocking the H $_3$ R by selective antagonists or inverse agonists can reverse the histamine mediated inhibition of neurotransmitter release. The cognition-enhancing effects of H $_3$ R antagonists in multiple preclinical cognition models generated considerable interest in the development of H $_3$ R antagonists and support the therapeutic approach for the treatment of cognitive deficits, involving disruption of multiple neurotransmitters.

Design and structure activity relationship of a series of conjugated amides is carried out and the compounds from this series are found to be potent and selective histamine H $_3$ receptor antagonists with acceptable pharmacokinetic properties. The *in vitro* and preclinical efficacy data covering receptor occupancy, novel object recognition and microdialysis data will be disclosed in this poster presentation.

MEDI 170

1-Isopropyl-1H-pyrrolo[2,3-b]pyridine-6-carboxamide derivatives as 5-HT $_4$ receptor partial agonists

Abdul Rasheed Mohammed, rasheed@suven.com, Anil K. Shinde, Shankar Reddy Gagginapally, Kambhampati R. Sastry, Ramkumar Subramanian, Venkatreddy Mekala, Gopinadh Bhyrapuneni, Pradeep Jayarajan, Ramakrishna Nirogi. Discovery Research, Suven Life Sciences Ltd, Hyderabad, India

Alzheimer's disease (AD) is a neurodegenerative disease that has higher prevalence and incidence in older people. The need for improved AD therapies is unmet. The 5-HT $_4$

receptor may play a role in memory and learning. Activation of 5-HT₄ receptors has been reported to modulate acetylcholine release, promote α -secretase pathway thereby increases sAPP α and reduces toxic beta amyloid accumulation. It is postulated that activation of 5-HT₄ receptors will offer improved clinical efficacy and/or tolerability relative to acetylcholine esterase inhibitors. The 5-HT₄ receptor partial agonists may be of benefit for both the symptomatic and disease-modifying treatment for cognitive disorders associated with AD. The 1-isopropyl-1H-pyrrolo[2,3-b]pyridine-6-carboxamide derivatives as 5-HT₄ receptor partial agonists have been designed, synthesized and their *in vitro* potencies established. The 1-isopropyl-1H-pyrrolo[2,3-b]pyridine-6-carboxamide derivatives have shown potent *in vitro* affinities towards 5-HT₄ receptor when tested in CHO stable cell line expressing human 5-HT_{4e} receptor and reporter gene. Details of design, chemistry, structure activity relationship and *in vitro* potencies will be disclosed in this presentation.

MEDI 171

Synthesis of [¹¹C]MK-1064 as a new PET radioligand for imaging of orexin-2 receptor

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Orexin or hypocretin receptors (OXRs) in the brain are G-protein-coupled receptors, and they are implicated in diverse physiological and neuropsychiatric conditions and associated with brain diseases such as narcolepsy and insomnia. There are two variants, OX₁R and OX₂R. OX₁R is selectively expressed in the locus coeruleus and OX₂R is expressed in the tuberomammillary nucleus. OXRs represent attractive therapeutic targets for drug development, and orexin agonists and antagonists have been used to treat narcolepsy and insomnia, respectively. MK-1064 {5''-chloro-*N*-((5,6-dimethoxypyridin-2-yl)methyl)-[2,2':5',3''-terpyridine]-3'-carboxamide} recently developed by Merck is a highly potent and selective OX₂R antagonist with *K_i* (nM) and IC₅₀ (nM) 0.5 and 18, 1584 and 1789 for OX₂R and OX₁R, respectively, and SI (selectivity index, *K_i*/IC₅₀) 3168/99 for the treatment of insomnia [Roecker, A. J. et al. *ChemMedChem* **2014**, 9, 311-322]. OXR is an interesting imaging target as well. A suitable radioligand would help to examine the relationship between the therapeutic effect and receptor occupancy of this new class of OX₂R antagonists. Here we report the design and synthesis of [¹¹C]MK-1064 {5''-chloro-*N*-((5-[¹¹C]methoxy-6-methoxypyridin-2-yl)methyl)-[2,2':5',3''-terpyridine]-3'-carboxamide} as a new candidate radioligand for imaging of OX₂R using biomedical imaging technique positron emission tomography (PET). The reference standard MK-1064 was synthesized from methyl 2-chloro-5-iodonicotinate and 5-(chloropyridin-3-yl)boronic acid in 4 steps with 32% overall chemical yield. The precursor desmethyl-MK-1064 {5''-chloro-*N*-((5-hydroxy-6-methoxypyridin-2-yl)methyl)-[2,2':5',3''-terpyridine]-3'-carboxamide} for radiolabeling was synthesized from 2-bromopyridin-3-ol and 5''-chloro-[2,2':5',3''-terpyridine]-3'-carboxylic acid in 6 steps with 15% overall chemical yield. The target tracer [¹¹C]MK-1064 was prepared by *O*-[¹¹C]methylation of its corresponding precursor desmethyl-MK-1064 with [¹¹C]CH₃OTf under basic condition (2 N NaOH) and isolated by a simplified solid-phase extraction (SPE) method in 50-60% decay corrected radiochemical yields based on [¹¹C]CO₂ at end of bombardment (EOB). The overall synthesis time from EOB was 23 min, the radiochemical purity was >99%, and the specific activity at end of synthesis (EOS) was 185-555 GBq/μmol.

MEDI 172

Tunable pH-sensitive linker for controlled release

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We have developed a novel pH-sensitive linker based on a phosphoramidate scaffold that can be tuned to release amine-containing drug molecules at various pH values. The pH-triggered phosphoramidate-based linkers are responsive to pH alone and do not require intracellular enzymatic action to initiate drug release. Key to the pH-triggered amine release from these linker is a proximal acidic group (e.g., pyridinium or carboxylic acid) to promote the hydrolysis of the phosphoramidate P-N bond, presumably through an intramolecular general-acid type mechanism. Phosphoramidate hydrolysis is largely governed by the pKa of the leaving amine (e.g., primary, secondary, aniline). However, the proximity of the neighboring acidic group attenuates the stability of the P-N bond to hydrolysis, thus allowing for control over the release of an amine from the phosphoramidate center. Based on the model scaffolds examined, phosphoramidate-based linkers could be selected for particular properties for controlled-release applications such as amine type, stability under physiological conditions, or release rates at various pH values such as intracellular endosomal conditions. The tunability of the phosphoramidate scaffold is expected to find broad applicability in various controlled drug-release applications such as antibody or small-molecule drug conjugates, drug-eluting stents, prodrug activation, as well as intracellular trafficking studies in which pH changes can trigger the release of turn-on dyes.

MEDI 173

Technologies for assessing target engagement and their applications in drug discovery

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Drug discovery is currently experiencing a paradigm shift due to multiple challenges that the pharmaceutical industry is facing, in particular the low number of new drug approval in spite of the high level of R&D investment. In order to improve drug discovery effectiveness and increase the probability of success, it is crucial to demonstrate sufficient target engagement of a potential drug to its given target both pre-clinically and clinically. Therefore, at Eli Lilly and Company, we have enabled different chemical biology technologies for target engagement and integrated them into the flowscheme of various steps of drug discovery process from as early as Hit Declaration all the way to Candidate Selection. Herein, we illustrated several strategies and technologies including Cellular Thermal Shift Assay (CETSA) and Activity-Based Protein Profiling (ABPP) for accessing target engagement in cells or even in tissue samples and demonstrated their applications to different medicinal chemistry projects.

MEDI 174

Long-term storage stability problems of screening libraries for drug discovery

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A variety of structural alerts are available today for the identification of compounds that are unsuitable for being included in high-throughput screening libraries. These include compounds identified through medicinal chemistry knowledge as well as through results from HTS campaigns, such as the PAINS (Pan Assay Interference Compounds). We recently became interested in an additional group of problematic screening compounds that have not been reported in a large-scale study: compounds that despite stringent storage criteria (-20 °C, dry nitrogen) decompose during storage in DMSO solution. Such compounds are not only problematic to handle and store over an extended period of time; they may decompose or react more rapidly under assay conditions and can therefore provide screening results that are hard to follow up on, leading to wasted efforts and resources.

We report our results from a large-scale analysis of QC data from the NCATS SMR (formerly MLSMR) screening library which has been managed and developed at Evotec since 2004. Many compounds have undergone multiple QC analyses over the years, providing us with a unique opportunity to identify and analyze scaffold families that have decomposed in our storage over time. We describe the identification of these scaffold families and discuss our hypotheses on some of the reaction mechanisms and resulting products as well as the potential applications as mild reactions in compound synthesis. We furthermore compare our results to previously published structural alerts. The collection of potentially unstable scaffolds will be shared as SMARTS patterns with the scientific community.

MEDI 175

NCI small molecule screening libraries available to academic oncology HTS investigators

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The NCI Experimental Therapeutics Program (NExT) has previously made available our complete 83K NExT Diversity Screening Library to academic investigators for use in oncology HTS projects. The set is available in single-use, 384-well pre-plated format and was designed from commercially-available molecules with the goal of identifying small molecule leads for drug discovery programs. We are now also making available 2 smaller pre-plated subsets of this library, the NExT Diversity 3500 and NExT Diversity 3500 SAR. Each library contains 3,500 compounds: NExT Diversity 3500 is a diverse sampling across the entire 83K library while the NExT Diversity 3500 SAR is designed to facilitate rapid SAR follow-up by sampling only those compounds that have at least 5 close neighbors in the 83K library. The new libraries were designed by initially filtering

the full NExT Diversity Library for undesirable compounds such as PAINS. Both atom-pair based 2D pharmacophore fingerprints and ECFP6 topological fingerprints from ChemAxon were then employed to generate the two non-overlapping subset libraries. The NExT screening libraries are provided as a resource to academic oncology investigators through the NExT Program (see NExT Resources: <http://next.cancer.gov>). The design details and physicochemical characteristics of the two new sets, NExT Diversity 3500 and NExT 3500 SAR, and procedures and criteria for obtaining this resource are presented along with several other screening sets available through the NCI/Developmental Therapeutics Program (DTP).

MEDI 176

Methods for clean-up and enrichment of corporate screening collection

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Medicinal chemistry metrics such as ligand efficiency (LE), lipophilic ligand efficiency (LLE), and lipophilicity-dependent ligand efficiency (LELP) have gained wide-spread acceptance. Unlike LE and LLE, LELP is reported to be useful throughout the evolution of hits to the final drug candidates. We apply LELP under the assumption of a 10 microM potency. Compounds with LELP < 10 are further subjected to a set of published and in-house PAINS, structural alerts, and property filters before each structure is reviewed independently by at least two chemists. The poster outlines this strategy and presents structures deemed unattractive by chemists, but not removed by the computational filters.

Attempt to quantify the 'beauty' of small molecules has been published in recent years with the MPO and QED scoring functions among the most two notable examples of such algorithms. Both of them are based on approved drugs. Since typical screening hits are well-known to 'grow' during lead optimization, the application of MPO or QED scores in screening library design or hit evaluation can be called into question. We present our in-house version of multiparameter algorithms based on the MPO and QED principles but modified to be more applicable to hits.

MEDI 177

Directed evolution of PET imaging agents by scanning unnatural protease resistance (SUPR) mRNA display

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Ideal molecular imaging agents combine the affinity and selectivity of monoclonal antibodies with the rapid clearance and pharmacokinetics of small molecules. In this work, we describe the use of directed evolution to develop Scanning Unnatural Protease Resistant (SUPR) peptides as novel molecular scaffolds for *in vivo* PET imaging of the Her2 receptor. SUPR peptides were obtained through selection against Her2-expressing breast cancer cells using macrocyclic peptide libraries containing non-proteogenic N-methyl amino acids. These libraries were pre-selected against a panel of proteases prior to target binding to dramatically enhance the stability of the winning sequences. A lead peptide isolated from this selection (SUPR4) bound selectively to the Her2 receptor *in vitro* with low nanomolar affinity and showed rapid and selective tumor uptake by *in vivo* optical imaging. SUPR4 was found to be non-competitive with either Trastuzumab or Pertuzumab but did compete with a Her2 affibody. Sequence analysis of both SUPR4 and the affibody revealed a consensus LYDD motif that appears to be essential for high affinity Her2 binding. SUPR4 was efficiently labeled with ^{18}F via the copper catalyzed azide-alkyne cycloaddition and high specific activities were achieved without HPLC purification using a novel azide-derivatized scavenging resin. PET/CT showed rapid tumor uptake (<1 hour) with predominantly renal clearance and low liver uptake. To further improve the *in vivo* properties, we systematically replaced the two selected N-methyl norvaline residues in SUPR4 with both natural and N-methyl residues to determine the contribution of N-methylation and side-chain composition to affinity and protease stability. In parallel, affinity maturation selections of SUPR4 were carried out using a combination of Her2-positive and Her2-knockdown cell lines to simultaneously improve both affinity and Her2 selectivity. The resulting second generation SUPR peptides were evaluated for tumor uptake, biodistribution, and clearance rate by PET/CT. While SUPR peptides are selected from biological display libraries, they share similar chemical composition and structure with peptides derived from non-ribosomal peptide synthesis. We believe that this combination of affinity, stability, rapid clearance, and synthetic accessibility make SUPR peptide technology a general approach for generating highly potent compounds for targeted molecular imaging of cancer biomarkers.

MEDI 178

[^{18}F]JNJ-311, a novel tau PET ligand

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In order to identify novel Tau aggregate binders, a set of 4000 compounds was selected based on pharmacophore, 3D shape and 2D fingerprint similarity with known Tau

binders. By screening this set in a competitive binding assay using neurofibrillary tangles (NFTs) and β -amyloid isolated from brains of Alzheimer's Disease (AD) patients, a novel potent but non-selective Tau binder was identified. Further optimization introduced over 100 fold selectivity versus β -amyloid. In a next phase suitable positions to introduce the ^{18}F radionuclide were identified. This resulted in the discovery of [^{18}F]JNJ-311, a low molecular weight molecule that binds potently to neurofibrillary tangles (NFTs) with a pIC_{50} of 7.75 in the competitive binding assay with high selectivity versus aggregated β -amyloid ($\text{pIC}_{50} < 5$). Both JNJ-311 and its precursor are relatively straightforward to synthesize and bench stable. Radiosynthesis of [^{18}F]JNJ-311 is uncomplicated and high yielding. Selectivity was also confirmed by autoradiography, competition with Tau binder T808 and immunohistochemistry using the AT8 antibody on human AD sections (Figure 1A-D). Profiling on 52 receptors & ion channels (CEREP), 400 kinases (DiscoverRx), MAO-A and MAO-B did not detect any significant off-target activity. This was confirmed by subsequent μPET studies in both rat and rhesus monkey, where no retention was observed in any brain region. JNJ-311 has an excellent pharmacokinetic profile for a PET ligand. Microdosing in rat with cold JNJ-311, mouse biodistribution of [^{18}F]JNJ-311 and μPET studies in both rat and rhesus monkey confirmed that JNJ-311 is rapidly and abundantly taken up in the brain and shows a faster clearance than the currently most advanced Tau PET tracer [^{18}F]AV-1451 *aka* [^{18}F]T807 (Figure 1E).

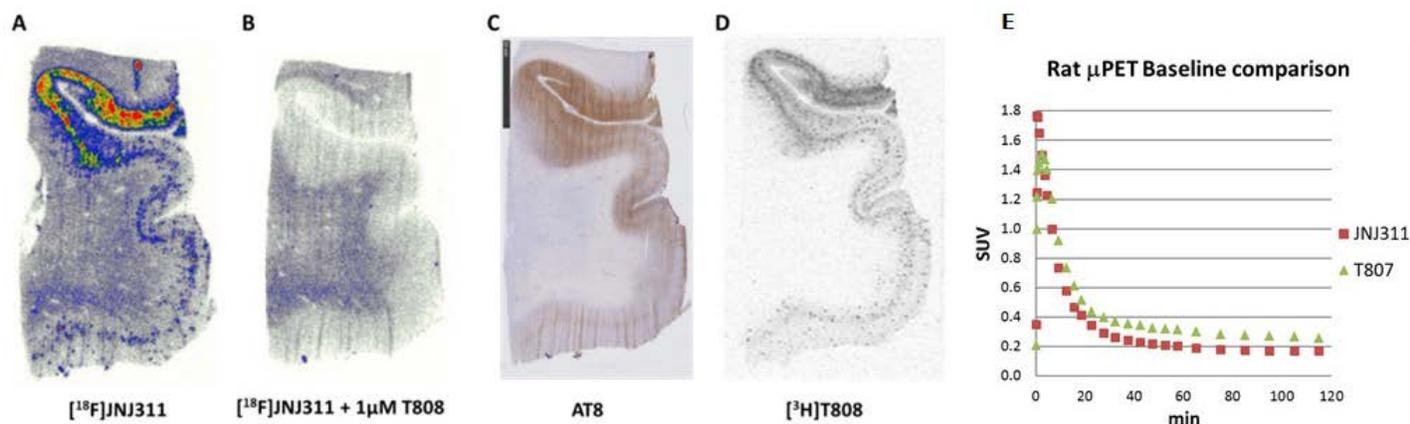


Figure 1: Autoradiographic image of [^{18}F]JNJ-311 (A), and (B) [^{18}F]JNJ-311 following a chase with cold T808. Tau pathology immunostaining (C) and [^3H]-T808 autoradiography (D) on adjacent sections of AD brain (Braak stage V-VI). μPET study of [^{18}F]JNJ-311 in Wistar rat: quantification (SUV) and comparative analysis with T807 (E)

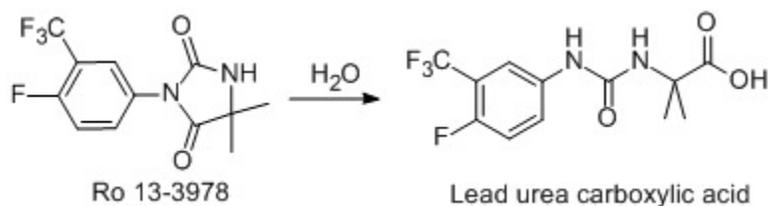
MEDI 179

Urea carboxylic acid derivatives as antischistosomal agents

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We recently discovered that 2-[3-[4-fluoro-3-(trifluoromethyl)phenyl]ureido]-2-methyl propanoic acid, the hydrolysis product of antischistosomal hydantoin Ro 13-3978, has significant activity in a *Schistosoma mansoni* mouse model. We now describe our initial efforts to optimize the synthesis, *in vitro* ADME and *in vivo* antischistosomal activity of this lead urea carboxylic acid.



MEDI 180

Discovery and evaluation of the first small molecules targeting GOAT inhibition *in vivo*

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Ghrelin O-Acyl Transferase (GOAT) is the only known enzyme that catalyzes the conversion of unacylated ghrelin (UAG) into acylated ghrelin (AG), its active form. The recent identification and characterization of GOAT introduces a new approach to attenuate the ghrelin receptor system, one of the most important mechanisms regulating feeding and energy balance. Despite the potential therapeutic benefits of inhibiting GOAT, only a few examples of peptide-based inhibitors or non-optimized small molecules have been reported. The effects of AG and UAG infusion on glucose and insulin levels have been assessed in clinical studies but the lack of orally available GOAT inhibitors has precluded the pharmacological intervention to modify the AG/UAG ratio. The lack of computational tools for structure-based drug design focused our efforts on screening strategies using an enzymatic assay. A few fragments and small molecules with common structural features were chosen as starting points to expand the chemical space. In parallel, we evaluated the optimal conditions for a cell-based assay that contributed to optimize molecules from an *in vitro* standpoint. Several SAR iterations evolved to identify a few advanced tools as high quality chemical probes for *in vivo* evaluation. The compounds presented in this poster inhibited the formation of plasma acylghrelin in a mouse PD model and confirmed the preclinical validation of the GOAT inhibitor hypothesis.

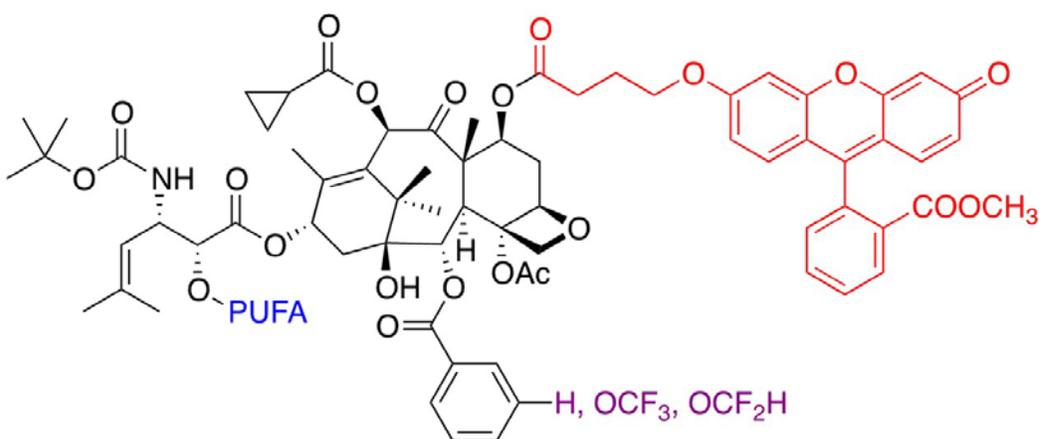
MEDI 181 – Withdrawn.

MEDI 182

Design, synthesis, and applications of novel PUFA-taxoid probes for fluorescence imaging and ^{19}F NMR analysis

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A PUFA-taxoid conjugate (PUFA = omega-3 polyunsaturated fatty acid), DHA-SBT-1214, has exhibited excellent efficacy *in vivo* against DLD-1, Panc-1, CFPAC-1, H460, and A121 xenografts in mice, as well as exceptional activity against cancer stem cells. Currently, an advanced preclinical study of its nanoemulsion formulation (NE-DHA-SBT-1214) is underway toward IND filing. However, the internalization mechanism of NE-SBT-1214 into cancer cells needs to be studied. For this study, we have synthesized novel fluorescence probes of DHA-SBT-1214 and LNA-SBT-1214 by attaching a fluorescein tether at the C7 position of the taxoid moiety. Furthermore, we also designed and synthesized orthogonal dual probes of fluorine-containing third-generation taxoids, such as SB-T-121405 and SB-T-121406, with a fluorescein tether. ^{19}F probe for ^{19}F NMR and fluorescein probe for fluorescence imaging, which are built in the taxoid component, can be exploited for the monitoring of the behavior of NE-PUFA-taxoids in cancer cells (especially for internalization process), cell culture medium, and blood plasma. The chemical synthesis and biological applications of these novel probes of PUFA-taxoids and NE-PUFA-taxoids will be presented.



MEDI 183

Studies of influence of plasma-activation of compounds on melanogenesis and tyrosinase activity

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It has been documented recently that plasma can enhance the biological activity of natural material naringin. This material can showed increased tyrosinase inhibition and anti-microbial activities after plasma treatment. The main challenge for medicinal chemist is the discovery and development of the drugs with greater activities for rapid and safer treatment and cure of respective malfunctioning. Enhancement of efficacy and quality of the medicine is the most popular interest among medicinal chemists. Many chemists around the world are focused to synthesize or extract natural products in order to find the most active drugs. We synthesized new eugenol derivatives (ED) and then treated them with an N₂ feeding gas atmospheric pressure plasma jet (APPJ) to increase their utility. We studied the tyrosinase-inhibition activity (activity test) and structural changes (circular dichroism) of tyrosinase with ED and plasma activated eugenol derivatives (PAED) in a cell-free environment. Molecular docking studies was carried out study the possible interaction sites of ED and PAED compounds with tyrosinase enzyme. Docking computation helped to investigate and compare the effect of binding mode of the ED and PAED compounds to get a deeper insight of binding mode inside the active site of tyrosinase. PAED for the field of plasma medicine. Moreover, we studied the possible effect of ED and PAED on melanin synthesis and its mechanism in melanoma (B16F10) cells. Additionally, we investigated the structural changes that occurred in activated ED after plasma treatment using nuclear magnetic resonance (NMR). Hence, this study provided a new perspective on plasma activated compounds for the plasma medicine field.

MEDI 184

Crystallographic study of metalloenzyme inhibitors

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Metalloenzyme inhibitors are underrepresented in FDA approved therapeutics, relative to other clinical targets. Our understanding of metal-inhibitor interactions, particularly those that dictate selectivity and potency of inhibitors has improved recently, but continued investigation is needed for further inhibitor development. Interactions that may influence inhibitor binding include active site structure, identity of the active site metal, and identity of the coordinating atoms of the inhibitor.

We use the mono-nuclear metalloenzymes Carbonic Anhydrase and Thermolysin along

with the dinuclear metalloenzyme Methionine Aminopeptidase as model systems, to explore the role of metal-inhibitor interactions. Mutational studies with Carbonic Anhydrase showed that coordinating atoms respond differently to a mutation which decreased steric crowding in the enzyme active site. The selectivity of different ligands between Thermolysin and Carbonic Anhydrase has been investigated. The active sites of these two metalloenzymes differ greatly, revealing the role active site structure and topology plays in inhibitor selectivity. To probe the affect metal identity plays in ligand preferences, the catalytically active metallo-isoforms of Methionine Aminopeptidase were studied. To observe inhibitor selectivity, a library of metal binding pharmacophores was screened. In all cases X-ray crystallography has been used to determine the origin of observed selectivity. The results reported herein can guide the development of potent and selective metalloenzyme inhibitors.

MEDI 185

Chemoenzymatic synthesis and characterization of multifunctional fluoresceins for breast cancer diagnosis

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Fluorescein exhibits excellent luminescent properties to be used as a diagnostic agent for the detection of malignant cells. This work highlights the synthesis of four tetra-functional fluoresceins by very efficient chemo-enzymatic catalysis. First pure fluorescein diacrylate (FL-DA) was prepared with high efficiency. Subsequently tetra-allyl, tetra-ester and tetra-hydroxy fluoresceins were synthesized via Michael addition of the corresponding functional *secondary* amines, catalyzed by *Candida antarctica* lipase B. The structure of the products was confirmed using ¹³C and ¹H-NMR. MS (ESI) was used to quantify the purity of the crude products: 100%, 96% and 91% for the tetra-hydroxy, tetra-ester and tetra-allyl fluorescein. These multifunctional fluoresceins are good candidates for the synthesis of imaging agents for a wide variety of applications.

MEDI 186

Evaluation of silica stability in methanolic solvents

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For those who have used flash chromatography with mobile phases consisting of DCM and MeOH there has been reports over many years of silica found in collected fractions.

In this poster we examine solubility of two silica types used in chromatography, an irregular 40-63 micron and a spherical 60 micron media.

MEDI 187

Effective cannabinoid purification by flash chromatography

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With the legalization of cannabis compounds many entrepreneurial companies are seeking to capitalize on what could be a large market for these products. Though many firms just repackage the raw plant components, those that are more serious about marketing a quality product are turning chromatography to separate and isolate the various cannabinoids of interest. In this poster we will show how reversed-phase flash chromatography can provide highly pure cannabinoids.

MEDI 188

Chemical make-up of plants used in herbal remedies and their applications

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With nearly half of all currently used medications being based off of compounds found in plants, it can be reasoned that many more medicinal compounds can be discovered by studying the different herbal remedies around the world. Examples include the chemical ephedrine, derived from the plant *Ephedra sinica*, which is used as a decongestant in Sudafed, and the chemical paclitaxel, derived from the Pacific yew, and used in the anti-cancer treatment drug, Taxol. Our focus will be on comparing the chemicals used in local plant remedies and tropical plant remedies, and their applications towards the alleviation of ailments they are used to treat. Using a generalized method of addition of solvents, compounds will be separated into organic and aqueous layers, then isolated from each other. Another route of extraction using Pressurized Liquid Extraction (PLE) will be explored. Once the desired chemicals are isolated from the rest of the organic matter, they will be analyzed, identified, and quantified using techniques such as Gas Chromatography-Mass Spectrometry (GC/MS), HPLC, and NMR. A comparison of the findings will be done to determine which method of extraction is optimized to give the highest yield of the chemical with medicinal properties.

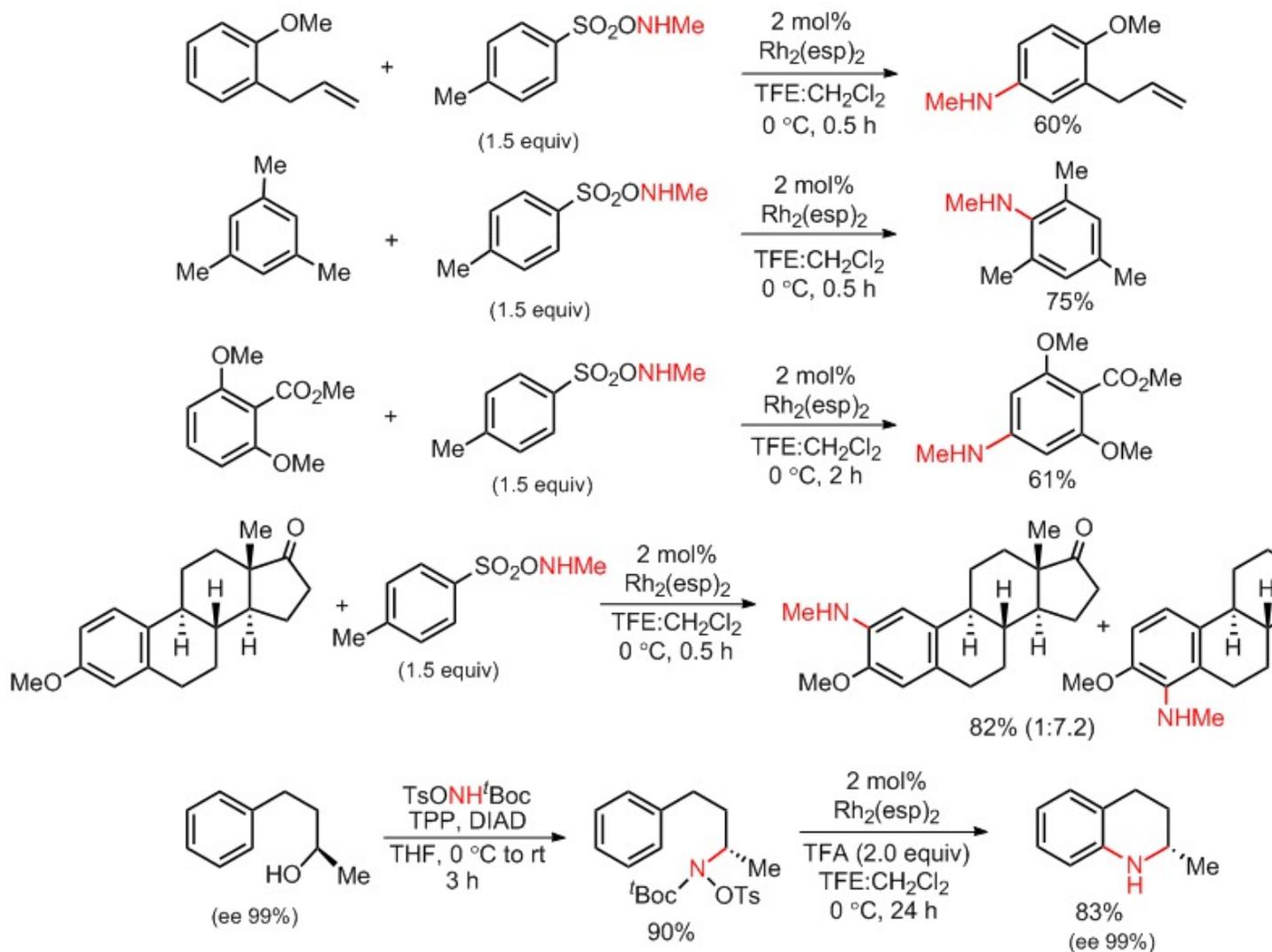
MEDI 189

Dirhodium catalyzed direct aryl amination

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Nitrogen is one of the key components of many natural products, pharmaceuticals, agrochemicals, and dyestuffs as well as many reagents and catalysts. It has been estimated that among all natural products, the average number of nitrogens per molecule is 0.7, while for drugs, this number rises to 3.0. Therefore, development of direct and practical methods for the introduction of nitrogens is of immense importance for both synthetic and medicinal chemists.

Creation of an aryl C(sp²)-N bond employing most traditional methods requires multiple steps and/or harsh reaction conditions. Consequently, these procedures have been largely supplanted by the more efficient palladium catalyzed Buchwald and Hartwig procedures. However, the latter suffer a common constraint, the need for pre-functionalized arenes. Herein, we describe an operationally simple, mild, and regioselective dirhodium catalyzed direct arene amination suitable for both inter- and intra-molecular applications.



MEDI 190

Synthesis of novel photoaffinity probes of antiangiogenic homoisoflavonoids

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Based on the natural antiangiogenic compound cremastranone, three types of photoaffinity probes were designed and synthesized in which benzophenone and biotin were attached to homoisoflavanonoids using PEG linkers on either the C-3' or C-7 position. Notably, the photoaffinity probe retains excellent activity of inhibiting retinal endothelial cell proliferation with GI50 = 230 nM.

MEDI 191

Peptide-based capsules for protein delivery

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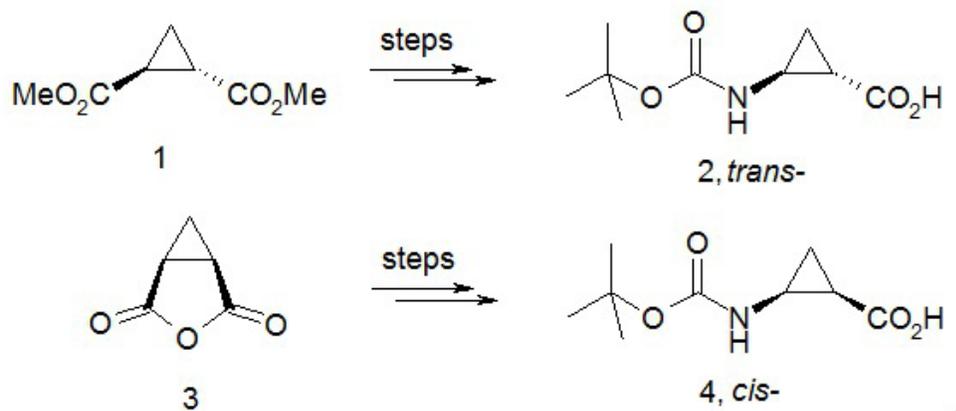
Protein delivery has been largely limited by its physical and chemical instability in the physiologically environment. Effort to develop advanced protein delivery systems has been continuously increasing over the past decade, highlighting the critical and unmet needs to address these challenges. Given their biodegradable, biocompatible, and self-assembling characteristics, peptide-based systems provide some unique and advantageous properties for delivery of biologics. In this work, we show that rationally designed amphiphilic peptides can be triggered to assemble upon contact with a protein-containing solution. This induced interfacial co-assembly enables the creation of capsules of tunable sizes that can incorporate the protein of interest with nearly 100% encapsulation efficiency. We used electron microscopy and confocal microscopy to characterize the co-assembly process, as well as the assembled capsule structures. Our studies on the release kinetics revealed a sustained-release profile of the encapsulated proteins over a long period of time.

MEDI 192

Synthesis of *cis/trans*-2-tert-butoxycarbonylamino-cyclopropanecarboxylic acid

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β -aminocyclopropanecarboxylic acid (β -ACC), the carbocyclic analogue of β -alanine, has received increased attention from synthetic community in recent years. Due to their inherent ring strain and coexistence of donor-acceptor functional groups, β -ACCs are extremely prone toward ring opening. To our knowledge, the synthesis of *cis*- and *trans*-2-tert-butoxycarbonylamino-cyclopropanecarboxylic acid has never been disclosed. We report herein our synthetic approach toward those compounds from readily available starting materials (**Scheme 1**).



Scheme 1. Synthesis of *cis*-/*trans*-2-tert-butoxycarbonylamino-cyclopropanecarboxylic acid

MEDI 193

Delivering phytochemical therapeutics through polymer nanofibers

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Many compounds obtained from natural sources exhibit therapeutic properties *in vitro*, but yield lackluster results *in vivo*. Two such compounds are curcumin and rosmarinic acid. Both of these phytochemicals display significant anti-cancer, anti-microbial, and other therapeutic activities *in vitro*, but have low bioavailability and are quickly eliminated from the body when administered *in vivo*. However, drug delivery systems which permit prolonged release can greatly increase the efficacy of such therapeutic compounds with poor bioavailability. Poly (lactic-co-glycolic acid) (PLGA) is a biodegradable polymer which has been studied extensively for localized, customizable drug delivery. We have generated curcumin- and rosmarinic acid- loaded PLGA nano fibers via a simplified and economical solution blow spinning process, for use as an implant for the treatment of cervical cancer and microbial infections. These nano fibers are able to slowly degrade *in vitro* and *in vivo* to provide a chronic release of the embedded therapeutic compounds, as well as to solubilize the curcumin in aqueous solutions, increasing efficacy of delivery. We have characterized the physical properties of nano fibers by scanning electron microscope for morphology, and confocal laser scanning microscopy for curcumin embedding. Moreover, we demonstrate anti-cancer activity using the WST assay and anti-bacterial activity via an agar diffusion assay. Our current findings provide evidence that loaded PLGA nano fibers hold great potential for drug delivery within a variety of medical applications.

MEDI 194

Strategic exploration of the magic methyl effect in drug design

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Construction of the smallest possible molecule that possesses a desired balance of physical and biological properties can optimize the chance of success in drug discovery. Interestingly, very small structural features can often provide profound effects. For example, the addition of a methyl group at a critical position has been shown to influence conformational preferences and hydrophobic and de-solvation effects, and to result in an improved DMPK profile and 100-fold or more improvement in IC50 value. This talk will review examples of the “magic methyl” effect and discuss strategic use of this approach in the Lead ID and Lead Optimization process.

MEDI 195

Challenges and opportunities of implementing halogen bonds in molecular design

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Particularly in life sciences and drug discovery, halogen bonding has gained a lot of attention in recent years. Boosting the affinity and potency of ligands by introduction of a halogen atom seems to be a desirable goal. We extensively used QM model calculations to systematically map the relationship between strength and geometry of halogen bonds to different interaction partners (carbonyl backbone, sulfur contacts, nitrogen contacts, carboxylates, π -systems, ...) in a binding site. We evaluated the potential for molecular design of additional halogen bonds in existing protein-ligand complexes by applying *XBScore*, our first QM-derived scoring function for the recognition of contacts to the carbonyl backbone. Based on experimental case studies, opportunities and challenges in applying such molecular design of halogen bonds to the field of kinase drug discovery will be discussed. In addition, we have also pioneered the generation of halogen-enriched fragment libraries for identifying fragments where halogen bonds are an integral part of the ligands binding mode. The contrast to halogen bonds by molecular design will be highlighted and strategies to harness the potential of halogen bonding in drug discovery will be suggested. Possibilities to use our tools and models are available at www.halogenbonding.com.

MEDI 196

Some applications of fluorine in drug design

Nicholas A. Meanwell, *nicholas.meanwell@bms.com*. Bristol Myers Squibb, Wallingford, Connecticut, United States

The applications of fluorine in drug design and development have grown rapidly in parallel with an increased knowledge and understanding of its unique properties and how they are best exploited. The judicious deployment of fluorine in a molecule can influence several factors of importance to drug properties including modulating conformation, moderating the basicity of amines, enhancing intrinsic potency, facilitating membrane permeability, and influencing metabolic pathways and pharmacokinetic properties. In this presentation, we will provide instructive examples of the productive effects of strategically incorporating fluorine in drug molecules.

MEDI 197

Improvement in aqueous solubility via small structural modifications

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Medicinal chemists typically rely on acidic, basic or highly-polar functional groups to improve aqueous solubility, often at the expense of activity, absorption, distribution and metabolism. However, significant changes in solubility can be achieved by small structurally conservative modifications which yield comparatively large improvements in solution and solid-state properties without having a negative impact on pharmacology. This presentation provides examples of this approach as well as describes the underlying chemical principles in order to enable application to new molecules.

MEDI 198

Strategies to reduce glucuronidation through structural modification

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Glucuronidation, one of the major Phase II drug metabolism pathways, takes place mainly in the liver to form highly polar glucuronide conjugates from xenobiotics. Glucuronidation reactions are catalyzed by a family of enzymes called uridine 5'-diphospho-glucuronosyltransferases (UDP-glucuronosyltransferases, UGTs) using uridine 5'-diphospho-glucuronic acid (UDPGA) as a glucuronide donor. A wide spectrum of nucleophiles can be glucuronidated including alcohols, phenols, thiols, carboxylic acids, and amines. Once conjugated to glucuronic acid, increased polarity, molecular weight, and ionization potential result in enhanced clearance and thus low exposure. Indeed, glucuronidation is involved in the metabolism of a number of marketed drugs as well as investigational drugs in development and early-stage projects, often presenting complex and/or undesirable pharmacokinetic profiles. However, in contrast to CYP-mediated oxidations, there has not been a systematic analysis aimed at establishing the correlation of molecular structures with susceptibility to glucuronidation. This presentation will provide an overview of the glucuronidation pathway with a particular focus on the molecular features preferred by UGTs as substrates. Various strategies

taken to reduce glucuronidation through drug design will be also discussed in depth including a recent case study by our research group, which led to the identification of D-amino acid oxidase (DAAO) inhibitors highly resistant to glucuronidation through minor structural modifications.

MEDI 199

Testing new therapeutics in SLE: Unmet needs and strategies

Anne Davidson, *adavidson1@nshs.edu*. Feinstein Institute, Manhasset, New York, United States

Systemic lupus erythematosus (SLE) is a chronic disease in which autoantibodies directed to nucleic acid antigens cause inflammation and tissue injury. Only one new drug has been approved for the treatment of lupus in the last 50 years. Major unmet therapeutic needs include lupus nephritis, neurologic decline, fatigue, anti-phospholipid syndrome, and premature atherosclerosis. Not all of these manifestations are adequately modeled in animals.

Lupus nephritis affects 30-60% of SLE patients and many challenges remain in its management. These include a low rate of complete remissions, recurrent flares and a high rate of progression to ESRD. Lupus nephritis can be modeled in mice, but there are substantial differences between strains, reflecting the heterogeneity of human disease. Responses to therapy vary with strain, disease stage and inflammatory load and the best outcomes occur when therapy is started prior to disease onset.

Other lupus manifestations can also be modeled in mice. Skin disease may occur spontaneously or may be induced with an innate stimulus. Similarly, anti-phospholipid syndrome may occur spontaneously or may be induced in normal mice following the transfer of human sera. Such models have led to the discovery of complement activation as a major effector mechanism in pregnancy loss and also allow testing of therapeutics that protect injured blood vessels. CNS lupus is difficult to model in mice but recent studies show that autoantibodies directed to brain antigens can directly damage brain cells and that autoantibody access to brain tissue is enhanced by damage to the blood brain barrier. The increased incidence of premature atherosclerosis in lupus patients can also be partially modeled by using genetic models of lupus and a high fat diet.

The multiple failures of clinical trials for lupus may be due to the inclusion of late stage patients with established immune abnormalities, the contribution of non-immune mechanisms to tissue damage, patient heterogeneity, the concomitant use of immunosuppression, as well as intrinsic differences between mouse and man. Early intervention in humans may be required to achieve the maximal potential of immune modulation. Many new targets remain to be tested including innate immune effectors, inflammatory cytokines, signaling molecules, complement and metabolic pathways. As

better testing of biomarkers is developed, targeting of the appropriate drug to the appropriate disease manifestation may become possible.

MEDI 200

Translational studies evaluating Btk inhibition as a therapeutic strategy for the treatment of SLE

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Bruton's tyrosine kinase (Btk) is expressed in a wide variety of immune cell types and regulates B cell receptor signaling as well as Fc receptor signaling. The significant role that Btk plays in immune function is underscored by loss of function mutations that cause X-linked agammaglobulinemia (XLA) in humans and X-linked immune defect (XID) in mice. Given the central role that Btk plays in immune function, it is not surprising that previous work using Btk inhibitors and Btk deficient mice has demonstrated that blocking Btk is a promising approach for treating autoimmune diseases. Herein, we report on studies which utilized tool Btk inhibitors to explore the therapeutic efficacy of Btk inhibition for treating systemic lupus erythematosus (SLE). We have used in vitro systems employing various immune cell types and mouse models of lupus with varying etiology and pathogenesis. In vitro studies using primary human immune cells revealed that Btk inhibition can block not only B cell activation, but also activation of macrophages by immune complexes and TLRs which contributes to tissue damage in SLE. In several mouse lupus models (IFN-accelerated NZB/W F1, BXSB-Yaa, pristane-DBA/1), Btk inhibition proved efficacious by inhibiting not only B cell activity and autoantibody production, but also end organ damage. Combined, the in vitro and in vivo results suggest that Btk inhibition may be efficacious through multiple mechanisms of action. Having multiple mechanisms of action is particularly important for treating lupus as the disease is extremely heterogeneous in symptomology and pathogenesis from patient to patient. Overall, our results provide translational insight into how Btk inhibition may provide therapeutic benefit to a variety of SLE patients by affecting both BCR and FcR signaling.

MEDI 201

Discovery of pyridine amide based inhibitors of interleukin receptor-associated kinase 4 (IRAK4) for the treatment of lupus

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IRAK4 is a member of the IRAK family of serine-threonine kinases which signals downstream of toll-like receptors (TLRs) and IL-1 receptors. TLRs are centrally critical to innate immune system defense due to their ability to recognize molecular patterns associated with various bacterial and viral pathogens. In addition, these pattern recognizing receptors also detect damage-associated molecular pathogens (DAMPs) generated in the course of chronic autoimmune disease states such as lupus. Activation of the TLRs (except TLR3 and to a lesser degree TLR4) by these DAMPs starts a complex signaling cascade of adaptor protein recruitment and IRAK4 activation. Subsequent signaling via downstream kinases leads to NF- κ B activation and cytokine expression. The location of IRAK4 downstream of these innate immune system signaling receptors has resulted in significant interest in therapeutic targeting of IRAK4 in autoimmune diseases. Herein, we present the discovery, SAR, and potency optimization of a pyridine amide series of IRAK4 inhibitors. The structural basis of inhibition will be detailed with X-ray co-crystal structures of select compounds. Additionally, kinase selectivity optimization and in vivo efficacy in a murine model of lupus will be presented.

MEDI 202

Structure-based design of potent and selective inhibitors of NF- κ B inducing kinase (NIK)

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NF- κ B-inducing kinase (NIK) is a protein kinase central to the non-canonical NF- κ B pathway and mediates the NF- κ B signal through IKK α activation and p100 processing to nuclear transcription factors p52 and RelB. This non-classical pathway is downstream from multiple TNF receptor family members including BR3/BAFF-R, CD40, LT- β R, OX40, RANK, CD27, and Fn14 (TWEAK-R) which have been associated with B cell survival and maturation, dendritic cell activation, secondary lymphoid organ development, and bone metabolism. Increased serum BAFF levels are associated with autoimmunity and disorders such as lupus erythematosus, and inhibition of BAFF signaling has been shown to be efficacious in murine models of lupus. It is thought that inhibition of NIK could provide additional benefit over BAFF inhibition in the blockade of the non-canonical NF- κ B pathway through modulation of signaling of multiple receptors at once.

A lead chemical series was identified through the optimization of a high throughput screening hit. Structure-based design led to the identification of several potent and

selective NIK inhibitors which reach past the methionine-471 gatekeeper residue. These compounds exhibited selective inhibition of LT β R-dependent p52 translocation and transcription of NF- κ B2 related genes. The identification and optimization of these compounds will be described with an emphasis on structure-based drug design.

MEDI 203

E6887: A novel and selective inhibitor of toll-like receptors 7 and 8

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The toll-like receptors (TLRs) play a key role in vertebrate immune recognition of pathogen-associated molecular patterns (PAMPs) and of tissue damage independent of infection (DAMPs). Various ligands can act as “danger signals” detected by this component of the innate immune system. TLR7 and 8 are located in the endosomes of various immune subpopulations, and are activated by single-stranded RNA from viruses. More recently it was found that TLR7 and TLR8 are also activated by autologous RNA fragments bound to immune complexes, inducing the generation of cytokines such as interferons (specifically IFN- α), known to be associated with autoimmune diseases such as systemic lupus erythematosus (SLE) and psoriasis. We describe here the discovery and development of E6887, a novel and selective small molecule TLR7/8 antagonist for the treatment of SLE. The hit-to-lead SAR efforts and molecular mechanism of action studies will be described for this tetrahydropyrazolo-pyrimidine series of compounds. Potent activity was observed in vitro in TLR-specific reporter systems (IC₅₀ of ~100 nM) and in primary human blood cells (IC₅₀ of 50-500 nM across various ligands and cytokine readouts). Finally, oral availability and in vivo pharmacodynamics were determined to be suitable for exploration of therapeutic activity in disease models.

MEDI 204

SLC transporters in drug response

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Solute Carrier (SLC) transporters, including 395 membrane proteins that are grouped by homology into 52 families, transport a wide array of substrates. Because of their key roles in solute homeostasis and human biology, transporters in the SLC superfamily are frequently associated with human disease. Genomewide association studies have implicated polymorphisms in over 150 SLC transporters in human disease and variation in drug response. Further, NextGen Sequencing studies have revealed mutations in SLC transporters that are causative for monogenic disorders and contribute to inter-individual differences in therapeutic and adverse drug response. Transporters on the plasma membrane may be excellent targets for both large and small molecules and new

drugs targeted to SLC transporters are currently in various stages of clinical development. This overview will begin with an overview of the SLC superfamily and then describe the roles of various transporters in human physiology and disease as revealed through human genetic studies. Recent information on transporters in the SLC superfamily, which are implicated in variation in drug response will be provided. Human genetic studies suggest that transporters in this superfamily constitute a large and diverse group of potential new targets for the prevention and treatment of disease.

MEDI 205

Structure-based ligand discovery for nutrient transporters

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Alterations in cell metabolism support rapid growth and proliferation of cells and are key Hallmarks of Cancer. Solute Carrier (SLC) transporters are membrane proteins that transport solutes such as metabolites and drugs across membranes, and play a major role in mediating nutrient delivery in reprogrammed cancer metabolism networks. For example, the amino acid transporters LAT-1 (SLC7A5) and ASCT2 (SLC1A5) are upregulated in multiple cancer types such as Glioblastoma Multiforme, where they supply the growing tumor cells with essential amino acids that are used as nutrients to build biomass and signaling molecules to enhance proliferation. Here, we describe a structure-based discovery approach to identify small molecule modulators for human SLCs and apply this approach to characterize ASCT2 and LAT-1, which function cooperatively in cancer metabolism and are highly expressed in the blood-brain-barrier (BBB). In particular, we first perform a comprehensive comparison of the human SLC transporters, to inform attempts to model their atomic structures, a prerequisite for structure-based ligand discovery. We then use homology modeling, virtual screening, and experimental testing with electrophysiology and other cell-based assays, to identify small molecule ligands for LAT-1 and ASCT2. Initial hits are then refined through iteration of computational modeling and experimental testing. Our results may explain some of the pharmacological effects (i.e., efficacy and/or side effects) of known drugs via polypharmacology, and rationalize the enhanced brain permeability of drug-like molecules. Finally, our top hits inhibited proliferation of various cancer cell lines via distinct molecular mechanisms, providing useful chemical tools to characterize reprogrammed metabolic networks, as well as a framework for developing efficacious lead compounds against these key targets and other human SLC transporters.

MEDI 206

Discovery of a non-absorbable ASBT inhibitor clinical candidate for treatment of type 2 diabetes

Jon L. Collins^{1,2}, jon.l.collins@gsk.com. (1) Discovery Partnerships with Academia, GlaxoSmithKline R&D, Research Triangle Park, North Carolina, United States (2) Metabolic Pathways and Cardiovascular Disease, GlaxoSmithKline R&D, King of Prussia, Pennsylvania, United States

The apical sodium-dependent bile acid transporter (ASBT) transports bile salts from the lumen of the gastrointestinal (GI) tract to the liver via the portal vein. Multiple pharmaceutical companies have exploited the physiological link between ASBT and hepatic cholesterol metabolism, which led to the clinical investigation of ASBT inhibitors as lipid-lowering agents. Whilst modest lipid effects were demonstrated, the potential utility of ASBT inhibitors for treatment of type 2 diabetes has been relatively unexplored. We initiated a lead optimization effort that focused on the identification of a potent, nonabsorbable ASBT inhibitor starting from the first-generation inhibitor 264W94. Extensive SAR studies culminated in the discovery of GSK2330672 as a highly potent, nonabsorbable ASBT inhibitor which lowers glucose in an animal model of type 2 diabetes and shows excellent developability properties for clinical evaluation. The discovery of GSK2330672 and effects in type 2 diabetic patients will be presented.

MEDI 207

Blocking lactic acid transport: A cancer metabolism-based strategy for finding new antitumor agents

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Since the pioneering work of Warburg we have understood that malignant cells are reliant upon glycolysis, thus they produce lactic acid, resulting in local extracellular acidification. Most tumor types rely upon the transporters MCT1, MCT4, or both to actively transport lactate. We have demonstrated the antitumor efficacy of MCT inhibitors in multiple structural series and will describe the discovery, early structural optimization, and assessment of these inhibitors.

MEDI 208

Development of selective uric acid reabsorption inhibitors (SURIs) for the treatment of gout

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A number of drugs are known to reduce serum urate levels by increasing the excretion of uric acid in the urine. These drugs are not selective for uric acid transporters in the kidney, and they tend to be associated with either significant drug-drug interactions or

other adverse events associated with their lack of selectivity, which significantly limits their use. Some of these drugs were discovered more than 50 years ago, and for many years, uric acid transport inhibitors were not actively targeted for drug discovery. We reported previously the serum urate reduction effect of RDEA594 (lesinurad), a recently discovered SURI. This drug was shown to selectively inhibit URAT1 and OAT4, without affecting other generic ion transporters like OAT1 and OAT3 in vivo. Lesinurad was approved by the FDA in December 2015, in combination with a xanthine oxidase inhibitor, for the treatment of hyperuricemia associated with gout in patients who have not achieved target serum uric acid levels with a xanthine oxidase inhibitor alone. We also reported the development of RDEA3170 (verinurad), a second generation SURI with an improved potency allowing lower daily dosing. In this presentation, we will describe the discovery and developments of lesinurad and verinurad.

MEDI 209

Overview of the progression of Pfizer's SGLT2 inhibitor program from the discovery of ertugliflozin (PF-04971729) to successful POC

Vincent Mascitti, vincent.mascitti@pfizer.com. Pfizer, Groton, Connecticut, United States

Inhibition of sodium-dependent glucose cotransporter 2 (SGLT2; SLC5A2), a transporter located in the kidney, is a mechanism that promotes glucosuria and therefore, reduction of plasma glucose concentration. Since the mechanism operates in a glucose-dependent and insulin-independent manner, and is associated with weight loss, it has emerged as a very promising approach to the pathophysiologic treatment of type 2 diabetes. In this presentation, we will describe the medicinal chemistry rationale that led to the rapid identification of Ertugliflozin (PF-04971729), an anti-diabetic agent belonging to a new class of SGLT2 inhibitors. Emphasis will be placed on the rapid progression of Ertugliflozin in the early phases of development, from first in human (FIH) to successful completion of phase 2 trials. Ertugliflozin is currently being developed (phase 3 trials) in partnership between Merck and Pfizer.

MEDI 210

Computational analysis of molecular scaffolds

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The scaffold concept is popular in chemical informatics and medicinal chemistry to represent core structures of bioactive compounds. Interests in scaffolds often reflects the desire to identify structural classes that are associated with specific biological activities or organize biologically active compounds on the basis of characteristic structural motifs and their relationships. Although the scaffold concept has limitations

and is often viewed differently from a chemical and computational perspective, it has provided a basis for a systematic exploration of molecular cores, going beyond the analysis and comparison of individual compound series. Alternative scaffold definitions and organization schemes have been introduced and scaffolds have increasingly been explored on a large scale.

Scaffold representations are often used to organize compounds in a hierarchical manner, classify structures, associate scaffolds with different biological activities, or predict active compounds. Computational approaches for scaffold generation and analysis are introduced, milestones in scaffold research highlighted, and recent developments discussed that impact medicinal chemistry.

MEDI 211

Spirocyclic scaffolds in drug discovery

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Spirocyclic drugs have been known for over 50 years. In recent years, spirocycles have enjoyed increased attention as scaffolds both in library-based approaches to drug discovery and in structure based-drug design. Spirocycles composed of 6-membered and smaller rings are either rigid or have a limited number of well-defined conformations. For structure based drug design, spirocyclic cores can be decorated with appropriate substituents to form favorable interactions with a binding site of interest, in a predictable manner. Furthermore, the obligatory sp³ character of spirocycles may favor increased water solubility compared to heavily (hetero)aromatic systems. Finally, spirocycles may offer greater scope for IP protection compared to well-explored monocyclic systems. We will present examples from our own work and from the literature to illustrate the applications of spirocyclic scaffolds in drug discovery.

MEDI 212

Malaria as a proof of concept of how natural products have inspired the development of preclinical and clinical candidates with diverse mechanisms of action

Felix Calderon Romo, *felix.r.calderon-romo@gsk.com*. Tres Cantos Medicines Development Campus, GlaxoSmithKline, Tres Cantos, Spain

From a drug-development perspective, natural products can be troublesome as starting points for optimization programs, as they are commonly associated with complex synthetic routes, high cost of goods (CoGs), and poor druglike properties due to their low ligand efficiency and high molecular weight. However, Natural products have played a pivotal role in different therapeutic areas.

In particular, malaria chemotherapy has benefit successfully from natural products

progressing from quinine and artemisinin to ozonide-based compounds. Many of these natural products have served as template for the design and development of antimalarial drugs currently in the clinic or in the development phase. In this talk malaria will be used as proof of concept of how some of these privileged scaffolds have guided medicinal chemistry efforts yielding molecules that have reached the clinic.

MEDI 213

Macrocyclic peptide scaffolds: Passive permeability and oral absorption beyond the rule of 5

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

The prospect that macrocyclic peptides that lie well outside the Rule of 5 can have drug-like, passive cell permeability has stimulated much effort toward understanding the physical basis for the behavior of such outliers. I will discuss our latest results from a series of systematic studies using a variety of synthetic, biophysical, and analytical tools, designed to probe the specific structural and physicochemical constraints that govern ADME behavior in macrocycles in the MW~1000 range. I will focus on our results using ultra-high sensitivity mass spectrometry to analyze complex library mixtures of scaffolds for passive permeability, and the optimization of cyclic peptide-peptoid hybrids (peptomers) for membrane permeability in non-biological model systems as well as cell-based assay systems.

MEDI 214

LipMetE assessment of bioisosteres in medicinal chemistry

Antonia F. Stepan, *Antonia.Stepan@pfizer.com*. Pfizer, Brookline, Massachusetts, United States

The lipophilic metabolism efficiency (LipMetE) parameter was introduced as a means to relate the microsomal metabolic stability of a drug molecule to its lipophilicity. LipMetE can aid medicinal chemists in the identification of analogs with sufficient metabolic stability at the required lipophilicity level of a particular target. LipMetE separates the contribution of lipophilicity to microsomal stability from factors such as chemical stability and series with high LipMetE values are preferred since sufficient metabolic stability can be achieved within a wider lipophilicity range. Project applications of this design parameter will be discussed as well as an assessment of bioisosteres with regards to their probability of modulating LipMetE.

MEDI 215

Chemical probes for target validation

Mark Bunnage, *mark.bunnage@pfizer.com*. Worldwide Medicinal Chemistry, Pfizer, Cambridge, Massachusetts, United States

It is arguable that improving the quality of target selection is the single most important factor to enhance the productivity of Pharmaceutical R&D and bring innovative new medicines to patients. High quality chemical probes are essential tools to support preclinical target validation. Unfortunately, many chemical probes reported in the literature are poorly characterized and their value in target validation is thus questionable.

In this presentation we will discuss the key characteristics for high quality chemical probes and describe the '4 Pillars' framework for their use in target validation experiments.

The presentation will also describe an open innovation collaboration led by the Structural Genomics Consortium (SGC) that is seeking to develop novel chemical probes for target validation in areas such as epigenetic proteins and kinases. Some of the recent progress in this collaboration will be described, highlighting the central role of medicinal chemistry in chemical probe design.

MEDI 216

Selective modulation of p97-dependent protein homeostasis networks

Michelle Arkin, *michelle.arkin@ucsf.edu*. Pharmaceutical Chemistry and Small Molecule Discovery Center, University of California-San Francisco, San Francisco, California, United States

The AAA ATPase p97/VCP is a master regulator of protein homeostasis, coupling with different 'adaptor' proteins to support protein degradation and trafficking in multiple organelles. Misregulation of p97 has been implicated in aging, cancer, and neurodegeneration; an ATP-competitive inhibitor of p97 has recently entered the clinic. Using high-throughput screening and fragment-based drug discovery, we set out to develop alternative mechanisms for inhibiting p97, including inhibition of protein-protein interactions and allosteric inhibition of ATPase activity. Here, we will describe the methodologies and mechanisms, including the first high-resolution cryo-EM structure of p97 bound to an allosteric inhibitor. This collaborative program, involving the University of Pittsburgh, Caltech, UCLA, UCSF, Leidos Biomedical Research, and the National Cancer Institute, is a fruitful example of the potential for academic drug discovery centers to drive innovative translational science.

MEDI 217

DrugTargetSeqR: An interdisciplinary approach to dissect the mechanisms of action of drugs and chemical probes

Tarun Kapoor, *kapoor@rockefeller.edu*. The Rockefeller University, New York, New York, United States

Determining a drug's physiological target is a major challenge in chemical biology and drug discovery. Current strategies to find drug targets fall into two broad categories: model organism-based approaches and affinity-based methods (e.g. pull-downs). However, many drugs are not active in model organisms and affinity-based approaches are effective only when the drug is potent and the target is reasonably abundant in vivo. Further, establishing the physiological target of a drug depends on frequently unreliable correlations between phenotypes associated with candidate target protein knockdown and chemical inhibition of activity. To address these limitations, we designed and validated a new approach at the interface of chemistry and genomics to identify the physiological target of a drug. Briefly, multiple drug-resistant clones are isolated from cells grown in culture and transcriptome sequencing is used to find genetic alterations (e.g. mutations or gene over-expression) present in each clone, but absent in the cell populations from which the clones are isolated. CRISPR/Cas9 genome editing is then used to examine if any of the identified genetic alterations, which recur across multiple clones, are sufficient to confer drug resistance when introduced into a drug-sensitive cell-line. Further biochemical and cell-based analysis of the drug resistance-conferring mutations can lead to the physiological targets of the drug, as indicated by our proof-of-concept studies of cytotoxic anticancer drugs. Our approach, which we have named 'DrugTargetSeqR', has advantages over other target identification methods as the methodology is not biased to one cellular pathway or protein family (e.g. kinases), chemical modifications of the drug are not required, and cell-type specific analysis is possible. Importantly, we can achieve 'gold standard' target validation, i.e. when a mutation in the target reduces drug sensitivity in cellular contexts and in biochemical assays. The use of DrugTargetSeqR can reveal drug targets in disease cells, unanticipated targets in healthy cells, and cellular mechanisms of resistance. These data can help design chemical modifications to improve drug efficacy and reduce toxicity.

MEDI 218

Sirtuin inhibitors as anticancer agents

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Sirtuins are known as nicotinamide adenine dinucleotide (NAD)-dependent deacetylases. They regulate aging, transcription, and metabolism, and are considered important targets for treating several human diseases. There are seven sirtuins in humans, SIRT1-7. Four of them (SIRT4-7) have very weak deacetylase activity, which have caused many confusions and debates in the biological community. My laboratory has recently discovered several novel enzymatic activities, such as desuccinylation and defatty-acylation, for several sirtuins with no robust deacetylase activity. This has led to the identification of previously unknown protein posttranslational modifications

(PTMs) and revealed new regulatory mechanisms of biology. Furthermore, this finding has enabled us to develop compounds that can inhibit particular sirtuins selectively. Some of the selective sirtuins inhibitors can kill cancer cells in cell culture and inhibit tumor formation in mouse models. In particular, we found SIRT2-selective inhibitors can promote the degradation of an important oncoprotein, c-Myc. Promoting the degradation of c-Myc contributes to the anticancer effects. As c-Myc is upregulated in more than 50% of human tumors, SIRT2 inhibitors may be useful for treating many human cancers.

MEDI 219

Designed covalent inhibitors as chemical biology probes and drug development candidates

Timothy D. Owens, *tim.owens@principiabio.com*. Principia Biopharma, South San Francisco, California, United States

Drugs that exert their pharmacological effects via covalent inhibition of proteins, either rationally designed or identified serendipitously, have found wide utility across many therapeutic areas. The clinical success and relative safety of many of these drugs has heightened interest in utilizing covalent strategies for targeted pharmacological intervention. Targeted covalent inhibition holds promise for the rapid identification of extremely selective inhibitors. Additionally, covalent inhibition can impart pharmacological effects without the need for prolonged plasma exposure, minimizing the need for extensive PK optimization. Principia has pioneered the development of covalent reversible drugs targeting non-catalytic cysteines. We will discuss our cysteine targeting approach with both covalent irreversible as well as covalent reversible inhibitors. We have used this approach to design and study selective inhibitors of proteins of therapeutic interest such as Bruton's tyrosine kinase (BTK), fibroblast growth factor receptors (FGFR), and interleukin-2-inducible T-cell kinase (ITK). These highly selective inhibitors serve as excellent chemical biology probes to study the catalytic function of these proteins and, as in the case of our clinical BTK and FGFR inhibitors, potentially best in class therapeutics.

MEDI 220

Chemical and proteome-wide reactivity profiling of covalent serine hydrolase inhibitor chemotypes

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The human serine hydrolase superfamily includes over 250 members making it one of the largest known enzyme classes. Through the production or degradation of a wide range of bioactive molecules, these enzymes operate in diverse physiological arenas including metabolism, inflammation, neurotransmission, and blood clotting. Covalent

inhibition through active-site directed electrophiles has proven to be an effective strategy for serine hydrolase drug development as well as in the design of tool molecules to study the biochemical and physiological functions of the many members of this class that remain biochemically and physiologically uncharacterized. While many covalent serine hydrolase inhibitor chemotypes have been described, their selectivity against the serine hydrolase class as well as the broader proteome remains underexplored. Here, we describe techniques including direct- and competitive-activity-based protein profiling (ABPP) to systematically explore the steric and electronic features that contribute to serine hydrolase inhibitor activity and specificity. We then systematically explore the relationship between chemical reactivity of serine hydrolase inhibitors and their biochemical (proteome-wide) reactivity. This work shows how an integrated understanding of chemical reactivity and protein reactivity is essential for design and use of covalent inhibitors, and provides a general framework for the evaluation and optimization of covalent drugs.

MEDI 221

Tuning the chemical properties of XNA nucleotides and oligomers for therapeutics and diagnostics

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Xeno nucleic acids (XNA) are backbone-modified nucleic acids in which the “X” can be replaced by a specific letter (or letters) to indicate a particular modification. Because XNAs do not occur in nature, they are generally poor substrates for naturally occurring enzymes such as nucleases. This imparts a high level of biostability that can be particularly beneficial for applications in therapeutics and diagnostics. Our lab has explored both peptide nucleic acids (PNA) and threose nucleic acids (TNA), with a focus on further modifying these unnatural scaffolds in order to modulate their biological and physicochemical properties. Specifically, we have found that the overall affinity and salt dependence of PNA can be modulated by altering the electrostatic properties of side chains integrated into the PNA backbone. We have also explored modifications to the β,γ -linkage in TNA nucleoside triphosphates as a means to improve the efficacy of chain termination by HIV reverse transcriptase.

MEDI 222

RNAi therapeutics: From base pairs to bed side

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Our laboratory has pioneered the systemic delivery of therapeutic siRNAs to liver hepatocytes by subcutaneous administration by conjugating chemically modified short-interfering RNAs (siRNAs) to multivalent *N*-acetylgalactosamine (GalNAc) residues that

are recognized by the asialoglycoprotein receptor (ASGPR). siRNA-GalNAc conjugates efficiently target and silence disease-causing genes produced in liver hepatocytes. Using this conjugation platform, Alnylam is advancing several RNA interference (RNAi) agents specific for liver targets through pre-clinical and clinical development to treat diseases with highly unmet medical need. Our progress with the chemistry of siRNAs, their conjugates, and applications of these drug constructs in several therapeutic areas will be presented.

MEDI 223

Expanding the chemical diversity of therapeutic oligonucleotides for the treatment of neurodegenerative disorders

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RNA interference has revolutionized human functional genomics and therapeutics, but its impact on neuroscience research has been limited by the lack of simple and efficient methods to deliver oligonucleotides to primary neurons and the brain. We show that primary neurons rapidly internalize fully stabilized, hydrophobically modified siRNAs (hsiRNAs) added directly to the culture medium and that hsiRNAs induce potent and long-lasting silencing *in vitro*. A single injection of unformulated hsiRNA (cholesterol-conjugated) into mouse brain silences locally with great potency and longevity. Limited distribution from the site of administration precludes direct use of this type of chemistry for modulation of gene expression in larger brains and potential therapeutic development.

Using fully chemically modified siRNA scaffolds, we systematically screened a wide range of bioactive conjugates and demonstrated that the chemical nature of the conjugation modality has a major impact on brain tissue retention, distribution and cellular internalization. We have identified several novel chemical classes of conjugates that demonstrate markedly improved brain distribution and robust *in vivo* efficacy. Direct conjugation of a fully chemically modified siRNA to docosahexaenoic acid (DHA), the most abundant poly-unsaturated fatty acid in the brain, results in improved tissue retention with wide distribution and robust efficacy in the striatum and cortex after a single injection. Most importantly, DHA-hsiRNA conjugates do not induce neural cell death or measurable innate immune activation following administration of concentrations 20-fold over the efficacious dose, establishing a new approach toward development of RNAi-based therapeutics for a wide range of neurodegenerative disorders.

MEDI 224

Development of nucleoside analogs as broadly active antiviral agents

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RNA viruses constitute a broad class of pathogens containing ribonucleic acid as their genetic material. They are the most common class of pathogens identified as causes of emerging and reemerging human disease. Many RNA viruses are transmitted to humans via mosquito vectors, and, as mosquito populations have grown, the incidence of viral infectious disease has consequently increased. In an effort to develop antiviral agents that act broadly against a number of RNA viral infections, we have targeted a highly conserved virus-encoded enzyme essential for replication of their genomes. RNA viruses encode an RNA-dependent RNA polymerase (RdRp) that catalyzes this replication process using genomic RNA as the template, and host-cell generated ribonucleotide 5'-triphosphates as substrates. Inhibition of the RdRp blocks viral replication and stops the pathogenesis of viral disease. Here we report the pre-clinical development of a cytidine analog, EIDD-1931, that acts broadly as a potent competitive, alternative substrate inhibitor of the RdRps encoded by multiple members of the genus *Alphavirus*, a genus of RNA arboviruses that causes significant human morbidity and mortality. EIDD-1931 is transported into infected cells where it is efficiently anabolized to its corresponding 5'-triphosphate, the active antiviral form of the compound. Incorporation of the 5'-monophosphate of EIDD-1931 5'-triphosphate into nascent chain viral RNA by the RdRp blocks genome synthesis in cell models of infection. EC₅₀ values in Vero cells for two targeted *Alphaviruses* are 1.09 μM for Chikungunya virus and 1.40 μM for Venezuelan Equine Encephalitis virus. EIDD-1931 has favorable pharmacokinetics and is distributed to key organs in the pathogenesis of disease where it is efficiently anabolized to its active 5'-triphosphate. The compound has shown dose-dependent activity in an animal model of Chikungunya infection and is being aggressively pursued as a broad-spectrum antiviral drug.

MEDI 225

Sequence-based design of small molecules targeting RNA

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RNA is an important biomolecule for small molecule intervention yet small molecules that selectively target RNA are sparsely known. In this talk, we briefly describe the development and implementation of several novel approaches to rationally design small molecules that target RNA based only on the sequence of the target. Rational design is enabled by using two-dimensional combinatorial screening (2DCS) that is an approach to identify and annotate a database of RNA fold-small molecule binders. This information is then used in conjunction with RNA structure annotation programs via Inforna to identify druggable RNA targets in the transcriptome in a target agnostic manner. These strategies will be applied towards in vivo targeting of RNAs that cause incurable orphan disease and also difficult to treat cancers. Importantly, these studies

advance a paradigm in which a disease-associated RNA is validated and its structure used to design and optimize small molecules in a rapid fashion.

MEDI 226

Concepts in the design of intestinally targeted drugs

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Intestinal targeting of drugs offers a way to increase the therapeutic index of certain therapies targeting the gut by limiting systemic exposure. Undesired effects can be avoided by maximizing the intestinal concentration at the site of action, while minimizing the concentration of drug in the anti-tissue. There are several approaches that can be considered to rationally design an intestinally targeted, or non-absorbable, drug. Physicochemical property manipulation toward large, polar chemical space favors minimizing intestinal absorption. Alternatively, driving properties into lipophilic space can increase first-pass metabolism and reduce systemic exposure. Designing for substrate-recognition by either uptake or efflux transporters in the intestinal enterocytes, or the hepato-biliary interface is another strategy. Finally, low absorption prodrugs that are cleaved by gut bacterial enzymes have been successfully applied, particularly for colonic-targeting. These differing strategies will be highlighted, using known examples of drugs and drug candidates to elucidate each approach.

MEDI 227

Discovery of TGR5 agonists with gut restricted action

Jason G. Lewis, jlewis@ardelyx.com, Tao Chen, Jeff W. Jacobs, Patricia Finn, David Rodriguez, Jill Kohler, Kenji Kozuka, Limin He, Christopher Carreras, Samantha Koo-McCoy, Jocelyn Tabora, Jeremy Caldwell . Ardelyx Inc., Fremont, California, United States

The epithelium of the GI tract is a sentinel interface capable of modulating physiology throughout the body. Bile acid signaling and metabolism in the gut have wide ranging influences on systemic disease. TGR5, a GPCR with broad tissue distribution, is one of the major effectors in bile acid sensing with demonstrated effects on inflammation, metabolism and endocrine processes. The pharmacologic utility of TGR5 has been limited due to inhibition of gallbladder contractility and other systemic target-related effects.

Here we demonstrate a series of gut-restricted TGR5 agonists that result in a sustained release of incretins into systemic circulation. In addition to gut-restricted activity, we were able to impart improved TGR5 potency by modulating polarity in regions of our

chemotypes. NTX3460, an exemplar identified in this study has activity in animal models of type 2 diabetes, oncology supportive care and in a model of inflammatory bowel disease when combined with Sitagliptin.

MEDI 228

Physical-property based design of gut-selective CCK1 receptor agonists

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The design of drugs that selectively target one tissue over another is a strategy that may be employed to improve the therapeutic index of a drug series. Achieving higher drug concentrations at the desired site of action relative to the undesired safety tissue has proved a successful approach as indicated by a number of tissue-selective drugs that are on the market today (e.g. statins). A number of key targets in the gastrointestinal tract have the potential to impact diseases such as obesity and diabetes. The ability to enhance the exposure of drugs within the gastrointestinal tract is therefore of interest. We will describe a physical property based approach used to identify cholecystokinin-1 receptor (CCK1R) agonists. The application of these design tactics led to the identification of a prototype compound that was progressed through Phase 2 clinical trials. In addition, our approach for identification of a backup compound with improved properties for gut retention will be described.

MEDI 229

Gut restricted oral peptides as therapeutics for inflammatory bowel disease

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Peptides have been shown to be effective therapeutics by blocking or stimulating protein-protein interactions, similar to antibodies. Unlike antibodies however, peptides can be chemically engineered to be orally stable, and therefore they are potential oral replacements to injectable antibodies. For therapeutic targets located in gastrointestinal (GI) tissues, oral peptides are ideal because they accumulate to high concentrations in the GI, but have low systemic exposure. We have developed a technology platform based on orally stable scaffolds. The platform involves synergistic integration of rational drug design, diversity oriented computational tools, phage display libraries, ex vivo oral stability assays, and peptide/medicinal chemistry techniques. We have used this platform to develop oral antagonists of $\alpha 4\beta 7$ integrin and IL-23 receptor (IL-23R), both clinically validated targets for inflammatory bowel disease (IBD). PTG-100, a selective oral peptide antagonist of $\alpha 4\beta 7$ integrin, is currently in Phase 1 clinical trials for patients

with moderate to severe ulcerative colitis. In preclinical animal models, PTG-100 alters trafficking of gut homing T cells, and its potency and selectivity are similar to that of the approved anti- $\alpha 4\beta 7$ antibody vedolizumab. Pharmacokinetic studies in rodents or cynomolgus monkeys show that PTG-100 exposure in the blood is <0.1% of dose, but >10% of dose in the small intestine and colon and up to 40% in feces, which indicate PTG-100 is orally stable and largely gut restricted. PTG-200 is an orally stable peptide antagonist of IL-23R. It was identified using a combination of phage display technology and medicinal peptide chemistry. In rat TNBS (2,4,6-trinitrobenzenesulfonic acid)-induced acute colitis models, PTG-200 significantly improved disease outcomes as assessed by histopathology, reduced local neutrophil activity, and decreased production of IL-22 and IL-17A in the colons of diseased animals. We will present models to explain the mechanism of peptide transport across the gut epithelial barrier.

MEDI 230

Power of the gut microbiome

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The importance of the gut microbiome on human health and disease is now apparent. We are rapidly learning more about the composition of a 'healthy' microbiome and realize that dysbiotic states are associated with disease. The efficacy of fecal microbiota transplantation as a treatment for recurrent *Clostridium difficile* infection provides proof-of-principle that restoration of a healthy gut microbiome can reestablish health.

It is now understood that the far-reaching effects of the gut microbiota are via the metabolites that these microbes produce. Collectively, these metabolites are referred to as the gut metabolome. It is the gut metabolome that impacts distant organ systems including the heart, liver, brain and others. Diet plays a major role in determining both the composition of gut microbiota as well as the resulting metabolites. However, evidence suggests that microbiota composition determines the level of metabolites produced in response to a specific diet and in some cases a specific composition may be 'permissive' or 'restrictive'. In depth understanding of these processes and the impact of specific metabolites on specific organs and diseases stands to provide new and novel preventative and therapeutic strategies for human disease. As we acquire more knowledge regarding the specific bacterial enzymes responsible for the production of detrimental metabolites, directed approaches to preventing and treating disease will be through 'drugging the bug' and not altering microbiota composition in some cases.

MEDI 231

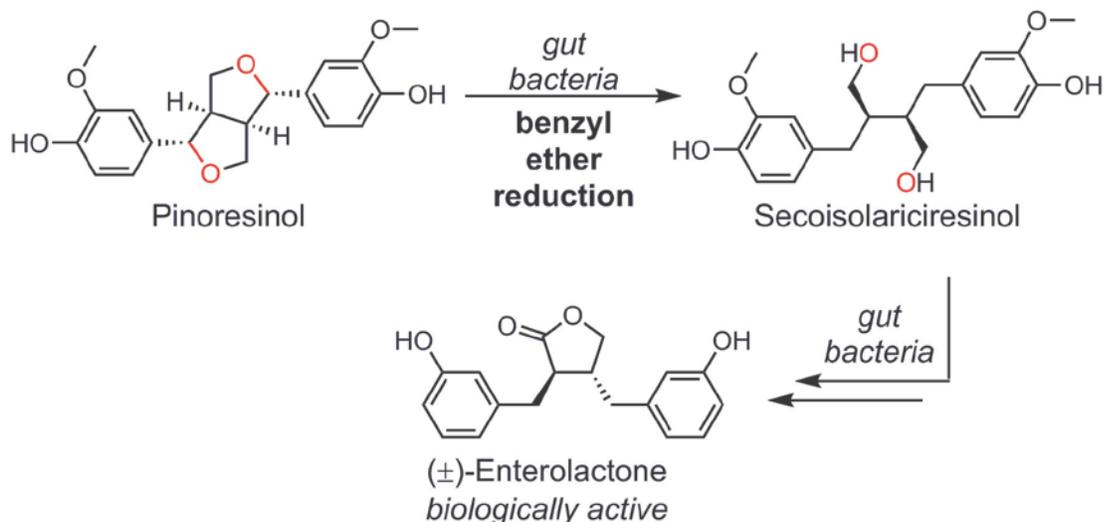
Strategies to investigate the xenobiotic-metabolizing capabilities of the human gut microbiome

Elizabeth Bess, *elizabeth.n.bess@gmail.com*, Jordan Bisanz, Peter Spanogiannopoulos, Peter Turnbaugh. UCSF, San Francisco, California, United States

Small molecules consumed in food and pharmaceuticals are frequently metabolized in the body, changing their function. Liver metabolism of these compounds has been extensively evaluated, leading to the development of algorithms to predict their pharmacokinetics. However, these models have largely neglected an aspect of metabolism that has significant implications for drug efficacy and toxicity: the human gut microbiome. Encoding an estimated 100-times more genes than the human genome, the 10^{14} microbial cells in the human gastrointestinal tract—the gut microbiota—are a vast potential source of small-molecule metabolism. We address this gap at the intersection of pharmacology and the gut microbiome by identifying microbial genes responsible for chemical metabolism.

The microbiome's metabolism of dietary molecules is dependent on an individual's unique set of gut microbial genes, which varies between individuals. Identifying these genes can serve as a tool to predict the chemical transformations performed by an individual's gut microbes. One such transformation mediated by gut bacteria is benzyl ether reduction. Benzyl ethers are found in numerous food and drug compounds, including the commonly consumed plant lignan pinoresinol (PINO). Benzyl ether reduction of PINO precedes additional bacteria-mediated transformations to produce the uniquely microbial metabolite enterolactone, the systemic circulation of which is linked to a decreased breast-cancer risk. Because genetic variation exists amongst the bacterial species known to metabolize PINO, we explore how the bacterial genes responsible for this chemical reduction may be used as a diagnostic tool to predict the potential to bioactivate and benefit from dietary lignans.

This work provides a foundation for characterizing the chemical reactions that are encoded by the microbiome. Identifying the types of substrates that are modified by microbes will inform and improve models of drug disposition. We envisage a revolution in drug-development strategies as the impact of the microbiome on small-molecule metabolism is integrated into the pharmacokinetics paradigm.



Bioactivation of dietary lignans via the bacteria in the human gut microbiota.

MEDI 232

Applications of bioisosteres in drug design

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Bioisosteres are widely applied in drug design in order to improve the intrinsic properties of molecules and address a range of developability issues. Structurally interesting and bioisosteric relationships continue to be explored as a means of modulating potency, improving pharmacokinetic properties, and addressing metabolic activation pathways and toxicity *in vivo*. In this presentation, we will examine some of the recent applications of bioisosterism in drug design with a focus on both problem solving and unconventional structural mimicry.

MEDI 233

New computational methods to support bioisosteric replacement and molecular library design

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Methods for bioisosteric replacement, and the sub-field of scaffold hopping, are of key importance in the rational design of new chemical entities. Their appropriate application to identify the most relevant replacements whilst covering the diversity of replacements has been demonstrated to reduce the time taken to the synthesis of clinical candidates. Many computational methods exist for the identification of bioisosteric and scaffold replacements and new approaches and analyses continue to be published, a reflection

of the complexity and synergy of potential replacements. There remains a great number of challenges that require careful consideration, both learning from what exists and what can be predicted *in silico*.

Here, a number of new methods are considered covering: design of diverse fragment libraries to cover the available chemical space, appropriate multiobjective optimization in the design of new molecules, and the emergence of three-dimensionality in drug-like molecules. A recent method for fragment replacement is presented in the context of multiobjective *de novo* design, the Rapid Alignment of Topological Scaffolds (RATS). We demonstrate that this is comparable in performance to extant three-dimensional methods which are more computationally expensive and also prone to noise due to the conformer problem.

MEDI 234

Novel building blocks for discovery chemistry: New vistas and opportunities with bioisosteres

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The design and efficient synthesis of novel, medicinally relevant building blocks is of fundamental importance to modern drug discovery. In recent years, a significant expansion of accessible chemical and pharmacological space has been achieved through the invention and application of novel scaffolds, including most prominently spirocycles. In an effort to further expand the chemical diversity we are developing methods that enable the synthesis of intensively functionalized structures and bioisosteres. We will present synthetic difficulties and successes intercepted while attempting to access unexplored small-molecule bioisostere chemical space. Additionally, recent developments in the generation of new building blocks and studies of their physicochemical properties of interest to drug discovery will be discussed.

MEDI 235

Strategies towards increasing the 3-dimensionality of the medicinal chemistry design space

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Drug molecules with an increased bond saturation and 3-dimensionality have been linked to a higher chance of clinical success. Aliphatic bioisosteres are therefore attractive replacements for moieties of high aromaticity. An example of a non-classical phenyl bioisostere rich in sp³ character is the bicyclo[1.1.1]pentane system and its application to both avagacestat and imatinib demonstrated improvements in compound properties, most notably with regards to solubility. Despite favorable attributes,

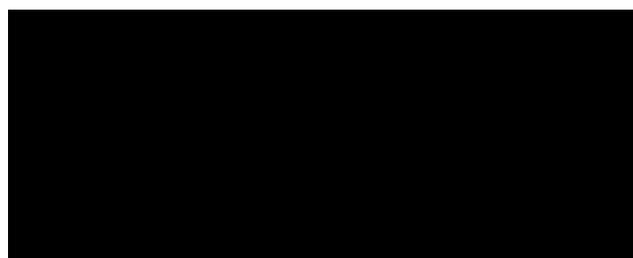
utilization of these unusual moieties is still rare, largely due to synthetic difficulty and a limited possibility for rapid diversification that allows for further lead optimization. Recent progress towards developing chemistry for this underexplored 3-dimensional design space will be discussed.

MEDI 236

Cubane: A benzene isostere!

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The synthesis of cubane (**1**), achieved in 1964 by Eaton, was long predicted to be impossible, due to the immense strain of the molecular structure. Since then a large array of chemical transformations have been performed on the cubane ring system demonstrating the framework to be both a stable and robust building block. Studies into the physical properties of cubane gave further insight, showing that the distance across the cube (the body diagonal) is 2.72 Å, which is almost equivalent to the distance across the benzene ring i.e. 2.79 Å. This similarity is best viewed from one of the 8 apexes (see apex representation **2**). Eaton observed that a number of other similarities exist between cubane (**1**) and benzene (**3**), for example the enhanced s orbital character of the C-H bond and the similar spatial relationships to *ortho* and *para* substituents (i.e. 1,2- and 1,4-disubstitution). However, whilst the physical, or spatial, appearance of cubane is similar to benzene, spectroscopically, cubane has both proton and carbon peaks much further up-field in the nuclear magnetic resonance (NMR) spectrum (^1H ~4ppm ^{13}C ~50ppm) suggesting obvious electronic differences. This lecture will disclose our recent efforts to explore, using known drugs, whether cubane can act as a benzene ring surrogate, based on the above similarities and differences profile displayed by both ring systems.



MEDI 237

Isosteric replacement by catalytic fluorination, fluoroalkylation, borylation, silylation, and amination

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The direct or formal replacement of hydrogens and hydroxyl groups with fluorine and amino groups can modulate the physical properties and pharmacokinetics of organic molecules for medicinal application. Our group has developed cross-coupling reactions and C-H bond functionalization reactions that create the ability to form complex molecules or molecular building blocks containing fluorine or nitrogen in place of hydrogen or oxygen in medicinally relevant structures. Selected examples of these methods will be presented in this lecture.

MEDI 238

Late-stage functionalization of marketed drugs: Synthesis and use of tetrazolones as a carboxylic acid bioisostere

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The frequent use of carboxylic acids in biologically-active molecules has led to the identification of numerous acid bioisosteres. One potential acid bioisostere, the tetrazolone (tetrazol-5-one) group, is related to the more common tetrazole motif. In this paper, we describe the late-stage functionalization of marketed drugs to make tetrazolone congeners of pharmaceuticals containing a carboxylic acid. We continue, by describing some attractions of using a tetrazolone as an acid bioisostere, and discuss the biological profile with tetrazolone analogs of marketed drugs such as Telmisartan (AT1 antagonist), monomethylfumarate (activator of Nrf2), and Bexarotene (RXR agonist), amongst others.

MEDI 239

Development of synthetic methods for the construction of isosteres

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The creation of novel building blocks and their incorporation into unique platforms present outstanding opportunities to increase chemical diversity in druggable space. The research described provides a means to facilitate the efficient incorporation of a variety of novel substructures through the development of effective synthetic routes that are tolerant of sensitive functional groups. These methods allow access to diverse families of molecules possessing novel chemical architectures.

MEDI 240

Optimization of a benzoxazepinoindazole series for human African trypanosomiasis

Dana Klug¹, *klug.d@husky.neu.edu*, **Rosario Diaz-Gonzalez**³, **Carlos Cordon-Obras**³, **Domingo Rojas-Barros**³, **Miguel Navarro**³, **Michael P. Pollastr**². (1) Northeastern University, Brighton, Massachusetts, United States (2) Department of Chemistry & Chemical Biology, Northeastern University, Waltham, Massachusetts, United States (3) Consejo Superior de Investigaciones Cientificas, Granada, Spain

Human African trypanosomiasis (HAT), also known as African sleeping sickness, is one of 17 neglected tropical diseases (NTDs) designated by the WHO. Despite the fact that HAT is 100% fatal if left untreated, the current drugs are less than ideal and new treatments are desperately needed. In resource-limited settings such as academic labs, where most drug discovery for NTDs is done, repurposing is a common drug discovery strategy. One such tactic is “lead repurposing,” wherein lead compounds against a human target are screened in a high-throughput manner against the disease-causing parasite. In 2014, a high-throughput screen of a kinase-inhibitor-based library was undertaken in collaboration with the OpenLab at GlaxoSmithKline, which identified 797 sub-micromolar hits that inhibited *T. brucei* growth and were >100-fold selective over HepG2 cells. The initial hits were grouped into clusters by structural similarity, their physicochemical properties were analyzed, and the clusters were prioritized on the basis of potency, physicochemical properties, rate and reversibility of trypanosome killing, and predicted CNS penetration. We now report the initial SAR exploration efforts for a prioritized cluster of benzoxazepinoindazole compounds, including the synthesis of analogs with improved solubility and LLE.

MEDI 241

Synthetic lethal targeting: A new anticancer strategy

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One of the greatest challenges facing the development of improved cancer therapeutics is the need to selectively kill all of the cancer cells in a patient without harming normal cells. To achieve this high level of selectivity, the genetic concept of synthetic lethality offers a promising strategy. This concept is based on the observation that mutations in two different genes that both contribute to an essential biochemical pathway, such as the genes BRCA1/2 and PARP, can be exploited to make certain cancers uniquely sensitive to anticancer agents. In this mechanism, disruption of either gene alone does not affect cellular viability, but agents or mutations that affect both genes are lethal. We are working to extend the concept of synthetic lethality to the targeting of pairs of growth factor receptors that drive the proliferation of highly aggressive cancers. To accomplish this objective, we are investigating novel antibody conjugates designed with the unique ability to synergistically kill cancer cells that express two distinct cell surface receptors. One targeting antibody is linked via a disulfide to a cell-impermeable cytotoxin that is incapable of unaided passage across cellular membranes. When this first antibody binds a specific growth factor receptor on the cell surface, and is internalized by endocytosis, the stability of the disulfide, in conjunction with the cell-impermeability of

the cytotoxin, causes entrapment in membrane-sealed endosomes. This entrapment prevents toxicity unless membranes of these endosomes are disrupted by co-administration with a secondary agent. To synergistically kill cancer cells, this antibody conjugate is co-administered with a second anti-growth factor antibody linked to a non-toxic endosome disruptive peptide. Release of this peptide from the second antibody forms pores in endosomal membranes. These pores enable cytosolic glutathione to enter endosomes, break the disulfide bond linking the toxin to the first antibody, and activate toxicity by enabling escape of the cytotoxin into the cytoplasm. These unique antibody conjugates are designed to provide high selectivity for killing specific cancer cells that express two distinct cell surface receptors without affecting normal cells that express one of these two target proteins. We termed this novel approach synthetic lethal targeting, and we will describe preliminary studies of this strategy using antibodies that bind the growth factor receptors EGFR and HER2.

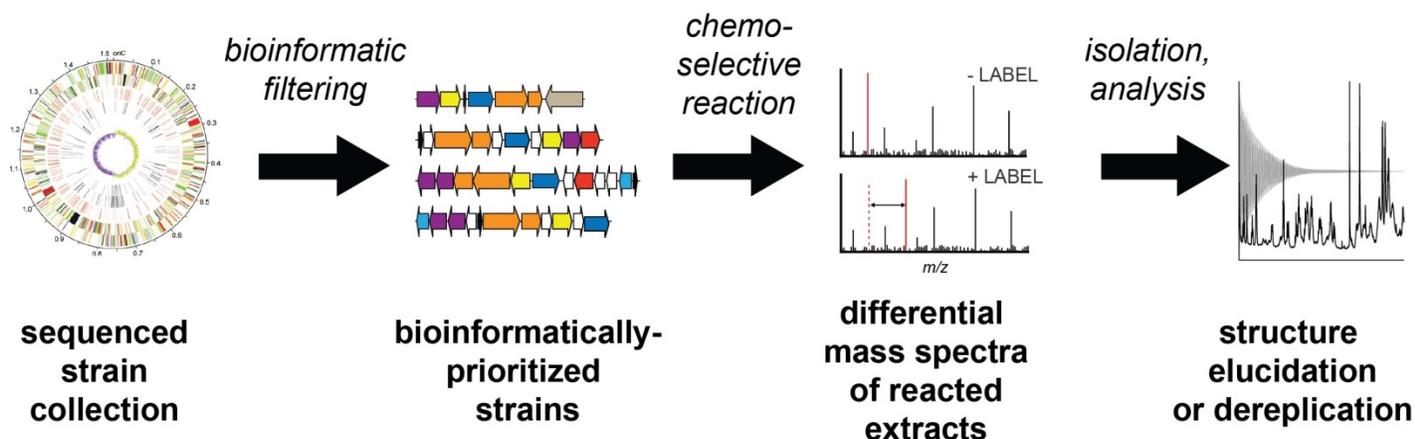
MEDI 242

Reactivity-based and genome-guided natural product discovery

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Natural products empowered the golden age of antibiotic discovery and represent privileged chemical scaffolds that interact with complex biological targets. However, the disproportionate abundance of certain natural products among frequently-screened organisms leads to unacceptably high re-isolation and re-elucidation rates in discovery campaigns, wasting substantial time and money. With insights gleaned from genome mining (now cheap and fast), interest in natural product discovery has been reinvigorated. In particular, biosynthetic gene clusters can be identified readily, enabling prediction of chemical substructures and the presence of specific functional groups. Reactivity-based screening is a method for accelerating the discovery of novel natural products via a combination of chemoselective covalent labeling and bioinformatics-based organism prioritization. In this method, thousands of microbial genomes are mined for the presence of key biosynthetic genes corresponding to the production of compounds bearing specific functional groups. A chemoselective reaction between a chemical probe and the target functional group is then performed in the context of extracts of chosen bacteria, and metabolites of interest are indicated by comparative mass spectrometry. This technique was used for the discovery of thiopeptide and hygrobafilomycin antibiotics and their corresponding gene clusters, among other targets. This strategy has also been diversified using a variety of nucleophilic, electrophilic, and cycloadditive chemical probes. We have additionally prepared a series of functionalized screening probes bearing reporter moieties aimed at improving the detection of low abundance metabolites in complex extracts. Our approach is further bolstered by RODEO, a bioinformatics software tool we have developed to aid in gene cluster discovery and structural prediction. The use of reactivity-based screening has

enabled faster discovery rates and also greatly eased structure elucidation of new compounds.



MEDI 243

Discovery of an agouti-related protein (AGRP) octapeptide macrocycle derivative with equipotent antagonist pharmacology at the mouse melanocortin-4 receptor as AGRP(87-132)

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Antagonists of the melanocortin-3 and -4 receptors (MC3R and MC4R) may be valuable appetite stimulants for negative energy balance states including cachexia associated with cancer. Agouti-related protein (AGRP) is the endogenous antagonist of the MC3R and MC4R. While AGRP potently induces a feeding response in rodents, the length of the 46-residue C-terminal domain [AGRP(87-132)] that possesses the active Arg-Phe-Phe pharmacophore of AGRP precludes the use of AGRP in a clinical setting. Previous attempts to truncate the highly structured AGRP(87-132), containing 5 disulfide bonds, resulted in peptides with diminished activity. It was hypothesized that stabilizing the beta-hairpin loop of AGRP containing the purported pharmacophore might result in potent and selective antagonists. A small macrocycle library was synthesized to explore different loop stabilization strategies. The most potent scaffold, c[Pro-Arg-Phe-Phe-Asn-Ala-Phe-DPro], was subjected to further structure-activity relationship studies exploring the two Phe positions in the pharmacophore and the Asn residue. Substitution at the Phe positions did not result in increased antagonist potencies. In contrast, substituting the Asn position with Gly or basic residues increased antagonist potencies at the MC4R. The most potent Asn to diaminopropionic (Dap) substitution, resulting in c[Pro-Arg-Phe-Phe-**Dap**-Ala-Phe-DPro], was as potent as AGRP(87-132) at the mMC4R, 160-fold selective for the mMC4R over the mMC3R, only partially activated the mMC1R at up to 100 μ M concentrations, and was unable to stimulate the mMC5R.

MEDI 244

Small molecule induced degradation of bromodomains

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Small molecule induced degradation is an attractive strategy to irreversibly inhibit all functions of a protein simultaneously. After developing methodology to hijack E3 ligases to induce the degradation of targeted proteins, we describe the development of the first-in-class degrader of BET bromodomains, dBET1. Further optimization has led to a more potent and broadly efficacious molecule, dBET6. The use of these probes demonstrates that BET degradation has wide-reaching biological effects beyond BET inhibition with small molecule inhibitors that bind to the acetyl-lysine binding pocket of BETs, such as JQ1. These effects include increased efficacy in cell lines that have limited response to BET inhibitors, as well as a pronounced global effect on transcription. These data suggest not only that BET degradation is a potential strategy for the development of therapeutics, but also demonstrate the potential benefits of small molecule induced degradation of multi-domain proteins over domain-specific inhibitors.

MEDI 245

Drug design for addiction: Targeting the dopamine D₃ receptor

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The dopamine D₃ receptor (D₃R) is a target for the development of medications to treat substance use disorders. D₃R-selective compounds with high affinity and varying efficacies have been discovered, providing critical research tools for cell-based studies that have been translated to *in vivo* models of drug abuse. D₃R antagonists and partial agonists have shown especially promising results in rodent models of psychostimulant relapse-like behavior, including stress-, drug- and cue-induced reinstatement of drug seeking. However, to date, advancement to human studies has been limited to nicotine addiction. Using the high resolution D₃R crystal structure in combination with small molecule structure activity relationships (SAR) a high affinity ($K_i = 6$ nM) and selective (~2700-fold selectivity over D₂ receptors) dopamine D₃R antagonist, *N*-(4-(4-(3-chloro-5-ethyl-2-methoxyphenyl)piperazin-1-yl)-3-hydroxybutyl)-1H-indole-2-carboxamide, (VK4-116) was discovered. In rats trained to self-administer the prescription opiate oxycodone, under an FR1 schedule, VK4-116 attenuated self-administration, inhibited oxycodone seeking during extinction testing and blocked oxycodone-induced reinstatement to drug seeking. VK4-116 also significantly attenuated naloxone-precipitated conditioned place aversion in chronic oxycodone treated rats. These data suggest that D₃R antagonists may be suitable alternatives or adjunctive to opiate-based medications currently used clinically, in treating opiate addiction. As VK4-116 proved successful in this model, we then explored its potential for treatment of cannabis

dependence. Due to the lack of a reliable THC self-administration model in rodents, we tested VK4-116 in squirrel monkeys self-administering THC under an FR10 schedule. VK4-116 dose-dependently blocked THC self-administration without affecting food self-administration. In addition, VK4-116, in the same dose range, blocked both cue- and THC-induced reinstatement of drug seeking in these animals suggesting that D₃R antagonists may be useful in treating marijuana abuse. These data support the D₃R as a medication target for opiate or marijuana use disorders and that the highly D₃R-selective VK4-116 is a new lead molecule for development.

MEDI 246

Novel immunomodulators that target toll-like receptors

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The protein-protein and protein-RNA interfaces have been regarded as “undruggable” despite their importance in many biological processes. The toll-like receptors (TLRs) provide exciting targets for a number of infectious diseases, pain management, and cancers. Using multidisciplinary approaches, we have successfully developed novel exogenous probes that were shown to be competitive inhibitors or activators of various TLRs with high affinity and specificity. Some of the lead compounds are currently in the pipeline for further drug discovery.

MEDI 247

Targeting the regulatory enzymes in protein ubiquitination

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Protein ubiquitination is a wide-spread form of protein post-translational modification that controls diverse cellular functions. Ubiquitin ligases and deubiquitinases regulate the dynamic processes of protein ubiquitination by catalyzing the forward and reverse ubiquitin conjugating reactions. Despite extensive studies of these enzymes in the past two decades, how to effectively harness their therapeutic potentials remains an open question. In this presentation, I will highlight our recent structural studies, which help reveal the mechanisms by which ubiquitin ligases are regulated by a variety of naturally occurring hormones and metabolites. I will also present our most recent structural analyses of deubiquitinase complexes, which shed light on the activation mechanisms of a cohort of ubiquitin-specific proteases by their regulatory binding partners. I will finish my presentation with a discussion on the pharmacological implications of these findings.

MEDI 248

New paradigm in drug action: Differentiated gain of function amongst IMiD® analogues binding the E3 ubiquitin ligase, CRL4^{CRBN}

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The immunomodulatory agents thalidomide, lenalidomide, and pomalidomide have numerous cellular effects and demonstrate significant activity in a wide range of hematologic cancers. Binding of these compounds to CRBN, a component of the CRL4 E3 ubiquitin ligase complex, triggers the activation of CRL4^{CRBN}, and the subsequent ubiquitylation and degradation of proteins such as Aiolos (IKZF3), Ikaros (IKZF1), and CK1 α . These proteins are structurally and functionally distinct, since IKZF3 and IKZF1 are zinc finger transcription factors and CK1 α is a protein kinase. Differential degradation of these proteins has been observed between IMiD® agents. This opens the possibility that chemical analogues may be found that possess altered protein degradation profiles. Developing an understanding of this paradigm has enabled rational development of a next generation of compounds.

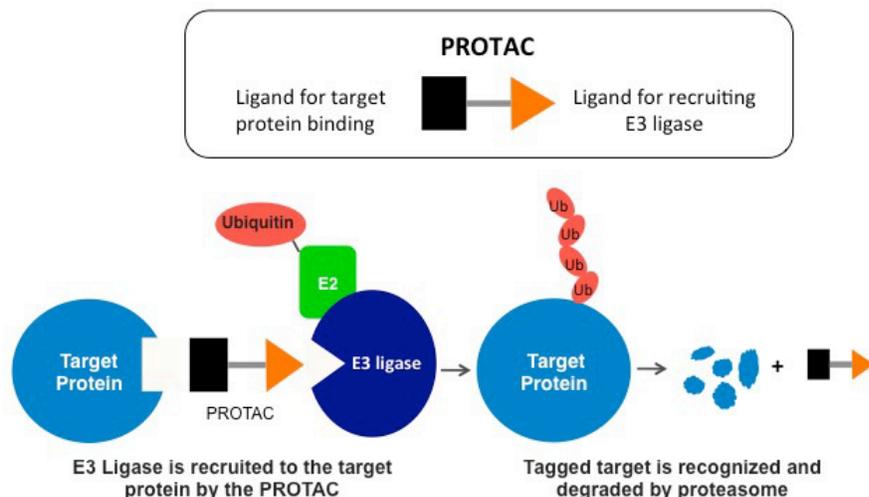
MEDI 249

Proteolysis targeting chimera (PROTACS): Recruiting proteins to the cellular quality control machinery

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Enzyme inhibition has proven to be a successful paradigm for pharmaceutical development, however, it has several limitations. As an alternative, for the past 16 years, my lab has focused on developing Proteolysis Targeting Chimera (PROTAC), a new 'controlled proteolysis' technology that overcomes the limitations of the current inhibitor pharmacological paradigm. Based on an '*Event-driven*' paradigm, PROTACs offer a novel, catalytic mechanism to irreversibly inhibit protein function, namely, the intracellular destruction of target proteins. This approach employs heterobifunctional molecules capable of recruiting target proteins to the cellular quality control machinery, thus leading to their degradation. We have demonstrated the ability to degrade a wide variety of targets (kinases, transcription factors, epigenetic readers) with PROTACs at picomolar concentrations. Moreover, the PROTAC technology has been demonstrated with multiple E3 ubiquitin ligases, included pVHL and cereblon.

PROTAC: PROteolysis TARgeting CHImera



MEDI 250

Structure based design of COP9 directed inhibitors

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The COP9 signalosome (CSN) is the platform for assembly and disassembly of cullin-RING E3 ubiquitin ligases (CRL), which comprise the largest enzyme family of the ubiquitin proteasome system (UPS) in humans. Over 200 CRL complexes are implicated in the regulation of almost all cellular processes including cell cycle progression, transcription, and apoptosis, and aberrant CRL activity is frequently associated with cancer. Since CSN functions as the protease which cleaves the ubiquitin-like protein Nedd8 from CRLs and thereby initiates their remodelling, we believed that inhibitors of the catalytic subunit CSN5 have therapeutic potential for the treatment of human diseases.

Here, we will present how CSN5 directed inhibitors were found and validated.

Structure based drug design was used to generate selective and orally available small molecule inhibitor of CSN5. The compound traps CRLs in the neddylated, active state leading to the autoinactivation of a subset of CRLs, e.g. SCFSkp2, by inducing the degradation of their substrate recognition module (SRM). As a result, the corresponding CRL substrates are stabilized, e.g. tumour suppressors p21 and p27.

MEDI 251

MEDI 251

Discovery of TAK-243: An investigational, first-in-class inhibitor of the ubiquitin activating enzyme

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The ubiquitin-proteasome system (UPS) regulates the bulk of protein turnover and plays a critical role in establishing and maintaining protein homeostasis in eukaryotic cells. Protein substrates are targeted for degradation by conjugation to polyubiquitin chains mediated by a multi-enzyme cascade consisting of E1, E2, and E3 enzymes. The Ubiquitin Activating Enzyme (UAE) is an E1 enzyme that activates ubiquitin as the key initiating step in this process. Due to the critical role of UAE in cell signaling and protein homeostasis, this enzyme was identified as an attractive target for oncology. This presentation will discuss structure activity relationships of a novel series of pyrazolo-pyridine compounds which led to the identification of TAK-243, an inhibitor of UAE currently in a Phase I clinical trial.

MEDI 252

From a novel HTS hit to a series of potent, selective, orally bioavailable KDM5 inhibitors: A success story utilizing structure- and property-based design

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

Lysine demethylase KDM5 (JARID1) family enzymes are 2-oxoglutarate (2-OG)-dependent hydroxylases that play critical roles in removing the methyl group(s) from histone H3K4Me3. Recently, we described our SAR effort on a series of pyrazolo[1,5-a]pyrimidin-7(4H)-one KDM5 inhibitors that culminated in identification of an in vivo tool compound for biological studies.

In parallel, we pursued optimization of a second, structurally distinct hit **1**, identified from an HTS of Genentech compound library. Biochemical and biophysical characterizations of **1** suggested it competed with 2-OG and the substrate peptide, a novel mechanism that has not been reported for KDM5 inhibitors.

Lacking guidance from a co-crystal structure or a plausible binding model, initial SAR around **1** was unproductive, as no improvement or a complete loss of potency was observed with many modifications. This SAR effort was also restricted by the synthetic challenge of constructing the C-C bond between the pyrrolidine and pyridine. To simplify analog synthesis and expedite SAR, we replaced the C-C with a C-N bond, as exemplified by 3-Br pyrazole **2**. A crystal structure of **2** complexed with KDM5A was obtained, which showed a novel bidentate chelation of the pyrazole N and carbonyl O with active-site Ni (a surrogate of Fe). Further optimization, guided by the structure- and

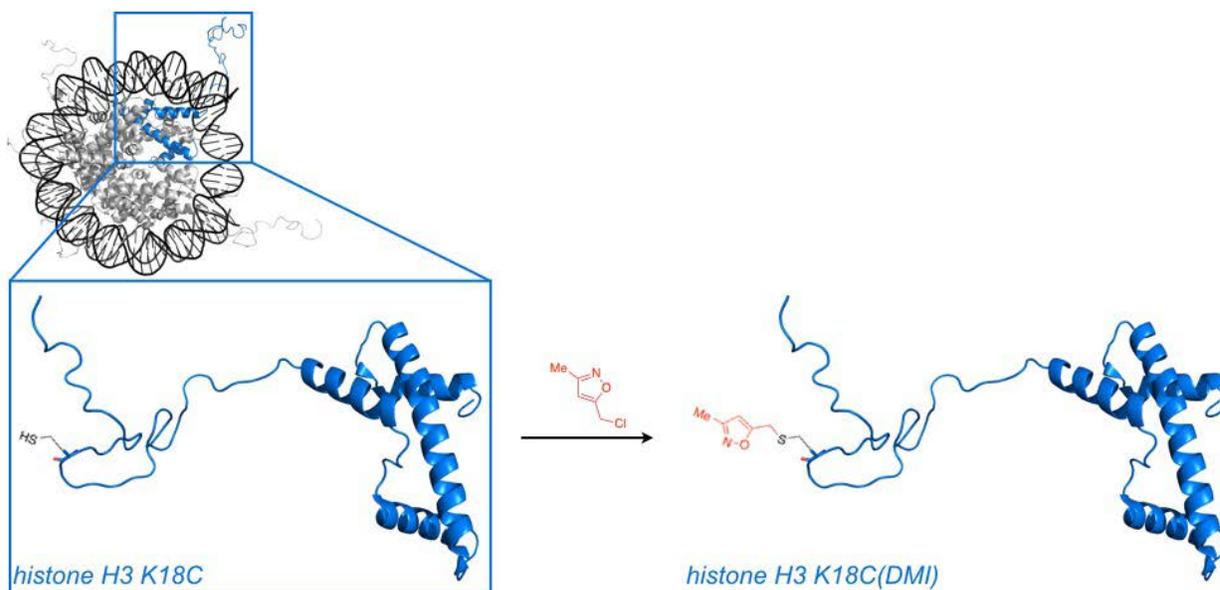
property-based design, led to amide **3**-a potent, selective and orally bioavailable KDM5 inhibitor in mouse, suitable for target validation in vivo.

MEDI 253

Isoxazole-derived amino acids are bromodomain-binding acetyl-lysine mimics when incorporated in histone H4 peptides and histone H3

*Angelina R. Sekirnik , David Hewings, Natalie H. Theodoulou, Lukass Jursins, Katie Lewendon, Laura Jennings, Timothy Rooney, **Stuart J. Conway**, stuart.conway@chem.ox.ac.uk.* Department of Chemistry, University of Oxford, Oxford, United Kingdom

A range of isoxazole-containing amino acids has been synthesized, which displace acetyl-lysine-containing peptides from the BAZ2A, BRD4(1), and BRD9 bromodomains. Three of these amino acids were incorporated into a histone H4-mimicking peptide and their affinity for BRD4(1) was assessed. Affinities of the isoxazole-containing peptides are comparable to those of a hyperacetylated histone H4-mimicking cognate peptide, and demonstrated a dependence on the position at which the unnatural residue was incorporated. An isoxazole-based alkylating agent was developed to selectively alkylate cysteine residues *in situ*. Selective monoalkylation of a histone H4-mimicking peptide, containing a lysine to cysteine residue substitution (K12C), resulted in acetyl-lysine mimic incorporation, with high affinity for the BRD4 bromodomain. The same technology was used to alkylate a K18C mutant of human histone H3. The complementary approaches that we report, which enable incorporation of effective and stable KAc mimics in a site-selective manner, will allow precise activation of KAc-mediated protein-protein interactions, facilitating study of their downstream effects in an unprecedented manner. We envisage that this approach could be of high value in developing peptide ligands for bromodomains with no known binding partners, underpinning epigenetic probe- and drug discovery.



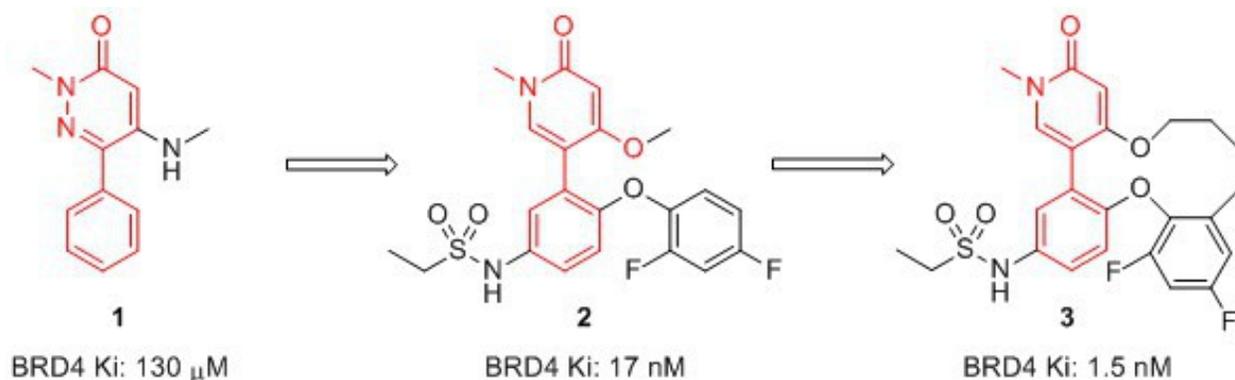
An isoxazole-based alkylating agent was developed to selectively alkylate cysteine residues *in situ*. This reagent was used to alkylate a K18C mutant of human histone H3.

MEDI 254

Fragment-based, structure-enabled discovery of novel pyridone and pyridazinone macrocycles as potent selective BET family bromodomain inhibitors

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Phenotypic cell-based screening assays combined with affinity chromatography and mass-spectrometry identified the BET family of bromodomains as a potential target for blocking proliferation in a variety of cancer cell lines. A pyridazinone fragment (**1**, K_i 130 μM) was identified as a weak BRD4 bromodomain inhibitor via a 2-dimensional NMR screen. The protein X-ray co-crystal structure of compound **1** with BRD4 domain 2 confirmed that it occupied the acetyl-lysine binding site. Medicinal chemistry efforts, aided by protein crystallography, rapidly led to the discovery of potent BET inhibitors such as compound **2**. Macrocycles such as compound **3** provided BET inhibitors with further improved biochemical and cellular activity. This presentation will describe the synthesis, x-ray analysis, SAR studies, pharmacokinetics, and *in vivo* efficacy studies for these novel BET inhibitors.

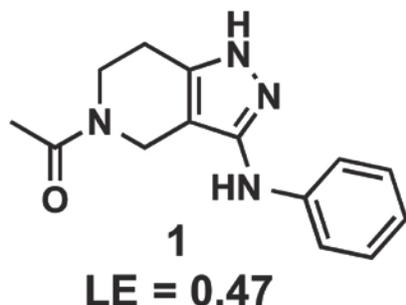


MEDI 255

Discovery of an *in vivo* probe for the bromodomain of CBP that is efficacious in a MOLM-16 AML xenograft model

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While significant progress has been made on identifying selective *in vitro* probes for CBP, there remains the challenge of identifying a probe suitable for *in vivo* studies. Screening of CBP with a property-restricted diversity set resulted in the identification of compound **1** as a selective, ligand efficient hit. A co-crystal structure of compound **1** with the bromodomain of CBP enabled structure-based design. Optimization of the interactions of **1** with the BC loop and LPF binding regions coupled with optimization of physicochemical properties resulted in GNE-272, a potent and selective CBP bromodomain inhibitor (CBP IC_{50} = 0.021 μ M, CBP BRET cell IC_{50} = 0.41 μ M, BRD4 IC_{50} = 12 μ M). GNE-272 was shown to be active in a Myc MOLM-16 PK/PD model and is efficacious in a MOLM-16 AML xenograft model.



CBP IC_{50} = 0.62 μ M
BRD4(1) IC_{50} = 60 μ M
Other BRDs = >20 μ M

MEDI 256

Discovery and structure-based optimization of novel allosteric inhibitors targeting the epigenetic methyltransferase PRC2

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Histone methyltransferases are involved in epigenetic regulation of early development and cell growth as well as the repression of tumor suppressor genes. Inhibitors of such enzymes were described to display anti-tumor efficacy and are currently being evaluated in clinical trials. Many of the disclosed compounds are either competitive with the SAM co-factor or enzyme substrate. Among them, inhibitors of the multi-subunit transferase PRC2 are known to target the catalytic subunit EZH2 and achieve their activity through a SAM-competitive mechanism. Here, we present the identification and optimization of small molecule inhibitors which modulate the activity of PRC2 via one of the non-catalytic subunits. Deconstruction of a larger screening hit to a fragment-sized molecule as well as structure-guided design and careful property modulation were employed to achieve a compound with sub-micromolar inhibition in functional assays and with cellular activity, revealing a novel allosteric mechanism. The molecular target will be disclosed in the presentation.

MEDI 257

Discovery of first-in-class reversible dual inhibitor of DNA methyl transferases and histone methyl transferase (G9a) with *in vivo* activity in different cancer models

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Using a knowledge- and structure-based approach, novel chemical probes that simultaneously inhibit the activity of epigenetic targets G9a and DNMT1 were designed and synthesized. *In vitro* treatment of hematological neoplasia (Acute Myeloid Leukemia-AML, Acute Lymphoblastic Leukemia-ALL and Diffuse Large B-cell Lymphoma-DLBCL) and hepatocellular carcinoma (HCC) with the lead compound CM-272 inhibited tumor cell proliferation and promoted apoptosis at nanomolar concentrations, at least in part by inducing interferon stimulated gene expression and immunogenic cell death. *In vivo*, treatment with CM-272 significantly prolonged survival of xenogeneic models of AML, ALL and DLBCL and reduced tumor growth in HCC. Our

results represent the discovery of first-in-class dual reversible inhibitors of G9a and DNMT1 and establish this chemical series, as a promising therapeutic tool for unmet needs in hematological and solid tumors.

MEDI 258

Dual screening using protein-observed fluorine NMR uncovers the first selective inhibitor for BPTF

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We describe a (19)F NMR dual screening method for detecting bromodomain-ligand interactions using fluorine-labeled aromatic amino acids incorporated into two different bromodomains. Bromodomains are integral protein domains in chromatin biology which provide enzymatic and scaffolding function to accessible DNA for transcription. Overexpression of bromodomain-containing proteins has been linked to disease states such as cancer and cardiac hypertrophy, so the ability to selectively inhibit bromodomain interactions could result in new therapeutic treatment options. Due to the excellent chemical shift dispersion of (19)F resonances within fluorine-labeled proteins, two fluorinated bromodomains were analyzed simultaneously with a single NMR experiment. Over 200 small molecules were screened against the first bromodomain of Brd4 and the bromodomain of BPTF and analyzed for their effects of the fluorine resonances. We report the first small molecule binder selective for BPTF over Brd4, in addition to two new classes of molecules that bind to the first bromodomain of Brd4. These hits were validated in a complementary differential scanning fluorimetry assay, and potency determined via fluorescence polarization for Brd4 and isothermal titration calorimetry for BPTF. The structure-activity-relationship was assessed for the top selective hit for BPTF, and in-cell studies are underway for assessing the role of BPTF in cancer. The speed, ease of interpretation, and low concentration of protein needed for protein-observed fluorine NMR binding experiments affords an excellent method to discover and characterize new and selective ligands for bromodomain-containing proteins.

MEDI 259

Structure-based drug design of aminobenzisoxazoles as orally available factor IXa (FIXa) inhibitors

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Thrombosis is caused by a clot of blood and it has been treated by anticoagulants such as warfarin and heparins. However these classical anticoagulants have major bleeding risk which leads life-threatening situations. Recently some factor Xa inhibitors have been launched for the treatment of thrombosis, but major bleeding risk is still key concern. The blood coagulation system is regulated by many coagulation factors and it is divided into three pathways: intrinsic pathway (FIXa, etc.), extrinsic pathway (FVII, etc.), and common pathway (FXa, etc.). Since FIXa works in the intrinsic pathway, extrinsic pathway could work regardless of FIXa and we assumed selective FIXa inhibitors could enhance safety margin of desired anticoagulation over major bleeding risk. Starting from HTS hit compounds, we have explored novel FIXa inhibitors by structure-based drug design. As a result, we have discovered aminobenzisoxazole derivatives as orally available FIXa inhibitors. Profile of advanced compounds will be presented.

MEDI 260

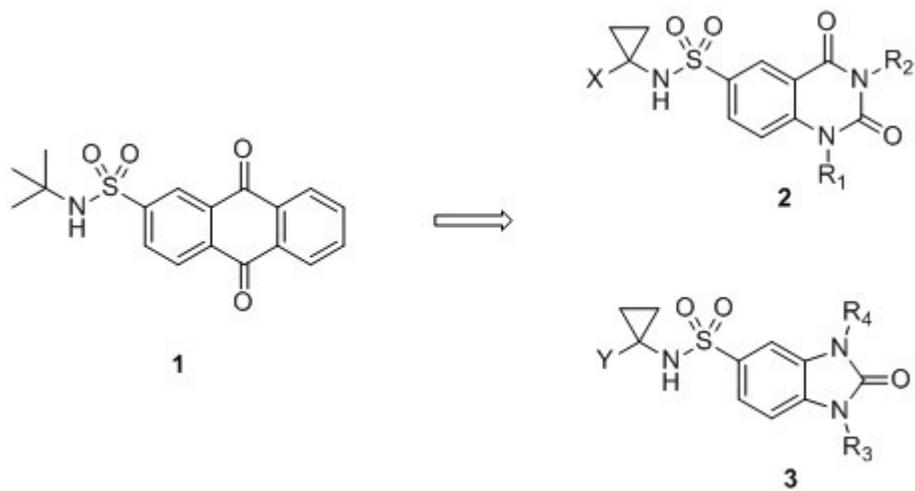
Discovery and optimization of the first sub-micromolar, cell permeable, small molecule inhibitors of poly(ADP ribose) glycohydrolase (PARG)

Alison McGonagle¹, *alison.mcgonagle@cruk.manchester.ac.uk*, Ben Acton¹, Jonathan Ahmet¹, Phil Chapman¹, Emma Fairweather¹, Louise Griffiths¹, Nicola Hamilton¹, Niall Hamilton¹, James Hitchin¹, Colin Hutton¹, Dominic James¹, Cliff Jones², Stuart Jones¹, Allan M. Jordan¹, Daniel Mould¹, Donald Ogilvie¹, Kate Smith¹, Alex Stowell¹, Helen Small¹, Julie Tucker², Ian Waddell¹, Bohdan Waszkowycz¹. (1) Drug Discovery, Cancer Research UK Manchester Institute, Manchester, United Kingdom (2) AstraZeneca, Macclesfield, United Kingdom

In recent years, many proteins involved in DNA repair have received considerable attention as potential cancer therapies, and several small molecule modulators have progressed to clinical evaluation or FDA regulatory approval in this field. However, the DNA repair protein poly(ADP ribose) glycohydrolase (PARG), which plays a critical role in DNA single strand break repair, has so far eluded a successful drug discovery campaign. PARG is involved in the hydrolysis of poly(ADP ribose) chains, which are synthesized by poly(ADP ribose) polymerase enzymes (PARPs), and serve to recruit repair proteins upon DNA damage. The dearth of potent, selective, cell permeable inhibitors of PARG has greatly limited research into the function and biological roles of this interesting target.

The poor druggability of PARG was evident from the low hit rate observed in the high-throughput screen (HTS). Although HTS hit **1** displayed off-target toxicity at 72 h, it exhibited an unexpected binding mode to human PARG which was exploited by a program of focused virtual screening and structure-based design to deliver several novel drug-like scaffolds. We disclose a series of quinazolinones (**2**) and benzimidazolones (**3**) as the first reported sub-micromolar cell permeable inhibitors of PARG. Non-cytotoxic, selective, drug-like chemical probes with low nanomolar cell

activity will be presented, and SAR will be discussed with reference to their binding interactions to human PARG as observed by X-ray crystallography.



MEDI 261

Discovery of ozanimod (RPC1063): A S1P_{1R} and S1P_{5R} selective agonist for the treatment of autoimmune disease

Junko Tamiya, jtamiya@celgene.com, Esther Martinborough, Adam R. Yeager, Liming Huang, Brahmachary Enugurthi, Manisha Moorjani, Marcus Boehm, Fiona Scott, Bryan Clemons, Jennifer Brooks, Rachel Powell, Harry Dedman, Hans Desale, Gregory Reinhart, Gregg Timony, Robert Peach. Receptos, a wholly owned subsidiary of Celgene Corp., San Diego, California, United States

Sphingosine 1-phosphate (S1P) receptors (S1P_{1-5R}) influence multiple physiological systems including lymphocyte trafficking and cardiac function. Agonists of the S1P_{1R} sequester lymphocytes in peripheral lymphoid organs and have thus garnered attention as effective agents against autoimmune diseases such as relapsing multiple sclerosis (RMS) and inflammatory bowel disease (IBD). Ozanimod (RPC1063) is a selective agonist of S1P_{1R} and S1P_{5R} currently in Phase 3 clinical trials for RMS and IBD. We will describe the medicinal chemistry efforts leading to the discovery of Ozanimod, its pharmacology, pharmacokinetics, and *in vivo* efficacy.

MEDI 262

Structure-based design of highly potent and selective small-molecule reversible factor D inhibitors blocking *in vivo* alternative complement pathway activation

Juergen K. Maibaum¹, juergen_klaus.maibaum@novartis.com, Karen Anderson², Anna Vulpetti¹, Edwige Lorthiois¹, Nils Ostermann¹, Sha-Mei Liao², Stefan Randl¹, Olivier Rogel¹, Erbel Paul¹, Bernd Gerhartz¹, Upendra A. Argikar², Irene Müller¹, Ulrich Hommel¹, Bernd Kinzel¹, Fabrice Kolb¹, Simon Rüdissler¹, Frederic Cumin¹, Richard C.

Sedrani¹. (1) Novartis Institutes for BioMedical Research, Novartis Pharma AG, Basel, Switzerland (2) Novartis Institutes for BioMedical Research, Novartis Pharma, Cambridge, Massachusetts, United States

The highly specific S1 serine protease Factor D plays a central role in the amplification of the complement alternative pathway (AP) of the innate immune system. Strong genetic associations in humans have implicated AP activation in age-related macular degeneration (AMD), and dysregulation of the AP predisposes individuals to diverse rare disorders such as paroxysmal nocturnal hemoglobinuria (PNH) and atypical hemolytic uremic syndrome (aHUS). Therefore, a selective Factor D inhibitor targeting the first and rate-limiting step of this pathway is considered to be a promising concept for the treatment of complement-dependent diseases. The atypical structural features of its catalytic site have hampered the design of potent reversible non-covalent inhibitors of Factor D to date. The lack of validated hits from two HTS campaigns has prompted us to conduct a small-sized library design approach based on target enzyme family hopping in combination with *in silico* docking of a focused fragment collection and subsequent hit evaluation by ligand/protein-observed NMR. These efforts and further optimisation of a proline-based lead structure, guided by X-ray crystallography, led to the discovery of selective Factor D inhibitors with drug-like properties including oral bioavailability. Optimized compounds showed strong inhibition of AP-mediated membrane-attack complex (MAC) formation *in vitro* in normal human blood, and demonstrated sustained oral efficacy in a model of systemic complement activation in mice expressing human Factor D.

MEDI 263

Potent, gut-restricted inhibitors of divalent metal transporter 1 (DMT1): Preclinical efficacy against iron overload and safety evaluation

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Divalent metal transporter 1 (DMT1) is the primary uptake mechanism for non-heme bound iron by enterocytes. DMT1 functions to transport ferrous ions into cells by co-transport with protons. Excessive iron uptake is normally prevented by tight regulation of DMT1 expression. However, in diseases such as hereditary hemochromatosis and thalassemia intermedia, control of DMT1 expression is lost and its upregulation leads to iron hyperabsorption and overload. Inhibiting DMT1 in enterocytes by small molecules represents a potential therapeutic approach for the treatment of iron overload disorders.

Divalent metal transporter 1 (DMT1) is the primary uptake mechanism for non-heme bound iron by enterocytes. DMT1 functions to transport ferrous ions into cells by co-transport with protons. Excessive iron uptake is normally prevented by tight regulation of DMT1 expression. However, in diseases such as hereditary hemochromatosis and thalassemia intermedia, control of DMT1 expression is lost and its upregulation leads to iron hyperabsorption and overload. Inhibiting DMT1 in enterocytes by small molecules represents a potential therapeutic approach for the treatment of iron overload disorders. Our design strategy is to develop gut-restricted compounds that act by locally blocking DMT1-mediated non-heme iron absorption in the intestine. This approach limits the systemic exposure of the DMT1 inhibitor and should result in a highly favourable safety profile. We report a lead small molecule inhibitor of DMT1, known as XEN602 that potently blocks the transport of both divalent cations and protons. XEN602 strongly inhibits dietary iron uptake in both rats and pigs, yet has negligible systemic exposure at a 100-fold higher dose. Efficacy is maintained for >2 weeks in a rat subchronic dosing assay. The talk will also reveal *in vitro* and *in vivo* safety assessments for XEN602. Overall, XEN602 represents a powerful pharmacological tool for understanding the physiological function of DMT1 and consequences of its pharmacological inhibition. This presentation will highlight the lead optimization activities that led to XEN602 as well as studies with XEN602 which elucidate the potential of DMT1 inhibition as a therapeutic approach for iron overload disorders.

MEDI 264

Development of novel, selective and irreversible PI3K δ inhibitors

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Phosphoinositide 3-kinase δ (PI3K δ) is involved in T-cell receptor signalling, B-cell development and neutrophil trafficking into inflamed tissues, making it an attractive target for the treatment of inflammatory and autoimmune diseases. A number of selective **reversible** PI3K δ inhibitors have been developed, most notably ZydeligTM, which has recently been approved by the FDA for the treatment of chronic lymphocytic leukemia. Wortmannin and its structurally related analogues (e.g. PX-866) are, to date, the only reported PI3K δ inhibitors which covalently bind to the kinase *via* reaction with a conserved lysine residue situated in the ATP binding site. However, these compounds show poor selectivity, especially for the various PI3K isoforms, restricting their use to specific indications.

By modifying selective **reversible** PI3K δ inhibitors with carefully positioned electrophilic moieties, it was proposed that selective **irreversible** PI3K δ inhibition could be achieved, despite the conserved nature of the targeted lysine residue within the PI3K family. The design and development of such compounds will be discussed, with data supporting their potency and selectivity in biochemical assays. Full characterisation of the covalent

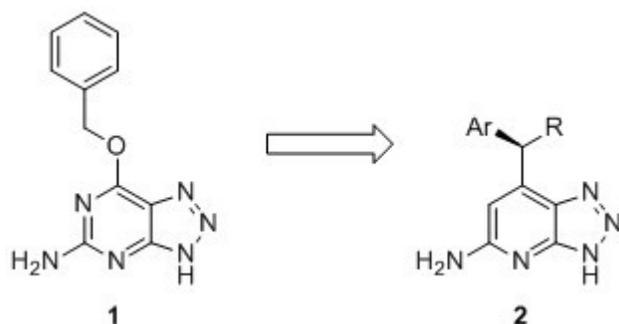
reaction using mass spectrometry, time-course experiments, and X-ray crystallography will also be presented. To our knowledge, this work represents the first established evidence of selective **irreversible** PI3K δ inhibitors, and constitutes a novel conceptual approach in this area with the potential to assist in the strategic design of related medicinal chemistry programmes.

MEDI 265

C-linked benzyl triazolopyridine inhibitors of myeloperoxidase

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Myeloperoxidase (MPO) is a heme peroxidase present in neutrophils, monocytes and macrophages and catalyzes the oxidation of chloride into the antimicrobial agent hypochlorous acid. Elevated MPO levels have been implicated in increased cardiovascular risk and other inflammatory diseases. The HTS lead **1** was a reversible MPO inhibitor with moderate potency, but exhibited poor stability of the benzyl ether to acid and enzymatic debenzylation. Transitioning to the C-linked benzyl triazolopyridine-based inhibitors (**2**, R = H) solved both problems, but at the expense of decreased selectivity for thyroid peroxidase (TPO). The addition of diverse branched substituents (**2**, R \neq H) lead to compounds with improved MPO potency and excellent selectivity against TPO while revealing a secondary hydrophobic binding pocket in MPO.



MEDI 266

Systematic study of the glutathione (GSH) reactivity of N-arylacrylamides

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Success in the design of targeted covalent inhibitors depends in part on a knowledge of the factors influencing electrophile reactivity. In an effort to further develop such structure-reactivity relationships, we determined glutathione (GSH) reaction rates for two complementary groups of acrylamides: unsubstituted *N*-arylacrylamides independently bearing different functional groups at *ortho*-, *meta*-, and *para*-positions, and *N*-phenylacrylamides independently bearing different substituents at nitrogen as well as the alpha- and beta- carbons of the acrylamide. Density functional theory calculations reveal a correlation between computed activation parameters and experimentally determined reaction rates. We believe that this comprehensive study will allow for more informed strategies for targeted covalent inhibitor design.

MEDI 267

Discovery of BMS-212 as a potent, liver-selective glucokinase activator clinical candidate

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Glucokinase (GK) is mainly expressed in pancreatic β -cells and liver parenchymal cells and catalyzes the conversion of glucose to glucose-6-phosphate (G6P), the first step in glucose metabolism. GK has a dual mode of action in both pancreas and liver to regulate blood glucose levels and GK activation has thus been explored as one of the novel mechanisms in recent years for the treatment of type 2 diabetes (T2D). One challenge that has been associated with GK as a diabetes target, however, is the potential mechanism-based hypoglycemia, which is likely due to pancreatic GK activation and the resultant non-glucose-dependent insulin secretion. Transgenic mouse models have shown that overexpression of GK in liver alone is sufficient to provide significant antihyperglycemic efficacy, suggesting that a liver-selective GK activator could be an effective antidiabetic agent. In this presentation, the effort to discover liver-selective GK activators at BMS will be discussed, which culminated in the identification of the development compound BMS-212, which is a potent, liver-selective GK activator. The synthesis as well as the in vivo efficacy and ADME profile of this compound will be detailed.

MEDI 268

Discovery of AG-120: A first-in-class inhibitor of IDH1 mutant enzymes for the treatment of cancers harboring IDH1 mutations

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Somatic point mutations at a key arginine residue (R132) within the active site of the metabolic enzyme isocitrate dehydrogenase 1 (IDH1) confers a novel gain-of-function in cancer cells resulting in the production, and accumulation, of high levels of D-2-hydroxyglutarate (2-HG), an oncometabolite. Elevated levels of 2-HG is implicated in epigenetics alterations and impaired cellular differentiation. IDH1 mutations have been described in an array of hematological malignancies and solid tumors. This presentation will recount the discovery of AG-120, a first-in-class, potent, reversible, selective, orally active inhibitor of the IDH1 mutant enzyme. AG-120 has an acceptable safety profile and exhibited early indication of antitumor activity in a Phase 1 clinical trial in patients with cancers harboring an IDH1 mutation.

MEDI 269

JNJ-54175446: A P2X7 receptor antagonist clinical candidate for major depressive disorders

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This report discloses the discovery of the clinical candidate JNJ-54175446 and the SAR of other 1H-[1,2,3]triazolo[4,5-c]pyridin-5(4H)-yl derived P2X7 receptor (P2X7R) antagonists. The P2X7 receptor is an ATP-gated ion channel expressed abundantly on microglial cells in the CNS. Activation of P2X7R by increased levels of ATP results in the secretion of IL-1beta and other pro-inflammatory cytokines. Literature reports support that antagonists of P2X7R would reduce central IL-1beta levels and could function as a useful treatment for depression. Although a few clinical trials of P2X7R antagonists for immune mediated disorders have appeared, none of those compounds are reported to have CNS penetration. This presentation will focus on efforts that led to the discovery of highly selective, potent, brain penetrant P2X7R antagonists. It will describe the evolution of a series of 1H-[1,2,3]triazolo[4,5-c]pyridin-5(4H)-yl compounds from early SAR through to key tool compounds and ending with a description of the clinical candidate JNJ-54175446.

MEDI 270

SAGE-217: A next-generation neuroactive steroid GABA_A receptor positive allosteric modulator for the potential treatment of seizure disorders

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Neuroactive steroids (NASs), such as allopregnanolone, have been shown in animal studies to be potent positive modulators of ionotropic GABA_A receptors (GABA_ARs). Several NASs are being studied for their potential in the treatment of a variety of CNS conditions, including status epilepticus (SE), certain neurological conditions characterized by a high seizure burden, and essential tremor. Next-generation GABA_AR-potentiating NASs exhibit substantially reduced off-target liabilities in animal models compared to the known first-generation analogs of similar structure, and furthermore, can be optimized for different routes of delivery (e.g. oral, fast on/off intravenous (i.v.)).

SAGE-217 is a novel next-generation NAS that acts as a positive allosteric modulator (PAM) of GABA_ARs. SAGE-217 potentiates the activity of both synaptic and extrasynaptic GABA_ARs, demonstrates highly potent and selective activity at GABA_ARs and, in contrast to endogenous NASs, has an optimized pharmacokinetic (PK) profile intended to support once daily, low dose oral administration.

In multiple animal models, SAGE-217 has demonstrated anti-seizure and anxiolytic activity, including potent activity in chronic (mesial temporal lobe epilepsy) and genetic (Fragile-X syndrome) pre-clinical epilepsy models. SAGE-217 is being developed as a treatment for epilepsies characterized by a high seizure burden and other GABA_A dysfunction-related disorders, such as essential tremor. A Phase 1 clinical program of SAGE-217 is ongoing, which includes a single ascending, double blind, placebo-controlled trial to evaluate the safety, tolerability, PK, and pharmacodynamic effects of SAGE-217 administered orally in approximately 80 healthy adult volunteers.

The structure activity relationship efforts, preclinical data, and early clinical results of SAGE-217 will be presented.

MEDI 271

Inhibition of autoimmune pathways with dual inhibition of JAK1 and TYK2:

Discovery of PF-06700841

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The Janus (JAK) kinases are a family of four non-receptor tyrosine kinases that modulate cytokine signaling through the Signal and Transduction of Transcription (STAT) pathways. The JAK kinases (JAK1, JAK2, JAK3 and TYK2) are important in both the innate and adaptive immune system, in a variety of cell types, for example lymphocytes, hematopoietic cells and structural cells (keratinocytes and fibroblasts). The current work describes the discovery of a series of selective JAK1/ TYK2 inhibitors for a range of inflammatory disorders such as inflammatory bowel disease, systemic lupus erythematosus and psoriasis. Balancing the in-family kinase selectivity is important to optimize the inhibition of pathogenic cytokines while limiting immune suppression, as well as to limit effects driven by JAK2 signaling through EPO and other molecules important in hematopoietic cell differentiation.

An important part of our program has been our understanding of PK:PD developed from our extensive experience with tofacitinib (Xeljanz™) in the clinic and in preclinical animal models. This has been important in setting lab objectives for projecting efficacious target cover and dose: in particular to understand selectivity ratio targets to minimize effects on JAK2 signaling and the hematopoietic system, whilst maximizing efficacy. We identified a series of ATP competitive pyrimidines from an early library lead, and through a structurally enabled program drove the biological profile and property space to a point where we could advance the lead compound (PF-06700841) into the clinic. The role of primary cell assays has been key to understanding the properties of the lead molecules, corroborated by PK:PD evaluation in-vivo. The lead is a well behaved molecule with excellent *in-vitro* potency and a superior off-target poly-pharmacology profile. PF-06700841 is currently in Phase 1 clinical study.

MEDI 272

Discovery of a pseudokinase domain ligand as an allosteric inhibitor of TYK2 for the treatment of autoimmune diseases

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The Janus kinase (JAK) family of non-receptor tyrosine kinases, which includes tyrosine kinase 2 (TYK2) and JAKs 1, 2 and 3, are intimately involved in the signaling pathways (JAK-STAT) of a variety of cytokines and have been clinically validated with now marketed kinase inhibitors for several autoimmune and oncologic indications. A challenge limiting the development of JAK inhibitors for a broader group of indications and patients is the limited selectivity of these inhibitors, particularly within the JAK family itself, and is a reflection of the high sequence homology of the ATP binding pocket of their catalytic (JH1) domains. Selective inhibition of TYK2 is of particular interest for the treatment of autoimmune diseases, as it mediates the signaling of a select subset of pro-inflammatory cytokines (IL-12, IL-23, and type 1 interferons). We have discovered that selective ligands of the pseudokinase (JH2) domain of TYK2 stabilize the unactivated state of the enzyme and thereby inhibit signaling of these pro-inflammatory cytokines. This presentation will disclose the identification and pre-clinical evaluation of BMS-986165, a TYK2 pseudokinase domain ligand that we have advanced to Phase 1 clinical trials.

MEDI 273

Discovery of NVP-HDM201: Identification of a next-generation Mdm2 inhibitor with superior characteristics

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Activation of p53 by blocking the p53-Mdm2 interaction using non-peptidic small-molecule inhibitors has been pursued for many years as a promising cancer therapeutic strategy.

We describe the identity of NVP-HDM201, a novel, highly optimized and selective inhibitor of the p53-Mdm2 interaction. NVP-HDM201 binds to human Mdm2 protein with a sub-nanomolar K_i value, activates p53 and induces robust p53-dependent cell cycle arrest and apoptosis in human p53 wild-type tumor cells. The activity and selectivity of NVP-HDM201 have been tested and confirmed across a panel of cancer cell lines and the molecule displays desirable pharmacokinetic and pharmacodynamic profiles in animals together with excellent oral bioavailability. Application of NVP-HDM201 using various dosing schedules triggers rapid and sustained activation of p53-dependent pharmacodynamic biomarkers resulting in tumor regression in multiple xenografted models of p53 wild-type human cancers.

We report here how a promising lead series was discovered and how innovative medicinal chemistry efforts led to further optimization of the potency and physico-chemical properties, culminating in the discovery of NVP-HDM201. The superior characteristics of the compound allowed the fast progression of the compound into the clinic where NVP-HDM201 is currently in Phase 1 clinical trials both as a single agent and as a combination partner in patients pre-selected for p53 wild-type tumors.

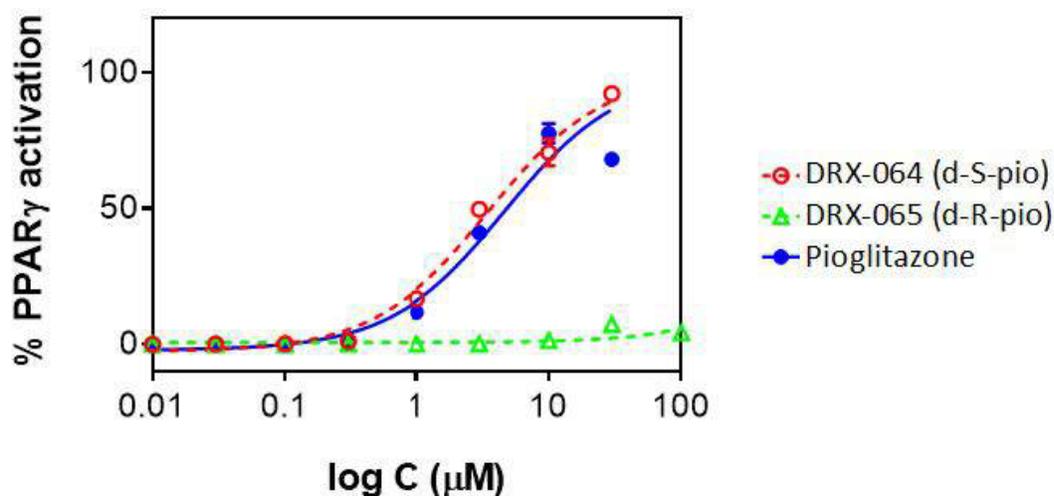
MEDI 274

Discovery of DRX-065: Characterizing the non-PPAR γ , mitochondrial function modulation and anti-inflammatory activity of thiazolidinedione (TZD) enantiomers using deuterium

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TZDs have been approved for Type 2 diabetes and are believed to act by activation of the PPAR γ nuclear receptor. Recent literature reports have demonstrated that TZDs also exert pharmacological activity through additional, non-PPAR γ -related mechanisms involving modulation of mitochondrial function and anti-inflammatory activity. These non-PPAR γ mechanistic properties have contributed to benefits in animal and/or human studies of Alzheimer's disease, nonalcoholic steatohepatitis (NASH), adrenoleukodystrophy (ALD), chronic obstructive pulmonary disease (COPD), etc.

The chemical structure of TZDs contains a chiral center that is prone to racemization. This has prevented the characterization of the individual enantiomers. By stabilizing the enantiomers of pioglitazone with deuterium we discovered that the PPAR γ activity is restricted to the (S)-enantiomer. The (R)-enantiomer is devoid of PPAR γ agonist activity and is responsible for the mitochondrial function modulation and anti-inflammatory activities. The deuterated (R)-enantiomer, DRX-065, is being advanced into clinical trials for the adult form of ALD, adrenomyeloneuropathy (AMN).



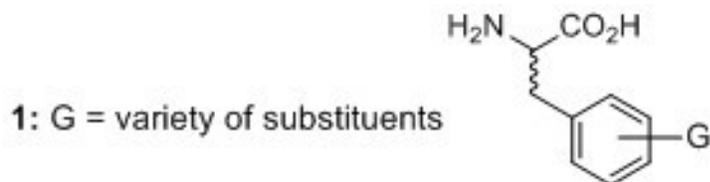
MEDI 275

Distributed drug discovery (D3) in action: Finding inhibitors of *P. aeruginosa*

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The Distributed Drug Discovery (D3) program educates student scientists in applied synthesis and biological evaluation. As they learn theory and practice they become a large, distributed research resource for the discovery of drugs to treat neglected diseases. *Pseudomonas aeruginosa* is a major cause of debilitating and deadly infections in patients with the orphan disease, Cystic Fibrosis. Using simple, inexpensive and powerful synthesis and biology laboratory procedures students made and tested, against *P. aeruginosa*, large numbers of unique unnatural amino acids and their derivatives. The D3 synthetic and testing procedures employed for these compounds will be described. Through this distributed process we have identified and will report several potent inhibitors of *P. aeruginosa*. The active compounds **1** are racemic, unnatural analogs of phenylalanine and its carboxyl derivatives. The structure activity studies presented will define key structural features required for their activity. In addition, the individual enantiomers of three of the active racemic compounds were separated by chiral chromatography. Biological evaluation showed that in each case the biological activity resided almost exclusively in a single enantiomer. Literature precedent indicates these active compounds are functioning as antimetabolites, with implications for potential toxicity in humans. We are further modifying the active molecules to seek derivatives that will have a greater therapeutic ratio.



MEDI 276

Discovery of potent HCV NS5A inhibitors with pan-genotype activity

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that does not rely on direct interaction with the GHSR1a. Screening of the Lilly compound collection in enzymatic and cellular assays provided attractive small molecule GOAT inhibitor starting points. Optimization of these hits to improve potency and ADMET properties led to the identification of LY3073084, a potent and selective GOAT inhibitor that demonstrates robust in vivo pharmacodynamic effects.

MEDI 278

Biotin carboxylase inhibitors with improved antibacterial activity against gram-negative pathogens

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Understanding the physicochemical properties that enable Gram-negative membrane penetration remains a challenge for new antibiotic discovery. Here we redesign pyridopyrimidine inhibitors of biotin carboxylase (BC) by applying some of the lessons from early attempts to identify these physicochemical properties. BC is a cytoplasmic enzyme involved in fatty acid biosynthesis and represents an exciting target for developing an antibiotic with a novel mechanism of action for which pre-existing resistance should be negligible. We sought to improve the antibacterial potencies of previously identified pyridopyrimidine BC inhibitors by introducing groups that were hypothesized to enhance membrane penetration and maintain BC target affinity. This was accomplished by using a two-step, Sandmeyer-type hydrolysis and chlorination, followed by S_NAr aminations. This approach led to the synthesis of compounds with minimum inhibitory concentrations (MICs) up to 64-fold lower against *E.coli* (ATCC 25922) and >8-fold lower against *P. aeruginosa* (ATCC 27853) compared to the parent compound. MICs conducted in the presence and absence of the membrane permeabilizing agent polymyxin B nonapeptide (PMBN) enabled an assessment of potential changes in membrane penetration properties of the redesigned BC inhibitors. To measure the BC inhibitor's ability to avoid efflux, MICs were compared between strains with native and compromised efflux activity. IC₅₀ value measurements and x-ray crystallography were used to determine compound target affinities and binding geometries. Spontaneous resistance to the improved pyridopyrimidine BC inhibitors in *P. aeruginosa* occurs at frequencies between 10⁻⁸ to 10⁻⁹. While these frequencies are comparable to other effective antibiotics, resistant isolates had relatively high MIC shifts (16 to >128-fold) compared to the parent strain. Whole genome sequencing of resistant isolates revealed that both BC target mutations and efflux pump up-regulation provide opportunities to develop high level resistance. These findings underscore the

challenges of designing effective antibiotics targeting BC and single-copy enzyme targets in general.

MEDI 279

Discovery of allosteric WNK inhibitors and in vivo proof-of-concept as anti-hypertensive agents

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Protein kinases are known for their highly conserved ATP-binding site, rendering the discovery of selective inhibitors a major challenge. In theory, allosteric inhibitors can achieve high selectivity by targeting less conserved regions of the kinases, often with an added benefit of retaining efficacy under high physiological ATP concentration. Although often overlooked in favor of ATP-site directed approaches, performing a screen at high ATP concentration and/or stringent hit triaging with high ATP concentration offer conceptually simple methods of identifying ATP non-competitive, allosteric inhibitors. Here we applied this approach to the With-No-Lysine (K) (WNK) kinases to discover lead molecules for a next-generation anti-hypertensive that requires a stringent safety profile. This strategy yielded several ATP non-competitive pan-WNK inhibitors. Their optimization enabled co-crystallization with WNK1, revealing allosteric binding modes consistent with the exquisite WNK-family kinase selectivity observed. For optimization towards in vivo proof-of-concept, we applied scaffold morphing strategies to several ATP non-competitive hits, guided by both crystal structure and an overlay hypothesis based on preliminary SAR. The effort resulted in an allosteric WNK inhibitor with a good balance of selectivity, cellular potency and pharmacokinetic profile. When dosed orally, the optimized compound reduced blood-pressure in mice overexpressing human WNK1 and induced diuresis, vasodilation and blood-pressure reduction in spontaneously hypertensive rats, confirming that inhibition of WNK kinase activity is effective at regulating cardiovascular homeostasis.

MEDI 280

Optimization of a heteroaryl sulfonamide series of potent, selective and efficacious Nav1.7 inhibitors

Matthew M. Weiss¹, *mmweiss@amgen.com*, **Alessandro Boezio**¹, **John Butler**¹, **Thomas A. Dineen**¹, **Russell Graceffa**¹, **Charles Kreiman**¹, **Thomas Kornecook**¹, **Daniel S. La**¹, **Isaac E. Marx**², **Benjamin Milgram**¹, **Brian Sparling**¹, **Bryan Moyer**¹. (1) Amgen Inc., Cambridge, Massachusetts, United States (2) Amgen Inc, Cambridge, Massachusetts, United States

Over the past decade, there has been considerable interest in the development of subtype selective Nav1.7 inhibitors. There is a wealth of human genetic data implicating Nav1.7 in the pain processing pathway. This comes in the form of both gain of function

and loss of function mutations in SCN9A, the gene that encodes for Nav1.7. This presentation will describe our efforts to identify potent (<10 nM) and isoform selective Nav1.7 inhibitors that demonstrate robust efficacy in a number of pain models, including mouse UVB and capsaicin. Included is a discussion of the quantitative pharmacology associated with this series of inhibitors as well as a review of the medicinal chemistry strategies used to optimize pharmacokinetics and address the numerous metabolic liabilities that have plagued this series of inhibitors.

MEDI 281

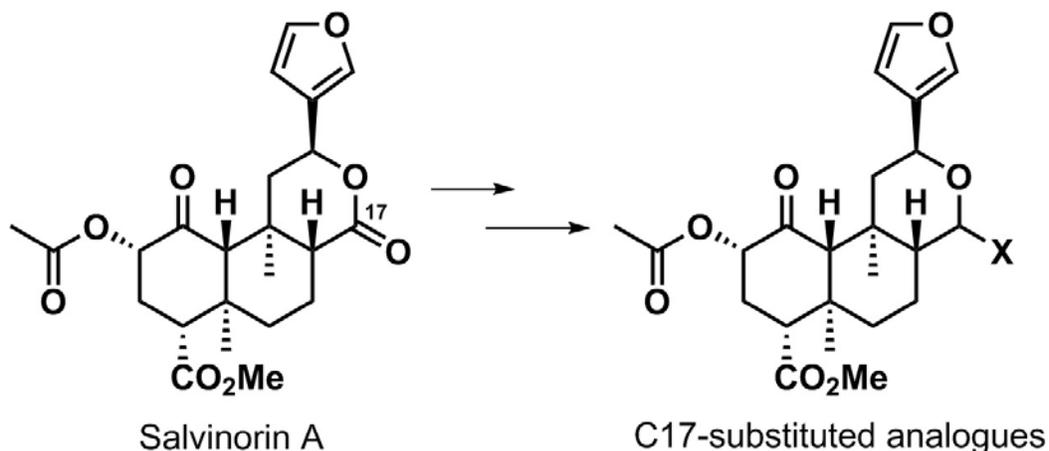
Natural product-based drug abuse therapies through the investigation of salvinorin A

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Drug overdose deaths are at an all-time high, with nearly 23 million Americans in need of treatment for a drug or alcohol addiction each year. Treatment options are very limited for opioid and alcohol abuse, and no approved medications for stimulant abuse exist. In animal models of drug abuse, kappa opioid receptor (KOR) agonists have been successful in treating abuse-related behaviors.

The natural product salvinorin A (SVA) is a potent and selective KOR agonist. It is a structurally-unique opioid, as it lacks a basic nitrogen once believed essential for opioid activity. SVA also contains a variety of chemically sensitive functional groups and stereocenters that pose a challenge to chemical modifications. While SVA has an interesting behavioral profile in animal models of drug abuse, its pharmacological utility is limited by its poor pharmacokinetic properties such as low water solubility and bioavailability. Through semisynthetic structural modifications, we are probing the structure-activity relationships at KORs with the aim of identifying a point on the molecule through which these pharmacokinetic shortfalls can be addressed without losing KOR activity.

Previous work described the lactone of SVA as being tolerant to minimal modifications but did not probe this position further. Development of synthetic strategies has allowed for extensive exploration of this position, and evaluation of these analogs for their KOR activity has led to the development of a well-understood structure-activity relationship for the lactone position. Results indicate that substitutions are tolerated, with a preference for sterically small, polar groups. As the goal of this work is to develop water soluble compounds, the fact that polar groups are not only tolerated but preferred is very promising. Currently, efforts are continuing in identifying potent compounds with high water solubility.



MEDI 282

Development of a new class of ALK2 inhibitor for the study of the most aggressive paediatric brain cancer, DIPG

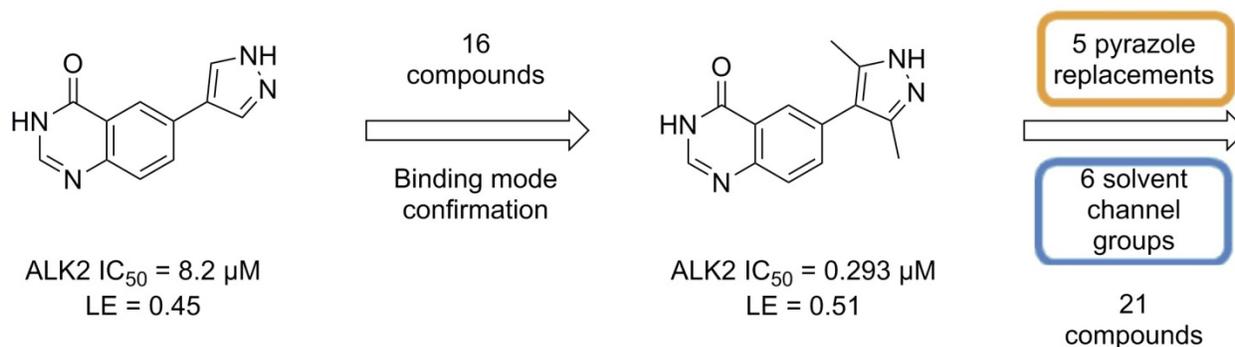
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ALK2 is a transmembrane serine/threonine kinase and member of the TGF β superfamily of signalling proteins. Mutations in ALK2 are known to cause fibrodysplasia ossificans progressiva (FOP); an extremely rare condition where patients are completely disabled by the growth of a second skeleton. This often occurs in response to injury, hence surgery accelerates the condition and no other treatments are available. More recently, many of the ALK2 mutants observed in FOP patients have been observed in approximately 25% of diffuse infiltrative pontine glioma (DIPG) cases. DIPG is the most aggressive paediatric brain cancer – representing only 10% of cases but responsible for 80% of deaths. As with FOP surgery is considered impossible due to the infiltrative nature of these cancers and their location in a vital region of the brain. Furthermore there is no beneficial treatment for DIPG (which has a median age of mortality of 9; less than 12 months from diagnosis).

The vast majority of reported ALK2 inhibitors share the same chemotype, and although some show promise in FOP models they are ineffective in DIPG models. Independent chemical tool compounds are needed to study the role of mutant ALK2, primarily in the context of DIPG.

We identified a 6-pyrazolo-quinazalinone fragment from another kinase project as a low micromolar inhibitor of ALK1, which shares 79% identity with ALK2. Screening of this and related quinazalinones strongly suggested the binding mode and confirmed that sub-micromolar inhibitors were possible for ALK2 with excellent ligand efficiencies (L.E. ~0.50). We prepared analogues with a range of central pocket pyrazole replacements, as well as a range of solvent channel groups – leading to inhibitors with low nM IC₅₀s – an increase in potency 390x the initial hit fragment.

Crystallography is ongoing with our collaborators at the SGC to aid the design of more potent inhibitors. Pleasingly, some early compounds are effective in killing DIPG cell lines with mutant ALK2 and have typical dose response curves in the low μM EC_{50} range – which is rarely recapitulated by existing compounds. Additional tests are ongoing.



MEDI 283

Leukotriene A_4 hydrolase aminopeptidase activity as a new target for chronic obstructive pulmonary disease

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Chronic obstructive pulmonary disease (COPD) is a lung disease with substantial morbidity and mortality due to irreversible respiratory failure. Accumulating evidence implicates the aminopeptidase activity of the leukotriene A_4 hydrolase (LTA_4H) enzyme in promoting the development of COPD through persistent pulmonary neutrophilia. LTA_4H is an epoxy hydrolase that elicits a pulmonary inflammatory response by catalyzing the hydrolysis of the epoxide leukotriene A_4 to the diol leukotriene B_4 . However, a secondary LTA_4H aminopeptidase activity promotes clearance of the inflammatory response by catalyzing the hydrolysis of the tripeptide proline-glycine-proline. Herein, we present 4-methoxydiphenylmethane as an orally available small molecule that augments the LTA_4H aminopeptidase activity. Enzyme characterization, structural studies, and preclinical evaluation in murine models for COPD will also be presented.

MEDI 284

Discovery of a novel, selective and orally bioavailable allosteric PRC2 inhibitor with robust anti-cancer efficacy

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The human Polycomb Repressive Complex 2 (PRC2) enables chromatin compaction and gene silencing through catalyzing the methylation of lysine 27 on histone 3 (H3K27). EZH2 is the catalytic subunit of PRC2, and aberrant trimethylation of H3K27 is oncogenic in a broad spectrum of human cancers. In clinical, EZH2 inhibitors demonstrated improved responses with continued treatment in patients with acceptable safety profile as single agents. Herein, we disclose the discovery of a first-in-class, potent, selective and orally bioavailable compound, which inhibits methyltransferase activity of PRC2 complex through a novel allosteric mechanism. The *in vivo* tool compound was discovered by fragmentation of a HTS hit, followed by proper regrowth and multi-parameter optimization. The compound induces robust and sustained tumor regression in EZH2^{MUT} DLBCL xenograft model in mice.

MEDI 285

Efficient discovery of lead molecules for hundreds of target proteins in parallel via DNA encoded chemical library: A platform for prioritizing therapeutic targets in a single experiment

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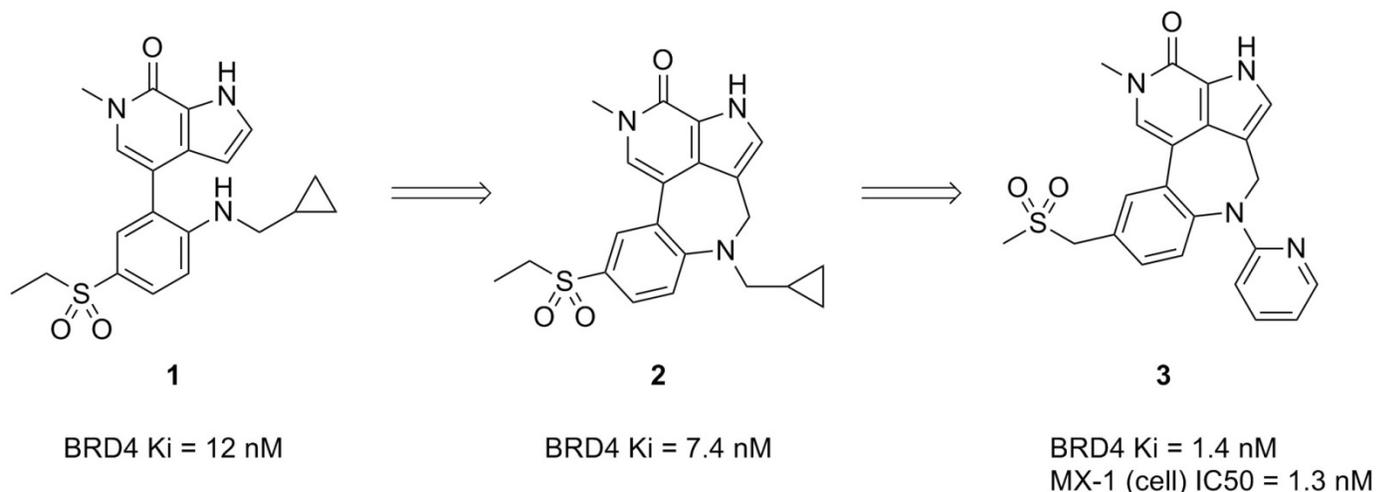
This presentation will entail an overview of encoded library technology (ELT) from the inception of the technology to current integration into one of the hit/lead identification platforms at GlaxoSmithKline (GSK). We will disclose some milestones during the platform development journey including technical advances in library chemistry, selection methodology, data analysis, and off-DNA decision making and hit confirmation for a number of chemical series. One of the recent developments in the use of ELT is efficient and rapid assessment of chemical tractability of multiple target proteins in parallel, a major hurdle in drug discovery. We have established a new paradigm to allow for rapid prioritization and streamlining of target proteins based on their ligandability prior to heavy investment in early stage therapeutic targets. We will disclose several examples of this panel screening approach and its output including hit/lead series for each tractability panel as a proof of concept of paradigm output.

MEDI 286

Design and optimization of novel tetracyclic pyrrolopyridone BET family inhibitors

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The BET family of bromodomain-containing proteins was identified as a potential target for inhibiting proliferation in a variety of cancer cell lines. In the course of prosecuting pyrrolopyridone inhibitors of BET family proteins (**1**), novel tetracyclic compounds were designed and prepared (**2**), aided by protein x-ray co-crystallography of compound **1**. Further refinements resulted in excellent potency and improved ADME properties (**3**). Compound **3** demonstrated excellent activity in cellular proliferation assays, as well as efficacy in *in vivo* tumor models. This presentation will describe the synthesis, x-ray analysis, SAR studies, pharmacokinetics, and *in vivo* efficacy studies of these novel BET inhibitors.



MEDI 287

Potential of silibinin derivatives in prostate cancer managements

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Silibinin, a flavonolignan extracted from a well-known traditional European medicine named as milk thistle, has been demonstrated to be a promising anti-prostate cancer lead compound. To potentiate its anti-proliferative effects in human prostate cancer cell

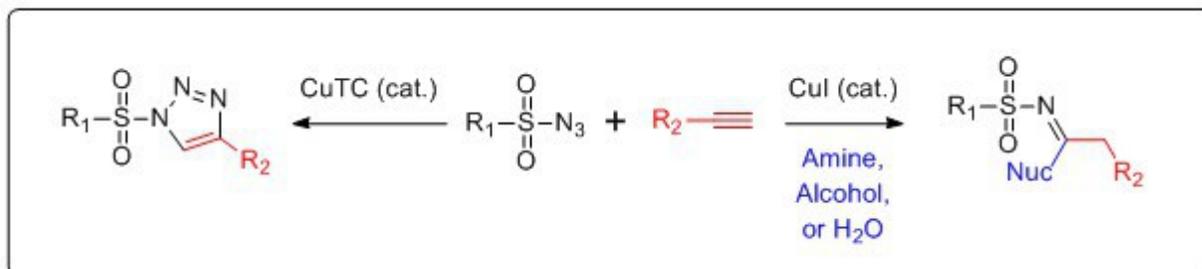
lines, we systematically explored the chemical modifications on its phenolic hydroxyl groups and C2-C3 bond. So far, we have synthesized eight groups (over sixty silibinin derivatives) for the evaluation of their anti-proliferative activity toward prostate cancer cells. The WST-1 cell proliferation assay indicates that six out of eight groups including 7-O-alkylsilibinins, 3-O-alkyl-2,3-dehydrosilibinins, 7-O-alkyl-2,3-dehydrosilibinins, 20-O-alkyl-2,3-dehydrosilibinins, 7-O-aminoalkyl-2,3-dehydrosilibinins, and 3-O-aminoalkyl-2,3-dehydrosilibinins can significantly and consistently increase the anti-proliferative potency against three human prostate cancer cells. However, 3,7-O-dialky-2,3-dehydrosilibinins and 5,20-O-dialky-2,3-dehydrosilibinins show similar or decreased potency. The cell proliferation inhibitory ability of 2,3-dehydrosilibinin and 7-O-1,2-dehydrosilibins is associated with prostate cancer cell arrest at G₀/G₁ phase and cell apoptosis induction. Interestingly, the cell proliferation inhibitory ability of 3-O-2,3-dehydrosilibins is linked with arresting prostate cancer cell at G₀/G₁ phase but not inducing cell apoptosis. The structure-activity relationships between eight groups of silibinin derivatives, as well as the action of mechanism, will be presented.

MEDI 288

Synthesis and SAR of sulfonyl azide-derived NDM-1 inhibitors

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New Delhi Metallo-β-Lactamase 1 (NDM-1) is a zinc-dependent metallohydrolase found in bacteria that confers resistance to commonly-administered antibiotics, including penicillins, cephalosporins, and carbapenems. Horizontal gene transfer has enabled the *bla*_{NDM-1} gene to spread between species, facilitating the development of multi-drug resistant bacterial strains. Bacteria carrying the *bla*_{NDM-1} gene have been found on all continents, and consequently, NDM-1 has gained international attention as a clinically relevant pharmaceutical target. We have employed copper(I)-catalyzed couplings of sulfonyl azides and alkynes to generate a diverse set of NDM-1 inhibitors including sulfonyl amidines, acyl sulfonamides, and sulfonyl triazoles with low-micromolar potency. Docking studies suggest that these inhibitors are acting as chelating ligands which interact with both Lewis acidic zinc atoms in the active site. Synthetic details and SAR from each series will be described.



Synthetic route to active NDM-1 inhibitors.

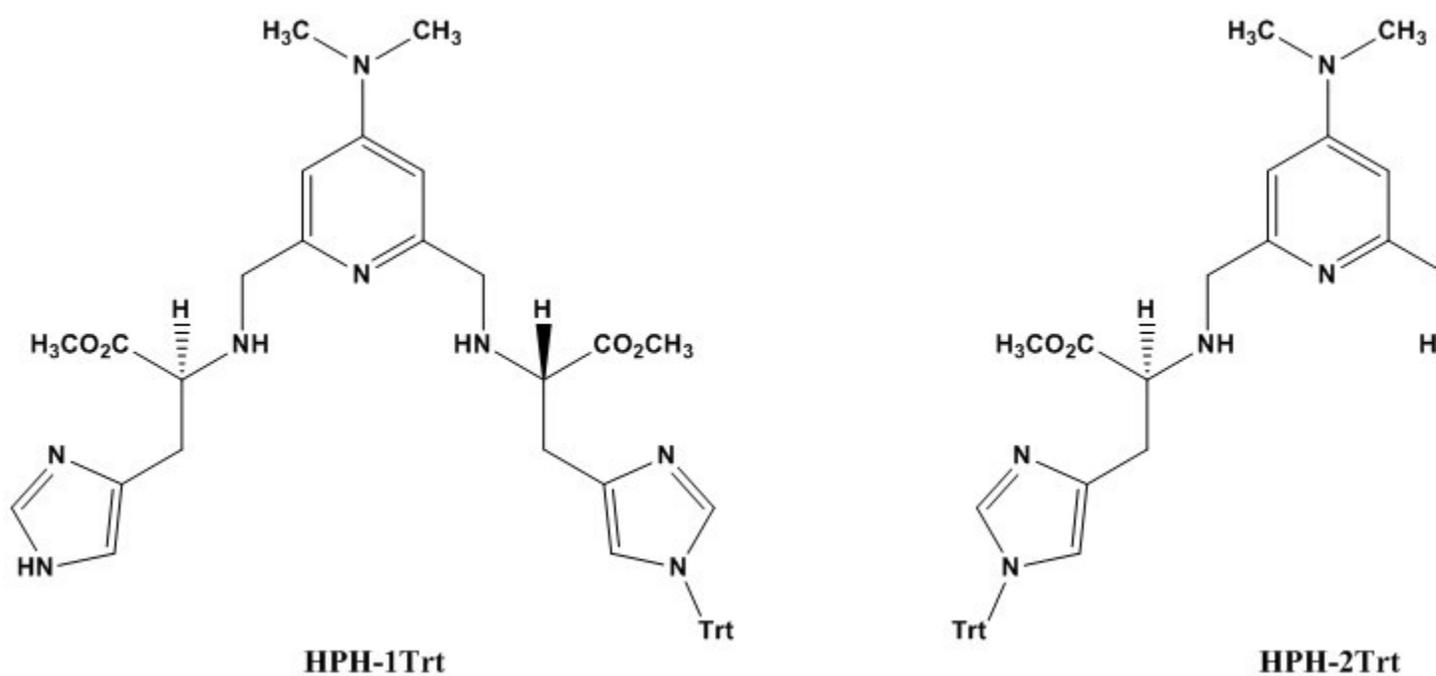
MEDI 289

Design and synthesis of small molecule Hsp70 inducers

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Chaperone protein, Hsp70, plays a pivotal role in folding and refolding of proteins *in vivo*. Induction of Hsp70 is a promising strategy for treatment of many neurodegenerative diseases that caused mainly by protein misfolding or aggregation such as Amyotrophic lateral sclerosis. We recently reported a series of Histidine-pyridine-histidine (HPH) derivatives, which are similar to the bleomycin iron-binding site

(T. F. S. Ali *et al.*, *Bioorg. Med. Chem.*, 23, 5476-5482, 2015.). Two of these compounds, namely HPH-1Trt and HPH-2Trt, have anticancer activity with oxygen activation ability. In the present study, we investigate the ability of these HPH derivatives to induce Hsp70. The results show that HPH-1Trt is able to induce Hsp70 in both HSC-F and PC-12 cells at 10 μ M. However, the parent compound, bleomycin, and the Hsp70 inducer, geranylgeranylacetone, as well as other HPH derivatives do not have the ability to induce Hsp70 at the same concentration. Because HPH-1Trt is cytotoxic at 20 μ M, we are currently synthesizing new HPH-1Trt analogues aiming to decrease its cytotoxicity and increase its Hsp70 induction capability. Furthermore, a molecular mechanism study of how HPH-1Trt induces Hsp70 is in progress. In summary, our preliminary study sheds the light on HPH derivatives as novel small molecule Hsp70 inducers with potential therapeutic application.



Structure of HPH-1Trt and HPH-2Trt

MEDI 290

Discovery of ABI-231 analogs as a new generation of tubulin inhibitors targeting the colchicine binding site

Qinghui Wang, Kinsie Arnst, Duane D. Miller, Wei Li, wli@uthsc.edu. University of Tennessee HSC, Memphis, Tennessee, United States

Despite recent advances in both targeted therapy and immunotherapy, acquired drug resistance often develops quickly and the overall survival for malignant cancers including melanoma remains unsatisfactory. Our ongoing research in developing new generations of tubulin inhibitors (Figure 1) have resulted in several sets of unique

structures from our initial thiazole analog that: 1) target the colchicine binding site in tubulin and have broad spectrum of potent anticancer activity; 2) effectively circumvent major drug resistance mechanisms that hinder the clinical efficacy with existing tubulin inhibitors; 3) are orally bioavailable and have excellent drug-like properties; and 4) are efficacious against both drug sensitive and drug resistant melanoma tumors *in vivo*, with ABI-231 as the best lead compound. Further structure optimization provided more than 40 new analogs, with QW-III-179 being the most potent analog, with GI50 values approaching pM range in some cell lines based on our in-house assays and the NCI-60 cell line assays. Mechanistic studies indicated that these compounds effectively inhibit tubulin polymerization, strongly induce cancer cell apoptosis and cancer cell colony formation, arrest cancer cells in G2/M phase, have minimal potential off-target effects as demonstrated by the SafeScreen44 assay, and potently inhibit melanoma tumor growth *in vivo*. We have also successfully obtained very high resolution X-ray crystal structures (2.2 to 2.5 Å) for the most active compounds in complex with tubulin, further confirmed their molecular interactions with tubulin and their mechanism of actions. These compounds represent a unique scaffold as orally bioavailable tubulin inhibitors, and are currently being developed for future clinical application for a variety of cancer types.

PO_AUC (min* $\mu\text{g/mL}$):

Rat *in vivo* oral exposure at 10 mg/kg

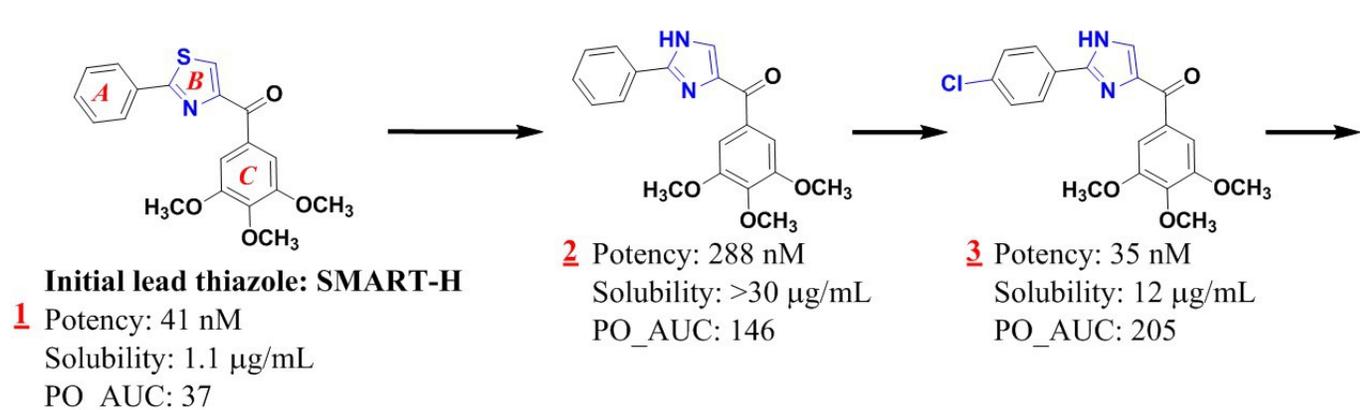


Figure 1. Average anticancer potency over 7 cancer cell lines and ADME driven compound progression from initial lead SMART 1 to new best analogs ABI-231 (4). Further structural optimization lead to the discovery of QW-III-179 with potency approaching pM range.

MEDI 291

Biologically active ferrocene based guanidines: Synthesis, antimicrobial, and anti-cancer potential

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Introducing new candidates for various biological targets is a prime characteristic of the present day medicinal research and development. Guanidines are the important bioactive compounds and are well recognized for their diverse biological activities, especially as anticancer, antimicrobial and antioxidant agents. A series of ferrocenyl guanidines have been synthesized via multi step protocol. Their structures were established by using elemental analysis, UV-visible, multinuclear (^1H and ^{13}C) NMR and FTIR spectroscopy, and X-Ray diffraction analysis. Due to the biological importance of guanidines; these ferrocenyl guanidines were screened for the different biological targets like antibacterial, antifungal, antioxidant and DNA binding. Their anticancer potential is under assessment. Current results revealed that the ferrocene incorporation to guanidines enhances their DNA binding ability and other tested biological applications.

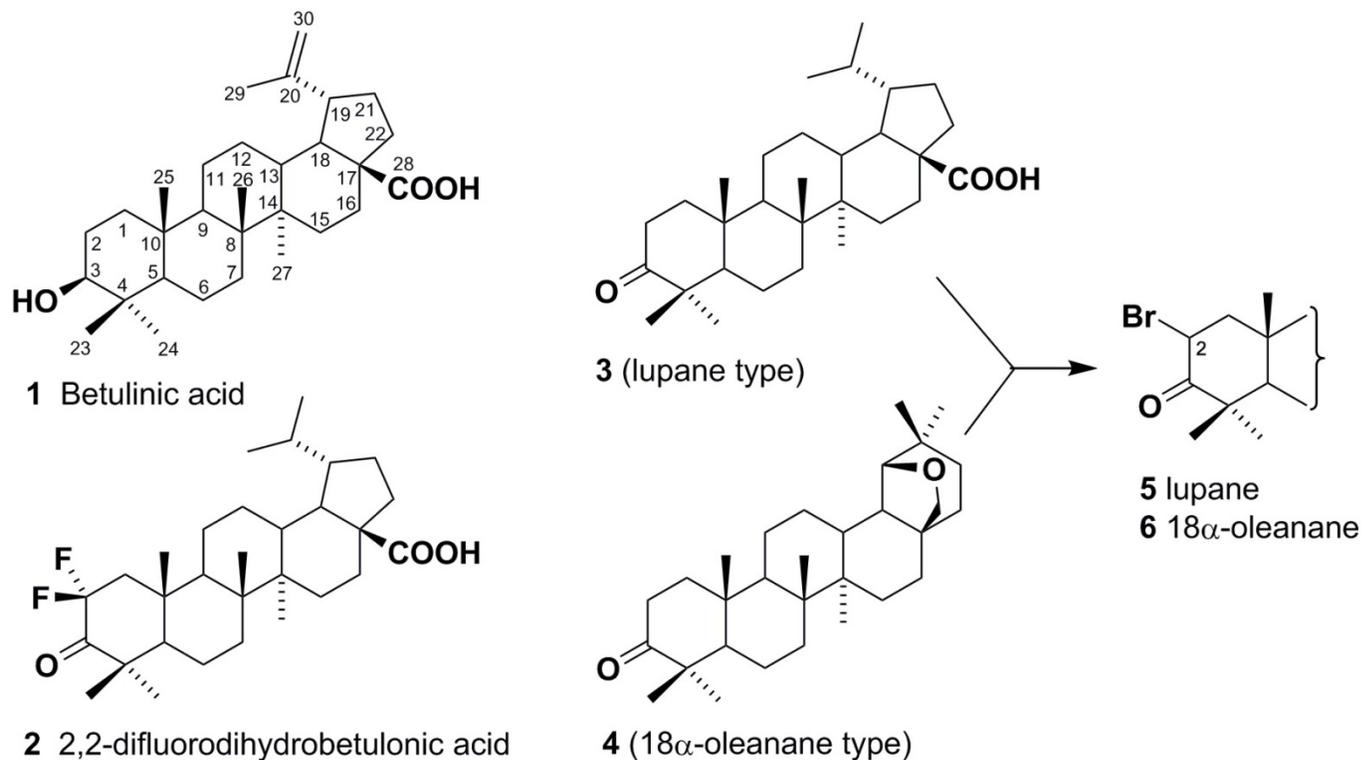
MEDI 292

Cytotoxic triterpenoids substituted in the position 2

Lucie Borkova¹, borkova.luc@gmail.com, **Jan Sarek**¹, **Jiri Rehulka**¹, **Petr Dzubak**¹, **Marian Hajduch**¹, **Milan Urban**^{1,2}. (1) IMTM, Palacky University, Faculty of Medicine and Dentistry, Olomouc, Czech Republic (2) Organic Chemistry, Palacky University, Faculty of Science, Olomouc, Czech Republic

Triterpenoids are natural compounds that are produced as secondary metabolites by numerous living organisms, mainly plants, marine organisms, or fungi; they are often used in natural medicine in Asia. Triterpenes have various biological activities; derivatives of betulinic acid **1**, for example, have strong anti-HIV and anti-cancer activities.

There are several known derivatives of lupane with high cytotoxicity and many of them contain an electronegative substituent at the position 2 of the skeleton such as compounds **5** or **7**. It appeared that their cytotoxicity is driven by the electronegativity of the substituent. In order to confirm or refute this theory, a small library of lupane and 18α -oleanane triterpenoids modified at C-2 was synthesized and their cytotoxic activities were investigated. First of all, a set of difluoroderivatives (e.g. **2**) was prepared as examples of the ultimately electronegative substituent. Secondly; a series of C-2 substituted triterpenes (e.g. **8** – **17**) was obtained mostly by nucleophilic substitution of bromoketones **5** and **6**. The *in vitro* cytotoxicity was screened on CCRF-CEM cell line, the most active derivatives were tested on a panel of seven cancer cell lines with/without MDR phenotype and non-tumor MRC-5 and BJ fibroblasts. Results of the biological screening and basic assumptions about structure-activity relationships will be discussed.



MEDI 293

Host-guest formulations of novel isozyme-selective carbonic anhydrase inhibitors for colon cancer detection and treatment

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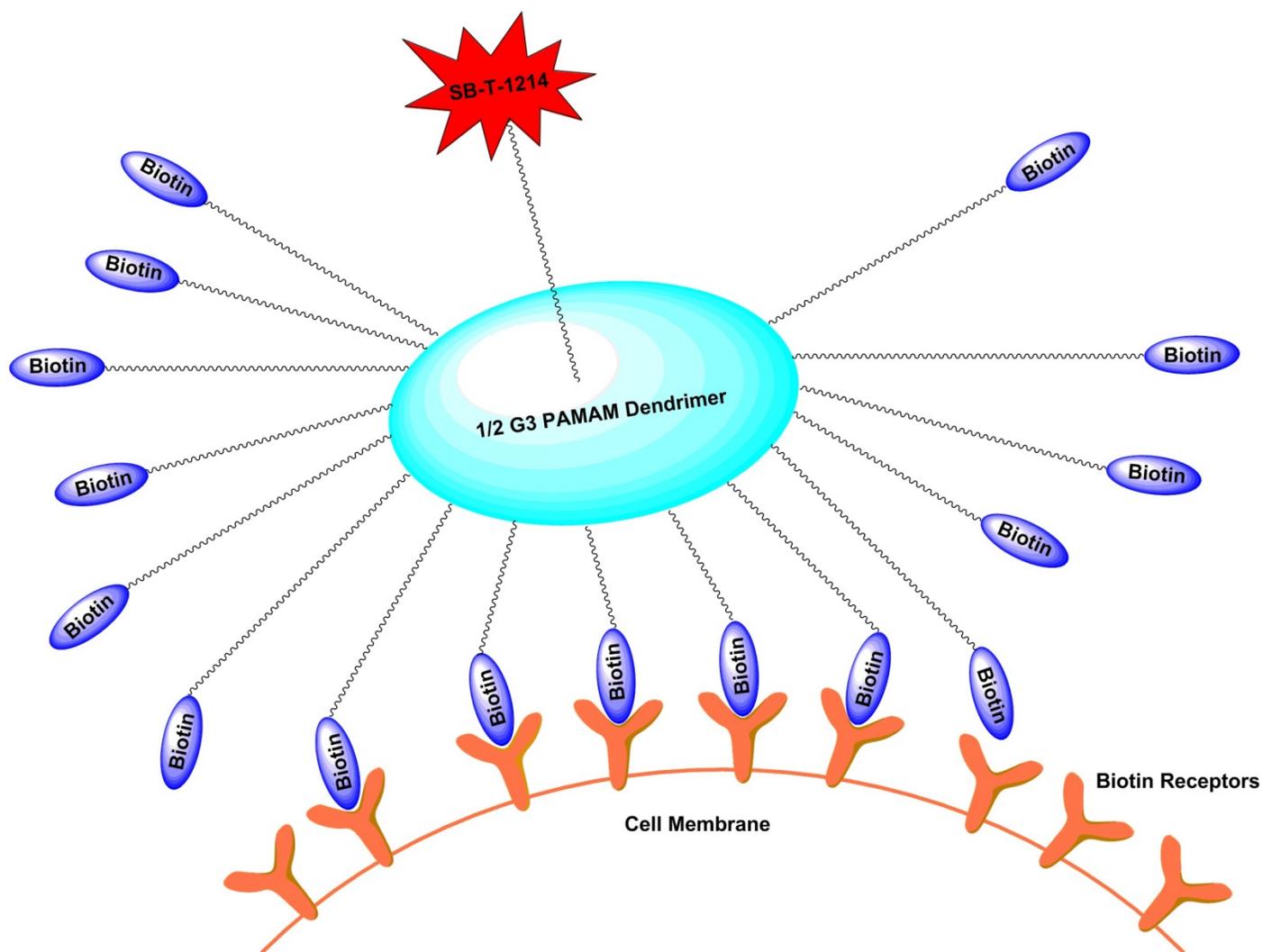
The purpose of the study was to generate and characterize novel theranostic host-guest complexes of pyridinium-sulfonamides as selective and efficient carbonic anhydrase inhibitors (CAIs) and their evaluation as colon cancer detection and treatment (theranostic) systems. We will present our most recent results in the complexation of pyridinium sulfonamides with several classes of hosts mentioned above. We will also present in a comparative manner the toxicity induced by different hosts and by their complexes with the pyridinium sulfonamide guests in vitro, using 2D and 3D cellular models, and we will correlate this toxicity of the host-guest complexes with the physicochemical properties of these nanocarriers for drawing structure-property relationships. The results of translating these technology into animal models of colon cancer will also be presented, emphasizing the most promising host-guest complexes for colon cancer early detection and treatment.

MEDI 294

Design, synthesis, and biological evaluation of novel PAMAM dendrimer-based tumor-targeted drug delivery systems

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Polyamidoamine (PAMAM) dendrimers are attractive anticancer drug delivery vehicles because of their well-defined structures and biocompatible properties. We have been developing novel tumor-targeted drug delivery systems using PAMAM dendrimers with cystamine cores and amino surfaces. Unlike directly attaching different functional groups onto dendrimer surface, which usually gives a mixture of products with wide distributions, we adopted a different strategy by fully functionalize PAMAM dendrimer surface with one functional group first, and then cleave the cystamine core with reducing agent TCEP, followed by attaching this functionalized half dendron to other functional groups using a proper bifunctional spacer. Using this strategy, we have successfully constructed PAMAM dendrimer-based tumor-targeting drug conjugates bearing a 2nd-generation taxoid SB-T-1214 as warhead, and 4 or 16 biotins on a half G1 or G3 PAMAM dendron as tumor-targeting modules respectively. We also constructed such tumor-targeting conjugates bearing fluorescent probes instead of cytotoxic warheads for imaging purposes. Biological evaluations (MTT, CFM and flow cytometry analyses) of these PAMAM dendrimer-based conjugates together with biotin-linker-taxoid (BLT) and biotin-linker-FITC as controls against various cancer cell lines, overexpressing biotin receptors (BRs), indicated 16 biotin units are better than 1 or 4 biotin units for targeting overexpressed BRs *via* multivalent binding effect. In addition, we have successfully constructed asymmetric bow-tie PAMAM dendrimer-based multifunctional conjugates by connecting a half G3 dendron with 16 biotins and a half G1 dendron with 4 taxoids or fluorescent probes. Biological evaluations of these conjugates exhibited excellent BR-specific cytotoxicity, and substantially enhanced receptor-mediated endocytosis (RME) *via* multivalent binding effect. The design, synthesis, and biological evaluations of these novel PAMAM dendrimer-based tumor-targeting drug conjugates will be presented.



MEDI 295 – Withdrawn.

MEDI 296

Selective DDRs inhibitors as novel therapeutic agents for human cancers and pulmonary fibrosis

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Discoidin domain receptors (DDR) are members of the transmembrane receptor tyrosine kinase (RTK) super-family which are distinguished from others by the presence of a discoidin motif in the extracellular domain and their utilization of collagens as internal ligands. The DDRs (DDR1 and DDR2) play important roles in the regulation of fundamental cellular process, such as proliferation, survival, differentiation, adhesion, and matrix remodeling, and are closely linked to a number of human diseases, including various fibrotic disorders, atherosclerosis and cancer. Thus, DDRs have been

considered as novel potential molecular targets for drug discovery and increasing efforts are being devoted to the identification of new small molecule inhibitors targeting the receptors. However, given the structural similarity of DDRs to the other kinase, it is highly challenging to identify selective small molecule DDR1/DDR2 inhibitors. We have identified a series of highly selective and orally bioavailable DDR1 inhibitors. One of the most promising compounds inhibited the enzymatic activity of DDR1 with an IC_{50} value of 6.8 nM, but was significantly less potent in suppressing the kinase activities of DDR2, Bcr-Abl and c-Kit. Further study revealed that the compound bound with DDR1 with K_d value of 0.6 nM, while was significantly less potent to the other 455 kinases tested. The S(35) and S(10) selectivity scores was 0.035 and 0.008, respectively. The compound also potently inhibited the proliferation of cancer cells expressing high levels of DDR1 and strongly suppressed cancer cell invasion, adhesion and tumorigenicity. Preliminary pharmacokinetic studies suggested that they possessed good PK profiles, with oral bioavailabilities of 67.4% and 56.2%, respectively. By using the novel inhibitor as a research tool, we further demonstrated DDR1 inhibitors displayed promising in vivo antitumor efficacy in a variety of pancreatic cancer and non-small cell lung cancer models. Another DDR1/DDR2 inhibitor also demonstrated promising therapeutic effect on pulmonary fibrosis. Our results may provide some basis for further validation of DDR1/DDR2 as novel molecular target for future drug discovery.

MEDI 297

Preparation of fenbufen boronopinacol, *meta*- and *ortho*- $[^{18}F]$ fluorofenbufen boronopinacol and $[^{18}F]$ fluorocelecoxib for boron neutron capture therapy of cholangiocarcinoma

Chun-Nan Yeh², Chi-Wei Chang³, I-Shio Chung⁴, Yong-Ren Chen¹, Tsong-Wen Chen², Ying-Cheng Huang⁵, Hsin-Er Wang⁶, Shi-Wei Tien¹, **Chung-Shan Yu¹**, *csyu@mx.nthu.edu.tw*, You-Cheng Chou¹, Che-How Liu¹, I-Ting Hsie¹. (1) Dept. of Biomedical Engineering and Environmental Sciences, National Tsinghua University, Hsinchu, Taiwan (2) Dept. of Surgery, Chang-Gung Memorial Hospital, Taoyuan, Taiwan (3) Dept. of Nuclear Medicine, Taipei Veterans General Hospital, Taipei, Taiwan (4) Dept. of Nuclear Medicine, Chang-Gung Memorial Hospital, Taoyuan, Taiwan (5) Dept. of Neurosurgery, Chang-Gung Memorial Hospital, Taoyuan, Taiwan (6) Yang-Ming University, Taipei, Taiwan

We have prepared a borono NSAIDs analog for boron neutron capture therapy (BNCT). Uptake selectivity of fenbufen ester borono pinacol in liver tumor could be referred to the signals of region of interest of Positron Emission Tomography data from fluoro analogs, ^{18}F -labeled *m*-fluorofenbufen ester borono pinacol (T/N = 1.38) and *o*-fluorofenbufen ester borono pinacol (T/N = 1.70). The radiofluoro analog of COX-2 selective inhibitor Celecoxib, ^{18}F -fluorocelecoxib, was also prepared for comparison. Binding assay of ^{18}F -tagged *m*-fluorofenbufen ester borono pinacol for COX-1 and COX-2 enzymes generated the IC_{50} values of 2.52 ± 2.16 mM and 10.56 ± 9.74 mM, respectively, comparing with 12 nM (COX-1) and 0.1 nM (COX-2) by ^{18}F -fluorocelecoxib. The uptake dose of *m*- $[^{18}F]$ fluorofenbufen ester borono pinacol in tumor

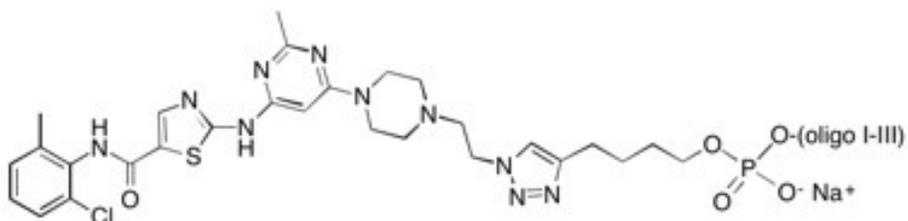
was 1.09 ± 0.13 (%ID/g) and the selectivity ratio was 1.38 ± 0.12 (T/N) which was comparable to 1.38 ± 0.23 (T/N) for ^{18}F -Fluorocetecoxib. The superior selectivity of *o*-fluorofenbufen ester borono pinacol (1.70 , T/N) was clarified by NIH-shift mediated metabolism. In the light of the fair half-life (30 min) and the marginal toxicity ($\text{IC}_{50} > 100$ mM), BNCT was performed from 40 min to 55 min after administration of fenbufen ester borono pinacol (20-30 mg). The two cholangio carcinoma (CCA) rats receiving BNCT and the control group of two CCA rats receiving NCT reduced the tumor growth in $26.42 \pm 2.575\%$ (N=8) and $17.37 \pm 3.921\%$ (N=6), respectively, with a P value of 0.0336.

MEDI 298

Synthesis and evaluation of drug-DNA conjugated gold nanoparticles activated by cancer cell specific mRNA

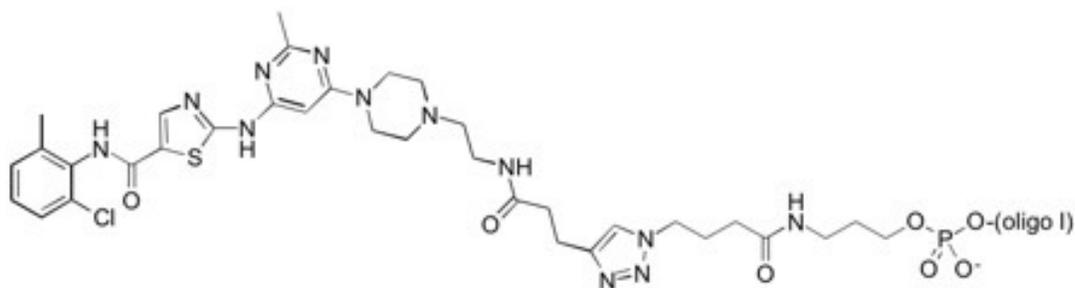
Nan-Sheng Li¹, *nanshengli@yahoo.com*, Nathan P. Gossa^{2,3}, Jordan Naumann^{2,3}, Peter M. Gordon^{2,3}, Joseph A. Piccirilli¹. (1) Biochemistry and Molecular Biology, and Chemistry, University of Chicago, Chicago, Illinois, United States (2) Pediatrics, Division of Pediatric Hematology and Oncology, University of Minnesota, Minneapolis, Minnesota, United States (3) Masonic Cancer Center, University of Minnesota, Minneapolis, Minnesota, United States

Two synthetic approaches to the dasatinib-DNA conjugates via click chemistry either by (1) the reaction of azido dasatinib derivative with 5'-(5-hexynyl) tagged DNAs or by (2) the reaction of alkynyl linked dasatinib with 5'-azido tagged DNA are described. The second approach using alkynyl derived dasatinib and 5'-azido tagged DNA yielded the corresponding dasatinib-DNA conjugates in higher yield. These drug-DNA conjugates were then used to prepare the bifunctional gold nanoparticles that effectively targeted leukemia cells while exhibiting less toxicity against hematopoietic stem cells or T cells than dasatinib alone. Fundamental to this approach is the observation that the amount of drug released from the Au-NP is proportional to both the presence and abundance of the cancer cell specific mRNA in a cell. As proof-of-principle, we demonstrate the selective release of the multi-kinase inhibitor dasatinib from Au-NPs in leukemia cells with resulting efficacy *in vitro* and *in vivo*. This approach has the potential to improve the therapeutic efficacy of a drug and minimize toxicity while being highly customizable with respect to both the cancer cell specific mRNAs targeted and drugs activated.



Dasatinib-DNA conjugates (I-III)

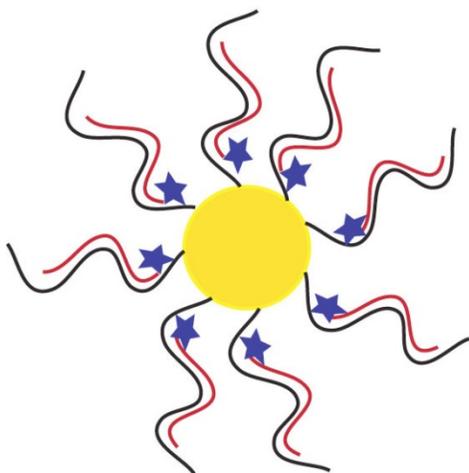
oligo I: TCA AGG AGC TGG AA
 oligo II: CTT GGA TGA TCG GTT
 oligo III: TCA AGG AGC TG



Dasatinib-DNA conjugate (IV)

oligo I: TCA AGG AGC TGG AA

Structures of drug-DNA conjugates



Model of drug-DNA conjugated gold nanoparticles (AuNPs)

MEDI 299

Discovery of hepatoselective inhibitors of diacylglycerol acyltransferase 2 (DGAT2)

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Diacylglycerol acyltransferase 2 (DGAT2) is one of the major enzymes that catalyze the terminal step in triacylglycerol biosynthesis. In preclinical species, small molecules and antisense oligonucleosides (ASO) have been used to demonstrate the potential of DGAT2 inhibition to treat a spectrum of metabolic diseases such as hyperlipidemia, non-alcoholic steatohepatitis (NASH), and type 2 diabetes. IONIS-DGAT2_{Rx}, an ASO developed by IONIS pharmaceuticals, is the most advanced clinical asset in this target class which is reported to be in phase I clinical trials for NASH indication. This presentation will describe the discovery and optimization of novel hepatoselective small molecule DGAT2 inhibitors. Hit-to-Lead medicinal chemistry efforts, preclinical pharmacology, pharmacokinetics and safety profile will be discussed for the representative hepatoselective DGAT2 inhibitors.

MEDI 300

Discovery of human NMUR2 selective hexapeptidic agonists

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Neuromedin U (NMU) is a bioactive peptide possessing various physiological functions. The common C-terminal amidated heptapeptide sequence (FLFRPRN-amide, **1**), among the mammals, is responsible for activation of type 1 (NMUR1) and type 2 (NMUR2) NMU receptors. Recently, the anti-obesity effect of NMU has attracted attention in drug development. However, development of small and selective peptide agonists to human NMU receptors has not yet been performed although several structure-activity relationship (SAR) studies have been already reported. Hence, in this study, a series of peptide derivatives based on **1** were prepared by Fmoc-based solid-phase peptide synthesis and their selectivity to both human NMUR1 and NMUR2 were evaluated in a calcium-mobilization assay with receptor-expressing cells. As a result, we discovered a novel hexapeptidic agonist **2** that selectively activates NMUR2 without significant NMUR1 activation. This peptide only showed a 2.5-fold decrease in agonistic potency compared to human NMU (hNMU, 25 aa). Further investigations *in vivo*

suggested that agonist **2** could be used as an anorexigenic drug. Therefore, agonist **2** would contribute to the promotion of anti-obesity drug development as well as the understanding of endocrinological significance of NMU.

MEDI 301

Synthetic chemistry core at Albert Einstein College of Medicine. Recent contributions to chemical biology and drug discovery

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The Synthetic Chemistry Core Facility at Albert Einstein College of Medicine provides synthesis capabilities to the scientific community at Einstein and for our external collaborators. This poster will highlight several of our recently completed projects in drug discovery and chemical biology.

We have recently synthesized a library of chloroquine (CQ) analogs for use as autophagy inhibitors. Inhibition of autophagy has emerged as a viable strategy in cancer treatment. CQ has been used in multiple clinical trials, but suffers from low potency. Our lead compound is 8 times more potent than CQ against lung- and pancreatic-cancer cell lines. An azide-alkyne cyclization was just for late stage derivatization with a library of azides. Ongoing studies focus on optimization of the alkyne coupling partner as well as pharmacokinetic studies.

Our contributions to chemical biology include ligands for CuAAC click reactions, and synthesis of several azide and alkyne containing unnatural monosaccharides for studies of various glycans.

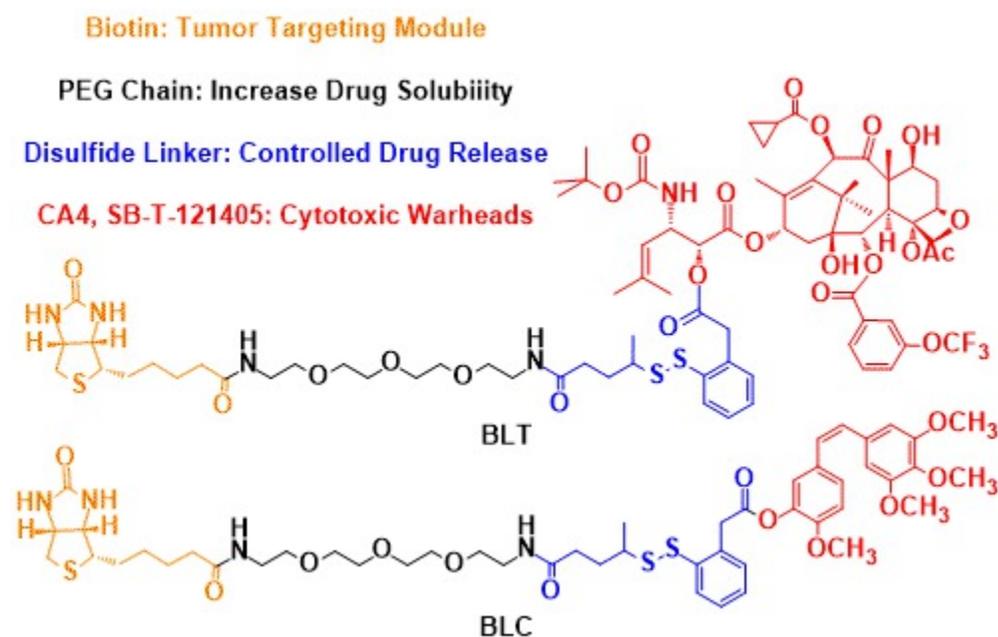
MEDI 302

Design, synthesis, and biological evaluation of novel tumor-targeted drug delivery systems for a third-generation taxoid, combretastatin and their combination

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Novel tumor-targeted drug delivery systems (TTDDSs), BLT (Biotin-Linker-SB-T-121405) and BLC (Biotin-Linker-Combrestastin A4), which consist of a cytotoxic warhead (SB-T-121405 or Combrestastin A-4), a self-immolative disulfide linker for drug release, and biotin as the tumor-targeting module have been designed and synthesized. Tumor-targeted drug delivery and controlled drug release have been used widely to achieve effective cancer treatment with reduced toxicity. It has been shown that biotin receptor (BR) is overexpressed on various cancer cells and thus BR serves as an excellent biomarker for TTDDS. We and others verified highly efficient receptor-mediated endocytosis (RME) of biotin-drug conjugates, targeting BRs on cancer cells.

Controlled drug release can be achieved by using a mechanism-based disulfide linker. Glutathione or other endogenous thiols can trigger the disulfide linker cleavage via a disulfide-thiol exchange reaction. Because the concentration of glutathione in tumor tissue (2-8 mM) is 1,000 times higher than that in bloodstream (1-2 μ M), the stability in blood stream and tumor specific cleavage of drug conjugate are both achieved by the use of this linker. As the warheads, a third-generation taxoid, SB-T-121405, and combrestastin A-4 were used. SB-T-121405 exhibits high potency against various cancer cell lines. Combrestastin A-4 is a potent antimitotic and antiangiogenic agent. The potency of individual drug delivery system and the combination effect of those two TTDDSs will be presented.



Chemical structures of BLT and BLC

MEDI 303

Molecular mimics of classic P-glycoprotein as dual cytotoxic/MDR auto-suppressors or in combination with Paclitaxel

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P-glycoprotein (Pgp) is a membrane bound efflux pump found on the cell membrane of variety of tumor cells and considered as a main component of multidrug resistance (MDR) to chemotherapies. Based on common structural features between curcumin and verapamil, two classic inhibitors of Pgp, three groups of compounds (imidazolone, oxazolone and vinyl dipeptide derivatives) were designed and synthesized aiming to develop a molecular framework that effectively suppresses MDR. We examined their

ability to inhibit Pgp pump activity due to Pgp efflux site blocking or Pgp ATPase subunit inhibition. Four compounds coded **4a**, **5**, **3a**, and **3b** significantly decreased remaining ATP concentration indicating Pgp substrate site blocking. On the other hand, **4b** and **4e** significantly increased remaining ATP concentration, which is indicative of Pgp ATPase inhibition. The cytotoxicity of synthesized compounds was examined against Pgp expressing/highly resistant colorectal cancer cell line (LS-174T) using sulfarhodamine-B assay. Among synthesized compounds, only **4a** and **4b** showed considerable cytotoxicity against LS174T cells with IC₅₀'s of 7.6 and 8.9 μM, respectively. Equitoxic combination of PTX and **4b** greatly diminished resistant cell clone of LS174T from 45.7±3.6% to 2.5±1.1%, albeit with a slight drop in potency from IC₅₀ of 7.9±0.53 nM to IC₅₀ of 23.8±8.1 nM. On the other hand, combination of PTX and the non-cytotoxic **5** (10 μM) significantly decreased the IC₅₀ of PTX to 3.8±0.43 nM as well as the resistant fraction to 16.2±6.6%. Both **4b** and **5** (10 μM) significantly influenced Pgp substrate cellular pharmacokinetics and increased the cellular entrapment of Pgp probe (doxorubicin) elevating its intracellular concentration from 1.9±0.3 pmole/cell to 3.0±0.45 and 2.9±0.3 pmole/cell respectively.

MEDI 304

Novel selective estrogen receptor downregulators developed using endocrine-independent breast cancer cells lines

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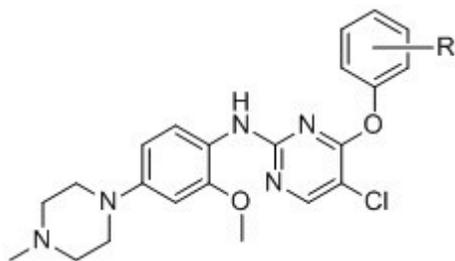
Approximately 70% of breast cancer patients are estrogen receptor positive (ER+). Aromatase inhibitors and the selective estrogen receptor modulator (SERM), tamoxifen, are the first line treatments for these patients; however, almost 50% of patients either do not respond or acquire resistance. Multiple mechanisms, including mutations of the *ESR1* gene, contribute to resistance via ligand-independent constitutive activation of ER. Selective estrogen down-regulators (SERDs) that block ligand-dependent and independent ER signaling by ablation of ER, offer a therapeutic approach to treatment-resistant, advanced stage and early stage ER+ breast cancer. Therapeutic use of the first generation SERD, fulvestrant (Faslodex), has largely remained 2nd and 3rd line, because of poor physiochemical/pharmacokinetic properties. Novel benzothioophene based SERDs were designed, synthesized, and optimized and assayed in three tamoxifen-resistant (TR), endocrine-independent ER+ MCF-7 and T-47D cell lines. Cell viability, ERE-luciferase response, and ER degradation was measured and compared to parent endocrine-dependent MCF-7 and T-47D cell lines in 2D and/or 3D spheroid cell cultures and compared to SERDs, fulvestrant and GDC-0810. Pharmacokinetic analysis was used to select novel SERDs for xenograft studies.

MEDI 305

Design, synthesis and evaluation of WZ4002 analogues as EGFR inhibitors

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The clinical efficacy of epidermal growth factor receptor (EGFR) kinase inhibitors such as gefitinib and erlotinib is limited by the development of drug resistance. WZ4002 has been shown to be effective against resistance mediated by the *EGFR* T790M mutation. WZ4002 is 30-100 fold more potent against *EGFR* T790M and up to 100 fold less potent against wild type EGFR compared to the quinazoline based EGFR inhibitors, gefitinib and erlotinib, in *in vitro* studies. A series of twenty one analogues with electrophilic, non-electrophilic and photolabile substitutions on the terminal phenyl ring has been synthesized and these analogues are being evaluated for antiproliferative effects against various cancer cell lines which include PC9, PC9GR, H460, NIH-3T3, BaF3-WT and BaF3-T3151. Among the compounds evaluated thus far, the *para* substituted acetamide derivative was found to be the most potent with IC_{50} values similar to that of the parent WZ4002.



MEDI 306

Exploring EGFR kinase-ligand interactions for optimizing dual action inhibitors

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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 researches 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 307

Synthesis of substituted trifluoromethyl ketone targeted antifolates as potential purine synthesis inhibitors

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Folic acid is the indispensable cofactor for purine *de novo* synthesis, and involves a formyl group transfer from N^{10} -formyl tetrahydro folate to two intracellular substrates, glycylamide ribonucleotide (GAR) and aminoimidazole carboxamide (AICAR). The transition state of these processes consist of a tetrahedral intermediate. To mimic this transition state without formyl transfer activity, we incorporated a trifluoromethyl ketone moiety into our previously established potent and tumor selective antifolates. In aqueous solution, the trifluoromethyl ketone moiety exists predominantly as the hydrated diol form, which mimics the tetrahedral center in the transition state for formyl transfer.

The enantioselective synthesis of these compounds started from trifluoromethyl ketone methyl benzoate, followed by chiral hydrazone formation, conjugate addition/ alkylation, deprotection of hydrazone, TMSCH₂N₂ assisted α -bromoketone formation, cyclization, and finally an amide coupling with *L*-glutamate. The design, synthesis, tumor uptake, selectivity and potency of these compounds will be discussed.

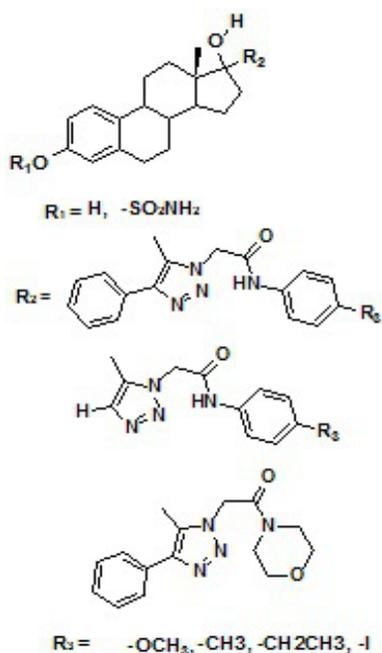
MEDI 308

In silico design and synthesis of novel estrone analogs utilizing click chemistry targeting colorectal cancer

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Colorectal cancer (CRC) is the third leading cause of cancer-related death in USA when men and women are considered separately, and the second leading cause when both sex are combined. It is expected to cause 50,000 death during 2016. There is a crucial need for new drugs that overcome the resistance of current chemotherapy, needless to say the urgency of new multi-target drug treatment of CRC. EGFR (Epidermal Growth Factor Receptor) is a tyrosine kinase receptor and expressions of its downstream

pathways are responsible for several tumors, such as colorectal cancer. Current research in our lab is investigating estrone analogs targeting several types of cancer with promising potent anti-proliferation activities. Our hypothesis that in silico approach for designing novel estrone analogs targeting EGFRs and utilizing click chemistry to introduce different 1,2,3-triazoles entities (Figure 1) could lead to estrone analogs that overcome the resistance of CRC chemotherapy. Molecular docking study of a virtual library of 400 analogs using OpenEye^o software on molecular targets 1M17, 1a52, 3q18 and 20JG was conducted. Data showed that 288 virtual analogs that have the 1,2,3-triazoles moieties showed a better affinity towards EGFRs than the current inhibitors such as Erlotinib. Data showed that triazoles moieties enhanced affinity to target proteins. Organic synthesis of estrone analogs, which showed top, calculated EGFR affinity towards target proteins was conducted and their anti-proliferation activities will be presented.



MEDI 309

Novel 6-substituted pyrrolo[2,3-d]pyrimidine classical antifolates as selective folate receptor substrates and antitumor agents

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Folates are involved in one-carbon transfer reactions, required for DNA synthesis. In mammals, there are three major folate transport systems, the reduced folate carrier (RFC), folate receptors (FRs), and proton-coupled folate transporter (PCFT). Currently used antifolates, such as methotrexate (MTX), pemetrexed (PMX), and pralatrexate (PDX) are transported by the ubiquitously expressed RFC that results in dose-limiting toxicity, due to non-selective drug uptake into normal tissues. FRs and PCFT have a limited expression in human tissue and are over-expressed in tumor cells. Antifolates that are selectively taken up by FR and/or PCFT into tumors would circumvent the dose-limiting toxicities of currently used clinical antifolates. We previously reported 6-substituted pyrrolo[2,3-*d*]pyrimidine classical antifolates that have superior FR and/or PCFT selectivity over RFC and show more potent (sub-nanomolar) inhibitory activity in FR/PCFT over-expressing human tumor cells (KB) compared to the clinically used antifolates. In this study, 6-substituted pyrrolo[2,3-*d*]pyrimidine classical antifolates with methylated thienyl regioisomers in the side chain were designed and synthesized to evaluate the effects of methylation in the side chain on transport by RFC, FR and/or PCFT and as inhibitors of human KB tumor cells (IC₅₀) targeting the folate utilizing enzymes involved in the purine and/or pyrimidine biosynthetic pathway. This report will discuss the molecular modeling, design, synthesis and evaluation of these compounds.

MEDI 310 – Withdrawn.

MEDI 311

Design and synthesis of antifolates as targeted antitumor agents: Exploring the benefits of fluorine substitution on the side chain (het)aryl ring for improved selectivity and potency

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The need for effective cancer chemotherapeutics devoid of dose limiting toxicities and tumor resistance is acute. There is ample clinical evidence to support the use of antifolates as targeted antitumor agents due to their selective uptake by two transporters overexpressed in tumor cells, namely the folate receptors (FR) and the proton-coupled folate transporter (PCFT). However, currently marketed antifolates such as methotrexate (MTX) and pemetrexed (PMX) are transported non-selectively by a ubiquitously expressed folate transporter, the reduced folate carrier (RFC) and as such, their use is severely limited due to dose-limiting toxicities. We have already reported a number of 6-substituted pyrrolo[2,3-*d*]pyrimidine classical antifolates that are more potent (they inhibit FR/PCFT over-expressing KB and IGROV1 tumor cells at sub-

nanomolar IC₅₀ values) and are selectively transported by FRs and/or by PCFT, over clinically used antifolates. In this report we provide an extension of the ligand-based drug design of classical 6-substituted pyrrolo[2,3-*d*]pyrimidines with/without fluorine atom/s on the side chain (het)aromatic ring for SAR exploration of effects caused by fluorine substitution. Fluorine substitution altered both the potency and selectivity of our targeted 6-substituted pyrrolo[2,3-*d*]pyrimidine antifolates, possibly due to structurally altered electronics and/or conformational effects. The molecular modeling, synthesis, *in vitro* evaluation and SAR of these compounds as substrates for folate transporters FR and PCFT over RFC and as potent inhibitors of human KB tumor cells (IC₅₀) due to inhibition of glycinamide ribonucleotide formyl transferase (GARFTase), aminoimidazole carboxamide ribonucleotide formyl transferase (AICARFTase), thymidylate synthase (TS) and/or dihydrofolate reductase (DHFR) will be presented and discussed.

MEDI 312

Small molecule mimics of a conserved TWXE/DFL motif targeting G- α -i3

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Heterotrimeric G proteins act as molecular switches that modulate numerous cellular signaling pathways. G- protein signaling is initiated and mediated by the binding of guanine nucleotide Exchange Factors (GEFs) to inactive G-proteins which accelerates the rate of exchange of GDP for GTP. Gai proteins have been demonstrated to enhance Akt activation, remodel the actin cytoskeleton, and mediate cell migration, making them a desirable pharmacological target for inhibiting cancer metastasis. A GDP-selective Gai binding peptide, KB-752, has previously been demonstrated to enhance spontaneous nucleotide exchange of Gai subunits. Several specific contacts between a conserved TWXE/DFL and Gai1 have been shown to be critical for nucleotide exchange. An intramolecular hydrogen bonding network within the α -helical TWXE/DFL motif involving threonine 4 (T4) and aspartate 7 (D7) serve to orient both tryptophan (W5) and phenylalanine (F8) toward the Ga binding face of the peptide, burying W5 within a hydrophobic pocket formed by F215, L249, and I253 of Gai1, while F8 likewise resides within a hydrophobic environment established by W211, I212, and F215 of Gai1. Based on this structural data, a library of peptidomimetic small molecules utilizing a tryptophanyl core structure was constructed. Computer-assisted drug discovery (CADD) focused the library to twenty compounds which were found to bind Gai1 in a fashion similar to the tryptophanyl moiety of KB-752. The small molecules are being synthesized and prepared for analysis.

MEDI 313

Development of functionalized aminobenzoboroxoles as anti-cancer agents

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Boronic acids often function as enzyme inhibitors owing to their unique properties and structural similarities to carboxylic acids. Bortezomib (proteasome inhibiting anti-cancer drug used for the treatment of multiple myeloma) and tavaborole (fungal enzyme leucyl-tRNA synthetase inhibitor, used for the treatment of onychomycosis) are couple of boronic acid based drugs that are currently in the market. Benzoboroxoles are cyclic boronic acids that have garnered attention in the last decade because of their excellent therapeutic potential as anti-fungal, anti-bacterial, and anti-cancer agents. We have developed several methodologies for the synthesis of functionalized benzoboroxoles starting from o-boronobenzaldehyde as the precursor. We have prepared oxaborole ring substituted derivatives employing Baylis-Hillman reaction, Barbier allylation, Passerini reaction, and aldol reaction as the key steps. While the synthesis of these derivatives was quite facile, we noticed that the substitution at the benzylic carbon in the oxaborole unit invariably led to the loss of biological activity and most of the compounds synthesized seemed to be fairly non-toxic even at higher concentrations (100 μ M). Hence, we are currently investigating the synthesis of benzoboroxoles with substitution on the aromatic ring while leaving the oxaborole ring unbranched. This presentation will deal with the synthesis and evaluation of few series of lead derivatives that we have been able to identify for detailed SAR analysis and future development.

MEDI 314

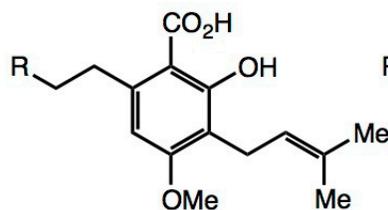
Synthesis and biological evaluation of amorfrutin analogs: A unique class of natural product that modulates PPAR γ activity

Sabesan Yoganathan, *yoganats@stjohns.edu*. Department of Pharmaceutical Sciences, College of Pharmacy and Health Sciences, St. Johns University, Jamaica, New York, United States

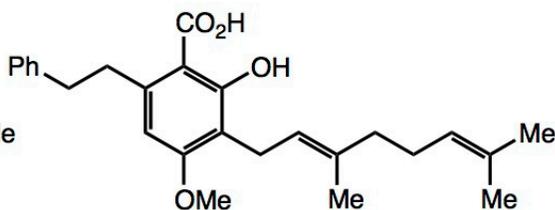
Natural products hold great potential as therapeutic agents for the treatment of several life-threatening diseases such as infections, cancer and metabolic diseases. Although medicinal natural products are privileged scaffolds, their structure needs to be fine tuned to identify analogs with desired pharmacological properties. PPAR γ (peroxisome proliferator-activated receptor gamma) has emerged as a highly sought target for metabolic diseases and inflammation associated health conditions. There are two main reasons why it is of considerable interest to develop novel therapeutics that target PPAR γ : (a) current drugs that target PPAR γ tend to exhibit adverse effects; and (b) due to physiological importance of PPAR γ , selective modulators may alleviate commonly observed side effects with full agonists. Amorfrutins (**1 - 3**), a unique class of prenylated 2,4-dihydroxybenzoic acid derivatives are potent modulators of PPAR γ . Due to their promise in being partial agonist, and low μ M activity, research efforts have been

focused on developing structural derivatives with improved pharmacological properties, and low toxicity. We have devised a research program to investigate a direct and scalable synthetic approach to generate a structurally diverse library of amorfrutins. Our synthetic plan begins with the preparation of the methyl or ethyl ester of 4-methoxysalicylic acid, and subsequent protection of 2-OH group as a silyl ether. The prenyl-group is installed via an electrophilic aromatic substitution reaction. Current effort is focused on optimizing a directing group assisted C-H functionalization of the intermediate. This method enables us to readily functionalize the aromatic ring with various aliphatic or aromatic motifs at both 3-position and 6-position. Since there have been no extensive studies done to evaluate the structure activity relationship (SAR), the first generation analogs we synthesize will shed light on the SAR. We plan to evaluate the anti-diabetic property, and anti-inflammatory property in pertinent *in vitro* and *in vivo* models. As toxicity associated with PPAR γ modulators is always a concern, we plan to investigate the hepatotoxicity profile of the analogs as well. The bioactivity data will assist us in designing the subsequent generation of analogs.

Natural amorfrutins:

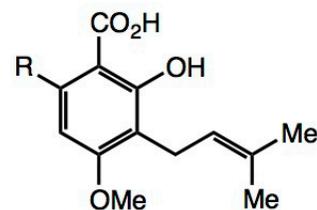


1: Amorfrutin 1(A)
(R = Ph; K_i = 0.24 μ M)
2: Amorfrutin 2
(R = *n*Pr; K_i = 0.29 μ M)



3: Amorfrutin B (K_i = 0.019 μ M)

Synthetic amorfrutin analogs:



4: R = H
5: R = alkyl group
6: R = aryl group

MEDI 315

Discovery of an inhibitor of the Rpn11 proteasome subunit

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The proteasome is a protein complex found in all eukaryotes and it is a major component of the ubiquitin-proteasome system (UPS). The proteasome plays a crucial role in degradation of abnormal or unwanted proteins required to maintain cell homeostasis. Inhibitors of the proteasome have been validated in the treatment of multiple myeloma, with multiple FDA-approved proteasome inhibitors. Inhibition of the UPS pathway represents an attractive oncology target with other avenues of untapped therapeutic potential. Among these targets is the Rpn11 subunit, which is a Zn²⁺-dependent metalloisopeptidase that hydrolyzes ubiquitin from tagged proteins that are

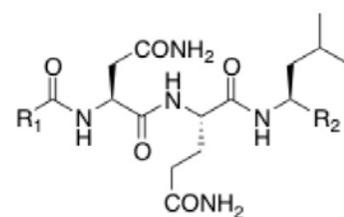
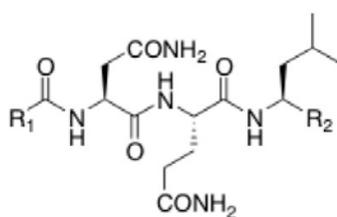
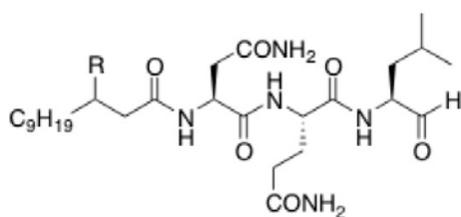
trafficked to the proteasome for degradation. We utilized a fragment-based drug discovery (FBDD) approach to identify fragments with activity against Rpn11. Screening of a focused library of metal-binding pharmacophores (MBPs) revealed that 8-thioquinoline (IC₅₀ value ~2.5 μM) displayed strong inhibition of Rpn11. Further synthetic elaboration of 8-thioquinoline yielded a small molecule compound (IC₅₀ value ~200 nM) that is a potent and selective inhibitor of Rpn11 that induces apoptosis in multiple myeloma cells in culture. This effort shows that a metalloenzyme-focused FBDD effort can produce a first-in-class inhibitor of Rpn11.

MEDI 316

Chemical modification and structure activity relationship (SAR) of fellutamide B, a natural product with anticancer and anti-tuberculosis activity

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Fellutamides (A-D) are a class of lipopeptides, which have been isolated from a marine fungi, and they exhibit promising proteasome inhibitory activity. Drugs, which usually inhibit proteasome activity, emerge as promising candidates for the treatment of cancer, and inflammatory diseases. In specific cases, proteasome inhibitors have also been shown to be effective anti-fungal or anti-bacterial compounds. Similar to the clinically used drugs bortezomib and carfilzomib, fellutamides are also of peptide in nature. Among these natural products, fellutamide B (**1**) has been shown to be the most potent member, and exhibits promising anticancer activity against several types of cancer cell lines. To date, very limited structure activity relationship (SAR) studies have been done to better understand the activity profile of **1**; hence our group is set to synthesize a library of structural analogs to study the role of various functional groups within **1**. Our aim is to understand the role of lipid-chain as well as the electrophilic aldehyde group. We also want to substitute the aldehyde motif with a more stable functional group to restrain the extent of epimerization of the C-terminal leucinal residue. We have synthesized a series of analogs (**2** - **9**) by modifying the N-terminus with a C-10, C-12 or a C-14 acyl chain, and the C-terminus with an aldehyde bioisostere like nitrile or benzimidazole. Currently, we are preparing a series of benzimidazole, and boronic acid derivatives. All the synthetic analogs will be tested for cytotoxicity against a set of cancer cell lines. The results from this study will guide us in designing a library of second-generation analogs with improved chemical stability and pharmacological properties.



3: $R_1 = C_9H_{19}$, $R_2 = CHO$

4: $R_1 = C_{11}H_{23}$, $R_2 = CHO$

5: $R_1 = C_{13}H_{27}$, $R_2 = CHO$

6: $R_1 = C_9H_{19}$, $R_2 = CN$

7: $R_1 = C_{11}H_{23}$, $R_2 = CN$

8: $R_1 = C_{13}H_{27}$, $R_2 = CN$

9: $R_1 = C_9H_{19}$, $R_2 = \text{benzimidazole}$

10: $R_1 = C_{11}H_{23}$, $R_2 = \text{benzimidazole}$

11: $R_1 = C_{13}H_{27}$, $R_2 = \text{benzimidazole}$

MEDI 317

Scaffold replacement & 3D ligand optimization applied to the discovery of tyrosine kinase inhibitors

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Point mutations within the BRC-ABL tyrosine kinase domain give rise to imatinib-resistant mutants. Designing next generation ligands to counteract TK inhibitor resistance remains a challenging problem. Scaffold replacement is applied to the imatinib framework where the 2-amino-pyrimidine fragment is exchanged through a scaffold screen to produce a number of related congeneric series. 3D ligand optimization is subsequently performed on one of the hits yielding a structurally related isomer of ponatinib, a known selective high affinity tyrosine kinase inhibitor.

MEDI 318

Design, synthesis, and anti-proliferation activity of cucurbitacin-inspired estrone analogs targeting pancreatic cancer

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Pancreatic cancer will be the fourth leading-cause for cancer related deaths in 2016. Moreover, the advance in increasing survival of pancreatic cancer has been slow compared to the steady increase for most cancer types, which suggests the urgent need to discover new agents for treatment. Recent studies in our lab showed cucurbitacin inspired estrone analogs targeting melanoma has shown promising anti-proliferative activity against several pancreatic cancer cell lines. Also a report of cucurbitacin E has shown a promising antiproliferative activity against human pancreatic cancer cells PANC-1 in a dose- and time- dependent manner via inhibiting STAT3 phosphorylation.

Our hypothesis is to carry out in silico drug design of inspired-cucurbitacins estrone analogs targeting proteins expressed in pancreatic cancer will lead to novel candidates based on our success in other areas of drug discovery. To test our hypothesis, a docking study of 400 virtual library of estrone analogs modified in 3, 11, 16, and 17 positions based on bioisosters approach in drug design, OpenEye[®] software and proteins (STAT 3, KRASG13D, JAK2 and erb1) expressed in pancreatic cancer. Analogs with higher calculated affinity, Figure 1, were promoted for organic synthesis and cytotoxicity biological test.

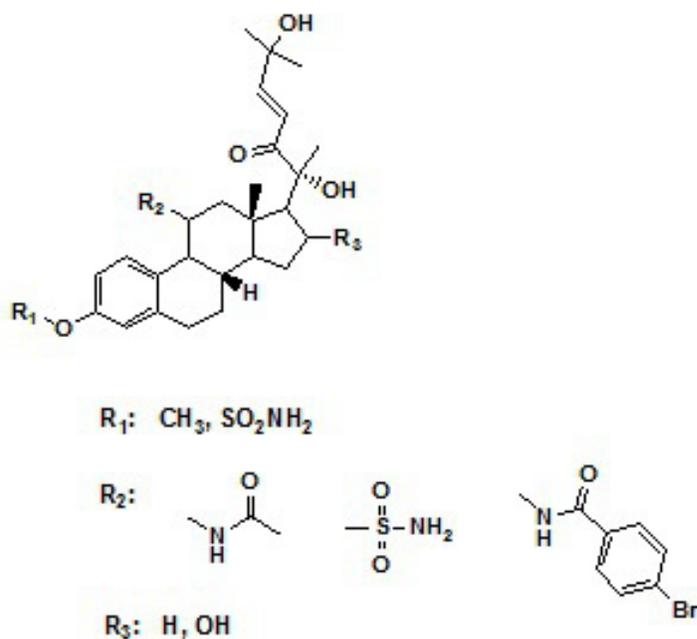


Fig. 1: Parent Analogues of cucs-like estrone derivatives.

MEDI 319

New cephalotaxane derivatives for TKI resistant CML

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Omacetaxine mepesuccinate was first isolated from Chinese yew tree in 1970s and was widely used in China for the treatment of acute myeloid leukemia (AML) and chronic myeloid leukemia (CML) before tyrosine kinase inhibitors, like imatinib, were launched. With the emergence of resistance to the targeted therapies, there is a revived interest in this natural product with unique mechanism of action. In 2012, Omacetaxine mepesuccinate was approved in US for CML patients with resistance or intolerance to

TKIs. New analogs with modifications on both pentacyclic moiety and ester side chain have been designed, synthesized and tested on T351I mutant cell line. The lead analogs were further evaluated in ADME/PK and in vivo efficacy. Detailed biological activity and synthesis will be presented.

MEDI 320

Compounds designed to elevate reactive oxygen species (ROS) and their antiproliferative implications

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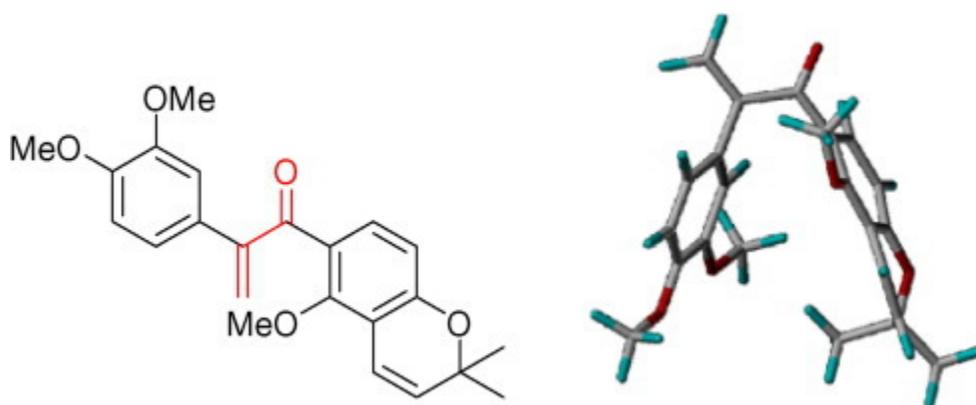
Opposite to the dark side of acrolein, a highly toxic Michael Acceptors (MAs), investigations confirmed that acrolein causes inhibition of the β -subunit of the proliferative anti-apoptotic protein NF κ B by covalent binding with the nucleophilic residues. Therefore, two approaches can be adopted to increase the therapeutic index of MAs, first; make it larger compound and second; make it less reactive. In this venue, we designed a library of MAs with enriched molecular features and tested for their antiproliferative activities on both cancer as well as normal cell lines. To study the anti-cancer activity of the newly synthesized compounds, they were evaluated against a panel of three human colon cancer cell lines including, HCT-116, HT-29 and CACO-2. The results of MTT assay revealed that most of the compounds displayed high level of antitumor activities with $IC_{50} = 0.89 \pm 0.04 \mu\text{M}$. Moreover, compounds showed clear SAR implicating their target specificity. Mechanistic studies revealed that these compounds induced oxidative stress as it decreased GSH level with concomitant increase in lipid peroxidation and significant decrease in the activities of antioxidant enzymes, superoxide dismutase and catalase in time-dependent manner. Furthermore, a representative compound induced apoptosis in HCT-116 cell line, and arrested the cell cycle at the G1 phase with concurrent decrease in S phase cell population. Interestingly, our MAs increased the expression level of cleaved caspase-3 protein expression. Moreover, the cytotoxic assay (SRB method) against C166 mouse skin fibroblasts (non-cancerous cells) revealed that many of our most potent compound are non-cytotoxic on normal cells up to 100 μM . In conclusion, the newly synthesized compounds induced- cancer cell death can be, at least partially, excreted through reactive oxygen species (ROS) production and induction of apoptosis.

MEDI 321

Identification of the small molecule inhibitor for STAT3 pathway through chemical structure focused library screening

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A series of novel STAT3 inhibitors consisting of Michael acceptor as a common electrophilic moiety has been identified through assays of the focused in-house library, which consist of a variety of scaffolds derived through long-term medicinal chemistry works. In addition, their mode of action and structural feature responsible for the STAT3 inhibition were investigated. In particular, enone analog revealed promising inhibitory activity in STAT3-driven luciferase expression in HeLa cells. We confirmed that the enone moiety of the analog, which is less-hindered exo-olefin, is essential for the direct interaction with the nucleophilic cysteine residue of STAT3 via Michael addition. The analog also exhibited selective inhibition of STAT3 phosphorylation without affecting STAT1 phosphorylation and cytostatic effect in human breast epithelial cells (MCF10A-ras), which supports cancer cell-specific inhibitory properties. To investigate the mode of action for the analog, STAT3 phosphorylation was examined by Western blot analysis using H-ras transformed MCF10A (MCF10A-ras), which seemed to serve as an adequate model for studies on mammary carcinogenesis. Intensive studies on the analog including elucidation of its precise inhibition and development of more potent STAT3 inhibitors based on the current results are making good progress.



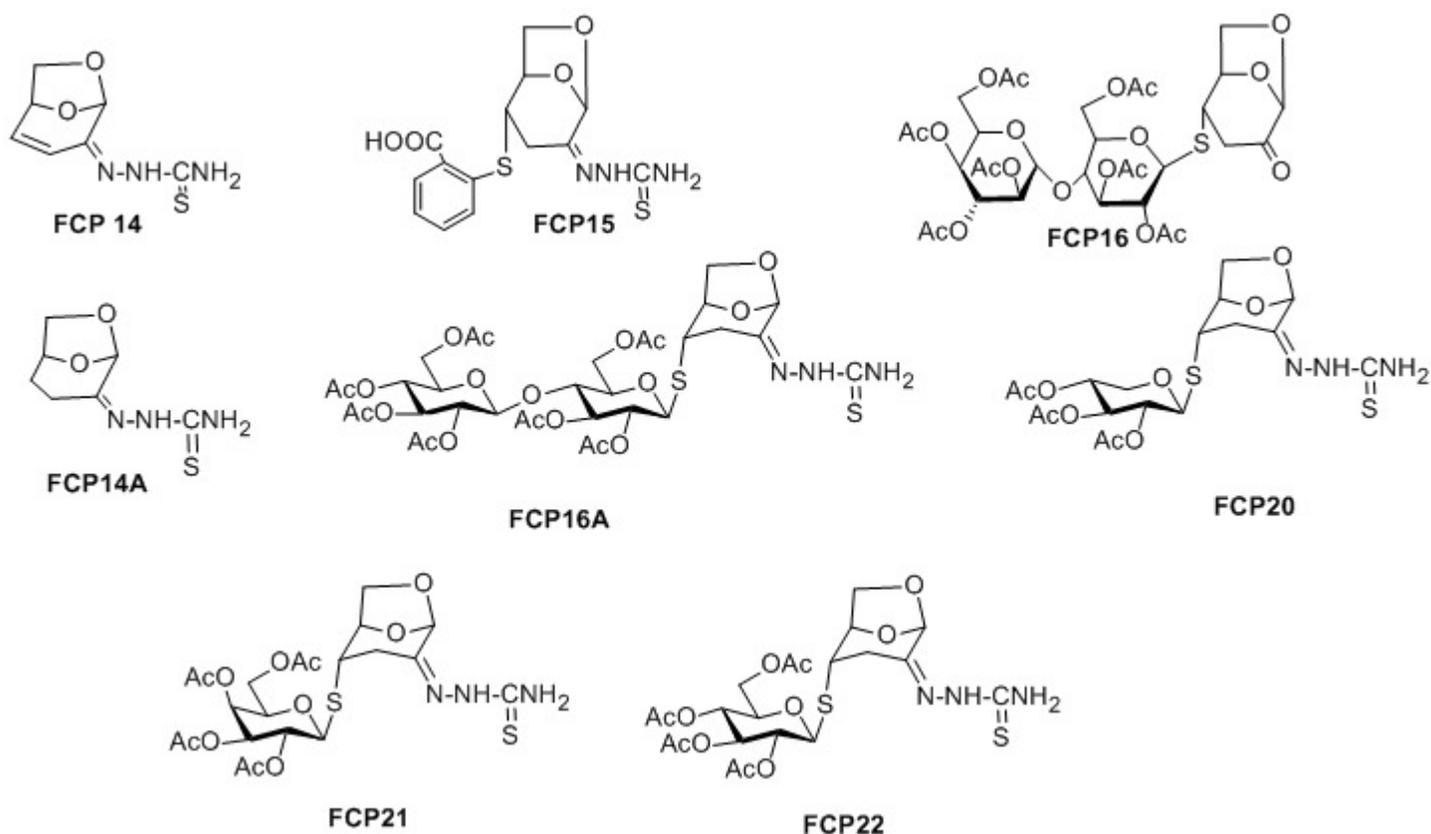
MEDI 322

Synthesis and cytotoxic effects of novel glycosylated thiosemicarbazides and their analogs as anticancer agents

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Thiosemicarbazides and their analogs are well known for their antifungal, antibacterial, antiviral and anticancer activity.

We designed and synthesized a series of eight novel compounds representing several carbohydrate moieties linked *via* S-bridge with core anhydro template derived from levoglucosenone and functionalized at C-2 as thiosemicarbazides (namely FCP14, 14A, 15, 16, 16A, 20, 21 and 22). To evaluate the structure-activity relationships, all synthesized thiosemicarbazides were evaluated for their *in vitro* cytotoxic activity against seven cancer cell lines, brain (MO59J, MO59K), breast (MCF-7), cervix (HeLa), ovarian (A2782, A2780CIS) and colon (LoVo). Cancer cells were incubated in the presence of increased concentrations of glycosylated thiosemicarbazides or their analogs for 72h. Next, the cytotoxic properties of tested compounds were determined by evaluating the amount of living cells via colorimetric cell viability assay CCK-8. The most potent of the studied compounds were FCP20 and FCP21. They evoked concentration-dependent decreased cell viability on HeLa, A2780 and LoVo cells at concentrations up to 1mM. FCP16 and FCP16A showed moderate anticancer properties and FCP16A were shown to be considerably less cytotoxic than its analog (FCP16). FCP16 displayed significant anticancer activities against all seven tested cancer cell lines while FCP16A show cytotoxic properties only on HeLa, A22780 and A2780CIS cells. The remaining compounds (FCP22, FCP14, FCP14A and FCP15) didn't induce a significant decrease in viability of all studied cancer cell lines. All results showed that the cytotoxic effect of glycosylated thiosemicarbazides and their analogs was structure and cancer cell line dependent. The data obtained clearly suggests that all functionalized thiosemicarbazides have the potential to augment or even replace current anticancer methods, however, their mechanism of action still must be evaluated.



MEDI 323

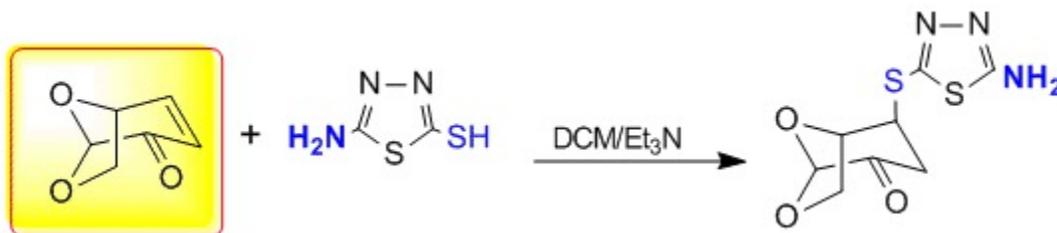
Synthesis, characterization, molecular modeling, and potential anticancer activities of novel 1,3,4-thiadiazole derivative

Anna Czubatka-Bienkowska², Anna Maciejka², Joanna Sarnik², **Zbigniew J. Witczak**¹, zbigniew.witczak@wilkes.edu, Tomasz Poplawski². (1) Dept of Pharm SCI Sch of Phar, Wilkes Univ, Wilkes Barre, Pennsylvania, United States (2) Department of Molecular Genetics, University of Lodz, Lodz, Poland

Derivatives of 1,3,4-thiadiazoles are known to exhibit antibacterial, antifungal, antidiabetic, anti-inflammatory and anti-tuberculosis activities. In an effort to establish new drug candidates with improved anticancer activities, we report the synthesis and *in vitro* biological evaluation of the first novel carbohydrate S-functionalized 1,3,4-thiadiazole derivative (FCP23). The target molecule was synthesized in 85% yield by stereoselective base catalyzed thio-click Michael addition of 1,3,4-thiadiazole thiol to levoglucosenone. The NMR and molecular model data clearly confirmed stereoselectivity of the thio-bridge at C-4. Measuring the S-C bond distances proved the previously established geometry of S-thiodisaccharides and their S-functionalized analogs.

This compound exhibited good potency, with IC₅₀ values of 41 μM and 111 μM against A2780 and A2780CIS ovarian cancer cells, respectively. We also investigated its

genotoxicity using comet assay. FCP23 evoked dose-dependent DNA damage in both ovarian cancer cells as evaluated by the alkaline comet assay and induce more than 30% of DNA damage at a 10 μ M concentration after 1h treatment with FCP23. These results clearly showed that FCP23 exerts cyto- and genotoxic action on both studied cancer cells. Thus it could be used as leads for further optimization.



MEDI 324

Bicycloheptylamines and cyclohexylamines as σ_2 receptor ligands: Potential use as anticancer agents

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Since σ_2 receptors have been found to be over-expressed in a variety of human tumor cell lines and induce endocytosis and apoptosis via multiple pathways, it was of interest to examine the effects of novel and potent ligands that are selective for those receptors. We were also interested in obtaining fluorescent ligands for further assessment of these receptors. Novel and selective σ_2 receptor ligands were synthesized in our lab, including fluorescently-labelled and doxorubicin-conjugated compounds. Target compounds were evaluated to determine binding affinities on several receptors. Assessment of cytotoxicity was performed using the MTT cell viability assay on the human pancreatic adenocarcinoma cell line PANC-1 and the colon cancer cell line HT-29 which are known to over-express the σ_2 receptor. Testing included the co-administration of σ_2 receptor ligands with doxorubicin to examine consistency with literature with regard to σ_2 receptor ligands having chemo-sensitization effects to DNA-targeting anticancer drugs. Also, comparisons between σ_2 receptor ligands, conjugates, and doxorubicin were performed. K_i values for compound displacement of [³H]DTG from σ_2 receptors were as low as 5.5 nM. Combination of σ_2 receptor ligands with doxorubicin was found to have synergistic cytotoxic effects. Fluorescent σ_2 receptor ligands were found to retain good affinity and selectivity towards the receptor. Doxorubicin-conjugated ligands were shown to be superior to doxorubicin in terms of cytotoxicity. In conclusion, σ_2 receptor ligands synergistically increased the sensitivity of PANC-1 cells to doxorubicin. Doxorubicin-conjugated σ_2 receptor ligands show potent toxicity. Fluorescently-labelled σ_2 receptor ligands may prove useful for tumor imaging and assessment of uptake into cancer cells.

MEDI 325

Synthesis and characterization of some new emetine amide derivatives for studies in prostate and breast cancer cells

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Emetine, an isoquinoline alkaloid from the ipecac species, has shown interesting medicinal properties including anti-viral, anti-parasitic and anti-cancer activities. In efforts to improve the pharmacological properties associated with emetine, we are exploring the chemical modification of the N-2' position of emetine with the aim to synthesize compounds with improved efficacy but reduced host toxicity. A series of new emetine amide derivatives were synthesized in our research group and will be examined for activity against breast and prostate cancer cells compared to normal cells.

MEDI 326

Encapsulation and delivery of trastuzumab into human breast cancer cells using cholestosomes

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According to the American Cancer Society, 1 in 8 (12%) of women in the United States develop invasive breast cancer. Among those individuals, approximately 25 to 30% of breast cancer cells exhibited elevated HER2 levels. HER2 positive breast cancers identified by a pathologist typically exhibit amplification of the HER2 gene resulting in an overexpression of HER2 receptors. The HER2 receptor (Human Epidermal Growth Factor Receptor 2) is a member of the epidermal growth factor family important for the intracellular signaling and regulation of cell growth. Trastuzumab (Herceptin®) is an IgG1 monoclonal antibody that has been proven to be effective in HER2 positive patients. Trastuzumab binding to HER2 interferes both directly and indirectly with downstream intracellular signaling pathways. Unfortunately, less than about 35% of patients benefit from treatment with Trastuzumab while the remainder exhibit initial or acquired resistance to treatment. Importantly, brain metastasis occurs more frequently in Trastuzumab treated patients. This population of resistant patients inspires efforts towards a more effective delivery system for Trastuzumab, including across the blood-brain barrier. This laboratory has developed a neutral lipid based vesicle (the CholestosomeTM), that uses naturally occurring lipids for the delivery of a wide variety of therapeutics, including small molecules, antibiotics, peptides, and proteins. Previous

work has shown Cholestosome-mediated delivery of FITC-labelled peptides into various mouse tissues (including brain) after oral administration. Cholestosomes can therefore potentially be used to orally deliver compounds for which intravenous administration is the only effective dosing route. Cholestosomes have been used to encapsulate. In the present studies, Trastuzumab has been encapsulated in Cholestosomes and characterized using an in vitro breast cancer model.

MEDI 327

Discovery and optimization of triazole compounds as novel BCL6 inhibitors

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The BCL6 transcriptional repressor is the most commonly misregulated oncogene in diffuse large B-cell lymphomas (DLBCLs) and blockade of the BCL6 BTB domain can suppress the BCL6-positive lymphoma cells both in vitro and in vivo. We combined computer-aided drug design with functional assays to identify triazole compounds that bind to the lateral groove of the BCL6 BTB domain. The optimal compound exhibited potent activity in alphaLISA assay and microscale thermophoresis assay, with IC₅₀ values of 2 μM and 3 μM, respectively, and it also showed activity in reporter assay under 50 μM. The triazole compounds are promising BCL6 inhibitors and more work are under progress.

MEDI 328

Synthesis and pharmacological evaluation of new compounds useful to treat sickle cell disease

Thais Regina Ferreira de Melo², *trfmelo@gmail.com*, **Karina Pereira Barbieri**², **Rafael C. Consolin Chelucci**², **Carolina Lanaro**¹, **Chung M. Chir**², **Jean L. Santos**². (1) State University of Campinas, UNICAMP, Campinas, SP, Brazil (2) UNESP - Univ Estadual Paulista, Ararquara, SP, Brazil

Sickle Cell Disease (SCD) is a hematologic disease characterized by a single mutation in the β-hemoglobin gene. This alteration promotes hemoglobin polymerization and changes in the structure of the erythrocyte cytoskeleton, which contribute to vaso-occlusive process. The disease is complex and it has been also associated with chronic inflammation. High levels of pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), are described for SCD patients. The only drug approved by FDA to the treatment is hydroxyurea, whose beneficial effects are associated with nitric oxide (NO) formed after metabolism. Therefore, in continuing efforts to discover new drugs for SCD, we reported herein the design, synthesis and pharmacological evaluation of drug candidates for the treatment of sickle cell anemia symptoms. Four hybrid compounds containing the phthalimide (TNF-α inhibitory subunit) and furoxan subunits (nitric oxide

donor subunit) were synthesized at global yields ranging from 25 – 55%. All compounds demonstrated ability to release NO at levels ranging from 11-40%. In SCD, NO plays beneficial effects such as vasodilation, inhibition of platelet aggregation and induction of fetal hemoglobin (HbF). In addition, all compounds were able to inhibit TNF- α at levels ranging from 20-65%. The most potent TNF- α inhibitor was the compounds I that inhibit 65% of TNF- α production. Compound I was able to decrease γ -globin gene expression measured by real time PCR using K562 cells at different times (24, 48, 72, and 96h) and different concentrations (5, 30, 60, and 100 μ M). After 72 hours and at 5 μ M, this compound was able to increase the gene expression of gamma globin by two times compared to control (vehicle). Moreover, all hybrid compounds inhibited ADP-induced platelet aggregation at levels ranging from 43 and 95%. In conclusion, these new hybrid compounds have shown anti-inflammatory, antiplatelet and ability to induce HbF and can be considered as promising drug candidates for sickle cell disease treatment.

MEDI 329

Synthesis and biological activity of new hybrids phthalimide-furoxan derivatives useful to treat sickle cell disease symptoms

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Sickle Cell Disease (SCD) is one of the most common hematological and genetic disease caused by a single mutation in the β -globin gene, responsible to produce the abnormal hemoglobin S (HbS) despite of normal hemoglobin A (HbA). At low oxygen tension, erythrocytes containing HbS polymerize and acquire a sickle shape leading to the vaso-occlusion process. SCD is also characterized by a chronic inflammatory process with high plasmatic levels of pro-inflammatory cytokines, such as, tumor necrosis factor alpha (TNF- α). The only drug available to treat SCD is hydroxyurea (HU). *In vivo* HU is metabolized into nitric oxide, whose benefits include vasodilation, inhibition of platelet aggregation and induction of gene expression of γ -globin. However, long-term treatment with HU has several limitations, including: lack of response in up to 30% of patients, myelo suppression and genotoxicity. Therefore, herein we described the synthesis and pharmacological evaluation of six hybrid derivatives containing the following pharmacophores subunits: phthalimide (responsible for TNF- α inhibition) furoxan (responsible for nitric oxide release). All compounds were synthesized at global yields ranging from 24 – 91%. The hybrid derivatives (RC-2 and RC-6) have shown anti-inflammatory effect by reducing the TNF- α levels at 33% (6.25 μ M) and 53% (50 μ M) using murine macrophage cells. RC-2 and RC-6 were also able to inhibit platelet aggregation induced by ADP at 50 and 48%, respectively. While, for RC-3 and RC-5 the

platelet aggregation induced by collagen was inhibited by 44% and 30%, respectively. The bleeding time for compounds RC-2, RC-5 and RC-6 was increased at levels superior of that of acetylsalicylic acid (ASA), used as control. RC-1, RC-3 and RC-4 have similar bleeding time compared to ASA. *In vivo* genotoxic effect was evaluated through micronucleus assay using mice. All compounds did not demonstrate genotoxic effect and average frequency of micronucleated reticulocytes (MNRET) were similar to that of negative control (vehicle). In conclusion, our findings show that all hybrid compounds have promising pharmacological activity useful to treat SCD symptoms by reducing the inflammatory process and inhibit the platelet aggregation. In addition, these compounds can be an alternative for current treatment with HU.

MEDI 330

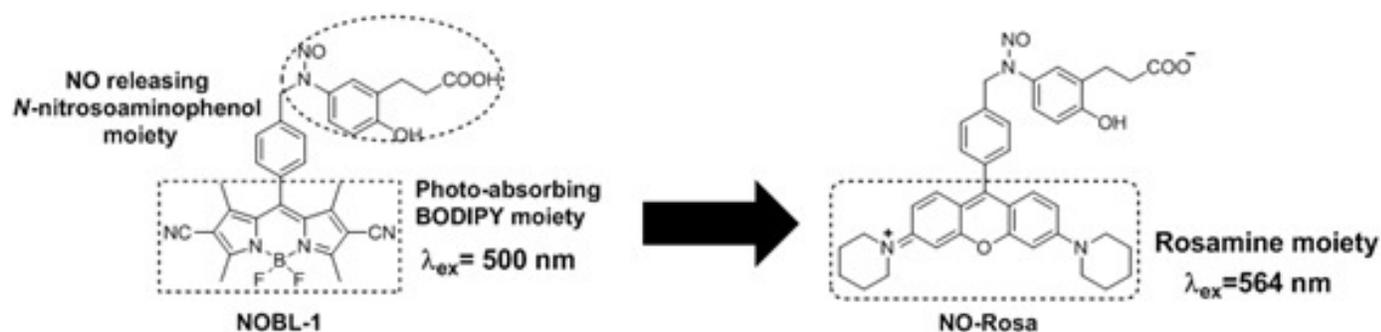
Nitric oxide donor controllable with yellowish green light

Hana Okuno, p112512@phar.nagoya-cu.ac.jp, Naoya Ieda, Yuji Hotta, Mitsuyasu Kawaguchi, Kazunori Kimura, Hidehiko Nakagawa. Pharmaceutical Sciences, Graduate School of Nagoya City University, Nagoya, Aichi, Japan

Countless researches have shown that nitric oxide (NO) works as an essential mediator in regulating vasodilation, neurotransmission, and biodefence. Photocontrollable NO donors, which enable controlling NO release in a temporal and spatial manner, attract further research on NO physiology and potential therapeutic application.

Although we have reported a nitric oxide donor, **NOBL-1** (Ieda, N. et al. *J. Am. Chem. Soc.* 2014, 136, 7085-7091.), controllable with blue light, photocontrol with longer wavelength is required for effective biomedical application. Since mechanism of NO release from **NOBL-1** is supposed to be via photoinduced electron transfer from NO releasing *N*-nitrosoaminophenol moiety to blue light absorbing BODIPY moiety, we have expected that control of NO release with longer wavelength light is possible by modifying its light absorbing moiety.

Herein, we report design, synthesis and evaluation of a novel nitric oxide donor, **NO-Rosa**, controllable with yellowish green light. Based on **NOBL-1**, we designed **NO-Rosa** by modifying light absorbing moiety from BODIPY to rosamine, which absorbs yellowish green light. We found that yellowish green light induces generation of NO from **NO-Rosa** by using Fe-MGD complex as a spin trap for NO, which gives Fe-MGD-NO complex and shows a typical triplet signal at around 330 mT in 1-GHz ESR spectroscopy. We also demonstrated photomanipulation of vasodilation of a rat aortal slice.



Molecular Design of NO-Rosa

MEDI 331

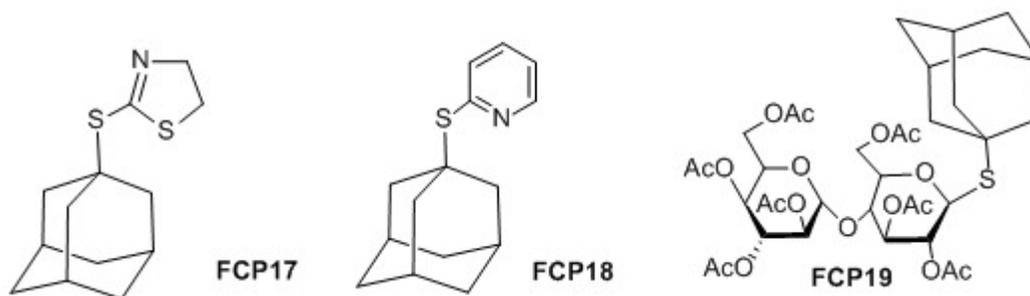
Novel adamantane derivatives efficiently inhibit cisplatin resistant ovarian cancer cell line growth

Anna Czubatka-Bienkowska², Joanna Sarnik², Anna Macieja², **Zbigniew J. Witczak¹**, zbigniew.witczak@wilkes.edu, Tomasz Poplawski². (1) Dept of Pharm SCI Sch of Phar, Wilkes Univ, Wilkes Barre, Pennsylvania, United States (2) Department of Molecular Genetics, University of Lodz, Lodz, Poland

Adamantane derivatives have been of interest to chemistry and medicine since the early 1940s. The adamantane group is present in therapeutic agents currently used for the treatment of Parkinson's and the flu. Moreover, adamantane derivatives have antiviral properties against HIV and the cytotoxic properties of functionalized adamantane derivatives inhibit colon, lung, brain and other cancer cell line growth. These reports prompted us to investigate the anticancer properties of three novel synthesized adamantane derivatives described as FCP17, FCP18 and FCP19 on human ovarian carcinoma (A2780) and cisplatin-resistant ovarian carcinoma cell line (A2780CIS). Cytotoxic properties of adamantane analogs were tested by using colorimetric assay - Cell Counting Kit-8 (CCK-8). Alkaline modification of comet assay was performed to evaluate genotoxic properties of studied adamantane derivatives. The most cytotoxic was FCP19, which decreased ovarian cancer cell viability in a micromolar concentration. The observed effect was more pronounced in A2780CIS cell lines (more than 50% decrease in A2780CIS cancer cell viability vs 10% in A2780 cells at 0.1mM). It also induced dose-dependent DNA damage. The remaining two studied compounds were not cytotoxic in micromolar concentration. Adamantane with pyridine moiety (FCP18) caused a 53% decrease in cisplatin-resistant ovarian cancer cell viability and 21% in A2780 cells at 1mM, whereas the second derivative with two sulfur atoms (FCP17) did not exhibit cytotoxic activity on either cancer cells. Both of these adamantane derivatives induce about 5% DNA lesions measured as % tail DNA at 0.25mM.

Our results indicate that functionalized carbohydrate adamantane analogs exhibit *in vitro* geno- and cytotoxicity at low concentrations in comparison to other studied adamantane derivatives. In addition, A2780CIS cells were more sensitive to

carbohydrate adamantane analogs than A2780 cells. It suggests that carbohydrate adamantane analogs could be a useful candidates in cisplatin-resistant ovarian carcinoma therapy.



MEDI 332

Discovery of dihydrobenzofuran substituted chromene analog as a potent anticancer agent for ovarian cancer

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Past several years, chemotherapy has evolved significantly and currently involves a combination of paclitaxel (PTX) with platinum drugs. Even though it has shown encouraging results in patients, long term survival rates generally remain poor. This highlights the urgent need for development of new therapies. In continuation of our effort to find new potent and selective chemotherapeutic agents for the advanced ovarian cancer, we identified dihydrobenzofuran substituted chromene analog (**SP2-9**) as a highly effective anticancer agent. This compound was screened for anti-cancer effect on cisplatin sensitive (A2780, SKOV-3, TOV112D) and cisplatin resistant (OVCAR-3, A2780-cisR, TOV112D-cisR) ovarian cancer cells. *In vitro* cell viability was assessed by fluorescence based Alamar Blue assay. Our preliminary studies indicate efficacy of **SP2-9** as a potent anticancer agent towards human ovarian cancer cell lines (IC_{50} range: 0.258 ± 0.159 - 3.61 ± 0.049 μ M). This compound exhibited anticancer efficacy towards both cisplatin sensitive (IC_{50} : 1.35 ± 0.88 μ M) and cisplatin resistant ovarian cancer cell lines (IC_{50} : 2.09 ± 1.7 μ M). Notably, **SP2-9** exhibited least cytotoxicity towards normal ovarian epithelial cells (IC_{50} : 75.77 ± 5.37 μ M). Additionally, compound **SP2-9** demonstrated potent growth inhibition against 60 cell lines in the NCI panel including seven ovarian cancer cell lines. Among the ovarian cancer cell lines, OVCAR-3 and NCI/ADR-RES cell lines have exhibited high sensitivity towards **SP2-9** (GI_{50} : ≤ 10 nM; OVCAR-3 and NCI/ADR-RES).

MEDI 333

Design and synthesis of a library of 8-quinolinethiol based Rpn11 inhibitors

Yuyong Ma¹, yum015@ucsd.edu, **Jing Li**², **Christian Perez**¹, **Raymond Deshaies**², **Seth Cohen**¹. (1) Chemistry and Biochemistry, UC San Diego, San Diego, California, United States (2) Biology and Biological Engineering, California Institute of Technology, Pasadena, California, United States

The ubiquitin-proteasome system (UPS) plays a key role in regulating numerous cellular processes by maintaining protein homeostasis. The 26S proteasome is the molecular machine that executes the degradation of proteins that are marked for destruction by the covalent attachment of polyubiquitin chains. Rpn11 is a Zn(II)-dependent subunit of 26S proteasome, which cleaves the polyubiquitin chain from protein to be degraded. The normal activity of Rpn11 is essential for the UPS, which make it an attractive target for pharmacologic intervention. Recently, 8-quinolinethiol was found to be a potent scaffold by fragment-based screening. However, the syntheses of 8-quinolinethiol and its derivatives are not widely reported, which hinders the development of 8-quinolinethiol based Rpn11 inhibitors. Here, we describe a convenient synthetic route to the preparation of a library of 8-quinolinethiol and its derivatives with substituents at C3, C4, and/or C6. In addition, 1,5-naphthyridine-4-thiol, an N5-analog of 8-quinolinethiol, and its derivatives have also been prepared in a concise fashion.

MEDI 334

Design, synthesis, and biological evaluation of novel metabolically stable (+)-discodermolide analogues

Boying Guo², guoby@email.arizona.edu, **Nan Zhang**³, **Hayley M. McDaid**⁴, **Susan B. Horwitz**⁴, **Amos B. Smith**¹. (1) Dept of Chemistry 6323, University of Penn, Philadelphia, Pennsylvania, United States (2) university of pennsylvania , Ridley Park, Pennsylvania, United States (3) Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania, United States (4) Albert Einstein College of Medicine, New York, New York, United States

Senescence, an underappreciated cell dormancy phenotype, occurs upon treatment of diverse cancer cell lines with many cytotoxic drugs, including Taxol. (+)-Discodermolide, a promising anti-cancer agent demonstrating comparable and/or superior potency to Taxol in many cell lines, unfortunately failed evaluation in an early phase I clinical trial (2004) due to pneumotoxicity, which we believe is due to the onset of reactive oxygen species (ROS) mediated senescence caused by discodermolide metabolites in lung tissue. The design, synthesis and evaluation of potent, metabolically stable discodermolide congeners specifically selected to circumvent the onset of senescence would thus hold the promise for the treatment of recalcitrant cancers in first-line treatment and the metastatic setting. To date we have completed a thorough investigation of all previously published discodermolide analogues. The result of our analysis, combined with our long-time experience in this field, has enabled the elucidation of the diene moiety, 7-hydroxy group and the lactone fragments as

candidates for further refinement.

The design, synthesis and evaluation of discodermolide analogues that have demonstrated improved potency in multi-cancer cell lines will be presented.

MEDI 335 – Withdrawn.

MEDI 336

Development of diketopiperazine-type antitumor agent plinabulin prodrug with an IgG binding peptide for generating a tumor selective non-covalent-type antibody-drug conjugate

*Kyohei Muguruma, Fumika Yakushiji, Ryosuke Kawamata, Daichi Akiyama, Risako Arima, Takuya Shirasaka, Akihiro Taguchi, Kentaro Takayama, **Yoshio Hayashi**, yhayashi@toyaku.ac.jp.* Department of Medicinal Chemistry, Tokyo University of Pharmacy Life Sciences, Hachioji, Tokyo, Japan

Although several approaches for antibody-drug conjugate (ADC) production have been developed, it has yet to be reported that an antibody binding peptide (such as Z33 derived from protein A) is utilized as the pivotal unit to generate the non-covalent-type ADC (NC-ADC). Our goal is to establish a novel probe for NC-ADC by synthesizing the Z33-conjugated antitumor agent plinabulin, a diketopiperazine (DKP) derivative, which was developed by our group from a natural DKP phenylahistin as a potent microtubule depolymerization agent. Due to the different solubility of two components (hydrophobic plinabulin and hydrophilic Z33), an innovative method with a solid-supported disulfide coupling reagent is required for the synthesis of the target compound with prominent efficiency. The synthesized hybrid exhibits a binding affinity against anti-HER2 (Herceptin) and anti-CD71 (6E1) antibodies (K_d : 46.6 nM and 4.5 mM, respectively) in SPR assay. In cell-based assays, the hybrid showed a significant cytotoxicity in the presence of Herceptin against HER2 over-expressing SKBR-3 cells, but not against HER2 low-expressing MCF-7 cells. Similar results are obtained with the 6E1 antibody. In summary, the data obtained in the present study indicate that NC-ADC has potential as the next-generation of antitumor agents.

MEDI 337

Towards a universal Mu-agonist template for alignment modeling of opioid ligands

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Template alignment modeling (TAM) is a newly developed ligand-based modeling approach for drug design, where a rigid molecular template is applied that ligands of

diverse structures are to be aligned with it. By doing so, it is possible to reveal clearly and effectively the structural features as well as the structural correlations among the diverse ligands. This modeling approach can also render insights for the structure-activity relationships (SARs) studies of ligands.

Opioid ligands are a large group of GPCR ligands with high structural diversity. And it has been a great challenge for medicinal chemists to recognize the potential structural correlations among the ligands, the critical information needed for new opioid drug design. Through TAM approach we previously proposed three receptor-subtype related pharmacophore models of opioid ligands, where morphine was used as the key template (PMID: 21488692). Although the models were quite helpful for us to understand better the complex SARs of opioid ligands, the scope and efficacy of the modeling were still limited, which was mainly attributed to the small size of morphine template. Therefore, establishment of a larger sized template for TAM modeling is highly desired in order for the improved applications on opioid ligands.

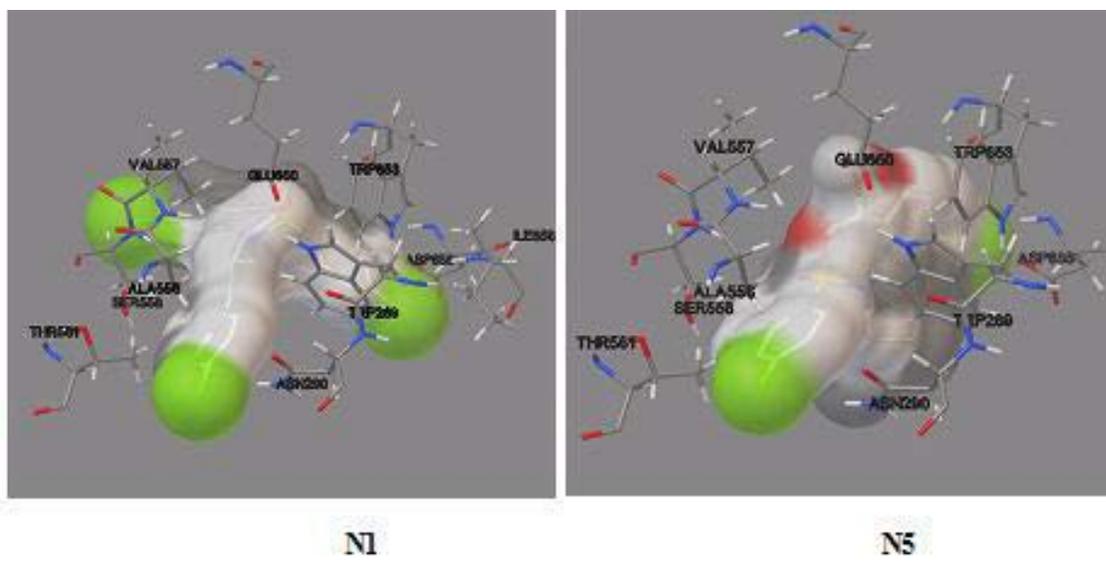
Herein, we wish to report the preliminary results of our recent efforts in establishing universal opioid ligand templates for TAM modeling. The current example illustrated is a mu-agonist specialized template, which was constructed and validated with a wide variety of mu-specific agonists, including both peptide and non-peptide ligands. Typical examples will be discussed regarding construction, validation, and potential applications of the template in opioid ligand SARs studies.

MEDI 338

Designing of selective gamma-secretase inhibitory benzenesulfonamides through comparative *in vitro* and *in silico* analysis

Neeraj Masand³, Satya Prakash Gupta¹, R L Khosa², **Vaishali Patil²**,
vaishuwise@gmail.com. (1) Dept of Applied Sciences, National Institute of Technical Teachers Training and Research, Bhopal Madhya Pradesh, India, Bhopal, India (2) Bharat Institute of Technology, Meerut, India (3) Department of Pharmacy, Lala Lajpat Rai Memorial Medical College, Meerut, UP, India

The series based on 3-chloro-2-hydroxymethyl-benzenesulfonamides was optimized and significant correlation has been obtained through MLR with gamma-secretase inhibition. Calculated Molar Refractivity and Surface Tension were found to be contributing along with indicator parameters. Applicability analysis revealed that the derived model [$\log(1/IC_{50}) = 0.835(\pm 0.561)CMR - 0.027(\pm 0.025)(CMR)^2 + 0.056(\pm 0.036)ST + 1.442(\pm 0.298)I - 5.306(\pm 3.436)$; $n = 34$, $r = 0.911$, $r_{cv}^2 = 0.897$, $s = 0.366$, $F_{4,29} = 35.46(4.05)$, $r_{pred}^2 = 0.827$, $CMR_o = 11.22$] has acceptable predictability ($r_{pred}^2 = 0.827$). Based on the MLR inferences, some substituted benzenesulfonamides have been synthesized and evaluated as gamma-secretase inhibitors followed by interactions at the active site of gamma-secretase (Figure 1). The results of *in silico* and *in vitro* investigation were helpful in exploring the novel scaffold.



MEDI 339

Exploiting solvent effects in drug design and optimization

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

When a ligand binds a protein, the structured solvent molecules in the binding pocket and around the unbound ligand become displaced or rearranged. These molecular reorganizations, in terms of desolvation, have a large impact on binding energy, influence ligand design requirements and can introduce elements of ambiguity in traditional SAR analysis. Typically, computing the energetics of these reorganizations requires lengthy and expensive simulations. This talk presents a fast and easy-to-use method (3D-RISM) which computes, in the order of minutes, the thermodynamic effects of solvent reorganization without explicit simulations. The method can help locate regions of organized solvent and their corresponding free energies. Application of the method to ligand optimization is demonstrated using case studies which highlight the unique insights that can be gained from this type of analysis.

MEDI 340

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

Raul Alvarez, ralvarez@chemcomp.com, **Joe Leonard**. Chemical Computing Group, Montreal, Quebec, Canada

Bioisosteric replacement and the functional group optimization of a lead are well-established and important medicinal chemistry methods applied in drug discovery. In silico methods for performing these medicinal chemistry transformations can

significantly expand the chemistry for a project, and increase the chance of success. Previous *in silico* methods for performing these transformations are typically limited to 2D space, ignore the receptor, or rank molecules using simplistic descriptors. In this work, a new method for performing the transformations in the context of the 3D receptor and ranking the results using energy scores and synthetic feasibility is presented.

MEDI 341

Property assessment of medium size molecules: Connecting drug-like properties from *in vitro* to *in vivo*

Maria J. Blanco-Pillado, *blanco_maria@lilly.com*, **Isabel C Gonzalez Valcarcel**, **Prashant Desai**, **Jaclyn Barrett**, **Geri Sawada**, **Tatiana N. Vetman**. Eli Lilly and Company, Indianapolis, Indiana, United States

Recently, compounds with MW in the range of 500-1500 Da have received significant attention from pharmaceutical industry for novel targets as protein-protein interactions (PPI). Macrocycles and cyclopeptides possess several features that make them suitable to tackle “difficult” targets with extended binding sites. Because of their size and complexity, they can engage targets through multiple spatially distributed binding interactions, thus increasing both binding affinity and selectivity. However, because of the high MW, the compounds seemed to be outside “rule of five” for achieving oral bioavailability. In this poster, we will describe thorough analysis of physicochemical properties, as well as solubility and pharmacokinetic profile of known oral marketed compounds in this chemical space.

MEDI 342

Problem-based learning in drug discovery with MOE

Audrey Bonin, *abonin@chemcomp.com*. Chemical Computing Group, Montréal, Quebec, Canada

Problem-Based Learning (PBL) is a pedagogical method which incorporates hands-on, active learning centered on the investigation and resolution of difficult, real-world problems. Some of the defining characteristics of PBL include: 1. A guided learning process with challenging open-ended problems where there are multiple solutions and 2. An environment where students work as self-directed, active investigators and problem-solvers. Here we demonstrate the effectiveness of the Molecular Operating Environment (MOE) in a PBL setting to teaching students about the advantages and limitations of the modeling tools that are used in the forefront of early stage drug design.

MEDI 343

What rings do medicinal chemists use, and why?

Mark D. Mackey, *mark@cresset-group.com*, *Tim Cheeseright*, *Rae Lawrence*. Cresset, Cambridgeshire, United Kingdom

The vast majority of small molecule drugs contain at least one ring. The rigidity, synthetic accessibility and geometric preferences of rings mean that medicinal chemistry series are usually defined in terms of which ring or rings they have at their core. However, ring systems are more than just scaffolds waiting to be elaborated: the electrostatic and pharmacophoric properties of ring systems are usually crucial to the biological activity of the molecules that contain them.

We have conducted an investigation into the most common ring system and substitution patterns in the recent medicinal chemistry literature, as derived from the ChEMBL database. For each of these rings, the electrostatic potential has been calculated allowing the chemist to see at a glance the electronic properties of each system. In addition, applying the Spark bioisostere evaluation metric to the rings database reveals the best bioisosteric replacements for each ring system.

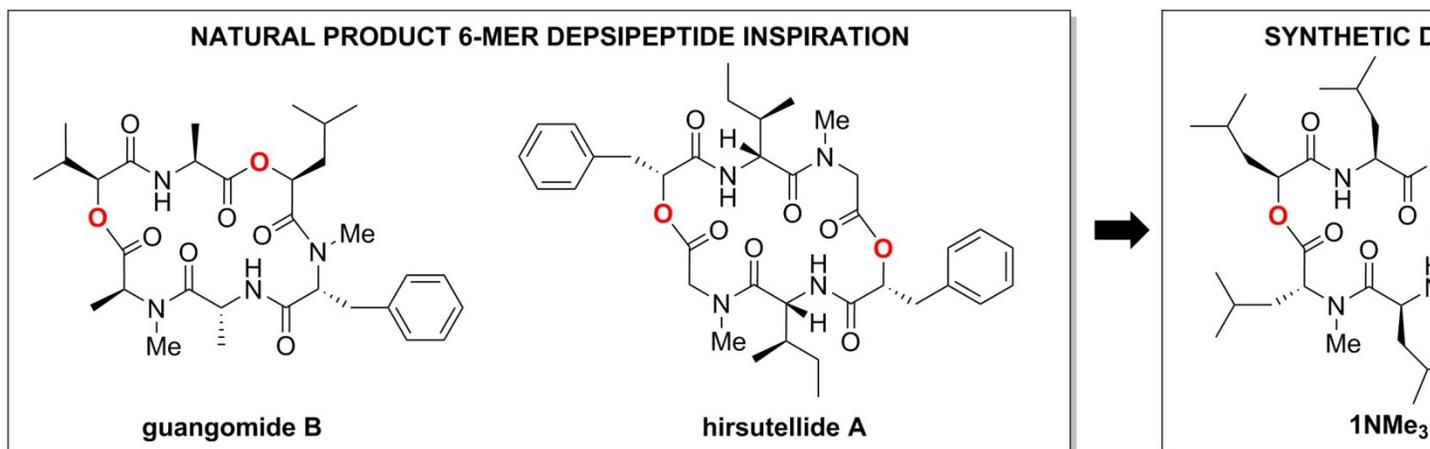
In this poster, selected entries from the rings database are shown and discussed. The full data set is an invaluable aid to the medicinal chemist looking to understand the properties of their lead molecule and the opportunities for variation of its core.

MEDI 344

Amide-to-ester substitutions modify the permeability and ADME properties of natural and synthetic cyclic peptides

Matthew Naylor^{1,2}, *mnaylor@ucsc.edu*, *Andrew Ly*², *Joshua Schwocher*², *Prashant Desai*¹, *Isabel C Gonzalez Valcarcel*¹, *Jaclyn Barrett*¹, *Geri Sawada*¹, *Maria J. Blanco-Pillado*¹, *Scott Lokey*². (1) Eli Lilly and Company, Indianapolis, Indiana, United States (2) Chemistry & Biochemistry, University of California Santa Cruz, Santa Cruz, California, United States

The natural product literature reveals an abundance of cyclic depsipeptides (peptides with at least one backbone ester) produced by a large diversity of organisms, suggesting functional advantages to this structural moiety—despite the lability normally expected by drug discovery. Although individual structures have been attained and studied synthetically, there has been little thorough elucidation of the physicochemical and ADME significance of specific amide-to-ester substitutions. Following our interest in the influence of N-methylation and conformational rigidity on the permeability of cyclic peptides towards beyond-Ro5 pharmaceutical targets, we performed an ester scan on a well-established permeable cyclic scaffold (1NMe3). In this poster, we will describe the physicochemical properties, solubility, plasma stability and ADME profile for a series of systematically synthesized depsipeptides.



MEDI 345

Structure-based drug design of macrocyclic factor XIa inhibitors

James R. Corte, james.corte@bms.com, Tianan Fang, Honey Osuna, Donald Pinto, Karen Rossi, Alan Rendina, Jeffrey Bozarth, Steven Sheriff, Joseph Myers, Timothy Harper, Zhen Lou, Joanna Zheng, Joseph Luettgen, Dietmar Seiffert, Patrick Y. Lam, Ruth R. Wexler, Mimi L. Quan. Bristol-Myers Squibb, Princeton, New Jersey, United States

Factor XIa (FXIa), a trypsin-like serine protease, functions in the intrinsic pathway of the blood coagulation cascade and plays a key role in the amplification of thrombin production which leads to the growth and maturation of thrombi. Inhibitors of FXIa have demonstrated excellent efficacy in a variety of preclinical thrombosis models with no effect on bleeding and are therefore promising novel anticoagulants. Based on the X-ray crystal structure of a phenyl imidazole lead bound in the active site of FXIa, a novel series of macrocyclic FXIa inhibitors was designed. Optimization of the macrocyclic linker, aided by structure-based drug design, resulted in the discovery of a substituted amide linker, which afforded picomolar FXIa inhibitors with excellent potency and selectivity.

MEDI 346

Optimization of BTK inhibitors to mitigate kinase selectivity, PK and off target shortcomings

Deodial Guiadeen¹, deodialsingh_guiadeen@merck.com, Jian Liu¹, Arto Krikorian¹, Xiaolei Gao¹, James Wang¹, Sobhana B. Boga¹, Abdul-Basit Alhassan¹, Jiayi Xu¹, Joseph Kelly¹, Rajan Anand¹, Younong Yu¹, Oleg Selyutin¹, Wensheng Yu¹, Shilan Liu³, Chundao Yang³, Hao Wu³, Jiaqiang Cai³, Kevin M. Maloney¹, Viktor Honak¹, Ying-Duo Gao¹, Sriram Tyagarajan¹, Thierry Fischmann¹, Jeremy Presland², My Mansueto², Zangwei Xu², Erica Leccese², Jie Zhang-Hoover², Ian Knemeyer², Nathan Bays², Peter Stivers², Philip Brandish², Alexander Hicks², Ronald Kim¹, Joseph A. Kozlowski¹. (1)

Department of Early Development and Discovery Sciences, Merck Research Laboratories, Rahway, New Jersey, United States (2) Department of Early Development and Discovery Sciences, Merck Research Laboratories, Boston, Massachusetts, United States (3) WaiGaoQiao Free Trade Zone, WuXi PharmaTech Co. Ltd, Shanghai, China

Bruton's Tyrosine Kinase (BTK) is an intracellular signaling kinase classified under the Tec family that is critical for the activation, proliferation and differentiation of B-cells. BTK's mode of action is mediated through B-Cell Receptor (BCR) activation and inhibition of BTK results in inhibition of B-Cell maturation. It has also been found to overexpress in B-lineage lymphoid malignancies and as such, also, a target for leukemias and lymphomas. In this context, POC has been achieved with the approval of IbrutinibTM for the treatment of B-lineage lymphoid malignancies. Following from its expression in B-cells and myeloid cells, BTK is of interest as a target for the treatment of Rheumatoid Arthritis (RA). In this presentation, we will report our efforts to design a novel series of BTK inhibitors with a focus on optimizing kinase selectivity, off-target activity and pharmacokinetic profiles.

MEDI 347

Discovery of low clearance PI3Kd templates for the treatment of respiratory disease

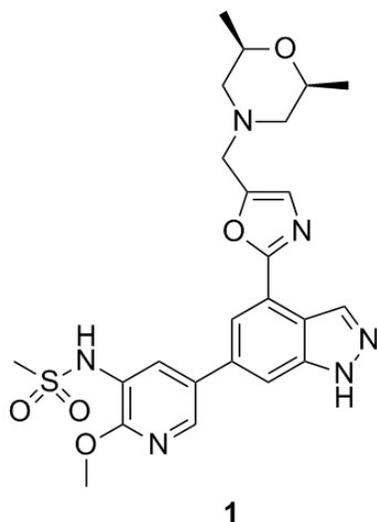
Simon Peace, *simon.2.peace@gsk.com*. GlaxoSmithKline, Stevenage, United Kingdom

The phosphoinositide-3-kinases (PI3Ks) are a family of lipid kinases that are implicated in the development of a range of diseases, including respiratory inflammation.^{[1],[2]} Of the known isoforms, PI3Kd is found to be over-expressed in neutrophils, suggesting it is a promising target for the treatment of asthma,^[3] COPD,^[4] and autoimmune disorders.^[5] We recently reported the discovery of an inhaled clinical candidate **1** as a potent and selective PI3Kd inhibitor as a potential therapy for respiratory indications.^[6] Herein we will describe the results of our efforts to identify low clearance, efficient and selective alternative series of inhibitors suitable for once-daily oral administration. We will describe the following process:

- i) Careful and considered analysis of all existing data to predict efficacy in the biophase
- ii) Deconstruction of **1** to identify critical features and provide an efficiency baseline
- iii) Replacement of vulnerable moieties at a fragment level using the principles identified in (i)

iv) Re-growth using efficient and flexible Ruthenium-catalysed trimerisation chemistry to explore numerous vectors and deliver advanced compounds in very short order.

This process led efficiently and directly to the discovery of PI3Kd inhibitors with excellent pharmacokinetic properties and *in vivo* efficacy from oral administration. There will be several transferable observations regarding the efficient planning and execution of medicinal chemistry strategies in addition to the first disclosure of advanced chemical matter of specific interest to those working in the lipid kinase field.



MEDI 348

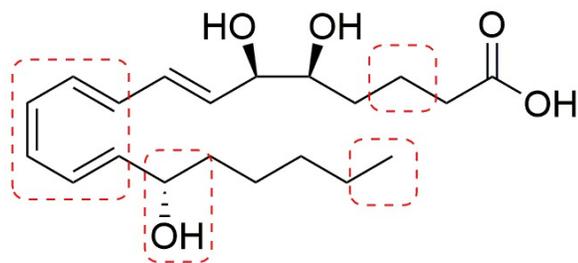
Synthesis of heteroaromatic lipoxin analogues for treatment of chronic inflammation

Catherine Tighe¹, catherine.tighe@ucdconnect.ie, **Monica de Gaetano**², **Catherine Godson**², **Patrick J. Guiry**¹. (1) Centre for Synthesis and Chemical Biology, University College Dublin, Dublin, Ireland (2) Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Dublin, Ireland

Inflammation is associated with a number of diseases including asthma, rheumatoid arthritis, atherosclerosis, psoriasis, periodontal disease and cystic fibrosis. Our research group is focused on developing new drug candidates for treating inflammation based on natural lipoxins. Lipoxins are eicosanoids that possess potent and selective anti-inflammatory activity. The two native lipoxins; LXA₄ and LXB₄, were first isolated from human leukocyte cells. They activate the ALX receptor on polymorphonuclear leukocytes (PMNs) and monocytes preventing the migration of neutrophils to sites of inflammation thus acting as stop signals. Due to this anti-inflammatory activity, lipoxins have the potential to be developed into drug candidates for treating inflammation. However, chemical and metabolic instability *in vivo* limits their therapeutic use.

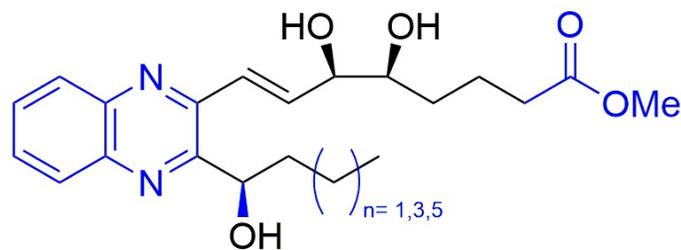
The aim of our research is to stabilise the triene core by replacement with aromatic or heteroaromatic moieties. Thus far, we have reported the introduction of a benzene and pyridine core and esterification of the acid which significantly increased phagocytosis of apoptotic PMN's and suppressed key cytokines compared to native LXA₄. Encouraged by these results, a range of quinoxaline analogues have been synthesised *via* a novel synthetic route and have shown improved biological activity which is presented in this poster.

susceptible to metabolism



Native LXA₄

improved stability and bioactivity



Quinoxaline analogues



MEDI 349

Discovery of novel and orally active quinolyl oxazole-based PDE4 inhibitors for the treatment of chronic obstructive pulmonary disease and asthma

Rongze Kuang, rongze.kuang@merck.com, Ho-Jane Shue, David Blythin, Pauline C. Ting, Neng-Yang Shih, Li Xiao, Xiao Chen, Danlin Gu, John Schwerdt, Jianhua Cao, Heping Wu, Dan B. Prelusky, Steve Sorota, Ping Wu, Ji Zhang, Xiang Zhang, Chander Celly, Motasim Billah, Peng Wang. Merck Research Laboratories, Rahway, New Jersey, United States

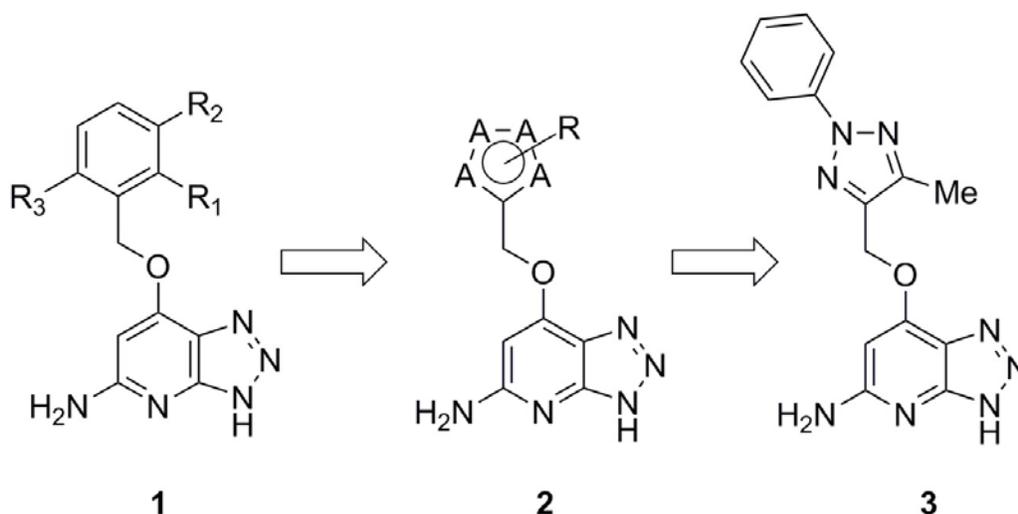
Phosphodiesterase 4 (PDE4), one of the cAMP-specific PDE isozymes, is highly expressed in inflammatory and immune cells. Inhibition of PDE4 effectively increases the intracellular cAMP level, which in turn provides critical negative regulation of various cellular functions in these cells. The anti-inflammatory effects of PDE4 inhibitors have been demonstrated in various animal models of airway diseases as well as in other biological disorders. This poster describes our efforts in the development of a novel class of PDE4 inhibitors based on quinolyl oxazole core for the treatment of chronic obstructive pulmonary disease and asthma. (S)-5-(1-amino-2-hydroxyethyl)-N-(2,4-difluorobenzyl)-2-(8-methoxy-2-(trifluoromethyl) quinolin-5-yl)oxazole-4-carboxamide was identified as an orally active and highly efficacious PDE4 inhibitor. The pharmacological profile of this compound will be discussed.

MEDI 350

7-Heteroaryl-methoxy-triazolopyridines as potent inhibitors of myeloperoxidase

Meriah Valente, *meriah.valente@bms.com*, Nicholas Wurtz, Andrew Viet, Scott Shaw, Dilger Andrew, Javed Khan, Sutjano Jusuf, Gayani Fernando, Xiaoqin Liu, Gregory Locke, Lisa M. Kopcho, Lynn Abell, Ji Gao, Ashok Dongre, Ruth R. Wexler, Franck Duclos, Ellen K. Kick. Bristol-Myers Squibb, Pennington, New Jersey, United States

Myeloperoxidase (MPO), a heme peroxidase highly expressed in neutrophils, and to a lesser extent in monocytes and macrophages, participates in immune defense through the formation of reactive oxidants and diffusible radical species. There is considerable evidence that under pathological conditions, MPO-derived oxidants contribute to tissue damage and the initiation and propagation of acute and chronic vascular inflammatory diseases, such as atherosclerosis. *Ortho*-substitution on benzyl ether substituted triazolopyridines (**1**) was essential to prevent off-target methyl guanine methyl transferase (MGMT) inhibition, and additional *meta*-substitution provided potent, reversible MPO inhibitors. Exploration of heteroaryl replacements (**2**) resulted in the identification of potent and selective MPO inhibitors, such as triazole **3**. Crystallography with multiple analogs showed the triazolopyridine consistently binding above the heme, while the ether moiety can adopt two distinct binding orientations.



MEDI 351

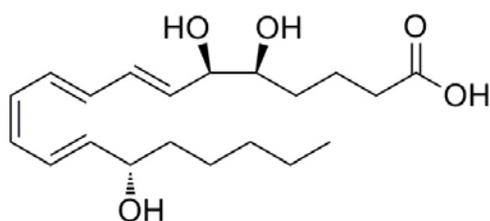
Stable lipoxin analogues for biological evaluation

Denise Moran¹, *denise.moran08@gmail.com*, **Monica de Gaetano**², **Catherine Godson**², **Patrick J. Guiry**¹. (1) Centre for Synthesis and Chemical Biology, University College Dublin, Dublin, Ireland (2) UCD Conway Institute of Biomolecular & Biomedical Research, Dublin, Dublin, Ireland

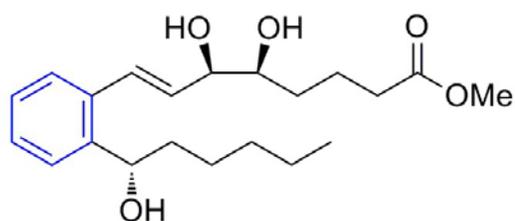
Promotion of the resolution of inflammation is a promising strategy for the treatment of chronic inflammatory diseases such as asthma, psoriasis, cystic fibrosis and rheumatoid arthritis. Lipoxins are a class of bioactive molecules which have been found to act as

endogenous mediators of the resolution of inflammation. The two native types of lipoxins, LXA₄ and LXB₄, are derived from arachidonic acid and are produced at the site of inflammation where they act as stop signals. They regulate the chemotaxis, adhesion and transmigration of polymorphonuclear leukocytes (PMNs) while stimulating the phagocytosis of apoptotic PMNs by macrophages. However, the therapeutic potential of the native lipoxins is greatly hindered by their rapid metabolism *in vivo*.

The focus of our research is the design of LXA₄ analogues that possess a greater resistance to metabolism while retaining the inherent anti-inflammatory activity. We have reported that the replacement of the metabolically labile triene moiety of LXA₄ with an benzene ring displayed improved biostability and increased potency compared to the native LXA₄. Following from this success, this talk will present the synthesis and biological evaluation of novel heteroaromatic analogues of LXA₄ and report their promising activity in resolving inflammation.



Native LXA₄



Benzo-LXA₄

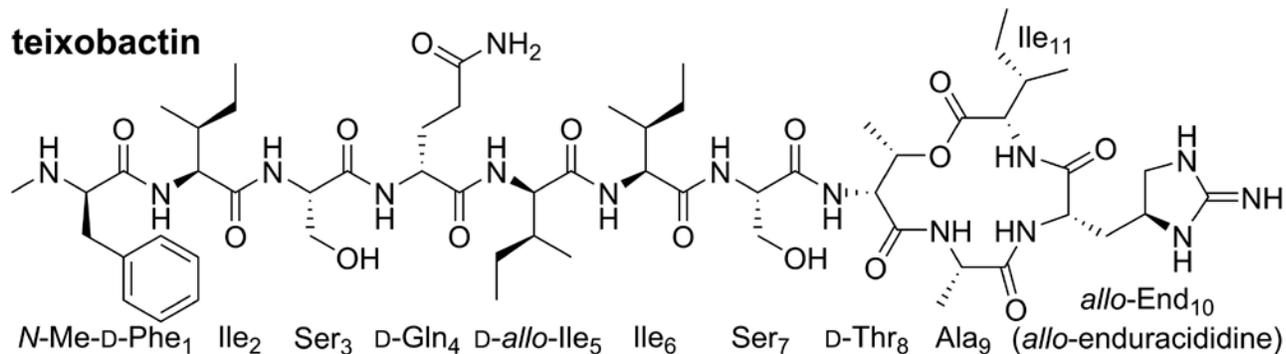


MEDI 352

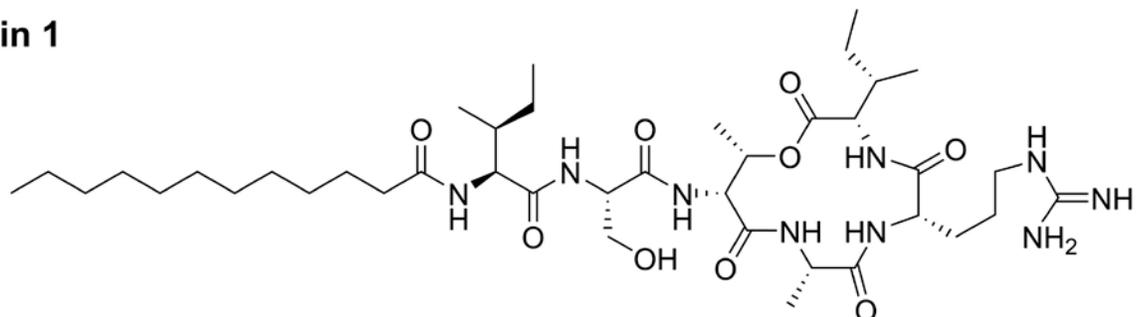
Lipobactins: A new class of antibiotics against gram-positive bacteria

Hyun Jun Yang, yanghj1@uci.edu, Kevin H. Chen, James S. Nowick. University of California, Irvine, California, United States

This presentation will describe the development of lipobactins, a new class of antibiotics against gram-positive bacteria. The lipobactins are homologues of teixobactin in which *N*-terminal residues are replaced with a lipid tail. The presentation will describe the elucidation of the teixobactin pharmacophore and show how it led to the discovery of simpler homologues, such as lipobactin 1.



lipobactin 1



MEDI 353

Design, synthesis, antimicrobial evaluation and molecular modeling studies of 4-(5-(2-aminothiazol-4-yl)-1,4-dihydro-2,6-dimethyl-4-aromatic substituted pyridin-3-yl)thiazol-2-amine derivatives

Parthasarathy Tigulla, *sarathychem@gmail.com*, *srinivasarao vankadari*. Osmania University, Hyderabad, Military: AP, India

In the present investigation, 4-(5-(2-aminothiazol-4-yl)-1,4-dihydro-2,6-dimethyl-4-aromatic substituted pyridin-3-yl)thiazol-2-amine derivatives were synthesized from reaction of 1,4-dihydropyridine derivatives with thiourea in the presence of iodine as a catalyst. In the first step, a series of 1,4-dihydropyridines were synthesized from treatment of acetyl acetone, substituted aromatic aldehyde and ammonium acetate. These were characterized by IR, ¹H NMR and mass spectral studies. The *invitro* antimicrobial activity (antibacterial and antifungal) was evaluated. Among the tested, compounds **3a**, **3c**, **3e**, **3f**, **3h** and **3i** showed excellent antibacterial activity with inhibitory zone >20 mm compared to standard drug ciprofloxacin. The compounds **3a**, **3c**, **3e**, **3f** and **3i** showed excellent antifungal activity with inhibitory zone >20 mm compared to standard drug itraconazole. The compounds **3a**, **3c**, **3e**, **3f** and **3i** exhibited both antibacterial and antifungal activity against all the selected pathogenic bacteria and fungi, and emerged as potential molecules for further development. The molecular docking simulation was also carried out to give a potent prediction binding mode between the small molecule and *E. coli* FabH enzyme and *Candida albicans*.

MEDI 354

Exploring structural importances on penetration of the first line tuberculosis prodrug: Pyrazinamide

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According to the World Health Organization (WHO), about one third of the world's population is infected with latent tuberculosis. To treat tuberculosis, there is a series of four first line drugs used including, rifampicin, ethambutol, isoniazid, and pyrazinamide where out of these four drugs, only the major mode of action of pyrazinamide is still debated. What is thought to occur is that pyrazinamide passively diffuses across a membrane where it is then hydrolyzed to its active form pyrazinoic acid. This initial passive diffusion has not yet been thoroughly investigated. To investigate the diffusion of pyrazinamide across a membrane along with determining important structural characteristics the interactions of pyrazinamide and analogs with model membranes were examined. Through this research, it was found that aromatic nitrogen placement on pyrazinamide is a key component for the interactions with membranes.

MEDI 355

Ribosome templated azide-alkyne cycloadditions: Synthesis of potent macrolide antibiotics screening by in situ click chemistry

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In medicine, the discovery and development of antibiotics has been a great success story wherein countless lives have been saved in the past century. However, there is an urgent need for new sources of antibiotics to keep pace with the rapid, inevitable onset of antibiotic resistance. Macrolide antibiotics comprise one of the most effective drug classes in medicine. For example, Solithromycin developed in 2005 by Optimer Pharmaceuticals, via Cu(I) catalyzed combinatorial click chemistry, is now in phase 3 clinical trials and has proved to be the best-in-class ketolide developed to date. To explore a larger scope of antibiotics, *target-guided in situ click chemistry* was implemented using either E.coli 70S ribosomes or 50S ribosomal subunits with azide and alkyne precursors. This methodology allowed for five and fifteen alkyne competition experiments to rapidly identify hit compounds. To evaluate the methodology's ability to identify more selective and potent antibiotics, dissociation constants, determined by fluorescence polarization, and minimum inhibitory concentration (MIC) assays were employed. In conclusion, *ribosomal in situ click chemistry* has shown to be a powerful drug discovery platform.

MEDI 356

Discovery of iguratimod as a selective, steroid-sparing MIF inhibitor via specificity-guided screening

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Macrophage migration inhibitory factor (MIF) is a pleiotropic cytokine that has been implicated in a broad range of inflammatory and oncogenic disease conditions. MIF is unique among cytokines in terms of its release profile and its ability to override the anti-inflammatory effects of glucocorticoids, and additionally possesses a catalytic tautomerase activity amenable to the design of highly affine small molecule inhibitors. Although several classes of these compounds have been identified, few have been well characterized biologically, and notably no studies have been undertaken examining off-target effects of these molecules. In this study, we used *in vitro* assays to characterize representative molecules from several classes of MIF inhibitors. We determined that MIF inhibitors can exhibit distinct profiles of anti-inflammatory activity, and that these activities can be MIF-independent. We also investigated a single molecule with low off-target anti-inflammatory activity--the clinically approved anti-rheumatic drug iguratimod--as a selective MIF inhibitor with glucocorticoid-sparing activity both *in vitro* and *in vivo*. Our work highlights the importance of considering specificity in target-based drug discovery, and also identifies iguratimod as a valuable new candidate for clinical application in MIF-relevant diseases.

MEDI 357

Elaboration of indole frameworks in the development of allosteric HIV-1 integrase inhibitors

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HIV-1 integrase (IN) has been recognized as a potentially important target for HIV therapy due to the role it plays in the incorporation of viral double stranded DNA into the host chromosomal DNA. To date, three IN inhibitors that target the active site of this protein have received FDA approval, but resistance to two of these agents, raltegravir and elvitegravir, has already been observed. An alternative approach to IN inhibition involves targeting of an allosteric site of integrase involved in the interaction of IN and its cellular cofactor LEDGF/p75. A number of compounds built around central six-membered heterocyclic cores, specifically quinolones and pyridines, have been shown to bind to the IN LEDGF/p75 binding site. Mechanistic studies of these compounds have shown multiple effects on integrase activity, including the promotion of aberrant IN multimerization. In order to more fully explore the steric and electronic features required

for the inhibition of IN activity and ultimately promotion of IN multimerization, the central scaffold of these systems has been varied through the use of alternative heterocyclic cores. To this end, several 5- and 6-membered heteroaromatic ring systems, including thiophenes, pyrroles, pyrazoles, and indoles, have been synthesized in our labs and tested for activity against IN. Many of the indole analogues synthesized during the course of this work have shown very promising IN inhibitory activity. Although the potencies of the indole compounds synthesized to date are weaker than previously published quinolones and pyridines, the ability to rapidly synthesize and functionalize the indole core is considered an advantage in analogue development and lead optimization, leading to a library of structurally diverse compounds. The syntheses of these indole compounds, their activity against IN, and X-ray crystallography data will be reported.

MEDI 358

Parallel inhibition of amino acid efflux and parasite growth of erythrocytic *Plasmodium falciparum* by mefloquine and open-ring analogs: Implication for the mechanism of antimalarial action

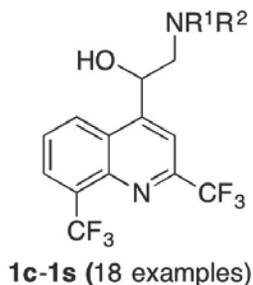
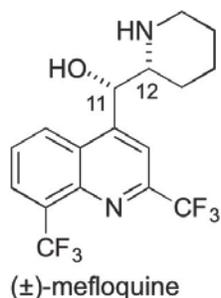
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Malaria is caused by an infection from a protozoan parasite of the genus *Plasmodium*; in 2014 it resulted in an estimated 438,000 deaths among 214 million cases of disease. Mefloquine (MQ) is one of several drugs used for both malaria prophylaxis and treatment. Its activity against all human malaria parasite species, its relative safety in pregnancy and less frequent dosing makes MQ an appealing prophylactic drug. However, the neuropsychiatric side effects experienced by some who take MQ have motivated the exploration of open-ring analogs of MQ (e.g. **1c-1s**).

A common mechanism of action has been put forward for quinoline-containing antimalarials. Chloroquine (CQ) is proposed to kill *Plasmodium sp.* by inhibiting the process by which toxic heme is eventually converted to a non-toxic heme polymer (hemozoin) in the acidic food vacuole of the parasite. Although MQ has been shown to imitate CQ by associating with parasite-derived hemozoin, and reducing hemozoin in vivo, a number of observations suggest that MQ's principal mode of antimalarial action is not inhibition of hemozoin formation. Resistance selection experiments with CQ and MQ suggest that the antimalarial target of MQ lies outside of the food vacuole. MQ significantly inhibits cytosomal endocytosis of host erythrocyte hemoglobin and endocytic tracers. In contrast CQ was found to only weakly inhibit endocytosis, but inhibited vesicle trafficking. Thus it has been proposed that the antimalarial action of MQ derives from inhibition of hemoglobin endocytosis.

In this report we use a recently developed amino acid efflux assay to determine the effect of MQ and 18 open-ring analogs on hemoglobin endocytosis and catabolism of hemoglobin in *Plasmodium*-infected erythrocytes. Among these closely related

compounds, an excellent correlation over nearly 4 log units is seen for inhibition of leucine (Leu) efflux and parasite growth (SYBR Green). These data are consistent with the hypothesis that the antimalarial action of these compounds derives from inhibition of hemoglobin endocytosis.



<p>Leu efflux IC₅₀ values = 9 to >20,000 nM</p> <p>Growth inhibition IC₅₀ values = 5 - >20,000 nM</p> <p>Log-Log correlation R² = 0.98</p>
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MEDI 359

Luminescence assay for natural product inhibitors of the *Mycobacterium tuberculosis* proteasome

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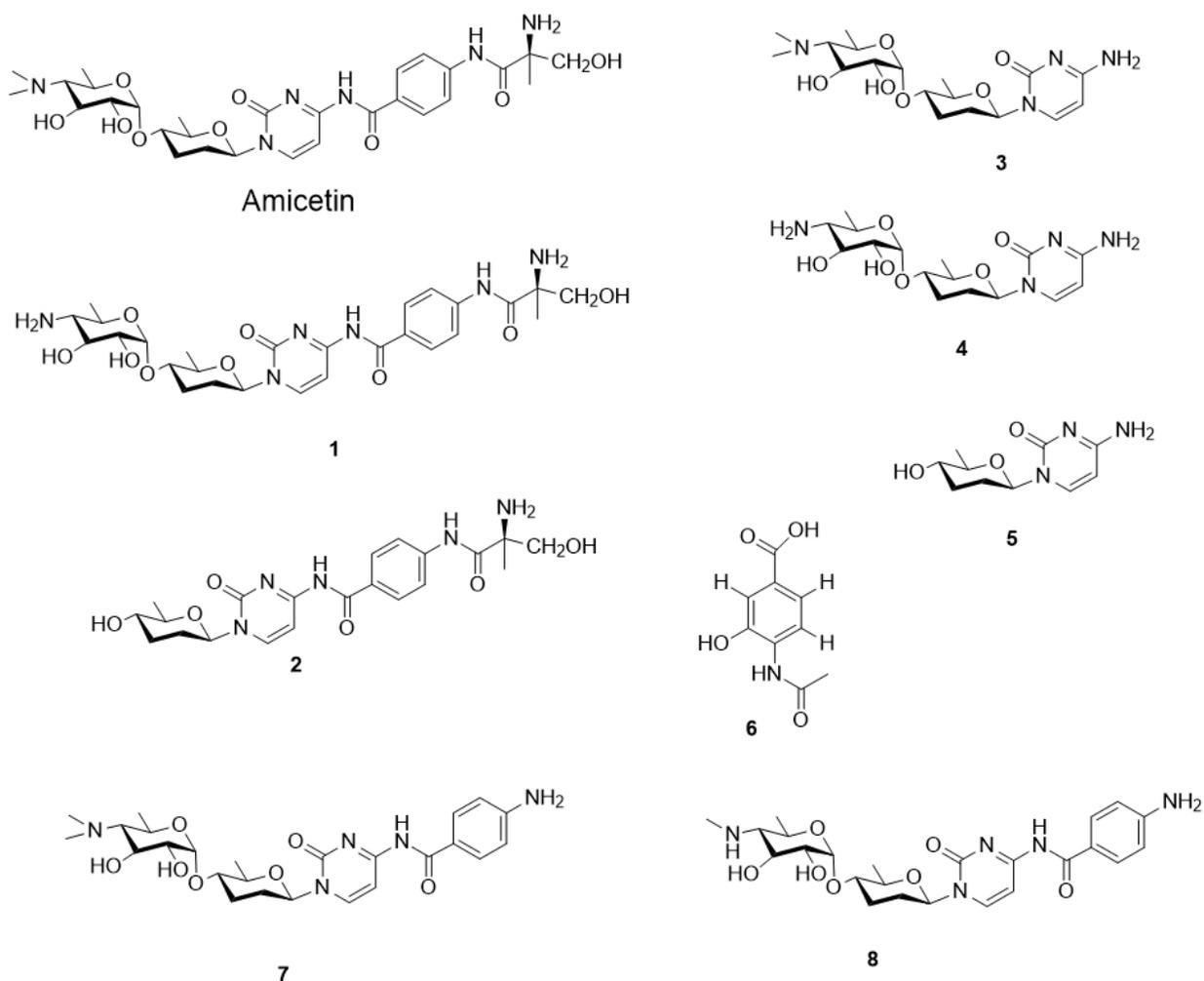
Tuberculosis, caused by the pathogen *Mycobacterium tuberculosis* (Mtb), causes 1.3 million deaths annually. The mycobacterial proteasome plays an important role in the defense mechanism of Mtb. We have investigated fluorescence and luminescence assays as potential screening methods to determine their respective robustness and repeatability for use in screening natural product extracts as Mtb proteasome inhibitors. The goal of this project is to develop and validate the luminescence assay as a novel method to screen proteasome inhibitors. The luminescence assay has been used to screen a small set of plant test extracts. The fluorescence assay is subject to interference by the natural fluorescence of compounds in many of the extracts; the luminescence approach is free of this interference. Luminescence is the more suitable assay for screening natural product extracts.

MEDI 360

Biosynthetic intermediates of amicetin produced by engineering mutants

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Amicetin is a disaccharide pyrimidine nucleoside antibiotic produced by *Streptomyces vinaceusdrappus* and *Streptomyces fasciculatis*, with potent activity against a number of both Gram-negative and Gram-positive bacteria. Four genes, *amiH*, *amiF*, *amiG*, and *amiR* were predicted to be involved in the biosynthetic pathway of amicetin. With the advantage of biological engineering technology, we found that the above mentioned mutants can produce amicetin and 8 biosynthetic intermediates (1-8). These intermediates provided essential chemical evidences for elucidating the biosynthetic pathway of amicetin. Compound 7 (plicacetin) showed potent inhibitory activity some pathogens such as *Streptococcus pneumoniae* (MIC₅₀=1.56 µg/ml), MRSA (MIC₅₀=6.25 µg/ml), and *Mycobacteria smegmatis* (MIC₅₀=6.25 µg/ml).



MEDI 361

Multicationic quaternary ammonium compounds (MultiQACs): Potent antimicrobial and antibiofilm agents arising from a variety of scaffolds

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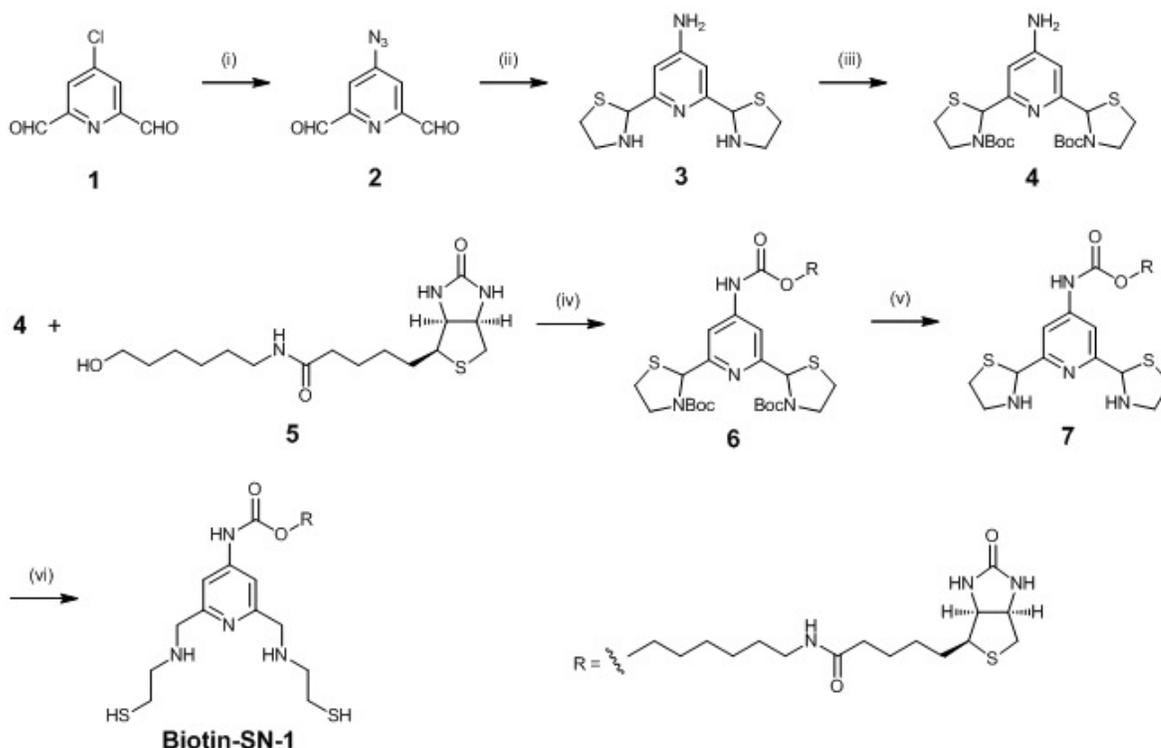
Quaternary ammonium compounds (QACs) are a historically prominent class of surface disinfecting agents. Taking inspiration from commercial disinfectants and antimicrobial natural products, we have derivatized a structurally diverse array of tertiary amines to generate several different classes of QACs with multiple cationic groups (multiQACs). We have synthesized over 300 novel QAC structures to date, many of which exhibit potent antibacterial and antibiofilm activity. Analysis of the structure-activity relationship of these amphiphiles have led to the facile production of QACs that display micromolar to (more recently) sub-micromolar MIC values against a suite of bacteria, and furthermore do not appear to trigger bacterial resistance systems in methicillin-resistant *Staphylococcus aureus* (MRSA). Antibiofilm activity is amongst the best reported to date.

MEDI 362

Zinc-mediated binding of a low-molecular-weight stabilizer of the host anti-viral factor APOBEC3G

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Previously, we reported compound SN-1, a zinc chelator that increases steady-state expression level of APOBEC3G (A3G) in the presence of Vif. In this study, we synthesized Biotin-SN-1, a biotinylated derivative of SN-1, according to the scheme below, to study the SN-1–A3G interaction. A pull-down assay revealed that Biotin-SN-1 bound A3G. A zinc-abstraction experiment using EDTA indicated that SN-1 binds to the zinc site of A3G. We carried out a SN-1–A3G docking study using Molecular Operating Environment 2014.09. The calculations revealed that SN-1 binds to the C-terminal domain through Zn²⁺, H²¹⁶, P²⁴⁷, C²⁸⁸, and Y³¹⁵. Notably, SN-1-binding covers the H²⁵⁷, E²⁵⁹, C²⁸⁸, and C²⁹¹ residues that participate in zinc-mediated deamination, and the ubiquitination regions of A3G. The binding of SN-1 presumably perturbs the secondary structure between C²⁸⁸ and Y³¹⁵, leading to less efficient ubiquitination and protection from proteasomal degradation of A3G. The A3G bound by SN-1 would inhibit viral reverse transcription by A3G deaminase-independently.



(i) NaN_3 , DMF, $70\text{ }^\circ\text{C}$, 2.5 h, (ii) 2-aminoethanethiol hydrochloride, Et_3N , MeOH, r. t., overnight, (iii) di-*tert*-butyl dicarbonate, Et_3N , CH_2Cl_2 , $40\text{ }^\circ\text{C}$, 4 h, (iv) triphosgene, CH_2Cl_2 , $0\text{ }^\circ\text{C}$, 1 h, then **5** in DMF, r. t., overnight, (v) *p*-TsOH·H₂O, CH_2Cl_2 , toluene, $40\text{ }^\circ\text{C}$, (vi) NaCNBH_3 , 0.1M HCl/MeOH, r.t..

Synthesis of Biotin-SN-1

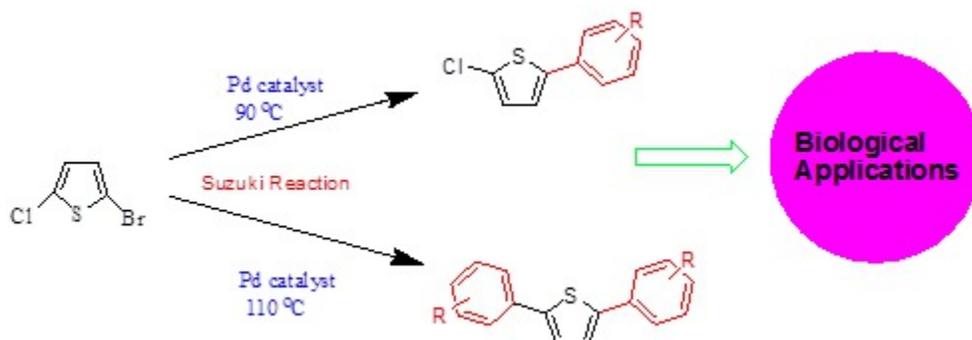
MEDI 363

Arylation of 2-bromo-5-chloro thiophenes with aryl boronic acids, their structural investigations (X-ray and DFT), and in vitro antibacterial and scavenging activities

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we report synthesis of various 2-aryl-5-chlorothiophenes and 2,5-biarylthiophenes starting from 2-bromo-5-chloro thiophenes. In Suzuki coupling reactions, different boronic acids/esters react with 2-bromo-5-chloro thiophenes in the presence of palladium catalyst. DFT investigations proved that a strong correlation exists among experimental and simulated results. X-ray and simulated geometric parameters of **2d** and **2f**, corroborate to each other very nicely. FMOs analysis revealed the reactivity of all synthesized compounds, and found that some molecules have lowest energy gap i. e. 3.96 eV (kinetically less stable). Reactive sites and electronic effect of group attached to benzene ring was investigated by ESP analysis. By noting the results of this study it

is revealed that some of the synthesized compounds of 2-bromo-5-chloro thiophenes can be used as a potent antibacterial and anti scavenger molecule.



MEDI 364

Synthesis of novel allosteric inhibitors of HIV-1 integrase that bind to the LEDGF/p75 site

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The incorporation of viral double stranded DNA into host chromosomal DNAs is mediated by HIV-1 integrase (IN). This protein has recently been targeted for the development of a number of FDA approved drugs which bind at the active site of IN. Raltegravir was the first FDA approved IN inhibitor, although resistance to this drug in the clinic has since been observed. With this in mind, second generation integrase inhibitors, including elvitegravir and dolutegravir, were subsequently developed and marketed. An alternative approach to targeting HIV-1 IN is the development of allosteric integrase inhibitors (ALLINIs) that target the binding site of the cellular cofactor LEDGF/p75 and promote aberrant IN multimerization, ultimately leading to inactivation of the protein. A number of ligands that bind at the IN CCD dimer interface have been explored and have allowed structure-activity relationships to be developed, establishing the structural components necessary for effective IN inhibition. In this work, a series of analogues will be presented which 1) probe interactions with the subunits of IN, allowing us to explore the mechanism of HIV-1 IN multimerization and 2) explore additional pockets within the LEDGF binding site through both steric and electronic manipulation of the substitution of these molecules.

MEDI 365

Synthesis and biological studies of dihydropyrido pyrimidinones

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The 1,3 nitrogenous bicyclic frameworks have been illustrious in drug discovery. Many pyrido pyrimidinone scaffolds have occupied privileged positions in medicinal chemistry due to their wide range of biological activities. It is a key constituent of numerous natural products passing wide range of biological activities including antitumor, anti-influenza, oxidative burst inhibitory, lipid droplet synthesis inhibition, and obesity properties. Some of the pyrimidinone have been marketed and used as antipsychotic drugs. The recent studies have revealed that the molecules bearing pyrimidinones as an integral part of their structure have been in different phases of drug discovery with a wide range activity against different disease including anticancer, hypertension control, anti-oxidant etc. Some of them have been recognized as aldose reductase inhibitors, efflux pump inhibitors and activity against hepatitis C virus NS3 protease including marketed drugs for antidepressant and anti-asthmatic. We report the development of new methodology for the synthesis of pyrido pyrimidinone by using sustainable approach. We also tested these molecules against bacterial growth.

MEDI 366

Design, synthesis, and evaluation of novel anti-DENV compounds

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Dengue Virus (DENV) is the pathogen responsible for Dengue fever, one of the most widespread diseases in the world. No antiviral treatment is currently available against this infection.

DENV NS5 RNA-dependent RNA polymerase (NS5 RdRp) is the protein responsible for the replication of the viral genome. Four allosteric sites can be identified within its structure. Among them, the allosteric site D is located in proximity of residue Trp795, which is essential for RNA synthesis initiation. This site represents a valid target for the identification of new inhibitors of DENV replication.

A previous computer-aided approach, carried out in our research group to target DENV NS5 allosteric site D, led to the identification of different compounds with low micromolar activity in a polymerase inhibition assay. These compounds did not show significant antiviral activity in cellular CPE assays.

With the aim to improve the antiviral activity of the original hits, a series of new potential allosteric inhibitors of DENV polymerase was designed. Molecular docking studies were performed on the structure of a homology model built for DENV RdRp. All the newly

designed compounds showed the ability to fit the allosteric site D in close proximity to Trp795.

Based on these results, the most promising compounds were synthesised. The rational and chemistry leading to their preparation, along with their antiviral behaviour in cell-based CPE assays, will be discussed in this presentation.

MEDI 367

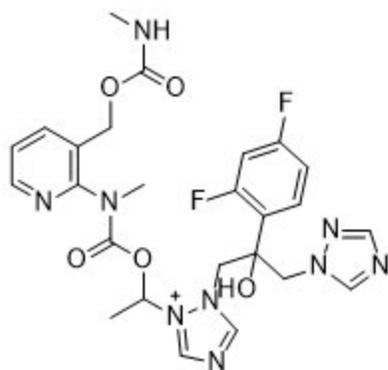
Design and synthesis of 1-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)propyl)-2-(1-((methyl(3-(((methylcarbamoyl)oxy)methyl)pyridin-2-yl)carbamoyl)oxy)ethyl)-1H-1,2,4-triazol-2-ium

Lee Peyton¹, lpeyton0@gmail.com, Scarlett Gallagher¹, Eric Allen¹, Nathan Herrmann¹, Mehrnoosh Hashemzadeh^{2,1}. (1) Chemistry, Pima College, Tucson, Arizona, United States (2) medicine, university of Arizona, Tucson, Arizona, United States

Purpose: The purpose of this project was to develop a novel approach to a new derivative of fluconazole that could potentially broaden the application and administration of the drug as an antifungal therapeutic. The goal of this project was to synthesize a molecule that had the potential for intramuscular administration. IM administration would allow for the treatment of mycotic diseases in hard to reach locations along with the treatment of livestock, specifically ruminant animals, who might otherwise go untreated due to the large amount of oral medication required to treat mycotic infections.

Introduction: Fluconazole (FLCZ) is a first generation antifungal used to treat a variety of fungal pathogens. FLCZ is available in capsules, tablet, powdered, or injectable forms (2mg FLUZ/9 mg NaCl). The low IV solubility of FLCZ has led to the development fosfluconazole, the phosphate ester prodrug of fluconazole which increased the solubility and allowed for bolus administration of this therapeutic. To date these are the only 2 formulations of this pharmaceutical. Offering a third formulation (our approach) for a FLCZ prodrug will allow for wider application of this antifungal.

Research: Our group is in the process of synthesizing our target molecule, which should be cleaved by a nondescript esterase in plasma. It is hoped this target molecule has a solubility greater than that of the phosphate ester prodrug fosfluconazole thereby allowing for IM administration. We have successfully synthesized and characterized our molecule of interest, and are awaiting testing results of our compound.



1-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)-2-(1-((methyl(3-((methylcarbamoyl)oxy)methyl)pyridin-2-yl)carbamoyl)oxy)ethyl)-1*H*-1,2,4-triazol-2-ium

MEDI 368

Some selected metal complexes of proguanil-sulphadiazine mixed ligands: Synthesis, characterization, and antimicrobial studies

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Mixed ligand metal complexes of the antimalarial drug – Proguanil and sulphonamide antibiotics were prepared by using Co(II) and Ni(II) metal acetates. The complexes were reported and characterized by elemental analysis, melting point determination, molar conductivity, metal content analysis, some physical properties and spectroscopic techniques such as Atomic Absorption Spectroscopy, Ultra-Violet spectroscopy and Infrared. Based on the analytical and spectroscopies data, the complexes were proposed to have the formulae $[(M)_2L_1L_2(\mu\text{-CH}_3\text{COO})_2(\text{H}_2\text{O})_4]$ (where L_1 = proguanil while, L_2 = sulphadiazine). The spectroscopic data proposed both L_1 and L_2 to acts as bidentate ligands in coordinating to the central metal ions along with the water and acetate groups. Conductivity measurement values supported the non-electrolytic nature of the complexes. The complexes have been tested in vitro against both fungi (*Aspergillus flavus*, *Aspergillus niger*, *Penicillium spp*) and bacteria (*Escherichia coli*, *Bacillus subtilis* and *Klebsiella pneumonia*) isolates. The activity data showed the metal complexes to be more potent antibacterial than the parent drugs against the three bacteria species. However, the antifungal activity data is not enhanced when compared. The results generally indicated that more potent compounds with better physical properties and enhanced antimicrobial activities upon complexation have been prepared.

MEDI 369

Synthesis of benzoxaborole-metronidazole based compounds for *Clostridium difficile*

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Clostridium difficile (*C. difficile*) is a gram positive spore forming bacteria that does not cause much symptoms in people with normal gut flora. However, patients who undergo prolonged treatment with broad spectrum antibiotics may have reduced gut flora resulting in opportunistic infections with *C. difficile*. These infections lead to severe diarrhea, abdominal pain and sometimes, fatal inflammation of the colon. Currently, mild to moderate *C. difficile* infections are treated with metronidazole and severe infections are treated with vancomycin. With the development of resistance of such common pathogens to these antibiotics, novel candidate compounds to treat these infectious diseases are urgently needed. Benzoxaboroles are structurally unique boron containing heterocyclic compounds that have excellent chemical and metabolic stability. Several derivatives of benzoxaboroles have been found to exhibit inhibitory activity against bacterial and fungal pathogens. In this regard, we have recently designed and synthesized several novel metronidazole based aminobenzoboroxole derivatives. Biological evaluation against *C. difficile* (ATCC BAA-1805, vegetative state) has shown good inhibitory activity with some of our synthesized compounds. Our synthetic and biological evaluation results will be presented in this poster.

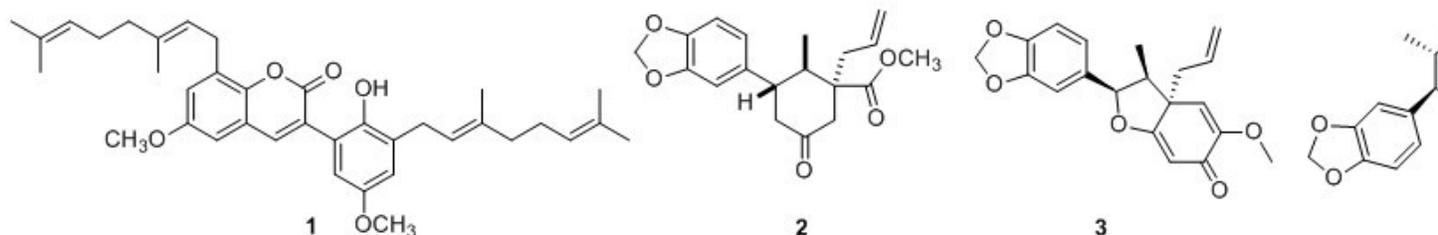
MEDI 370

Antiplasmodial and other compounds from an *Aniba* sp.

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Malaria is a neglected tropical disease that has a disproportionate effect in poor and underdeveloped countries. As part of our ongoing search for new antimalarial compounds from Nature, an extract of a plant of the *Aniba* genus from the former Merck Repository now at the Natural Products Discovery Institute was found to have good antiplasmodial activity, with an IC₅₀ value less than 1.25 µg/mL against the drug-resistant Dd2 strain of *Plasmodium falciparum*. Bioassay guided fractionation using liquid-liquid partition, chromatography on Sephadex LH-20, diol open column and C-18 reverse phase HPLC separations yielded the new bioactive coumarin **1**, the new neolignan **2** and six known neolignans, including compounds **3** and **4**. The structures of the new and known compounds were determined by using NMR spectroscopy, MS and CD. Among these compounds, the coumarin **1** had the most potent antiplasmodial

activity.



Selected compounds isolated from an *Aniba* sp.

MEDI 371

Overcoming PK limitations via prodrugs to advance a second generation of HIV-1 integrase strand transfer inhibitors

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The identification of next-generation HIV Integrase strand transfer inhibitors (InSTIs) continues to be a dynamic area of drug discovery research. Efforts are focused around developing inhibitors that show broad resistance profiles and are amenable to once-daily (QD) dosing. Herein, we present our recent efforts toward this goal, which have focused on a novel 5-membered aminal lead class based on the 2-pyridone core of MK-0536. Optimization within the aminal series led to the identification of lead molecules that demonstrated excellent antiviral activity and showed preclinical pharmacokinetic profiles supportive of QD human dosing. However, the advancement of this class was challenged due to limited plasma exposure in non-rodent dose escalation studies, hindering progress into late preclinical toxicological evaluation. To address this

limitation, an innovative prodrug strategy was applied. This strategy utilized two different synthetically accessible hydroxyl groups of the leading compounds to introduce promoieties. Prodrugs were designed to independently address the limited aqueous solubility and limited permeability of parent compounds, likely due to the phenolic hydroxyl functional group inherent to the minimal pharmacophore. Synthetic approaches were optimized to incorporate solubility- and permeability-enhancing prodrugs such as phosphates, aminoesters, and carbonate acetals. Our efforts culminated in a phosphate/carbonate acetal “double–prodrug” that achieved high parent plasma exposures and provided a path to further advance the new class of InSTI.

MEDI 372

Optimization of macrocyclic peptide triazole HIV-1 inactivators

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HIV-1 entry inhibition remains an urgent need for AIDS drug discovery and development. We previously reported the discovery of cyclic peptide triazoles (cPT's) that retain the HIV-1 irreversible inactivation functions of the parent linear peptides (PT's). We also showed that the proteolytic susceptibility of cPT's was massively reduced compared to that of the corresponding linear PT's. Here, we have followed up with structural optimization and minimization to produce a next generation of cPT's. We identified more potent (low nanomolar range) cPT analogues by replacing the Trp and Ile residues in the pharmacophore of first generation cPT's. We also identified smaller 5-residue cPT's that are weaker but still retained the dual host cell receptor binding antagonism properties of the 6-residue cPT's. These optimization/minimization steps have led to understanding the key structural requirements for PT's activities, in general. In turn, this understanding is being used to identify smaller scaffolds that could lead to a third generation cPT-derived peptidomimetics that still retain the unique phenotypes of PT's.

MEDI 373

Design and synthesis of novel nucleotide analogues targeting HCV NS5B

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The development of new agents against Hepatitis C Virus (HCV) is a high priority because of the increasing incidence of viral infection, which makes HCV a major cause of liver transplant which is associated with high cost medical expenses. The current standard of care is moderately effective and causes a lot of side effects to patients. Additionally, newly approved medications are expensive (\$84,000-94,500 for a typical

12-week course of treatment). The recent publication of the crystal structure of the HCV RNA-dependent RNA-polymerase (RdRp) NS5B with an inhibitor in the active site has enabled elegant development of new agents targeting the NS5B enzyme. Nucleoside analogues modified at the 2' position have been proven to be effective against the HCV RdRp NS5B as a chain terminator. Herein, we present the design and synthesis of new nucleoside analogues starting from unmodified nucleosides in a few steps. The synthesis is in the process of optimization and will be extended to the synthesis of phosphoramidate prodrugs. These modified nucleotides will be tested against HCV using the Replicon assay.

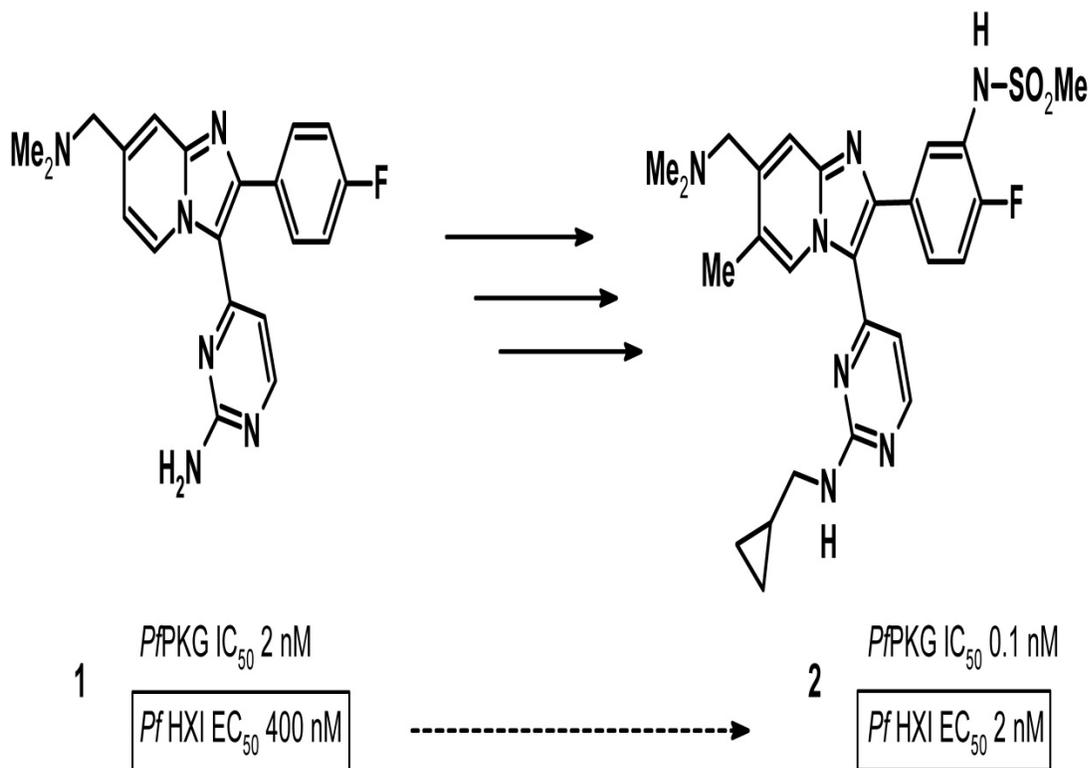
MEDI 374

Potent, selective and orally efficacious inhibitors of *Plasmodium falciparum* protein kinase G (*Pf*PKG)

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Malaria is one of the most prevalent infectious diseases of the developing world, whose primary causative agent in humans is the protozoan parasite *Plasmodium falciparum*. It is currently responsible for almost 0.5 million deaths per year, with both young children and pregnant women in sub-Saharan Africa particularly at risk. There is significant concern about widespread and rapidly growing resistance to current standard malaria drugs; hence the development of structurally and mechanistically novel malaria treatments is urgently required to maintain control and advance eradication of the disease.

We have developed a class of inhibitors of *Pf*PKG, using known compounds (eg. **1**) with activity against the *Eimeria tenella* PKG homologue as chemical starting points. Our new compounds show excellent *Pf*PKG enzyme affinity together with potent cell inhibition against the parasite, are highly selective against a human kinase panel and possess ADME profiles which translate to good levels of *in vivo* efficacy in rodent models of malaria. Development of both structure activity relationships and compounds which overcame pharmacokinetic and toxicity issues will be shown, together with some interesting aspects of *in vivo* protocol design. Key program compounds have been assessed in a range of malaria life cycle assay platforms, output from which continues to inform areas of PKG target biology as an approach to anti-malarial drug design. Some of the newest analogues such as **2** have shown very high levels of cell potency, improving on those of some known malarial drugs.



MEDI 375

Trisubstituted thiazoles as potent and selective inhibitors of *Plasmodium falciparum* protein kinase G (PfPKG)

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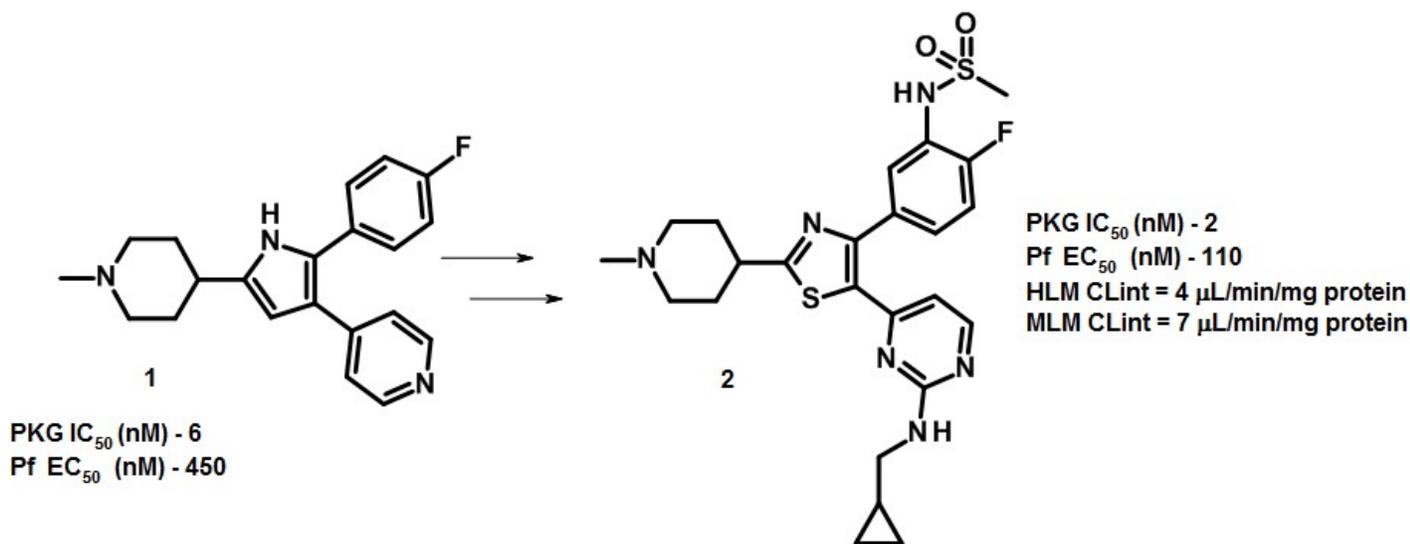
Malaria is one of the most prevalent infectious diseases of the developing world, whose primary causative agent in humans is the protozoan parasite *Plasmodium falciparum*. It is currently responsible for nearly 0.5 million deaths per year, with both young children and pregnant women in sub-Saharan Africa particularly at risk. There is significant concern about widespread and rapidly growing resistance to current standard malaria drugs; hence the development of structurally and mechanistically novel malaria treatments is urgently required to maintain control and advance eradication of the disease.

We have been developing a class of inhibitors of PfPKG, starting from previously reported compounds (such as **1**) with activity against the *Eimeria Tenella* PKG homologue.

Compound **1** showed several undesirable traits: a pyrrole, an unflanked 4-pyridyl, and potent activity against human p38a MAPK and PKA kinases and this poster will

describe our efforts to moderate or remove these traits while retaining good anti-parasitological activity.

Substitution of the pyrrole for a thiazole and SAR modifications on the side chains led to a series of potent anti-malarials. Analogues such as compound **2** possess potent enzyme affinity and *in vitro* anti-parasite activity, coupled with excellent selectivity against human kinases as well as good ADME properties.

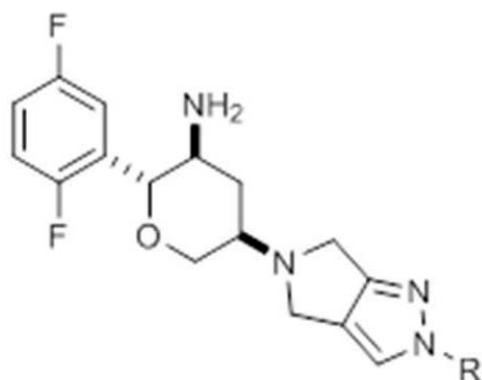


MEDI 376

Structure-activity-relationship of alkyl and alcohol analogs of omarigliptin, long acting DPP-4 inhibitors

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Omarigliptin (MARIZEV™) is a once-weekly oral DPP-4 inhibitor which was approved in Japan last year for the treatment of T2DM. The 2-methyl propanol analog (**12B**) of omarigliptin showed a similar long acting property in both rats and dogs. The SAR of alkyl and alcohol analogs of omarigliptin, pharmacokinetic properties and synthetic schemes are discussed in this poster.



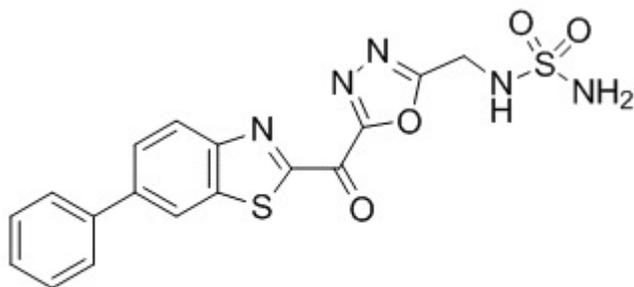
R = SO₂Me, **Omarigliptin**
R = 2-methyl-propanol **12 B**

MEDI 377

Reversible small molecule inhibitors of endothelial lipase (EL) which increase high density lipoprotein (HDL) concentration *in vivo*

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High density lipoprotein cholesterol (HDL-C) concentration is inversely related to cardiovascular disease (CVD) risk (Framingham Study). HDL-C plays a central role in reverse cholesterol transport (RCT), a process where cholesterol is removed from the periphery (e.g., atherosclerotic plaques in blood vessels) to the liver for elimination. HDL-C may also reduce blood vessel injury through its anti-oxidant and anti-inflammatory functions. Endothelial Lipase (EL) hydrolyzes the phospholipids in HDL ultimately resulting in a reduction in plasma HDL particles (presumably by increasing HDL clearance). Studies with murine transgenic, KO or loss-of-function variants strongly suggest that inhibition of EL will lead to a sustained HDL-C increase and potentially a reduced CVD risk. **Compound 1** is an example of a potent inhibitor of EL which demonstrated robust PD effects in murine models of HDL-C elevation. SAR and lead optimization for potency and selectivity of this series will be disclosed.



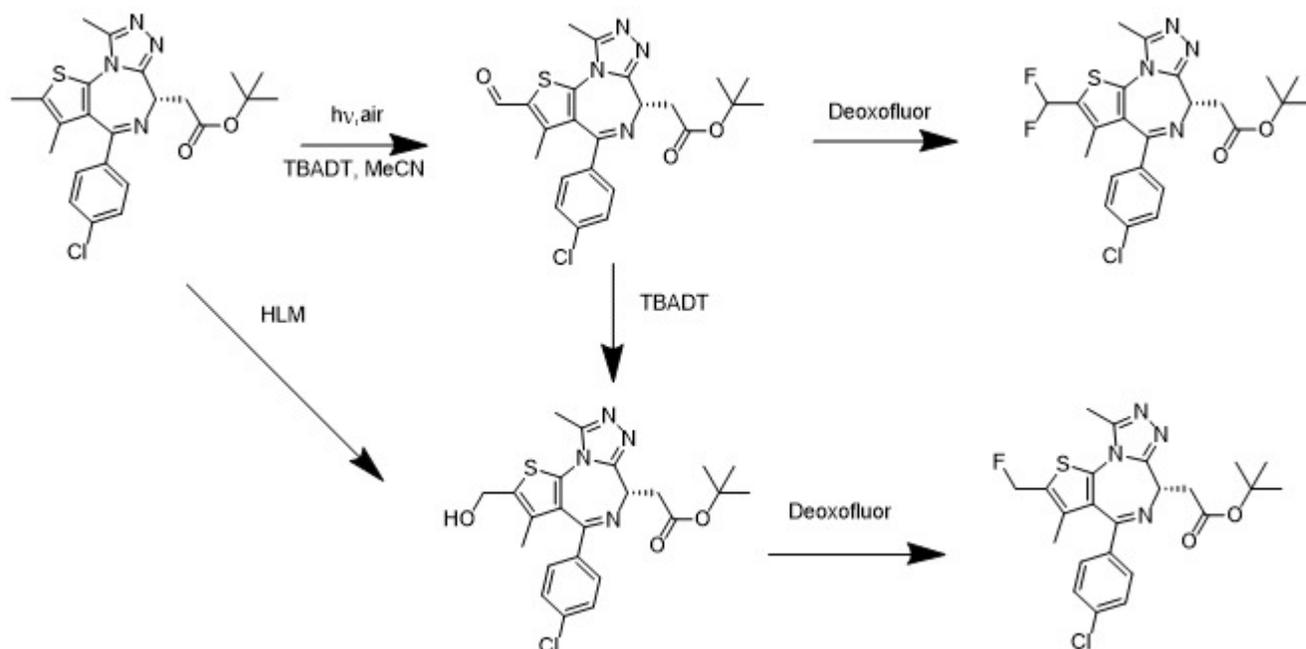
Compound 1
(EL human serum IC₅₀ 92 nM)

MEDI 378

Fluorination of JQ1 slows its metabolism

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JQ1 is a small molecule inhibitor of the Bromodomain and Extra-terminal (BET) family, including bromodomain testis-specific (BRDT). As a BRDT inhibitor, JQ1 is used as a probe compound for a non-hormonal male contraceptive. However, JQ1 is known to have a short in vivo half-life. Pharmacokinetic studies have shown that metabolism of JQ1 by human liver microsomes (HLM) produces a major monohydroxylated metabolite. A photochemical oxygenation of JQ1 catalyzed by tetrabutylammonium decatungstate (TBADT) was used to synthetically produce an aldehyde precursor to the major metabolite as confirmed by MS/MS. Following the oxygenation reaction, treatment of the aldehyde or derived alcohol with Deoxofluor™ produces mono- and di-fluoro JQ1 analogs. Preliminary studies of the analogs show there is at least a 2.5-fold increase in the half-life of the fluorinated JQ1 analogs with retained enzymatic activity against BRDT when fluorine is introduced to the metabolic soft spot.



MEDI 379

Finding hits for designing new antidiabetic drugs. Inhibition of protein tyrosine phosphatase 1B

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Currently there are 387 million diabetics in the world, but by the year 2035 this number could increase around 600 million. Type II diabetes is the most common, representing 90 to 95% of all cases. In the search for new antidiabetics, one of the main strategies is to promote the action of insulin. A negative regulator of the insulin-signaling cascade, protein tyrosine phosphatase 1B (PTP1B), has been recognized as a good molecular target. The aim of this work was to obtain new PTP1B inhibitors and to characterize their mode of action through a kinetic and structural analysis. To this end, around 1000 compounds were assessed as PTP1B inhibitors. Three molecules were identified with significant inhibition potency, PTSB6, JM151, and TCBZ. Kinetic studies revealed that compounds showed mixed type inhibition with a K_i value of 5, 4, and 41 μM , for PTSB6, JM151, and TCBZ, respectively. Indicating that they can recognize both the free enzyme and the enzyme-substrate complex. According to molecular dynamics simulations, these inhibitors interacted with the second aryl phosphate-binding site of the enzyme, an exclusive site in PTP1B with respect to other phosphatases. Theoretical physicochemical and ADMET parameters indicated that these molecules have the characteristics to be considered as potential drug candidates. Binding energies

comparisons between PTP1B and its closest homologous TCPTP, suggested that the inhibitors could be selective for PTP1B. Therefore, inhibitors reported here represent a new chemical scaffold to design novel drugs against type II diabetes.

MEDI 380

Synthesis and SAR of triazole analogs as potent glucokinase activator

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Glucokinase (GK) belongs to the hexokinase family of enzymes and is expressed primarily in the pancreatic beta-cells and liver parenchymal cells. Liver-selective GK activation should result in an increase in hepatic glucose utilization with a corresponding decrease in hepatic glucose production and may reduce the risk for hypoglycemia vs. systemic GK activation (resulting from undesired pancreatic insulin secretion even at low glucose levels). A series of 4-amino-1,2,3-triazole amides incorporating different phosphorus-containing moieties have been designed and synthesized as potent glucokinase activators. The SAR of this series showed that most of these triazole phosphonate and phosphinate analogs display excellent GK in vitro potency and functional activity. This effort resulted in the identification of potent liver-selective GK full activators which showed excellent glucose lowering in Diet-Induced Obese (DIO) Mice without increase in insulin levels even at high doses.

MEDI 381

Optimization of sulfonamide based GPBAR1 (TGR5) agonists

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G-protein coupled bile acid receptor 1 (GPBAR1, also known as TGR5) agonism increases energy expenditure and lowers blood glucose, thus GPBAR1 has attracted interests as a target for treating human metabolic diseases. A sulfonamide based chemical series was identified as an attractive starting point from screen of our ECLiPS compound collection. Since TGR5 activation was linked to gallbladder filling and some cardiovascular side effects, the goal of our lead optimization effort was to identify potent agonists with minimal systemic exposure. This led to some potent and minimally exposed TGR5 agonists.

MEDI 382

GPBAR1 (TGR5) agonists with low systemic exposure

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Activation of the Takeda G-protein receptor 5 (TGR5), also known as G-protein coupled bile acid receptor 1 (GPBAR1), was shown to improve glucose homeostasis and insulin sensitivity, as well as increase in energy expenditure. Initial screen of our ECLiPS compound collection identified multiple hit series, one of which contains a pyrimidine scaffold. The reported association between TGR5 activation with gallbladder filling and cardiovascular effects led us to pursue TGR5 agonists with low systemic exposure. Optimization of this pyrimidine series for potency as well as reduced plasma exposure led to potent and efficacious compounds in animal models.

MEDI 383

Design, synthesis, and evaluation of (2S, 4R)-ketoconazole sulfonamide analogs as potential treatments for metabolic syndrome

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Metabolic syndrome, also referred to as Syndrome X” or “Insulin Resistance Syndrome,” remains a major, unmet medical need despite over 30 years of intense effort. Recent research suggests that there may be a causal link between this condition and abnormal glucocorticoid processing. Specifically, dysregulation of the hypothalamic-pituitary-adrenocortical (HPA) axis leads to increased systemic cortisol concentrations. Cushing’ syndrome, a disorder that is also typified by a marked elevation in levels of cortisol, produces clinical symptomology that is similar to those observed in MetS, and they can be alleviated by decreasing circulating cortisol concentrations. As a result, it has been suggested that decreasing systemic cortisol concentration might have a positive impact on the progression of MetS. This could be accomplished through inhibition of enzymes in the cortisol synthetic pathway, 11 β -hydroxylase (Cyp11B1), 17 α -hydroxylase-C17,20-lyase (Cyp17), and 21-hydroxylase (Cyp21). We have identified a series of novel sulfonamide analogs of (2S, 4R)-Ketoconazole that are potent inhibitors of these enzymes. In addition, selected members of this class of compounds have pharmacokinetic properties consistent with orally delivered drugs, making them well suited to further investigation as potential therapies for MetS.

MEDI 384

Discovery of 2-thio-5-thiomethyl substituted imidazoles as potent and orally efficacious TGR5 receptor agonists for treatment of type 2 diabetes

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TGR5 (also known as GPBAR1, M-BAR, or GPCR19) is a G-protein coupled receptor broadly expressed in human tissues, such as the GI tract, gall bladder, spleen, lung, brown adipose tissue and placenta. Activation of the TGR5 receptor upon binding of bile acids (BAs) transduces signal through G_s protein-mediated cyclic adenosine monophosphate (cAMP) accumulation and downstream mitogen-activated protein kinase pathways. It has been reported that stimulation of TGR5 with BAs in murine enteroendocrine cell line STC-1 increased glucagon like peptide-1 (GLP-1) secretion, which could improve glucose homeostasis via various mechanisms including stimulation of pancreatic insulin secretion and inhibition of glucagon secretion. Additionally, activation of TGR5 can also induce type 2 iodothyronine deiodinase (D2) in brown adipose tissue in mice, which leads to increased energy expenditure. Therefore, a small molecule TGR5 agonist may be beneficial for the treatment of type 2 diabetes with simultaneous management of glucose levels, body weight, and associated complications. The TGR5 gene is highly expressed in gallbladder. Activation of TGR5 in gallbladder epithelium causes smooth muscle relaxation, stimulation of gallbladder filling, and an acute increase in gallbladder volume, which becomes the main obstacle to the clinical application of TGR5 agonists.

Herein we describe our efforts to discover a series of 2-thio-5-thiomethyl substituted

imidazoles as potent and orally efficacious TGR5 agonists. To minimize the side effect on the gallbladder, we sought to discover non-systemic TGR5 agonists specifically targeting the intestinal lumen. We report structure activity relationship studies on this series to identify a lead compound (**I**) showing in vivo antidiabetic activity with low systemic exposure. In cAMP assays with enteroendocrine cells, (**I**) displayed an EC₅₀ value of 2.0 µM in the murine STC-1 cell line and an EC₅₀ value of 42.6 µM in the human NCI-H716 cell line. A single oral dose of (**I**) (50 mg/kg) significantly reduced blood glucose levels and caused a 16% reduction in the area under the blood glucose curve (absolute AUC)₀₋₁₂₀ min during an acute oral glucose tolerance test (OGTT) in C57 mice with no sign of adverse effects on the gallbladder. A two-week sub-chronic model in db/db mice revealed that (**I**) improved glucose disposal but also stimulated gallbladder filling. Further studies on non-systemic TGR5 agonists are needed to develop clinically useful agents.

MEDI 385

Ghrelin O-acyl transferase (GOAT) inhibitors: Optimization of the 6-chloro-2-methyl-5-[2-(4-piperidyl)ethyl]pyrimidin-4-amine scaffold

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Ghrelin O-Acyl Transferase (GOAT) is the enzyme that transfers *n*-octanoic acid to the third serine residue of ghrelin peptide. Unacylated ghrelin (UAG) requires this modification into acylated ghrelin (AG) to exhibit optimal activity at GHS-R1a (growth hormone secretagogue receptor). GOAT is co-localized with ghrelin in the stomach enabling the production of active ghrelin. The hypothesis that inhibition of GOAT in humans will lead to improved metabolic control has not yet been proven due to the lack of clinical compounds inhibiting GOAT. In a previous poster we presented the identification of a few advanced compounds of key value to validate the preclinical GOAT hypothesis. These compounds were considered as suitable starting points for medicinal chemistry optimization based on potency and PK profile refinement. In this poster we will describe the SAR expansion and the small modifications that provided better affinity inhibiting GOAT with optimal PK characteristics. The synthesis, *in vitro* and *in vivo* profile of a few pre-clinical development compounds will be presented.

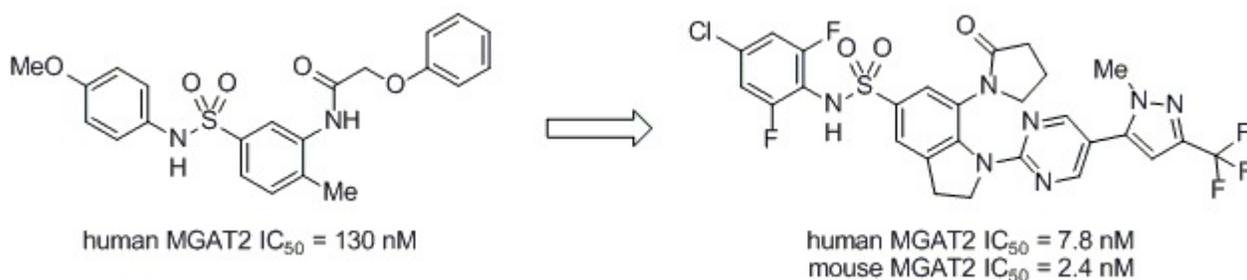
MEDI 386

Discovery of a novel series of *N*-phenylindoline-5-sulfonamide derivatives as potent, selective, and orally bioavailable acyl CoA: monoacylglycerol acyltransferase-2 inhibitors

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Acyl CoA:monoacylglycerol acyltransferase-2 (MGAT2) catalyzes the synthesis of diacylglycerol from monoacylglycerol and fatty acyl CoA, and is considered to play an important role in dietary fat absorption in the intestine. Recent research using MGAT2 gene knockout mice suggested that the inhibition of MGAT2 could serve as a novel peripheral target for the treatment of obesity and metabolic disorders by modulation of lipid metabolism.

Starting from a HTS hit *N*-phenylbenzenesulfonamide with moderate potency for MGAT2 inhibition, we identified *N*-phenylindoline-5-sulfonamide derivatives which displayed much improved potency, by rearrangement of a hydrophobic group to the adjacent position combined with introduction of a bicyclic central core to restrict the substituent orientation. Further optimization efforts resulted in the discovery of *N*-(4-chloro-2,6-difluorophenyl)-1-{5-[1-methyl-3-(trifluoromethyl)-1*H*-pyrazol-5-yl]pyrimidin-2-yl}-7-(2-oxopyrrolidin-1-yl)-2,3-dihydro-1*H*-indole-5-sulfonamide with potent MGAT2 inhibitory activity ($IC_{50} = 7.8$ nM), high selectivity against related acyltransferases (DGAT1, DGAT2, and ACAT1), and excellent ADME-Tox profiles. In a mouse oral fat tolerance test, this compound effectively and dose-dependently suppressed the elevation of plasma triacylglycerol levels after oral administration at doses of 1 and 3 mg/kg. In this presentation, we will describe the design, synthesis, and biological evaluation of a novel series of *N*-phenylindoline-5-sulfonamide derivatives as potent, selective, and orally bioavailable MGAT2 inhibitors.



MEDI 387

Design, synthesis, and mechanism of action determination of flupirtine derivatives with enhanced neuroprotective activity

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Neurodegenerative diseases such as Amyotrophic Lateral Sclerosis (ALS) and the Neuronal Ceroid Lipofuscinoses (NCLs) afflict patients across all ages from infant to adult. No effective drug therapy is available for either disease resulting in a clear urgent and unmet medical need to develop novel therapeutics.

The pathophysiology of both ALS and NCL are poorly understood. While both diseases have distinctly different pathophysiology, symptoms and presentation two small molecules have been identified to offer protective effect in in vitro models of both diseases, perhaps offering a common target for therapeutic exploitation.

The non-opioid analgesic flupirtine and its bioisostere, retigabine, were shown to increase the in vitro survival of SOD1^{A4V/+} ALS motor neurons. Flupirtine has separately been shown to ameliorate apoptosis in NCL patient-derived CLN3-deficient lymphoblasts. No medicinal chemistry structure-activity relationship (SAR) studies have been conducted to investigate the potential of these compounds as leads for neurodegenerative drug discovery. The leads are known to induce the anti-apoptotic protein Bcl-2 (a function of the mutated CLN3 gene) and function as Kv7 potassium channel agonists, resulting in reduction of hyperexcitability. However, both compounds have a range of additional mechanisms that result in neuroprotective activity. The actual mechanism(s) of action has never been determined.

We are applying a chemical genetics approach to identify the mechanism of action of chemical probes derivatives of the lead compounds. A unique library of derivatives abort etoposide-induced and serum starvation induced-apoptosis in neuron-like PC12 cells with several compounds possessing greater protective effect than the leads. These greater efficacy compounds provide protective effects in phenotypic CLN3^{-/-} knockdown and SOD1^{G93A} cell lines, with further screening in a patient-derived stem cell model pending. We have demonstrated that derivatives with greater neuroprotective ability do not function to ameliorate reactive oxygen species.

MEDI 388

Discovery and quantitative pharmacology of novel azetidide-containing PDE10A inhibitors

Qingyian Liu, qingyian@amgen.com, Albert K. Amegadzie, Jian J. Chen, Ning Chen, Michael J. Frohn, Essa H. Hu, Matthew R. Kaller, Vu Ma, Tom Nguyen, Alex Pickrell, Wenyuan Qian, Shannon Rumpf, Robert M. Rzasa, Kristin Andrews, Sharon Zhao, Carl Davis, Jessica Able, Jianxia shi, Geraldine Hill Della Puppa, Mary Dovlatyan, Hang chen, Silke Miller, James Treanor, Thomas Kornecook, Wenge Zhong, Jennifer R. Allen. Amgen Inc., Thousand Oaks, California, United States

Phosphodiesterase 10 A (PDE10A) is a member of the phosphodiesterase family of enzymes that is intimately involved in the regulation of the cyclic nucleotides, cAMP and cGMP. It is predominantly expressed in medium spiny neurons in the striatum of the brain and is believed to play an important role in the pathophysiology of schizophrenia.

As a result, selective inhibition of PDE10A is thought to represent a novel mechanism for the treatment of positive, negative, and cognitive symptoms of schizophrenia. AMG 579, a potent and selective PDE10A inhibitor, was identified as our first generation clinical candidate. In this poster, we report the identification and development of azetidine-containing PDE10A inhibitors as a novel and structurally distinct back-up chemical series to AMG 579. Structure activity relationship (SAR) studies were performed to increase PDE10A potency, improve selectivity over PDE2 and PDE4, reduce hERG liability, and improve *in vitro* ADME properties. Target occupancy (TO) assays were used to screen inhibitors for *in vivo* PDE10A potency. This lead optimization led to several azetidine-containing PDE10A inhibitors that demonstrated high TO by both *ex vivo* and *in vivo* LC-MS/MS TO methods. The potent lead 4-(3-(1-(7-chloroquinazolin-2-yl)azetidin-3-yl)pyrazin-2-yl)-N-methylpicolinamide (PDE10A IC₅₀ = 1.4 nM) was determined to have a TO ED₅₀ in rats of 1.0 mg/kg, corresponding to an unbound EC₅₀ of 5.1 nM. A quantitative pharmacology relationship between *in vivo* TO and unbound plasma concentrations of these inhibitors compared to their *in vitro* PDE10A potency will also be discussed.

MEDI 388

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unbound EC₅₀ of 5.1 nM. A quantitative pharmacology relationship between *in vivo* TO and unbound plasma concentrations of these inhibitors compared to their *in vitro* PDE10A potency will also be discussed.

MEDI 389

Design, synthesis and pharmacological evaluation of benzamide derivatives of 1,3,4-thiadiazole as acetylcholinesterase inhibitors for cognitive dysfunction

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Alzheimer's Disease (AD) is a progressive debilitating neurodegenerative disorder characterized by loss of intellectual and cognitive functionalities of an individual. Cognition refers to a combination of capabilities of a person to discern, observe, understand, reminisces and rationalize any particular situation or event. Research undertaken during the past two decades has led to a better understanding of the underlying pathophysiology, suggesting the role of deficit cholinergic neurotransmission in AD. In pursuit of bringing out potential lead molecules of interest for the therapeutics of cognitive decline, 1,3,4-thiadiazole seems to be a propitious scaffold that has a propensity to be developed as acetylcholinesterase inhibitors for the treatment of cognitive dysfunction.

The present study fosters the design and synthesis of novel therapeutically useful 1,3,4-thiadiazole derivatives for treating cognitive impairments associated with AD. The novel 1,3,4-thiadiazole derivatives were synthesized by carrying out suitable structural modifications at the 2nd and 5th position of the heterocycle. Mixture of aryl carboxylic acid (2-chloro benzoic acid and p-toluic acid) and thiosemicarbazide were refluxed in phosphorus oxychloride to afford cyclic 1,3,4-thiadiazoles. The amino group at 2nd position of 1,3,4-thiadiazole scaffold was derivatised into amide by refluxing with different acid chlorides in tetrahydrofuran (THF) according to the principles of Schotten Baumann Reaction (**PA-7 to PA-14**). These newly synthesized compounds were evaluated against behavioural alterations using morris water maze and passive avoidance (laca mice), at a dose of 0.5mg/kg with reference to the standard, Rivastigmine. Biochemical estimation of different makers for cholinergic activity and oxidative stress has also been carried out.

The synthesised compounds were characterized using spectral and elemental analysis. All the compounds (**PA-7 to PA-14**) exhibited promising activity comparable to that of standard, Rivastigmine. The pharmacological and biochemical evaluation of the synthesized compounds suggested that 1,3,4-thiadiazole derivatives can be further traversed as promising leads for the therapeutics of cognitive decline.

MEDI 390

GC-MS and GC-IRD studies on S cathinones: Bath salt-type aminoketone designer drugs related to MDPV

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This presentation will describe our research efforts to evaluate the structure-retention, structure-fragmentation and other structure-property analytical relationships for a large series of substituted aminoketones related to the cathinone-type drugs.

Our research has focused on the development of regioisomer specific methods for the identification of ring substituted aminoketone compounds (cathinone derivatives). The work includes the chemical synthesis of homologous and regioisomeric forms of selected aromatic ring substituted aminoketones; generation of analytical profiles for each compound; chromatographic studies to separate/resolve homologous and regioisomeric aminoketones having overlapping analytical profiles, and design and validation of confirmation level methods to identify individual compounds.

Based on the structure of the unsubstituted cathinone molecule, designer modifications are possible in three distinct regions of the molecule: the aromatic ring, the alkyl side chain and the amino group. Example compounds from all three of these areas of designer modification have been reported as components of clandestine drug samples. Commercially available precursor aldehydes, alkyl halides and amines can be converted to a wide variety of cathinone-type compounds. Legal control of a specific molecule often provides the driving force for clandestine development of additional substituted cathinone designer molecules.

The synthesis, GC-MS, IR and related spectroscopic properties will be presented for several series of substituted cathinone derivatives.

MEDI 391

Syntheses and evaluations of arylbicycloalkylamines as NMDAR antagonists

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Glutamate is an excitatory neurotransmitter in the CNS that promotes signal transduction. When activity of glutamate becomes excessive, cellular damage can ensue, leading to apoptosis (programmed cell death) or necrotic cell death due to oxidative stress. NMDAR (*N*-methyl-D-aspartate receptor) over-activity has been associated with pathological observations that stem from excess glutamate during states such as ischemic stroke, neuropathic pain, epilepsy, and Alzheimer's disease. A

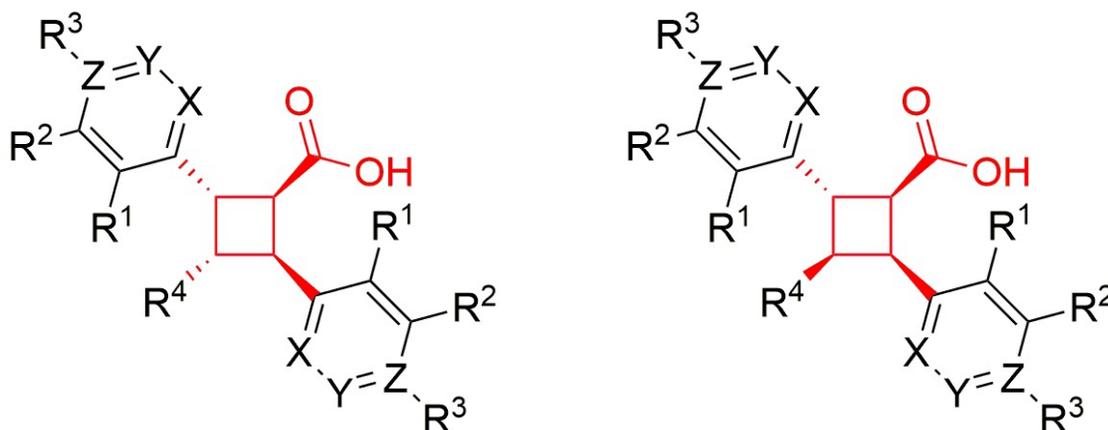
developing treatment strategy is to use NMDAR antagonists to help alleviate excessive glutamate agonism. Uncompetitive antagonists can act by occupying the NMDAR cation channel and prevent excessive activation. The purpose of this study is to synthesize and evaluate the pharmacological properties of arylbicyclo[2.2.2]octane amines as NMDAR probes. These target compounds were designed based on phencyclidine (PCP) as lead. The synthesis utilizes a nine-step procedure to produce novel arylbicycloalkylamines. The reaction scheme begins with a Diels Alder reaction to generate the bicyclic ring, a Grignard reaction to introduce the aryl group, and a reduction to generate the amine. Finally the amine is substituted using a microwave assisted S_N2 reaction. The bindings of target compounds to NMDAR and other CNS receptors will be evaluated to take advantage of complementary polypharmacological factors influencing pathology.

MEDI 392

Synthesis and optimization of truxillic acid-based fatty acid binding protein inhibitors as anti-nociceptive and anti-inflammatory drugs

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The endocannabinoid anandamide (AEA), is an antinociceptive lipid that is linked to the regulation of stress, pain, and inflammation. It is inactivated through cellular uptake and subsequent catabolism by fatty acid amide hydrolase (FAAH). Fatty acid binding protein 5 (FABP5) and fatty acid binding protein 7 (FABP7) are intracellular carriers that deliver AEA and related *N*-acylethanolamines (NAEs) to FAAH for hydrolysis. Inhibition of FABP5 and FABP7 will lead to higher extracellular anandamide levels, resulting in anti-inflammatory and anti-nociceptive effects. Based on these observations, FABP5 and FABP7 would make good drug targets. Prior work from our laboratories has demonstrated α -truxillic acid 1-naphthylmono-ester (SBFI26) to exhibit strong binding to FABP5 and potent anti-nociceptive effects in mice. We designed a series of α - and γ -truxillic acid-based FABP5 inhibitors using Autodock software, based on the co-crystal structure of FABP5-SB-FI-26. These compounds were synthesized to optimize potency and pharmacological properties. The analogues include substitutions on the phenyl, heteroaromatic, and carboxylic acid moieties. The design and synthesis of α - and γ -truxillic acid-based inhibitors, their biological evaluations, and SAR studies will be presented.



MEDI 393

Synthesis & evaluation of a dopamine D3 receptor-selective positron emission tomography probe

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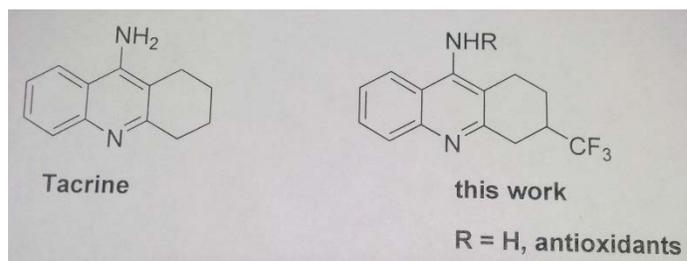
Dysfunction in dopamine transmission is implicated in several neurological and subsequent neurodegenerative and psychiatric disorders. Specifically, this includes depression, schizophrenia, and Parkinson's disease, with evidence supporting a role in drug abuse. There are five dopamine receptors, G-protein coupled receptors (GPCRs): D-1 like (D1, D5), and D2-like (D2, D3, D4), which mediate dopamine transmission. These GPCRs have proven to be useful drug targets for a variety of disorders – most notably the D3R, which is targeted by D3 partial agonist aripiprazole for treatment in schizophrenia and manic depression. A challenge here lies in the high sequence homology between receptor subtypes D2 and D3, primarily at the helical transmembrane section, or orthosteric dopamine binding site. The current understanding and characterization of the D3R is incomplete as its pharmacology is yet to be fully explored, and its utility in further therapeutic application requires investigation. Positron Emission Tomography (PET) is a non-invasive, *in vivo* imaging modality for the direct investigation of the appropriate drug target and subsequent action of the drug, but with less unwanted effects due to dosing below that required to elicit a pharmacological effect. The availability of PET radiotracers for the study of D3R are limited and lack selectivity for either D2 or D3 receptors, including [¹¹C]raclopride and [¹⁸F]fallypride; even the more recent [¹¹C](+)-PHNO is a D3 preferring radioligand with only ~20-fold selectivity. Herein we report progress exploring the synthesis and evaluation of novel radiolabeled (Fluorine-18 or Carbon-11) bioactive molecules with improved selectivity for the Dopamine D3R to better exploit it as a pharmacological target.

MEDI 394

Synthesis and biological evaluation of novel fluorinated tacrine hybrids against Alzheimer's disease

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Alzheimer's disease (AD) remains an unmet medical need despite global efforts to identify drugs that are potent, safe and selective. Unfortunately AD is a multifactorial disorder in which several factors impact both its etiology and pathogenesis. In particular, decreased levels of acetylcholine (ACh), accumulation of β -amyloid plaques, and neurofibrillary tangles, including oxidative stress have been identified as symptoms of AD. Studies involving the inhibition of acetylcholinesterase, the enzyme that hydrolyzes ACh led to the approval of tacrine for the treatment of AD. Later, tacrine was found to be unselective, since it inhibits both AChE and butyrylcholinesterase (BuChE). In addition, tacrine was found to induce oxidative stress. Rather than relying on one molecule one target paradigm current approach involves multi-target ligand design (MTLD) with the goal of finding a single molecule capable of modulating multiple targets. This approach is expected to be a more effective and safe strategy than the one drug one target approach. The primary objective of this study is to design novel fluorinated tacrine hybrids that contain pharmacophores for enzyme inhibition, and antioxidant properties, and to evaluate them for possible inhibition of the above enzyme. We will incorporate a trifluoromethyl group on the cyclohexane ring of tacrine. Since the drug is destined to the CNS, the trifluoromethyl group is expected to enhance blood brain barrier (BBB) penetration and result in better drug-target interaction. The synthesis, characterization, and enzyme inhibition studies will be presented.



Fluorinated Tacrine Hybrid

MEDI 395

Quinazoline and quinoline derivatives as inhibitors of adaptor associated kinase

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The effective treatment of chronic pain, in particular neuropathic pain, is an area of significant unmet medical need. A mouse gene knock out approach combined with evaluation of pain behavior resulted in the identification of adaptor associated kinase 1 (AAK1), also known as AP2-associated protein kinase 1, as a potential novel therapeutic target for neuropathic pain. AAK1 is a member of the Ark1/Prk1 family of serine/threonine kinases and plays a role in modulating receptor endocytosis, but was not previously associated with neuropathic pain. In a mouse PD marker assay, dose dependent inhibition of phosphorylation of AAK1 substrate $\mu 2$ in the brain was observed. An aryl amide-based AAK1 inhibitor was previously identified as a brain penetrant, AAK1-selective compound that was active in animal models for neuropathic pain. Additional structure-activity relationship (SAR) studies were conducted by constraining the aryl amide to form either a quinazoline or quinoline ring system. The synthesis, SAR, and in vivo evaluation of a series of quinazoline and quinoline derivatives as AAK1 inhibitors will be described.

MEDI 396

Synthesis & optimization of vinyl sulfone compounds as Nrf2 activator

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Although the etiology of Parkinson's disease (PD) remains elusive, recent studies suggest that oxidative stress contributes to the cascade leading to dopaminergic (DAergic) neurodegeneration.

The Nrf2 signaling is the main pathway responsible for cellular defense system against oxidative stress. Nrf2 is a transcription factor that regulates environmental stress response by inducing expression of antioxidant enzyme genes. We have synthesized novel vinyl sulfone derivatives and carried out a target-specific functional cellular assay

for Nrf2 translocation. Among them, compound **28** was confirmed to activate Nrf2 and induce expression of the Nrf2-dependent antioxidant enzymes NQO1, GCLC, GLCM, and HO-1, at both mRNA and protein levels in DAergic neuronal cells. In this work, we have focused our attention onto the optimization for microsomal stability, plasma stability, hERG channel safety and mutagenicity. Novel vinyl sulfone compound **43** exhibited potent activity, excellent hERG safety and no mutagenic potential.

MEDI 397

Synthesis of imidazobenzodiazepine oxazole bioisosteres as potential alpha 2, 3 selective GABA(A) receptors agonists with improved antiepileptic and antinociceptive efficacy

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A series of new imidazobenzodiazepine oxazole bioisosteres with various substituents were designed, synthesized, and evaluated for their alpha 2, 3- subtype selective GABA(A) receptor efficacy with improved brain penetrability and associated antiepileptic and antinociceptive effects. Originally a 2'-F oxazole bioisostere was synthesized as a byproduct during the preparation of an ethyl ester. We have now successfully synthesized imidazobenzodiazepine oxazole bioisosteres with improved product yields from 9 % to 70 % using a newly designed synthetic route. One analogue (**KRM-II-81**) in particular was the most promising candidate of the series, and this lead compound (**KRM-II-81**) is a better anxiolytic than our best compounds (HZ-166 and XHe-II-53, Fischer, Rowlett et al., 2010) and has a great PK profile in mice/rats. Positive allosteric modulators of GABA_A receptors transduce a host of beneficial effects but can have motor and sedative liabilities. A benzodiazepine, HZ-166, had previously been characterized as an alpha 2,3-selective GABA(A) receptor modulator with anticonvulsant, anxiolytic, and anti-nociceptive properties with no motor effects. However, the ester moiety in HZ-166 was quickly metabolized in rodents to the carboxylic acid leading to variable exposures of ester in plasma and brain. The compound was stable in human liver, blood and brain tissue as well as in primates. Moreover, the acid of HZ-166 possessed significant *in vitro* activity and may well contribute to efficacy seen in *in vivo* models. We have identified five membered ring heterocycles (oxazoles) as competent ester replacements which lend themselves to comparable *in vitro* potency, reduced metabolic complications, consequently leading to an overall increase in unbound central concentration and thus increased efficacy in animal models of pain and epilepsy.

MEDI 398

Syntheses and pharmacological characterizations of arylbicycloheptylamines as uncompetitive NMDAR antagonists

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The N-methyl-D-aspartate receptor (NMDAR) plays a role in synaptic plasticity, a process required for cognitive functions such as memory and learning. NMDAR antagonists may aid symptomatic and behavioral relief in many CNS disorders including Alzheimer's disease, depression, and neuropathic pain. Few drugs with this mechanism of action are clinically approved; many fail in clinical trials due to poor tolerability. We have been investigating the arylbicycloheptylamine class of compounds which bind NMDAR as uncompetitive antagonists. Our hypothesis is that chemical properties necessary for the desired activities can be separated from those leading to poor tolerabilities. Functional group manipulations have led to discovery of lead compounds that are tolerable in mice and rats, including 3,4-methylenedioxyphenylbicycloheptylamine (3,4-MD-PBCHA). The goal of the project is to synthesize 3,4-MD-PBCHA and derivatives; and examine their pharmacological properties. The compounds were synthesized in gram quantities using modifications of the Geneste route as previously reported by our laboratory. Chromatography methods, MS, NMR, and elemental analyses were used to characterize structures and confirm purities. NMDAR bindings of the compounds were assessed using [³H]MK-801 competitive binding assays. The compounds were assessed on over 40 CNS receptors to ascertain activities and selectivities. Several compounds with desired profiles have been obtained. Enantiomeric resolutions of the compounds have been embarked upon.

MEDI 399

Targeting ion channels, transporters, and GPCRs with monoclonal antibodies

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Integral membrane proteins are important drug targets and monoclonal antibodies (MAbs) directed against them are highly sought for research, diagnostic, and therapeutic purposes. However, the complex structure of membrane proteins makes the discovery and characterization of these MAbs especially challenging. We have implemented a robust approach for the generation and characterization of antibodies against complex epitopes on many structural classes of membrane proteins including G protein-coupled receptors, transporters, and ion channels. Our approach combines DNA and virus-like particle (VLP) immunizations with B cell cloning and phage display to generate antibodies with specific biological properties. As a specific example, we have generated potent antibodies targeting P2X3, a ligand-gated ion channel and a validated

target for neuropathic pain. Thorough characterization of the P2X3 MAbs reveals that they bind diverse conformational epitopes on P2X3 with high specificity. Using VLPs coupled to an optical biosensor, we measured the sub-nanomolar affinity and kinetics of the binding of the MAbs to P2X3. The P2X3 MAbs inhibited ATP-mediated calcium influx in transfected cells and, more importantly, blocked 90% of neuronal currents in primary cells *ex vivo*, enabling further development of lead MAb candidates. We have also generated antibodies against the glucose transporter GLUT4 and demonstrate its utility as a critical reagent for studying the up-regulation of glucose transport by insulin.

MEDI 400

Novel sigma-2 receptor modulators for the treatment of Alzheimer's disease

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Disease modifying therapies for Alzheimer's disease (AD) continue to be a significant unmet medical need. According to the CDC, AD was the 5th disease-related cause of death in people over age 65 in 2010. There currently are only five drugs approved for AD, and all are palliative treatments that slow cognitive decline by regulating neurotransmitters. The lack of validate targets is a major factor contributing to the paucity of available AD treatments. The soluble oligomeric form of the A β protein (A β O), is widely considered to be a viable target. The oligomer hypothesis of AD postulates that A β O are the most potent neurotoxic form of the A β protein. A β O bind specifically and saturably to a receptor site on neurons, rapidly inhibiting long term potentiation (LTP) by altering glutamate receptor trafficking to the plasma membrane. This leads to a transient synaptic spine regression and inhibition of learning and memory. Recent published work demonstrates that sigma-2/PGRMC1 (sigma-2) modulates the binding of A β O to receptors on neurons and that this protein can be targeted with small molecules to inhibit the binding and synaptotoxic effects of A β O. This study demonstrated that small molecules directed towards the sigma-2/PGRMC1 receptor can prevent and displace binding of A β oligomers to neurons. By stopping the initiating event in the oligomer cascade, these first-in-class small molecule drug candidates block downstream synaptotoxicity and restore memory in aged transgenic mouse models of AD. Recently, we have identified a novel series of arylpiperazinyl butyrolactones sigma-2 binders that have the significant potential for further advancement as part of drug discovery program. To date, over 100 compounds have been prepared in this new series, and many examples exhibit potent Sigma-2 binding (IC₅₀ < 100nM). The synthesis, biological activity and in vitro ADME of this series will be discussed.

MEDI 401

Synthesis and profiling of CNS prodrugs of 5-lipoxygenase (5-LO) inhibitors

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Purpose and hypothesis: Pharmacological blockade of 5-LO through inhibitors like zileuton has been shown to reduce both misprocessing of amyloid precursor protein and over-phosphorylation of tau protein in transgenic mice, two hallmarks of Alzheimer's disease. However, zileuton suffers from poor brain penetration due to its polar hydroxyurea group. Prodrugs of zileuton that deliver high concentrations of the parent molecule to the brain could overcome this problem. We designed and synthesized 3 types of brain penetrable prodrugs for 5-LO inhibitors: lipophilic, chemical delivery systems (CDS), and transporter mediated prodrugs.

Methods: 1) Synthesis: the lipophilic prodrugs were synthesized by incorporating lipophilic pro-moieties into the drug through ester, carbonate, and carbamate bonds; CDS prodrugs were prepared by incorporating linked trigonelline or dihydroquinoline into the drug; the transporter mediated prodrugs were synthesized by adding transporter-recognized small molecules such as glucose, lysine, and tyrosine onto the drug. 2) Solubility and stability Assays: aqueous solubility was assessed at a concentration of 200 μ M and pH 7.4. Aqueous and plasma stability was tested at 1 μ M in C57BL/6 mouse plasma for 1 hr. GI tract stability assay was tested in 10 μ M in simulated gastric fluid for 1 hr and in simulated intestinal fluid for 3 hrs.

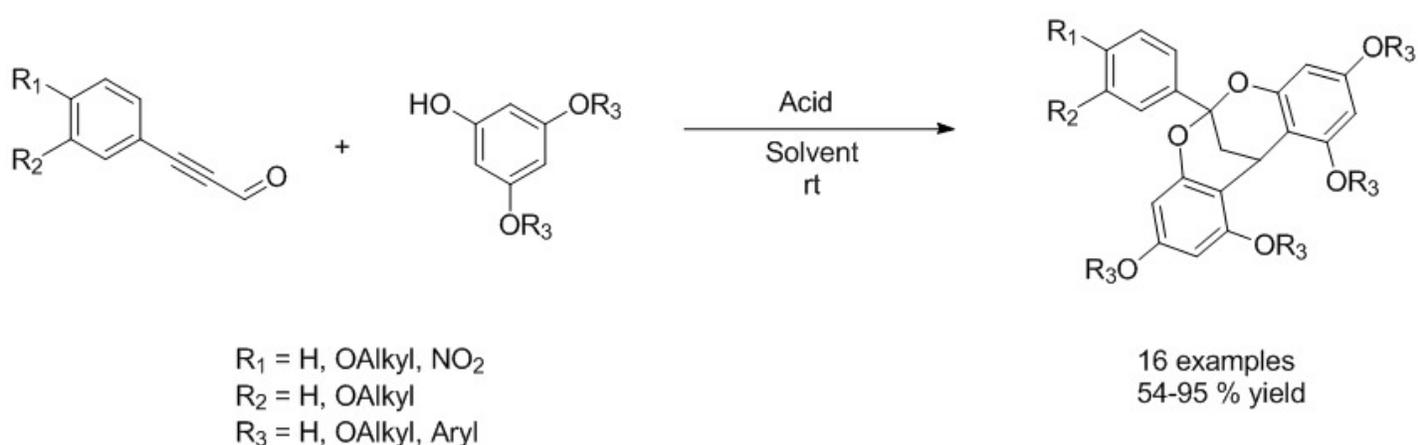
Results, discussion, future direction: We synthesized 128 prodrugs. Most of them have good solubility, in which 15 prodrugs (carbamates, CDS with amide bonds) showed good plasma stability and GI tract stability. These data will be used to prioritize prodrugs for CNS stability studies, in vivo pharmacokinetic assessment and, ultimately, in vivo evaluation.

MEDI 402

New synthetic approach to procyanidins

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Procyanidins are naturally occurring biopolymers which are present in a variety of plants and vegetables. They have been found to exhibit a broad scope of biological activities including antioxidative, antitumor, anti-inflammatory, and free-radical scavenging activity. Present work introduces a new synthetic approach for A-type procyanidins. Condensation of phloroglucinol with phenylpropargyl aldehydes afforded A-type procyanidin core structures in 54-95 % yield.

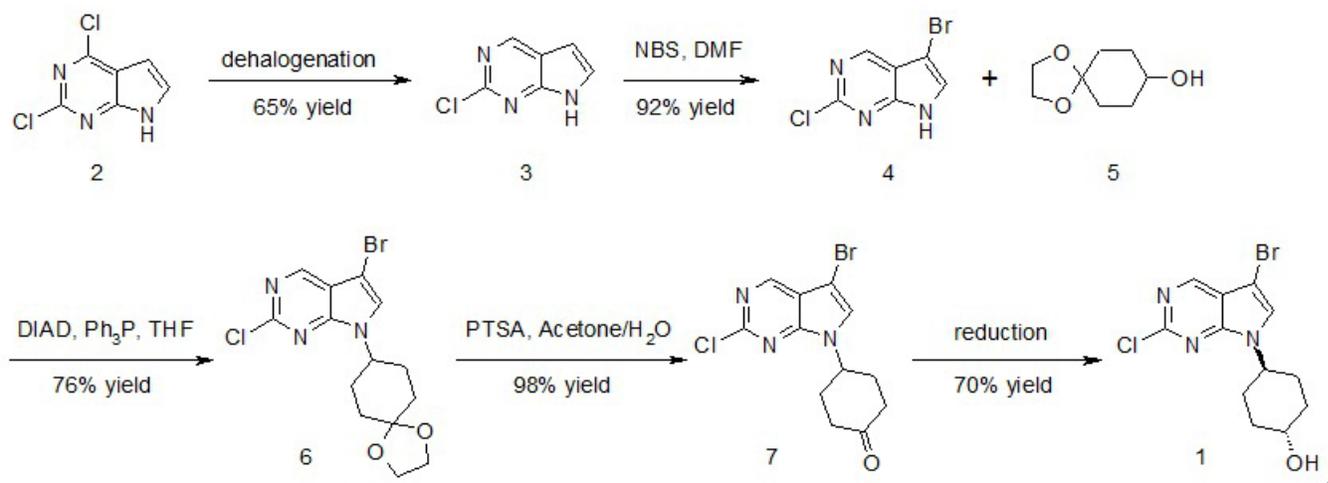


MEDI 403

Development of a multikilogram scale synthesis of *trans*-4-(5-bromo-2-chloro-pyrrolo[2,3-d]pyrimidin-7-yl)-cyclohexanol: A key intermediate for MER/FLT3 dual inhibitors

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A process route for *trans*-4-(5-Bromo-2-chloro-pyrrolo[2,3-d]pyrimidin-7-yl)-cyclohexanol (**1**), the key intermediate for MER/FLT3 dual inhibitors is developed. The pivotal steps are to increase the selectivity of dechlorination in the first step, and to obtain pure *trans*-**1** in the last step. With this process in hand, greater than 5 kg of pure *trans*-**1** can be obtained in one batch.



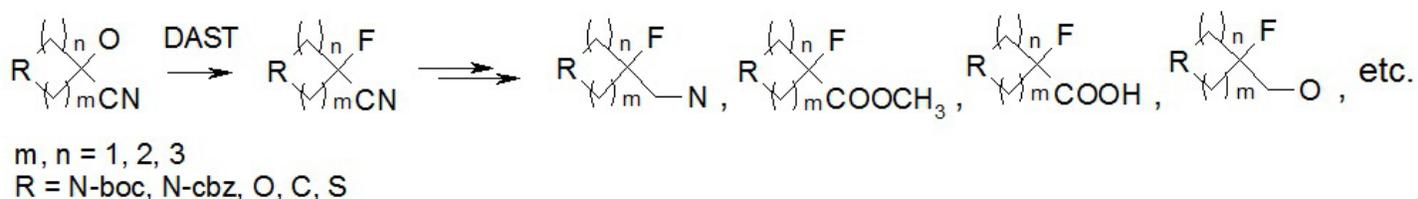
Scheme 1. Multikilogram scale synthesis of pure *trans*-**1**

MEDI 404

Synthesis of α -fluoro nitriles and derivatives

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α -Fluoro nitriles and their derivatives are privileged structure motif found ever increased application in medicinal and agrochemical chemistry. We have developed a very convenient method to prepare them from corresponding cyanohydrins with DAST. Our protocol features inexpensive starting materials, benign reaction conditions, as well as broad substrate scopes. With this method, a series of cyclized aliphatic α -fluoro nitriles, α -fluoro esters, and their derivatives are obtained in good to excellent yields.



Scheme 1. Synthesis of α -fluoro nitriles and derivatives

MEDI 405

Drug patent lifecycle management through follow-on patents, extending the term of patents and regulatory exclusivities

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The pharmaceutical industry spends huge amount of investment to bring a drug to the market. The cost for developing a new drug is enormous because of high attrition rate as they proceed through laboratory, preclinical, clinical and market as well as the huge cost involved in regulatory approvals. The cost to place a drug in the market is estimated as \$2,558 million. As the cost involved in drug development is huge the pharma companies should protect their innovations adequately at right time. Therefore, the drug patent lifecycle management is most important tool for the pharmaceutical companies to recoup the expenditure incurred in the drug discovery research, maximizing the drug product values and make better business decisions. Herein, we discuss the strategies to extend the life of a drug product by various types of patents at different stages of drug developmental process and different regulatory exclusivities of drug product available in various countries and their relation to the patents. The benefits of the patent lifecycle management to the pharmaceutical companies developing a drug will also be presented.

MEDI 406

Microneedles for transdermal delivery of traditional Chinese medicine

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Traditional Chinese medicines (TCM) have more than 3000 years of history and include many bioactive phytochemicals derived from plants. Decoction and oral administration remain common for TCM making it inconvenient and unpopular to younger generation of patients. The possibility of formulating TCM for transdermal delivery is attractive and microneedle could provide an avenue for effective administration of TCM. This work explores the delivery of ferulic acid (FA) derived from TCM, *Danggui (Angelica sinensis)* by microneedle. Ferulic acid is shown to be an antioxidant with pro-apoptotic effects on cancer cell. The microneedle was made of pure silica zeolites that have good hardness and strength, and can cut through skin with minimal peripheral damages. The delivery of ferulic acid was monitored in an ex-situ experiment using pig skin as model skin surface to measure the efficiency of the microneedle transdermal delivery system.

MEDI 407

Synthesis of hollow mesoporous silica nanoparticles and their application for delivery of multiple peptides for melanoma immunotherapy

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Hollow mesoporous silica nanoparticles (HMSNs), as one of the most promising drug carriers, have attracted much attention due to their uniform mesopores, large surface area, excellent biocompatibility, improved loading capacity and easy modification, etc. HMSNs can act as carriers for drug delivery and vaccine adjuvants, markedly improving the antitumor immunity.

We will introduce a facile yet effective method to generate monodisperse HMSNs and load multiple peptides, including hydrophobic and hydrophilic peptides. To improve the biocompatibility of the HMSNs, the peptides loaded HMSNs were coated with lipid bilayers (HMLBs) containing monophosphoryl lipid A adjuvant. Through this experimental design, the resulting HMSNs displayed improved cellular uptake ability and enhanced activation of both tumor specific CD8+ and CD4+ T lymphocytes. In addition, the formed HMLBs greatly inhibited tumor growth and lung metastasis in murine melanoma models. Thus, HMLBs provides a promising platform for co-delivery of multiple peptides, adjuvant and enhancement of anti-tumor efficacy of conventional vaccinations.

MEDI 408

Synthesis of d-labeled and unlabeled ethyl succinic anhydrides and application to quantitative analysis of peptides by MALDI and ESI mass spectrometry

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Quantitative analysis of relative amounts of expressed proteins and identification of the proteins in combination with mass spectrometry are central to proteomics studies. We have thus far reported several kinds of reagents that react with specific amino acid residues and their d- or ¹³C-labeled versions which can successfully measure the relative amounts of peptides or proteins in combination with electrophoresis and soft ionization mass spectrometry such as MALDI or ESI. However, most of the reagents we reported in the past rely on the existence of cysteine residues. Here we report synthesis of a new modifier, 2-ethyl succinic anhydride (ESA), which reacts with the amino group and its d₅-labeled version, and the application of their combination to quantitative analysis of peptides. For this quantitative analysis, we utilized both MALDI TOF and ESI mass spectrometry.

This study finds that either MALDI or ESI mass spectrometer enables quantitative analysis of model peptides with high accuracy. As this method does not require the existence of particular amino acid residues, it is expected to be applicable to quantitative analysis of a wider variety of proteins. These modifiers can also be prepared relatively inexpensively from readily available sources. This study provides an additional method for quantitative analysis of peptides and proteins, which is important for proteomics studies.

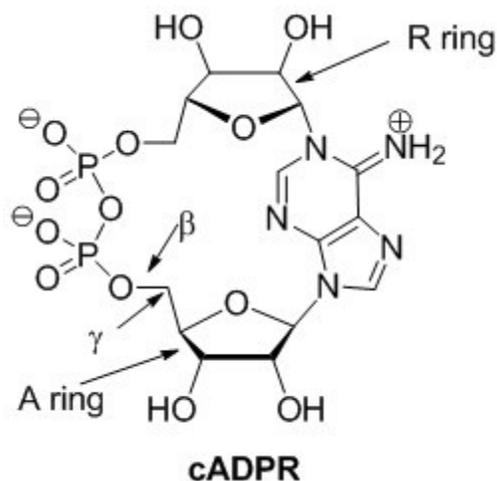
MEDI 409

On the structure-activity relationship of cADPR and cADPR analogs: a high-field NMR study

Steven M. Graham², *grahams@stjohns.edu*, **Janez Plavec**¹, **Uroš Javornik**¹. (1) NMR Ctr Nat Inst of Chem, Ljubljana, Slovenia (2) Chemistry, St Johns University, Jamaica, New York, United States

Numerous cellular processes are regulated by the flow of calcium ions. Cyclic adenosine diphosphate ribose (cADPR), a cyclic metabolite of NAD⁺, is a second messenger that causes release of calcium from intracellular stores. Unraveling the structure-activity relationships in cADPR and cADPR analogs, in particular the conformation of the 5-membered furanose rings, requires access to high-quality ¹H NMR data, particularly ¹H-¹H coupling constants. At lower fields (300- and 400 MHz),

several ^1H signals are overlapped, greatly complicating extraction of the needed coupling constants. This talk will focus on high-field (600- and 800 MHz) NMR studies of cADPR and analogs thereof, as well as the corresponding conformational analysis using the PSEUROT program.



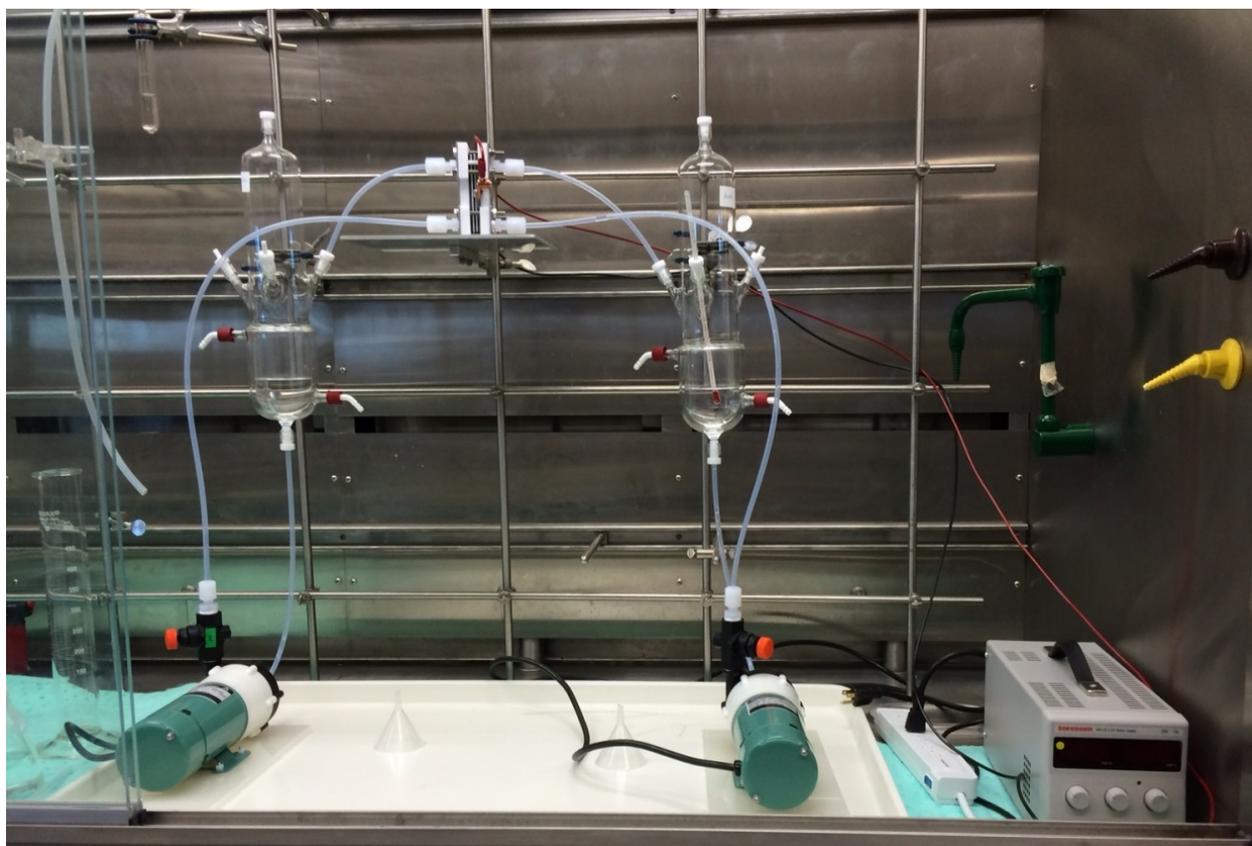
MEDI 410

Electrochemical halogenation: A new method to synthesize intermediates for tritium labeling

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Electrochemistry offers an alternative method for selective oxidation, halogenation, or reduction of organic molecules that yield products quite different from those obtained with chemical reagents. Use of Electrochemistry is more environmentally friendly than the traditional methods that frequently employ transition metal oxidants and harsh reaction conditions. In addition, electrochemical synthesis often uses less toxic raw materials, safer solvents, employs more atom-efficient processes, greatly reduces waste generation, and allows more facile isolation of products.

We explored electrochemical halogenation with NaCl or NaBr with a separate micro-flow cell with recycling provided by mechanical pumps. Aryl halide intermediates are widely used as substrates for tritium labeling. Tritium-labeled molecules have important applications in drug discovery, which include the measurement of affinity binding to target protein and the evaluation of drug clearance and metabolism. Electrochemical halogenation of MK-8457, MK-4618, Sch 48973 and Tenofovir[®] was attempted, and good conversion and yields were obtained. Different regio-selectivity compared to traditional synthetic approaches employing NBS or NCS was observed in some cases. Subsequent tritium-labeling reactions using these intermediates yielded equal or better specific activity. Our results showed that electrochemical halogenation provides a novel, alternative, and greener way to make halide intermediate for tritium-labeling.



separate micro-flow cell

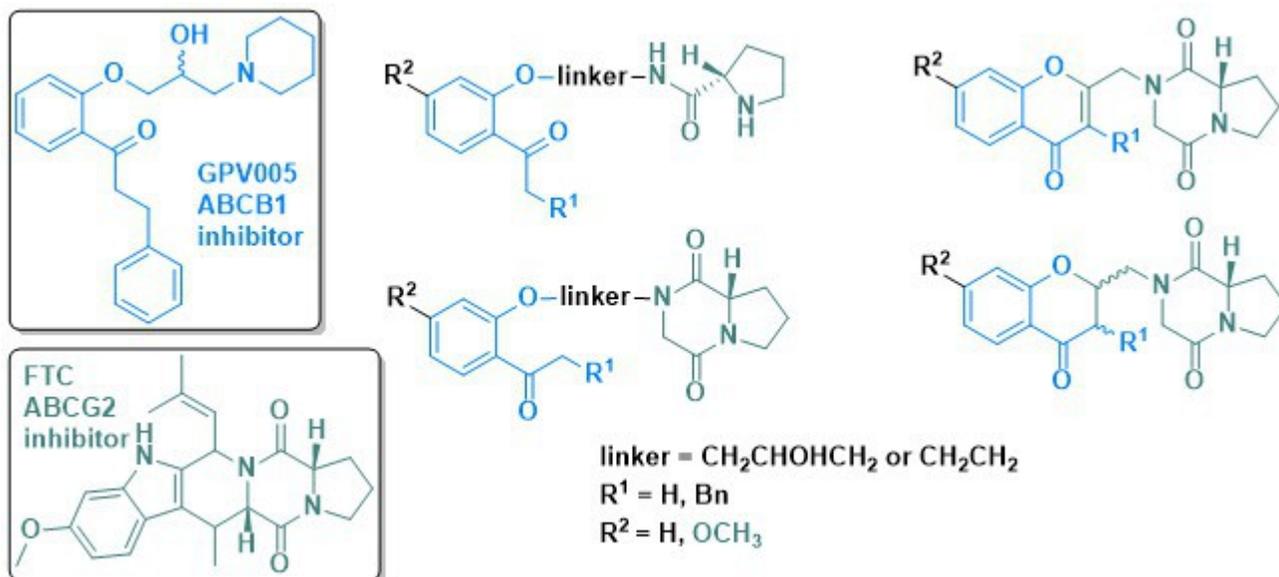
MEDI 411

Synthesis of biotinylated triterpenes and their use in target identification

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During our past research, we discovered several triterpenoids with high and selective cytotoxic activity against multiple cancer cell lines. To better understand their mechanism of action, we are developing proteomics pull-down affinity experiments with the ultimate goal of identifying their molecular targets. For such experiments, we needed to connect active terpenes to biotin through a defined linker (Fig. 1). In this work, we developed a simple and fast solid-phase technique, which we used to biotinylate betulinic acid (of which many molecular targets are known) from three different sides, which we are using as a standard for pull down experiments optimization and validation. In addition, a set of four highly cytotoxic (IC_{50} 0.5-5.0 mM on CCRF-

C, we hope to identify features which lead to selectivity or to general inhibition. In parallel, we established a classification model for predicting the inhibitor profile of the compounds planned for synthesis. Results indicate that the basicity of the nitrogen atom as well as the rigidity of its substituents influence the activity and selectivity of the compounds. Furthermore, the results are generally in good accordance with the *in silico* model.



MEDI 413

Novel architectures for multicationic quaternary ammonium compounds (multiQACs)

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Research in our laboratories has been expanding the structural variety of multicationic quaternary ammonium compounds (multiQACs) via a scaffold-hopping approach. We have identified a number of commercially available bis-, tris-, and tetraamines and performed derivatization to generate some of the most potent antimicrobials reported, with strong inhibition of both Gram-positive and Gram-negative bacteria. These structures also show strong ability to eradicate pre-existing biofilms, including those of methicillin-resistant *S. aureus* (MRSA). Our progress in this area will be reported, with a focus on multi-pyridinium structures with unusual (submicromolar) antimicrobial activity in light of their straightforward assembly. Computational analysis is likewise being performed, both in regards to structural conformation and toxicity approximation.

MEDI 414

Tunable polymersomes: Towards enzyme delivery through the blood-brain barrier

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Although neurological disorders account for 7% of global burden of disease, greater than AIDs and cancer, modern medicine has fallen short of treating the brain due to the presence of the blood-brain barrier (BBB), which prevents passage of 98% of drugs from the blood into the brain. Our work involves the creation of self-assembled polymeric vesicles, nano-polymersomes, from block co-polymer polyethylene glycol-b-poly(lactic acid) (PEG-b-PLA). Our nano-polymersomes show promise towards enzyme delivery to the brain for the first time. Injection method formation led to polymersomes with an overall average diameter of 147.2 ± 24.1 nm, with 88% formed in a deliverable range. Polymersomes around 200 nm in diameter demonstrate transport through the BBB *in vitro*.

The missing enzyme in a neuropathic disease called GM1 gangliosidosis, β -galactosidase (β -gal), was encapsulated into polymersomes during formation at a payload of 0.075 ± 0.030 mg β -gal/mg block co-polymer. An encapsulation efficiency of $86.2 \pm 12.2\%$ of introduced β -gal was determined. Enzyme needs to maintain activity during encapsulation to remain efficacious. In our case, β -gal remained active during and after loading in water at around 100% efficiency, with 650.7 ± 192.9 units of activity/mg β -gal loaded. Release of active β -gal occurs quickly in acidic buffer compared to neutral buffer, demonstrating tunable release from PEG-b-PLA carriers.

Attachment of targeting ligands, CF350 amine and apolipoprotein E (ApoE), were mitigated through homobifunctional PEG, NHS-PEG(2000)-NHS, during polymersome formation. Dynamic light scattering confirmed attachment with a statistical increase in polymersome diameter to 210.5 ± 8.6 nm. Fluorescence microscopy and flow cytometry showed $83.2 \pm 14\%$ of polymersomes both loaded with molecules and targeting ligands. ApoE targets low-density lipoprotein receptors (LDLR) which are upregulated at the BBB during brain inflammation.

Delivery of β -gal into feline GM1 fibroblasts expressing LDLR demonstrates the potential of this carrier to deliver enzymes with appropriate levels of activity into the brain. Results demonstrate strict control over carrier size formed, therapeutic payload, release location, and ligand attachment possible when utilizing polymersomes as an

enzyme delivery vehicle. This novel carrier provides a brain delivery platform for currently untreatable diseases and has the potential to cause a paradigm shift in the way we treat the central nervous system.

MEDI 415

γ -Radiation generates active chlorine species (ACS) in physiological solutions. A novel mechanism of radioprotection by ACS scavengers

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Chloride anions (Cl^-) consumed >90% of hydroxyl radicals ($\cdot\text{OH}$) in physiological solutions produced by γ -radiation resulting in active chlorine species (ACS) formation. Primarily formed ACS are radical, namely chlorine atoms ($\text{Cl}\cdot$) and dichloro radical anions ($\text{Cl}_2\cdot^-$). Hypochlorite (ClO^-) is a secondary ACS formed by the disproportionation of the chlorine molecule in water. Exposure to γ -radiation resulted in increased taurine chlorination, indicative of ClO^- generation.

Secoisolariciresinol diglucoside (SDG) and its simplified structural analog dopamine are effective ACS scavengers.

Proton NMR studies revealed that the ACS scavenging mechanism includes radical substitutive chlorination of benzylic protons in SDG and dopamine, while hypochlorite chlorinates amino group and aromatic rings in dopamine. In the absence of Cl^- , γ -radiation of dopamine solution in water resulted in aldehyde formation as a result of oxidative cleavage of carbon-carbon or/and carbon-nitrogen. Thus, the chloride anion plays the role of a scavenger of reactive oxygen species (ROS).

Importantly, SDG scavenged hypochlorite- and γ -radiation-induced ACS. In addition, SDG blunted ACS-induced fragmentation of calf thymus DNA and plasmid DNA relaxation. SDG treatment before or after ACS exposure decreased the ClO^- or γ -radiation-induced chlorination of 2-aminopurine (2-AP), a fluorescent analog of 6-aminopurine.

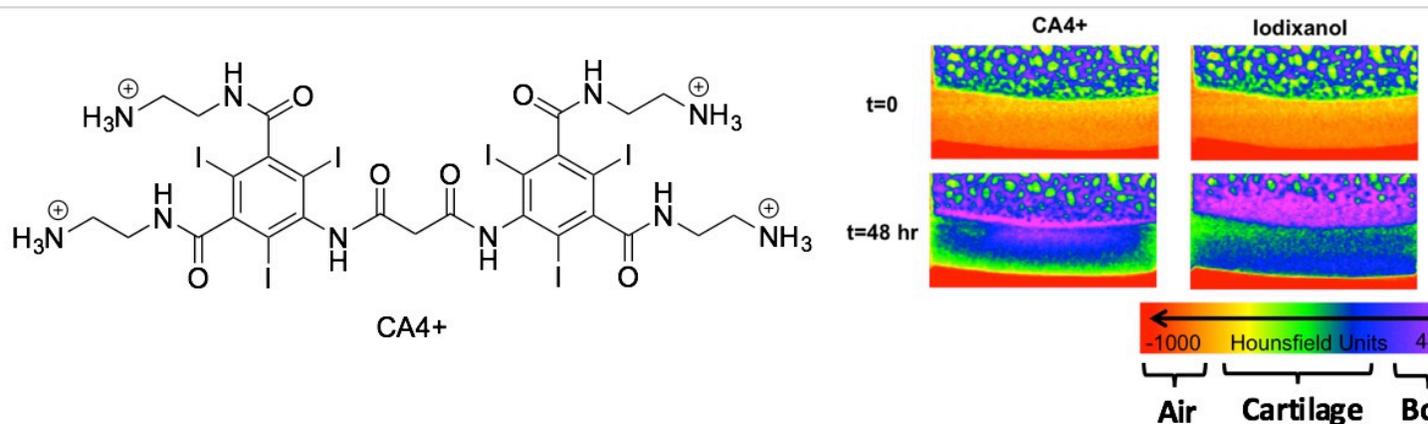
MEDI 416

Large-scale synthesis and pre-clinical characterization of a cationic iodinated imaging contrast agent (CA4+) and its use for quantitative computed tomography of ex vivo human hip cartilage

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Radiology, Boston University School of Medicine, Boston, Massachusetts, United States
(5) Department of Chemistry, Boston University, Boston, Massachusetts, United States

Contrast agents are readily employed in X-ray computed tomography (CT) to visualize tissues to provide better demarcation of tissue boundaries. Contrast agents that go beyond qualitative visualization and enable quantitative assessments of functional tissue performance represent the next generation of clinically useful imaging tools. One clinical area where such contrast agents can have a significant impact is in the diagnosis of soft-tissue-based diseases like osteoarthritis (OA). OA is a prevalent and growing cause of disability worldwide. Development of a sensitive, minimally-invasive diagnostic technique for early OA may enable researchers and clinicians to develop and implement therapeutics to slow or mitigate OA progression, thereby improving patient care. Herein, we describe a cationic iodinated contrast agent (CA4+) for use with CT imaging in an *in situ* assay for biomarkers of cartilage health. CA4+ can be synthesized in bulk and at high purity. *Ex vivo* cartilage experiments indicate that its use in CT imaging affords sensitive, quantitative measurements of cartilage biochemical content and biomechanical functioning, both of which are key indicators of cartilage health.



Contrast Agent (CA4+) and μ CT images of a bovine osteochondral plug visualized in color maps. The contrast agents diffuse into cartilage over time and the color turns from yellow to green to blue indicating higher CT attenuation.

MEDI 417

1,2,3-Triazole inhibitors of *Porphyromonas gingivalis* biofilm formation

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Biofilms allow the establishment and survival of cooperative communities of pathogenic oral bacteria. The binding of *Porphyromonas gingivalis* to *Streptococcus gordonii* biofilms is an initial step toward infection of the subgingival pocket and erosion of gum

tissue. Small polypeptides are capable of inhibiting the binding of *P. gingivalis* to *S. gordonii* and small-molecule peptidomimetics would constitute an improvement in potential therapy owing to stability and cost. 'Click' compounds containing an oxazole backbone, triazole linker and hydrophobic ends were designed as easily-synthesized mimics of the inhibitory peptide. An array of 2-(azidophenyl)- and 2-(azidoalkyl)-4,5-disubstituted oxazoles were prepared and reacted with substituted arylacetylenes to afford the 1,2,3-triazole 'click' products. New strategies in forming the trisubstituted oxazole azide click partners were developed and utilized in the synthetic scheme. Substituted triazines and other heterocyclic-substituted click acetylenes were developed while most of the simpler acetylenic click partners were commercially-available. The title compounds were evaluated for their ability to inhibit *P. gingivalis* adherence to streptococci and several were found to be inhibitory when assayed in vitro. The multi-step syntheses and bioassay data of the biofilm-inhibitory 'click' candidates will be presented.

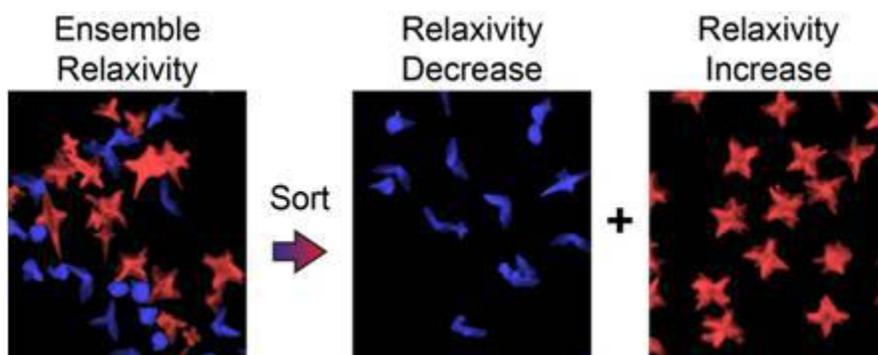
MEDI 418

Shape-dependent relaxivity of nanoparticle-based MRI contrast agent

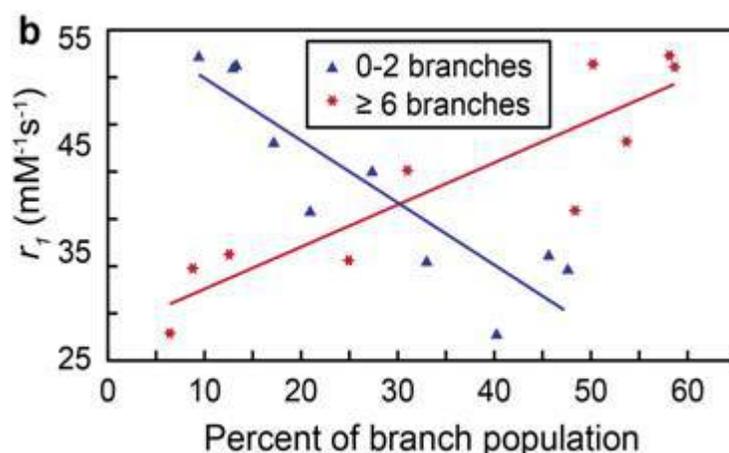
Yu Jin Shin¹, yujinshin@u.northwestern.edu, **Kayla Culver**¹, **Matthew Rotz**², **Thomas J. Meade**², **Mark Hersam**^{1,2}, **Teri W. Odom**^{1,2}. (1) Materials Science and Engineering, Northwestern University, Evanston, Illinois, United States (2) Chemistry, Northwestern University, Evanston, Illinois, United States

When functionalized with Gd, highly branched gold nanoparticles (*i.e.*, gold nanostars) have shown significant promise as contrast agents for magnetic resonance imaging (MRI). However, the size and shape polydispersity of as-synthesized gold nanostar samples have confounded efforts to develop rigorous relationships between the gold nanostar structure (e.g., number of branches) and relaxivity of surface-bound Gd. Here, we found a centrifugal separation method that produces structurally refined subpopulations of gold nanostars and is compatible with subsequent biomolecule surface functionalization. Subsequent characterization with transmission electron microscopy, optical absorbance spectroscopy, and dynamic light scattering shows that increasing nanostar branch number leads to enhanced relaxivity.

In detail, this separation allows the proportion of many- (greater than 6 branches) and few-branched (0-2 branches) gold nanostars to be varied by over a factor of 2, resulting in the relaxivity of the population enriched in many-branched gold nanostars to reach levels as high as 57.3 mM⁻¹s⁻¹. Overall, this work provides insight into the underlying relaxivity mechanisms for Gd-functionalized gold nanostars, thereby informing ongoing efforts to develop high-performance MRI contrast agents.



TEM images of (left) polydisperse unsorted gold nanostars and (right) sorted nanostars by branch numbers via density gradient centrifugation.



Relationship between percent of gold nanostar branch population and relaxivity

MEDI 419

Formulation of insulin for oral dosing

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Diabetes is a chronic condition that causes high blood sugar levels that are potentially fatal if left unmanaged. Type 1 diabetes requires treatment with daily insulin hormone injections, while Type 2 diabetes usually requires treatment with insulin as the disease progresses. Injection of insulin is therefore the primary treatment against this disease. Unfortunately a daily intramuscular injection regimen can be painful and tedious while daily subcutaneous injection, manually or via a pump, is a less efficient delivery mode. Orally available insulin would be a positive development in the treatment of diabetes.

However orally dosed insulin has not been developed yet due to insulin's inability to survive both the acidic environment of the stomach, as well as be absorbed through the intestinal membrane. Work from this laboratory describes the development of a neutral lipid based vesicle (the CholestosomeTM), that uses naturally occurring lipids, for delivery of problematic therapeutics. In this formulation, insulin dose is limited by solubility in the aqueous buffer prior to encapsulation. The present study was undertaken to develop higher dose insulin formulations for CholestosomeTM encapsulation by examination of parameters affecting solubility of insulin. Parameters of pH and ionic strength were systematically tested for effects on encapsulation efficiency in order to optimize insulin dose. Formulations were encapsulated and characterized for size, insulin and lipid content. These formulations have been tested in rats and the data has shown oral availability of insulin.