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J. Macor, Program Chair

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MEDI 1

Development of second generation epigenetic agents

***Philip Jones**, pjones3@mdanderson.org.Institute of Applied Cancer Science, University of Texas MD Anderson Cancer Center, Houston, Texas 77054, United States*

DNA in the nucleus of eukaryote cells is packaged in the nucleosomes around histone proteins. This is highly organized and tightly regulated to control gene transcription. While gene expression patterns are directed by transcription factors which bind to specific promoter and enhancer sequences, it has now been recognized that the chromatin structure also plays a significant role in determining gene expression patterns. Histone proteins are not static; the histone tails undergo a wide variety of dynamic post-translational modifications that regulate gene transcription, as well as other cellular process that require access to DNA, for example DNA repair. There is now ample evidence that these patterns are aberrantly regulated in multiple disease states – most notably in cancer and inflammation.

A wide array of selective, enzyme catalyzed, covalent histone post-translational modifications have been reported, ranging from acetylation and methylation of the lysine residues, as well as ubiquitinylation and sumoylation. Similarly, arginine residues undergo methylation; while serine, threonine and tyrosine residues are subjected to phosphorylation. Furthermore, glutamate and arginine residues in the histone tails can undergo ADPribosylation.

It is now known that multiple classes of proteins control the writing, reading and removal of these covalent histone modifications. The first generation of agents that target some of these histone modifying enzymes have been approved for use in humans, such as vorinostat and romidepsin histone deacetylase inhibitors.

This introduction for Recent Advances in Modulating the Epigenome will focus on the enzymes that modulate the epigenome - the writers, readers and erasers of the histone code. Attention will then be given to the emerging therapeutic opportunities for small molecule drug discovery in this area, and touch upon some of the recent developments in the next generation of novel modulators of the epigenome, which will then be expanded upon in the subsequent session.

MEDI 2

Drug discovery efforts toward the identification and optimization of potent and selective EZH2 inhibitors

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EZH2 (Enhancer of Zeste Homolog 2) is a histone methyltransferase which specifically methylates K27 of histone H3 through the use of the S-adenosylmethionine (SAM) cofactor. This methylation, in concert with other epigenetic mechanisms, modulates gene expression at specific genetic loci and EZH2 has been implicated in many processes including tumor initiation and progression. Therefore it is expected that inhibition of EZH2 catalytic activity will provide potential benefit in the treatment of various cancers. Herein we describe our drug discovery effort in the identification and optimization of potent selective inhibitors with nM potency, their enzymatic mechanism of inhibition, and application in cellular assays is also presented.

MEDI 3

From protein to candidate: Discovery of EPZ-5676, a potent and selective inhibitor of the histone methyltransferase DOT1L

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The clinical candidate EPZ-5676, a DOT1L inhibitor for treatment of MLL-rearranged leukemia, is described. The lead series was discovered *via* structure guided design. Crystallography aided optimization of the pharmacokinetic properties and potency of this series led to EPZ-5676.

MEDI 4

Interrogating epigenomic regulators inside living cells with metabolite mimics

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Protein methyltransferases (PMTs) orchestrate epigenetics through posttranslational methylation and their dysregulation has been frequently implicated in diseases including developmental abnormalities, neurological disorders and cancer. Uncovering the context-dependent targets of PMTs is pivotal toward elucidating their roles in normal physiology and disease states. Unfortunately, few prior tools were available for mapping proteome-wide and genome-wide methylation events in an unambiguous manner. To address this situation, the Luo laboratory recently developed BPPM (Bioorthogonal Profiling of Protein Methylation) technology for profiling the histone and nonhistone targets of multiple PMTs inside living cells. Here, human SAM (*S*-adenosyl-*L*-methionine) synthetase was engineered to process metabolite mimics (terminal-alkyne-containing methionine analogs), thus allowing in situ production of the corresponding SAM analogs. Upon coupling with engineered PMTs, the SAM analogs will be processed to label the histone and nonhistone targets of the corresponding PMTs. The labeled substrates can then be readily enriched via alkyne-azide click chemistry for further analysis. Since only engineered PMTs recognize the SAM analogs, the resultant labeled targets can be assigned unambiguously to the designated (engineered) PMTs. As exemplified here, this unprecedented tool enables to define and dissect dynamic epigenetic events of multiple cancer-relevant PMTs.

MEDI 5

Novel chemical tools for epigenetic targets

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Research into the role of epigenetics in disease could be significantly accelerated if cell active chemical probes for such targets were available to the research community, through a collaborative, open innovation model. Pfizer is a member of a public-private partnership led by the Structural Genomics Consortium (SGC) to help identify a suite of high-quality chemical probes for epigenetic targets. This collaboration has yielded quality chemical tools for bromodomains and histone methyl transferases and the latest developments in these programs will be reported.

MEDI 6

Transcriptional architecture of the circadian clock in mammals

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The circadian clock mechanism in animals involves an autoregulatory transcriptional feedback loop in which CLOCK and BMAL1 activate the transcription of the Period and Cryptochrome genes. The PERIOD and CRYPTOCHROME proteins then feedback and repress their own transcription by interaction with CLOCK and BMAL1. Recently, we

have focused on the biochemical mechanisms of the core circadian transcriptional regulators and have used structural biology and genomics to study the CLOCK:BMAL1 complex and its genomic targets. Using x-ray crystallography, we have solved the three-dimensional structure of the CLOCK:BMAL1 heterodimeric complex. In addition, we have interrogated on a genome-wide level the cis-acting regulatory elements (cistrome) of the entire CLOCK:BMAL1 transcriptional feedback loop. This has revealed a global circadian regulation of transcription factor occupancy, RNA polymerase II recruitment and initiation, nascent transcription and chromatin remodelling. In addition, we have used cell-based circadian rhythms to screen for small molecules that perturb the clock system.

MEDI 7

Chronobiology of inflammation

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We are interested in applying the principles of chronobiology to the development of anti-inflammatory drugs through optimization of their time of day dosing. Asthma and rheumatoid arthritis are known to display circadian symptomatology, with increased morbidity in the early morning. To shed light on the molecular basis for these observations, we have identified components in the lung and inflammatory signaling pathways that are under circadian control. The application of these insights to the design of chronotherapeutics will be discussed.

MEDI 8

Characterization of ligands targeting RAR-related orphan receptor alpha (RORα)

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All animals have evolved complex systems of gene regulation in response to environmental cues such as the 24hr light-dark cycle, which has influenced many aspects of animal behavior and physiology including feeding and metabolism. In mammals, the central clock, located in the Suprachiasmatic Nucleus (SCN) of the hypothalamus, is entrained by light. In turn, the SCN entrains peripheral clocks that are maintained, even in the absence of a light-dark cycle, by a web of interacting gene transcription feedback loops, tightly controlling gene expression with 24hr periodicity.

Two transcription factors, CLOCK and BMAL1, activate gene expression of Per (Period) and Cry (Cryptochrome). The protein products of Per and Cry form a heterodimeric complex that translocates back into the nucleus and inhibits BMAL1 and CLOCK expression, forming a negative feedback loop. Superimposed upon this transcriptional loop are two nuclear hormone receptors, RORα and Rev-erbα, which are ligand dependent transcription factors that have recently been deorphanized. The endogenous ligand for RAR-related orphan receptor alpha (RORα) has been identified as 7α-hydroxycholesterol while Rev-erbα requires heme. Both receptors are constitutive, RORα as an activator and Rev-erbα as a repressor, and they compete for the same binding elements near the transcription start sites of metabolically important genes. Here we describe synthetic ligands for RORα, co-crystal structures of these ligands bound to the ligand binding domain, and protein hydrogen deuterium exchange (HDX) studies of ligand binding. Using these compounds, we identified RORα target genes and demonstrated activity *in vitro* and *in vivo*.

MEDI 9

Synthetic REV-ERB ligands: Tools for probing the chemical biology of the circadian clock

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Physiological processes including metabolism and behavior are governed in a circadian rhythm. This 24h rhythm is maintained by a cell autonomous transcriptional/translational feedback loop composed of the transcription factors BMAL1 and CLOCK and their target genes, PER and CRY. The nuclear receptor REV-ERB also plays an important regulatory role in maintaining the circadian oscillator by direct regulation of the *Bmal1* and *Clock* genes. Appropriate oscillations in this molecular clock are required for normal physiological function and behavior. In fact, abnormal clock function has been associated with a range of disorders including metabolic diseases, sleep disorders, mental disorders and cancer. Using a chemical biology approach, we recently demonstrated that synthetic compounds that modulate the circadian expression of clock genes also alters metabolic processes. We demonstrated that synthetic REV-ERB agonists increase the metabolic rate of mice leading to decreased fat mass. Diet induced obese mice also display weight loss when the REV-ERB agonists is administered and, additionally, demonstrate improved plasma lipid profiles. Here, I will describe recent results examining the effects of REV-ERB agonists in additional models of metabolic disorders as well as models of sleep and anxiety. In summary, our data indicate that drugs that modulate clock activity, REV-ERB agonists in particular, may have utility in treatment of human diseases.

MEDI 10

KLF15 links the circadian clock to arrhythmogenesis

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Sudden cardiac death (SCD) secondary to ventricular arrhythmia is the most common cause of mortality from cardiovascular disease worldwide. Despite decades of investigation, a thorough understanding of triggers and effective pharmacologic treatments for SCD are lacking and the primary treatment remains mechanical defibrillation. This sobering reality has led to the view that a complete re-examination of the fundamental mechanisms underlying the development of SCD is needed to provide a foundation for the development of novel, effective therapies.

Biological processes that oscillate with a 24-hour periodicity are termed circadian. All cells have a circadian clock that synchronizes changes in gene expression with rhythmic patterns of daily life, i.e. eating or sleeping. The observation that SCD exhibits a peak during early morning, is increased in shift workers suggests that circadian influences may be operative. However, a direct link between the circadian clock, metabolism, and cardiac electrical activity was lacking. In this regard, recent studies from our laboratory identified the first molecular link between endogenous circadian rhythms and SCD. Specifically, we reported that a specific cardiac ion channel that controls myocyte repolarization exhibited circadian oscillation under the control of the clock-dependent oscillator KLF15. Alterations in KLF15 levels in vivo rendered animals susceptible to SCD. Finally, the observation that KLF15 is altered in subjects with heart failure and Brugada syndrome suggests that the study of this pathway may have implications for human disease.

MEDI 11

Succeeding in an unprecedented CNS target space: Discovery of selective casein kinase (CK1δ/ε) inhibitors for the treatment of circadian rhythm disorder

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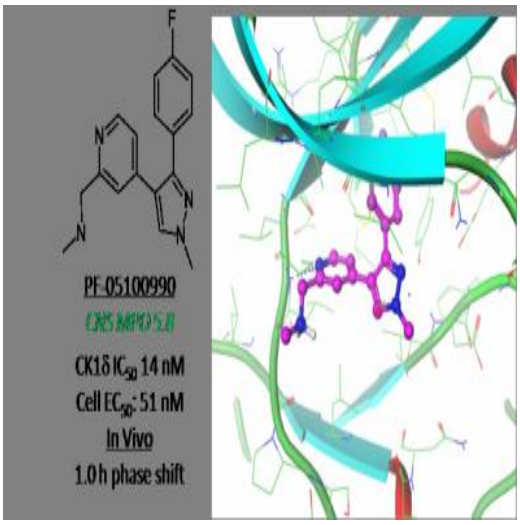

CK1 delta (CK1δ) and CK1 epsilon (CK1ε) are closely related members of a family of seven mammalian serine/threonine protein kinases previously known as casein kinases. The CK1δ and CK1ε isoforms are highly expressed in the suprachiasmatic nucleus (SCN) where they form an essential component of the mammalian biological clock. Selective inhibitors of this class of Kinases will provide tools to study the role of circadian clock in CNS disorders such as: morning lark, bipolar or unipolar depression. A challenge with targeting Kinase inhibitors for chronic CNS indications is a seemingly insurmountable task because of the need to achieve good brain penetration for efficacy and high selectivity for safety. Through a combination of structure-based design, computational chemistry, and innovative medicinal chemistry we have identified a series of highly selective, brain penetrant CK1δ inhibitors which demonstrated phase shift in mouse and cynomolgus monkey models of circadian rhythm. These inhibitors also provided excellent therapeutic index in exploratory toxicology studies. This presentation will highlight the discovery of such compounds as well as general guidelines for targeting kinases for CNS disorders.

MEDI 12

Structure-based design and synthesis of novel pyrimidine analogs as Mer kinase inhibitors in the treatment of cancer

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Mer kinase is a member of the TAM (*T*yro3, *A*xl, *M*er) family of receptor tyrosine kinases. Recent studies showed Mer kinase could be a novel therapeutic target for the treatment of ALL and other Mer-related diseases either by the small molecule inhibitors or the combination with chemotherapeutic agents. Based on the X-ray co-crystal structure on Mer protein of our previous bicyclic pyrazolo[3,4-d]pyrimidine scaffold, we used pseudo ring design strategy and designed two novel monocyclic pyrimidine scaffolds. The recently resolved X-ray co-crystal structures on Mer protein of this pyrimidine scaffolds have confirmed our structure-based design strategy. The Structure-Activity Relationships (SAR) on both pyrimidine scaffolds have been well studied; lead compounds with nanomolar to subnanomolar IC50 activity against Mer kinase in both enzymatic and cell-based assays were also identified. We will discuss the properties of our specific Mer inhibitors, as well as their selectivity profile.

MEDI 13

Discovery of a potent anti-breast cancer agent from expanded chemical space of pyrrolo[3,2-*f*]quinazoline-1,3-diamines

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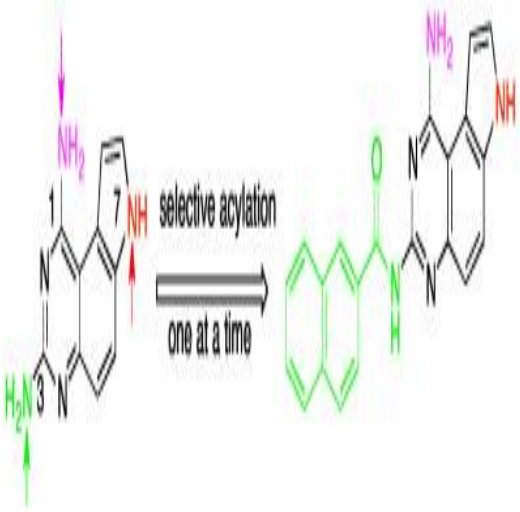
Phenotypic screening has played a fundamental role in drug discovery by providing new molecular entities. In order to sustain further discoveries by phenotypic screening, we are in great need to access novel chemical space. Privileged chemical scaffolds are potential ligands to a diverse array of receptors and thus novel chemical space resulting from privileged chemical scaffolds can provide the basis to discover novel bioactive compounds with drug-like properties. Pyrroloquinazoline-1,3-diamine



is a privileged chemical scaffold with significant biological activities. However, the currently accessible chemical space is rather limited. In particular, the regioselectively mono-*N*-acylated chemical space has been untapped likely due to the significant challenges in rapidly preparing these compounds. However, such compounds are expected to significantly modulate the electrostatics of the structure core and are the potential chemical space to discover novel biologically active leads. In this presentation, we will describe our efficient methodology to rapidly synthesize mono-*N*-acylated products at N^1, N^3 and N^7 of pyrroloquinazoline-1,3-diamine. This methodology effectively exploits the distinct differences in nucleophilicity and pK_a of the three ionizable nitrogen atoms. To demonstrate the biological utility of this expanded chemical space, a phenotypic anti-breast cancer screen was performed against the newly synthesized library. Distinct structure-activity relationship patterns were observed with these regioisomers. A potent and nontoxic anti-breast cancer agent with N^3-(2-naphthoyl) group was identified with a unique mechanism of action.

MEDI 14

Design of high-affinity stapled peptides to target the RAP1-TRF2 protein-protein interaction

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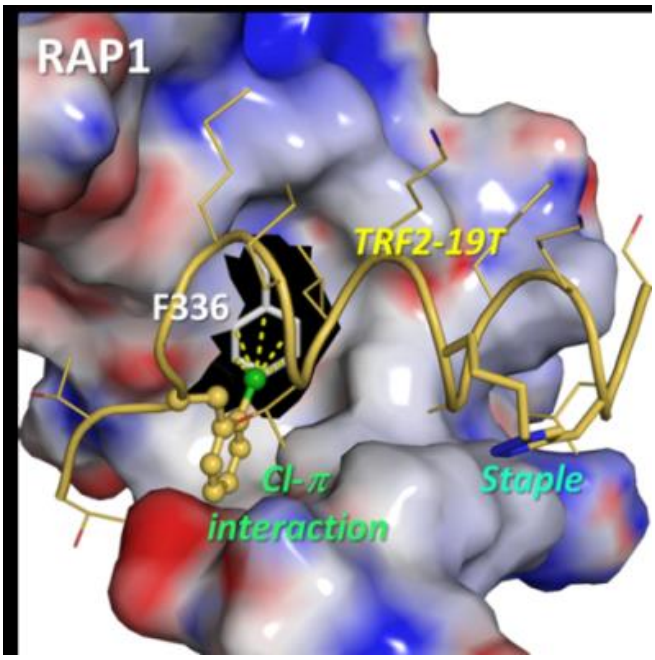
RAP1 is a shelterin subunit and has a key role in modulation of telomere stability, gene transcription and NF-κB regulation. Telomeric repeat-binding factor 2 (TRF2) binds to a well-defined surface grove in RAP1 via an a-helix and modulates the function of RAP1. Based upon the crystal structure of TRF2 in complex with RAP1, we have designed and synthesized a series of TRF2 peptides through mutations and stapling. Our most potent TRF2 stapled peptide binds to RAP1 with a K_i value of 7nM and ~300-times more potent than the wild-type TRF2 peptide. Further optimization of this potent TRF2 stapled peptide has the potential to generate a set of high-affinity and cell-permeable pharmacological tools to investigate the role of RAP1-TRF2 in a variety of cellular processes.



Peptide	Sequence	K_i (μM)
TRF2-WT	Ac-^{281}TTIGMMITLKAAFKTLS-NH_2	2.0±0.1
TRF2-19T	Ac-^{281}TT(F 2-Cl)GMMTLK*AFK^LS-NH_2	0.007±0.001

*^Triazole stapled sites

MEDI 15

Design, synthesis, and biological evaluation of isoquinoline derivatives of naltrexamine as MOR selective ligands

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Globally, nearly 4% of people are estimated to be associated with alcohol use disorders (AUDs), i.e. alcohol abuse or dependence. Yet only a few medications are available to treat AUDs. Studies have shown that opioid receptors are implicated in AUDs development. Our effort on identifying the mu opioid receptor (MOR) selective ligands revealed that NAQ, a 6α-N-substituted-naltrexamine derivative, was more efficacious

and less susceptible to tolerance than naltrexone in reducing high concentration alcohol consumption in C57BL/6J mice. In order to explore the structure-activity relationship of NAQ, a series of its analogues were designed, synthesized and evaluated in the radioligand competition binding assay and the ^{35}S-GTP[γS] functional assay. Several new compounds with minimum MOR agonism and improved MOR selectivity over the kappa and delta opioid receptors were identified and subjected to further in vivo evaluations.

MEDI 16

Silent agonists for alpha7 nicotinic acetylcholine receptor

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The α7 nicotinic acetylcholine receptor (nAChR) is currently a drug target for Alzheimer's disease, schizophrenia, and inflammatory disorders. Traditionally, the function of the α7 receptor was linked to its ion channel activity; however growing evidence supports a metabotropic function that may modulate inflammatory response in non-neuronal cells. We introduce the term silent agonist to describe receptor ligands that are competitive antagonists, are not agonists on their own in the sense of ion channel activity, but place the receptor in a desensitized state that can be revealed in the presence of type II positive allosteric modulator (PAM). We have identified three structurally unique groups of silent agonist pharmacophores. One group is exemplified by benzylidene anabaseine type molecules, the second group features bulky quaternary alkyl ammonium compounds, and the third group is represented by newly designed and synthesized compounds KC-1, KC-5, KC-7. When tested in *Xenopus* oocytes, the compounds showed no response on their own but when co-applied at 100 μM with 10 μM PNU-120596, the net charge response relative to 60 μM ACh was 18, 12, and 21-fold higher for KC-1, KC-5, KC-7, respectively). Because these molecules convert the α7 nAChR from a resting state selectively into a desensitized state that can be probed by type II PAM, they are of interest as a tool to study the functions of the α7 nAChR that do not involve ion conducting states, and may constitute a new alternative for the development of α7 nAChR therapeutics.

MEDI 17

Structure-activity relationship studies of novel guanidine based inhibitors of Sphingosine kinase-2

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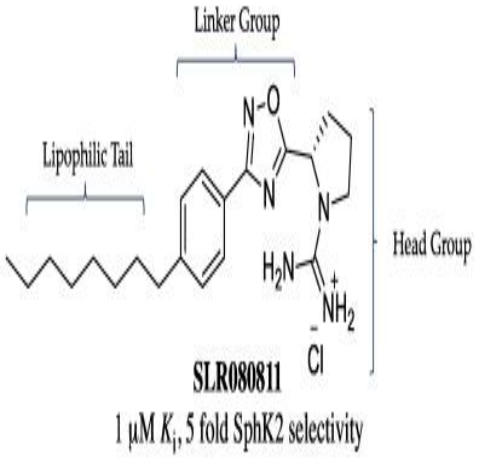
Sphingosine kinase (SphK) has emerged as an attractive target for various therapeutics due to its prominent role in processes such as cell proliferation, apoptosis etc. SphK exists in two isoforms: SphK1 is localized in the cytosol while SphK2 is localized in the nucleus. These enzymes phosphorylate sphingosine to sphingosine-1-phosphate, which has been shown to signal intracellularly via HDACs and BACE1, and extracellularly via interactions with the five G-protein coupled receptors $S1P_{1-5}$. This signaling pathway has recently been associated with a variety of different diseases. Recently, the Santos group has developed a novel guanidine based lead compound **SLR080811** that selectively targets SphK2 with good selectivity and potency at a low micromolar K_i. The structural scaffold contains three regions that could be diversified into a variety of derivatives to improve selectivity: the head, linker and tail regions. In this presentation, we will highlight our current efforts towards developing different head, tail and linker group analogs of **SLR080811** . The *in vitro* and *in vivo* activity of these inhibitors will be discussed.



MEDI 18

Drug resistance: Multisite targeting of some new drug leads

***Eric Oldfield**, eo@chad.scs.uiuc.edu.Chemistry, University of Illinois, Urbana, Illinois 61801, United States*

Drug resistance is a major problem. Here, I consider the mechanisms of action of two classes of drugs/drug leads: the anti-tuberculosis drug SQ-109 and its analogs, and members of the bisamidine class of anti-infectives. Both have multiple sites of action and I will present several crystallographic structures, as well as presenting growth inhibition results against a very broad range of infectious organisms. The results

indicate that: multiple-site targeting is responsible for the difficulty in generating resistance to these drugs in bacteria; other organisms are susceptible, due to multi-site targeting, and that combination therapies utilizing such poly-pharmaceuticals offer one route to resistance-resistant therapeutics.

Supported by NIH AI074233, GM065367 and CA158191, and by the American Heart Association, Midwest Affiliate

MEDI 19

Structure and inhibition of the drug-resistant mutants of the M2 proton channel of influenza A virus

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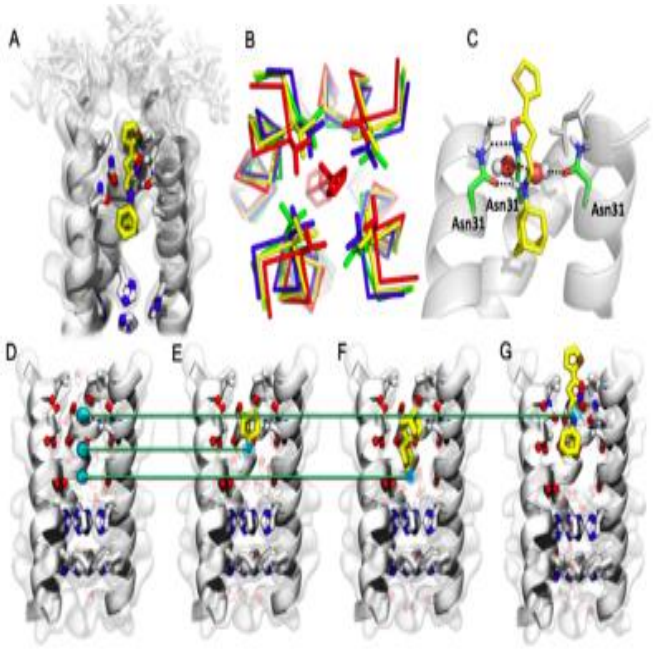
The influenza A virus M2 proton channel (A/M2) is the target of the antiviral drugs, amantadine and rimantadine, whose use have been discontinued due to widespread drug resistance. Among the handful of drug-resistant mutants of M2, S31N, V27A and L26F were found in more than 99% of the currently circulating viruses. Discovery of inhibitors targeting these M2 mutants has been hampered by the lack of structural information and their limited sizes, polarity, and dynamic nature of their drug binding sites. Nevertheless, using an integrated approach including medicinal chemistry, molecular dynamics simulation, solid/solution-state NMR, X-ray crystallography, and pharmacological characterizations, we have discovered small molecule drugs that inhibit mutant M2 (S31N, V27A and L26F) with potencies greater than amantadine's potency against WT M2. A few compounds exhibiting EC50 around 100 nM are advanced to mice model studies. Structural characterization of S31N drug binding by NMR shows the drug bound in the homotetrameric channel, threaded between the side chains of Asn31.



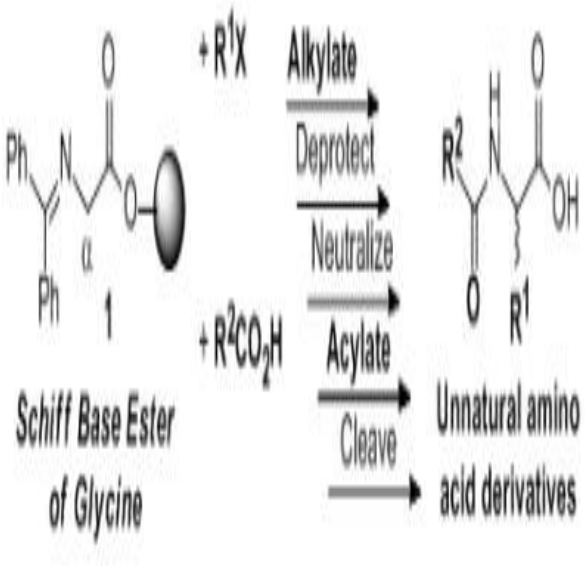
The S31N inhibitors, like other potent WT M2 inhibitors, contain a charged ammonium group. The ammonium binds as a hydrate to one of three sites aligned along the central cavity that appear to be hotspots for inhibition. These drug binding hotspots along the channel axis provide a general model of M2 inhibition that can be used to guide the design of other channel blockers.

MEDI 20

Distributed drug discovery (D3): Virtual D3 biomimetic catalogs, tested molecules, hit follow-up, drugs

William L. Scott*, wscott@iupui.edu, Ryan E. Denton, Richard W. Harper, J. Geno Samaritoni, Martin J. O'Donnell.Department of Chemistry and Chemical Biology, Indiana University Purdue University Indianapolis, Indianapolis, Indiana 46202, United States*

Distributed Drug Discovery (D3) (*J. Comb. Chem.***2009,** *11*, 3-13) employs simple yet powerful synthetic procedures, inexpensive equipment and distributed resources to enable scientists world-wide to readily and reproducibly synthesize new biomimetic molecules as potential drug leads. Molecules to be made using particular D3 based synthetic procedures (e.g. Scheme) are chosen either for their inherent biomimetic nature or through computational analysis of virtual D3 catalogs enumerated from proven D3 synthetic protocols and accessible reagents.



D3 molecules have been submitted to the NIH small molecule repository and have been tested in a variety of NIH high-throughput screens. Several hits have been identified through these screens. The flexible nature of D3 protocols permitted the rapid re-synthesis of these hits, with the simultaneous replicated synthesis of analogs. Examples are given for molecules reported to be potentiators at the oxytocin receptor or to cause delayed death in malarial parasites.

The ability of D3 accessible biomimetic molecules to lead to marketed drugs will be demonstrated through a retrospective analysis of the discovery of nateglinide, a drug for type II diabetes. A molecule made through the D3 process solely because of its biomimetic nature was found in the literature to be a key part of the SAR that led to nateglinide. This validates the potential for molecules in the biomimetic class represented by D3 virtual catalogs to be synthesized and lead to the discovery of drugs.

MEDI 21

Investigating markers of acute and chronic inflammation in murine models using fluorescence optical imaging

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Inflammation is an underlying pathologic feature common to many diseases, and activated immune pathways associated with inflammation may serve as appropriate

targets for *in vivo* molecular imaging of disease progression. Recent optical imaging studies have applied this rationale with the use of activatable and activity-based fluorescent probes to track biological processes associated with disease pathophysiology and pharmacological intervention in a non-invasive manner. Neutrophil elastase (NE) has been established in the etiology of several acute inflammatory disorders; in the current study, we investigated NE activation as a marker for both aggressive and chronic, low-grade inflammation using an optical imaging approach. By using fluorescence signal from an NE-specific optical imaging agent we studied induction, magnitude, progression, and amelioration of inflammation in an endotoxin-induced intra-tracheal (IT) model of acute lung injury (ALI) and a high-fat diet (60% HFD) induced obese (DIO) mouse model. Amelioration of ALI upon IT administration of a therapeutic was indicated by decrease in NE-specific probe signal compared to untreated animals. In the DIO model, NE activity/signal in adipose tissue increased up to 6 months of high-fat feeding and returned to significantly lower levels upon switching to regular chow. Taken together, this study demonstrates *inter alia* the utility of optical imaging and fluorescence assisted sensing of molecular events in i) evaluating *in vivo* activity of a therapeutic following inhalation and, therefore, the efficiency of this delivery approach, and ii) establishing NE as an important participant in the pathophysiology of metabolic inflammation, with implications in insulin resistance and diabetes.

MEDI 22

Major structure-based discoveries en route to clinical PI3K-inhibitor GDC-0032

***Steven T Staben**, stevents@gene.com, Timothy P Heffron, Alan G Olivero.Discovery Chemistry, Genentech, Inc., South San Francisco, CA 94080, United States*

The phosphoinositide 3-kinase (PI3K) pathway plays a crucial role in driving cell growth, migration and survival in many tumor types. Given the prevalent occurrence of mutation or activation of this pathway in cancer patients, the PI3Ks have become popular targets for small molecule inhibition. Starting from a lipophilic 2-amido-thienobenzopyran HTS hit, this presentation will detail major structure and property based changes that improved potency, selectivity and pharmacokinetic properties leading to clinical PI3K inhibitor GDC-0032. In addition, a novel hypothesis for the PI3Kβ sparing activity of GDC-0032 and similar inhibitors will be disclosed.

MEDI 23

Chemical probes for bromodomains outside the BET family

***Paul E Brennan**[1,4], paul.brennan@sgc.ox.ac.uk, Sarah J Martin[1], Octovia Monteiro[1], Oleg Fedorov[1], Stefan Knapp[1,4], Duncan Hay[1,2], Chris Wells[1,3], Panagis Filippakopoulos[1,5], Sarah Picaud[1], Susanne Muller-Knapp[1], Tracy Keates[1], Clarence Yapp[1,3], Martin Philpott[1], Chris Schofield[2], Nicola Burgess-Brown[1], Leela Shrestha[1], Claire Strain-Damerell[1]. (1) Structural Genomics Consortium, University of Oxford, Oxford, United Kingdom (2) Department of Chemistry, University of Oxford, Oxford,*

United Kingdom (3) Botnar Research Centre, University of Oxford, Oxford, United Kingdom (4) Nuffield Department of Medicine, Target Discovery Institute, University of Oxford, Oxford, United Kingdom (5) Nuffield Department of Medicine, The Ludwig Institute for Cancer Research, University of Oxford, Ox, United Kingdom

Epigenetics is the study of heritable changes in phenotype that are not encoded in an organism's DNA. Epigenetic effects due to persistent changes in gene transcription have been linked to chemical modification of DNA and the proteins that package and regulate DNA in the nucleus, histones. One of the major post-translational modifications of histones is acetylation of lysine residues prevalent in histone tails. The enzymes that add and remove lysine acetylation marks, HATs and HDACs, have been extensively researched in, but very few potent inhibitors that modulate the recognition process between acetylated histones and transcription have been described.

The principal readers of histone acetyl lysine marks are bromodomains (BRDs), which are a diverse family of over sixty evolutionary conserved protein-interaction modules. The conserved BRD fold contains a deep, largely hydrophobic acetyl lysine binding site, which represents an attractive pocket for the development of small, pharmaceutically active molecules. Proteins that contain BRDs have been implicated in the development of a large variety of diseases, including cancer and inflammation.

Although a number of inhibitors of the BET subfamily of bromodomains, which includes BRD4, have been described, few inhibitors for other bromodomains are known. In order to decipher the complex biology of bromodomains and provide evidence linking specific bromodomains to disease, we are discovering selective, cell active small molecule inhibitors of bromodomains outside the BET family.

MEDI 24

Chemical biology of methyl-lysine: Discovery of a chemical probe for L3MBTL3

***Stephen Frye**, svfrye@email.unc.edu.Center for Integrative Chemical Biology and Drug Discovery, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599-7363, United States*

Methyl-lysine (Kme) recognition domains play a central role in epigenetic regulation during cellular differentiation, development, and gene transcription with more than 200 known “reader” domains in the human proteome. We describe our target-class approach to ligand design and the discovery of UNC1215, a potent and selective chemical probe for the Kme reading function of L3MBTL3, a member of the malignant brain tumor (MBT) family of chromatin interacting transcriptional repressors. UNC1215 binds the MBT domains of L3MBTL3 with a Kd of 120 nM, competitively displacing mono- or dimethyl-lysine containing peptides. This probe is greater than 50-fold selective versus other members of the human MBT family and also demonstrates selectivity against more than 200 other Kme reader domains examined. Using X-ray crystallography we identified a novel 2:2 polyvalent mode of interaction inwhich two

UNC1215 molecules bridge two L3MBTL3 molecules. In cells, UNC1215 is non-toxic and binds directly to L3MBTL3 via the Kme-binding pocket of the MBT domains. UNC1215 increases the cellular mobility of GFP-L3MBTL3 fusion proteins and point mutants that disrupt the Kme binding function of GFP-L3MBTL3 phenocopy the effects of UNC1215. Finally, we used UNC1215 to demonstrate a novel Kme-dependent interaction of L3MBTL3 with BCLAF1, a protein implicated in DNA damage repair and apoptosis. The potency, specificity, and cellular effects of UNC1215 establish it as the first cell-active antagonist of a Kme reader domain and a useful chemical probe for biological studies of the function of L3MBTL3.

MEDI 25

Mechanism-based irreversible inhibitors of the lysine demethylase LSD1

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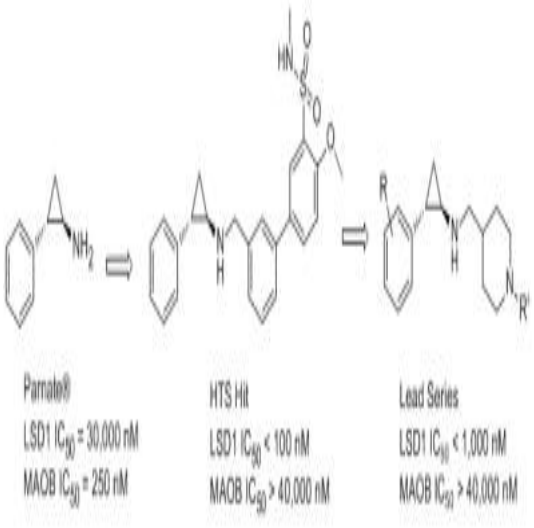
LSD1 (*lysine specific demethylase I*), a flavin-dependent histone demethylase that oxidatively removes methyl groups from mono- and di-methylated Lys-4 of histone H3 (H3K4), is a component of various transcriptional corepressor complexes that often include HDAC1/2 and CoREST. LSD1 is a key regulator of the epigenome, modulating gene transcription at both histone and DNA levels, making it an interesting target in oncology. High-throughput screening of the GSK compound collection led to the identification of validated hits based on tranylcypromine (Parnate®), a known irreversible LSD1 inhibitor. Lead optimization, with a focus on physicochemical properties, produced highly potent, selective, mechanism-based inhibitors of LSD1 with good oral bioavailability.



MEDI 26

Novel cell active inhibitors of histone lysine demethylases (KDMs): Progress and challenges in the discovery of tool inhibitors of JumonjiD2 (JmjD2) and JumonjiD3 (JmjD3)

Susan M Westaway, *sue.m.westaway@gsk.com.Epinova DPU, GlaxoSmithKline, Stevenage, Herts SG1 2NY, United Kingdom*

There is currently considerable interest in developing an understanding of the roles of epigenetic processes in the development and progression of disease and therefore, in elucidating the potential for modulation of these processes to provide therapeutic benefit. Small molecule inhibitors of epigenetic proteins such as the 'writers', 'erasers' and 'readers' of histone post-translational modifications provide avenues for exploring how modulation of the activities of such proteins affects disease processes. Many of the Jumonji (Jmj) class of proteins have been shown to be members of the histone lysine demethylase class of epigenetic 'erasers'. In this presentation we will report on our efforts towards the discovery of inhibitors of the H3K27 and H3K9 demethylases, JmjD3 and the JmjD2 family respectively, that have demonstrated cellular activity and therefore have potential utility as tool compounds to probe the role of these enzymes in disease.

MEDI 27

Histone demethylases: A chemist's perspective

Xiang Wang, *Xiang.Wang@colorado.edu.Department of Chemistry and Biochemistry, University of Colorado at Boulder, Boulder, CO 80309-0215, United States*

Histone demethylases (HDMs) are the most recently discovered class of histone-modifying enzymes. These often display tissue-specific expression and play critical roles in a wide range of cellular processes, including gene expression, meiosis, and embryonic stem cell self-renewal, as well as disease processes, such as those leading to the development of cancer and mental retardation. However, many important questions remain elusive in this field, such as the cellular functions, substrate specificity, and “druggability” of HDMs. We have designed, synthesized, and characterized a series of highly selective probes to address these questions.

MEDI 28

Targeting epigenetic mechanisms involved in neuroplasticity and memory

***Stephen Haggarty**, haggarty@chgr.mgh.harvard.edu.Departments of Neurology & Psychiatry, Massachusetts General Hospital,, Boston, MA 02114, United States*

Developing novel therapeutics and diagnostic tools to improve the treatment and ultimately the prevention of CNS disorders with cognitive deficits, such as Alzheimer's disease and schizophrenia, is of critical importance given the burden of these disorders to individuals and society as a whole. Recent preclinical molecular, cellular, and behavioral findings have begun to reveal the importance of epigenetic mechanisms that alter chromatin structure and dynamically regulate patterns of gene expression in the regulation of neuroplasticity in both health and disease. To better understand the regulation of these epigenetic mechanisms in the CNS, and to develop small molecule-probes and genetic tools to validate therapeutic targets, we have established a panel of high-throughput, neuronal cell-based, and biochemical assays reporting on key pathways and histone modifications implicated in neuroplasticity and cognition. A major focus of these efforts have been towards selectively targeting class I histone deacetylase (HDAC) complexes, in particular HDAC2, that our work and that of others has shown plays a key role in regulation of neuronal gene transcription, synaptogenesis, and learning and memory. Beyond HDACs, we have also developed selective inhibitors of the histone demethylase LSD1 (KDM1A), which is a major component of HDAC2 complexes in brain, and begun to use these small molecule-probes to determine the role of LSD1 in neuroplasticity and cognition. Collectively, these studies will advance our understanding of the potential for targeting mechanisms of chromatin-mediated neuroplasticity for novel treatments of neurodegenerative and neuropsychiatric disorders.

MEDI 29

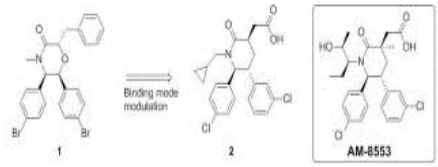
Rational design and binding mode duality of MDM2-p53 inhibitors

***Felix Gonzalez-Lopez de Turiso**[1], felgonza@amgen.com, Daqing Sun[1], Yosup Rew[1], Michael D. Bartberger[3], Hilary P. Beck[1], Jude Canon[4], Ada Chen[1], David Chow[1], Tiffanny L. Correll[3], Xin Huang[5], Lisa D. Julian[1], Frank Kayser[1], Mei-Chu Lo[1], Alexander M. Long[5], Dustin McMinn[1], Jonathan D. Oliner[4], Tao Osgood[4], Jay P. Powers[1], Anne Y.*

Saiki[4], Steve Schneider[5], Paul Shaffer[5], Shou-Hua Xiao[1], Peter Yakowec[5], Xuelei Yan[1], Qiuping Ye[2], Dongyin Yu[4], Xiaoning Zhao[1], Jing Zhou[1], Julio C. Medina[1], Steven H. Olson[1]. (1) Department of Therapeutic Discovery, Amgen Inc., South San Francisco, CA 94080, United States (2) Department of Pharmacokinetics and Drug Metabolism, Amgen Inc., South San Francisco, CA 94080, United States (3) Department of Therapeutic Discovery, Amgen Inc., Thousand Oaks, CA 91320, United States (4) Department of Oncology Research, Amgen Inc., Thousand Oaks, CA 91320, United States (5) Department of Therapeutic Discovery, Amgen Inc., Cambridge, MA 02142, United States

The tumor suppressor protein p53 plays a key role in the control of cell cycle arrest and apoptosis. In normal cells p53 activity is regulated by the oncoprotein MDM2 which binds to p53 and inhibits its intra-nuclear transcription activity via three different mechanisms: 1) direct binding to the N-terminal transactivation domain, 2) activating proteosomal degradation via ubiquitination through E3 ligase activity and 3) promoting the transport of p53 from the nucleus to the cytoplasm. In approximately 50% of tumors p53 is mutated or deleted, and in wild type p53 tumors, this protein can be inactivated by over expression or amplification of MDM2. It is proposed that inhibition of the MDM2-p53 interaction could be a promising therapeutic strategy for activating the p53 pathway in a wide variety of p53 wildtype tumors.

This presentation will describe the initial rational design studies which lead to the identification of tetrasubstituted morpholinones as inhibitors of the MDM2-p53 interaction. The X-ray co-crystal structure of **1** bound to MDM2 lead to a bidirectional optimization approach which ultimately resulted in the discovery of piperidinone **2** after modulation of the binding mode of the initial morpholinone series. This work provided the foundation for the discovery of the potent MDM2 inhibitor **AM-8553** .

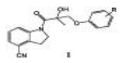


MEDI 30

Novel class of selective androgen receptor modulators (SARMs) for the treatment of muscle frailty

***Eugene P Chekler**, piatnits@gmail.com.BioTherapeutics Medicinal Chemistry, Pfizer, Cambridge, MA, United States*

We present a novel series of Selective Androgen Receptor Modulators (SARMs) which shows excellent biological activity and physical properties. 1-(2-Hydroxy-2-methyl-3-phenoxypropanoyl)indoline-4-carbonitriles (**1**) show potent binding to the androgen receptor (AR) and activate AR-mediated transcription *in vitro*. Representative compounds demonstrate diminished activity in promoting the intramolecular interaction between the AR carboxyl (C) and amino (N) termini. This N/C termini interaction is useful as a biomarker to decouple undesired androgenic response from anabolic activity. In castrated rats, the daily administration of a lead compound shows anabolic activity by increasing *levator ani* muscle weight. Minimal effects were observed on the prostate, and seminal vesicles along with minimal effects repressing circulating luteinizing hormone (LH) levels. A lead compound completed a two week rat toxicology study, and it was well tolerated at dosages up to 100 mg/kg/day for 14 consecutive days, the highest dosage evaluated.



MEDI 31

Rapid approach to identification of a PDE10A tracer to support development of PDE10A inhibitors in preclinical and clinical studies

***Essa Hu Harrington**[1], ehu@amgen.com, Ji Ma[2], Christopher Biorn[2], Dianna Lester-Zeiner[2], Robert Cho[2], Shannon Rumfelt[1], Roxanne Kunz[1], Thomas Nixey[1], Klaus Michelsen[1], Silke Miller[1], Jianxia Shi[2], Geraldine Hill Della Puppa[1], Santosh Talreja[2],*

Jessica Able[2], Dah-Ren Hwang[1], Mark Slifstein[4], Balu Easwaramoorthy[4], Stephen Hitchcock[1,3], Amy Porter[1], Jennifer Allen[1], David Immke[1], James Treanor[1], Hang Chen[2]. (1) Department of Small Molecule Chemistry, Department of Neuroscience, Department of PKDM, Department of Molecular Structures, Department of Medical Sciences, Amgen Inc., Thousand Oaks, CA 91320, United States (2) Department of Neuroscience, Department of PKDM, Amgen Inc., South San Francisco, CA 94080, United States (3) Envoy Therapeutics, Jupiter, Florida 33458, United States (4) Department of Psychiatry, Columbia University, New York, NY 10032, United States

To support the development of our PDE10A inhibitor program for the treatment of schizophrenia, we report the identification of a PDE10A specific radiotracer. Using preselected in vitro criteria for CNS penetration we identified a subset of candidates that were further profiled in vivo using LC-MS/MS technology and culminated in the discovery of tracer candidate AMG7980. AMG7980 was utilized for measurement of PDE10A CNS receptor occupancy (RO) in both ex vivo RO and in vivo LC-MS/MS RO studies in rodents. These RO measurements provided a direct means to evaluate target coverage during our lead optimization efforts. To facilitate translation of preclinical findings into clinical settings, we then developed [^{11}C] labeled AMG7980 for potential use as positron-emission-tomography (PET) tracer of PDE10A CNS target occupancy in humans. Kinetic profile and RO measurements using our PDE10A PET tracer in non-human primates will be described.

MEDI 32

GPCR structure-based drug discovery: Identification of dual orexin antagonists and orexin-1 selective antagonists for the treatment of insomnia and addiction disorders

***John A Christopher**, john.christopher@heptares.com.Department of Medicinal Chemistry, Heptares Therapeutics Limited, Welwyn Garden City, Hertfordshire AL7 3AX, United Kingdom*

G-protein coupled receptors (GPCRs) play crucial roles in disease and are the site of action of a large percentage of current drugs. Despite this rich history many opportunities remain for clinical intervention, as successful NCEs have not been developed for several validated targets, and high quality molecules are scarce for challenging GPCR sub-families. Heptares has solved X-ray crystal structures of multiple ligands in orexin-1 (OX1) and orexin-2 (OX2) neuropeptide receptors stabilised into antagonist conformations and is leveraging these in structure-based drug discovery programs.

Instability of GPCRs when removed from their membrane environment has severely limited the application of structure-based and fragment-based drug discovery techniques. The Heptares approach to GPCR drug discovery nucleates around a unique ability, using stabilised receptor (StaR®) technology, to mutationally stabilise GPCRs in precisely defined biologically-relevant conformations. Subsequent use to

design efficient small-molecules focuses on structure-based approaches to problematic targets. StaRs® are amenable to techniques that cannot be readily used with wild-type GPCRs, including fragment screening, biophysical kinetic profiling and crystallography.

The orexins are two neuropeptides produced in the hypothalamus which bind to OX1 and OX2 GPCRs. Antagonism of the receptors has utility in numerous areas including insomnia, migraine, addiction and panic. OX1 and OX2 StaRs® have been generated and have facilitated rapid progression of a dual orexin receptor antagonist (DORA) series to a pre-clinical candidate. Structural insights into the DORA series will be presented, as will the use of information from multiple chemotypes in the structure-based design of an advanced series of OX1 antagonists.

MEDI 33

Spirocyclic lactams as BACE-1 inhibitors for the treatment of Alzheimer's disease

Michael A Brodney[1]*, michael.a.brodney@pfizer.com, Claude Ambroise*[1]*, Curt Christoffersen*[1]*, Eva Hajos-Korcsok*[1]*, Gabriela Barreiro*[1]*, Louis Leung*[2]*, JianHua Liu*[2]*, Yasong Lu*[2]*, Stephen Noell*[2]*, Charles Nolan*[1]*, Christine Oborski*[1]*, Brian T. O'Neill*[2]*, Christopher J Helal*[2]*, Kevin Ogilvie*[1]*, Felix Vajdos*[3]*. (1) Neuroscience Research Unit, Pfizer Global Research and Development, Cambridge, MA 02139, United States (2) Neuroscience Research Unit, Pfizer Global Research and Development, Groton, CT 06340, United States (3) Center of Chemistry Innovation and Excellence, Pfizer Global Reserach and Development, Groton, CT 06340, United States*

A major pathophysiology of Alzheimer's disease (AD) is the presence of amyloid plaques which are primarily composed of the Aβ peptide. The formation of the Aβ peptide is the result of sequential enzymatic cleavage of the Amyloid Precursor Protein (APP) by β-secretase (BACE) and subsequently by γ-secretase. Small molecule BACE1 inhibitors would be expected to prevent the generation of the Aβ peptides and consequently reduce the detrimental effects of Aβ toxicity and the formation of amyloid plaques in the brain. We will describe the identification and optimization of a novel series of spirocyclic lactam BACE-1 inhibitors that bind to the catalytic aspartic acids *via* water mediated hydrogen bond. A strategy to align a set of physicochemical properties, CNS penetration, and selectivity into a single molecule led to the discovery of PF-05297909. The efficacy of PF-05297909 to decrease amyloidogenic peptides in multiple species and in-vivo paradigms provided rationale to advance the compound into human following a single dose exploratory IND study.

MEDI 34

Lead optimization of aminoheterocyclic xanthenes: Identification of potent, CNS-penetrant BACE inhibitors for the treatment of Alzheimer's disease

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Vijay Gore[2], Dean Hickman[6], Zihao Hua[1], Hongbing Huang[1], Jason Human[1], Ted Judd[2], Yuan-Hon Kiang[8], Chuck Kreiman[1], Daniel S La[1], Qingyian Liu[2], Patricia Lopez[2], Jon Low[2], Vu Ma[2], Matthew Martin[1], Isaac Marx[1], Ana Minatti[2], Nick Paras[2], Vinod F Patel[1], Timothy S Powers[2], Wenyuan Qian[2], Robert C Wahl[7], Matthew M Weiss[1], Paul Wen[5], Douglas A Whittington[3], Stephen Wood[5], May Xue[2], Bryant Yang[2], Chester Yuan[2], Xiao Mei Zheng[1], Wenge Zhong[2], Robert T Fremeau[5]. (1) Department of Medicinal Chemistry, Amgen, Inc., Cambridge, MA 02142, United States (2) Department of Medicinal Chemistry, Amgen, Inc., Thousand Oaks, CA 91320, United States (3) Department of Molecular Structure, Amgen, Inc., Cambridge, MA 02142, United States (4) Department of Molecular Structure, Amgen, Inc., Thousand Oaks, CA 91320, United States (5) Department of Neuroscience, Amgen, Inc., Thousand Oaks, CA 91320, United States (6) Department of Pharmacokinetics and Drug Metabolism, Amgen, Inc., Thousand Oaks, CA 91320, United States (7) Department of HTS and Molecular Pharmacology, Amgen, Inc., Thousand Oaks, CA 91320, United States (8) Department of Pharmaceutics, Amgen, Inc., Thousand Oaks, CA 91320, United States

Alzheimer's disease (AD) is the most common neurodegenerative disease, representing one of the largest unmet medical needs in neuroscience today. A key factor in AD pathogenesis is the accumulation of beta-amyloid peptides (Ab) in the brain, where formation of these peptides is initiated via proteolytic cleavage of amyloid precursor protein (APP) by the aspartyl protease BACE1 (b-Secretase). Although significant efforts have focused on the inhibition of BACE1 as a possible disease-modifying therapy for AD, identifying orally available, CNS-penetrant BACE1 inhibitors has proven particularly challenging. We report the structure-based optimization of amino-heterocyclic xanthene BACE inhibitors. The use of extensive X-ray cocrystallographic information combined with effective control of physiochemical properties to maximize CNS-exposure led to the identification of potent, selective, and orally available inhibitors which show robust reduction of brain (Ab) in preclinical species.

MEDI 35

Discovery of novel and highly selective allosteric inhibitors of PAK1

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Protein kinases mediate a variety of intracellular pathways and their aberrant activity is often associated with tumor progression. Many kinase inhibitors have been shown to be very powerful therapeutics with Glivec being the most prominent example used for the treatment of chronic myelogenous leukemia (CML). Most of kinase inhibitors discovered to date block the ATP binding site which is highly conserved among different kinases and as a result such compounds suffer very often from the lack of selectivity and a very congested chemical space. Design of inhibitors targeting novel allosteric kinase sites is still considered to be very challenging. The authors will describe their strategy towards finding allosteric hits of PAK, including the biochemical and biophysical approaches chosen to support hit finding and validation. They will disclose for the first time the X-Ray structures of allosteric PAK1 inhibitors binding to a hydrophobic pocket induced by the compounds. Optimization of the hits yielded very potent inhibitors with single-digit nanomolar PAK1 IC50s without making use of interactions to the hinge. Furthermore, the allosteric inhibitors modulated PAK1 at the cellular level. Compounds presented by the authors may be used as valuable research tools to study biological functions of the PAK kinases.

MEDI 36

MK-3102 (omarigliptin): A novel DPP-4 inhibitor for once weekly treatment of type 2 diabetes

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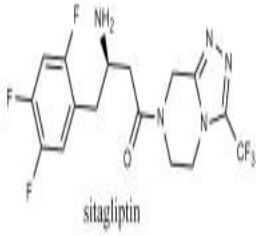
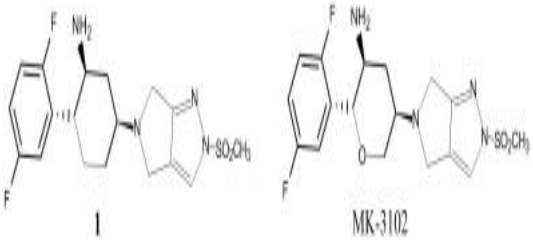
JANUVIA™ (sitagliptin phosphate) is the first DPP-4 inhibitor approved by the FDA for the treatment of patients with Type 2 diabetes. The objective of the 2nd generation DPP-4 program is to identify a long acting DPP-4 inhibitor for a once weekly dosing. Based on X-ray crystallography of sitagliptin bound to DPP-4, we proposed that a cyclohexylamine group could be an appropriate replacement for the central β-amino butanoyl portions of sitagliptin, providing a ring constrained analog such as **1** . Compound **1** proved to be a potent, selective DPP-4 inhibitor with excellent pharmacokinetic profile in preclinical species. Further modification of **1** and continued effort in this area provided **MK-3102** as a clinical candidate, that is currently in Phase 3

clinical studies. In this presentation, the discovery of **MK-3102** and its biological profile including efficacy in reducing glucose will be discussed.



sitagliptin



MEDI 37

Structure-guided design of novel small molecule inhibitors of bacterial t-RNA-(N^1G37) methyl transferase (TrmD)

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The t-RNA-(N^1G37) methyl transferase (TrmD) is essential for growth and conserved in both Gram-positive and Gram-negative bacterial pathogens. Since, in addition, TrmD is very distinct from its human ortholog TRM5, it is a suitable target for the design of novel antibacterials. Screening of a small-molecule fragment collection using *Haemophilus influenzae* TrmD identified a series of compounds that were competitive with S-adenosyl methionine (SAM), the natural methyl donor. Guided by co-crystal structures, these fragments elaborated into a nanomolar inhibitor of a broad range of Gram-negative TrmD isozymes. This is the first report of nano-molar inhibitors of bacterial TrmD.<ins cite="mailto:phill" datetime="2013-03-01T10:47">

MEDI 38

Using small molecules to engineer and explore human immunity

***David A Spiegel**, david.spiegel@yale.edu.Departments of Chemistry and Pharmacology, Yale University, New Haven, CT 06510, United States*

Antibody-based therapeutics have become critical instruments in treating diseases ranging from rheumatoid arthritis to cancer in recent years. However, antibodies and other therapeutic proteins are limited in therapeutic applications by their chemical structures: because they are peptide-based, they require intravenous administration, are often highly immunogenic or allergenic, and treatment regimens are often very costly.

This talk describe recent research efforts in our laboratories toward the design, chemical synthesis, and biological characterization of small molecule antibody recruiting therapeutics against prostate cancer, *Staphylococcus aureus*, and the human immunodeficiency virus (HIV). These are bifunctional small molecules designed to redirect antibodies already present in the human bloodstream to the surfaces of pathogenic cells, such as cancer cells, bacteria, and virus particles. The ternary complex formed between these agents, endogenous antibodies, and target cells will lead to immune-mediated pathogen destruction. In theory, this strategy would exploit many of the advantages of biologics, while circumventing the disadvantages, by capitalizing on the chemical properties of small molecules (e.g., high oral bioavailability, facile synthesis, and low cost).

It is our hope that this small molecule-based strategy will serve as starting point toward entirely novel scientific insights and therapeutic approaches relevant to a wide range of disease states.

MEDI 39

Developing antibody-based agents specific for difficult targets in cancer: Our experience with the MIlerian Inhibiting Substance Type II Receptor

***Gregory P Adams**, gp_adams@fccc.edu, Heidi Simmons, Calvin Shaller.Developmental Therapeutics Program, Fox Chase Cancer Center, Philadelphia, PA 19111, United States*

Antibodies are now well-established agents for the treatment of a variety of diseases including cancer. A number of anti-tumor antibodies have been licensed by the U.S. F.D.A. and many more are currently being evaluated in advanced clinical trials. However, it is often believed that the cancer antigens most readily targeted by antibodies are being rapidly exhausted and the remaining relevant target epitopes are difficult to develop functional antibodies against due to high degrees of conservation between species or the lack of a structure that is readily targeted by antibodies. One such target is the Müllerian Inhibiting Substance Type II Receptor (MISIIR). MISIIR is a functional hormone receptor that is involved in the regression of the female reproductive tract in the developing male fetus in response to exposure to its hormone, the Müllerian Inhibiting Substance (MIS), which is released from the developing testis. MISIIR

expression persists in the ovarian surface epithelium and ovarian cancers frequently express MISIIR and apoptose in response to treatment with MIS. We have been developing anti-MISIIR antibodies using a variety of techniques including phage display and hybridomas but to date have failed to isolate agonistic antibodies. We have recently begun employing homology modeling of the MISIIR/MIS complex and using this information to guide the engineering of antibodies that incorporate key contact residues into their CDR loops. Our first generation antibodies are capable of blocking the ligand receptor interaction.

MEDI 40

Chemical tools to monitor and manipulate the immune system

***Thomas Kodadek**, kodadek@scripps.edu.Chemistry & Cancer Biology, Scripps Research institute, Scripps Florida, Jupiter, FL 33458, United States*

We have developed technology that allows high throughput screens to be done for compounds that target the antigen-binding sites of antibodies. This can be accomplished with known, monoclonal antibody targets of interest or in a mode where antibody biomarkers for a given disease and compounds that bind them selectively are discovered simultaneously. The application of this technology to the diagnosis of a variety of important disease will discussed. We will also present preliminary attempts to develop therapeutic agents targeted to malignant B cells in chromic lymphoid leukemia (CLL).

MEDI 41

Identification and optimization of a pteridinone toll-like receptor-7 (TLR-7) agonist

***Paul A. Roethle**[1], paul.roethle@gilead.com, Ryan M. McFadden[1], Hong Yang[1], Paul Hrvatin[1], Hon Hui[1], Jessica Chao[1], Joseph Hesselgesser[2], Paul Duatschek[2], Jim Zheng[3], Bing Lu[3], Jason Perry[4], Daniel Tumas[2,5], Randall L. Halcomb[1]. (1) Department of Medicinal Chemistry, Gilead Sciences, Foster City, CA 94404, United States (2) Department of Biology, Gilead Sciences, Foster City, CA 94404, United States (3) Department of Drug Metabolism, Gilead Sciences, Foster City, CA 94404, United States (4) Department of Structural Chemistry, Gilead Sciences, Foster City, CA 94404, United States (5) Department of Drug Safety Evaluation, Gilead Sciences, Foster City, CA 94404, United States*

Pteridinone-based TLR-7 agonists were identified as potent and selective alternatives to the previously reported adenine-based agonists, leading to the discovery of GS-9620. Analogs were optimized for the immunomodulatory activity and selectivity versus other toll-like receptors (TLRs), based on induction of key cytokines including interferon-alpha (IFN-a) and tumor necrosis factor-alpha (TNF-a). In addition, physicochemical properties were adjusted to achieve desirable pharmacokinetic and pharmacodynamic

properties *in vivo.* GS-9620 is currently in clinical evaluation for the treatment of chronic hepatitis B (HBV) infection.

MEDI 42

Chemologics: Using medicinal chemistry to augment monoclonal antibody and vaccine modalities

Lyn H Jones*, lyn.jones@pfizer.com.WorldWide Medicinal Chemistry, BioTherapeutic Chemistry, Pfizer, Cambridge, MA 02140, United States*

Our group harnesses medicinal chemistry design and synthesis to create novel immunogens that elicit antibodies to specific epitopes. In the area of chemical vaccinology, various features of the carrier-linker-hapten construct were explored and optimized to create an immunopharmacotherapeutic anti-nicotine vaccine clinical candidate. Similarly, rational design of synthetic immunogens has enabled multiple monoclonal antibody projects targeting membrane proteins that are difficult to drug using traditional methods such as cell-based immunizations. The synergy between chemologics, chemical biology and molecular biology technologies will be presented, including methods that enable the creation of complex biomolecular architectures necessary for next generation immunogen design.

MEDI 43

Exploiting protein–carbohydrate interactions for tolerance and immunity

Laura L Kiessling*, kiessling@chem.wisc.edu.Departments of Chemistry and Biochemistry, University of Wisconsin - Madison, Madison, Wisconsin 53706, United States*

The immune system depends upon multivalent binding to control both tolerance and immunity. We used chemical synthesis to direct the immune system to tumor cells. Our strategy takes advantage of low affinity, multivalent interactions to kill tumor cells. Alternatively, we have generated compounds that inhibit autoimmune responses by exploiting inhibitory receptors. This presentation will focus on our recent results in the synthesis and application of new types of ligands that exploit key carbohydrate-binding proteins in the immune system.

MEDI 44

Design and optimization of new 3,5-disubstituted piperidines as renin inhibitors

Yasuyuki Ogawa*, ogawa.yasuyuki.cm@daiichisankyo.co.jp, Yuji Nakamura, Chie Sugita, Teppei Fujimoto, Yutaka Mori, Akiyoshi Mochizuki, Shojiro Miyazaki, Kazuhiko Tamaki, Yumi Matsui, Mizuki Takahashi, Takahiro Nagayama, Masumi Ueno-*

Kanemitsu, Mina Nishi, Yoko Nagai, Akifumi Kurata, Takahide Nishi.Daiichi Sankyo Co., Ltd., Shinagawa, Tokyo 140-8710, Japan

The design and optimization of new 3,5-disubstituted piperidines as orally active renin inhibitors are described. Introduction of our original structure, 2,2-dimethyl-4-phenylpiperazin-5-one, at the C-5 position of the piperidine ring resulted in a lead compound, which showed single-digit nanomolar renin inhibitory activity (IC_{50} = 7.7 nM). Utilizing the X-ray crystal structure analysis of this lead compound and renin complex, we focused on further chemical modification and successfully acquired the most potent compound, **1** (IC_{50} = 1.3 nM), which possesses a 5-fluoro-2-pyridyl ring at the C-3 position. Compound **1** showed significant blood pressure lowering effects in monkeys as well as dTG rat models by oral administration. We will present details of the discovery and pharmacological effects of **1** including pharmacokinetic data.

MEDI 45

Effect of ligand rigidity on binding cooperativity between hydrophobic side chains: A case study with thrombin inhibitors

***Ahmed M Said**, ahmedmoh@buffalo.edu, David G Hangauer.Department of Chemistry, University at Buffalo, Buffalo, NY 14260-3000, United States*

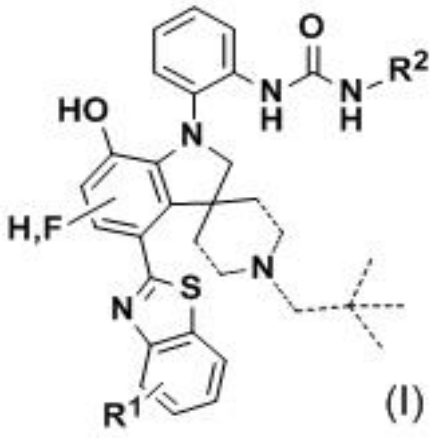
One of the underappreciated factors affecting ligand binding affinity is the potential cooperativity (positive or negative) between different ligand functional groups that interact with the protein host. In this study we report a new type of ligand functional groups cooperativity, i.e. a strong positive hydrophobic ligand side chains cooperativity (4.86 kJ/mol) within a series of thrombin inhibitors. We also evaluated the effect of the ligand's rigidity on the magnitude of this positive cooperativity. It was found that ligand rigidity significantly enhances the magnitude of cooperativity among the hydrophobic side chains. Understanding the factors affecting cooperativity, and quantifying the ligand functional groups cooperativity, is important for gaining a better understanding of the factors controlling ligand binding affinity.

MEDI 46

Synthesis and SAR of 4-benzothiazole-7-hydroxy indolinyl diaryl urea analogs as $P2Y_1$ antagonists

***Tammy Wang**[1], tammy.wang@bms.com, Jennifer X Qiao[1], Carol Hu[1], Dora M Schnur[3], Steve A Spronk[3], Ji Hua[2], Laura A Price[2], Linda Matusick-Kumar[2], Hong Shen[4], Christine Huang[4], Robert Rehfuss[2], Ruth R Wexler[1], Patrick Y.S. Lam[1]. (1) Department of Medicinal Chemistry, Bristol-Myer Squibb, Pennington, NJ 08534, United States (2) Department of Discovery Biology, Bristol-Myer Squibb, Pennigton, NJ 08534, United States (3) Department of CADD, Bristol-Myer Squibb, Pennington, NJ 08534, United States (4) Department of PCO MAP, Bristol-Myer Squibb, Pennington, NJ 08534, United States*

Thrombosis remains the major cause of cardiovascular disorders in the Western countries. The continued impact of thrombotic diseases on morbidity and mortality has led to extensive efforts to discover and develop novel antithrombotic agents. Preclinical data suggests that $P2Y_1$ and $P2Y_{12}$ inhibition provide similar antithrombotic efficacy, while targeting $P2Y_1$ may have the potential for reduced bleeding liability. In this presentation, the synthesis and SAR of a series of 4-benzothiazole-7-hydroxy indolinyl based $P2Y_1$ antagonists (I) will be disclosed. In particular, several analogs were potent in the *in vitro* platelet aggregation assay (PA IC_{50}< 0.5 μM) and showed low clearance and small volume of distribution in rat pharmacokinectic studies.



(I)

MEDI 47

Design and optimization of novel macrocyclic FVIIa inhibitors

Jeremy M Richter[1]*, jeremy.richter@bms.com, J Alex Bates*[1]*, Daniel L Cheney*[1]*, Anzhi Wei*[2]*, Pancras C Wong*[1]*, Joseph M Luettgen*[1]*, Alan R Rendina*[2]*, Timothy W Harper*[1]*, Dietmar Seiffert*[2]*, Ruth R Wexler*[1]*, E Scott Priestley*[1]*. (1) Department of Medicinal Chemistry, Bristol-Myers Squibb, Hopewell, New Jersey 08534, United States (2) Unaffiliated, United States*

Inhibitors of the tissue factor-Factor VIIa complex have demonstrated excellent efficacy and minimal bleeding liability in preclinical antithrombotic models. We have previously reported macrocyclic inhibitors of FVIIa based on a 16-membered, para-linked phenylglycine core. Herein we describe the design and optimization of a novel series of macrocyclic FVIIa inhibitors based on a phenylglycine core. Molecular modeling and crystallography were utilized to optimize the size, constitution, and substitution of the macrocyclic ring. The inhibitors were further improved to single digit nanomolar potency, with good clotting activity and good selectivity.

MEDI 48

WITHDRAWN

MEDI 49

Discovery of inhibitors of *Burkholderia pseudomallei* methionine aminopeptidase with antibacterial activity

Phumvadee Wangtrakuldee[1]*, pwangtrakuldee@niu.edu, Matthew S Byrd*[2]*, Cristine G Campos*[2]*, Micheal W. Henderson*[2]*, Ali Masoudi*[3]*, Peter J Myler*[3]*, Peggy A Cottter*[2]*, James R Horn*[1]*, Timothy J Hagen*[1]*. (1) Chemistry and Biochemistry, Northern Illinois University, DeKalb, Illinois 60115, United States (2) Microbiology and Immunology, University of North Carolina, Chapel Hill, North Carolina 27599, United States (3) Global Health and Biomedical Informatics and Medical Education, Seattle Biomedical Research Institute, Seattle, Washington 98195, United States*

Burkholderia pseudomallei is the causative agent of melioidosis, a severe and often fatal infection that manifests as pneumonia or septicemia. It is a significant cause of morbidity and mortality in several countries in southeast Asia. Evaluation of a series of MetAP inhibitors in an *in vitro* enzyme activity assay led to the first identification of potent molecules that show significant growth inhibition against *Burkholderia pseudomallei*. We discovered that nitroxoline displayed excellent inhibition potency in the BpMetAP1 enzyme activity assay (IC_{50} = 60 nM) and completely inhibits the growth of *B. pseudomallei* and *B. thailandensis*. We have synthesized a series of nitroxoline analogs and determined their ability to inhibit BpMetAP1 and inhibit growth of *B. thailandensis*. The poster will report the design, synthesis and activity of the BpMetAP1 inhibitors.

MEDI 50

Stereoselective synthesis of rhodotorulic acid analogs with potential antibacterial activities

Marine Pillon, Alexandra Dassonville-Klimpt, ***Alexia Jonet****, alexia.jonet@u-picardie.fr, Emmanuel Baudrin, Pascal Sonnet.UFR de Pharmacie, Amiens, France*

The development of antibacterial resistance to antibiotics implies to propose therapeutic strategies to overcome this problem. One of the antimicrobial therapies consists to use iron chelators that can interact with the bacterial iron uptake pathways. Rhodotorulic acid (RA) is a tetradentate siderophore produced by *Rhodotorula pilimanæ*. It is a 3,6-di-alkylated piperazine with particular stereochemistry (3*S*, 6*S*) and possesses two hydroxamate ligands to chelate ferric iron. In previous studies, we have proposed an efficient and stereoselective synthesis of di-substituted 2-oxopiperazines. Herein, we describe the synthesis of RA analogues in order to study their potential antibacterial properties.

MEDI 51

Mechanism of action of SQ-109 analogs in a variety of organisms

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Tuberculosis, caused by *Mycobacterium tuberculosis* , is one of the three major deadly diseases. The improvement of hygiene condition and the marketing of the current first line anti-tuberculosis drug—isoniazid, pyrazinamide, ethambutol and rifampin— in the 1950-1960s have signficanlty prevented and treated or cured the disease. However, the outbreak of multi-drug-resistant tuberculosis (MDR-TB) in the 1980s and the emergence of extensive-drug-resistant tuberculosis (XDR-TB) in 2006 call for new chemotherapies interacting with new drug targets or multiple drug targets. SQ-109, developed by Sequella Inc., is a promising TB drug candidate with activity against MDR-TB strains and XDR-TB strains that is in clinical trials. But the mechanism of action of SQ-109 is somewhat unclear. We synthesized a series of SQ-109 analogs to explore the mechanism of action in a variety organisms.

MEDI 52

Bisamidines targeting prenyl synthases and DNA

Wei Zhu[1], *weizhu1@illinois.edu, Yonghui Zhang*[2], *Kai Li*[2], *Ke Wang*[2], *Yi-Liang Liu*[2], *Xinxin Feng*[2], *William Sinko*[3], *Steffen Lindert*[3,4], *J. Andrew McCammon*[3,4,5,6], *Dean Crick*[7], *Eric Oldfield*[1,2]. *(1) Center for Biophysics and Computational Biology, University of Illinois, Urbana-Champaign, URBANA, Illinois 61801, United States (2) Department of Chemistry, University of Illinois, Urbana-Champaign, Urbana, IL 61801, United States (3) Department of Pharmacology, University of California San Diego, La Jolla, California 92093, United States (4) NSF Center for Theoretical Biological Physics, University of California San Diego, La Jolla, California 92093, United States (5) Howard Hughes Medical Institute, University of California San Diego, La Jolla, California 92093, United States (6) Department of Chemistry & Biochemistry, University of California San Diego, La Jolla, California 92093, United States (7) Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, CO 80523, United States*

Using *in silico* high-throughput screening we have identified a number of high nano-molar/low micro-molar bisamidine inhibitors of farnesyl diphosphate synthase (FPPS), undecaprenyl diphosphate synthase (UPPS) and decaprenyl diphosphate synthase (DPPS). Several of these compounds also had potent activity in cell-based assays, and in one case *in vivo*, in a mouse model of infection. We also find that these compounds bind to DNA, increasing their activity and decreasing the likelihood of resistance occurring. Specifically, DSC experiments showed that upon inhibitor binding, the melting temperature (Tm) of DNA shifted by more than 20 degrees, and a crystallographic investigation revealed that the inhibitor bound to the central AATT site located in the minor groove of the DNA dodecamer $d(CGCGAATTCGCG)_2$ duplex. These results provide new leads for antibacterial development based on a poly-pharmaceutical approach targeting DNA and isoprenoid biosynthesis. We also find synergistic activity with methicillin in a *MRSA* strain, suggesting the future possibility of combinations of poly-pharmaceuticals to help decrease the occurrence of drug resistance.

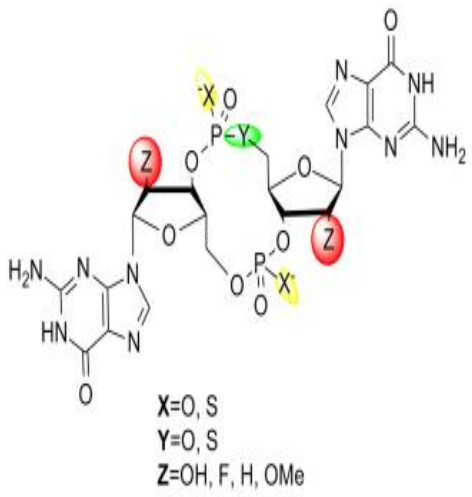
MEDI 53

Polymorphism and binding studies of c-di-GMP analogs

***Jie Zhou**, jzhou@umd.edu.Department of Chemistry and Biochemistry, University of Maryland, College Park, College Park, MD 20742, United States*

For an organism to survive, it must sense its environment and coordinate metabolism to a changing environment. In bacteria, cyclic diguanylate (c-di-GMP) is a universal second messenger that is synthesized in the cytosol, in response to a changing bacterial environment, to regulate bacterial physiology. C-di-GMP has been shown to regulate biofilm formation as well as virulence gene expression in a variety of bacteria. Analogs of c-di-GMP have the potential to be used as chemical probes to study c-di-GMP signaling and could even become drug leads for the development of anti-biofilm compounds to treat persistent bacterial infections. Herein we report the synthesis and biophysical studies of a series of c-di-GMP analogs, which have both phosphate and sugar moieties simultaneously modified. We used computational methods to predict the relative orientation of the guanine nucleobases in c-di-GMP and analogs. DOSY NMR was used to characterize the aggregation states of the c-di-GMP and analogs. Binding studies with various c-di-GMP effector molecules (both proteins and RNAs) revealed that conservative modifications to the phosphate and 2'-positions of c-di-GMP dramatically affected aggregative behavior and binding properties to effector molecules.

Effect of modification of c-di-GMP on binding to proteins and RNAs



MEDI 54

Progress towards antibacterial and antifungal fluoride toxicity agonists

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Due to the continued increase of bacterial resistance to both commonly used antibiotics, as well as antibiotics of last resort, new antibacterial strategies are needed. We recently discovered a riboswitch class whose representatives selectively bind fluoride and control the expression of numerous genes involved in mitigating the toxic effects of fluoride, an inhibitor of numerous metabolic enzymes. Most commonly, fluoride riboswitches activate the expression of genes coding for fluoride transporters, which act to lower intracellular levels of fluoride. We hypothesized that small molecules that either block these channels or otherwise enhance fluoride toxicity by some other mechanism could serve, in conjunction with fluoride, as a new type of topical antibiotic formulation. To identify such compounds, we performed a high-throughput screen of small, drug-like compounds, using a fluoride-responsive reporter to assess their ability to increase intracellular fluoride concentrations. Several different series of compounds were identified that sensitize Escherichia coli to fluoride. Preliminary SAR analysis is indicative of the existence of specific pharmacophore structures. We also demonstrate that a series of known antibacterial or antifungal compounds that compromise membrane integrity, and thereby allow fluoride to enter cells, are more effective antibiotics when supplied in combination with fluoride.

MEDI 55

Identification of selective sulfonamide tubulin inhibitors as antiproliferative agents in African trypanosomiasis

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Tryapanosoma brucei are parasitic protozoan that causes Human African Trypanosomiasis (HAT) or sleeping sickness, a life-threatening disease endemic in sub-Saharan regions of Africa. Current drugs available for the treatment of HAT exhibit drawbacks such as high toxicity to the hosts due to their poor parasitic cell selectivity, difficult routes of drug administration, narrow anti-trypanosomiasis spectrum and high costs of hospitalization. There is a need for potent drugs with efficient bioavailability and the ability to cross the blood-brain-barrier for treating complex stages of the disease. Tubulin plays a central role in parasitic cell growth due to their rapid rate of cell proliferation. In addition, microtubule within the flagellum of the parasite allows for locomotion via oscillations, which is vital for their survival thereby suggesting the potential advantages of tubulin inhibitors for the treatment of trypanosomiasis. Based on the differences between the parasitic and mammalian tubulins, a class of sulfonamide tubulin inhibitors previously developed as anti-cancer agents, were evaluated on *T. brucei* for the identification of candidates selective for parasitic cells over mammalian cells. Tubulin-inhibitor compounds evaluations suggested a difference in the colchicine-binding domains between the parasitic and mammalian cells. The predicated *T. brucei* tubulin structure revealed that several b sheets of the bovine and parasitic tubulin do not overlap in the colchicine domain supporting the difference in the specific binding domains that leads to selectivity of tubulin inhibitors. Several lead compounds from the sulfonamide tubulin inhibitors library (colchicine domain binders) selectively inhibited *T.brucei* cell growth. The pharmacophore of tubulin inhibitors with better activity on mammalian cell growth were different to those promoting *T. brucei* cell growth inhibition. This study therefore provides with a unique molecular scaffold that selectively targets *T. brucei* tubulin and has elucidated efforts for development of new lead compounds targeting tubulin for the treatment of sleeping sickness.

MEDI 56

Discovery of imidazole based IspF inhibitors

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The methylerythritol phosphate (MEP) pathway, a non-mevalonate isoprenoid biosynthetic pathway, is essential for certain bacteria and other infectious disease organisms including *Plasmodium falciparum*. One highly conserved enzyme in the MEP pathway is 2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase (IspF). Inhibitors of IspF may result in potential anti-malarial activity with minimal host toxicity. Fragment based screening of IspF from *Burkholderia pseudomallei* yielded a co-crystalline structure of FOL955. Imidazole analogs of FOL955 have been synthesized and X-ray crystal structure of an analog was obtained and used for computational modeling. The ability of compounds to inhibit IspF was determined by SPR (surface plasma resonance) and anti-malarial activity was obtained at Walter Reed Amy Institute of Research.

MEDI 57

Cyclophilin A inhibitors as potential anti-HIV agents

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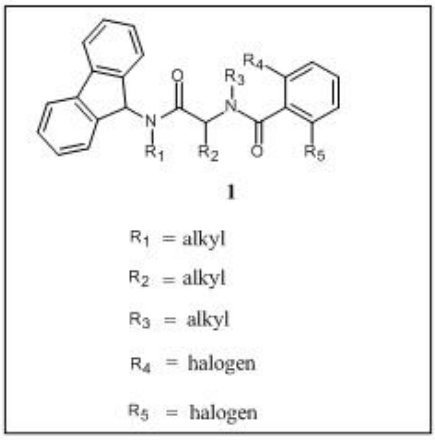
Cyclophilins are host cellular factors which have enzymatic *cis-trans* Peptidyl-Prolyl Isomerase activity (PPIase). The cyclophilin family of enzymes has 17 human isoforms. Among these, cyclophilin A is the most abundant form and is the best studied. Cyclophilin A has been shown to bind to Cyclosporine (**PDB ID: 1CWB**), this complex recruits calcineurin, which ultimately leads to immunosuppressive activity. It has been further demonstrated that cyclophilin A inhibition without calcineurin recruitment can result in anti-HIV and anti-HCV activity in cell culture. Using X-ray crystal structures and docking studies, we have designed a series of novel compounds represented by **1** for analysis of cyclophilin A inhibition. This presentation will include the design, synthesis and biological activity of selected compounds.



1

R_1 = alkyl

R_2 = alkyl

R_3 = alkyl

R_4 = halogen

R_5 = halogen

MEDI 58

Novel inhibitors of DENV NS2B/NS3pro protease

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Dengue virus (DENV) NS2B/NS3pro protease is a non-structural dengue protease that plays a pivotal role in viral maturation and has been identified as a viable drug target for treating dengue viral infections. Currently, there is no vaccine or antiviral drug available to treat these infections which are fatal in a small number of cases and debilitating in a majority of cases. A substrate-based peptide aldehyde inhibitor (PhAc-Lys-Arg-Arg-CHO) targeting DENV3 NS2B/NS3pro protease has been reported [1], with a moderate inhibitory activity (IC_{50} = 10 μM). Analogues of this compound are three orders of magnitude more potent inhibitors of the corresponding West Nile Virus NS2B/NS3pro enzyme [2]. The hydrophilic nature of this highly charged peptide leads to low cell-permeability, rendering it undesirable as a drug candidate.

We present some approaches to generating potent, selective and more drug-like DENV NS2B/NS3pro inhibitors evaluated in *in vitro* enzymatic assay. Selected compounds and their activities will be described and discussed.

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MEDI 59

Synthesis of pyoverdine analogs with potential antibacterial properties

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Because of its resistance to classical antibiotics, *Pseudomonas aeruginosa* has become an important public health problem. *P.aeruginosa* needs iron, present in low quantity in biological media for its development. To obtain it, *P. aeruginosa* produces Pyoverdine (Pvd) that is the principal siderophore secreted into the extra-cellular environment where it binds Fe^{3+} ions. Then these newly formed pyoverdine-iron complexes are transported back into the cell *via* specific receptor proteins, namely FpvA. Our objective is to synthesize analogues of Pvd showing a significant siderophore activity and able to antagonize the FpvA receptor or/and carry antibiotics.

MEDI 60

Lead optimization of novel benzimidazoles for efficacious antitubercular agents targeting FtsZ

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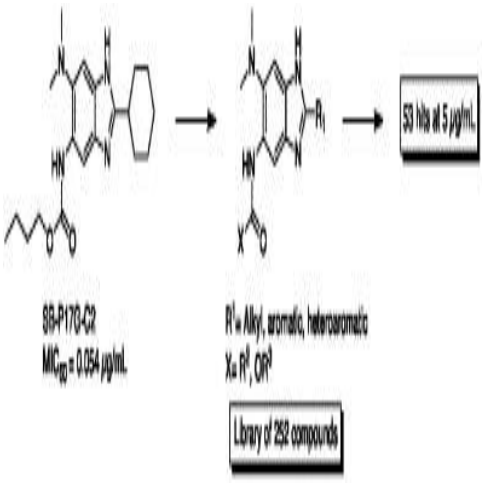
Recent statistics suggest that there is a significant increase in the number of MDR-TB and XDR-TB cases, suggesting the pressing need for the development of new anti-TB agents with novel mechanism of action. In this context, FtsZ, a crucial bacterial cell division protein is a potential target for the development of new anti-TB agents. Previously a library of 2,5,6- and 2,5,7-trisubstituted benzimidazoles were synthesized and tested for their anti-TB activity. A large number of molecules from this library displayed excellent activities with MIC_{50} 0.054-6.1 μg/mL. The lead molecules inhibited the assembly of *Mtb*FtsZby enhancing the GTPase activity. Based on the SAR studies a new series of 2,5,6-trisubstituted benzimidazole library containing a dimethylamino group at the 6-position and various modifications at the 2-position was designed and synthesized. The synthesis and biological evaluation of the new library of 2,5,6-trisusbstituted benzimidazoles will be presented.



MEDI 61

Design and evaluation of novel 8-oxo-pyridopyrimidine Jak1/2 inhibitors

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The Janus kinases (Jak1, Jak2, Jak3, and Tyk2) are intracellular protein tyrosine kinases with essential roles in immune function and hematopoiesis. Inhibition of the Jaks has shown promise against hematopoietic and immunologic diseases, as evidenced by recent approvals of the pan-Jak inhibitors ruxolitonib and tofacitinib for treatment of myelofibrosis and rheumatoid arthritis (RA), respectively. Our interest in the identification of Jak inhibitors led to the design of an 8-oxo-pyridopyrimidine which exhibited good Jak1 potency (235 nM) and excellent ligand efficiency (LE = 0.51). Optimization of this template resulted in inhibitors with low nanomolar enzyme potencies and good cellular potencies in related assays for both Jak1 and Jak2 isoforms. A crystal structure of an optimized inhibitor bound to Jak1 isoform was also obtained.

MEDI 62

Furopyridines, discovery of a novel, potent, and selective class of spleen tyrosine kinase inhibitors with in vivo efficacy in a mouse collagen antibody-induced arthritis model

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Spleen Tyrosine Kinase (SYK) is a promising target for the treatment of autoantibody-mediated diseases such as RA and SLE. Here we present a new and promising scaffold for orally available SYK inhibitors. SYK is a non-receptor tyrosine kinase. It mediates FcR (FcgRI, II, III, FceRI, FcaR) and BCR signaling. These mechanisms are involved in rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). Few SYK inhibitors are in clinical testing, however, they have some safety issues. Therefore, there is a strong need to develop safe and selective SYK inhibitors.

We designed a target specific library of 50 novel compounds and screened it during a HTS campaign. One of the first hits showed a good potency of 390 nM (compound I). By rational drug design we could improve the potency to 20 nM. However, this compound inhibited 19 % of tested kinases with an IC50 < 1 µM. By specific structural changes, the KDR and SRC selectivity could be improved, leading to compounds like no II, which was 21 or 76 fold more active on SYK than on KDR and SRC, respectively. The overall kinase selectivity was 10 % (IC50< 1 µM, 168 kinases tested). Mouse PK parameters were unfavourable with an in vivo clearance of 3.8 L/h/kg and bioavailability of 14%, respectively. During the next optimization cycle, we identified compound III with 10 nM SYK potency, favourable selectivity (5 % of the kinases with an IC50 < 1 µM), including KDR and SRC. This compound is soluble (935 µg/ml) and has favourable PK profile in mice (Clearance 1.5 L/h/kg, bioavailability of 45 %). After oral administration in mice, compound III showed dose dependent efficacy in the ERK phosphorylation assay and Passive Cutaneous Anaphylaxis model, respectively. Furthermore, the compound showed inhibition of disease parameters in the mouse Collagen-Antibody Induced Arthritis model.

MEDI 63

Discovery of ASP4058, a potent and selective sphingosine-1-phosphate receptor 1 and 5 agonist

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Sphingosine-1-phosphate (S1P) acts as an extracellular ligand for the sphingosine-1-phosphate G-protein coupled receptors (S1P1-5). S1P1 agonism induces receptor internalization, which attenuates T-cell response to S1P gradients, preventing their egress from secondary lymphoid tissues. S1P5 receptor is predominantly expressed in

oligodendrocytes and may contribute to remyelination, whereas activation of S1P3 has been reported to cause heart rate reduction and pulmonary epithelial leakage.

A non selective S1P receptor agonist, FTY720 (Gilenya^{TM}) was approved for the treatment of relapsing remitting multiple sclerosis. The 2nd generation of selective S1P1 agonists and S1P1/5 agonists are in clinical development.

This presentation will detail the discovery and SAR of a potent and selective series of benzimidazole based S1P1/5 agonists. ASP4058 discovered from this series demonstrated efficacy when administered orally in the experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis.

MEDI 64

Cell penetrating peptides targeting the Nrf2/Keap1 protein-protein interaction

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Nuclear factor-erythroid 2 related factor 2 (Nrf2) is a redox-regulated transcription factor, able to induce production of a wide range of phase II and antioxidant enzymes. Increasingly, Nrf2 has been found to be a key mediator in the resolution of inflammation and the progression of chronic diseases. As a result, the induction of Nrf2-mediated genes is an attractive therapeutic target. Its negative regulator, Kelch-like ECH-associated protein 1 (Keap1) sequesters Nrf2 and targets it for ubiquitination. Upon detection of stress by key cysteine residues of Keap1, Nrf2 is released and translocates to the nucleus where it induces gene transcription. Known inducers of Nrf2 act by modification of Keap1 cysteine residues, altering the shape of the protein. The exact mechanism of this induction has not been determined and the off target interactions of these inducers are unknown. Therefore development of a non-covalent, highly specific inducer of Nrf2 is desirable, both for pathway elucidation and therapeutic use. One method to achieve this is to competitively block the Nrf2 binding site of Keap1. Previously, Lo et al. identified several peptides, based upon the Nrf2 protein, which show nanomolar binding to the Keap1 Kelch domain. Our current research has developed modified Nrf2 binding sequence peptides with cell penetrating sequences for the non-covalent induction of the Nrf2/Keap1 pathway. The ability of cell penetrating peptides (CPPs) to transport various cargos across cell membranes makes these Nrf2 binding sequence peptides bioavailable, and useful as both biological probes and therapeutic agents. Data from *in vitro* experiments in THP-1 monocytes suggest that the peptides enter cells rapidly, inducing downstream genes and reducing pro-inflammatory mediators in an established model of bacterial sepsis. Recent work has employed several routes that vary the CPPs utilised or remove the need for them, in order to increase both potency and stability.

MEDI 65

Novel S1P1 receptor agonists with unique intracellular signaling

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Several S1P receptor agonists have been studied for treating relapsing-remitting multiple sclerosis (MS) and other autoimmune diseases. Immuno-modulating effect of S1P1 receptor agonists is based on reducing the number of circulating lymphocyte through agonist's binding to the S1P1 receptor on Lymphocyte then inducing internalization of the receptor via beta-arrestin signaling (functional antagonism). Though reported S1P1 agonists have shown high efficacy in clinical trials, none of them could avoid adverse effects including heart rate reduction. These unfavorable effects of S1P1 agonists are presumably caused by the stimulation of S1P1 receptor on cardiomyocytes via G-protein (Gi) signaling and sequential GIRK channel activation. We hypothesized that a novel S1P1 agonist that has sufficient receptor internalization activity via beta-arrestin signaling but with weaker GIRK activity via Gi signaling activity is expected to be a next-generation S1P1 agonist having a broader therapeutic window. In order to create the compound with our aim, we optimized our thiazole derivatives based on in vitro (receptor internalization assay and Ca influx assay) and in vivo (lymphocyte reduction and cardio-toxicity in rats) tests. One of the optimized compounds CP9531 has impressive efficacy both in vitro and vivo tests and improved safety profile. Interestingly, CP9531 and its some derivatives have a potent Ca influx activity that is distinct from other S1P1 agonists in clinical trials. We speculated that CP9531 derivatives activate not only Gi, but other G-proteins (such as Gq) that weaken the Gi signaling in the cell, and consequently moderate the cardio-toxicity derived from the S1P1 receptor stimulation by agonists. Additionally, CP9531 induced faster receptor internalization than BAF-312 (siponimod). These results indicate that our compounds are expected to have broader therapeutic window and faster onset than other S1P1 receptor agonists. Detailed SAR will be discussed in the presentation.

MEDI 66

Novel tricyclic modulators of sphingosine-1-phosphate receptor 1 (S1P1)

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Sphingosine-1-phosphate (S1P) is the endogenous ligand for a family of G-protein coupled receptors (S1P1-5) and evokes a variety of cellular responses through their stimulation. Clinical validation of S1P receptor modulation therapy was recently achieved with the approval of fingolimod (Novartis) as the first oral disease modifying treatment for relapsing remitting multiple sclerosis. While the phosphorylated metabolite of fingolimod was found to be a non-selective S1P receptor agonist, agonism specifically of S1P1 is responsible for the peripheral blood lymphopenia believed to be key to its efficacy. Identification of modulators that maintain activity on S1P1 while sparing activity on other S1P receptors could offer equivalent efficacy with reduced liabilities. This presentation will detail the discovery and SAR of a potent and selective series of tricyclic modulators of S1P1. Compounds in this series were highly active in a pharmacodynamic model (suppression of circulating lymphocytes) and demonstrated impressive efficacy when administered orally in a rodent model of arthritis.

MEDI 67

Acylureas as thiadiazole amide isosteres in the 5*H*-chromeno[2,3-b]pyridine (azaxanthene) series of glucocorticoid receptor agonists

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<h2>We have disclosed structurally novel 2-aryl-5*H*-chromeno[2,3-b]pyridines (azaxanthenes), such as (*S*)-4-(5-(1-((1,3,4-thiadiazol-2-yl)amino)-2-methyl-1-oxopropan-2-yl)-5H-chromeno[2,3-b]pyridin-2-yl)-2-fluoro-N,N-dimethylbenzamide (BMS-776532) and its methylene homologue (BMS-791826), to be selective glucocorticoid receptor (GR) ligands which display a broad range of pharmacologic profiles. [D. Weinstein et al. JMC, 2011, 54, 7318] In this presentation we report that acylureas are good isosteres for 2-acylaminothiadiazole in the azaxanthene-based GR agonists. It has been found that primary acylureas showed not only good GR potency and selectivity, but also improved CYP inhibition and pharmacokinetic profile over the thiadiazole amides. General methods for synthesis of acylureas are discribed. An improved method for synthesis of primary acylureas from a hindered acid will also be discussed.</h2>

MEDI 68

Identification of a novel benzimidazole derivative as a highly potent NPY Y5 receptor antagonist with an anti-obesity profile

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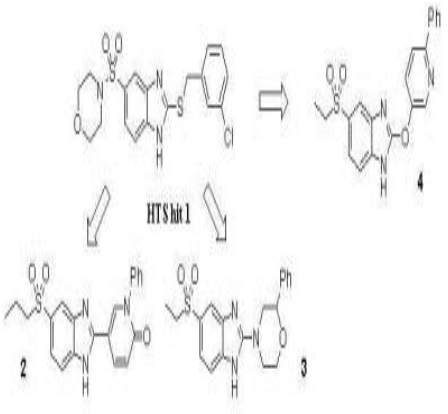
Neuropeptide Y (NPY) is a 36-amino acid peptide which is widely distributed in the central and peripheral nervous systems. The biological effects of NPY are mediated through its interaction with G-protein coupled receptors (Y1, Y2, Y4 and Y5). Among them, the Y5 receptor is thought to play a key role in the central regulation of food intake and energy balance. Therefore, antagonism of the Y5 receptor represents an attractive target for potential therapeutic application against obesity.

Recently, we carried out optimization of HTS hit **1** , with a main focus on modification at the C-2 and C-5 positions of the benzimidazole core. We identified three types of novel benzimidazole derivatives (**2** , **3** , and **4**) as highly potent NPY Y5 receptor antagonists. Among them, derivative **4** exhibited an acceptable PK profile and inhibited food intake induced by the NPY Y5 selective agonist, which resulted in reduction of body weight gain in DIO mice.

In this presentation, we will describe how we successfully identify the novel potent and orally available NPY Y5 receptor antagonist.



MEDI 69

Discovery of arylsulfonyl 3-((1,3-dioxo-2,3-dihydro-1H-pyrrolo[3,4-c]pyridin-4-yl)oxy)-anilines as novel GPR119 agonists

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GPR119 is a G protein-coupled receptor predominantly expressed in human insulin-secreting pancreatic islets and incretin releasing cells in the gastrointestinal (GI) tract. Phospholipids and lipid amides, such as oleoylethanolamide (OEA), have been suggested as its endogenous agonists. Activation of GPR119 with small molecule agonists has been shown to stimulate glucose-dependent insulin secretion and may have beneficial effects on the health of pancreatic islets. Although the physiological role of GPR119 remains unknown and needs to be answered clinically, new oral agents that increase endogenous insulin secretion through activation of GPR119 in a glucose-dependent manner still have the potential to deliver robust efficacy for Type 2 diabetes treatment with much lower risk of hypoglycemia. In the poster, we describe the study of a high-throughput screen (HTS) hit, compound **1** , which led to the identification of arylsulfonyl 3-((1,3-dioxo-2,3-dihydro-1H-pyrrolo[3,4-c]pyridin-4-yl)oxy)-anilines, such as compound **40** , as potent and efficacious GPR119 agonists with good PK properties.

[Figure]

MEDI 70

Identification and design of a novel series of MGAT2 inhibitors

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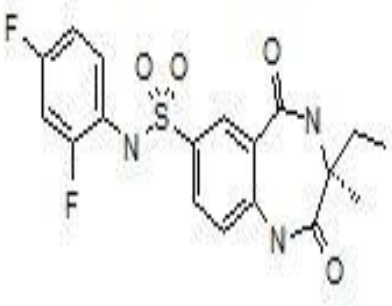
Acyl CoA:monoacylglycerol acyltransferase 2 (MGAT2) is of interest as a target for therapeutic treatment of diabetes, obesity and other diseases which together constitute the metabolic syndrome. In this poster we report our discovery and optimisation of a novel series of MGAT2 inhibitors. The development of the SAR of the series in lead generation phase as well as the development of the different chemistry routes will be disscussed. The *in vivo* results from an oral lipid tolerance test (OLTT) using the MGAT2 inhibitor (*S*)-**10** , shows a significant reduction (68% inhibition relative to naïve, $p<0.01$) in plasma triacylglycerol (TAG) concentration



(*S*)-10

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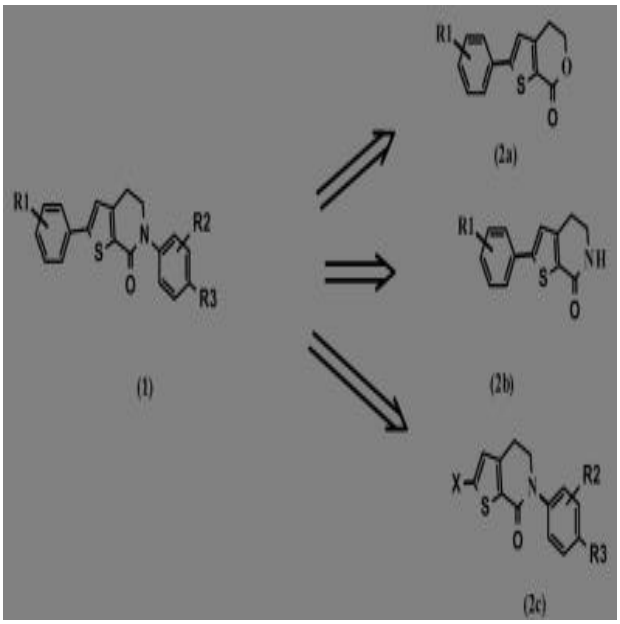
MEDI 71

Exploration of synthetic routes to novel thienolactam MCHR1 antagonists

Steven J Green*, green_steven_j@lilly.com, Erik J Hembre, Yen Dao, Jianliang Lu, Macklin B Arnold, Todd Grote, Steven D Kahl, Saba Husain.Lilly Research Laboratories, Eli Lilly and Co, Indianapolis, IN 46285, United States*

The thienolactam moiety was incorporated into our design of MCHR1 antagonists. Multiple synthetic routes were explored for accessing this novel core. The synthesis of key intermediates 2a, 2b and 2c allowed for flexibility in expanding the SAR via multiple routes. The strategic use of these routes to allow late stage diversification of the different regions of the thienolactam (1) will be discussed. Data will also be presented demonstrating the affinity of these molecules as MCHR1 antagonists.



MEDI 72

Discovery and SAR study of furan-2-carbohydrazides as orally active glucagon receptor antagonists

Futoshi Hasegawa[1], *futoshi-hasegawa@ds-pharma.co.jp, Akihito Fujii*[1], *Kazumi Niidome*[1], *Daisuke Tanaka*[1], *Chiaki Migihashi*[1], *Yasunao Inoue*[1], *Shigeo Ueda*[1], *Tomoki Omodani*[1], *Makoto Murata*[1], *Tomoyuki Hirata*[2], *Kaori Kato*[2], *Takafumi Matsumoto*[2]. *(1) Department of Chemistry Research Laboratory, Dainippon Sumitomo Pharma Co., Ltd., Konohana-ku, Osaka 554-0022, Japan (2) Department of Pharmacology Research Laboratory, Dainippon Sumitomo Pharma Co., Ltd., Konohana-ku, Osaka 554-0022, Japan*

Glucagon, a peptide hormone consisting of 29 amino acid residues produced in the alpha-cells of the pancreas, acts primarily in the liver where it binds to the G-protein coupled glucagon receptor (GCGR) to initiate gluconeogenesis and glycogenolysis. Past studies have demonstrated that secreted glucagon levels in blood of Type II diabetic patients are higher than those of healthy people. These evidences suggest that overstimulation of GCGR in diabetic patients causes serious hyperglycemia. Therefore, the GCGR antagonists have the potential to modulate the rate of hepatic glucose output, resulting in a decrease in plasma glucose levels in diabetics. In our search for novel potent GCGR antagonists, we conducted a hit-to-lead campaign and found a series of furan-2-carbohydrazides as novel GCGR antagonists. Additionally, our medicinal chemistry efforts have identified an orally active GCGR antagonist **1** . In this report, we describe the design, synthesis, SAR study and biological activity of our novel GCGR antagonists.

MEDI 73

Synthesis of mukanadin B and analogs as possible neuroprotective agents

***Michelle Brothers**, mvan126@aucklanduni.ac.nz, David Barker.School of Chemical Sciences, University of Auckland, Auckland, New Zealand*

Glutamate is an important amino-acid that acts as an excitatory neurotransmitter in the central nervous system (CNS), demonstrating effects described as being both beneficial and detrimental. The beneficial effects can be seen by its regulation of neuronal function, growth, and differentiation. However, due to glutamate being an excitatory amino acid it has effects that could be injurious to brain tissue. These effects result from large amounts of glutamate or aspartate being released into the extracellular space, leading to hyperactivation of glutamate receptors. This hyperactivation, termed 'excitotoxicity', has been seen in patients with ischemia, hypoglycaemia, epileptic seizures, and in neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis.

For this reason, glutamate antagonists may prove to be neuroprotective through their actions of inhibiting the hyperactivation of glutamic receptors which occurs in the presence of excessive glutamate.

Recently members of the mukanadin family, 9-hydroxy mukanadin B (1) and 9-methoxy mukanadin A (2), were found to possess potent glutamate antagonism. We therefore present our progress towards the synthesis of mukanadin B (3), mukanadin B pyrrole analogues and C-9-substituted mukanadin B derivatives in order to determine what effect different substitutions have on glutamate antagonism and general bioactivity.

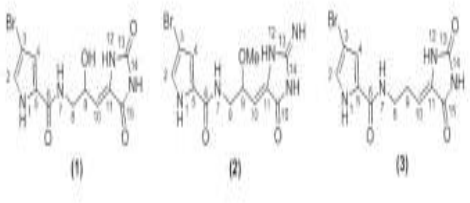


Figure 1. 9-Hydroxy mukanadin B (1), 9-methoxy mukanadin A (2) and mukanadin B (3).

MEDI 74

Descriptors from structural biology predict biological activity of BACE1 inhibitors

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Beta-site amyloid precursor protein cleaving enzyme-1 (BACE1) is a target of interest for treating patients with Alzheimer's disease. Inhibition of BACE1 may prevent amyloid-beta plaque formation and the development or progression of Alzheimer's disease. The number of long hydrophobic contacts between the inhibitor and the active site and the number of short hydrogen-bonds explain 75 percent of the variants in IC50 values.

MEDI 75

Exploratory studies on (*S*)-3,4-dicarboxyphenylglycine, a subtype selective mGlu8 receptor agonist

***Junliang Hao**[1], haoju@lilly.com, Matt Reinhard[1], V Scott Sharp[1], Nicole Hicks[1], Megan A Gokey[1], John T Catlow[1], Steven Swanson[1], Manuel F Molina[2], Beverly Heinz[1], Thomas J Beauchamp[1], Eric P Seest[1], Mark G Bures[1], David B Shaw[1], Cecilia Mur[2], Joan H Carter[1], Amy B Vandergriff[1], Mallorie R Bracey Walker[1], Edda F Roberts[1], Brian G Getman[1], Chuanxi Xiang[1], James A Monn[1], David L McKinzie[1], Jeffery M Schkeryantz[1]. (1) Lilly Research Laboratories, Eli Lilly & Company, Indianapolis, IN 46285, United States (2) Centro de Investigación Lilly, S.A., Alcobendas, Madrid 28108, Spain*

L-Glutamate is the major excitatory neurotransmitter in the mammalian central nervous system. The glutamate receptors include ionotropic glutamate (iGlu) receptors (ligand-gated ion channels) and metabotropic glutamate (mGlu) receptors (Class C G protein-coupled receptors). There are three classes of iGluRs, namely, AMPA, kainate, and NMDA receptors. Eight mGluRs are also further divided into three groups: group I (mGlu1/5), group II (mGlu2/3) and group III (mGlu4/6/7/8). (*S*)-3,4-dicarboxyphenylglycine ((S)-DCPG) was first identified in 2001 as a highly selective and potent mGlu8 receptor agonist (EC_{50} = 31±2 nM). It has been investigated as an anticonvulsant, anxiolytic, or antipsychotic agent in animal studies. Chemically, (*S*)-DCPG belongs to racemization-prone arylglycines, and the question whether (*S*)-DCPG racemizes in vivo following systemic administration has not been previously addressed. This is particularly relevant owing to the reported AMPA-receptor antagonist activity of the *R*-isomer of this molecule. To ensure that physiologic responses following systemic administration of (*S*)-DCPG in rodents are not mediated by in vivo racemization and production of the *R*-isomer, we have developed highly sensitive analytical methods for detecting both enantiomers of this molecule and applied this method to assess racemization following systemic dosing of (*S*)-DCPG in vivo. We also report here a large-scale synthesis of (*S*)-DCPG and a homology model of (*S*)-DCPG docked with the extracellular domain of human mGlu8 receptor.

MEDI 76

Design and synthesis of piperidine derivatives as potent gamma-secretase modulators

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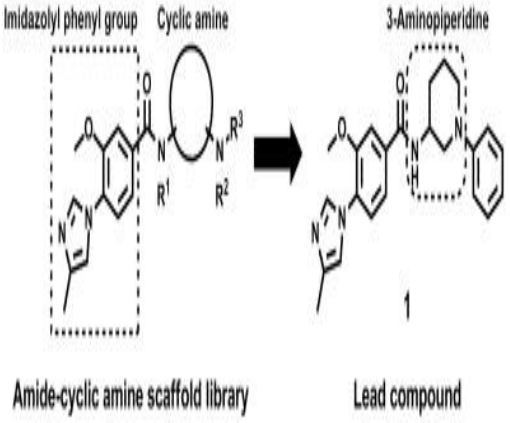
Gamma-Secretase Modulators (GSMs) are compounds capable of allosteric modulation of gamma-secretase, a protease responsible for the final cut of the amyloid beta-peptide precursor protein (APP) to produce the amyloid beta-peptide implicated in the pathogenesis of Alzheimer's disease. Several researchers have reported GSMs with the imidazolyl phenyl group as a key pharmacophore for gamma-secretase modulating activity. In order to discover a new chemotype of GSMs, we have constructed the amide-cyclic amine scaffold library containing the imidazolyl phenyl moiety. From in vitro screening this library, we have found a lead compound (1) which has a 3-aminopiperidine structure. In this presentation, we describe the GSM library design and the optimization studies base on the lead compound 1.



MEDI 77

High-yield synthesis of T808 and T808P for preparation of [^{18}F]-T808, a tau PET tracer for Alzheimer's disease

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[^{18}F]-T808 is a highly selective and specific PET (positron emission tomography) tracer for imaging of tau pathologies in Alzheimer's disease (AD), recently developed and patented by Siemens. The importance of this compound as a PET AD imaging agent is well recognized, and broader research investigation to fully explore and validate the

utility of neuroimaging tool [^{18}F]-T808-PET is important. However, the limited commercial availability, complicated and patented synthetic procedure, and high costs of starting materials and precursor can present an obstacle to more widespread evaluation of this intriguing agent. Wishing to study this compound in our PET center, we decided to make our own material by modifying the literature methods. The authentic standard T808 and its corresponding mesylate precursor T808P were synthesized from ethyl vinyl ether and trichloroacetyl chloride in 6 and 6 steps with 35% and 55% overall chemical yield, respectively. Significant improvements in the multiple-step organic synthesis of T808 and T808P included eliminating the use of microwave reactor, increasing the yields, and enlarging the reaction scale from mg-grade to g-grade. Following the literature method, [^{18}F]-T808 can be prepared from T808P by the nucleophilic substitution with K[^{18}F]F/Kryptofix 2.2.2 and isolated by HPLC combined with SPE (solid-phase extraction) purification.

MEDI 78

Asymmetric synthesis of ^{18}F-FP-(+)-DTBZ as potential PET imaging agent for vesicular monoamine transporter

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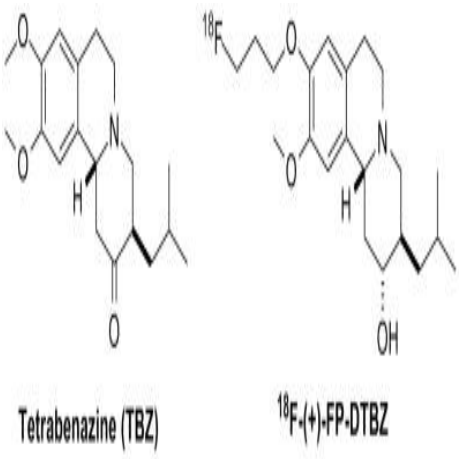
In August 2008, racemic tetrabenazine (3-(2-methylpropyl)-9,10-dimethoxy-1,3,4,6,7,11b-hexahydro-2*H*-benzo[a]quinolizin-2-one, (±)-TBZ) was approved for the treatment of chorea associated with Huntington's disease in USA. The primary pharmacological action of TBZ and its active metabolites, such as dihydrotetrabenazine (DTBZ), is to deplete the levels of monoamines within the central nervous system by inhibiting the human vesicular monoamine transporter 2 (VMAT2). Therefore, radiotracers derived from TBZ can be potential biomarkers of VMAT2 for positron emission tomography (PET) imaging. (2*R*, 3*R*, 11b*R*)-9-Fluoropropyl-(+)-dihydrotetrabenazine (FP-(+)-DTBZ) was found to have nanomolar binding affinity to VMAT2 (K_d = 6.76 nM) and could differentiate normal controls from Parkinson's disease subjects. In this report, asymmetric synthesis of ^{18}F-FP-DTBZ and PET imaging study using ^{18}F-FP-(±)-DTBZ and ^{18}F-FP-(+)-DTBZ on male Sprague–Dawley rats are presented.



MEDI 79

Identification of a series of novel hydroxyacetophenones containing dual pharmacology at the mGluR2 (potentiator) and CysLT1 (antagonist): SAR, ADME properties and effects in an in vivo model of migraine

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The amino acid glutamate is the major excitatory neurotransmitter in the mammalian brain exerting its effects through ionotropic and metabotropic glutamate receptors. The family of metabotropic glutamate receptors is divided into eight subtypes and classified into three groups based on their sequence homology, signal transduction and pharmacology. Group I (mGluR1 and 5) are positively coupled to phospholipase C whereas group II (mGluR2 and 3) and group III (mGluR4, 6, 7 and 8) receptors are negatively coupled to adenyl cyclase. Due to the high degree of homology between group II mGluR's, very few selective agonists for mGluR2 vs. mGluR3 have been discovered. Therefore, another strategy entails finding a positive allosteric modulator (PAM) of the mGlu2 receptor, which does not bind to the glutamate binding site, but rather binds to the transmembrane domain and increases the affinity of the mGlu2 receptor for glutamate. Allosteric potentiators of mGluR2 have been shown to inhibit glutamate neurotransmisson under conditions of enhanced, but not normal glutamate activity with potential therapeutic use in certain CNS conditions like anxiety, schizophrenia and pain/migraine. A high-throughput screen (HTS) identified the hydroxyacetophenone series as one class of compounds acting as mGluR2 PAMs.

Moreover, these compounds also demonstrated antagonist activity at the CysLT1 (LTD_4) receptor and it was hypothesized that this dual combination of pharmacologies would prove beneficial as a treatment for migraine pain. The SAR, ADME characteristics and *in vivo* pharmacology leading to the identification of clinical candidate LY2300559 possessing these dual pharmacologies will be presented.

MEDI 80

Synthesis and neurotrophic activity evaluation of amaryllidaceae alkaloids

*Huairong Luo[2], Maomao He[1], Jinmei Zhu[1], Chunfeng Wu[2], Zixin Deng[1], **Xuechuan Hong**[1], xhy78@whu.edu.cn. (1) School of Pharmaceutical Sciences, Wuhan University, Wuhan, Hubei 430072, China (2) State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan 650201, China*

Neurotrophic factors are best known for their roles in both development and continued maintenance of the nervous system. Their strong potential to elicit pro-survival and pro-functional responses in neurons of the peripheral and central nervous system make them good drug candidates for treatment of neurodegenerative disorders. Developing low molecular natural products having a typical neurotrophic property has become one of the hot topics in modern drug development. This topic mainly focuses on the total synthesis of a library of amaryllidaceae alkaloids and their derivatives, and the study of their biological activities and their neuroprotective mechanism through Cell Biology, C. elegans mutants, knockout mice and other technical tools.

MEDI 81

Isoquinolinone-4-carboxamides as NK3 receptor antagonists: Discovery of development candidate Lu AE61035

***Karsten Juhl**[1], kaju@lundbeck.com, Nikolay A. Khanzhin[1], Christoffer Bundgaard[1], Dorrit B. Larsen[1], Björn Steiniger-Brach[2], Søren M. Nielsen[2], Klaus B. Simonsen[1]. (1) Discovery Chemistry and DMPK, H. Lundbeck, Valby, Denmark (2) Discovery Pharmacology Research, H. Lundbeck, Valby, Denmark*

The identification and structure activity relationships of isoquinolinone-4-carboxamides as novel NK_3 receptor antagonists are presented. The compound series was optimized to give compounds that show **antipsychotic potential** in the amphetamine induced hyperactivity model in guinea pigs.

SAR on NK_3/NK_2 selectivity, brain/plasma ratios and *in vivo* binding to the NK_3 receptor in guinea pigs are discussed.

MEDI 82

Novel positive allosteric modulators (PAMs) of the metabotropic glutamate 2 receptor and cysLT1 antagonists for migraine treatment

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Glutamate is the major neurotransmitter in the central nervous system, and abnormal glutamate neurotransmission has been implicated in many neurological disorders, including schizophrenia, Alzheimer's disease, Parkinson's disease, addiction, anxiety, depression, epilepsy, migraine and pain. Metabotropic glutamate receptors (mGluRs) activate intracellular signaling cascades in a G-protein dependent manner, which offer the opportunity for developing drugs that regulate glutamate neurotransmission in a functionally selective manner.

Lilly was one of the first to disclose a PAM of the mGlu2 receptor with in vitro and in vivo data reported on the *meta*-pyridylsulphonamide series. We have also identified several hydroxyacetophenone series with compounds that are not only potent mGluR2 PAMs but also possess significant CysLT1 (cysteinyl leukotriene receptor 1) antagonist activity. Here we will describe a novel structure-activity relationship (SAR) of this series modulating dual pharmacological activity, optimization for potency, PK properties and efficacy in a dural plasma protein extravasation (PPE) model of migraine.

MEDI 83

Stereochemical requirements within the tetrahydroquinoline scaffold for the positive allosteric modulation at alpha7 nicotinic acetylcholine receptors

Abhijit R Kulkarni[1]*, kulkarni.abh@husky.neu.edu, Jeffrey Deschamps*[3]*, Roger L Papke*[2]*, Ganesh A Thakur*[1]*. (1) Pharmaceutical Sciences, Northeastern University, Boston, MA 02115, United States (2) Pharmacology and Therapeutics, University of Florida, Gainsville, FL 32610, United States (3) Naval Research Laboratory, Washington, DC 20375, United States*

Activation of a7-nicotinic acetylcholine receptor (nAChRs) has broad therapeutic potential for the treatment of cognitive deficits associated with schizophrenia, Alzheimer's disease, Parkinson's disease, and attention-deficit/hyperactivity disorders as well as inflammation and neuropathic pain. Although activation of a7 nAChR ion channels is primarily controlled by the binding of ligands at conventional orthosteric site, it is also regulated in either positive or negative ways by binding of ligands to an allosteric site in the transmembrane domains which is topographically distinct from orthosteric site. Among the Type II Positive Allosteric Modulators (PAMs) of a7 nAChRs,

4BP-TQS (4-(4-bromophenyl)-3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[c]quinoline-8-sulfonamide)

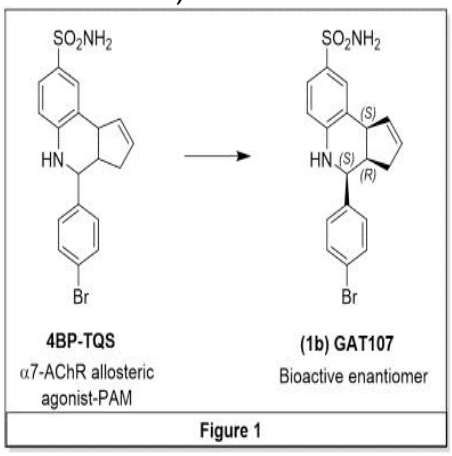

Figure 1

is unique in a sense that in addition to being a potent PAM, it also behaves as an allosteric agonist. In this work, we have developed an expeditious microwave-assisted synthesis of 4BP-TQS, performed separation of its enantiomers by chiral HPLC followed by their biochemical evaluations. Initial electrophysiological characterization in *Xenopus* oocytes revealed that the biological activity almost exclusively resided in the (+)-enantiomer **1b** (GAT107). The (-)-enantiomer **1a** had negligible activity as an allosteric agonist or PAM and it did not affect ago-PAM activity of (+)-enantiomer **1b** , when applied together. X-ray crystallography studies revealed the absolute stereochemistry of the active enantiomer **1b** to be 3a*R*, 4*S*, 9b*S*. This enantiomer represents the most potent ago-PAM of a7 nAChRs and is considered as a candidate for further evaluation in vivo.

MEDI 84

Novel positive allosteric modulators of CB1 cannabinoid receptor for the treatment of anorexia nervosa

Pushkar M Kulkarni[1]*, pm.kulkarni@neu.edu, Torbjorn Jarbe*[2]*, Steven Goldberg*[3]*, Nicole r Barbarich-Marsteller*[4]*,* ***Ganesh A Thakur***[1,2]*, g.thakur@neu.edu. (1) Pharmaceutical Sciences, Northeastern University, Boston, MA 02115, United States (2) Center For Drug Discovery, Pharmaceutical Sciences, Northeastern University, Boston, MA 02115, United States (3) Preclinical Pharmacology Section, Biomedical Research Center, National Institute on Drug Abuse, Baltimore, MD 21224, United States (4) Preclinical Eating Disorders Research Laboratory, Columbia University, New York, NY 10032, United States*

The endocannabinoid system (ECS) comprising of two cannabinoid receptors (CB1 and CB2), endogenous cannabinoids (eCB), enzymatic machinery in-charge of eCB synthesis and inactivation has been recognized as one of the most important modulatory system in the brain. It is known to impact neurobiology of reward processing

and feeding behavior. ECS is underactive in individuals with anorexia nervosa (AN) leading to compensatory chronic upregulation of CB1 in cortical and subcortical brain areas. Moreover, an increase in plasma levels of anandamide, an endogenous CB1 agonist, has also been shown in these individuals.

Δ9-Tetrahydrocannabinol (THC), a psychoactive component of marijuana and a CB1 agonist, is known to increase food intake and improve hedonic properties of food. Recently, THC has been shown to attenuate weight loss in a rodent model of AN. Currently THC is in phase-III clinical trial for the treatment of AN. However, THC or other CB1 direct-acting agonists are associated with undesirable CNS side effects, strong abuse and addiction potential which limit their use as medications. Accomplishing CB1 agonism through a different pharmacological mechanism such as positive allosteric modulation may provide a safer alternative. Our novel approach involves use of positive allosteric modulators (PAMs) for the treatment of AN. PAMs bind to a topographically distinct (allosteric) CB1 site, positively modulate the eCB signaling. We have synthesized a novel series of CB1 PAMs and evaluated their potential in-vitro and in-vivo. In functional assays (cAMP and b-arrestin) PAMs show a potentiation of agonist response. In our microdialysis studies PAMs showed negligible rise in dopamine level in nucleus accumbens shell, thus, indicating less abuse liability. In activity based anorexia model one of the PAMs showed a decrease in hyperactivity. Therefore, PAMs alone or in combination with lower doses of THC/CB1 agonist would maintain an acceptable therapeutic value in the treatment of AN.

MEDI 85

Synthesis of kojic acid derivatives as secondary binding site probes of D-amino acid oxidase

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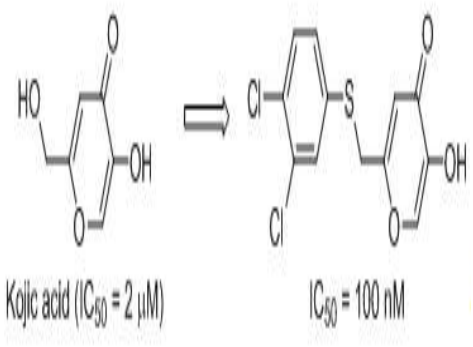
D-Serine is an endogenous co-agonist at the glycine modulatory site of the *N*-methyl-D-aspartate (NMDA) receptor. D-Serine degradation is catalyzed by the flavoenzyme D-amino acid oxidase (DAAO) producing the corresponding imino acid, along with ammonia and hydrogen peroxide as by-products. Oral administration of D-serine in combination with a DAAO inhibitor could improve D-serine pharmacokinetics and provide a new therapeutic option for the treatment of disorders associated with NMDA receptor hypofunction such as schizophrenia. As part of our continuous efforts to identify a new structural class of DAAO inhibitors, a series of kojic acid (5-hydroxy-2-hydroxymethyl-4*H*-pyran-4-one) derivatives were synthesized and evaluated for their ability to inhibit DAAO. Various substituents were incorporated into kojic acid at its 2-hydroxymethyl group. These analogs serve as useful molecular probes to explore the

newly recognized secondary binding site, which can be further exploited in designing more potent DAAO inhibitors.



MEDI 86

Discovery of LSN2535717, a selective, orally active mGluR2 positive allosteric modulator (PAM): Lead generation SAR and effects in animal models of anxiety

Daniel R. Mayhugh*, mayhugh_daniel_r@lilly.com, Albert Khilevich, James A. Knobelsdorf, David A. Hay, Kjell A. Svensson, Julie F. Falcone, Steve Swanson, John T. Catlow, Linda Rorick-Kehn, John C. Hart, Beverly Heinz, Xushan Wang, Mark J. Benvenga, Stephen Chaney, Jeffrey M. Witkin, Xia Li, Jeffrey M. Schkeryantz.Lilly Research Labs, Eli Lilly & Company, Indianapolis, Indiana 46285, United States*

The amino acid glutamate is the major excitatory neurotransmitter in the mammalian brain exerting its effects through ionotropic and metabotropic glutamate receptors. The family of metabotropic glutamate receptors is divided into eight subtypes and classified into three groups based on their sequence homology, signal transduction and pharmacology. Group I (mGluR1 and 5) are positively coupled to phospholipase C whereas group II (mGluR2 and 3) and group III (mGluR4, 6, 7 and 8) receptors are negatively coupled to adenyl cyclase. Compounds that activate Group II (mGlu2 and 3) receptors have the potential to treat several disorders of the central nervous system (CNS) including anxiety, schizophrenia and pain/migraine. Herein we describe the lead generation efforts around a series of compounds containing the hydroxyacetophenone moiety as selective mGluR2 positive allosteric modulators (PAMs). Our initial series of compounds containing a hydroxyacetophenone functionality identified LY2300559 possessing dual pharmacology (mGluR2 PAM and CysLT1 antagonist) with therapeutic potential in chronic migraine. However the initial compounds possessed limited brain penetration. A description of the SAR leading to increased brain penetration while maximizing potency to generate the advanced lead compound LSN2535717 will be

discussed. In addition, it's ADME and *in vivo* effects in animal models of anxiety will be presented.

MEDI 87

Discovery of LY2607540: A novel and selective mGluR2 positive allosteric modulator (PAM) demonstrates activity in animal models of anxiety and depression

***Albert Khilevich**, khilevich_albert@lilly.com, Daniel R. Mayhugh, David A. Hay, Deyi Zhang, Bin Liu, Kjell A. Svensson, Julie F. Falcone, Beverly A. Heinz, Xushan Wang, Jeff M. Witkin, Kurt Rassmussen, Linda Rorick-Kehn, Mark J. Benvenga, Xia Li, John C. Hart, Stephen Chaney, John T. Catlow, Steve Swanson, Jeffrey M. Schkeryantz.Lilly Research Labs, Eli Lilly & Company, Indianapolis, Indiana 46285, United States*

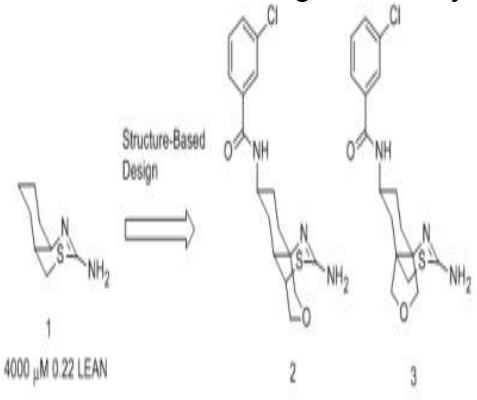
The amino acid glutamate is the major excitatory neurotransmitter in the brain. The family of metabotropic glutamate receptors is divided into eight subtypes and classified into three groups based on their sequence homology, signal transduction and pharmacology. Group I (mGluR1 and 5) are positively coupled to phospholipase C whereas group II (mGluR2 and 3) and group III (mGluR4, 6, 7 and 8) receptors are negatively coupled to adenyl cyclase. The mGlu2 receptors are mainly located presynaptically and negatively regulate glutamate release from nerve terminals. Allosteric potentiators of mGluR2 have been shown to inhibit glutamate neurotransmisson under conditions of enhanced, but not normal glutamate activity with potential therapeutic use in certain CNS conditions like anxiety. Our initial series of hydroxyacetopheneone containing compounds identified LY2300559 which contained dual pharmacology (mGluR2 PAM and CysLT1 antagonist) with therapeutic potential in chronic migraine. However this compound possessed limited brain penetration. Addition SAR focusing on improving brain penetration led to the discovery of LSN2535717, which showed efficacy in multiple mood disorder assays. Unfortunately LSN2535717 underwent metabolism through a unusual *in vivo* Baeyer-Villiger like oxidation to form significant amounts of catechol metabolites. The SAR to diminish the undesired catechol metabolites as well as biological data which led to the discovery of the clinical candidate LY2607540 will be presented.

MEDI 88

Highly regio- and diastereoselective syntheses of conformationally constrained nonpeptidic β-secretase inhibitors

***Leonard L Winneroski**[1], lwinneroski@lilly.com, James P Beck[1], Brian M Mathes[1], Kevin J Hudziak[1], Brain M Watson[1], Matthew A Schiffler[1], Patrick C May[1], Leonard N Boggs[1], David E Timm[1], Stephanie L Stout[1], Jon A Erickson[1], Robert D Boyer[1], Richard A Brier[1], Anthony R Borders[1], Scott A Monk[1], James E Audia[2], Dustin J Mergott[1]. (1) Lilly Research Laboratories, Eli Lilly & Co., Indianapolis, IN 46285, United States (2) Constellation Pharmaceuticals, Inc., Cambridge, MA 02142, United States*

Fragment screening efforts led to the identification of cis-fused 4a,5,6,7,8,8a-hexahydro-4*H*-1,3-benzo[*d*]thiazin-2-amine (1), a 4000 µM inhibitor of β-secretase. X-Ray analysis of fragment (1) bound in the active site of hBACE1 helped inform the design of conformationally constrained analogues (2) and (3), which required the development of independent synthetic strategies. Tricycle (2) was prepared *via* a 17-step linear sequence featuring a palladium-catalyzed pi-allyl cyclization to build the cyclohexane core, a diastereoselective iodine-promoted cyclization of a highly functionalized thiourea to establish the cis-fused [6,6] ring system, and a reductive alkylation to construct the final tricyclic ether ring system. Inhibitor (3) was prepared in 21 steps, using a diastereoselective [4 + 2] cycloaddition reaction to introduce the cis-fused hexahydroisobenzofuran, followed by cyclization of a highly functionalized thiourea to assemble the thiazine ring. The retrosynthetic strategies and evolution of the synthetic routes that enabled the preparation of compounds (2) and (3) will be discussed. The biological activity of these β-secretase inhibitors will also be presented.



MEDI 89

Identification of novel, selective CDK5/p25 inhibitor: Structure based virtual screening, synthesis, biological evaluation and SAR studies

Arindam Chatterjee[1], *achatter@go.olemiss.edu, Stephen J Cutler*[1,4], *Robert J Doerksen*[1,4], *Ikhlas A Khan*[2,3], *John S Williamson*[1,4]. *(1) Department of Medicinal Chemistry, University of Mississippi, University, MS 38677, United States (2) Department of Pharmacognosy, University of Mississippi, University, MS 38677, United States (3) National Center for Natural Product Research, University of Mississippi, University, MS 38677, United States (4) The Research Institute of Pharmaceutical Sciences, University of Mississippi, University, MS 38677, United States*

Cyclin dependent kinase 5 (CDK5) is a proline directed Ser/Thr kinase, which is expressed primarily in the central nervous system (CNS). CDK5 regulates neuronal development rather than cell division and is deregulated by its neurotoxic activator p25. As a cascade mechanism, tau protein becomes hyperphosphorylated and produces deposits of neurofibrillary tangles (NT). The deregulation of CDK5 is believed to be responsible for several neurodegenerative conditions including Alzheimer's disease, Parkinson's disease, stroke and Huntington's chorea. Because of its involvement in NT

formation, the inhibition of CDK5-p25 complex has been identified as a potential therapeutic target for Alzheimer's disease. CDK5 has a high level of structural homology (~60%) with its mitotic counterpart CDK2, which has made it difficult to design selective CDK5 inhibitors. We employed structure based virtual screening of a commercial database containing 2.5 million compounds to identify a group of probable hits. Subsequently, we employed the selectivity constraints from docking scores and identified a limited number of CDK5 selective compounds. These compounds were evaluated in ^{33}P labeled functional assays in order to validate the computational model. A novel, selective and non-ATP competitive CDK5-p25 inhibitor has been identified. The present study highlights the computational model, novel synthetic strategy and further SAR studies of the lead compound.

MEDI 90

Discovery and synthesis of novel 3-alkoxy azetidines as PDE10A inhibitors

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Cyclic nucleotide phosphodiesterases (PDEs) constitute an 11-membered family of bimetallic hydrolase enzymes that regulate cell signaling mediated by the ubiquitous second messengers, cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP). Specifically, cyclic nucleotide PDEs modulate intracellular levels of cAMP and cGMP by specifically hydrolyzing the 3'-5'phosphodiester bond producing AMP and GMP, respectively. PDE10A is a member of this family that is predominantly expressed in the medium spiny neurons of the striatum and modulates intracellular concentrations of cAMP and cGMP. Due to this localization in the striatum, PDE10A has been the target of extensive research efforts for the treatment of neurological disorders. The goal of our studies was the development of a small molecule PDE10A inhibitor with low, nanomolar PDE10A activity, good selectivity against other PDEs and improve solubility. Improved solubility was obtained by substitution of a phenyl ether from our first generation scaffold with a 3-alkoxy-azetidine ring. In general, this scaffold also improved solubility properties. Synthesis and structure activity relationships surrounding this 3-alkoxy-azetidine moeity will be presented.

MEDI 91

Design, synthesis, and biological evaluation of multifunctional molecules as potential anti-Alzheimer agents

***Shin Yu Lai**, f88423011@ntu.edu.tw, Chen Wei Huang, Pei Teh Chang, Chao Wu Yu, Nagendra B Kondekar, Ravindra Ramesh Deore, Sheang Tze Fung, Qing Qing Ye, Ji Wang Chern.School of Pharmacy and Center for Innovative Therapeutic Discovery, National Taiwan University, Taipei, Taiwan Republic of China*

Drug development based on blocking amyloid cascade is the mainstream strategy in recent decade. The biometal dyshomeostasis like Cu^{2+} and Zn^{2+} were regarded as important cofactors of amyloid cascade in AD.However, there are no compounds in the clinical trial targeting on Aβ demonstrate significant benefits in cognitive improvement. Therefore, we designed and synthesized a multifunctional lead compound that could interact with metal resulting in anti-Aβ aggregation, ROS elimination and neurotrophic activities. The muti-functional compound also proved to improve the cognition in AD mice. Hereafter, series derivatives were synthesized to optimize their biological profiles. The results showed that several compounds could inhibit Aβ aggregation, dissolute Aβ aggregates and trigger neurotrophic events. These multi-functional molecules might serve as promising anti-Alzheimer disease agents.

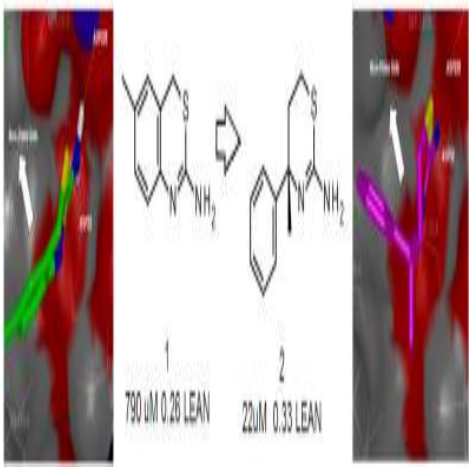
MEDI 92

Fragment based design of aminodihydrothiazines as βeta-secretase inhibitors

*Patrick C May[1], Robert A Dean[1], Stephen Lowe[4], Ferenc Martenyi[1], Scott M Sheehan[1], Leonard N Boggs[1], Scott A Monk[1], Jose E Lopez[1], Brian M Mathes[1], Dustin J Mergott[1], Grant M Vaught[1], **Brian M Watson**[1], watson_brian_m@lilly.com, Leonard L Winneroski[1], Stephanie L Stout[1], David E Timm[1], Richard A Brier[1], Anthony R Borders[1], Theresa Day[1], John M Strelow[1], Elizabeth Smith LaBell[1], Celedon R Gonzales[1], Masako Nakano[8], Stanford S Jhee[5], Mark Yen[5,9], Larry Ereshefsky[5,6], Terry D Lindstrom[3], David O Calligaro[1], Patrick J Cocke[1], D. Greg Hall[1], Stuart Friedrich[1], Martin Citron[7], James E Audia[2]. (1) Lilly Research Laboratories, Eli Lilly & Co, Indianapolis, IN 46285, United States (2) Constellation Pharmaceuticals, Inc, Cambridge, MA 02142, United States (3) Retired—Lilly Research Laboratories, Eli Lilly & Co., Indianapolis, Indiana, United States (4) Lilly-National Uneversity of Singapore Centre for Clinical Pharmacology, Singapore 11697, Singapore (5) PAREXEL International Early Phase Los Angeles, Glendale, California 91206, United States (6) University of Texas Health Science Center, San Antonio, Texas 98284, United States (7) UCB Pharma, Brussels, Belgium (8) Eli Lilly Japan K.K., Kobe 651-0086, Japan (9) California Clinical Trials Medical Group, Glendale, California 91206, United States*

High concentration fragment screening, supported by X-ray crystallography, identified 6-methyl-4H-3,1-benzothiazine **1** , a 790 μM inhibitor of BACE1 which binds to the catalytic diad (Asp 228 and Asp 32) in the BACE1 active site. Using structure based design, **1** was deplanerized to the aminodihydrothiazine **2** which proved to be a versatile platform to gain access to the non-prime cavities of BACE1. Optimized

aminodihydrothiazine inhibitors achieved sub-micromolar activity for BACE1, and were brain penetrant. This poster will discuss the structure guided growth of **2** designed to optimize ligand-protein interactions while maintaining good physiochemical properties, and the in-vivo PD effect on BACE1 relevant biomarkers.



MEDI 93

Design, stereoselective synthesis, and biological evaluation of novel tricyclic compounds as inhibitor of apoptosis proteins (IAP) antagonists

Moriteru Asano[1]*, moriteru.asano@takeda.com, Kentaro Hashimoto*[1]*, Bunnai Saito*[1]*, Zenyu Shiokawa*[1]*, Hiroyuki Sumi*[1]*, Masato Yabuki*[1]*, Mie Yoshimatsu*[1]*, Kazunobu Aoyama*[1]*, Teruki Hamada*[1]*, Nao Morishita*[1]*, Douglas R Dougan*[2]*, Clifford D Mol*[2]*, Sei Yoshida*[1]*, Tomoyasu Ishikawa*[1]*. (1) Pharmaceutical Research Division, Takeda Pharmacetical Company Limited, Fujisawa, Kanagawa 251-8555, Japan (2) Structural Biology, Takeda California, Inc., San Diego, California 92121, United States*

Inhibitors of apoptosis proteins (IAPs) are anti-apoptotic regulator which blocks cell death. The cellular IAP (cIAP) modulates tumor necrosis factor-alpha (TNFα) related death receptor signaling pathway, and the X−linked IAP (XIAP) suppresses apoptosis by binding to effectors, caspase-3 and -7, and an initiator, caspase-9. An apoptosis induction by inhibition of both cIAP and XIAP is considered to be a new therapeutic approach to treat cancer. Our previous synthetic studies on IAP antagonists led to the identification of novel octahydropyrrolo[1,2-*a*]pyrazine **A** , which showed strong IAP binding inhibition along with significant anti-tumor efficacy in MDA−MB−231 (human breast cancer) xenograft model in mice. However, compound **A** displayed insufficient PK profiles due to its low metabolic stability and thus we decided to further investigate to generate IAP antagonists with improved PK profiles. Based on the co-crystal structure of **A** in complex with cIAP, we designed novel tri-cyclic compounds, octahydro-1*H*-cyclopropa[4,5]pyrrolo[1,2-*a*]pyrazines **1** and **2** (diastereomer of **1**). The additional cyclopropane moiety was aimed to block the predicted metabolic site of compound **A**

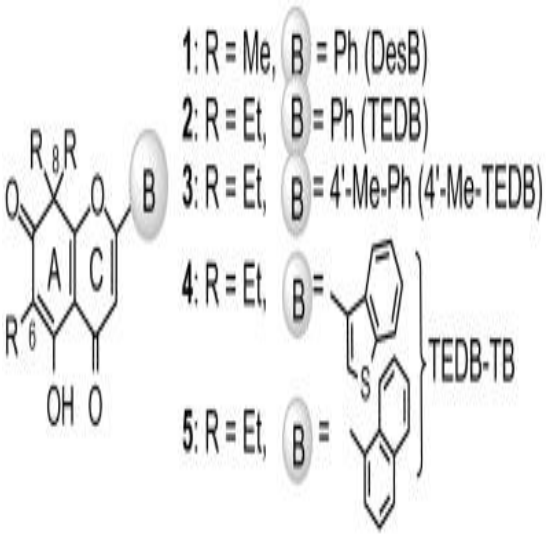
without detriment to the binding affinity against cIAP. These novel cyclopropane structures were constructed by diastereoselective Simmons−Smith reaction of 2,3-dihydro-pyrrole which contains asymmetric functional groups at *C*-2 position. Following sequential formation of requisite amide side chains provided target compounds (**1** , **2**). Compounds **1** and **2** showed strong growth inhibition in MDA−MB−231 cell line with improved metabolic stability compared with that of **A** . Furthermore, one diastereomer of these compounds exhibited significant in vivo PD effect to increase TNFα mRNA induction in a dose dependent manner. In this poster presentation, design, stereoselective synthesis and biological evaluation of novel octahydro-1*H*-cyclopropa[4,5]pyrrolo[1,2-*a*]pyrazine derivatives will be discussed.

MEDI 94

TEDB-TB analogs as new classes of tubulin inhibitors

Kyoko Nakagawa-Goto[1]*, kngoto@p.kanazawa-u.ac.jp, Ernest Hamel*[2]*, Emika Ohkoshi*[3]*, Bastow F Kenneth*[3]*, Kuo-Hsiung Lee*[3]*, Masuo Goto*[3]*. (1) Department of Pharmaceutical Science, Kanazawa University, Kanazawa, Ishikawa 920-1192, Japan (2) Devision of Cancer Treatment and Diagnosis, National Cancer Institute, Frederick, Maryland 21702, United States (3) Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599, United States*

Desmosdumotin B (DesB, **1**) has a unique flavonoid skeleton with an unusual non-aromatic trimethylated ring-A (**Fig. 1**). DesB (**1**)and its triethylated analogs (TEDBs), including **2** and **3** , inhibited only P-gp overexpressing multidrug resistant (MDR) tumor cell growth, leading to significant nonMDR/MDR selectivity. Interestingly, analogs with a bicyclic aryl ring-B (TEDB-TB), such as benzo[*b*]thiophene **4** and naphthalene **5** , displayedadramaticallydifferentbioactivity profile**,** with potent cell growth inhibition against multiple cancer cell lines. These analogs inhibited tubulin polymerization, partially through the colchicine binding site. Importantly, none of the analogs were P-gp substrates, and thus, they were effective against MDR tumors. In structure-activity relationship studies of bicyclic ring-B analogs, various functional groups were installed on ring-B of **4** and **5** to provide better solubility. The newly synthesized analogs were evaluated for cytotoxicity, and their effects on microtubule dynamics. Regardless of the position or character of the functional group, all cytotoxic analogs inhibited the onset of metaphase. While clinically successful tubulin inhibitors target both the mitotic spindle and interphase microtubules in chemosensitive cancer cells, the new analogs effectively inhibited bipolar mitotic spindle formation without interfering with interphase microtubule dynamics in both chemosensitive and MDR cancer cells. Therefore, these TEDB analogs might be promising candidates leading to a new chemotherapeutic drug targeting tubulin, and offer the possibility of overcoming MDR obstacles and reducing unexpected effects in normal cells at interphase.



MEDI 95

Nonpeptide macrocyclic histone deacetylase inhibitors derived from clarithromycin

***Idris O Raji**, iraji3@gatech.edu, Subhasish Tapadar, Shaghayegh Fathi, Eric Raftery, Adegboyega Oyelere.Department of Chemistry and Biochemistry, Parker H. Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, Georgia 30332-0400, United States*

Ever since the discovery that reversal of aberrant epigenetic regulation can be exploited in drug development for cancer and other diseases, efforts have been focused on developing compounds with such capability. Inhibition of Histone deactylases (HDAC), an epigenetic regulator, has since been validated as a viable therapeutic route, with the Food and Drugs Administration (FDA) approving two HDAC inhibitors (HDACi) (Suberoylanilide hydroxamic acid (SAHA) and Romidepsin or FK228) for treatment of Cutaneous T-Cell Lymphoma. Despite their success, SAHA and FK228 suffer from lack of efficacy against solid tumors and off-target toxicity. Towards obtaining tissue selective HDACi, we have designed a series of clarithromycin-capped hydroxamic acid-based HDACi following similar pharmacophoric model with SAHA. We designed and synthesized two series of these compounds having HDACi moiety attached to the desosamine and the cladinose sugars of clarithromycin. Linker lengths in each series were varied to determine the optimum length. Furthermore, we evaluated the HDAC isoform selectivity of these compounds as well as their antiproliferative activity in breast cancer cell line (MCF-7).

MEDI 96

Design and synthesis of pyridone-oxindole derivatives as novel Aurora-B kinase inhibitor

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Hepatocellular carcinoma (HCC) is an aggressive and life-threatening disease which caused enormous number of deaths worldwide. Limited success of current treatments reveals an urgent need for a more effective therapeutics. Literature has highlighted Aurora kinases as potential targets because they frequently overexpress in HCC patients and the expression is associated with poor clinical outcome. Our aim is to develop novel small-molecule inhibitorsagainst Aurora kinases with the hope to ameliorate disease burden. On the basis of our previous work on multikinase inhibitors, scaffold hopping strategy led to discovery of new bioisostere of critical ureido linker. Resultant molecules had different activity spectrum to parent arylureidooxindoles. The IC_{50} against Aurora-B kinase was submicromolar to nanomolar range but showed inactive to other screened tyrosine kinases except Flt-3. Cellular activity was demonstrated by selected compounds in which they decreased phosphorylation level of histone H3 in Hep3B and Huh-7 cells. Herein, we report series of oxindole based compounds as novel Aurora-B kinase inhibitor that showed cross inhibition to Flt-3, and provide a new lead for anti-cancer drug development.

MEDI 97

Syntheses and anticancer activities of N^1, N^3-dialkyl-N^1, N^3-di-(alkylcarbonothioyl)malonohydrazides: The discovery of Elesclomol

***Shoujun Chen**, schen@syntapharma.com, Lijun Sun, Keizo Koya, Noriaki Tatsuta, Zhiqiang Xia, Zhenjian Du, Timothy Korbut, Guiqing Liang, Jim Wu, Mitsunori Ono, Dan Zhou, Andy Sonderfan.45 Hartwell Ave., Synta Pharmaceuticals Corp., Lexington, MA 02421, United States*

Despite the tremendous progress made in cancer chemotherapy, cancers of various kinds still remain among the leading causes of mortality worldwide. Exploiting the unique features of tumor cells and examining the specific molecular pathways or cellular targets that differentiate cancer cells from normal cells are among the key focuses for modern oncology drug discovery.Scientific evidence reveals that elevated levels of reactive oxygen species (ROS) are present in almost all cancer cells. It is known that an extremely high level of ROS, beyond the survival threshold in cancer cells, can trigger apoptosis. In contrast, the relatively low level of ROS in normal cells can be effectively detoxified by the endogenous antioxidants, and normal cells are much more resistant to ROS induction. In parallel, very high levels of HSP70, a 70 kDa heat shock protein, were found in malignant human tumors of various origins, and the expression of HSP70 in cells has been positively correlated with elevated ROS generation. Recent research and observations have suggested that HSP70 can act as a biomarker of oxidative injury. We initiated a program by screening selected compounds from Synta's unique compound library using an HSP70 induction assay together with a cytotoxic assay using

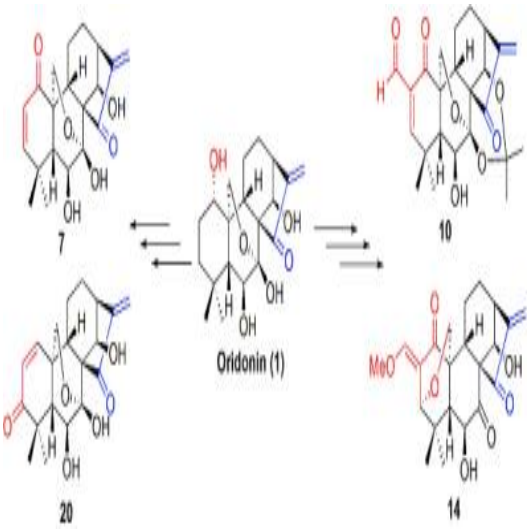
the MES-SA/DX5 cell line, a well-established drug resistant cell line useful for compound screening. Herewith we disclose the discovery, syntheses, and SAR studies of a series of N'[1],N'[3]-dialkyl-N'[1],N'[3]-di(alkylcarbonothioyl) malonohydrazides as anticancer agents, which culminated in the discovery of Elesclomol® (STA-4783) - a novel small molecule that has been evaluated in a number of clinical trials and showed promising results with patients having low level of lactate dehydrogenase (LDH).The hit generation, lead optimization, process chemistry and final candidates' *in vitro*/*in vivo* pharmacologic profiling will be discussed.

MEDI 98

Oridonin ring A-based diverse constructions of enone functionality: Identification of novel dienone analogs for treatment of highly aggressive breast cancer

*Chunyong Ding[1], Yusong Zhang[2], Haijun Chen[1], Zhengduo Yang[2], Christopher Wild[1], Lili Chu[2], Mark A. White[1], Qiang Shen[2], **Jia Zhou**[1], jizhou@utmb.edu. (1) Chemical Biology Program, Department of Pharmacology and Toxicology, Sealy Center for Structural Biology & Molecular Biophysics, University of Texas Medical Branch, Galveston, TX 77555, United States (2) Department of Clinical Cancer Prevention, Division of Cancer Prevention and Population Sciences, The University of Texas M.D. Anderson Cancer Center, Houston, TX 77030, United States*

Highly aggressive and drug-resistant breast cancer remains an unmet medical need and represents an important clinical challenge. Oridonin, isolated from the herb *rabdosia rubescens* that is often used in Chinese traditional medicine, has attracted considerable attention in recent years due to its remarkably unique and safe anticancer pharmacological profile. Nevertheless, it is not very effective against highly aggressive and drug-resistant breast cancer cells such as triple-negative MDA-MB-231. Herein, we disclose our facile and efficient synthetic approaches to generating novel oridonin dienone analogues with the enone functionality diversely installed in the A-ring through a series of selective functional group transformations starting from the natural product. These new analogues have demonstrated superior anticancer effects against aggressive and drug-resistant breast cancer *in vitro* and *in vivo*, while exhibiting comparable or less toxicity to normal human mammary epithelial cells in comparison with oridonin.



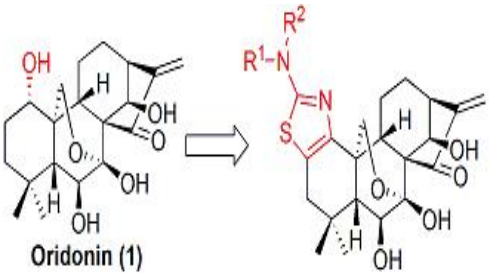
MEDI 99

Tweaking the natural product: Protecting group-free synthesis of novel nitrogen-enriched oridonin analogs with thiazole-fused A-ring and their potent antiproliferative effects against cancer cells

*Chunyong Ding[1], Yusong Zhang[2], Haijun Chen[1], Zhengduo Yang[2], Christopher Wild[1], Lili Chu[2], Huiling Liu[1], Qiang Shen[2], **Jia Zhou**[1], jizhou@utmb.edu. (1) Chemical Biology Program, Department of Pharmacology and Toxicology, Sealy Center for Structural Biology & Molecular Biophysics, University of Texas Medical Branch, Galveston, TX 77555, United States (2) Department of Clinical Cancer Prevention, Division of Cancer Prevention and Population Sciences, The University of Texas M.D. Anderson Cancer Center, Houston, TX 77030, United States*

Oridonin (**1**), a complex *ent*-kaurane diterpenoid isolated from traditional Chinese herb *Rabdosia rubescens*, has demonstrated great potential in the treatment of various human cancers due to its unique and safe anticancer pharmacological profile. However, the clinical development of oridonin for cancer therapy has been hampered by its relatively moderate potency, limited aqueous solubility and bioavailability. Up to now, the oridonin-based structural modifications and relevant structure-activity relationship remain sparse likely because of synthetic challenges and its structural complexity. Herein, we report the concise synthesis of a series of novel nitrogen-containing oridonin derivatives with thiazole-fused A-ring through an efficient protecting group-free synthetic strategy. Most of them including compounds **7** , **8** , **9** , **10** , **11** , **13** and **14** exhibited potent antiproliferative effects against breast, pancreatic and prostate cancer cells with low micromolar to nanomolar IC_{50} values, as well as markedly enhanced aqueous solubility. Moreover, these new analogs obtained by tweaking the natural product have been demonstrated not only to significantly induce apoptosis against estrogen receptor

(ER)-negative breast cancer MDA-MB-231 cells, but also drug-resistant ER-positive MCF-7 clones.



Oridonin (1)

- Improved aqueous solubility
- Enhanced anticancer potency
  e.g. 14 (R^1 = H, R^2 = allyl): IC_{50} = 0.18 μM
  (MDA-MB-231, 163-fold better than oridonin)
- Effective for adrimycin-resistant MCF-7 clones

MEDI 100

Synthesis and biological evaluation of combretastatin water-soluble amino acid prodrugs

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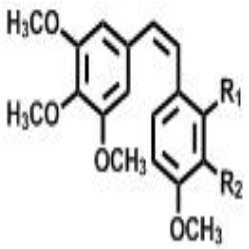
Tumor vasculature represents an important therapeutic target in the treatment of cancer. Tumor vessels are significantly different in structure from normal vasculature and are subject to rapid proliferation of endothelial cells and formation of highly disorganized microvessels. Combretastatin A-1 (CA1) and combretastatin A-4 (CA4) are among a class of vascular disrupting agents (VDAs) isolated from the bushwillow tree *Combretum caffrum* by George R. Pettit (Arizona State University). Water-soluble phosphate prodrug salts of these compounds have been prepared. VDAs selectively damage existing tumor vasculature leading to blood flow reduction and tumor necrosis. Many combretastatin analogues have been prepared synthetically, and nitrogen-bearing derivatives are especially noteworthy. The 3-amino-combretastatin serinamide prodrug (AVE8062, compound 4) is one example. In an effort to discover new VDAs with good water solubility, amino acid prodrugs of diamino-CA1 (3) and its fluorine substituted congeners were synthesized. These compounds were evaluated in terms of their ability to inhibit tubulin polymerization and for their cytotoxicity (*in vitro*) against selected human cancer lines. The hydrochloride salt (KGP322) of compound 3 demonstrated significant disruption of a capillary-like network of tubules (from HUVECs) at a

concentration of 0.1 μM and caused a > 90% reduction in blood flow (at 2 h) as evidenced by bioluminescence imaging (BLI) in a SCID mouse model at a dose of 80 mg/kg, thus demonstrating the vascular damaging capability of this compound.



(1) Combretastatin A-1 (CA1): R_1=R_2=OH
(2) Combretastatin A-4 (CA4): R_1=H, R_2=OH
(3) Diamino-CA1: R_1=R_2=NH_2
(4) AVE8062: R_1=H, R_2=NH-L-serine • HCl

MEDI 101

Synthesis and evaluation of novel pyrimidine-based dual EGFR/Her-2 inhibitors

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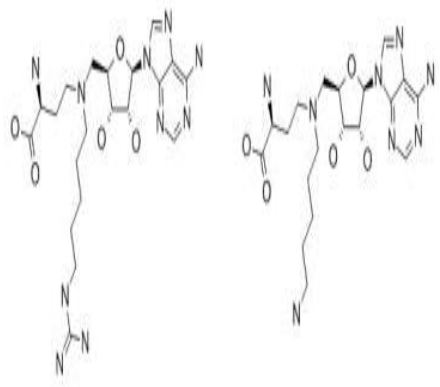
Epidermal growth factor receptor EGFR (ErbB-1) and Her-2 (ErbB-2), members of the Type 1 receptor tyrosine kinase family, play a key role in tumor proliferation. Therefore, simultaneous inhibition of these kinases has been expected to provide effective antitumor activity. Our structure-activity relationship study for dual EGFR/Her-2 inhibitor has resulted in the identification of novel 4-anilino-5-alkenyl or 5-alkynyl-6-methylpyrimidine derivatives that have exhibited effective inhibitory potency against both EGFR and Her-2. The presence of 5-alkenyl or 5-alkynyl moiety with terminal hydrophilic group played important role for the potent cellular inhibition. Several compounds in this series demonstrated effective activity against Her-2 dependent tumor cell line (BT474), and the representative compound exhibited significant antitumor potency in a mouse xenograft model.

MEDI 102

High resolution structures of SMYD2 in complex with inhibitors that occupy the lysine substrate channel and SAM cofactor domain

David Mendel[1]*, mendel_david@lilly.com, Stephen Antonysamy*[2]*, Laura Pelletier*[2]*, J. Spencer Emtage*[2]*, Tarun Gheyi*[2]*, Anh-Quan Hannah Nguyen*[1]*, Mary Mader*[1]*. (1) Lilly Research Laboratories, Indianapolis, IN, United States (2) Lilly Research Laboratories, San Diego, CA, United States*

The lysine methyltransferase SMYD2 is a maturing target in the area of epigenetics. Known substrates for SMYD2 include histone H3 and the tumor suppressor proteins p53 and Rb. Furthermore, SMYD2 overexpression associates with poor survival in esophageal and other cancers and so represents a potential target for anticancer drug development. We found that several “bisubstrate” class inhibitors originally targeted against arginine methyltransferases inhibit SMYD2 in the 200 nanoM to low microM IC50 range and then devised a method to obtain high resolution (<2Å) co-complex structures of these compounds with SMYD2.



This class of inhibitors was found to occupy both the SAM cofactor binding site and the channel that orients substrate lysine sidechains for methyl group transfer. Here we describe in detail the important interactions between SMYD2 and the bisubstrate type inhibitors and how those structural details may be used to design inhibitors with more drug-like properties.

MEDI 103

Discovery of a multi-arm polymer conjugated taxane with improved efficacy in a tumor xenograft model

***Zhongxu Ren**, mren@nektar.com, Wen Zhang, Antoni Kozlowski, Dennis Fry, Ute Hoch, Christine Brew, Steve Lee, Fadil Dahhani, Timothy Riley, Stephen Harrison, Jennifer Riggs.Nektar Therapeutics, San Francisco, CA 94158, United States*

The taxanes (including paclitaxel, docetaxel, and cabazitaxel) are widely used anticancer drugs for the treatment of metastatic tumors. Taxanes disrupt cellular architecture by stabilizing microtubules and inhibiting their disassembly, thus inhibiting cell division and preventing the propagation of rapidly dividing cells. Despite the success of taxanes in chemotherapy, there would be value in improving their efficacy and reducing their toxicity. Nektar's proprietary Advanced Polymer Conjugate Technology platform has demonstrated utility in improving the safety and efficacy of chemotherapeutic agents. For example, Nektar's etirinotecan pegol, a polymer conjugate of the topoisomerase I inhibitor irinotecan, is currently being evaluated in a Phase 3 clinical study. Here we report application of this technology to the improvement of efficacy of a taxane. Two synthetic methods applying direct conjugation or multi-step synthesis were successfully developed to facilitate introduction of the specific linkers into the designed conjugates. A series of multi-arm polyethylene glycol (PEG)-taxane conjugates with different PEG sizes and biodegradable linkers was prepared with acceptable drug loading. These conjugates showed comparable trends of drug release rates in mouse and human plasma *in vitro,* with the rates depending on the PEG size and conjugate-linker structure. Following intravenous administration (q7dx3) of the conjugates in a NCI-H460 xenograft model, a lead conjugate, PEG-TX1, was identified. PEG-TX1 delayed tumor growth by 336% vs. saline vehicle, causing 90% partial and 10% complete tumor regressions. In comparison, cabazitaxel (CBZ) delayed tumor growth by 213% and caused only 40% partial tumor regressions and 0% complete regressions. PEG-TX1 also showed greater tumor growth inhibition following a single administration of PEG-TX1 vs. CBZ. PK/PD experiments showed PEG-TX1 has a significantly altered pharmacokinetic profile, resulting in higher and sustained exposure of the released taxane in the tumor and thus correlated with the improved tumor growth delay.

MEDI 104

Synthesis and investigation of the anticancer activity of imidazole-based new compounds

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Heterocyclic compounds containing imidazole moiety have shown therapeutic applications in diverse disease areas such as, anthelmintic, analgesic, anti-bacterial,

anti-fungal, antiviral, antitubercular, anti-cancer etc. Thus, imidazole-based compounds are attractive targets in the design of novel chemical structures for the discovery of new drugs. In the current study, we have designed/synthesized a host of compounds bearing imidazole, by multi-component reaction using the corresponding aldehydes, amines/ammonium acetate and diketo compounds i.e. benzil and pyridil. These compounds were tested for antitumor activity on the National Cancer Institute's 60 human cancer cell lines panel. This panel is organized into nine subpanels representing diverse histologies: leukemia, melanoma, and cancers of lung, colon, kidney, ovary, breast, prostate, and central nervous system. The screening showed several compounds to be highly active against different cancer types, causing growth inhibition at micro- or nano-molar concentrations. To understand the mode of action of the displayed cytotoxicity, A549 cells were treated with four compounds in dose and time dependent manner. Also, in-vitro assays were performed to learn about the effect of these compounds on A549 cells for apoptosis, migration, anchorage independent growth and cellular senescence. Results of these investigations suggest cell migration and induced activation of caspase 3&9. Further studies for the cell cycle analysis and, to understand how intracellular signaling pathways that are known to be implicated in cancer progression might be altered by these compounds, are in progress.

MEDI 105

Synthesis and anticancer activity of C_5-curcuminoids

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Curcumin has been implicated as beneficial in numerous medicinal applications. These include inhibition of tumor propagation, protection against Alzheimer disease and anti-inflammatory properties. It has been found that the modification of the central β-diketone moiety of curcumin to mono-keto group leads to C_5-curcuminoids with greater stability, low toxicity and enhanced cancer inhibitory activity. Based on these observations, in the present investigation we have synthesized 18 new symmetrical C_5-curcuminoids for evaluating their anticancer. The synthesis was achieved by nucleophilic of the NH group of 4-piperidone hydrochloride, followed by Claisen-Schmidt condensation of the intermediate with a variety of halo substituted benzaldehydes to obtain the desired target molecules. A set of the curcuminoids were tested for anti-cancer activity on 60 human cancer cell lines, which represent diverse histologies. The screening showed some of the compounds to be highly active against different cancer types, causing growth inhibition at nano-molar dose. A mechanistic study using COLO205 cells showed these compounds to cause apoptosis. The Annexin V, DNA Fragmentation, Cell Cycle Effects and Mitochondrial Membrane Permeabilizationtests provide further insight into the mechansim.

MEDI 106

Synthesis and investigation of the antitumor activity of 4-aminoquinoline and C_5-curcuminoids hybrids

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The 4-aminoquinoline scaffold based compounds are found in the majority of drugs commonly used for the treatment of malaria. While, the natural product 'curcumin' has been shown wide range of biological activity including anti-cancer potential. We anticipated that covalent hybridization of these two pharmacophore may lead to molecules with better anticancer activity. To test the potential of such 4-aminoquinoline & Curcumin hybrids, we have covalently attached these chemical moieties via triazole linker. The hybrid compounds were tested for anti-cancer activity on 60 human cancer cell lines, which represent diverse histologies. Our study has indentified a set of these hybrids that show excellent growth inhibition at nano-molar concentrations. The mechanistic investigations through series of assays show apoptotic induction to cause anti-cancer activity.

MEDI 107

In vitro cytotoxicity of new coumarin derivatives in human lung (A549) cancer cell line

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Coumarins are classified as a member of the benzopyrone family of compounds with diverse and interesting biological activities. They have been used as therapeutic agents in the treatment of various diseases. In the present study, we evaluated the *in vitro* cytotoxic activity of eleven (11) newly synthesized coumarin derivatives against human lung (A549) cancer and normal lung (MRC-9) cell lines at various concentrations for 48 h. *In vitro* mechanism of the most active compound toxicity was examined by cell cycle analysis, reactive oxygen species (ROS) production and mitochondrial membrane potential (MMP). Our findings indicate that 8-(acetyloxy)-3-(4-methanesulfonylphenyl)-2-oxo-*2H*-chromen-7-ylacetate (**11**) showed the highest cytotoxic activity in A549 (LD_{50} = 24 µM) and selectivity in comparison to MRC-9 (LD_{50} > 100 µM**;** inactive) cell lines.

Furthermore, compound **11** caused significant cells arrest ($p<0.05$) in the –S and $–G_1$ phases, an increase in ROS production and decrease in MMP, in dose dependent manner. These findings suggest that compound **11** could serve as new leads for the development of novel synthetic compounds with enhanced anticancer activity.

MEDI 108

Discovery of ASP9521, a novel, potent, selective 17*β*-HSD5 inhibitor

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Type 5 17*β*-hydroxysteroid dehydrogenase (17*β*-HSD5), also known as aldo-keto reductase 1C3 (AKR1C3), is a member of the aldo-keto reductase superfamily of enzymes and is expressed in the human prostate. One of the main functions of 17*β*-HSD5 is to catalyze the conversion of the weak androgen, androstenedione, to the potent androgen, testosterone. The concentration of intraprostatic 5*α*-dihydrotestosterone (DHT) in patients following chemical or surgical castration has been reported to remain as high as 39% of that of healthy men, with 17*β*-HSD5 shown to be involved in this androgen synthesis. Inhibition of 17*β*-HSD5 therefore represents a promising target for the treatment of castration-resistant prostate cancer (CRPC).

We obtained a lead compound having a non-steroidal scaffold by high throughput screening (HTS) approaches for targeting enzyme activity of 17*β*-HSD5. After optimizations of the lead compound, we found ASP9521 as a potent, selective, and orally bioavailable 17*β*-HSD5 inhibitor.

Oral administration of ASP9521 to castrated nude mice bearing the CWR22R xenograft resulted in the suppression of androstenedione-induced intratumoral testosterone production. ASP9521 also demonstrated good isoform selectivity, minimal inhibitory activity against either CYP or hERG, and preferable pharmacokinetic and physicochemical properties.

MEDI 109

Novel phthalazine hedgehog pathway antagonists

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The Hedgehog (Hh) signal pathway plays a critical role in the development and homeostasis of many organs and tissues. Hh regulates embryonic pattern formation and adult tissue maintenance by directing cell differentiation and proliferation. Hh signaling has recently attracted considerable interest based on the discovery that aberrant activation of Sonic Hedgehog (Shh) signaling leads to the formation of various tumors, e. g., medulloblastoma, basal cell carcinoma, pancreatic cancer, small cell lung cancer, and prostate cancer.

While Hh antagonists have been evaluated clinically for the treatment of locally advanced metastatic basal cell carcinoma, treatment resistant and poor tolerability remains as major concerns. There still exists a need for potent hedgehog pathway inhibitors, particularly those having desirable pharmacodynamic, pharmacokinetic, and toxicology profiles.

This presentation will highlight the discovery of potent, orally bioavailable 1,4-disubstituted phthalazine Hh antagonists derived from High Throughput Screening and their evaluation in a number of preclinical assays demonstrating antitumor *in vivo* activity.

MEDI 110

Synthetic routes to neurotoxic and antiangiogenic acetogenins

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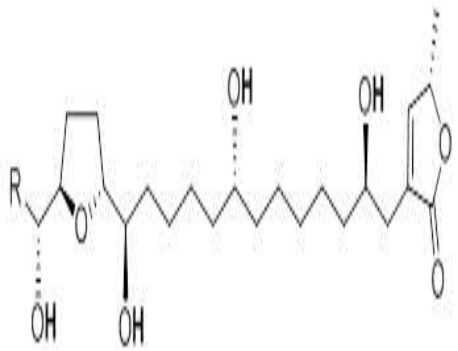
Annonacin (1) is an annonaceous acetogenin which is part of a group of natural products isolated from the leaves, fruit and bark of *Annona muricata* (soursop), and the fruit and/or bark of *Asimina triloba* (the North American pawpaw). These naturally-occurring compounds act as protective agents for the plant, but exhibit a range of biological activities in many bioassays including inhibition of mitochondrial complex I.Accordingly, the extracts and pure compounds isolated from annonaceous plants have been investigated for their potential as cancer treatments, but also for neurotoxicity. Annonacin causes cell death and tau pathology in mesencephalic cultures and neurodegeneration of the basal ganglia and brainstem after its chronic, intravenous administration to rats. More recent reports in the cancer arena cite annonacin as an inhibitor of angiogenesis although no mechanistic studies in that regard have been thoroughly investigated. We are interested in developing a total synthesis of annonacin whereby multigram quantities of the compound will be available for rapid analogue preparation and molecular target investigation. The typical retrosynthetic disconnections entail the C12 lipid portion, the disubstituted tetrahydrofuran portion and the butenolide portion. We have embarked on a strategy which involves a stereoselective de-novo synthesis of the butenolide portion and both stereoselective and chiral pool-derived syntheses of the tetrahydrofuran portion. The strategies and synthetic chemistry together with some recently-investigated biological activity will be presented.



(1) R=$C_{12}H_{25}$-n

MEDI 111

Structure-based design and synthesis of novel 2,4-diaminopyrimidine analogs as Mer kinase inhibitors in the treatment of cancer

Weihe Zhang[1]*, zhangwh@email.unc.edu, Andrew McIver*[1]*, Dmitri B Kireev*[1]*, Michael A Stashko*[1]*, Deborah DeRycke*[3]*, Michael Miley*[2]*, Mischa Machius*[2]*, Jacqueline L Norris-Drouin*[1]*, William Janzen*[1]*, H. Shelton Earp, III*[2]*, Doug K Graham*[3]*, Stephen Frye*[1,2]*, Xiaodong Wang*[1]*. (1) Center for Integrative Chemical Biology and Drug Discovery, Division of Chemical Biology and Medicinal Chemistry, Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, United States (2) Lineberger Comprehensive Cancer Center, Department of Medicine and Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, United States (3) Department of Pediatrics, School of Medicine, University of Colorado at Denver, Aurora, CO 80045, United States*

Mer kinase is a member of the TAM (Tyro3, Axl, Mer) family of receptor tyrosine kinases. Mer is ectopically expressed in acute lymphoblastic leukemia (ALL), and other cancers such as lymphoblastic and myeloid leukemia, glioblastoma, non-small cell lung cancer, melanoma, and pediatric rhabdo-myosarcoma. When Mer is inhibited through si/sh-RNA knockdown, cells are more susceptible to death after chemotherapy treatment, and the onset of disease in a xenograft mouse model of leukemia is significantly delayed. Therefore Mer provides a novel therapeutic target for the treatment of ALL and other Mer-related diseases either by the small molecule inhibitors or the combination with chemotherapeutic agents. Based on the X-ray co-crystal structure on Mer protein of our previous bicyclic pyrazolo[3,4-d]pyrimidine scaffold, we used pseudo ring design strategy and designed a novel monocyclic pyrimidine scaffold. The recently resolved X-ray co-crystal structures on Mer protein of this monocyclic pyrimidine scaffold have confirmed our structure-based design strategy. The Structure-

Activity Relationships (SAR) on this novel scaffold has been well studied; lead compounds with nanomolar to subnanomolar IC50 activity against Mer kinase in both enzymatic and cell-based assays were also identified.

MEDI 112

Molecular dynamics studies of the bromodomain family

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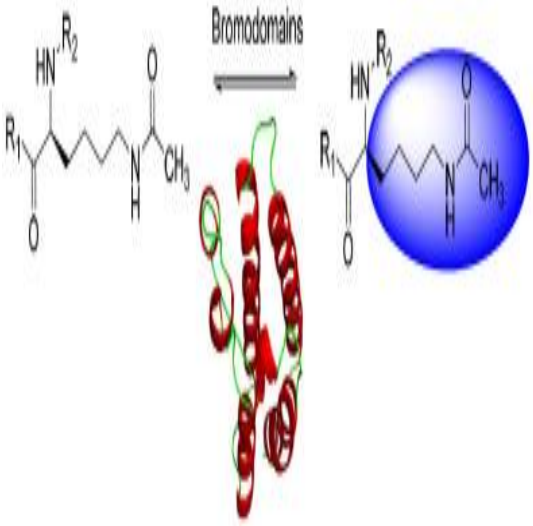
Lysine acetylation is a frequently occurring modification in proteins and plays an important role in chromatin remodeling, cell-cycle control and DNA damage. There are 46 diverse human proteins containing 61 bromodomains. In 2010, Filippakopoulos and coworkers reported JQ1, a selective inhibitor of BET bromodomains, which not only demonstrated the feasibility of abrogating protein-protein interactions with small molecules, but also identified bromodomains as potential drug targets in cancer therapy. Despite the large amount of recent work reported on bromodomain inhibition, there are no potent inhibitors of many bromodomain families. Here, a large-scale molecular dynamics study of all bromodomains is reported that investigates protein flexibility, conformational change, pocket water conservation, and protein-ligand interaction. The findings can provide a basis for the design of selective bromodomains inhibitors as well as to understand the function of the different bromodomains.



MEDI 113

Novel DNA alkylating agents, indolizino[6,7-b]indoles, suppress the growth of human nonsmall cell lung cancer cells

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Non-small cell lung cancer (NSCLC) is the one of the most common issues in global health problem due to its prevalent, poor prognosis, and lowest 5 year survival rate. Although chemotherapy drugs (such as cisplatin and carboplatin) and tyrosine receptor inhibitors (TKI, such as cetuximab and gefitinib) are commonly used clinically, the outcomes are still poor and TKI resistances are frequently emerged. Developing new therapeutic agent against NSCLC is essential. In this study, we demonstrated that indolizino[6,7-b]indoles, novel class of DNA crosslinking agents, effectively kill NSCLC lines containing various genetic mutations, including KRas, TP53, and EGFR mutations, with the concentration range of μM of the IC_{50} values.

	H460	A549	H1299	PC9	PC9/gef B4	CL141T	HEL299
BO-1922	0.38±0.09	0.64±0.16	2.89±0.35	0.43±0.02	0.57±0.16	0.51±0.16	0.61±0.28
BO-1978	3.11±0.38	3.15±1.20	3.02±0.65	2.10±1.06	2.45±1.21	1.32±0.47	4.23±2.22
BO-1972	0.73±0.35	2.97±1.00	2.07±0.55	0.92±0.23	0.87±0.22	0.79±0.33	0.99±0.48
BO-1973	2.81±0.46	5.32±1.01	1.87±0.13	2.28±0.48	2.68±0.90	1.40±0.30	ND
BO-1974	1.51±0.33	2.64±0.72	0.85±0.16	1.52±0.43	1.40±0.43	1.88±0.59	ND
Cisplatin	8.88±1.86	31.10±3.03	16.53±0.90	8.12±1.14	36.50±2.55	2.80±0.36	1.80±0.41
Gefitinib	28.41±7.44	17.65±1.55	27.87±2.34	0.35±0.07	14.15±4.13	20.56±3.34	ND

Table 1. The cytotoxicity of Indolizino[6,7-b]indoles against NSCLC cells growth *in vitro* (IC_{50}, μM)

The selected derivatives, BO-1922 and BO-1978, were shown to induce DNA double-strand crosslink by alkaline gel shift assay and modified comet assay, and are be capable of causing DNA damage using Western blotting assay and immunofluorescence assay. By aid of flow cytometric assay, we found that exposure of H460, CL141T, PC9, and PC9/gef B4 cells to BO-1978 delay cell cycle progression at the G1 and S phase and trigger cell apoptosis death pathway. Importantly, the intravenous injection of BO-1922 and BO-1978 significantly suppressed the growth of H460, PC9, and PC9/gef B4 xenograft tumors. Our present results revealed that BO-1922 and BO-1978 may have potential against NSCLC lines.

MEDI 114

Nonpeptide macrocyclic histone deacetylase inhibitors derived from azithromycin

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Histone deacetylase inhibitors (HDACi) hold great promise as agents of choice, either as stand-alone therapeutics or in combination with other traditional chemotherapeutic agents, in the fight against cancer scourge. In fact, HDAC inhibition has recently been clinically validated as a novel therapeutic strategy for cancer treatment with the FDA approval of Suberoylanilide hydroxamic acid (SAHA) and Romidepsin or FK228 for treatment of Cutaneous T-Cell Lymphoma. However, SAHA, FK 228 and many HDACi currently in clinical trials suffer from lack of efficacy against solid tumors and off-target toxicity. Toward obtaining tissue selective HDACi, we have designed a series of azithromycin-capped hydroxamic acid-based HDAC inhibitors following the standard three-pharmacophoric model of HDACi. We designed and synthesized two series of these compounds having HDAC inhibition moiety attached to the desosamine and the cladinose sugars of azithromycin. Linker lengths in each series were varied to determine the optimum length. Furthermore, we evaluated the HDAC isoform selectivity of these compounds as well as their antiproliferative activity in MCF-7 breast cancer cell line. The impact of these SAR on in vitro and whole cell HDAC inhibition will be discussed.

MEDI 115

Design, synthesis, and biological evaluation of a novel class of SENP1 inhibitors

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Prostate cancer is one of the most prevalent types of malignant cancers in men and has a high mortality rate among all male cancers. Previous studies have demonstrated that Sentrin/SUMO-specific protease 1 (SENP1) plays an important role in the occurrence and development of prostate cancer, and has been identified as a novel drug target for development of small molecule drugs against prostate cancer. In this paper, we used virtual screening and docking to identify a novel class of compounds inhibiting SENP1, from SPECS library. We further investigated the SAR (structure–activity relationship) of the benzoate substituents of this novel class of compounds. Our compounds are the high potent SENP1 small molecule inhibitors discovered up to date, and further lead optimization may lead to a series of novel anti-SENP1 agents.

MEDI 116

Tricyclic FOXO modulators

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The PI3K-AKT and RAS-MAPK oncogenic kinase pathways are responsible for the phosphorylation, inactivation, and cytoplasmic sequesteration of tumor suppressor - “FoxO1”. In a cell based FOXO localization screen reported by Kau et al, it was discovered that tricyclic neuroleptics (chlorpromazine, trifluoperazine) restore FoxO1 to the nucleus in tumor cells. As part of our program to develop FoxO1 modulators, devoid of the dose limiting CNS toxicities characteristic of the parent tricyclic neuroleptics, seven novel *N*-(3',4',5',6',10,11-hexahydrospiro[dibenzo[*a*,*d*][7]annulene-5,2'-pyran]-4'/5'-yl)benzenesulfonamides were synthesized and evaluated. The synthesis commenced from dibenzosuberone, and involved, among key steps - Grignard addition, Grubbs-I catalyzed intramolecular ring closing metathesis, and hydroboration-oxidation reactions. Compounds were tested in H1650 cell viability assay at 1, 10, 20, 30, 40 micromolar concentrations. The most potent compound was tested in the clonogenicity assay; it also demonstrated coordinated down regulation of Akt and Erk. The synthesis and *in vitro* biological activity of these compounds will be presented in this report.

MEDI 117

"Traceless" prodrugs for peptidomimetic caspase inhibitors

***Steven J Durrant**, steven_durrant@vrtx.com, Jean-Damien Charrier, John Studley, Linda Lawes, Peter Weber.Vertex Pharmaceuticals (Europe) Ltd, Abingdon, Oxfordshire OX14 4RY, United Kingdom*

The use of prodrugs as a method for improving the ADME properties of a potential drug candidate are well documented. However, in some cases the liberation of a stoichiometric amount of the pro-moiety can lead to toxicity concerns. To overcome this potential pitfall a series of prodrugs based on a 6,6a-dihydrofuro[3,2-*d*]oxazol-5(3a*H*)-one motif were designed as 'traceless' prodrugs for caspase inhibitors. *In vivo* hydrolysis of the lactone functional group is sufficient to liberate the active inhibitor *in vivo* and *in vitro* without producing any cleavage by-product. When dosed orally in rats an improvement in oral availability is observed. These results suggest such prodrugs may have the potential to be used to enhance the systemic exposure of peptidomimetic caspase inhibitors in indications where oral administration is desirable.

MEDI 118

Mechanistic studies on the anticancer activity of imidazolium-based ionic liquids

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In the development of anti-cancer drugs the 'toxicity' could be a desirable property as has been seen with many toxins which were originally used as poisons but found to be medically important. Understanding and managing the toxicity of small molecule toxins is a major challenge. Once the mechanism of compound toxicities is understood, it can be used to advantage. Ionic liquids have emerged as new chemical class whose 'toxicities' are yet to be fully understood. However, the possibility to tailor their physiochemical properties motivated us to investigate their potential for development as anti-cancer drug. Our study on a range of imidazolium-based ionic liquids on the 60 human cancer cell lines representing diverse histologies has identified compounds which show potency at nano-molar dose. The mechanistic studies show these molecules activate an extrinsic apoptotic pathway due to activity of the initiator caspase 8 and activation of effector caspase 3. Also, the Annexin V, DNA Fragmentation, Cell Cycle Effects and Mitochondrial Membrane Permeabilizationtests provide further insight into the mechansim. These studies and gene expression analysis merits the potential for further development of ionic liquids for therapeutic use.

MEDI 119

Synthesis and screening for anticancer activity of sempervirine analogs

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Sempervirine is an indolo[2,3-*a*]quinolizine based alkaloid isolated from the roots of *Gelsemium sempervirens*, and known to cause anti-proliferative activity. Earlier, in a high throughput screening (HTS) campaign of natural products, Sempervirine was discovered as a MDM2 E3 ubiquitin ligase inhibitor, which stabilizes p53 tumor suppressor protein levels by blocking its proteasomal degradation *via* ubiquitin-dependent pathway. Thus, cancer cells carrying wild-type p53 when treated with this compound induce stabilization of p53 leading to apoptosis. Sempervirine is also implicated to intercalate DNA and inhibit DNA topoisomerase I, therefore, it is a potential lead for development of anticancer drug. This alkaloid was first isolated in 1916 from plant, and since then several routes have been developed for its preparation. Interestingly however, there is no report on the synthesis and biological properties of sempervirine analogs with modified substituents. To explore such possibility, we synthesized a host of Sempervirine analogs and screened them on National Cancer Institute's 60 human cancer cell lines. The screening identified a set of analogs that are highly active against different cancer types. To understand the mechanism of the cytotoxicity, A549 cells were treated with a representative compound in dose and time dependent manner. Also, in-vitro assays were performed to learn about the effect of these compounds for apoptosis, migration, anchorage independent growth and cellular

senescence. Further studies for the cell cycle analysis and how intracellular signaling pathways that are known to be implicated in cancer progression might be altered by Sempervirine analog, are in progress.

MEDI 120

Structural basis for stabilization of Nrf2 by chemopreventive agents and oxidative stress

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Transcription factor Nrf2 is a key regulator of a genetic program that protects cells from reactive chemical species. Nrf2 is normally sequestered in the cytoplasm by a protein known as Keap1 that binds Nrf2 and promotes its proteosomal degradation by functioning as an adaptor for Cul3-based E3 ligase. Chemopreventive agents and oxidative stress allow Nrf2 to escape from Keap1-mediated repression, although the molecular mechanisms responsible for activation of Nrf2 are not yet understood. Two critical cysteine residues in Keap1, C273 and C288, are required for Keap1-dependent ubiquitination of Nrf2. In our effort to elucidate the role of C273 we built structural models of Keap1 and its complex with E3 ubiquitin ligase Cullin-3. We have also explored a possible dimerization of Keap1 through the IVR domain mediated by coordination of Zn by C273 and H272. the newly developed models provides a rational for stabilization of Nrf2 by Chemopreventive Agents and Oxidative Stress.

MEDI 121

Novel ROS activated prodrugs: A therapeutic approach to selectively target cancer cells

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Reactive Oxygen Species (ROS) are chemically reactive molecules formed as a natural byproduct of metabolism in cells. These species play an important role in cell signaling and homeostasis. Recent literature studies have shown that bulk cancer cells, including cancer stem cells, have elevated levels of ROS when compared to normal cells. Based on the literature support, our lab is designing novel ROS activated pro-drugs that target cancer cells over normal cells. We recently synthesized a new scaffold which undergoes oxidation in presence of ROS to activate. We hypothesize that upon oxidation, this new agent may undergo a cyclic transition state that may interact with DNA or act as a strong electrophile which may react with anti-oxidants in the cell to cause its depletion, leading to cell death. We are synthesizing a new series of these agents with modifications at various locations in the lead molecule to understand

structure activity relationship (SAR). This study will help us design agents with increased specificity and cytotoxicity.

MEDI 122

Structure-activity study of a new class of inhibitors of the uPAR-uPA protein-protein interaction that impair breast cancer cell invasion

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The urokinase receptor (uPAR) is a cell-surface receptor that engages several proteins through transient and tight protein-protein interactions. As a result, a diverse set of cellular processes that include cell adhesion, migration and invasion are implicated with the receptor. Here, a substructure search of commercial libraries based on the core of previously-identified pyrazole-based compounds led to **1** (IPR-993). The pyrrolone-based compound inhibited binding of uPAR to its high-affinity serine proteinase ligand the amino-terminal fragment of the urokinase-type plasminogen activator (uPA_{ATF}) with an IC_{50} of 44 μM. Further substructure search of more than 500 derivatives led to derivatives **2** (IPR-1109) and **3** (IPR-1110) with IC_{50} of 25 and 17 μM, respectively. Synthesis of additional derivatives through modifications of the substituents on the pyrrolone core resulted in new inhibitor with higher inhibition. A structure-activity analysis provided deeper insight into the properties of the binding sites they occupy. The data guided computational studies that predicted the binding mode of the compound. In cancer cells, IPR-993 impaired breast MDA-MB-231 and pancreatic PANC-1 invasion in a concentration-dependent manner without exhibiting any cytotoxicity.

MEDI 123

Novel nucleoside and nucleobase anticancer prodrug candidates: Synthesis and investigation of their biological activity in human cancer cell lines

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Contrary to normal cells, cancer cells undergo rapid, abnormal, and uncontrolled division, which warrants a constant need for DNA replication. Therefore, interfering with this process affects them preferentially, and represents a plausible approach to cancer

chemotherapy. We are designing and synthesizing anti-cancer agents that disrupt DNA replication. Recently discovered base-modified nucleotides undergo incorporation into a partial double-helix primer by natural DNA polymerases better than natural nucleotides, yet they terminate further DNA synthesis upon a single incorporation event. Given that nucleosides and nucleobases undergo conversion into nucleotides within the cell through series of enzymatic transformations, it was hypothesized that treatment of cancer cells with base-modified nucleosides (or nucleobases thereof) whose 5'-triphosphates terminate DNA synthesis would obstruct their DNA replication process. A library of new nucleoside and nucleobase prodrug candidates was synthesized and tested for their effect on cell viability of MCF7 (breast) and HeLa (cervical) human cancer cells. Cell viability was measured by the reduction of resazurin, which produces the highly fluorescent product resorufin. Cell plating density was optimized to permit observation of cell growth in the linear range. To examine the effect of novel nucleosides and nucleobases on cell proliferation, cells were plated at 5,000 per well when grown in 96-well plates for 24 hr then treated with variable compound concentrations and incubated for 2-3 days. Cell growth was measured by addition of resazurin dye followed by fluorescence reading. The results revealed significant activity of only those modified nucleobases and nucleosides whose 5'-triphosphates terminate the *in-vitro* DNA synthesis upon a single incorporation event, which supports the central hypothesis of this project. Further elucidation of structure-activity relationship and investigation of the mechanism of action is underway.

MEDI 124

4-Aminoquinoline -triazine hybrids: Synthesis and cytotoxicity study for anticancer activity

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Designing molecules with potential known activities against multiple targets has always been an attractive strategy. Since, molecules containing the 4-aminoquinoline and triazine core are active in several therapeutic areas, we synthesized hybrid molecules with both the 4-aminoquinoline and triazine core. A library of 48 new 4-aminoquinoline-triazine hybrids with different substitution pattern were synthesized and tested for anti-cancer activity on 60 human cancer cell lines. A set of hybrids molecules showed excellent growth inhibition at low micromolar concentrations. The mechanistic investigations through series of assays on COLO205 cells show apoptotic induction to cause anti-cancer activity.

MEDI 125

WITHDRAWN

MEDI 126

Lipoplexes with pH-sensitive conformational swtich for siRNA delivery

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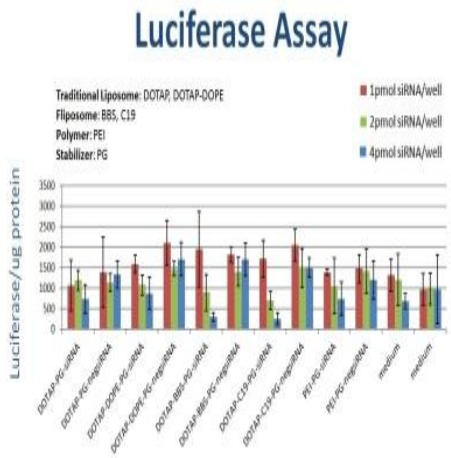
siRNA represents a promising category of therapeutic agents for the treatment of viral and genetic diseases. However, successful gene knockdown by siRNA depends on its efficient delivery into target cells. Previously, we have reported pH-sensitive liposomes with conformational switch (Fliposomes) as a potential delivery system for small-molecule drugs and biomacromolecules. In this study, we prepared complexes of cationic Fliposomes and siRNA to transfect T47D-KBluc cells (human breast cancer), which stably express firefly luciferase. The cationic Fliposomes and their complexes with siRNA were prepared either with or without poly-L-glutamic acid (PG) as a stabilizer. Electrophoresis studies indicated that PG improved the stability of the Fliposome-siRNA complexes. Significantly more efficient knockdown of the luciferase activity was achieved by the Fliposome/siRNA complexes compared to liposome/siRNA complexes containing the common cationic lipid DOTAP.



PG also enhanced the transfection efficiency of the complexes in T47D-KBluc cells. Two other mammalian cell lines expressing GFP (green fluorescent protein) were also investigated as potential cell lines to evaluate the efficiency of siRNA delivery by Fliposomes.

MEDI 127

Identification of ubiquitin-specific protease 2 (USP2) inhibitors that promote cell cycle arrest via cyclin D1 destabilization

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Recent approval of bortezomib for use in multiple myeloma has validated the ubiquitin-proteasome system (UPS) as a druggable target, increasing interest in developing small molecule therapeutics for the UPS. Ubiquitin-specific protease 2 (USP2) has attracted a large degree of attention from the scientific community as a potential anti-cancer target. It has been linked to the stabilization of many cancer-associated proteins such as cyclin D1, fatty acid synthase, and MDM2 as well as a number of circadian rhythm enzymes. Herein, we describe the identification of a novel USP2 inhibitor from a quantitative high throughput screen, synthesis of analogs with improved potency and ADME properties, and preliminary characterization of their biological activity. These compounds will prove valuable in validating downstream protein targets of USP2 and in understanding the role USP2 in various diseases.

MEDI 128

Lead optimization of tubulin inhibitors and further mechanism investigation

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Microtubules are the basic components of cell structure, which take part in a wide number of pivotal cellular functions. Drugs that are able to modulate the microtubule assembly either by inhibition of tubulin polymerization or by blocking microtubule assembly are of great interest in anti-cancer therapy. In the past few years, several small synthetic molecules that have indole nucleus as a core structure have been identified as tubulin inhibitors. Indomethacin, a well-known NSAID, with an indole nucleus was chosen for our study. In order to investigate the Structure Activity Relationship (SAR) between the anti-cancer effect and some potentially relevant chemical properties, we prepared a series of indole derivatives, where the structure of Indomethacin was gradually modulated, varying the carboxylic group nature by substitution with different amide groups and the amide hydrogen nature by introduction of different benzoyl substituents. Interestingly, these modifications resulted to a set of compounds exhibiting higher potency than the parent compound. One of the Indomethacin analogue showed stronger antiproliferative activity against multiple cancer cell lines via interfering with tubulin polymerization. Tubulin polymerization assay indicated that this compound inhibited tubulin assembly at high concentrations, but promoted this process at low concentrations. The binding mode of this compound in tubulin was predicted using the molecular docking simulation. This compound was selected as the lead compound for further SAR study, devoted to the optimization of the cytotoxic effect and other mechanism investigation.

MEDI 129

Development of small molecules as Mer kinase inhibitors for Mer related diseases

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Ectopic expression of Mer Tyroine kinase is associated with the development of certain types of cancers like Acute Lymphoblastic Leukemia (ALL), Brain cancer, Non-small-cell Lung Carcinoma (NSCLC) etc. Following this principle, Mer kinase was targeted and its inhibitors were developed as drug candidates for ALL and other Mer related diseases in our lab. Based on the structure-related design and synthesis, herein, we present a series of small molecules with a new pyridine-pyrimidine scaffold, which are potent inhibitors of Mer kinase. A detailed SAR study was performed and a cocrystal structure of Mer with a pyridine-pyrimidine small molecule is presented.

MEDI 130

Structure-based optimization of potent, aminothiadiazole inhibitors of Akt1

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The serine threonine kinase Akt regulates cell survival, inhibits apoptosis and is often up-regulated or over-expressed in a number of human tumors. Consequently, interfering with signaling along the PI3K/Akt pathway has been a topic of interest in drug discovery. Herein, we describe the identification and SAR exploration of a thiadiazole series of Akt kinase inhibitors. Guided by structure-based design, the series was optimized to afford

Akt inhibitors with single digit nanomolar activity. Pathway inhibition and anti-proliferative effects of these inhibitors have been demonstrated, both in vitro and in vivo.

MEDI 131

Design and synthesis of chromene based novel antiglioma agents

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Gliomas are the common malignant primary brain tumors that arise within the central nervous system in adults and they account for more than 80% of all brain tumors. Glioblastoma Multiforme (GBM) is the most aggressive form of the gliomas. A common approach for the treatment of GBM involves surgery, radiation therapy, and concomitant and adjuvant chemotherapy with temozolomide. Despite advance standard therapy the prognosis for patients with GBM remains poor. In an effort to discover new chemotherapeutics, we recently identified the new lead antiglioma agent **SP-6-27** in racemic form. In this study, we have separated and screened the isomers **SP-6-27-FI** and **SP-6-27-FII** against human glioma cell lines. Additionally, it has been further selected for 5 dose NCI 60 cell line testing. Based on **SP-6-27** , we designed, synthesized and screened new chromenes for antiglioma activity. Results are encouraging and these new analogs will be developed as novel GBM therapeutic agents.

MEDI 132

Synthesis and in vitro evaluation of novel 1,2,3,4-tetrahydroisoquinoline derivatives as potent anti-glioma agents

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Glioblastoma Multiforme (GBM) is the most common and deadliest of malignant primary brain tumors in adults. In the United States, approximately 20,000 patients are diagnosed with GBM each yearand the mean life expectancy of these patients is approximately one year. Among the recent clinical therapy approaches, only the combined therapy of Temozolomide and radiation treatment produced encouraging clinical results in long term survival of patients. Therefore, there is a substantial need for the development of new and more effective chemotherapeutic agents to treat GBM. In continuation of our effort to identify new anti-glioma agents, we synthesized a novel 1,2,3,4-Tetrahydroisoquinoline **(EDL-155)** analogs and screened them for their anti-glioma activity in four established human glioma cell lines (T98, U87, LN18 and A172). We discovered the **EDL-358** as a potent and selective anti-glioma agent (A172 IC_{50}:

8.25 ±0.42 μM), which displayed a similar cytotoxicity profile as standard Temozolamide in human glioma cell lines.

MEDI 133

Novel phenanthroindolizidine alkaloids with potent antitumor activity: A CoMFA study of derivatives of DCB-3503

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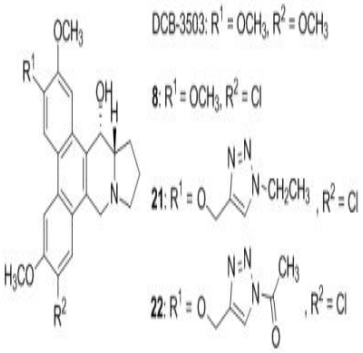
Tylophorine analogues are a group of phenanthroindolizidine alkaloids isolated from plants that show potent antitumor activities. DCB-3503, a novel tylophorine analogue and a lead compound, has shown potent growth inhibition (GI_{50} ~10^{-8} M) against a large number of NCI's 60 human-derived cell lines. Its mode of action is via inhibition of NF-κB and associated nuclear proteins. In this study, a CoMFA model to predict the biological activities of several DCB-3503 analogues was built as a learning set based on the pGI_{50} of several tylophorine analogues against a HepG2 cell line. The results show that the biological activities of DCB-3503 can be increased if R^1 is modified with sterically bulky groups or electron-withdrawing groups, or R^2 with electron-withdrawing groups. Compounds **8** , **21** and **22** shown in the figure were selected as the modified target compounds aiming at increasing the biological activities from DCB-3503. Synthesis of these compounds was carried out, and their structures were confirmed by NMR spectroscopy and crystallography.



MEDI 134

Design, synthesis, and in vitro cytotoxicity evaluation of seven-membered cyclic sulfamides

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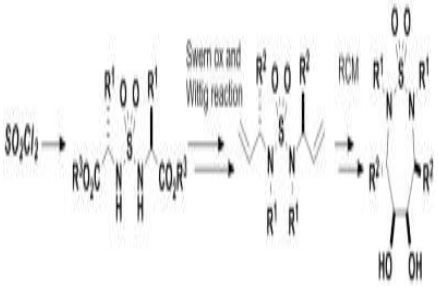
Cyclic urea-based compounds have demonstrated antiviral activity and there are prominent examples of highly potent HIV protease inhibitors developed by pharmaceutical industry. Previous studies have elucidated the effect of sustituents, absolute and relative stereochemistry, hydrophobicity etc, on the hydrogen bonding and catalytic aspartate interactions with enzyme, and thereby, overall inhibitor potency. It is well known that modification with sulfamide functional group provides an attractive and versatile opportunity for the selective and potent modula-tion of protein function. These observations inspired us to explore the potential of cyclic sulfamide analogs of ureas, for anti-cancer activity. We synthesized several of seven member cyclic C_2-symmetric and unsymmetric compounds through ring closing metathesis. These compounds were tested on NCI's 60 human cancer cell lines panel, which represents diverse histologies. The screening identified a set of these compounds showing high activity against different cancer types. Four such compounds were further tested in dose and time dependent manner. To understand the mechanism of the observed cytotoxicity, investigations with a representative compound are underway to learn about the effect for apoptosis, migration, anchorage independent growth and cellular senescence.



MEDI 135

Comparative in vitro phototoxicity response of irradiated prostate cancer cells

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Photodynamic therapy (PDT) is a minimally invasive form of treatment used for various types of cancer, including prostate cancer. PDT consists of two steps for treatment: introducing a photosensitizer (PS), exposing the PS with radiant energy of the appropriate wavelength in order for the PS to produce singlet oxygen, and then destroying the targeted cells. To limit the effect of PDT to malignant tissues is a major challenge since this anticancer treatment contains side effects which include necrosis due to nonselective tissue targeting of the photosensitizers. The ultimate goal of this research is to study the mechanism of cell death pathway such as apoptosis and necrosis during photoirradiation. Two known water-soluble photosensitizers, chlorin-e6

and Zn-phthalocyanine, were incubated in non-tumorigenic (*PNT1A*) and malignant (*DU-145*) prostate cancer cells to compare their photosensitizing abilities. MTT assay was performed to determine the extent of cell survival upon irradiation with 650 nm light (fluence rate of 0.72 J/cm^2). The number of viable cells which is directly proportional to the absorbance was determined using a BioRad 550 Microplate Reader. Cell survival assay indicated that cell destruction increased with PS concentration and with increasing light dosage for the two photosensitizers used for both normal prostate and tumorigenic prostate cancer cells. Cell morphological changes associated with apoptosis or necrosis will be determined by fluorescence microscopy technique.

MEDI 136

Radiolabeling and in vivo evaluation of cubosomes andhexosomes for lymphatic targeting

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The main goal applying targeted therapy is to achieve high concentrations of the therapeutic agent in the tissue of interest, while reducing its relative concentration in the remaining tissues. Targeted therapy is of upmost interest when it comes to anticancer treatments, as it is well known that cytotoxic drugs kill both cancerous cells and healthy proliferating cells. In the present contribution, our main attention is to design radiolabeled cubosomes and hexosomes and to investigate the potential utilization of these nanostructured liquid crystalline particles as drug delivery systems in relation to drug targeting to the lymphatic system after subcutaneous (s.c.) injection. Cubosomes and hexosomes display nanostructures closely related to biological systems and have unique physicochemical properties that set the stage for promising potential applications in the area of drug nanotechnology.

To our knowledge, this is the first report in literature on the successful radiolabeling and lymphatic targeting of cubosomes and hexosomes using the 99mTc polyamine method. Injections of radiolabeled cubosomes and hexosomes were made to the flank of mice. The nanoSPECT/CT imaging technique providing high quality small animal imaging was utilized for investigating the in vivo biodistribution of the labeled nanoparticles in mice. The obtained results demonstrate that these radiolabeled nanoparticulate systems are able to specifically target the lymphatic system, with neglectable distribution to other organs/tissues. These nanoparticulate matrices could provide a new promising approach in cancer therapy for specific targeting of anticancer drugs to the lymphatic system.

MEDI 137

Discovery of a potent inhibitor of CREB-mediated gene transcription with growth inhibitory activity in triple-negative breast cancer

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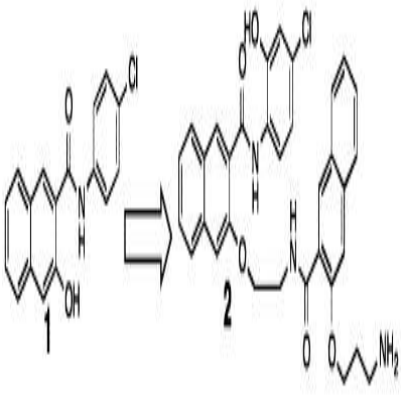
Breast cancer is a heterogeneous group of diseases with distinct and complex mechanisms of pathogenesis. Triple-negative breast cancers (TNBC) form a subgroup of breast cancers with poor prognosis. TNBCs lack the expression of estrogen receptor (ER), progesterone receptor (PR) or HER2 and no targeted therapies exist. The cAMP response element binding protein (CREB) is a stimulus-induced transcription factor activated by multiple extracellular signals through phosphorylation. The phosphorylated CREB (p-CREB) can then bind the mammalian transcription co-activator, CREB-binding protein (CBP). This binding event will further recruit other transcriptional machinery to the gene promoter to initiate CREB-dependent gene transcription. CREB is overexpressed in breast cancer tissues compared to normal mammary tissues and the level of expression inversely correlates with disease-free survival. We present here the discovery of a potent small molecule inhibitor of CREB-mediated gene transcription with in vitro and in vivo activity in TNBCs. Starting from **1**



, which is a low micromolar and cell-permeable inhibitor of CREB-mediated gene transcription, we designed and synthesized **2** . Compound **2** potently inhibited CREB-mediated gene transcription with IC_{50} ~80 nM. This compound potently inhibited proliferation of MDA-MB-231 and MDA-MB-468 cells. In contrast, compound **2** was not toxic to normal human cells. In vivo, compound **2** completely suppressed the growth of MDA-MB-468 cells without toxicity. These results suggest that compound **2** is a potent CREB inhibitor warranting further development as an anti-breast cancer agent.

MEDI 138

Differential loss of cell viability after exposure of MCF-7 breast cancer cells and normal human mammary fibroblast to *S*-nitroso-arylamides

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It is well-established that nitric oxide (NO), a gaseous free radical participates in cellular signaling events. Among other NO donors, *S*-nitrosothiols have been implicated as major transducers of NO bioactivity, acting as both potent vasodilators and inhibitors of platelet aggregation. However, the mechanisms by which these reactive nitrogen species affect cellular functions are not fully established. *S*-nitroso-*N*-acetyl-penicillamine (SNAP) is a widely characterized nitrosothiol whose functionality and unique aqueous stability suggest that penicillamine derivatives are good substrates for the synthesis of novel and stable *S*-nitrosothiols. Recently we synthesized a series of *S*-nitroso-arylamides (**1** -**4**) derivatives of penicillamine that are fairly water soluble and therefore suitable for biological testing. The use of other classes of NO donors as potential cancer chemotherapeutic agents is fairly documented. However, the use of S-nitrosothiols for this purpose has not been explored.

In this communication we determine the potential anticancer activity of *S*-nitroso-aryl-amides (**1** -**4**) by assaying the cytotoxic effects of these compounds (Figure 1). MCF-7 breast cancer cells and normal human mammary fibroblasts were exposed to increasing concentrations of the S-nitroso-arylamides. Increasing concentrations of the compounds were increasingly cytotoxic to MCF-7 cells. Furthermore, the **2** and **3** compounds were preferentially cytotoxic to MCF-7 cells as compared to normal human mammary fibroblasts.

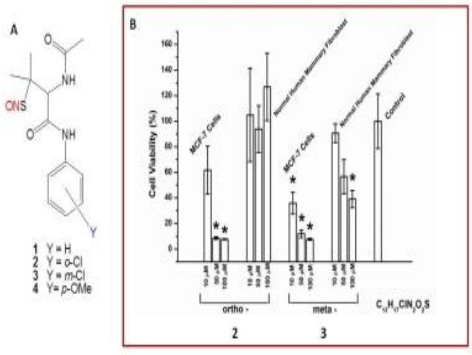

Figure 1. (**A**) Structure of *S*-nitroso-arylamides (**1**-**4**); (**B**) cell viability assay for MCF-7 breast cancer cell line and normal human mammary fibroblast exposed to **2** and **3** derivatives.

MEDI 139

Fragment-based approach to covalent inhibitors of catalytic cysteines

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We have developed a general method to discover covalent inhibitors of catalytic cysteines using fragment-based drug discovery. NMR rate studies were used to compare several electrophiles in order to identify one which is moderately reactive with cysteine and which demonstrates similar reactivity regardless of the structure of the fragment it is attached to. Electrophiles were also tested for their ability to exclusively label the catalytic cysteine of an enzyme with multiple surface cysteines. (E)-methyl 4-aminobut-2-enoate was identified as an electrophile which meets these criteria, although other electrophiles are still being investigated. This amine handle of this electrophile was then coupled to a library of fragments containing a carboxylic acid group. This library was selected on the basis of meeting the “rule of three” criteria as well as maximizing structural diversity. Pseudo first-order NMR kinetics studies with *N*-acetyl cysteine methyl ester have confirmed that all members of this library have rate constants within a factor of 2 of each other.The electrophilic fragments can then be screened in batches of ten against the enzyme of interest, and any binders can be identified by mass spectrometry. Whole protein ESI-MS is used to identify any hits based on the mass shift of the protein peak. Selectivity for the catalytic cysteine is confirmed by tryptic digestion and MALDI-TOF MS of the tryptic peptides. Our choice of electrophile ensures that any hits will be selected based on their ability to bind to the active site of the enzyme and not simply due to their greater reactivity. We have chosen the HECT E3 ubiquitin ligase Nedd4-1 as a model enzyme to validate this methodology, and screening against this target is currently underway. However, we anticipate that this technology will be applicable to all enzymes with catalytic cysteines, including cysteine proteases.

MEDI 140

Development of Pd-catalyzed reaction conditions to support medicinal chemistry using an Automated Synthesis Lab (ASL): Med-chem with a view

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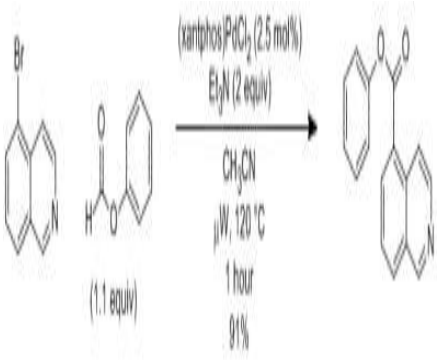
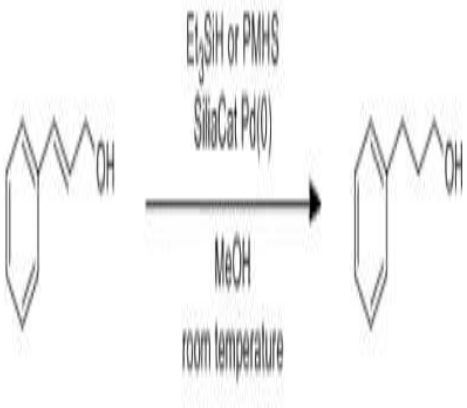
The Automated Synthesis Lab (ASL) has become a powerful tool for synthetic chemists at Eli Lilly to increase their productivity by running remotely guided chemistry. This poster will focus on our efforts to develop “automation friendly” conditions for some of the most popular Pd-catalyzed reactions. We will discuss our experiences with single

component pre-catalysts such as (dtbpf)$PdCl_2$ and Buchwald palladacycles for Suzuki and Buchwald-Hartwig cross-couplings.

For carbonylations, we will disclose microwave conditions that allow us to employ just 1.1 equivalents of phenyl formate as the source of CO.



Finally, we will present our work on olefin reductions utilizing the non-pyrophoric SiliaCat Pd(0) combined with silyl hydride reagents.



MEDI 141

Direct screening of functional aptamers capable of influenza hemagglutination inhibition by epitope-specific SELEX

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In addition to playing roles of antibody surrogates in diagnostics, aptamers, a unique class
of nucleic acid ligands, also exhibit great niches in therapeutics through exerting regulatory function on the target molecule. However, the ordinary *in vitro* selection method SELEX does not guarantee obtaining a “functional” aptamer for therapeutics. Here, we present a specially designed approach called epitope-specific SELEX (ES-SELEX) for direct screening functional aptamers and use this approach to obtain the aptamers capable of blocking recombinant influenza (A/New Caledonia/20/99) hemagglutinin (HA)'s function. The ES-SELEX starts with five rounds of ordinary (protein-specific) SELEX against the entire HA and then follows four additional rounds of epitope-specific selection, in which antagnoistic aptamers are evolved through fetuin competition on the sialic acid receptor-binding epitope of HA (Fig. 1(a)). After the selection process, we choose five aptamer candidates based on the structural stability and find four of them showing complete or partial antagonistic functionality against HA (Fig. 1(b)). A further study shows that aptamer 536 can inhibit HA function at a low picomole dose (6.25 pmol). The above results demonstrate that the concept of ES-SELEX is promising for direct
screening functional aptamers for therapeutics.

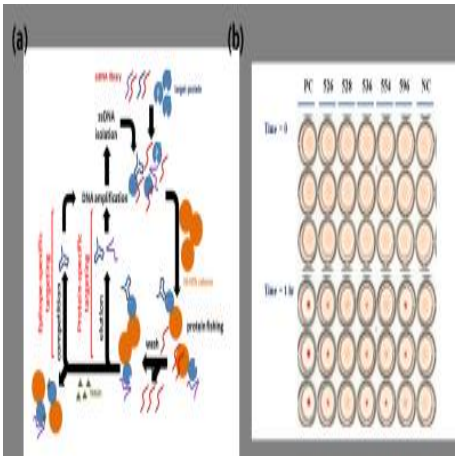

Figure 1 (a) ES-SELEX against the sialic acid receptor-binding epitope of HA. (b) Hemagglutination inhibition assay with the selected aptamers.

MEDI 142

Microstructural modulation of DNA by small molecule complexes in the minor groove

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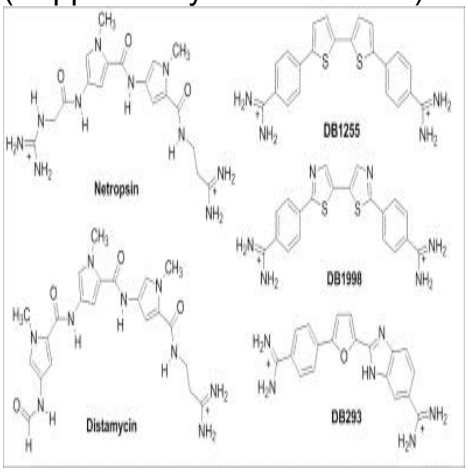
Minor groove binders (MGB) have been designed to recognize various DNA sequences with high affinity due to their potential use as therapeutics and in biotechnology. They have displayed excellent anti-parasitic and anti-viral activities, and also the capability to modulate DNA transcription by inhibiting transcription factors binding. These inhibition effects MGBs are closely related to binding affinity and also to the ability to alter the local geometry of DNA. Therefore, the conformational effects of MGB on DNA are important aspects for the design of therapeutic candidates and establishing the molecular recognition basis of MGB-DNA complexes.

Some different MGBs can target the same DNA sequence but in distinct binding modes: monomer or dimer. The conformational effects of MGBs on DNAs have been systematically evaluated in this study using a polyacrylamide gel electrophoresis - ligation ladder assay. This method is very sensitive to analyze global and local DNA structure, as well as to detect the MGB binding induced DNA conformational changes. The results show that both the monomeric binding of netropsin and the dimeric binding of distamycin can bend ATATA sites by ~ 20 degree/helical turn, but towards opposite directions. For the GC base pair containing site, ATGA, monomer complexes of DB1255 and DB1998 bend DNA more significantly than the dimer complex of DB293. The MGBs display clear DNA sequence and binding mode dependent structural effects on DNA. (Supported by NIH AI064200).



MEDI 143

Remotely-guided automated synthesis lab: A novel paradigm in advancing productivity in drug discovery synthesis

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Herein is described a unique automated synthesis lab directed at supplementing the work a medicinal chemist does at the bench. Traditionally automation technologies have

been mainly directed to support parallel library synthesis in a centralized lab under the full direction of a highly-trained and specialized group of chemists. In contrast, chemical synthesis effort in this lab allows chemists to remotely submit and guide chemical reactions in a highly integrated and automated synthesis lab in a manner that resembles how work is done at their bench - i.e. the pursuit singletons at >= 100 mg scale, in addition to small libraries and multi-step synthetic sequences with complete workflow automation from reagent gathering and mixing to purification and characterization. This poster describes the layout of the lab, how it functions and is supported, and how it is going about impacting and transforming our approach to doing chemical synthesis in the pursuit of drug discovery.

MEDI 144

Radiolabeling a PSMA-specific RNA aptamer with the PET tracer zirconium-89

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Aptamers, which are short synthetic single-stranded oligonucleotides that specifically bind to various molecular targets, are an attractive alternative for *in vivo* oncological use. While a relatively recent arrival relative to amino acid-based candidates, aptamers' popularity stem from exhibiting low immunogenicity, superior tumor penetration, and rapid clearance, making them attractive in vivo molecular probes with the prospect of rapid clinical translation. Positron emission tomography (PET) is widely utilized clinically because of high resolution due to the short range of the positron in tissue and high sensitivity due to coincidence detection. Recently, a prostate specific membrane antigen (PSMA)-specific RNA aptamer was generated with 2'-fluoro substitutions to impart *in vivo* stability. The cancer biomarker prostate specific membrane antigen (PSMA) is an attractive target due to its expression on neovasculature of almost all solid tumors, while being markedly absent from normal neovasculature.

The chelating agent desferroxamine-maleimide (DFO-mal) was conjugated to both a 3'thiol-functionalized PSMA-specific RNA aptamer and a non-functional control oligo (C36) and radiolabeled with the PET tracer zirconium-89. The A9-DFO-^{89}Zr aptamer showed a specific activity comparable to monoclonal antibodies and stability in serum (>85% over four days). Immunoreactivity assays with were evaluated on PSMA transfected PC-3 cells and wild-type PC-3 cells as a negative control, and showed sustained binding activity. Competitive binding assays were completed with an excess of cold A9 aptamer, and showed reduced binding of the radiolabeled A9 aptamer. The first reported *in vivo* PET imaging of RNA aptamers is ongoing.

With its high specificity for the integral membrane glycoprotein PSMA and ease of synthesis and radiolabeling, the PSMA-specific A9 aptamer could ultimately be used for facile PET imaging of PSMA-positive prostate cancer, with subsequent improved clinical outcome. Furthermore, due the modularity of aptamers, this radiolabeling procedure has the potential for facilitating pharmacokinetic studies of a multitude of RNA aptamers.

MEDI 145

Fully automated SPE-based high-yield synthesis of *N*-[^{11}C]methyl-laudanosine for imaging of small-conductance Ca^{2+}-activated K^+ channels

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Small-conductance Ca^{2+}-activated K^+ channels (SK_{Ca}) are widely expressed in different tissues such as the brain, peripheral nervous system, skeletal muscle, smooth muscle, heart and cancers, and regulate cognitive dysfunction, neuronal hyperexcitability, dopamine-related disorders, depression, hormone secretion from endocrine cells, and repolarization of cardiac action potentials. *N*-methyl-laudanosine (6,7-dimethoxy-1-(3,4-dimethoxybenzyl)-2,2-dimethyl-1,2,3,4-tetrahydroisoquinolinium iodide, NML) is a reversible and selective SK_{Ca} channel blocker/inhibitor with high affinity. *N*-[^{11}C]methyl-laudanosine (6,7-dimethoxy-1-(3,4-dimethoxybenzyl)-2-[^{11}C]methyl-2-methyl-1,2,3,4-tetrahydroisoquinolinium triflate, [^{11}C]NML) was synthesized as a potential PET (positron emission tomography) SK_{Ca} imaging agent. The precursor 6,7-dimethoxy-1-(3,4-dimethoxybenzyl)-2-methyl-1,2,3,4-tetrahydroisoquinoline was commercially available, and the methylation of the tertiary amine precursor with methyl iodide provided NML in 88% yield. The target quaternary ammonium tracer [^{11}C]NML was prepared by the *N*-[^{11}C]methylation of its corresponding precursor using [^{11}C]CH_3OTf and purified by a simplified solid-phase extraction (SPE) method using a cation-exchange CM Sep-Pak cartridge in 50-65% radiochemical yield based on [^{11}C]CO_2 and 20 min overall synthesis time from end of bombardment (EOB). The radiosynthesis was performed in a home-built automated multi-purpose ^{11}C-radiosynthesis module. The specific activity, radiochemical purity and chemical purity of [^{11}C]NML were determined by analytical HPLC as 6-20 Ci/μmol, >99% and >87%, respectively.

MEDI 146

Synthesis, characterization, and pharmacodynamics of vitamin B_{12} conjugated exendin 4

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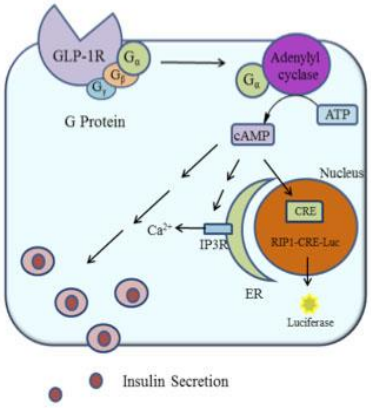
Vitamin B_{12} (B_{12}) is actively being explored as a method for oral drug delivery. The advantage of using B_{12} is that the dietary uptake pathway may be exploited to deliver

medication orally to the blood stream. Previous work in our group conjugated glucagon-like peptide-1 (GLP-1) to B_{12}. This conjugate was tested as a cAMP elevating agent using HEK-GLP-1R cells and reported an EC_{50} value of 4.1 nM, which shows that B_{12} does not affect the binding capabilities of GLP-1. We were also able to show that B_{12}-GLP-1 is able to potentiate GSIS in human pancreatic islets when glucose levels rise by producing a 3.2 fold stimulation of insulin secretion compared to glucose alone (GLP-1 control produced a 3.3 fold increase). Exendin 4 (Ex-4) is a DPP-IV resistant GLP-1 analog. The resistance to DPP-IV makes Ex-4 a viable medication by extending the lifetime of the peptide in vivo. Similar GLP-1 analogs are currently being used to treat type 2 diabetes via subcutaneous injection. Herein we discuss the synthesis, purification, and pharmacodynamic properties of a new B_{12}-Ex-4 conjugate. Also studied was the impact of B_{12}-Ex-4 binding to intrinsic factor on pharmacodynamics.



MEDI 147

Zeolite microneedles for controlled transdermal drug delivery

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Skin provides a protective barrier against harmful chemicals and pathogens, but also hinders transdermal delivery of therapeutic drugs, particularly large molecules of low lipophilicity. Skin poration methods including iontophoresis, phonophoresis, chemical,

laser and RF ablation and microneedles have been used to improve transdermal drug delivery with minimal pain and skin trauma. The key feature of this work is the use of porous zeolite microneedles for regulating the transport and delivery of drug molecules (i.e., diclofenac and insulin). Pure silica zeolites with composition similar to glass were used because of their inert properties and good biocompatibility. The zeolite was formed into needle lumen and its drug permeability was adjusted by controlling the intra- and inter-crystalline pores to deliver from 20 to 100 kDa. The zeolite microneedles are sharp and strong, and can easily penetrate the stratum corneum, without damage. Unlike polymer materials, the greater mechanical strength of zeolite makes it less susceptible to deformation and damage from osmosis and fluid flow. The biocompatibility of the zeolite microneedles was tested in animal models with no observable skin irritation or redness. Both in vitro and in vivo studies of insulin delivery by the zeolite microneedles were carried out. A diabetic rat was used for the in vivo study wherein the blood glucose and insulin levels were monitored during the duration of the studies.

MEDI 148

Synthesis of phosphorodithioate-based hydrogen sulfide donors and their biological effects

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Hydrogen sulfide (H_2S) is known for its characteristic smell of rotten egg. It has been recently recognized as the third physiological gaseous mediator along with CO and NO involved in several physiological processes including hypertension, inflammation, pain perception, and anticancer effects among others. These findings have fueled the development of a wide range of H_2S donors in order to understand the physiological roles of H_2S and its therapeutic applications. Our group is interested in the development of a controllable H_2S donor. We recently synthesized several phosphorodithioate-based H_2S donors and performed tests to evaluate their biological effects. I will present some detailed results of these H_2S donors including syntheses, H_2S concentration measurements, and biological effects.

MEDI 149

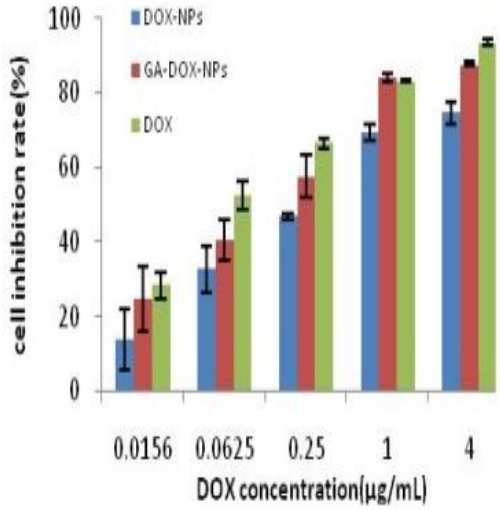
Doxorubicin-loaded glycyrrhetinic acid-modified recombinant human serum albumin nanoparticals for targeting liver tumor chemotherapy

Wen Wen Qi, Hui Guo, Zhi Ming Wang, Jun Lou, Yu Ling Xiao, ***XIANMING Hu****, xmhu@whu.edu.cn.department of medicinal chemistry, Wuhan University School of Pharmaceutical Sciences, Wuhan, Hubei Province 430071, China*

Introduction: Over-expression of glycyrrhetinic acid receptor in liver cancer could offer glycyrrhetinic acid as good targeting ligand for anti-cancer therapy. Glycyrrhetinic acid-

modified human serum albumin nanoparticles for targeting liver tumor cells may result in increased therapeutic efficacy and decreased adverse effects of cancer drugs. Doxorubicin(DOX)-loaded glycyrrhetinic acid-modified recombinant human serum albumin nanoparticles (rHSANP-GA NPs) were prepared for targeting therapy for liver cancer. **Methods:** The preparation procedure of the rHSANP-GA NPs were performed. GA was covalently conjugated to recombinant human serum albumin nanoparticles, which could efficiently deliver DOX into liver cancer with higher loading content. The rHSANP-GA NPs were characterized by ^{1}H NMR spectra, dynamic light scattering (DLS) and transmission electron microscopy (TEM). Cellular uptake study and Cytotoxicity assay were conducted by flow cytometry, confocal laser scanning microscopy and MTT assay, respectively. **Results:** The resultant rHSANP-GA exhibited uniform spherical in shape with diameter around 170nm and high stability in plasma with fixed negative charged (~ -25 mV). DOX was loaded into rHSANP-GA with a maximal encapsulation efficiency of 75.8%. Moreover, DOX-loaded nanoparticles showed increased cytotoxic activity in liver tumor cell than the free DOX. Therefore, our experimental data suggest that the DOX-loaded rHSANP-GA could be developed as a potential effective delivery nanoplatform for targeted anticancer drug into liver tumor cells (Fig. 1). *In vivo*biodistribution, pharmacokinetics and antitumor effect are under investigation.

*In vitro*cytotoxicity of free DOX, DOX-NPs and GA-DOX-NPs at 48h.



MEDI 150

Development of fluorescent retinoid X receptor ligands as a ligand screening tool

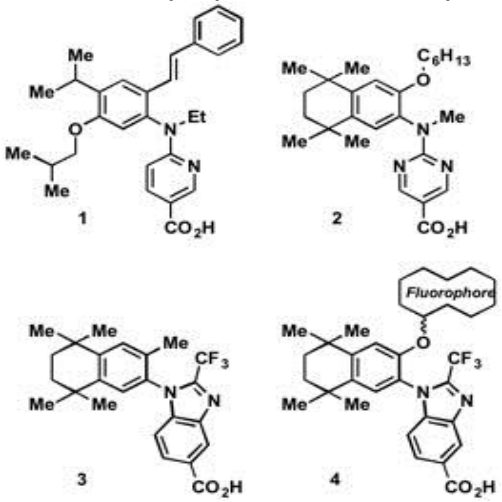
Shoya Yamada[1,2]*, gph422007@s.okayama-u.ac.jp, Mariko Nakayama*[1]*, Makoto Makishima*[3]*, Hirotaka Naitou*[4]*, Hiroki Kakuta*[1]*. (1) Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama, Japan (2)*

Research Fellow of the Japan Society for the Promotion of Science, Japan (3) Department of Biomedical Sciences, Nihon University, Japan (4) Graduate School of Nutritional and Environmental Sciences, University of Shizuoka, Japan

Retinoid X receptor (RXR) agonists are candidate agents for the treatment of type 2 diabetes and Alzheimer's disease. For the screening of RXR ligands, the binding assay using radiolabeled compounds and the reporter gene assay are performed. The former method needs cumbersome radioisotope usage. The later one requires cost and time. These backgrounds prompted us to create a simple and inexpensive RXR ligand screening system. As a simple and inexpensive system, fluorescence polarization (FP) assay is known. Thus, we created fluorescent RXR antagonist NEt-SB (**1** ; *p*A2: 8.23) usable for FP assay. However, its fluorescence intensity is not optimal for the FP assay. In this research, we developed novel fluorescent RXR ligands for the purpose of the improvement of the problem.

Since **1** , which possesses stilbene structure, and PA452 (**2** ; *p*A2: 7.11) showed strong RXR antagonistic activity, we expected that compounds possessing fluorophore in the similar position work as RXR antagonists. Having a potent RXR agonist CBTF-PMN (**3** ; *p*D2: 7.82) and referring to the side chain length of **2** , we designed **4** possessing fluorophore. Compound **4** was synthesized and its antagonistic activity was evaluated by using reporter gene assay. As a result, we found that antagonistic activity of novel fluorescent RXR antagonist **4** (*p*A2: 7.36) was similar to that of **2** .

In this presentation, design, syntheses, and the activities of fluorescent RXR ligands, fluorescent properties will be reported.



MEDI 151

Use of the Automated Synthesis Lab (ASL) for frequently used transformations in medicinal chemistry

***Hong Hu**, hu_hong@lilly.com, Miles G Siegel, Jothirajah Marimuthu, Jayana P Lineswala, Angela L Marquart, Alexander G Godfrey.Discovery Chemistry Research & Technology, Eli Lilly& Co, Indianapolis, IN 46285, United States*

The Automated Synthesis Lab (ASL), which integrates state-of-the-art synthesis, analysis, isolation, evaporation, and information management technologies into one suite, provides globally accessible synthetic solutions to Lilly scientists and our collaborators worldwide. The ASL has proven utility in many synthetic transformations routinely used in drug discovery efforts, such as C-C bond cross coupling, C-N bond formation, oxidation, reduction, and heterocycle formation. This poster highlights the application of the ASL and how the automated synthesis would speed up innovation in searching therapeutics to meet unmet medical needs.

MEDI 152

Molecular recognition in a diverse set of protein-ligand interactions studied with molecular dynamics simulations and end-point free energy calculations

***Bo Wang**[1,3], dadiaobo@gmail.com, Liwei Li[2,3], Samy O Meroueh[1,2,3,4]. (1) Department of Chemistry and Chemical Biology, Indiana University Purdue University Indianapolis, United States (2) Department of Biochemistry and Molecular Biology, Indiana University, United States (3) Center for Computational Biology and Bioinformatics, United States (4) Stark Neurosciences Research Institute, United States*

End-point free energy calculations using MM-GB(PB)SA provide a detailed understanding of molecular recognition in protein-ligand interactions. The binding free energy can be used to rank-order protein-ligand structures in virtual screening for compound or target identification. Here, we carry out free energy calculations for a diverse set of 11 proteins bound to 14 small molecules using more than a microsecond of explicit-solvent MD simulation. The structure of these complexes was previously solved by crystallography and their binding studied with isothermal titration calorimetry (ITC) data enabling direct comparison to the MM-GB(PB)SA calculations. Four MM-GBSA and three MM-PBSA calculations reproduced the ITC free energy within 1 kcal•mol^{-1}. MM-GBSA exhibited better rank-ordering with a Spearman r of 0.68 compared to 0.40 for MM-PBSA. The SVRKB scoring function (derived using Support Vector Regression using binding affinity and structural data) applied to MD snapshots resulted in excellent rank-ordering (r = 0.81). Calculations of the configurational entropy using normal mode analysis resulted in free energies that correlated significantly better to the ITC free energy than the MD-based quasi-harmonic approach, but the computed entropies showed no correlation with the ITC entropy. When the adaptation energy is taken into consideration by running separate simulations for complex, apo and ligand (MM-PBSA$_{ADAPT}$), there is less agreement with the ITC data for the individual free energies, but remarkably good rank-ordering is observed (r = 0.89). Interestingly, filtering MD snapshots by pre-scoring protein-ligand complexes with a machine learning-based approach (SVMSP) resulted in a significant improvement in the MM-PBSA results from r = 0.40 to r = 0.81. Finally, the non-polar components of MM-

GB(PB)SA, but not the electrostatic components, showed strong correlation to the ITC free energy.

MEDI 153

Remote synthesis of a DOS library on the automated synthesis lab (ASL)

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The Automated Synthesis Lab (ASL), Lilly's globally accessible platform for organic synthesis, analysis, and isolation, affords a unique opportunity for collaboration between remote research groups. This poster highlights our partnership with the Broad Institute to create a library of complex macrocycles with potential for further medicinal chemistry exploration via ring closing metathesis (RCM) and Huisgen cycloaddition chemistry. The library was realized using a Diversity-Oriented Synthesis (DOS) approach for library design, and the ASL, accessed remotely by Broad Institute researchers, for library execution.

MEDI 154

Synthesis and evaluation of pH sensitive folate targeted probes

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Folate receptor (FR) levels are overexpressed on cells associated with numerous diseases including cancers (e.g. ovarian, lung) and inflammatory conditions (e.g. atherosclerosis, arthritis). Folic acid binds with its cognate FR with high affinity and thus serves as a useful targeting moiety for both diagnosis and treatment of diseases. This report describes the synthesis and evaluation of a pH sensitive folate conjugate that increases in fluorescence upon endocytosis, which facilitates its use for fluorescence imaging and fluorescence based analytical detection systems. As evidenced during fluorescence microscopy, the folate based probe was readily taken up via endocytosis by FR+ L1210 leukemia cells. Uptake in controls was readily blocked by excess folic acid indicating folate specific targeting. Given that small-molecule targeted imaging agents constitute a new type of diagnostic strategy, this probe, with its pH sensitive nature, may prove useful in both in vivo and in vitro diagnostic applications.

MEDI 155

Employing potency data in computational lead optimization by the means of automated Free-Wilson analysis

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Lead optimization efforts are guided by a combination of factors, among which, the lead's potency, and its ADME/Tox properties play the major roles. Each drug discovery project aims at optimizing activity against specific target, however, computational models for the multitude of target affinity endpoints are not readily available. Consequently, conventional in silico lead optimization techniques can only be used for ADME/Tox profiling, while potency is neglected. In this work we present an Auto-SAR approach to overcome this issue by incorporating user-defined potency data in analog profiling. This approach is based on automatic Free-Wilson type SAR analysis on a series of known compounds with a common scaffold and varying substituents, to evaluate the influence of substituents in different positions on the considered property. The substituents are represented by their contributions to major physicochemical properties, such as size, lipophilicity, ionization, and hydrogen bonding. Exploring physicochemical dependences allows obtaining feasible, mechanistically interpretable class-specific SAR models from small data sets (several tens of compounds with measured potency data). Modeling involves special statistical methods to capture the nonlinearities in the relationship between the dependent property and used descriptors. The obtained class-specific models can be utilized to gain better understanding of substituent effects, evaluate target activities of new compounds of the same class, and guide lead optimization efforts to the most promising candidates. Finally, we present several case studies based on published lead optimization articles, where the structural analogs suggested by the software are compared to those proposed by the authors of the original studies.

MEDI 156

Emerging role of aldehyde oxidase in drug metabolism and drug discovery

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Mammalian aldehyde oxidases (AOXs) are cytosolic molybdo-flavoenzymes requiring FAD and a molybdopterin for their catalytic activity. Different animal species are characterized by a distinct complement of active AOX genes which varies from one in humans (*AOX1*) to four in mice (*Aox1*, *Aox3*, *Aox4* and *Aox3l1*). The broad substrate specificity of mammalian AOXs, the ability of the enzymes to oxidize different types of heterocycles, which often constitute the building blocks of new pharmacophores, and the presence of high levels of human AOX enzymatic activity in the liver make this class of enzymes interesting for drug metabolism and discovery.

AOX-dependent biotransformation of new drug candidates is an emerging problem, as new strategies of chemical synthesis aimed at reducing CYP450-dependent metabolism tend to enrich for pharmacophores which are AOX substrates and are inactivated by this enzyme. This calls for the development of new approaches to predict and test AOX-dependent metabolism particularly during the pre-clinical development of new drugs.

In silico methodologies to predict whether a new organic molecule is a potential AOX substrate are required. The situation is likely to change, because of the recent availability of the crystal coordinates and the structure of the first mammalian AOX, mouse AOX3.

Robust *in vitro* systems allowing the design of appropriate high- or medium-throughput screening tests to identify AOX substrates are required. Current efforts are focusing on the development of new technologies for the expression and purification of human AOX1 and other mammalian AOXs with high catalytic activity.

In vivo studies on AOX-dependent metabolism in animal models is highly problematic, as the complement of liver AOXs in humans and popular experimental animals is different. Although not optimal, the best human proxies in terms of liver AOX expression are represented by the guinea pig and the Rhesus monkey.

MEDI 157

Challenges to predicting clearance of aldehyde oxidase substrates in humans: Species differences and subject variability

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The cytosolic molybdo-flavoprotein aldehyde oxidase (AO) has emerged as a key drug-metabolizing enzyme, with an increased role in the biotransformation of heterocyclic-containing drugs. Due to subcellular location and profound species differences in activity, conventional metabolism screening assays using human liver microsomes and pharmacokinetic studies in rat and dog have failed to identify a role for AO in pre-clinical studies. The result is several drug candidates failing to achieve adequate exposure in clinical studies, and in one case, acute renal toxicity due to an AO-mediated metabolite. This is a costly and unfortunate result for a clinical program. This presentation will discuss the complicating aspects of studying AO, and provide examples of clinical failures, including a retrospective analysis for a candidate from Boehringer-Ingelheim, BIBX1382, an EGFR inhibitor investigated for the treatment of cancer. An integrated strategy will be discussed in order to confirm a role for AO in the metabolism of a candidate drug, as well as identify a surrogate species for pharmacokinetic studies towards predicting pharmacokinetic properties in humans. From an in vitro metabolism model standpoint, pooled cryopreserved human hepatocytes have been demonstrated to be suitable in a drug discovery setting to investigate AO-mediated metabolic

clearance (Hutzler et al, *Drug Metab Dispos* 40(2): 2012), albeit some under-predictions have been observed. More recent data where AO enzymatic activity has been compared in freshly isolated human hepatocytes from 10 individual donors over the initial 24 hours following hepatocyte isolation, in effort to understand the impact of AO enzyme stability, will be presented. Finally, literature reports have demonstrated donor-to-donor variability in AO activity in liver cytosol. Our findings from analysis of activity variability across a larger donor population (>50 human donors) using cryopreserved hepatocytes will be presented to identify the potential clinical pharmacokinetic variability expected for a substrate metabolized and cleared primarily by AO.

MEDI 158

Identification of a disproportionate metabolite of a novel 5HT4 partial agonist (5HT4 PA): Adherance to the MIST strategy by application of old tools using new approaches

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Metabolite profiling of novel 5HT4PA in monkey plasma suggested possible active moieties in addition to the parent molecule in monkey plasma. This led us to a plausible iminium metabolite [**M2**] demonstrating activity at the 5HT4 receptor. Understanding the formation of this active metabolite in humans was important a) to support receptor occupancy projections in the clinic and b) to address MIST (Metabolites In Safety Testing) strategy for the program, going forward. Hence, the human plasma obtained from subjects administered a single dose of 40 mg, P.O. of 5HT4PA, was analyzed, fractionated and tested for **M2** ; the results of this analysis demonstrated a large abundance of **M2** in human plasma, relative to the parent. Application of semi-quantitative analysis of the metabolite in humans and pre-clinical species ensured coverage of this new metabolite in multiple dosing studies up to projected efficacious doses of the parent at 15 mg, Q.D., without significant efforts on metabolite synthesis. The current approach has played a significant role in accelerating timelines towards initiation of POC studies without a significant lag (6-8 months) during the late stage development of this program. In the meantime, an effort to synthesize a standard of the metabolite, in a diasteromeric mixture reflective of that in human plasma, was accomplished using microbial biosynthesis. This permitted measurement of the **M2** at the target and off-target potencies, and plasma protein binding, and these values were used to estimate contribution to receptor occupancy at 5HT4 receptor. Current availability of authentic standard of metabolite **M2** can support simple in vitro studies to understand the formation/clearance/accumulation of the circulating **M2** ; in addition, development of the pre-clinical and clinical GLP assay will help us understand the *ivivc* of **M2** pharmacokinetics in humans and pre-clinical species. Thereby a mass balance study may be deferred to post-POC.

MEDI 159

Engineered human knockout cell lines for discovery screening of NCEs: Transporter, xenobiotic sensor, and drug metabolism pathways

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Adverse drug-drug interactions (DDIs) are an increasing concern for the development of new medicinal products. These may occur as a result of the utilization of the same transporter, drug metabolism and/or induction pathways when two or more drugs are taken concurrently. New chemical entities (NCEs) are typically screened for potential DDIs by a variety of specific assays, including 1) transporter studies in Caco-2 or other cell lines, 2) cytochrome p450 (CYP) induction assays assessing interactions with xenobiotic sensors, and 3) CYP profiling assays for determining the specific isozymes involved in the metabolism of the drug candidate. Many of these assays depend on the use of substrates or inhibitors which, however, may not be as target-specific as desired. For example, substrates in the Caco-2 transporter assay are often recognized by multiple transporters at different affinities, and the specificity of inhibitors is often unknown or poor, leading to ambiguous interpretations. In an effort to help improve and clarify the data generated in these assays we utilized genomic editing tools (zinc finger nucleases) to create modified cell lines containing individual and multiple knockouts of these targets. Data generated using single and double efflux transporter knockouts in Caco-2 cells will be discussed as well as progress toward generating xenobiotic sensor and drug metabolism knockouts in hepatocytes. Improved data from these novel discovery screens should provide better feedback to medicinal chemists on appropriate strategies to mitigate DDI concerns.

MEDI 160

Biotransformation of novel allosteric modulators of GPCRs: Challenges in efficacy modulation, receptor selectivity, and mechanism based adverse events

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Allosteric modulators for G-protein-coupled receptors (GPCRs) offer the potential for enhanced receptor subtype selectivity, reduced desensitization, and improved therapeutic index. Despite these advantages, research focusing on promising family A and C GPCR targets has revealed within some classes of allosteric modulators, unanticipated modulation of efficacy with small structural modifications ('molecular switches'), including generation of ligands with dual allosteric agonism and potentiation (ago-PAM). Ago-PAM ligands of the metabotropic glutamate receptor subtype 5 (mGlu5), for example, induce epileptiform activity and behavioral convulsions in rodents, whereas mGlu5 positive allosteric modulators (PAMs) do not induce these adverse effects. Such mode switching phenomenon has major implications for safety and

development of GPCR allosteric modulators since by analogy through drug metabolizing enzymes metabolites may be capable of presenting a range of pharmacological activity (e.g. NAM, PAM, ago-PAM) at the target and/or target family receptors. Examples and strategies for mitigating formation of active allosteric metabolite-ligands will be presented.

MEDI 161

Why macrocycles are important for drug discovery: Identification of small molecule synthetic macrocycle antagonists of human IL17A

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Macrocycles are found widely in nature and several of these natural products are marketed as effective drugs with good drug-like properties. Synthetic macrocycles by contrast have been generally underexploited for drug discovery despite the opportunities they present for addressing challenging targets.

IL17A has been demonstrated to be a key pro-inflammatory cytokine in human rheumatoid arthritis and psoriasis. Small molecule macrocyclic compounds (Ensemblins) have been discovered at Ensemble Therapeutics that are nanomolar inhibitors of the interaction of the IL17A cytokine with its receptor. These compounds act as inhibitors of IL17A-stimulated IL-6 production in RASF and HT29 cells, and are also anti-inflammatory in an IL17-directed murine delayed-type hypersensitivity model. Unlike the IL17 biological therapies currently in clinical development, Ensemblins can be optimized to have desirable drug-like properties including membrane permeability and oral bioavailability.

MEDI 162

Charting islands of bioavailability beyond the Rule of 5: What can we learn about cyclosporine A and other rule-breaking cyclic peptide natural products?

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There has been a renewed interest in bioactive cyclic peptides and peptidomimetics, and new synthesis and screening technologies have enabled the discovery of potent macrocycles against a wide variety of biological targets. But while many cyclic peptides found in nature have suprisingly good cell permeability and even oral bioavailability, endowing synthetic cyclic peptides with “drug-like” permeability and pharmacokinetic properties has been relatively hit-or-miss. Cyclic peptide natural products often share a common chemical feature, backbone N-methylation, which serves to enhance lipophilicity and proteolytic stability, and our group has been interested in uncovering the relationship between N-methylation and membrane permeability. I will present new

chemical methods for the selective, on-resin N-methylation of cyclic peptides to generate compounds with improved membrane permeability. I will also show unpublished results detailing the interplay of N-methylation and side chain functionality on cell permeability in cyclic octapeptides with molecular weights over 1000.

MEDI 163

NICAMs: Development of novel cyclosporine-based cyclophilin inhibitors

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Isotechnika Pharma's discovery-stage NICAM compounds are a broad platform of cyclophilin inhibitors for the treatment of cyclophilin-mediated disorders including viral, cardiac, neurological, and inflammatory diseases. The compounds are synthetically novel chemical modifications of the cyclosporine molecule which yield non-immunosuppressive high affinity cyclophilin inhibitors. The structure-activity relationships of the compounds vis-à-vis their potency, toxicity, drug interactions, and pharmacokinetics will be discussed.

MEDI 164

SOM230: A new therapeutic modality for Cushing's disease

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The somatostatin (SRIF, somatotropin release inhibiting factor) field has been a success story in terms of medicinal chemistry and drug discovery offering a variety of therapeutic opportunities, e.g. acromegaly, gastrointestinal neuroendocrine tumors, whole body imaging and radiotherapy. Indeed, a rational medicinal chemistry approach capitalising on structure activity relationships led to the discovery of SOM230, a stable cyclohexapeptide somatostatin mimic which exhibits unique binding to human SRIF receptors (sst1-5). This approach involved transposing functional groups, in the form of unnatural amino acids, from SRIF-14 into the stable, reduced size cyclohexapeptide template. Further, the hydroxyproline urethane extension of SOM230 has been functionalized with the chelators DTPA and DOTA, which is a necessary prerequisite for the possible development of ligands which could be used for whole body imaging. Uniquely, SOM230 exhibits binding with a 30 to 40 times higher affinity than Sandostatin® to the sst1 and sst5 receptors and exhibits higher efficacy in preclinical models in lowering Growth Hormone, Insulin-Like Growth Factor-1, ACTH and corticosterone than Sandostatin®. Recently, phase III clinical studies have established

the therapeutic potential of SOM230 / Pasireotide (Signifor®), as the first pituitary directed medical therapy for Cushing's disease[1] leading to registration of SOM230 by both EMEA and FDA in 2012.

[1] A 12-Month Phase 3 Study of Pasireotide in Cushing's Disease, Annamaria Colao, Stephan Petersenn, John Newell-Price, James W. Findling, Feng Gu, Mario Maldonado, Ulrike Schoenherr, David Mills, Luiz Roberto Salgado and Beverly M.K. Biller for the Pasireotide B2305 Study Group, N Engl J Med 2012;366:914-24.

MEDI 165

Nontraditional macrocyclic peptide discovery accelerated by the RaPID system

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The genetic code is the law of translation, where genetic information encoded in RNA is translated to amino acid sequence. The code consists of tri-nucleotides, so-called codons, assigning to particular amino acids. In cells or in ordinary cell-free translation systems originating from prokaryotes, the usage of amino acids is generally restricted to 20 proteinogenic (standard) kinds, and thus the expressed peptides are composed of only such monomers. To overcome this limitation, we recently devised a new means to reprogram the genetic code, which allows us to express non-standard peptides containing multiple non-proteinogenic amino acids in vitro. This lecture will describe the most recent development in the genetic code reprogramming technology that enables us to express natural product-like non-standard peptides. The technology involves (1) efficient macrocyclization of peptides, (2) incorporation of non-standard amino acids, such as N-methyl amino acids, and (3) reliable synthesis of libraries with the complexity of more than a trillion members. When the technology is coupled with an in vitro display system, referred to as RaPID (Random non-standard Peptide Integrated Discovery) system, the non-standard macrocyclic peptide libraries with a variety ring sizes and building blocks can be screened (selected) against various drug targets inexpensively, less laboriously, and very rapidly, leading to the next generation of peptide drugs.

Recent featured readings:

• J. Morimoto, Y. Hayashi, H. Suga* “Discovery of macrocyclic peptides armed with a mechanism-based warhead that isoform-selectively inhibit a human deacetylase SIRT2” **Angewandte Chemie International Edition** *51*, 3423-3427 (2012).

• Y. Hayashi, J. Morimoto, H. Suga* “*In Vitro* Selection of Anti-Akt2 Thioether-Macrocyclic Peptides Leading to Isoform-Selective Inhibitors” **ACS Chemical Biology** *7*, 607-613 (2012).

MEDI 166

Structure-based discovery of novel indenoquinolones targeting wild-type and camptothecin-resistant R364H mutant of topoisomerase I

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DNA topoisomerase I (Top1) has been established as an effective antitumor target, since the classic Top1 inhibitors camptothecin derivatives, including Irinotecan and Topotecan, have been widely used in chemotherapy. Several limitations of camptothecins, such as low solubility and stability, high toxicity, and the occurrence of resistance, have encouraged the development of non-camptothecin Top1 inhibitors. Several non-camptothecin compounds from the indenoisoquinoline and indolocarbazole classes of Top1 poisons have already entered clinical trials and demonstrated promising antitumor activities. Unfortunately, crystallographic studies of the human Top1-DNA complex bound with representative compounds of the camptothecin, indenoisoquinoline and indolocarbazole classes of top1 poisons demonstrated that all these three classes of compounds have a characteristic hydrogen bonding with Arg364. Therefore R364H Top1 mutant, which was initially discovered from camptothecin-resistant prostate cancer cell lines, will render resistance to all the three classes of drugs. Thus, there is an urgent need to develop second-generation Top1 inhibitors targeting wild-type and R364H mutant of Top1, which also presents a huge challenge to drug discovery. Using structure-based design, quantum chemistry guided scaffold hopping and medicinal chemistry approaches, we firstly discovered a novel series of indenoquinolone derivatives that have shown potent activities against both wild-type and R364H mutant of Top1. This study also demonstrated that targeting R364H mutant could be achievable by an elegant re-engineering of current Top1 inhibitors scaffolds and provided a new starting point to design second generation Top1 inhibitors that address the problem of drug resistance of current Top1 inhibitors that are currently used or investigated in the clinic.

MEDI 167

Characterization and targeting of leukemia stem cells

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The past decade has witnessed a major surge in research related to the potential relevance of stem cells in cancer. Prevalent in much of this work has been the analysis of malignant stem cells in hematologic malignancies such as chronic and acute forms of myeloid leukemia. Of particular interest, recent reports have begun to define molecular pathways controlling self-renewal and survival of leukemia stem cells (LSCs), thereby

affording novel opportunities for therapeutic intervention. Importantly, selective eradication of leukemia stem cells has been demonstrated in several preclinical models, and has been accomplished with minimal toxicity to normal tissue.

Perhaps the most significant challenge in targeting LSCs is the well known cellular and molecular heterogeneity found in primary tumor populations. While many targeted strategies have been described in recent years, few have been show to provide broad efficacy towards the many subtypes of leukemia that can emerge as a consequence of intrinsic diversity or chemotherapy-induced selection. This seminar will provide a discussion of the molecular and cellular properties most relevant to the growth/survival of malignant stem cells, with an emphasis on leukemia. In addition, ongoing efforts to develop improved therapeutic regimens will be described.

MEDI 168

Identification of the tumor initiating cell and treatment strategies for its eradication by inhibiting embryonic pathway signaling

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Tumor relapse and metastasis remain major obstacles for improving overall cancer survival. Tumor initiating cells (TICs) also referred to as cancer stem-cells (CSCs) may be responsible for invasion, metastasis and tumor heterogeneity. TICs exhibit tumorigenic properties and the ability to self-renew, form differentiated progeny, and develop resistance to therapy. TICs use signaling pathways that are found in normal stem cells, such as Wnt, Notch, and Hedgehog (Hh), as well as other signaling pathways and modifications to the microenvironment. The origin of TICs is not understood, but data suggest that they develop from a primordial cell, or possibly other cancer cells that develop aberrant genetic events early in cancer development. Therapeutic targeting of TICs and bulk tumor populations may provide a strategy to suppress tumor re-growth. Development of agents that target critical steps in the Wnt, Notch, and Hh pathways will be complicated by signaling cross-talk. The role that embryonic signaling pathways play in the function of TICs, the development of new anti-TIC therapeutic agents, and the complexity of signaling cross-talk will be described and the controversies related to “stem cells” discussed.

MEDI 169

Targeting FAK and PI3K/mTOR: Clinical candidates that preferentially target cancer stem cells

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Verastem is focused on development of small molecule drugs that preferentially target cancer stem cells (CSCs). Through screening of more than 300,000 compounds for CSC-specific effects, we have identified Focal adhesion kinase (FAK) and PI3K/mTOR as key druggable pathways to target CSCs. FAK is a non-receptor tyrosine kinase that orchestrates cellular signaling through integrins and growth factor receptors and plays an essential role in multiple steps of tumorigenesis. The PI3 kinase/mTOR pathway is a signal transduction pathway central to cancer cell proliferation and survival. Furthermore, both the FAK and PI3 kinase pathways have been shown to be critical for the maintenance of cancer stem cells, which are rare cancer cells that are endowed with tumor initiating capability and responsible for metastasis, relapse and resistance to chemotherapy. We present here that Verastem's FAK and PI3K/mTOR inhibitors preferentially target cancer stem cells both *in vitro* and *in vivo* and exhibit potent anti-cancer activities in xenograft models. Translational insights/strategies for selection of cancer patient populations most likely to respond to FAK inhibitors will also be discussed. All of our data taken together provide strong rationale for the clinical development of Verastem's FAK and PI3K/mTOR inhibitors for cancer to achieve durable clinical responses. Consequently, we have designed CSC-targeted clinical trials which are planned to initiate this year for 3 small molecule drugs targeting these key pathways.

MEDI 170

Targeting glioblastoma stem cells through BMX inhibition

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Glioblastoma (GBM) is the most lethal and common type of primary brain tumor that displays remarkable cellular hierarchy with tumorigenic GBM stem cells (GSCs). We previously demonstrated that GSCs promote tumor angiogenesis, cancer invasion and therapeutic resistance, suggesting that therapeutic targeting of GSCs may significantly improve GBM treatment. We recently found that the non-receptor tyrosine kinase BMX is differentially expressed in GSCs relative to non-stem cancer cells and neural progenitor cells (NPCs). We demonstrated that BMX mediates STAT3 activation to maintain self-renewal and tumorigenic potential of GSCs. Disrupting BMX by shRNA potently suppressed STAT3 activation, expression of GSC transcription factors, and growth of GSC-derived intracranial tumors. In contrast, STAT3 signaling in NPCs is activated by the Janus kinases (JAK2) but not by BMX. Importantly, pharmacological inhibition of BMX kinase activity by its irreversible inhibitor potently inhibited GSC tumorsphere formation, STAT3 activation and tumor growth in vivo but showed no significant effect on NPCs. These findings indicate that BMX kinase represents a GSC-specific molecular target and therapeutic targeting of GSCs through BMX inhibition may effectively block GBM tumor progression.

MEDI 171

Novel approaches to define distinct pathways in cancer stem cells

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Human cancer stem cells are currently being identified and characterized from many tissue types. This characterization normally uses strategies involving phenotypic analysis combined with subsequent FACS-based purification to exclude or include the subpopulation responsible for tumor initiating capacity in human-mouse xenografts. Since xenografting is both complex and limited by the availability of human tissue, other methods to analyze cancer stem cell characteristics are required. Equally important is comparing the properties of cancer stem cells to normal counterparts in order to define distinct features that may allow more specify targeting of the cancer stem cell population without harming normal healthy stem cells required for tissue repair and homeostasis. We have recently explored the use of human embryonic stem cells (hESCs) and novel transformed (t-hESCs) counterparts to help identify pathways that may distinguish cancer stem cells from normal stem cells. Since both of these pluripotent sources of human stem cells have well-defined culture conditions and are, thus amenable to high content screening, we have further investigated whether chemical compounds with differential effects may better characterize and describe differences in cancer stem cells compared to normal stem cells as an alternative strategy to candidate phenotypic characterization. We will describe these assays and our ongoing progress in this regard, which has allowed us to identify unique targets specific to cancer stem cells that would have been difficult to ascertain using traditional methods to describe cancer stem cells in the human.

MEDI 172

Using fragments alongside HTS – the best of both worlds

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The past decade has seen tremendous developments in the experimental methods of “Fragment-Based Lead Discovery”, FBLD, with many compounds now in clinical trials and the first compound derived from FBLD already on the market. The core of the FBLD approach is that the drug discovery process begins with the identification of small (<250 MW), low affinity hits. By restricting the size of the initial ligands, a large area of chemical space can be sampled whilst retaining a high degree of binding efficiency. These low affinity ligands provide a rich source of chemical matter.

In the early days of FBLD, the emphasis was on optimisation of these fragments to hits and leads by structure based design. As the technique begins to mature, it is becoming clear that fragments can also be combined with chemical information provided by HTS hits to generate new ideas for lead series.

In this presentation, we will discuss the essential features of a fragment screening platform. In FBLD, low affinity ligands are used as startpoints for lead discovery, so it is important that there is a high level of confidence in the initial fragment hits to avoid potentially misleading or costly artefacts. We will briefly review the issues of library curation and provide our current view on the pros and cons of different screening techniques. This will include some discussion of common pitfalls which can bedevil an FBLD campaign.

We will also discuss how fragment hits can be assessed and combined with HTS hits to identify promising series for optimisation. This will include a discussion of how to triage the 10 to 100 chemically diverse fragments that are typically found as hits from screening 1k-10k fragments. We will include examples where fragment hits have been combined with hits from other screening techniques to generate novel lead compounds.

MEDI 173

How do you know what you know: Modern developments in biophysical hit assessment to enhance confidence in the output of lead generation campaigns

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The ultimate goal of an experimental lead finding campaign is to produce a dataset of sufficient breadth and quality to justify and focus medicinal chemistry resources on developing early lead series. The utilization of a variety of biophysical techniques in lead finding has been on the rise. Due to limited throughput compared to modern HTS infrastructure biophysical assessment has tended to be limited to later stages of hit characterization and the screening of small libraries. Through refinement of our methodology and the advancement of modern optical biosensor hardware and software we have been able to provide biophysical assessment of HTS hits during the HTS screening cascade, rather than later in hit triage or after repurchase/resynthesis. In addition we are utilizing the non-enzymatic nature of the sensor to provide rapid mechanism of action assessment on larger numbers of both HTS and fragment screening hits, which can be useful in situations where MOA by biochemical approaches can be technically challenging. We are also using new cheminformatic tools to cluster fragments by their properties in addition to chemical structure, and provide chemists with a preliminary priority score based on a variety of properties. Through tighter and more coordinated multidisciplinary efforts we are continuing to find ways to enhance confidence and understanding of hits emerging from different lead generation platforms.

MEDI 174

Colloidal aggregates in vitro and in vivo (or how I learned to stop worrying and love the bomb)

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At micromolar and sub-micromolar concentrations, many drug-like organic molecules aggregate into colloids in aqueous media. These aggregates sequester protein targets without specificity, inhibiting them. The colloids are sensitive to assay conditions and target concentration, which can give an illusion of specificity, but this illusion can be rapidly dissipated by a few well-controlled experiments. I will summarize the range of molecules that can behave this way, and recent expansion of the number of assays and milieus in which colloids can be active, including membrane-bound receptor assays and cell-culture assays. Recent research on their affects on drug distribution in vivo will also be considered.

MEDI 175

Protein-family virtual screening for drug discovery: Accurate enzymatic and cellular activities and selectivities – without crystal structures, and with or without training data

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Virtual screening with accuracy comparable to experimental High-Throughput Screening (HTS) has long been a goal of computational chemists. Protein Family Virtual Screening (PFVS) is finally achieving this for kinases and several other protein families. The three PFVS methods achieve unprecedented speed and accuracy by including massive amounts of IC50 and structural data from previous targets into models for each new family member: the 2D “Profile-QSAR” meta-QSAR, the Kinase-Kernel chemogenomic model, and the 3D Surrogate AutoShim docking method. Between the methods, 2 billion activity predictions have been made for 4 million internal and commercial compounds across 500+ kinases, so initial kinase virtual screening is now a table lookup. The methods have been applied to over 4 dozen active Novartis projects, with external R^2=0.35–0.7 and enrichments of 20x–60x. PFVS has recently been extended to non-kinase adenosine-binding proteins, GPCRs, and to serine and cysteine proteases, in all now covering half of the estimated druggable genome.

The methodologies will be described and examples will be presented from all stages along the drug discovery pipeline, from finding tool compounds for early target validation to finding backup chemistries for successful projects going into the clinic.

MEDI 176

Chemical property bias in molecular screening

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Candidate attrition is the major issue facing the Pharma industry and a number of recent studies have shown that, concomitant with increases in drug development costs and failure rates, properties of drug candidates have trended towards more bulky, greasy, flat (i.e. achiral) molecules. Whether this relationship is causal or not, it has appropriately focused attention on improving the properties of future candidates to make them more like the drugs of the past.

One obvious approach to improving the properties of candidate molecules is to try to find better starting points (i.e. screening hits). This can be distilled into two critical factors- improvement of the property and diversity space occupancy of the chemical libraries to be tested and, secondly, identification of hits with the most favorable combinations of chemical properties and biological response. This presentation will focus on ways in which the latter objective can be achieved.

Virtually all current hit calling procedures applied in high throughput screening are based entirely on the apparent potency of compounds and take little or no account of their chemical properties. Here, we will present an analysis of over 300 HTS campaigns conducted at GSK and demonstrate that marking hits in this fashion introduces significant size and lipophilicity biases to HTS hit progression, mirroring the trends seen in studies of drug candidate attrition. Additionally, we will present alternative hit marking models in which chemical properties may be taken into account and show how these methods yield hits which are substantially improved with respect to the balance of physiological response and chemical properties, e.g. ligand efficiencies.

MEDI 177

From data to decisions – a holistic approach to the analysis of HTS results

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The choice of one or more lead series based on high throughput screening, and subsequent follow-up data, is one of the most critical decisions in the course of a drug discovery program. The proper selection of a chemical series is a complex process which requires a drug discovery team to evaluate multiple factors. In addition to considering the potency and/or efficacy of compounds against the target of interest, the team must consider whether a particular series is selective for the target of interest or generally promiscuous. Teams can also consider factors such as the presence of preliminary SAR, physical properties, and pharmacokinetics of related compounds. Other factors such as intellectual property and literature reports of off-target activity can also come into play. The information required to fully assess a chemical series is often scattered among multiple systems, making an optimal assessment difficult or even

impossible. An appropriate computational infrastructure can significantly ease the burden of evaluating HTS data, and can enable drug discovery teams to make better decisions. This presentation will focus on computational tools we are developing to integrate internal as well as external data, with the objective of providing teams with a more holistic view of the chemical series being considered for lead optimization

MEDI 178

Antibody drug conjugates

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Antibody conjugates are diverse therapeutics consisting of a cytotoxic agent linked covalently to an antibody or antibody fragment directed toward a specific cell surface target expressed by tumor cells. The main approaches using antibodies to target cytotoxic agents to malignant cells: antibody-protein toxin (or antibody fragment–protein toxin fusion) conjugates, antibody-chelated radionuclide conjugates, antibody–small-molecule drug conjugates (ADCs). Although ADCs are highly selective, they are quite inefficient in delivering drugs to tumor and require very potent cytotoxins as the drug component. The drugs used on most ADCs in clinical trials are dolastatin 10 analogs, monomethylauristatin E or F, maystansine analogs, calicheamicin, or duocarmycin derivatives. The drugtargets are either tubulin or DNA. Most ADCs have 4 drug molecules per antibody molecule; however, several methods of binding 20 or more drug molecules per antibody molecule through branched polymeric linkers are being explored. At least 25 ADCs are currently in clinical trials. Brentuximab vedotin (Adcetris) was approved by the US Food and Drug Administration as a treatment of Hodgkin lymphoma and anaplastic large cell lymphoma in 2011. Recently, FDA approved trastuzumab emtansine (Kadcyla), which is a treatment of HER2+ breast cancer. Preclinical pharmacokinetic and pharmacodynamic assays indicate that there are several drug metabolites present in xenograft tumor tissue that reflect lysosomal degradation of the ADC and that the role of the cell surface target in vivo can be proven by negating the ADC therapeutic activity by administration of excess naked antibody. Several antibody formats including bispecific antibodies, minibodies, Fabs, diabodies, scFv fragments are under investigation as methods for delivery of radionuclides for imaging and for radiation therapy. ADCs are chemotherapeutics that will be used in combination treatment regimens.

MEDI 179

Engineering next generation antibody drug conjugates with the improved therapeutic index

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Antibody drug conjugates (ADCs) are attractive targeted chemo-therapeutic molecules as they combine ideal properties of both antibodies and cytotoxic drugs by targeting potent cytotoxic drugs to the antigen-expressing tumor cells, thereby enhancing their anti-tumor activity. ADC is a three component molecule and all three components (antibody, linker and cytotoxic drug) are equally important in building a successful ADC therapeutic for a given tumor specific antigen. In this presentation, I will review three conjugation methods - conventional lysine and cysteine and engineered site-specific conjugation methods, used in current ADC development for pre-clinical and clinical studies. I will present results on how engineered site-specific ADCs would improve ADC manufacturing, define drug-to-antibody ratio, and therapeutics compared to conventional ADCs. In addition, the presentation also highlights the role of conjugation site in the therapeutic activity of ADCs and discusses a detail biochemical mechanism for the stability of cysteine-maleimide based antibody conjugates in plasma *in vitro* and *in vivo*.

MEDI 180

Discovery of new auristatins for the use on antibody drug conjugates for the treatment of cancer

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This talk will provide a comprehensive overview about the medicinal chemistry efforts that led to the discovery of novel auristatins as payloads for Antibody Drug Conjugates (ADC), including the lead compound **PF-06380101** . The design strategy that was the foundation for the initial SAR studies aimed to identify novel structural elements on the natural product while maximizing potential receptor interactions will be outlined. Furthermore, assays that were developed that aided in the medicinal chemistry efforts to identify new and potent analogs will be discussed. The talk will also give insight about how compounds were evaluated, triaged and selected for optimization. The enablement of these novel auristatins as ADC payloads along with the *in vivo* efficacy and tolerability studies will also be described. A particular emphasis will be placed on how cell based assays and cell receptor expression profiles differentiate ADC's in regard to *in vitro* and *in vivo* potencies as a function of structural modifications of the

linker/payloads. Finally, it will be described how the development of the auristatins enabled a more general understanding of payload design. These aspects include considerations about target expression profiles, target binding affinities and *in vitro* potencies, physicochemical properties, ADME characteristics and the relation of these parameters to the challenges that are presented by the nature of the tumor physiology.

MEDI 181

Holistic approaches to analytical characterization of ADCs

***John Valliere-Douglass**, jdouglass@seagen.com, Lucy Pan, Nathan Ihle, Oscar Salas-Solano.Department of Process Sciences, Seattle Genetics, Bothell, WA 98021, United States*

Antibody-drug conjugates (ADCs) harness the specificity of antibodies to deliver a conjugated cytotoxic agent directly to tumor cells thus avoiding the systemic toxicity associated with standard chemotherapy. The recent approval of ADCETRIS® (brentuximab vedotin) which is comprised of a CD30-targeted antibody conjugated to the antimitotic agent monomethyl auristatin E (MMAE) highlights the potential impact of these novel agents as cancer therapeutics. This presentation will review Seattle Genetics' current portfolio of MMAE and monomethyl auristatin F (MMAF)-based ADCs as well as our pyrrolobenzodiazepine (PBD) dimer EC-mAb (engineered cysteine) based technologies. We will highlight recent analytical advancements that allow for comprehensive top-down analysis of ADCs by mass spectrometry and other large molecule structure based techniques which augment our capacity to rapidly develop therapeutically relevant molecules for the treatment of cancer.

MEDI 182

Unnatural amino acids in novel antibody conjugates

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We use genetically encoded non-natural amino acids as a chemical “handle” to site-specifically couple small molecules and protein components. Antibody-drug conjugates to a single UAA are homogenous and highly active. DNA coupled to antibody constant regions enables very sensitive immune-PCR for biodetection applications. Protein-nucleic acids (PNAs) coupled to different antibody fragments allow basepairing to rapidly form bispecific or higher order heteromultimers, and open unique avenues for protein engineering.

MEDI 183

Antibody drug conjugates (ADCs): From bench to bedside

***John M Lambert**, john.lambert@immunogen.com.ImmunoGen, Inc, Waltham, Massachusetts 02451, United States*

Oncologists viewed monoclonal antibody technology with great optimism when the technology was first developed, since they offered the promise of targeted elimination of tumor cells without the systemic toxicity associated with chemotherapy. However, despite considerable effort spanning over three decades of clinical research, application of monoclonal antibody technology has had only modest success in improving treatment outcomes in patients with solid tumors. In general, the immunological mechanisms for cell elimination induced upon antibody binding to cell surfaces have not proven effective against solid tumors without some other mechanism for enhanced potency.

Enhancing the cancer cell-killing activity of antibodies through conjugation to highly potent cytotoxic “payloads” to create ADCs offers a strategy for developing anti-cancer drugs of great promise. As with many simple ideas, its successful execution has proved to be challenging. Early ADCs exhibited side-effect profiles similar to those of “classical” chemotherapeutic agents, and their performance in clinical trials in cancer patients was generally poor. However, with the recent clinical development of ADCs utilizing highly potent cytotoxic agents as “payloads” designed specifically for antibody-targeted delivery, interest in the ADC field has been reinvigorated. With the approval of brentuximab vedotin for treatment of Hodgkin lymphoma in 2011, and the approval of ado-trastuzumab emtansine for the treatment of HER2-positive breast cancer in 2013, it has become apparent that ADC technologies utilizing potent tubulin-acting agents are able to generate highly active, well-tolerated, anticancer agents that fulfill the long-awaited promise of ADCs. Several more ADCs, including those developed with ImmunoGen's maytansinoid technologies, have shown encouraging efficacy in clinical trials in both solid tumors and hematologic malignancies. In creating effective, well-tolerated, ADCs, each element in its design, from target selection, selection of the antibody, the cytotoxic “payload”, and the linker, is important, and will be exemplified in the presentation.

MEDI 184

Sequence-defined oligomers as shuttles for targeted nucleic acid and protein delivery

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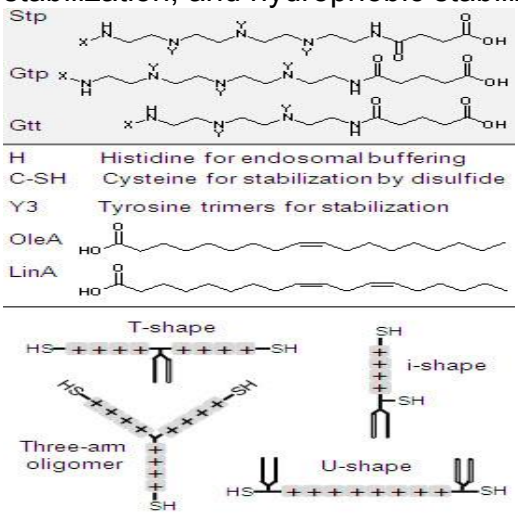
For intracellular macromolecular drugs such as proteins or nucleic acids, dynamic carriers for extra- and intra-cellular transfer are required. Like natural viruses, such carriers contain subdomains for facilitating the various delivery steps, including packaging and protecting the therapeutic cargo during extracellular delivery, receptor targeting for specific cell attachment and intracellular active domains. Like shuttles the

carrier should release the cargo at the right intracellular location. Standard multifunctional conjugates suffer from lack of precision with regard to conjugation sites, modification degrees, and polydispersity. We applied solid phase supported synthesis and novel artificial oligoamino acids for design of >600 sequence-defined cationic oligomers. Small chemical motifs useful in various delivery steps were incorporated in defined topologies (Fig.1), including protonatable amines, cysteines for covalent stabilization, and hydrophobic stabilizing fatty acids or tyrosine trimers.



Nanosized polyplexes were formed by complexation or reversible covalent linkage in case of cargo proteins. Targeting and endosomal escape are key requirements for delivery of such polyplexes. The carrier protonation capacity and pH maximum in the endolysosomal range (pH 5 to 7.4) could be fine-tuned by incorporation of histidines and oligoethanamines. This resulted in improved cytosolic delivery and up to >100-fold enhanced gene transfer. PEG-ligands for shielding and targeting cellular receptors such as the folate receptor or the HGF receptor/c-met were found to effectively mediate targeted pDNA and siRNA delivery.

MEDI 185

Delivery strategies for RNA interference (RNAi) based therapeutics

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Therapeutic agents that act through the RNA interference (RNAi) pathway are specific and potent inhibitors that may be designed to disease pathways previously considered “non-druggable”. Numerous proof-of-concept studies in animal models of human disease demonstrate the broad potential of RNAi. Recently, Alnylam has demonstrated both proof-concept and proof-of-mechanism of RNAi therapeutic agents in clinical trials in disease areas such as liver cancer, respiratory syncytial virus, hypercholesterolemia,

and transthyretin-mediated amyloidosis. The major challenge for the successful development of systemically delivered RNAi therapeutics has been to identify delivery approaches that could be translated into the clinic. Tremendous progress has been made in this area, and importantly, clinical trials are underway with several RNAi therapeutic candidates using lipid nanoparticles (LNPs) for intravenous administration. We have also discovered an siRNA-GaNac conjugate platform which utilizes asialoglycoprotein receptor (ASGPR) targeting that enables subcutaneous delivery of RNAi therapeutics against genes expressed in hepatocytes. Alnylam is advancing several RNAi agents specific for liver targets to address genetically defined diseases with high unmet medical need. The recent advances with ALN-TTR, an RNAi therapeutic for the treatment of transthyretin-mediated amyloidosis, a fatal, autosomal dominant, multisystem disease caused by abnormal extracellular deposits of transthyretin amyloid fibrils, and ALN-AT3 targeting antithrombin for the treatment of hemophilia will be discussed.

MEDI 186

Evalution of the EPR effect in dogs with spontaneous tumors and its implications on nanotherapies

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Nanoparticles are well established as effective drug delivery systems and have potential in biomedical imaging as a diagnostic tool. We have recently developed a highly efficient method for utilizing liposomes as agents in positron emission tomography (PET) imaging giving high resolution images and allowing direct quantification of liposome tissue distribution and blood clearance. Our approach is based on remote loading of a copper-radionuclide (^{64}Cu) into preformed liposomes and copper entrapment by an encapsulated copper-chelator. We show that the ^{64}Cu-liposomes provide quantitative *in vivo* imaging in canines with spontaneous tumors using PET. Seven canines with spontaneous tumors were included in the study where the main focus was to evaluate the EPR effect in large animals with spontaneous tumors and the performance of the developed liposome imaging agent. None of the included dogs displayed any anaphylactic, toxic or adverse reactions. Liposome circulating half-life ranged from 24.2 hours to 54.2 hours, with a mean half-life of 35.0 ± 4.24 hours. The study showed that the EPR effect assures substantial tumor accumulation in some but not all spontaneous tumors in canines. The included carcinomas displayed higher mean and maximum uptake levels of liposomes relative to the included sarcomas. The ^{64}Cu-liposomes have potential as a diagnostic tracer in cancer diagnostics. We envision that the ^{64}Cu-liposomes will be an important tool for evaluating liposome performance in

future and may become an important tool in selection of cancer patients for nanoparticle based chemotherapy.

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MEDI 187

Development of lipid-based nanotherapeutics for treating solid tumors

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Using highly efficient intraliposomal trapping agents, small molecule drugs can be stably encapsulated in the liposome interior resulting in long circulation lifetimes, sustained drug exposure at the site of the tumor, and an increased capacity for molecular targeting using antibodies specific for cell surface receptors overexpressed preferentially on cancer cells. Here we provide examples of two clinical stage drugs, a nontargeted nanoliposomal CPT-11 (MM-398) and an ErbB2(F5)-targeted pegylated liposomal doxorubicin (F5-PLD or MM-302), including their therapeutic design, preclinical rationale, and current clinical status. F5-PLD has recently completed Phase I trials as a monotherapy in ErbB2-overexpressing breast cancer and relies on tumor cell internalization of the targeted cytotoxic to significantly improve drug delivery and therapeutic activity. Nanoliposomal CPT-11 is currently in Phase III trials for pancreatic cancer and displays a slow-release rate in the circulation ($T_{1/2}$=56.4 h) that results in tumor-specific conversion of the inactive CPT-11 prodrug to the 1000-times more active SN-38 metabolite by tumor associated macrophages. The result is long duration sustained exposure to the active metabolite compared to treatment with the unencapsulated or free form of the drug. Phase II studies in gastric, colorectal, and pancreatic cancers are either enrolling or have recently been completed. We also describe some novel modes of administration or uses of the technology, such as convection enhanced delivery (CED) to bypass the blood brain barrier and increase the exposure of drug in the brain while minimizing systemic exposure.

MEDI 188

Multiscale modeling of drug delivery nanosystems: Liposomes and cross linked nanospheres

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Drug delivery nanosystems play a crucial role in improving the formulation, targeting, cell and tissue uptake, and bloodstream circulation while reducing the toxicity of therapeutic and imaging agents. However, a molecular level understanding of the structures of these nanocarriers and the biophysical factors that control drug release in nanocapsule transporters are still incomplete. To address these issues novel multiscale simulation techniques are introduced. These techniques are applied to DPPC liposomes loaded with doxorubicin and Cross-linked Hyaluronic Acid-PEG nanospheres loaded with BHQ3 quencher and Ce6 photosensitizer. An understanding of how changes in pH, temperature and salinity can produce changes in the structure of these nanocapsules and trigger payload release is sought. The objective is to achieve computer aided design of drug delivery nanomaterials. Multiscale MD simulations are performed using an approach that preserve atomic detail and makes use of interatomic force fields. Therefore specific effects of payload-nanocapsule interaction are accounted for. The simulation techniques integrate multiscale perturbation theory, Langevin dynamics, and Trotter factorization, all of which are implemented in our simulator DMS. With this technology, it is possible to proceed orders of magnitude faster than conventional MD, allowing for parameter studies (i.e. across payload, nanocapsule and host medium chemistries). Since the approach provides atom level resolution, one can investigate the effect of dressing a nanocapsule with transporter molecules to target and facilitate entry into cells and tissues and to achieve nanocarrier biodegradation.

MEDI 189

Protein polymer nanoparticles with multivalent avidity for small molecules or cell-surface receptors

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INTRODUCTION

Protein polymers are an emerging platform for engineering biodegradable cancer nanomedicines. A class of protein polymers, elastin-like-polypeptide (ELP) has tunable solubility that allows them to stabilize either the core or corona of monodisperse nanoparticles. We recently discovered two novel approaches to arm these nanoparticles: i) by decoration of their surface with the cognate protein target of

Rapamycin (Rapa), which promotes specific and sustained release *in vivo*; and ii) to develop antibody-core protein polymer nanoparticles (APPNs) as multivalent CD20-targeted therapeutics. Rapamycin analogues are under clinical evaluation across a spectrum of cancers, while anti-CD20 therapies like Rituximab are approved for B-cell Lymphoma.

EXPERIMENTAL METHODS

Synthetic ELP genes were fused with genes encoding for the FK506 binding protein, which binds Rapa (FSI-Rapa), and a single chain antibody (scFv) that binds CD20. Nanoparticles were optimized to assemble stable particles with diameters below 100 nm. FSI-Rapa was evaluated for release rate using HPLC and its anti-proliferative potential in a mTOR (mammalian target of rapamycin) sensitive cell line. Anti-CD20 APPNs were characterized for their binding specificity and apoptotic cell signaling. Both FSI-Rapa formulations and APPNs were evaluated for suppression of human xenograft models.

RESULTS

Both fusion proteins assemble protein nanoparticles with significant biological activity. FKBP decorated FSI nanoparticles have a 5-fold slower rate of Rapa release than free FKBP and 30-fold slower release than unmodified ELP nanoparticles. Drug-loaded nanoparticles reduced side-effects compared to free Rapa; furthermore, they halt tumor progression *in vivo*.The APPNs induce CD20 dependent apoptosis in a B-cell lymphoma *in vitro* and halt the *in vivo* growth of tumor xenografts better than Rituximab.

CONCLUSIONS

Protein polymer nanoparticles offer multiple opportunities to control assembly, presentation, and release of biologically functional molecules.

FUNDING

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MEDI 190

Synthesis, characterization, and evaluation of pluronic-based β-cyclodextrin polyrotaxanes for mobilization of accumulated cholesterol from Niemann-Pick type C fibroblasts

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Several lines of evidence suggest that β-cyclodextrin (β-CD) derivatives initiate the efflux of accumulated, unesterified cholesterol from the late endosomal/lysosomal compartments in Niemann Pick C (NPC) disease models. Unfortunately, repeated injections or continuous infusions of current β-CD therapies are required to sustain suppression of symptoms and prolong life. In an effort to make CD treatment a more viable option by boosting efficacy and improving pharmacokinetics, a library of Pluronic surfactant-based β-CD polyrotaxanes has been developed using biocompatible PEG-PPG-PEG triblock copolymers. These compounds carry multiple copies of β-CD as shown by ^{1}H NMR, 2D nuclear overhouser effect spectroscopy, gel permeation chromatography/multi-angle light scattering, analytical ultracentrifugation analysis, Matrix Assisted Laser Desorption/Ionization Mass Spectrometry and diffusion-ordered spectroscopy. Analysis of free β-cyclodextrin contamination in the compounds were made by reverse phase high pressure liquid chromatography. Dethreading kinetics were studied by reverse phase high pressure liquid chromatography, UV/Vis, and ^{1}H NMR analysis. Filipin staining studies using *npc2*$^{-/-}$ fibroblasts show significant reversal of cholesterol accumulation after treatment with polyrotaxane compounds. The rate and efficacy of reversal is similar to that achieved by equivalent amounts of monomeric β-CD alone.

MEDI 191

Computational design of polymer matrices for formulation of poorly soluble drugs

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We discuss in detail methods for calculating drug-polymer interactions in the important case of formulating poorly soluble drugs as amorphous solid dispersions. Amorphous drugs yield an apparent solubility advantage but are thermodynamically unstable relative to its crystalline counter-part and are at risk of crystallization. Stabilization of amorphous drugs in a polymer matrix is a common formulation strategy. Experiments to understand the physical stability of drug-polymer solid dispersions are time consuming, expensive, yet yield minimal molecular-level understanding of the system. Computational methods that speed up and steer experimental screening in a rational way can be a great and practical tool. As example and proof of concept we demonstrate the methods using two generic drugs (Nifedipine, and Indomethacin) and a few common polymers (PVP, PVAc, and PVP-VA). We tested various approaches: (1) calculating pair energies of interaction between isolated molecules; (2) Molecular Dynamics (MD) methods using atom detailed force fields; (3) rapid screening protocols derived from

engineering thermodynamics (quasi-chemical COSMO approach). We compared the theoretical findings with Flory-Huggins parameters extracted from measured melting-point depression curves. Some methods fail: simply calculating pair energies in vacuum may be rapid, but inaccurate. Likewise MD using Dreiding force fields was not sufficiently accurate. However, we do find quite reasonable numbers from the MD approach using a modified OPLS force field, and also the engineering thermodynamics method. In all, the conclusion is that by combination of proper protocols, it is indeed possible to find valuable windows of stability, by computational design only.

MEDI 192

Development of doxorubicin loaded chitosan based injectable hydrogel for image guided transarterial chemoembolization in liver

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Trans-arterial chemoembolization (TACE) is a minimally invasive procedure in which tumor feeding arteries are catheterized and a chemotherapy mixture is injected followed by microbead or drug eluding beads are injected until antegrade flow ceases. An ideal embolic agent should be image-able, elute anticancer drugs, and achieve complete vessel occlusion but also be resorbable to allow re-intervention. Furthermore, it should be small enough to occlude capillaries without passing through the venous system, to prevent undesirable pulmonary embolization. Although there are solid and liquid embolic agents in widespread use for TACE, they lack either image-ability to guide the embolization procedure, drug eluting properties, or provide minimal tumor coverage or penetration. To address these critical needs, we designed and developed a temperature sensitive drug eluting hydrogel, made of chitosan and hydroxy methyl propyl cellulose (CH-HMPC) containing iodinated contrast Iodixanol, and Doxorubicin (Dox), that transitions into a gel state at body temperature (37 °C)

CH-HMPCs were optimized for sol-gel transition time by varying the wt. % of HMPC (0, 5 and 10%). Results demonstrated gelling times of ˜120, 90 and 43 seconds at 37 °C for 0, 5 and 10% of HMPC, respectively. These CH-HMPC formulations encapsulated 5.5 mg/ml of Dox and 17% of Iodixanol (v/v) and released ˜20% of Dox in 7 days and ˜96% of Iodixanol over 4 days in physiologic buffer and temperature.

In this study, we formulated injectable temperature sensitive drug eluting hydrogels loaded with a widely prescribed anticancer drug and contrast agent. These hydrogels demonstrate a very slow release of encapsulated agents at early time points, which may

minimize systemic side effects and provide image-ability during the TACE procedure. In addition, it may have a potential of occluding nearby capillaries to the tumor and increase bioavailability of drug or drug coverage. This technology has potential for clinical translation.

MEDI 193

Adventures in allosteric drug discovery

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This talk will focus on over a decade of drug discovery efforts and medicinal chemistry research focused on developing allosteric ligands to modulate kinases, GPCRs and phospholipases and highlight tool compounds and clinical candidates. Without question, targeting allosteric sites provides unique mechanisms for target modulation as well as unprecedented levels of selectivity. I will discuss the benefits, issues and challenges with the allosteric approach for target modulation, with particular attention placed on class A, B and C GPCRs. Issues concerning 'molecular switches', subtle structural modifications that modulate either the mode of pharmacology of family subtype selectivity, will be discussed as well as the need to identify scaffold that possess 'molecular locks'. Related to this concept, the need to fully evaluate metabolites of allosteric ligands is critical, and I will highlight examples where metabolites have proven both detrimental and beneficial by virtue of 'molecular switches' derived from oxidative metabolism. I will also address the concept of allosteric agonism and highlight the issues governing this approach; moreover, data will be presented that supports targeting positive allosteric modulation over allosteric agonism is a variety of contexts. Finally, I will showcase a number of examples where allosteric approaches have provided highly selective tools that enabled the dissection of the contributions of discrete receptors to the efficacy of *pan*-orthosteric ligands for a number of CNS disorders including schizophrenia, Parkinson's disease and Alzheimer's disease.

MEDI 194

Development of novel chemotypes for endocannabinoid hydrolase inhibition

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Endocannabinoids are endogenous lipid signaling molecules that activate the cannabinoid receptors, CB_1 and CB_2, and mediate a wide range of physiological and pathological processes. Direct modulators of the cannabinoid receptors have well established therapeutic properties; however, their undesirable effects on cognition and motor control have encouraged the pursuit of alternative strategies to modulate EC signaling. One useful strategy for modulating EC signaling is through the inhibition of

the EC hydrolases FAAH and MAGL, which elevates brain AEA and 2-AG levels, respectively, thereby, enhancing CB-signaling. While FAAH inhibitors have already advanced to clinical trials, the therapeutic potential of MAGL inhibitors are just beginning to be realized. Using competitive and click chemistry ABPP, which utilizes active site-directed chemical probes to determine the functional state of large numbers of enzymes in native proteomes, we have profiled various carbamate chemotypes for their proteome-wide reactivity and identified several unique classes of inhibitors for MAGL, including *O*-hexafluoroisopropyl and *O*-(*N*-hydroxysuccinimidyl) carbamates, which provide distinct advantages over previously developed inhibitors, including improved selectivity, potency and activity across orthologous forms of MAGL. In this talk, I will discuss the development of these inhibitors and how they offer new contexts in which to study these enzymes.

MEDI 195

Drug discovery in academia: A student's perspective

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Current research is focused on two translational medicinal chemistry projects involving the rational design, synthesis, and biological evaluation of small molecules for the treatment of colon cancer and Alzheimer's disease. Project 1 details the development of compounds that modulate PGE_2 production, devoid of COX-1/2 activity, as a novel approach to treat cancer. Microsomal prostaglandin E synthase-1 (mPGES1) and PGE_2 are up-regulated in various cancers, particularly colon cancer. Two series of small molecules will be discussed with associated *in vivo* activity in mouse xenograft models.[1-2] Project 2 details a knowledge based design effort to identify inhibitors of dual specificity tyrosine phosphorylation regulated kinase-1A (DYRK1A). DYRK1A has been shown to play a pathological role towards the cognitive deficits associated with Down's syndrome and neurodegenerative diseases, particularly Alzheimer's.[3] To date, most promising inhibitors are highly active in H4-neuroglioma cells with IC_{50} values in the nanomolar range, and display selectivity against a small panel of kinases.

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MEDI 196

Discovery and optimization of novel c-Myc inhibitor JY-3-094

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c-Myc is an intrinsically disordered (ID) protein that functions as a transcription factor of genes involved in cell proliferation, growth, and survival. The over-expression of c-Myc has been reported in multiple cancers such as prostate, breast, lung, and neuronal tumors. c-Myc becomes transcriptionally active only upon binding its obligatory partner, Max. This transcriptionally active heterodimeric c-Myc–Max complex binds the palindromic hexanucleotide sequence 5'-CACGTG-3' in the major groove of dsDNA, and subsequently recruits DNA transcriptional machinery. Agents that can interfere with c-Myc–Max heterodimerization may provide an approach to inhibit the oncogenic activity of c-Myc. Indeed, several groups have validated the disruption of the c-Myc–Max heterodimer with small-molecules by electrophoretic mobility shift assays (EMSA) in vitro, which generally correlates well with viabilities of c-Myc-overexpressing cells, although inhibitory profiles remain in the low micromolar range. In the absence of Max, c-Myc assumes an ID monomeric form that does not exhibit any recognizable secondary structure or motifs, highlighting the difficulty of c-Myc targeted drug design. In an effort to develop more potent c-Myc inhibitors, we conducted a structure–activity relationship (SAR) study on the known c-Myc inhibitor *N*-([1,1'-biphenyl]-2-yl)-7-nitrobenzo[*c*][1,2,5]oxadiazol-4-amine (10074-G5), which exhibits modest potency for disruption of the c-Myc–Max heterodimer in vitro (IC_{50} = 146 μM). Our efforts led to the discovery of the new lead compound JY-3-094, which exhibits almost five-fold greater inhibition of c-Myc–Max dimerization (IC_{50} = 33 μM) than parent compound 10074-G5, along with negligible activity against Max–Max homodimers (IC_{50} > 100 μM). Together, these data demonstrate that JY-3-094 is amongst the most potent and selective c-Myc inhibitors reported in the literature. Finally, with several opportunities for improvement, we will present our ongoing efforts towards the further optimization of JY-3-094 in vitro and in c-Myc-overexpressing HL60 and Daudi cells.

MEDI 197

Structure-based design of novel inhibitors of sex steroid biosynthesis targeting metastatic prostate cancer

***Charlie Fehl**, cfehl@ku.edu, Elyse Petrunak, Emily Scott, Jeffrey Aubé.Department of Medicinal Chemistry, University of Kansas, Lawrence, KS 66049, United States*

Prostate cancer is the leading cancer diagnosis in men, and the second-leading cause of male cancer-related death in America. Once this disease reaches its metastatic form, fatal progression is rapid and inevitable. These carcinomas require androgens for both growth and survival pathways. Targeting this, the androgen analog abiraterone acetate was approved in 2011 as a first-in-class treatment targeting the biosynthesis of androgen precursors via inhibition of the enzyme cytochrome P450 17A1 (CYP17), extending median survival time by ca. 5 months. Using abiraterone to stabilize CYP17, the Scott lab was able to solve a protein crystal structure of this complex. We set out to use this information to develop more effective and selective agents for this target.

Specifically, polar active site residues R239 and D298 lie within 5 Å of the A and B rings of abiraterone's steroid backbone. Polar substitution revealed high-nanomolar inhibitory activity, but no improvement upon abiraterone. A ring-expansion strategy to better orient these substituents resulted in a crystal structure of improved resolution (2.25 Å, from 2.7 Å with abiraterone), revealing several key structural waters. Although the original hit lost activity, potency was increased through the addition of polar substituents able to make novel contacts with the protein backbone (confirmed with further crystallography), restoring abiraterone-like inhibitory activity.

Our process of structure-based ligand design, compound synthesis & profiling using inhibition assays, and protein crystallography are presented in an effort to identify novel binding interactions for CYP17-selective inhibitors.

An improvement we seek to make upon abiraterone-based CYP17 inhibition is to circumvent a virtually irreversible coordination with the active site metal, which is recognized as a common motif in nearly all cytochrome P450-targeted inhibitors. We are currently using the interactions identified through our inhibitor studies to design compounds that maintain activity despite the loss of this potent but nonselective Fe interaction.

MEDI 198

Development of novel PAC-1 derivatives for the treatment of brain tumors

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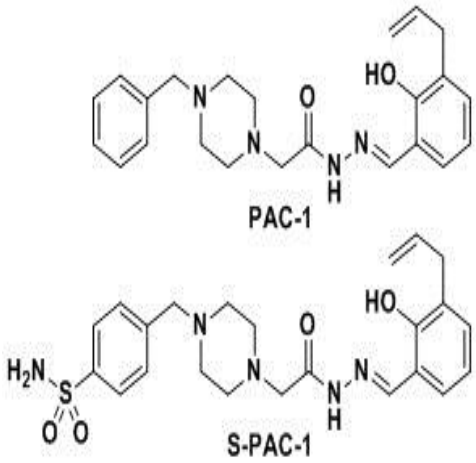
PAC-1 is an *ortho*-hydroxy-*N*-acylhydrazone that induces apoptosis in cancer cells by chelation of antiapoptotic zinc, activating procaspase-3. Preliminary results indicate the potential for significant anticancer efficacy with PAC-1, which is currently being investigated in various mouse, rat, and dog models of cancer. One limitation of PAC-1 is the neurotoxicity observed in animals at elevated doses. A safer derivative, S-PAC-1, does not penetrate the blood-brain barrier (BBB) to a meaningful degree, and a Phase 1 canine clinical trial with S-PAC-1 showed outstanding safety and promising anticancer efficacy. However, due to the relative paucity of therapeutic agents available for the treatment of CNS tumors and the observed efficacy of PAC-1 in animal models of glioma, the development of a well-tolerated, BBB-permeable PAC-1 derivative would represent a significant advance. In order to probe the relationship between BBB permeability and efficacy of PAC-1 derivatives, a library of 45 compounds was synthesized, covering a broad spectrum of predicted BBB permeabilities. The predicted BBB permeability was calculated for each compound, and the metabolic stability (liver microsomes), cell culture potency (cancer cell lines), and neurotoxicity (mice) of the compounds were evaluated. Candidate compounds were identified with promising properties, including improved cell culture potency and tolerability *in vivo*. The BBB permeability of a small subset of compounds was evaluated in mice and compared to the predicted values. Efforts toward investigating anticancer efficacy in murine tumor models are underway.



MEDI 199

Challenges associated with targeting cancer metabolism

***Matthew Vander Heiden**, mvh@mit.edu.Koch Institute at MIT, United States*

Cells adapt metabolism to meet distinct physiological needs. To proliferate, cancer cells must adapt metabolism to support anabolic processes and allow the accumulation of biomass while still generating sufficient ATP to maintain homeostasis. However, tumor cells also experience periods of stress, and metabolic plasticity to shift metabolism

toward efficient ATP production from available nutrients is also needed for tumor progression. Cancer cells can catabolize a variety of nutrients, including extracellular protein, depending on their genetic and environmental context. Regulation of key reactions in the metabolic network also impact the metabolic phenotype of tumor cells, and how available nutrients are metabolized impacts whether cells are able to proliferate. These findings suggest a framework to consider how the regulation of metabolism is regulated to support tumor initiation, growth and progression. Numerous challenges exist to understand how metabolism is regulated in different physiological contexts, and using animal models to study cell metabolism will be discussed, as will the impact of our findings on efforts to target metabolism for cancer therapy.

MEDI 200

Discovery of mutant IDH2 inhibitors for the treatment of cancer

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Isocitrate dehydrogenases (IDH1/2) convert isocitrate to alpha-ketoglutarate (aKG) as part of the TCA cycle. In cancer cells, somatic point mutations of arginine residues in the active site of IDH1/2 leads to a neomorphic activity mutant enzyme to reduce aKG, which results accumulation of the onco-metabolite R(-)-2-hydroxyglutarate (2HG). To gain insight into how inhibition of mutant IDH can affect tumorigenesis, we sought potent and selective inhibitors of these enzymes. From a high-throughput screen, we identified a class of diaryl-ureas that we further optimized to yield the IDH2 R140Q mutant inhibitor AGI-6780. In cellular assays AGI-6780 lowers 2-HG, reverses DNA and histone hypermethylation, and reverses differentiation block. These results suggest that inhibitors against mutant IDH2 may provide a novel targeted differentiation therapy and have an impact in the clinic. This presentation will focus on structure-based inhibitor design of this chemical series, in vitro properties, and 2-HG lowering in murine PKPD models.

MEDI 201

Structure and fragment-based design of novel nicotinamide phosphoribosyltransferase (NAMPT) inhibitors

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Nicotinamide phosphoribosyltransferase (NAMPT) catalyzes the rate-limiting event in the two-step conversion of nicotinamide (NAM) to the enzyme co-factor nicotinamide adenine dinucleotide (NAD) and thus plays a key role in maintaining NAD levels required for cell survival. Blocking NAMPT activity is therefore expected to impair the growth of tumor cells, which are often highly reliant on NAD-dependent processes, and this approach is currently viewed as a novel strategy for the development of new anticancer agents. Accordingly, we conducted fragment-based screening activities to identify leads which could be elaborated into novel NAMPT inhibitors. Structure-guided optimization of an efficient fragment lead (mw = 202, NAMPT K_D = 5.2 uM, LE = 0.47) afforded several independent series of potent NAMPT inhibitors (NAMPT BC IC_{50} <0.020 uM) which exhibited nanomolar antiproliferation activities in cell culture. Detailed biological characterization (MOA, PK/PD relationships, xenograft efficacy) of these entities, along with other potent NAMPT inhibitors will also be discussed.

MEDI 202

Experiences and challenges in lead generation against metabolism targets

***Mark D Charles**, mcharles@cancertechnology.com.Cancer Research Technology, United Kingdom*

Since the discovery by Otto Warburg in the 1920s that cancer cells switch from using glucose for oxidative phosphorylation to using glycolysis, cancer metabolism has been of utmost interest to researchers in the field of oncology. However the complex metabolic pathways, coupled with the challenges in developing inhibitors and screening platforms for these targets, have hampered the development of cancer metabolism targets. AstraZeneca and Cancer Research Technology have recently extended their multi-project cancer metabolism alliance. Aiming to develop a drugs pipeline targeting cancer metabolism, the alliance further builds on efforts to identify new agents to target cancer cells' dependence on altered metabolic pathways for their survival.

This talk will focus on the challenges that we have experienced over the last 4 years in trying to develop small molecule inhibitors targeting cancer metabolism. We will cover how we assess the druggability of novel targets in order to prioritise them. In particular, we will cover how we select types of screening technologies, focussed screening libraries and the deconvolution strategies employed to provide us with the best possible chance of success.

MEDI 203

Synthesis and biological evaluation of allosteric glutaminase inhibitors targeting cancer cell metabolism

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Kidney-type glutaminase (GLS) plays a critical role in glutaminolysis as an important energy source for rapidly proliferating malignant cells. Bis-2-(5-phenylacetamido-1,2,4-thiadiazol-2-yl)ethyl sulfide (BPTES) is an allosteric GLS inhibitor that binds to the GLS tetramer at each of the two dimer interfaces. This unique binding mode makes BPTES an attractive molecular template to design allosteric GLS inhibitors of therapeutic interest. This presentation will discuss our SAR studies on BPTES analogs as well as the therapeutic utility of GLS inhibitors in targeting cancer cell metabolism.

MEDI 204

Novel series of metabolic activators of PKM2 alter oncogene-meditated changes in tumor cell growth and metabolism

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Human tumor cells primarily utilize aerobic glycolysis to metabolize glucose instead of relying on oxidative phosphorylation for the generation of ATP (the Warburg effect). Pyruvate kinase catalyzes the rate-limiting step of glycolysis converting phosphoenolpyruvate (PEP) to pyruvate. The M1 isoform of pyruvate kinase (PKM1) is the principal isoform in most adult differentiated tissues, while the M2 splice variant is the main isoform in embryonic tissues and in all cancer cells. PKM2 is found in cells as an inactive dimer under normal physiological conditions and tetramerization of PKM2 requires binding of the allosteric activator fructose-1,6-bisphosphate (FBP), an upstream glycolytic intermediate, resulting in a fully active enzyme. Regulation of PKM2 activity in cancer cells may allow glycolytic intermediates to be diverted into other biosynthetic pathways necessary for biomass production. PKM2 expression enhances tumorigenicity of cells while PKM1 expression represses it. This suggests that activators of PKM2 may have anti-tumor properties by forcing PKM2 to act more like PKM1. We have a series of small molecule PKM2 activators that exhibit low nM activation activity in biochemical and cell-based assays. These compounds increase pyruvate kinase activity in cancer cells and lead to an increase in pyruvate and ATP production. Our studies show that PKM2 activators inhibit the growth of lung cancer cell lines in vitro and in vivo and can reverse the metabolic changes induced by oncogenes such as k-Ras and c-Myc in lung cancer cells. The current lead compound was tested in established subcutaneously implanted A549 lung adenocarcinoma xenografts, where we observed a statistically significant decrease in tumor growth, with no observable toxicity. These

data suggest that this class of PKM2 activators is effective as tumor cell metabolic regulators with anti-tumor activity for lung cancer and potentially other malignancies.

MEDI 205

Conformational aspects of sulfur in drug design

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Recently, we described pyrazol-3-yl amino nicotinonitrile (**AZ960**) as an ATP-competitive Jak2 inhibitor where the pyrazol-3-yl amine group occupies the ATP binding site and interacts *via* hydrogen bonds with the Jak2 hinge. The suggested binding motif of three hydrogen bond interactions in a *cis*-donor/acceptor/donor fashion is believed to be possible because of an intra-molecular hydrogen bond between the C_4-H of the pyrazole and the adjacent nitrogen of the pyridine ring.

Further we showed that locking the two rings in a co-planar conformation to permit efficient interaction of the hinge binder with the protein backbone can also be accomplished by an isosteric replacement of the pyrazole ring with thiazole. Here co-planarity is sustained *via*a favorable electrostatic interaction between the nitrogen (δ-) of the B-ring and the sulfur of the thiazole (δ+).

In this presentation a general account for the role of sulfur in conformation aspects of drug design will be discussed and how the use of sulfur-containing molecules in medicinal chemistry permits optimal interactions with targeted proteins.

MEDI 206

Optimization of amide conformation in small molecule drug discovery: Case studies from PI3-kinase inhibitor programs

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Case studies from PI3K inhibitor programs will be presented where optimization of amide conformation (or isosteric replacement) led to improved potency, isoform selectivity, and/or DMPK properties. In this context, general torsional and angular preferences for benzanilides and N-acylanilines will be discussed.

MEDI 207

Conformational preferences of the aryl-X-aryl motif: Evolution of potent next generation HIV-1 nonnucleoside reverse transcriptase inhibitors (NNRTI's) that contain a biaryl ether

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NO ABSTRACT SUPPLIED

MEDI 208

Structure-based de novo design of novel inhibitors of the MDM2-p53 interaction

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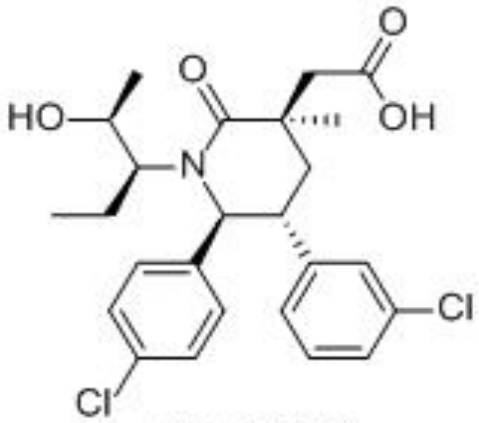
The tumor suppressor protein p53 plays a pivotal role in protecting cells from malignant transformation. It activates the transcription of numerous genes involved in cell cycle arrest, DNA repair, senescence, and apoptosis. Recent studies demonstrate that restoring endogenous p53 function causes tumor regression *in vivo.*

The MDM2 (murine double minute 2) oncogene is an important negative regulator of p53. MDM2 is transcriptionally activated by p53, and the activity of p53 is regulated by MDM2 through various mechanisms. All of these mechanisms would be blocked by neutralizing the MDM2-p53 interaction. This therapeutic strategy could potentially be applied to the ~50% of tumors that are $p53^{WT}$ (~725,000 patients are diagnosed annually with $p53^{WT}$tumors in the US alone).

This presentation will describe a successful approach for designing new scaffolds of MDM2 inhibitors based on the binding mode of known inhibitors with MDM2 protein. Through a combination of X-ray crystallography, molecular modeling, and iterative medicinal chemistry, we have identified potent inhibitors of the MDM2-p53 interaction. The affinity of these compounds for MDM2 was improved through conformational control of both the piperidinone ring and the appended *N*-alkyl substituent.

Our optimization efforts resulted in the discovery of AM-8553, a highly potent selective MDM2 inhibitor (Surface Plasmon Resonance K_d = 0.4 nM, SJSA-1 EdU cell proliferation IC_{50} = 72 nM), with excellent pharmacokinetic properties and *in vivo* anti-tumor activity inthe SJSA-1 osteosarcoma xenograft model.



AM-8553

MEDI 209

Conformational tuning of the furanose ring in antisense oligonucleotides to achieve allele selective silencing of mutant Huntingtin in the CNS

***Punit Seth**, pseth@isisph.com.Medicinal Chemistry, Isis Pharmaceuticals, Carlsbad, CA 92010, United States*

Antisense Oligonucleotides (ASO) are short (12 to 25 base-pairs) chemically modified oligonucleotides which bind to their complementary mRNA in cells by Watson-Crick base-pairing and modulate gene expression via multiple pathways. Over the years, a large number of 2'-modified nucleotides have been investigated to improve the drug-like properties of ASOs. For example, introducing electron withdrawing groups at the 2'-position shifts the conformational equilibrium of the furanose ring towards the C3'-endo sugar pucker and improves ASO binding affinity for complementary RNA. Further conformational restriction of the 2'-substituent into the 4'-position of the furanose ring or completely replacing the furanose ring with a hexitol or a cyclohexenyl ring system can provide dramatic to moderate improvements in binding affinity of the modified oligonucleotides for complementary nucleic acids. We recently undertook the rational design of RNase H active antisense oligonucleotides (ASOs) targeting single nucleotide polymorphisms (SNPs) for allele selective silencing of mutant Huntingtin protein in Huntington's disease (HD) patient-derived fibroblasts and in the CNS of a completely humanized mouse model of HD. We found that position specific incorporation of 2'-modified nucleotides, which differentially bias the conformation of the furanose ring, within the DNA gap-region of the ASO can profoundly improve allele selectivity without compromising activity against the mutant allele. The modified ASOs were also well tolerated after injection into the CNS of wild-type animals suggesting that their tolerability profile is suitable for advancement as potential allele-selective HD therapeutics. Our findings highlight the effect of subtle structural changes to modulate ASO behavior and lay the foundation for efficient allele-selective silencing of gene

expression using ASOs – an outcome with broad application to dominant genetic disorders.

MEDI 210

Using conformationally restricted odorant ligands to probe the olfactory GPCRs

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Close to half of the human G-Protein Coupled Receptors (GPCRs) belong to the chemosensory GPCRs, and most of these are odorant receptors (ORs). Olfactory sensory neurons choose to express only one OR family member, and the chosen receptor determines the pharmacologic behavior of the neuron as it monitors inhaled air for volatile organics. Although some ORs are broadly tuned and become activated by diverse ligands, many receptors, such as the rat OR-I7, are selective for specific functional groups and carbon skeleton features. We are using the OR-I7 receptor to understand how a selective OR recognizes and distinguish among small molecule odorants. The primary natural product ligand for OR-I7 is octanal, a flexible molecule affording many opportunities for designing analogs with restricted rotation. Using rotationally constrained octanal analogs we deduce the conformation of octanal that best activates OR-I7, and describe features that differentiate aldehyde agonists from antagonists. We also present evidence that OR-I7 and several other ORs specific for aldehydes recognize the aldehyde group by virtue of its ability to convert to a 1,1-*gem*-diol.

MEDI 211

Progress and challenges in developing therapeutics for Alzheimer's disease

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In the past 20 years, tremendous strides have been made toward understanding the molecular basis of Alzheimer's disease, with considerable evidence supporting the amyloid β-peptide as an essential initiator of the disease process. However, several different approaches to targeting this peptide have failed in the clinic in recent years, raising concerns that intervening at this level may not be sufficient for Alzheimer's prevention or treatment. A number of other targets are being pursued and will be reviewed here, particularly the microtubule-associated protein tau, which forms pathological neuronal filaments in Alzheimer's and related dementias. Strategies toward

targeting tau at the mRNA level, especially modulation of tau pre-mRNA splicing, will be discussed.

MEDI 212

Inhibitors of glutaminyl cyclase prevent pE-Aβ mediated neurotoxicity: A new concept for the treatment of Alzheimer's disease

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N-terminally truncated Ab peptides constitute a major part of Aβ accumulating in Alzheimers disease (AD). In particular, pyroglutamate (pE, pGlu)–modified Aβ, Aβ3pE-40/42 and Aβ11pE-40/42, have been shown to correlate with disease progression and being overrepresented in early-onset forms of inherited AD. Recently, we could show that Aβ3pE-40/42 form oligomeric structures with considerable surface hydrophobicity which, in turn, mediates a significantly enhanced neurotoxicity. Glutaminyl cyclase (QC, isoQC) has been identified to catalyze the conversion of glutamic acid into pGlu, thereby mediating the formation of pE-Ab. QCs represent single-zinc metalloenzymes, which are localized in the secretory pathway of mammalian cells. QC activity is particularly high in neuronal tissue. In addition, the enzyme expression is further upregulated in human AD, thus correlating with appearance of pE-Aβ. The crucial involvement of QC in pE-Aβ formation has been genetically validated in transgenic mouse models with AD-like pathology. The cognitive deficits correlated with the amount of Aβ3pE-42, which could be modulated by knock-out or overexpression of QC. These data make QC-inhibition an atractive therapeutic approach. Probiodrug is developing competitive inhibitors of QC in order to suppress the formation of Aβ3pE-40/42 and Aβ11pE-40/42. The treatment of transgenic mice with PQ912 attenuated the pE-Aβ pathology and ameliorated behavioral impairment in prophylactic as well as therapeutic settings. PQ912 is the first QC-inhibitor being applied to humans, has nearly completed Phase I being very safe with an attractive PK/PD profile and is ready to go into patient studies

MEDI 213

Targeting anti-Alzheimer's therapeutics via small-molecule inhibitors of RAGE-amyloid beta peptide binding

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The Receptor for Advanced Glycation Endproducts (RAGE) mediates transport of amyloid beta peptide (Aβ) across the blood-brain barrier, and as such is a potential target for the development of therapeutic agents for Alzheimer's disease. Building on the results of a 5000-compound diversity library screen, we developed a pharmacophore model for the inhibition of RAGE-Aβ binding. Synthesis and screening of a focused library based on this model allowed the identification of a potent small molecule inhibitor; this compound was found to dramatically decrease brain levels of Aβ in a mouse model of Alzheimer's disease. Recent efforts to further enhance the potency and pharmacokinetic properties of this lead compound will be discussed.

MEDI 214

Identification of PDE9 clinical candidate PF-4447943 for the treatment of Alzheimer's disease utilizing prospective design and synthetic enablement

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Alzheimer's disease is a neurodegenerative disease with high unmet medical need. There are clear pathological features of the disease including amyloid deposition and synaptic loss. While the deposits of amyloid occur significantly prior to cognitive impairment, synaptic loss correlates most closely with disease progression. We believed that inhibition of PDE9 will improve synaptic transmission and stabilize vulnerable synapses leading to an improvement in treating patients.

Our strategy involved utilizing prospective design, the development of novel library protocols and coupled with structure based drug design we were able to rapidly identify a lead series which built our confidence in rationale. By enabling the synthetic chemistry we were able to quickly improve selectivity over PDE1 and optimize physicochemical properties to identify a clinical candidate with good predicted pharmacokinetic properties and preclinical safety. We developed a deeper understanding of CNS drug property space, which we expanded by minimizing molecular weight, hydrogen bond donor count and adjusting fractional polar surface area. Our PDE9 clinical candidate has shown excellent human pharmacokinetic properties, clear elevation of CSF cGMP and has been tested a 13-week mild to moderate Alzheimer's trial. The discovery of PF-4447943 and the clinical results will be presented.

Figure 1. PF-4447943 bound in PDE9

MEDI 215

O-GlcNAc as a potential target for disease modifying therapy in Alzheimer disease

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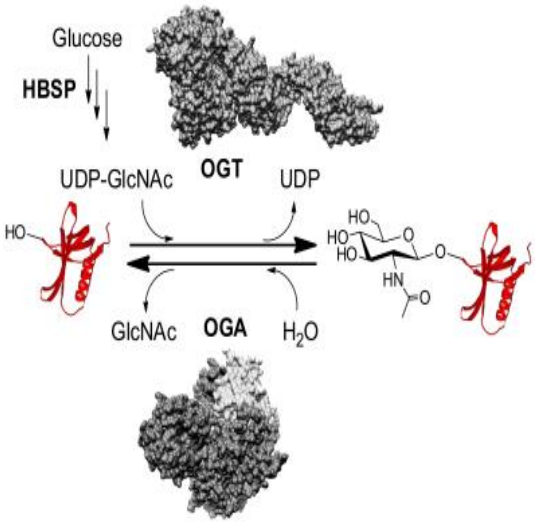
The development of disease modifying therapies targeting Alzheimer disease (AD) is a topic of intense interest. The two pathological hallmarks of AD are proteinaceous aggregates deposited in the brain that are known as tangles and plaques. These aggregates form from inappropriately post-translationally modified forms of the microtubule associated protein tau and peptide fragments, known as Aβ, which are generated by proteolytic cleavage of the amyloid precursor protein (APP). New strategies that can be exploited to decrease the expression of tau and Aβ as well as modulate their toxicity continue to be uncovered.

We have recently proposed one new potential approach to block disease progression by targeting these species. We have focused on an unusual form of glycosylation found on nuclear and cytoplasmic proteins that involves the attachment of O-linked N-acetylglucosamine (O-GlcNAc) to serine and threonine residues. This reversible post-translational modification is found on hundreds of proteins but is regulated by only two enzymes. O-GlcNAc transferase (OGT) installs O-GlcNAc and O-GlcNAcase (OGA) removes this modification. O-GlcNAc levels respond to changes in glucose availability and this modification is sometimes reciprocal to protein phosphorylation; implicating O-GlcNAc in diverse biological processes. In this presentation I introduce O-GlcNAc and discuss our efforts to validate OGA as a target for AD. Studies ranging from the rational design of various transition state analogues and derivatives through to animal studies of efficacy will be described.



MEDI 216

Alpha7 agonists for the treatment of cognitive disorders

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MEDI 217

Selective and potent morpholinone inhibitors of the MDM2-p53 interaction

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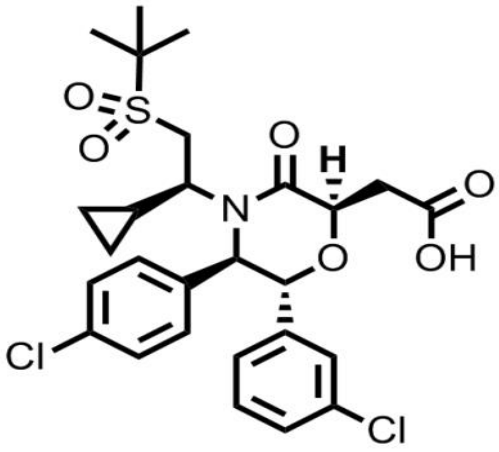
Recognized as the “guardian of the genome” the transcription factor p53-protein is the cell's main tumor suppressor. Upon cellular stress, p53 triggers a lethal response by regulating the expression of multiple target genes that control cell cycle arrest, apoptosis, senescence and DNA repair. In about 50% of cancer cells, p53 inactivation takes place through mutations within the p53 gene (*TR53*) or by translational modifications of its gene product. In those tumors that retain wild-type p53 function, loss of activity is achieved by other means such as direct inhibition by its natural agonists. The oncogene MDM2-protein has been identified as p53's main antagonist. As a result, disruption of this MDM2-p53 protein-protein interaction emerged an attractive strategy for the activation of the p53 pathway. To date, several small molecule inhibitors of the MDM2-p53 interaction have been tested in the clinic for the treatment of cancer.

The title presentation will describe our continued quest towards finding new inhibitor scaffolds. These efforts lead to the discovery of a new class of morpholinone MDM2 inhibitors. Among these, **(1)** is a highly potent inhibitor (EdU IC_{50} = 22 nM), with remarkable pharmacokinetic properties and *in vivo* anti-tumor activity in the SJSA-1 osteosarcoma xenograft model (ED_{50} = 41 mg/kg). In addition, we will discuss the important differences in potency and divergent metabolism pathways observed between this new class of morpholinone inhibitors and their previously reported piperidinone counterparts.



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MEDI 218

Discovery and optimization of potent and brain-penetrant isoquinoline and naphthyridine inhibitors of cAbl

***Emily Hanan**, hanan.emily@gene.com, Lewis Gazzard, Samuel Kintz, Bianca Liederer, Patrick Lupardus, Shiva Malek, Anatoly Nikolaev, Hans Purkey, Steve Sideris, Lan Trinh, Monica Wetzel, Christine Yu, Joseph Lyssikatos.Genentech, Inc., South San Francisco, CA 94080, United States*

Published cAbl inhibitors in general have very poor brain exposure of free drug. A highly potent, selective and brain permeable inhibitor of cAbl was desired to probe the role of cAbl in various neurodegenerative diseases. A medicinal chemistry program was initiated to identify such an inhibitor with a suitable pharmacokinetic profile for use in various *in vivo* neurodegeneration models. 7-aryl-3-amido-isoquinoline was identified as a highly ligand efficient scaffold for our lead optimization effort. The high lipophilicity and amphiphilicity of the leads precluded these compounds from being candidates for *in vivo* efficacy studies. A campaign was carried out to address, in parallel, poor metabolic stability, undesirable physicochemical properties (poor solubility, high LogD, amphiphilicity), and modest kinase selectivity while maintaining good brain permeability and sub-nanomolar enzyme potency. A large “cell-shift”, presumably driven by P-cAbl's low Km for ATP, necessitated very high potency in the enzyme assay. Structure-based design was enabled with x-ray crystallography and utilized concurrently with calculated multi-parameter optimization models. Synthetic chemistry development allowed late-stage diversification of an unusual naphthyridine scaffold that provides an attractive physicochemical profile. These strategies enabled rapid optimization in multiple dimensions and the ultimate success of the medicinal chemistry program. Several CNS-permeable lead compounds were identified with excellent potency, kinase selectivity, and sustained *in vivo* exposure of free-drug several multiples above the free-cellular IC_{50}s. These compounds are currently being studied in mouse neurodegeneration models to enable further understanding of the biological target.

MEDI 219

Development of a potent and ALK2 selective bone morphogenetic protein receptor (BMP) inhibitor

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A fast-track chemistry effort was initiated to evaluate the structure-activity relationship of the 3- and 6-positions of the pyrazolo[1,5-a]pyrimidine scaffold of the known BMP inhibitors. This medicinal chemistry effort led to the identification of a potent and selective compound for ALK2 versus ALK3. The potency contributions of several 3-position substituents were evaluated with subtle structural changes leading to significant changes in potency. From these studies, a novel 5-quinoline molecule was identified and designated an MLPCN probe molecule, ML347, which shows >300-fold selectivity for ALK2 and presents the community with a selective molecular probe for further biological evaluation.

MEDI 220

4-Aryl-7-hydroxylindoline based $P2Y_1$ antagonists as novel antiplatelet agents

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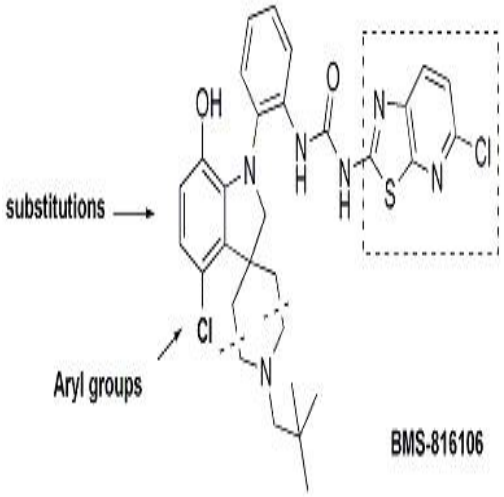
$P2Y_{12}$ antagonists such as clopidogrel and prasugrel are marketed antiplatelet drugs. The efficacy of $P2Y_{12}$ antagonists can be limited by increased bleeding at higher doses. New antiplatelet agents with an improved therapeutic index are desired. Targeting the $P2Y_1$ receptor with antagonists has been shown to have the potential to provide equivalent antithrombotic efficacy as $P2Y_{12}$ inhibitors with reduced bleeding from

preclinical animal model studies. We have previously reported the identification of BMS-816106 from a 7-hydroxyindoline chemotype which showed potent $P2Y_1$ inhibition and was orally bioavailable in all preclinical species. This presentation describes further optimization of BMS-816106 by introducing 4-aryl groups, which resulted in analogs with excellent potency and desired PK profiles (low clearance, low volume of distribution). The lead compound from the 4-aryl-7-hydroxylindolineseries also demonstrated similar antithrombotic efficacy with less bleeding compared with known $P2Y_{12}$ antagonist clopidogrel in the rabbit model of electrically-induced carotid artery thrombosis and cuticle bleeding. The results from these studies supports $P2Y_1$ receptor antagonism as a promising drug target for the development of new antiplatelet agents.



MEDI 221

Novel inhibitors of the *Pseudomonas* type three secretion system

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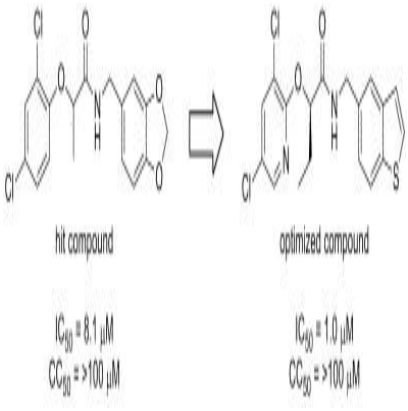
The type three secretion system (T3SS) is a bacterial virulence factor found in many gram negative pathogens that acts as a molecular machine, capable of introducing toxins directly into host cells. Although not essential for survival, the presence of a functional T3SS is highly correlated with poor clinical outcomes in *Pseudomonas* infections, particularly in cystic fibrosis patients. Through an internal HTS campaign, we have identified a novel set of highly selective inhibitors, based on a phenoxyacetamide core, that inhibits the proper functioning of this multi-protein system. We have thus far optimized the structure of the phenoxyacetamide to provide inhibitors with low μM activity in a T3SS toxin/β-lactamase fusion protein secretion assay. Development of a comprehensive set of SARs in the phenoxyacetamide series will be presented.



MEDI 222

Structures of bacterial diterpene and isoprenoid synthases: Targeting virulence and biofilm formation

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We report the X-ray structures of two bacterial terpene synthases involved in virulence factor or biofilm formation: tuberculosinol/(13R,S)-*iso*-tuberculosinol synthase (Rv3378c) from *Mycobacterium tuberculosis,* involved in virulence factor formation, and YisP from *Bacillus subtilis*, involved in biofilm formation. Both enzymes contain the DDXXD domains found in most enzymes involved in terpene biosynthesis and act on terpene diphosphate substrates. Rv3378c acts as a phosphatase and its structure is unique for a terpene synthase, closely resembling that of the *cis*-isoprenoid diphosphate synthases involved in bacterial cell wall biosynthesis. We solved structures with bound substrates and an inhibitor and these results combined with site-directed mutagenesis lead to a mechanism of action in which two Tyr residues activate water molecules for nucleophilic attack on the tuberculosinol diphosphate substrate. The BsYisP structure closely resembles that of dehydrosqualene synthase (CrtM, used in formation of the *S. aureus* virulence factor staphyloxanthin), but it also acts as a phosphatase and produces farnesol. We show that both tuberculosinol and farnesol (as well as staphyloxanthin) affect membrane structure in a cholesterol-like manner that may be associated with their effects on virulence or biofilm formation. The results are of broad

general interest in the context of mechanistic enzymology as well as in drug discovery in which membrane structure modulators are targeted.

MEDI 223

Building a successful reaction optimization group in drug discovery: Lessons learnt from Pfizer Worldwide Medicinal Chemistry

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In modern drug discovery projects, the synthetic chemistry team faces a constant tension between timely target delivery, investigation of new synthetic space and the optimization of the synthetic sequence used to deliver those compounds. In Pfizer Worldwide Medicinal Chemistry, a small team was charged with the specific role of optimizing bottleneck reaction steps to enable compound scale up and high speed analog synthesis. The presentation advocates for the employment of dedicated synthetic excellence teams in drug discovery projects through short examples of optimization work completed for the Mineralocorticoid Receptor, Ghrelin, Glucokinase Activator projects in CVMED, and Monocarbam project in the Antibacterials therapeutic area. Within the discussion, the talk shares the techniques used and lessons learnt from the successful execution of these optimization projects.

MEDI 224

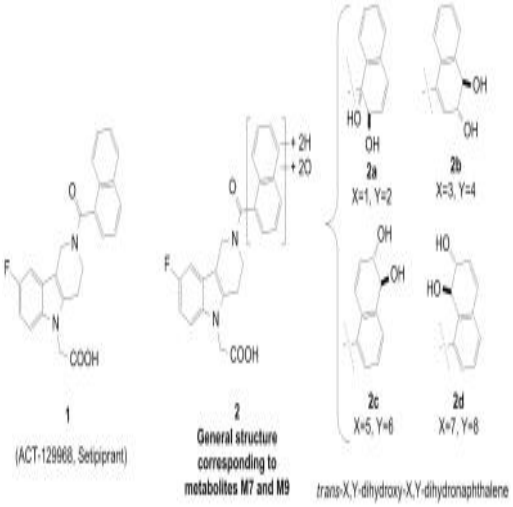
Structure elucidation of two major metabolites of CRTh2 antagonist 2-(2-(1-naphthoyl)-8-fluoro-3,4-dihydro-1*H*-pyrido[4,3-*b*]indol-5(2*H*)-yl)acetic acid (ACT-129968, setipiprant)

Philippe Risch[1]*, Heinz Fretz*[1]*, Thomas Pfeifer*[2]*,* ***Julien Pothier***[1]*, julien.pothier@actelion.com. (1) Drug Discovery Chemistry, Actelion Pharmaceuticals Ltd., Allschwil, Switzerland (2) Preclinical Pharmacokinetics and Metabolism, Actelion Pharmaceuticals Ltd., Allschwil, Switzerland*

Number, nature as well as the concentration of metabolites appearing in the blood and organs after administering a drug candidate to a living creature may significantly affect drug development. As a result, early identification of metabolites has become of utmost importance in drug discovery and development.

Two major metabolites **M7** and **M9** were detected by LC-MS/MS after incubating ^{14}C labeled CRTh2 antagonist **1** with human hepatocytes. Analysis of the mass spectra (MS/MS) indicated that the tetrahydropyridoindole core of **1** remained intact whereas its

naphthyl ring seemed to be converted to two distinct dihydroxy-dihydronaphthalene regioisomers as shown with **2** .



Based on literature precedence, conversion of **1** to **M7** and **M9** in two consecutive enzymatic steps was assumed: an initial cytochrome P450 catalyzed epoxidation of the naphthyl ring is followed by an epoxide hydrolase catalyzed epoxide ring opening to form two distinct vicinal *trans*-dihydroxy-dihydronaphthalene regioisomers. Consequently, the four most plausible regioisomers **2a-d** were synthesized in racemic and enantioenriched form. Structure and absolute stereochemistry of the two metabolites **M7** and **M9** could unequivocally be assigned by comparing analytical and spectral data of the synthetic with the biological samples.

MEDI 225

Design and optimization of pyrazinecarboxamide-based inhibitors of diacylglycerol acyltransferase 1 (DGAT1) leading to the clinical candidate AZD7687

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A new series of pyrazinecarboxamide DGAT1 inhibitors was designed to address the need for a candidate drug with good potency, selectivity, and physical and DMPK properties combined with a low predicted dose in man. Rational design and optimization of this series led to the discovery of the compound AZD7687, which met the project objectives for potency, selectivity, in particular over ACAT1, solubility, and preclinical PK profiles. This compound showed the anticipated excellent pharmacokinetic properties in human volunteers.

MEDI 226

Discovery of BMS-767778, a highly potent and selective DPP4 inhibitor for the treatment of diabetes mellitus

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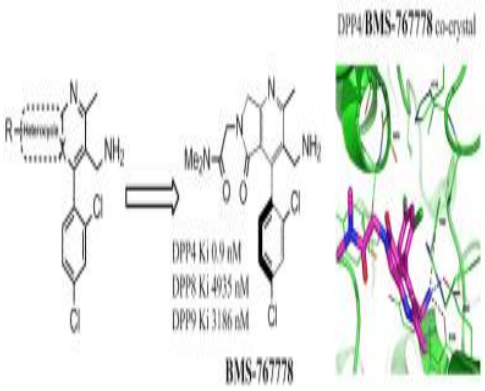
Mitigation of hERG and CYP liabilities in the 5-oxopyrrolopyridine series via exploitation of the solvent-exposed region of the active site of DPP4 yielded BMS-767778 with an overall activity, selectivity, efficacy, PK, and developability profile suitable for progression into the clinic. Stucture-Activity relationships in the series as well as characterization of BMS-767778 is described.



MEDI 227

Construction validation and application of an artificial template for the template-based alignment modeling of N-terminal ATP-binding-site inhibitors of Hsp90

***Zhijun Wu**, zj1234508536@yahoo.com.ABC Resources, Plainsboro, New Jersey 08536, United States*

The template-based alignment modeling (TAM) is an innovative molecular modeling approach for SARs studies, featuring alignment of different ligands with a special molecular template so as to reveal the potential structural correlations among the ligands. This approach proved to be straight and effective in our recent opioid modeling studies (*J. Chem. Inf. Mod.* **2011** , *51* (5), 1151-1164). And it also showed to be applicable to several other types of ligands.

The N-terminal Hsp90 inhibitors are a class of structurally diverse ligands and all bind at the N-terminal ATP-binding site of Hsp90 along with different binding conformations. Recently when analyzing a cluster of the X-ray crystal structures of a group of the Hsp90-ligand complexes, we recognized a unique structural correlation pattern among the different ligands. Based on this information we constructed an artificial template for use in the template-based alignment modeling. In this presentation I will talk about the detailed process of template construction and validation as well as examples of application in the related SAR studies.

MEDI 228

Discovery of AMG 747, a novel glycine transporter type-1 (GlyT1) inhibitor, as a potential treatment for negative, cognitive and positive symptoms of schizophrenia

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Taborn[3], Sara Rao[3], Narender Gavva[3], Darren Reid[5], Dean Hickman[4], Faye Hsieh[4], Jiyun Chen[4], Tom Kornecook[3], Randall Hungate[1], James Treanor[3], Jennifer Allen[1]. (1) Department of Medicinal Chemistry, Amgen, Thousand Oaks, CA 91320, United States (2) Department of Molecular Structure and characterization, Amgen, Thousand Oaks, CA 91320, United States (3) Department of Neuroscience, Amgen, Thousand Oaks, CA 91320, United States (4) Department of Pharmacokinetics and Drug Metabolism, Amgen, Thousand Oaks, CA 91320, United States (5) Department of Pharmaceutics, Amgen, Thousand Oaks, CA 91320, United States

Evidence suggests that N-methyl d-aspartate (NMDA) receptor hypofunction is involved in the pathogenesis of schizophrenia. The NMDA receptor is activated by two co-agonists, glutamate and glycine. Elevating brain glycine levels, by inhibiting GlyT1, is a potentially novel way to enhance NMDA receptor function. AMG 747 is a highly selective, orally bioavailable, and brain-penetrant small molecule inhibitor of GlyT1. In preclinical rodent species, AMG 747 demonstrates promising pharmacokinetic (PK) properties and produces an increase in cerebrospinal fluid (CSF) glycine content, in both a dose- and time-dependent fashion. Additionally, in rats, AMG 747 significantly improves performance in the novel object recognition (NOR) task. In mice, AMG 747 reduces the hyperactivity caused by NMDA receptor antagonism in a manner similar to that observed with known antipsychotic drugs. These results are consistent with other published data on GlyT1 inhibitors and warrant further investigation of AMG 747 as a potential treatment for negative, cognitive, and positive symptoms in schizophrenia. AMG 747 is currently in phase 2 clinical trials. Our supporting preclinical data, the chemical synthesis and structure activity relationships (SAR) leading to the discovery of AMG 747 and related analogs will be disclosed.

MEDI 229

Exploiting novel bacterial topoisomerase inhibitors (NBTIs) as a new class of antibiotic

***Neil D Pearson**, neil.pearson@gsk.com, Michael N Gwynn.Antibacterial DPU, GlaxoSmithKline, Collegeville, Pennsylvania 19426, United States*

NBTI's (novel bacterial topoisomerase inhibitors) are a new class of gyrase and topoisomerase IV inhibitors which act by a different mechanism to fluoroquinolones, and avoid target mediated cross-resistance. Since GSKs original disclosure of this novel antibiotic class, multiple companies have now reported discovery efforts.Lead optimization in the NBTI class had to address antibacterial potency and spectrum in addition to multiple toxicity issues including hERG inhibition, genetic toxicology and species specific retinal pigment epithelium effects.

GSK solved the first X-ray structure of a NBTI inhibitor in complex with *S.aureus*DNA gyrase and DNA providing unprecedented knowledge for lead optimization and the design of novel inhibitors.

Optimization of the Gram positive selective early leads led to new series which afforded good activity versus some Gram negative pathogens and identification of development candidates from the NBTI class encompassing pathogens implicated in hCAP and SSTI. Two NBTIs were progressed to early Phase I studies and GSK2140944 was selected as the optimal molecule for continued progression.

GSK2140944 has good activity versus a range of biothreat pathogens and exploitation of this molecule was part-supported by the US DoD Defense Threat Reduction Agency (DTRA). GSK2140944 is being evaluated for use versus both biothreat and conventional pathogens.

Acknowledgements

This work was part supported by the Transformational Medical Technologies Program (HDTRA1-07-9-0002) from the Department of Defense, Joint Chemical and Biological Defense Program through the Defense Threat Reduction Agency (DTRA). The views expressed in this article are those of the authors and do not necessarily reflect the official policy or position of the US Department of Defense or the US Government.

MEDI 230

From PF-04971729 to ertugliflozin: An overview of the progression of Pfizer's SGLT2 inhibitor program from FIH to successful POC

***Vincent Mascitti**, vincent.mascitti@pfizer.com.Pfizer Global R&D, Groton, CT 06340, United States*

This presentation will provide an update since the first disclosure of ertugliflozin (PF-04971729) at the 2010 American Chemical Society national meeting held in Boston; particular 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 Phase 3 ready, with trials expected to begin later in 2013, and is being evaluated for the treatment of type 2 diabetes.

MEDI 231

Synthesis and biological evaluation of novel asymmetric bow-tie PAMAM dendrimer-based tumor-targeting drug conjugates

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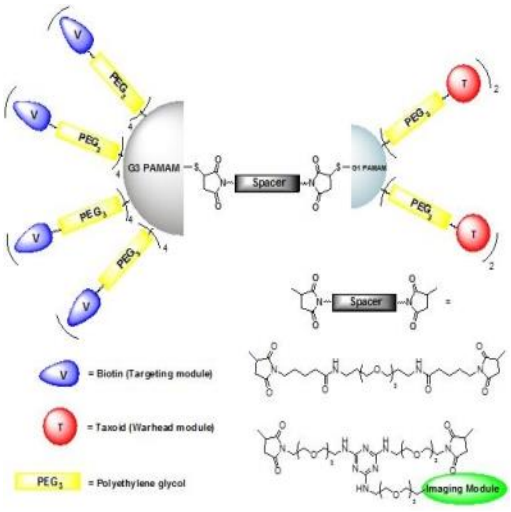
Poly-(amidoamine)(PAMAM) dendrimers have been studied as macromolecular carriers to deliver drugs, resulting in selective accumulation in tumor tissues due to EPR effect.

The use of PAMAM derivatives with a cleavable cystamine core enables the assembly of different generations of half dendrons modified with different functionalities into single molecule. Thus, we designed novel asymmetric bow-tie PAMAM dendrimers, bearing a PEGylated bis-maleimido spacer, as the vehicles for tumor-targeting drug conjugates. The synthesis and biological evaluation of the novel dendrimer-based drug conjugates, bearing a vitamin as the tumor-targeting module and a new-generation taxoid as the warhead will be presented.



MEDI 232

Discovery of NSC765844, a novel, potent, and orally efficacious dual inhibitor of PI3K and mTOR

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The phosphoinositide 3-kinases (PI3Ks) are one of the most frequently activated enzymes in a wide range of human cancers, and thus inhibition of PI3K represents a promising strategy for cancer therapy. Herein, we have designed and synthesized a series of nitrogen-containing sidechain substituted arylsulfonamides as dual inhibitors of PI3K and mTOR through a strategy integrating focused library design and virtual screening, resulted in the discovery of NSC765844, which possesses IC50 values of 1.3 nM and 3.8 nM against PI3Kα and mTOR, respectively. NSC765844 was further tested by NCI for in vitro anticancer screening, and was found to be active against 60 human tumor cell lines with mean GI50 values of 18.62 nM. NSC765844 demonstrated very promising antitumor activity when administered orally in the A549 xenograft model. It was currently selected by Biological Evaluation Committee (BEC) of NCI for further evaluations.

MEDI 233

Metabolism in vitro of the microtubule perturbers ceratamine A and B

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Disruption of microtubule dynamics results in anti-mitotic activity that can ultimately lead to cell death, especially in highly proliferative cells. This target has been of great interest in cancer drug discovery and was deemed successful with the approval of the effective anti-cancer agent Taxol®. Ceratamine A and B are natural products isolated from the marine sponge *Pseudoceratina* sp. They behave as microtubule perturbers, resulting in anti-mitotic activity with IC_{50} values in the low micromolar range. Studies of *in vitro* metabolism were performed to begin to understand the pharmacokinetics of the ceratamines. Each compound was incubated within rat and human liver microsomes. Initial analysis was performed in a qualitative manner with LC-MS/MS techniques used for structure elucidation. Ceratamine A was converted to at least eight phase I metabolites by rat liver microsomes. The metabolites were the result of demethylations, at the secondary amine, tertiary amide, or methoxy groups, and aromatic hydroxylation. A similar metabolic profile was determined for ceratamine B, with five metabolites being formed by rat liver microsomes. Human liver microsomes, converted the parent drugs to four and three phase I metabolites, for ceratamine A and B, respectively. These metabolites were consistent with those already identified.

MEDI 234

WITHDRAWN

MEDI 235

OTL-0038: A potent folate receptor (FR)-targeted NIR dye

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The surgical removal of solid tumor has been the foundation of treatment for most types of cancers. While combination of chemo- and radiotherapy cure less than 5% of all cancer patients, surgery cures 50% of patients with solid tumors in the US. However, out of over 700,000 patients undergoing cancer surgery in each year in the US, 40% of them have a recurrence of the disease within 5 years. Therefore, there is an unmet medical demand to develop techniques to remove primary malignant mass with entirely

negative margins and remove all lymph nodes having metastatic cancer cells. Motivated to achieve these goals, we recently have developed a novel FR-targeted near-infrared (NIR) fluorescence probe (OTL-0038) for use in image-guided tumor surgery. Herein we show that the lead optimization, structure activity relationships, scale-up synthesis, and chiral analysis of OTL-0038. Moreover, the binding and specificity of OTL-0038 for FR using (1) FR+ cancer cells in culture, (2) in vivo whole body imaging and ex vivo biodistribution in mice with FR+ or FR- tumor xenografts, (3) dose escalation studies in nude mice, and (4) safety studies will be demonstrated. OTL-0038 can be synthesized from milligram to multi gram scale in high purity and high yield. It is compatible with both organic and aqueous solvents, easy to purify and characterize, and stable during synthesis and storage. Finally, OTL-0038 demonstrates ~20 nM affinity for FR and accumulates exclusively in FR expressing tumors. In near future we anticipate the examination of OTL-0038 in human clinical trials with appropriate stability and toxicity data.

MEDI 236

Binding ensemble profiling with (f)photoaffinity labeling (BEProFL): Mapping the binding sites and poses of metabotropic glutamate receptor type 2 (mGlu2) positive allosteric modulators (PAMs)

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Metabotropic glutamate receptor type 2 (mGlu2) positive allosteric modulators (PAMs) have recently garnered significant attention as potential anti-addiction therapeutics. Our specific aim is to develop and utilize photoaffinity probes based on mGlu2 PAMs for application of a Binding Ensemble Profiling with Photoaffinity Labeling (BEProFL) approach, which rationally couples photoaffinity labeling with molecular modeling in order to map the binding sites and poses of these compounds within mGlu2. Once it is known how mGlu2 PAMs interact with their target protein, lead compounds can then be rationally manipulated to improve therapeutic outcomes associated with drug addiction. A large number of photoprobes have been rationally designed in our lab from known potent mGluR2 PAMs. These compounds contain a photoreactive group that can covalently link to mGlu2 upon UV irradiation. Furthermore, a terminal alkyne in these probes can serve as a clickable handle allowing attachment of a tag for visualization and enrichment of probe-labeled mGlu2. Such photoprobes are currently being synthesized, pharmacologically evaluated, and subjected to photoaffinity labeling experiments. Our approach is expected to aid in characterizing the 3-D structure and functions of mGluR2, guide ligand optimization of drug candidates, plus enable virtual screening and structure-based drug design of anti-addiction therapeutics.

MEDI 237

In vitro delivery and pharmacodynamics of the appetite-suppressing peptide PYY(3-36) through the vitamin B_{12} uptake pathway

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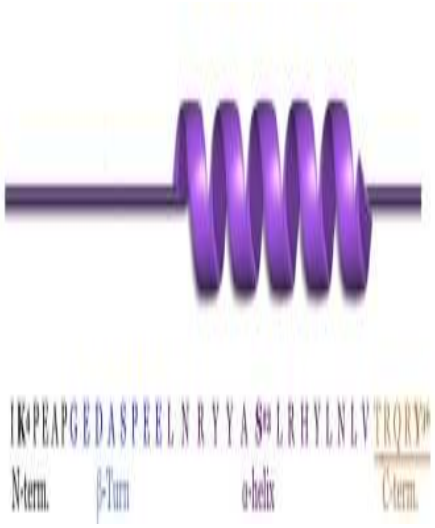
It has been shown that peptide tyrosine tyrosine (PYY) plays a key role in appetite regulation. Release of PYY(3-36), a PYY derived analogue, creates an anorectic effect, which may be of value as a therapeutic in the worldwide struggle with obesity. There are almost 80 million obese adults in the United States alone, and that figure reaches over 1 billion when regarding obese persons worldwide. Research with PYY(3-36) is constantly expanding and the use of vitamin B_{12} (B_{12}) as an oral delivery agent is also being explored, with its own record of successes. A successful oral delivery of clinically relevant levels of PYY(3-36) via B_{12} has been established in vivo. Herein we describe the full pharmacodynamic studies through selectivity and agonism of B_{12}-PYY(3-36) with its Y2 receptor in vitro through a calcium-induced calcium response (CICR) assay and ^{3}H-inositol assay. Synthesis of a B_{12}-PYY(3-36) conjugate will be produced from B_{12}coupled to PYY(3-36) through the K4 lysine position using a Sharpless/Huisgen Cu(I)-catalyzed cycloaddition reaction. The results of this study provide a direction for the potential of pharmaceutical development of B_{12} peptide conjugates.



MEDI 238

4-Aminoquinoline: Enantioselective synthesis and antiplasmodial evaluation

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Malaria is a devastating parasitic disease and it is responsible for approximatively 800.000 deaths each year, particularly children under five years old. According to the World Health Organization, malaria is still endemic in 100 countries. Malaria is due to *Plasmodium* and among the five species that infect human beings, the most dangerous one is *Plasmodium falciparum.* These parasites are transmitted through a female Anophele bite.

Research of new antimalarial chemotherapies has become urgent because of the emergence of drug resistance.

Hence, our laboratory is interested in the synthesis of new antimalarial drugs in particular mefloquine analogs. Mefloquine is used for therapy as a racemic mixture, the mefloquine (*+*)-*erythro* enantiomer is supposed to be the most active form and side effects (brain damage) may essentially come from the (-)-*erythro* enantiomer.

Recent studies have been done on the pharmacomodulations of 4-aminoquinoline and particularly concerning the importance of the stereoselectivity.

The goal of the present study is to synthesize new enantiomeric 4-aminoquinolines to observe the potential influence of the asymmetrical centers on both the side effects and the antimalarial activity.

The achievement of the stereoselective synthesis of oxiran precursors of 4-aminoquinolines, in four steps allows to obtain global yields about 50% and excellent enantiomeric excesses up to 90%. Consequently a library of 4-aminoquinolines was synthesized and biological tests were done. The results show inhibiting concentrations (IC_{50}) to be of the nanomolar range, and that the (*S*) configuration of the compounds seems to be more efficient than their (*R*) analogs. These compounds are patented and are among the most active antimalarial products.

These tests validate our strategy as they show that the stereoselectivity of the compounds have a high influence on antimalarial activity. It also remains to be demonstrated whether the same can be observed with side effects.

MEDI 239

Collaborative drug discovery paradigm: A modern approach to drug research informatics

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There are hundreds of technologies for small molecule drug discovery – each with its own scope and limitations. Secure, hosted collaborative technologies, like the CDD Vault®, for chemical registration and SAR have become important. The number of researchers who trust a secure, hosted platform for chemical registration and structure

activity relationships is growing geometrically, with a remarkable 99.98% uptime since 2009 and a perfect security record since 2004. A fundamentally more economical, collaborative process for generating valuable pre-publication, pre-patent SAR has emerged. Representative case studies include:

- NIH Neuroscience Blueprint consortia: Including AMRI, CDD, Southern Research Institute, and SRI International working with seven leading academic biology laboratories and the NIH.

- The *Bill* & *Melinda Gates Foundation* CDD TB (Tuberculosis) Database Project: 250 users, 58 labs, 20 collaborations. Three projects are currently teamed with seven big pharmas.

- MM4TB EU funded collaboration with 25 partners in 13 countries including two large pharmas working together as if one organization with data partitioning in a single CDD Vault.

- UCLA campus-wide and Rockefeller University for secure inter-campus collaborations.

- Acetyton Pharmaceuticals: Harvard spinout company managing academic-industry and China CRO collaborations advancing a selective HDAC inhibitor into the clinic.

MEDI 240

Discovery of ML326: The first sub-micromolar, selective M_5 PAM

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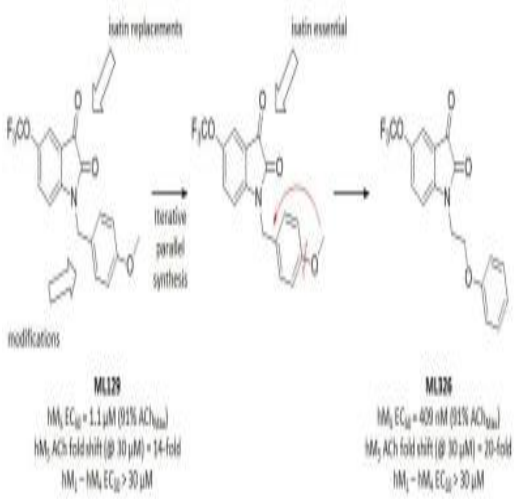
The muscarinic acetylcholine receptors (mAChR) consist of five subtypes ($M_{1\text{-}5}$) that are broadly expressed in the central nervous system and periphery of mammals. These receptors and their endogenous agonist, acetylcholine (ACh), play an important role in regulating a wide range of physiological functions. Recent advances in the discovery of highly subtype-specific ligands for M_1 and M_4 has enabled researchers to begin pharmacological characterization of the discrete functions of these subtypes; however, discovery of M_5-selective ligands has remained challenging.

Consistent with M_5 expression in midbrain dopamine pathways and throughout the cerebrovascular system, phenotypic studies of M_5-knockout mice have suggested that activation of M_5 may be therapeutically relevant for the treatment of chronic cerebrovascular diseases, acute ischemic stroke, Alzheimer's disease, and drug addiction.



We recently reported the discovery of the first subtype-selective M_5 positive allosteric modulators (PAMs) based on ML129 (VU0238429; hM_5 EC_{50} = 1.1 μM), but their modest potency and poor physiochemical profiles limit their *in vivo* utility as pharmacological probes and all attempts at further optimization were unsuccessful. A subsequent HTS campaign discovered several weak M_5 PAMs structurally related to ML129, but with unique peripheral pharmacophores. Although these new compounds were unable to be optimized further, the juxtaposition of the peripheral pharmacophores with ML129's core resulted in a prodigious increase in potency, leading to the first sub-micromolar, M_5-selective PAM, ML326 (VU0467903; hM_5 EC_{50} = 409 nM).

MEDI 241

In-depth analysis for the cooperative contributions of ligand functional groups to binding using thrombin as a model system

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Accurately predicting ligand binding affinities to their protein hosts with scoring functions remains one of the most significant challenges facing medicinal and biological chemists. One of the underappreciated reasons for this is the fact that individual protein-ligand non-covalent interactions are not always contributing to binding affinity in an additive fashion due to cooperativity. Cooperativity among ligand functional groups is the mutual modulation of the ligand's non-covalent interactions in such a way that the binding energy obtained from these interactions together is more (positive cooperativity) or less (negative cooperativity) than the sum of the binding energies obtained from them individually. In previous studies we revealed a positive ligand groups cooperativity between a hydrogen bond and aliphatic side chains for a series of thrombin inhibitors[1], as well as a water-mediated positive cooperativity between a thermolysin inhibitor

carboxyl group and an aliphatic side chain[2]. In the present study, using thrombin as a model system, we present different types of ligand functional groups cooperativity. Also, we reveal how factors such as ligand rigidity, bioisosteric replacement of functional groups, and additional H-bonds can affect the magnitude of the cooperativity. Understanding the fundamental factors affecting cooperativity, and quantifying various ligand functional groups cooperativity as we are doing using model systems, is important for building an experimental foundation for the improvement of scoring functions.

(1) Muley, L.; et al., J. Med. Chem., 53, 2126-2135 (2010)

(2) Nasief, N.; et al., J. Med. Chem., 55, 8283-8302 (2012)

MEDI 242

Antimalarial drug leads targeting isoprenoid biosynthesis

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Targeting the isoprenoid biosynthesis pathway is a potentially important approach to treating malaria. Here, we synthesized 30 lipophilic bisphosphonates with various chain lengths along with the current clinically used drug zoledronate (Zometa®), and tested them in malaria parasite killing (inhibiting *Plasmodium* geranylgeranyl diphosphate synthase, GGPPS) as well as in human Vγ2Vδ2 T cell activation (inhibiting human farnesyl diphosphate synthase, FPPS). In *Plasmodium* GGPPS, we found short to medium chain-length species had most activity. Similar effects were observed against human FPPS. In malaria parasite killing, optimal activity was found with ~C_{10} alkyl chain species, which was shown to be best in enzyme inhibition and in parasite cell membrane and red blood cell penetration. Shorter chain-length species had low activity because of the poor membrane permeability. In addition, we determined the crystal structure of one of the potent inhibitors (C_4) bound to a human FPPS. The results are of interest since they suggest a combined chemo/immuno-therapeutic approach to antimalarial drug development targeting both direct malaria parasite killing as well as Vγ2Vδ2 T cell activation.

MEDI 243

Copper-binding antimicrobial peptides

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Antimicrobial peptides hold promise against antibiotic resistant pathogens. Here, we report the conjugation of a copper binding motif to the antimicrobial peptides buforin II, tritrpticin, and anoplin to afford potent antibacterial agents effective against both Gram-positive and Gram-negative bacteria.

MEDI 244

Design and synthesis of long residence time inhibitors and its application in radioimaging and assay development

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The substrate-binding pocket of InhA, the enoyl-ACP reductase from *Mycobacterium tuberculosis*, is large in comparison to homologous enoyl-ACP reductases (FabI) from different organisms. This presents a unique opportunity to probe the chemical space in this region and determine if residence time is correlated to size of the inhibitor side chain. A series of inhibitors based on the diphenyl ether scaffold have been synthesized using click chemistry and exhibit good minimum inhibitory concentrations (MICs). The compound with the most potent MIC has the longest residence time of all InhA inhibitors known to date. The residence time, or the lifetime of a drug-target complex has important implications on therapeutic efficacy but can also be exploited as an informative chemical tool in applications ranging from radioimaging to assay development. Using the best 'click chem' analog as a starting point, efforts are underway to incorporate radiolabelled ^{18}F into the structure for the purpose of imaging infection and for extracting important pharmacokinetic information. Residence time is also being exploited to address the question of target occupancy, which will provide direct information on a target's vulnerability.

MEDI 245

Efficient Schiff bases of indoline-2,3-dione (isatin) derivatives against single resistant strains of Mycobacterium tuberculosis

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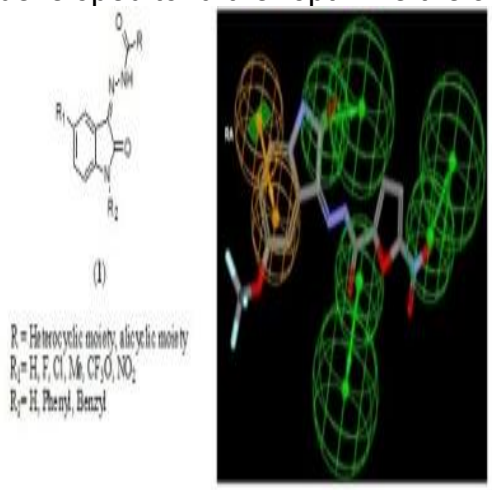
Tuberculosis (TB) remains among the world's great public health challenges. Worldwide resurgence of TB is due to two major problems: the AIDS epidemic, which started in the

mid-1980s, and the outbreak of multi-drug resistant (MDR) TB and extensively multi-drug resistant (XMDR). Thus, there is an urgent need for anti-tubercular (anti-TB) drugs with enhanced activity against MDR strains. In recent years, Schiff bases of 1H-indole-2,3-diones are
reported to exhibit anti-TB activity. In the current study, Schiff bases of indoline-2,3-dione with the general structure (**1**) were synthesized by the reaction of isatin or its derivatives (1- / 5- / 1,5-substituted isatins) with a series of acid hydrazides in mild conditions. The target Schiff bases were screened for their anti-TB activity against *Mycobacterium tuberculosis* H37Rv. Compounds with promising and potent anti-TB activity were further screened against single resistant strains of *M. tuberculosis* and a minimum bacteriostatic (MBC) assay against the pan-sensitive *M. tuberculosis* H37Rv. Results revealed that these compounds can be strongly recommended as potential candidates as promising leads against resistant strains of *M. tuberculosis* with MIC and MBC values in the low micromolar range (0.156). A pharmacophore model was developed to further optimize the activity among this series of novel compounds.



MEDI 246

In vitro and in vivo anti-hepatitis B virus activities of novel 2-pyridone derivatives

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We designed and synthesized a series of novel 2-pyridone derivatives and evaluated their anti-hepatitis B virus activities and cytotoxicities both in vitro and in vivo. Moderate to good activities against HBV DNA replication were observed in the target compounds. Among them, the most potent ones are compounds **11d** and **11g** , with profound inhibitory activity against HBV DNA replication (IC_{50} = 0.61 and 0.11 mM, respectively) and remarkably high selectivity (selectivity index 483.3 and = 1600.0, respectively). The in vivo study conducted on Pekin ducklings showed that compound **11d** significantly reduced plasma and hepatic DHBV DNA levels in a dose-dependent manner with low

toxicity. Compound **11d** hence represents a promising drug candidate for the cure of HBV infections.

MEDI 247

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

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Opportunistic infections caused by *Pneumocystis jirovecii* (pj), *Toxoplasma gondii* (tg) are two of the major reasons for morbidity and mortality associated with patients suffering from Autoimmune Deficiency Syndrome (AIDS). Dihyrofolate reductase (DHFR) is an important enzyme which catalyzes the reduction of dihyrofolate to tetrahydrofolate, a co-factor required in *de novo* synthesis of purines, thymidylate and certain amino acids. We have previously reported 2,4-diamino-5-methyl-6-substituted thieno[2,3-*d*]pyrimidines as potential DHFR inhibitors more selective for pathogen DHFR as compared to mammalian DHFR. Additional compounds were designed and synthesized as a part of a SAR study within this series with a variety of aryl substitution at the 6-position. The design, synthesis, biological activities and SAR of these compounds will be presented.

MEDI 248

Antiviral 1-glycosyl-1,2,3-triazoles

*Natalia Spitha, **Erland P Stevens**, erstevens@davidson.edu.Department of Chemistry, Davidson College, Davidson, NC 28035, United States*

The discovery of new antiviral nucleoside analogues is hampered by a lack of heterocyclic scaffolds that imitate purine and pyrimidine bases. An appropriately substituted 1,2,3-triazole ring should be able to serve in place of a purine ring system. A number of new triazole-based nucleoside analogues have been prepared. Each has a sugar or sugar analogue off N1 of the triazole ring with a group off C4 that can engage in Watson-Crick base pairing. Synthetic procedures and preliminary screening results will be presented.

MEDI 249

Lactamase inhibitors with antimicrobial activity: The case of MN-2-261

*Micheal Nottingham[1], Christopher R. Bethel[2], Marissa L. Winkler[4], **Weirui Chai**[1], wchai@smu.edu, Piet de Boer[4], Paul R. Carey[3], Focco van den Akker[3], Robert A. Bonomo[2,4], John D. Buynak[1]. (1) Department of Chemistry, Southern Methodist University, Dallas, TX 75275-0314, United States (2) Department of Research Service, Louis Stokes Cleveland Department of Veterans Affairs Medical Center, Cleveland, Ohio 44106, United States (3) Department of Biochemistry, Case Western Reserve University, Cleveland, Ohio 44106, United States (4) Department of Microbiology and Molecular Biology, Case Western Reserve University, Cleveland, Ohio 44106, United States*

It is highly unusual for inhibitors of ß-lactamase to also possess independent antimicrobial activity (i.e. ability to bind key bacterial penicillin-binding proteins, PBPs). We prepared and evaluated a focused library of 2ß-substituted-6ß-hydroxymethylpenicillin sulfones, and found that one compound, MN-2-261, possessed independent antimicrobial activity. The PBP binding of this compound will be presented, together with a model of how the appended functionalities may be contributing to PBP affinity.

MEDI 250

High-throughput screening for small molecules with efficacy against *Coccidioides* infection

Nathalie Meurice[1], *meurice.nathalie@mayo.edu, Joachim L Petit[1], Pooja Narang[1], Michael Valentine[2], Elizabeth Driebe[2], Jolene Bowers[2], Paul Keim[2], Dave Engelthaler[2]. (1) Department of Research, Mayo Clinic, Scottsdale, AZ 85259, United States (2) Pathogen Genomics Division, TGen North, Flagstaff, AZ, United States*

Currently there are few treatment options for Valley Fever. Concerns about current antifungals include toxicity and fungistatic limitations. Drug discovery pipelines for Valley Fever are scant, as cost *vs* return to discover and deliver new treatments are deterrents. At TGen North, in collaboration with Mayo Clinic, a low cost, high throughput, primary screening assay has been developed for the discovery of new compounds efficacious against *Coccidioides* in a BSL2 setting, using the avirulent strain *Coccidioides posadasii* Δ*cts2*/Δ*ard1*/Δ*cts3*. The method is a high-throughput 96-well culture array, and uses spectrophotometry to determine whether there are effects of the small molecule library against the growth of this strain.

Mayo Clinic researchers designed a pilot *Coccidioides* small molecule library evaluated for activity in the assays established by the TGen team under the BSL2 conditions. This test library (~ 1800 compounds) includes 1200 approved drugs (Prestwick Chemical Library). An additional ~600 compounds were selected from the Mayo Clinic Collection (MCC) using a knowledge-driven approach, capitalizing on the *Coccidioides* structural data available in the Protein Data Bank. Virtual screening workflows utilizing a blend of 2D scaffold-based and 3D structure-based techniques were used to interrogate the

MCC and identify compounds of potential interest for primary screening in the *Coccidioides* assay workflow established by the TGen team.

This method is a first step towards a workable BSL3 protocol allowing screening of virulent *Coccidioides immitis* and *posadasii*. The ability to screen thousands of molecules relatively quickly and inexpensively may result in more options for effective Valley Fever treatments.

MEDI 251

Strategic analysis of the physicochemical properties of small molecules that can cross the outer membrane of Gram-negative bacteria

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Multi-drug resistant Gram-negative bacteria are a growing problem in the clinic. These bacteria possess an outer membrane that is nearly impermeable to small molecules, and this trait presents a significant challenge in the discovery of new antibiotics to combat these pathogens. Compounds that do traverse this membrane do so through water filled channels called porin proteins; while there is some information about the polarity and molecular weight requirements for compound passage through porins, many of these requirements remain a mystery. Utilizing a novel method to rapidly generate complex and diverse compounds with natural product-like properties, we have constructed a series of complex molecules where polarity and other physicochemical properties have been systematically varied; these compounds are being tested for their ability to cross the outer membrane of Gram-negative bacteria. A thorough analysis of the physicochemical properties that allow certain small molecules to cross the outer membrane of Gram-negative pathogens will be critical to the identification of novel antibacterial agents.

MEDI 252

Synthesis of small molecules as inhibitors of the TAT pathway in *Campylobacter jejuni*

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Campylobacter jejuni a zoonotic pathogen, is among the top five pathogens contributing to foodborne illnesses in humans. The Twin-argenine Translocation (TAT) pathway is an important virulence mechanism in many bacterial pathogens and is critical for *C. jejuni* survival. Chickens and mammals do not have proteins or receptors that are

homologous to bacterial TAT proteins which make it a potential antimicrobial target. The successful use of high throughput small molecule screens for the discovery of antibacterials has been previously described in *V. cholera* and *Salmonella*. A HTS effort for TAT system inhibition was therefore used to identify potential antimicrobial therapies against *C. jejuni* and resulted in identification of a number of hits. As part of the our study, predictive method have been used to analyze the hit set and to identify structurally diverse compounds with drug-like properties for further screening against the TAT pathway. The syntheses of several structurally diverse hits from the library have been accomplished and have been used to develop structural activity relationship studies. A series of these analogues have already demonstrated modulation of toxicity in healthy cells.

MEDI 253

Metalloprotein cross-inhibition and metal ion removal by chelating inhibitors: The impact of metal binding groups on selectivity

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Metalloenzymes are prevalent throughout the body and serve a wide array of functions *in vivo*. These roles range from regulating blood pH and inflammation to controlling DNA expression and superoxide dismutation, to name but a few. Given the importance of these functions, metalloenzyme misregulation can play a significant role in human disease.

Typically, *de novo* designed metalloenzyme inhibitors incorporate a metal binding group (MBG) to ligate the catalytic metal and a backbone optimized for the desired metalloenzyme through structure-activity or structure-based drug design approaches. Currently most inhibitors incorporate a hydroxamate, carboxylate, or thiolate MBG. The use of only a few metal binding moieties raises concerns for off-target inhibition of metalloenzymes.

A study on the cross-inhibitory activities of known metalloenzyme inhibitors against a panel of metalloproteins has been performed. The inhibitors studied use atypical MBGs such as hydroxyurea and hydroxpyridinonate, as well as the more common hydroxamates and carboxylates. The panel of metalloproteins included several matrix metalloproteinases, carbonic anhydrase, tyrosinase, histone deacetylase, angiotensin converting enzyme, and holo-transferrin. This panel allowed for the comparison of zinc, copper, and iron metalloprotein cross-inhibition and metal removal. Results indicate that this assortment of inhibitors and metalloenzymes remain extremely selective for their intended targets, even at high concentrations.

These findings will help guide the design of novel metalloenzyme inhibitors that are specific as well as efficacious.

MEDI 254

Structurally simple cartilage probes constructed with ε-lysine oligomers targeting chondroitin sulfates

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Osteoarthritis (OA) or rheumatoid arthritis (RA) require early diagnosis because destruction of cartilage tissue continues and advances these diseases. Since X-ray apparatus are placed in many medical facilities, high sensitive cartilage X-ray probes are thought to be useful and valuable. And these probes are useful for not only diagnostic product but also evaluation in animal experiments for drug discovery. So far, we found that arginine oligomers can act as cartilage selective probes. Comparing binding abilities to cartilage by arginine oligomeric numbers with 4, 8, 12, and 16, the octamer (R8) was found to be most suitable as a cartilage probe. However, for the application of R8 to X-ray cartilage probes, since R8 has high-molecular-weight character (molecular weight without signaling moieties such as fluorescent group is more than 1200), the imaging ability by a signaling moiety per weight is expected to be low. Thus, we aimed to create less low-molecular-weight cartilage probes. R8 was revealed to target mainly chondroitin sulfates widely existing in cartilage tissue by using several cartilage proteoglycan-degrading enzymes. This time, we came up with lysine oligomers connected by their ε-amino and α-carboxyl groups. The binding affinity with chondroitin sulfates was assessed using fluorescent polarization and fluorescent imagery with mouse knee joint tissue, suggesting that tetra or more oligomers (the molecular weight without signaling moieties of the pentamer and tetramer are about 530 and 660, respectively) showed more potent binding affinity than less oligomers. In addition, the ε-lysine pentamer possessing a triiodobenzene moiety was discovered as a high sensitive cartilage X-ray probe. In this presentation, molecular design, syntheses, and the activities of cartilage probes will be reported.

MEDI 255

Development of small-molecule autophagy modulators for the study of Crohn's disease

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Human genetic studies have revealed the importance of autophagy in the pathogenesis of Inflammatory Bowel Diseases (IBD). Autophagy is a cellular disposal system that directs cargoes into lysosomes, where the substrates, ranging from proteins to pathogens, are subjected to proteolytic cleavage. Several Crohn's disease (CD) susceptibility genes identified in genome wide association studies, such as ATG16L1, IRGM, and NOD2 have been implicated in the regulation of autophagy. Failure of autophagosome formation, maturation and cargo recognition have been correlated with CD-relevant phenotypes, such as impaired clearance of intracellular bacteria and prolonged pro-inflammatory responses, in mouse models as well as in patient-derived samples. These data have led to the hypothesis that small-molecule enhancers of autophagy may correct specific immune phenotypes caused by genetic variants observed in IBD patients. Our current research focuses on the investigation of the cause of Crohn's disease by identifying highly selective small-molecule probes that target autophagy through a high-throughput screen (HTS), by using the resulting lead compounds in mechanism of action (MOA) studies to identify cellular targets to further our understanding of the disease biology, and by testing the therapeutic potential of these compounds in animal models of the disease phenotype. In addition to providing insight into the cause and treatment of Crohn's disease, a collection of autophagy probes with known MOAs could drive future experiments to address the role of autophagy in neurodegeneration and cancer, where this process has been implicated yet remains poorly understood. Our efforts to develop selective, small-molecule modulators of autophagy, including synthetic and medicinal chemistry endeavors, autophagic flux assays, bacterial co-localization and clearance experiments, inflammatory cytokine measurements, and mechanism of action studies, will be highlighted.

MEDI 256

Toward more orally bioavailable inhibitors of phospholipase A_2 GIIA (pla2g2a) to treat chronic inflammation

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Phospholipases A_2 (PLA_2) specifically hydrolyse the *sn2* ester of membrane phospholipids to release bioactive fatty acids and lysophospholipids. PLA_2 enzymes have been implicated in the pathogenesis of many inflammatory conditions, including rheumatoid arthritis. To date, no PLA_2inhibitors have successfully progressed through clinical trials to market [1].

Our research group previously reported a substrate analogue (KH064) as a potent and subtype-selective small molecule inhibitor of PLA_2 GIIA (pla2g2a) [2]. KH064 has demonstrated potent *in vitro* (IC_{50} 29 nM) and *in vivo* activity in numerous rat and mouse models of inflammatory and metabolic disease. KH064 is orally active but has non-optimal drug-like properties, being very hydrophobic (ClogP 6.9) and highly flexible (10 rotatable bonds). Extensive structure-activity relationships of the *sn1*, *sn2* and *sn3* sidechains of KH064 have led to pla2g2a inhibitors with improved drug properties. Some of these compounds and their activities will be described in this presentation.

References:

1. Dennis, E. A. *et al. Chem. Rev.* **2011** , *111*, 6130-6185.

2. Hansford, K. A. *et al. ChemBioChem* **2003** , *4*, 181-185.

MEDI 257

Mandelamides as novel agonists of sphingosine-1-phosphate 1 (S1P1)

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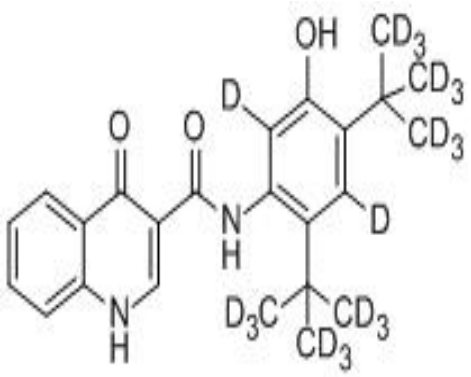
S1P1 is a membrane bound receptor that is expressed on lymphocytes and is a member of the G-protein coupled receptor family. The endogenous ligand for S1P1 is the lipid signaling molecule sphingosine-1-phosphate (S1P), which binds to S1P1 to elicit effects within the immune system. The S1P1 receptor is the most widely expressed member of the S1P receptor family, and S1P/S1P1 signaling is required for the egress of immune cells from the thymus and lymph nodes. S1P1 receptor modulators are able to block lymphocyte migration out of lymphoid tissue and into the lymphatic and blood circulation, thereby reducing peripheral lymphocyte counts. As a result, S1P1 agonists hold promise as therapeutics to treat a variety of autoimmune disorders. Clinical validation of S1P receptor modulation therapy was recently achieved with the approval of fingolimod (FTY720), the phosphorylated metabolite of which is a non-selective S1P receptor agonist, as the first oral disease modifying treatment for relapsing remitting multiple sclerosis. In this communication, we disclose mandelamides as potent and selective S1P1 receptor agonists.

MEDI 258

Design and synthesis of deuterated analogs of ivacaftor with enhanced pharmacokinetic properties

***Adam J Morgan**, amorgan@concertpharma.com, Sophia Nguyen, Changfu Cheng, Gary Bridson, Vinita Uttamsingh, Lijun Wu, Philip B Graham, Scott Harbeson.Concert Pharmaceuticals, Inc., Lexington, MA 02421, United States*



110

As part of an ongoing effort to apply the Deuterated Chemical Entity Platform (DCE Platform®) to clinically validated drugs, several deuterated analogs of the CFTR potentiator ivacaftor (Kalydeco™) have been prepared. The devised synthetic routes allowed for site selective deuterium incorporation with high levels of isotopic purity. Due to the fact that ivacaftor was poorly metabolized in standard liver microsome assays under the conditions tested, human CYP3A4 Supersomes™ were used to assess and compare the *in vitro* metabolic stability of ivacaftor and the DCEs. In this manner, multiple deuterated analogs displaying marked levels of *in vitro* metabolic stabilization have been identified. One such analog, compound **110** , exhibited a 55% increase in half-life vs. ivacaftor. Synthetic routes to the individual isotopologs along with metabolic stabilization data using human CYP3A4 Supersomes™ will be presented.

MEDI 259

Structure-based drug design of TRAF6 inhibitors

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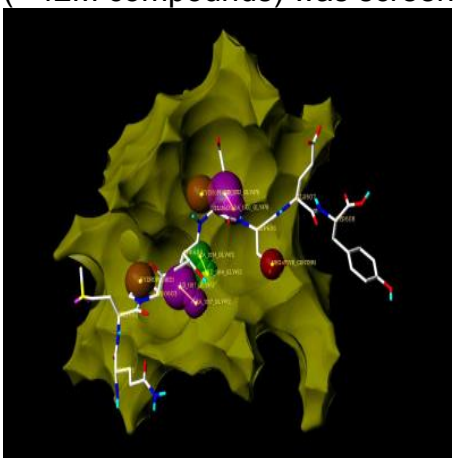
Tumor-necrosis factor (TNF) receptor-associated factor 6 (TRAF) has recently been identified as an oncogene and is an important adaptor molecule involved in multiple aspects of immunity, inflammation, and bone homeostasis. A structure-based drug design approach was used to identify compounds targeting the C-terminal adaptor function of TRAF6 in complex with its binding peptide. Based on the key interactions of the peptide with TRAF6 and a filter for drug-like properties, a PubChem chemical library

(~42M compounds) was screened that led to the identification of 670 structures.



Examination by two well-known docking programs, Surflex and Glide with Prime MM-GBSA re-scoring, revealed several compounds from one particular structural core that scored high with both docking methods and had consistent binding modes. A synthesis was developed for that core that would lead to a diverse set of compounds. A library of 260 structures were docked and then processed via workflow software that resulted in 29 compounds of interest after consideration of ADME type properties. Several compounds from this library have been synthesized and then tested in a split-luciferase assay to assess their TRAF6 inhibitory activity. The design and synthesis of this novel library of compounds and the results of the biological studies will be reported.

MEDI 260

Toll-like receptor-8 agonistic activities in C2, C4, and C8 modified 2-alkylthiazolo[4,5-*c*]quinolines

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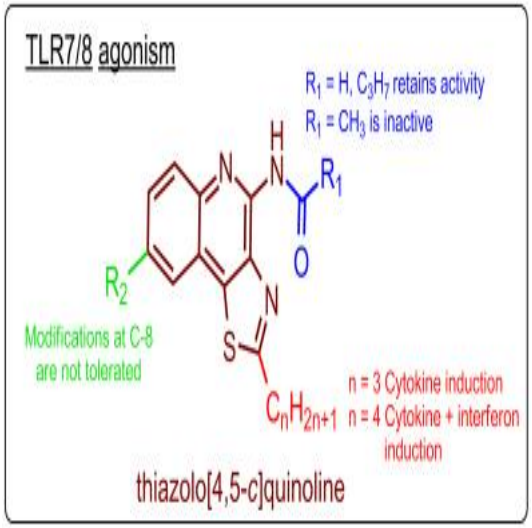
Toll-like receptor (TLR)-8 agonists typified by the 2-alkylthiazolo[4,5-*c*]quinolin-4-amine (CL075) chemotype are thought to be uniquely potent in activating adaptive immune responses by inducing robust production of T helper 1-polarizing cytokines, suggesting that TLR8-active compounds may be promising candidate vaccine adjuvants, especially for neonatal vaccines. Analogues with methyl, ethyl, propyl and butyl groups at C2 displayed comparable TLR8-agonistic potencies; activity diminished precipitously in the C2-pentyl compound, and higher homologues were inactive. The C2-butyl compound was unique in possessing substantial TLR7-agonistic activity. Virtually all modifications at C8 led to abrogation of agonistic activity. Alkylation on the C4-amine was not tolerated, whereas *N*-acyl analogues with short acyl groups (other than acetyl) retained TLR8 agonistic activity, but were substantially less water-soluble. Immunization in

rabbits with a model subunit antigen adjuvanted with the most potent TLR8 agonist showed dramatic enhancements of antigen-specific antibody titers.



MEDI 261

Discovery of cycloalkenyl aryl derivatives for cholesteryl ester transfer protein inhibitor

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Cholesteryl ester transfer protein (CETP) is a plasma protein that mediates the transfer of cholesteryl ester (CE) from HDL to apolipoprotein B (apoB)-containing lipoprotein (VLDL and LDL) in exchange for triglyceride (TG). Inhibition of CETP is expected to reduce cardiovascular risk due to increased level of the high-density lipoprotein-cholesterol (HDL-c). A structurally novel cycloalkenyl aryl derivative, CKD-519, was identified as a potent and orally available CETP inhibitor which is currently at the preclinical stage. This poster describes the SAR studies towards the discovery of CKD-519, which demonstrated strong inhibitory activity against human CETP in vitro. Orally administrated CKD-519 has increased the HDL-c level significantly in the hamster PD model.

MEDI 262

Discovery of a novel selective 11β-HSD1 inhibitor DSP-0011

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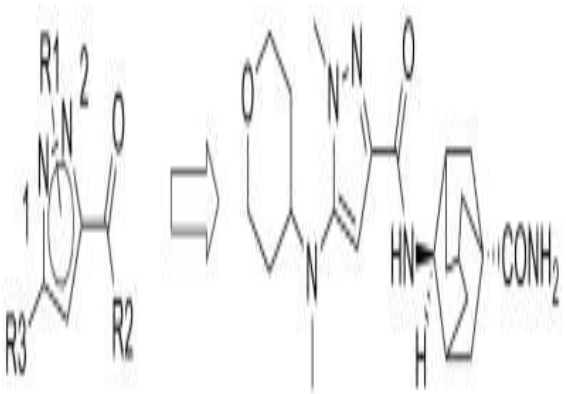
11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) is the reductase converting inactive glucocorticoid hormone cortisone to active glucocorticoid hormone cortisol. 11β-HSD1 inhibitor has been explored as potential therapeutics, such as type 2 diabetes, obesity and dyslipidemia by regulating the amount of cortisol. In our 11β-HSD1 inhibitor project, we found a novel aminopyrazoleamide derivative as a lead compound. The further lead optimization study gave DSP-0011 which exhibited IC_{50} values of 5.2, 4.2 and 10.3 nM, respectively, for human, mouse and rat 11β-HSD1 and presented good pharmacokinetic and safety profiles.



DSP-0011

MEDI 263

Identification of synthetic fragments for the vitamin D receptor by hydrogen-deuterium exchange (HDX)

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The vitamin D receptor (VDR) is a ligand activated gene transcription factor and a member of the nuclear receptor superfamily. Upon binding with the seco-steroidal hormone vitamin D_3 (VD3, calcitriol), VDR heterodimerizes with the retinoid x receptor (RXR) resulting in recruitment of coactivator proteins. The resulting complex then regulates calcium homeostasis and bone formation by binding to the promoter region of mineral and bone metabolic genes. Calcitriol and seco-steroidal synthetic analogs have been designed for the treatment of osteoporosis, but serum and hypercalcemic side effects limit their therapeutic value. We and others have hypothesized that a non-seco steroidal ligand would bind to VDR and induce a ligand-protein conformation leading to selective pharmacology (i.e. bone building in favor of hypercalcemia). One component of our lead generation strategy included fragment based drug design. Screening of ligands in a VDR fluorescence polarization assay and a RXR/VDR conformations sensing assay resulted in the identification of multiple fragment hits (lean > 0.300). These fragment scaffolds were subsequently evaluated for interaction with the VDR ligand binding domain using hydrogen-deuterium exchange (HDX) mass spectrometry. Significant protection to H-D exchange is observed for some fragments in helices 3, 7, and 8 of the ligand binding domain, regions which are similar to those seen for VD3, the natural ligand. The fragments appear to mimic the A-ring of VD3 thereby providing viable starting points for synthetic expansion.

MEDI 264

Multiparameter optimization of pharmaceuticals: The big-data way

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Reliable approaches to multi-parameter optimization in drug-discovery remain one of the intractable problems in the field; the cynical might even suggest that mono-parameter optimization remains an unsolved problem. Recent advances in matched molecular pair analysis include its broader application by computer algorithms which do not require human oversight. This has begun to provide examples of the successful application of this technique to suggest structural changes that achieve optimization of more than one parameter at a time. Examples taken from programs concerning the optimization of compounds in diabetes (glucose kinase activators), obesity (Ghrelin receptor inverse agonists) and oncology (aromatase inhibitors) will all be described. The future for this kind of analysis should involve larger and more diverse sets of compounds to permit the generation of more structurally specific insights that are more likely to be successful when applied to a problem molecule. Bringing together large datasets from several pharmaceutical companies, encoded according to *changes* in

structure and property is allowing the contributing companies to explore the potential for this kind of technique without taking the risk of exposing their intellectual property or critical data. Progress and the challenges involved in achieving this will be described.

MEDI 265

Inactivation of protein tyrosine phosphatase 1B by exo-affinity labeling agents

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Protein tyrosine phosphatases (PTPs) comprise a large family of proteins that work in tandem with protein tyrosine kinases (PTKs) to control multiple signaling pathways. PTP1B is a negative regulator of the insulin and leptin signaling pathways and inhibitors of this enzyme could be used in the treatment of type II diabetes. However, traditional reversible inhibitors of this PTP1B suffer from lack of selectivity and/or poor bioavailability. Here we describe our efforts to pursue a novel exo-affinity labeling strategy for inactivation of PTP1B. Toward this end, we have synthesized compounds consisting of a known phosphotyrosine isostere combined with a biocompatible electrophile. We will present evidence that these agents inactivate PTP1B via a mechanism involving noncovalent association of the phosphotyrosine isostere with the active site, followed by covalent modification of residues outside the enzyme active site. Exploiting the covalent reactivity of protein functional groups outside the enzyme's catalytic pocket represents a new approach for the selective knockdown of PTP activity.

MEDI 266

Discovery of pyrazolopyrrolidinone-derived melanin concentrating hormone receptor-1 antagonists as anti-obesity agents

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Discovery Analytical Group, Bristol-Myers Squibb Research and Development, United States (7) Syngene, India

Melanin Concentrating Hormone (MCH) is a cyclic 19 amino acid peptide predominantly expressed in the CNS. Knock out animal studies suggested that MCH and MCH receptors (MCHRs) play a role in feeding and energy homeostasis. This presentation describes the design, synthesis, and SAR of a pyrazolopyrrolidinone-derived class of MCHR1 antagonists. A selective amine analog from this series with a Ki of 1 nM showed significant body weight loss and reduction of food intake in a chronic animal model.

MEDI 267

Synthesis and evaluation of non-absorbable ASBT inhibitors for the treatment of type 2 diabetes

***Yulin Wu**, Yulin.X.Wu@gsk.com, Christopher Aquino, David Cowan, Michael J. Bishop, Bert Yao, Lihong Chen, Don Anderson, Maggie McIntyre, Lindsey Harston, Shane Roller, Jon L. Collins.EE DPU, GlaxoSmithKline, Durham, NC 27709, United States*

Several classes of ASBT inhibitors were synthesized and evaluated in both in vitro assays and animal models. To minimize the potential side effects caused by systematic drug circulation, efforts were made to identify gastrointestinally (GI)-restricted ASBT inhibitors for the treatment of type 2 diabetes. A panel of parameters, including potency, selectivity, permeability, solubility, stability, and fecal drug recovery were used in the optimization and selection process. One of the GI-restricted ASBT inhibitors, GSK2330672, was identified as a highly potent, non-absorbable ASBT inhibitor with excellent drug development properties.

MEDI 268

Molecular switch strategy delivers distinct group II mGluR allosteric modulator families

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GPCRs have proven to be a valuable target family for drug discovery and development with more than 30% of marketed drugs acting through this receptor superfamily. However, numerous GPCR members remain challenging with no selective and druggable ligands being successfully developed. For these difficult targets, a novel strategy consisting in developing allosteric modulators (AMs) is now emerging. AMs positively or negatively modulate GPCR activity through interaction with binding sites topologically distinct from the orthosteric binding sites.

Metabotropic glutamate receptors (mGluRs) belong to family C GPCRs and are characterized by a large extra-cellular domain named the “Venus flytrap” containing the orthosteric binding site and a 7TM domain where allosteric modulators bind. mGluRs represent attractive drug targets for numerous indications such as neurodegenerative or psychiatric disorders. They are divided into three groups based on their structures, signal transductions and pharmacologies.

AMs of group II mGluRs (mGluR2 and mGluR3) are currently studied to discover new treatments for schizophrenia or anxiety (mGluR2 PAM), Alzheimer disease or depression (mGluR2/3 NAM), Parkinson disease (mGluR3 PAM) and glioblastoma (mGluR3 NAM). The development and screening of a FRET-based binding assay at Domain led to the identification of a novel family of mGluR2/3 NAMs. Medicinal chemistry efforts including molecular switch strategy (subtle structural modifications leading to drastic changes in the pharmacology), enable the discovery of novel families of selective mGluR2 NAMs, mGluR3 PAMs and mGluR3 NAMs.

MEDI 269

Novel and potent 5-$HT_{2B/7}$ receptors antagonists for the treatment of IBS

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5-HT_{2B} and 5-HT_7 receptors exist in smooth muscle and neurons in gastrointestinal tract, and are involved in abnormal bowel movement and abdominal pain of IBS (irritable bowel syndrome). Therefore, dual antagonists of 5-$HT_{2B/7}$ receptors are expected to improve of the symptoms of IBS. In an effort to develop 5-$HT_{2B/7}$ receptors antagonists, we have synthesized a series of novel indole derivatives. Among these compounds, 3-ethyl-1-[(4-fluorophenyl)methyl]-2-methyl-N-[(*3S*)-1-methylpyrrolidin-3-yl]-1H-indole-5-carboxamide (ASP1017) showed potent antagonistic activity to 5-$HT_{2B/7}$ receptors and high selectivity. And also, ASP1017 inhibited stress-induced defecation and abdominal pain in rats. Synthesis and structure-activity relationships of a novel series of indole derivatives, including in vivo evaluation of ASP1017 will be presented.

MEDI 270

Synthesis and preliminary evaluation of radiolabeled substrates and inhibitors for in vivo monoamine oxidase B imaging

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Monoamine oxidase B in the brain is predominantly expressed in astrocytes and increased MAO-B activity has been proposed as a biomarker of gliosis. Prior successful imaging agents for MAO-B have been based on the reaction of irreversible inhibitors, such as [^{11}C]deprenyl and related radiotracers. The objective of this work is the synthesis and evaluation of radiolabeled non-toxic N-methyl-1,2,3,6-tetrahydropyridines that would be trapped in the brain in proportion to MAO-B catalyzed oxidation to the corresponding dihydropyridinium salts; as well as, the synthesis and evaluation 1,5-Diphenylpenta-2,4-dien-1-one competitive inhibitors. The oxidation of the dihydropyridine can produce non-toxic products provided the group substituted at the 4 position is susceptible to hydrolysis upon oxidation by MAO-B that additionally could provide a means for more favorable pharmacokinetics of the radiolabel for imaging studies. The generation of a competitive substrate or inhibitor of MAO-B should provide data not only on MAO-B expression levels but also its kinetics *in vivo*.

MEDI 271

Synthesis and evaluation of PET probe targeting metal-Aβ aggregates

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Amyloid-β (Aβ) and tau-neurofibrillary tangles are the proposed pathological markers of Alzheimer's disease (AD). With the goal of early diagnosis of AD, and differentiation from other clinically overlapping neurodegenerative disorders, this project aims to further understand amyloid pathology in AD by using PET imaging to evaluate the role of metal ions known to accumulate in high quantities in Aβ plaques. Carbon-11 labeled radiopharmaceuticals based upon *N'*,*N'*-dimethyl-N4-(pyridin-2-ylmethyl)benzene-1,4-diamine, a scaffold recently demonstrated to bind Aβ plaques and sequester metal ions, have been synthesized. This presentation will report chemical and radiochemical syntheses, quality control testing, and preliminary evaluation of proof-of-concept *in vivo* using rodent and primate microPET imaging experiments, and *in vitro* using autoradiography experiments with post-mortem brain samples from AD patients.

MEDI 272

Synthesis of heparan sulfate hexa- to dodecasaccharides as inhibitors of the Alzheimer's disease target β-secretase

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Heparan sulfates (HS) are a class of sulfated polysaccharides which function as dynamic biological regulators of the functions of diverse proteins. The structural basis of

these interactions however remains elusive, and chemical synthesis of defined structures represents a challenging but powerful approach for unravelling the structure-activity relationships of their complex sulfation patterns. HS has been shown to function as an inhibitor of the beta-site cleaving enzyme β-secretase, a protease responsible for generating the toxic A-beta peptides that accumulate in Alzheimer's disease (AD), with 6-O-sulfation identified as a key requirement. Here we demonstrate a novel generic synthetic approach to HS oligosaccharides applied to production of a library of 16 hexa- to dodeca-saccharides, targeted at β-secretase inhibition. Screening of this library has provided new insights into structure-activity relationships for optimal β-secretase inhibition, and yielded a number of potent non-anticoagulant inhibitors with potential for development as leads for treatment of AD through lowering of A-beta peptide levels.

MEDI 273

Synthesis of novel cannabinoids that significantly decrease the reinforcing and conditioning effects of ethanol

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Recent studies have demonstrated that development and/or maintenance of alcohol dependence may be due to an overactive endocannabinoid system. It was recently reported that a mono-hydroxylated metabolite of the synthetic cannabinoid JHW-073 exhibits neutral antagonist activity at CB1Rs and can therefore serve as a promising lead in the search for novel treatments for alcohol abuse. In the current study, we show that systematic modification of the JWH-073 scaffold identified two new compounds with dual CB1R antagonist/CB2R agonist activity. In a similar manner to the CB1R antagonist/inverse agonist rimonabant, analogue **23** was shown to decrease oral alcohol self-administration, without affecting total fluid intake or body weight. Analogue **26** was shown to block the development of alcohol-conditioned place preference similarly to rimonabant. These initial findings suggest that systematic modification of aminoalkylindoles may lead to the development of novel cannabinoid ligands with dual CB1R antagonist/CB2R agonist activity and potential for alcohol abuse therapies.

MEDI 274

Optimized and improved larger scale synthesis of opioid macrocyclic tetrapeptides with potential for drug development

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Kappa opioid receptor (KOR) ligands have demonstrated potential as therapeutic agents in the treatment of various diseases including drug abuse and pain. The natural product macrocyclic tetrapeptide KOR ligand CJ-15,208 (Figure 1, Saito *et al*., *J. Antiobiot*. **2002,** *55*, 847) can prevent reinstatement of cocaine seeking behavior *in vivo* following oral administration (Aldrich *et al.*, *J. Nat. Prod.* **2013,** *76*, 433). These macrocyclic peptides are potential candidates for drug development because of their low molecular weight and expected metabolic stability *in vivo*, but the small 12-membered ring size can make their synthesis difficult, resulting in low yields and dimeric macrocyclic octapeptides as the major products. We are synthesizing analogs of CJ-15,208 by modifying our initial synthetic protocol (Ross *et al*., *Tetrahedron Lett.* **2010,** *51*, 5020) to prepare larger quantities of these macrocyclic tetrapeptides for detailed pharmacological evaluation *in vivo* following systemic administration. The macrocyclic tetrapeptides were synthesized by a combination of solid phase synthesis of the linear peptide precursors, followed by cyclization in solution. Optimization of the crucial cyclization step and the use of normal-phase column chromatography increased the yields of the final products, providing sufficient material for extensive characterization *in vivo*. Pharmacological results for selected macrocyclic tetrapeptides will also be presented. These novel macrocyclic tetrapeptides are promising candidates for the development of potential peptide KOR therapeutic ligands. Research supported by NIDA grants R01 DA018832 and R01 DA 023924.

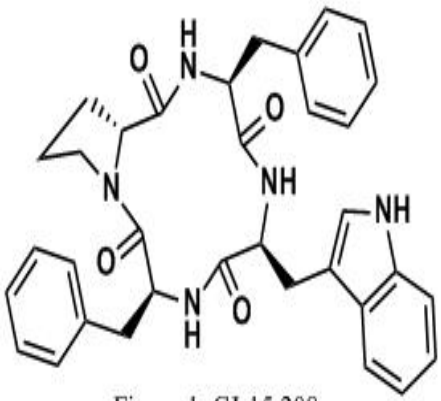

Figure 1. CJ-15,208

MEDI 275

Backbone conformations and hydrogen bonding patterns of analogs of the macrocyclic tetrapeptide CJ-15,208

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Narcotic analgesics such as morphine, which act primarily through mu opioid receptors (MOR), have been widely used clinically for the treatment of severe pain. However, their use is limited by severe side effects such as respiratory depression and drug dependence. Kappa opioid receptor (KOR) ligands, both agonists and antagonists, have potential as therapeutic agents in the treatment of various diseases including drug abuse and pain. The natural macrocyclic tetrapeptide CJ-15,208 (*cyclo*[Phe-D-Pro-Phe-Trp]) was reported to be a KOR antagonist with modest affinity and selectivity for KOR (Saito *et al.*, *J. Antiobiot.* **2002,** *55*, 847). We have demonstrated that CJ-15,208 and its analog [D-Trp]CJ-15,208 are active after oral administration (Aldrich *et al.*, *J. Nat. Prod.* **2013,** *76*, 433; Eans *et al., Br. J. Pharmacol.* **2013,** in press) and therefore are excellent lead compounds for further development. One major advantage of these peptides is the limited number of possible conformations because of their macrocyclic structure. In this work we performed extensive 1D and 2D NMR spectroscopic analysis of CJ-15,208 and selected analogs, and used interproton distances measured in the 2D ^{1}H-^{1}H ROESY, as well as dihedral angles calculated from $^{3}J_{HH}$, to define the secondary structural elements and backbone conformations (e.g. *cis* vs. *trans* amide bonds) of the peptides. Additionally, circular dichroism spectroscopic data provided information on turns in the backbones of these macrocyclic tetrapeptides. Linking the structural/dynamic characteristics of these ligands with their observed biological activity assists in developing pharmacophore models that will be helpful in the design of future analogs. This research was supported by NIDA grants R01 DA018832 and R01 DA023924.

MEDI 276

Design and synthesis of melatonin receptor 1 ligands

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The aim of this project is to make small organic molecules that selectively bind melatonin receptor 1 (MT1) in relation to melatonin receptor 2 (MT2). MT1 knockout studies have established a relationship between melatonin receptor 1 and Depression, Huntington's disease, Insomnia, Insulin resistance – to mention a few. However, there are hardly any selective ligands (where MT1/MT2 ≤ 0.01) available to study MT1 and possibly develop into medicines. Some of the problems associated with finding useful MT1 ligands are lack of X-ray crystal structures of the receptor and its low sequence similarity with GPCR's having known crystal structures. In the absence of these tools, it becomes challenging to determine useful allosteric sites in a receptor. Medicinalkey is employing Ligand Based Drug Discovery to make ligands which bind the orthosteric site and explores undiscovered allosteric sites in the melatonin receptor 1. These compounds are popularly referred to as bitopic ligands. Many of the synthesized ligands were non-selective for MT1 or MT2 (where selectivity for MT1 is defined as MT1/MT2 ≤

0.01 and selectivity for MT2 is MT1/MT2 ≥ 100), but had higher binding affinity at one or the other receptor (for example MT1/MT2 = 0.2 and MT1/MT2 = 5) – that is enhancing subtype selectivity. The structural information of the ligands which promoted subtype selectivity was noted. This information was then used to improve MT1 selectivity (by approximately 20X, that is MT1/MT2 = 0.05). The plan is to have MT1/MT2 ≤ 0.01 by exploring all the identified factors, which enhance MT1 selectivity.

MEDI 277

Procognitive potential of novel aryl and heteroaryl amides as potent and selective histamine H_3 receptor antagonists

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The histamine 3 receptor (H_3R) belongs to G-protein-coupled receptor (GPCR) family, which plays a major role in controlling various physiological processes. H_3R is a presynaptic auto receptor as it regulates the release of the various neurotransmitters like serotonin, histamine, acetylcholine and dopamine. As these neurotransmitters play important role in the modulation of cognition and mood, the H_3R antagonists have become the promising drug target for the treatment of neurodegenerative diseases like Alzheimer's disease, schizophrenia, obesity, attention-deficit hyperactivity disorder (ADHD), epilepsy, narcolepsy as well as pain. Keeping in mind the H_3R pharmacophore model, we have developed the novel series of aryl/heteroaryl amide compounds as highly potent and selective H_3 receptor antagonists with favorable pharmacokinetic properties and with no hERG liability. The design, synthesis, SAR and pharmacological profile along with neurochemical profile of these NCE's is the subject matter of this poster.

MEDI 278

Design and synthesis of newer amide derivatives as 5-HT_4 receptor ligands for the treatment of Alzheimer's disease

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Alzheimer's disease (AD), the most prevalent type of dementia in the elderly, is characterized by the appearance of amyloid plaques (Aβ), synaptic loss and cholinergic hypofunction. Serotonin 5-HT_4Rs are widely expressed throughout the body, but in all species studied so far, the highest density of 5-HT_4R is observed in the CNS. Several literature evidences suggest a role of 5-HT_4 agonist in cognition. 5-HT_4 receptor partial agonist shifted the equilibrium of APP processing from amyloidogenic to non-amyloidogenic pathway by activating alpha secretase enzyme and demonstrated excellent pro-cognitive profile in various animal models. We have designed and synthesized a new series of amide compounds which showed potent agonism towards 5-HT_4 receptor and selectivity over closely related receptors. In general the series has adequate ADME properties and efficacy in animal models of cognition. Details will be presented in the poster.

MEDI 279

Novel indolizine derivatives as potent 5-HT4 partial agonists and their efficacy in animal models of cognition

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A series of novel indolizine derivatives have been designed and synthesized which have shown potent partial agonist activity towards 5-HT4 receptors. The SAR was done to obtain the compounds which have favorable ADME properties and potent efficacy in animal models of cognition. These compounds can be used in treating cognition deficits associated with Alzheimer's disease (AD), which is the most prevalent type of dementia in the elderly and is characterized by the appearance of amyloid plaques (Aβ), synaptic loss and cholinergic hypofunction. Serotonin 5-HT_4Rs are widely expressed throughout the body, but in all species studied so far, the highest density of 5-HT_4R is observed in the CNS. Several literature evidences suggest a role of 5-HT_4 agonist in cognition.

MEDI 280

Conformationally constrained pyrrolidine derivatives as α4β2 nicotinic acetylcholine receptor ligands and their efficacy in animal models of depression

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A series of conformationally constrained pyrrolidine derivatives were synthesized and evaluated for their affinity at α4β2 nicotine acetylcholine receptors and for antidepressant properties in rats. The introduction of rigidity in the pyrrolidine ring exerted a profound influence on both receptor binding and antidepressant effects. Substitution of different groups in the heteroaromatic group which is connected to the pyrrolidine ring through ether linkage has also shown the influence on the antidepressant properties. These results demonstrated that structural requirements for receptor binding and functional are distinctively different. In our quest in finding the novel α4β2 modulators, we have designed and synthesized a series of conformationally constrained pyrrolidine derivatives with favorable ADME properties. The design, synthesis, SAR and pharmacological profile of these novel compounds in animal models of depression will be presented.

MEDI 281

New analogs of pawhuskin A

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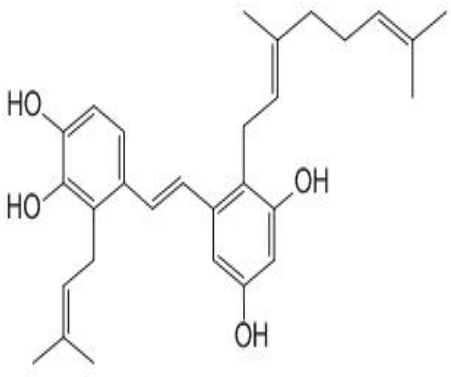
Pawhuskin A (**1**), isolated from the purple prairie clover by Belofsky and co-workers, is a rare example of a non-nitrogenous natural product that binds selectively to an opioid receptor as a competitive antagonist. Since the pawhuskins were identified, we have synthesized two of the natural products along with a number of pawhuskin analogues intended to elucidate the novel pharmacophore. Compounds which are very similar structurally to ones with weak to moderate binding affinity as competitive antagonists may bind with equal or greater affinity than synthetic pawhuskin A to a negative allosteric site(s). The chemical syntheses of several new pawhuskin analogues will be presented, in addition to on-going efforts to discover the various opioid receptor interaction(s) through further compound design and synthesis.



Pawhuskin A (1)

MEDI 282

Synthesis and biological evaluation of 4-aryl-4-arylmethoxy piperidine analogs on monoamine transporters

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A series of 3α-arylmethoxy-3β-aryltropane and piperidine derivatives have been found to be exhibit potent affinity (nM) for dopamine transporters (DAT) and serotonin transporters (SERT) in rat brain tissue, respectively. The aim of the present study is to synthesize and evaluate 4-aryl-4-arylmethoxy piperidine derivatives that would have affinity towards multiple transporters. We have synthesized a series of target compounds via a three step process from 4-Boc-piperidone. The binding affinity for DAT, SERT and nor epinephrine transporters (NET) has been determined in rat brain tissue. The synthetic details and results of this SAR study will be presented.

MEDI 283

3-Aryl-3-arylmethoxyazetidines: A new series of high affinity ligands for monoamine transporters

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A series of 3-aryl-3arylmethoxyazetidine analogs were found to be active (nM) for the serotonin transporter (SERT) receptor in rat brain tissue. The aim of the project is to find a dually active compound at both the SERT and dopamine (DAT) transporter receptors, which could be used in the field of psychotherapeutics. A four- or five-step synthesis starting from 1-Boc-3-azetidinone was used for production of these 3-aryl-3-arylmethoxyazetidinone derivatives. Binding studies for SERT and DAT were completed by competitive inhibition against [^{3}H]citalopram and [^{3}H]WIN 35,428 , respectively, in rat brain tissue. To date, the 3-phenyl-3-(3,4-dichlorophenyl)methoxyazetidine analog (R =H, X = H, Y = 3,4-Cl_2) was found to be the most active at the SERT receptor (K_i = 4.5 nM) in in vitro binding studies, while having little DAT affinity (K_i = 2914 nM). These preliminary SAR studies indicate that the 3-aryl-3arylmethoxyazetidine scaffold is feasible for the development of SERT ligands with variable DAT affinity.

MEDI 284

Discovery and structure-activity relationship of a novel choline transporter inhibitor (ML352)

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Acetylcholine (ACh) is a major neurotransmitter that modulates multiple biological processes including periphereal (cardiovascular) and central nervous system (motor function, attention disorders and addiction). A major course of research has been targeted at potentiation of the cholinergic signaling through the use of acetylcholinesterase inhibitors (AChE) – which is the predominant course of treatment strategy for Alzheimer's disease (AD). Another unexplored therapeutic target is the high-affinity choline transporter, which is presynaptic and could lead to the development of novel tool compounds with which to further explore these cholinergic pathways. Unfortunately, the only small molecule available that targets CHT is hemicholinium-3, a quaternary ammonium containing molecule, making this less than ideal as a tool compound. In order to identify more appropriate small molecule inhibitors, we utilized a high-throughput screening (HTS) campaign followed by an iterative medicinal chemistry approach. Through this, we discovered ML352 which shows nanomolar inhibition at both high (10 mM) and low (100 nM) concentrations of applied choline, 290 nM and 90 nM, respectively. The discovery of this molecule should enable further investigation of the cholinergic pathways.

MEDI 285

Novel mutants of human butyrylcholinesterase as therapeutic enzymes in metabolizing cocaine, norcocaine, and cocaethylene

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Cocaine abuse is a major medical and health concern. There is no FDA-approved medication for treating cocaine overdose/addiction. Butyrylcholinesterase (BChE) catalyzed hydrolysis is the primary pathway for (-)-cocaine detoxification mammals, but native BChE has a very low catalytic efficiency against it. Rationally designed mutations of human BChE in this laboratory have led to ~2000 fold improved catalytic efficiencies against (-)-cocaine.Clinical trials show that the high-activity mutants of human BChE, known as cocaine hydrolases (CocHs), are promising therapeutic agents for treating cocaine abuse.

Hydrolysis *via*BChE is not the only pathway for cocaine removal; ~5% (-)-cocaine is metabolized in the liver to produce norcocaine, which is still toxic to the body. Statistical data report that 73% of cocaine users also consume alcohol. Alcohol can react with (-)-cocaine to produce a significantly more cytotoxic compound, cocaethylene. It is

unknown whether native human BChE or any of CocHs can efficiently hydrolyze norcocaine or cocaethylene.

The kinetic parameters of ***native human BChE*** and ***CocHs*** against norcocaine and cocaethylene have been determined ***for the first time*** in the present study. The most effective enzymes have at least a ~500-fold improved catalytic efficiency against all of the substrates (cocaine, norcocaine, and cocaethylene).

Although the catalytic efficiencies at which norcocaine and cocaethylene substrates can be hydrolyzed by CocHs are relatively (3~20 times) lower than these compared to (-)-cocaine, the novel enzymes can effectively hydrolyze all three cytotoxic compounds produced from the simultaneous abuse of cocaine and alcohol in both addiction and overdose models.

Supported by the NSF CHE-1111761, NIH R01DA032910, and NIH R01DA035552.

MEDI 286

Centrally active positive allosteric modulators (PAMs) of metabotropic glutamate receptor 5 (mGluR5) for traumatic brain injury (TBI)

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Traumatic brain injury (TBI) causes long-term neuroinflammation characterized by microglial activation and leading to progressive neurodegeneration. Our recent results show that the neuroinflammation process can be targeted as late as 1 month after experimental TBI in rodents and still result in significant attenuation of the progressive neurodegeneration. This was accomplished using a novel strategy targeting the metabotropic glutamate receptor type 5 (mGluR5) in microglia. Stimulation of mGluR5 results in significant inhibition of microglia activation, which represents a key pathway for modulation of microglia activation. A series of mGluR5 PAMs have been designed using a combination of computer-aided drug design (CADD) and medicinal optimization. The synthesized compounds were evaluated in multiple assays involving cell lines, primary neurons, and neuronal microglial co-cultures. The optimal drug candidates were tested for their effectiveness in reducing lesion volume and neurological dysfunction in a mouse CCI model.

MEDI 287

Structure-based design, synthesis, and evaluation of imidazo[1,2-*b*]pyridazine and imidazo[1,2-*a*]pyridine derivatives as dual inhibitors of c-Met and VEGFR2 kinases

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To identify compounds with potent anti-tumor efficacy against various human cancers, we designed and synthesized dual inhibitors of c-Met and VEGFR2 kinases. The design of dual c-Met and VEGFR2 inhibitors were based on our VEGFR2 inhibitor which contains imidazo[1,2-*b*]pyridazine scaffold as a hinge binder. After lead optimization of this scaffold to enhance c-Met and VEGFR2 activities, one compound of particular note was the imidazo[1,2-*a*]pyridine derivative **A** bearing a 2-pyridone ring, which strongly inhibited enzyme activities of both c-Met and VEGFR2 (IC_{50} = 1.9, 2.2 nM). It potently suppressed proliferation of c-Met-addicted MKN45 cells and VEGF-stimulated human umbilical vein endothelial cells (GI_{50} = 5.0, 1.8 nM). Compound **A** exhibited dose-dependent anti-tumor efficacy in a MKN45 mouse xenograft model (T/C = 4% at a dose of 5 mg/kg qd). In this poster, details of the design, synthesis, and biological evaluation of the imidazo[1,2-*b*]pyridazine and imidazo[1,2-*a*]pyridine derivatives will be presented.

MEDI 288

Cytotoxic conjugates of Disorazol Z with the LHRH receptor agonistic peptide D-Lys^6-LHRH as approach to targeted therapy of LHRH-R positive tumors

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Background

In search for innovative treatment options we are investigating the highly potent natural compound Disorazol Z as a novel cytotoxic component in a drug-targeting approach for the treatment of LHRH receptor overexpressing cancers. Different Disorazol Z conjugates with D-Lys^6-LHRH as LHRH receptor targeting moiety have been subjected to preclinical evaluation. Here we present the selection of the preclinical development candidate AEZS-138 based on in vitro characterization and with respect to PK/PD parameters and provide evidence that LHRH receptor targeting contributes to the mechanism of action.

Results

Disorazol Z is a potent tubulin binding agent with outstanding cellular cytotoxicity with EC_{50} values in the subnanomolar range. For all Disorazol Z - D-Lys^6-LHRH conjugates,

the tubulin binding activity compared to Disorazol Z was either unaltered or just slightly diminished. LHRH receptor binding and activation mediated by the targeting peptide D-Lys^6-LHRH was attenuated by conjugation, but still in the low nanomolar EC_{50} range. The conjugates showed varying cytotoxic activity from low nanomolar to three digit nanomolar EC_{50}values. Comparison in ovarian and endometrium cancer xenograft models revealed potent inhibition of tumor growth for the conjugates, whereas equimolar dosing of Disorazol Z failed to reach statistical significance. PK analysis showed substantial plasma levels for the conjugates with only minor release of Disorazol Z, pointing to stabilization by conjugation and demonstrating reasonable half-life of the intact conjugates as prerequisite for tumor targeting. The tumor models employed have been analysed regarding LHRH-R positivity by mRNA in situ hybridisation.

Conclusions

All Disorazol Z – D-Lys^6-LHRH conjugates analysed so far demonstrate a high potential regarding the treatment of LHRH receptor positive tumors. Based on in vitro characterization and with respect to PK/PD parameters AEZS-138 has been chosen as preclinical development candidate. Preclinical development of AEZS-138 was initiated in Q2/2013.

MEDI 289

Design and synthesis of potential 5α-reductase inhibitors for the management of benign prostaic hyperplasia

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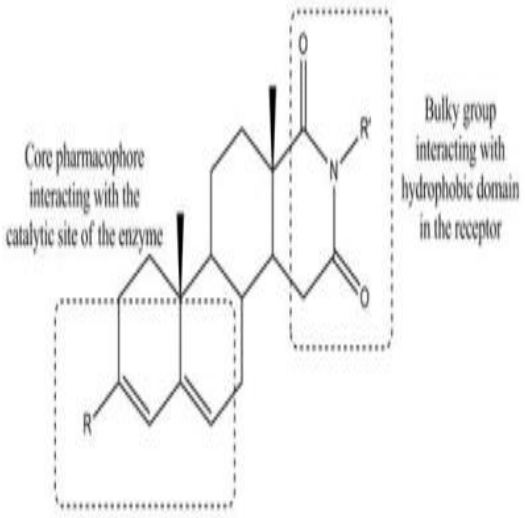
Human steroid 5α-reductase is a NADPH dependent enzyme that catalyzes the irreversible conversion of 4-en-3-oxo-steroid testosterone to the more potent 5α-H-3-oxo-steroid dihydrotestosterone which is involved in several disease states such as acne, hirsutism and benign prostatic hyperplasia. In the present study we have designed some potent inhibitors of the enzyme using 3 D QSAR CoMFA & CoMFA approach. The designed molecules were further synthesized using appropriate strategy. The 3D-QSAR models were developed using CoMFA and CoMSIA methodologies using a series of 4-azasteroid inhibitors. The developed models were found to be reliable and significant with good predictive r2 value. The contour plots obtained has shown a favourable effect of bulkier groups at C-17 position. Further on the basis of results obtained we have synthesized the molecules keeping in mind to increase the bulk at C-17 postion which would certainly enhance the enzyme inhibition by binding into the hydrophobic pocket. The synthesized compounds were characterized using melting point, IR, ^{1}HNMR, ^{13}C NMR etc. The general structure of designed and synthesized compounds in the figure 1



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MEDI 290

Cell-based studies of new prostate cancer inhibitors

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Prostate cancer is one of the most common forms of neoplasia in men, especially over 40 year-old. This cancer is hormone-dependent, being androgen receptor (AR) activation the triggering cellular signaling for cell growth and cancer development. Despite the existence of some anti-AR molecules (bicalutamide, flutamide, nilutamide) the therapeutic approaches are limited, given the opportunity to the discovery and development of novel compounds, like MDV3100 (enzalutamide) which is efficacious in cancer bearing mutated AR.[1] Besides the androgen-driven growth, metastatic prostate cancer can be resilient to androgen ablation therapy. Many approaches are underway to discover bioactive molecules besides anti-AR compounds, which include kinase and survivin inhibitors.[2] Selective and mixed kinase inhibitors are now under evaluation for prostate cancer, among them anti-mTOR molecules.[3]

The approach of this work consisted on the in silico studies of molecules by ligand and target-based virtual screening. Fifty molecules were acquired for in vitro evaluation of the potential anticancer activity in three cell lines. This report is going to present the biological results of novel AR inhibitors in cell-based assays using LNCaP (AR-dependent) DU-145 and PC-3 (AR-independent) that are leading to SAR analyses.

1. Tran, C. et al. Science 2009, 324, 787

2. Nakahara, T. et al. Cancer Res. 2007, 67, 8014

3. Hsieh, A.C. et al. Nature 2012, 485, 55

MEDI 291

Functionalization and modification of 2-hydroxymethyl-5, 8-dimethoxy-1, 4-naphthaquinone as HER2 inhibitors

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HER2 overexpression in breast cancer tumors predicts lower overall survival. Because of the aggressive nature of HER2 tumors and the association with metastatic disease, the HER2 receptor holds great promise as a therapeutic target in metastatic breast cancer. We are developing small molecule inhibitors that bind to the ATP binding site of the tyrosine kinase domain in order to inhibit tyrosine autophosphorylation. This process controls biological pathways that mediate the cell growth. In normal cells this process is highly controlled. We are targeting the modification of the side chain of the hydroxymethyl group of 2-hydroxy methyl-5, 8-dimethoxy-1, 4-naphthaquinone. These compounds should inhibit the tyrosine kinase cascade of reactions thereby suppressing the overexpression of HER2 shutting down the tumor growth. In this poster, we will present the inhibition and synthesis of a series of 2-hydroxy methyl-5, 8-dimethoxy-1, 4-naphthaquinone analogs.

Acknowledgement

This work was supported by

Department of Defence Breast Cancer Research Program, Partnership Training Award (W81XWH11-1-0105) and Western Kentucky University.

MEDI 292

Structural variations on anthranillic amides and their effect on mesenchymal transition

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Extracellular stress and mitogens activate mitogen activated protein kinase (MAPK) pathways that mediate various intracellular events including cell differentiation, cell proliferation, and cell death. The MEK-5/ERK-5 pathway is up-regulated in breast and prostate cancer and is involved in tumor progression and development of resistance. While screening a series of compounds for inhibition of MEK5 activity and proliferation/viability, we observed a concomitant reversal of the epithelial-to-mesenchymal transition (EMT) by one of our compounds. Consistent with the acquisition of a more epithelial morphology, qPCR analysis confirmed an increased in E-Cadherin gene expression and altered expression of associated EMT related genes. We will present how structural variations on anthranillic amides modify this transition.

MEDI 293

Use of core modification to identify a new series of mTOR kinase inhibitors and CC214-2, an orally available, selective inhibitor of mTOR kinase

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The mammalian target of rapamycin (mTOR) is a highly conserved serine/threonine protein kinase that regulates cell growth, metabolism, proliferation and survival by integrating growth factor signaling with cellular nutritional status and energy use. The PI3K/Akt pathway is frequently mutated in many cancers, leading to hyperactivation of mTOR signaling,making mTOR an attractive drug target. Rapamycin analogs such as temsirolimus and everolimus, which target only the mTORC1 complex, have shown some clinical activity. It is hypothesized that mTOR kinase inhibitors, blocking both mTORC1 and mTORC2 signaling, should have expanded therapeutic potential. We report here the discovery of a novel series of selective mTOR kinase inhibitors and the identification of CC214-2, a compound with demonstrated anti-tumor activity upon oral dosing in a PC3 prostate cancer xenograft model. A series of 4,6-disubstituted-3,4-dihydropyrazino[2,3-b]pyrazine-2(1H)-ones were discovered through a core modification of our original compound series. Analogs from this series have excellent mTOR potency and maintain selectivity over the related PI3Kα lipid kinase. Compounds such as CC214-2 were found to block both mTORC1(pS6) and mTORC2(pAktS473) signaling in PC3 cancer cells, in vitro and in vivo.

MEDI 294

Total synthesis of tubulysins and their folate-receptor targeting conjugates: A new chemical reaction leads to analogs with enhanced cytotoxicity

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Tubulysins are natural products isolated from myxobacterial species and are mitotic poisons which exceed the cell growth inhibition of any clinically relevant traditional chemotherapeutic. Structurally, tubulysins are linear tetrapeptides comprised of *N*-methyl pipecolic acid (Mep), isoleucine (Ile), the novel amino acid tubuvaline (Tuv), and the novel tyrosine analogue tubutyrosine (Tut). All isolated tubulysins possess an acid- and base-sensitive *N*-acyloxymethyl substituent not previously found in nature. This *N,O*-acetal of formaldehyde is attached as a side chain to the amide *N*-atom of the Tuv fragment. The isolation of natural tubulysins from culture extracts provides only limited quantities. Recently, we reported a large scale total synthesis of natural tubulysin B. Among the multiple synthetic and stereochemical challenges, the most striking were: a) the incorporation of the labile *N*,*O*-diacyl *N,O*-acetal and b) the regioselective hydrolysis of the *C*-terminal methyl ester (OMe) in the tripeptide Mep-Ile-Tuv-OMe.

Herein, we present a convergent total synthesis of tubulysin analogues incorporating an alkoxymethyl side chain. Such molecular architecture allows for compounds that are more base and esterase inert, thus providing additional metabolic/catabolic stabilization. The key step in our synthetic strategy relies on use of dibutyltin oxide for the efficient conversion of the *N*-acyloxymethyl substituent to the novel side chain groups with concomitant facilitated hydrolysis of the *C*-terminal methyl ester. Following LiOH-based hydrolysis of the *C*-terminal ester, the Tut fragment is added resulting in novel tubulysin analogues. This novel process opens the door to tubulysin analogs with improved potency for treating cancer.

MEDI 295

Novel water soluble N-mustard-benzene conjugates with potent antitumor activity, synthesis, and biological activity

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The solubility of compound is one of the important factors for determining the success of the agent during drug development. We have previously reported a series of water-

soluble N-mustards-benzamide conjugates having hydrophilic side-chain at the *meta*- or *para*-position of the carboxamide group via a urea spacer. Of these derivatives, BO-1055 HCl exhibits a broad spectrum of antitumor activity and potent therapeutic efficacy against various human solid tumor xenografts. Recently, we have synthesized a series of novel water soluble *N*-mustard-benzene conjugates prepared by linking phenyl *N*-mustard pharmacophore with benzene moiety through urea linker. The benzene ring bears a variety of ω-*N*,*N*-dialkylaminoalkylamide or ω-cyclicaminoalkylamide side-chains located to the *meta*- or *para*-position of the urea linker. The tertiary amino function on the side-chain can be converted into a variety of water-soluble salts with various acids. The newly synthesized derivatives were subject to evaluate their antitumor activities both in vitro and in tumor xenograft model. The results showed that these conjugates exhibit a broad spectrum of antitumor activity against variety of human leukemia and solid tumor cell growth in culture. Among these derivatives, **BO-2094** was selected for further antitumor evaluation. The results revealed that this agent exhibited potent antitumor activity several human tumor xenografts in animal model. Studies on the mechanism of action revealed that **BO-2094** is able to induce DNA cross-linking and arrest cell cycle at G2/M phase. The present studies suggest that **BO-2094** is a promising candidate for preclinical antitumor studies.

MEDI 296

Bioconjugation strategies for improving pharmacokinetics of MTI-101 for the treatment of multiple myeloma

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Myeloma (MM) is an incurable malignancy due to unsuccessful elimination of minimal residual disease (MRD). Current standard chemotherapeutic treatments that target apoptotic cell death pathways have proven to be unsuccessful in curing this disease, due to emergence of drug resistance. Recently Hazlehurst and co-workers have reported an all d-amino acid peptide HYD1 that induces necrotic cell death in MM. Several strategies aiming to enhance the potency of this linear d-amino acid peptide led to the discovery of a novel cyclic peptide we named MTI-101. MTI-101 and its analogs bind to the extracellular domain of CD44 and kills MM cells as a single agent via a necrosis pathway. According to surface plasmon resonance experiment MTI-101 binds to an ectodomain of CD44 with high affinity ($K_D \sim 10$ nm). To optimize the PK of MTI-101, we have identified a site for peforming bioconjugation of pegylating reagents and specific multimers of MTI analogs. Herein, we report several bioconjugation strategies that aim to increase the circulating half-life of peptide MTI-101 analogs for the treatment of MM.

MEDI 297

Synthesis and biological evaluation of substituted 3,5-diaryl indole-5-carboxylic acid derivatives as anticancer agents

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Indole represents one of the most important pharmacodynamic nucleus which has been actively explored in discovery and development of new drugs. Indole-based compounds exhibit broad range of biological properties viz., anti-inflammatory, anticonvulsant, cardiovascular, anti-HIV, antibacterial etc. In our efforts of designing new compounds, we synthesized a series of novel 3,5-diaryl indole-5-carboxylic acid derivates. These compounds were screened on National Caner Institute's 60 human cancer cell line panel which covers diverse histologies. A subset from this chemical library when tested in dose dependent manner showed anti-cancer activity against multiple cell lines. To understand the mechanism of activity, we performed further studies on the non-small cell lung cancer 'A 549 cells'. The in vitro assay using Annexin V-FITC showed early apoptosis. The immunofluorescence test showed reduction in Ki-67 proliferation marker but significant increase in phosphor P38 MAPK. This suggests new compounds to induce apoptosis in A549 cells by decreasing cellular proliferation and activation of P38 MAPK, ultimately leading to DNA fragmentation.

MEDI 298

Prostate-specific membrane antigen targeted tubulysin conjugates for cancer therapy

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Prostate-specific membrane antigen (PSMA) is a cell-surface marker for prostate cancer. Recent findings suggest that PSMA is also abundantly expressed on newly formed blood vessels which supply most non-prostatic solid tumors; including lung, colon, breast, renal, liver and pancreatic carcinomas, but not on normal vasculature. These studies have provided solid support for the concept of PSMA-targeted cancer therapy. Here we report the design and synthesis of a series of PSMA-targeted tubulysin conjugates. Structural optimization is also discussed based on SAR studies.

MEDI 299

3,4-diaryl substituted isoxazole and pyrazole derivatives: Synthesis and evaluation of antitumor activity

*Vineet Kumar[1], Pradipkumar Chandubhai Patel[2], D. Kishore[2], Nitin Joshi[3], Shahdeep Kaur[3], Rinti Banerjee[3], Bhavani Singh[2], **Sanjay V. Malhotra**[1], malhotrasa@mail.nih.gov. (1) Laboratory of Synthetic Chemistry, SAIC-Frederick Inc., Frederick National Laboratory for Cancer Research, Frederick, MD 21702, United States (2) Department of Chemistry, Banasthali Vidyapith, Banasthali, Rajasthan 304022, India (3) Department of Biosciences & Bioengineering, Centre for Research in Nanotechnology & Science, Indian Institute of Technology Bombay Powai, Mumbai, Maharashtra 400076, India*

Derivatives of Isoxazole and pyrazole have played important role as synthon and pharmacophore in the design and discovery of new compounds for diverse biological applications. Compounds derived from these heterocycles have exhibited anti-viral, antitumor, antibacterial, anti-inflamatory, analgesic, fungistatic, and anti-hyperglycemic activity. In our efforts of designing new compounds containing both these core structures, we synthesized a series of 3,4-diaryl substituted isoxazole and pyrazoles. These compounds were screened for anticancer activities on 60 human cancer cell lines which represent leukemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostate and breast caner histologies. Some of the compounds showed activity against multiple cell lines at nano- and/or low micromolar concentrations. To understand the mechanism of their action, the experiments in vitro assay on non-small cell lung cancer 'A 549 cells' using Annexin V-FITC indicated early apoptosis. While, the immunofluorescence test showed reduction in Ki-67 proliferation marker but significant increase in phosphor P38 MAPK. This suggests new compounds to induce apoptosis in A549 cells by decreasing cellular proliferation and activation of P38 MAPK, ultimately leading to DNA fragmentation.

MEDI 300

Discovery of oral allosteric AKT kinase inhibitors for the treatment of cancer

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AKT or protein kinase B (PKB) is a serine/threonine kinase that plays an important role in signaling within the phosphatidylinositol-3 kinase (PI3K) pathway. Activation and deregulation of the PI3K pathway are common to many cancers and contribute to cell survival, proliferation and growth. Therapies directed against several targets in this pathway have been the focus of intense research and AKT inhibitors could have broad utility in the treatment of cancer. Enhanced AKT activity has been correlated to cancer drug resistance and survival in a number of human cancers. A major challenge in developing ATP-competitive kinase inhibitors for AKT has been a lack of kinase

selectivity due to the highly conserved nature of the ATP-binding site. An alternate strategy to achieve excellent kinase selectivity has been the development of allosteric AKT kinase inhibitors. Previous publications from these labs have described the discovery of allosteric AKT inhibitors with potent *in vitro* and *in vivo* activity in xenograft models of human cancers after parenteral administration.

The identification of oral allosteric AKT inhibitors suitable for clinical development will be presented. The key structural modifications that led to improved physical properties and oral bioavailability over earlier leads involved truncation and optimization of the central core. Ultimately these efforts led to the identification of MK-2206, the first oral allosteric AKT kinase inhibitor that is currently in Phase 2 clinical trials in cancer patients.

MEDI 301

Discovery of BMS-871: A potent Notch inhibitor as an anticancer agent

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The Notch signaling pathway regulates cell fate decisions and many other cellular properties, such as proliferation, apoptosis, stem cell self-renewal and angiogenesis. Activation of Notch signaling pathway has been implicated in the pathogenesis of various solid tumors. Thus, Notch inhibition is an attractive anticancer strategy. We have identified a novel series of benzodiazepines with succinamide side chains as potent Notch inhibitors. BMS-871 demonstrated excellent *in vitro* potency and robust *in vivo* antitumor activity in TALL-1 Leukemia and MDA-MB-157 triple negative breast cancer xenograft models. The chemical synthesis and structure-activity relationships for this series as well as pharmacokinetics and *in vivo* efficacy data for select lead compounds will be presented.

MEDI 302

Design and synthesis of novel curcumin analogs for antiprostate cancer

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A series of curcumin analogues has evolved within the last decade, leading to the attention of synthesizing and SAR (structure activity relationship) modification on favored regions a key component on the natural compound. Curcumin isolated in rhizomes from the plant *curcuma longa*, has shown to be a dietary supplement and

often as food coloring due to its yellow pigment. Curcumin analogues with modifications on the phenyl rings and beta-diketone region have shown to be potential anti-prostate cancer agents on androgen receptors. We designed and synthesized over 20 new analogues classified into 3 series: heterocyclic analogues, hydrophobic analogues, and hydrophilic analogues all having varying substituents in the different regions. These newly synthesized compounds were tested for cell viability on prostate cancer cells and molecular docking studies were performed to confirm the binding site of the androgen receptor. This study was designed not only to establish novel synthesize curcumin analogue for anti-tumor activity but also to provide evidence that these newly synthesized analogues may be implemented in the treatment of other types of cancer because of its scaffold modification.

MEDI 303

Kinesin spindle protein (KSP) inhibitors with carbazole and diaryl amine scaffolds

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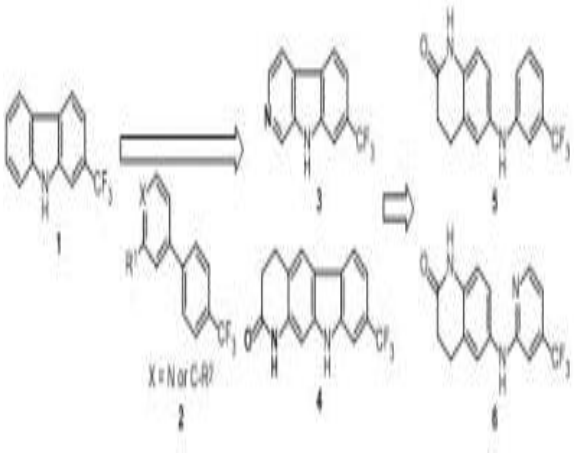
The kinesin spindle protein (KSP) is a motor protein which is essential in the bipolar spindle formation and maintenance during mitosis. Because KSP inhibition induces mitotic arrest in prometaphase without affecting microtubules, resulting in apoptotic cell death, the inhibitors could be therapeutic agents with reduced side effects for the treatment of malignant tumors. Recently, we reported that substituted carbazole such as **1** is a core scaffold for KSP inhibitory activity.[1)] The structure–activity relationship studies of the scaffold combined with the known biaryl-type KSP inhibitors **2** also demonstrated that b-carboline **3** and lactam-fused carbazole **4** exhibited potent KSP inhibition and cytotoxicity.[2)] However, the low aqueous solubility rendered these derivatives undesirable development candidates. In an attempt to overcome this problem, various diaryl amine derivatives such as **5** and **6** were designed and synthesized. Evaluation of bioactivity and physicochemical properties of these derivatives revealed that positively charged and nonplanar diaryl amine structure is a promising novel scaffold for KSP inhibitors with improved aqueous solubility.



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MEDI 304

Novel methodology for the synthesis of methyl substituted 4-(4'-methoxyphenylamine)-5, 6-dihydropyrrolo[2,3-*d*]pyrimidines as water-soluble microtubule targeting agents that circumvent multiple drug resistance

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Intrinsic and acquired multidrug resistance (MDR) is a limitation in the clinical efficacy of many anticancer drugs including those that target microtubules. We previously reported water soluble antitubulins N-(4'-methoxyphenyl)-N, 2, 6-trimethyl cyclopenta[*d*]pyrimidin-4-amine **1** (IC_{50}=17 nM) and N-(4'-methoxyphenyl)-N, 2-dimethyl pyrrolo[2,3-*d*]pyrimidin-4-amine **2** (IC_{50}=183 nM) as inhibitors of the proliferation of human cancer cells (MDA-MB-435) in culture, as well as parental and Pgp expressing SKOV-3 cell lines (IC_{50} 38.6 nM and 278 nM respectively). In this report we present the novel synthesis and biological activities of the 5, 6-dihydropyrrolo[2,3-*d*]pyrimidine scaffold as cytotoxic agents. This scaffold possesses both the flexibility of the fused cyclopenta ring in **1** and the hydrogen bonding ability of the fused pyrrole ring in **2** . The novel synthesis and cytotoxic activities of methyl and desmethyl substituted 4-(4'-methoxyphenylamine)-5, 6-dihydropyrrolo[2,3-*d*]pyrimidines will be presented and discussed.

MEDI 305

Triazole-based inhibitors of geranylgeranyl transferase II

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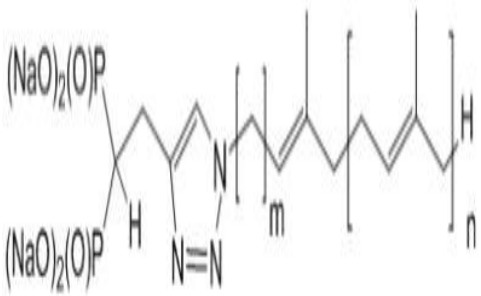
Addition of the 20-carbon geranylgeranyl chain to Rab protein family members is catalyzed by the enzyme geranylgeranyltransferase II (GGTase II or RabGTase), and is essential for proper cellular localization of these important proteins. In an effort to develop new inhibitors of this enzyme, we have prepared a number of triazole derivatives bearing a geminal bisphosphonate to mimic the pyrophosphate of geranylgeranyl diphosphate, a triazole moiety that may complex with the active site zinc, and an isoprenoid chain that might occupy the distal hydrophobic pocket. While triazoles substituted with true isoprenoids (e.g. **1**) have been obtained as mixtures of olefin isomers, the homoisoprenoid series (e.g. **2**) can be prepared as single olefin isomers. The synthesis of these compounds and their activity as inhibitors of GGTase II will be presented.



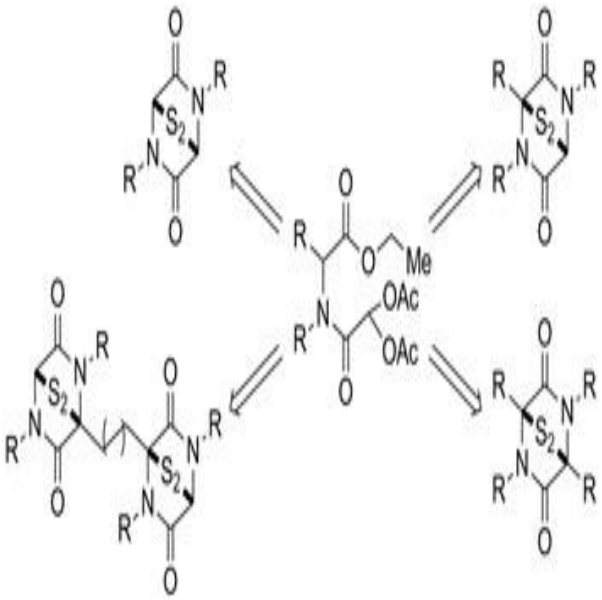
1 m = 1, n = 2
2 m = 2, n = 2

MEDI 306

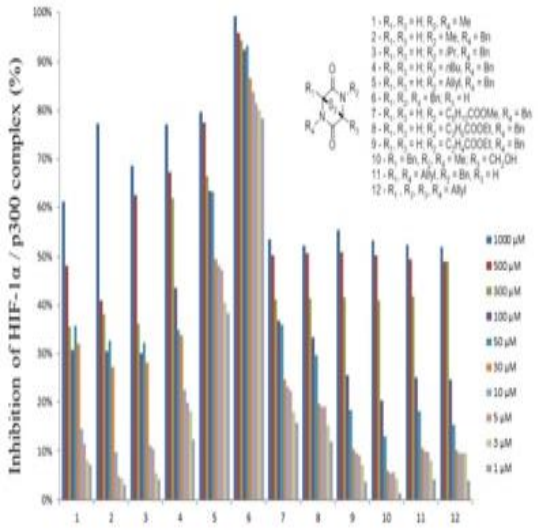
ETPs as anticancer agents: Synthesis and biological activity

Bruno C. Sil*, bruno.santos.11@ucl.ac.uk, Stephen T. Hilton.Department of Pharmaceutical and Biological Chemistry, UCL School of Pharmacy, London, London WC1N 1AX, United Kingdom*

Natural products containing sulfur represent a promising group of chemo-preventives showing both *in vitro* and *in vivo* antiproliferative effects. One of these family of natural products, the epi-3,6-dithio-2,5-diketopiperazines (ETPs), are a group of structurally diverse compounds defined as containing one or two sulfur bridged diketopiperazine rings. Our newly developed simple approach to synthesise and functionalize these molecules enabled us to obtain a library of compounds (Figure 1).



Hypoxia Inducible Factor-1 (HIF-1) is a transcription factor that regulates the expression of a large number of genes, including those responsible for cell survival. Under normal oxygen conditions, levels of HIF-1α are tightly regulated but in a deficient oxygen environment found in many tumours, HIF-1α accumulates causing transcription activation and hence tumour growth. Inhibition of protein-protein interactions associated with the *locus* where ETPs express their biological activity – the HIF-1α / p300 co-activator complex – highlighted the potent activity of these compounds (Figure 2).



MEDI 307

JS-K as a broad-spectrum anticancer agent: Lead optimization strategies

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JS-K is an arylated diazeniumdiolate of structure ArON=N(O)-NR_2 (Ar = 2,4-dinitrophenyl, NR_2 = 4-[ethoxycarbonyl]piperazin-1-yl) currently in late stage development as a promising anticancer agent. JS-K has demonstrated in vivo activity in rodent models of prostate cancer, leukemia, liver cancer, multiple myeloma, lung cancer, and glioma. Its mechanism of action is initiated by S_NAr attack of cellular nucleophiles such as glutathione (GSH), leading to irreversible consumption of reducing equivalents and production of the $^-$ON=N(O)-NR_2 anion. The latter species spontaneously hydrolyzes to generate two molecules of cytolytic nitric oxide (NO). Here we detail our recent efforts to improve on this lead. One direction involves adding a second ON=N(O)-NR_2 group meta to the first, doubling the NO payload and providing an additional route to GSH consumption in the form of cross-linking glutathionylation. To increase the drug's lifetime in the blood, we have replaced one or the other of the nitro groups with cyano. Transition state modeling has led to second-generation structures that are preferentially metabolized by glutathione S-transferase (GST) isoform GSTP1, which is overexpressed in many cancers thus leading to selective activity against the tumor cells relative to their normal counterparts. Finally, drug combinations are being

systematically studied for possible synergies in pursuit of improved activity. Of special interest is a hybrid molecule we have developed, which on metabolism generates both NO and a poly-ADP ribose-polymerase (PARP) inhibitor.

MEDI 308

Optimization of benzoic acid derived inhibitors of the cyclin groove of CDK2 using the REPLACE strategy

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The cyclin dependent kinases (CDKs) play a significant role in controlling cell proliferation. Inhibition of CDK2/Cyclin A in G1/S phase of the cell cycle leads to selective apoptosis of cancer cells. Currently available CDK inhibitors primarily target the ATP binding site which is conserved across the family of 516 protein kinases and targeting this site can lead to cross reactivity, side effects and toxicity. In our current study, we are utilizing an alternative approach to inhibit only cell cycle CDKs by targeting the protein-protein interaction on the Cyclin binding groove (CBG) which is distinct from the ATP binding pocket. The CBG is a hydrophobic groove found only in cell cycle CDKs (Cyclin A, Cyclin D and Cyclin E) through which cyclins recruit substrates and inhibitory proteins. CBG is recognized by a sequence of amino acids present in CDK substrates and potent and selective inhibition of CDK2 is achieved through the octapeptide, HAKRRLIF. In this study, the N-terminal tetrapeptide was replaced by benzoic acid derivatives using the REPLACE (REplacement with Partial Ligand Alternatives through Computational Enrichment) strategy. Based on these, a series of fragment ligated inhibitory peptides (FLIPs) capable of retaining the majority of the interactions of the parent peptide's N-terminal region were synthesized. Benzoic acid derived FLIPs substituted with basic groups interacting with Glu224 of cyclin A, were among the potent derivatives in the series with IC_{50} values of less than 10μM. The small molecule N-caps were designed using the LigandFit docking method in Discovery studio 3.5, FLIPs were synthesized by ligating the N-caps to the peptide sequence by solid phase peptide synthesis and further tested for their potency in a fluorescence polarization competitive binding assay. The REPLACE strategy has been successfully applied for the development of more drug-like and non-ATP competitive CDK2 inhibitors as anti-tumor therapeutics.

MEDI 309

Design and synthesis of cyclopropylamide analogs of combretastatin-A4 as novel microtubule-stabilizing agents

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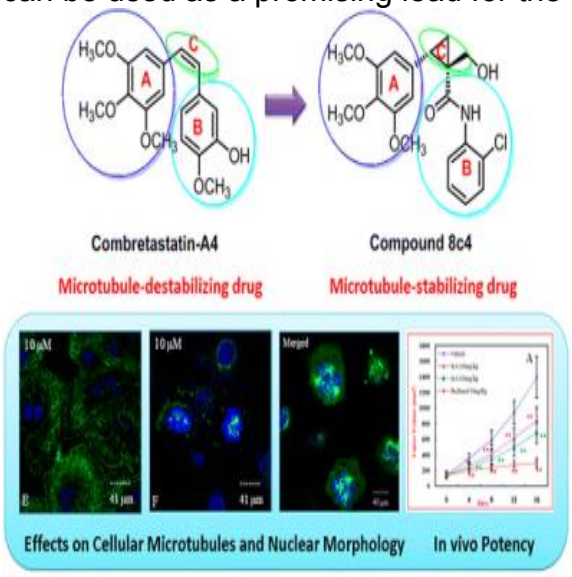
A series of novel cyclopropylamide analogues of combretastatin-A4 (CA-4) were designed and synthesized. Most of them had significant in vitro antiproliferative activities, particularly for compounds 7i4, 7c4, 8a4, and 8c4. Moreover, compound 8c4 was also equally potent against paclitaxel resistant cancer cells. Interestingly, the novel cyclopropylamide analogues had different binding mechanisms from CA-4. Instead of inhibiting tubulin polymerization, these CA-4 derivatives were able to stimulate tubulin polymerization. Flow cytometry revealed that compound 8c4 arrested A549 cancer cells in the G2/M phase and resulted in cellular apoptosis. Further immunofluorescence assays revealed that compound 8c4 induced mitotic arrest in A549 cells through disrupting microtubule dynamics. In addition, compound 8c4 also effectively inhibited the tumor growth in the A549 xenograft model without causing significant loss of body weight. Compound **8c4** represents a novel class of microtubule-stabilizing agent and can be used as a promising lead for the development of new antitumor agents



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MEDI 310

Synthesis and biological study of boron-containing alkene derivatives as potential therapeutics for the treatment of mantle cell lymphoma(MCL)

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Mantle cell lymphoma (MCL) is an aggressive and incurable B cell malignancy accounting for 5% of non-Hodgkin's lymphomas. Current treatment for MCL includes multi-agent chemotherapy such as CHOP or Hyper-CVAD4 with stem cell transplantation in selected patients, but prognosis for most patients remains poor, with median survival around 2-4 years. The exact mechanism of lymphoma genesis in MCL is not known, but it is believed that MCL arises from naïve B cells in the mantle zone of lymph node follicles and is characterized by the chromosomal translocation t(11,14) leading to over-expression of CCND1. However, murine models over-expressing CCND1 in the absence of other oncogenes including MYC do not develop lymphoma, implying that additional mechanisms may be involved in lymphoma genesis in MCL. Based on the literature, in the context of an ongoing chemical biology project, we focused on the Transforming Growth Factor-beta (TGF-beta) pathway, considering a possible biological mechanism involving cross-talk between TGF-beta and Notch1 signaling pathways for genesis of MCL. Based on our previous work implicating stilbene derivatives in modulating TGF-beta signaling, we synthesized boron-containing stilbene derivatives and tested their biological activity as potential therapeutic agents for MCL. From preliminary screening in cell-based assays using HBL-2 and Jeko-1 cell lines, we found that compound 1g[Figure1] specifically inhibits cell growth (IC50 6.4 μM). We are in the process of further derivatizing our lead hit compound 1g to analyze the mechanism of action including apoptosis assays and changes in expression of Notch signaling components and downstream Notch1 regulated genes. These pinacolylboronate-substituted alkene derivatives may be developed as potential therapeutic agents for MCL.

MEDI 311

Design, synthesis, and biological evaluation of substituted monocyclic pyrimidines with dual antiangiogenic and cytotoxic antitubulin activities as antitumor agents

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Microtubules play a major role in mitosis, and this makes microtubule disrupting agents like paclitaxel highly active cancer chemotherapeutic agents. Resistance due to overexpression of Pgp and/or β-III-tubulin severely limits the clinical utility of these drugs as anticancer agents. The combination of cytotoxic antitubulin activity with

cytostatic antiangiogenic activity might provide agents with chemotherapeutic potential which in turn could circumvent or delay tumor cell resistance. Such dual acting agents also need not be highly potent so as to circumvent serious toxicity issues compared with potent cytotoxic agents. We reported analogs with the pyrrolo[3,2-*d*]pyrimidine scaffold with such dual activities. To simplify this scaffold, conformationally flexible substituted monocyclic *N*-methyl-4-methoxyanilino pyrimidines were designed with tubulin inhibition (cytotoxic) as well as VEGFR-2 kinase inhibition (antiangiogenic) in single molecules. The design, synthesis and structure activity relationship of these agents will be presented.

MEDI 312

Discovery and SAR exploration of a novel series of [1,2,4]triazolo[1,5-a]pyridines as potent and selective RSK inhibitors

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The 90 kDa ribosomal S6 kinase (RSK) family of proteins is a highly conserved group of serine/threonine protein kinases that regulate cell growth, cell motility, cell survival and cell proliferation. RSK1, one of four human isoforms, is amplified in breast and prostate tumors and implicated in other human diseases. We report here a novel series of [1,2,4]triazolo[1,5-a]pyridines, identified through structure based optimization of a high throughput screening hit, that are potent and selective RSK inhibitors. Synthesis, SAR, and x-ray crystallographic analysis for this series will be described.

MEDI 313

Structure-based drug design of pyruvate kinase inhibitors

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Pyruvate kinase (PK) is an important enzyme in the glycolytic pathway that catalyzes the conversion of phosphoenolpyruvate to pyruvate. One of the isoforms, PKM2, has been shown to have an important role in cancer cell metabolism and growth. As a result, there has been interest in developing inhibitors of this enzyme. We utilized a structure-based design approach to identify novel inhibitors of PKM2. Our design

strategy started with the crystal structure of PKM2 with the bound ligand, FBP. We developed a docking model and analyzed the interaction of several known inhibitors, which allowed us to propose a modification that would presumably interact better with the binding site. This modified designed was used as a reference structure in a two-stage similarity search of the NIH Pubchem. In the first stage, there were approximately 39,000 structures identified and this was reduced to 1,314 in the second stage. We identified several compounds having high scores and consistent binding modes, which led to the selection of two cores. After proposing a viable synthesis, virtual libraries were created for each of those cores and then evaluated in the docking model. Workflow software was utilized to process the docking scores, filter by ADME properties, and check for a match to a 3D query based on the binding site. Three novel compounds were initially synthesized and one was found to have an IC50 on a similar scale to the known inhibitor. In an effort to improve on the design of these and other novel inhibitors, we identified the need to incorporate protein flexibility in the docking model. We will report on the design, synthesis, and testing of these compounds for inhibition of PK activity.

MEDI 314

Synthesis of NAM, NAS, and NAP based telomerase inhibitors

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Telomerase is a reverse transcriptase enzyme that adds a specific DNA

sequence to the ends of the chromosomes. There are experimental evidences that

telomerase is overly expressed in almost 90% of human cancers but not in normal

somatic cells. This selective activity of telomerase in cancer cells makes it a

potential therapeutic target to treat cancer disease.

Acridine derivatives are one of the oldest and most successful classes of bioactive

agents. Recent studies showed that some of the acridine derivatives such as

amsacrine and nitracrine have the anticancer activity. Here in we are presenting

the synthesis of NAM (N-Acridyl Maleimide), NAS (N-Acridyl Succinimide), and NAP (N-Acridyl

Phthalimide) derivatives as anticancer drugs.

MEDI 315

Structure-based drug design and synthesis of salt-inducible kinase 2 (SIK 2) inhibitors

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Salt-Inducible Kinase 2 (Sik2) is a centrosome kinase that has recently been reported as a potential target for therapy in ovarian cancer. As there was no structure available for SIK2 at the time of this work, we generated a three plausible homology models based on a single template, Mark3PAR-1. Kinase targeted libraries from several vendors were screened against these homology modes, but this resulted in very few structures that could adopt typical kinase binding modes. This led us to seek an alternative strategy for the designs. Aurora A kinase was identified as a possible structural template during the homology building process and one series of inhibitors of that kinase were selected and utilized as a starting point for design of Sik2 inhibitors. There appears to be less room available in the ATP binding site of Sik2 and many of the attached groups for the Aurora inhibitors would tend to bump into residues if not modified substantially. Therefore, one side of the structure was reduced in size so that it would better interact with nearby residues. A library was generated based on the modified template and the enumerated library was docked using Surflex and Glide XP with MM-GBSA post-processing. The resulting scores and structures from docking were fed into a workflow that combined the results and calculated ADME style parameters for each compound. A series of filtering cutoffs led to the top scoring compounds. Both docking programs led to reasonable binding modes expected of typical kinase inhibitors. There were eleven compounds that met the analysis criteria and five of those were synthesized and tested for inhibition of phosphorylation of SIK2. The design, synthesis, and biological testing of these compounds will be reported.

MEDI 316

Steric induced conformational restriction: Design, synthesis, and biological evaluation of novel pyrrolo[3,2-*d*]pyrimidines as water soluble antitubulin agents with antiangiogenic and antitumor potential

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One of the major limitations of cancer chemotherapy is multidrug resistance (MDR), which reduces the efficacy of many microtubule disrupting agents, including the taxanes and vinca alkaloids. A study involving the binding modes of agents with the potential to inhibit multiple receptor tyrosine kinases led to the discovery of a series of highly potent antimitotic agents that bind to the colchicine site on tubulin. These compounds are able to overcome clinically relevant modes of drug resistance to other microtubule disrupting agents, including overexpression of the P-glycoprotein (Pgp) drug efflux pump and the βIII-isotype of tubulin. Several strategies of conformational restriction were adopted in an attempt to optimize their microtubule specific effects. Analogs of the pyrrolo[3,2-*d*]pyrimidine lead compounds were designed by inducing conformational restriction using alkyl substituents. Molecular modeling suggested that conformational flexibility was indeed restricted. The synthesized analogs inhibited microtubule polymerization and demonstrated GI_{50} values in the nanomolar range in the NCI60 human tumor preclinical drug screen. The enhanced aqueous solubility of these compounds and their ability to overcome Pgp and βIII-tubulin mediated drug resistance mechanisms afford advantages over currently used antimitotic drugs. The compounds also showed potential antiangiogenic effects, which are possibility mediated via receptor tyrosine kinase inhibition. Single agents with both antiangiogenic and cytotoxic activities could circumvent the pharmacokinetic problems associated with delivery of multiple agents, avoid drug-drug interactions, alleviate toxicity, and delay or prevent tumor cell resistance. The design, synthesis and biological activities of these analogs will be presented.

MEDI 317

Derivation of hydroquinone to create selective oxidatively activated anticancer agents

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Cancer is a leading cause of death in industrialized nations. Reactive oxygen species are elevated in cancers such as leukemia and renal cell carcinoma. Our group sought to synthesize DNA-modifying agents activated by reactive oxygen species, which would increase selectivity and retain high cytotoxicity. Cell studies confirm that our agents are over three times more selective for leukemia cancer cells than for normal blood cells. These agents contain a nucleophile tethered to an oxidizable hydroquinone. Due to the unique structure of the molecule, selective activation of the hydroquinone occurs in the presence of reactive oxygen which results in a highly reactive electrophilic molecule that adds to DNA. Therefore, design of clinically relevant and selective DNA damaging agents may be possible using an oxidatively activated strategy.

MEDI 318

Targeting B-cell lymphoma 6 (BCL6) for the treatment of diffuse large B-cell lymphomas (DLBCLs)

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BCL6 is the most commonly involved oncogene in diffuse large B-cell lymphomas (DLBCLs), and constitutive expression of BCL6 in GC B-cells causes DLBCL in mice. It is also frequently expressed in follicular lymphomas (FLs), and may be required for survival of these tumors as well. BCL6 binds to the SMRT co-repressor through a unique interaction mediated by the *N*-terminal BTB/POZ domain of BCL6. Peptides that mimic the SMRT interface can displace SMRT from BCL6. We have identified small molecules that bind to the BCL6 BTB domain and displace SMRT its repression complex with BCL6. These compounds specifically re-activate BCL6 target genes, kill BCL6 dependent DLBCL cells *in vitro*, suppress already established DLBCL tumors in mice, kill primary DLBCL cells from human patients *ex vivo*, and are non-toxic in animals. The design and synthesis, as well as the crystallographic analysis and biological evaluation of the new inhibitors will be detailed.

MEDI 319

Design, synthesis, and biological studies of Ru(II) complexes of some nitrogen chelating ligands

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In cancer chemotherapy, metal-based complexes have been recognized as the most promising means of inhibiting cancer growth due to the successful application of *cis*-platin and its derivatives above many of the existing organic anticancer agents. However, one of the greatest challenges that are preventing rational drug design of metal-based anticancer complexes is complexity of the mechanism of their operations due to lack of proper knowledge of their targets. In this study, some ruthenium(II) complexes have been synthesized and characterized by elemental analysis and spectroscopic studies. Computational docking methods were used to predict their most probable targets and the mechanism of their activities. The interesting features of the binding of the complexes showed that some of the complexes preferentially target specific macromolecule than the other which is an indication of their specificity and possibility of their therapeutic combination without severe side effect that may come from competition for the same target. Both experimental and theoretical results were

compared. It was found that introduction of some unusual ligands significantly improve the activities of most of the complexes studied

MEDI 320

Small molecule mediated relief of procaspase inhibition enhances the potency of cancer chemotherapeutics

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Manipulation of the apoptotic pathway has long been recognized as an effective strategy for the eradication of cancer cells. This work targets the labile zinc pool, a physiological inhibitor of apoptosis utilizing a small molecule, PAC-1. Labile zinc inhibits the conversion of procaspase-3 to caspase-3, preventing the activation of this key apoptotic executioner. Aiming to harness the potential of active caspases for the selective treatment of cancer, we developed PAC-1, a mild zinc chelator ($K_d \sim 42$ nM) to facilitate caspase activation. PAC-1 rapidly enters cells, elicits a reduction in the labile zinc pool, induces apoptotic cell death, and has shown efficacy in multiple animal models of cancer. Recently we have used PAC-1 to prime cells for more effective treatment by targeted and conventional cancer chemotherapeutics. By relieving the zinc-mediated inhibition of the procaspases, cancer cells are more responsive to cytotoxins and cell death signals. This synergistic potential and increase in potency, driven by the primed and uninhibited procaspase-3, has been demonstrated mechanistically, shown in cell culture, and validated in animal models of cancer. Thus, PAC-1, a well-tolerated compound with significant single agent anticancer activity, has the capacity to increase the efficacy of a diverse panel of standard-of-care chemotherapeutics. Procaspase priming may therefore serve as a general strategy to increase the apoptotic potential of the cell and be a general method for potentiation of chemotherapeutic agents *in vivo.*

MEDI 321

Synthesis and evaluation of small molecule inhibitors of replication protein A

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Cisplatin and carboplatin impart their chemotherapeutic effect by forming Pt-DNA adducts that block DNA replication and transcription, culminating in apoptosis. Repair of those Pt-DNA adducts via nucleotide excision repair (NER) or homologous

recombination repair (HRR) can substantially reduce the effectiveness of the Pt therapy. Thus, inhibition of these repair pathways holds the potential to sensitize resistant cancer cells to Pt treatment. While most therapies are focused on enzyme-substrate interactions, our approach addresses protein-DNA disruption. In particular, we are interested in replication protein A (RPA), a single-stranded DNA binding protein involved in the aforementioned repair mechanisms. Having identified a small molecule inhibitor (SMI) with promising activity, we are in the process of developing analogs of the lead compound to test their potential to be used in combination therapy.

MEDI 322

Madindoline A as a lead for the development of new class of IL-6/GP130 homodimerization inhibitors

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IL-6 has emerged as a potential target for the development of antitumor agents. IL-6 acts as a growth factor or antiapoptotic factor in various malignancies, including multiple myeloma, mesothelioma, and renal cell carcinoma and numerous other cancers. IL-6/GP130 homodimerization triggers the JAK2/STAT3 signaling cascade via interaction with the extracellular domain of IL-6R and GP130, resulting in homodimerization of the heterotrimeric IL-6/IL-6R/GP130 complex. Thus, this complex plays a key role in tumor development in IL-6 dependent cancer cells, the interaction of the IL-6 with GP130 may be a therapeutic target in these IL-6-related diseases. Madindoline A, a natural product isolated from the fermentation broth of *Streptomyces nitrosporeus* K93-0711 is reported as an antagonist to homodimerization of GP130 in IL-6 dependent cancer cells. The existing synthetic routes to madindoline A show limited applicability in drug discovery efforts and moreover the extraction from its natural source is no longer possible due to mutation in the bacterial strain. Our efforts focus both on simplification of the madindoline A structure and incorporation of functional groups predicted to provide additional binding interactions with the receptor in order to increase synthetic feasibility and develop potent synthetic analogues. MDL-16 has been identified and validated as a potent IL-6 antagonist with significant in vitro antitumor potency based on the natural product Madindoline A. In the current study, further modification of MDL-16 has led to a new class of analogues inhibiting the IL-6/JAK/STAT pathway. Furthermore, SAR optimization of these compounds and exploration of SAR is ongoing to develop novel and potent IL-6/GP130 inhibitors.

MEDI 323

Synthesis, optimization, and evaluation of dialkylated curcumin analogs as inhibitors of the JAK2/STAT3 pathway

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The JAK2/STAT3 pathway is frequently upregulated in both blood cancers and solid tumors as compared to normal cells and plays an essential role in transmitting signals involved with cell growth, differentiation, and apoptosis. Inhibitors of the pathway are considered attractive targets for the prevention and therapy of cancer. Curcumin, isolated from the rhizome of *Curcuma longa,* has previously been shown to inhibit both JAK2 and STAT3, while displaying little toxicity *in vivo.* As a drug, however, curcumin is severely limited due to poor bioavailability and rapid metabolism. Therefore, the aim of this study has been to develop curcumin derivatives as potential therapeutic agents. Analogs were designed to increase JAK/STAT selectivity, increase potency, and improve pharmacological profiles. FLLL31 and FLLL32, analogs synthesized early in the process, are two of our most extensively studied analogs and demonstrate selective inhibition of JAK2/STAT3 in a variety of *in vitro* assays. FLLL32 was also shown to decrease tumor growth *in vivo,* although with limited success, presumably due to poor water solubility. Our computational models also suggested the possibility of enhancing binding to STAT3 through the synthesis of non-symmetric analogs designed to target the hot spots of the SH2 domain. With this in mind, an efficient route to a series of non-symmetric curcumin analogs for SAR studies was achieved through two analogous acylation procedures. A water soluble pro-drug, FLLL100P, was also synthesized as a part of this effort and initial *in vivo* results showed a drastically improved plasma concentration and better drug-like properties.

MEDI 324

Construction and functionalization of the core cyclopenta[*b*]benzofuran ring system of silvestrol and SAR studies on the flavagline class of natural products

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Rocaglamide derivatives (flavaglines) from the genus *Aglaia* that possess a highly substituted cyclopenta[*b*]benzofuran core ring systems have garnered significant synthetic attention due not only to their structurally complexity, but also potent biological activities. Silvestrol is a novel flavagline due to the presence of an unprecedented dioxanyloxy side chain, which has demonstrated potent cytotoxic activity (low nM IC_{50} values) *in vitro* against numerous human cancer cell lines. Importantly, silvestrol has displayed this potent cytotoxicity against acute and chronic lymphoblastic leukemia while exhibiting selectivity. Our goal has been to develop a versatile and modular synthesis of silvestrol which would permit deep-seated structural changes to the natural product, functional group manipulation, and structural simplification. This ongoing effort

has facilitated the synthesis of two classes of structurally distinct flavagline analogues designed to explore the structure-activity relationship, delineate the mechanism of action, and ultimately improve the pharmacological profile of silvestrol.

MEDI 325

Design, synthesis, and biological activities of novel oligoamines containing 3-5-3 linkers as epigenetic modulators

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One of the key epigenetic modulators, lysine-specific demethylase 1 (LSD1), catalyzes the oxidative demethylation of mono- and dimethyl- lysine 4 of histone H3 (H3K4me and H3K4me2 respectively), thereby controlling gene transcription and regulation. Since LSD1 is over expressed in various cancers such as lung, prostate, breast and bladder, LSD1 inhibitors have potential as novel anticancer agents. Potent, oligoamine-based (bis)urea and (bis)thiourea LSD1 inhibitors, as well as small molecule amidoximes that have been synthesized in our laboratory inhibit LSD1, increase the levels of H3K4me and H3K4me2, and promote the re-expression of aberrantly silenced genes. We now report the synthesis and evaluation of oligoamine compounds containing 3-5-3 linkers. These analogues outperformed all of our previously synthesized compounds. When tested in a recombinant demethylase assay, three compounds displayed at least 95% inhibition at 10 μM. One of the best compounds (Compound **14**) displayed 96% inhibition in a chemiluminescence assay with an IC_{50} of 5.5 μM in a recombinant assay. Interestingly, when Calu6 lung cancer cells were treated with this compound there was a statistically significant increase in the mRNA expression of various aberrantly silenced tumor suppressor genes such as SFRP2, HCAD, GATA4, and p16. Molecular docking using the GOLD software package predicted the role of Asp 555 and Asp 556 in forming hydrogen bonds with the inhibitor. The selectivity of the best compounds against other monoamine oxidases has also been measured, and is presented herein. The compounds described in this presentation represent an expanded series of epigenetic modulators with the potential for use as antitumor agents.

MEDI 326

Novel bivalent ligands for the estrogen receptor: Design, synthesis, and biological study

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Estrogens play a crucial role in reproductive system, breast, bone and cardiovascular system *etc*. Estrogens exert their physiological effects through the regulation of two estrogen receptors, ERα and ERβ[1]. The regulation of gene expression through ERs basically involves the following two steps: receptor dimerization induced by ligand binding to ERα or ERβ, translocation of dimers to the nucleus and recognition of Estrogen Response Elements (EREs) on DNA to control gene transcription. Hence, dimerization is prerequisite for ERs-mediated physiological responses[2]. Therefore, it was suggested that bivalent ligands might be a good target to study the dimerization of ERs and their biological activity.

In this series, we prepared a small library of 19 bivalent ligands (e.g., estradiol, cyclofenil, ferrocifen *etc*) tethered by flexible spacers of polyethylene glycol with varying lengths or rigid squaramide linker, and evaluated their binding affinities for both ER subtypes and their biological activities in cell lines. As a global observation, an optimum affinity for ERα was shown at about 23 Å, which is considerably identical with 23.5 Å distance measured in the ERα homodimer crystal structure of 4-hydroxytamoxifen. The biological activity and a rather detailed structure-activity relationships (SARs) for these bivalent ligands were investigated. It's worth noting that the introduction of squaramide motif showed evidence in improving the overall binding affinities. The generation of these new series of bivalent ligands provides important insight into the diversity of structures that can function as ligands for the estrogen receptors. Detailed study on biological activity and structural biology of these bivalent ligands with ERs will be presented in the conference.

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MEDI 327

High-throughput combination screening identifies novel polypharmacologies for small molecules from a mechanistically defined library

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Our labs have recently described a novel platform for high-throughput screening of small molecule combinations for the rapid and systematic identification of synergistic and antagonistic drug combinations. This platform was used to determine drug-drug combinations for the Bruton's tyrosine kinase (BTK) inhibitor ibrutinib against two established cell lines (TMD8 and HBL1) within the ABC sub-type of diffuse large B-cell lymphomas (DLBCL). These results highlighted synergies between ibrutinib and inhibitors of the PI3K-AKT-mTOR signaling cascade through the use of a mechanistically annotated small molecule library. Here we describe the use of this platform for the identification of new polypharmacologies for known small molecules. Specifically, we investigated the influence of Lyn and Lck inhibition for the activity of ibrutinib by comparing its matrix profile to the combination profiles of known Lyn and Lck inhibitors, DCC-2036 and AMG-47a, respectively. Furthermore, using a mechanism annotated library allows us to compare combination profiles for small molecules with the same reported mechanisms of action, and unique profiles within these can be attributed to undisclosed polypharmacologies. In this manner, we discovered that the 'VEGFR' inhibitor vargatef possesses activity against checkpoint kinase 1 (Chk1) at a potency of 105 nM. This finding was validated by comparing the combination profile of vargatef and ibrutinib to the profiles of known Chk1 inhibitors (AZD-7762 and PF-477736) and ibrutinib. Additional small molecule polypharmacologies were similarly discovered by making comparisons between combination profiles generated using this novel screening platform.

MEDI 328

Targeting of near infrared (NIR) dyes to cancer folate receptors and comparative analysis of their candidacy toward optical imaging of cancer tumors

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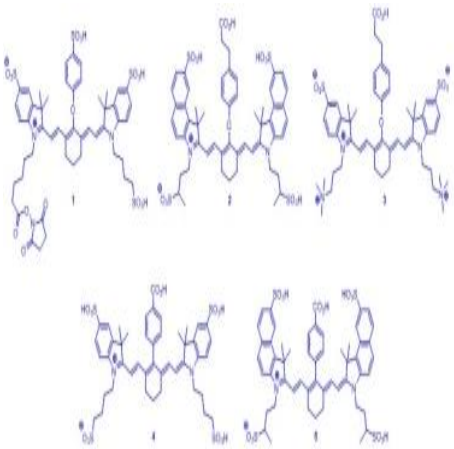
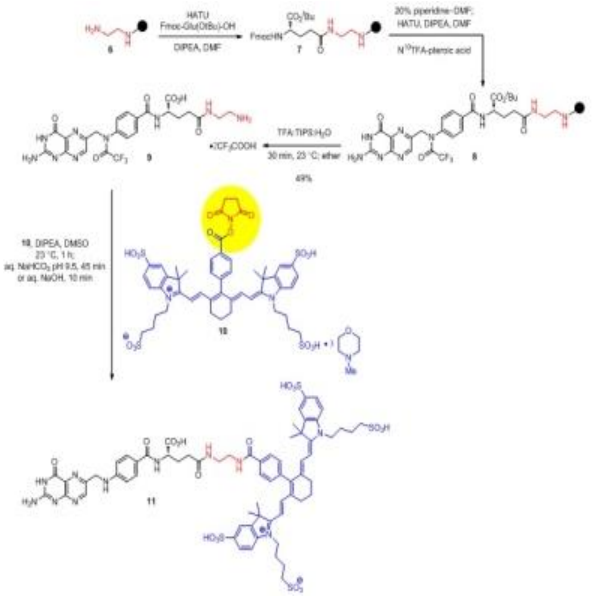
Targeting of multiple NIR dyes towards cancer tumors was achieved via their derivatization as folates with ethylene diamine (EDA) or lysine (Lys) as linkers. The employed NIR dyes included IR800CW (1), ZW800 (2), ZW800 analogue (3), LS288 (4), Kodak IRD28 (5), DyLight 750, and Alex Flour 750 (Figure 1).



Solid phase syntheses of folate–linkers commenced from EDA– and lysine–resins. Couplings with glutamic acid then with N^{10}–trifluoroacetylpteroic acid were followed by cleavages from the resins employing trifluoroacetic acid (TFA). Large scale production of ligand–linker(s) was facilitated by precipitation of the TFA–lysates in anhydrous ether (Scheme 1). Transformation of NIR dyes to their *N*–Hydroxysuccinimide esters was followed by coupling to folate–linker(s) (Scheme 1).



Highly pure conjugates were tested *in vitro* and *in vivo* in order to evaluate their brightness as well as bioavailability and chemical stability. The brighter dyes have strong potential in image–guided surgery especially for the detection of the deeply seated tumors.

MEDI 329

Evaluation of pteroyl-amino acid-NIR dye conjugates for tumor targeted fluorescence guided surgery

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Surgical resection of malignant disease is one of the effective treatments for cancer. However, the inability of surgeons to distinguish diseased tissue from adjacent healthy tissue leads to incomplete tumor resection and tumor recurrence. Optical image-guided surgery is gaining traction, especially when near infrared (NIR) fluorophores are targeted to tumor tissues. Thus, imaging in the NIR region (700-900 nm) avoids autofluorescence that primarily resides in the visible range and targeting to tumor tissues eliminates non-specific uptake by healthy tissues that resulting to higher signal-to-back ground ratio. While multiple NIR dyes are commercially available for conjugation to a tumor-targeting ligand, each of them displays major deficiencies related to conjugation efficiency, product stability, or fluorescence quantum yield. To obtain the optimal tumor-targeted NIR dye for eventual clinical use, we have conjugated pteroic acid to S0456 (a commercially available NIR dye) via amino acids that can undergo addition-elimination reaction with vinyl chloride moiety in S0456. Amino acids such as cysteine, tyrosine, lysine, serine, tyramine, amino proline, etc. were screened as possible candidates to improve binding affinity of pterioc acid to folate receptor as well as enhance fluorescence intensity of S0456. Of the multiple imaging agents considered, pteroyl-tyrosine-S0456 exhibits a quantum yield equal to the best NIR dyes available, but can be synthesized in just two steps at 90% overall yield in multi-gram quantities. Moreover, the conjugate displays high affinity and specificity for folate receptors (FR) and accumulates in FR+ expressing cancers in mice with FR+ tumors. Finally, histopthathological analysis demonstrated that pteroyl-tyrosine-S0456 exhibits excellent safety profile in mice. Taken together, FR specific NIR probe demonstrates significant potential for use in fluorescent guided surgery by aiding in the complete resection of diseased tissue.

MEDI 330

Synthesis of novel prodrugs of highly potent doxorubicin analogs and their conjugates

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Doxorubicin is an antineoplastic agent widely used in the treatment of leukemia, lymphoma, breast and ovarian carcinoma, and many other solid tumors. The clinical

application of this anthracycline drug is, however, limited by its toxic dose-related side effects, such as myelosuppression, gastrointestinal disorders, stomatitis, cumulative cardiotoxicity, and extravasation. To overcome these limitations, we synthesized a folate conjugate of doxorubicin to target cancer cells which express high levels of folate receptor (FR). However, the cytotoxicity of doxorubicin itself is not high enough to ensure robust biological activity of the folate conjugate. Therefore we designed a series of novel pro-drugs of known highly potent doxorubicin analogs and their folate conjugates exhibiting superior *in vitro* cytotoxicity. All compounds in this group possess a structural motif, a latent aldehyde functional group, designed to release after (FR)-mediated targeted delivery to the cancer cell. Subsequent attack on the aldehyde by the *N*-atom of the daunosamine moiety forms a cyclic iminium species, predicting the enhanced alkylating activity of this class of molecules. These highly potent analogs and their conjugates show greater than a six orders of magnitude improvement in their IC_{50}-values on (FR)-positive KB cell over their unmodified doxorubicin counterparts.

MEDI 331

Tuneable radical cyclisations: Synthesis of biologically active heterocycles to target neuroinflammation

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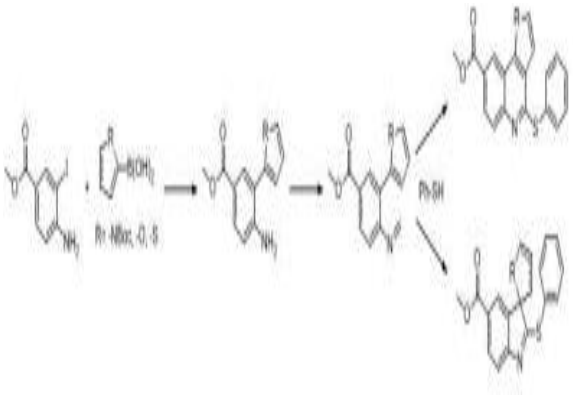
Radical chemistry has received little attention in medicinal chemistry due to the fact that most reactions use organotin derivatives under high dilution conditions producing low amounts of product. Our research is based on the development of novel non-toxic tuneable methodology towards biologically active heterocycles, as shown below (Scheme 1).



Scheme 1

We have successfully demonstrated that our non-toxic thiyl radical/isocyanide approach can produce diverse structures for various disease targets. Using this approach, we have been able to synthesize a large number of biologically active compounds, as shown (Table 1).

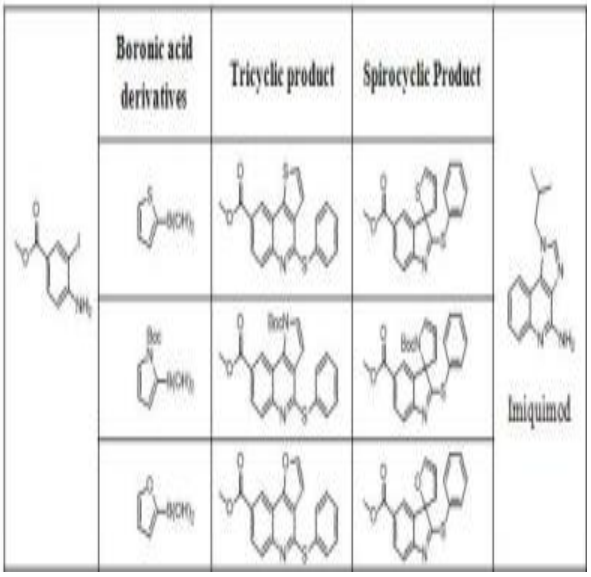


Table 1

Imiquimod is used for treating various cancers such as skin and melanomas. It has recently attracted attention due to its neuroinflammatory modulation on several demyelinating diseases, including multiple sclerosis (MS). Our compounds being analogues to imiquimod they could be potential therapeutic candidates. Therefore the next stage of our research will focus on their effects on multiple sclerosis and our synthetic approach to these diverse classes will be described.

MEDI 332

Synthesis of a carbon-11-labeled PIM1 inhibitor as a new potential PET agent for imaging of PIM1 in cancer

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Proto-oncogene serine/threonine-protein kinase (PIM1) is an emerging target for cancer therapy, and PIM1 inhibitors have been developed as anticancer drugs. (*Z*)-2-((1*H*-indazol-3-yl)methylene)-6-methoxy-7-(piperazin-1-ylmethyl)benzofuran-3(2*H*)-one is a novel potent and selective PIM1 inhibitor with PIM1 IC_{50} 3 nM. Here we report the

synthesis of (*Z*)-2-((1*H*-indazol-3-yl)methylene)-6-[^{11}C]methoxy-7-(piperazin-1-ylmethyl)benzofuran-3(2*H*)-one as a new potential PET agent for imaging of PIM1 in cancer. The reference standard, intermediate (*Z*)-*tert*-butyl 4-((2-((1*H*-indazol-3-yl)methylene)-6-methoxy-3-oxo-2,3-dihydrobenzofuran-7-yl)methyl)piperazine-1-carboxylate and its Boc-protected precursor (*Z*)-*tert*-butyl 4-((2-((1*H*-indazol-3-yl)methylene)-6-hydroxy-3-oxo-2,3-dihydrobenzofuran-7-yl)methyl)piperazine-1-carboxylate for radiolabeling were synthesized from 6-hydroxybenzofuran-3(2*H*)-one and *tert*-butyl piperazine-1-carboxylate in 4, 3 and 2 steps with 29%, 35% and 44% overall chemical yield, respectively. The precursor was labeled with [^{11}C]CH_3OTf under basic conditions (2 N NaOH) through *O*-[^{11}C]methylation to provide a radiolabeled intermediate (*Z*)-*tert*-butyl 4-((2-((1*H*-indazol-3-yl)methylene)-6-[^{11}C]methoxy-3-oxo-2,3-dihydrobenzofuran-7-yl)methyl)piperazine-1-carboxylate, which was then quickly de-protected under acidic conditions (1 N HCl) to give the target tracer in 20-30% decay corrected radiochemical yield and 370-740 GBq/μmol specific activity at end of bombardment (EOB). The two-step radiolabeling reaction was performed in a home-built automated ^{11}C-radiosynthesis module, and the target tracer was purified by HPLC method.

MEDI 333

Potent antioxidant dendrimers devoid of pro-oxidant effects

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Antioxidants neutralize free radicals and prevent oxidative stress-mediated disorders. However, antioxidants in the presence of transition metal ions may produce pro-oxidant effects, which may irreversibly damage biomolecules. We report unique dendritic antioxidants. The surface of the dendrimers consists of phenol rings with electron donating groups. Their interior is composed of functional groups that can chelate metal ions and thus prevent pro-oxidant effects. These novel materials exhibited highly potent radical scavenging activities based on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical assay (IC_{50} < 2μM). The antioxidants also demonstrated strong protective effects on human low-density lipoprotein and DNA against free radical damage by 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH). The novel antioxidants showed no pro-oxidant effects on DNA in the presence of physiological amount of copper ion (10 μM). The toxicity of these antioxidants was evaluated on Chinese hamster ovary cells. Cell viability over 5 days was unaffected in the presence of up to 50 μM antioxidant.

MEDI 334

Synthesis of an imidazopyridine library using the automated synthesis laboratory (ASL)

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Beyond the technology innovation, we were convinced that there is a need to influence the future of the pharmaceutical industry, by breaking with traditional research paradigm of medicinal chemistry practice and allow chemists to remotely guide chemical syntheses as a virtual extension of their own lab. During assembly of the automated Synthesis and purification Laboratory (ASPL) at Eli Lilly and Co., we recognized novel opportunities to tap into the creative synthetic talents of chemists from anywhere in the world. This poster highlights our partnership with University of Notre Dame group for the design and syntheses of imidazopyridines with impressively potent anti-tuberculosis activity and how the inception of this tool coincided well with a renewed focus on the collaborative research model through the Fully Integrated Pharmaceutical network (FIPNet) model. While preliminary SAR studies were carried out using traditional manual synthetic techniques, the chemistry was readily adapted to the ASPL and allowed further expansion of SAR. Collaborative efforts, including high throughput screening (University of Illinois, Chicago), development and utilization of separate sensitive assays (IDRI) and determination of in vivo pharmacokinetics at Lilly, have accelerated studies. The combined results indicate that imidazo[1,2-a]pyridine-3-carboxamides are an exciting new class of potent, selective anti-TB agents that merit additional development opportunities

MEDI 335

Synthesis of d-labeled and unlabeled ethyl succinic anhydride and application to quantitative analysis of peptides by isotope differential 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 five kinds of reagents that react with specific amino acid residues and their d- or ^{13}C-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 d_5-labeled version, and the application of their combination to quantitative analysis of peptides in combination with MALDI TOF mass spectrometry.

This study finds that this combination 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 336

Molecular umbrella conjugates for the in vivo delivery of siRNA

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siRNA mediated gene silencing continues to hold great potential as a therapeutic modality. The central challenge in this area remains the ability to deliver siRNA *in vivo* in a potent and safe manner. One attractive approach is the use of siRNA conjugated to specific molecules which are designed to facilitate siRNA delivery to the cytosol via either cellular or endolysosomal membrane translocation. This requires the design of conjugates which possess properties amenable to both hydrophilic and hydrophobic environments. One potential system is the cholic acid based “molecular umbrella”, which possess two or more cholic acids attached via lysine linkers. The dynamic amphiphilic nature of this system allows it to create both a hydrophilic exterior in a hydrophilic environment and a hydrophobic exterior in a hydrophobic environment. This work will detail the design and application of “molecular umbrella” conjugates of siRNA for *in vitro* and *in vivo* delivery.

MEDI 337

Open innovation drug discovery: An example of symbiotic industry-academia collaboration

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The Lilly Open Innovation Drug Discovery program (OIDD) was established to provide a platform for the engagement of investigators worldwide in the submission of novel

compounds for therapeutic potential evaluation. OIDD has been embraced by the scientific community, both scientists and technology transfer professionals alike. To date, more than 300 academic institutions and small biotech companies in over 30 countries have become affiliated with the program. Thousands of compound samples have been evaluated in the phenotypic and target-based disease modules included in the OIDD screening panel, providing important scientific value to small companies and resource-constrained faculty.

In 2011, Lilly evaluated a collection of compounds submitted to OIDD by the University of Valencia. From this collection, three compounds with good drug-like properties displayed a profile of interest in the area of angiogenesis [cord formation IC50 (μM) 2.1, 1.7, and 3.2]. These compounds formed the basis of a one-year collaborative agreement between Lilly and the University of Valencia, wherein a post-doctoral fellow was hired by the University to continue synthesizing new and diverse molecular entities designed by Lilly. This was the first collaboration of Lilly with an academic institution in Europe that resulted from the OIDD program. Using this unique model of partnering, Lilly and the University of Valencia integrated medicinal chemistry, computational chemistry, synthetic chemistry, and rapid parallel synthesis to produce a wide variety of new compounds based on the original molecular scaffold of interest.

In this poster we provide details regarding the OIDD business model, and describe scientific and operational aspects of the collaboration between Lilly and the University of Valencia with an emphasis in the description of new synthetic methodologies.

MEDI 338

Analysis and comparison of active expansion methods: Which method can most efficiently retrieve series worthy of wet-chemistry investment?

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Iterative screening of corporate collections is an important and effective way of identifying chemical starting points for pharmaceutical targets. In this situation, a diverse representation of the collection is plated and assayed against a potential target of interest. Once initial actives have been identified, multiple follow up assays and physical evaluation are done on these compounds to confirm them as actives. Typically, confirmed actives are used as starting points to collect follow-up compounds in a process we call “Active Expansion”. These follow up sets are plated and assayed to collect additional information from the corporate collection. Many different cheminformatic methods can be used for active expansion. Historically, these methods are compared and evaluated based on their performance against each other in terms of an “active rate”, retrospectively and prospectively. In this study we propose to focus on the ability of each method to retrieve the series which were candidates for lead discovery efforts. To this extent we collected 236 compounds from 71 projects that

represent the chemical series that were followed up with wet-chemistry. We will show results on the ability of different actives expansion methods to retrieve these series from the screening collection.

MEDI 339

Novel building blocks for ^{18}F-radiolabeling of bioactive peptides for PET imaging

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Positron emission tomography (PET) is a valuable diagnostic imaging method. Many methods have been reported for labeling peptides and proteins with ^{18}F for PET imaging of cancer and other diseases. Traditional methods for labeling ^{18}F-labeled peptides for diagnostic imaging rely on carbon-fluorine bond formation and are complex and inefficient. Recently, several prosthetic groups (such as boron-, aluminium- and silicon-containing) labeled with ^{18}F for peptides have been reported but require multistep reaction sequences and are time-consuming. Herein, a new linker has been discovered that allows very efficient ^{18}F conjugation to specifying ligands under mild aqueous conditions with high efficiency, which simplifies the synthesis of peptide-based molecular probes for PET imaging. We believe these novel ^{18}F radiolabeled analogs are potential candidates as PET molecular imaging agents.

MEDI 340

RGDyK conjugates of NETA-based bifunctional chelators for integrin $\alpha_v\beta_3$-targeted radiotherapy using β-emitting radionuclides

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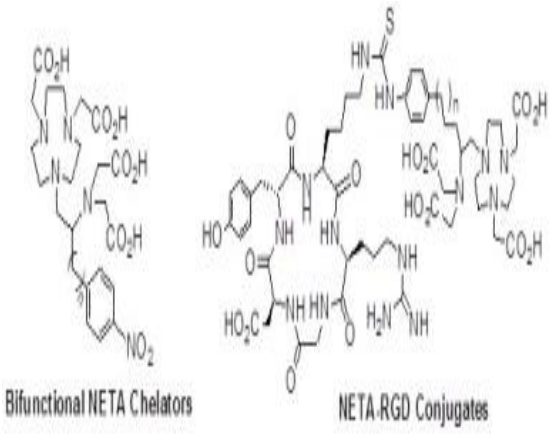
We report novel bifunctional chelators and their biological evaluation for integrin $\alpha_v\beta_3$-targeted radiotherapy using a cyclic peptide (Arg-Gly-Asp-D-Tyr-Lys, RGDyK). The novel bifunctional analogues of an effective chelator (NETA) were efficiently prepared via nucleophilic ring opening of aziridinium ions. The bifunctional NETA chelators were evaluated for radiolabeling and in vitro complex stability with β-emitting radionuclides, ^{90}Y and ^{177}Lu. The bifunctional chelators rapidly bound to ^{90}Y or ^{177}Lu and presented an excellent serum stability profile with no loss of the radioactivity over 14 days. The bifunctional NETA chelators were conjugated to a cyclic Arg-Gly-Asp-D-Tyr-Lys (RGDyK) peptide targeting integrin $\alpha_v\beta_3$ thatis over-expressed on many different cancer

cells. The in vitro data indicate that the corresponding NETA-*c*(RGDyK) conjugates bound to ^{90}Y or ^{177}Lu nearly completely at the starting point of the radiolabeling reaction, and ^{90}Y or ^{177}Lu-NETA-*c*(RGDyK) remained stable without no measurable dissociation of the activity. Binding affinity of the RGD-NETA conjugates radiolabeled with ^{90}Y or ^{177}Lu to integrin $\alpha_v\beta_3$ andbiodistibution of ^{90}Y or ^{177}Lu-radiolabeled RGD-NETA conjugates will be described.



MEDI 341

Synthesis and biological evaluation of new bifunctional chelators for targeted positron emission tomography (PET) imaging using ^{64}Cu

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^{64}Cu ($t_{1/2}$ = 12.7 h, E_{max}^{b+} = 656 keV; E_{max}^{b-} = 573 keV; E_{max}^{g} = 511 keV) is one of the most useful radionuclides for positron emission tomography (PET) imaging of cancers due to its ideal physical properties. Its half-life allows optimal biodistribution of antibody conjugates with slow clearance, and the low β^+ maximal energy results in good intrinsic image resolution. Clinical and Preclinical evaluations of various ^{64}Cu-labeled antibody conjugates as targeted PET agents have shown encouraging results. However, less progress has been made on development of bifuncitonal chelators of ^{64}Cu. An effective chelator must be empolyed to minimize transchelation of ^{64}Cu to the metal-binding proteins. We have synthesized new bifunctional chelators for PET applications using^{64}Cu. The chelators were conjugated to a tumor-targeting moiety, trastuzumab, a bile acid, or transferrin. Trastuzumab targets HER2 receptor over-expressed in various cancer and was approved in the treatment of metastatic breast cancer. Transferrin and bile acid are known to target transferrin receptor and bile acid transporter over-expressed in colon cancer. Amphiphilic bile acids including cholic acid (CA),

deoxycholic acid (DCA),or chenodeoxycholic acid (CDCA) have been investigated as a shuttle for therapeutic and diagnostic drugs. The new bifunctional chelators were conjugated to the targeting moiety, trastuzumab, transferrin, or bile acid. The corresponding conjugates were evaluated for their radiolabeling efficiency with ^{64}Cu. Comparative complex stability of the ^{64}Cu-labeled conjugates in human serum was also evaluated. The conjugated chelators were rapid in binding to ^{64}Cu, and radiolabeled conjugates displayed excellent *in vitro* stability. The result of the biological evaluation indicates that the new bifunctional chelators are promosing candidates for PET applications using ^{64}Cu, and further biological evaluation of the conjugates using animals is warranted

MEDI 342

Efficient bifunctional chelators for radioimmunotherapy applications

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^{90}Y ($t_{1/2}$ = 64.1 h) and ^{177}Lu ($t_{1/2}$ = 6.7 days) are potent radionuclides for radioimmunotherapy (RIT). The therapeutic efficacy of ^{90}Y or ^{177}Lu-radiolabeled antibody conjugates has been demonstrated in clinical trials. Despite great potential of RIT as proven by the first RIT drug, Zevalin, a limited progress has been made on improvement of chelation chemistry of ^{90}Y and ^{177}Lu. Our continued research on chelation chemistry of the radionuclides led to the design of bimodal chelators in the NETA and DEPA series. The new bifunctional chelators were synthesized using novel synthetic method centered on the ring opening of aziridinium ions and subsequently were conjugated to an antibody, either trastuzmab or panitumumab. Trastuzumab is known to target HER2 overexpressed on metastatic breast cancer, while panitumumab is a fully humanized mAb approved for treatment of colorectal cancers and known to rapidly internalize upon its binding to EGFR with high specificity and affinity. The corresponding trastuzumab conjugates were evaluated for radiolabeling kinetics (pH 5.5), and the radiolabeled antibody conjugates were studied for serum staibility and *in vivo* biodistribution and tumor uptake using LS174T tumor bearing mice. The chelators conjugated with antibody instantly bound ^{90}Y and ^{177}Lu. The corresponding radiolabeled trastuzumab conjugates were stable in human serum and displayed excellent *in vivo* stability as evidenced by low organ uptake and high tumor targeting. In this report, we describe the synthesis of a series of bifunctional chelators for RIT applications using ^{90}Y and ^{177}Lu and comparative radiolabeling efficiency and *in vitro* serum stability of their trastuzumab or panitumumab conjugates with ^{90}Y and ^{177}Lu. *In vivo* biodistribution and tumor uptake of ^{90}Y and ^{177}Lu-radiolabeled trastuzumab and panitumumab conjugates will be also reported.

MEDI 343

Iterative rapid lead optimization in silico

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With drug discovery pipelines under enormous pressure, it is important to have reliable software tools to support synthetic lead optimization to open up new idea paths and avoid researching in the wrong direction.

Our software LeadIT helps the Medicinal Chemist develop a multitude of lead derivatives through an iterative process within the matter of an afternoon (or a cup of coffee), with subsequent prioritization of compounds based on whether they will likely be a good binder or not. Visual feedback tells the researcher where a compound needs to be optimized.

The talk will highlight the scientific details of the backbone tools of LeadIT.

MEDI 344

Comparative evaluation of automated flash chromatography and preparative HPLC for bench-scale purification of a broad range of sample types

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Delivering large quantities of high purity compounds in the shortest possible time is the goal of a purification chemist. Two of the most popular purification techniques are Automated Flash Column Chromatography (AFCC) and Preparative HPLC. Traditionally, AFCC is characterized by the ability to load large amounts of material and short purification times, while Prep HPLC is valued for high resolution separations resulting in very pure products. As a result, AFCC is typically used as a complementary technique whereby the crude sample is enhanced to a higher level of purity before final purification using Prep HPLC.

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

In this study, we evaluate the productivity advantages of AFCC over Prep HPLC, in terms of time, solvent and cost savings. We also demonstrate the benefits of AFCC both as a complementary technique to Prep HPLC, as well as a highly versatile stand-alone technique to deliver high purity separations in a cost-effective manner.

MEDI 345

Towards chromatography-free Mitsunobu reactions with the versatile and water-soluble Mitsunobu reagent azodicarbonyl dimorpholide (ADDM)

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One of the most powerful reactions in organic chemistry is the Mitsunobu reaction, which, due to its simplicity, mildness and convenience, has become an extremely important tool for the conversion of an alcohol to a variety of functional groups, such as esters, ethers, sulfonamides and imides. Indeed, the Mitsunobu reaction features heavily in the syntheses of our α-helix mimetics designed to inhibit the oncoprotein Mcl-1. The Mitsunobu reaction employs an azodicarbonyl species (typically diisopropyl azodicarboxylate (DIAD)) and a phosphine (typically triphenylphosphine (PPh_3)) as co-reagents to generate the requisite betaine intermediate *in situ*. However, the major caveat of the Mitsunobu reaction is purification, as reduced DIAD (DIAD-H_2) and oxidized PPh_3 ($Ph_3P{=}O$) often contaminate the product. As part of our continued interest in the Mitsunobu reaction, we have investigated the scope of the water-soluble Mitsunobu reagent azodicarbonyl dimorpholide (ADDM). Furthermore we will present optimized conditions for a chromatography-free Mitsunobu reaction with ADDM.

MEDI 346

Understanding the effect of solvation during lead optimization

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

MEDI 347

Rationalizing nonstandard interactions in ligand design: The duality of halogens

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Non-standard intermolecular interactions such as CH donors, halogen bonds, close sulfur contacts and cation-p interactions have recently been recognized as significant factors in protein-ligand binding. However, exploiting these interactions in structure-

based drug design projects (SBDD) has been difficult, because they are inadequately modeled using MM force-field based methods. Atom-centered charges typically used in force-fields cannot capture the anisotropic charge distributions responsible for some non-standard interactions. To address these challenges, a model of intermolecular interactions based on Extended Hückel Theory (EHT) is proposed, which accounts for the effect of electron delocalization and geometry on interaction strength. The qualitative and semi-quantitative accuracy of the model is demonstrated using case studies that highlight the importance of non-standard interactions in a number of systems, including native ligands of the thyroid hormone receptor.

MEDI 348

MOE in education: A pedagogical tool for the chemical sciences

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Molecular modeling is fast becoming a fundamental research and teaching aide in academia. One of the most predominant molecular modeling suites in industry today that facilitates rational drug discovery is MOE, Molecular Operating Environment. Here we present a brief introduction into the wide range of MOE applications available to academics as well as examples of how MOE has been used as a teaching aide in the life sciences such as Computational and Medicinal Chemistry, Pharmacology, and Biology.

Chemical Computing Group has a solid history of supporting research and pedagogic programmes in academia through sponsored initiatives and awards including the CCG Excellence Awards at the ACS. MOE's comprehensive, integrated platform, and open-source code provides a variety of scientific researchers with an all in one drug discovery tool that is easily integrated, customizable and a fundamental research and teaching aide.

MEDI 349

Collaborative drug discovery technology applied to neglected, infectious and CNS diseases

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Layering unique collaborative capabilities upon requisite drug discovery database functionality *unlocks and amplifies* latent synergy between biologists and chemists. The application of **collaborative** technologies to interrogate potency, selectively, and therapeutic windows of small molecule enzyme, cell, and animal study data will be presented. An example combining integrated bioinformatics and chemoinformatics with *in vitro* experimental validation to identify two leads against putative new Tuberculosis

targets (in collaboration with SRI's Computer Science + Bioscience Divisions), a second example to overcome malaria chloroquine resistance (see: Hohman, M. *et al*, Drug Discov Today. 2009 Mar;14(5-6):261-70), and a third example broadly across CNS therapeutic areas (in collaboration with the NIH Neuroscience Blueprint) will demonstrate the general concept that a more effective collaborative model is possible today using secure, web-based collaborative technologies to bring together complementary, specialized expertise.

MEDI 350

WITHDRAWN

MEDI 351

Optimized flash chromatography gradient methods

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Purifying compounds require two processes; the actual purification and drying the sample afterwards. Several gradient methods were evaluated on a reaction mixture to evaluate run time and number of fractions to collect the desired compound. Reducing the volume of fractions is important to reduce the time required to dry the purified material. On large runs, drying time may be equal to, or greater, than the purification time. An optimized gradient is a combination of short run times and sharp peaks that reduce the solvent used and evaporated. Tests showed that the most efficient gradient for one-time purifications was a linear gradient. A linear gradient with an isocratic hold was found to be efficient when purifying compounds that eluted close to each other on TLC plates. For repeated purifications an optimized step gradient was found to be most efficient, but the time required to optimize the gradient must be evaluated versus the time saved during the purification procedure. Optimized step gradients are best used when the purification needs to be repeated, such as in a production environment.

MEDI 352

Elucidation of the associations of mercury(II) with cysteinyl peptides

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Current clinical chelation therapy of mercury poisoning generally uses thiol compounds, including cysteine. The present study is conducted to elucidate the associations of mercury(II) with cysteinyl peptides. The effect of increasing the number of cysteinyl residues in a peptide chain on mercury(II) binding and complex type formations was

investigated. Three series of di-, tri-, and tetra-cysteinyl peptides, $E[CDG]_nCG$, $E[CEG]_nCG$, and $D[CGD]_nCG$, where n = 1,2, or 3, were prepared by microwave-assisted solid phase peptide synthesis. Their mercury(II) binding constants were evaluated by isothermal titration calorimetry. Complexes formed in different relative ratios of mercury(II) to cysteinyl peptides were also characterized by LTQ orbitrap mass spectrometry. The results from this study show that tetracysteinyl peptides are effective in binding mercury(II) ($K_b > 10^{10}$ M) and they bind more than one mercury ion per peptide (Hg_2(peptide). They may present more attractive options for mercury chelation therapy or environmental heavy metal remediation.