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248th ACS National Meeting, San Francisco, CA, August 10-14, 2014

J. Macor, Program Chair

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MEDI 1

Leveraging peptide homology to access polypharmacology: Novel GLP1R and GCGR dual agonists

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Stabilization of the endogenous incretin peptide, GLP-1, and injection of synthetic analogues have both proven effective at lowering glucose in diabetic patients. Activation of the GLP-1 receptor (GLP1R) on the pancreatic β-cell stimulates glucose dependent insulin secretion and can reduce food intake, resulting in modest improvements in body weight.

Glucagon, which derives from the same precursor preproglucagon gene, shares almost fifty percent sequence homology with GLP-1. However, glucagon stimulates hepatic glucose production and has classically been considered the glycemic counterpart of insulin. Activation of the glucagon receptor (GCGR) has also been shown to act to suppress appetite, increase energy expenditure, alter body mass and lower circulating lipids. Several clinical and preclinical studies have now shown that through activation of both the GLP-1 (GLP1R) and glucagon (GCGR) receptors one can access the positive anti-obesity effects of both peptides.

We have leveraged the high degree of sequence homology to design peptides that simultaneously activate both the GLP1R and GCGR. These peptides were tested in lean, obese and spontaneously diabetic non-human primates to demonstrate the translatability of this mechanism. The results suggest a novel approach to treating diabetes and obesity that involves activation of the glucagon receptor.

MEDI 2

"Weighty matters": Fixed dose combinations for obesity

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Body weight is regulated by complex neurohormonal interactions between endocrine signals of long-term adiposity (e.g., leptin, a hypothalamic signal) and short-term satiety (e.g., amylin, a hindbrain signal). Ultimately, active weight loss and maintaining a weight-reduced state engage counter-regulatory responses that render monotherapy-based diet drugs relatively ineffective. This presentation will highlight potential strategies for overcoming these responses with biologic combinations. As an example of synergy, translational research findings from the development of pramlintide (an amylin receptor agonist) and recombinant leptin [figure 1] as a weight-loss combination will be highlighted. Pharmacologic, mechanistic and practical considerations and challenges encountered during development will be discussed.

MEDI 3

Discovery of triple reuptake inhibitors (TRIs) for the treatment of depression

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The discovery of triple reuptake inhibitors (TRIs) for the treatment of depression

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Major depressive disorder (MDD) remains an inadequately treated disease in spite of the advances of past decades. Clinical evidence based on combination studies suggests that adding dopamine transporter inhibition can potentially augment the efficacy of selective serotonin and serotonin/norepinephrine reuptake inhibitors (SSRIs and SNRIs). Hence, triple reuptake inhibitors (TRIs) that appropriately block DAT, NET and SERT function hold promise as new antidepressant therapies. BMS-820836 is a novel triple uptake inhibitor that was advanced to Phase II clinical trials. The design, synthesis, pre-clinical pharmacology and Phase I biomarker results (PET) for BMS-820836 will be presented. TRIs from related series with different triple inhibition profiles will also be described.

MEDI 4

Development of bifunctional mu opioid receptor (MOR) agonist/delta opioid receptor (DOR) antagonist peptides and peptidomimetics

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The observation that DOR antagonists can mitigate some adverse effects of MOR agonists – such as the development of tolerance and dependence – has motivated several groups to pursue bifunctional MOR agonist/DOR antagonist ligands. Using structure-based design we have developed several peptide ligands with similar affinity toward MOR and DOR and that function as MOR agonists and DOR antagonists. Members of this series possess advantages over previously reported examples of MOR agonist/DOR antagonist ligands, but, like most peptides these analogs poorly penetrate the blood brain barrier. Consequently, our focus has turned to improving the bioavailability of these bifunctional ligands using two complementary approaches: 1. Modifying our lead MOR agonist/DOR antagonist peptides to improve membrane penetration, while preserving their pharmacological profile and 2. Transferring the key pharmacophore elements of these peptides to more drug-like peptidomimetic scaffolds. Successes, failures, and observations from these parallel approaches toward the development of opioid analgesics with reduced tolerance and dependence liability will be described. Supported by NIDA grant DA003910 and Fellowship support from NIDA Training Grants DA007281 (JPA) and DA007267 (LY).

MEDI 5

BET bromodomains interact with diverse kinase inhibitors

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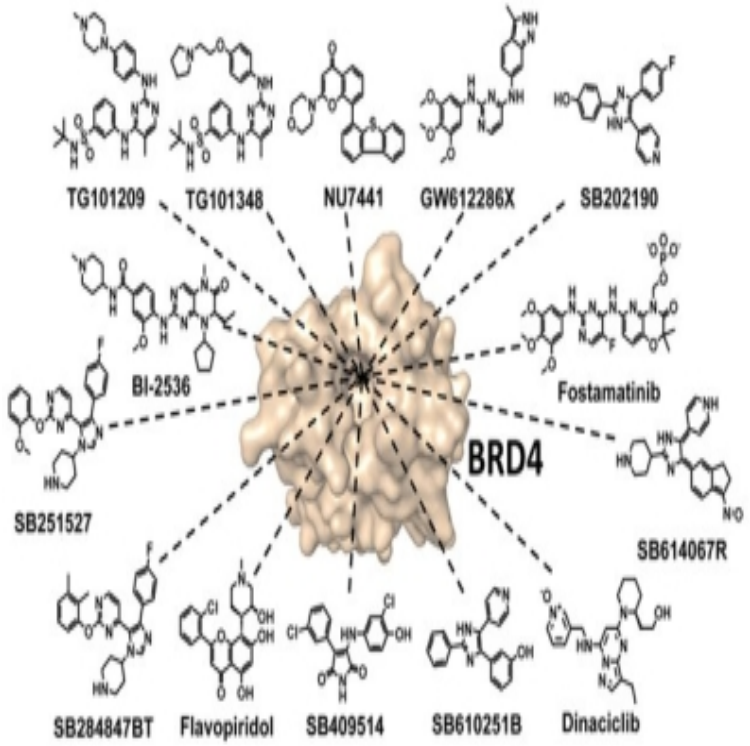
Members of the bromodomain and extra terminal (BET) family of proteins are essential for the recognition of acetylated lysine (KAc) residues in histones and have emerged as promising drug targets in cancer, inflammation and contraception research. In co-crystallization screening campaigns using the first bromodomain of BRD4 against kinase inhibitor libraries, we identified and characterized 14 kinase inhibitors (10 distinct chemical scaffolds) as ligands of the KAc binding site. Among these, the PLK-1 inhibitor BI2536 and the JAK2-FLT3 inhibitors TG101209 and TG101348 displayed strongest inhibitory potential against BRD4 (IC_{50} values of 25 nM and 130 nM, respectively) and high selectivity for BET bromodomains. Comparative structural analysis revealed markedly different binding modes of kinase hinge-binding scaffolds in the KAc binding site. The results suggest that BET proteins are potential off-targets of diverse kinase inhibitors, the knowledge of which could significantly impact kinase drug development campaigns. BI2536 and TG101348 inhibit the intended kinase targets and BET proteins simultaneously and effectively at relatively low concentrations. Knapp and colleagues recently demonstrated that both inhibitors potently suppressed c-Myc expression (a cellular marker of BET inhibition) in multiple myeloma cells, and TG101348 potently inhibited proliferation of AML cells driven by BET bromodomains and mutant FLT3. Combined, these findings provide a new structural framework for the rational design of

next-generation BET-selective and dual kinase-BET inhibitors able to target multiple disease pathways with improved efficacy over traditional combination therapies



MEDI 6

Application of multivalent ligand design for the treatment of prolonged pain and addiction without toxicities

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Prolonged and neuropathic pain affects 100 million Americans and costs the economy over 500 billion dollars annually. Current treatments are inadequate and there is an epidemic of deaths from the misuse of prescription drugs for pain, with tens of thousands dying annually. Clearly, more efficacious and safer drugs are needed. Based on insights from genomic, proteomic and mechanistic studies of prolonged and neuropathic pain in the central and peripheral nervous systems, we have suggested that the design and synthesis of multivalent ligands that address the disease states will provide unique opportunities to design ligands for prolonged and neuropathic pain. At the same time, these ligands will have greatly reduced or none of the toxicities of current opiate drugs including constipation, respiratory depression, development of addiction, dependence and tolerance. We will discuss the design, synthesis and biological evaluation of a number of peptidomimetic ligands that have agonist activities

at mu and delta opioid receptors and antagonist activities at neurokinin-1 receptors with highly potent analgesic effects in acute and neuropathic pain, cross the blood brain barrier, do not develop tolerance, have no addiction potential and do not have the toxicities of current opioids.

MEDI 7

PAINful lessons: Philosophical remarks, structure interference relationships, and compound natural histories

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PAINS ('Pan-Assay Interference Compounds') are rapidly becoming the “irreversible inhibitors” of the second decade of the 21st century. That is, while the wave of fear concerning covalent inhibitors has crested and slowly receded, PAINS and their like appear to be the next tsunami on the horizon of the medicinal chemistry landscape, particularly at the pre-clinical stages of the academic drug discovery process. Three challenges face the world of academic drug discovery: (1) high-throughput screening (virtual or real) has now become the de facto approach for chemical matter discovery; (2) there appears to be a general lack of knowledge regarding PAINS compounds in the medicinal chemistry community; and (3) too little is known about the mechanism(s) of interference by some of these well-disguised bad actors. We will report on our investigations into structure-interference relationships (SIR) that were prompted by our recent riding out of a perfect storm assay. Many of the interference compounds we discovered appeared to have reproducible relative activities that some might interpret as SAR of a more positive nature. Additionally, we will report on a well-trodden series of compounds that interfere by a 'triple threat' of interference mechanisms (including instability in assay buffer, thiol reactivity and redox activity), leading us to question the utility of certain bioactivity results reported in the literature. Finally, we will make a request that researchers carefully consider the “natural histories” of the hits they report and consider rigorous structural prioritization in their project screening trees.

MEDI 8

Cryptic covalents and other unknown knowns: Useful or not?

***Daniel Erlanson**, derlanson@carmot.us. Carmot Therapeutics, Inc., San Francisco, CA 94158, United States*

Covalent modifiers represent one of the largest categories of pan-assay interference compounds, compounds that appear as hits in screening assays but in fact do not interact specifically with protein targets. Though many drugs act covalently – in some cases on multiple targets – the possibility of covalent binding is often not recognized when hits are identified from a screening campaign. In some cases, the covalent modification was only determined during the course of the investigation. In other cases, despite being flagged in the literature as problematic, these molecules are still reported as selective inhibitors. Knowing precisely what a given molecule is doing is critical for answering biological questions. This presentation will consider several molecules that modify proteins. It will describe how to identify and exclude covalent modifiers as false positives while acknowledging that even promiscuous covalent modifiers can occasionally provide insights into protein structure and function.

MEDI 9

PAINS at Penn

***Jeffrey D Winkler**, winkler@sas.upenn.edu. Department of Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania 19104, United States*

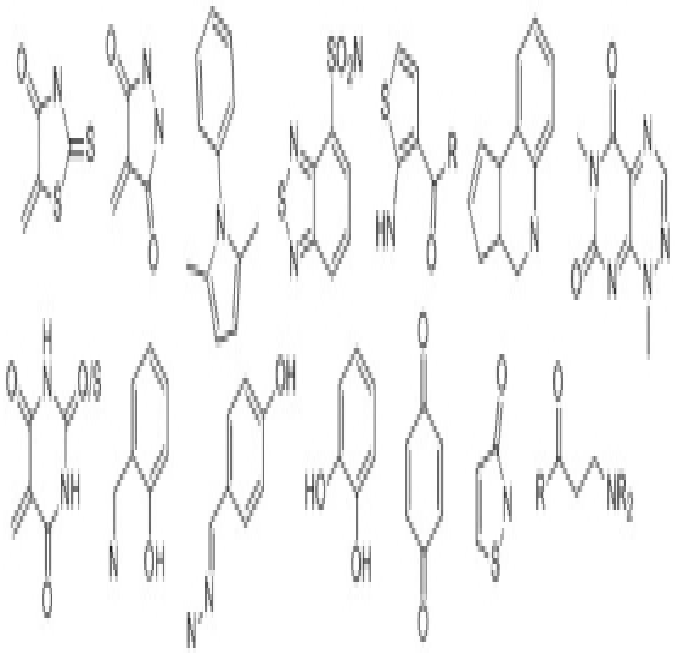
Recent work from our laboratory in collaboration with researchers at the School of Medicine at the University of Pennsylvania, the Wistar Institute, and the Children's Hospital of Philadelphia, will be described. These investigations are directed toward the development of both small molecule probes and lead compounds, starting from natural products and library-derived hits.

MEDI 10

PAINS: Are they always painful?

***Jonathan Baell**, Jonathan.Baell@monash.edu. Medicinal Chemistry Theme, Monash Institute of Pharmaceutical Sciences, Parkville, VICTORIA 3052, Australia*

With increasing access to high throughput screening, academic drug discovery is being accompanied by a plethora of publications that report screening hits as good starting points for drug discovery or as useful tool compounds, whereas in many cases this is not so. These compounds may be protein-reactive but can also interfere in bioassays via a number of other means, many of which may remain unknown, and it can be very hard to prove early on that they represent false starts.[1-4]Publications arise only to mislead others. Examples of such compound cores are shown in Figure 1.



If the reader of this abstract is working on any such compounds, he or she is strongly advised to desist from further progress: these compounds will only cause problems and will be highly unlikely to lead to anything useful. Wider awareness and recognition of these problematic compounds will help the academic drug-discovery community focus on and publish genuinely optimizable screening hits. This will be of widespread and general benefit. But are there occasions where a recognizable PAIN may actually be progressable? This presentation will delve into such issues.

1. Baell JB & Holloway GA. New substructure filters for removal of pan assay interference compounds [PAINS] from screening libraries and for their exclusion in bioassays. *J. Med. Chem*. **53** (2010) 2719-2740.

2. Baell JB. Observations on Screening-Based Research and Some Concerning Trends in the Literature. *Future Med. Chem.* **2** (2010) 1529–1546.

3. Baell JB. Redox active nuisance screening compounds and their classification. *Drug Discov. Today* **16** (2011) 840-841.

4.Baell JB, Ferrins L, Falk H, Nikolakopoulos G. PAINS: Relevance to Tool Compound Discovery and Fragment-Based Screening. *Aust. J. Chem*. **66** (2013) 1483-1494.

MEDI 11

Biophysical characterization of promiscuous protein binding by marketed drugs

***Donald Huddler**, donald.p.huddler@gsk.com, Constantine Kreatsoulas, Susan Chen. Department of Molecular Discovery Research, GlaxoSmithKline, Collegeville, Pennsylvania 19426, United States*

Over the last decade, there has been an increasing focus on the origins of non-specific screening hits. In parallel, there have been significant efforts to simplify the post screening hit qualification campaign by prospectively identifying non-specific hits among the set of all screening hits using computational and experimental approaches. A common theme of this research is that many non-specific hits do not engage targets in one-to-one binding interactions. Based on these data, we sought to (1) interrogate the assumption that successfully developed and marketed compounds lacked this non-specific binding propensity and (2) evaluate several biophysical and computational approaches to identify compound behaviors and properties that correlate with non-ideal target engagement. Based on our results, we suggest two rapid assays and a simple computational approach to identify suspect compounds among screening hits.

MEDI 12

Sometimes PAIN is a good thing: Some examples of tractable electrophilic leads from screening

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MEDI 13

Discovery of inhibitors of the hepatitis C virus using a cell-based infection assay

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Due to the lack of a culture system for infectious hepatitis C virus (HCV), the search for new HCV drugs has been greatly hampered. Cell-based screens for HCV inhibitors in use today are based on the HCV replicon system, which only targets the RNA replication step of the viral lifecycle and does not encompass viral entry, processing, assembly or secretion. High-throughput screening (HTS) with an infectious HCV system would cover the complete spectrum of potentially druggable targets in all stages of the HCV lifecycle, and thus have more biological relevance than other cell-based assays.[1] Moreover, targeting several key processes in the viral life cycle may not only increase antiviral efficacy; more importantly, it may also reduce the capacity of the virus to

develop resistance to the compound.[2] Here we describe the development of **ML391** , a potent HCV inhibitor developed using the infectious screening platform above and possessing a novel mode of action different from known antivirals for HCV.

1. Hu, Z; Lan, K.H; He, S.; Swaroop, M.; Hu, X.; Southall, N.; Zheng W.; and Liang, T.J. Novel cell-based hepatitis C virus infection assay for quantitative high-throughput screening of anti-hepatitis C virus compounds. *Antimicrob. Agents Chemother*. **2014** , *58*, 995-1004.
2. Liang, T.J.; Ghany, M.G. Current and future therapies for hepatitis C virus infection resistance to direct antiviral agents in patients with hepatitis C virus infection. *N. Engl. J. Med.* **2013** , *368*, 1907-1917.

MEDI 14

Site-specific antibody-drug conjugates via a trapped-Knoevenagel ligation

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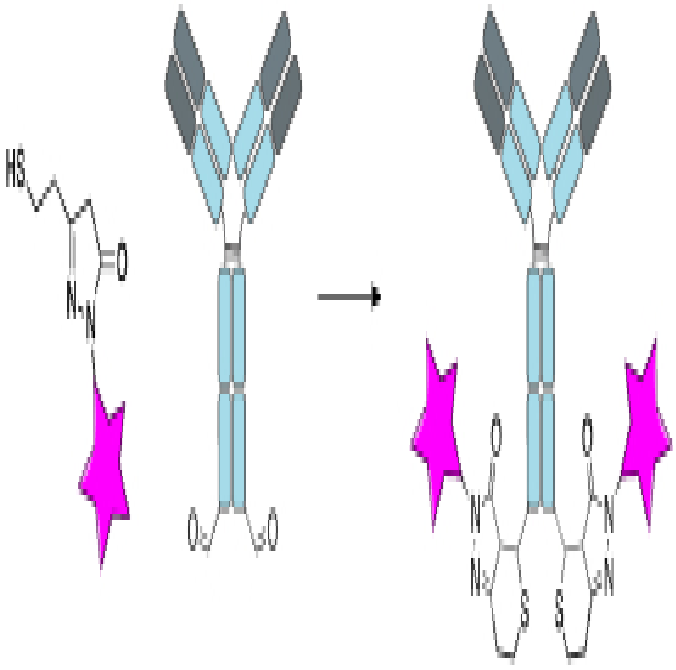
As synthetic protein conjugates grow in therapeutic importance, there is a need to expand current bioconjugation chemistries to allow for greater architectural and synthetic control. Chemistries that allow for site-specific modification and overcome the limitations of conventional ligation techniques, which create stochastic conjugates, will expand the potential of this field. One method to generate site-specific bioconjugates is via incorporation of aldehyde-functionalized biomolecules. Here, we introduce a new aldehyde-specific bioconjugation technique, the Trapped-Knoevenagel Ligation. The reaction proceeds quickly at near neutral pH to yield bioconjugates with high in vitro stability ($t_{1/2}$~38 days) in human plasma. To demonstrate the utility of this ligation technique, we generated an antibody drug conjugate (ADC) and studied it in vitro and in vivo. When compared to an ADC prepared by conventional lysine conjugation, our ADC demonstrated equal efficacy in vivo with half the drug loading and displayed superior pharmacokinetic properties.



MEDI 15

Identification and optimization of tertiary sulfonamides as RORc inverse agonists

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Screening a nuclear receptor (NR) compound subset in a NR-related orphan receptor-gamma (RORγ or RORc) biochemical binding assay revealed a benzylic tertiary sulfonamide hit. Using structure-based drug design principles, we created compounds with improved RORc biochemical inverse agonist activity and cellular potencies (<100 nM). These improved compounds also possessed appreciable selectivity for RORc over other nuclear receptors (>100-fold).

MEDI 16

Effect of fluorine introduction on hydrogen bond donating capacity of alcohols

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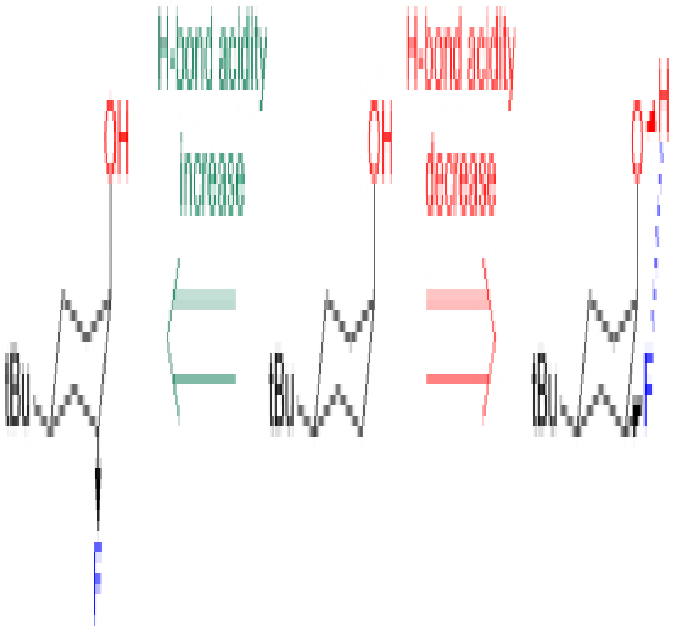
The fluorine atom is widely incorporated in bioactive compounds with the aim to improve their biological properties. This approach had great successes as shown by the number of fluorinated drugs on the market.[1] In fact, the fluorination of an organic compound can significantly modify its physical properties. Among these, the hydrogen bond (H-bond) is one of the most important interactions which is implicated in the binding affinity of a drug with its biological target. The strong inductive effect of fluorine is generally considered to lead to an increase in hydrogen bond donating capacity of an adjacent hydrogen bond donor.[3]

We have shown that this is not always the case for fluorohydrins and that a reduction in hydrogen bond donating capacity is also possible. This study demonstrates that the influence of fluorine is strongly dependent of the relative orientation between the fluorine atom and the hydroxyl group (see scheme below).[3]

This presentation will give an overview of the recent results obtained, with a tentative rationale for the observed effects.



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1) a) K. Müller, C. Faeh, F. Diederich, *Science*, **2007** , 317, 1881-1886, b) S. Purser, P. R. Moore, S. Swallow and V. Gouverneur, *Chem. Soc. Rev.*, **2008** , *37*, 320-330.

2) B. E. Smart, *J. Fluorine Chem.*,**2001** , *109*, 3-11.

3) J. Graton, Z. Wang, A.-M. Brossard, D. Goncalves Monteiro, J.-Y. Le Questel, B. Linclau, *Angew. Chem. Int. Ed.*, **2012** , *51*, 6176-6180.

MEDI 17

Inhibitory effect of various zinc binding groups on HDAC1–11

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Histone deacetylases (HDACs) covalently modify ε-N-acetylated lysines on histone tails and contribute to regulation of gene activation. Dysfunction of the enzymes is known to be related to many cancer forms. Over the last decade it has been recognized that a whole range of non-histone proteins are substrates to this family of enzymes and HDAC dysfunction is observed in a number of clinical conditions beside cancers, such as neurodegenerative disorders, memory loss, and cystic fibrosis. HDAC inhibitors, which can assist in rectifying such dysfunction is therefore an interesting target for drug development[1].

In the work presented here, scaffolds from known medicinally relevant HDAC inhibitors were the basis for a small array of potential HDAC inhibitors which were synthesized and profiled. The classical pharmacophore of HDAC inhibitors includes a capping group, a linker and a zinc binding group. The library was designed to compare novel zinc binding groups as well as zinc binding groups from established toolboxes. Silanediol and sulfonamides among other were incorporated as novel zinc binding groups which were compared to ketones, trifluoromethyl ketones, hydroxamic and carboxylic acids in the library. The compounds were tested *in vitro* against the full panel of recombinant human HDACs and expanded the current arsenal of zinc binding motifs for future design of HDAC inhibitors[2].

1. Arrowsmith, C. H.; Bountra, C.; Fish, P. V.; Lee, K.; Schapira, M., Epigenetic protein families: a new frontier for drug discovery. *Nat Rev Drug Discov* **2012,** *11* (5), 384-400.

2. Madsen, A. S.; Kristensen, H. M. E.; Lanz, G.; Olsen, C. A., The Effect of Various Zinc Binding Groups on Inhibition of Histone Deacetylases 1–11. *ChemMedChem* **2014,** *9* (3), 614-626.

MEDI 18

Hsp90 inhibitor drug conjugates (HDCs): Proof of concept in preclinical studies

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One of the major challenges in cancer treatment is to selectively deliver oncology drugs directly to tumors, and thus spare normal tissues. One successful approach to meet this challenge is demonstrated by the development of Antibody Drug Conjugates (ADCs).

It has been shown in human cancer patients and mouse xenografts that heat shock protein 90 (Hsp90) is overexpressed in tumors, and inhibitors of Hsp90 are preferentially retained in tumor tissue in contrast to their rapid clearance from normal tissues. We have developed a small-molecule drug conjugate platform technology using these unique properties of Hsp90 proteins and Hsp90 inhibitors. Hsp90-Inhibitor Drug Conjugates (HDCs) offer many of the advantages of antibody-driven targeted delivery with potentially broader applicability.

To date, we have conjugated over 40 payloads representing various oncology drug categories to Hsp90 inhibitors. Conjugates with payloads like SN-38 and docetaxel have been advanced into preclinical studies and have been shown to prolong intratumoral drug exposure in mouse xenografts, reduce on-target adverse effects, and confer superior efficacy in a variety of tumor types.

In HDCs, we have created a promising platform technology which will result in many novel anticancer agents in the near future.

MEDI 19

Cocrystals and APIs: Latest advances in "in silico" methods for predicting cocrystal coformers

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The formulation, design, and implementation of active pharmaceutical ingredients (API) is an area of great interest. Changing the structure and composition of an API by cocrystalizing it with a coformer can have a significant influence on the properties and bioavailability of the drug. Cocrystals can influence the pharmacokinetics through dissolution during drug release. Such modifications offer the potential for new patents and associated revenues.

The traditional experimental approach to screening for possible new coformers and measuring their properties is time-consuming and expensive. However, in silico methods are potentially orders of magnitude faster and are becoming more accurate, such that it is now possible to prescreen huge libraries of potential coformers in minutes or hours on a laptop computer, to determine the most likely candidates. This enables

the chemist to take into account a much wider range of possible candidates and then focus the experimental work on the most promising ones, so greatly improving the chances of success in the lab.

This presentation will review the capabilities and limitations of some of the available in silico tools for predicting cocrystal coformers and their properties, and compare them with experiment, including group contribution methods, quantum chemistry, and statistical thermodynamics. In addition, the latest algorithmic and method advances for improving the prediction of compounds that can form cocrystals and their properties in a range of solvents, will be discussed.

MEDI 20

Discovery and characterization of ML204, a novel inhibitor of the TRPC4 and TRPC5 ion channels that has been shown to protect the kidney filter

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Herein is reported the synthesis and biological action of ML204, a novel inhibitor of the TRPC4 and TRPC5 ion channels. Futher, data will be presented showing that the ion channel TRPC5 mediates kidney filtration barrier injury. A faulty filter can lead lead to albuminurea, the presence of plasma albumin into the urine, of which there are no specific therapies for this condition. Many times, albumineria is a consequence of metabolic disease (i.e., diabetes) and cardiovascular disease. Thus targeting the TRPC5 ion channel may provide a path toward a therapy for this important condition. ML204 provide an important tool compound to further validate this mechanism.

MEDI 21

Pre-clinical development and safety findings of two novel mGluR5 negative allosteric modulators

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MEDI 22

Revising the Topliss decision tree based on 30 years of medicinal chemistry literature

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In a landmark paper, Topliss [1] described a decision tree approach to guide a medicinal chemist to the most potent analogue by rational analysis of the activity order observed so far. One of the decision trees involved substituted phenyl rings. After synthesizing and measuring the potency of the phenyl and 4-chlorophenyl derivatives, subsequent molecules to synthesize and test were suggested based on the observed potency order of those already measured.

Topliss derived the tree based on a consideration of the sigma, pi and steric effects of the substituents. Now that large amounts of published activity data are available from databases such as ChEMBL, it is possible to compare the suggestions of Topliss with those obtained by analyzing activity data across many different scaffolds and targets.

We present a Topliss-like decision tree derived from activity data in ChEMBL [2] and compare its predictions to those of the original tree. An advantage of our approach is that it is possible to apply it to any situation for which sufficient data is available, for example, a tree based on data just from kinases. Furthermore, it is possible to inspect the data on which predictions are based.

[1] Topliss, J. G. *J. Med. Chem.* **1972** , *15*, 1006-1011.

[2] O'Boyle, N.M.; Boström, J.; Sayle, R.A.; Gill, A. *J. Med. Chem.* **2014** , In press. DOI:10.1021/jm500022q

MEDI 23

Discovery of orally active, brain-penetrant C6-substituted aminothiazine BACE1 inhibitors

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Beta-Site amyloid precursor protein cleaving enzyme1 (BACE1) is one of the key enzymes involved in the processing of the amyloid precursor protein (APP) and formation of amyloid-beta peptide (Abeta) in the brain. Thus, inhibition of BACE1 to prevent Abeta formation is potentially a disease-modifying approach for the treatment of Alzheimer's disease. Here, we report structure-based optimization of a series of C6-substituted aminothiazine BACE1 inhibitors. X-ray studies show that the C6-substituents of the aminothiazine core interact with threonine 72 and glutamine 73 of the flap region of BACE1. This study culminated in the discovery of several potent BACE1 inhibitors that significantly reduced brain Aβ42 levels when administered orally to rats.

MEDI 24

Drug discovery for challenging targets

***James A Wells**, jim.wells@ucsf.edu. Pharmaceutical Chemistry, UCSF, San Francisco, CA 94158, United States*

I will present work from our lab for two classes of challenging targets: protein-protein interfaces and novel allosteric sites, both to activate and inhibit protein function. We use a site-directed fragment-based discovery method, called Tethering, coupled to HTS approaches that are well-suited to probing these challenging surfaces. These approaches have led to the discovery of potent and selective compounds and some with surprising properties that will be discussed.

MEDI 25

Accessing therapeutically important proteins with millamolecules

***Carl P Decicco**, carl.decicco@bms.com. Research and Development, Bristol-Myers Squibb, Princeton, New Jersey 08543, United States*

The discovery of promising new compounds for difficult to drug, therapeutically relevant proteins will be highlighted. A powerful example of the use of chemical genetics screening which led to the elucidation of a new class of rule-breaker drug candidates will be described. The creation of highly diverse millamolecular libraries have led to the discovery of important new molecules that are active against non-traditional targets. The discovery, the advancement of these molecules and their significance as new therapeutic agents will be discussed.

MEDI 26

Chemical approach to regenerative medicine

***Peter G Schultz**, schultz@scripps.edu. Department of Chemistry, The Scripps Research Institute, La Jolla, CA 92037, United States*

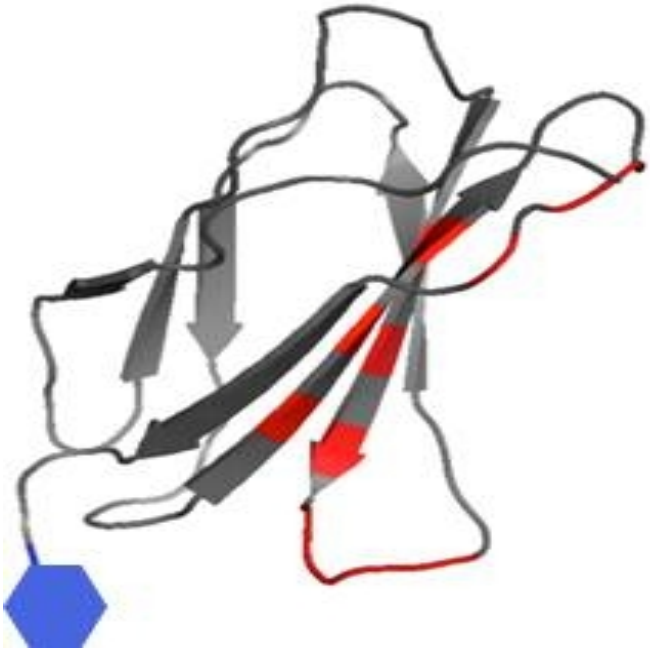
We are using a variety of cell based screens to identify and characterize the mechanisms of small molecules that affect stem cell self renewal and differentiation, and the reprogramming of somatic cells. Examples will be discussed including: the expansion of cord blood derived HSCs; the selective differentiation of MSCs to chondrocytes, OPCs to oligodendrocytes, and neural stem cells to neurons; reversible beta cell proliferation; hepatic stellate cell transdifferentiation; and human ESC self renewal and differentiation.

MEDI 27

Exploiting the biophysical properties of centyrins for targeted delivery

***Karyn ONeil**, koneil@its.jnj.com, Shalom Goldberg, Drew Elias, Donna Klein, Lavanya Peddada. Centyrex, Janssen R&D, Springhouse, PA 19477, United States*

Alternative scaffold proteins represent an emerging class of protein therapeutics. Such proteins have been explored for a variety of applications where it is difficult to use antibodies. We have designed novel consensus FN3 domains, called Centyrins, based on the sequence of the extracellular matrix protein, human Tenascin C. Centyrins are 10kDa domains that combine the specificity properties of antibodies with the stability, solubility and tissue penetration properties of small molecules. We have established a robust platform for selection of binders to a variety of therapeutically relevant targets and are exploring the robustness of the Centyrin platform for novel therapeutic applications that may address outstanding challenges in antibody drug conjugate technology and targeted nanoparticle delivery. The excellent biophysical properties of Centyrins make them ideal for conjugation technologies across an array of applications designed to combine small molecule and large molecule therapeutics. Our data suggest that the good *in vivo* stability and tolerability properties of Centryins together with their excellent site specific conjugation efficiency can be used to address some of the outstanding challenges for targeted drug delivery.



MEDI 28

Glycan assembly pathways

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Each cell on earth displays glycans on its surface. The glycans of pathogens can be essential; therefore, the pathways responsible for their assembly provide new opportunities for generating anti-infective agents. Despite the benefits of targeting these pathways, few inhibitors of glycan assembly pathways have been identified. We are exploring how the cell surface carbohydrate coat of microbes is built and how it can be detected by human lectins. Specifically, we are elucidating the molecular mechanisms that control polysaccharide localization, length, and patterning by studying the incorporation of furanose sugars (e.g., galactofuranose or arabinofuranose) in mycobacteria, corynebacteria, and fungi. Our recent investigations will be described, which are focused on elucidating the mechanisms underlying glycan biosynthesis in microbes and on understanding how human lectins interact with these “foreign” sugars.

MEDI 29

Inhibitors for amyloid fibers and oligomers

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Soriaga[1,2,3,4], Meytal Landau[1,2,3,4], Poh K. Teng[1,2,3,4], Duilio Cascio[1,2,3,4], Lin Jiang[1,2,3,4], Stuart Sievers[1,2,3,4], Alice Soragni[1,2,3,4], Lorena Saelices-Gomez[1,2,3,4], Smriti Sangwon[1,2,3,4], Elizabeth Guenther[1,2,3,4], Michael Hughes[1,2,3,4]. (1) Department of Biological Chemistry, University of California-Los Angeles, Los Angeles, CA, United States (2) Department of Chemistry & Biochemistry, University of California-Los Angeles, Los Angeles, CA, United States (3) Howard Hughes Medical Institute, United States (4) UCLA-DOE Institute for Genomics and Proteomics, United States

Amyloid diseases, including Alzheimer's, Parkinson's, and the prion conditions, are each associated with a particular protein in fibrillar form. At the morphological level, these fibers appear similar and are termed “amyloid.” We found that the adhesive segments of amyloid fibers are short protein sequences which form pairs of interdigitated, in-register beta sheets. These amyloid fibrils are probably the agents of systemic amyloid diseases and some cancers. We have shown that amyloid fibers can be inhibited. In contrast, evidence suggests that in the neurodegenerative diseases, smaller, often transient and polymorphic oligomers are the toxic entities. We have identified a segment of the amyloid-forming protein, alphaB crystallin, which forms an oligomeric complex exhibiting properties of other amyloid oligomers: beta-sheet-rich structure, cytotoxicity, and recognition by an anti-oligomer antibody. The X-ray-derived atomic structure of the oligomer reveals a cylindrical barrel, formed from six anti-parallel, out-of-register protein strands, which we term a *cylindrin*. The cylindrin structure is compatible with sequence segments from the Abeta protein of Alzheimer's disease and from other amyloid proteins. Cylindrins offer models for the hitherto elusive structures of amyloid oligomers, and are distinct in structure from amyloid fibrils. From structure-based design, we have discovered small molecules that inhibit oligomer toxicity. The influence of Bill DeGrado, my longest-term scientific collaborator, runs deep in all of these studies

MEDI 30

Discovery of novel small molecule inhibitors of diacylglycerol acyltransferase 2 (DGAT2)

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Diacylglycerol acyltransferase 2 (DGAT2) catalyzes the terminal step in triacylglycerol (TAG) synthesis. Studies performed with antisense oligonucleotides (ASO) in rodent models have shown beneficial effects of DGAT2 blockade on both glycemic control and plasma lipid profile, suggesting the potential utility of DGAT2 inhibitors for the treatment of metabolic disease. However, to date, the pharmacologic effects of DGAT2 inhibitors have not been described. This presentation will describe hit-to-lead medicinal chemistry efforts to identify novel DGAT2 inhibitors which ultimately led to the discovery of the pre-clinical candidate. To our knowledge, the pre-clinical candidate discovered is the first small molecule DGAT2 inhibitor to be evaluated in preclinical models of metabolic disease. Our findings demonstrate that inhibition of DGAT2 has the potential to impact multiple components of metabolic disease. Preclinical characterization, including *in vitro*/*in vivo* pharmacology, ADME, and safety profiles, of the preclinical candidate, will be discussed.

MEDI 31

Discovery of BMS-986118, a dual MOA GPR40 agonist that produces glucose-dependent insulin and GLP-1 secretion

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GPR40 is a G-protein coupled receptor for long-chain fatty acids which is expressed in pancreatic beta cells and enteroendocrine cells in the gastrointestinal tract. The receptor can contribute considerably to overall glycemic control by acting directly on pancreatic β-cells to enhance glucose-stimulated insulin secretion and by stimulating the release of incretins (GLP-1, GIP) from enteroendocrine cells. Throughout the discovery program, efforts were made to advance the chemotype via increases in polarity (log P), SP^3 character, and chirality with the goal of reduced off-target activity. These efforts led to the optimization of a dihydropyrazole series of GPR40 agonists culminating in the discovery of BMS-986118, which has been extensively characterized in vitro and in preclinical in vivo models of diabetes.

MEDI 32

Lead optimization in the 1,7-diazacarbazole class of inhibitors of checkpoint kinase 1

***Lewis J Gazzard**, lgazzard@gene.com. Department of Discovery Chemistry, Genentech Inc, South San Francisco, CA 94080, United States*

Checkpoint kinase 1 (Chk1) is a serine/threonine kinase which functions as a central mediator of the S and G2/M phase cell cycle checkpoints, blocking the G2/M transition to allow for repair of damaged DNA. Inhibition of Chk1 is an emerging strategy for selectively potentiating the cytotoxicity of chemotherapeutic agents in checkpoint defective tumor cells while minimizing toxicity to normal, checkpoint competent cells.

We have previously described how the application of structure-based design to a low micromolar potency hit series from a high throughput screening campaign resulted in the evolution of the novel and potent 1,7-diazacarbazole class of inhibitors of Chk1. Initial 1,7-diazacarbazole leads demonstrated encouraging rodent pharmacokinetic properties, including high oral bioavailability. However significant undesirable activities versus both kinase and non-kinase off-targets were identified. We will describe how through a series of SAR investigations we were able to optimize biochemical potency and identify the structural features responsible for these liabilities. Subsequently we were able to transition to a new series with improved physical properties, notably reduced lipophilicity, leading to a greatly improved off-target profile and ultimately a clinical candidate.

MEDI 33

Tuning of halogen bonds and integration into binding motifs

***Frank M. Boeckler**, frank.boeckler@uni-tuebingen.de, Andreas Lange, Johannes Heidrich, Markus O. Zimmermann. Department of Pharmacy and Biochemistry, Eberhard Karls University Tuebingen, Tuebingen, BW 72076, Germany*

Halogen bonding has recently experienced a renaissance, gaining increased recognition as a useful molecular interaction in the life sciences. Halogen bonds are favorable, fairly directional interactions between an electropositive region on the halogen, the σ-hole, and a number of different nucleophilic interaction partners. Some aspects of halogen bonding are not yet understood well enough to take full advantage of its potential in drug discovery. The placement of chlorine, bromine, or iodine in aryl halides and heteroaryl halides, as well as the occurrence of other substituents can strongly modify the strength and geometry of halogen bonds. Based on their complex nature, halogen bonds may consist of different contributions of electrostatic, dispersive, polarization and charge transfer effects. Such contributions will probably change based on the chemical and biological environment of the halogen bond. Particularly tuning effects and integration of halogen bonds in efficient and selective binding motifs are topics most interesting for both, fundamental academic research and industrial applications. In this talk, I will highlight tools and studies helping to address these topics with the aim of making halogen bonds more easily applicable in drug discovery campaigns.

MEDI 34

Discovery and early development of AZD2927: A selective blocker of the inwardly rectifying acetylcholine activated potassium channel (K_{ACh}) for the treatment of atrial fibrillation

Kenneth L Granberg[1], *kenneth.granberg@astrazeneca.com, Björn Holm*[1], *Ulf Fahlander*[1], *Alma Redzic*[1], *Maria Petersson*[1], *Hans Emtenäs*[1], *Yantao Chen*[1], *Mickael Mogemark*[2], *Mikael Sellén*[1], *Jan Lindberg*[1], *Krystle da Silva Andersson*[1], *Jonas Boström*[1], *Ola Fjellström*[1], *Henrik Sörensen*[1], *Cecilia Ericsson*[3], *Carina Kärrman-Mårdh*[2], *Leif Carlsson*[1], *Göran Duker*[1], *Gunilla Linhardt*[1], *Ulrika Svensson*[1], *Jan-Arne Björkman*[1], *Sara Laine*[1], *Charlotte Tropp*[1], *Boel Löfberg*[1], *Marianne Ridderström*[1], *Emma Lindhardt*[1], *Monika Sundqvist*[1], *Helene Hallberg*[3], *Kerstin Kenne*[3], *Jenny Ottosson*[4], *Hans van Giezen*[4], *Steffen Ernst*[4], *Anders Berggren*[5]. *(1) Cardiovascular and Metabolic Diseases, Innovative Medicines, AstraZeneca, Mölndal, Sweden (2) Respiratory, Inflammation and Autoimmunity, Innovative Medicines, AstraZeneca, Mölndal, Sweden (3) Drug Safety and Metabolism, Innovative Medicines, AstraZeneca, Mölndal, Sweden (4) Global Medicines Development, AstraZeneca, Mölndal, Sweden (5) Early Clinical Development, Innovative Medicines, AstraZeneca, Mölndal, Sweden*

There is a significant unmet medical need of a safe and effective antiarrhythmic agent for management of atrial fibrillation (AF) which is the most common sustained arrhythmia encountered in clinical practice. The US Centers for Disease Control and Prevention estimates that more than 2.7 million Americans had AF in 2010, and expects that as many as 12 million people will have AF in 2050. AF contributes markedly to population morbidity and mortality conferring to a 5-fold increase risk of stroke and a doubled mortality rate independently of other known predictors of death.

Aim was to identify selective blockers of the inwardly rectifying acetylcholine activated potassium channel (K_{ACh}) and the associated current (IK_{ACh}) as a mean to maintain sinus rhythm (SR) control. The K_{ACh} channel is a heterotetrameric complex made up of two Kir3.1 and two Kir3.4 subunits. In the heart, these are predominantly expressed in atrial tissue and blockade of IK_{ACh} is therefore a promising approach for AF treatment as the risk for ventricular proarrhythmia would be low.

The original hit was identified through a HTS and lead optimization aimed at reducing opioid kappa agonism, $Na_v1.5$ blockade, time dependent CYP3A4 inhibition, CYP2D6 inhibition and reactive metabolites. This led to the identification of AZD2927 ((S)-4-fluoro-N-(1-(3-hydroxyazetidin-1-yl)-3-methylbutan-2-yl)-N,3-dimethylbenzamide) fulfilling our criteria of an agent selectively blocking IK_{ACh} *in vitro* and possessing potent electrophysiological and antiarrhythmic efficacy *in vivo*. AZD2927 was nominated as the clinical candidate for a Proof-of-Principle study in atrial arrhythmia patients undergoing an invasive electrophysiological procedure.

MEDI 35

Discovery of GDC-0339 as a bioavailable and efficacious PIM inhibitor for multiple myeloma

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Pim kinase inhibition is emerging as a promising rational for the treatment of multiple myeloma, a progressive hematological cancer with no cure. This talk will describe our efforts in discovering a potent, selective, bioavailable and efficacious pan-Pim inhibitors. This is the first time that the structure of an early development candidate, GDC-0339, will be fully disclosed. Structure based drug design using co-crystal structures and property based drug design will be discussed.

MEDI 36

Discovery of VX-787: A novel, first-in-class, orally bioavailable azaindole inhibitor of influenza PB2

***Michael P Clark**, michael_clark@vrtx.com. Department of Chemistry, Vertex Pharmaceuticals, Inc, Boston, MA 02210, United States*

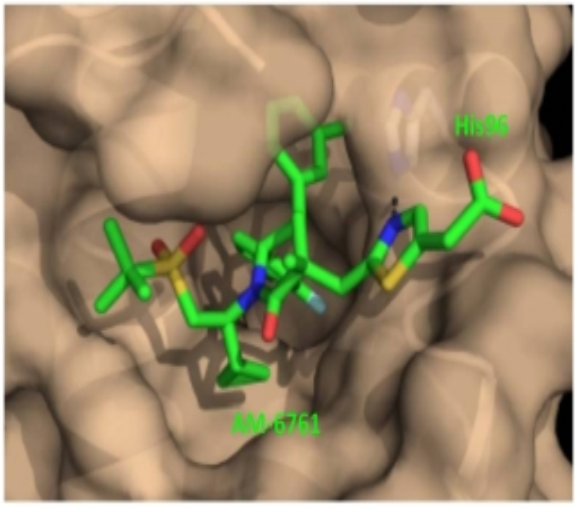
In our effort to develop agents for the treatment of influenza, a phenotypic screening approach utilizing a cell protection assay identified a series of azaindole based inhibitors of the cap-snatching function of the PB2 subunit of the influenza A viral polymerase complex. Using a bDNA viral replication assay[1] in cells as a direct measure of antiviral activity, we discovered a set of cyclohexyl carboxylic acid analogs, highlighted by VX-787. VX-787 shows strong potency versus multiple influenza-A strains, including pandemic 2009 H1N1 and avian H5N1 flu strains, and shows a efficacy profile in a mouse influenza model even when treatment was administered 48h post infection. VX-787 represents a first-in-class, orally bioavailable, novel compound that offers potential for the treatment of both pandemic and seasonal influenza and has a distinct advantage over the current standard of care treatments including potency, efficacy and extended treatment window

MEDI 37

Novel inhibitors of the MDM2-p53 interaction featuring carboxylic acid isosteres

***Ana Z Gonzalez**, anagonza@amgen.com, Zhihong Li, Hilary P Beck, Jude Canon, Ada Chen, David Chow, Jason Duquette, John Eksterowicz, Brian M Fox, Jiasheng Fu, Xin Huang, Jonathan Houze, Lixia Jin, Yihong Li, Yun Ling, Mei-Chu Lo, Alexander M Long, Lawrence R McGee, Joel McIntosh, Jonathan D Oliner, Tao Osgood, Yosup Rew, Anne Y Saiki, Paul Shaffer, Sarah Wortman, Peter Yakowec, Xuelei Yan, Qiuping Ye, Dongyin Yu, Xiaoning Zhao, Jing Zhou, Steven H Olson, Daqing Sun, Julio C Medina. Therapeutic Discovery, Amgen Inc., San Francisco, California 94080, United States*

Activation of the pro-apoptotic protein p53 is a promising approach towards the treatment of cancer. p53 activation leads to the transcription of multiple downstream genes that regulate cell cycle control, apoptosis, DNA repair and senescence. About half of all human cancers progress either by mutation or deletion of p53. However, for the half that retains $p53^{wt}$ activity, survival is achieved by other mechanisms such as upregulation of one of its natural antagonists, MDM2. We and others aimed to design small molecules that bind to MDM2 impeding it's interaction with p53, reactivating its pathways. From these, several inhibitors have emerged and are now being tested in the clinic.



We recently reported the discovery of potent and selective morpholinone and piperidinone inhibitors of the MDM2-p53 interaction, including our clinical candidate AMG 232. These inhibitors have in common a carboxylic acid moiety that engages in an electrostatic interaction with MDM2-His96. This presentation discusses our continued search for diverse inhibitors leading to the discovery of novel replacements for these acids and uncovering new interactions with the MDM2 protein. In particular, using pyridine or thiazole as carboxylic acid isosteres resulted in very potent analogues. For example, piperidinone-thiazole AM-6761 has remarkable biochemical (HTRF IC_{50} = 0.1 nM) and cellular potency (SJSA-1 EdU IC_{50} = 16 nM), favorable pharmacokinetic properties and excellent anti-tumor activity in vivo in the SJSA-1 osteosarcoma mouse xenograft model with an ED_{50} of 11 mg/kg. Optimization efforts towards the discovery of these inhibitors as well as the difference in clearance pathways between AM-6761 and AMG 232 will be discussed.

MEDI 38

Fragment based design of protein-protein interaction antagonists: A case history

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Binding sites of protein-protein interaction (PPI) targets are often solvent exposed and poorly defined, presenting significant challenges to the development of antagonists. Fragment based drug discovery (FBDD) has been successfully used to identify hits for PPI targets, providing alternatives to more traditional methods like high throughput screening or peptidomimetics. The presentation will describe the use of FBDD to identify potent dual antagonists of XIAP and cIAP1, members of the inhibitor of apoptosis protein (IAP) family. IAPs are key regulators of anti-apoptotic and pro-survival signaling pathways and are often overexpressed in cancer, being associated with tumour progression and resistance to treatment. A defining feature of IAPs is the presence of three Baculoviral IAP Repeat (BIR) domains in the sequence. A mitochondrial protein, second mitochondrial activator of caspases (SMAC) deactivates the anti-apoptotic function of IAPs through a protein-protein interaction with the IAP-BIR domains mediated by binding of a tetrapeptide motif (AVPI) within the SMAC N-terminal region. Various groups have reported SMAC mimetic compounds based on AVPI for the treatment of cancer. These compounds are selective for cIAP1 over XIAP, but there is evidence that the full pro-apoptotic response requires high affinity for XIAP. Using our fragment-based screening platform (Pyramid^{TM}) we identified a non-alanine fragment hit with > 5 mM affinity for XIAP. This molecule was optimized into a series of sub-10 nanomolar, balanced dual cIAP1/XIAP antagonists, structurally distinct from previously reported alanine-based peptidomimetics. Key factors in the success of the optimization were (i) use of multiple X-ray crystal structures of co-complexes (ii) maximization of the electrostatic potential complementarity between protein and ligand and (iii) understanding of conformational preferences of ligands in solution in order to increase binding efficiency. This approach led to the discovery of an advanced lead, designated AT-IAP, having oral bioavailability and activity in mouse xenograft models.

MEDI 39

Virtual screening strategies for the discovery of new inhibitors of cruzain from *Trypanosoma cruzi*

Mariana Laureano de Souza[1]*, marianalaureano@gmail.com, Rafaela S Ferreira*[2]*, Renata Krogh*[1]*, Glaucius Oliva*[1]*, Luiz C Dias*[3]*, Adriano D Andricopulo*[1]*. (1) Department of Physics and Interdisciplinary Science, University of Sao Paulo - USP, Sao Carlos, Sao Paulo, Brazil (2) Department of Biochemistry and Immunology, Federal University of Minas Gerais - UFMG, Belo Horizonte, Minas Gerais, Brazil (3) Department of Chemistry, State University of Campinas - UNICAMP, Campinas, Sao Paulo, Brazil*

Chagas' disease is a major cause of morbidity and mortality in Latin America. The limited drugs available for the treatment of this parasitic disease have severe limitations, including poor efficacy and high toxicity. The enzyme cruzain, the major cysteine protease from *Trypanosoma cruzi*, is an attractive target for the development of a new generation of trypanocidal agents. In order to search for novel classes of cruzain inhibitors, structure-based virtual screening (SBVS) approaches were employed considering two subsets of componds (lead- and fragment-like) from ZINC. Initially, the compounds were screened with the program DOCK, and the top hits from each subset were subsequently evaluated by the GOLD and Surflex methods. A number of computational hits was inspected and selected among the best ranked molecules from each docking strategy. The biochemical evaluation of 18 compounds possessing substantial structural diversity against recombinant *T. cruzi* cruzain allowed the identification of a potent (IC_{50} = 580 nM, K_i = 100 nM) and non-covalent inhibitor (LE = 0.53 kcal mol^{-1} per non-H atom), which was also active against *T. cruzi* whole cell cultures. These results provide a promising starting point for lead optimization efforts. The detailed results and *in vitro* anti-*T. cruzi* properties of these compounds will be presented.

MEDI 40

Rapid identification and understanding of selectivity cliffs

***Tim Cheeseright**, tim@cresset-group.com, Mark Mackey, Rae Lawrence. Cresset, Cambridge, Cambridgeshire SG80SS, United Kingdom*

Selectivity has always been important in drug discovery. However, in the age of genetics it has an enhanced focus for the discovery of efficacious small molecule therapeutics. In particular it has become increasingly important to engineer selectivity for one member of a close family of biological targets. To do this effectively medicinal chemists must find, understand and exploit features of their series that impart the desired selectivity.

Activity cliffs are an established and useful method for rapidly finding critical regions in the structure activity (SAR) landscape. These are regions of a molecule where small changes in the structure cause significantly larger changes in the activity of the series. We recently presented a method for the identification and understanding of activity cliffs using the shape and especially the electrostatic character of the molecules.

In this poster we present an extension to our previous method where we rapidly identify molecule features that are critical to the improvement of the selectivity of a lead series. Critical to this approach is clear visualisation of large datasets that can contain many activity measurements. Once identified, the reason for the selectivity change must be thoroughly understood in order to improve on the observed selectivity and progress the series through to candidate. To do this we present the use of 3D shape and electrostatic difference maps that clearly and concisely communicate the important changes in the binding characteristics of the molecules involved in the selectivity cliff.

MEDI 41

ACD/Percepta structure design engine: Virtual enumeration and screening of physchem properties for 10^{16} compounds in real time

Pranas Japertas[1,2]*, pranas.japertas@acdlabs.com, Kiril Lanevskij*[1,2]*, Andrius Sazonovas*[1,2]*. (1) VsI [quot]Aukstieji algoritmai[quot], Vilnius, Lithuania (2) ACD/Labs, Inc., Vilnius, Lithuania*

The efforts of lead optimization projects are directed towards analogs that have favorable ADME profiles and are devoid of safety concerns whilst retaining target activity. Recently, we have developed a novel computational platform called ACD/Structure Design Engine (SDE) to aid such projects by generating virtual analog libraries in the physicochemical space regions compatible with the desired biological characteristics. SDE is implemented on top of ACD/Percepta software platform that couples virtual analog generation to their physicochemical, ADME/Tox profiling and ranking by conformance to the particular project objectives. While enumeration of structural analogs falling within the desired physicochemical property ranges is quite straightforward in case of one varying substituent position, most real-world projects are focused on optimizing multiple substituents in different parts of the molecule. To address this issue, we present a new generation of SDE that would enable extensive enumeration of substituent property space in accordance with specific constraints defined by the user, and that would be able to account for up to four simultaneously varying substituent positions. Several optimization techniques allowed to bring complexity of this task from $O(n^4)$ to approximately $O(n^2)$. In such case, with a built-in database of more than 10^4 building blocks for each substitution position, this leads to exploration of up to 10^{16} virtual analogs in seconds, which allows to work with such virtual set of compounds interactively and greatly enhances the potential of encountering new compounds with the most favorable property profiles.

MEDI 42

ACD/Percepta portal: Crowdsourcing in medicinal chemistry projects

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In-house knowledge has always been considered a valuable asset for any company in the pharmaceutical industry. Adequate management of this wealth of intellectual information, facilitating re-use of existing expertise instead of redundant experimental studies, is among the primary objectives of any organization striving to make drug development process more cost-effective. This work introduces ACD/Percepta Portal – a new platform that combines well established components of the ACD/Percepta software (predictive algorithms, structure design engine, databases, etc.) with the

flexible network based deployment, raising software interactivity to a new level and offering some exciting features.

A particular focus of this presentation will fall on the components of the ACD/Percepta Portal that enable utilizing wisdom of the crowd within the company. The workflow starts in Drug Profiler that provides a quick summary of all relevant information about the posted chemical structure, and brings up a virtual discussion board in order to get a live feedback from the company peers working in other departments and involved in various projects. The response comes in the form of favorable/unfavorable votes that can be accompanied by comments containing any arguments for a particular opinion. The size of the "crowd" involved as well as the weight of the input from a particular group of voters can be easily adjusted, for example, depending on the project phase. The voting feedback can subsequently be used in compound ranking, alongside their PhysChem & ADME/Tox profiles and any other in-house data. Wrapped up in an intuitive visual interface, this medicinal chemistry "social network" adds a new dimension to the process of knowledge sharing within the company. As a result, ACD/Percepta Portal transforms the usual drug-discovery project workflow within a dedicated group into an interactive company-wide endeavor.

MEDI 43

Diketopiperazine-based inhibitors of protein-protein interactions

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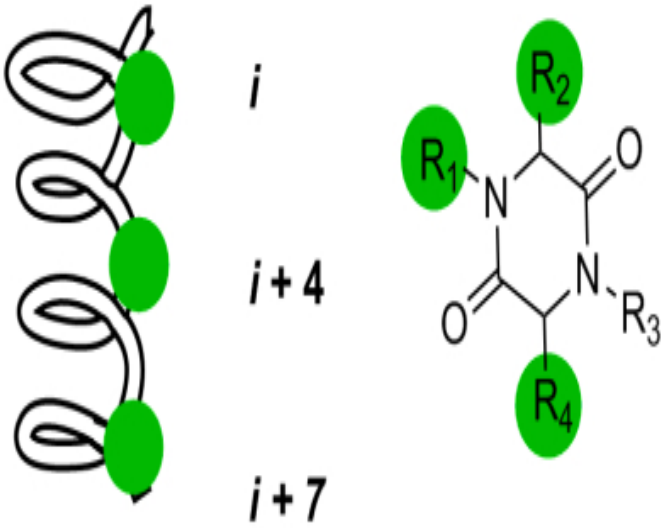
A large number of important regulatory pathways in the human body are controlled by protein-protein interactions (PPIs). Inhibition of PPIs would provide possibilities to dissect such pathways and also identify new drug targets[1]. Since short α-helical structures have been shown to play an important role in several PPIs, they are attractive targets for the design of molecular mimetics[2]. We are investigating if diketopiperazines (DKPs) with the general structure **1** can be used as α-helix mimetics and disrupt PPIs. Computational studies have revealed that **1** (figure 1) place the interacting substituents (R-groups) in a similar way as residues at the *i*, *i* + 4, *i*+ 7 positions of a α-helix, illustrated as green sphere in figure 1.



1

Figure 1. General structure 1 and how structure 1 mimic a α-helix.

Acknowledgement. Thanks to the Swedish Research Council for financial support.

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(2) Peczuh, M. W.; Hamilton, A. D. *Chemical Reviews.***2000** , 7, 2479.

MEDI 44

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

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Bioisosteric replacement and the functional group optimization of a lead are well established and important medicinal chemistry methods applied in drug discovery. *In silico* methods for performing these medicinal chemistry transformations can significantly expand the chemistry for a project and increase the chance of success. Previous *in silico* methods for performing these transformations are typically limited to

2D space, ignore the receptor, or rank molecules using simplistic descriptors. In this work, a new method for performing the transformations in the context of the 3D receptor and ranking the results using energy scores and synthetic feasibility is presented.

MEDI 45

Novel antifibrinolytic strategy: Discovery of potent and safe antihemorrhagic agents with new mechanism of action

*Josune Orbe[1], Jose Antonio Rodriguez[1], Juan Antonio Sánchez[2], Lara Montori[1], Agustina Salicio[1], Miriam Belzunce[1], Ana Ugarte[2], Haisul C. Y. Chang[3], Obdulia Rabal[2], Jose Antonio Páramo[1], **Julen Oyarzabal**[2], julenoyarzabal@unav.es. (1) Laboratory of Atherosclerosis, Division of Cardiovascular Sciences, Center for Applied Medical Research (CIMA) - University of Navarra, Pamplona, Spain (2) Small Molecule Discovery Platform, Center for Applied Medical Research (CIMA) - University of Navarra, Pamplona, Spain (3) Experimental Hepatology, Center for Applied Medical Research (CIMA) - University of Navarra, Pamplona, Spain*

New therapeutic strategies are needed to reduce bleeding and blood transfusions associated with increased morbidity and mortality in surgical and trauma patients. The most urgent clinical need is to discover potent and safe agents capable of reducing hemorrhage with a success rate better than current therapies by antifibrinolytic lysine analogs (e.g current standard of care: tranexamic acid, TXA), since it could have a huge socioeconomical impact. Transcriptome analysis of human endothelial cells identified MMP10 as a new pro-fibrinolytic agent. Thus, we focused on designing a promising new class of small molecules, potent and soluble, that inhibited fibrinolysis by targeting metalloproteases (MMPs) a novel mechanism of action leading to acute therapeutic application for MMP inhibition. Our lead compound CM-352 inhibited fibrinolysis in a functional assay in whole blood (thromboelastometry), was efficacious in mouse tail-bleeding model at a 30,000 times lower dose than TXA (Figure 1), and it also reduced bleeding in a liver hepatectomy model, highlighting the potency of this compound. Additionally, CM-352 displays optimal pharmacokinetic and safety profiles, even at 10 times higher dose, with no evidence of thrombosis or hemostatic impairment. Together, our data indicate that CM-352 is a promising new clinical candidate for the treatment of bleeding.

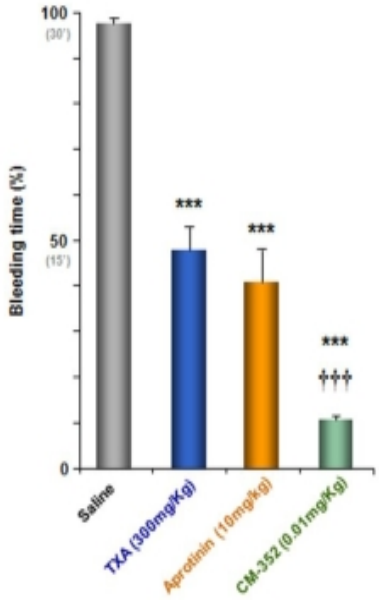


Figure 1. Mean±SME; n≥10 per group; where *** p<0.001 vs saline and ††† p<0.001 *vs* TXA or Aprotinin

MEDI 46

Discovery of a highly potent and selective Liver X Receptor β agonist, K-20770-2b

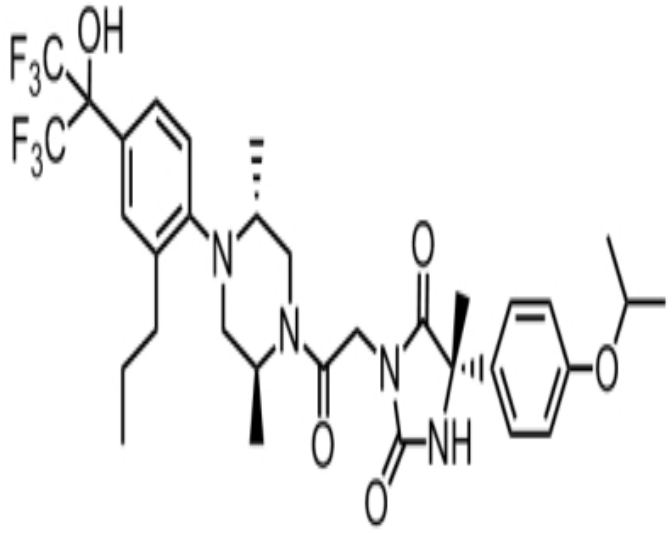
***Minoru Koura**, m-koura@kowa.co.jp, Hisashi Sumida, Kimiyuki Shibuya, Takayuki Matsuda, Yuichiro Watanabe, Takashi Enomoto, Yasunobu Yoshinaka, Hiroyuki Yamazaki, Sohei Tanabe. Tokyo New Drug Research Laboratories, Kowa Company Ltd., Higashimurayama, Tokyo 189-0022, Japan*

Liver X Receptors (LXRs) α and β are ligand activated transcription factors of the nuclear receptor superfamily involved in cholesterol metabolism, lipogenesis and glucose homeostasis. LXRα is the dominant subtype in the liver, while LXRβ is distributed ubiquitously. Elevation of triglyceride (TG) in plasma and liver in animal models is an adverse effect reported for many other LXR agonists, and is thought to be caused by up-regulation of SREBP1-C following LXRα activation in liver.

Therefore in our attempt to differentiate between these two LXR subtypes and discovering LXRβ selective agonists for the treatment of atherosclerosis, we have synthesized an LXRß agonist, K-20770-2b, with high potency and selectivity (EC_{50} for LXRα:250 nM, EC_{50} for LXRβ: 3.5 nM, α/β ratio: 71).
In a 10-week multiple oral dose study in low-density lipoprotein receptor knockout mice, K-20770-2b enhanced blood ABCA1 mRNA expression and suppressed lipid

accumulation in aortic valve areas by 20% at a dose of 0.1 mg/kg/day without elevation of plasma TG. In contrast, the non-selective compound GW3965 (10 mg/kg) also prevented aortic lipid
deposition, but markedly increased plasma TG. K-20770-2b enhances the reverse cholesterol transport system without affecting TG levels and provides a novel approach for the treatment of atherosclerosis.

The synthesis, structure-activity relationships and pharmacological effects of K-20770-2b are
presented.



K-20770-2b

MEDI 47

Development of selective GRK2 inhibitors for the treatment of heart failure

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Heart failure is one of the leading causes of death in the Western world with a 50% mortality rate after five years. In the failing heart catecholamine levels are increased leading to stimulation of the β-adrenergic receptors. As a result, signaling of these receptors is increased. G-protein coupled receptor kinases (GRKs) regulate the β-adrenergic receptors through the process of desensitization. Elevated levels of G-protein coupled receptor kinase 2 (GRK2) have been found in cardiac muscle of animal models subjected to transverse aortic constriction. In addition, inhibition of GRK2 using a peptide inhibitor and through gene knockout has been shown to have cardioprotective effects in mice models. Previously, a small molecule inhibitor, the FDA approved selective serotonin reuptake inhibitor, paroxetine, was shown to inhibit GRK2 and improve cardiac performance in animal models. Through the use of structure based drug design we have made a library of paroxetine derivatives that have shown potency and selectivity for GRK2.

MEDI 48

Synthetic study of lanost-8-en-3β-ol-7,11-dione, an inhibitor of cholesterol biosynthesis

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This paper represents the chemical synthesis of ketolanosterol to be a potent inhibitor of HMG-CoA reductase activity

MEDI 49

Allylic and benzylic oxidations of steroids with chromiunm (VI) reagents

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Complexation of chromium reagent has been found to be effective for allylic and benzylic oxidation. This paper represents the allylic oxidation of steroids to yield the corresponding α, β-unsaturated ketones.

MEDI 50

Synthetic study of methylhydrazinoacetic acid for inhibitory activity against bacteria

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This paper represents a facile procedures of synthetic strategy for methylhydrazinoacetic acid from the acyclic homoallylamine as an intermediate

MEDI 51

Deeper insight on physicochemical determinants of hERG inhibitor specificity

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Drug-induced inhibition of hERG potassium channels is a major obstacle in drug discovery due to the risk of severe cardiac adverse effects. Applying in silico techniques for early identification of potential hERG blockers is an attractive approach, but the usability of available tools is still quite limited. Despite a few relative successes in hERG inhibition modeling, many recently published models suffer from inherent complexity and lack of interpretability, while simple and well-known physicochemical rules remain mostly qualitative. In this study we attempt to overcome these issues by constructing a large and thoroughly curated hERG inhibition database spanning a range of >2500 diverse chemicals, and utilizing these data to classify compounds as hERG blockers/non-blockers solely on the basis of principal physicochemical determinants such as lipophilicity, ionization, aromaticity, molecular size and flexibility. The proposed classification model was built using Gradient Boosting statistical method known for its ability to account for complex nonlinear relationships and low sensitivity to outliers. The model was able to produce correct classification for almost 80% of validation set compounds indicating that the major part of variation in hERG inhibition propensity can be conveyed by general physichochemical trends, in full consistence with broad ligand specificity of hERG channel. In addition to evaluation and visualization of the physicochemical effects, the obtained model can be used as a baseline predictive tool for more detailed analysis, e.g., exploring the potential of discrete structural modifications to further attennuate hERG liability of candidate compounds.

MEDI 52

Structure activity relationship studies on a vascular endothelial growth factor binding peptide

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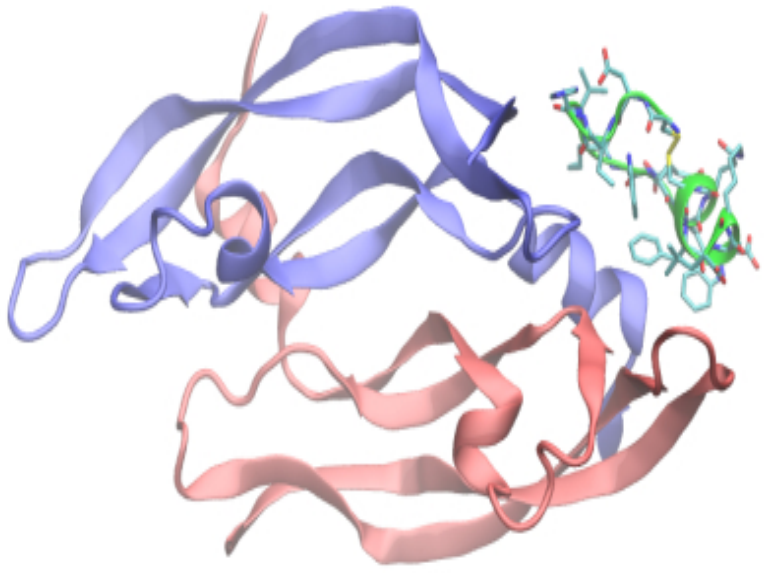
The deregulation of angiogenesis is involved in cancer and inflammatory diseases. The development of molecules able to control angiogenesis is therefore the subject of intense research. Vascular endothelial growth factor A (VEGF) is the main pro-angiogenic factor. Peptides able to bind the VEGF and disrupt its interaction with the cellular receptors VEGFR1 and VEGFR2 have been identified by Genentech. In particular, the peptide v114* (VEPNCDIHV^{n}LWEWECFERL-NH_2, with the two cysteines involved in a disulfide bond) was chosen as a starting point for our SAR studies, because a potent $K_i$of 60 nM has recently been determined.

A detailed SAR study of the peptide v114* will be presented, including the incorporation of non-natural amino-acids, the variation of the macrocycle ring size and the introduction of additional side chains covalent constraints for the stabilization of an α-helical segment. Biochemical activity evaluation was made by Elisa type displacement assays of the VEGFR1 / btVEGF interaction, completed by ITC measurements with $(VEGF_{11-107})_2$, allowing the determination of thermodynamic parameters of binding. The efficiency of the strategy was further supported by X-ray diffraction analysis of $(VEGF_{11-107})_2$co-crystallized with one of the constrained analogs (figure).



These findings provide a strong basis for further optimization of VEGF binding peptidomimetics with increased rigidity and protease stability, decreased size, and overall better drug-like properties.

MEDI 53

Discovery of highly potent and selective PoA MK2 inhibitors

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Mitogen activated protein kinases such as p38 MAPK (p38) and MAPKAP-K2 (MK2) are attractive targets for inflammatory diseases such as rheumatoid arthritis (RA), Crohn´s disease, inflammatory bowel syndrome (IBS) and chronic obstructive pulmonary disease (COPD). Targeting the MK2 pathway downstream of p38 has been hypothesized to provide a therapy with p38-like efficacy, but with an improved safety profile. This is due to the dual role of p38, which not only activates MK2 but also another kinase, MSK1. It has been hypothesized that inhibition of p38 decreases not only the pro-inflammatory mediator TNFα which signals through MK2, but also the anti-inflammatory mediator IL-10, through inhibition of the MSK1 pathway, which might contribute to the side-effects. Thus, inhibiting MK2 should block the production of TNFα, whilst sparing the anti-inflammatory mediator IL-10. To achieve this, our aim was to identify substrate selective p38α inhibitors which bind only to the heterodimeric complex of p38α-MK2, and inhibit the phosphorylation of non-activated MK2 by p38α via a prevention of activation (PoA) mechanism. This attractive PoA approach provides the possibility to identify novel binding modes in order to mitigate issues associated with standard kinase inhibition, such as selectivity and potency.

The development of a novel lead series having excellent potency, good physicochemical properties and kinase selectivity profile will be described. Furthermore, human cell results leading to the invalidation of the hypothesis of sparing IL-10 will be presented.

MEDI 54

Synthesis and evaluation of tetracyclic compounds as selective glucocorticoid receptor agonists

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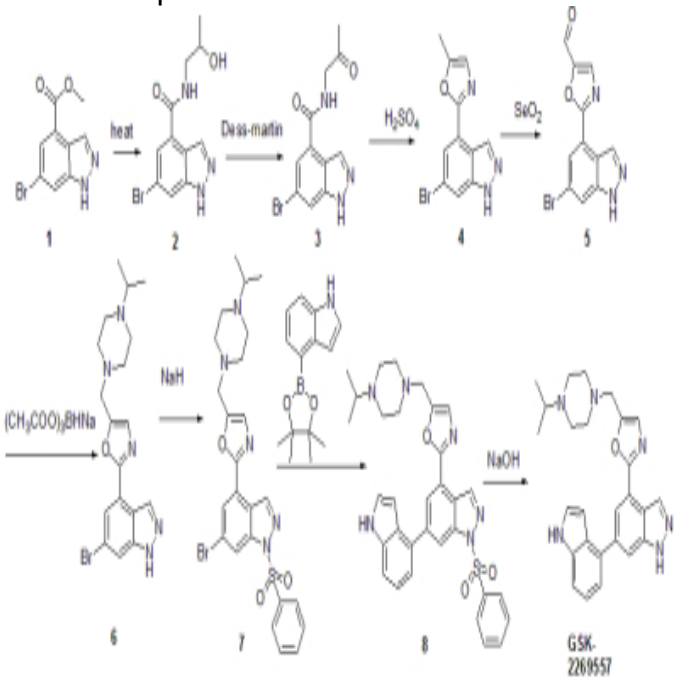
Glucocorticoid receptor (GR) agonists exert anti-inflammatory effect by GR-mediated transrepression (TR) via inhibition of several transcription factors. On the other hand, they also cause multiple side effects by GR-mediated transactivaton (TA). We disclosed structurally novel tetracyclic compounds as potent GR agonists. Further structure optimization resulted in the improvement of TR/TA selectivity in vitro. Several compounds were selected for in vivo evaluation and examined their anti-inflammatory effects by oral administration.

MEDI 55

Novel synthetic method of GSK2269557

***Zhu Xingyong**, zhu_xingyong@PharmaBlock.com, **Wu Xihan**, Wu_xihan@pharmblock.com, **Yang Minmin**, Yang_minmin@pharmablock.com. department of chemistry, PharmaBlock (Nanjing) R&D Co. Ltd., nanjing, jiangsu 210061, China*

GSK2269557 is a potent inhalable PI3Kd-selective inhibitor being developed by GSK for
the treatment of inflammatory airway diseases. Here we are reporting an efficient route to this compound.



Starting from methyl 6-bromo-1H-indazole-4-carboxylate **1** , the key oxazole intermediate **5** was obtained through aminolysis, Dess-Martin oxidation, dehydration and subsequent benzylic oxidation. Reductive amination followed by protecting group manipulation and Suzuki-coupling afforded the title compound **GSK2269557** in24.8% overall yield after eight steps. Our route avoids the use of expensive starting material and toxic organtin reagent in the key Stille-coupling in the prior report. In addition, all intermediates could be easily purified by slurrying in proper solvents and no column chromatography was needed.

MEDI 56

Kv1.3 inhibitors: En route to clinical trials

***Stefan Tasler**, stefan.tasler@4sc.com, Tobias Dreker, Ilga Krimmelbein, Svetlana Hamm. 4SC Discovery GmbH, Planegg-Martinsried, Germany*

The voltage-gated potassium channel Kv1.3 represents a promising target for the treatment of autoimmune diseases like multiple sclerosis, rheumatoid arthritis and psoriasis as it is a crucial player in maintaining the activation signal within T-cells, potentially allowing for a selective suppression of autoimmunity and thus minimizing the potential for opportunistic infections during therapy. The Lead Optimization Program for two small molecule hit classes resulted in a fine-tuning of activity, selectivity and physicochemical and PK properties. Selected representatives display highly encouraging ameliorative effects within a set of animal models relevant in the context of autoimmune diseases, comparable or even favourable over positive controls like methotrexate, betamethasone or tacrolimus. Based on promising PK/PD data, IND enabling studies shall be initiated.

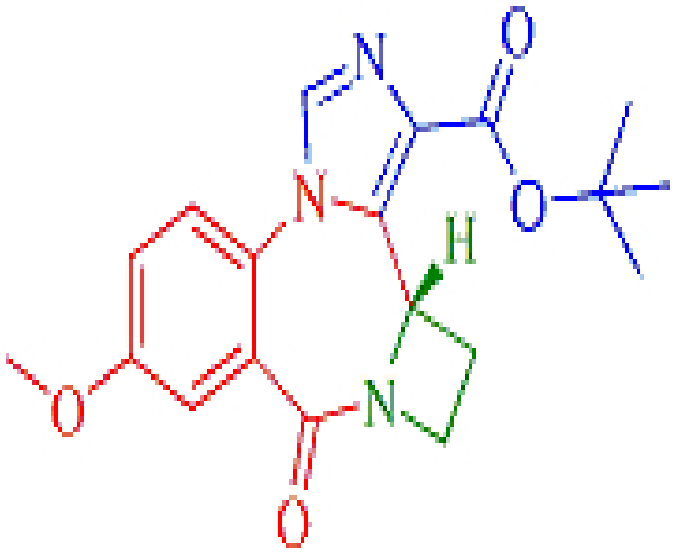
MEDI 57

Inducing airway smooth muscle relaxation by targeting the restricted α-subunit repertoire of $GABA_A$ receptors using CMD-45

***Michael Rajesh Stephen**[1], stephenm@uwm.edu, Rajwana Jahan[1], George Gallos[2], Charles W Emala[2], James M Cook[1]. (1) Department of Chemistry and Biochemistry, University of Wisconsin-Milwaukee, Milwaukee, Wisconsin 53211, United States (2) Department of Anesthesiology, College of Physicians and Surgeons of Columbia University, New York, New York 10032, United States*

Asthma is a condition in which airway smooth muscles(ASM) become constricted and swell. The extra mucus created reduces the flow of air in and out of the lungs. Medications are available to promote ASM relaxation and/or suppress local immune/ inflammatory processes by inhaling corticosteroids and β-adrenergic antagonists and out of them promoting airway smooth muscle relaxation would be clinically valuable.[1] Activation of ASM $GABA_A$ receptors with agonist (8-methoxy imidazobenzodiazepine

(CMD-45)) selective for these subunits resulted in appropriate membrane potential changes, chloride currents and promoted relaxation of ASM. Given the absence of α6 subunit expression this drug truly targeting the α4 containing $GABA_A$receptors. Recently a series of new subtype selective Bz/GABAergic ligands have been developed based on the SAR of CMD-45 and will be presented in this poster.



CMD-45

MEDI 58

Structure activity relationship study of citronellol type compounds and their derivatives

*Tairan Yang[1], Yumi K Honda[2], Meen-Woon Hsiao[3], **Hongfei Yin**[4], yinhf2010@gmail.com, Tsao-Yi Wei[4], Hiroshi Honda[4], Tianshu Liu[4]. (1) Department of Chemistry, University of Toronto, Toronto, Ontario M5S3H6, Canada (2) School of Pharmacy, Loma Linda University, Los Angeles, CA 92350, United States (3) Department of Applied Chemistry, Chung Shan Medical University, Taichung, TW 40201, Taiwan Republic of China (4) Department of Bioengineering, Northwestern Polytechnic University, Fremont, CA 94539, United States*

This paper represents the chemical synthesis of citronellol type compounds and their structure activity relationship study of anti PPO activity

MEDI 59

Synthesis of flexible purine analog inhibitors of NCp7

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In recent years, the use of FDA approved Highly Active Antiretroviral Therapies (HAARTs) has shown great promise in the treatment of Human Immunodeficiency Virus Type 1 (HIV-1). A large majority of FDA approved HIV-1 chemotherapies target the viral enzymes essential for viral replication. Unfortunately, monotherapy-based treatments have been plagued with rapid loss of efficacy due to the virus' ability to adapt and mutate. Thus, the use of combination therapies is now the preferred mode of treatment of HIV-1 since the rate of mutation development decreased significantly in the presence of multiple drugs, although it has not been completely halted. Consequently, the development of new drugs that work through alternative modes of action are needed. The objective of this project is to synthesize a series of flexible guanine nucleobase analogues (Flex-bases) to selectively inhibit the highly conserved nucleic acid (NA) binding domain of the HIV-1 nucleocapsid protein (NCp7). The rotational flexibility of the Flex-base analogues is installed by “splitting” the purine base into its imidazole and pyrimidine counterparts with a single C-C bond. Previous results obtained by the Seley-Radtke lab suggest that this flexibility may endow the nucleobase with the ability to overcome active site mutations in NCp7 as well as adapt to potential binding site repositioning.

Previously reported NCp7 inhibitors have not been entirely successful due to significant toxicity, caused by their non-specific zinc-ejecting properties. Preliminary computational studies have indicated that the target compounds should work through an alternative mechanism that will not involve zinc ejection. This should endow them with significant advantages over known NCp7 inhibitors. We propose to synthesize several series of these Flex-bases using palladium-catalyzed coupling techniques in order to fully study the effects of flexibility on this new class of NCp7 inhibitors.

MEDI 60

Discovery and optimization of indazoles as potent and selective interleukin-2 inducible T cell kinase (ITK) inhibitors

Richard Pastor*, rmpastor@gene.com, Jason Burch, Steven Magnuson, Zhonghua Pei, Daniel Ortwine, Charles Eigenbrot, Lawren Wu, Yichin Liu, Kelly De La Torre, Adam Johnson, Yuan Chen, Xiao Ding, Marya Liimatta, Steven Shia. Discovery Chemistry, Genentech Inc., South San Francisco, CA 94080, United States*

There is evidence that small molecule inhibitors of the non-receptor tyrosine kinase ITK, a component of the T-cell receptor signaling cascade, could represent a novel asthma therapeutic class. Moreover, given the expected chronic dosing regimen of any asthma treatment, highly selective as well as potent inhibitors would be strongly preferred in any potential therapeutic. Here we summarize hit-to-lead optimization of a series of

indazoles that demonstrate sub-nanomolar inhibitory potency against ITK with strong cellular activity and good kinase selectivity. We also elucidate the binding mode of these inhibitors by solving the X-ray crystal structures of the complexes.

MEDI 61

Synthesis and evaluation of Praziquantel derivatives as a potential pharmacological chaperone for Mucopolysacharidosis VI (Marateaux-Lamy syndrome)

***Trisha A Russell**, trisharussell@whitworth.edu. Chemistry, Whitworth University, Spokane, WA 99218, United States*

Mucopolysacharidosis VI (Marateaux-Lamy syndrome) is a lysosomal storage disease that is caused by the deficiency of *N*-acetylgalactosamine-4-sulfatase (aryl sulfase B, ASB). The deficiency in ASB causes a build-up in the glycosaminoglycan chondroitin sulfate and dermatan sulfate resulting in debilitating multisystemic organ damage. Pharmacologic chaperone therapy uses small molecules to rescue the mutant lysosomal enzyme and facilitate its delivery to the lysosome. In this study, derivatives of praziquantel were synthesized and evaluated as potential small molecule pharmacological chaperones of ASB using enzymatic activity assays. Studies on the effect of the derivatives of praziquantel on the enzymatic activity of ASB will be presented.

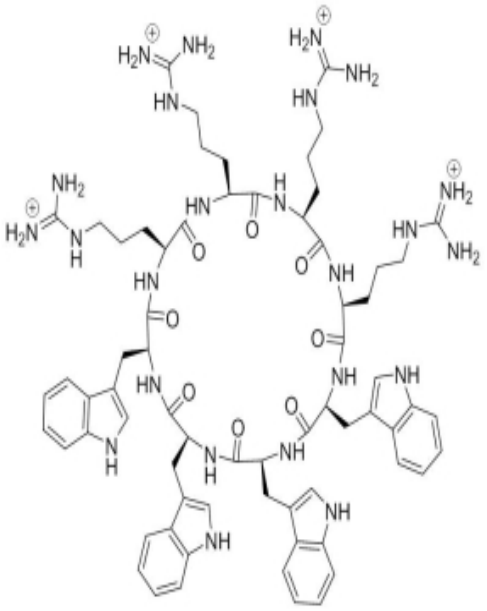
MEDI 62

Antibacterial activities of amphiphilic cyclic cell-penetrating peptides against multidrug resistant pathogens

*Donghoon Oh[1], Jiadong Sun[1], **Amir Nasrolahi Shirazi**[1,2], anshirazi@gmail.com, Kerry L. LaPlante[3], David C. Rowley[1], Keykavous Parang[1,2]. (1) Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, Kingston, Rhode Island 02881, United States (2) School of Pharmacy, Chapman University, Irvine, CA 92618, United States (3) Department of Pharmacy Practice, College of Pharmacy, University of Rhode Island, Kingston, Rhode Island 02881, United States*

Antimicrobial peptides (AMPs), a major class of antibacterial agents, share amphiphilicity and cationic structural properties with cell-penetrating peptides (CPPs). Herein, several amphiphilic cyclic and linear CPPs containing tryptophan and arginine along with their analogs were synthesized and exhibited potent antibacterial activities against multidrug resistant pathogens. Among them, a cyclic peptide containing arginine and tryptophan namely [R_4W_4] showed the highest antibacterial potency against methicillin-resistant *Staphylococcus aureus* (MRSA, exhibiting a minimum inhibitory concentration (MIC) of 2.67 μg/mL). Furthermore, the cyclic [R_4W_4] peptide and its linear counterpart R_4W_4 exhibited the MIC values of 42.8 and 21.7 μg/mL, respectively,

against *Pseudomonas aeruginosa*. Cyclic [R_4W_4] also showed cell-penetrating property as expected. This peptide exhibited less than 16% cytotoxicity at 15 μM (20.5 μg/mL) concentration in three different human cell lines. This study suggests that there is a strong correlation between cell penetrating and antimicrobial properties in this class of amphiphilic peptides.



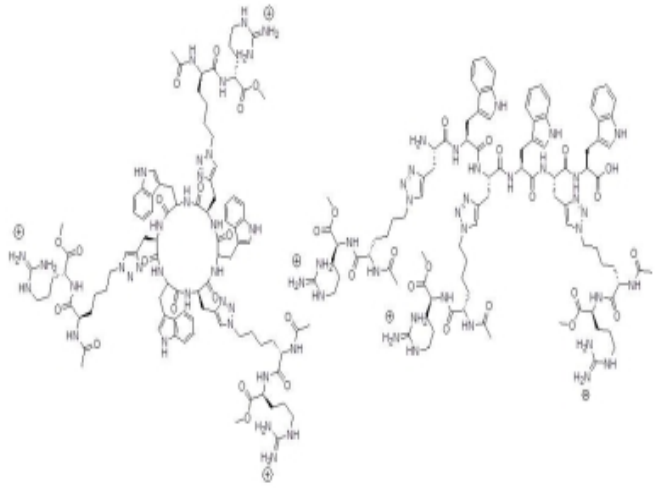
MEDI 63

Synthesis of amphiphilic triazolyl peptides and evaluation of their cytotoxicity

*Naser Sayeh[1], **Amir Nasrolahi Shirazi**[1,2], anshirazi@gmail.com, Rakesh Kumar Tiwari[1,2], Donghoon Oh[1], Keykavous Parang[1,2]. (1) Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, Kingston, Rhode Island 02881, United States (2) School of Pharmacy, Chapman University, Irvine, CA 92618, United States*

Peptides have unparalleled potential applications as nano-materials, surfactants, and drug delivery systems. Here, a new class of amphiphilic triazolyl peptides were designed and synthesized with functionalized peptide-based building blocks containing alkyne and azide functional groups namely linear $(W(pG))_3$, cyclic $[W(pG)]_3$, and Ac-$K(N_3)R$-NH_2 where W, R, K, (pG) represent tryptophan, arginine, lysine, and propargyl glycine residues, respectively. The linear $(W(pG))_3$ and cyclic $[W(pG)]_3$ peptides were conjugated with Ac-$K(N_3)R$-NH_2 through click chemistry in the presence of $CuSO_4.5H_2O$, Cu (powder), sodium ascorbate, and DIPEA in methanol:water to afford amphiphilic triazolyl peptides including linear $(WG(triazole\text{-}KR\text{-}NH_2))_3$ and cyclic [WG(triazole-KR-

$NH_2)]_3$. CD spectroscopy exhibited that although the secondary structures of both peptides are moderately α-helix, the pattern of the cyclic peptideis slightly different from the linear one. TEM imaging showed that l(WG(triazole-KR-NH_2))$_3$ and c[WG(triazole-KR-NH_2)]$_3$ form nano-sized structures in the size range of 50-100 nm and 50-80 nm, respectively. Both peptides showed higher toxicity in cancer cells compared to normal cells at a concentration of 100 μM.



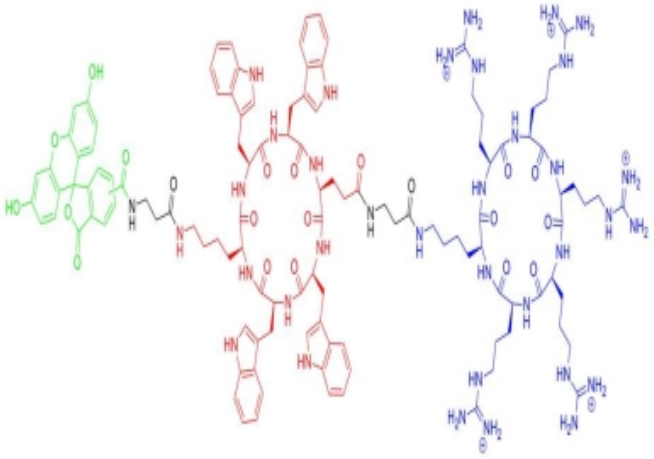
MEDI 64

Amphiphilic bicyclic peptides as cellular delivery agents

*Donghoon Oh[1], Shaban Anwar Darwish[1], **Amir Nasrolahi Shirazi**[1,2], anshirazi@gmail.com, Rakesh Kumar Tiwari[1,2], Keykavous Parang[1,2]. (1) Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, Kingston, Rhode Island 02881, United States (2) School of Pharmacy, Chapman University, Irvine, CA 92618, United States*

Cell-penetrating peptides (CPPs) have been studied as molecular transporters because of their cellular translocation properties. Among CPPs, cyclic peptides take advantage of their higher serum stability compared to the linear counterparts. In addition, the presence of positively charged arginine and hydrophobic tryptophan amino acids were found to be critical due to their characteristic interactions with phospholipid membranes. Here, two bicyclic peptides [W_5G]-(triazole)-[KR_5] and [W_5E]-(β-Ala)-[KR_5] composed of tryptophan and arginine residues were synthesized from monocyclic peptide building blocks and evaluated as cellular delivery agents. [W_5G]-(triazole)-[KR_5] and [W_5E]-(β-

Ala)-[KR_5] containing triazole and β-alanine linkers improved the cellular delivery of fluorescein-labelled phosphopeptide, F′-GpYEEI by 7.6 and 19.3-fold, respectively, in human ovarian adenocarcinoma (SK-OV-3) cells. Confocal microscopy showed that the corresponding fluorescein-labelled bicyclic peptide F′-[KW_4E]-(β-Ala)-[KR_5] was localized in the cytosol and nucleus. Studying the cellular uptake of F′-[KW_4E]-(β-Ala)-[KR_5] in the presence of endocytosis inhibitors indicated that the clathrin- and caveolin-dependent endocytosis were the main pathways for cellular uptake. The bicyclic peptide was able to improve antiproliferative activity of doxorubicin by 20%. These data suggest that amphiphilic bicyclic peptides containing tryptophan and arginine residues can be utilized as a new class of cell-penetrating peptides and potential cellular delivery tools.



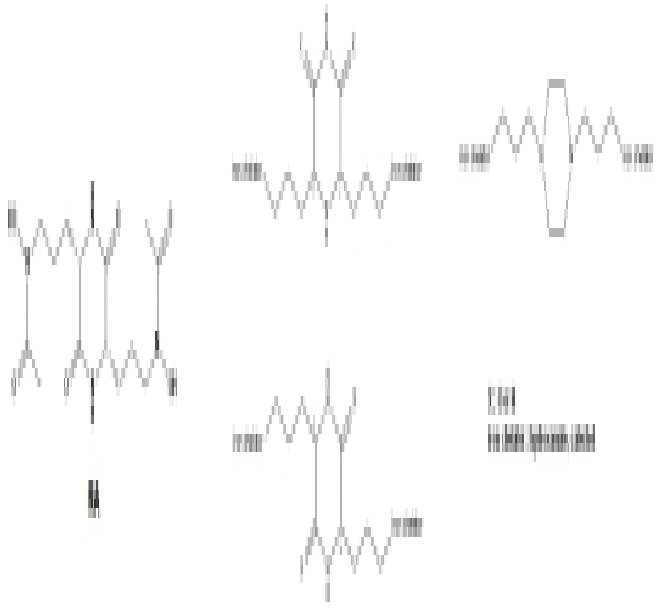
MEDI 65

Enantioselective synthesis and antibacterial activities of rhodotorulic acid siderophore analogs

Marine Pillon, Alexandra Dassonville-Klimpt, ***Christine Cezard****, christine.cezard@u-picardie.fr, Emmanuel Baudrin, Pascal Sonnet. UFR de Pharmacie - Université de Picardie Jules Verne, LG2A - FRE-CNRS 3517, 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.

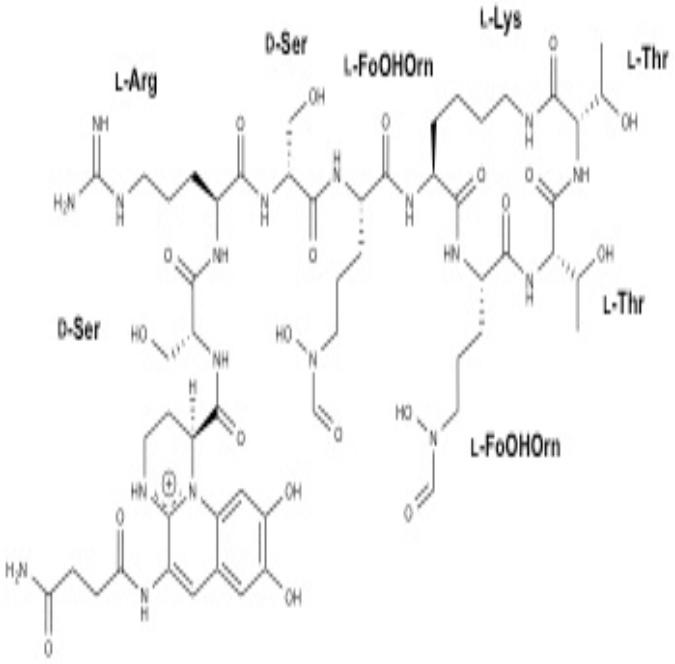


MEDI 66

Pyoverdine analogs: Trojan horse strategy against *Pseudomonas aeruginosa*

Natacha Farvacques, Viviane Silva-Pires, ***Christine Cezard****, christine.cezard@u-picardie.fr, Pascal Sonnet. UFR de Pharmacie - Université de Picardie Jules Verne, LG2A - FRE-CNRS 3517, Amiens, Picardie 80037, France*

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, see figure), its primary siderophore, which is 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 67

Asymmetric synthesis of new aryl methanols as antimalarial drugs

Guillaume Bentzinger, Alexia Jonet, ***Christine Cezard****, christine.cezard@u-picardie.fr, Alexandra Dassonville-Klimpt, Pascal Sonnet. UFR de Pharmacie - Université de Picardie Jules Verne, LG2A - FRE-CNRS 3517, Amiens, France*

Nowadays, microbial diseases are one of the most lethal diseases for human. *Malaria*, due to a *Plasmodium* protozoa is the 5th most lethal infections in the world, mainly located in tropical and subtropical countries (40% of the world population).[1] To overcome the problem of resistance to the actual drugs, there is an urgent need to search new and more efficient treatments. Our team is involved in the design and the synthesis of new antimalarial drugs. Recently, we have described the asymmetric syntheses and the biological activities of aminoquinolinemethanols 1, derivatives of the mefloquine 2.[2,3] Some structure-reactivity relationships have been highlighted: i) importance of the absolute configuration of asymmetric carbons, ii) importance of the nature of the amines (aliphatic *vs.*aromatic amines). Following these results, we will present the the synthesis of new amino-aryl alcohols.

Bibliographic references:

[1] WHO. *World Malaria Report*. WHO: Geneva, Switzerland, 2009.

[2] A. Jonet, A. Dassonville-Klimpt, S. Da Nascimento, J.-M. Leger, J. Guillon, P. Sonnet, *Tetrahedron : Asymmetry* 2011, *22*, 138.

[3] C. Mullié, A. Jonet, C. Desgrouas, N. Taudon, P. Sonnet, *Malar J.* 2012, *11*, 65.

MEDI 68

Innovative approaches for secure commercial and neglected drug discovery collaborations via the CDD Vault

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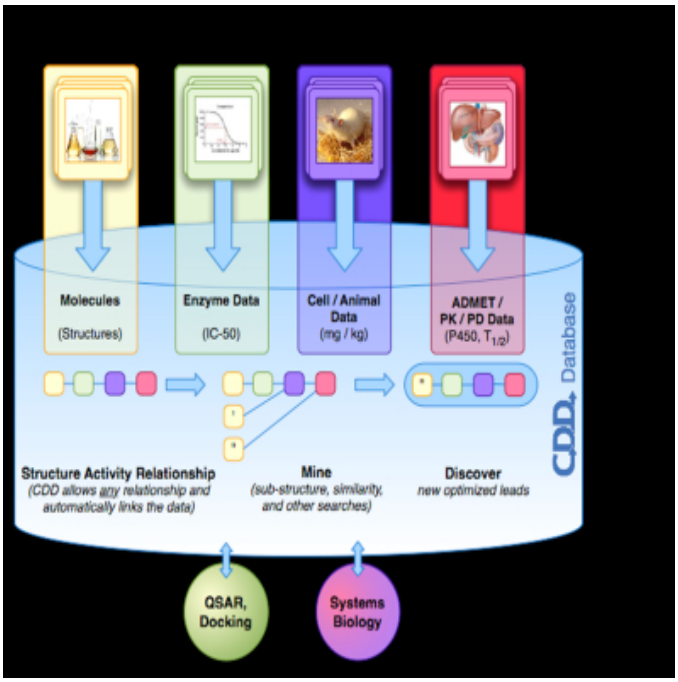
Currently, infectious diseases of the developing world (e.g., malaria, tuberculosis) represent a global health challenge of the 21st century and require new approaches that would allow scientists to do research more effectively. As a result of the development of web-database technologies (CDD Vault®), a collaborative approach to research on infectious diseases of the developing world and global health has emerged. The major components of effective scientific-community based research include: (1) unifying goal or focus on common therapeutic areas/diseases; (2) multiple research areas/expertise; (3) uniform database platform for effective data accumulation and management; (4) easy access and sharing of information; (5) potential for unlimited growth. The Collaborative

Drug Discovery (CDD) Vault® was built by utilizing innovative web technologies in order to provide a platform that allows scientists to archive, mine, and securely share research data with a focus on infectious diseases of the developing world. This new collaborative technology allows researchers to build up networks of technical experts around therapeutic or target areas thus advancing research facilitating the discovery of new drug candidates. It also allows scientists to speed up research by sharing unpublished data providing new hope in the race to overcome drug resistance. An example illustrating how potential chemosensitizers that address chloroquine resistance could be identified by using the CDD Vault® database platform is presented.



MEDI 69

Synthesis and evaluation of antibacetrial activity of of analogs of novel ansamycins- divergolides and hygrocins

Sivappa Rasapalli[1], *srasapalli@umassd.edu, Gopalakrishna Jarugumilli*[1], *Timoth Opperman*[2]. *(1) Chemistry and Biochemistry, UMass Dartmouth, North Dartmouth, MASSACHUSETTS 02747, United States (2) Microbiotix, Inc.,, Worcester,, Massachusetts 01605, United States*

Divergolides A-D and hygrocins A-F , novel ansmayicins i.e. deivatived of 3-amino-5-hydroxy benzoic acid (AHBA) derived polyketide natural products, hqave been shown to posess antibiotic and anticancer propoerties. We have been involved in synthesis of analogues of these families. We have screened the structural fragments and the synthetic intermediates to decipher if the SAR relations of this family in collaboration with Microbiotix. The results of our efforts will be shared.

MEDI 70

Stereoselective synthesis and evlautaion of cephalosol for antibacterial acivity

Sivappa Rasapalli[1], *srasapalli@umassd.edu, Umair Javed*[1], *Ronald Mastrolia*[1], *Timothy Opperman*[2]. *(1) Chemistry and Biochemistry, UMass Dartmouth, North Dartmouth, MASSACHUSETTS 02747, United States (2) Microbiotix, Inc.,, Worcester,, MASSACHUSETTS 01605, United States*

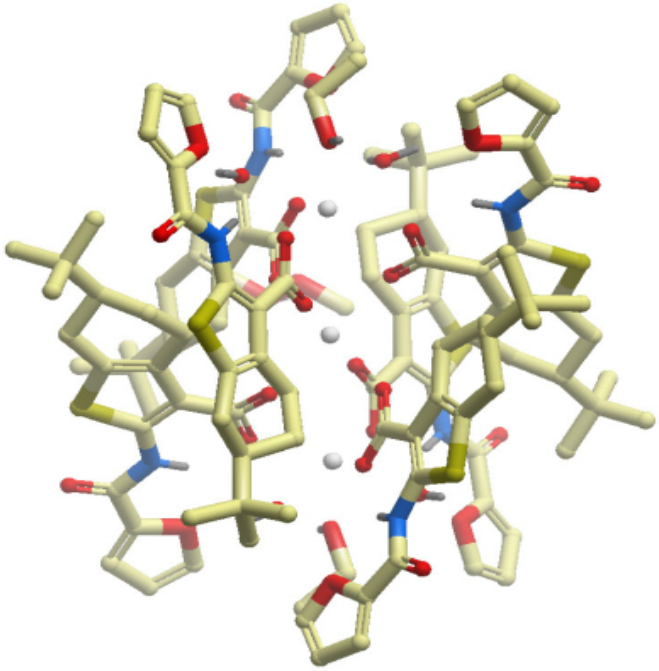
Cephalosol, a potent antimicrobial secondary metabolite with a new carbon skeleton, was characterized from the culture of endophtic *Cephalosporium acremonium* IFB-E007. Its structure and absolute configuration were unambiguously determined by spectroscopic and computational approaches. We have developed a stereoselective synthesis of cephalosol and its analogues via Shrapless AD and intramolecular aldol condensation. We have been involved in evaluation of their antibacterial activity in collaboration with Microbiotix, Inc. and the resultes will be disclosed.

MEDI 71

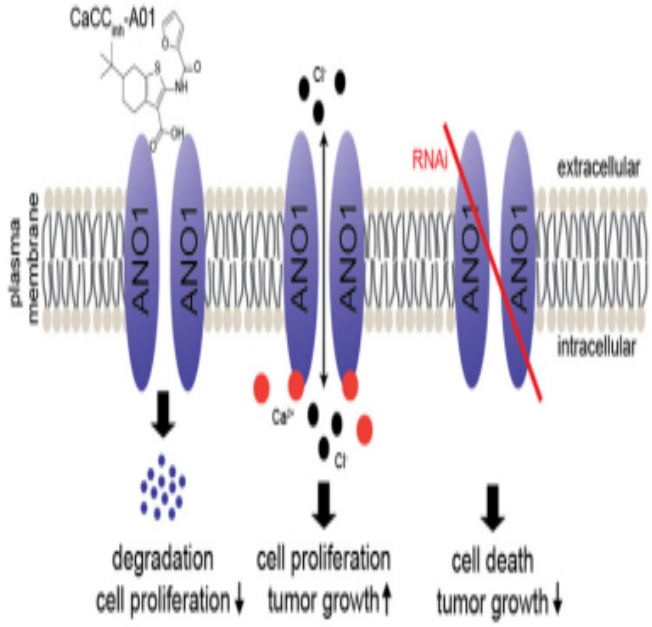
Small molecule facilitated degradation of ANO1 - a new targeting approach for anticancer therapeutics

*Anke Bill[2], Michelle Lynn Hall[1], Jason Borawski[2], Catherine Hodgson[4], Jeremy Jenkins[2], Philippe Piechon[3], Mariana Oana Popa[4], Christopher Rothwell[2], Pamela Tranter[4], Scott Tria[1], Trixie Wagner[3], **Lewis Whitehead**[1], lewis.whitehead@novartis.com, Alex Gaither[2]. (1) Global Discovery Chemistry, Novartis Institutes for Biomedical Research, Cambridge, MA 02139, United States (2) Developmental Molecular Pathways, Novartis Institutes for Biomedical Research, Cambridge, MA 02139, United States (3) Global Discovery Chemistry, Novartis Institutes for Biomedical Research, Basel, Switzerland (4) Respiratory Diseases, Novartis Institutes for Biomedical Research, Horsham, West Sussex RH12 5AB, United Kingdom*

ANO1, a calcium-activated chloride channel, is highly expressed and amplified in human cancers and is a critical survival factor in these cancers. We explored the mechanism behind CaCC inh -A01's inhibitory effect on cell proliferation using a



combined experimental and *in silico* approach. We report that CaCC inh -A01 reduces ANO1 protein levels by facilitating endoplasmic reticulum-associated, proteasomal turnover of ANO1. Washout of CaCC inh -A01 rescued ANO1 protein levels and



resumed cell proliferation. Proliferation of newly derived CaCC inh -A01 resistant cell pools was not affected by CaCC inh - A01 as compared to the parental cells. Consistently, CaCC inh -A01 failed to reduce ANO1 protein levels in these cells while ANO1- currents were still inhibited by CaCC inh -A01, indicating that CaCC inh -A01 inhibits cell proliferation by reducing ANO1 protein levels. Furthermore, we employed *in silico* methods to elucidate novel biological functions of ANO1 inhibitors. Specifically, we derived a pharmacophore model to describe inhibitors capable of promoting ANO1 degradation and report new inhibitors of ANO1-dependent cell proliferation. In summary our data demonstrate that inhibition of ANO1's channel activity is not sufficient to inhibit ANO1- dependent cell proliferation indicating that ANO1's role in cancer only partially depends on its function as a channel.

MEDI 72

Automated ligand and structure based protocol for in-silico prediction of human serum albumin binding

*Michelle Lynn Hall[1], William L Jorgensen[2], **Lewis Whitehead**[1], lewis.whitehead@novartis.com. (1) Global Discovery Chemistry, Novartis Institutes for Biomedical Research, Cambridge, MA 02139, United States (2) Department of Chemistry, Yale University, New Haven, Connecticut 06520, United States*

Plasma protein binding has a profound impact on the pharmacodynamic and pharmacokinetic properties of drug candidates and thus is integral to drug discovery. Nevertheless, extant methods to examine small-molecule interactions with plasma protein have various limitations, thus creating a need for alternative methods. Herein we present a comprehensive and cross-validated in silico workflow for the prediction of small-molecule binding to Human Serum Albumin (HSA), the most ubiquitous plasma protein. This protocol reliably predicts small-molecule interactions with HSA including binding affinity via a multiple linear regression, binding site using a naïve Bayesian classifier, and binding pose using induced fit docking. Further, this workflow is implemented in a portable and automated format that can be downloaded and used by other end users either as is or with customization.

MEDI 73

Biophysical characterization of novel thiopeptide-derived inhibitors of bacterial elongation factor Tu

*Gejing Deng[1], Lac Lee[1], Gregg Chenail[1], Jennifer A Leeds[1], Christine Luu[2], Matthew J LaMarche[3], Deborah J Palestrant[4], Meena Sachdeva[1], Robert Yu[1], **Lewis Whitehead**[3], lewis.whitehead@novartis.com. (1) Infectious Diseases, Novartis Institutes for Biomedical Research, Cambridge, MA 02139, United States (2) Infectious Diseases, Novartis Institutes for Biomedical Research, Emeryville, California 94608, United States (3) Global Discovery Chemistry, Novartis Institutes for Biomedical Research, Cambridge, MA 02139, United States (4) Center for Proteomic Chemistry, Novartis Institutes for Biomedical Research, Cambridge, Massachusetts 02139, United States*

GE2270 A is a macrocyclic thiopeptide natural product that inhibits bacterial protein synthesis by targeting prokaryotic amino-acyl tRNA chaperone elongation factor Tu (EF-Tu). Medicinal chemistry approaches were used at this institute to generate synthetic derivatives of GE2270 A with improved physicochemical properties, including increased solubility, which retained the antibacterial potency of the natural product. In this study, we investigate and compare the biophysical characteristics and interactions of GE2270A and these novel, optimized semi-synthetic thiopeptide derivatives with EF-Tu using surface plasmon resonance (SPR), and calorimetry. We found the novel thiopeptides behave differently from GE2270 A in their binding to EF-Tu, including a binding preference for the EF-Tu·GTP form, and changes in the thermodynamics of association. GE2270 A resembled these novel compounds in that binding did not change the affinity of EF-Tu·GDP for nucleotide exchange factor Ts (EF-Ts), nor did it inhibit the nucleotide exchange reaction. A hypothesis explaining this data is presented based on the previously published EF-Tu X-ray crystallographic data for thiopeptide binders. LFF571 is one of the semisynthetic analogs of GE2270 A with greatly improved aqueous solubility. LFF571 is being investigated in humans for safety and efficacy in the treatment of Clostridium difficile infection. The biophysical methods described in this report confirm the binding of LFF571 to *C. difficile* EF-Tu.

MEDI 74

Optimization and activity of the argyrins for EF-G inhibition in gram negative pathogens

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Argyrins are natural products that have antibacterial and anti-tumor activities. Their spectrum includes certain Gram negatives such as P. aeruginosa and B. multivorans. Our institute recently identified the cellular target of argyrin B as elongation factor G (EF-G), enabling further examination of the spectrum and resistance characteristics of these molecules. Here we present the medicinal chemistry optimization strategies employed on the Argyrin natural product template.

MEDI 75

Structure-based drug design of anthrax toxin lethal factor inhibitors

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Anthrax poses a significant threat to society as a biowarfare agent; antibiotics such as fluoroquinolones are effective against the causative agent *Bacillus anthracis*, but must be administered early in the disease cycle due to rapid exotoxin secretion and consequent host death. The anthrax toxin lethal factor (LF) is the toxin component chiefly responsible for cytotoxicity and has garnered significant attention as a target for small-molecule inhibitor design. As part of our LF-targeted discovery effort, we have obtained structural biology data incorporating the active LF inhibitor **MK-31** (**Fig.1**) indicating that the underexplored LF S2' binding pocket is important for ligand binding and biological activity against LF. Here we present key SAR, binding requirements, and design principles for the LF S2' subsite obtained via molecular modeling, synthesis, biochemical evaluation, and structural biology, featuring 30 original analogs of **MK-31** that wespecifically designed to explore this key LF subsite. We are currently applying these design principles to optimize novel LF inhibitor scaffolds identified via large-scale high-throughput screening (HTS).

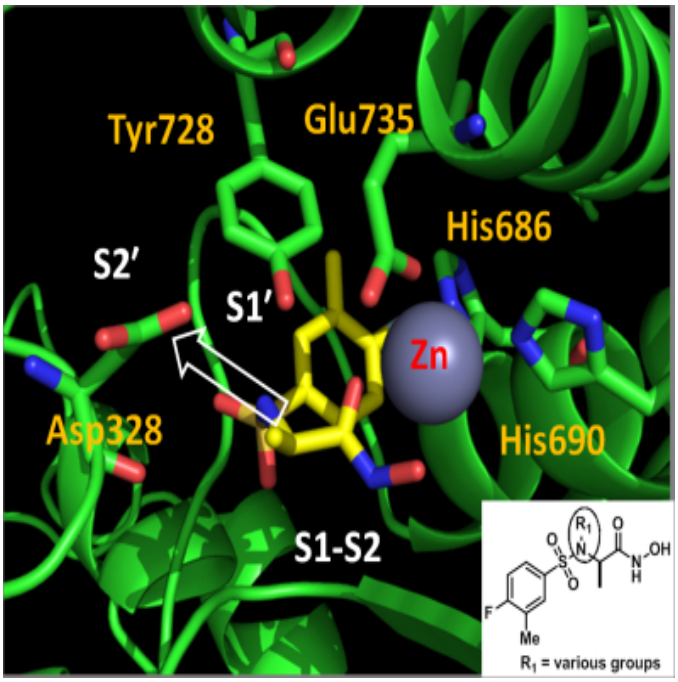

Figure 1. LF active site with catalytic Zn^{2+} (blue sphere) co-crystallized with **MK-31** (R_1=H).

MEDI 76

SAR study for the lead optimization of trisubstituted benzimidazoles as new-generation antitubercular agents, targeting FtsZ

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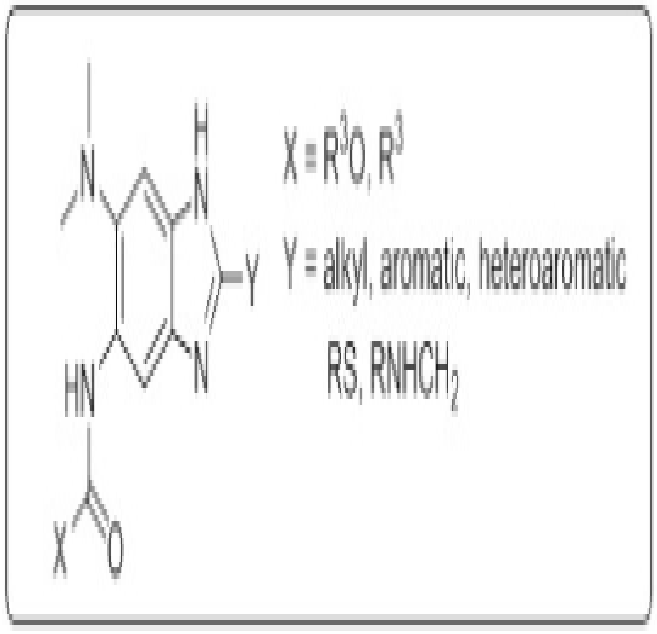
Tuberculosis, which is responsible for 1.3 million deaths worldwide in 2012, is treated using first and second line drugs. The repertoire of treatments has remained virtually static for decades, but the prevalence of drug resistance has increased. The rise of MDR- and XDR-TB has rendered current treatments ineffective, and new anti-tubercular agents with unique mechanisms of action are needed to overcome this hurdle. Filamenting temperature-sensitive mutant Z (FtsZ) is an essential bacterial cell division protein and a promising target. Inhibition of this protein will disrupt cell division, killing the bacterial cells. Libraries of 2,5,6- and 2,5,7-trisubstituted benzimidazoles were previously synthesized and tested against *Mtb* H37Rv. Lead compounds enhanced

GTPase activity, inhibit polymerization and promotes depolymerization of FtsZ. For further optimization studies and to diversify our database, a new library of 2,5,6-trisubstituted benzimidazoles, containing a dimethylamino substitution at the 6-position and different substitutions at the 2-position, was synthesized and tested for its activity in vitro. The synthesis, biological evaluations, and SAR of these novel benzimidazoles will be presented.



MEDI 77

Antimalarial 2-aminopyridine MMV390048 clinical candidate: Discovery and target identification studies

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Australia (9) Centre for Drug Candidate Optimisation, Monash University, Parkville, Australia

2-Aminopyridines were identified from phenotypic whole cell high-throughput screening of a commercially available SoftFocus kinase library as promising selective *in vitro* antiplasmodial hits. The selected hits were validated through re-synthesis, retesting, physico-chemical and *in vitro* metabolism screens and showed attractive properties for a “Hit to Lead” medicinal chemistry program. **MMV017007** , was identified as a lead compound possessing good *in vivo* efficacy in mice *Pf* SCID (ED_{90} 3.6 mg/kg) and pharmacokinetics properties in rat (F = 83% and half-life 8.7 hrs). However, **MMV017007** showed potential cardiovascular risks through hERG inhibition (IC_{50} 5.5 µM) and a high predicted human dose. **MMV017007** was subjected to a lead optimization program resulting in a late Lead and pre-clinical candidate, compound **MMV390048** . This pre-clinical candidate showed impressive *in vitro Pf* activity and overcame cardiovascular risks having low hERG inhibition (IC_{50} >11 µM). **MMV390048** completely cured *P. berghei-infected* mice at low oral doses (4 x 3 mg/kg or 1 x 30 mg/kg) and ED_{90} of 0.57 mg/kg in *Pf* SCID mouse model. The data suggested that **MMV390048** has potential to be a component in a single dose cure. The results from toxicological, safety, pharmacology and pharmacokinetic studies demonstrate that **MMV390048** may be safely administered to humans. Phase I FIM single rising dose studies of this compound are scheduled to start in 2014.

Studies using both genomics and chemoproteomic techniques that identified *Pf*PI4K as the target of **MMV390048** will be described.

MEDI 78

Synthesis and characterization of novel tetrahydropyran-based bacterial topoisomerase inhibitors

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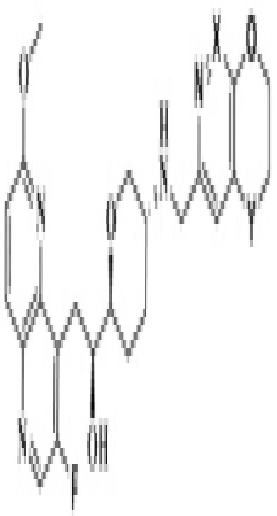
There is an urgent medical need for novel antibacterial agents effective against methicillin-resistant *Staphylococcus aureus* (MRSA)*,* a pathogen responsible for thousands of deaths every year in the US. Bacterial type II topoisomerases (DNA gyrase and topoIV) are essential enzymes intervening during the bacterial replication process. While fluoroquinolones block DNA topoisomerases by interacting with structurally closely related GyrA and ParC subunits of DNA gyrase and topoIV respectively, the emergence of resistant mutants has limited their clinical utility. Exploiting a novel binding site present in GyrA and ParC, allows to overcome resistance to fluoroquinolones. In continuation of our antibacterial program dedicated to novel bacterial topoisomerase inhibitors (NBTIs), we present a novel series of tetrahydropyran-based topoisomerase inhibitors that display potent enzyme-inhibitory

activity and potent anti-staphylococcal antibacterial activity. *In vitro* and *in vivo* parameters illustrating the potential utility for human use of this series will be presented.



X	Y	MIC_{90} S.aureus (μg/mL)	
CH	O	0.06	(50 strains)
N	S	0.06	(48 strains)

MEDI 79

Discovery of thienoimidazole-based HCV NS5A genotype 1a and 1b inhibitors

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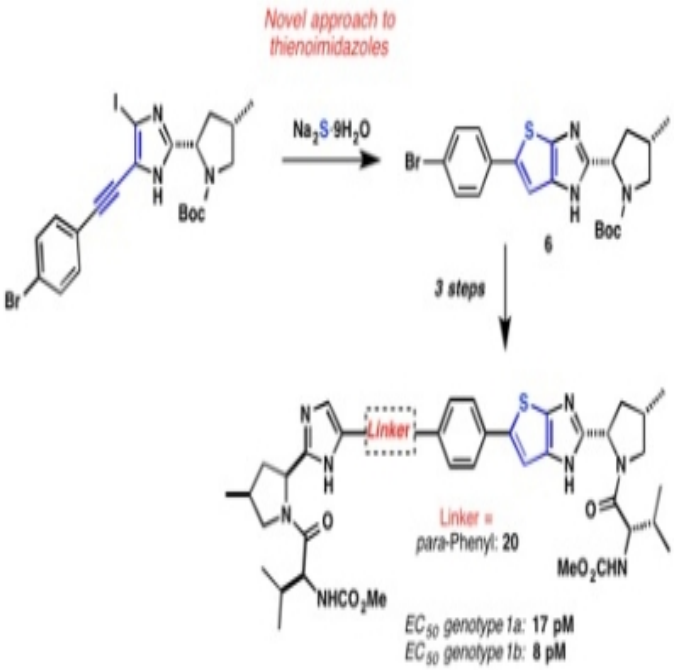
The discovery of potent thienoimidazole-based HCV NS5A inhibitors is herein reported. A detailed account of the progression from early C_2-symmetrical compounds with unfavorable genotype 1a potencies to non-symmetrical compounds with high potencies for both genotype 1a and 1b is presented. Additionally, a novel method to access the thienoimidazole [5,5]-bicyclic system is disclosed. This method gave access to a common key intermediate (**6**) that was engaged in Suzuki or Sonogashira reactions with coupling partners bearing different linkers. A detailed study of the structure-activity-

relationship (SAR) of the linkers revealed that aromatic linkers with linear topologies are required to achieve high potency for both 1a and 1b HCV genotypes. Compound **20** , with a *para*-phenyl linker, was identified as a potential lead displaying potencies of 17 pM and 8 pM against genotype 1a and 1b replicons, respectively.



MEDI 80

Facile synthesis of β-lactam from amino acids by intramolecular condensation using a condensing reagent

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This paper represents the application of condensing reagent for the formation β-lactam compounds from amino acids

MEDI 81

Discovery of a novel lactam-containing non-nucleoside class of HCV NS5B inhibitors

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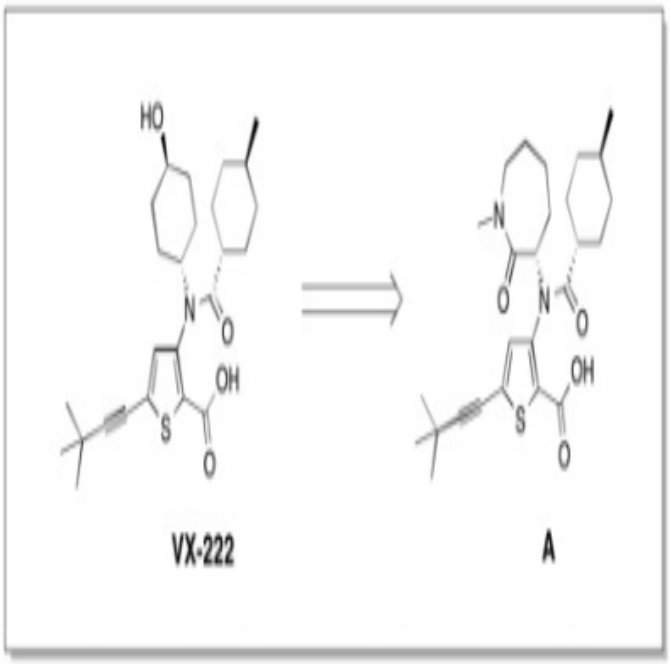
Abstract



VX-222 is a non-nucleoside allosteric inhibitor of the hepatitis C virus NS5B polymerase with demonstrated efficacy in clinical trials. Our back up program has focused on further improving the anti-viral activity, physicochemical properties, and pharmacokinetics within this class of inhibitor. Based on the X-ray structures of NS5B polymerase with VX-222 and other analogs, a series of lactam derivatives were designed to create additional interactions between polymerase residue Arg501 and the lactam moiety. As a result, this series exemplified by Compound A indeed provided 3-5-fold improvement in potency against NS5B polymerase (genotype 1a and 1b), as measured by replicon assays. The synthesis, structure-activity relationships and ADME characterization of this novel series are discussed herein.

MEDI 82

Non-antibacterial azithromycin derivatives for COPD

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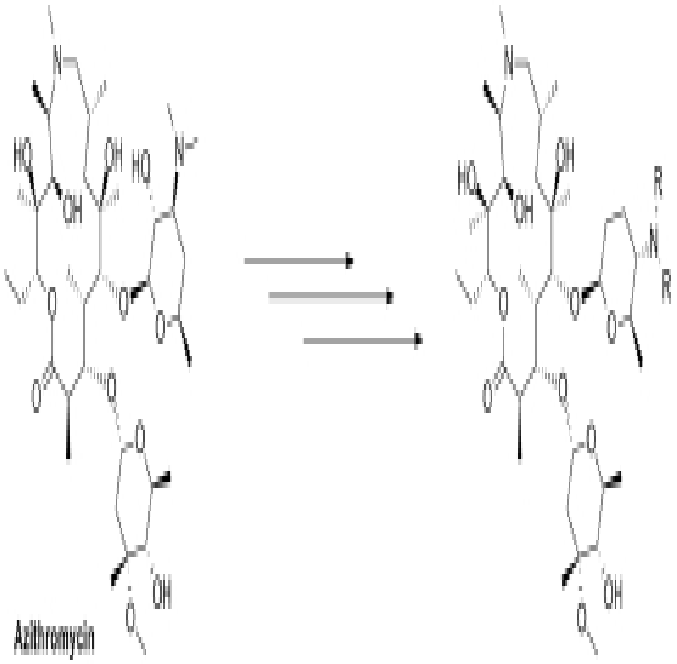
Macrolide antibiotics are a class of natural products that have been integral to treatment regimens for over three decades. In addition to their antibacterial effects, macrolides such as azithromycin are known to have antiinflammatory and immunomodulatory properties. Prophylactic treatment with azithromycin has been shown to decrease the frequency of exacerbations in patients with chronic obstructive pulmonary disease (COPD), but concerns about developing microbial resistance remain. As part of our program to develop non-antibacterial yet anti-inflammatory macrolides, which would hopefully demonstrate a decrease in exacerbations but not affect microbial resistance in COPD patients, azithromycin derivatives lacking a 2'- hydroxyl group and a shifted 3'-amine were prepared. These compounds were evaluated for MUC5AC activity and antibacterial activity.

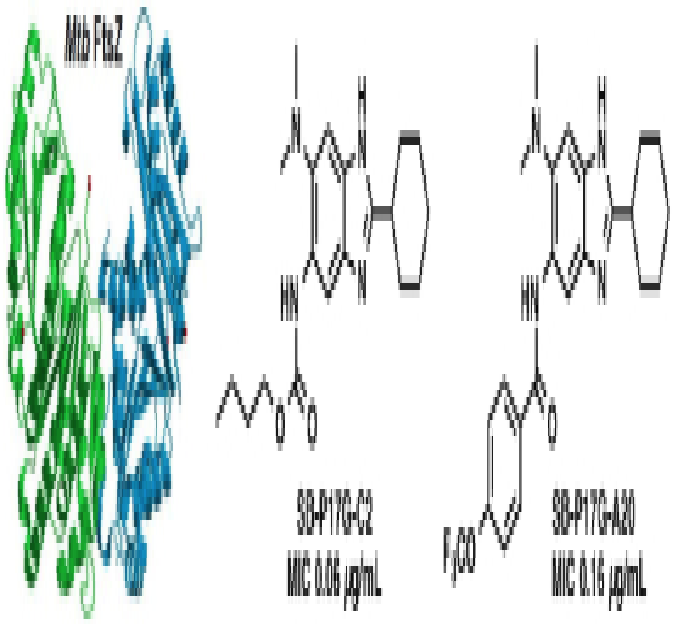


MEDI 83

Trisubstituted benzimidazoles as a new class of antitubercular agents targeting FtsZ with high in vitro activity and in vivo efficacy

Krupanandan Haranahalli[1]*, krupa.haranahalli@stonybrook.edu, Divya Awasthi*[1]*, Kunal Kumar*[2]*, Susan Knudson*[3]*, Laurent Goullieux*[4]*, Alexandra Carreau*[4]*, Laurent Fraisse*[4]*, Hélène Vermet*[5]*, Sylvie Klieber*[5]*, Richard A Slayden*[3]*, Iwao Ojima*[1,2]*. (1) Department of Chemistry, Stony Brook University, STONY BROOK, NEW YORK 11794-3400, United States (2) Institute of Chemical Biology & Drug Discovery, Stony Brook University, Stony Brook, New York 11794-3400, United States (3) Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, Colorado 80523-1682, United States (4) Infectious Diseases Thérapeutique Stratégie unit, Sanofi, Toulouse, France (5) Deposition Safety and Animal Research Department, Sanofi, Montpellier, France*

Current drugs for TB primarily target cell wall biosynthesis or DNA/RNA synthesis. Rising number of resistant strains of MDR- and XDR-TB display an alarming need for new anti-tubercular agents with unique mode of action. FtsZ, a homolog of tubulin and a ubiquitous bacterial cytokinesis protein is a promising target. Inhibition of FtsZ assembly has shown to affect the cell division process. In our laboratory, we have successfully designed and synthesized novel series of trisubstituted benzimidazoles that displayed excellent antitubercular activity by inhibiting Ftsz. Hit to lead optimization studies resulted in the identification of compounds with good *in vivo* activities (up to 6.5 $\log_{10}$ reduction of CFU in lung and spleen) in the rapid mouse model of TB infection. The lead compounds were also found to be bactericidal and were active against *Mtb* grown under hypoxic conditions (LORA). Herein, we present the SAR study and biological evaluation of the lead compounds.



MEDI 84

3D QSAR studies for a series of antichagasic agents using comparative molecular similarity indices (CoMSIA)

Leonardo L G Ferreira[1]*, leonardo@ifsc.usp.br, César M Fioravanti*[1]*, Rafael V C Guido*[1]*, Marco A Dessoy*[2]*, Luiz C Dias*[2]*, Glaucius Oliva*[1]*, Adriano D Andricopulo*[1]*. (1) Laboratory of Medicinal and Computational Chemistry, University of Sao Paulo, Sao Carlos, Sao Paulo 13563-120, Brazil (2) Laboratory of Organic Synthesis, University of Campinas, Campinas, Sao Paulo 13083-970, Brazil*

Chagas' disease is endemic in Latin America, affecting around 10 million people. Caused by the protozoan *Trypanosoma cruzi*, it is classified as a neglected tropical disease of high priority by the World Health Organization. The two available drugs were discovered in the 1970s and present several problems, including low efficacy and high toxicity. This scenario shows the urgent need for new therapeutic agents for the treatment of the disease. In this work, quantitative structure-activity relationships (QSAR) were investigated for a series of 88 pyridine derivatives bearing anti-*T. cruzi* activity. 3D QSAR models were generated employing the Comparative Molecular Similarity Indices Analysis (CoMSIA) approach. The generated CoMSIA models have high internal statistical consistency (r^2 = 0.84 and q^2 = 0.74), as well as predictive power for untested compounds (r^2_{pred} = 0.83), covering substantial structural diversity. The 3D isocontour surface maps indicated the essential structural features of the data set compounds related to biological activity. The results are valuable in suggesting interesting ways to the design and optimization of new structurally related analogs with improved potency.

MEDI 85

1-Glycosyl-1,2,3-triazole-4-carboxamides as nucleoside analogs

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Nucleoside analogs are generally prepared by attaching an unnatural sugar to one of the natural nucleobases. The development of new, unnatural nucleobases would expand the number of nucleoside analogs with potential biological activity. 1,2,3-triazole-4-carboxamide contains nearly all the functionality of purine nucleobases. The preparation of a number of nucleosides constructed around this unnatural nucleobase will be reported. Preliminary biological activity will also be disclosed.

MEDI 86

Design, synthesis, and antibacterial activity of azaindole inhibitors of ParE/GyrB with improved properties for the treatment of gram positive bacterial infections

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DNA gyrase and topoisomerase IV are known targets in antibacterial drug discovery. Inhibitors of the B subunits of gyrase (GyrB) and topoiosmerase IV (ParE) were designed specifically to block the ATP binding site. The azaindole scaffold was discovered through virtual screening of Kinase/ATP-binding scaffolds. Herein we present synthesis and structure-activity relationship of azaindole-naphthyridone inhibitors of ParE with improved *in vivo* clearance.

MEDI 87

Decreased ring size in cyclotriazadisulfonamide (CADA) analogs with preserved CD4 down-modulating and anti-HIV activity

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Cyclotriazadisulfonamide (CADA) prevents HIV entry into target cells by down-modulating human CD4 via blocking co-translational translocation of nascent protein across the ER membrane. The current goal is to determine if changing the size of the 12-membered ring might modulate CD4 down-modulation potency. A series of 11-membered ring analogs were synthesized with methoxy substituents on benzenesulfonyl side arms and benzyl or cyclohexylmethyl tail groups. Evaluation of CD4 down-modulation performed in CHO cells transfected with a fluorescent CD4 fusion protein revealed enhanced activity for ES-US2 (IC_{50} = 0.33 μM), an 11-membered analog with combined cyclohexylmethyl tail and methoxybenzenesulfonyl side arm. In addition, CD4 down-modulation of all 11-membered analogs in the T-lymphoid cell line MT-4 correlated well with anti-HIV-1 potency. Cytotoxicities of all the new analogs were found to be negligible (CC_{50} > 75 μM). These data demonstrate that ring size variation of CADA is feasible with preservation of CD4 receptor down-modulating activity.

MEDI 88

Discovery of novel pyridine-2-carboxyamides as liver-preferring glucokinase activators for treatment of type II diabetes mellitus

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Discovery Science, and Preclinical Development, Merck & Co, Rahway, NJ 07065, United States

Glucokinase (GK) is a hexokinase that catalyzes the reaction from glucose to glucose 6-phosphate. It also works as a glucose sensor and plays a key role in glucose homeostasis in humans. Physiologically, glucokinase is localized mainly in the liver, pancreas and brain. Systemically acting glucokinase activators have been demonstrated in clinical trials to effectively lower blood glucose levels in type II diabetic patients. However, hypoglycemia has also been found to be a potential risk factor that can limit the therapeutic application of this mechanism. Presumably, if the hypoglycemia risk is caused by pancreatic insulin secretion at low glucose level trigged by the activation of GK in beta cells, restricted activation of GK in hepatocytes may offer a promising solution to the issue. Herein, we report the discovery of a novel series of carboxylic acid containing GKAs based on pyridine-2-carboxyamide. These GKAs exhibit preferential activity in vitro in hepatocytes versus pancreatic INS1 cells. SAR studies led to the identification of a potent and orally active compound which demonstrated robust glucose lowering efficacy in HFD mice at 10 mpk with over 120-fold liver to pancreas ratio of tissue drug distribution, and no effect on insulin secretion. The design, synthesis, and biological evaluation of these novel GKAs will be discussed.

MEDI 89

Evaluation of the effect of amount of sunlight on concentration of steroids and flavonoids in *Pyrostegia venusta*

***Erika Guadalupe Escobedo Avellaneda**, akire287@hotmail.es, Elisa Leyva Ramos, Silvia Elena Loredo-Carrillo, Gabriela G Navarro-Tovar. Department of Quimica, Universidad Autónoma de San Luis Potosi, San Luis Potosi, San Luis Potosí 78210, Mexico*

The use of synthetic estrogen to decrease menopause symptoms causes side effects on females such as obesity, headache, insomnia and a higher risk of cancer. *Pyrostegia venusta* is a not genotoxic plant used on traditional medicine to reduce the symptoms of menopause without secondary effects. This biochemical property seems to be related to flavonoids and sterols composition.

Some plants produce flavonoids and steroids through secondary metabolism, synthetic routes of these compounds may be affected or regulated by the amount of sunlight received by plants. For example, it has been observed that light plays a central role in the synthesis of some secondary metabolites such as alkaloids because the light is needed to activate enzymes in the final steps of the biosynthetic pathway.

In this work, the amount of steroids and flavonoids was determined in several parts of *Pyrostegia venusta*, the effect of amount of sunlight was also investigated.

MEDI 90

Discovery of a first activator of 17β-hydroxysteroid dehydrogenases from diversity oriented synthesis of fused steroidal azacycle derivatives

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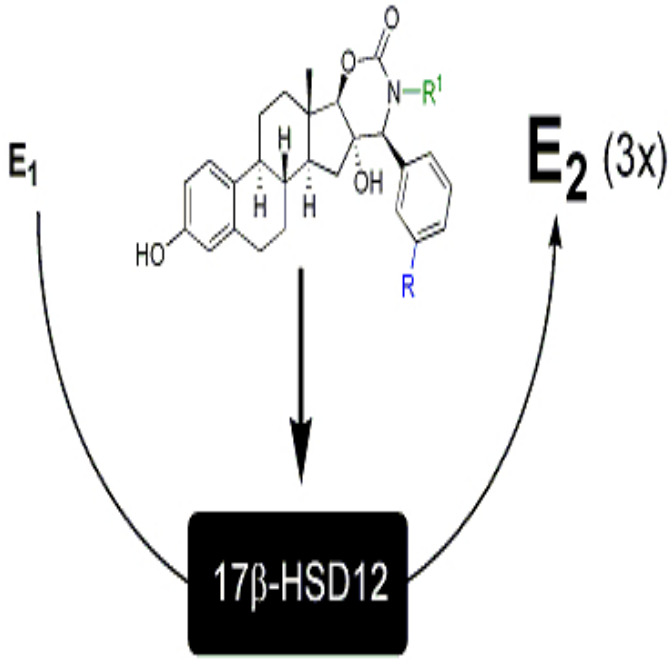
A new diversity-oriented synthesis (DOS) methodology has been developed to generate different types of azacycles (oxazinones, oxazolidinones, and morpholines) from β-aminodiol steroid templates bearing a ketone functionality. A library of 16β,17β-oxazinone-estradiol derivatives obtained by this DOS approach unexpectedly provided a series of activator of 17β-hydroxysteroid dehydrogenase type 12 (17β-HSD12) (Figure 1). In fact, small molecule activators that directly modulate the activity of an enzyme are uncommon entities and such activators had never yet been identified for any 17β-HSD's. We hereby report the fortuitous discovery of a steroid derivative that caused an up to a 3-fold increase in the activity of 17β-HSD12. The stimulation of estrone (E_1) to estradiol (E_2) conversion has been characterized in intact and homogenized stably transfected HEK-293 cells and has also been observed in T-47D breast cancer cells. Structure-activity relationships closely linked to the nature of the substituent on the oxazinan-2-one ring of an E_2 derivative emerged from this study. This activator will therefore be a useful tool to study this relatively unknown enzyme as well as the possible activation of other 17β-HSD family members.



MEDI 91

Development of a new inhibitor of steroid sulfatase that combine oesteogenic and antiestrogenic properties for treatment of breast and prostate cancers

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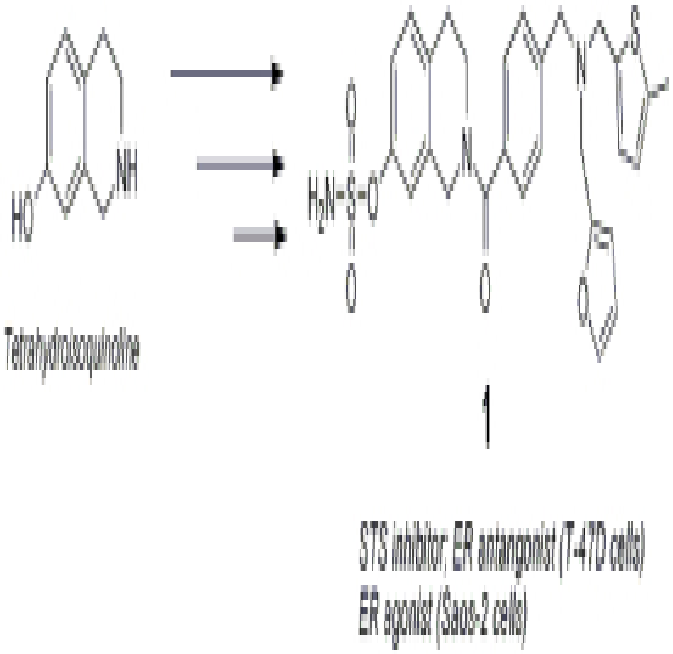
Steroid hormones play a crucial role in the growth of estrogen-sensitive and androgen-sensitive cancers, which constitute about 35% of all cancers in women and 25% of all cancers in men, respectively. Blocking both biosynthesis of active steroids as well as their action on hormonal receptor allowed to develop new therapies. The use of specific and better tolerated therapies than chemotherapy has led to clinical outcomes of interest particularly in the treatment of breast cancer (using an antiestrogen) and prostate cancer (using an antiandrogen). Steroid sulfatase (STS) is a key enzyme involved in the production of estrogens and androgens in humans. This enzyme is an attractive therapeutic target to block the biosynthesis of estrogenic and androgenic hormones. Recently, we obtained a multiple-action inhibitor of STS (**1** , Figure 1). In fact, in addition to strongly block in vitro the synthesis of sex hormones (estrone from estrone sulfate; IC_{50}= 3.9 nM), this inhibitor also blocks the estrogen receptor (ER) in the manner of an antiestrogen (in T-47D breast cancer cells) and stimulates in the same time the ER in bone cells (Saos-2 cells). Considering the important role of estrogens in breast cancer and their recently reported potential role in prostate cancer, this new family of tetrahydroisoquinoline derivatives opens the door to an innovative hormonal treatment of these diseases.



MEDI 92

Design and syntheses of multiple fluorescent tocopherol probes

Zhen-Dan Shi[1]*, shizh@mail.nih.gov, Biying Xu*[1]*, Olga Vasalatiy*[1]*, Gary L. Griffiths*[1]*, Wei Zheng*[2]*, Rolf E. Swenson*[1]*. (1) Imaging Probe Development Center, National Heart, Lung, and Blood Institute, Rockville, Maryland 20850, United States (2) Therapeutics for Rare and Neglected Diseases, National Center for Advancing Translational Sciences, Rockville, MD 20850, United States*

Niemann-Pick type C (NPC) is a lysosomal storage disease caused by mutations of the NPC1 and NPC2 genes with an estimated frequency of 1:150,000 people. Deficiency in functions of NPC1 or NPC2 proteins results in accumulation of unesterified cholesterol in lysosomes of defective cells. Currently there are no FDA-approved therapies for NPC. Recent studies showed that tocopherol, an antioxidant agent, effectively decreased lysosomal cholesterol accumulation and lysosomal volume as well as increased cholesterol efflux. Reduction of these abnormalities may be mediated through a tocopherol-induced intracellular Ca^{2+} response and subsequent increment of lysosomal exocytosis. In order to further investigate mechanisms of cellular cholesterol trafficking, several fluorescent tocopherol probes were designed and synthesized by utilization of different dyes, linkers and labeling positions. Herein we present the detailed background, design and syntheses of the fluorescent tocopherol probes.

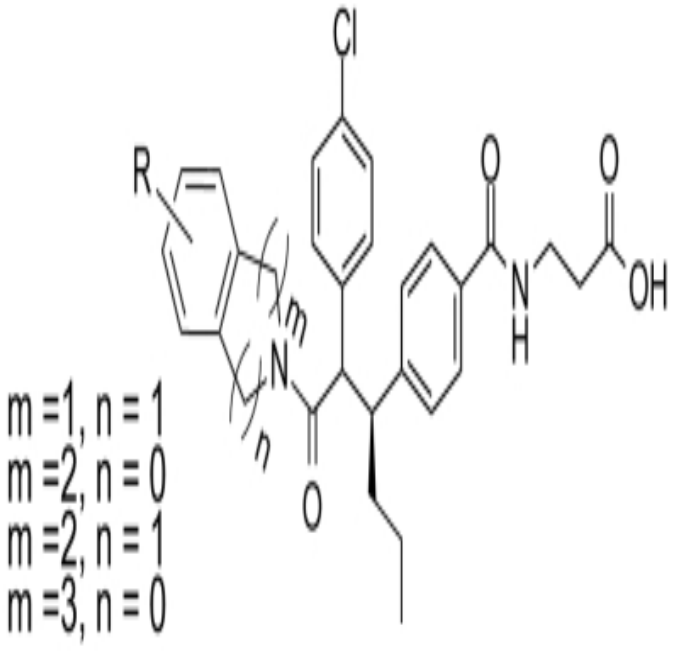
MEDI 93

Novel glucagon receptor antagonists bearing a cyclic tertiary amide for the treatment of type II diabetes

Songnian Lin*, songnian.lin@merck.com, Sheryl D Debenham, Xibin Liao, Roman Kats-Kagan, Edward Metzger, Sunita V Dewnani, Tracey Spencer, Guoqiang Jiang, Sajjad A Qureshi, Xiaodong Yang, Gary G Chicchi, Laurie Tota, Alka Bansal, Edward Brady, Maria Trujillo, Gino Salituro, Corey Miller, James R Tata, Bei Zhang, Emma Parmee. Early Development and Discovery Science, Merck Research Laboratories, Kenilworth, NJ 07033, United States*

Blood glucose levels are maintained by the balance of glucose production in the liver and glucose uptake in peripheral tissues. An inappropriately high rate of hepatic glucose production (HGP) is the predominant cause of fasting hyperglycemia and a major contributor to the postprandial hyperglycemia characteristic of type 2 diabetes (T2DM). The glucagon receptor is predominantly located in the liver and upon activation stimulates hepatic glycogenolysis and gluconeogenesis. Studies in T2DM patients have demonstrated a causal role for glucagon in promoting excessive HGP. Glucagon receptor antagonists (GRAs) therefore have the potential to reduce HGP and be effective anti-diabetic agents.

This presentation will describe the synthesis and biological evaluation of a novel series of GRAs bearing a cyclic tertiary amide, designed based on an earlier GRA lead MK-3577.



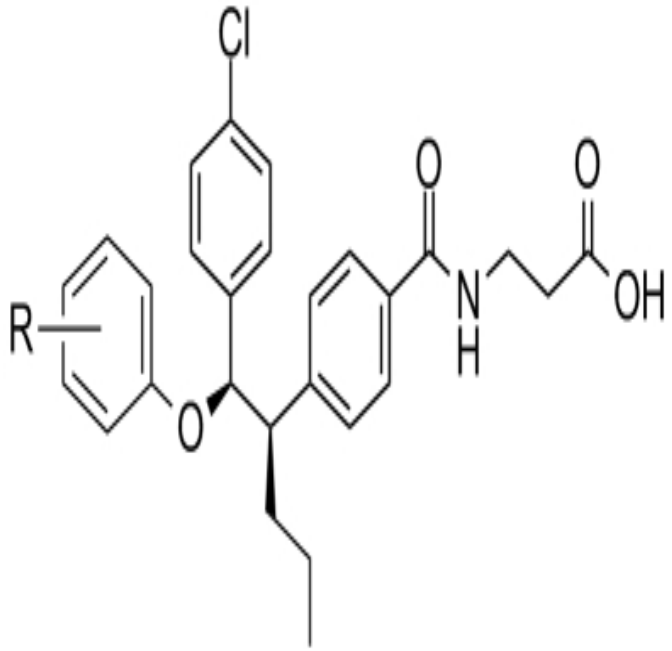
MEDI 94

Novel glucagon receptor antagonists bearing an ether linkage for the treatment of type II diabetes

Songnian Lin*, songnian.lin@merck.com, Xibin Liao, Roman Kats-Kagan, Guoqiang Jiang, Sajjad A Qureshi, Xiaodong Yang, Gary G Chicchi, Laurie Tota, Alka Bansal, Edward Brady, Maria Trujillo, Gino Salituro, Corey Miller, James R Tata, Bei Zhang, Emma Parmee. Early Development and Discovery Science, Merck Research Laboratories, Kenilworth, NJ 07033, United States*

Blood glucose levels are maintained by the balance of glucose production in the liver and glucose uptake in peripheral tissues. An inappropriately high rate of hepatic glucose production (HGP) is the predominant cause of fasting hyperglycemia and a major contributor to the postprandial hyperglycemia characteristic of type 2 diabetes (T2DM). The glucagon receptor is predominantly located in the liver and upon activation stimulates hepatic glycogenolysis and gluconeogenesis. Studies in T2DM patients have demonstrated a causal role for glucagon in promoting excessive HGP. Glucagon receptor antagonists (GRAs) therefore have the potential to reduce HGP and be effective anti-diabetic agents.

This presentation will describe the synthesis and biological evaluation of a novel series of GRAs bearing an ether linkage, designed based on an earlier GRA lead MK-3577.



MEDI 95

Thiazaheterocycles with antidiabetic activity: Design, synthesis, and in combo pharmacological studies

Gabriel Navarrete-Vazquez[1]*, gabriel_navarrete@uaem.mx, Samuel Estrada-Soto*[1]*, Guadalupe Morales-Vilchis*[1]*, Sergio Hidalgo-Figueroa*[1]*, Julio C. Almanza-Perez*[2]*, Scott P. Webster*[3]*. (1) Facultad de Farmacia, Universidad Autonoma del Estado de Morelos, Cuernavaca, Morelos 62209, Mexico (2) Ciencias de la Salud DCBS, Universidad Autónoma Metropolitana, Iztapalapa, Mexico City 09340, Mexico (3) Centre for Cardiovascular Science, The Queen's Medical Research Institute, University of Edinburg, Edinburgh, Edinburg EH16 4TJ, United Kingdom*

Thiazaheterocycles such as thiazole and thiazolidine-2,4-dione, are privileged structures which shows several pharmacological activities. In this research, we used these scaffolds in order to get new antidiabetic compounds. A series of thiazolidine-2,4-dione (TZD) and related bioisosteres were prepared and their in vitro relative expression of PPAR alpha, PPAR gamma were determinate. Some compounds showed an increase in the mRNA expression of both PPAR isoforms, as well as the GLUT-4 levels and FATP-1. Thiazole derivatives were tested as 11β-HSD1 inhibitors. The antidiabetic activity of the most active compounds of each series was determined at 50 mg/Kg

single oral dose using a non-insulin dependent diabetes mellitus rat model. The results indicated a significant decrease in plasma glucose levels.

MEDI 96

Synthesis and biological evaluation of a retinoid X receptor agonists identified and derived from lead optimization of a peroxisome proliferator-activated receptor γ- ligand

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(E)-3-[5-di(1-methyl-1H-indol-3-yl)methyl-2-thienyl]acrylic acid (BI-1051) is a retinoid X receptor (RXR) agonist derived from a peroxisome proliferator-activated receptor γ (PPARγ) ligand 1-Di(1H-indol-3-yl)methyl-4-trifluoromethylbenzene (DIM-Ph-4-CF3). This RXR agonist represents one of the first compound with an indole as the hydrophobic ring and an additional indole moiety as the bridge substituent. However, docking pose of BI-1051 lacks important hydrophobic interactions between W305, N306 and functional group in the bridge part, which is unveiled in the crystal structure of the human RXR alpha ligand binding domain bound to the synthetic agonist compound BMS 649. Computational modeling study showed that the exchange of the position of double bond and thiophene ring improved the ligand-protein interactions with hydrophobic interfaces between methyl group in one of the indole rings and W305, N306, as well as stronger hydrogen bond between carboxylic acid and R316, A327. The multi-step syntheses and competitive RXRa binding assay of (E)-5-(3,3-bis(1-methyl-1H-indol-3-yl)prop-1-en-1-yl)thiophene-2-carboxylic acid (UI-MY45) were studied to evaluate binding affinity and selectivity towards RXR. Detailed characterization and biological data will be presented.

MEDI 97

Novel 2,5,6-trisubstituted indoles as highly potent glucokinase activators for treatment of type II diabetes mellitus

***Jiayi Xu**, jiayi_xu@merck.com, Emma R. Parmee, Roman Kats-Kagan, Sunita Malkani, Brian Campbell, Libo Xu, Fengqi Zhang, Jennifer Zhang, Qinghong Fu, Daniel R McMasters, Robert W Mayers, Wen Feng, Nadine Elowe, Michael Kavana, George H. Addona, Michele J. Pachanski, Maria Trujillo, George J. Eiermann, Husuan_shen Chen, Zhesheng Chen, Gino M Salituro, Xinchun Tong, Kaushik Mitra, Brian T Farrer, Joel P Berger, **Songnian Lin**, songnian_lin@merck.com. Early Development and Discovery Science, and Preclinical Development, Merck Research Laboratories, Rahway, New Jersey 07065, United States*

Type II diabetes mellitus (T2DM), a metabolic disease characterized by high blood sugar, has become a rapidly expanding epidemic worldwide. Despite the existing therapies, many patients are still having difficulty to safely achieve healthy glycemic

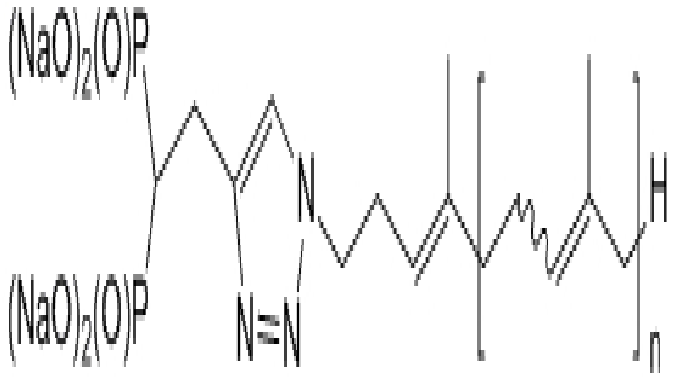
control and suffering from a number of severe diabetic complications. Glucokinase (GK) is a unique hexokinase that plays a center role in glucose homeostasis in humans. Glucose phosphorylation by GK in beta-cell is the rate-limiting step that controls glucose-regulated insuline secretion. In diabetic patients, its activity is likely to be impaired. Small molecule GK activators can increase the activity of GK by allosterically binding to the enzyme. Therefore, it is a promising method for the treatment of T2DM and has attracted many attentions from both academic and industrial researchers. Herein, we report SAR studies on a series of novel 2,5,6-trisubstituted indole derivatives as highly potent GKA's. Orally administrated, many compounds showed robust glucose-lowering effect in preclinical species.

MEDI 98

Homoisoprenoid triazole bisphosphonates as inhibitors of geranylgeranyldiphosphate synthase

Veronica S. Wills[1]*, veronica-wills@uiowa.edu, Ella J. Born*[2]*, Sarah A. Holstein*[2]*, David F. Wiemer*[1]*. (1) Department of Chemistry, University of Iowa, Iowa City, Iowa 52242-1294, United States (2) Department of Internal Medicine, University of Iowa, Iowa City, Iowa 52242, United States*

In the isoprenoid biosynthetic pathways, addition of the 5-carbon compound isopentenyl diphosphate to the 15-carbon farnesyl diphosphate is catalyzed by the enzyme geranylgeranyl diphosphate synthase (GGDPS), and results in formation of the 20-carbon geranylgeranyl diphosphate (GGPP). Because protein prenylation via reaction with GGPP is critical for proper cell signaling and protein trafficking, there has been interest in GGDPS inhibitors for their anti-proliferative effects. In an effort to discover new inhibitors of this enzyme, we have prepared a number of triazole derivatives bearing a geminal bisphosphonate (e.g. **1** –**4**). The triazoles are substituted with homoisoprenoid chains of varying lengths and have been prepared as single olefin isomers. The synthesis of these compounds and their activity as inhibitors of GGDPS will be presented.



1 n = 0
2 n = 1
3 n = 2
4 n = 3

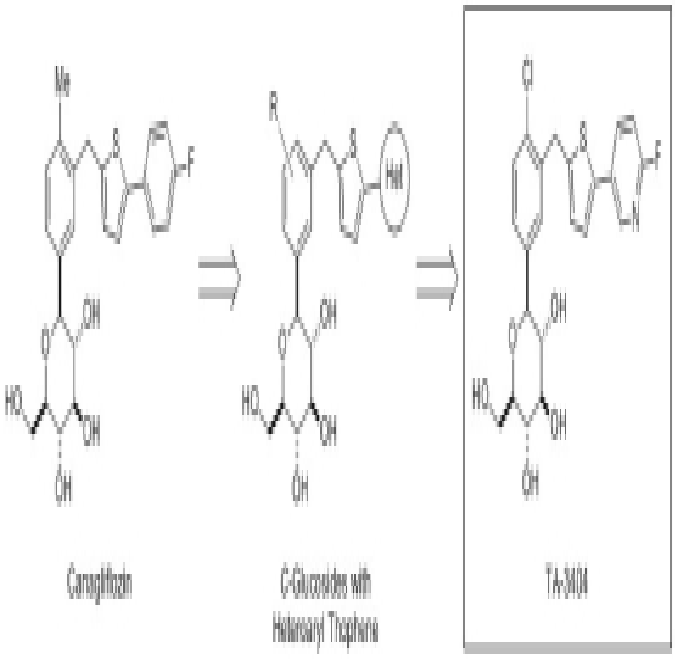
MEDI 99

C-Glucosides with heteroaryl thiophene as novel sodium glucose co-transporter 2 inhibitors

Sumihiro Nomura[1], *nomura.sumihiro@mw.mt-pharma.co.jp, Yuichi Koga*[1], *Shigeki Sakamaki*[1], *Mitsuya Hongu*[1], *Eiji Kawanishi*[1], *Toshiaki Sakamoto*[1], *Yasuo Yamamoto*[1], *Kiichiro Ueta*[2], *Hirotaka Kimata*[2], *Keiko Nakayama*[2], *Yasuaki Matsushita*[2], *Chiaki Kuriyama*[2], *Minoru Tsuda-Tsukimoto*[3]. *(1) Medicinal Chemistry Research Laboratories, Mitsubishi Tanabe Pharma Corporation, 2-2-50 Kawagishi, Toda, Saitama 335-8505, Japan (2) Pharmacology Research Laboratories, Mitsubishi Tanabe Pharma Corporation, 2-2-50 Kawagishi, Toda, Saitama 335-8505, Japan (3) DMPK Research Laboratories, Mitsubishi Tanabe Pharma Corporation, 2-2-50, Kawagishi, Toda, Saitama 335-8505, Japan*

Sodium glucose co-transporter 2 (SGLT2) mediates glucose reabsorption into the kidney. Inhibition of SGLT2 in vivo results in increased urinary glucose excretion and controls blood glucose levels in hyperglycemic animals. In previous studies, T-1095 was identified through optimization of the natural phenol-*O*-glucoside product phlorizin, producing the first orally active SGLT2 inhibitor. T-1095 enhanced urinary glucose excretion and lowered blood glucose levels independent of the action of insulin in various animal models of diabetes. We recently reported that aryl-*C*-glucoside analogues, including canagliflozin, are metabolically stable SGLT2 inhibitors that exhibit excellent in vivo potency. Canagliflozin has been launched in the USA. Further investigations of the structure–activity relationships of a series of *C*-glucosides with

heteroaryl thiophene led to the identification of the fluoropyridyl thiophene derivative TA-3404 as a potent SGLT2 inhibitor. In the present study, we developed novel synthetic methods and produced pharmacological data for *C*-glucosides with heteroaryl thiophene and analyzed their potential as novel antihyperglycemic agents.



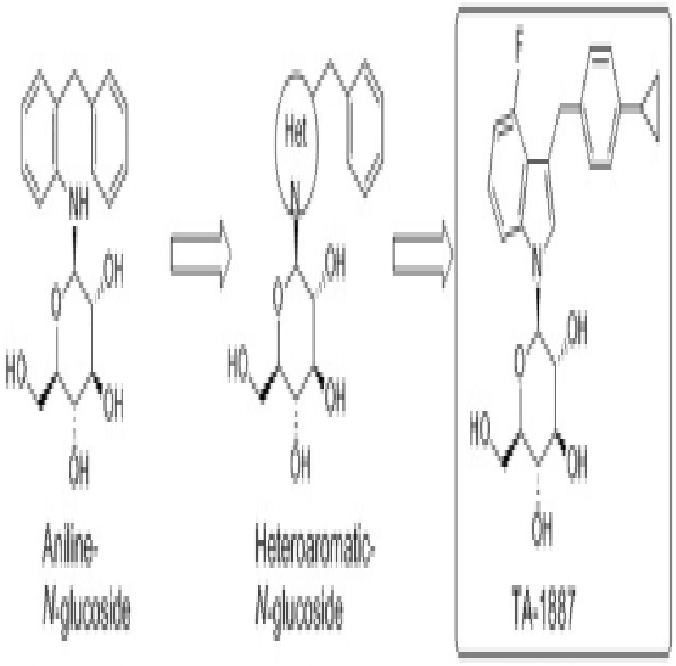
MEDI 100

Discovery of the novel indole-*N*-glucoside TA-1887 as a sodium glucose co-transporter 2 inhibitor

Yasuo Yamamoto[1]*, yamamoto.yasuo@ms.mt-pharma.co.jp,* ***Sumihiro Nomura***[1]*, nomura.sumihiro@mw.mt-pharma.co.jp, Yousuke Matsumura*[1]*, Kiyomi Ohba*[1]*, Shigeki Sakamaki*[1]*, Eiji Kawanishi*[1]*, Yuichi Koga*[1]*, Toshiaki Sakamoto*[1]*, Kiichiro Ueta*[2]*, Hirotaka Kimata*[2]*, Keiko Nakayama*[2]*, Yasuaki Matsushita*[2]*, Chiaki Kuriyama*[2]*, Minoru Tsuda-Tsukimoto*[3]*. (1) Medicinal Chemistry Research Laboratories, Mitsubishi Tanabe Pharma Corporation, 2-2-50 Kawagishi, Toda, Saitama 335-8505, Japan (2) Pharmacology Research Laboratories, Mitsubishi Tanabe Pharma Corporation, 2-2-50 Kawagishi, Toda, Saitama 335-8505, Japan (3) DMPK Research Laboratories, Mitsubishi Tanabe Pharma Corporation, 2-2-50 Kawagishi, Toda, Saitama 335-8505, Japan*

The incidence of type 2 diabetes is markedly increasing in western countries and some developing countries, leading to a strong demand for novel drugs with improved efficacy and safety. Plasma glucose is filtered and reabsorbed in the kidney via sodium glucose co-transporter 2 (SGLT2). In a previous study, the natural phenol-*O*-glucoside product

phlorizin was optimized to the first orally active SGLT2 inhibitor. The resulting compound T-1095 enhances urinary glucose excretion and lowers blood glucose levels independent of the action of insulin. We recently demonstrated that the aryl-*C*-glucoside canagliflozin is a metabolically stable SGLT2 inhibitor with excellent in vivo potency, and it has been launched in the USA. Further investigations of the potential of a series of glucosides demonstrated SGLT2 inhibitory activity of novel aniline- and heteroaromatic-*N*-glucosides. Through optimization of these compounds, we identified 3-(4-cyclopropylbenzyl)-4-fluoroindole-*N*-glucoside (TA-1887), as a highly potent and selective SGLT2 inhibitor. Oral administration of TA-1887 exerted pronounced antihyperglycemic effects in high-fat diet-fed KK (HF-KK) mice. Analysis of the structure–activity relationships revealed the potential of indole-*N*-glucosides as antihyperglycemic agents that inhibit renal SGLT2.



MEDI 101

Presentation of the peptide sequence, RGD, (arginine, glycine, aspartic acid) tethered to polyethylene glycol hydrogels as examined by VSFG spectroscopy

Mark R Watry[1]*, mwatry@franciscan.edu, Derek Doroski*[2]*, Rosita Asawa*[2]*, Holly Baca*[2]*, Daniel Schmitt*[2]*. (1) Department of Chemistry and Physics, Franciscan University of Steubenville, Steubenville, Ohio 43952, United States (2) Department of Biology, Franciscan University of Steubenville, Steubenville, Ohio 43952, United States*

A primary goal of tissue engineers is to develop a material that can be injected into a wound that directs and speeds up the body's own repair mechanisms, that minimizes

the risk of infection and immunogenic reaction, and that metabolizes away when the job is finished. The RGD sequence of amino acids is known to effect cell adhesion and cell mobility. Artificial scaffolds like PEG hydrogels can be used to fill the space in a wound where we want new cells to accumulate. We have synthesized PEG hydrogels functionalized with pendant RGD groups. Mesenchymal stem cells adhere to the functionalized hydrogel. In this work, we use Vibrational Sum Frequency Spectroscopy (VSFG), a nonlinear optical spectroscopy that probes the vibrational spectrum of molecules at interfaces, to determine the orientation and conformation of the hydrogel and the RGD sequence.

MEDI 102

Western-style Chinese (Kampo) medicine targeting retinoid X receptors (RXRs)

***Hiroki Kakuta**, kakuta-h@cc.okayama-u.ac.jp. Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Okayama 700-8530, Japan*

Western-style Chinese (Kampo) medicine is defined by the presenter as a methodology to exert medical effects like Chinese (Kampo) medicine by synthetic compounds (western medicine) targeting receptors or enzymes. This time I would like to propose this methodology by targeting retinoid x receptors (RXRs).

So-called modern diseases, such as diabetes, cancer and AD, are closely related to lipid metabolism, which is likely to have been greatly affected by lifestyle changes in recent years. Therefore, improvement of the internal bodily environment might be an effective therapeutic approach for modern diseases. Traditional Chinese medicine (TCM) developed in China and Kampo medicine derived from TCM and progressed independently in Japan both emphasis the whole patient, focusing on the integrity of the human body and the close relationship between the human body and its environment.

Retinoid X receptors (RXRs) act as homo- or heterodimers with other nuclear receptors that regulate glucose and lipid metabolism, such as PPAR, LXR and FXR. Modulating the transcriptional activities of these heterodimers by theirselves (permissive effects), RXR ligands can produce wide-ranging therapeutic effects, which other nuclear receptor ligands cannot produce. However, previously reported RXR agonists cause several adverse effects including blood TG elevation, hepatomegaly and hypothyroidism. Since these RXR agonists are full agonists, we hypothesized that these side effects are caused by the excessive activation of RXRs and moderate activation of RXR is enough for the desired medical effects.

Our group developed RXR partial-agonists CBt-PMN (**1** ; EC_{50} = 143 nM, E_{max} = 75%) and NEt-4IB (**2** ; EC_{50} =169 nM , E_{ma} = 55%) and demonstrated these compounds to exert blood glucose-lowering and anti-inflammatory effects without body weight gain, blood TG elevation or hepatomegaly. In this presentation, our research concept and these compounds will be introduced.

MEDI 103

Discovery of novel alkoxybenzamides, alkoxypicolinamides, and alkoxybenzofuran-2-carboxamides as potent AMPK activators

***Ihab S. Darwish**, idarwish@rigel.com, Rajinder Singh, Jiaxin Yu, Hui Hong, Sambaiah Thota, Xiang Xu, Sarkiz D. Issakani, Tian-Qiang Sun, Wei Li, Sacha Holland, Vadim Markovtsov, Yonchu Jenkins, Yingwu Li, Alison Pan, Gerald Uy, Yasumichi Hitoshi, Gary Park, David Sweeny, Rachel Basile, Maurice Standlee, David Lau, Donald Payan. Rigel Pharmaceuticals, Inc., South San Francisco, CA 94080, United States*

Adenosine monophosphate-activated protein kinase (AMPK) is an essential regulator of cellular energy homeostasis. It exists as a heterotrimeric protein consisting of three subunits (α, β, and γ) making up the functional enzyme and is expressed in a number of tissues, including the brain, liver and skeletal muscle. AMPK activation stimulates hepatic fatty acid oxidation, inhibits cholesterol and triglyceride synthesis and modulates insulin secretion. We have identified a series of low nanomolar small molecule activators of AMPK. In both cell culture and *in vivo* studies, our lead compound, structure 25, reduces liver fat content, enhances glucose uptake in cells and increases Glut4 expression in muscle tissue resulting in lowering blood glucose and insulin levels in both *db/db* and diet-induced-obesity (DIO) mice.

MEDI 104

Pyrimidine derivatives as mGlu5 receptor negative allosteric modulators

***Guiying Li**[1], guli@lundbeck.com, Myriam April[2], Xiaosui Pu[3], Michelle A Uberti[4], Robbin M Brodbeck[3], Maria D. Bacolod[5], Gamini Chandrasena[6], Darío Doller[1]. (1) Discovery Chemistry & DMPK, Lundbeck Research USA, Paramus, NJ 07652, United States (2) Novartis, Cambridge, MA, United States (3) Molecular Pharmacology, Lundbeck Research USA, Paramus, NJ 07652, United States (4) ETHOS Health Communications, Newtown, PA 18940, United States (5) Celgene Avilomics Research, Bedford, MA 01730, United States (6) Bioanalysis, Lundbeck Research USA, Paramus, NJ 07652, United States*

Several mGluR5 negative allosteric modulators (NAMs) containing an alkyne as a key structural motif are being evaluated in the clinic, including mavoglurant for fragile X syndrome (FXS), dipraglurant for levodopa-induced dyskinesia in Parkinson's disease (LID-PD) and dystonia, and RO4917523 for FXS and depression. The termination of the development of ADX10059 due to liver function abnormalities in clinical studies for chronic indications motivated the exploration of alternative chemotypes lacking the alkyne moiety. We wish to report the discovery of pyrimidine derivatives, a novel series of mGluR5 NAMs, which demonstrated tractable SAR and good potency *in vitro*. A representative compound from this series has good in silico CNS drug-like properties (MW 356, tPSA 72Å, cLogP 2.4, MPO 5.8), had Ki value of 31 nM and IC_{50} value of 4.2 nM in human mGluR5 binding and functional assays, respectively.

MEDI 105

Discovery of pyrrolo-benzo-1,4-diazines as potent Nav1.7 sodium channel blockers

Ginny D. Ho[1], Deen Tulshian[1], Ana Bercovici[1], Zheng Tan[1], Jennifer Hanisak[1], Stephanie Cooke[1], Julius Matasi[1], Charles R Heap[2], William G Earley[2], Brandy Courneya[2], Xiaoping Zhou[3], Terry Bridal[3], Diane Rindgen[4], Lynn Hyde[3], Mary Cohen-Williams[3], Steve Sorota[3], ***Shu-Wei Yang****[1], shu-wei.yang@merck.com. (1) Discovery Chemistry, Merck Research Laboratory, Kenilworth, New Jersey 07033, United States (2) Department of Medicinal Chemistry, AMRI Global, Albany, New York 12203, United States (3) Department of Neuroscience, Merck Research Laboratory, Kenilworth, New Jersey 07033, United States (4) Pharmacokinetics, Pharmacodynamics and Drug Metabolism, Merck Research Laboratory, Kenilworth, New Jersey 07033, United States*

Nav1.7 is a voltage-gated sodium channel that is highly expressed in nociceptive neurons and sympathetic ganglion. It plays a critical role in the generation and conduction of electrical signaling in small and medium-diameter sensory afferent nerves. Knockout mice lacking Nav1.7 in NaV1.8 expressing neurons displayed reduced pain response to mechanical or inflammatory pain. Humans lacking Nav1.7 exhibit a profound loss of all pain sensation. Consistent with the human phenotype, a more complete loss of pain sensation was observed in a pan-neuronal Nav1.7 knockout mouse. In contrast to the human and mouse knockout phenotype gain of function mutations in Nav1.7 causes pain syndromes in humans, either primary erythromelalgia or paroxysmal extreme pain syndrome. On balance these observations indicate that Nav1.7 is an excellent target for pain relief.

During high-throughput screening of our chemical library, some analogs with the pyrrolo-benzo-1,4-diazines skeleton were identified to display Nav1.7 inhibitory activity and modest selectivity against cardiac sodium channel Nav1.5. This poster will describe structure-activity relation of this class of compound vs. Nav1.7.

MEDI 106

Fully automated synthesis of a new ^{18}F-labeled bexarotene analog for PET imaging retinoid X receptor and apolipoprotein E

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Retinoid X receptor (RXR) is a member of nuclear hormone receptor family proteins, and it is closely linked to the apolipoprotein E (APOE), a cholesterol transport protein. Allelic variation in APOE gene is the most influential genetic risk factor for sporadic Alzheimer's disease (AD). Bexarotene (tradenamed Targretin, binding affinity K_i to RXR 21 nM) is a selective RXR agonist approved by FDA as an anticancer drug, and it is

being explored as a potential drug against AD in 3 murine models of AD, although the results remain controversial. RXR is a promising therapeutic target, a novel series of bexarotene analogues have been recently developed as selective RXR agonists by Wagner et al., and representative compound 2-fluoro-4-(1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)vinyl)benzoic acid exhibited higher binding affinity to RXR (*Ki* 12 nM) than its parent compound bexarotene. RXR is also an attractive imaging target. Here we report the synthesis of a ^{18}F-labeled bexarotene analogue, 2-[^{18}F]fluoro-4-(1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)vinyl)benzoic acid for use in biomedical imaging technique positron emission tomography (PET) to image RXR and APOE in cancer and AD. The reference standard 2-fluoro-4-(1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)vinyl)benzoic acid was synthesized from 2,5-dimethyl-2,5-hexanediol and 2-fluoro-4-methylbenzoic acid in 10 steps with 3% overall chemical yield. The precursor 2-nitro-4-(1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)vinyl)benzoic acid was synthesized from 2,5-dimethyl-2,5-hexanediol and dimethyl-2-nitroterephthalate in 7 steps with 2% overall chemical yield. The target tracer 2-[^{18}F]fluoro-4-(1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)vinyl)benzoic acid was synthesized from its nitro-precursor by the nucleophilic substitution with K[^{18}F]F/Kryptofix 2.2.2 in DMSO at 140 ^{o}C and isolated by HPLC combined with solid-phase extraction (SPE) purification in 20-30% radiochemical yield with 37-370 GBq/mmol specific activity at end of bombardment (EOB). The radiolabeling reaction was performed in a home-built automated ^{18}F-radiosynthesis module.

MEDI 107

Parallel enabled synthesis of 3,4-dihydroisoquinolin-1-ones as gamma secretase modulators

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Two synthetic routes are discussed for a series of 5-fluoro-3,4-dihydroisoquinolin-1-ones **1** having promising potency within the Gamma Secretase Modulator (GSM) program for the treatment against Alzheimer's disease. While early effort, utilizing the Bischler-Napieralski cyclization, provided the initial targets, further improvement was needed to facilitate diversification of the lactam *N*-substituent. A new route was developed which employed a one-pot reductive amination-intramolecular amidation on the key ester-aldehyde intermediate **2** . This approach allowed us to explore the SAR at a key position via parallel synthesis and taking advantage of the diverse set of available amines.

figure1

MEDI 108

Development of an agonist of the TGF-beta signaling pathway to treat Alzheimer's disease

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Alzheimer's disease (AD) is a common and devastating neurodegenerative disorder for which no cure exists. AD often results in complete incapacitation, profound impairment of the quality of life, enormous burdens on family caregivers and society, and ultimately death from one of a variety of complications of the disease. In the US alone there are over 5 million people with AD and this figure is expected to grow to over 16 million by 2050 with costs exceeding $1.1 trillion. The cytokine transforming growth factor (TGF)-β1 is an organizer of the brain's response to injury and has been shown to have both immunomodulatory and neuroprotective effects in models of brain injury and degeneration. Since TGF-β receptor expression is reduced in human AD brains possibly leading to reduced signaling in the neurons as well. Increasing TGF-β signaling may reduce neurodegeneration and be beneficial for the treatment of individuals with mild to moderate AD. From a screening program to identify small molecules that mimic the beneficial effects of TGF-β, a hit, SRI-011273, was discovered. An optimization program was undertaken resulting in SRI-011381, a small molecule with promising *in vitro* and *in vivo* efficacy and safety data, which will be described.

MEDI 109

Synthesis and biological evaluation of some new thiazolodiazepine analogs as CNS active agents

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The present investigation describes the synthesis of a new derivative of thiazolo[3,2-a][1,3]diazepine analogues in continuation to previous patented efforts in this area (compound 1).1,2 The polarity of the free acid metabolite 2, rushes the drug out of the brain and this might be the reason for the ultra-short duration of action. It was thought to replace the ethyl carboxylate moiety of 1 by a methyl group in position 2- or 3- (3). Meanwhile, a bromine atom was introduced to position 2- (4) to increase lipid solubility and to be used as a handle for attaching S bridge to cyclohexyl or phenyl moieties representing alicyclic and aryl functions, (5-7). In order to evaluate the necessity of the 8-oxo function to the pharmacophoric requirements, it was planned to be reduced into 8-hydroxy function (6) or to be removed (7) to find out to what extent it will affect the biological activity. These derivatives (3-7) exhibited a promising and varying range of

CNS activities, including, hypnotic and anticonvulsant activities. A structure activity relationship SAR), which correlates the variation in chemical structure to the activity, was established. These new findings are currently under patency

MEDI 110

Facile synthesis of haloindoles for the development of ergot alkaloids compounds

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This paper represents the development of haloindoles for an alternative approach to the study of lysergic acid.

MEDI 111

Development of selective chemical inhibitors for the two-pore potassium channel, KCNK9, through a HTS and hit refinement strategy

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The two-pore domain potassium channel, KCNK9 (or TASK-3), is widely expressed in the nervous system and is important to maintaining resting state membrane potential. Using a high throughput screen, a thiotriazole chemotype was identified with preliminary selectivity over KCNH2 (hERG), KCNQ2, KCNJ2 ($K_{ir}2.1$) and closely related KCNK3 (TASK-1) channels. A subsequent structure-activity relationship study yielded **ML308** , a potent inhibitor of KCNK9 in both a thallium influx fluorescent assay (IC_{50} = 130 nM) and an automated electrophysiology assay (EC_{50} = 413 nM). Relative to KCNK3, 51-fold and 8-fold selectivities were observed for KCNK9 in the thallium-based and Qpatch assays, respectively. The potency and selectivity profile of **ML308** and the supporting

analogs derived thereof are useful pharmacological probes for the study of KCNK9 function.

MEDI 112

Synthesis of T807 for preparation of [^{18}F]T807, a tau PET imaging agent for Alzheimer's disease

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[^{18}F]T807 (also known as [^{18}F]AV-1451) 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, and early clinical PET imaging results with this radioligand have been reported. 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]T807-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 T807 was synthesized from (4-bromophenyl)boronic acid and 3-bromo-4-nitropyridine in 3 steps (Suzuki coupling, cyclization and Suzuki coupling) with 30% overall chemical yield. The cyclization reaction conditions including temperature and time were optimized. The second Suzuki coupling reaction was modified by changing the catalyst and reaction solvent. Significant improvements in the multiple-step organic synthesis of T807 included increasing the yields and enlarging the reaction scale. Following the literature method, [^{18}F]T807 can be prepared from its either nitro-precursor or quaternary ammonium salt precursor 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 113

Development and PET imaging of atypical antipsychotics for the treatment of schizophrenia

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Schizophrenia is a mental disorder that affects over 24 million people worldwide according to the World Health Organization and has a significant economic impact on society in terms of healthcare and indirect costs. There is currently no cure for schizophrenia, but there are several antipsychotics on the market that treat the

symptoms. The first atypical antipsychotic developed for the treatment of schizophrenia, clozapine, was approved by the FDA over 20 years ago and while it remains one of the most effective drugs, it is prescribed sparingly due to its potentially severe side effects. Several alternative antipsychotics with fewer side effects have been developed, but none have as high of a therapeutic effect as clozapine. The goal of this project is to synthesize derivatives of clozapine, screen them against key schizophrenia receptors for biological activity and ultimately perform PET imaging studies with the most promising compounds to observe distribution in the brain.

MEDI 114

Facile synthesis of oxazolidinone molecules and the attachment of imidazole for their biological activity study

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This paper represents a facile synthesis of oxazolidinone molecule for biological study with a phenylfluorenyl protection to reduce the risk of racemization in the aldehyde stage.

MEDI 115

Design and synthesis of newer amide derivatives as 5-HT_4 receptor ligands

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Dementia is a prevalent disease with estimated 36 million people currently affected. The most common disease representing such patient pool is Alzheimer's disease (AD). AD is a progressive neurodegenerative disorder with ageing as the major risk factor. Extracellular beta amyloid deposits and intracellular tau tangles in brain provide the best differentiator of AD from other forms of dementia. Current therapies treat only the disease symptoms and are associated with modest efficacy, offset by dose-limiting side effects. 5-HT_4 receptor agonists have demonstrated excellent pro-cognitive profile in various animal models. In addition, 5-HT_4 receptor agonists shift the equilibrium of APP processing from amyloidogenic to non-amyloidogenic pathway by activating alpha secretase enzyme.

A new series of heteroaryl amide compounds was designed. The synthesized compounds are potent and selective 5-HT_4 receptor agonists. A poster covering these aspects along with SAR, ADME and *in vivo* profiling (both in animal models of cognition and neurochemistry) will be presented.

MEDI 116

Discovery and optimization of indoline derivatives as new LXR agonists

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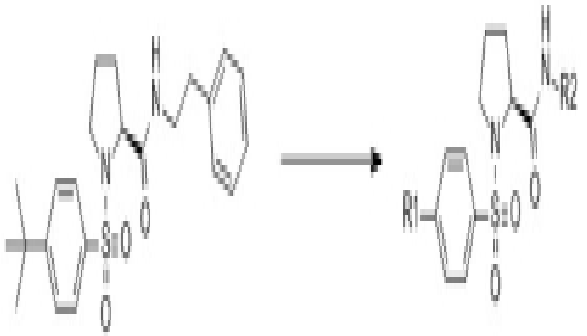
Liver X receptors (LXRs) are members of the nuclear receptor superfamily of ligand activated transcription factors. Two LXR isoforms have been identified: LXRα (NR1H3) and LXRβ (NR1H2).
Our drug discovery process, aimed at developing LXR agonists started with an HTS screening. Substituted proline **1** was identified as human LXR agonists in a transactivation assay. A first round of hit to lead optimization of **1** led to indoline analogs **2** with significant improvement in potency.
The design, synthesis and *in vitro* biological evaluation of this series **2** will be discussed. Compounds with different LXR α/β selectivity profiles and with good PK properties have been obtained. Derivatives bearing an acidic moiety on the R2 part were shown to be LXRα selective, while the LXRβ component was restored with tertiary amino groups on R2, leading to dual LXRα/β compounds or to some analogs with a tendency towards LXRβ selectivity.



MEDI 117

Synthesis and characterization of 17β-heteroaryl-substituted analogs of ganaxolone as modulators of $GABA_A$ receptors

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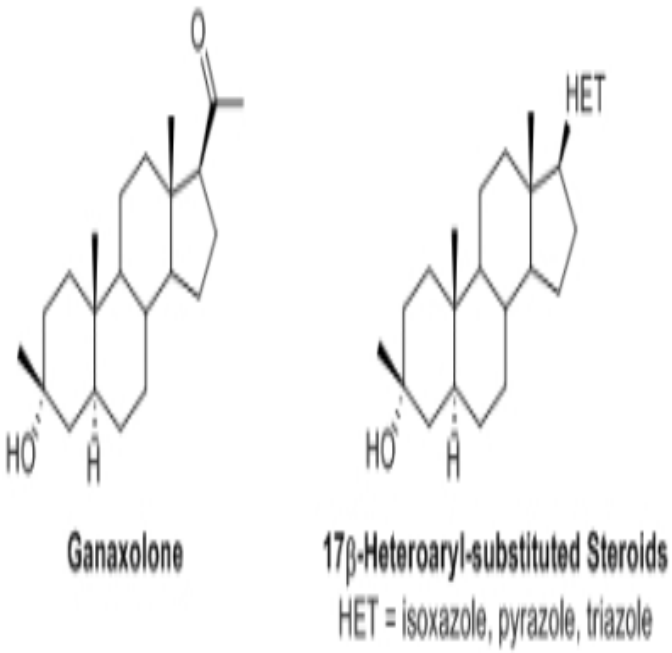
Ganaxolone (3α-hydroxy-3β-methyl-5α-pregnan-20-one) is a neuroactive steroid with potent activity as a positive allosteric modulator (PAM) of γ-aminobutyric $acid_A$ receptors ($GABA_ARs$). The compound has shown activity clinically as an oral anticonvulsant and trials examining its activity in Fragile X syndrome and post-traumatic stress disorder (PTSD) are underway. Bioisosteric replacement of the 17β-acetyl group in ganaxolone with 5-membered heteroarenes resulted in compounds that retained activity as PAMs of $GABA_ARs$ based on their ability to potentiate the effect of GABA on human $GABA_ARs$ expressed in *Xenopus* oocytes. Structure-activity relationship (SAR) studies examined the effect of changes to the heteroaryl group on the *in vitro* activity of the steroids. The neuroactive steroids were synthesized from ganaxolone or from 3β-hydroxy-5α-androstan-17-one. Select compounds were tested *in vivo* for their activity as anxiolytics and anticonvulsants.



MEDI 118

Modeling, synthesis, and biological evaluation of potent retinoid-X-receptor agonists: Novel analogs of bexarotene, LGD100268, and CD3254

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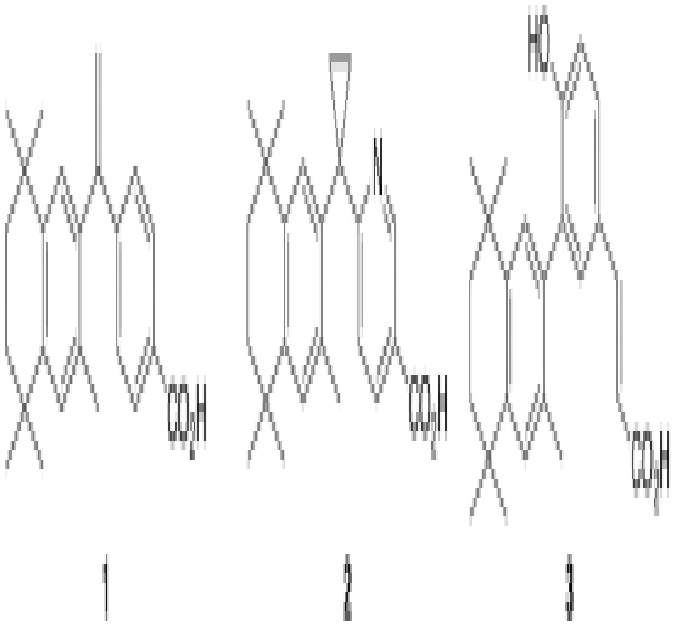
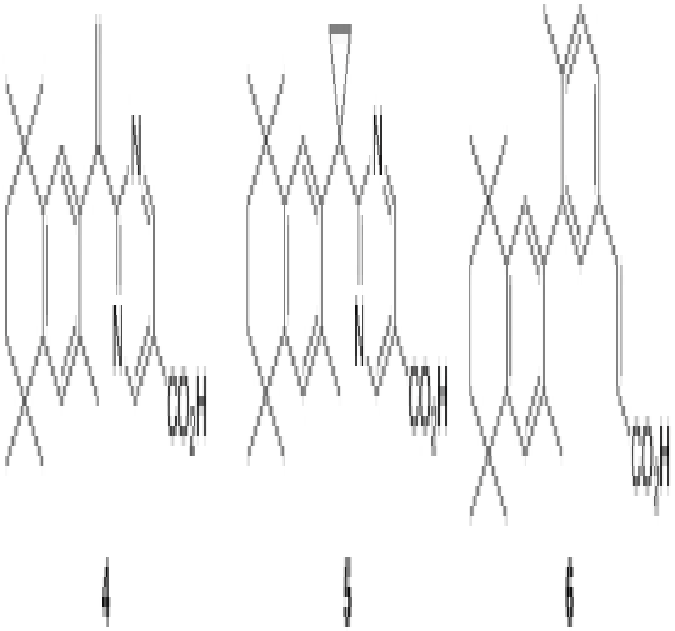
Bexarotene (**1**), LGD100268 (**2**) and CD3254 (**3**) are all potent retinoid-X-receptor agonists.



Bexarotene (**1**) is an FDA approved drug for the treatment of cutaneous T-cell lymphoma, but it is ofen used off-label to treat other cancers, and it has recently attracted interest as a potential treatment for Alzheimer's Disease. Pyrimidine analogs of bexarotene (**4**) and LGD100268 (**5**), as well as a methyl-substituted analog of CD3254 (**6**) were modeled, synthesized and evaluated alongside four other novel analogs for RXR agonism in mammalian 2 hybrid and RXRE-mediated assays in HCT-116 human colon cancer cells.



Many of the novel analogs were observed to be more potent than **1** and are currently being characterized in additional assay systems.

MEDI 119

Attachment of a peptide on a gold surface, and the temperature dependence of reversible self-assembly of the protein, abeta-142 in DMSO solvent: Investigation into the mechanisms of Alzheimers disease

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The amyloid beta (AB) 1-42 sequence is insoluble in water and is known to aggregate easily. This has prevented us from studying the reversible self-assembly process of AB1-42 monomers as well as the properties of an intermediate protein structure in this process. For the first time, our research group was able to capture the intermediate involved in the reversible self-assembly by placing the AB1-42 over the nanoscale gold colloid in dimethyl sulfoxide, in which AB1-42 is soluble. The length of nanoscale gold colloid used was 80 nm and 40 nm in diameter. However, the reversible self-assembly process did not repeat more than 10 cycles indicating that a denaturing process was going on simultaneously. Currently, absorption spectroscopy is being used to investigate temperature dependence ranging from 0-50°C in gold nanoparticles of various sizes. So far it has been established through trial and error that there is a non-linear relationship between the pH and the temperature of the amyloid beta 1-42 complex.

MEDI 120

γ-Aminobutyric acid$_A$ receptor positive allosteric modulators potentiate α7 nicotinic acetylcholine receptor mediated nootropic-effect

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Sensory gating deficits and deficiencies in executive function in neurological diseases, such as schizophrenia, have been linked to pathological deficits in γ-aminobutyric acid$_A$ ($GABA_A$) and α7 nicotinic acetylcholine (nACh) receptors. We have previously described a series of selective type I positive allosteric modulators (PAMs) of α7 nACh receptors that are active in animal models of cognition. We have found that this effect can be potentiated in vivo with a positive modulator of $GABA_A$ receptors. Modifications to this series of compounds resulted in a series of PAMs that have dual activity at both $GABA_A$ and α7 nACh receptors. A structure activity relationship developed around these dual modulators led to varying ratios of modulation of α7 nACh and $GABA_A$ receptors. This

directed polypharmacy is proposed to be advantageous in diseases of a polygenic origin, such as schizophrenia.

MEDI 121

Design, synthesis, and pharmacological evaluation of novel multifunctional dopamine D_2/D_3 agonists with iron chelation property: Potential implication in symptomatic and neuroprotective treatment of Parkinson's disease

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Parkinson's disease (PD) is the second most common form of neurodegenerative disorders that results from the progressive loss of dopaminergic neurons in the midbrain substantia nigra pars compacta (SNpc) triggering profound motor perturbation, as well as cognitive, sensory and mood deficits. Although extensive research has been done to elucidate the underlying molecular events leading to neuronal death, yet the cause and individual steps in the pathogenesis of the disease are still not understood well and thus, PD remains a progressive and incurable condition. It is generally believed that oxidative stress, neuroinflammation, compromised natural antioxidant defense, protein aggregation and impaired mitochondrial functions are the mainstream predisposing factors implicated in the pathogenesis of PD. Due to complexity of the pathogenesis of PD, it is increasingly evident that drugs targeting only a single site may not be sufficient to slow the disease progression and alleviate motor dysfunction at the same time. In our overall goal to develop multifunctional drugs as neuroprotective treatment agents for PD, we designed novel dopamine D_2/D_3 agonist molecules with a capacity to address some underlying pathological factors in PD including chelating iron to reduce oxidative stress. The molecules exhibited high affinity for both D_2 and D_3 receptors where as in GTPγS functional assay, the lead compound (-)-**D-583** showed potent agonist activity at both D_2 and D_3 receptors (EC_{50} (GTPγS); D_2 = 3.14 nM and D_3 = 0.50 nM). Furthermore, the lead molecules demonstrated potent antioxidant activity in DPPH assay and also exhibited iron chelation property. In PD animal model study, both lead molecules (-)-**D-583** and (-)-**D-607** exhibited potent in vivo activity in reversing hypolocomotion in reserpinized rats. In cell culture study, the selected compounds demonstrated significant reduction of toxicity induced by treatment with 6-hydroxy dopamine, thereby, producing neuroprotection effect. This work is supported by grants from NINDS (NS 047198, AKD).

MEDI 122

Further structure activity relationship (SAR) study of novel hybrid N^6-(2-(4-(1*H*-indole)piperazine-1-yl)ethyl)-N^6-propyl-4,5,6,7-tetrahydrobenzo[*d*]thiazole-2,6-diamine analogs: Development of highly potent and selective D_3 receptor preferring agonist molecules

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Parkinson's disease (PD) is an age-related and progressive movement disorder that is characterized by dopaminergic neuronal loss in the substantia nigra region of the brain. Dopamine modulates movement, cognition, and emotion through activation of dopamine receptors in the brain. PD is a multifactorial disease caused by oxidative stress and mitochondrial dysfunction in neuronal cells with subsequent reduction of the dopamine level. An interesting development in the use of dopamine receptor agonists for the treatment of PD is that some of them may prove to be neuroprotective such as D_3 receptor preferring agonists. Targeting dopamine D_3 receptor, a subfamily of dopamine D_2-class of receptors, for various CNS disorders is drawing much attention because of its unique location of D_3 receptor. It has also been shown that selective D_3 receptor agonists can provide neuroprotection in PD by inducing brain derived neurotrophic factors (BDNF). Much of the pharmacological actions mediated by D_3 receptor are still unresolved because of the lack of highly potent and selective D_3 receptor agonist. In our previous communication, we described the development of a series of hybrid molecules for D_2 and D_3 receptors that showed high agonist potency by combining pharmacophoric elements of aminothiazole and piperazine molecular fragments derived from known dopamine receptor agonist and antagonist molecules. In our present SAR study, we used N^6-(2-(4-(1*H*-indole)piperazine-1-yl)ethyl)-N^6-propyl-4,5,6,7-tetrahydrobenzo[*d*]thiazole-2,6-diamine analogues to design such drugs. In these molecules, we specifically introduced various indole derivatives as accessory binding sites which led to the discovery of highly potent and selective molecules for D3 receptor. Compounds were characterized both in the in vitro binding and functional assays. Synthesis and in vitro binding and functional characterization will be presented. This work is supported by NS047198 (AD).

MEDI 123

Development of pharmacophore model and asymmetric synthesis of novel tetrahydrofuran derivatives enroute to triple reuptake inhibitors as anti-depressant agents

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Unipolar depression, caused by an imbalance of monoamine neurotransmitters in brain, is ranked as the most prevalent of all somatic and psychiatric illness. It is estimated that about 40 % of patients remains refractory to treatment thereby limiting the use of current anti-depressant drugs. Moreover, because of relapse and unwanted side effects of existing drugs there is an unmet need to discover novel agents for the treatment of this

devastating mental disorder. Current treatment aims at alleviating extraneuronal concentration of serotonin (5-HT) and norepinephrine (NE) but do not include dopaminergic element, which is also implicated in depression, into the therapy. Tremendous efforts are underway to inhibit all the three neurotransmitters with an aim to discover drugs with broader spectrum and faster onset of action. In our pursuit of effective drugs to treat depression, we have developed novel di- and tri-substituted pyran derivatives with balanced inhibitory activities at all the three transmitters that demonstrated anti-depressant effect in *in vivo* animal models. In this study, we report a triple monoamine uptake inhibitor (TUI) pharmacophore model that revealed a distinct 'folded' conformation and suggests features common to inhibitors that exhibited a TUI profile. Furthermore, the distances between the benzhydryl moiety and the N-benzyl group as well as the orientation of the secondary nitrogen were also important for TUI activity. We have validated our findings by synthesizing and testing novel asymmetric pyran analogs. Furthermore, a series of asymmetric tetrahydrofuran derivatives that fitted the pharmacophore model were synthesized and evaluated as a novel class of TUI as anti-depressant agents. Supported by MH84888 (AD).

MEDI 124

Hsp90 c-terminal inhibitors that manifest neuroprotective activity

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Novobiocin, a naturally occurring antimicrobial agent that inhibits DNA gyrase also binds to the C-terminus of Hsp90, and exhibits anti-proliferative effects. Through structural changes, the biological effect of novobiocin binding can be modified. Replacement of the benzamide side chain of novobiocin with an acetamide results in neuroprotective activity. A small molecule inhibitor of Hsp90, KU-32, is based on novobiocin and contains the acetamide side chain, which results in its neurodegenerative protective effects. In order to further improve the affinity and efficacy of KU-32, molecular modeling studies were carried out. A homology model was used to identify possible key residues that might be responsible and important in inhibitor binding to the C-terminus. The coumarin core of KU-32 was substituted for a biaryl ring system; in order improve the ease of exploration of the binding pocket. A second generation of KU-32 novologues containing this new biaryl ring system was synthesized, and one of the compounds, KU-596, was found to have comparable activity to KU-32. KU-596 was used as a parent compound in the generation of a new library of small molecule inhibitors, in which modifications were designed to gain more favorable interactions within the binding pocket. These compounds were synthesized and biologically evaluated, and will be presented.

MEDI 125

Synthesis, optimization, and pharmacological evaluation of TREK-1 activators as novel analgesics

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Analgesics used today date from the 19th century. Morphine remains the analgesic of reference for the treatment of pain (*nociception*), but it is also responsible for serious adverse effects. Research studies have showed that animals deprived of potassium channels (TREK-1) were over-sensitive to pain[1]. More recently, it has been demonstrated that the TREK-1 K^+ channel is a crucial contributor of morphine-induced analgesia in mice, while it is not involved in morphine-induced constipation, respiratory depression and dependence[2]. These results suggest that the TREK-1 channels constitute targets of interest for the design of novel analgesics without opioid-like adverse effects.

Previous studies within our consortium led to the identification of different families of TREK-1 activators exhibiting analgesic activity *in vivo*[3] ,[4]. We present here the synthesis and pharmacological evaluation of the third generation of analogues to confirm their therapeutic interest.

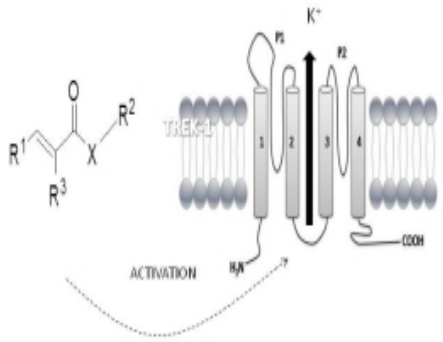

Figure 1 : Development of TREK-1 activators as novel analgesics

[1]A. Alloui, K. Zimmermann, J. Mamet, F. Duprat, J. Noel, J. Chemin, N. Guy, N. Blondeau, N. Voilley, C. Rubat-Coudert, M. Borsotto, G. Romey, C. Heurteaux, P. Reeh, A. Eschalier, M. Lazdunski, *EMBO J.,* **2006,** 25(11)**,** 2368-2376.

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[3]S. Ducki, K. Bennis, A. Eschalier, J. Busserolles, F. Lesage, N. Rodrigues, D. Vivier, **2013** , WO 2013098416/A2

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MEDI 126

Design and synthesis of the first tetrameric tuftsin aminopterin conjugate for active targeting therapy of autoimmune diseases

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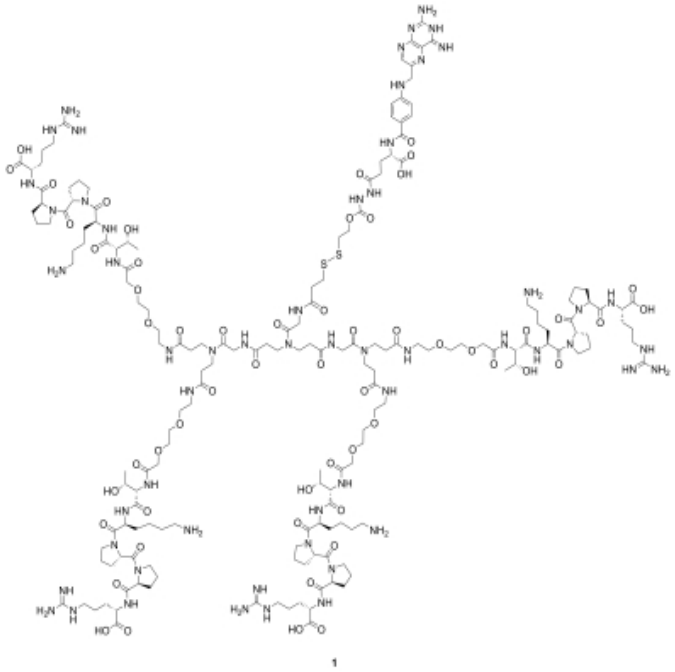
Tuftsin is an immunostimulatory tetrapeptide (TKPR) that binds to specific receptors on the surface of macrophages and polymorphonuclear leukocytes, stimulating their migration, phagocytic, bactericidal, and tumoricidal activity. Soon after its discovery, a pentapeptide tuftsin analog (TKPPR) was synthesized as an antagonist of tuftsin receptors to bind 20-fold stronger than tuftsin. Since enhanced affinity can be achieved by multivalent binding of ligands, a tetrameric TKPPR was recently reported with 45-fold stronger binding than TKPPR.

Aminopterin (AMT) is an antineoplastic drug with immunosuppressive properties. Attaching AMT to target-specific ligands may improve the efficacy of the drug through increased selectivity and reduced toxicity.

Here we report the design and synthesis of the first tetra-tuftsin aminopterin conjugate for treating inflammation. TKPPR was prepared by solution phase synthesis. After connecting the TKPPR subunits to the iminodipropionic acid-based symmetric central core, the intermediate was attached to AMT via a disulfide-based cleavable linker to afford the final conjugate (**1**).



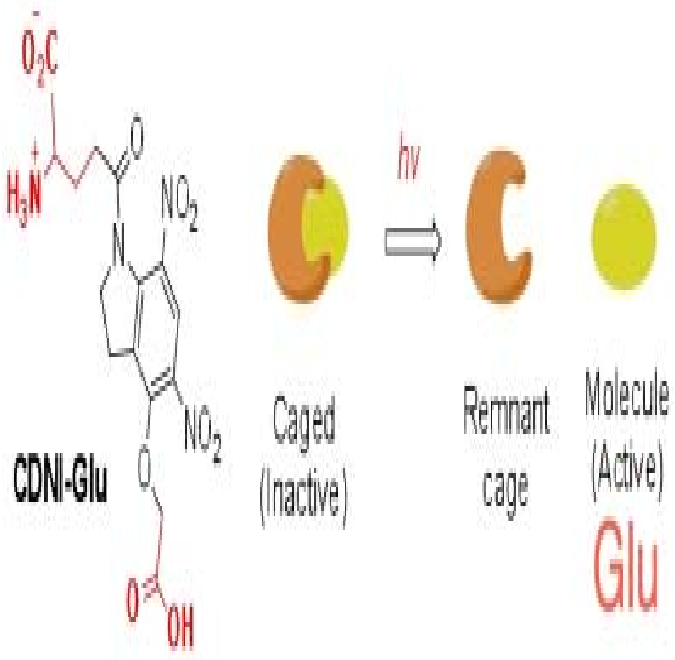
1

MEDI 127

Evaluation of photoactive cleavable neurotransmitters in the elucidation of neural networks

***Nasri Nesnas**, nesnas@fit.edu, Richard L. Comitz, Yannick P. Ouedraogo. Chemistry, Florida Institute of Technology, Melbourne, FL 32901, United States*

The brain's neural network represents the epitome of complex processing. To date, this challenge remains amongst the most of elusive investigations much needed to unravel mysteries in neural disorders, including but not limited to: Alzheimer's, Parkinson's, epilepsy, autism, and various forms of depression. While electrical stimulation of neurons has been historically well established and investigated, recent advances in the chemical designs of neurological tools have enabled a more precise stimulation of single synapses using laser technologies. A design of such neurological photoactive cleavable neurotransmitters will be presented alongside methods developed for studying their photocleavable efficiencies. Studies on the mechanisms of photocleavage will also be included to facilitate the potential for the design of more efficient photoactive neurological tools.



MEDI 128

Design and synthesis of novel β-carbolines as $GABA_A$ subtype selective agents for the treatment of alcohol abuse

***V. V. N. Phani Babu Tiruveedhula**[1], tiruvee2@uwm.edu, Kashi Reddy Methuku[1], Kaitlin T Warnock[2], Harry L June[2], James M Cook[1]. (1) Department of chemistry and Biochemistry, University of Wisconsin-Milwaukee, Milwaukee, Wisconsin 53211, United States (2) Department of Psychiatry and Behavioral Science, Howard University College of Medicine, Washington, DC 20059, United States*

Alcoholism plays a significant role in public health concerns, impacting physical and mental well-being, family structure and occupational stability. β-carboline-3-carboxylate-*t*-butyl ester [βCCt] and 3-propoxy-β-carboline hydrochloride [3-PβC·HCl] function as mixed benzodiazepine receptor agonist-antagonists, and they selectively bind at benzodiazepine $GABA_A$ α-1 receptor. The results have shown that systemic and direct infusion of βCCt or 3-PBC·HCl into the ventral pallidum produces remarkably selective reduction in alcohol responding in nondependent alcohol preferring (P) and high alcohol drinking (HAD) rats. They were also weakly anxiolytic in these genetic rat lines but not anxiolytic in normal rats. This indicates that these types of β-carbolines may represent a non-addicting treatment for human alcoholics. Initially βCCt and 3-PBC were synthesized via 5 step (35 % yield) and 8 step (8 % yield) protocols, respectively. In an attempt to replace this time consuming syntheses a new route involving 3 steps was developed. This new route involved two palladium catalyzed Buchwald-Hartwig coupling and an intramolecular Heck reaction as key steps. The later reaction lead to two regio isomers β- and d- carbolines . Among these two, the β- isomer ratio is increased by introducing the BOC protecting group. This 3-step protocol decreased the number of steps and improved the overall yields 3-PBC and βCCt to 39% and 50 %, respectively. Using this protocol a number of analogs of 3-PBC and Aza β-Carbolines were synthesized. Among these analogs, 3-ISOPBC.HCL showing potential lead anti-alcoholic agent. The development, application of this synthetic route and *in vivo* studies are presented.

MEDI 129

Synthesis of polyheterocyclic antagonists of GPR55

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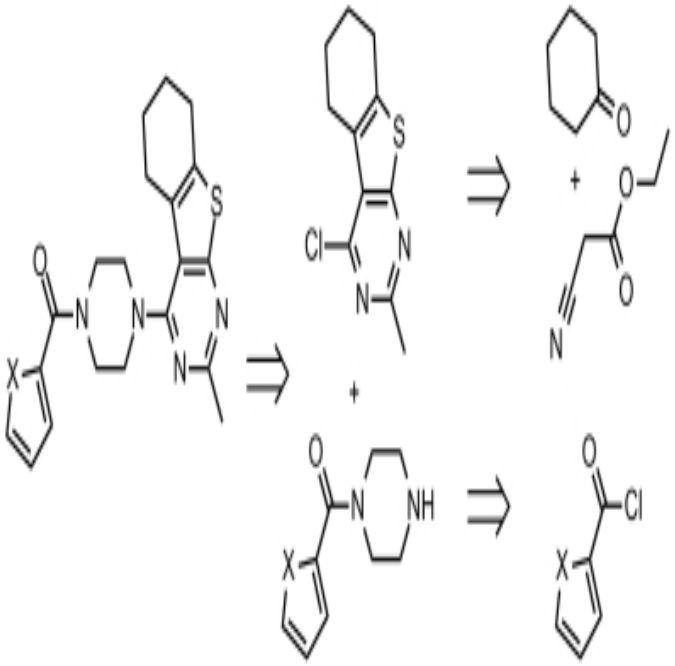
G protein-coupled receptor 55, also known as GPR55, was previously considered a cannabinoid receptor. GPR55 is expressed widely throughout the body, especially in the brain and small intestines along with cells that regulate bone cell function. GPR55's specific physiological role is still unclear; however, it has been linked to involvement in several processes including neuropathic/ inflammatory pain, cancer, and bone physiology. With implicated roles in such important areas of medical interest, it is desirous to synthesize effective antagonists in order to properly characterize the physiological role(s) of GPR55. Computational modeling of GPR55 was utilized to identify lead antagonist molecules. A method of assembly was devised retrosynthetically as shown in, and the synthesis of a number of analogs is currently under way to better map out GPR55's function and purpose. The results of these synthetic efforts will be herein presented.



MEDI 130

Development of non-isatin M1 positive allosteric modulators

Joseph D. Panarese*, Joseph.D.Panarese@Vanderbilt.edu, Jerri M. Rook, Michael S. Poslusney, Bruce J. Melancon, Thomas M. Bridges, Hyekyung P. Cho, Jonathan Dickerson, Corey Hopkins, Michael R. Wood, Zixui Xiang, Ryan Morrison, Shaun R. Stauffer, J. Scott Daniels, Colleen Niswender, Carrie K. Jones, P. Jeffrey Conn, Craig W. Lindsley. Vanderbilt Center for Neuroscience Drug Discovery, Nashville, Tennessee 37232, United States*

Muscarinic acetylcholine receptors (mAChRs) are G-protein-coupled receptors (GPCRs) that consist of five different subtypes (M1-M5). All participate in binding of the neurotransmitter acetylcholine (ACh) at a highly conserved orthosteric site. When stimulated by ACh, the mAChR signal transduction pathway is activated leading to release of calcium. Clinical studies with mAChR agonists suggest that this mechanism improves cognitive function in patients displaying impairments. Importantly, genetic studies indicate that M1 is the subtype responsible for procognitive effects. As a result, attention has been focused on selective activation of the M1 pathway using positive allosteric modulators (PAMs) due to the lack of subtype specificity of mAChR agonists. We describe a series of structurally unique M1 PAMs derived from a functional HTS of 160,000 compounds leading to the identification of MLPCN probes ML137 and ML169. Continued optimization of these probe molecules led to non-isatin M1 PAMs with excellent selectivity and improved DMPK profiles. Synthesis and structure-activity-

relationships (SAR) from these efforts will be presented in addition to efficacy and modified Irwin profiles for isoindolinone tool compound VU0453595.

MEDI 131

Novel oxazolidinone CGRP receptor antagonists for the acute treatment of migraine

Brendan M. Crowley[1], *brendan_crowley@merck.com, Hal G. Selnick*[1], *Craig A. Stump*[1], *Shawn J. Stachel*[1], *Craig M. Potteiger*[1], *Diem N. Nguyen*[1], *Amy G. Quigley*[1], *Ashley Nomland*[1], *Joseph G. Bruno*[3], *Amanda L. Kemmerer*[3], *Karsten Menzel*[4], *Rebecca B. White*[4], *Dan Cui*[4], *Eric L. Moore*[2], *Andrew Danzinger*[5], *Dan V. Paone*[1], *Christopher S. Burgey*[1], *Mark E. Fraley*[1], *Christopher A. Salvatore*[2], *Scott D. Mosser*[3], *Christine Fandozzi*[4], *Stefanie A. Kane*[2], *Samuel L. Graham*[1]. *(1) Department of Medicinal Chemistry, Merck Research Laboratories, West Point, PA 19486, United States (2) Department of Pain & Migraine, Merck Research Laboratories, West Point, PA 19486, United States (3) Department of In Vitro Pharmacology, Merck Research Laboratories, West Point, PA 19486, United States (4) Department of Pharmacokinetics, Pharmacodynamics, & Drug Metabolism, Merck Research Laboratories, West Point, PA 19486, United States (5) Department of In Vivo Pharmacology, Merck Research Laboratories, West Point, PA 19486, United States*

Calcitonin gene-related peptide (CGRP) is a 37 amino acid neuropeptide widely expressed in the peripheral and central nervous system that has been implicated in the pathogenesis of migraine headache. Antagonism of the CGRP receptor has been clinically validated to provide effective acute migraine relief comparable to the triptans with an improved adverse event profile. In our efforts to develop low-dose successors to telcagepant, our first-generation oral clinical candidate, we investigated a series of novel oxazolidinone-based CGRP receptor antagonists. The development of potent and selective compounds with low potential human doses will be described. Favorable pharmacokinetic and off-target profiles, including excellent ion channel selectivity, were achieved by a design strategy that took physiochemical properties into careful consideration. Conformational restriction as a means to improve potency and strategies to address potential genotoxicity concerns will also be discussed.

MEDI 132

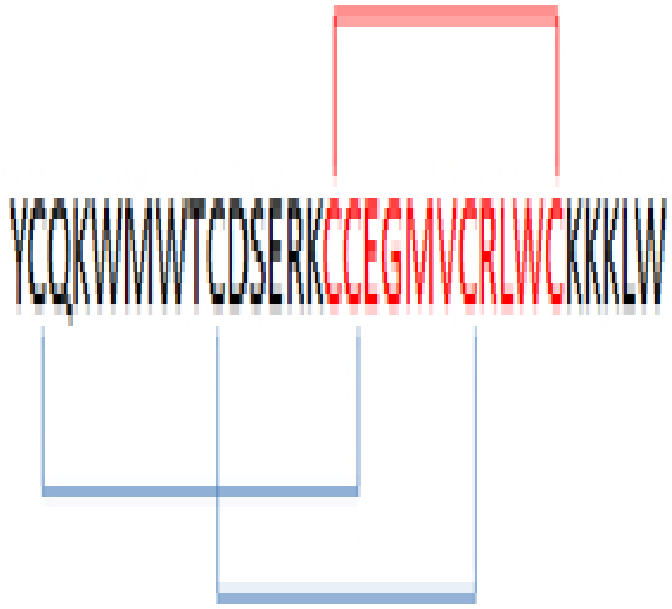
Probing pain receptors with cysteine knot peptides

Zoe V F Wright[1], *zoe.wright.10@ucl.ac.uk, Alethea B Tabor*[1], *Adrian Hall*[3], *Erik Arstad*[1], *Martin Koltzenburg*[2]. *(1) Department of Chemistry, University College London, London, United Kingdom (2) Institute of Neurology, University College London, London, United Kingdom (3) Eisai Ltd, Hatfield, Hertfordshire AL10 9SN, United Kingdom*

Chronic pain affects almost 10 million people in the UK[1] but despite this, few effective treatments exist. Research has shown that targeting the $Na_v1.7$ ion channel provides a

novel approach to treatment.[2] ProTx-II, a 30 amino acid peptide isolated from Tarantula venom, is highly selective for the channel (IC_{50} value - 0.3nm *in vitro*) but *in vivo*results were less promising.[3]

ProTx-II contains three interlocking disulphide bonds connected in a distinctive pattern. To investigate the structure-activity relationship between the peptide and the ion channel, truncated analogues based on the individual cysteine rings were synthesised.



We have investigated the effect of replacing the disulphide bond with a thioether linkage through the incorporation of a novel diastereomer of the non-natural amino acid lanthionine to produce hydrolytically stable compounds. Lanthionine can be thought of as two alanine residues connected by a thioether linkage at the β-carbon.[4] We have investigated novel methodologies for the incorporation of a new diastereomer of lanthionine containing just L-amino acids using a new microwave-based methodology. The biological properties of these analogues will be discussed.

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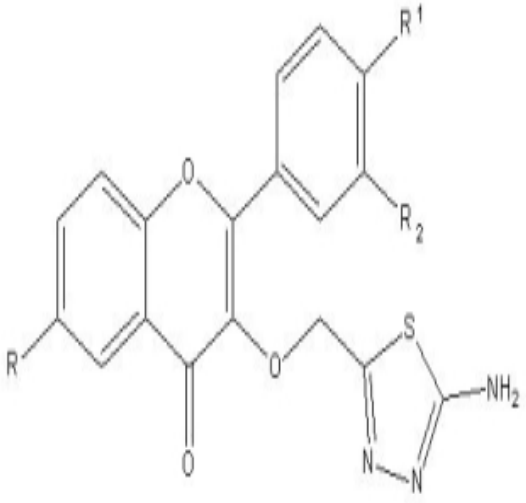
MEDI 133

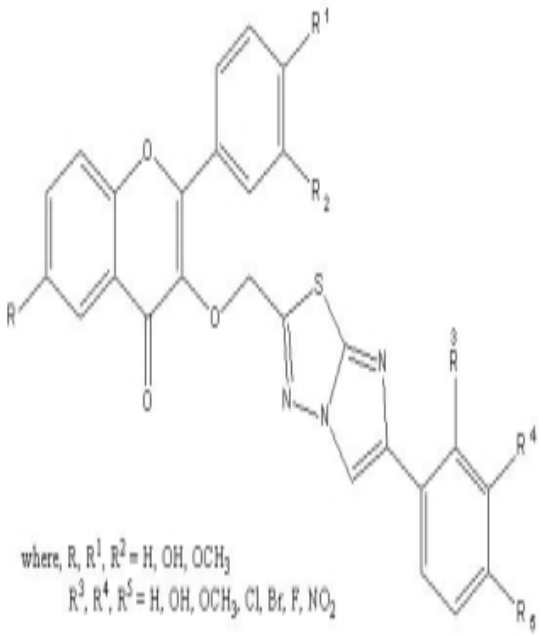
Synthesis of new flavone derivatives against estrogen dependent cancers

Vanrajsinh Jitendrasinh Thakor[1]*, vanraj7777@gmail.com, Jalpa N Kher*[2]*, Bhavini P Atodaria*[1]*, Fenil D Bhayani*[1]*, Malleshappa N Noolvi*[1]*, Jinal J Bharucha*[3]*. (1) Department of Pharmachemistry, Shree Dhanvantary Pharmacy college, Surat, Gujarat 394130, India (2) Department of Biotechnology, Ashok & Rita Patel institute of Biotechnology, New Vallabh Vidyanagar, Gujarat, India, Vallabh Vidyanagar, Anand, Gujarat 388001, India (3) Pharmaceutical Management & Regulatory Affairs, K.B. Institute of Pharmaceutical Education and Research, Gandhinagar, Gujarat 382010, India*

Aromatase and 17-ßHSD inhibitors are main target of pharmacological interest for the treatment of estrogen dependent cancers. Chalcones, Coumarins, Flavones, Isoflavones have been reported for such inhibition and are used for treatment of breast tumors. Flavone derivatives containing Imidathiadiazole, Thiadiazole, Triazole and benzimidazole hetrocycles derivatives are synthesize by using simple laboratory reagents like 2-Hydroxy Acetophenone and 4-Hydroxy Benzaldehyde to convert chalcone leads to formation of Flavones by cyclization using Microwave and followed by attachment of different hetrocycles to form Flavone derivatives and which can be characterize by IR, ^{1}H NMR, ^{13}C NMR spectroscopy and elemental analysis.



where, R, R^1, R^2 = H, OH, OCH_3

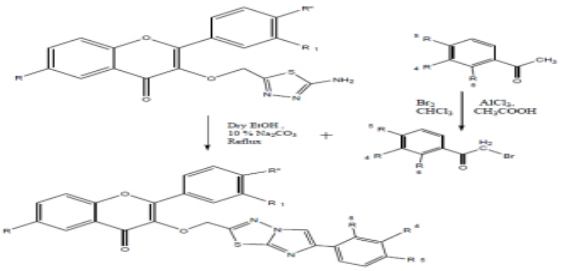


MEDI 134

Synthesis of new flavone, flavanone, and isoflavone derivatives as potential anticancer agents

Vanrajsinh Jitendrasinh Thakor[1], *vanraj7777@gmail.com, Jalpa N Kher*[2], *Fenil D Bhayani*[1], *Jinal J Bharucha*[3], *Malleshappa N Noolvi*[1]. *(1) Department of Pharmaceutical Chemistry, Shree Dhanvantary Pharmacy college, Surat, Gujarat 394130, India (2) Department of Biotechnology, Ashok & Rita Patel institute of Biotechnology, Surat, Gujarat 394120, India (3) Pharmaceutical Management & Regulatory Affairs, K.B. Institute of Pharmaceutical Education & Research, bharuch, Gujarat 392002, India*

Aromatase and 17-ßHSD inhibitors are main target of pharmacological interest for the treatment of estrogen dependent cancers. Coumarins, Flavones, Isoflavones have been reported for such inhibition and are used for treatment of brest tumers. So in this topic, Flavone derivatives containing Imidathiadiazole, Thiadiazole, Triazole and benzimidazole hetrocycles were synthesised by using simple laboratory reagents like 2-Hydroxy Acetophenone and 4-Hydroxy Benzaldehyde to convert chalcone leads to formation of Flavones by cyclazation using Microwave and followed by attachment of different Hetrocycles and charactrarized by IR, ^{1}H NMR, ^{13}c NMR spectroscopy and elemantal analysis. This Flavone derivatives were found to exhibit moderate to high inhibitory activity against Estrogen dependent cancers.



Derivative Id.	R	R^1	$R^{1'}$	R^2	R^3	R^4	R^5	R^6
2g(h)-A/A1/ A2/ A3/A4	H / OCH_3	H / OCH_3	H / OCH_3	H	H	-	-	-
2g(h)-B /B1/ B2 / B3/B4	H / OCH_3	H / OCH_3	H / OCH_3	OCH_3	H	-	-	-
2g(h)-C / C1/ C2 /C3/C4	H / OCH_3	H / OCH_3	H / OCH_3	H	OCH_3	-	-	-
2g(h)-D /D1 /D2/ D3/D4	H / OCH_3	H / OCH_3	H / OCH_3	H	OH	-	-	-
2g(h)-E /E1 /E2 /E3/E4	H / OCH_3	H / OCH_3	H / OCH_3	OCH_3	OH	-	-	-
3g(h)-A / A1/ A2/ A3/A4	H / OCH_3	H / OCH_3	H / OCH_3	-	-	H	H	H
3g(h)-B / B1/ B2 / B3/B4	H / OCH_3	H / OCH^3	H / OCH^3	-	-	OH	H	H
3g(h)-C / C1/ C2 /C3/C4	H / OCH_3	H / OCH_3	H / OCH_3	-	-	H	H	OH
3g(h)-D /D1 /D2/ D3/D4	H / OCH_3	H / OCH_3	H / OCH_3	-	-	H	OH	OH
3g(h)-E / E1/ E2/ E3/E4	H / OCH_3	H / OCH_3	H / OCH_3	-	-	H	Cl	H
3g(h)-F / F1/ F2/ F3/F4	H / OCH_3	H / OCH_3	H / OCH_3	-	-	H	F	H
3g(h)-G / G1/ G2/ G3/G4	H / OCH_3	H / OCH^3	H / OCH^3	-	-	H	Br	H
3g(h)-H / H1/ H2/ H3/H4	H / OCH_3	H / OCH^3	H / OCH^3	-	-	H	OCH_3	H
3g(h)-I / I1/ I2/ I3/I4	H / OCH_3	H / OCH_3	H / OCH_3	-	-	H	NO^2	H

Where, A = R, R^1 = H
A1 = R = H and R^1 = OCH_3
A2 = R = OCH_3 and R^1 = H
A3 = R = OCH_3 and R^1 = OCH_3
A4 = $R^{1'}$ = OCH_3

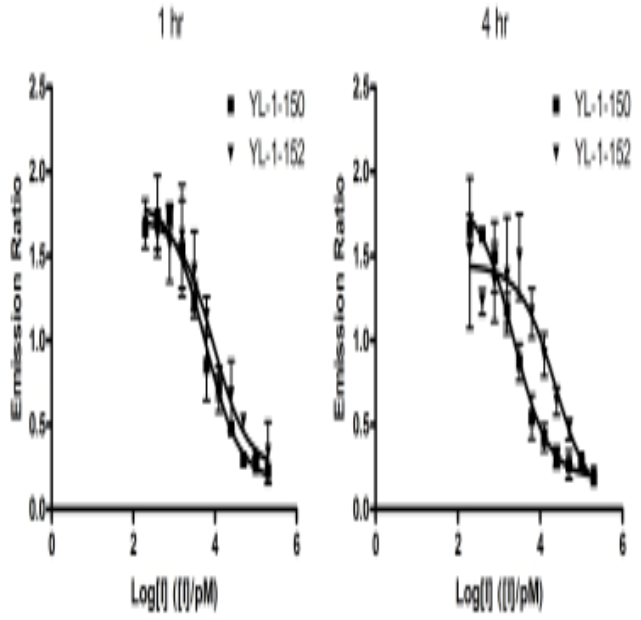
NOTE: i) for 2g and 3g series of derivatives, $R^{1'}$ must be –OH
ii) for 2h and 3h series of derivatives, No –OH group should free at R^1 and $R^{1'}$.

MEDI 135

Development of an irreversible inhibitor of EphB3 kinase

Yangbing Li[1], *ybsli@umich.edu, Frank E. Kwarcinski*[1], *Matthew B. Soellner*[1,2]. *(1) Department of Medicinal Chemistry, Univeristy of Michigan, Ann Arbor, Michigan 48109, United States (2) Department of Chemistry, University of Michigan, Ann Arbor, Michigan 48109, United States*

The Eph receptors have been implicated in variety of human cancers. Most of the current researches on the small molecule inhibitors of Eph receptors are focusing on the inhibition of EphA2, A4, B2 and B4 receptors. EphB3 receptor has been found overexpressed in several cancer cells and tumor tissues, such as pancreatic cancer, non-small-cell lung cancer and rhabdomyosarcoma; but few inhibitors were designed to target it. Here we have developed the first irreversible inhibitor of EphB3 kinase by modification of Dasatinib with a Michael acceptor to generate covalent bonding with the kinase. It has been demonstrated that the irreversible inhibitors are significantly more potent than their reversible counterparts after four-hour incubation and they have shown a time-dependent inhibition with EphB3 kinase.



MEDI 136

WITHDRAWN

MEDI 137

Substituted diarylamine compounds with antineoplastic activity

*Huichao Li, **Aiying Guan**, guanaiying@sinochem.com, Changling Liu. state Key Laboratory of the Discovery and Development of Novel Pesticide, shenyang Research Institute of Chemical Industry, shenyang, liaoning, China*

Cancer, also called malignant tumor, is a serious threat to public health and life. One of the most common types of malignant tumor, lung cancer, is a leading cause of cancer death worldwide. Studies on anticancer drugs never stopped. Recently, a series of substituted diarylamine compounds were discovered with potent antineoplastic activity in our research program. The study was started with a kind of novel substituted diphenylamine compounds, which were synthesized with the aim to discover new fungicides used in agriculture field. To our surprise, some of these compounds showed good antineoplastic activity. Then studies on two directions to increase the anticancer activity were carried out: 1) Replacing one of the phenyl group of diphenylamine with another aryl group such as pyridyl, pyrimidyl, and so on; 2) Replacing both of the phenyl groups with other aryl groups. As a result, a series of titled substituted diarylamine compounds were synthesized. The test results indicated that several *N*-pyridylaniline

compounds showed much better activity against A549 and H460 lung adenocarcinoma cells, T24 and J82 bladder carcinoma cells and LNCap and PC3 prostate cancer cells.

MEDI 138

Natural coumarin and methoxyacrylate hybrids: Anticancer activity, SAR, and mode of action studies

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Cancer is a leading cause of death worldwide, accounting for 8.2 million deaths in 2012 (International Agency for Research on Cancer (IARC)), lung cancer is the most common cause of cancer-related, and is responsible for 1.59 million deaths. Natural coumarin and methoxyacrylate hybrids have a broad-spectrum of fungicidal activity in agriculture field, particularly, coumoxystrobin has already been launched in 2011. In order to find novel structure pharmaceuticals to overcome increasing resistance to currently available anticancer drugs, a serial of coumarin and methoxyacrylate anologues were screened recently, fortunately, some of them were found with good anti tumor activity against human lung cancer cell lines A549, H460 and H520, showing a greatly improvement compared to the developing controls AZD6244, iressa and cisplatin, almost equal to docetaxel. A potent candidate (SYP-333) was identified with promising activity for further development. The primary mechanisms results indicated that SYP-333 has some effect on p21, Akt and Erk in A549 cell line, while there is no significant effect on Stat3. The present work provides strong reference for further development of these coumarin and methoxyacrylate hybrids as potential antitumor agents for the treatment of lung cancer.

MEDI 139

6,8-Disubstituted purines as inhibitors of anaplastic lymphoma kinase (ALK)

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In several human cancer diseases dysregulation of the receptor tyrosine kinase anaplastic lymphoma kinase (ALK) has been detected.[1] For instance, the oncogenic NPM (nucleophosmin) ALK fusion gene is associated with anaplastic large cell lymphoma (ALCL).[2] A similar fusion gene, EML4-ALK, is identified in 5% of all non-small cell lung carcinoma (NSCLC).[3] The first available dual ALK/cMet inhibitor crizotinib was approved by the FDA in 2011 for the treatment of ALK positive NSCLC. A major issue observed during crizotinib treatment is emerging resistance which appears with a median of 10.5 months.[3]The design and development of additional ALK

inhibitors structurally unrelated to crizotinib is a rational concept to overcome such resistances. The poster will present the synthesis, putative binding mode and kinase inhibitory activities of the title compounds as a new class of ALK inhibitors.

References:

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MEDI 140

Targeting the p53-MDM2 interaction: Synthesis of novel chlorofusin analogs

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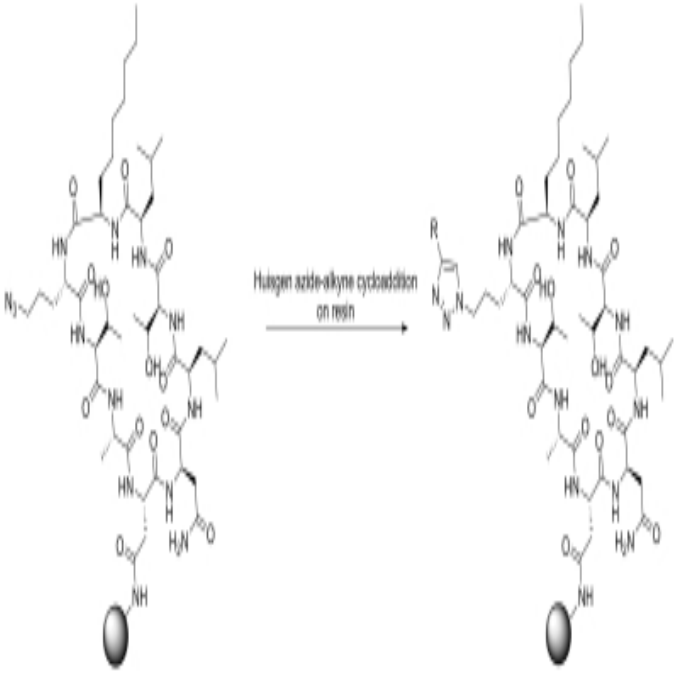

Protein-protein interactions (PPIs) regulate a large number of pathways within cells and are now recognised as potential targets for therapeutic intervention. The p53/MDM2 interaction is a paradigm for PPI inhibition in cancer, with multiple compounds shown to inhibit the interaction and to have an antitumour effect, although no compounds have yet progressed to the clinic. Chlorofusin was the first natural product shown to inhibit the p53-MDM2 interaction. It is a cyclic nonapeptide conjugated to a bicyclic azaphilone through an ornithine side chain. Although the total synthesis of chlorofusin has been achieved and a small number of analogues have been explored, there is still little information with regard to the binding site or mechanism of action of the natural product. The purpose of this research was to produce novel analogues of chlorofusin to further explore the structure-activity relationships associated with MDM2 binding. Using our Fmoc-based solid phase approach to the production of the peptide, an azide was introduced in place of the ornithine amine enabling us to introduce defined, novel conjugation partners to the peptide through click chemistry. The results of the synthesis and biological studies of these compounds will be of interest in the design of new PPI inhibitors targeting p53/MDM2.

MEDI 141

Discovery of quinazolinedione derivatives as potent PARP1 inhibitors

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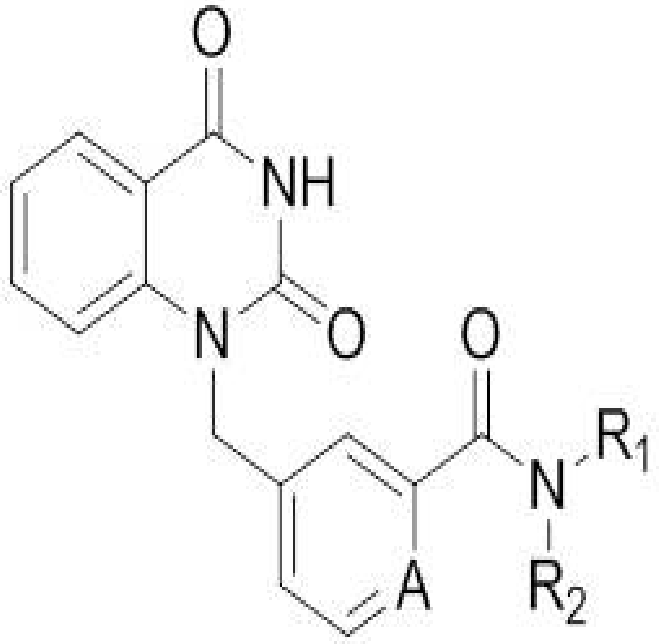
Poly(ADP-ribose) polymerases 1 (PARP1) is the most abundant and well characterized protein of PARP family members, catalyzing the polymerization of poly(ADP-ribose) on target proteins. PARP1 participates in a variety of cellular functions, including chromosome stability, signal transduction and the regulation of gene transcription. Inhibiting PARP1 prevents the repair of damaged DNA, leading to cell death. Small molecule PARP1 inhibitors have showed anti-tumor activity not only in combination with standard chemotherapy, but also in single therapy in the treatment of tumors with DNA repair defect such as breast tumors with BRCA1/2 mutations.

We identified a series of quinazolinedione derivatives as potent PARP1 inhibitors. Our lead compounds have potent in vitro activities, good DMPK profiles and significant in vivo efficacy in SW620 xenograft model. In this presentation, we will discuss synthetic method, the structure activity relationship (SAR) and pharmacological data of this series.



MEDI 142

Growth inhibition of malignant melanoma by glutathione disulfide liposomes

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The incidence of cutaneous malignant melanoma has been rising during the past decade. Once disseminated, malignant melanoma is associated with a very poor prognosis. Despite a large number of clinical trials with various chemotherapeutic agents and biological modifiers, an effective treatment is still lacking. Glutathione disulfide (GSSG) is an endogenous, cell membrane impermeable, oxidized form of glutathione (GSH). We have developed a GSSG cationic liposome formulation that effectively delivers GSSG into cells leading to a 20 fold increase in intracellular GSSG. We found that the GSSG liposomes effectively inhibited the growth of murine B16F10 melanoma cells. An *in vivo* experiment with B16F10 melanoma cells implanted subcutaneously in syngeneic C57BL6 mice demonstrated that the GSSG liposomes effectively inhibited the tumor progression and doubled the survival rate.

MEDI 143

Anti-metastatic effects of glutathione disulfide liposomes

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Cancer metastasis is associated with more than 90% of cancer mortality, hence considered the terminal stage for the patient. Despite extensive research efforts, no effective anti-metastatic drugs are available. Glutathione disulfide (GSSG) is an endogenous and cell membrane impermeable peptide. We have developed a cationic liposome formulation that effectively delivers GSSG into cells leading to a 20 fold increase in intracellular GSSG. Through the use of B16F10 murine melanoma cells, we found that the GSSG liposomes completely prevented key steps involved in cancer metastasis, namely cell detachment and migration. GSSG liposomes also significantly inhibited cell invasion *in vitro*. Through a well-established murine melanoma metastasis model with C57BL6 mice and B16F10 cells, it was shown that GSSG liposomes completely prevented pulmonary metastasis. The results reveal GSSG liposomes could be very effective in the treatment of metastatic cancer.

MEDI 144

Mikania laevigata: Chemical characterization and selective cytotoxic activity of extracts on tumor cell lines

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Cancer is the second major cause of mortality worldwide, losing only to cardiovascular disease. Nowadays, around 50% of antineoplastic drugs were discovered and isolated by indications of plants in folk medicine. In Brazilian flora there are many species of plants which have great therapeutic importance, highlighting the *Mikania laevigata* (Asteraceae) that has been used for their valuable properties, especially in the respiratory tract. In the present study, the compounds of *M. laevigata* extracts were characterized by High Resolution Mass Spectrometry (HRMS) and Gas Chromatography with Mass analysis (GC/MS-EI). Therefore, the presence of some compounds with promising biological properties as antitumor activity was detected. Coumarin (1,2-benzopyrone) was previously reported as responsible for some biological activities of this plant species. Here, the extracts were evaluated by their cytotoxic activity against tumor (Hep-2, HeLa) and non tumor (MRC-5) cell lines, presenting significant inhibitory activity of cell growth in all extracts analyzed, chloroform, ethyl acetate, hexane, ethanol, which is related to its chemical composition. From the four different extracts here tested, two of them, hexane and ethanol, presented a clear selectivity against both tumor cells lines investigated. This can be explained by variances and increase of phenolic compounds in the ethanol fraction and an association of molecules with coumarin found in the hexane fraction.

MEDI 145

MAGMAS inhibition in hepatocellular carcinoma

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Objective : The purpose of our study is to evaluate the efficacy of a MAGMAS inhibitor synthesized from Dr Bhaskar Das, a member of our group, in HCC cell lines HepG2, SNU449 and Huh7.

Research Plan : MAGMAS (mitochondria-associated, granulocyte-macrophage colony stimulating factor signaling molecule) gene has been shown to be overexpressed in certain tumors, with its effect being anti-apoptotic in nature. In addition, we found that MAGMAS protein is expressed in HCC cell lines. We hypothesize that using a novel MAGMAS inhibitor may be effective in hepatocellular carcinoma cell lines.

Methods : HCC cell lines will be treated with MAGMAS inhibitor at various concentrations *in vitro*. For analysis, we will use Vybrant MTT Cell Proliferation Assay Kit at 24 and 48 hours, per manufacturer's protocol. We will test MAGMAS inhibitor effect on apoptosis/necrosis, colony formation and microtubule destabilization in HepG2 as well.

Clinical Relevance : Liver cancer is the third most common cause of cancer-related death in the world, behind lung cancer and gastric cancer. 80-90% of liver cancer is due to hepatocellular carcinoma (HCC). In early stage HCC, a surgical approach, including liver transplant, and/or local hepatic interventions can be quite effective. Unfortunately, a majority relapse within five years, despite these aggressive approaches. Sorafenib, an oral multi-targeted tyrosine kinase inhibitor, is the only FDA-approved drug for advanced HCC. In the landmark SHARP trial, sorafenib proved superior to placebo by 3 months in radiologic progression-free survival and overall survival objectives in patients with HCC with preserved liver function yet cancer too advanced for surgical or local approaches aforementioned. Finding a more effective means of treating advanced HCC is paramount.

MEDI 146

SAR study on novel androgen receptor pan-antagonists

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Prostate cancer (PCa) is the second cause of cancer-related death among the male population of Western society and androgen-deprivation therapy (ADT) represents the first line in PCa treatment. In spite of androgen receptor (AR) expression throughout the various stages of PCa, ADT frequently fails and prostate cancers progress towards the androgen-independent prostate cancer (AIPC) or the hormone-refractory prostate cancer, also known as castration-resistant prostate cancer (CRPC). Clinical evidence suggests that the classical AR antagonists such as Casodex®, also known as bicalutamide, are un-effective for the treatment of advanced prostate cancers.

In this study we will discuss the diastereoselective synthesis of a novel class of bicalutamide-like molecules bearing different molecular fragments at the C2-position that was achieved starting from inexpensive and commercially available starting materials. Molecular modeling studies along with *in vitro* and *in vivo* biological profiling will be provided to demonstrate that small chemical changes in the structure of non-steroidal AR ligands sensibly change the molecular mechanisms underling the pharmacological responses of AR and its mutated forms.

MEDI 147

Novel inhibitor of Notch signaling for the treatment of cancer

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The Notch pathway is a highly conserved signaling system that plays an important role in development and tissue homeostasis. Inhibition of Notch signaling may provide an attractive targeted cancer therapeutic strategy. We have identified and characterized LY3039478, a novel small molecule that is an exquisitely potent inhibitor of Notch-1 intracellular domian (N1ICD) cleavage with an IC_{50} of approximately 1 nM in most of the tumor cell lines tested. In a xenograft tumor model, LY3039478 inhibited N1ICD cleavage and expression of Notch-regulated genes in the tumor microenvironment. To mitigate the mucoid gastroentropathy caused by Notch inhibition, PK/PD data were incorporated in devising dosing strategies that identified an optimal intermittent dosing schedule without negatively impacting efficacy. In summary, we have characterized an orally bio-available small molecule Notch inhibitor that may provide therapeutic benefit to cancer patients with deregulated Notch signaling. LY3039478 is specifically designed to potently inhibit Notch signaling and is being investigated in phase 1.

MEDI 148

C-Jun N-terminal kinase 3 (JNK3) as target for halogen bonding

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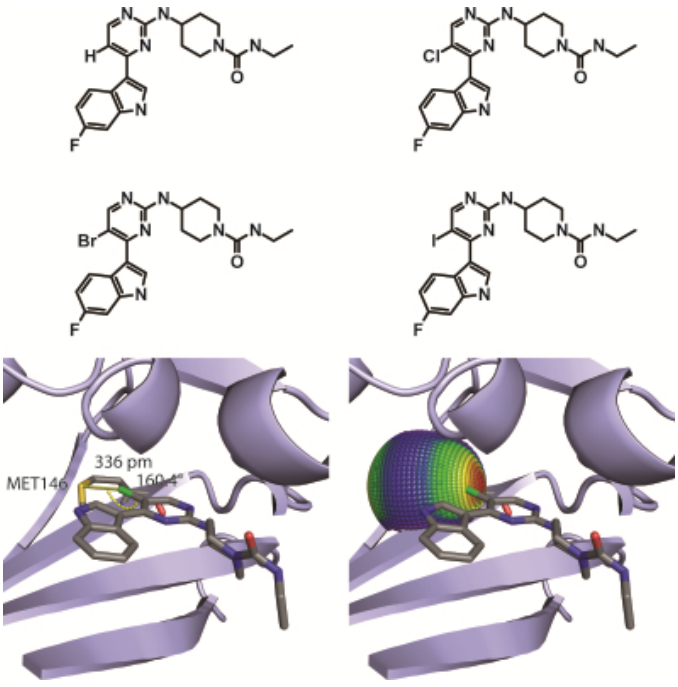
For a broader recognition of halogen bonding in molecular design, we have recently studied halogen bonding contacts with different interaction partners in protein binding sites [1-4]. One of our targets was the accessibility of methionine in the protein binding pocket through halogen bonding [1]. For the experimental prove of this theory we found in the c-Jun N-terminal kinases 3 (JNK3) an interesting target. JNK3 is part of the family of serine/threonine protein kinases and is a member of the mitogen-activated protein kinases (MAPK) family. It is involved in various processes such as neuronal proliferation, differentiation, and programmed cell death. In the binding pocket of JNK3 the methionine 146 (MET146) could be addressed by halogen bonding (PDB 2p33) [5]. Here, chlorine is in a very favorable distance (336 pm) and σ–hole angle (160.4°) to the methionine (see below). In the picture below the iodine interaction sphere is plotted on the methionine, representing a very favorable region for halogen bonding interaction. Our study [1] suggests that an exchange of chlorine with bromine or iodine should be beneficial for the strength of this halogen bond. Experimental studies to test this hypothesisare ongoing.



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5. Alam et al., *Bioorg. Med. Chem. Lett.*, **2007** , *17*, 3463-3467

MEDI 149

Discovery of potent and bioavailable pan-Pim inhibitors for treatment of cancer

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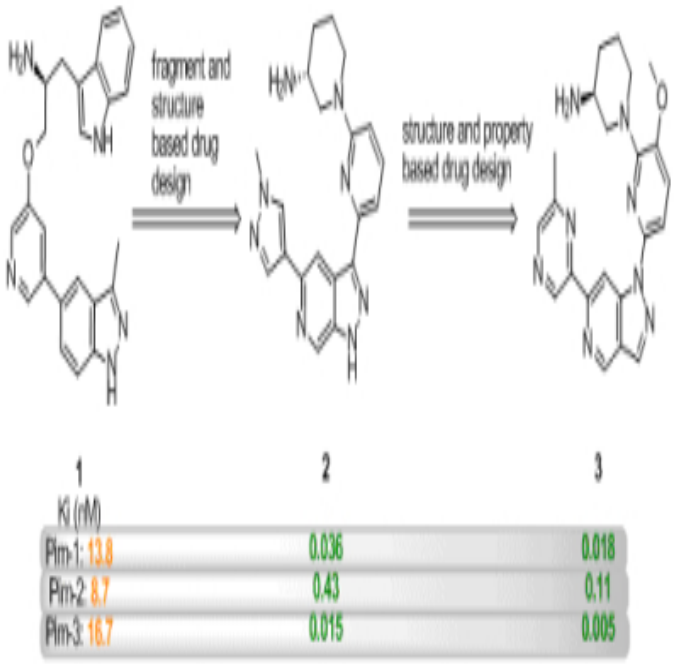

Pim kinases are emerging targets for cancer therapeutics.[1] A number of research organizations are developing Pim inhibitors, and AZD-1208 and LGH447 are the two most advanced experimental drugs currently in phase-1 clinical trials for treatment of hematological and solid tumors.[2] In this poster communication, we report our research efforts in the discovery of potent and bioavailable pan-Pim inhibitors for the treatment of Multiple Myeloma (MM). An X-ray co-crystal structure of an indazole screening hit **1** bound to Pim-1 kinase revealed the key binding interactions within the ATP binding site. Using compound **1** as a template, screening of analogous core fragments identified a 6-azaindazole moiety as a new core for further development. Structure- and fragment-

based drug design led to identification of compound **2** as potent pan-Pim inhibitor with low picomolar biochemical potency on all three Pim kinase isoforms. To improve the bioavailability of compound **2** , compound **3** was discovered which has excellent potency, good oral bioavailability, and improved kinase selectivity. In this presentation, structural activity relationship, syntheses and co-crystal structures of this series will be described.

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MEDI 150

Multicomponent synthesis and in vitro screening of new prodrug derivatives of 5-aminolaevulinic acid for Photodynamic Therapy (PDT)

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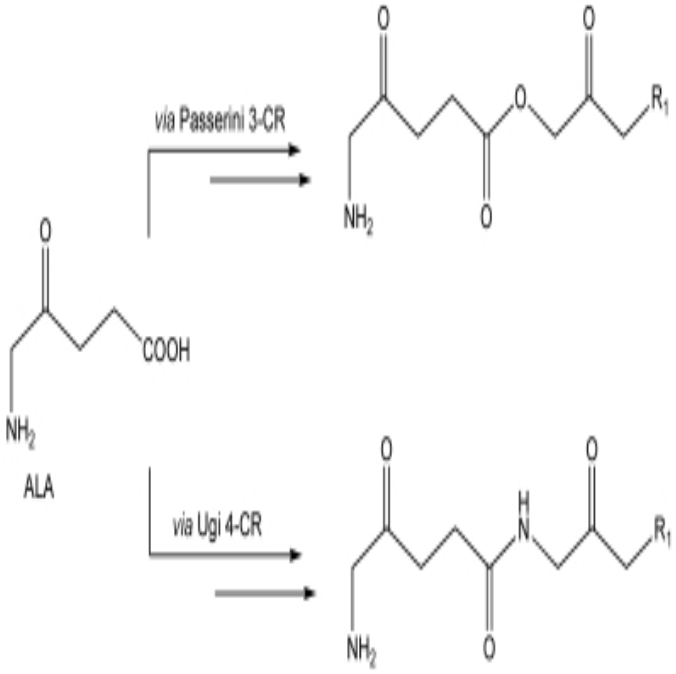
The exogenous administration of 5-aminolaevulinic acid (ALA) is a relatively new approach in PDT since it is a naturally precursor of protoporphyrin IX (PPIX) which is an intermediate in the haem biosynthetic pathway. PDT treatment is carried out using red light to activate the PPIX, resulting in the generation of cytotoxic reactive oxygen species. The exogenous administration of ALA can induce significant intracellular levels of PPIX, which is an effective photosensitiser.

At present, the main clinical PDT application of ALA is the treatment of basal cell carcinomas (BCCs), using topical administration. However, ALAis a zwitterion at physiological pH and therefore has low lipid solubility, which limits its clinical application. More lipophilic ALA prodrugs are expected to cross cellular membranes more easily than ALA itself, resulting both in an enhanced depth of penetration and a shorter topical application time. It has been reported that long chain ALA esters are taken up, desterified, and converted into PPIX with higher efficiency than ALA, leading to higher photosensitiser levels both *in vivo* and *in vitro*.

In this work, the synthetic strategy of multicomponent reactions (Passerini and the Ugi reactions) to achieve new ALA prodrugs, and screening of the new compounds as

potential pro-photosensitizers for PDT of cancer cell lines of different tissues will be shown.



MEDI 151

Synthesis and identification of oxidative metabolites of birinapant/TL32711, a novel smac-mimetic for the treatment of cancer

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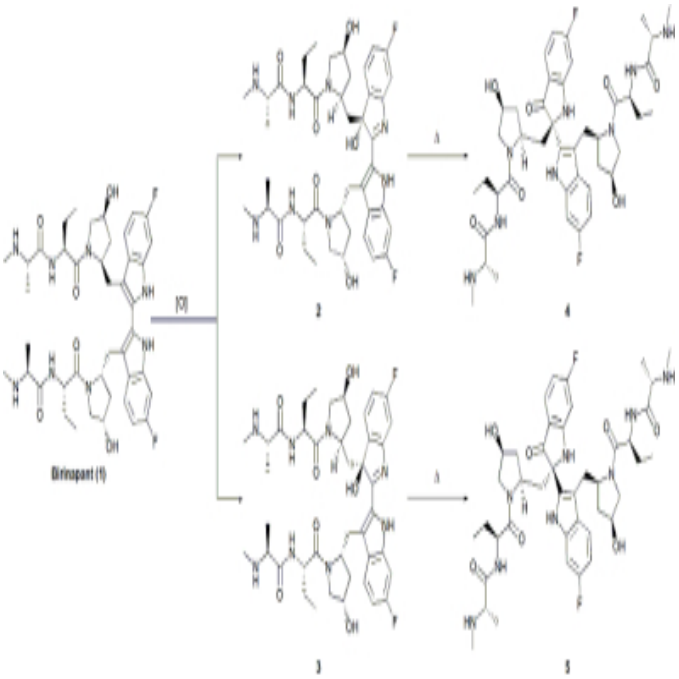
Birinapant/TL32711 (**1**), is a bivalent inhibitor of the inhibitor of apoptosis (IAP) family of proteins and was designed to mimic the *N*-terminal tetrapeptide of the second mitochondria-derived activator of caspases (Smac). Birinapant binds to the BIR3 domains of cIAP1, cIAP2, and XIAP with K_i values of 1 nM, 36 nM and 45 nM, respectively. Birinapant-induced activation of cIAP1 resulted in cIAP1 autoubiquitylation and degradation and correlated with inhibition of TNF-mediated NF-kB activation, tumor cell death *in vitro*, and tumor regression *in vivo*. Birinapant is being evaluated in Phase I/II trials for the treatment of cancer. At accelerated storage conditions, birinapant drug product (1 mg/mL, 50 mM citrate) afforded four degradants in >0.1% abundance by HPLC analysis. The primary degradants (**2** and **3**) formed via initial oxidation of the biindole core, while the secondary degradants (**4** and **5**) arose via rearrangement of **2** and **3** . Under forced degradation, **2** through **4** were prepared on gram-scale. Novel deuterated analogs of **1** were prepared to determine the primary site of oxidation and

NMR experiments confirmed the structure of **4** and **5** . The *de novo* synthesis of **2** through **4** confirmed these experimental findings. This presentation discusses the isolation, identification and synthesis of these degradation products, and presents a mechanistic rationale for their formation. These results were essential to developing the manufacturing process and aided in the selection of alternative drug product presentations.



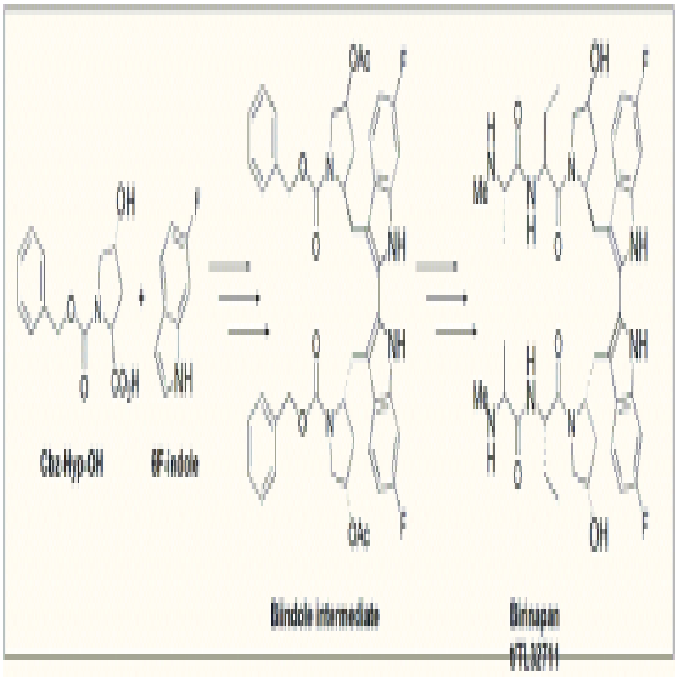
MEDI 152

Process development of birinapant/TL32711: A novel smac-mimetic for the treatment of cancer

Yijun Deng[1]*, yijun.deng@tetralogicpharma.com, Qiuzhe Xie*[2]*, Anna T. A. Chasnoff*[2]*, Seth A. Putrelo*[2]*, Debasis Patra*[2]*, Jun Yan*[2]*, Antonovich S. Robert*[2]*, Arthur J. Cooper*[3]*, Susan R. Rippin*[1]*, Thomas Haimowitz*[1]*, Yu-Hua Lee*[1]*, Matthew G. LaPorte*[1]*, Stephen M. Condon*[1]*. (1) Department of Chemistry, TetraLogic Pharmaceuticals, Malvern, PA 19355, United States (2) Albany Molecular Research, Inc., Albany, NY 12212, United States (3) Ricerca Biosciences, Corcord, OH 44077, United States*

Birinapant/TL32711is a novel bivalent antagonist of the inhibitor of apoptosis (IAP) family of proteins. The IAPs block tumor cell apoptosis (or, programmed cell death) by inhibiting the activation of caspase enzymes. IAP activity is modulated by the second mitochondria-derived activator of caspases (or, Smac). The *N*-terminal tetrapeptide of Smac, i.e., Ala-Val-Pro-Ile (AVPI), binds to the IAPs which allows for caspase activation. Birinapant was thus designed to mimic the AVPI tetrapeptide motif of Smac. Birinapant

treatment induced the rapid degradation of cIAP1, which correlated with inhibition of TNF-mediated NF-kB activation, caspase activation and tumor cell apoptosis *in vitro* and *in vivo*. Birinapant is currently undergoing clinical development for the treatment of both solid and hematological cancers. To support our clinical program, a GMP process for the large-scale synthesis of birinapant was required. The synthesis was divided into two sections: i. conversion of Cbz-Hyp-OH into the biindole-containing intermediate; and, ii. addition of the two requisite dipeptide chains to furnish birinapant API. Using this process, birinapant API has been prepared multiple times under good manufacturing practice (GMP) at >500 g-scale. The evolution of this process from the initial discovery route as well as a detailed discussion of several key observations will be described.



MEDI 153

Identification of intermediates in the stepwise reduction of 1,3-dichloro-6-nitroisoquinoline to 6-aminoisoquinoline

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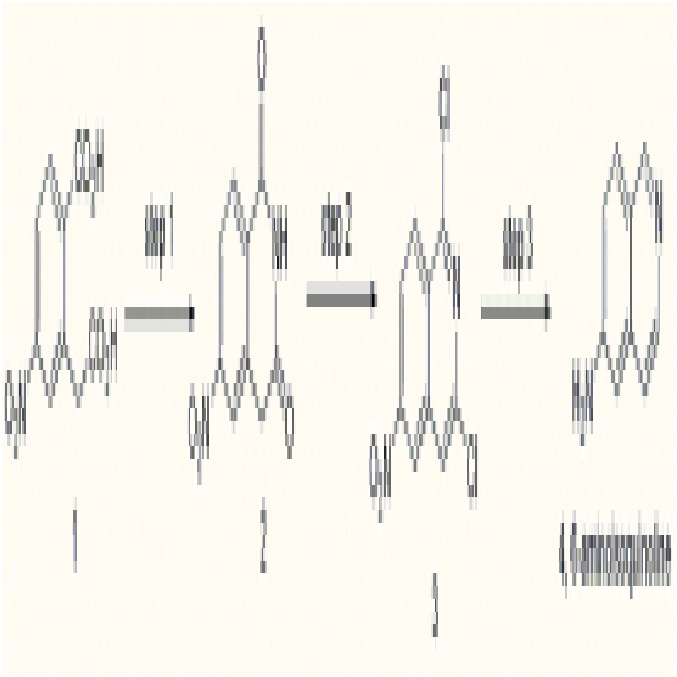
Rho kinase inhibitor (ROCK) AR-13324 lowers the intraocular pressure (IOP) in both human and animal models of glaucoma by increasing the aqueous humor outflow through the trabecular meshwork, by reducing the fluid production and by reducing the episcleral venous pressure (EVP). A key step in the synthesis of AR13324 is coupling the (*S*)-3-(tert-butoxycarbonylamino)-2-(4-((2,4-dimethylbenzoyloxy)

methyl)phenyl)propanoic acid with 6-aminoisoquionline. 6-Aminoisoquinoline, in turn, is prepared in 3 steps from 2-(carboxymethyl)-4-nitrobenzoic acid (1). The final step involves a 4-part reduction of 1,3-dichloro-nitroisoquinoline (3) using Pd/C and either hydrogen or hydrazine. To better understand this synthesis, and to identify the intermediates formed in the reaction towards 6-aminoisoquinoline, this step was studied in detail.



MEDI 154

Thermodynamic properties of membrane-binding peptides

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Drug resistance is a major concern not only for antibiotic drugs but also for anticancer drugs. Instead of targeting specific proteins or signaling pathways, the disruption of the plasma membrane of microbial or cancer cells offers an opportunity for the discovery of new classes of antibacterial or anticancer agents. We present the biophysical characterization of lipid membrane-selective antimicrobial peptides (AMPs) and anticancer peptides (ACPs) [1]. For a deeper understanding of the processes involved we performed a comprehensive experimental study of the binding of natural and computer-generated AMPs and ACPs to lipid vesicles by nano isothermal titration calorimetry (ITC) and liquid nano differential scanning calorimetry (DSC). These investigations were complemented by observations made in other experiments e.g.

membrane disruption assays and atomic force microscopy. The results show that membrane binding of the natural peptides is mostly entropy-driven, with only a small fraction of enthalpic contribution. Most of the designer peptides showed remarkably different thermal signatures, and a completely entropy driven process seems to facilitate binding or even the disruption of the membrane.

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MEDI 155

Design and synthesis of GTx-134, a highly selective dual IGF-1R and insulin receptor (IR) inhibitor

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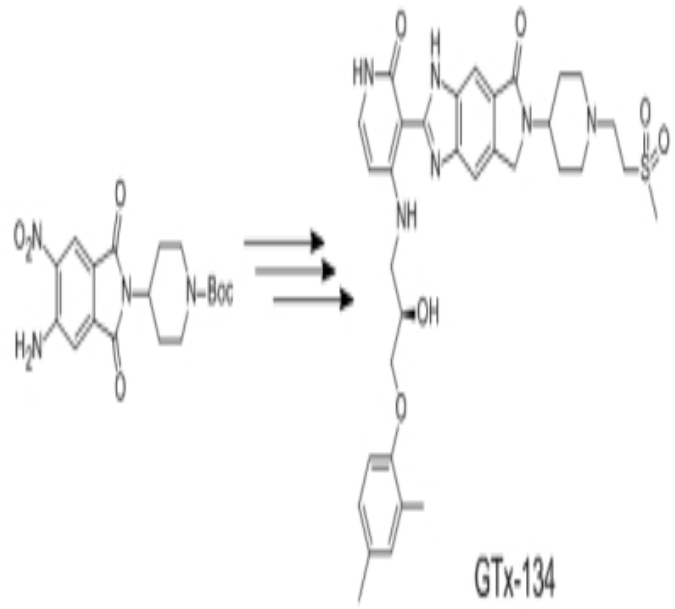
Multiple myeloma (MM) is a cancer of plasma cells, a type of white blood cells normally responsible for producing antibodies.In MM, collections of abnormal plasma cells accumulate in the bone marrow where they receive proliferative, survival, and migratory signals from the bone marrow microenvironment. Insulin-like growth factor 1 receptor (IGF-1R) is a receptor tyrosine kinase (RTK) widely expressed in normal tissues where it functions in growth regulation. Studies showed that IGF-1R stimulates the proliferation and survival of MM cells as well as their migration, adhesion, and invasion. Therefore, inhibition of IGF-1R represents an attractive therapeutic target for MM. We designed and synthesized a novel small-molecule IGF-1R inhibitor GTx-134 on the basis of IGF-1R protein-inhibitor co-crystal X-ray structure and computational modeling. GTx-134, as a dual inhibitor of IGF-1R and insulin receptor (IR), reduces autophosphorylation of its target receptor and inhibits signaling through the PI3k/Akt pathway. GTx-134 induces apoptosis of primary patient MM cells and effectively reduces tumor burden in a HMCL xenografts model.



MEDI 156

Identification of new vitamin D receptor-coregulator inhibitors among nuclear receptor ligands

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The vitamin D receptor is a nuclear hormone receptor that regulates cell proliferation, cell differentiation, calcium homeostasis and immunomodulation. The receptor is activated by the vitamin D metabolite, 1,25-dihydroxyvitamin D_3, which induces a cascade of events including the recruitment of coactivators that activate transcription of specific VDR target genes. Thousands of VDR agonists have been synthesized based on the secosteroid scaffold of 1,25-dihydroxyvitamin D_3. However, most of these ligands are metabolically unstable, have sub-optimal drug-like properties, and induce hypercalcemia *in vivo.* The limited numbers of VDR antagonists reported bear the same secosteroid scaffold and thus exhibit the same problems. In order to identify new more drug-like ligands for VDR, we applied virtual screening with a database of 14330 nuclear receptor ligands and their activities using the online available “Binding Database”. Two different screens were carried out using a stringent and less stringent pharmacophore model to filter ligand conformations. The two pharmacophore models were based on the spatial orientation of the hydroxyl functionalities of VDR's natural

ligands 1,25(OH_2)D_3 and 25(OH_2)D_3. The first screen identified 32 compounds. All but nordihydroguaiaretic acid (NDGA) were VDR ligands, which inhibited the interaction between VDR and coactivator peptide SRC2-3 with an IC50 of 15.8 μM. The second screen identified 162 compounds. Half of these compounds were VDR ligands followed by ERα/b ligands (26%), TRα/b ligands (7%) and LxRα/b ligands (7%). Thus, ligands developed for theses NRs might potentially bind VDR. Among the hit compounds we confirmed the VDR activity of H6036 (ERα ligand) and a homoserine analog of triiodothyronine (TRα ligand).

MEDI 157

Improved process for synthesis of 4-nitro-7-azaindole

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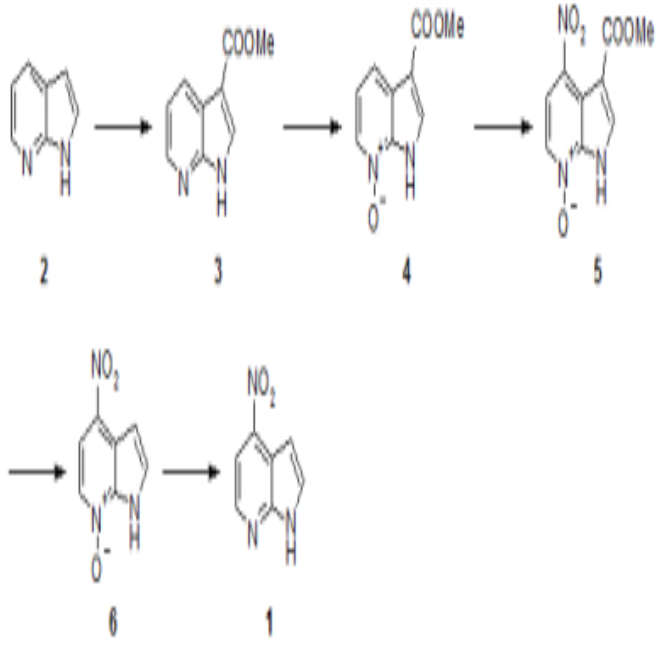
Direct nitration of the corresponding N-oxide of 7-azaindole (**2**) always provides 3- and 4-position regioisomers. Purification of the desired 4-position isomer requires tedious separation which results in a very low yield process. Herein we would like to report a new process toward the synthesis of 4-nitro-7-azaindole (**1**).Starting from 7-azaindole (**2**), > 1 kg of compound **1** can be obtained in a single batch after a five-step sequence, with a total yield of 45-50%.



MEDI 158

Novel synthetic method for key intermediate of sGC stimulators

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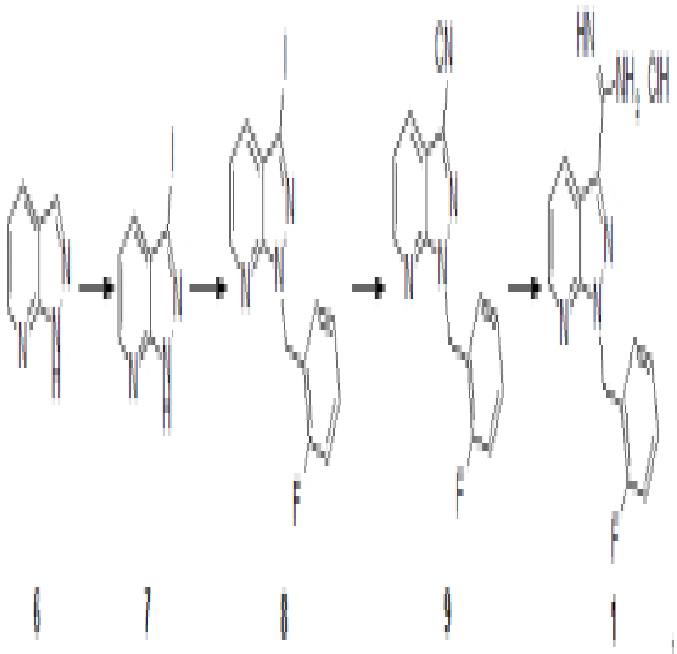
A novel and scalable process a key intermediate **1** for the synthesis of soluble guanylate cyclase (sGC) stimulators was developed. Starting from compound **6,** >1 kg of intermediate **1** was obtained in a single batch via four steps with a total yield of 40-50%.



MEDI 159

Synthesis and regio-selective esterification of Nemorubicin metabolite (PNU-159682), a highly cytotoxic warhead for targeted therapies, and their folate conjugates

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3'-Desamino-3'',4'-anhydro-[2''(S)-methoxy-3''(R)-oxy-4''-morpholinyl]doxorubicin (PNU-159682) is a major metabolite of nemorubicin (methoxymorpholinyl doxorubicin; MMDX) was isolated from an incubate of NADPH and liver microsomes from dexamethasone-induced male rats. PNU-159682 is remarkably more cytotoxic (>3000 fold) than MMDX and doxorubicin *in vitro* and was effective in the two *in vivo*tumor models tested, i.e.,

disseminated murine L1210 leukemia and MX-1 human mammary carcinoma xenografts. The clinical application of this anthracycline drug is, however, limited by its toxic side effects. Biotransformation of MMDX to PNU-159682, was achieved by CYP3A4, the major human cytochrome P450s (CYP) in human liver. However this process will provide only small quantities of PNU-159682 for further studies. Selective targeting of receptors over expressed on pathologic cells with ligand-drug conjugates provides an opportunity to reduce toxicity to normal cells. In this poster we present the design and synthesis of PNU-159682 folate conjugates (Fig.1) targeting folate receptor (FR) positive pathologic cells. Furthermore, we report the synthesis of a PNU-159682 esters utilizing regio-selective esterification of primary alcohol in the presence of phenol using pyridne-triazole acylating reagents.

MEDI 160

Regioselective synthesis of monoacylated cytarabine monopropionate by using fungi whole-cell biocatalyst in nonaqueous medium

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The freeze-dried *Aspergillus oryzae* cells was employed as an new type of biocatalyst for highly 3′-regioselective modification of a polar antitumor drug, 1-β-D-arabinofuranosylcytosinecytarabine (ara-C). The organic solvents showed an evident influence on the reaction catalyzed by the *A. oryzae* whole-cells. Except for hexane-

pyridine, the catalytic activity and regioslectivity of the whole-cells clearly increased with increasing the polarity of the hydrophobic organic solvents used. Among all the tested pure and binary solvents, the best results were observed in isopropyl ether (IPE)-pyridine system, in which the catalyst also showed good thermal stabilities. For the biocataylsis in IPE-pyridine, the optimal IPE concentration, VP/ara-C ratio, biocatalyst dosage, reaction temperature and shaking speed were 30% (v/v), 90, 60 mg/mL, 30 °C and 140-180 rpm respectively, under which the initial rate, yield and 3′–regioslectivity were 10.7 mmol/L·h, 88.3% and 70%, respectively. The fungus whole-cells also had environmental and cost advantages, which made them a promising alternative to the expensive free enzymes for modification of nucleoside drugs with high polarity.

MEDI 161

Synthesis of highly potent tubulysin analogs as payloads for targeting therapies of cancer

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Tubulysins are natural products isolated from myxobacterial species. They are potent mitotic poisons as they inhibit the polymerization of tubulin into microtubules. Structurally, tubulysins are linear tetrapeptides comprised of *N*-methyl pipecolic acid (Mep), isoleucine (Ile), tubuvaline (Tuv), and tubutyrosine (Tut). All isolated tubulysins possess an acid-, base-, and enzyme-sensitive *N*-acyloxymethyl substituent, which is essential for their potent cytotoxicity. Herein, we present the design and synthesis of more stable tubulysin analogs based on our reported synthesis of tubulysin B. These highly potent tubulysin analogs are less prone to degradation under acidic/basic conditions or by esterases, thus, making them easier to be developed as payloads for targeted therapy. Structural features, essential for the cytotoxicity of the tubulysin analogs, were established. Folate conjugates of these tubulysin analogs were shown to exhibit remarkable cytotoxicity against (FR)-positive KB cells.

MEDI 162

Imidazo[4,5-b]pyrazines as thiadiazole amide isosteres in the 5H-chromeno[2,3-b]pyridine (azaxanthene) series of glucocorticoid receptor(GR) agonists

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A series of Glucocorticoid Receptor(GR) ligands utilizing imidazo[4,5-b]pyrazine isosteres for acylaminothiadiazoles in the azaxanthene series is reported. These partial agonists (2-27) retain not only high GR affinity and selectivity (e.g., over progesterone receptor), but also display an improved pharmacokinetic profile over the lead thiadiazole amide (compound 1). SAR and general methods for synthesis are described.

MEDI 163

Synthesis, anticancer activities, and molecular docking studies of peptides and iso peptides

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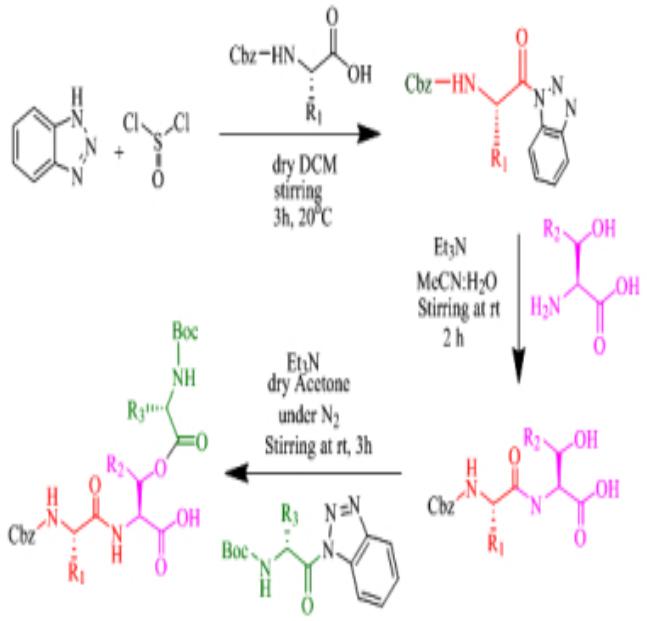
Chiral peptides and *iso*-peptides were synthesized in excellent yield in Benzotriazole mediated solution phase synthesis. Benzotriazole acted both as activating and leaving group, eliminating frequent use of protection and subsequent deportation. The procedure was based on the hypothesis that epimerization should be suppressed in solution due to faster coupling rate which is usual in case of SPPS.[1]

All the synthesized peptides showed compliance with Lipinski's Ro5 despite one violation in case of number of rotatable bonds. Inhibition of cell proliferation of cancer cell lines is one of the most commonly used methods to study the effectiveness of any anticancer agents. Synthesized peptides and *iso*-peptides were tested against three cancer cell lines (MCF-7, MDA-MB 231) to determine their anti-proliferative potential. NFkB was also determined. Molecular docking studies were also carried out to complement the experimental results.



Scheme: General scheme forsynthesis of *iso* peptide

References

1. Sohma, Y.; Yoshiya, T.; Taniguchi, A.; Kimura, T.; Hayashi, Y.; Kiso, Y. Development of O-acyl isopeptide method. *Biopolymers. Pept. Sci.* **2007** , *88*, 253-262.

MEDI 164

Screening, synthesis, and development of macrocyclic XIAP antagonists with drug like properties

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Ensemble Therapeutics has utilized its proprietary DNA-Programed Chemistry (DPC) platform for synthesis and screening of combinatorial macrocyclic libraries against a wide variety of protein-protein interaction (PPI) targets. Employing this platform, we have been able to find multiple hits and SAR information for numerous PPI targets. Despite being outside conventional small molecule Ro5 space, the macrocycles can be drug-like with properties including cell membrane penetration and oral bioavailability. Through library screening and medicinal chemistry effort we have developed several series of macrocyclic inhibitors of the BIR2 and BIR3 domains of XIAP. Inhibiting the sequestration of pro-apoptotic caspases by BIR2 and BIR3 induces apoptosis

selectively in cancer cell lines. After optimization for drug like ADME properties these macrocyclic inhibitors have shown efficacy in *in vitro* and in vivo models.

MEDI 165

Discovery of pyrazolo[1,5-a]pyrimidin-7-amine derivatives as mTOR kinase domain inhibitors: Hit to lead optimization

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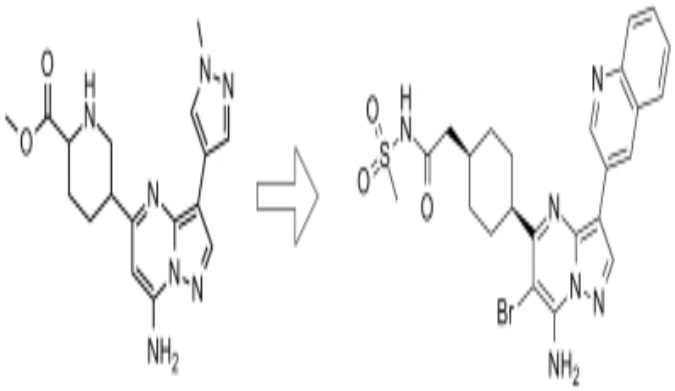
The mammalian target of rapamycin (mTOR) is a central regulator of cell growth and proliferation and plays a gate keeper role in the control of cell cycle progression and mediates mitogenic signals from PI3K/AKT through to the downstream target S6K1 and 4E-BP1 and to Ser 473 on AKT. In cancer, mTOR is frequently hyper-activated and is a clinically validated target of therapy. In our high-through-put screening campaign, we have identified several ATP-competitive mTOR inhibitors with different chemical structures. The initial evaluation revealed that pyrazolo[1,5-a]pyrimidin-7-amine derivative (**cpd-1**) has good PI3Kα selectivity and was selected as the major focus as selective mTOR inhibitors may be better tolerated. With the aide of computational modeling, the subsequent hit to lead optimization efforts improved the both enzyme and cellular potency. **Cpd-2** selected for in vivo study demonstrated good efficacy in cancer Xenograft models in nude mice.



Cpd-1
mTOR IC50 = 816 nM
PI3K IC50 > 3000 nM
pAKT IC50 > 1000 nM
pS6K IC50 > 1000 nM

Cpd-2
mTOR IC50 = 1 nM
PI3Kα IC50 = 2452 nM
IC50 (pAKT) = 23 nM
IC50 (pS6K) = 45 nM

The syntheses and structure-activity relationship of the representative compounds will also be presented.

MEDI 166

Synthesis of conjugates of a cell penetrating cyclic peptide with paclitaxel and camptothecin

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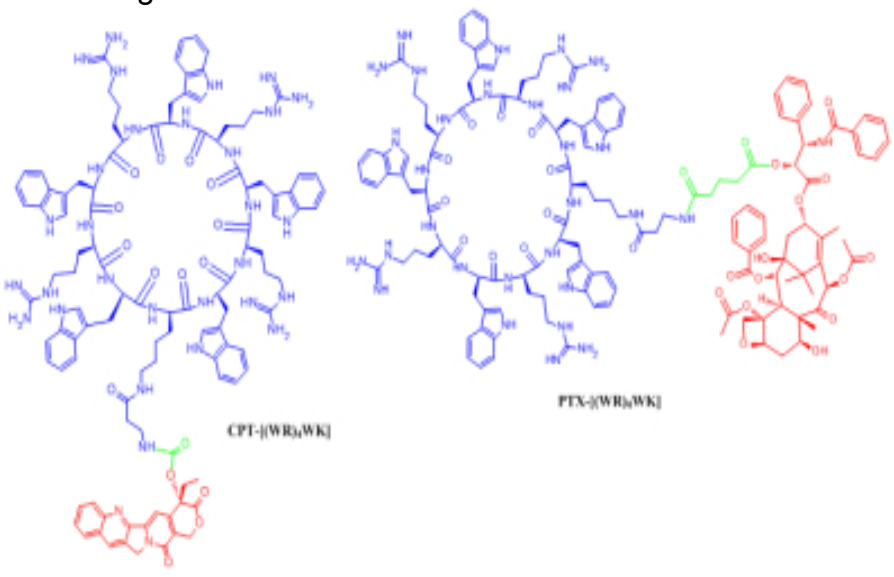
Despite the broad spectrum antitumor activity of paclitaxel (PTX) and camptothecin (CPT), their clinical applications have been hampered by their limited cellular permeability, lack of tumor selectivity, and/or hydrophobicity. Therefore, several strategies have been employed to improve PTX and CPT solubility, cellular delivery, and selectivity to overcome their clinical limitations. We have previously shown that amphiphilic cell-penetrating cyclic peptide [$(WR)_4WK$] is an efficient molecular transporter for doxorubicin. We investigated whether [$(WR)_4WK$] can act as a molecular transporter for other hydrophobic drugs, such as PTX and CPT. First, PTX was esterified at 2¢-OH position through reaction with glutaric anhydride. PTX-glutarate was then conjugated with [$(WR)_4WK$]. CPT was modified at C(20)-OH, by reaction with triphosgene, followed by peptide conjugation. Conjugation reactions with [$(WR)_4WK$] was carried out in the presence of PyBOP and HOBt/DIPEA to yield PTX-[$(WR)_4WK$] and CPT-[$(WR)_4WK$] conjugates. The final products were purified by reverse phase HPLC. The anticancer activity of drug-peptide conjugates and their cellular uptake will be investigated.



MEDI 167

Largazole analogs as selective histone deacetylase inhibitors

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Largazole is a selective anticancer agent isolated form a marine organism. It is a histone deacetylase (HDAC) inhibitor and can be used for epigenetic regulation of gene expression to develop anticancer drugs. HDAC inhibitors currently in clinical use suffer from undesirable side effects as they lack isoform selectivity. Isoform selective HDAC inhibitors are being developed to reduce these undesirable side effects. The depsipeptide ring of largazole interacts with the less conserved hydrophobic rim of the HDAC active site and can be targeted for developing isoform selective HDAC inhibitors. We have designed and synthesized largazole analogues by structurally altering its depsipeptide ring. The synthesis and biological activity of these novel largazole analogues will be presented.

MEDI 168

Trapping liquid drugs inside crystals

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The objective of this work is to find a systematic way to embed liquid or volatile drugs inside crystalline materials, with the multiple aims of stabilizing them, of tuning their possible ways of delivery in medicine, and to explore new regulatory and intellectual properties issues. Liquid or volatile formulations of active pharmaceutical ingredients (APIs) are intrinsically less stable and durable than solid forms; in fact most drugs are formulated as solid dosage because they tend to be stable, reproducible, and amenable to purification. Most drugs are manufactured and distributed as crystalline materials, and their action involves the delivery of the active molecule by a solubilization process either in the body or on the environment. The poor solubility of pharmaceutical active ingredients (API) is a problem often encountered in their formulation since these phenomena limit the bioavailability of the API. However some important compounds for the human health occur as liquids at room temperature.

The formation of co-crystals has been demonstrated as a means of tuning solubility properties of solid phases, and therefore it is widely investigated in the fields of pharmaceuticals. In spite of this extremely high interest towards co-crystallization as a tool to alter solubility, practically no emphasis has been paid to using it as a means to stabilize volatile or labile or low-melting products. In this work we trap and stabilize volatile and liquid APIs in crystalline matrices by engineering suitable co-crystals. These

new materials alter the physic state of the active ingredients allowing to expand the phase space accessible to manufacturing and delivery.

We have defined a benchmark of molecules relevant to human health that have been combined with suitable partners according to the well known methods of crystal engineering in order to obtain cocrystals. The first successful results will be discussed.

MEDI 169

BioSens-All™: Anew technology dedicated to GPCR biased ligand drug discovery

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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, novel strategies consisting in developing allosteric modulators or biased ligands (BLs) are now emerging. Indeed, all GPCR agonists do not always uniformly activate all signalling pathways mediated by a given receptor. BLs therefore constitute a novel approach to more selectively activate a receptor for the development of safer drug candidates. This concept of ligand bias, also referred to as functional selectivity, has now been validated *in vitro* and *in vivo*, and several GPCR BLs are currently under clinical investigations.

Despite these promising features, uncovering and profiling BLs still remains challenging as no specific technology has been proposed for this task. Cell-system dependency of the multiple functional analyses for instance makes it difficult to guide medicinal chemists in their optimization programs toward active BLs. To meet this expectation, the dedicated BioSens-All™ technology was generated. BioSens-All™ consists in a set of multiple biosensors each engineered to follow one specific functional pathway. The parallel characterization of GPCR orthosteric or allosteric ligands with theses biosensors, in a homogenous format, enables the generation of signalling signatures and consistent comparative data for chemists.

This poster will present and exemplify how BioSens-All™ can be used to support medicinal chemists in their quest for GPCR biased ligands.

MEDI 170

Quantification of proteins using $^{13}C_7$-labeled and unlabeled iodoacetanilide by nano liquid chromatography/nanoelectrospray ionization and by selected reaction monitoring mass spectrometry

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Quantitative analysis of proteins is essential for modern proteomics. We have thus far reported methodologies for the relative quantitative analysis of proteins and peptides with the use of combinations of stable isotope-labeled and unlabeled small organic molecules that react with specific amino acid residues combined with soft ionization mass spectrometry, such as matrix-assisted laser dissorption/ionization (MALDI) or electrospray ionization (ESI) by a so-called “shotgun approach.” Here, the combination of the cysteine-specific modifiers we reported previously, iodoacetanilide (IAA) and $^{13}C_7$-labeled iodoacetanilide ($^{13}C_7$-IAA), was applied to the absolute quantification of proteins. The selected reaction monitoring (SRM) with the use of nano liquid chromatography/nanoelectrospray ionization ion trap mass spectrometry (nano LC/nano-ESI-IT-MS) analysis was applied to the precise quantification of three commercial proteins. Good correlation was observed between the theoretical ratios and observed ratios for all these proteins. Due to efficient tagging, this method does not require separate synthesis of isotope-labeled peptides for the SRM studies unlike most other existing SRM studies. This method was also found to be effective for precise quantification of proteins even in a complex protein environment, such as in the clinical sample of nipple discharges. Therefore, this method is expected to be a useful tool for proteomics research.

MEDI 171

Accelerating the transition from drug discovery to FIH studies: Case examples in discovery stage reaction and route optimization

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Organic synthesis occupies a central role in medicinal chemistry research and is often the rate limiting step on the path to first in human (FIH) studies. In this context, synthesis enables the delivery of API across the drug discovery continuum from initial *in vitro* assays through *in vivo* profiling, toxicological safety studies, and finally the FIH milestone. While the existence of synthetic bottlenecks can hinder progression towards FIH, the application of a focused reaction optimization effort within the Discovery framework, with guidance from Process R&D, can remove these bottlenecks to facilitate production of bulk API for early toxicological studies through FIH. This presentation will cover in detail reaction optimization efforts undertaken at Pfizer for a number of projects within Neuroscience Medicinal Chemistry with the aim of developing scaleable routes to facilitate the transition to regulatory bulk synthesis.

MEDI 172

Lidocaine-ibuprofen ionic liquid for dermal anesthesia

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Local anesthesia in the skin occurs at least one hour after application of a commercial topical formulation of lidocaine and prilocaine prepared as a eutectic mixture. In this study, we prepared a lidocaine-ibuprofen ionic liquid and found that local anesthesia of skin was achieved within 15 min in rats with no apparent adverse effects to the skin. The preparation of an ionic liquid was verified by NMR, calorimetry and electrical conductivity. Addition of even a small amount of DI water appeared to cause dissociation of the ionic liquid into dissolved ions, as shown by an increase in electrical conductivity. We believe that the lidocaine-ibuprofen ionic liquid increased lidocaine absorption into the skin due to the high lidocaine concentration in the liquid and due to possible interactions between the ionic liquid and the skin to increase skin permeability. These findings suggest that ionic liquids may provide a novel approach to transdermal drug delivery.

MEDI 173

MOE in education: Problem-based learning in medicinal chemistry

***Petrina Kamya**, pkamya@chemcomp.com. Chemical Computing Group, Montreal, QC H3A2R7, Canada*

Problem-Based Learning (PBL) is an pedagogical method which incorporates hands-on, active learning centered on the investigation and resolution of difficult, real-world problems. Some of the defining characteristics of PBL include: 1. A guided learning process with challenging open-ended problems where there are multiple solutions and 2.An environment where students work as self-directed, active investigators and problem-solvers. Here we demonstrate the effectiveness of the Molecular Operating Environment (MOE) in a PBL setting to teaching students about the advantages and limitations of the modeling tools that are used in the forefront of early stage drug design.

MEDI 174

Synthesis of [^{11}C]GSK1482160 as a potential PET agent for imaging of P2X$_7$ receptor

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$P2X_7$ is an adenosine triphosphate (ATP)-gated ion-channel, which is found in the immune and central nervous systems, implicated in ATP-mediated cell death, regulation of receptor trafficking and inflammation, and associated with cancer, neurological and cardiovascular disorders. GSK1482160 is a potent $P2X_7$ antagonist with excellent in vivo activity in animal models of pain, developed by GlaxoSmithKline. $P2X_7$ is an attractive therapeutic target as well as imaging target. Here we report the synthesis of [^{11}C]GSK1482160 as a candidate radioligand for non-invasive evaluation of neuroinflammation using biomedical imaging technique positron emission tomography (PET). The reference standard GSK1482160 ((*S*)-*N*-(2-chloro-3-(trifluoromethyl)benzyl)-1-methyl-5-oxopyrrolidine-2-carboxamide) was synthesized from L-pyroglutamic acid or methyl L-pyroglutamate with 2-chloro-3-(trifluoromethyl)benzylamine in 3 steps with 21% overall chemical yield, using the modifications of the published methods by Abdi et al. The desmethyl precursor (*S*)-*N*-(2-chloro-3-(trifluoromethyl)benzyl)-5-oxopyrrolidine-2-carboxamide was prepared from L-pyroglutamic acid with 2-chloro-3-(trifluoromethyl)benzylamine in 83% chemical yield. The target tracer [^{11}C]GSK1482160 ((*S*)-*N*-(2-chloro-3-(trifluoromethyl)benzyl)-1-[^{11}C]methyl-5-oxopyrrolidine-2-carboxamide) was prepared from the desmethyl precursor with [^{11}C]CH_3OTf through *N*-[^{11}C]methylation under basic condition (NaH) and isolated by HPLC combined with solid-phase extraction (SPE) in 50-60% radiochemical yield, based on [^{11}C]CO_2 and decay corrected to end of bombardment (EOB), with 370-740 GBq/μmol specific activity at EOB.

MEDI 175

Electrochemical investigation and analytical determination of Hg in artificial saliva

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In the present study, a reliable and rapid electroanalytical method using anodic stripping voltammetry with macro- and microelectrodes for the determination of Hg in synthetic saliva matrix is developed.

Anodic stripping method is one of the sensitive methods for the detection of trace metals in human fluids. Hg is highly toxic metal and its accurate determination in the human body is extremely beneficial. Conventionally the sample is collected invasively as blood for trace element detection, yet in the present method, saliva as a non-invasive technique is used as a sample. The analysis is done on the synthetic saliva which has analogous characteristics of the natural saliva. This synthetic saliva is spiked with the targeted metal (Hg). Anodic stripping voltammetry is used to check the concentration of the added trace metals in the synthetic saliva.

Various electrode materials, e.g. graphite, gold and platinum, are tested, and various electrochemical parameters of the detection of Hg are optimized and will be presented.

The developed electroanalytical method can be used for simultaneous multi-element detection in synthetic saliva.

Acknowledgment

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MEDI 176

Exploring the duality of halogens as participants in non-bonded interactions during lead optimization

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Identification and rationalization of non-standard interactions presents many challenges during optimization of lead drug candidates. The typical approach of using SMARTS patterns to handle CH...O interactions is impractical as it requires a large set of patterns. An additional degree of difficulty is introduced when considering moieties such as halogen bonds, proton-p interactions, sulfur-aromatic and the like. In order to address these challenges, Extended Hückel Theory (EHT) is used to handle and account for the fundamentals of electron-withdrawal effects in relation to non-standard and hydrogen bond interactions. The EHT approach to modeling non-standard non-bonded interactions uses partial charges and bond orders from extended Huckel theory (EHT) to derive interaction strength parameters. Furthermore, it accounts for resonance and electron withdrawal effects in a true QM fashion, circumventing the need to encode subtle electronic differences with a myriad of SMARTS matches and atom types, which is typically used in most force-field calculations. Applying the EHT method is especially useful when dealing with CH donors, whose strengths are greatly influenced by local resonance and electron withdrawal. The Huckel strength parameters are interesting by themselves as input to QSPR equations, as they correlate well with physio-chemical properties such as solvation energy and pKa. Given that the EHT based non-bonded interaction model is a molecular orbital-based Lewis Acid/Lewis Base approach, it naturally extends to all manner of non-bonded interactions - CH donors, sulfur-O contacts, cation-pi, and H-pi interactions - and contrasts point-charge based methods, which cannot deal with the anisotropic charge distributions that give rise to many non-standard interactions. Here we also demonstrate the quantitative accuracy of our model, by showing how our computed halogen-bond energies correlate well with experimental activity differences in a congeneric series of halogenated ligands. The ability to better quantify non-standard non-bonded interaction strengths provides the opportunity to better predict and assess these interactions for explaining existing SAR, and to exploit these interactions in ligand design. Current non-standard interaction models do not consider the halogen bond interaction, and instead see it as a VDW clash. Using such models would be detrimental and potentially misleading in situations where the halogen bond contributes to ligand binding affinity. Our new framework for non-standard

interactions is a step towards their inclusion in SBDD projects and in the medicinal and computational chemistry arsenal of modeling applications.

MEDI 177

Free tools for molecular docking and ligand discovery: Recent developments

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This poster describes recent developments in our databases and sofware that we make available via our websites. 1) ZINC - a free public resource for ligand discovery. 2) UCSF DOCK and DOCK Blaster, a molecular docking program and a publically accessible interface to docking. We welcome users of these tools as well as prospective users to drop by the poster to see what is new and hear more about recent developments.

MEDI 178

Electrochemistry-HPLC-ESI-MS in mimicry of oxidative drug metabolism

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Bio-activation of drugs leading to reactive metabolites is now the major impeding factor in drug development. This has led to drug attrition as well as late stage compound failure during drug development. Idiosyncratic drug toxicities due to reactive metabolites have even resulted in withdrawal of drugs that had been on the market such as troglitazone and amodiaquine. With the emergence of a large pool of lead compounds from computational chemistry and synthetic organic chemists, early drug screening is necessary in order to weed out unsuitable compounds for further development. This also means efficient, cheap and fast techniques are being sought. In our lab we have started using electrochemistry coupled to HPLC and Mass spectrometer (EC-HPLC-MS/MS) as a tool for mimicry of oxidative metabolism and subsequent high throughput screening of metabolites. The ability of electrochemical techniques to mimic some of both Phase I and Phase II oxidation reactions that are catalyzed by CYP 450 enzymes makes it a suitable and complementary technique for *in vitro* experiments during drug discovery and development. The absence and hence non- interference of biological matrix which characterize the hepatocellular fractions makes EC-HPLC-MS/MS a convenient tool for screening those reactive metabolites that bind to hepatocellular matrix possible. In this paper we demonstrate the utility of EC-MS/MS, in oxidation of different drugs. By definition, reactive metabolites have a very short life-time. These short-lived metabolites were trapped with glutathione or methoxyalamine and the stable adducts obtained were analyzed either directly online as well as offline using ESI-MS.

Our results showed that most oxidation processes that are initiated by one electron transfer and hydrogen abstraction can be mimicked using the above method. Although slight variations were observed, most metabolites generated using EC-MS were comparable to those from perfused organs *in vitro*.

Key words electrochemistry, oxidation, metabolites,

MEDI 179

Development of non-peptide pyrazole-based proteasome inhibitors as anticancer agents

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The proteasome inhibitors bortezomib and carfilzomib have effectively revolutionized the treatment of multiple myeloma over the past decade. Unfortunately, despite many preclinical indications of activity and multiple clinical trials, these first-generation agents have proven relatively ineffective in solid cancers. We hypothesize that this poor *in vivo* activity stems from multiple limitations of bortezomib and carfilzomib including rapid metabolism, susceptibility to efflux transporters, and undesired off-target activities. We further believe that these limitations stem from common structural and functional characteristics: peptide-based structures and the use of reactive electrophilic warheads to mediate inhibition. Accordingly, we sought to develop novel proteasome inhibitors which were non-peptide in structure and did not rely on covalent bonding to mediate proteasome inhibition. We began with a thorough *in silico* screen of more than 340,000 structures and via *in vitro* screening we identified a 1,3,5-substituted pyrazole compound with potent *in vitro* activity and potent cytotoxic activity *in cellulo*. We have since explored the structure-activity relationship of this scaffold through the synthesis and testing of over 40 analogs and we have demonstrated that our lead compound is active *in vivo* in a mouse xenograft model of prostate cancer with no observed toxicity. We have also shown that these compounds act as reversible inhibitors and appear to be highly metabolically stable *in vitro*. Further preclinical development including experiments to validate the proteasome as the biological target of these inhibitors and efforts to identify any potential off-target activities of our lead compound are currently under way. We are also working to develop inhibitors with greater potency and improved physiochemical properties in the hopes of creating non-peptide proteasome inhibitors suitable for use in humans. It is our belief that the development of such a proteasome inhibitor with the correct pharmacokinetic and pharmacodynamics properties could prove to be a highly useful cancer therapy.

MEDI 180

LC/MS-guided investigation of natural products chemistry: A case study of African medicinal plants

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Natural products have historically been the most consistent source of lead compounds in drug discovery, but their utilization by the pharmaceutical industry has declined in recent years. This is largely because most of the "low-hanging fruit" has already been metaphorically picked and it has become more and more difficult to identify unique compounds. In recent decades, wide application of LC/MS technique enabled us to pre-screen the chemical profile of the matrices and identify the materials containing unique molecules with pharmaceutical interest prior to the time-consuming process of isolation and elucidation. In this study, a total of 49 plant samples from 22 species collected from Namibia on the basis of bioassay results and traditional application were subjected to in-depth chemical profiling using LC/MS. Under LC/MS guided screening, most species failed to yield unique/new compounds (most of natural compounds were identified as flavonoids, other common polyphenols or triterpenes), and only two plant species were found to contain unique/novel structures. This is actually a very promising ratio of return for the small number of plant species chemically profiled in this investigation. Under this model, we were able to successfully narrow down the plant list to facilitate further natural products investigation, which is presently still very laborious work.

MEDI 181

Alternative strategies for targeting HSP70

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High expression levels of the molecular chaperone HSP70 have been linked to poor prognosis in cancer.[1] Despite significant research effort, targeting HSP70 has proved challenging due to its flexible structure and complex catalytic cycle. Most published inhibitors have poorly characterised modes of action and only moderate to weak potency.[2]

Fluorescence Polarisation (FP) and Surface Plasmon Resonance (SPR) techniques were used to elucidate the mechanisms of action of reported HSP70 inhibitors, apoptozole[3-4] and VER-155008[5](Figure 1). Fluorescent probes derived from the inhibitors were designed and synthesised using new synthetic routes amenable to analogue synthesis and fluorophore attachment. An FP assay was established and used to interrogate interactions between the inhibitors and HSP70 or HSP70/co-

chaperone complexes. Finally, a screen for small molecule inhibitors targeting a novel HSP70 complex was established.

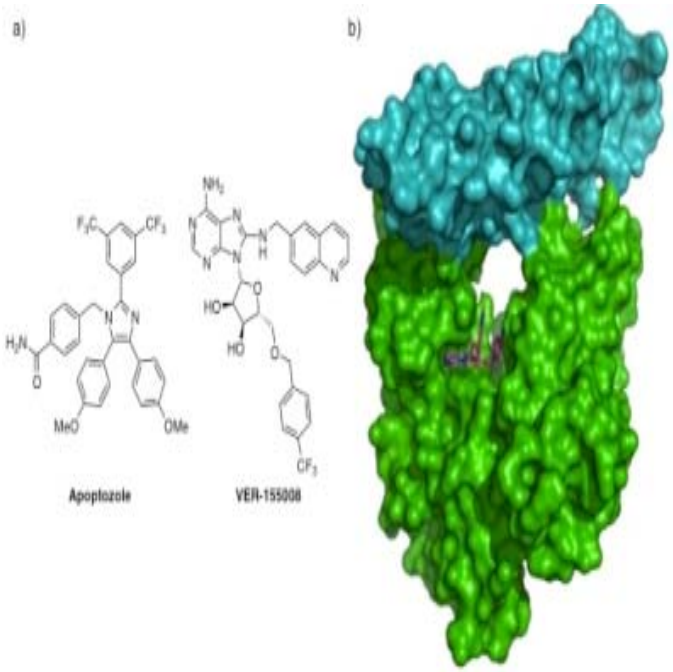

Figure 1 a. Structure of HSP70 inhibitors apoptozole and VER-155008; b. X-Ray crystal structure of VER-155008 (pink) bound to HSP70 (green) in complex with co-chaperone BAG1 (blue), PDB: 3FZM.[2]

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MEDI 182

Synthesis, evaluation, and docking of new 4-alkoxybenzyl bicyclic amine ethers as nicotinic acetylcholine receptor antagonists

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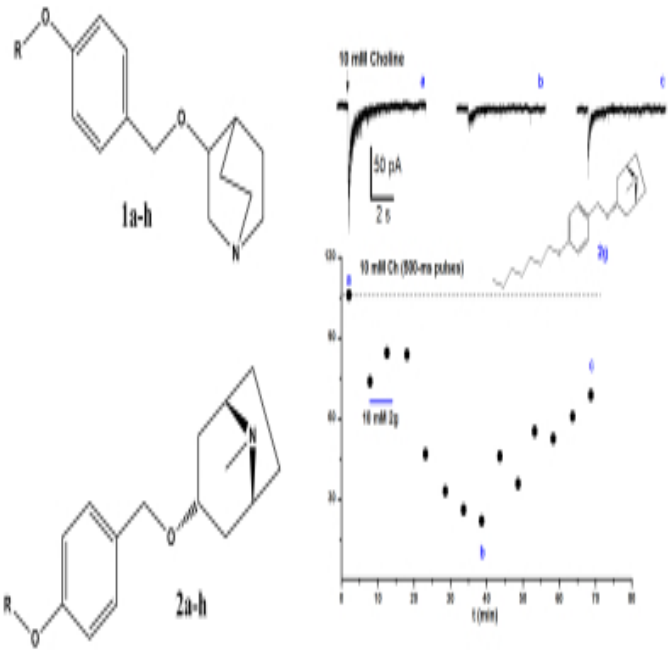
Development of selective agonists or antagonists for the nicotinic acetylcholine receptors (nAChR) can result in new and potentially useful therapeutic agents. It has been reported in the literature that potent and selective nAChR antagonists might be used for the treatment of nicotine dependence and may be efficacious as therapeutic adjuvants in many different oncologic protocols. We have now designed and synthesized a new series of aminoethers bincorporating a quinuclidine (1a-h) or tropine (2a-h) bicycle and a 4-alkyloxybenzyl moiety. The inhibitory activity of these compounds was determined vs. α7 nAChRs expressed on interneurons in rat hippocampus CA1 slices using the whole-cell patch-clamp. Compounds 1a-h and 2a-h inhibited choline-induced whole-cell currents at low micromolar concentrations (Figure1).



The design, synthesis, bioassay and docking studies of these new compounds are presented.

MEDI 183

Ferroptotic small molecules for the treatment of *Ras*-mutated cancers

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The *Ras* protein is a small GTPase involved in key cell signaling pathways regulating cell growth, differentiation, and programmed cell death. Mutations of the *Ras* protein occur in 30% of all cancers and are difficult to target due to the complex network of upstream and downstream regulators. A high priority has been to identify compounds that are *Ras*-selectively lethal (RSL) while ineffective against healthy cells or cells that are *Ras* wild type. Several RSL compounds, such as Erastin, Oncrasin-1, and Lanperisone, have been identified and kill cancer cells through a unique mechanism of action called ferroptosis. Ferroptosis is a non-apoptotic form of cell death that utilizes iron to increase the intracellular levels of reactive oxygen species (ROS). We have synthesized and identified a new class of small molecule anticancer agents that exhibit a ferroptotic mechanism of action. The synthesis and biological evaluation of the ferroptotic therapeutics will be presented.

MEDI 184

Dibenzazepine and dibenzocycloheptane based FOXO modulators

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The FOXO (Forkhead transcription factors, Class O) proteins are a group of tumor suppressors. “FOXO1” is rendered inactive on phosphorylation, and cytoplasmic localization by the PI3K-AKT and RAS-MAPK oncogenic kinase pathways. In a cell based FOXO sequestration screen reported by Kau et al, it was revealed that tricyclic neuroleptics (chlorpromazine, trifluoperazine) reinstate FOXO1 to the nucleus in tumor cells. As part of our program to develop FOXO1 modulators, devoid of the dose limiting CNS toxicities of parent tricyclic neuroleptics, we synthesized and evaluated novel dibenzazepine and dibenzocycloheptane based analogs. Some of the key steps in the synthetic schemes include an allyl-silane addition to dibenzosuberol, and opening of functionalized epoxides with a weak dibenzazepine nucleophile in base/lewis acid free microwave conditions. These compounds demonstrated anti-proliferative activities in lung cancer H1650 cell lines. The synthesis and biological activity of these compounds will be presented in this report.

MEDI 185

New flash chromatography silica increases purity of separated compounds

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A new, spherical, high surface area silica for flash chromatography has been developed by Biotage to increase purification efficiency. This novel media improves sample resolution and increases sample loading by up to 4x over standard silica. Data comparing compound purity following purification using the new silica cartridge, Biotage® SNAP Ultra to standard silica will be presented.

MEDI 186

Simple flash chromatography step-gradient process eliminates purification guesswork

***John R. Bickler**, bbickler@biotage.com, John Urh. Biotage, LLC, Charlotte, NC 28269, United States*

For many years flash chromatography has been performed using isocratic elution, linear gradients or a combination thereof. Often the elution methods and sample loading amounts are based on previous experience or gut-feel estimation, resulting in less than optimal purifications, manual purification monitoring, and repeated purification runs. New software developed by Biotage for its Isolera flash chromatography systems now provides an option to optimize purification methods for up to six different compounds by using as few as two TLC separations.

MEDI 187

Computerized drug design of novel class of flavone entities

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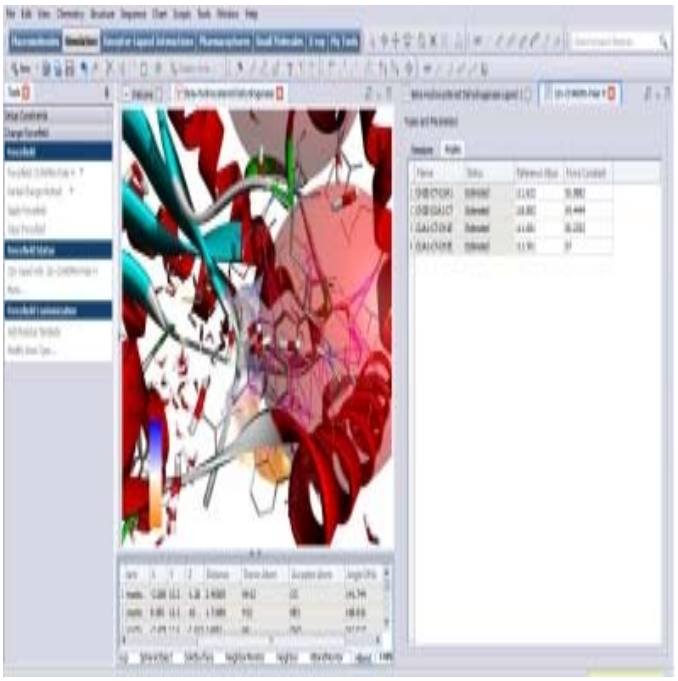
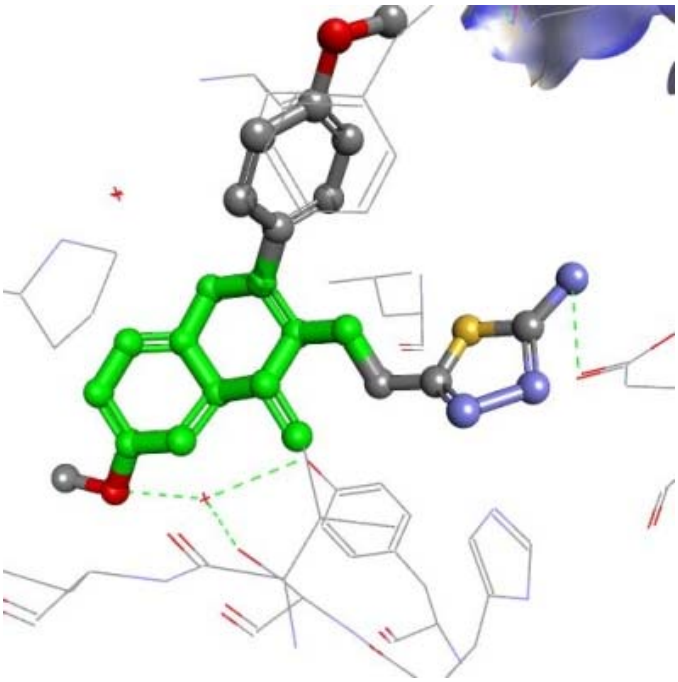
Flavanones are important precursors for synthesis of Flavones, isoflavones, flavonols and dihydroflavonols. The flavone skeleton is present in a wide range of synthetic and naturally occurring products exhibiting various interesting pharmacological activities. so here computerized drug design of different Flavone analogs are targeted. different pdb sequence were used. flavone analogs were applied under forcefield and molecular mechanics were checked to generate spheres and docking score has been meassured by libdock score. ligand based drug design has been carried out for all other molecules. and optimized with reference molecule.





MEDI 188

Aldehyde recognition and discrimination by mammalian odorant receptors via functional group-specific hydration chemistry

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The mammalian odorant receptors (ORs) form a chemical-detecting interface between the atmosphere and the nervous system. This large gene family is composed of hundreds of membrane proteins predicted to form as many unique small molecule binding niches within their G-protein coupled receptor (GPCR) framework, but very little is known about the molecular recognition strategies they use to bind and discriminate between small molecule odorants. Using rationally designed synthetic analogs of a typical aliphatic aldehyde, we report evidence that among the ORs showing specificity for the aldehyde functional group, a significant percentage detect the aldehyde through its ability to react with water to form a 1,1-*geminal* (*gem*)-diol. Evidence is presented indicating that the rodent OR-I7, an often-studied and -modeled OR known to require the aldehyde function of octanal for activation, is likely one of the *gem*-diol activated receptors. A homology model based on an activated GPCR X-ray structure provides a structural hypothesis for activation of OR-I7 by the *gem*-diol of octanal.

MEDI 189

Natural product inspired libraries for hit generation in drug discovery

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Natural product inspired building blocks can be potential starting points in drug discovery given their desirable physicochemical attributes. This poster highlights the use of a core generated from natural product *Asperparaline A* and synthesis of library of compounds. Pauson-Khand cyclization of enyne **4** enabled the rapid assembly of tricyclic core **6** and further elaboration provided structurally diverse compounds for screening.

MEDI 190

Design, synthesis, and biological evaluation of spiro-fused thiadiazoline inhibitors of the mitotic kinesin KSP

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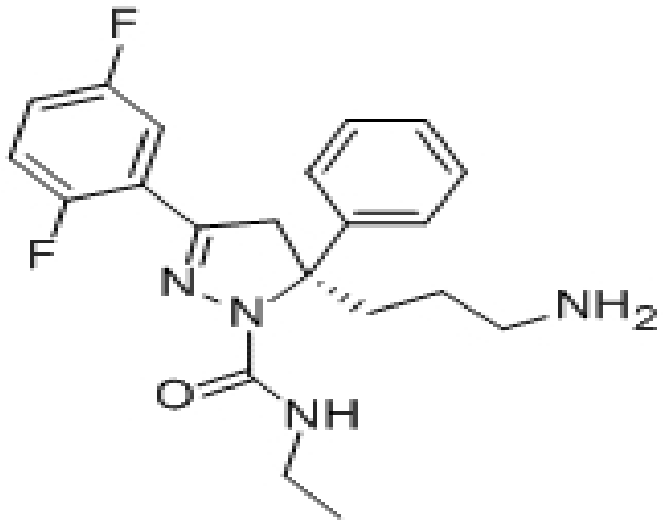
Kinesin spindle protein (KSP) belongs to the kinesin-5 family and is necessary for the formation of the bipolar mitotic spindle. Inhibition of this motor enzyme has been validated to result in mitotic arrest and apoptosis. Many reported KSP inhibitors are currently being tested in clinical trials for the treatment of cancer. However, these potential anti-cancer agents are delivered intravenously utilizing a variety of dosing schedules. Accordingly, the development of an orally bioavailable small molecule KSP inhibitor represents a promising means of providing a more convenient dosing schedule.

A medicinal chemistry effort to discover small molecule inhibitors of KSP was initiated following the disclosure of 3,5-diaryl-4,5-dihydropyrazole **1** and related analogues. Inspired by its potent biological activity, we were encouraged to envision compound **1** to serve as our initial structural platform from which to access a range of spiro-fused-5-membered ring scaffolds. This presentation will describe the synthesis, structure activity relationships, pharmacokinetic and pharmacological properties of these potent KSP inhibitors.



1

IC_{50} = 44 nM

MEDI 191

Identification of potent imidazoquinoline derivatives as antiglioma agents from screening

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Glioblastoma Multiforme (GBM) is a highly fatal disease and new chemotherapeutic agents are desperately needed to treat GBM patients. With the aim of identifying new antiglioma agents we employed the UC DDC library screening based on our finding of the antiglioma lead molecule **1 (SP-6-27)** and clinically used drug **2 (Azixa),** the combination ofwhich produced molecule **3 (S-94403)** as one of the most potent imidazoquinoline-based antiglioma agents (IC_{50} = 0.625 μM).

MEDI 192

Phenanthroline based metal complexes as G-quadruplex ligands

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Guanine-rich human telomeric DNA found at the ends of chromosomes plays an important role in maintaining the integrity of the genome. In normal somatic cells, telomeric DNA progressively shortens after each replication cycle due to the lack of template for fully replicating the 3'single-stranded overhang. Such shortening eventually triggers genomic instability and cellular senescence. In contrast, telomeric DNA in cancer cells can be maintained via a reverse transcriptase known as telomerase that is overly expressed in 80-85% cancer cells and is responsible for cancer cells' immortality. This remarkable difference in telomerase activity between normal and cancer cells has attracted much attention to exploit telomerase as a potential therapeutic target in oncology.

One of the efficient approaches to inhibit telomerase activity is to block the binding of telomerase via the formation of G-quadruplex structures in telomeric DNA. G-quadruplex structures are formed under physiological conditions and their formation can be greatly facilitated by suitable small molecules. In this present work, we investigated the binding of G-quadruplex DNA with a series of newly developed 5-substituted phenanthroline metal complexes. Their binding affinity and specificity towards G-quadruplex DNA were determined using biophysical techniques including thermal denaturation, fluorescence titration, UV absorbance, and circular dichroism. Our results demonstrated that phenanthroline-metal complexes can selectively bind to G-quadruplex over duplex with high affinities partly due to the enhanced planar complex aromatic surface. Substituents at the 5 positions of phenanthroline derivatives also count for the observed G-quadruplex stabilization effect.

MEDI 193

Synthesis of 2 amino benzothiozoles as potential anticancer agents

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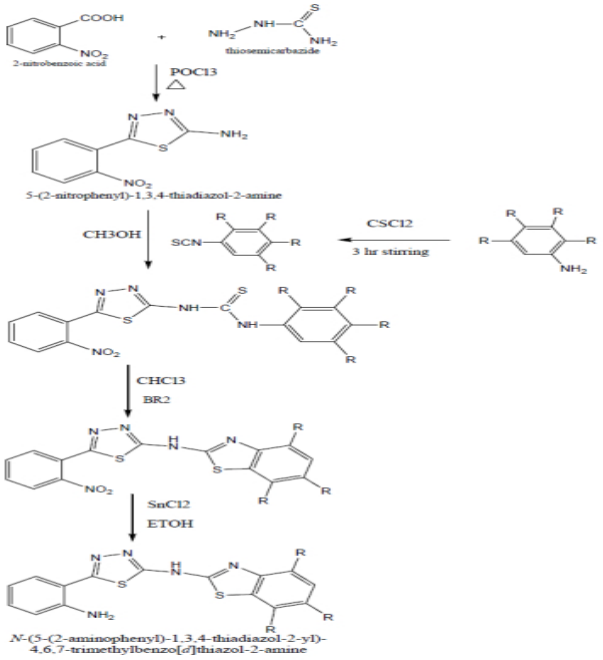
In the present study we have reported the synthesis of some novel heterocyclic derivatives comprising benzothiazole and 1,3,4-thiadiazole containing moiety. 2-amino-1,3-benzothiazole are of interest because of their diverse biological activities and clinical applications. We have reported the new series of 2-amino-1,3-benzothiazole analogsto target Vascular endothelial growth factor and receptor. The reaction was monitored by Thin-layer chromatography using suitable mobile phase. The R_f values were compared and determined the melting point of derivatives, found that they were different from each others. Further these derivatives were characterized and confirmed by IR, ^{1}H-NMR, ^{13}C-NMR, and Mass spectral (MS) studies. For anticancer activity,all the selected compounds submitted to National Cancer Institute (NCI) for *in vitro* anticancer assay were evaluated for their anticancer activity. Primary *in vitro* one dose anticancer assay was performed in full NCI 60 cell panel with the protocol of the NCI,USA.



MEDI 194

Chemical probes for studying cell-to-cell communication in bacteria

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The opportunistic pathogen *Pseudomonas aeruginonsa* has been shown to utilize quorum sensing to coordinate activation and repression of many unlinked genes in response to cell density. *P. aeruginonsa* uses small signaling molecules, which are synthesized continually throughout cell growth, to determine cell density. Once a critical threshold concentration of these molecules has been reached, binding to a specific receptor occurs resulting in synchronized changes in gene expression. Both virulence factor production and formation of antibiotic resistant biofilms are known to be under quorum sensing control leading to infections that are difficult to eradicate.

PQS, a quorum sensing signaling molecule used by *P. aeruginonsa*, and its biosynthetic precursor HHQ have been shown to bind the PqsR receptor. While PQS and HHQ bind PqsR, it is believed that they also interact with additional proteins regulating virulence factor production and biofilm formation.

We present the results of chemical proteomic studies in which immobilized PQS and HHQ probes were used to identify unknown macromolecular biological targets of the two molecules. The design and synthesis of second-generation photoaffinity-based PQS and HHQ chemical probes for use *in vivo* is also presented and the results from both proteomic studies are compared. These studies are the first to identify that PQS binds MexG, a component of the MexG-OpmD efflux pump, which has previously been linked to quorum sensing, antibiotic susceptibility and virulence. As well as improving the current understanding of bacterial communication these studies also have the potential to uncover new drug targets for combating antibacterial resistance.

MEDI 195

Structure – kinetics relationship analysis of a set of HSP90 inhibitors

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The kinetic aspect of interactions of small molecules with their protein targets is increasingly recognized as being highly relevant for successful drug development. In light of the IMI project K4DD- “Kinetics for Drug Discovery”, we aim for gaining a deeper understanding of the structure-kinetics relationships of compounds at selected targets. In the first attempt to elucidate general trends in kinetic behavior, statistical modeling techniques were applied to a dataset of 180 compounds of HSP90 inhibitors derived from Surface Plasmon Resonance. Methods include histogram analysis, principal component analysis, decision trees, as well as self organizing maps. The trends observed in histogram analysis are pointing towards an influence of the molecular weight, the number of single bonds and the number of rings. This is in accordance with previously observed trends [1]. Building decision trees using a set of ADME related descriptors, led to predictive models, which allow to separating fast- from slow-dissociating compounds. The performance of the model is mainly influenced by the

number of single bonds, number of hydrophobic atoms as well as number of aromatic atoms. (ROC: 0,852, MCC: 0,653) Further studies will focus on incorporation of descriptors encoding the flexibility of proteins.

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MEDI 196

Synthesis and evaluation of linoxepin analogs for topoisomarease inhibition

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Linoxepin, a novel lignin isolated by Schmidt and co-workers from the aerial parts of *Linum perenne L.* (Linaceae) with its previously undescribed carbon skeleton represents a new scaffold for evaluation of its potential bioactivites. We have set out to develop the synthesis of the derivatived of Linoxepin via novel sigmatropic rearrangement chemistry and in screening the analogues and intermediates towards topoisoamerase inhibition due to its similarities with currently known inhibitors. Resutlts will be presented.

MEDI 197

Development of a near infrared fluorescent probe for optical imaging of DNA base excision repair

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DNA repair mechanisms have evolved to ensure the integrity of DNA and maintain cellular functions in the body. However, these repair processes are the main causes of drug resistance to many chemotherapeutics designed to cause cell death through DNA damage. Therefore, there is a need for the development of imaging tools that can indicate where and to what degree DNA repair occurs and possibly reduces the efficacy of cancer treatment. In particular, new optical imaging probes could readily allow for

evaluating new drug candidates in a preclinical setting and monitoring patient response to therapy. To help address this need, we have developed a near infrared (NIR) fluorescent DNA repair-targeted compound for use in optical imaging. This small molecule imaging probe, which we have designed, synthesized, and characterized, binds to abasic (or AP for apurinic/apyrimidinic) sites, key intermediates in the base excision repair (BER) pathway. Taking advantage of the chemistry of BER, we have developed a fluorescent oligomer-based assay to evaluate the ability of this probe to bind to AP sites and stall further repair. Through this assay, we have shown that the probe binds specifically and rapidly to AP sites in DNA. In addition, using calf thymus DNA treated with heat and acid to induce AP sites, we have demonstrated that the probe is sensitive to the quantity of AP sites. We are currently evaluating the potential of the probe to detect DNA damage and repair in vivo.

MEDI 198

Utilisation of Fmoc solid phase chemistry as a novel approach to the generation of duocarmycin analogues

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Duocarmycin SA is the archetypal member of one of the most potent families of antitumor antibiotics yet isolated. Their antitumor activity is derived from non-covalent recognition of AT rich regions of DNA's minor grove, which induces a destabilising conformational change in the compounds structure, leading to the exceptionally efficient alkylation of adenine. The terminal ester and amide functionality of the duocarmycin alkylation subunit suggested that it would be suitable as a building block for solid phase synthesis. We describe the first synthesis of a duocarmycin subunit suitably substituted for Fmoc-based solid phase synthesis and preliminary studies of its use in the synthesis of duocarmycin analogues.

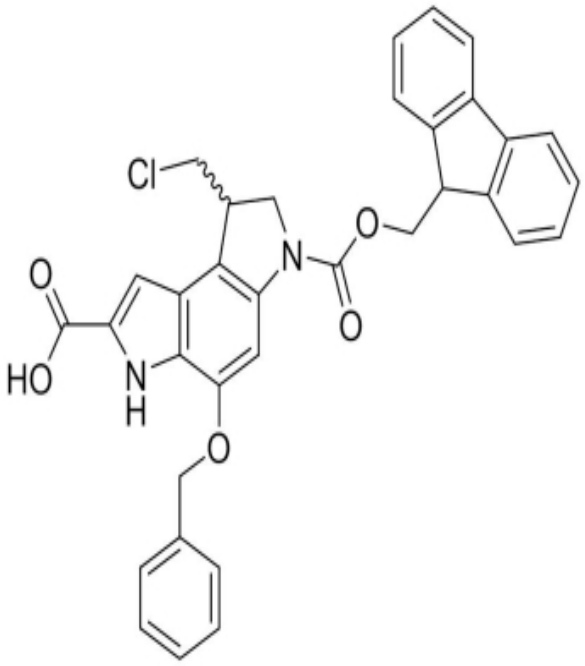

Figure 1: Structure of the duocarmycin alkylation subunit building block for Fmoc based solid phase synthesis.

MEDI 199

Discovery of new inhibitors of deoxyhypusine synthase (DHPS): Virtual screening and biochemical evaluation

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The enzyme deoxyhypusine synthase (DHPS) is responsible for the first step of the posttranslational modification in the protein eIF5A, which requires the polyamine spermidine and results in the unique aminoacid hypusine. Mature, hypusine-containing, eIF5A has been described as necessary to tumor progression and inflammatory response, therefore inhibitors of eIF5A function may become a new important strategy for the therapy of cancer and inflammatory diseases. Moreover, the hypusine modification in eIF5A by DHPS is very specific, which makes it a promissing drug target. The most potent DHPS inhibitor known so far is the N^1-guanyl-1,7-diamine-heptane (**GC7** , K_i = 9,7 nM), a substrate analog that harbors a guanidine group and acts as a competitive inhibitor. However, GC7 is not very specific to DHPS in the cell and

interferes with other aspects of polyamines metabolism. In this work, human DHPS was employed as a molecular target for the discovery of new inhibitors as lead candidates. On the basis of that, structure-based virtual screening was employed to assist the identification and selection of guanidine derivatives as inhibitor candidates. The virtual screening protocol used Surflex to model and evaluate the binding mode of the molecules into the active site of DHPS (PBD ID 1RQD). The DHPS inhibitory activity of the selected molecules were experimentally assessed. The procedure identified 10 guanidine derivatives that exhibited substantial *in vitro* inhibitory activity (> 30%) against DHPS. The most potent inhibitors showed IC_{50} values in the micromolar range. The new guanidine derivatives are promising lead candidates for future medicinal chemistry efforts aimed at developing new therapeutic alternatives targeting DHPS and eIF5A function.

MEDI 200

Synthesis and evaluation of 2-furanyl analogs of salvinorin A as kappa opioid receptor agonists

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The neoclerodane diterpene salvinorin A is the active hallucinogen in the recreational drug *Salvia divinorum.* Salvinorin A is a potent and selective kappa opioid receptor (KOR) agonist. Since modulation of KORs has been implicated as a potential target for illicit drug abuse and addiction therapy, salvinorin A is studied for its ability to modulate drug abuse. In fact, several salvinorin A analogs have effectively attenuated addictive behaviors in animals, and we aim to continue to optimize these behavioral responses by modifying the furan ring of salvinorin A. While the binding mode of salvinorin A at the KOR has not been fully elucidated, the furanyl oxygen is proposed to interact with the receptor through hydrogen bonding; therefore changing the placement of this oxygen should affect activity at KORs. We have recently developed a semisynthetic method to replace the 3-furanyl substituent of salvinorin A with a 2-furanyl substituent without affecting the other sensitive functionalities in the compound. A series of these 2-furanyl analogs has been synthesized, and pharmacological evaluation of this series for KOR activity has extended our understanding of how salvinorin A interacts at KORs.

MEDI 201

Potent [1,2,4]triazolo[1,5-a]pyridine and 1H-pyrazolo[3,4-d]pyrimidine inhibitors of polo-like kinase 1 and the structural basis for divergent SAR between the series

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Polo-like kinase 1 (PLK1) is a serine/threonine kinase which functions in mitosis and cytokinesis and as such is a target for anti-cancer therapeutics. Here we describe the discovery of 2 classes of potent PLK1 inhibitors: namely, the [1,2,4]triazolo[1,5-a]pyridine series (TPs) and the 1H-pyrazolo[3,4-d]pyrimidine series (PZPs). In this poster, we will show SAR comparisons and x-ray crystallographic analysis for the two series. The two chemical series have highly similar R-group trajectories and interactions; however, the 5/6- ring systems bind in opposing orientations. Our calculations demonstrate that intramolecular sterics originating from the inhibitor core in combination with steric effects from the PLK1 binding pocket contribute to the observed conformational differences.

MEDI 202

Toward well-defined antibody-drug conjugates

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A promising approach to enhance the antitumor activity of antibodies and reduce the systemic toxicity of small molecule anti-cancer drugs is to covalently attach the small molecule to the antibody, thus forming antibody-drug conjugates (ADCs). ADCs are commonly produced by chemically conjugating cytotoxic drugs to antibodies through lysine side chains or cysteine thiols generated by the reduction of inter-chain disulfide bonds. Both of these methods yield heterogeneous conjugates with complex biophysical properties. To limit these liabilities, we have designed, characterized, and validated antibody variants which allow precise control of the site of conjugation and a drug load of two or four drugs per antibody. These engineered antibody variants can be efficiently and site-specifically conjugated with payloads at the milligram and gram scale to yield homogenous ADC products.

The engineered antibody variants described have biochemical, biophysical, and manufacturability properties similar to their non-engineered parental antibodies. Upon conjugation to a cytotoxic drug with anti-mitotic activity, the described ADCs have potent *in vitro* and *in vivo* anti-tumor activity. Furthermore, the ADCs have been engineered to ablate FcγRs binding, thus offering the potential to minimize off-target toxicities. Conjugation at these sites has also been shown to improve the *in vivo* safety profile of ADCs in rats.

The strategies presented herein for engineering ADCs with defined sites for conjugation, controlled drug loading, potentially decreased off-target toxicity, and optimal serum stability are broadly applicable to any full-length IgG or Fc-containing therapeutics as they involve the Fc constant domain of the antibody.

MEDI 203

Discovery of potent small molecule inhibitors of Wnt-dependent transcription

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The Wnt signalling pathway is frequently deregulated in cancer due to mutations in APC, β-catenin and axin; indeed 80% of colon cancers have defects in the APC gene leading to high levels of β-catenin. Some colon cancers, endometrial cancers and melanomas that lack defects in the APC gene harbour β-catenin mutations that abrogate its degradation. We have screened a diverse small molecule chemical library against a cell-based luciferase reporter assay to identify small molecule inhibitors of Wnt signalling.[1] Here we report the medicinal chemistry optimisation of a pyridine-based series of inhibitors resulting in the discovery of potent, selective and orally bioavailable small molecule inhibitors of Wnt signalling.

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MEDI 204

Synthesis and investigation of C^4 & C^5 substituted imidazolium cations for anti-cancer applications

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The synthesis and structural characterization of 4-substituted (C^4) and 5- substituted (C^5) imidazolium cations (ICs) are reported. The paper explores the integration of the hydrophilic and hydrophobic groups substituted at the imidazole's 4 and 5 positions for tuning the compounds' efficacy and lipophilicity as potential anti-cancer agents.

Conjugated imidazolium cations with varied functionalities are synthesized from commercially available starting materials. The synthesis of N, N'-bisalkylated imidazole compounds, with a lypophilic aromatic head group, are explored and differ by the extent of hydrophobicity and length at the 4 and 5 positions. Variation of the substitutients, studied by the Youngs' group, demonstrates that the hydrophobicity of the substitution plays a role in the ICs efficacy against cancer cells. Analysis shows that the duality of the lipophilic N,N-bisalkylated head group and hydrophobic sterically involved substitution at the 4 and 5 positions yields a better anti-cancer agent.

MEDI 205

Inhibitors of sterol biosynthesis: A facile synthesis of ketosteroids and related compounds

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This paper represents the preparation of ketosteroids and potent inhibitors of sterol synthesis in L cells

MEDI 206

Structure-based design of HMG CoA reductase inhibitors as novel antimicrobials targeting drug resistant bacterial pathogens

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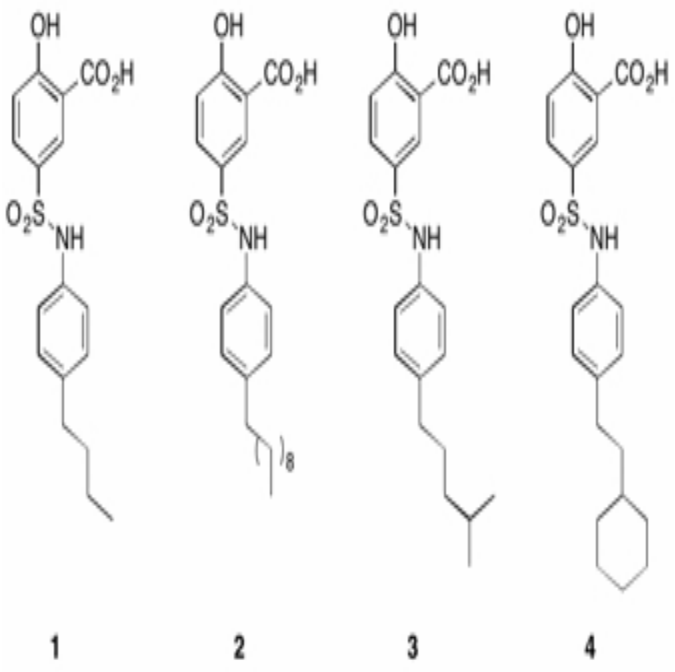
To combat the emergence of drug-resistant bacterial pathogens such as Methicillin-Resistant Staphylococcus Aureus (MRSA) and Vancomycin-Resistant Enterococcus (VRE) as serious threats to human health, a new class of antimicrobials that target the Class II HMG-CoA Reductase (HMGR) enzyme found in Gram-positive cocci has been developed. A lead HMGR inhibitor (**1**) discovered in a screen of a 30,000-member library was co-crystallized with HMGR and the crystal structure thus obtained was used as the basis for structure-based modifications to the original structure. Of the fourteen analogues initially synthesized, three (**2-4**) were identified that displayed both potent in vitro inhibition of HMGR and potent antimicrobial activity against MRSA and VRE.



Inhibitors **2-4** have been shown to have activity against clinical isolates of MRSA and VRE and against biofilms. They have also been shown to display selectivity for the Gram-positive pathogens as they do not affect the Gram negative bacterium E. coli. Inhibitors **2-4** also display no cytotoxicity toward cultured murine cells at a concentration an order of magnitude greater than their IC_{50} values. Inhibitors **2-4** have also been tested in a C. elegans model of infection. When tested at 100 mM concentration, inhibitors **2-4** were found to be 10-fold more effective at killing bacteria in C. elegans than the clinically used antibiotics vancomycin and linezolid tested at comparable concentration. Co-crystal structures of **2-4** with HMGR have been obtained and show that these inhibitors bind into the active in the same region as the substrate, making polar contact with a number of key active site residues. Prospects for more potent and selective HGMR inhibitors will also be discussed.

MEDI 207

Multifunctional nanoparticles for the targeted delivery of a coagulation amplifier to sites of internal hemorrhage

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Battlefield mortality is a pressing problem, and internal bleeding causes the majority of these deaths. Uncontrollable internal bleeding is primarily in the torso of the body and cannot be easily constrained with devices such as tourniquets. Based on the difficulty of access there is a need to develop a product that can target sites of injury to help

facilitate the coagulation cascade, stopping the loss of blood, until surgical help can be administered. Nanoparticles are currently a highly explored field for drug delivery, and appear promising to help tackle the problem of uncontrollable internal bleeding. Polymers have desirable properties for nanoparticles targeted at drug delivery, such as biocompatibility and biodegradability. Polymers can also be used to adjust the charge that nanoparticles carry along with particle size. Polyphosphates are of interest as a coagulation accelerator based on their biocompatibility, half-life of 90 min (biodegradability) and they are known to be a potent hemostatic agent. Short chain polyphosphates (P60-P100) have been found to interact with multiple steps in the coagulation cascade, and have been shown to enhance coagulation. Functionalized targeting peptide sequences, also known as protease degradable cross-linkers, such as the KGGLVPRGSGK sequence, can be incorporated into the nanoparticle shell in varying concentrations to help locate the particles at the site of injury. This specific peptide sequence is cleaved by Thrombin, which will only be present at the wound site, anchoring the particles to this area for degradation. Thrombin will cleave the sequence, breaking down the polymer shell and release polyphosphate so it can participate in the coagulation cascade and control the bleeding. Non-protease degradable cross-linkers will also be explored to make control particles. Particles have been successfully synthesized in the size range of 50-250 nm. This research has the potential to benefit not only the military but civilian trauma patients too.

MEDI 208

Novel synthetic methodology for 5- and 6-subsituted cyclopenta[*d*]pyrimidine nonclassical and classical antifolates as TS and DHFR inhibitors

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The essential role of folates in nucleic acid precursor biosynthesis has made folate metabolism an attractive antitumor target for decades. We have reported several thieno[2,3-*d*]pyrimidine, pyrrolo[2,3-*d*]pyrimidine, furo[2,3-*d*]pyrimidine classical antifolates as selective tumor targeting agents. These agents are selectively transported by the folate receptor (FR) and/or proton-coupled folate transporter (PCFT) rather than the ubiquitous reduced folate carrier (RFC). These compounds inhibit FR and PCFT expressing tumor cells (KB and IGROV1) at picomolar to nanomolar IC_{50} values. It is suggested in the literature for classical antifolates that a heteroatom in the 7- position of pyrimidine-fused five member rings or the 8- position of pyrimidine-fused six member rings play a crucial role in drug uptake. Although the literature is surfeit with the synthesis of fused pyrimidine bicyclic and tricyclic analogs, a viable synthetic method for substituted cyclopenta[*d*]pyrimidine analogs is conspicuously absent. As part of our continuing efforts to synthesize molecules with targeted tumor-specific attributes, we

report a novel synthetic methodology for 5- and 6-subsituted cyclopenta[*d*]pyrimidine nonclassical and classical antifolates.

MEDI 209

Substituted 4-hydroxy-6-phenyl-5,6-dihydropyridin-2-ones as potent inhibitors of human lactate dehydrogenase A

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Tumor cells rely on glycolysis for energy generation, in contrast to oxidative phosphorylation (TCA cycle) utilized by healthy cells, even in the presence of normal oxygen concentrations. Selective inhibition of the lactate dehydrogenase A (LDHA), which catalyzes the conversion of pyruvate to lactate, is believed to be beneficial in cancer therapy. Our interest in LDHA inhibitors led to the identification of 3-hydroxy-2-mercaptocyclohex-2-enone in a HTS campaign. Core change and structure-based design provided substituted 4-hydroxy-6-phenyl-5,6-dihydropyridin-2-ones with low nanomolar enzyme and low micromolar cellular potencies. A crystal structure of an optimized molecule bound to LDHA and associated mouse PK and efficacy studies will be discussed.

MEDI 210

Combining CoMFA models for the design dual inhibitors of receptor tyrosine kinases (RTK) and tubulin

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Tumor angiogenic mechanisms that are vital for tumor growth and metastasis are inhibited by antiangiogenic agents. Antiangiogenic agents that target receptor tyrosine kinases have found utility in the treatment of a variety of cancers but these agents are usually not cytotoxic and are mainly cytostatic. Microtubules play a vital role in mitosis and cell division and are a particularly attractive target for drug development as anticancer agents. Antimitotic agents have been successfully used the clinic for the treatment of various cancers. However, the utility of such agents are often limited by the emergence of tumor resistance due to overexpression of P-glycoprotein and/or *β*III-tubulin. Combination chemotherapy with antiangiogenic and cytotoxic agents has shown significant promise, and several clinical studies with such combinations are in progress.

A dataset of bicyclic RTK and/or tubulin inhibitors and tricyclic RTK inhibitors reported from our laboratory was used to develop topomer COMFA models that correlate chemical structure and inhibitory potency for RTK and tubulin. The details of these models and their potential predictive power will be reported.

MEDI 211

Design, synthesis, and SAR studies of novel survivin inhibitors with potent antiproliferative properties

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Survivin is a unique member of inhibitors of apoptosis proteins (IAP). Unlike other IAPs which are expressed in both cancer and normal cells, survivin is only highly expressed in cancers but not in differentiated normal tissues. Therefore, targeting survivin is an ideal approach to selectively treat cancer. UC112 was previously screened out from a library of compounds as a strong survivin inhibitor that can induce cancer cell apoptosis in our lab. The average IC_{50} of UC112 against several cancer cell lines is around 0.7 μM. Further optimization of UC112 resulted in a series of novel survivin inhibitors which showed excellent antiproliferative activity. We applied three approaches to make modifications on the structure of UC112. We first modified the linker between the B ring and C ring of UC112, trying to find the optimal linker. Then, we utilized a series of different substituted phenyl and heterocyclic rings to replace the C ring of UC112. Finally, we optimized the D ring moiety of UC112. All those compounds were synthesized and then tested against a panel of different cancer cell lines. The *in vitro* results showed that several compounds were very potent against all tested cancer cell lines including multi-drug resistant cell lines. The Structure–Activity Relationships (SAR) studies of this UC112 template were also discussed.

MEDI 212

Synthesis of pyridine and thiophene derivatives as potential antagonists of chemokine receptor type-4

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CXCR4 is a chemokine receptor that has been linked to various disease pathways including: HIV-1 proliferation, auto-immune disorders, inflammatory disease and cancer metastasis. The interaction of the C-X-C chemokine receptor type for (CXCR4) with C-X-C chemokine ligand 12 (CXCL12) plays an important role in triggering these disease related pathways. Various antagonists for CXCR4-CXCL12 interaction have been

synthesized and tested, but many are not useful clinically. This work focuses on the design and synthesis of a new class of compounds that show potential as CXCR4 antagonists. Upon synthesis, these compounds were tested using an affinity binding assay and a cell invasion assay to asses activity. A few hit compounds have been identified and all structures have been confirmed by spectroscopy analysis.

MEDI 213

Quality control methodology studies on *Tripterygium wilfordii* Hook. f. based on their anti-tumor effective substances

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Tripterygium wilfordii Hook. f. (TwHF) belongs to *Tripterygium* Celastraceae. This traditional Chinese medicine exhibits good clinical activity, such as anti-tumor, immunosuppressive and anti-inflammatory. Currently, quality control methods of TwHF can not fully reflect their chemical information and pharmacological activity, which limit their extensive application. In this study, quality control methods of TwHF were established based their main active substances. The main work was displayed as follows:

Firstly, under the direction of anti-tumor activity, medium pressure column chromatography and prepararive-high performance liquid chromatography purification were mainly used to obtain compounds of high purity and high anti-tumor activity. In total, 12 compounds were isolated and purified. This study deepen the understanding of effective substances of TwHF. Then, the central composite design-response surface methodology was employed to optimize the experimental conditions for extraction of anti-tumor active ingredients group from TwHF, which laid a good foundation for improvement of the TwHF preparations process and fully quality control of TwHF. Furthermore, the anti-tumor activity comparison of TwHF herbs, its products with the mixture of active ingredients group with the same content to corresponding herbs and products was conducted. The results indicated that the anti-tumor activity of active ingredients group was close to TwHF and its products to a certain extent and therefore provided pharmacological support for the following quality control methodology studies.

Based on ultra-high-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UHPLC-QTOF-MS), a simultaneous quantification method was developed for anti-tumor active ingredients group in TwHF and its products. This method is simple, rapid (4.5 min), high sensitivity (LOD>5.7 ng mL^{-1}, LOQ>19.0 ng mL^{-1}), wide linear range (20~20000 ng mL^{-1}, r^2>0.996) and stable. The method was applied

to quantitatively analyze five main bioactive ingredients with significantly differences of their contents in different parts of TwHF herbs, Duoganpian (DGP) and processed pieces. The results indicated that the method can achieve accurate qualitative analysis for active ingredients group to provide technical support for quality control methodology studies.

Finally, After accurate quantitative analysis of active ingredients group, UHPLC-QTOF-MS fingerprinting was established for TwHF, DGP and processed pieces, to investigate the full information of medicinal ingredients. Using chemometric methods of pattern recognition and variable selection, mass spectrometric fingerprintings of TwHF herbs from different locals, DGP from different manufacturers and processed pieces from different markets were analyzed to establish recognition model and to screen markers. The results show that the developed MS fingerprintings could reflect differences of chemical composition for TwHF and its products to provide more effective technical support for their quality control. The discovery of markers permits further study of medicinal efficacy of TwHF from different locals in the molecular mechanism.

MEDI 214

New heterocyclic scaffolds for drug discovery

***Lexie Winograd**, erstevens@davidson.edu, Yufei Wang, Erland P Stevens. Department of Chemistry, Davidson College, Davidson, NC 28035, United States*

While nitrogen heterocycles form the core of a large percentage of small molecule pharmaceuticals, the number of different heterocyclic systems used for this purpose is small. For this reason, intellectual property space around these commonly used heterocycles has become crowded through decades of pharmaceutical research. The discovery of new synthetic routes to previously unreported heterocycles will possibly facilitate the discovery of new drugs while minimizing intellectual property overlap. Toward this end, we will present general synthetic routes for the preparation of functionalized and previously unknown nitrogen heterocycles.

MEDI 215

Ugi four component reaction enables expedient synthesis and comparison of photoaffinity probes

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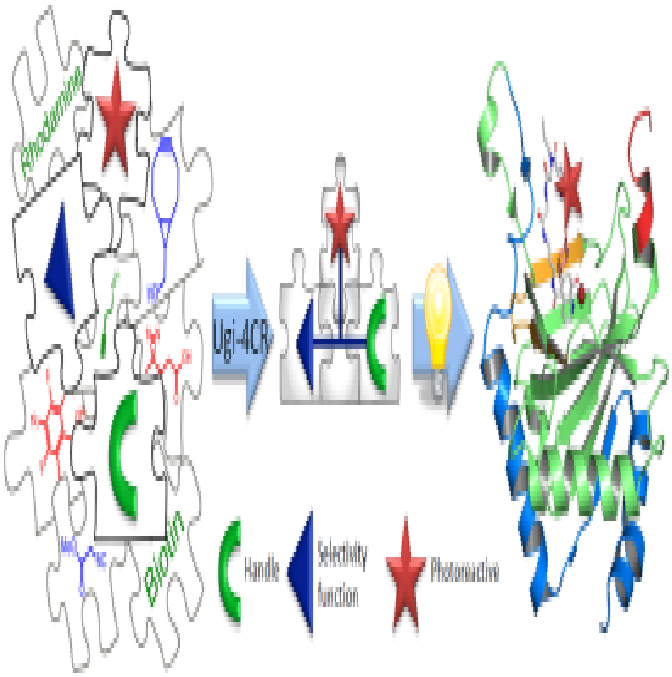
Photoaffinity probes are increasingly being used for the study of biological interactions; however, the lack of structure–activity relationship studies has hindered their rational application. We describe the use of the Ugi four-component reaction (U-4CR) for the expedient and versatile assembly of photoaffinity scaffolds that can be linked to small molecule probes. The rates, yields and sites of crosslinking of five commonly used

photoreactive groups comprising diazirines, aryl azides and a benzophenone, were compared using a human 2-oxoglutarate oxygenase as a model protein. The results reveal significant differences in the behavior of the probes and suggest that empirically guided optimization of probes for specific tasks is desirable. In the absence of such optimization it may be advisable to use a set of crosslinking probes/conditions; the U-4CR provides a convenient method for obtaining such a set.



MEDI 216

Synthesis of indazole based diarylurea derivatives and evaluation of their inhibition activity against human cancer cell growth

Wenbao Li*, wbli92128@163.com. School of Medicine and Pharmacy, Ocean University of China, Qingdao, Shandong 266003, China*

New series of indazole based diarylureas derivatives were synthesized and their anticancer activity against H460, A549, OS-RC-2, HT-29, Lovo, HepG2, Bel-7402, SGC-7901, MDA-MB-231 and PLC/PRF/5 were evaluated. In general, these derivatives showed superior or similar activity against most of these selected cancer cell lines with sorafenib as control. The effect of substituents on the indazole ring was investigated; the derivatives with trifluoromenthy or halogen substituent showed higher activity. Among them, Compound **1** , having 4-(trifluoromenthy)-1H-indazole and 4-(trifluoromenthy) benzene moieties, exhibited the most potent anticancer activity; its efficacy and mechanisms were investigated comparing with sorafenib. Compound **1** significantly inhibited PLC/PRF/5 human hepatocellular carcinoma growth. Flow

cytometry analysis indicated the induction of apoptosis and arrest of cell cycle in G1 phase. Western blotting showed the decrease of cyclin D1 and regulation of apoptotic proteins. Further analysis suggested that these effects of Compound **1** might arise from its roles in the inhibition of multi-kinases, including c-Kit and its downstream targets and the Wnt/β-catenin pathway in PLC/PRF/5 cells. In conclusion, Compound **1** could be developed as a potent anticancer agent that might supplant the use of sorafenib.

MEDI 217

Synthesis of new flavone derivatives against estrogen dependent cancers

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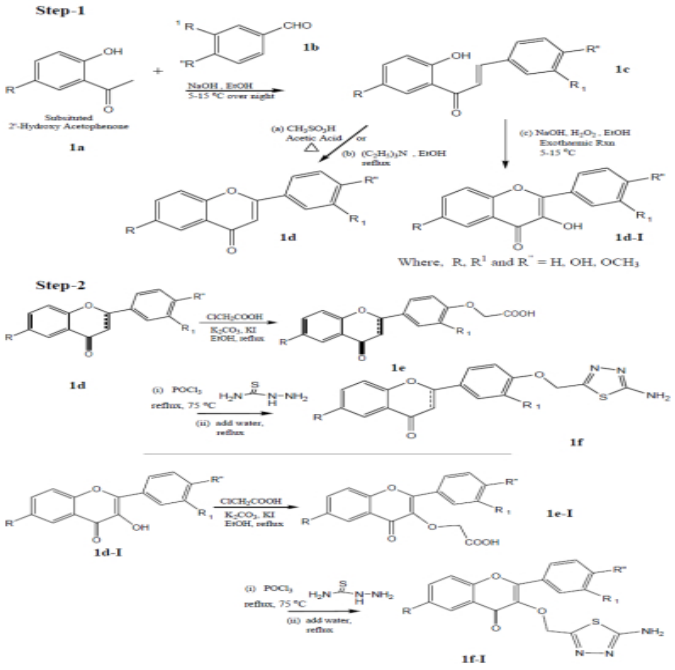
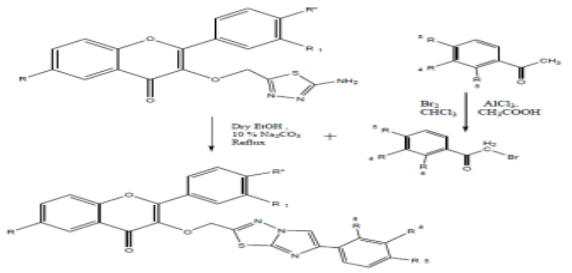
Antiestrogens, SERMs, Aromatase and 17-ßHSD inhibitors are main target of pharmacological interest for the treatment of estrogen dependent cancers. Coumarins, Flavones, Isoflavones have been reported for such inhibition and are used for treatment of brest tumers. So in this topic, Flavone derivatives containing Imidathiadiazole, Thiadiazole, Triazole and benzimidazole hetrocycles were synthesised and charactrarized by IR,^{1}H NMR, ^{13}c NMR spectroscopy and elemantal analysis. This Flavone derivatives were found to exhibit good IC_{50} value against different cell lines like MCF-7, SNB-75 and others.





Derivative Id.	R	R^1	R^{11}	R^2	R^3	R^4	R^5	R^6
2g(h)-A/A1/ A2/ A3/A4	H / OCH_3	H / OCH_3	H / OCH_3	H	H	-	-	-
2g(h)-B /B1/ B2 / B3/B4	H / OCH_3	H / OCH_3	H / OCH_3	OCH_3	H	-	-	-
2g(h)-C / C1/ C2 /C3/C4	H / OCH_3	H / OCH_3	H / OCH_3	H	OCH_3	-	-	-
2g(h)-D /D1 /D2/ D3/D4	H / OCH_3	H / OCH_3	H / OCH_3	H	OH	-	-	-
2g(h)-E /E1 /E2 /E3/E4	H / OCH_3	H / OCH_3	H / OCH_3	OCH_3	OH	-	-	-
3g(h)-A / A1/ A2/ A3/A4	H / OCH_3	H / OCH_3	H / OCH_3	-	-	H	H	H
3g(h)-B / B1/ B2 / B3/B4	H / OCH_3	H / OCH^3	H / OCH^3	-	-	OH	H	H
3g(h)-C / C1/ C2 /C3/C4	H / OCH_3	H / OCH_3	H / OCH_3	-	-	H	H	OH
3g(h)-D /D1 /D2/ D3/D4	H / OCH_3	H / OCH_3	H / OCH_3	-	-	H	OH	OH
3g(h)-E / E1/ E2/ E3/E4	H / OCH_3	H / OCH_3	H / OCH_3	-	-	H	Cl	H
3g(h)-F / F1/ F2/ F3/F4	H / OCH_3	H / OCH_3	H / OCH_3	-	-	H	F	H
3g(h)-G / G1/ G2/ G3/G4	H / OCH_3	H / OCH^3	H / OCH^3	-	-	H	Br	H
3g(h)-H / H1/ H2/ H3/H4	H / OCH_3	H / OCH^3	H / OCH^3	-	-	H	OCH_3	H
3g(h)-I / I1/ I2/ I3/I4	H / OCH_3	H / OCH_3	H / OCH_3	-	-	H	NO^2	H

Where, A = R, R^1 = H
A1 = R = H and R^1 = OCH_3
A2 = R = OCH_3 and R^1 = H
A3 = R = OCH_3 and R^1 = OCH_3
A4 = R^{11} = OCH_3

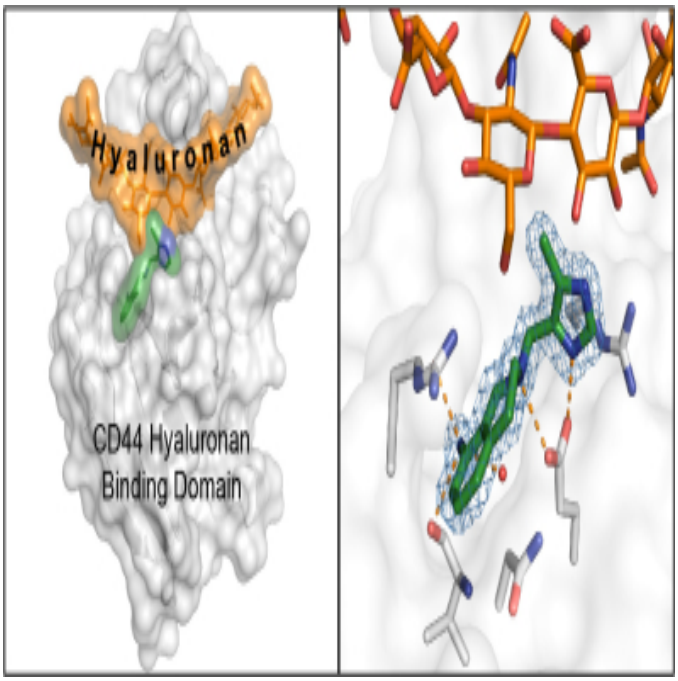
NOTE: i) for 2g and 3g series of derivatives, R^{11} must be –OH
ii) for 2h and 3h series of derivatives, No –OH group should free at R^1 and R^{11}.

MEDI 218

Substituted tetrahydroisoquinolines as selective inhibitors for hyaluronan binding to the tumor cell receptor CD44

***Li-Kai Liu**, liux0754@umn.edu, Barry Craig Finzel. Department of Medicinal Chemistry, University of Minnesota - Twin Cities, Minneapolis, MN 55455, United States*

CD44 is an important cell-surface receptor for hyaluronan (HA), a polymeric glycoamino-glycan that is a major component of the extracellular matrix. Selective inhibitors of HA binding to CD44 will have value as probes of CD44-mediated signaling and have potential as therapeutic agents in chronic inflammation, cardiovascular disease, and cancer. Using biophysical binding assays, fragment screening, and crystallographic characterization of complexes with the CD44 HA-binding domain, we have discovered an inducible pocket adjacent to the HA-binding groove into which small molecules bind. Iterations of fragment combination and structure-driven design have allowed identification of a series of 1,2,3,4-tetrahydroisoquinolines as the first non-glycosidic inhibitors of the CD44-HA interaction



(Liu & Finzel, *JMC* **2014**). X-ray crystallographic complexes of improved analogues are described and the affinity of these molecules for the CD44 HA-binding domain parallels their ability to interfere with CD44 binding to polymeric HA *in vitro*. To evaluate the potential effect of these compounds in living cells, we have established cell-based assays that are sensitive measures of CD44-dependent processes on tumor cell motility, growth and HA-retention.

MEDI 219

Metabolically fortified DPA-714 analogs for improved PET imaging of translocator protein (TSPO)

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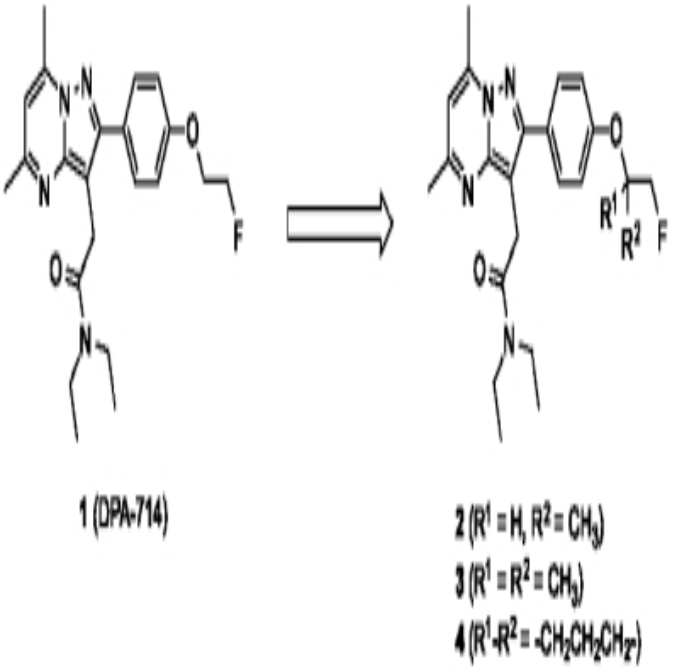
The translocator protein (TSPO) is an 18 kDa, five transmembrane domain protein subunit of a multimeric complex primarily located on the outer mitochondrial membrane. Although TSPO expression in the brain is low under normal physiological conditions, overexpression of TSPO is associated with microglial activation and neuroinflammation, and has prompted the investigation of this target as a biomarker for neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, and stroke.

[^{18}F]DPA-714 (**1** , Fig. 1) is a clinically important radiotracer for positron emission tomography (PET) imaging of TSPO.[1] However, *O*-dealkylation of [^{18}F]DPA-714 is a significant metabolic route in both baboon and human liver microsomes, which provide a level of complexity in developing methods quantitative image analysis .[1]

In an effort to improve the metabolic profile of [^{18}F]DPA-714 in PET imaging, several metabolically fortified analogs have been designed by increasing steric congestion around the alkyl ether. The synthesis and TSPO binding affinities of DPA-714 analogs featuring an α-methyl (**2**), α-*gem*-dimethyl (**3**), or α-cyclobutyl (**4**) substituent will be presented, along with the metabolic profiles of their fluorine-18 radioisotopes.



MEDI 220

Design, synthesis, and biological evaluation of fused heteroaryl derivatives as MEK5 inhibitors

Dhruv Shah[1]*, shahd@duq.edu, Sneha Potdar*[2]*, Patrick T. Flaherty*[1]*, Jane Cavanaugh*[2]*. (1) Department of Medicinal Chemistry, Duquesne University, PIttsburgh, PA 15282, United States Virgin Islands (2) Department of Pharmacology, Duquesne University, PIttsburgh, PA 15282, United States Virgin Islands*

MAPK (Mitogen Activated Protein Kinase) signaling cascade mediates external cell signaling events with internal cellular responses. They are involved in complex cellular functions like cell differentiation, proliferation and growth. MEK is activated by binding of numerous extracellular mitogens or ligands. This kinase cascade has multiple

interacting levels with the phosphorylation of a unique ERK (Extracellular signal-Regulated Kinases), by its unique activating kinase, or MEK, which is a unique parallel event. ERK5 is the only known substrate of MEK5. The ERK5/MEK5 signaling cascade is upregulated in specific cancers like breast cancer and prostate cancer. Novel fused analogs based on prior SAR and homology modeling of MEK5 were synthesized. Design, synthesis, and biological evaluation of novel hetero-aryl compounds as MEK5 inhibitors will be presented.

MEDI 221

Synthesis and biological evaluation of novel flavonoid derivatives as anticancer agents

Nelly Mateeva[1]*, nelly.mateeva@famu.edu, Donka Tasheva*[2]*, Krista Cabret*[1]*, Bereket Mochona*[1]*, Kinfe Redda*[3]*. (1) Department of Chemistry, Florida A&M University, Tallahassee, Florida 32307, United States (2) Department of Chemistry, Sofia State University [quot]St. Kl. Ohridski[quot], Sofia, Bulgaria (3) College of Pharmacy and Pharmaceutical Sciences, Florida A&M University, Tallahassee, FL 32307, United States*

Flavonoids – a broad family of polyphenolic compounds, have been identified as potent and promising anti-cancer agents. In this study, series of novel chalcones and flavones were synthesized and tested as inhibitors of cell growth and proliferation on several breast and prostate cancer cell lines. The compounds contain variety of substituents and the biological studies have shown a distinctive pattern of activities depending on the structure and mutual position of the functional groups. Polyhydroxylated derivatives exhibit lower activity compared to those bearing chlorine, fluorine, and nitro groups in ring “B”. 3-hydroxy flavones have comparable inhibition properties, however their solubility is considerably decreased. The alkyl substituents in ring “A” do not affect the inhibition properties of the compounds.

MEDI 222

Discovery of a KIT Exon 17 mutant-targeted inhibitor

Jack Lin*, jlin@plexxikon.com, Guoxian Wu, Marika Nespi, Yan Ma, Bernice Matusow, Ying Zhang, Garson Tsang, Katrina Chan, Heidi Carias, Chao Zhang, Gaston Habets, Wayne Spevak, Prabha Ibrahim, Gideon Bollag. Plexxikon, Inc., Berkeley, California 94710, United States*

The stem cell factor KIT is a type III receptor tyrosine kinase (RTK). Mutational activation of KIT has been well-documented in mastocytosis and a number of cancers. Inhibiting oncogenic KIT mutations is a validated therapeutic approach to treat gastrointestinal stromal tumors (GIST). The use of clinically approved KIT inhibitors such as imatinib and sunitinib frequently reveal secondary or acquired mutations that render these first-generation KIT inhibitors ineffective. Some of the acquired mutations

reside in exon 17, coding for amino acids in the activation loop of the folded protein. This set of mutations has been shown to constitutively activate the receptor. Using a scaffold-based drug discovery approach, we have successfully identified a series of novel KIT exon 17 mutant-targeted inhibitors that showed minimal cross-reactivity in a kinome wide screen. A lead compound has demonstrated both in vitro and in vivo anti-tumor activity, and currently is in preclinical toxicity testing.

MEDI 223

Towards dual kinase-bromodomain inhibitors for the treatment of acute myeloid leukemia (AML)

*Lijia Chen[1], Makoto Yoshioka[2], Jeffery Strovel[2], **Steven Fletcher**[1], sfletcher@rx.umaryland.edu. (1) Department of Pharmaceutical Sciences, University of Maryland, Baltimore, Baltimore, MD 21201, United States (2) ConverGene, Gaithersburg, MD 20878, United States*

Kinases that are dysregulated in cancer have proven highly effective targets towards the treatment of this disease through the focused blockade of key signaling pathways with small-molecules. However, the efficacy of kinase-selective inhibitors has not met with the expected promise, likely owing to the compensatory upregulation of other signaling pathways. Similarly, the bromodomain epigenetic reader proteins are also involved in tumorigenesis. Bromodomains (BRDs) are protein modules that recognize acetylated lysines of histones, and are involved in transcriptional control. BETs control the expression of genes that are essential for tumor growth, such as c-Myc, and survival, such as Mcl-1.

Acute myeloid leukemia (AML) is driven by FLT3 receptor tyrosine kinase and BRD4. The dual inhibition of kinases and bromodomains might, therefore, afford an especially potent treatment for cancers such as AML. It has recently been demonstrated that certain potent kinase inhibitors are also potent bromodomain inhibitors, for example BI-2536 and TG-101348. Herein we describe our efforts towards the optimization of the dual kinase-bromodomain inhibitor BI-2536 as a potential treatment for AML.

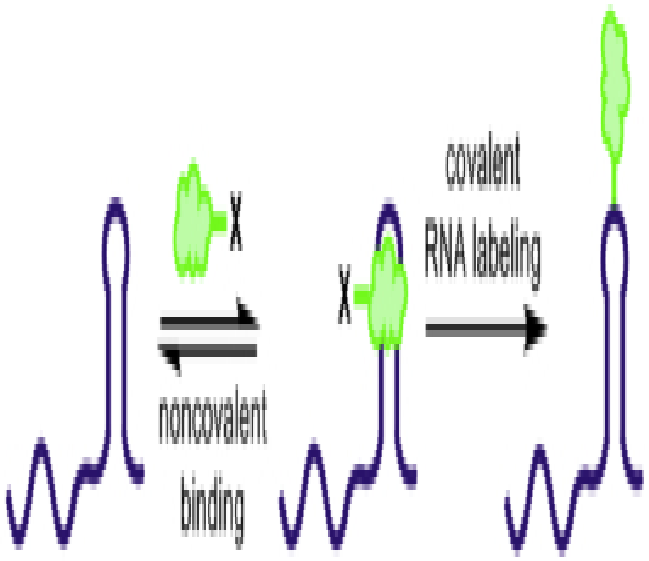
MEDI 224

Covalent mRNA labeling using fluorescently self-alkylating ribozymes

***April Anamisis**[1], anamisisa@gmail.com, Ashwani K. Sharma[1], Joshua J. Plant[1], Alex E. Rangel[1], Kirsten N. Meek[1], Jennifer M. Heemstra[1], Julie Hollien[2]. (1) Department of Chemistry, University of Utah, Salt Lake City, Utah 84106, United States (2) Department of Biology, University of Utah, Salt Lake City, UT 84112, United States*

Aberrant RNA localization plays a role in many diseases including cancer, Alzheimer's, and Huntington's disease. Therefore, developing techniques for visualizing mRNA localization in cells would provide insight into these diseases. Currently, the most

popular method for labeling mRNA involves using a genetically encoded green fluorescent protein (GFP) tag. This allows for direct visualization of mRNA in the cell.However, the protein fusion is large and can disrupt normal mRNA transport in the cell and native mRNA structure.We have developed a ribozyme that covalently self-alkylates with fluorescein and could be fused to an mRNA sequence of interest. Benefits of this covalent linkage include providing a robust complex that prevents signal loss, the ability to wash out any unbound fluorescein and reduce background fluorescence, and a broader fluorophore scope. This method may enable the rapid screening of small molecules for efficacy in modulating cellular RNA localization.



A general scheme of ribozyme labeling with a fluorescent probe, where x represents a leaving group.

MEDI 225

Small molecule inhibitors of the BCL6 BTB domain for DLBCLs

*Shaymaa Emam Kassab, Wenbo Yu, Alexander MacKerell, Jr., **Fengtian Xue**, fengtian.x@gmail.com. Pharmaceutical Sciences, University of Maryland Baltimore, Baltimore, MD 21201, United States*

BCL6 is the most commonly involved oncogene in diffuse large B-cell lymphomas (DLBCLs) and is frequently expressed in follicular lymphomas (FLs). BCL6 achieves its biological functions by binding to the SMRT co-repressor through a unique interaction mediated by the N-terminal BTB domain. Using computer-aided drug design (CADD),

we have designed small molecules to the BCL6 BTB domain to displace SMRT from its repression complex with BCL6. A new family of inhibitors has been synthesized and their activities have been determined by a variety of in biological assays. One inhibitor of the new family showed exceptional in profile both in vitro and in vivo.

MEDI 226

Synthesis, anticancer, and antioxidant activities of some pyrrolyl-1-3,4-oxadiazole and pyrrolyl-4-aminoantpyrine Schiff base analogs

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A series of 24 compounds that comprise pyrrolyl- 1,3,4-oxadiazole and pyrrolyl- 4-aminoantipyrine pharmacophores were subjected to Molinspiration virtual screening protocol, compounds with maximum predicted drug-likeness model score among the series were synthesized and screened for anticancer and antioxidative activities. The anticancer activity against a panel of prostate cancer cell lines and breast cancer cell lines displayed moderate inhibitory activities. Among the synthesized compounds, compound 4 and 7 with pyrrolyl-1,3,4-oxadiazole moiety emerged with moderate antioxidant (DPPH test) as well as anticancer agent for further investigation.

MEDI 227

Hydrogen peroxide responsive thiazolidinone-based prodrugs

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Prodrugs are inactive derivatives of drug molecules that become activated upon exposure to a specific chemical or enzymatic stimulus. Prodrug strategies often serve to improve the drug-like properties of molecules by increasing bioavailability and solubility, and minimizing off-target interactions. Reactive-oxygen species (ROS)-activated prodrugs have emerged as promising prodrug strategies, because they can facilitate targeted release to areas of inflammation or certain cancer cells. In this study, we explored a novel approach for ROS-activated prodrugs based on a thiazolidinone protecting group. This strategy can be used to mask carboxylic acid groups that are revealed upon exposure to H_2O_2. This work explored the kinetic response to H_2O_2, the stability of the prodrug in a simulated physiological environment, and the inhibitory profile of the prodrug compared to the ROS-activated drug of interest.

MEDI 228

5-Substituted Pyrimido[4,5-*b*]indoles with single agent combination chemotherapeutic potential

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Combination chemotherapy of cytotoxic agents with antiangiogenic agents have shown significant promise in cancer treatment, and several such clinical trials are currently underway. We previously reported a series of 5-(thioaryl)-9*H*-pyrimido[4,5-*b*]indole-2,4-diamines as inhibitors of vascular endothelial growth factor receptor-2 (VEGFR-2) and platelet-derived growth factor receptor-ß (PDGFR-ß) for antiangiogenic effects. These compounds remarkably also inhibit human thymidylate synthase (hTS) as cytotoxic agents. In a COLO-205 xenograft mouse model, one of these analogs demonstrated potent tumor growth inhibition, inhibition of metastasis, and antiangiogenic effects *in vivo*. In an effort to elucidate the structural requirements that influence dual antiangiogenic and cytotoxic effects of these single agents, we designed a series of 5-substituted aryl-9*H*-pyrimido[4,5-*b*]indoles with isosteric replacements of the linker at the 5-position. The design, synthesis and molecular modeling studies of these compounds will be reported.

MEDI 229

Conformationally restricted pyrrolo[2,3-*d*]pyrimidines as potential antimitotic and antitumor agents

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One of the most effective ways to treat tumors is to disrupt cellular microtubule. The taxanes and vinca alkaloids are classes of compounds which bind directly to tubulin/microtubules and have excellent antitumor and anticancer effects. However, multidrug resistance is a major limitation of these classes of drugs. We previously reported N-(4-methoxyphenyl)-N,2-dimethyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (**1**) (IC_{50}= 183 nM) as an inhibitor of the proliferation of human cancer cells (MDA-MB-435). Compound **1** inhibited tubulin polymerization and the binding of [^{3}H]colchicine to tubulin. It also circumvented P-glycoprotein (Pgp) and ßIII-tubulin mediated resistance that limits efficacy of paclitaxel and the vinca alkaloids. On the basis of the anti-tubulin activity of **1** , we designed conformationally restricted analogs with various substitutions in the 2'- and/or 6'-positions of the phenyl ring that effectively restricts the rotation of the phenyl ring. ^{1}HNMR studies suggest that the conformation is indeed restricted in these 2'- and/or 6'-phenyl substituted analogs compared to the phenyl unsubstituted compounds.

The synthesis, NMR spectra and biological activity of these analogs will be presented and discussed.

MEDI 230

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

***Chris Williams**, cwilliams@chemcomp.com. Chemical Computing Group, Montreal, QC H3A2R7, Canada*

The kinase activity of epidermal growth factor receptor (EGFR) is the target of a several commercial antineoplastic agents such as Tarceva and Iressa. Despite their efficacy, EGFR kinase inhibitors can be often plagued by moderate antiproliferative activity against certain tumour types in the clinic. To circumvent the traditional pathway and using structure based design techniques, dual action compounds termed "combi-molecules" were designed and investigated to examine a synergistic approach targeting both EGFR kinase and Src kinase or DNA.

MEDI 231

Improved automated clinical production of ^{68}Ga-DOTA-TATE for targeting somatostatic receptor-positive neuroendocrine tumors

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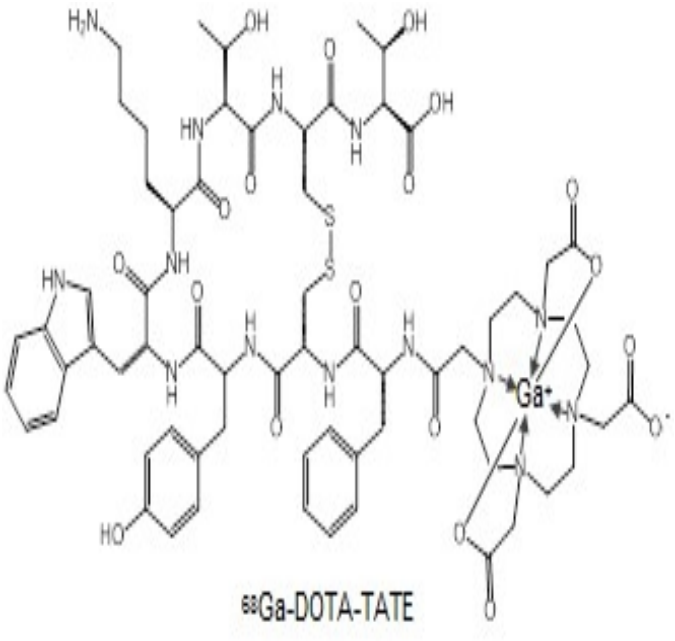
* Contributed equally to this work

Many previous studies indicate improved diagnostic capability of ^{68}Ga-labeled DOTA-somatostatin analogs (e.g., ^{68}Ga-DOTA-TATE) with positron emission tomography (PET) over conventional techniques (e.g., Octreoscan/MRI/CT) for imaging somatostatin receptor-expressing neuroendocrine tumors (NET). Since ^{111}In-octreotide imaging was the only NET-specific scan available in the United States until recently, we sought and obtained FDA approval for using ^{68}Ga-DOTA-TATE to examine patients with somatostatic receptor-positive tumors, and we report our first experience herewithin.

Unlike other North American sites that produce ^{68}Ga-labeled DOTA-TATE by manual methods, we produce ^{68}Ga-DOTA-TATE using an automated cassette-based platform that incorporates a new “acetone-free” purification step for ^{68}Ga, which is eluted from a ^{68}Ge/^{68}Ga generator via a cation exchange cartridge. Concentrated NaCl/HCl solution is used to elute the ^{68}Ga from the cartridge (as opposed to the prevailing acetone-based elution method) for the subsequent radiolabeling step. After the final product is purified by solid phase extraction, it is formulated and sterilized for injection. Quality control and

release criteria were set according to USP‹823›. The radiochemical yield was 75.6±6.6% (decay-corrected to start-of-synthesis, n=13 patients).

Clinical PET images obtained with ^{68}Ga-DOTA-TATE showed tremendous sensitivity for lesion detection compared to our current standard of care planar/SPECT imaging with ^{111}In-labeled octreotide. Thus, through relatively simple implementation of this novel automated clinical radiochemistry, we can now provide ^{68}Ga-DOTA-TATE as a new standard of care and treatment for our patients with somatostatic receptor-positive tumors.



^{68}Ga-DOTA-TATE

MEDI 232

Effects of curcumin analogs on proliferation and cell death of cervical and prostate cancer cells

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The investigation in our research group on curcumin aims to engineer curcumin analogs with improved bioavailability and enhanced potency as anti-cancer agents. Recently, a class of promising analogs, possessing better cytotoxicity than curcumin against two metastatic prostate cancer cell lines, has been reported by us in European Journal of Medicinal Chemistry. To explore the in-depth cell death mechanisms of these promising curcumin analogs, we started with a systematic investigation on their effects on cancer

cell plasma membrane integrity (compromised during necrosis), and cancer cell proliferation (compromised during both necrosis and apoptosis). These studies were performed using 2 cancer cell models: an aggressive cervical cancer cell (HeLa) line and a hormone-independent cancer cell (PC-3) line. The cytotoxicity of this panel of curcumin analogs (32 compounds in total) towards the aggressive cervical HeLa cancer cell line was tested using trypan blue dye exclusion method and a cell viability analyzer (Beckman Coulter). The in vitro antiproliferative activities of these synthetic analogs were measured with a WST-1 based assay in the HeLa cervical cancer and PC-3 prostate cancer cell lines. The effects of these curcumin analogs on necrosis and cell proliferation in the HeLa and PC-3 cell lines will be presented. The cell death pathways caused by these analogs will also be discussed in terms of stimulation of apoptosis or necrosis.

MEDI 233

Design, synthesis, and biological evaluation of 6-amino-5-chloro-2-methyl N^4-substituted pyrimidine analogs as potential antitubulin agents

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Agents that disrupt tubulin assembly are some of the most widely used cancer chemotherapeutics drugs. Inhibition of tubulin assembly can be achieved by targeting different sites of binding on tubulin, namely, the vinca binding site, the taxol binding site and the colchicine binding site (CBD). Currently CBD is of particular interest as there are no clinically approved agents available which bind to this site. In addition these agents provide the advantage of being effective against *β*-III resistant cancer cell lines. We have previously reported a series of 5-amino-6-chloro-2-methyl N^4-substituted pyrimidines as potent anti-tubulin agents. These compounds circumvent *β*-III tubulin and Pgp- mediated drug resistance. These monocyclic compounds also have the advantage of being water soluble. To further explore the SAR of monocyclic pyrimidines as potential anti-tubulin agents additional analogs were designed and synthesized by interchanging the 5-amino and 6- chloro groups in the parent scaffold to determine its effect on biological activity. The design, synthesis and biological activities of these compounds will be presented.

MEDI 234

Side chain modifications of mithramycin SA for improved anticancer activity

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Mithramycin (MTM) is a potent anticancer agent produced naturally by soil bacteria of the *Streptomyces* genus. MTM was used clinically in the 1960s but was abandoned due to unwanted side effects. More recently, genetic engineering to inactivate the last gene of the MTM biosynthetic pathway led to the development of the M7W1 mutant strain and the production of several new MTM analogues, SK, SDK, and SA. All of the analogues include a shortened aglycon side chain and MTM SK and SDK show improved anticancer activity. However, MTM SA drastically reduces the potency compared to the parent molecule. MTM SA differs from MTM SK and SDK in that the aglycon side chain of both MTM SK and SDK contain ketone functionalities while MTM SA is terminated with a carboxylic acid. The carboxylic acid is not favorable for the biological activity of the molecule but does offer a prime location to selectively modify the molecule through the coupling of a primary amine. To that end, MTM SA was functionalized with a variety of small molecules containing a primary amine to replace the terminal carboxylic acid and the anticancer activity investigated. Several of these modifications led to new derivatives with much improved anticancer activity. Interestingly, some of the bulkier modifications enhanced the potency beyond that of MTM SK and SDK. The ability to selectively modify a molecule in hopes of tailoring activity should allow increased cancer specificity and the decrease of unwanted side effects.

MEDI 235

Transmembrane domains of the bacterial cell division proteins FtsB and FtsL form a stable high-order oligomer: A FRET analysis

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FtsB and FtsL are two essential integral membrane proteins of the bacterial division complex or 'divisome', both characterized by a single transmembrane helix and a juxta-membrane coiled coil domain. The two domains are important for the association of FtsB and FtsL, a key event for their recruitment to the divisome that in turn enables recruitment of the late divisomal components and subsequent completion of the division process. We have previously established that the transmembrane domain of FtsB self-associates in Escherichia coli membranes using a biological assay in vivo. We hypothesized that the FtsB dimer forms a core for the lateral association of FtsL, leading to the the assembly of a higher-order oligomeric FtsB-FtsL complex. Here we present a biophysical analysis performed in vitro that further supports this hypothesis. Using FRET, we have measured the association of fluorophore-labeled transmembrane domains of FtsB and FtsL in both detergent and lipid. Our findings demonstrate that these helices form a very stable higher-order oligomeric complex in isolation. The data also suggest that the transmembrane component is likely to be a major contributor to the stability of the FtsB-FtsL complex.

MEDI 236

Computationally designed peptides targeting the amyloid precursor protein transmembrane domain

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The Amyloid Precursor Protein (APP) is a type-I transmembrane glycoprotein present at the neuronal synapses. The proteolytic cleavage by γ-secretase of its C-terminal fragment produces amyloid-β (Aβ) peptides of different lengths, the deposition of which is an early indicator of Alzheimer's disease (AD). The APP-TM contains two GXXXG and one GXXXA motif, which are believed to mediate dimerization in transmembrane proteins. Mutations in APP transmembrane domain affect significantly the length of Aβ peptides. The importance of APP dimerization and its impact on the cleavage remain however unclear.

Computational design peptides (CHAMP) have proven to be valuable method for binding specifically to transmembrane domains. We therefore implemented a fully automatized protocol for the design of CHAMP peptides. An empirical pairwise force field was optimized and our protocol was integrated to the ROSETTA software suite. Two different sets of CHAMP peptides were designed to target the different dimerization sites of APP. Taken together, our results allowed gaining insight into the importance of dimerization of APP and its impact on the processing by γ-secretase.

MEDI 237

Discovery of dual inhibitors targeting both WT and the S31N mutant of the influenza A virus M2 proton channel

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Influenza virus infection poses a continuously global health challenge that cause numerous deaths and millions of hospitalizations. One difficulty facing anti-flu drug development is the heterogeneity of the circulating flu viruses, which compose of different strains with variable sensitivity to antiviral drugs. For example, the wild type (WT) influenza A virus M2 proton channel (A/M2) is sensitive to antiviral drugs amantadine and rimantadine; while the S31N mutant of M2 is resistant to these drugs. Thus, antiviral drugs targeting both WT and the S31N are highly desired. We report our

design of a novel class of such dual inhibitors targeting both the WT and the S31N muant and their ion channel blockage and antiviral activities. The potency of the most active compound in inhibiting WT and the S31N mutant viruses is comparable with that of amantadine in inhibiting WT flu viruses. Solution NMR studies and molecular dynamics (MD) simulations of drug-M2 interactions reaffirmed our design hypothesis: the drug binds in the WT M2 channel with its aromatic headgroup facing down towards the C-termini; while the same drug binds in the S31N M2 channel with its headgroup facing up towards the N-termini. The discovery of this flipping mode of binding correlates with the structure-activity relationship we observed and has paved the way for the next round of rational design of antiviral drugs with broad specificity.

MEDI 238

Structure and function of bilayer stress-sensing transmembrane domains

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Many antibiotics work by disrupting the bacterial cell membrane. Histidine Kinase (HK) two-component systems are part of an essential stimulus-response circuit used by bacteria to sense antibiotic-induced stress and develop resistance.

A typical HK two-component system consists of a transmembrane kinase that senses the antibiotic-induced environmental change and relays the information to a cytoplasmic response regulator via a phosphorylation cascade. While much is known about the cytoplasmic parts of the HKs, the transmembrane domains that sense membrane stress remain structurally elusive.

We report cloning, purification and biophysical characterization of the transmembrane domain from a histidine kinase associated with Nisin resistance in *S. aureus*. Nisin is a lantibiotic that is a commonly used suppress pathogenic gram-positive species in food products. Structural characterization of the transmembrane helix dimer by analytical ultracentrifugation, circular dichroism and NMR are presented, and insights into the mechanisms by which transmembrane stress is sensed are discussed.

MEDI 239

Examining disease-associated Aβ mutants as conformational strains

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The major pathological hallmark of Alzheimer's disease is the accumulation of β-amyloid (Aβ) peptide within amyloid plaques in the brain. When forming the cross-β

structure typical of amyloid fibrils, there is some inherent flexibility regarding the intermolecular contacts that the Aβ peptide can adopt. As a result, the fibril structure may vary, resulting in distinct conformational strains. While a specific primary sequence is able to adopt multiple strain conformations, it is not known how disease-associated mutations occurring within that sequence may alter the fibril structure. We are using biophysical methods to determine whether certain Aβ mutants form unique fibril strains that propagate their particular structure irrespective of primary sequence.

MEDI 240

Modeling the antibody-eliciting conformation of the HIV-1 gp41 MPER

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The Human Immunodeficiency Virus (HIV-1), which causes Acquired Immunodeficiency Syndrome (AIDS), remains a serious health problem in the United States and around the world despite extensive research efforts into treatment and prevention. Most vaccine candidates thus far have failed to provide immunity, which occurs through the induction of broadly neutralizing monoclonal antibodies. The membrane proximal external region (MPER) of HIV's envelope glycoprotein (gp41) is a promising vaccine candidate, as it has high sequence conservation among strains and is transiently exposed before fusion with the host. Several broadly neutralizing monoclonal antibodies that recognize the MPER have been discovered to date, and binding of these antibodies irreversibly inactivates the virus. Further, the adjacent transmembrane domain (TM) and the viral membrane itself are involved in antibody binding and recognition. Unfortunately, there are limited structural data available on the MPER for use in rational vaccine design efforts, particularly in the context of the TM and viral membrane. Here, we present several μs-timescale, all-atom molecular dynamics simulations of the MPER+TM bound to broadly neutralizing antibodies (2F5, 4E10, 10E8, Z13e1) in a viral-like membrane in order to discern the antibody-eliciting conformations of the MPER.

MEDI 241

Stabilization of radicals with a *de novo* designed protein

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In nature organic radicals exist transiently as unstable intermediates due to their high reactivity. Quinones and hydroquinones are nature's most widely used electron carriers in redox processes (such as Coenzyme Q, Photosystem II, Vitamin K, Superoxide

Dismutase, and xenobiotics). Semiquinone ($SQ^{\bullet -}$) is the highly unstable reaction intermediate in the two electron reduction of quinone (Q) to hydroquinone (QH_2).

Enzymes of the broad genre of tyrosinases catalyze the oxidation of catechol (*ortho*-hydroquinone) to *ortho*-benzoquinone, presumably via an *ortho*-semiquinone ($SQ^{\bullet -}$) type intermediate; however, due to its transient nature, this intermediate has not been directly observed.

We developed a *de novo* designed protein (DFsc) that exhibits a binding pocket for providing a bimetallic site comprising an array of first-row transition metals, Zn(II), Fe(II), Cu(II), Ni(II), and Mn(II). This bimetallic site supported by the *de novo* protein provides an environment that captures and stabilizes the bound semiquinone ($SQ^{\bullet -}$) intermediate.

MEDI 242

Computational design of metallotransporter

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Protein design has significantly advanced to allow incorporation of desired artificial features to protein structure, assembly and function, yielding rich understanding in how structure relates to function. However, similar investigations with transmembrane proteins are extremely rare due to limited knowledge and technical challenges. Here, we present computational design of a transmembrane helix that self-assembles into a stable three-dimensional structure capable of generating conformational dynamics linked to co-transportation function. De-novo minimalist transporter features dual-subunit topology consisting a 25-residue-long anti-parallel homo-tetrameric bundle that uses proton gradient to drive Zn(II) up its gradient across the bilayer of lipid vesicles, indicated by solids NMR, solution NMR, molecular dynamics simulations and functional assay. Together with its subunit's crystal structures, the first for designed transmembrane domain, our data and simulations critically corroborate the mechanistic importance of structural inter-conversion proposed in alternating access theory for transporters and support gene-duplication proposed for evolution of their dual topology.

MEDI 243

Using de novo protein models to understand functional tuning in binuclear non-heme iron enzymes

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Correlations between catalytic function and the number and orientation of active site His residues in binuclear non-heme iron enzymes suggest that they play an important role in functional tuning. De novo four-helix bundle proteins such as DFsc are uniquely suited for studying these structure-function relationships due to their simplicity, stability, and ease of mutation. The recent alteration of function from oxidation to N-hydroxylation in DFsc through four mutations (one active site His and three supporting mutations) demonstrates the feasibility of using this scaffold for investigations into the geometric and electronic factors that influence catalytic tuning of di-iron active sites. To complement the existing 2-His/4-carboxylate and 3-His/4-carboxylate variants of DFsc, we have rationally redesigned the DFsc active site to mimic those found in nature that contain additional carboxylates (rubrerythrins, symerythrin) or additional histidines (FprA, MIOX). The secondary structure, stability, and metal-binding capacity of these new proteins have been characterized. In addition, their ability to catalyze a range of O_2- and H_2O_2-dependent reactions has been investigated. As the active sites of the proteins in this series differ stepwise by either a single His or carboxylate residue, comparisons of the spectroscopic and geometric properties of these structurally-similar but functionally distinct proteins provide insight into the roles of charge and coordination number in tuning catalytic activity.

MEDI 244

Polysaccharide lysae from *S. maltophilia* with unique, pH-regulated substrate specificity

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Anionic polysaccharides are of growing interest in the biotechnology industry due to their potential pharmaceutical applications in drug delivery and wound treatment. Chemical composition and polymer length strongly influence the physical and biological properties of the polysaccharide and thus its potential industrial and medical applications. One promising approach to determining monomer composition and controlling degree of polymerization involves the use of polysaccharide lyases, which catalyze the depolymerization of anionic polysaccharides via a b-elimination mechanism. A putative alginate lyase (Smlt1473) from *S. maltophilia* was heterologously expressed in *E. coli*, purified in a one-step fashion via affinity chromatography, and activity as well as specificity determined for a range of polysaccharides. Interestingly, Smlt1473 catalyzed the degradation of not only alginate, but poly-b-D-glucuronic acid and hyaluronic acid as well. Furthermore, the pH optimum for enzymatic activity is substrate-dependent, with optimal hyaluronic acid degradation

at pH 5, poly-b-D-glucuronic acid degradation at pH 7, and alginate degradation at pH 9. Additionally, single point mutations of His^{221} andArg^{312}, two putative substrate binding residues located in the active site cleft, resulted in increased activity and specificity towards poly-β-D-mannuronic acid and poly-b-D-glucuronic acid, respectively. Collectively, these results imply that Smlt1473 is a multifunctional PL that exhibits broad substrate specificity, but utilizes pH as a mechanism to achieve selectivity. Furthermore, mutation of substrate binding residues resulted in significant changes in substrate specificity, indicating the potential use of Smlt1473 as a platform to engineer lyases which generate products of a desired size and composition for various industrial applications.

MEDI 245

Transition metal catalysts as novel tools for intracellular enzyme inhibition

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Transition metals have found broad applicability for human health, most often as drugs and diagnostic imaging agents. In contrast, one of the most common commercial applications of small transition metals, catalysis, has been largely unexplored as a tool for the study and treatment of disease. This void in the field is attributable in part to long-held dogmas about the toxicity and instability of transition metal catalysts in biological environments. Only now, on the heels of important advances in biocompatible catalyst development by our lab and others, is small molecule transition metal catalysis inside living cells a possibility. We herein demonstrate a broadly applicable strategy for transition metal induced control of enzyme inhibition by catalytic uncaging of prodrugs. We also discuss the advantages of this method, specifically “On Demand” temporal control of prodrug uncaging, over current technologies. This technology will be demonstrated to be useful for the inhibition of kinases, which are key targets for the study and treatment of diseases ranging from cancer to Alzheimer's disease and diabetes. We also describe a method for the transition metal catalyzed intracellular synthesis of molecular libraries to streamline the drug discovery process by combining screens for cellular drug activity and toxicity into a single, high-throughput cellular assay. This technology is being developed using a protein tyrosine phosphatase model system, which is a highly important but underdeveloped class of enzyme for drug discovery.

MEDI 246

Integrin allbb3-mediated c-Src activation: Differential b3 binding to inactive and active c-Src

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It is currently thought that inactive tyrosine kinase c-Src in platelets binds to the cytoplasmic tail of the b3 integrin subunit via its SH3 domain. Although a recent NMR study supports this contention, it is likely that such binding would be precluded in inactive c-Src because an auto-inhibitory linker physically occludes the b3 tail binding site. Accordingly, we have re-examined c-Src binding to b3 by immunoprecipitation as well as NMR spectroscopy. In unstimulated platelets, we detected little to no interaction between c-Src and b3. Following platelet activation, however, c-Src was co-immunopreciptated with b3 in a time-dependent manner and underwent progressive activation as well. We then measured chemical shift perturbations in the ^{15}N-labeled SH3 domain induced by the C-terminal b3 tail peptide NITYRGT and found that the peptide interacted with the SH3 domain RT loop and surrounding residues. A control peptide whose last three residues where replaced with those of the b1 cytoplasmic tail induced only small chemical shift perturbations on the opposite face of the SH3 domain. Next, to mimic inactive c-Src, we found that the canonical polyproline peptide RPLPPLP prevented binding of the b3 peptide to the RT loop. Under these conditions, the b3 peptide induced chemical shift perturbations similar to the negative control. We conclude that the primary interaction of c-Src with the b3 tail occurs in its activated state and at a site that overlaps with PPII binding site in its SH3 domain. Interactions of inactive c-Src with b3 are weak and insensitive to b3 tail mutations.

MEDI 247

Effects of ionic liquids on protein folding-unfolding transitions

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Ionic liquids are fully ionic salts which exist as liquids at room temperature. Ionic liquids are well known to have negligible vapor pressures, are nonflammable, and have a wide range of solvent compatibility. As such, these ionic liquids have garnered much interest from analytical and physical chemists interested in exploiting the ionic properties in applications such as batteries fuel cells, co-solvents, and other electrochemical applications. Recent investigations into the compatibility with biomolecules in aqueous solutions (where the ionic liquids exist as strong electrolytes) have resulted in a range of results including both stabilization and destabilization of proteins, as well as enhancement and inhibition of enzymatic activity. The specific effects on activity are determined both by the cation/anion pair that comprises the ionic liquid as well as the model system it is interacting with. We previously showed that the aqueous solutions supplemented with the ionic liquid $BMIBF_4$ significantly destabilized the folded structure of myoglobin whereas aqueous solutions supplemented with the related ionic liquid EMIAc had minimal effect on the stability of the folded structure when analyzed using traditional GuHCl titration experiments. We've extended these studies to include a number of other denaturation approaches including traditional thermal denaturation as well as other common chemical denaturants such as urea and SDS. We monitored the

unfolding of myoglobin using circular dichroism, absorbency, and fluorescence spectroscopy (FRET). Our results show that the inclusion of relatively low concentrations of the ionic liquid $BMIBF_4$to neutral pH solutions of myoglobin similarly destabilized the folded protein structure in thermal and other chaotrope induced denaturations. The destabilization of proteins by these and related ionic liquids will provide routes to studying protein folding intermediates, hard to denature proteins, and gain insights into protein ligand interactions to enhance drug design and accessibility to occluded binding sites.

MEDI 248

Hydrophobic amino acid SAR in antimicrobial peptides

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Antimicrobial peptides are part of the innate immune system and have been an area of great interest due to their small size and broad-spectrum activity. Numerous studies on antimicrobial peptides from a variety of sources including humans, reptiles, insects, and other mammals have shown no conserved sequences but conserved gene including amphiphilicity, tendency to form Alpha helical structures, net positive charge, and a propensity to interact with lipid membranes. The proposed mechanism of action is through disruption of the bacterial membrane. This study investigates the peptide C 18 G, derived from a human platelet protein, by varying the hydrophobic amino acids in the sequence. The parent peptide was composed of solely leucine residues as the hydrophobics which were replaced with phenylalanine, isoleucine, valine, and the nonproteinogenic amino acid 2-aminoisobutyric acid. Peptide affinity for model lipid membranes was monitored by tryptophan fluorescence spectroscopy. The structure and topography of the peptide when membrane-bound was investigated using fluorescence quenching and circular dichroism spectroscopy. Antimicrobial efficacy and assays of bacterial membrane permeablization using nonnatural chromogenic substrates were also performed. The parent peptide C 18 G exhibited good binding affinity for an ionic lipid membranes, broad-spectrum antimicrobial activity against both gram-positive and gram-negative species, and a dose dependent ability to permeabilize bacterial membranes Results show that substitution of leucine with phenylalanine had little effect but substitution with isoleucine had a more pronounced effect on both membrane binding and antimicrobial efficacy. In contrast, substitution of leucine with either valine or 2-aminoisobutyric acid completely inhibited the interactions with the model lipid membranes and significantly reduced the antimicrobial efficacy.

MEDI 249

Understanding production of reactive oxygen species in amyloid plaques using small peptide models

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Amyloid-forming peptides can bind Cu(II) and mediate formation of reactive oxygen species (ROS), which in turn may contribute to amyloid toxicity in Alzheimer's disease. De novo designed 7-residue peptides assemble in the presence of Cu(II) into fibrils similar to those of Aβ. These fibrils are capable of generating ROS in the presence of H_2O_2 as evidenced by the oxidation of 2,6-dimethoxyphenol, ABTS and TMB. The effects of Cu(II) coordination environment in the fibrils on the ROS formation are discussed. These small water-soluble peptides in combination with redox active metals like Cu(II) and Fe(II) can be used to catalyze a number of oxidation reactions. Among advantages of this approach is simple preparation of catalyst, ability to screen multiple coordination spheres by mixing different peptides and environmentally benign solvent. This work will extend our current understanding of the role of Cu(II) in oxidative stress and Alzheimer's as well as provide fibril-based catalysts for oxidation reactions.

MEDI 250

Optimizing the efficacy of opioids by TLR4 blockade

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After years of neglect, glial cells are finally registering on drug developers' radar. Evidence has accrued that glia are activated by opioids and that this opioid-induced glial response suppresses opioid analgesia, resulting in the development of opioid tolerance and dependence. A literature has developed linking opiate efficacy and side effects to their influence on glial cells within the central nervous system via the signaling pathway mediated by TLR4. Recently, we reported the first direct evidence that morphine creates its neuroinflammatory effects by binding to the TLR4 accessory protein, MD-2, and inducing TLR4/MD-2 dimerization and subsequent TLR4 signalling activation in a similar fashion to LPS. Further, morphine induces neuroinflammation solely through its binding in a specific LPS-binding pocket of MD-2. We have shown that small molecule agents that disrupt the essential TLR4/MD-2 interactions can suppress morphine-induced neuroinflammation. These results suggest the TLR4/MD-2 complex as a novel target for improving the analgesic efficacy of opioids. To the best of our knowledge, these studies represent the first drug discovery approach attempting to regulate opioid-induced glial activation while almost all previous research focused on neurons.

MEDI 251

High-resolution crystal structures of the influenza A M2 proton channel in the lipidic cubic phase: Insights into water networks at high and low pH

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The M2 protein of influenza A is a pH-activated, homotetrameric proton channel whose function is necessary for viral replication. The smallest construct that retains function has 100 total residues. Because of its small size, the M2 channel has been studied as a model system for the transport of protons across a membrane. Protons are shuttled across water wires that start at the N-terminus of the channel, pass through gating His37 residues, then move protons to the C-terminus of the channel. Crystal structures to a resolution of 1.1 Å were determined for the AM2 proton channel transmembrane domain under high and low pH conditions in the lipidic cubic phase. Variations in the water network leading from the N-terminal half of the pore to residue His37 were observed with pH. Room temperature data collection was used to determine if and how the observed water networks were affected by the low temperature conditions of cryocrystallography. Molecular dynamics simulations were used to predict the positions of the water dipoles and the electrostatic potential of the channel at high and low pH.

MEDI 252

On-demand control of antimicrobial activity of methacrylate random copolymers using amphiphilic polysaccharide nanogel

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Synthetic amphiphilic cationic polymers exhibit potent antimicrobial activity against a broad spectrum of bacteria by disrupting bacterial cell membranes, which mimic naturally occurring antimicrobial peptides. In this study, we designed a new self-assembly system, in which the antimicrobial activity of polymers can be controlled by capture and on-demand release of polymer chains (Figure 1). We prepared a nano-sized particle (∼50 nm in diameter) in which polysaccharide pullulan chains modified with cholesterols (∼1 per 100 glucose unit) (CHPs) are physically cross-linked by association of cholesterol groups in water. The nano-particles have swollen pullulan polymer networks, providing a nano-sized polymer gel (nanogel) structure which is capable of capturing proteins and small drugs [*Chem. Rec.* 2010, 10, 366]. When the CHP nanogels were incubated with amphiphilic antimicrobial methacrylate copolymers (AMPs), the nanogels captured the AMPs by the hydrophobic interaction, and ∼6-7 of AMP chains were bound to one nanogel. The CHP-AMP complex did not show any significant reduction in the number of viable *E. coli*. However, once methyl-β-cyclodextrins (CDs) were added, the CHP nanogels were dissociated due to the capping of cholesterols by CDs and released the AMPs into solution. The released

AMPs showed potent bactericidal activity against *E. coli* (>99% killing in 120 minutes). This result demonstrates that the antimicrobial activity of AMPs can be controlled by modulating the non-covalent interactions and binding affinities between amphiphilic polymers and nano-particles in water.

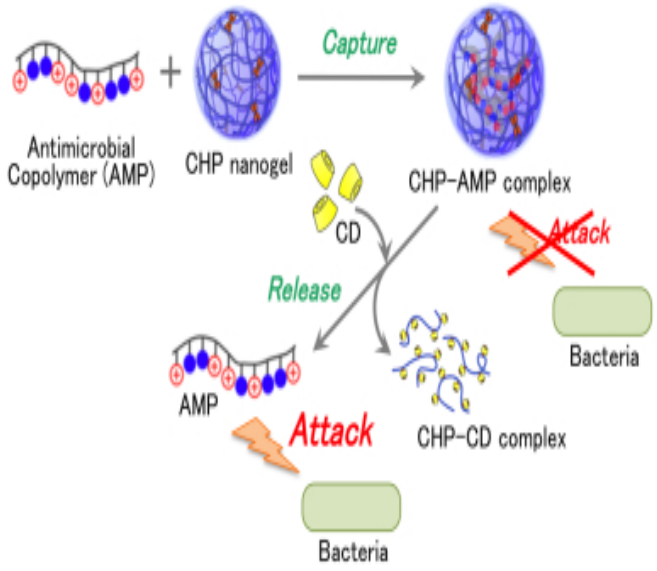

Figure 1. Schematic illustration of amphiphilic self-assembly system for on-demand control of antimicrobial activity of AMPs

MEDI 253

Deconstructing the pathways of ion conduction to describe the geometry of inhibition sites of the flu's proton channel

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The pore of the M2 channel from the influenza A virus is lined by a conserved histidine-tryptophan motif and by multiple backbone carbonyls, enclosing clusters of water molecules responsible for conducting protons and stabilizing other ions. By using molecular dynamics simulations of a small library of amine-based amphiphilic molecules (including both inactive compounds and active inhibitors), we observed sites of metastable energetic equilibrium along the pore of the channel. The relative equilibria between different sites are shifted upon the appearance of drug-resistant mutations. By applying a structural analysis of the local water network, we reproduce the high affinity

sites of known inhibitors, and propose quantitative geometric requirements to be used in the search for new scaffolds.

MEDI 254

Combining shape-based recognition and molecular simulation in drug discovery

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Incorporating computational effort within medicinal chemistry has become an established strategy to accelerate individual stages of drug discovery (Jorgensen, *Science* **2004**). However, the effectiveness of combining approaches that are highly optimized for specific workflows is not always guaranteed.
Here, we describe a strategy to integrate shape-based recognition (Zauhar et al., *J. Comput. Aided Mol. Des.* **2013**) with molecular dynamics as essential components of hit identification, as well as lead identification and optimization.
We used our approach to aid multiple stages of the search for small molecule inhibitors of three proteins involved in human disease. First, we validated our strategy using the androgen receptor (AR), a well studied oncological target. We then focused on the signal transducer and activator of transcription 5 (STAT5), a human protein essential in prostate cancer development and progression.
Lastly we study the M2 proton channel, an integral membrane protein of the influenza virus, which poses significant challenges to rational drug design due to the role played by water molecules in molecular recognition (Wang et al., *PNAS* **2013**).

MEDI 255

Synthesis and characterization of dendronized helix bundle assemblies

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Accurate control of the three-dimensional arrangement of atoms in molecular materials remains a challenge in synthetic and supramolecular chemistry. Our approach is to translate protein design rules from biological environments to supramolecular materials. Patterning of hydrophilic and hydrophobic residues has provided peptides that fold and self-assemble into protein-like structures, and that can further organize into crystals or gels. Atomic accuracy and long-range periodic order of molecular materials can be achieved by coupling amphiphilic dendrons which self organize into periodic arrays, to

peptides that fold and self-assemble into helical bundles. We are developing model peptides that fold and self-assemble into α-helical structures based on hydrophobic patterning. Mutant peptides have been synthesized whose surface residue positions have been modified to allow for the incorporation monodisperse dendrons via the copper-catalyzed azide-alkyne cycloaddition (CuAAC) 'click' reaction. The effect of these dendrons on the secondary structure of the peptide was evaluated. The presentation will discuss the design, synthesis, and characterization of the dendronized peptides.

MEDI 256

Toward an ensemble-centric view of structure for protein design

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Scoring the compatibility of a sequence with a given fold is a cornerstone task in computational protein design. Because vast sequence spaces are considered in design applications, scoring functions must be fast. On the other hand, as modern computer clusters become more ubiquitous and powerful, this speed requirement is continually softened. Unfortunately, this does not necessarily translate into better scores, as traditional scoring functions contain built-in assumptions and approximations that do not vanish at the infinite sampling limit. I propose a novel method, which can estimate the absolute free energy of a structural state using only information from a canonical sampling thereof (e.g. via Molecular-Dynamics or Monte-Carlo simulations). The method, dubbed VALOCIDY, is mathematically exact at the long sampling limit, whereas estimates based on limited amounts of simulation can be accurate enough to enable comparisons between very different structural states (e.g. folded and unfolded states or alternative folded conformations). I will briefly present the theoretical foundation of the approach and demonstrate several applications, including a small design problem. Tradeoffs between increased accuracy of VALOCIDY estimates, over traditional scoring, and added computational expense will need to be carefully examined, especially in larger systems, but the added physical interpretability is certainly a much needed property in the context of design applications.

MEDI 257

Mutational analysis of the PhoQ signal transduction pathway

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Bacteria use two-component systems (TCSs) to transduce signals across a lipid membrane. In Gram negative bacteria, the PhoPQ TCS confers virulence and is activated in the absence of Mg^{2+}, at low pH, or in the presence of antimicrobial peptides. While this response is well known, the structural rearrangements required to transmit these signals remain contested. This dimeric sensor histidine kinase (PhoQ) has multiple helical bundle domains that a signal must pass through to obtain a genetic response. Previously, we utilized disulfide-scanning and Bayesian inference to construct a model of PhoQ signal transduction. We described helical rearrangements associated with signaling. Here, we study PhoQ mutants by *in vivo* activity assays along with *in vitro* hydrogen/deuterium exchange studies to pinpoint the residues that are critical in this signaling cascade. These mutational scans highlight the importance of structural integrity within a particular domain as well as critical tertiary interactions between domains.

MEDI 258

Effect of post-translational modifications on secondary structure stability

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Post-translational modifications increase the complexity of the proteome compared to the genome. Such modifications serve to expand the chemical functionality of natural proteins. These modifications serve many important roles in a wide variety of biochemical processes. As such, overwhelming research efforts have focused on identifying the type and location of these post-translational modifications. The biochemical functional consequences of post-translational modifications have also been studied to some extent. In comparison, there have only been limited studies on the effect of post-translational modifications on structural stability. Since the secondary structures alpha-helix and beta-sheets are the basic three dimensional building blocks for protein structures, we have investigated the effect of various post-translational modifications on secondary structure stability. Modifications including Ser/Thr phosphorylation and glycosylation, Asn glycosylation, Arg methylation and deimination, and Lys methylation and acetylation were investigated. Alanine-based peptides were used to explore the effect of post-translational modifications on helix N-capping, C-capping, and propagation (propensity). All peptides were synthesized by solid phase peptide synthesis using Fmoc-based chemistry. The peptides were investigated by circular dichroism spectroscopy coupled with modified Lifson-Roig theory. Two series of hairpin (strand-turn-strand) forming peptides were used to explore the effect of post-translational modifications on sheet forming energetics. Complete sequence specific assignment for the peptides was achieved using 2D 1H NMR TOCSY, DQF-COSY, and ROESY spectra. The Hα chemical shifts were used to derive the folding energetics. These studies should complement functional studies on post-translational modifications and provide the foundation for predicting the effect of post-translational modification on protein structure stability.

MEDI 259

Imaging of bindings between chemical drug and its target protein kinases by redistribution assay in live cells

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For directly imaging the bindings between chemical drugs and their target protein kinases in live cells using a redistribution assay, we have developed a methodology. Briefly, the HaloTag protein (HT) (or Streptavidin (SA)) was fused to Protein kinase C (PKC), which enables the chemical drug and its target protein kinase to cotranslocate from the cytoplasm to the plasma membrane. PKC is well known to translocate from the cytoplasm to the plasma membrane in response to physiological stimuli, as well as exogenous ligands such as phorbol esters. The HT is a mutant of a hydrolase protein that efficiently forms a covalent bond with the HT ligands such as aliphatic halogenated compounds. In particular, we initially modified the chemical drugs (e.g. Dasatinib) with 6-chlorohexyloxyethane. The resulting compounds were cell-membrane permeable, and binding both HT and its target protein kinases. To verify our approach, the fusion construct PKC-mRFP-HT and each of the eGFP-tagged dasatinib target kinases (CSK, SRC, and LYN) were transiently cotransfected into HEK-293T cells and the cells were treated with Halotag Ligand-labelled dasatinib. When the exogenous ligand phorbol 12-myristate 13-acetate (PMA) was added, both PKC fusion and eGFP-tagged dasatinib target kinase were cotranslocated to the plasma membrane. To image the FKBP12-rapamycin-FRB complex, PKC-mRFP-SA/eGFP-FRB/TaqBFP-FKBP12 cotransfected cells were pretreated with biotin-labelled rapamycin before PMA treatment. As expected, treatment with rapamycin induced an interaction between FKBP12 and FRB, FKBP12 and FRB were cotranslocated to the plasma membrane.

MEDI 260

Stabilization of helical peptides by chemical crosslinking

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Protein-protein interactions play a key role in a myriad of biological processes. Utilization of peptide fragments in the interface as a modulator of such interactions has been widely pursued in chemical biology and medicinal chemistry field. However, the inherent conformational flexibility in small peptides has posed a big problem. Thus, chemical crosslinking strategies have been widely investigated to provide the required

conformational restriction in the peptide secondary structures. We previously demonstrated that the cysteine crosslinking at *i* and *i+4* positions of α-helix could be a versatile approach in design of protease inhibitor. However, the detailed structural information of the effect of crosslinking was not elucidated. In this study, we present the detailed structure of side-chain crosslinked peptide bound to Calcium channel β-subunit. In addition, another novel approach for helix stabilization - “capping motif stabilization” by chemical crosslinking is presented.

MEDI 261

Development of a small molecule inhibitor of integrin αvβ1

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Tissue fibrosis plays a central role in most chronic diseases that lead to organ failure, but there are no currently approved, effective therapies specifically targeting fibrosis in the US. Transforming growth factor β (TGFβ) is a central mediator of fibrotic processes, and its activation thorough av integrins has been increasingly important in therapeutic development. We have developed a potent and highly specific small molecule inhibitor of the αvβ1 integrin and show that this inhibitor completely inhibits TGFβ activation by primary fibroblasts from several organs. We also show that the inhibitor is therapeutically effective *in vivo* in mouse models of lung, liver and kidney fibrosis. This study suggests that αvβ1 inhibitors may be useful therapeutics for treating fibrotic diseases of multiple organs.

MEDI 262

Crystal structure of an amphiphilic foldamer reveals a 48-mer assembly comprising a hollow truncated octahedron

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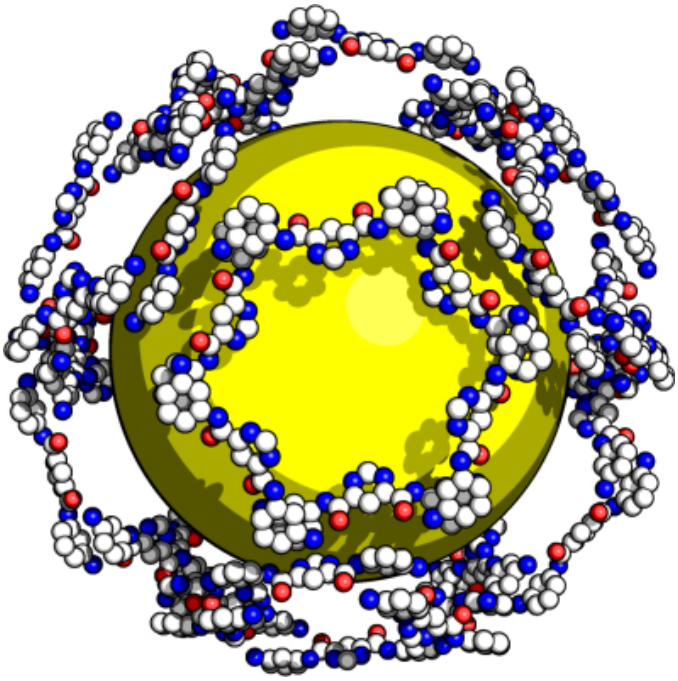
Foldamers provide an attractive medium to test the mechanisms by which biological macromolecules fold into complex three-dimensional structures, and ultimately to design novel protein-like architectures with properties unprecedented in nature. Here, we describe a large cage-like structure formed from an amphiphilic arylamide foldamer

crystallized from aqueous solution. Forty eight copies of the foldamer assemble into a 5 nm cage-like structure, an omnitruncated octahedron filled with well-ordered ice-like water molecules. The assembly is stabilised by a mix of arylamide stacking interaction, hydrogen bonding and hydrophobic forces. The omnitruncated octahedra tessellate to form a cubic crystal. These findings provide an important step towards the design of framework nanostructures in aqueous environments.



MEDI 263

Synthesis & kinome selectivity patterns of imidazo[4,5-*b*]pyridine-derived fragment libraries

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We are studying the influence of small structural changes to ATP-competitive kinase inhibitor scaffolds on binding mode and kinome-selectivity profile. To this end, we have developed synthetic routes to focused libraries of fragment-like molecules that comprise small structural modifications along multiple vectors of a single scaffold. We have investigated structure-selectivity profiles by biochemical screening (Caliper Profiler Pro® assay) and binding modes by X-ray crystallography. We anticipate that this knowledge can be applied to other kinase inhibitor scaffolds of interest, potentially accelerating the hit-to-lead phase of kinase chemical probe and drug discovery projects, and may contribute to the design of novel hinge-binding scaffolds.

We initially chose to investigate the imidazo[4,5-*b*]pyridine hinge-binding scaffold which has been elaborated in-house to give potent, orally bioavailable inhibitors of Aurora A kinase, and dual FLT3/Aurora kinase inhibitors for the treatment of Acute Myeloid Leukaemia. We will present synthetic methodology compatible with flexible elaboration along multiple vectors of the imidazo[4,5-*b*]pyridine scaffold, including the discovery of efficient direct arylation methods for C2 functionalisation and its incorporation into fragment-like multi-vector libraries.

Our results demonstrate that small changes to kinase hinge-binding motifs can have dramatic effects on both selectivity and kinase binding mode. We will present our profiling results and analysis of kinome selectivity patterns with reference to both kinase primary sequence and to protein-structural information. The extent to which the observed trends are replicated in alternative hinge-binding motifs is currently under study and application to the design of novel hinge-binding scaffolds is underway in our laboratories.

MEDI 264

Discovery of Tankyrase inhibitors: Combining structure-based drug discovery, fragments, and biophysical techniques

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Tankyrases are involved in telomerase function, mitotic spindle formation, and regulation of β-catenin signaling. Deregulation of Wnt/β-catenin signaling occurs in several cancers and is common in colorectal cancer. Tankyrase inhibitors capable of reducing β-catenin signaling may be beneficial as cancer therapies.

Screening the Vernalis fragment library by crystallography identified a diverse set of fragments which bound to two adjacent hot spots within the binding site. Common interactions with the protein were used to build a pharmacophore and prioritize starting points. Surface plasmon resonance was used to measure fragment affinities (affinity in solution) and to quickly improve the binding affinity of one fragment series (off-rate screening).

We have optimized multiple novel and potent chemical series which inhibit Tankyrase in binding, functional, and cellular assays. These compounds increase the levels of Axin, a member of the β-catenin destruction complex, and reduce the transcription of the β-catenin-responsive LEF/TCF promoter.

MEDI 265

Bicyclic pyrimidine modulators of Abeta production for the treatment of Alzheimer's disease

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Alzheimer's disease (AD) is a debilitating progressive neurodegenerative condition which results in memory loss, loss of independent function, and ultimately death. According to the current amyloid cascade hypothesis, the production and accumulation of the toxic soluble oligomers of Abeta1-42 are causal in the cascade of events leading to AD. Gamma-secretase is one of the two proteases responsible for producing the Abeta peptides, however inhibitors of this enzyme have not advanced to market due to toxicity attributed to inhibition of Notch processing. Gamma-secretase modulators (GSM's) are compounds which shift the production of Abeta1-42 to shorter, less toxic and less aggregation-prone species such as Abeta1-37 and Abeta1-38, while preserving normal Notch function. Our labs have advanced a pyrimidine-based, non-NSAID-like, Gamma-secretase modulator into the clinic. This talk presents our effort to optimize solubility, hERG, CYP_{3A4}, and PXR en route to the discovery of our clinical compound BMS-932481.

MEDI 266

Discovery and preclinical profile of BMS-932481, a gamma secretase modulator for the treatment of Alzheimer's disease

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Alzheimer's disease (AD) is the most prevalent cause of dementia, and is associated with accumulation of amyloid beta peptides (Abeta) in the brain. In particular, soluble oligomers of Abeta1-42 are thought to play a key neurotoxic role. Abeta1-42 levels can be decreased by gamma-secretase modulators (GSMs), which are small molecules that target gamma-secretase, an enzyme essential for Abeta production. GSMs lower Abeta1-42 levels by shifting processing to shorter forms of Abeta without affecting other key gamma-secretase functions including Notch processing. Our team has recently disclosed our efforts to optimize a bicyclic triazole GSM scaffold, resulting in the identification of BMS-869780, a potent and selective GSM. Further optimization around this chemotype resulted in the identification of a novel bicyclic pyrimidine scaffold. This talk will discuss the SAR of this chemotype and the ultimate identification of a lead molecule, BMS-932481, which was advanced to clinical studies for the treatment of AD.

MEDI 267

Use of non-traditional conformational restriction in the design of a novel, potent, and metabolically stable series of GK-GKRP inhibitors

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Amgen has maintained a long-term interest in the role of glucokinase (GK) in the treatment of metabolic disease, culminating in the discovery of a novel binding pocket in the glucokinase regulatory protein (GKRP) and the first reported small molecule inhibitors of the GK-GKRP interaction.[1] Subsequent screening and medicinal chemistry efforts have given rise to yet additional inhibitor classes. Early leads in a novel scaffold were complicated by high metabolic turnover. This presentation describes the role of computational techniques in addressing a severe potency-stability conundrum, by incorporation of an unconventional, *syn*-locked interaction between two adjacent rings. Quantum mechanical calculations were employed to characterize stabilizing interactions and conformational preferences, guiding the design of improved analogs in the series. This strategy resulted in a highly potent and stable modified series, ultimately paving the way for highly in vivo efficacious inhibitors of GK-GKRP.

1. http://www.nature.com/nature/journal/v504/n7480/full/nature12724.html

MEDI 268

Small molecule inhibitors for glucokinase–glucokinase regulatory protein (GK–GKRP) binding: Optimization for in vivo target assessment of type II diabetes

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Amgen, Inc. has previously disclosed the first orally active compounds that inhibit the GK–GKRP binding interaction by selectively engaging a formerly unknown allosteric binding pocket on GKRP (*Nature*, **504** , pp 437-440) with additional studies within this arylpiperazine series of compounds having also been recently published (*J. Med. Chem.,* **2014** , 57 (2), pp 309-338). The development of an alternative series of inhibitors began with a modestly potent (μM enzyme IC_{50}) screening hit with high microsomal clearance. Following computationally-designed coplanarization of neighboring heterocyclic rings, we created a modified series with improved activity and stability properties. From this new starting point, this presentation will discuss how the further use of X-ray crystallography of compounds bound to GKRP, along with structural alignment to the prior chemical series, and iterative medicinal chemistry design cycles yielded analogs with vastly improved activity (nM IC_{50} enzyme). In addition, we completed microsomal *in vitro* metabolite identification studies that aided in the discovery of lead compounds with pharmacokinetic profiles suitable for evaluation in diabetic rodent models. These new compounds were shown to induce *in vivo* GK translocation in rats and revealed pharmacodynamic blood glucose reduction in mice. This newly presented series expands the possibility to further explore GK-GKRP as a potential therapeutic target for type II diabetes.

MEDI 269

Discovery and synthesis of substituted benzimidazoles as 5-lipoxygenase-activating protein inhibitors for the treatment of inflammatory diseases

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Leukotrienes (LTs) play a critical role in both acute and chronic inflammation, and 5-lipoxygenase-activating protein (FLAP) is a key protein involved in leukotriene biosynthesis. In the first step of the leukotriene synthesis FLAP, a nuclear membrane protein, selectively transfers arachidonic acid (AA) to the enzyme 5-lipoxygenase (5-LO)

and facilitates the synthesis of leukotriene epoxide LTA_4. LTA_4 is then converted to either LTB_4 or LTC_4. These LTs can activate G protein-coupled receptors including BLT_1/BLT_2 and $CysLT_1$/$CysLT_2$ leading to an immune response. Inhibition of FLAP would prevent the biosynthesis of both LTB_4 and LTC_4 making it an attractive target for the treatment of asthma and other inflammatory diseases where LTs likely play a role. Previous programs targeting the nuclear membrane protein FLAP have been hampered by the lack of potency, developability challenges, and the potential for drug-drug interactions (DDI). This presentation will describe a de novo approach to small molecule lead generation for the Janssen FLAP inhibitor program that led to novel substituted benzimidazoles. Positive differentiation from known FLAP inhibitors and structure-activity relationships (SAR) for this series will be presented.

MEDI 270

Biaryl amino-heteroarenes as potent 5-lipoxygenase-activating protein inhibitors

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The development 5-lipoxygenase activating protein (FLAP) inhibitors has been an area of great interest to the pharmaceutical community due to their ability to interrupt the formation of a variety of pro-inflammitory leukotrienes (LTA_4 - LTE_4) that contribute to the pathophysiology of asthma and other inflammatory diseases. In our own effort to develop FLAP inhibitors, we have discovered a promising series of biaryl amino-heteroarenes. Structure activity relationships (SAR), cellular activity and species differences in potency will be presented.

MEDI 271

Discovery and synthesis of tetrasubstituted aryl aminopyrazines as potent 5-lipoxygenase-activating protein (FLAP) inhibitors

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After the body experiences an inflammatory stimulus, synthesis of the various pro-inflammatory leukotrienes is initiated. Increased leukotriene levels may contribute to the worsening of disease symptoms for various immune related pathologies, such as asthma. Interruption of portions of the leukotriene synthesis pathway have resulted in several marketed drugs for inflammatory diseases. 5-Lipoxygenase-activating protein (FLAP), a nuclear membrane protein, is a key constituent of the leukotriene synthesis cascade and is upstream of the enzymes responsible for the production of leukotriene A_4 – leukotriene E_4. FLAP inhibitors have shown proof of concept in allergen challenge studies, suggesting that FLAP inhibition could be a viable approach to treating asthma. Previous programs targeting FLAP have been hampered by a lack of human whole blood (hWB) potency, developability challenges, and the potential for drug-drug interactions (DDI). This presentation will describe a series of tetrasubstituted aryl aminopyrazines incorporating an ether linkage serving as the point of diversification. The potency, hWB activity, and pharmacokenetic properties were tunable through judicious selection of the ethereal substituent. The chemistry, structure-activity relationships (SAR), cellular and in vivo activity for this series will be presented.

MEDI 272

Phosphodiesterases: Validated targets for medicinal intervention

***Tom Chappie**, thomas.a.chappie@pfizer.com. Department of Worldwide Medicinal Chemistry, Pfizer, Cambridge, MA 02139, United States*

The phosphodiesterases (PDE) are a family of enzymes whose function is to control the levels of the signaling molecules cyclic adenosine monophosphate (cAMP) and/or cyclic guanosine monophosphate (cGMP) within the cell. The PDEs accomplish this by hydrolysis of the cyclic monophosphate to the silent ring-opened monophosphate. The development of PDE inhibitors has resulted in multiple non-selective and several selective PDE drugs. Based on the “drugability” of inhibitors of this enzyme family and expanded biological understanding of the targets, many efforts to develop selective inhibitors have been carried out over the past two decades. The successful drugs and the lead compounds from each of the PDEs will be presented.

MEDI 273

Fragments, crystals, and multiple scaffolds: How parallel hit-to-lead efforts enabled the discovery of optimized PDE10A inhibitors for the treatment of schizophrenia

***Christopher D. Cox**, chris_cox@merck.com. Department of Discovery Chemistry, Merck Research Laboratories, West Point, PA 19486, United States*

Currently prescribed antipsychotics attenuate the positive symptoms associated with schizophrenia, but are limited by suboptimal efficacy in some patients, numerous side-effects that reduce patient compliance, and a failure to address cognitive deficits and negative symptoms associated with the disease. Phosphodiesterase10A (PDE10A) is a member of a super family of enzymes that regulate intracellular signaling by deactivating the ubiquitous second messengers cAMP and cGMP. PDE10A is highly localized in the striatum, a region of the brain involved with properly integrating glutaminergic and dopaminergic inputs; this integration process, which relies upon cyclic nucleotide signaling, is hypothesized to be dysfunctional in schizophrenics, and substantial preclinical evidence now supports PDE10A inhibition as a mechanistically novel treatment that may rectify the positive and cognitive symptom domains in schizophrenia.

This presentation will highlight the lead-finding, hit-to-lead, and lead optimization strategies Merck employed that led to the discovery of multiple series of potent and selective inhibitors of PDE10A, as well as a novel PET tracer. By progressing multiple series in parallel, important lessons were learned within a particular lead class, and then applied across structural series; this approach enabled rapid advancement of certain lead series while facilitating informed NoGo's on others. Particular emphasis will be placed on Fragment Based Drug Discovery (FBDD), and how we employed X-ray crystallography and rational design, to create a novel series of pyrazolopyrimidine-based PDE10A inhibitors that show significant promise in preclinical models of psychiatric disorders.

MEDI 274

Structural biology and molecular pharmacology of phosphodiesterase 4 (PDE4) regulation

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The human genome contains four PDE4 genes that encode multiple transcripts which produce long, short and super-short forms of the enzyme. Long forms of the enzyme contain two upstream, N-terminal regulatory domains known as UCR1 and UCR2. The UCR1/UCR2 module normally holds the enzyme in a closed, partially inhibited conformer. Phosphorylation of UCR1 by protein kinase A in response to cAMP signaling activates the enzyme. We were the first to show that UCR2 contains a helical domain that closes across the active site, thereby locking the enzyme in a closed, inactive

conformation. The physiological relevance of this regulatory mechanism is highlighted by the recent discovery of human PDE4D mutations in acrodydosostosis, a developmental disorder that causes brachydactyly and intellectual disability. Acrodysostosis mutations destabilize closure of the UCR1/UCR2 negative regulatory module, thereby activating the enzyme, and this can be reversed by PDE4D allosteric inhibitors that selectively bind UCR2. Correspondingly, we have been able to design PDE4B allosteric inhibitors that bind a C-terminal regulatory domain that we term Control Region 3 (CR3). Amino acid sequence differences in these control regions allows us to design subtype-selective PDE4D and PDE4B inhibitors. The chemistry is modular and tunable. We are advancing a selective, PDE4D allosteric inhibitor towards human clinical trials for the treatment of cognitive impairment in neurodegenerative and psychiatric disorders.

MEDI 275

Identification of a brain penetrant, highly selective phosphodiesterase 2A inhibitor clinical candidate for treating cognitive impairment: In vivo efficacy and human pharmacokinetic data

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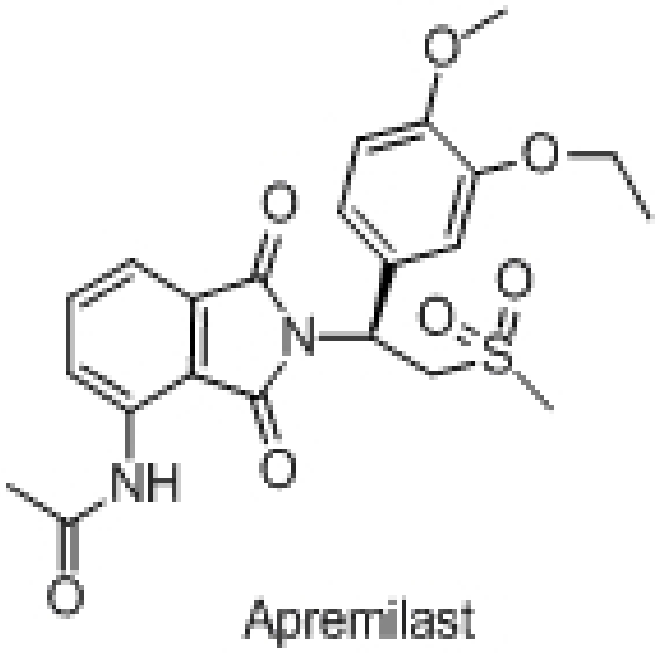
While treatments exist for positive symptoms of schizophrenia in the form of atypical antipsychotics, no approved therapies exist for the concomitant negative symptoms or cognitive impairment associated with schizophrenia (CIAS). The key second messenger molecules cyclic guanosine monophosphate (cGMP) and cyclic adenosine monophosphate (cAMP) have been implicated as playing a major role in cognitive processes. Phosphodiesterase 2A (PDE2A), which hydrolyzes both cGMP and cAMP, has highest levels of expression within limbic and basal ganglia brain circuitry found to be dysfunctional in schizophrenia. Inhibitors of PDE2A would increase cyclic nucleotide levels in these key brain regions and could thus potentially improve cognitive processes. This presentation will detail the identification of a hit series via high-throughput screening (HTS), and the strategic application of parallel synthesis, fragment and structure-based design to improve potency, selectivity, ADME and safety to yield a clinical candidate. The pre-clinical biological profile and first-in-human pharmacokinetic data will be presented.

MEDI 276

Discovery of Apremilast, a selective PDE4 inhibitor

***Hon-Wah Man**, hwman@celgene.com. Drug Discovery, Celgene Corporation, Summit, NJ 07901, United States*

Apremilast is an oral small molecule specific PDE4 inhibitor. It is being studied in multiple Phase III clinical trials for the treatment of psoriasis, psoriatic arthritis and other chronic inflammatory diseases with promising clinical efficacy in psoriasis and psoriatic arthritis. Herein, we describe the discovery of Apremilast starting from the optimization of a series of β-amino-propionic acid PDE4 inhibitors. SAR exploration of three moieties, dialkoxyphenyl, phthalimide, and sulfone, will be presented.



MEDI 277

Phosphodiesterase 10A inhibitors – targeting a highly ligandable binding pocket

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The basal ganglia specific metallohydrolase phosphodiesterase 10A (PDE10A), is a dual substrate enzyme recognizing both cAMP and cGMP [1]. However, it has a substrate preference for cAMP, likely caused by an internal hydrogen bond network, which locks the enzyme in a conformation favoring the hydrogen bonding pattern presented by adenine in cAMP.

The talk will discuss different conceptual strategies for targeting the PDE10A binding pocket arguing that it is a highly ligandable binding pocket. However, in spite of the

apparent high drugability of the PDE10A enzyme, specific issues relating to multiple possible binding modes, a shared pharmacophore with Cyp- and pgp proteins causing metabolic stability issues as well as brain penetration issues, has greatly challenged many PDE10A drug discovery programs.

The talk will discuss Lundbeck strategies and experience based principles for CNS drug discovery targeting PDE10A, exemplified with and benchmarking different scaffolds. Emphasis will also be on the importance of securing the development of methods enabling assessment of target engagement early on in the discovery process which was achieved by early development of a ligand for PDE10A in vivo binding and a PDE10A positron emission tomography (PET) ligand.

[1]. Kehler, Jan; Nielsen, Jacob. PDE10A inhibitors: novel therapeutic drugs for schizophrenia. Current Pharmaceutical Design (2011), 17(2), 137-150.

MEDI 278

Binding kinetics in drug action and discovery

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Binding kinetics are integral to a medicine's molecular mechanism of action (MMOA), and thereby influence the efficacy, safety, differentiation and duration of action. Binding kinetics include equilibrium dissociation constants, association rates and dissociation rates that can inform chemical optimization. Binding kinetics can impact PK/PD relationships depending on the equilibrium state of the system. Under equilibrium conditions thermodynamic control will drive PK/PD relationships, and equilibrium constants and fractional occupancy will define a pharmacological response. In contrast, competing kinetic rates will define the pharmacological response when a system that is not at equilibrium. The potential for mechanism-based toxicity (on-target toxicity) is a key determinate to differentiate between the value of equilibrium versus non-equilibrium binding kinetics. With no potential for mechanism-based toxicity, an insurmountable/irreversible kinetic mechanism is optimal. An insurmountable/irreversible mechanism is achieved by covalent bond formation or slow, reversible kinetics in a non-equilibrium system. The insurmountable/irreversible mechanisms will increase the therapeutic index by decreasing the drug concentrations required for efficacy. When there is potential for mechanism-based toxicity, equilibrium binding kinetics can contribute to a tolerable therapeutic index. This talk will cover 1) first principles (kinetic versus thermodynamic control) and 2) the effect of binding kinetics on PK/PD behavior.

MEDI 279

Medicinal chemistry optimisation of binding kinetics

***Michael J Waring**, mike.j.waring@gmail.com. Oncology Medicinal Chemistry, AstraZeneca, Macclesfield, Cheshire SK10 3JW, United Kingdom*

A significant proportion of drugs reaching the market exhibit non-equilibrium binding characteristics.[1] Intuitively, it is likely that this represents a significant enrichment in compounds of this type the proportion of such compounds entering development and so these compounds must have benefits with respect to attrition. This could be due to increased efficacy and / or reduced toxicity. However, the majority of them appear to have been discovered without knowledge of their kinetic behaviour at the time of compound selection and these properties were revealed later as they became of greater interest to the wider community and subject to more detailed studies.

It is often stated that association and dissociation rates cannot be manipulated rationally during optimisation. This talk will cover some of the principles of attempting to do so, highlighted with literature examples and data generated through our ongoing collaborative research project “Kinetics for Drug Discovery” (K4DD).[2] From these examples, some general observations give rise to hypotheses that may be of general relevance to the manipulation of kinetics and to a more detailed understanding of the underlying processes driving these observations at a molecular level.

1. Swinney, D. C. Biochemical mechanisms of new molecular entities (NMEs) approved by United States FDA during 2001-2004: mechanisms leading to optimal efficacy and safety. Current Topics in Medicinal Chemistry, 2006, 6, 461-478

2. http://www.k4dd.eu/

MEDI 280

Structure guided fragment evolution toward long residence time compounds exemplified for CDK8/CycC

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Despite the fact that residence time is an increasingly appreciated optimization parameter in drug discovery, molecular understanding of binding kinetics in order to facilitate efficient generation of compounds with tailor made residence time values remain a challenge. We have used the target CDK8/CycC to demonstrate one strategy how fast binding fragments can be evolved to long residence time compounds by combination of structural biology and kinetic profiling. Deep pocket binding fragments with fast binding kinetics were identified by a fragment screening campaign. After structure based examination of the binding mode, the fragments were gradually extended in order to establish an increasing number of compound-protein contacts. These contacts were systematically analyzed for their impact on residence time.

MEDI 281

GPCR drug-binding kinetics: Insights from explicit water network modeling

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G Protein-Coupled Receptors (GPCRs) are the largest protein family in the human genome, and as key regulators of many physiological processes, are also the family most often targeted by small molecule therapeutics. The wealth of co-crystal structures and biophysical data for GPCR-ligand complexes now available allows for “high-end” ligand design, taking into account explicit water networks as an additional dimension for structure based drug design. We will present structural and kinetic data for several small molecule GPCR binders. Using computational models of explicit water structure and dynamics we will rationalize this kinetic data. Explicit water structure is often the missing dimension that can be used to explain puzzling SAR data, and must be taken into account for prospective lead optimization. The use of explicit water networks to rationalize and predict small molecule energetics, potency and kinetics in the context of GPCR binding will likely contribute to safer and more efficacious drugs.

MEDI 282

Revealing molecular determinants of drug-receptor binding kinetics through atomic-level simulation

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The rates at which drugs associate with and dissociate from their receptors directly impact drug efficacy and safety, but the molecular determinants of drug-receptor binding kinetics remain poorly understood, and the rational modulation of binding kinetics thus remains challenging. Molecular dynamics simulations provide an increasingly powerful tool to elucidate the factors controlling binding kinetics at the atomic level. In particular, recent advances in simulation speed and accuracy have made possible simulations in which certain drugs spontaneously associate with their receptors to achieve bound conformations that match crystal structures almost perfectly, allowing one to examine their binding process in full atomic detail. I will describe several cases in which atomic-level simulations, together with complementary experimental data, have uncovered atomic-level determinants of drug binding kinetics, and I will discuss the implications of these findings for drug design.

MEDI 283

Discovery of AMG 900, a highly selective, orally bioavailable inhibitor of Aurora kinases with efficacy in preclinical antitumor models and activity against multidrug-resistant cancer cell lines

***Stephanie D. Geuns-Meyer**, MeyerS@amgen.com. Department of Medicinal Chemistry, Amgen Inc, Cambridge, Massachusetts 02142, United States*

The Aurora family of serine/threonine kinases (Aurora-A, -B, -C) regulate cell-cycle progression in mammalian cells. Whereas Aurora-C function appears restricted to meiosis in males, Aurora-A and -B are essential for proper chromosome congression, segregation, and cytokinesis during mitosis. These mitotic kinases have been implicated in tumorigenesis, with overexpression levels correlating to clinical staging of cancers and poor prognosis, and thus have been the subject of much interest as targets for anticancer therapy.

N-(4-((3-(2-amino-4-pyrimidinyl)-2-pyridinyl)oxy)phenyl)-4-phenyl-1-phthalazinamine was a key Aurora kinase inhibitor lead, possessing oral bioavailability in rats that was lacking in the anthranilamide compounds from which it was derived. This phthalazine compound possessed a key feature that was deemed important to maintain in a clinical candidate: potency against a model multidrug resistant (MDR) cell line (MES-SA Dx5) commensurate with its activity against cell lines that do not overexpress P-gp. Improved *in vivo* potency was desired, as measured by suppression of the phosphorylation of the Aurora-B substrate Histone H3 (Ser10) *in vivo*. SAR from targeting this improvement uncovered a delicate balance between pharmacokinetic parameters and cell potency in MES-SA Dx5 cells. AMG 900 was identified as a suitable candidate for clinical development based on its low single digit nanomolar potency against MDR cell lines, robust pharmacodynamic response, and high selectivity against other kinases. Oral administration of AMG 900 at well-tolerated doses using three distinct schedules significantly inhibited the growth of HCT116 tumor xenograft cells *in vivo*. AMG 900 is currently undergoing phase 1 clinical evaluation in patients with advanced cancers.

MEDI 284

Synthetic design and mechanistic studies as tools for the development of a genotoxic impurity control strategy

***Matthew M Bio**, mbio@amgen.com, Eric Fang, Karl Hansen, Sean Wiedemann. Department of Chemical Process Research and Development, Amgen, Inc, Thousand Oaks, CA 91320, United States*

Appropriate drug substance control strategy and specifications are best achieved when based on a understanding of the manufacturing process and a close collaboration of drug substance and product development teams. For one of Amgen's clinical candidates, controlling genotoxic impurities in the drug substance and product presented a challenge to the chemistry, manufacturing and controls team responsible for developing the clinical candidate. Control of one class of impurities, hydrazines, drove us to redesign the synthetic route to the drug substance. This redesign included the development of a practical and highly regioselective, thiophene metalation procedure which eliminated N,N-dimethylhydrazine from one step and the need for excess hydrazine in a subsequent step. The second genotoxic concern is an aniline

intermediate in the synthetic route. The aniline is also observed as a degradation product of the drug product, the formation of which has the potential to impact the shelf-life and/or storage conditions of the drug product. Control over the aniline content in drug substance and product was established through a three-pronged approach. First, a detailed mechanistic study of the final synthetic step, a SnAr with aniline as the nucleophile, led to the discovery that the step was autocatalytic. Understanding this mechanism enabled us to develop reaction conditions to minimize carryover of the aniline to the freebase drug substance. Second, the final salt-forming and crystallization process was optimized to reject the aniline within an established range and thereby set achievable specifications for aniline in the freebase. Finally, we studied the mechanism of drug product degradation which led us to identify parameters leading to the formation of aniline and propose changes to both the drug product process and the DS attributes to achieve the target product profile. This collaborative and 1st principles approach to developing a comprehensive genotoxic control strategy will be presented in detail.

MEDI 285

Identification of BMS-791325 from the preclinical drug discovery search for an allosteric NS5B replicase inhibitor for the treatment of hepatitis C

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Approximately 3% (170 million) of the world's population are infected with hepatitis C virus (HCV). Considerable resources have been directed to preclinical and clinical research aimed at achieving improved cure rate, shorter duration of treatment, improved tolerability, and Peg-IFNα-free combination regimens in the rapidly evolving, global effort to address this disease. This presentation will describe the preclinical research program, with an emphasis on strategy and the medicinal chemistry decisions, which enabled the discovery of BMS-791325, an allosteric inhibitor of HCV NS5B polymerase (replicase). This compound is a potent inhibitor of both genotype 1a and 1b HCV virus in replicon studies and displayed pharmacokinetics in three species and a preclinical profile which supported advancement to clinical development. BMS-791325 is currently

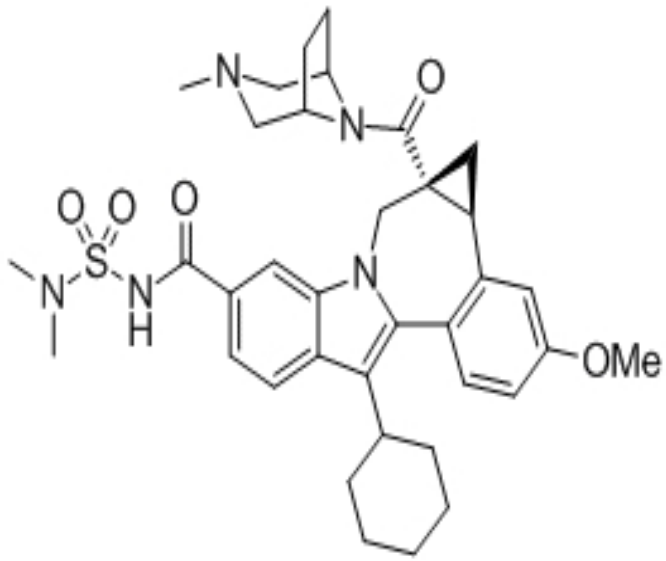
in Phase III clinical trials designed to explore its use in combination therapy against HCV in an IFNα-free regimen with daclatasvir and asunaprevir, two mechanistically distinct, direct acting antiviral agents.

MEDI 286

Route discovery and process development of BMS-791325: A pharmaceutical development candidate for the treatment of hepatitis

***Albert J DelMonte**, albert.delmonte@bms.com. Chemical Development, Bristol-Myers Squibb, New Brunswick, NJ 08903, United States*

This lecture will detail the route discovery and development efforts that culminated in a convergent synthetic route capable of producing commercial quantities of drug substance. The technical perspective will include discussion on an asymmetric approach as well as a more traditional resolution approach. In addition, an unusual Pd-catalyzed C-H arylation reaction on pilot scale will be discussed.



BMS-791325

MEDI 287

Discovery of PF-05212384/PKI-587: A phase 2 clinical PI3K/mTOR inhibitor

***Christoph M Dehnhardt**[1], cdehnhardt@xenon-pharma.com, Aranapkam M Venkatesan[1], Efren Delos Santos[1], Semiramis Ayral Kaloustian[1], Natasja Broojimans[1], Irwin Hollander[2], Zecheng Chen[1], Judy Lucas[1], Robert Mallon[2], Jeroen Verheijen[1], Arie*

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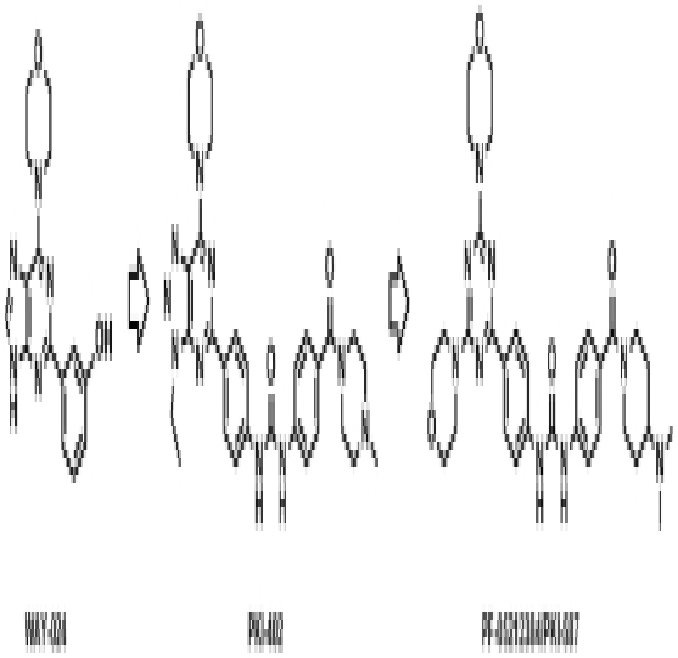
The phosphoinositol 3-kinase (PI3K) signaling pathway plays an important role in mitogenic signaling, cell survival, cell growth, proliferation and metabolic control. PF-05212384/PKI-587 is an intravenously (IV) administered pan-PI3K (class I)/mTOR inhibitor that is efficacious in humans and is currently undergoing phase 2 clinical evaluation. The presentation describes the lead optimization efforts, which culminated in the discovery of PF-05212384/PKI-587.

[figure 1]

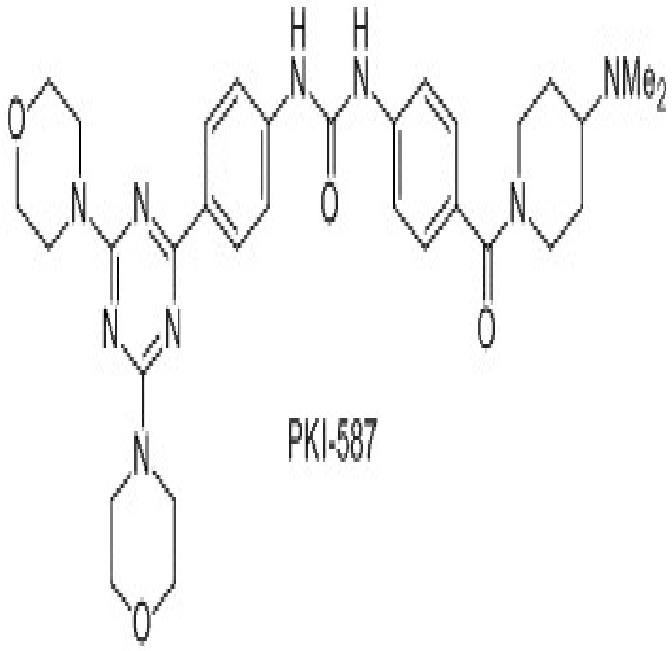


MEDI 288

Process development of the PI3K/mTOR inhibitor PKI-587

***Bob Dugger**, robert.w.dugger@pfizer.com. Department of Chemical Research and Development, Pfizer Inc, Groton, CT 06340, United States*

The original synthesis of PKI-587 was a linear 5 step synthesis. One step was a Pd catalyzed Suzuki reaction and another used a low melting isocyanate, both of which we would like to avoid in an improved route. We also saw an opportunity to make the synthesis convergent. The talk will present the different chemistry we explored to achieve these goals.



PKI-587

MEDI 289

Glutamate: Excitatory synapses deliver therapeutic potential

Jeffrey M Witkin*, witkin_jeffrey_m@lilly.com. Lilly Research Laboratories, Eli Lilly and Company, Inc., Indianapolis, Indiana 46285, United States*

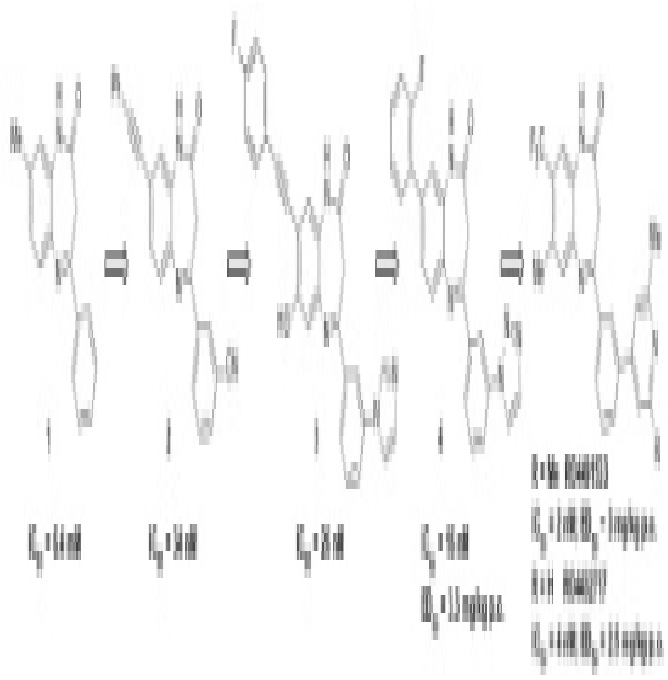
Multiple approaches to glutamate receptor therapeutics have been interrogated over the years. CNS penetrant small molecules have been designed to block major excitatory ion channel receptors including the AMPA, NMDA, and kainite channel subtypes. For example, the AMPA channel blocker Perampanel was launched in 2012 for the treatment of epilepsy. Proof of concept studies support blockade of NMDA receptors to relieve symptoms in patients suffering from treatment-resistant depression (TRD). In addition to ion channels, metabotropic glutamate (mGlu) receptors provide another series of proteins guiding chemical synthesis and medicinal chemistry refinement. Of the eight mGlu receptor subtypes, significant progress has been made in the areas of cognitive dysfunction, anxiety, and schizophrenia targeting mGlu2 and mGlu5 receptors. In the area of TRD, mGlu2 and mGluR3 receptors have been a primary focus. Taken together, deep knowledge of glutamate receptor biology combined with the development of selective pharmacological tools may lead to a number of novel therapeutics.

MEDI 290

1,3-Dihydro-benzo[b][1,4]diazepin-2-one derivatives as selective mGlu2/3 NAM alleviate pharmacological or age-related cognitive impairment in rodents and monkeys

***Thomas Woltering**, thomas.woltering@roche.com, T. M. Ballard, J. Wichmann, E. Goetschi, R. Moog, M. Schmitt, J. Huwyler, F. Knoflach, S. Gatti. Department of pRED Basel, F. Hoffmann-La Roche Ltd, Basel, Switzerland*

We have synthesized a series of 1,3-dihydro-benzo[b][1,4]diazepin-2-one derivatives, which are potent and selective negative allosteric modulators (NAM) of the metabotropic glutamate (mGlu) 2/3 receptor, and tested suitable compounds in cognition models in rodents and monkeys, e.g. an operant conditioning delayed match to position (DMTP) task and the Morris water maze. From the screening hit **1** the series evolved *via* compounds like **2** [1], **3** [2] and **4** [3] to the brain penetrant RO4491533 and RO4432717 [4].



They consistently showed *in vivo* activity after oral administration in both the DMTP task and in the Morris water maze. mGlu2/3 NAM dose-dependently attenuated the impairment of working memory induced by either the mGlu2/3 selective agonist LY354740 or scopolamine. Moreover, in combination studies with a cholinesterase inhibitor RO4491533 shows apparent synergistic effects on working memory impairment induced by scopolamine. For further profiling of mGlu2/3 NAM on cognition, RO4432717 was tested in aged Fischer 344 rats (22-24 months) and in a mouse model of Alzheimer's disease, PS2APP transgenic mice aged 12-13 months [5]. In the Morris water maze test RO4432717 significantly improved spatial acquisition in aged rats after

4 days of treatment and it rescued completely the spatial learning deficit observed in 12 month old PS2APP transgenic mice the after ten days of treatment. Finally, RO4432717 was shown to improve performance of a delayed matching to sample (DMTS) task in aged rhesus monkeys.

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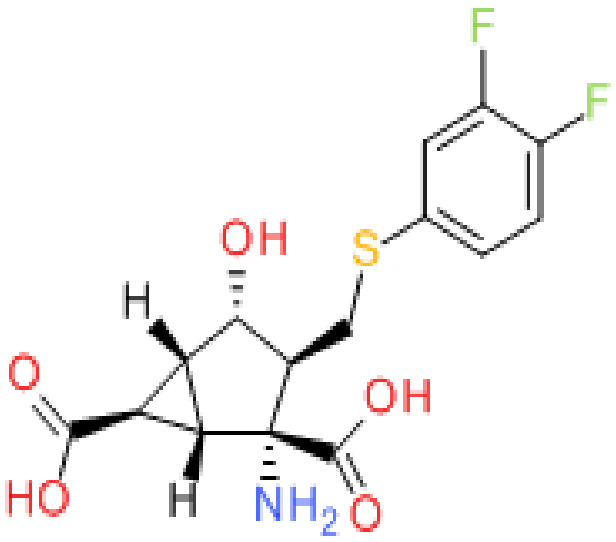
MEDI 291

Discovery, synthesis, and characterization of a potent antagonist of the metabotropic glutamate 2/3 receptors possessing anti-depressant and wake-promoting properties

Mark D. Chappell*, chappell_mark@lilly.com, Renhua Li, Stephon C. Smith, Bruce A. Dressman, Eric G. Tromiczak, Allie E. Tripp, Maria J. Blanco, Tatiana Vetman, Steven J. Quimby, James Matt, Thomas Britton, Adam M. Fivush, Jeffrey M. Schkeryantz, Albert Khilevich, Carlos Jaramillo, Mercedes Carpintero, Jose Eugenio de Diego, Mario Barberis, Susana Garcia, Jose F. Soriano, Jeffrey M. Witkin, Xia Li, Carl Overshiner, Keith A. Wafford, Wesley Seidel, Xu-Shan Wang, Beverly Heinz, Steven Swanson, John Catlow, David Bedwell, Paul Ornstein, Charles H. Mitch. Discovery Chemistry Research & Technologies, Eli Lilly & Company, Indianapolis, IN 46285, United States*

Metabotropic glutamate 2/3 receptors (mGlu2/3R) are class C G-protein coupled receptors that are highly expressed in the central nervous system and modulate neuronal excitability. Negative modulation of the mGlu2/3R receptors has demonstrated an anti-depressant profile and wake-promoting signature in preclinical animal models, thus making these receptors attractive therapeutic targets. This communication will discuss the synthesis and medicinal chemistry optimization of the bicyclo[3.1.0]hexane glutamic acid scaffold to produce selective orthosteric mGluR 2/3 antagonists, such as compound **1.**



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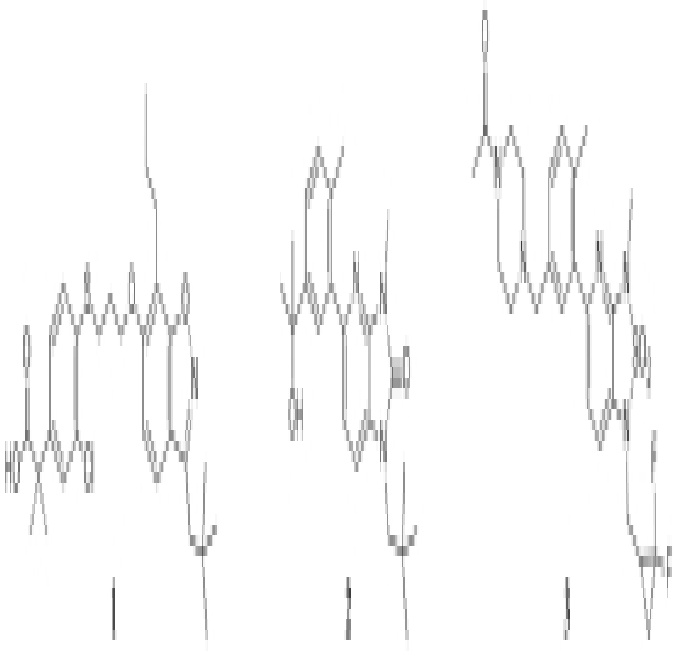
MEDI 292

Development of benzothiadiazole-diones as positive allosteric modulators of mGluR2 for the treatment of schizophrenia

Mark E Layton[1], *mark_layton@merck.com, Timothy J Hartingh*[1], *Alexander J Reif*[1], *Michael J Kelly*[1], *Joseph E Pero*[1], *Michael A Rossi*[1], *Hannah D Fiji*[1], *Kevin J Rodzinak*[1], *Vadim Y Dudkin*[1], *Kenneth L Arrington*[1], *Robert M Garbaccio*[1], *Marlene A Jacobson*[2], *Brian C Magliaro*[2], *Pete H Hutson*[2], *Julie A O'Brien*[3], *Sarah L Huszar*[4], *Jason M Uslaner*[4], *Sean Smith*[4], *R Pachmerhiwala*[4], *Kerry L Fillgrove*[5], *Cuyue Tang*[5], *Yuhsin Kuo*[5]. *(1) Department of Medicinal Chemistry, Merck & Co., Inc., West Point, PA 19486, United States (2) Department of Psychiatry Research, Merck & Co., Inc., West Point, PA 19486, United States (3) Department of In Vitro Sciences, Merck & Co., Inc., West Point, PA 19486, United States (4) Department of Central Pharmacology, Merck & Co., Inc., West Point, PA 19486, United States (5) Department of Drug Metabolism, Merck & Co., Inc., West Point, PA 19486, United States*

Modulation of excitatory glutamate neurotransmission through activation of the metabotropic glutamate receptor 2 (mGluR2) represents a promising approach for the treatment of positive symptoms associated with schizophrenia. Although the dual mGluR2/3 agonist LY2140023 has had mixed results in the clinic, positive allosteric modulators (PAMs) of mGluR2 may overcome the shortcomings of orthosteric agonists by achieving superior subtype selectivity and tuning physiological effects through promoting activation of receptors only in the presence of endogenous ligand. Herein we describe the optimization of an HTS hit benzisoxazole **1** to aza-benzimidazolone **2**

through truncation and identification of the key pharmacophore, followed by core modification to improve physico-chemical properties. Following oral administration, compound **2** was identified as an efficacious mGluR2 PAM in pre-clinical measures of antipsychotic-like effects at CSF exposures consistent with *in vitro* potency. In order to improve *in vivo* potency, compound **2** was further optimized with a focus on potency, physico-chemical properties and pharmacokinetics to provide benzothiadiazole-dione **3** as an orally bioavailable mGluR2 PAM. The in-depth characterization of compound **3** will be presented, including the PK/PD relationship between plasma concentrations, receptor occupancy, efficacy in rodent behavioral tests, as well as the attenuation of MK-801-induced glutamate release in the medial prefrontal cortex.



MEDI 293

Discovery of BMS-955829, a potent positive allosteric modulator of metabotropic glutamate receptor 5 (mGluR5) for the treatment of schizophrenia

Andrew P Degnan*, andrew.degnan@bms.com. Neuroscience Chemistry, Bristol-Myers Squibb, Wallingford, CT 06492, United States*

Schizophrenia is a debilitating illness which affects approximately 1% of the population. While current therapeutics, based on dopamine 2 (D2) inhibition, are highly effective at treating the positive symptoms of schizophrenia, they are of limited utility in addressing the negative and cognitive symptoms. It has been suggested that loss of N-methyl-D-aspartate (NMDA) receptor function may give rise to schizophrenia symptomology. Activation of NMDA receptors is mediated by metabotropic glutamate receptor 5

(mGluR5) which is co-expressed with them on GABA interneurons. High throughput screening identified a novel, potent positive allosteric modulator (PAM) of mGluR5. Optimization of the chemotype led to compounds with exquisite potency and a range of mGluR5 pharmacology profiles (high fold shift PAMs, low fold shift PAMs, and Ago-PAMs). Interrogation of these leads resulted in the identification of BMS-955829, a low fold shift PAM with efficacy in rodent cognition models and a favorable preclinical safety profile.

MEDI 294

PF-04958242: A novel AMPA receptor positive allosteric modulator for the treatment of cognitive impairments associated with schizophrenia

***Nandini C. Patel**, nandini.c.patel@pfizer.com. Neurosciences Medicinal Chemistry, Pfizer Inc., Cambridge, MA 02139, United States*

α-Amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors (AMPAR) mediate fast glutamatergic excitatory neurotransmission throughout the central nervous system. Changes in AMPAR density and subunit composition dynamically regulate synaptic efficacy underlying adaptive brain functions. Based on the intimate relationship between AMPAR and *N*-methyl-D-aspartate receptors (NMDAR) and the hypothesis that schizophrenia fundamentally results from dysfunction in NMDAR glutamatergic neurotransmission, positive allosteric modulation (“potentiation”) of AMPAR may overcome certain cognitive impairments in schizophrenia. This presentation will discuss the medicinal chemistry strategy, along with the preclinical pharmacology, pharmacokinetics and safety data, that led to the discovery of the novel AMPAR potentiator PF-04958242. PF-04958242 is currently in late-Phase 1 clinical trials; it has excellent safety, tolerability and pharmacokinetics in both healthy volunteers and subjects with schizophrenia.

MEDI 295

Matched molecular pair analysis: The right tool for which job?

***Andrew G Leach**, a.g.leach@ljmu.ac.uk. School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Liverpool, United KingdomMedChemica Ltd, Newcastle-under-lyme, United Kingdom*

Matched molecular pair analysis (MMPA) and related approaches have received much attention in the medicinal chemistry literature and some of the recent advances will be reviewed. These include the use of three-dimensional data in MMPA, the link to synthetic routes and the value of increasing the specificity of the chemical context in which structural changes take place. The more specific a set of matched pairs are, the less data will be available; this presents a dilemma that can only be resolved by using larger and more diverse datasets. The principle focus of this presentation will therefore be upon the different behavior to be expected for different sized datasets.

The value of MMPA as a compound design tool will particularly be highlighted. Real and simulated datasets will be used to explore the ability of MMPA to predict the best compounds to make. A further step of predicting the numerical values of the properties of those compounds provides a different kind of challenge. A probabilistic approach will be advocated for compound design and an informed balance of MMPA and QSAR for property prediction. The root causes of the different levels of accuracy and utility in these different applications will be explored.

The ability to convert the recommendation to employ ever larger datasets into practice requires changes in the size of the datasets available. Each large pharmaceutical company has available to it large databases containing the measured value of a range of pharmaceutically relevant properties for many thousands of molecules. By bringing these sets of data together, a step-change in the ability to make good predictions about which compounds to make can be brought about. A recent initiative in which large datasets have been shared among pharmaceutical companies will be presented.

MEDI 296

Fine-tuning ligand binding, conformation, and properties by local dipole changes

***Bernd Kuhn**, bernd.kuhn@roche.com. Molecular Design and Chemical Biology, F. Hoffmann-La Roche, Basel, Switzerland*

For successful drug design it is crucial to understand the interaction and conformation preferences of all molecular fragments in a ligand. An efficient, yet sometimes poorly characterized, approach to optimize target interactions, molecular preorganization and physicochemical properties is the modification of the local dipole moment of a ligand moiety. The talk highlights research in this area, namely a systematic investigation of the stacking of heterocycles with protein amide groups, the preferred protein environment of selected functional groups with substantial dipole moment, and the multiple effects of dipole modification by introducing fluorine atoms.

MEDI 297

Sulfoximines: A neglected opportunity in medicinal chemistry

***Ulrich TJ Lücking**, ulrich.luecking@bayer.com. Department of Medicinal Chemistry, Bayer HealthCare, Berlin, Berlin 13353, Germany*

The monoaza analogues of sulfones — sulfoximines — are an underrepresented pharmacophore in drug discovery approaches, even though they offer very interesting properties to the medicinal chemist.

In the discovery process of the pan-CDK inhibitor BAY 1000394 (roniciclib), for example, the unconventional proposal to introduce a sulfoximine group into the lead series initially led to raised eyebrows, since sulfoximines have seldom been used in

drug discovery. However, it was the introduction of the sulfoximine group that finally allowed the fundamental issues of the project to be overcome, culminating in the identification of the clinical sulfoximine pan-CDK inhibitor BAY 1000394.

Based on the case study of BAY 1000394, this presentation provides an overview on the properties of the sulfoximine group and its rather limited history in medicinal chemistry approaches, focusing on selected examples where the concept for its use as a pharmacophore and the outcome are available.

MEDI 298

Improving the plausibility of success in drug discovery with the use of inefficient metrics

***Michael D Shultz**, michael.shultz@novartis.com. Global Discovery Chemistry, Novartis Institutes for Biomedical Research, Inc., Cambridge, MA 02139, United States*

Over the past 15 years there have been extensive efforts to understand and reduce the high attrition rates of drug candidates with an increased focus on physicochemical properties. The fruits of this labor have been the generation of numerous efficiency indices, metric-based rules and visualization tools to help guide medicinal chemists in the design of new compounds with more favorable properties. This deluge of information may have had the unintended consequence of further obfuscating molecular optimizations by the inability of these scoring functions, rules and guides to reach a consensus on when a particular transformation is identified as beneficial. The difficulty in improving our chances of success may be caused by the inherent problem of properly understanding and utilizing probabilities in decision making. Several widely used efficiency metrics such as ligand efficiency can be demonstrated as invalid despite appearing as highly plausible methods. This presentation will discuss some possible reasons for irrational decision making in medicinal chemistry and why it is easier to improve the plausibility of success than the probability of success.

MEDI 299

Physical properties in drug discovery: Facts, patterns, and the principles of good medicinal chemistry practice

***Robert J Young**, rob.j.young@gsk.com, Darren VS Green, Michael M Hann, Alan P Hill, Paul D Leeson. Chemical Sciences, GlaxoSmithKline R&D, Stevenage, Hertfordshire SG1 2NY, United Kingdom*

Medicinal chemistry is a science, bordering on an art form, where chemists (more often than not trained in synthesis) interface with many complementary disciplines to drive the drug discovery process. The key to all of this is to understand the behaviour of prototype molecules in biological surroundings, observing and measuring data to

describe the various processes and interactions; fundamentally, many such phenomena are linked to the physical properties of the molecules.

The lipophilicity and solubility properties of molecules have come under particular scrutiny in recent years, particularly the significance of having these in a sub-optimal range and consequences this has had on risk and attrition. The factual background of analyses within GSK, where the impact of chromatographic hydrophobicity measures and aromatic ring count was shown to have profound impact, will briefly be reprised, followed by the recognition of patterns and formulation of probabilistic principles that guide lower risk strategies in drug discovery programmes. Finally, illustrative examples will be given of how better physical properties, combined with efficiency measures, can provide compelling differentiation within experimental molecules and drugs for particular targets.

MEDI 300

Breaking bad: Advantages of protein degradation over inhibition

***Craig M. Crews**, craig.crews@yale.edu. Departments of Chemistry; Pharmacology; and MCDB, Yale University, New Haven, CT 06511, United States*

While consisting of successful drugs, the current pharmacopeia also has inherent limitations based on its 'Occupancy-based' paradigm of pharmaceutical control. These limitations include: 1) the need to achieve/maintain high systemic exposure to insure sufficient in vivo protein inhibition, 2) the potential off-target side effects due to high in vivo concentrations, and 3) the need to bind to an active site, thus limiting the potential 'drug target space' to a fraction of the proteome. As an alternative pharmaceutical strategy, induced protein degradation lacks these limitations. Based on an 'Event-driven' paradigm, this approach offers a novel, catalytic mechanism to irreversibly inhibit protein function, namely, the intracellular destruction of target proteins. This is achieved via recruitment of target proteins to the cellular quality control machinery, i.e., the Ubiquitin/Proteasome System (UPS). For the past decade, the Crews lab has focused on developing different strategies for inducing protein degradation, including the PROTAC (Proteolytic Targeting Chimera) and HyT (Hydrophobic Tagging) technologies. These heterodimeric ligand approaches offer the potential to selectively knock down intracellular levels of specific proteins, irrespective of protein class, thus allowing one to target those proteins that are currently not '*pharmacologically vulnerable*'.

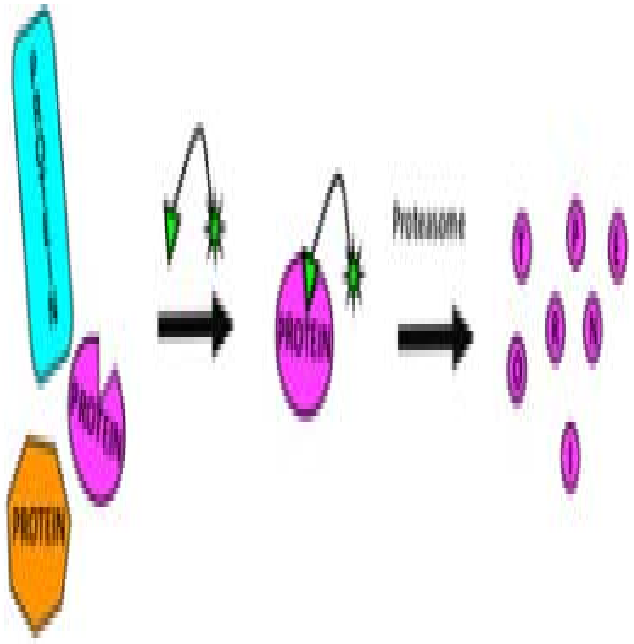
MEDI 301

Greasing the wheels of destruction: Hydrophobic tagging

***Lizbeth Hedstrom**[1], hedstrom@brandeis.edu, Marcus J.C. Long[2], Rory Coffey[4], Yuntao Shi[3], Arifa Ahsan[5], Kaitlin Hulce[5]. (1) Departments of Biology and Chemistry, Brandeis University, Waltham, MA 02453, United States (2) Graduate Program in*

Biochemistry and Biophysics, Brandeis University, Waltham, MA 02453, United States (3) Graduate Program in Chemistry, Brandeis University, Waltham, MA 02453, United States (4) Graduate Program in Molecular and Cellular Biology, Brandeis University, Waltham, MA 02453, United States (5) Department of Biochemistry, Brandeis University, Waltham, MA 02453, United States

We serendipitously discovered that the degradation of glutathione S-transferase is accelerated when treated with a covalent ligand, ethacrynic acid, linked to *tert*-butyl carbamate-protected arginine moiety (Boc_3Arg). Similarly, the degradation of *E. coli* dihydrofolate reductase is accelerated when treated with a noncovalent ligand, trimethoprim, linked to Boc_3Arg. The Boc_3Arg degron is another example of the hydrophobic degrons initially discovered by Crews and colleagues. Here we report progress on defining the affinity requirements of noncovalent ligands and further generalization of hydrophobic tagging to new targets and ligands.



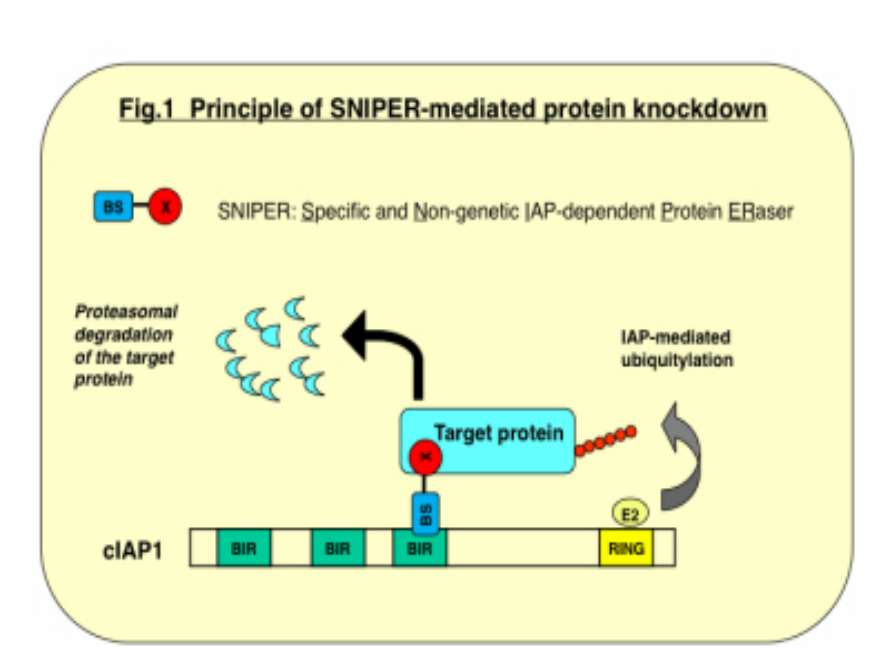
MEDI 302

SNIPER: Inducing protein degradation via recruitment to IAP

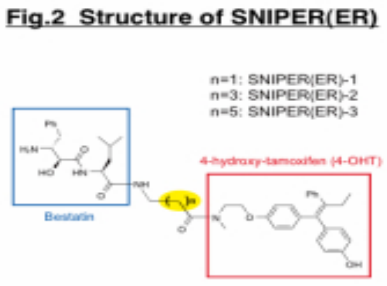
***Mikihiko Naito**, miki-naito@nihs.go.jp. Department of Biochemistry and Molecular Biology, National Institute of Health Sciences, Tokyo, Japan*

SNIPER (Specific and Nongenetic IAP-mediated Protein ERaser) is a hybrid molecule composed of a ligand for cIAP1 (bestatin, BS) linked to another ligand (X) for a target protein, which is designed to cross-link cIAP1 and the target protein, thereby inducing

IAP-mediated ubiquitylation and proteasomal degradation of the target protein (Fig.1).



We initially employed *all-trans* retinoic acid (ATRA) to target cellular retinoic acid binding protein-II (CRABP-II), and SNIPER(CRABP) reduced the CRABP-II protein in the cells within several hours. With its modular structure, SNIPER technology can be easily applied to other target proteins. By using tamoxifen as a ligand, we also developed SNIPER(ER) to target estrogen receptor-alpha (ERα) (Fig.2). The SNIPER(ER) induces degradation of ERα followed by necrotic cell death in breast cancer cells. Thus, SNIPER can target a variety of cytosolic proteins for degradation, which might be useful for biological researches and therapeutic purposes.



MEDI 303

PROTACS: Two heads are better than one

***Kyung Bo Kim**, kbkim2@uky.edu. Department of Pharmaceutical Sciences, University of Kentucky, Lexington, KY 40536-0596, United States*

PROTACs (PROteolysis TArget-ing Chimeric molecules) have been established as a useful investigative tool that can achieve degradation of targeted proteins at the post-translational level. PROTACs are comprised of a moiety for E3 ubiquitin ligase recognition on one end and a ligand for binding to a targeted protein on the other end, thereby recruiting targeted proteins to cellular ubiquitin-proteasome systems for degradation. Proof-of-concept studies demonstrated that PROTACs targeting estrogen receptor (ER) or androgen receptor (AR) can be effective as a potential cancer therapeutic strategy. However, further development efforts have been hampered due to concerns associated with poor cell permeability and relatively low catalytic efficiency of current PROTAC molecules. Here, we show our efforts to improve the catalytic efficiency of PROTACs using different strategies, such as optimization of linker length and introduction of two protein ligands into a single PROTAC molecule.

MEDI 304

Inducing protein degradation: A new paradigm for pharmaceutical intervention

***John D Harling**[1], john.d.harling@gsk.com, Giovanna Bergamini[3], Marcus Bantscheff[3], Sebastien A Campos[1], Paul S Carter[2], Ian Churcher[1], Laurens Kruidenier[1], Afjal Miah[1], Christina Pancevac[1], Ian E Smith[1], Christopher J Tame[1]. (1) Protein Degradation DPU, GlaxoSmithKline, Stevenage, Hertfordshire SG12NY, United Kingdom (2) Molecular Discovery Research, GlaxoSmithKline, Stevenage, Hertfordshire SG12NY, United Kingdom (3) Cellzome, GlaxoSmithKline, Heidelberg, Germany*

Small molecule induced protein degradation represents an exciting frontier in chemical biology. The development of small molecule protein degraders as therapeutics would significantly increase the accessible druggable space since a direct functional response to the binding of a molecule to a protein is not required. The challenge to translate the current tool molecules into potential therapeutics is, however, significant. This talk will outline the early work we have undertaken at GSK with bifunctional degraders (PROTACs) to develop the technology and to build a broader developability dataset with these molecules to build confidence that molecules with profiles consistent with progression through to candidate selection and beyond are achievable. As these molecules require simultaneous binding to both a target and E3 ligase complex, they will inevitably sit outside of perceived normal small molecule property space. Relying on measured data rather than *in silico* prediction has been a critical element to this work.

MEDI 305

Rational design of small molecules targeting RNA

Matthew D Disney*, disney@scripps.edu. Chemistry, The Scripps Research Institute, Jupiter, FL 33458, United States*

Herein we will describe the development of rational methods to design small molecules that target RNA. Specifically, the development of an RNA motif-small molecule database will be described as well as the use of this database to design small molecules targeting a variety of important RNA drug targets from their suquence such as repeating transcripts and microRNA precusors. Designer small molecules are potent and selective modulators of RNA frunction in cellular and animal models of disease.

MEDI 306

Synthesis and biological evaluation of compounds with activity against antibacterial resistant bacteria

Elizabeth I Parkinson[1]*, parknsn2@illinois.edu, Joseph Bair*[2]*, Brad Nakamura*[1]*, Hani Kuttab*[1]*, Emma Southgate*[3]*, Paul J Hergenrother*[1]*. (1) Department of Chemistry, University of Illinois at Urbana-Champaign, Urbana, IL 61801, United States (2) ExxonMobil, NJ, United States (3) Department of Chemistry, Carleton College, Northfield, MN, United States*

Antibiotic resistance is a global health crisis. This year in the United States, approximately 2 million people will acquire infections resistant to at least one antibiotic and of those an estimated 23,000 people will die. While better antibiotic stewardship is believed to be capable of slowing the spread of this resistance, in order to combat these infections novel antibiotics with activity against resistant strains are needed. Recently, a novel antibiotic with activity against antibacterial resistant bacteria was discovered. Herein we describe the total synthesis of this molecule as well as further evaluation of its antibacterial activity and mode of action.

MEDI 307

Discovery of quinazolinones as an antibiotic class active against methicillin-resistant *Staphylococcus aureus*

Renee Bouley[1]*, rbouley@nd.edu, Malika Kumarasiri*[1]*, Wei Song*[1]*, Lisandro Otero*[2]*, Zhihong Peng*[1]*, Mark A Suckow*[3]*, Valerie A Schroeder*[3]*, William R Wolter*[3]*, Elena Lastochkin*[1]*, Nuno Antunes*[1]*, Hualiang Pi*[1]*, Sergei Vakulenko*[1]*, Juan Hermoso*[2]*, Mayland Chang*[1]*, Shahriar Mobashery*[1]*. (1) Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN - Indiana 46556, United States (2) Department of Crystallography and Structural Biology, Consejo Superior de Investigaciones Cientificas, Madrid, Spain (3) Freimann Life Sciences Center and Department of Biology, University of Notre Dame, Notre Dame, IN - Indiana 46556, United States*

Methicillin-resistant *Staphylococcus aureus* (MRSA) has become a major threat worldwide due to its resistance to a wide range of antibiotics. MRSA encodes a unique penicillin-binding protein (PBP), termed PBP2a, which exhibits a low affinity to traditional β-lactam antibiotics. This low affinity is attributed structurally to the presence of a loop that creates a closed active-site conformation. Allosteric activation of PBP2a is required in order to induce an open state, which is catalytically functional. PBP2a is a target for MRSA antibacterial discovery. *In silico* docking and scoring of a 1.2-million compound library using the structure of PBP2a led to the discovery of a novel non-covalent inhibitor containing a quinazolinone core with promising antibacterial activity. Subsequent medicinal chemistry explorations of the structural space led to a quinazolinone with efficacy in a mouse model of infection. The elucidation of the structure of a complex of PBP2a with this quinazolinone showed binding to the allosteric site, as well as profound protein conformational changes at the active site. These conformational changes lead to enlargement of the active site and access to the site by the antibiotic. Further exploration of the antibacterial mechanism of action has been investigated through the use of macromolecular synthesis assays, which show inhibition of peptidogylcan biosynthesis by a quinazolinone. Enzyme activity assays have also shown that this quinazolinone inhibits PBP2a and PBP1, an essentail monofunctional transpeptidase. The current lead quinazolinone demonstrates excellent solubility and good pharmacokinetics in mice. Further investigations of the quinazolinone structural template have been explored in an attempt to discover an analog which balances potent *in vitro* activity with good pharmacokinetics and water solubility. Overall this class of antibiotics shows promise in utilizing PBPs as important biological targets in antibacterial drug development.

MEDI 308

Controlling KRAS gene expression through drug targeting of DNA secondary structures

Christine E Kaiser[1]*, cekaiser@email.arizona.edu, Laurence H Hurley*[1,2,3]*. (1) College of Pharmacy, University of Arizona, Tucson, Arizona 85721, United States (2) BIO5 Institue, University of Arizona, Tucson, Arizona 85721, United States (3) Arizona Cancer Center, University of Arizona, Tucson, Arizona 85724, United States*

KRAS is a well-validated drug target for anti-cancer therapy, yet no clinically useful drugs that directly inhibit its function currently exist. We aim to target KRAS at the transcriptional level, through DNA secondary structures. The proximal promoter of the KRAS gene contains a GC-rich nuclease hypersensitivity site with three potential DNA secondary structure-forming sequences: the Far-, Mid-, and Near-regions. As a result of transcription-induced negative superhelicity, these regions can open up to form unique secondary structures: G-quadruplexes on the G-rich strand and i-Motifs on the C-rich strand. While G-quadruplexes in the promoter region of proto-oncogenes have the potential to silence transcription, i-Motifs have the potential to act as transcriptional activators. Thus, compounds capable of binding to and stabilizing G-quadruplexes or destabilizing i-Motifs could decrease gene expression. To date, we have developed a

series of quindoline compounds that can either stabilize or destabilize the various G-quadruplexes forming in the KRAS promoter. Our results show that only those compounds that stabilize the Far-region G-quadruplex are capable of modulating KRAS gene transcription. We have also identified compounds capable of destabilizing the Mid-region i-Motif in favor of a hairpin species. These results will be discussed in terms of a strategy to silence KRAS gene expression by targeting either the G-quadruplex or the i-Motif structures.

MEDI 309

Development of small molecule inhibitors of the breast cancer oncoprotein APOBEC3B

Margaret E Olson[1]*, olso3706@umn.edu, Ming Li*[2]*, Angela L Perkins*[1]*, Aaron M Kempema*[1]*, Reuben S Harris*[2,3]*, Daniel A Harki*[1,3]*. (1) Department of Medicinal Chemistry, University of Minnesota, Minneapolis, MN 55455, United States (2) Department of Biochemistry, Molecular Biology & Biophysics, University of Minnesota, Minneapolis, MN 55455, United States (3) Masonic Cancer Center, University of Minnesota, Minneapolis, MN 55455, United States*

Genomic mutation lies at the crux of cancer development, propagation, and relapse. Cancer-causing mutation often arises from exogenous sources such as sunlight and chemical exposure; yet, *endogenous mutation can* also actively contribute to cancer development. Considerable evidence implicates APOBEC3B (A3B), a host C-to-U DNA deaminase, as a major source of mutation in breast cancer.[1] A3B is over-expressed in greater than 50% of primary breast cancers and 90% of breast cancer cell lines.[1] Tumors that over-express A3B have twice as many overall mutations as low A3B expressing tumors.[1] A3B knockdown studies directly link its activity to an increased frequency of genomic uracil content, C-to-T transitions, cell cycle deviations, and DNA fragmentation.[1]

As part of our on-going effort to test the hypothesis that small molecule inhibition of A3B will decrease the overall mutation rate in breast cancer cells and ultimately slow tumor evolution, the Harki and Harris Labs have completed the high-throughput screening (HTS) of approximately 500,000 small molecules. Previous work from our laboratories has identified both covalent and non-covalent inhibitors of related deaminase APOBEC3G (A3G).[2-4] This talk will highlight our ongoing efforts to develop first-in-class inhibitors of A3B through derivative synthesis of established A3G inhibitors as well as A3B-focused HTS.

[1]Burns, M. B.; Lackey, L.; Carpenter, M. A.; Rathore, A.; Land, A. M.; Leonard, B.; Refsland, E. W.; Kotandeniya, D.; Tretyakova, N.; Nikas, J. B.; Yee, D.; Temiz, N. A.; Donohue, D. E.; McDougle, R. M.; Brown, W. L.; Law, E. K.; Harris, R. S. *Nature* **2013** , *494*, 366.

[2]Li, M.; Shandilya, S. M. D.; Carpenter, M. A.; Rathore, A.; Brown, W. L.; Perkins, A. L.; Harki, D. A.; Solberg, J.; Hook, D. J.; Pandey, K. K.; Parniak, M. A.; Johnson, J. R.; Krogan, N. J.; Somasundaran, M.; Ali, A.; Schiffer, C. A.; Harris, R. S. *ACS Chem. Biol.* **2012** , *7*, 506.

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MEDI 310

Structure-guided design of inhibitors of the menin-mixed lineage leukemia (MLL) interaction for the treatment of MLL-associated leukemias

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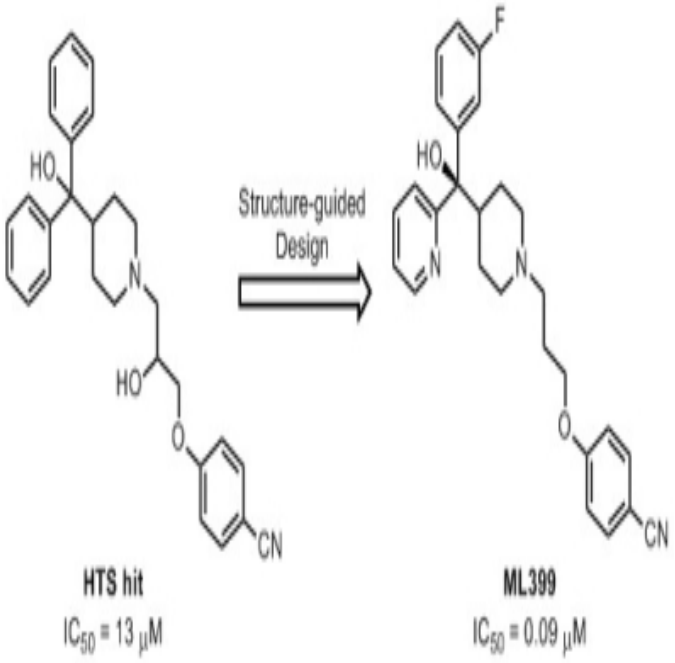
The development of a novel small molecule protein-protein interaction (PPI) inhibitor as a potential theurapeutic approach for the treatement of mixed lineage leukemia (MLL) will be discussed. The protein-protein interaction between menin and MLL is critical for oncogenic activity of MLL fusion proteins, resulting from translocations of the *MLL* gene. Patients with MLL-associated leukemias have very poor prognoses, with an estimated 35% overall five-year survival rate. Inhibition of this interaction with small molecule PPI inhibitors represents a novel therapeutic target.

Optimization of a hydroxymethyl piperidine series identified from a high-throughput screen of ~288,000 compounds through the NIH Molecular Libraries Program identified initial probe ML227, which closely mimics many key interactions of MLL with menin. Extensive crystallography studies enabled a structure-guided design approach for the optimization of this series. Each region was systematically studied to engage in favorable interactions with menin that were not observed in the HTS hit while addressing metabolic and pharmacokinetic liabilites. This resulted in the second generation probe ML399.



Improvements in potency and physicochemical properties to ML227 will allow for evaluation in animal models of mixed lineage leukemia and provides an example of PPI inhibition in the context of epigenetic regulation.

MEDI 311

Discovery of a novel class of potent, broad-spectrum antibacterial agents containing bis-thioureas and bis-ureas

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Rapidly emerging antibiotic resistance to currently available antibiotics represents a serious threat to global human health, and thus novel classes of compounds that are active against both drug-sensitive and drug-resistant bacterial strains need to be identified. In this presentation, we report a novel class of antibacterial agents containing oligoamine linkers attached to either (bis)ureas or (bis)thioureas. Several representative oligoamines with linker lengths ranging from 3 to 7 carbons have been chemically attached to (bis)ureas or (bis)thiourea functionalities. The minimum inhibitor concentration (MIC) values of 18 terminally substituted (bis)urea or (bis)thiourea compounds range between 2 to 256 μg/ml. Interestingly, isosteric replacement of the central amine with a methylene unit results in complete loss of antibacterial activity

thereby emphasizing the significance of two central cationic amine moieties. The terminal (bis)urea or (bis)thiourea moieties confer superior activity, as compared to the polyamines spermine (MIC >256 µg/ml) and spermidine (MIC >256 µg/ml). One of the most active compounds in the series, BP-107-5 (MIC 2 µg/ml) has greater antibacterial activity than gentamycin (MIC 8 µg/ml) or chloramphenicol (MIC 8 µg/ml). In the time-kill kinetics experiment, BP-107-5 exhibited a rapid, concentration-dependent killing of *Pseudomonas aeruginosa* (Pa), *Escherichia coli* (Ec) and *Staphylococcus aureus* (Sa) achieving a 6-log (99%) reduction in viable bacteria within 3 hours. When mixed with kanamycin, BP-107-5 produces a synergistic effect, whereas with norfloxacin it shows additive effects. Compound BP-107-5 displays selectivity of 5.3-fold against Pa, 16-fold against Ec and 5.3-fold against Sa when compared to cytotoxicity in normal kidney cells. Importantly, BP-107-5 displayed greater potency in biofilm inhibition and biofilm dispersal assays, in addition to being active against five clinical isolates of drug-resistant MRSA strains. Preliminary mechanistic studies suggest that BP-107-5 elicits antibiotic activity by causing membrane disruption. Overall, these results demonstrate that our compounds hold promise as a novel class of potent, broad-spectrum antibacterial agents with a unique mechanism of action.

MEDI 312

Synthesis of exceptionally potent tubulysins for drug-antibody conjugates

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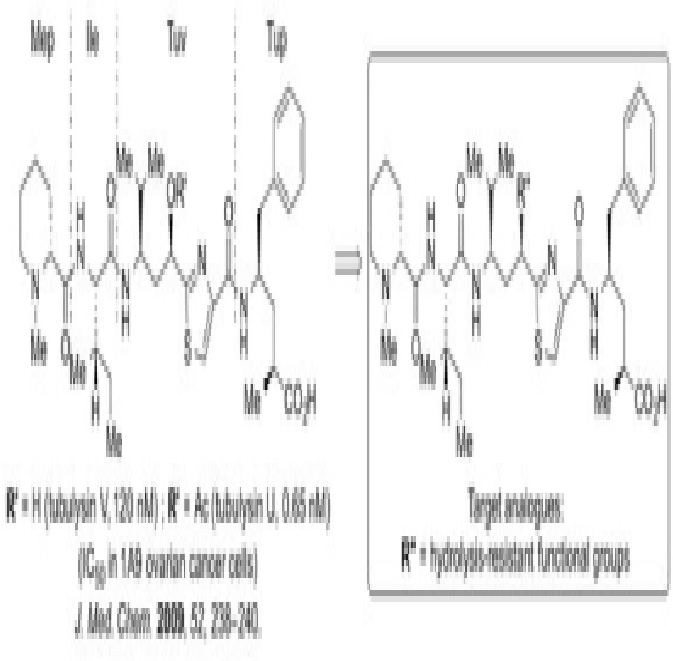
Tubulysins are antimitotic natural products with potent anticancer activity against multidrug-resistant (MDR) cancer cells, acting by inhibition of tubulin polymerization. The promising activity of these tetrapeptides makes them attractive leads, but the limited quantity available by fermentation and poor therapeutic index has hampered their clinical development. Synthetically accessible, highly cytotoxic tubulysins are therefore prime candidates for use in targeted delivery strategies such as drug-antibody conjugates. Comparison of the antiproliferative activities of tubulysins V and U exemplifies the importance of the tubuvaline (Tuv) acetate for potent activity. Yet, the potential *in vivo*efficacy of tubulysin U could be limited due to the lability of this acetate under mildly acidic or basic conditions. To address this issue, we have prepared two series of tubulysin analogues wherein the acetate in replaced with hydrolysis-resistant functional groups. The synthesis and early biochemical studies of these derivatives will be presented. Development of an optimized second-generation synthesis of tubulysin V enabled the synthesis of novel Tuv constructs, and rapid target analogue generation was possible using shared, late-stage intermediates. Initial evaluation of the target analogues' anticancer activity revealed some tolerance to changes at R'' with groups exhibiting increased hydrolytic stability. Several analogues have demonstrated exceptionally potent antiproliferative activity, and their potential for use in targeted delivery systems such as drug-antibody conjugates will be discussed.



MEDI 313

Expanding the bioorthogonal toolkit

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Bioorthogonal chemistries can be used to visualize and identify diverse classes of biomolecules in complex environments. Despite the tremendous success of these reactions in revealing new facets of cell and organismal biology, significant limitations remain: (1) many of the most common reactions are incompatible with one another, limiting their utility for studies of multiple biomolecules in tandem, and (2) many bioorthogonal reagents are too large for tagging native biomolecules in live cells. Thus, identifying new transformations that are not only suitable for use in vivo—but that also work well in tandem—remain important goals in chemical biology. We have developed new classes of bioorthogonal reactions using functionalized cyclopropenes. Cyclopropenes are attractive motifs for use in cells owing to their small size, biocompatibility, and diverse manifolds of reactivity. To date, we have identified three classes of small, strained cyclopropenes that exhibit unique reactivities and can be used concurrently for multi-component bioorthogonal labeling. Our progress in these areas will be discussed.

MEDI 314

Direct photocapture of bromodomains

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Medicinal chemistry techniques, including structure-based molecular design, fragment replacement and synthetic library enablement, were used to create potent inhibitors of BET and CREBBP bromodomains. One inhibitor featured the unusual tropolone methyl ether motif as a mimic of acetyl lysine. The intrinsic photoreactivity of the tropolone probe was harnessed successfully to directly photolabel bromodomains which inspired further development of an occupancy biomarker technology. These methods will facilitate target validation and drug discovery efforts in the bromodomain arena.

MEDI 315

Chemical tools for exploring bacterial virulence inhibitors

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The emergence of antibiotic-resistant bacteria and growing appreciation for beneficial commensal bacteria has motivated the development of small molecules that selectively target bacterial pathogens. Amongst the virulence mechanisms that have been discovered, type III secretion systems (T3SSs) are prime targets for novel anti-infective agents since these specialized protein secretion systems are often required for infection and conserved amongst many human Gram-negative bacterial pathogens such as *Salmonella*, *E. coli* and *Pseudomonas.* Here we present an efficient fluorescence assay for monitoring type III protein secretion, discovery of T3SS inhibitors from medicinal plants and the functional characterization of active compounds on *S.* Typhimurium type III protein secretion and infection. Notably, the generation of chemical probes based on these lead compounds revealed that T3SS inhibitors covalently target key bacterial proteins involved in *S.* Typhimurium invasion of host cells. Our results reveal novel lead compounds for targeting T3SSs and uncover an unpredicted mechanism of action for these small molecule inhibitors of bacterial virulence. Further development of T3SS inhibitors should afford new anti-infectives that preferentially attenuate bacterial pathogens and preserve the beneficial microbiota.

MEDI 316

Chemical proteomics sheds light on off-target drug-protein interactions

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LDK378 is an investigational selective inhibitor of Anaplastic lymphoma kinase (ALK) which has received Breakthrough Therapy designation by the FDA (US Food and Drug Administration) for the treatment of patients with ALK+ metastatic non-small lung cancer (NSCLC) who had progressed during treatment with, or were intolerant to, crizotinib.

The application of chemical proteomics to assess drug-protein interactions in naïve animal tissues, guided by specific target organ toxicities, represents a powerful complementary approach for identifying potential off-target binding proteins relevant for the observed toxicity phenotype. We have used a chemical proteomics strategy to investigate adverse events in preclinical animal studies associated with LDK378. A linker-modified drug was used for affinity-based pull-down experiments in tissue protein extracts. Off-target drug-protein interactors were identified via competition of binding using the parent drug molecule. To further investigate the role of candidate off-target binding proteins we assessed: 1) their function and possible association with toxicity phenotypes by combining preclinical animal study pathology, clinical chemistry, and transcriptomics with literature and pathway knowledge; 2) in vitro affinity- and/or functional drug-protein binding; 3) tissue-specific mRNA- and protein expression levels; and 4) off-target interactor localization in the relevant tissues. Together these combined molecular, biochemical and functional approaches provide novel insights into molecular mechanisms of toxicity.

Two Phase II clinical trials have been initiated in 2013 to further evaluate the compound in patient population with plans to initiate several Phase III clinical trials.

MEDI 317

Activity-based proteomics - applications for enzyme and inhibitor discovery

***Benjamin F Cravatt**, cravatt@scripps.edu. Department of Chemical Physiology, The Scripps Research Institute, La Jolla, CA 92037, United States*

Genome sequencing projects have revealed that eukaryotic and prokaryotic organisms universally possess a huge number of uncharacterized enzymes. The functional annotation of enzymatic pathways thus represents a grand challenge for researchers in the genome era. To address this problem, we have introduced chemical proteomic and metabolomic technologies that globally profile enzyme activities in complex biological systems. These methods include activity-based protein profiling (ABPP), which utilizes active site-directed chemical probes to determine the functional state of large numbers of enzymes in native proteomes. In this lecture, I will describe the application of ABPP and complementary proteomic methods to discover and functionally annotate enzyme activities in mammalian physiology and disease. I will also present competitive ABPP platforms for developing selective inhibitors for poorly characterized enzymes and

discuss ongoing challenges that face researchers interested in assigning protein function using chemoproteomic methods.

MEDI 318

Drug discovery innovation: The evolving role of medicinal chemistry

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

The Pharmaceutical Industry is facing significant challenges: a regulatory environment that has become more restrictive and where the safety bar is higher; substantial cost increases at the same time that R&D success rates have decreased and identifying highly validated targets has become even more difficult; and a system of payers worldwide trying to reduce costs and demanding more accountability. Medicinal chemists within the Industry have been particularly affected by changes made in response to these challenges.

Despite the headwinds, there is reason for optimism - not only because drug therapies will continue to be important for an aging population, but especially given considerable scientific advancements within Drug Discovery. For the medicinal chemist, the ability to rapidly evolve and adapt to new approaches and strategies will be key.

This presentation will be a personal view of the key innovations where Chemistry has the opportunity for greatest impact and will also highlight the attributes and capabilities needed by the future medicinal chemist.

MEDI 319

Multiple models for industrial-academic collaborations in CNS drug discovery

***Craig W Lindsley**, craig.lindsley@vanderbilt.edu. Vanderbilt Center for Neuroscience Drug Discovery, Vanderbilt Medical Center, Nashville, TN 37232-6600, United States*

This lecture will provide an overview of the Vanderbilt Center for Neuroscience Drug Discovery (VCNDD), focusing on how an Academic Drug Discovery Center was established and is maintained. I will discuss why we elected to focus on allosteric modulation of GPCRs and how we interact with corporate partners (both biotech and big Pharma) and the structure of licensing and sponsored research. Key to the success of the Center is an appreciation that there is no 'one size fits all' for collaborations with industry and biotech, and our Center has thus far formed seven unique collaborations. In this talk, I will discuss all of these, as well as the pros and cons of the different models. Finally, I will showcase an example from HTS to IND-enabling studies for a challenging target with a corporate partner that was successful due to the 'deep dive' into the basic science by our academic arm of the VCNDD.

MEDI 320

Drug discovery challenges and opportunities: Shaping the future of medicinal chemistry

***Karin Briner**, karin.briner@novartis.com. Novartis Institutes for BioMedical Research, Inc., United States*

Chemistry at the interfaces of various scientific disciplines plays a key role in innovative drug discovery. Challenges in pharmaceutical research and medicinal chemistry's role in addressing them will be presented with the intent to stimulate a discussion.

MEDI 321

Driving pharmaceutical research and development through leadership in chemistry

***Kevin Koch**, Kevin.koch@biogenidec.com. Drug Discovery: Chemical and Molecular Therapeutics, Biogen Idec Inc., United States*

Chemistry is the central science in the invention of small molecule drugs, therapeutic proteins and various hybrid drug discovery platforms. The discussion will include successful management practices and R & D strategies from my experience in Pharma, Biotech and VC backed start-up companies as well as the future role of chemists in these and other industries.

MEDI 322

Evolution of medicinal chemistry: Building and leading the global team

***Bruce D Roth**, roth.bruce@gene.com. Department of Discovery Chemistry, Genentech, South San Francisco, CA 94080, United States*

With the end of the “blockbuster era”, the pharmaceutical industry has come under enormous economic and scientific pressure. The response of the industry has been efforts to reduce cost (often through layoffs and site-closures), increase efficiency and reduce candidate attrition, while attempting to expand druggable target space. Offsetting the contraction of the industry, growing capabilities at many contract research organizations offer the opportunity to build an effective global network with reduced cost, while maintaining quality and efficiency. A working model of this network will be presented along with the evolving role, skill-set, challenges and opportunities for the medicinal chemist in this new world of drug discovery.

MEDI 323

Discovery and optimisation of 3-methoxybenzamide analogs as potent antibacterial inhibitors of FtsZ

***Neil R Stokes**, n.stokes@redxpharma.com. Redx Anti-Infectives, Alderley Park, Cheshire SK10 4TG, United Kingdom*

There is a compelling need to discover new antibacterial therapies to combat the problem of antibiotic drug resistance. One strategy to address this challenge is to develop inhibitors of previously-unexploited cellular targets. The bacterial cell division protein, FtsZ, is a novel and attractive target for small-molecule drug discovery. 3-methoxybenzamide, a low molecular weight ligand that is known to target FtsZ and inhibit cell division in *Bacillus subtilis*, was used as a fragment-based starting point for a medicinal chemistry programme. Initial synthetic modifications focussed on the structure-activity relationships of the benzamide moiety and extensions to the methoxy group to generate potent inhibitors of bacterial growth. Subsequent replacement of the alkyloxy chain with bicyclic substitutions led to the identification of a lead compound designated PC190723. PC190723 inhibited *Staphylococcus aureus*, including drug-resistant strains, with an average MIC of 1 µg/mL. Biochemical, cytological and genetic data confirmed the intracellular target of PC190723 to be FtsZ. Significantly, PC190723 was the first inhibitor of FtsZ to demonstrate efficacy *in vivo*. In the murine septicaemia infection model a single subcutaneous or intravenous administration of 30 mg/kg PC190723 resulted in 100 % survival of mice inoculated with a potentially-lethal dose of *S. aureus*. Further chemical optimisation of PC190723 led to the discovery of substituted oxazole-benzamide analogues that demonstrated enhanced antibacterial activity (MIC = 0.12 µg/mL against *S. aureus*), improved pharmacokinetics and efficacy in the murine thigh infection model. In summary, optimised derivatives of 3-methoxybenzamide have the potential to provide first-in-class small-molecule therapies for the treatment of drug-resistant staphylococcal infections.

MEDI 324

Identification of FtsZ modulators as broad-spectrum antibiotics: Challenges and lessons learned

***Alex Therien**, alex_therien@merck.com. Department of Infectious Diseases, Merck & Co., Inc., Kenilworth, NJ 07033, United States*

FtsZ has long been recognized as a potential therapeutic target for the treatment of bacterial infections. Because of the highly conserved nature of this protein across bacterial species, FtsZ inhibitors are thought to have potential as broad-spectrum antibiotics. This idea has led to the discovery, over the last 10 years or so, of several molecules described as having FtsZ inhibitory activity. These compounds are generally thought to target FtsZ because they induce bacterial filamentation (resulting from inhibition of bacillar cell division) and/or have direct effects on FtsZ GTPase activity or polymerization. However, the gold standard for demonstrating target engagement – that is, confirming a direct link between effects on the biochemical activity of the target and

on bacterial cell viability – is the ability of a compound to select for resistant clones that contain mutations in the putative target gene, or at least in genes involved in the related pathway(s). This is particularly important for a target like FtsZ for which biochemical assays lack robustness because of the complex and poorly understood interplay between GTPase activity and polymerization, and the requirement for high concentrations of purified FtsZ protein. Compounding the problem is the high rate of resistance emergence that is often associated with single-target agents and in the case of FtsZ, the emerging notion that biochemical activators as well as inhibitors may have antibacterial activity. The story of Merck's efforts to identify potent modulators of FtsZ activity as broad-spectrum antibiotics illustrates the difficulties involved and the importance of demonstrating target engagement as well as understanding the structural basis for compound interactions with FtsZ.

MEDI 325

Enhancing the efficacy of FtsZ-directed methoxybenzamides vs. staphylococcal infections and extending their activity to include Gram-negative pathogens

Daniel S. Pilch[1]*, pilchds@rwjms.rutgers.edu, Malvika Kaul*[1]*, Edmond J. LaVoie*[2]*, Ajit Parhi*[3]*, Yongzheng Zhang*[3]*, Lilly Mark*[3]*. (1) Pharmacology, Rutgers Robert Wood Johnson Medical School, Piscataway, New Jersey 08854-5635, United States (2) Medicinal Chemistry, Rutgers Ernest Mario School of Pharmacy, Piscataway, New Jersey 08855, United States (3) TAXIS Pharmaceuticals, Inc., North Brunswick, New Jersey 08902, United States*

The rise of infections caused by multidrug-resistant (MDR) bacterial pathogens, such as methicillin-resistant *Staphylococcus aureus* (MRSA) and extended-spectrum β-lactamase (ESBL)-producing enterobacteriaceae, has created an acute need for new antibiotics with novel mechanisms of action. The essential role that FtsZ plays in bacterial cell division makes it an attractive new antibiotic target that is not exploited by any current clinical agent. Methoxybenzamides represent a promising class of FtsZ-directed agents for the treatment of staphylococcal infections. However, their development as potentially useful clinical agents has been hampered by problems related to (i) drugability, (ii) frequency of resistance (FOR), and (iii) spectrum of activity. This presentation will focus on our approach to addressing each of these problems. We have developed Mannich base and carboximide derivatives with enhanced drugable properties and both oral and intravenous efficacy *in vivo* [1,2]. In addition, we have determined that the spectrum of activity of methoxybenzamide derivatives can be extended to include Gram-negative pathogens through concomitant efflux pump inhibition [3].

[1] M. Kaul, L. Mark, Y. Zhang, A.K. Parhi, E.J. LaVoie, D.S. Pilch, Pharmacokinetics and *In Vivo* Antistaphylococcal Efficacy of TXY541, a 1-Methylpiperidine-4-Carboxamide Prodrug of PC190723, Biochem. Pharmacol. 86 (2013) 1699-16707.

[2] M. Kaul, L. Mark, Y. Zhang, A.K. Parhi, E.J. LaVoie, D.S. Pilch, An FtsZ-Targeting Prodrug with Oral Antistaphylococcal Efficacy *In Vivo*, Antimicrob. Agents Chemother. 57 (2013) 5860-5869.

[3] M. Kaul, Y. Zhang, A.K. Parhi, E.J. LaVoie, D.S. Pilch, Inhibition of RND-Type Efflux Pumps Confers the FtsZ-Directed Prodrug TXY436 with Activity Against Gram-Negative Bacteria, Biochem. Pharmacol. (2014) doi 10.1016/j.bcp.2014.03.002.

MEDI 326

Targeting bacterial cell division protein FtsZ with small molecules and fluorescent probes

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Cell division protein FtsZ is the structural homolog of tubulin that directs the formation of the bacterial division ring. FtsZ is a target for new antibiotics, needed to fight the widespread emergence of resistant pathogens[1]. FtsZ assembles into dynamic filaments that hydrolyze GTP at the association interface between monomers, which can be selectively inhibited with modified nucleotides[2].

We have replaced FtsZ´s GTP with non-nucleotide synthetic inhibitors of bacterial cell division[3]. We tested compounds from the literature, virtual screening and a synthetic library. We identified three small molecules that bind *Bacillus subtilis* (Bs) FtsZ monomers with micromolar affinities, reduce the proportion of normal FtsZ rings at division sites, induce FtsZ delocalization into punctate foci, and effectively inhibit the growth of antibiotic-resistant *Staphylococcus aureus* (Sa) and *Enterococcus faecalis*. Chemical modification has defined their essential features for replacing GTP, leading to new analogs with improved affinity-activity that selectively inhibit FtsZ versus tubulin assembly.

Other essential zone in FtsZ is the cleft between the C-terminal and the nucleotide binding domains. In the crystal structure of Sa-FtsZ filaments[4], the antibacterial agent PC190723 binds as a wedge into the open interdomain cleft, allosterically stabilizing the filament. However, all structures of unassembled FtsZ from other species have a closed cleft. We have obtained a fluorescent PC190723 analog that binds to Bs- and Sa-FtsZ only in their assembled states, thus supporting the cleft-opening mechanism for the FtsZ assembly switch. This probe may be useful to screen for new antibacterials and permits imaging the FtsZ ring in live bacterial cells.

[1]Schaffner-Barbero C.et al. (2012) ACS Chem.Biol.7,269

[2]Marcelo F.et al. (2013) JACS 135,16418

[3]Ruiz-Avila L.B.et al. (2013) ACS Chem. Biol.8,2072

[4]Elsen N.et al. (2012) JACS 134,12342

MEDI 327

New generation anti-TB drug discovery targeting *Mtb*-FtsZ

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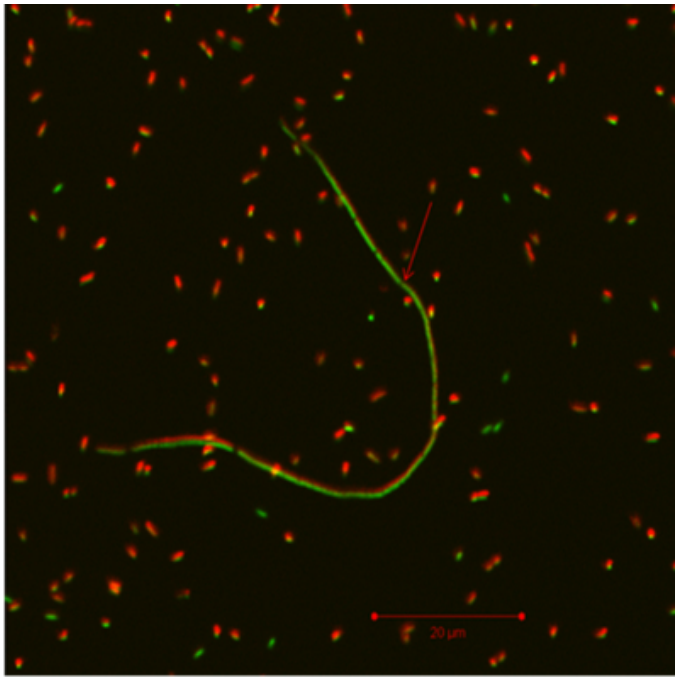
In 2012, WHO estimated that there were 8.6 million new cases of TB globally (13% co-infected with HIV) resulting in 1.3 million deaths. In addition, MDR-TB and XDR-TB are significant public health threats. Emergence of drug-resistant strains of *Mtb* makes many of the currently available anti-TB drugs powerless. Thus, there is an urgent need to develop new-generation anti-TB drugs with novel target to counter the bacterial resistance. FtsZ, bacterial homolog of tubulin, is a highly conserved and ubiquitous bacterial protein, playing an essential role in bacterial cytokinesis. The inhibition of proper FtsZ assembly causes the disruption of bacterial cell division, leading to cell lethality. Accordingly, *Mtb*-FtsZ inhibitors have a high potential to be next-generation anti-TB agents. Since FtsZ is an unexploited target for current antimicrobials, FtsZ inhibitors are active against clinical isolates of *Mtb* strains with various drug-resistance profile. This salient feature is highly promising against not only MDR-TB strains but also XDR-TB and TDR-TB strains. We have designed and synthesized novel classes of taxanes as well as benzimidazoles for creation of compound libraries as new chemical entities (NCEs) (>2,500 compounds). We have been leading the investigation in this field of research through productive collaboration with the Mycobacteria Research Laboratory, Colorado State University, as well as the Infectious Diseases Research Division of Sanofi. Two advanced lead benzimidazoles exhibited remarkable efficacy in the acute infection model using C57BL/6 mice via i.p. as well as p.o. route at 100 mg/kg dose/day for 10 days. SB-BI-38 reduced the bacterial load by 6.2 $\log_{10}$ CFU in lung and 4.0 $\log_{10}$ CFU in spleen (virtually no live bacterial counts) via p.o. route. SB-BI-42 was slightly less potent than SB-BI-38 in lung, but equally effective in spleen. The drug discovery and preclinical drug development of the next-generation anti-TB agents in our laboratory will be presented.

MEDI 328

Cationic oligo-*p*-phenylene ethynylenes form complexes with surfactants for long-term light-activated biocidal applications

Eric H Hill, ***Harry Craig Pappas****, hpappas@unm.edu, David G Whitten. Center for Biomedical Engineering, University of New Mexico, Albuquerque, New Mexico 87106, United States*

Cationic oligo-*p*-phenylene ethynylenes are highly effective light-activated biocides that deal broad-spectrum damage to a variety of pathogens, including bacteria. A potential problem arising in the long-term usage of these compounds is photochemical breakdown, which nullifies their biocidal activity. Recent work has shown that these molecules complex with oppositely-charged surfactants, and that the resulting complexes are protected from photodegradation. Recent studies have demonstrated the biocidal activity of an oligomer and a complex formed between it and sodium dodecyl sulfate. The complexes are able to withstand prolonged periods of irradiation, continuing to effectively kill both Gram-negative and Gram-positive bacteria, while the oligomer by itself loses its biocidal effectiveness quickly in the presence of light. In addition, damage and stress responses induced by these biocides in both *E. coli* and *S. aureus* have been observed. This work shows that complexation with surfactants is a viable method for long-term light-activated biocidal applications. Evidence suggests that the short, dim sections of the filament, such as that indicated by the red arrow in the figure below, arise from the “Z-ring” formed by FtsZ, an essential division protein that mediates septation.



MEDI 329

Optogenetic control of hypocretin/orexin neurons

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The hypocretins, also known as orexins, are neuropeptides expressed in a few thousand neurons in the lateral hypothalamus that have a key role in arousal stability. Hypocretin deficiency leads to narcolepsy, a sleep disorder characterized by intrusions of sleep into wakefulness. Hypocretin neurons project broadly throughout the brain and control the activity of numerous neurotransmitter and neuromodulator systems. We were first to use optogenetics to control the activity of hypocretin neurons with millisecond precision and established that a main function of the Hcrt system is to increase the probabilty of awakenings. We have now used opto and chemo genetic approaches to map the functional circuitry emanating from hypocretin cells in the lateral hypothalamus. The connectivity of Hcrt cells suggests a potential therapeutic use of selective and non-selective Hypocretin/orexin receptor antagonists in a variety of arousal disorders, including insomnia, anxiety, depression and drug addiction.

MEDI 330

Structure-activity-relationship, biological and pharmacological characterization of the proline sulfonamide ACT-462206, a highly potent, brain penetrant dual orexin 1/orexin 2 receptor antagonist

***Christoph Boss**, christoph.boss@actelion.com. Drug Discovery and Preclinical Research & Development, Actelion Pharmaceuticals Ltd, Allschwil, BL 4123, Switzerland*

The orexin system consists of two G-protein-coupled receptors, the orexin 1 receptor and the orexin 2 receptor, widely expressed in diverse regions of the brain and two peptide agonists orexin A and orexin B, which are expressed in a small assembly of neurons in the lateral hypothalamus. [1] The orexin system plays an important role in the regulation of sleep and wakefulness and particularly for the appropriate maintenance of wakefulness across a variety of conditions. Several compounds (almorexant, suvorexant) have been in advanced clinical trials for primary insomnia. [2, 3] ACT-462206 is a new, potent, and selective dual orexin receptor antagonist that inhibits the stimulating effects of the orexin A or B peptides at both the orexin 1 and 2 receptors. It decreases wakefulness and increases non-REM and REM sleep while maintaining natural sleep patterns in rat and dog EEG experiments. It is therefore a potential candidate for the treatment of insomnia.

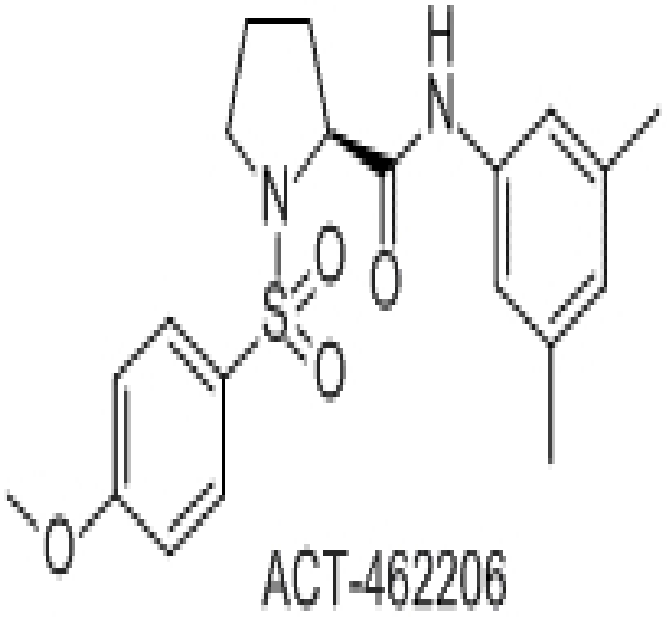

Fig 1: Structure of the Dual Proline Sulfonamide Orexin Receptor Antagonist ACT-462206

MEDI 331

Synthesis and evaluation of novel radioligands for positron emission tomography imaging of central orexin-2 receptor

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A positron emission tomography (PET) tracer for orexin receptors (OXRs) is of potential utility for assessing levels of these receptors in neuropsychiatric conditions, and for quantifying receptor occupancy by a candidate therapeutic drug, serving to link pharmacodynamics and pharmacokinetics of the drug toward estimation of its optimal dosage. However, no radiotracer is currently available for an *in vivo* PET assay of OXRs. The aim of this study is to establish a systematic strategy for developing a novel PET tracer for OXR type 2 (OX_2R). Novel classes of cyclopropane derivatives were identified as potent OX_2R ligands, and were subsequently screened for PET tracer candidates, based on their *in vitro* inhibitory constants for OX_2R, ^{11}C labelability and lipophilicity suitable for entry into the brain. The selected compounds were radiolabeled

with ^{11}C, and their performance was evaluated by *in vitro* autoradiographic and *in vivo* PET imaging of animal brains. These assessments have enabled us to find a new radioligand with potential as a lead for practical imaging agents, and to further determine properties of compounds, including ranges of affinity for OX_2R and flux ratio for efflux transporters, required for high-contrast PET.

MEDI 332

Discovery of 2*H*-1,2-4-benzothiadiazin-3(4*H*)-one 1,1-dioxide derivatives as orexin receptor antagonists by structure-based drug design

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The orexin receptors (OX_1 and OX_2) are G protein-coupled receptors (GPCRs) that are validated drug targets for the treatment of conditions such as sleep disorders and addictive or compulsive behaviours. Innovative medicinal chemistry approaches are required to develop CNS-active drugs for these receptors given that their endogenous ligands (orexin-A and orexin-B) are high molecular weight neuropeptides comprising 33 and 28 amino acid residues, respectively.

Heptares applies structure-based drug design approaches to GPCRs using its StaR platform. StaR proteins have a significantly increased thermostability when compared to the wild-type receptors and this enables the application of techniques such as X-ray crystallography, biophysical methods and high concentration fragment screening, which are typically not compatible with these challenging membrane-bound receptors. The 3D structures of many GPCRs have been determined by applying StaR technology, including the first crystal structure of a family B GPCR.

A case study will be presented in which these methods were applied to the discovery of a series of 2*H*-1,2-4-benzothiadiazin-3(4*H*)-one 1,1-dioxide derivatives as dual orexin receptor antagonists (DORAs). Co-crystal structures with OX_1 and OX_2 will be described, along with structure-based design aspects of the program, target engagement studies, and details of pharmacokinetics and in vivo efficacy of the lead molecules.

MEDI 333

Discovery and characterization of selective orexin receptor antagonists

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In 1998, De Lecea and Sakurai independently reported the existence of the orexin neuropeptides hypocretin-1 (hcrt-1) and hypocretin-2 (hcrt-2) which were also termed orexin-A and orexin-B. These neuropeptides were shown to originate in the hypothalamus from a common precursor and project widely to key areas of the central nervous system (CNS) hypothesized to control sleep-wake states, modulation of food intake, panic, anxiety, reward and addictive behaviors. The orexin neuropeptides mediate their effect by stimulating two distinct G-protein coupled receptors, orexin-1 (OX1) and orexin-2 (OX2). These receptors are co-located or selectively located in certain areas of the CNS suggesting differentiated roles. For example, OX1Rs are selectively expressed in the bed nucleus of the stria terminalis, amygdala, cingulate cortex and locus coeruleus which play a role in panic and anxiety. Conversely, OX2Rs are exclusively expressed in histaminergic neurons in the tuberomammillary nuclei which play a critical role in wake promotion. Interest in the orexin system has led to at least four dual orexin receptor antagonists entering human trials for the treatment of sleep related disorders. We have shown pre-clinically that selective antagonism of the OX2R is sufficient to initiate and prolong sleep in rodents. Selectively targeting the OX1R and its role in more complex emotional behavior (panic, anxiety) is now emerging. For example, sodium lactate infusion or acute hypercapnia, which causes panic in humans and are used as an animal model of panic, activates orexin neurons in the perifornical hypothalamus. This activation correlates with anxiety in the social interaction test or open field test. Blocking the activation with either siRNA or selective OX1 receptor antagonists attenuates these panic-like responses. In order to further validate the role of the OX1 receptor, characterization of improved tool compounds in animal models is required. Presented here will be the discovery, synthetic methods and SAR associated with novel selective orexin receptor antagonists.

MEDI 334

Discovery and characterization of OX_2R/OX_1R and OX_2R antagonists for the treatment of sleep disorders

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Orexin receptors are G-protein coupled receptors expressed within regions of the brain that regulate wakefulness through downstream pathways involving histaminergic, dopaminergic, and cholinergic activation. Orexin receptors bind excitatory orexin neuropeptides which are secreted by neurons projecting from the lateral hypothalamus. Clinical and preclinical studies suggest orexin receptor antagonists could provide a novel therapy for treating insomnia and other disorders in which sleep/wake cycles are disrupted. The identification of potent orexin receptor antagonists is an active area of investigation, and small molecule antagonists have recently progressed through late stage clinical development. We have disclosed the discovery of dual orexin receptor antagonists (MK-4305,suvorexant; MK-6096, filorexant) and OX_2R-selective antagonists (MK-1064). This presentation will highlight those discoveries and describe the preclinical properties of dual and OX_2R-selective antagonists.

MEDI 335

Leveraging developmental and cancer genetics to drug the Wnt pathway

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The secreted Wnt signaling molecules are essential to coordinated cell fate decision-making in developing and post-embryonic metazoans. Wnt ligand-dependent and –independent signaling is frequently exploited in a broad range of maladies that affect adult tissue homeostasis including cancer and aging. We discuss our efforts to delineate the mechanism of action for novel small molecules that disrupt Wnt signaling identified from a high throughput screen in cultured cells. We also present our progress in matching these chemical discoveries to therapeutic discovery agendas.

MEDI 336

Discovery of LGK974, a potent and selective Porcupine inhibitor targeting Wnt signaling

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Wnt signaling is tightly controlled during cellular proliferation, differentiation and embryonic morphogenesis. Aberrant activation of this pathway plays a critical role in a

variety of cancers. Blockade of Wnt signaling is therefore an attractive therapeutic approach for anticancer therapy. In this presentation, we will discuss our approach to search for inhibitors of Wnt ligand secretion. We developed and performed a cellular high-throughput screen using a co-culture system. Lead structure (GNF-1331) was identified and further target elucidation revealed Porcupine, a membrane bound O-acyl transferase, as its molecular target. Further structure-activity relationship studies led to the discovery of LGK974, a potent and specific Porcupine inhibitor. Treatment of LGK974 leads to tumor regression in a Wnt dependent MMTV-Wnt1 mouse model at well tolerated doses. LGK974 is currently in Phase 1 clinical trials.

MEDI 337

Potent and highly kinase-selective isonicotinamide GSK-3 inhibitors: In vivo activity, brain occupancy, and initial toxicity assessment

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GSK-3 is a serine/threonine kinase that has numerous substrates. Many of these proteins are involved in the regulation of many diverse cellular functions, including metabolism, differentiation, proliferation and apoptosis. Most prominently, GSK-3 is known to regulate nuclear levels of beta-catenin as part of the Wnt pathway, and is therefore involved in aspects of hyperplasia and stem cell self-renewal. Inhibition of GSK-3 may be useful in treating a number of diseases including Alzheimer's disease (AD), type II diabetes, mood disorders, and some cancers, but the approach poses significant challenges. Here we present a class of isonicotinamides that are potent, highly kinase-selective GSK-3 inhibitors, members of which demonstrated oral activity in

a triple transgenic mouse model of AD. We also disclose a well-behaved radioligand used to assess in vivo GSK-3 brain occupancy, and discuss initial toxicological results with an exemplary compound.

MEDI 338

Structure-based design of potent and selective tankyrase inhibitors to target the Wnt pathway

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The evolutionarily conserved Wnt/b-catenin (canonical) signaling transduction pathway plays a critical role in embryonic development and maintenance of homeostasis in mature tissues. Tankyrases are proteins in the poly-ADP-ribose polymerase (PARP) family. They have been shown to directly bind to axin proteins, which negatively regulate the Wnt pathway by promoting b-catenin degradation. Inhibition of tankyrases may offer a novel approach to the treatment of *APC*-mutant colorectal cancer. Hit compound was identified as an inhibitor of tankyrase through a combination of substructure searching of the Amgen compound collection based on a minimal binding pharmacophore hypothesis and high-throughput screening. Structure-based optimization of the hit compound led to the identification of more potent and selective tankyrase inhibitors with improved pharmacokinetic properties in rodents, which are well suited as tool compounds for further in vivo validation studies.

MEDI 339

Use of structure efficiency relationships to generate NVP-TNKS656, an orally active, highly potent and selective tankyrase inhibitor with enthalpy driven binding

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Tankyrase 1 and 2 have been shown to be redundant, druggable nodes in the Wnt pathway. As such, there has been intense interest in developing agents suitable for modulating the Wnt pathway in vivo by targeting this enzyme pair. By utilizing a combination of structure based design and LipE based structure efficiency relationships (SER), the core of XAV939 was optimized into a more stable, more efficient but less potent dihydropyran motif. This core was combined with elements of screening hits and resulted in highly potent, selective tankyrase inhibitors that are novel three pocket binders. NVP-TNKS656 was identified as an orally active antagonist of Wnt pathway activity in the MMTV-Wnt1 mouse xenograft model. With an enthalpy-driven thermodynamic signature of binding , highly favorable physicochemical properties and

high lipophilic efficiency, NVP-TNKS656 is a novel tankyrase inhibitor that is well suited for further in vivo validation studies.

MEDI 340

Discovery of a cardiac-selective late sodium current inhibitor (Nav 1.5) GS-462808

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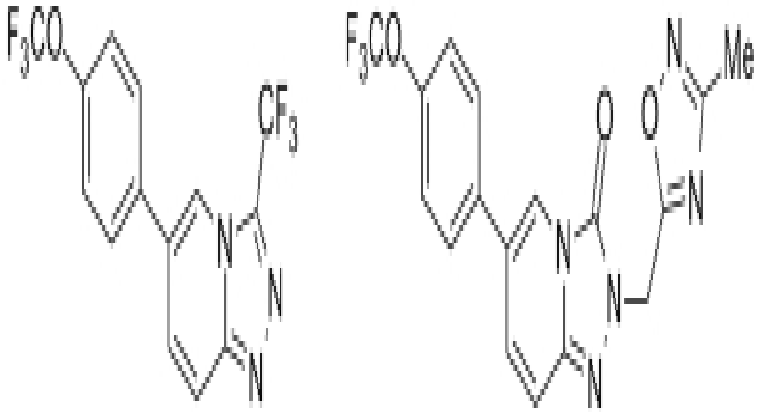
Ischemic heart disease (IHD) is associated with an increase in reactive oxygen species (ROS) which can modify Nav1.5 resulting in an enhanced Late Na^+ current (I_{Na}). As was previously disclosed, GS-458967 (**1**) selectively inhibits late I_{Na} compared to peak I_{Na}, but also exhibits use-dependent block (UDB) of other sodium channel isoforms, notably Nav1.1 and Nav1.2. Herein, we report the discovery of a potent and cardiac-selective Late I_{Na} blocker, triazolopyridinone (GS-462808, **2**), which has suitable PK consistent with QD dosing. Enhanced late I_{Na} generated by sea-anemone toxin (ATX-II) was inhibited by GS-458967 and GS-462808 with IC_{50}'s of 0.25 mM and 1.3 mM, respectively. Unlike **1** , that had similar potency for inhibition of Late I_{Na} and UDB of Nav1.1 and Nav1.2 I_{Na}, **2** was selective for the cardiac sodium channel with no significant inhibition of neuronal isoforms. GS-462808 represents a new class of isoform-selective Late I_{Na}.



1 GS-458967 2 GS-462808

MEDI 341

Aryl indanyl- and aryl hetaryl ketones as novel inhibitors of peptidyl-prolyl-cis/trans-isomerases

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Peptidyl-prolyl-cis/trans isomerases (PPIases) catalyze the rotation bout the imidic peptide bond in front of proline. Substances that act as PPIase inhibitors are considered as potential chemotherapeutics in the treatment of diseases with errors in the regulation of cell proliferation. Thus, PPIases emerged as promising targets in anticancer drug development.

[figure1.jpg]

Guided by the idea that compounds, which mimic the transition state **1** of the enzymatic rotation about the peptidic bond in front of proline, may act as PPIase inhibitors, we synthesized a series of aryl indanyl ketones **2** and aryl hetaryl ketones **3** and investigated their ability to inhibit Pin1 and cyclophilin.

Indeed, remarkable reversible inhibition of the PPIases Pin1 and cyclophilin by ketones **2** and **3** was detected. The cell-penetrating ketones displayed K_i values in the mono-digit micromolar range or lower.[1] A surprising selectivity in binding toward the iso-forms of cyclophilins was observed.[2] In addition, we have identified a non-immunosuppressive ketone **2** which does not inhibit calcineurin but maintains inhibition of endothelial cell proliferation and *in vivo* angiogenesis.[3]

[1] T. Hediger, W. Frank, M. Schumann, G. Fischer, M. Braun, *Chem. Biodiversity* **2012** , *9*, 2618 and references given therein. [2] S. Daum, M. Schumann, S. Mathea, T. Aumüller, M. A. Balsley, S. L. Constant, B. Féaux de Lacroix, F. Kruska, M. Braun, C. Schiene-Fischer, C., *Biochemistry* **2009** , *48*, 6268. [3] B. A. Nacev, W.-K. Low, Z. Huang, T. T. Su, Z. Su, H. Alkuraya, D. Kasuga, W. Sun, M. Träger, M. B., G. Fischer, K. Zhang, J. O. Liu*, J. Pharmacol. Exp. Ther.* **2011** , *338*, 466.

MEDI 342

Discovery of a novel series of phenylimidazole inhibitors of fXIa containing non-basic P1 groups

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Thrombotic disease continues to be a leading cause of mortality and morbidity in the world. FXIa has recently emerged as an antithrombotic target and its inhibition in preclinical models has demonstrated efficacy with low potential for bleeding. Support for inhibiting FXIa is supported by genetic evidence as individuals with congential FXI deficiency rarely experience spontaneous bleeding. Furthermore, epidemiologic studies support inhibiting FXIa as increased circulating FXI levels are associated with a higher incidence of thrombosis. Our labs have recently disclosed proof of concept (POC) studies demonstrating that inhibition of coagulation factor XIa provides robust antithrombotic efficacy with low potential for bleeding. Additionally, we also have disclosed a series of novel potent and efficacious (*S*)-2-phenyl-1-(4-phenyl-1H-imidazol-2-yl)ethanamine inhibitors of FXIa. This presentation will focus on a structure based approach with a focus on identifying novel non basic P1 groups for this series of compounds with a goal of improving oral bioavailability. The effort led to the discovery of several highly potent phenylimidazole FXIa inhibitors bearing neutral P1 moieties.

MEDI 343

Design and optimization of 2-aminooxazoline 4-azaxanthene inhibitors of BACE1 for the treatment of Alzheimer's disease: Discovery of AMG-8718

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Alzheimer's disease (AD) is the most common form of dementia and is characterized by cognitive decline, changes in thinking, memory loss, and ultimately death. The disease is thought to be a result of the formation and accumulation of Aβ peptides in the brain. Given that the process of Ab formation is initiated in the brain by the enzyme BACE1, considerable efforts across the industry and academia have focused on inhibiting this enzyme as a disease-modifying therapy for AD.

Our own efforts in this area led to the identification of the aminooxazoline xanthene scaffold, which enabled extension of side-chains into both the S3 and S2' pockets of BACE1. While compounds in this series produced robust reduction of central Aβ when dosed orally to rats and monkeys, advancement of this series was limited due to off-target cardiac effects, most notably prolongation of the QT interval as a result of hERG channel blockade. This talk will focus on our efforts to optimize the distribution of polar heteroatoms in our inhibitors to more efficiently inhibit BACE1 while simultaneously

decreasing off target cardiac ion channel activity and maintaining low Pgp-mediated efflux. These efforts resulted in the identification of the 4-azaxanthene series. Extensive use of the X-ray crystallography and a rat pharmacodynamic model enabled further optimization of these compounds, and the design of a novel P2'-side chains resulted in the identification of AMG-8718, a compound advanced to preclinical development.

MEDI 344

SAR and optimization of trioxoisothiazole-based liver receptor X (LXR) agonists leading to the clinical candidate AZD3971

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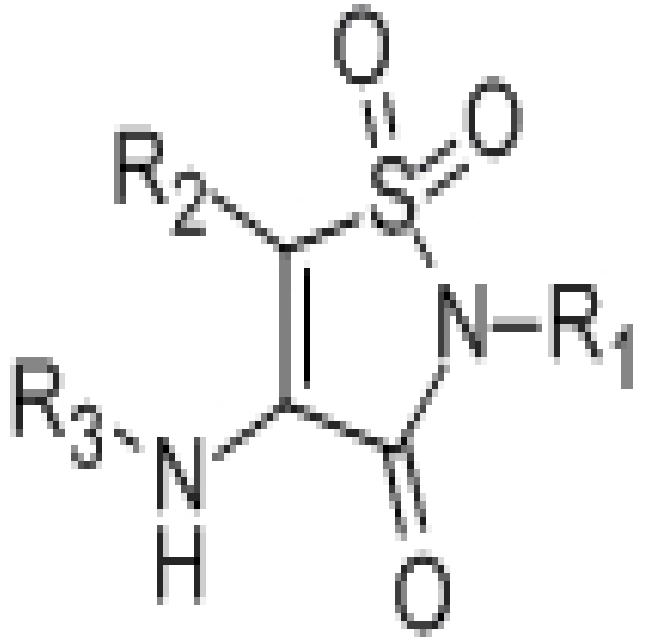
The liver X receptors (LXRα and LXRβ) are members of the nuclear receptor family of transcription factors. The activation of LXR induces genes involved in reverse cholesterol transport (RCT), which is believed to be the main effect of LXR agonists in the prevention or treatment of atherosclerosis. However LXR agonists have also been shown to cause hepatic steatosis and hypertriglyceridaemia. The ability to separate beneficial effects from negative effects has been a challenge that so far has hampered the development of LXR agonists for human use.

We herein describe the SAR and optimization of a series of trioxoisothiazole-based LXR agonists leading to compounds with nanomolar potencies and a separation of beneficial versus negative effects *in vivo*. This work ultimately led to the nomination of AZD3971 as a candidate for the treatment of atherosclerosis.



MEDI 345

Pyridones as highly selective, reversible inhibitors of T790M double-mutants of EGFR

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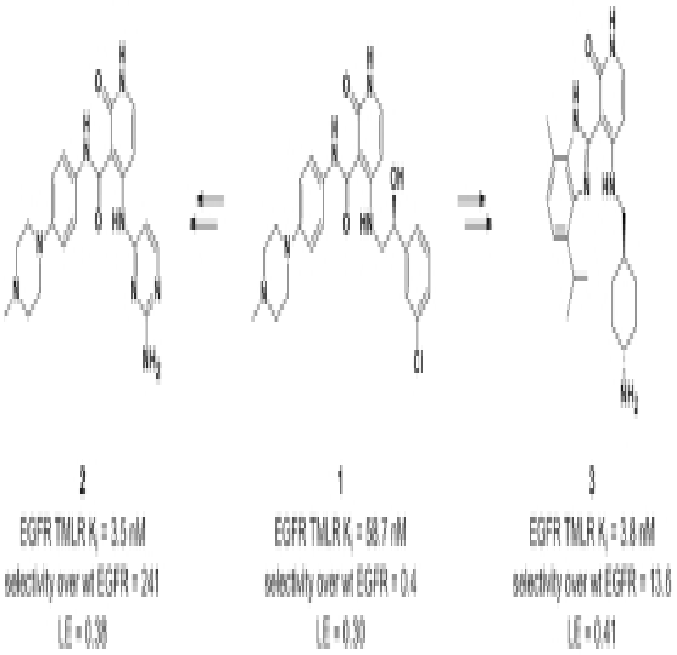
The advancement of a series of pyridones, initially identified from high-throughput screening and lacking selectivity for T790M resistance mutants over wild-type EGFR, to two potent and selective sub-scaffolds with improved ligand efficiency.



MEDI 346

Methyl urea-substituted pyridines as a new class of glucokinase activators

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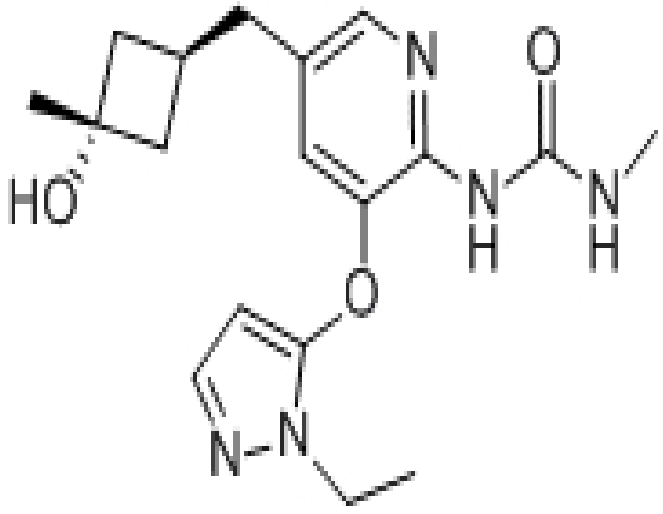
Despite the wide array of anti-diabetic therapeutics in current use, an unmet need for effective and safe pharmacotherapy for Type 2 diabetes still exists. Glucokinase (GK), a member of the hexokinase family, converts glucose to glucose 6-phosphate and functions as a key sensor of glucose both in hepatocytes and pancreatic islet b-cells. Gluocokinase activators (GKAs) have emerged as potentially attractive Type 2 diabetes therapeutics due to the central role that glucokinase plays in regulating glucose

homeostasis. We will disclose a novel methyl urea-substituted pyridine series of GK activators derived from our previously reported thiazolylamino pyridine series. We will describe our efforts in optimizing potency, stability and the induced kinetic parameters on GK that led to the identification of compound **AM-9514** , which showed a favorable combination of the previously listed parameters, a good pharmacokinetic profile in preclinical species, and efficacy in a rodent PD model.



AM-9514

Compound **AM-9514** also was tested for hypoglycemic potential and showed no hypoglycemia in mice after an oral challenge of **AM-9514** to fed mice and then food removed for 4 hour. **AM-9514** was progressed into additional preclinical studies.

MEDI 347

Discovery of ATR selective inhibitors for the treatment of cancer

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ATR is a key regulator of the cell response to replication stress, a potentially lethal form of DNA damage that arises when cells attempt to replicate damaged DNA. Drugs that induce such DNA damage have been the cornerstone for cancer therapy for decades. However, the clinical benefit of these agents is limited by poor differentiation between toxicity in cancer and normal cells, and because most cells are capable of repairing the damaged DNA. It has been shown that many cancer cells are more reliant on ATR for survival from DNA damage than normal cells and consequently, inhibitors of ATR could improve the efficacy of DNA damaging agents.

Herein we describe the discovery of selective ATR inhibitors. The relationships between the structure of these aminopyrazines, their potency and selectivity will be presented along with characteristics of the most advanced compound, VX-970 (VE-822). This compound inhibits the ATR kinase with Ki<0.3nM via an ATP competitive mechanism of action. It markedly enhances the cytotoxic activity of DNA damaging drugs in many cancer cells but is well tolerated by normal cells. In a panel of patient-derived mouse xenograft cancer models, administration of VX-970 dramatically improved cisplatin efficacy without increasing toxicity.

The pre-clinical profile for this series of compounds show that ATR inhibitors have great potential to improve the clinical benefit of a range of established DNA damaging drugs.

MEDI 348

Chemistry in water using micelles: Applications in the pharmaceutical industry

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The notion of doing **transition-metal-catalyzed** organic synthesis **in water** only at **ambient temperatures** is oftentimes dismissed out of hand, as most uncharged organic molecules are simply insoluble or unstable in water. One approach that circumvents this limitation is the use of catalytic amounts of nanoparticles derived from amphiphiles that self-aggregate spontaneously in water, thereby providing a micellar environment within which organic substrates and catalysts may readily interact. Such reactions are technically under homogeneous conditions, taking place within the lipophilic inner core of the micelle, while the water serves as the macroscopic medium that drives particle organization due to entropic factors. With limited levels of surfactant present, concentrations of reactants are typically quite high, in which case reaction rates at room temperature can be competitive with those commonly seen at elevated temperatures in organic solvents. In this oral presentation we will disclose applications of micellar catalysis for the most important reaction types performed in the pharmaceutical industry (e.g. transition-metal-catalyzed reactions such as Buchwald-Hartwig amination, Suzuki and Negishi couplings). This new methodology enables reactions to be performed under environmentally benign conditions by avoiding hazardous, toxic and expensive organic solvents.

MEDI 349

Discovery and characterisation of CRTh2 receptor antagonists suitable for clinical testing in allergic diseases

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The global disease burden of allergy remains a significant unmet medical need. For example, The World Health Organisation (WHO) estimates that the number of patients with asthma is currently 300 million and likely to rise to 400 million by 2025. The lipid mediator prostaglandin D_2 (PGD_2) is a key orchestrator in allergy, exhibiting increased levels in the airways of allergen challenged asthmatics, as well as in the nasal mucosa of allergic rhinitics and in the skin of atopic dermatitis patients. CRTh2 is a receptor for PGD_2, which mediates the activation and migration of Th2 cells and eosinophils, effector mechanisms which lie at the core of inflammatory process in allergic asthma. Consequently a CRTh2 antagonist represents an attractive target as a novel anti-inflammatory therapy for asthma and other allergic diseases. This presentation will describe identification of an initial development molecule NVP-QAV680 from a high throughput screening approach. Clinical studies with NVP-QAV680 established the safety, tolerability and efficacy of the compound in allergic rhinitis. The discovery and characterisation of a follow up molecule NVP-QAW039 exhibiting improved potency on human eosinophils and Th2 cells, together with a longer receptor residence time will also be discussed. As a potent and selective antagonist of the CRTh2 receptor with an acceptable pharmacokinetic profile, NVP-QAW039 is currently being evaluated in asthma clinical studies.

MEDI 350

Discovery of clinical candidate BMS-986115, an oral pan-Notch inhibitor for the treatment of cancer

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Deregulation of the Notch pathway has been shown to be oncogenic in numerous tissue types including T-cell acute lymphoblastic leukemia (T-ALL), breast cancer, non-small

cell lung cancer, and colorectal carcinoma. Notch signal activation can cause uncontrolled proliferation, restrict differentiation leading to increased self-renewal capacity, evasion of apoptosis, and enhancement of angiogenesis and metastasis. There is increasing evidence that Notch plays a role in the maintenance and survival of cancer stem cells. Gamma secretase mediates the Notch signaling pathway by releasing the Notch intracellular domain (NICD) which translocates to the nucleus and binds to the transcription factor CSL to activate transcription of various target genes. BMS-906024 is a potent pan-Notch inhibitor that demonstrated robust anti-tumor activity at tolerated doses in multiple tumor xenograft models. It is being evaluated in Phase 1 clinical studies. BMS-906024 is being administered IV (once weekly) in the clinic and the projected human efficacious dose is 4 - 6 mg. This presentation will describe further optimization of potency and PK properties in the 1,4-benzodiazepinone series that culminated in identification of BMS-986115 as a potent pan-Notch inhibitor suitable for oral dosing. Structure-activity relationships will be described along with in vivo evaluation of BMS-986115 in relevant tumor xenograft models. BMS-986115 is currently in Phase 1 clinical trials for the treatment of cancer.

MEDI 351

Discovery of TD-5959 (GSK-961081): A first-in-class dual pharmacology multivalent muscarinic antagonist and beta-2 agonist (MABA) for the treatment of COPD

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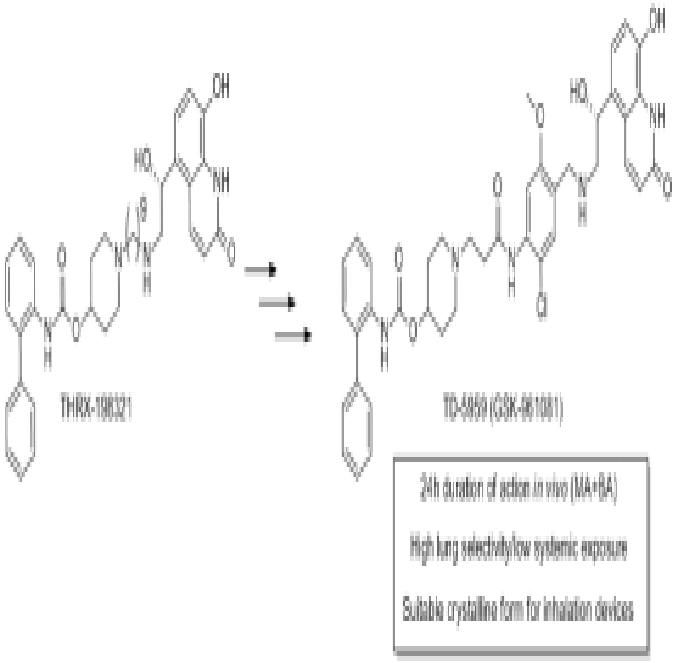
Through application of our multivalent approach to drug discovery we previously reported the first discovery of dual pharmacology MABA bronchodilators, exemplified by THRX-198321. Herein we describe the subsequent lead optimization of both muscarinic antagonist and β_2 agonist activities, through modification of the linker motif, to achieve 24h duration of action in a guinea pig bronchoprotection model. Concomitantly, we targeted high lung selectivities, low systemic exposures and identified crystalline forms suitable for inhalation devices. This presentation culminates with the discovery of our first clinical candidate TD-5959 (GSK-961081). In a Phase 2b trial, TD-5959 produced statistical and clinically significant differences compared to placebo and numerically greater improvements in the primary endpoint of trough FEV_1compared to salmeterol after 4 weeks of dosing in patients with moderate to severe COPD.



MEDI 352

Discovery of respiratory syncytial virus (RSV) fusion inhibitor GS-5806 and clinical proof of concept in a human RSV challenge study

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Respiratory syncytial virus (RSV) is a common seasonal virus that most severely affects infants, immunosuppressed patients, and the elderly with cardiopulmonary disease. Upper respiratory tract infection can progress to the lower respiratory tract causing bronchiolitis, pneumonia and respiratory failure. RSV is the leading cause of infant hospitalizations and has a mortality rate approximately 10x that of influenza in US infants. Currently there are no accepted effective antiviral options. GS-5806 is a novel, orally bioavailable RSV fusion inhibitor discovered following a lead optimization campaign on a hit originated from a phenotypic RSV antiviral high-throughput screen. Lead optimization focused on improving human plasma protein-binding adjusted antiviral potency, permeability, pharmacokinetic properties that would support once daily oral administration, and aqueous solubility properties to enable solution formulations for infants. GS-5806 is potent against RSV A and B clinical isolates (N=75, mean EC_{50}=0.43 nM). Oral bioavailability in preclinical species ranged from 46-100% and moreover, GS-5806 efficiently penetrated into the lung tissue of Sprague-Dawley rats. Dose-dependent (0-30 mg/kg) antiviral efficacy was observed in a cotton rat model of RSV infection. Oral GS-5806 appeared safe, and in healthy human volunteers experimentally infected with RSV, demonstrated a potent antiviral effect (at the time of mean peak viral load for placebo subjects, the mean viral load in GS-5806 treated subjects was 4.2log_{10} lower), and reduction in disease severity. In conclusion, GS-5806 is a potent, once daily, oral RSV fusion inhibitor with the potential to treat RSV infection in infants and adults.

MEDI 353

Optimizing in vivo probes for the BET bromodomains

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Bromodomains are acetyl-lysine recognition domains that mediate protein-protein interactions, often between histones and transcriptional machinery, playing an important role in the epigenetic transfer of information. Bromodomain-containing proteins play fundamental cellular roles, and show association with particular diseases including psychological diseases, obesity, inflammation and cancer [1, 2]. This wide range of biological roles emphasizes the importance of developing small molecule probes to assist in gaining a better understanding of bromodomain function *in vivo*.

Many protein-protein interactions are challenging to inhibit, because their interfaces are often heavily solvent-exposed, cover large areas, are structurally featureless and poorly defined. Conversely, bromodomains have a well-defined binding pocket that displays

characteristic interactions with acetylated lysine residues. Consequently these recognition domains have proved ligandable, with a range of ligands for the BET sub-family of bromodomains reported in the literature. Work within this group has led to the development of nanomolar potent inhibitors of the BET bromodomains. Here we report that these compounds have antiproliferative activity in a variety of cancer types and a promising profile in the NCI60 panel. We have synthesized a collection of analogues to understand the scope of SAR in the WPF region of the bromodomains, with the aim of increasing compound potency. Further optimization is being carried out to improve the physical and pharmacokinetic properties of this series of compounds, to develop a probe compound for use in animal models.

References

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MEDI 354

α7 nAChr state distribution controlled by quaternary ammonium ligands

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The minimum pharmacophore for activation of the human alpha 7 nicotinic acetylcholine receptor (nAChR) is the tetramethylammonium cation. Previous work demonstrated that larger quaternary ammonium compounds such as diethyl dimethylammonium cation or N-methyl quinuclidine were alpha7-selective partial agonists, but additional increase in the size of the N-alkyl group by a single carbon to an N-ethyl group led to a loss of this activity. We report that, while such compounds are ineffective at inducing the normal channel open state, they nonetheless regulate the induction of specific conformational states. We synthesized several panels of quaternary ammonium nAChR ligands that systematically varied the size of the substituents bonded to the central positively charged nitrogen atom. In these molecular series we found a correlation between the molecular volume of the ligand and the receptor's preferred distribution amongst conformational states including partial agonism, competitive antagonism, a non-conducting state that could be converted to an activated state by a positive allosteric modulator (PAM), or a PAM-insensitive non-conducting state. We hypothesize that the changes of molecular volume subtly impact interactions at the subunit interface constituting the orthosteric binding site in such a way as to determine state distribution. We define a new minimal pharmacophore for the class of compounds we have termed as “silent agonists”, which are able to induce allosteric modulator-dependant activation but not the normal activated state.

MEDI 355

Discovery of a clinical prodrug of a p38α MAP kinase inhibitor

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The discovery of a clinical prodrug of a p38α MAP kinase inhibitor will be presented. This prodrug, stable and soluble under both acidic and neutral conditions, features a unique carbamoylmethylene linked promoiety containing a combination of ester and dihydrogenphosphate functionality. It was completely bio-converted to the parent drug primarily during absorption, and significantly improved exposure of the parent drug *in vivo* in a dose response manner.

MEDI 356

Inhibitors of *Leishmania major* inositol phosphorylceramide synthase: New therapies for leishmaniasis

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Leishmaniasis is a Neglected Tropical Disease (NTD) caused by the protozoan *Leishmania spp*. Over 350 million people worldwide are at risk, with an estimated annual death toll of **60,000** . Treatment typically requires long, expensive courses of exposure to toxic medicines *via* parenteral administration. There is an urgent need for **novel treatments** that are inexpensive and free of side effects.

Our group has previously identified and validated the ***Leishmania major* inositol phosphorylceramide synthase** (*Lmj*IPCS) enzyme as an attractive drug target.[1,2] This membrane-bound **essential** enzyme has no mammalian equivalent, potentiating the development of **safe** , **selective** anti-leishmanials.

A set of **1200** pharmacologically active compounds (NINDS set supplied by MRCT) were screened against *Lmj*IPCS, **57** were found to exhibit **>70%** inhibition at 20 μM. A secondary assay against *L.major* promastigotes highlighted **14** compounds with **selective** cytotoxic effects. **Four** of these inhibitors displayed ED_{50} values lower than that of pentamidine (2.05 μM), a second-line treatment for leishmaniasis. Significantly these compounds have good **bioavailability** and mammalian **safety profiles** , making

them ideal for development into new drugs. A **hit-to-lead** study is being explored to elucidate further **structure-activity relationship data** . This approach has the scope to provide a wide variety of **chemical tools** that will deliver **potent** , **selective** leishmanicidal therapies.

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MEDI 357

Development of selective non-secosteroidal vitamin D receptor inhibitors

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The vitamin D receptor is a nuclear hormone receptor that regulates cell proliferation, cell differentiation, calcium homeostasis and immunomodulation. The receptor is activated by the vitamin D metabolite, 1,25-dihydroxyvitamin D_3, which induces a cascade of events including the recruitment of coactivators that activate transcription of specific VDR target genes. Thousands of VDR agonists have been synthesized based on the secosteroid scaffold of 1,25-dihydroxyvitamin D_3. However, most of these ligands are metabolically unstable, have sub-optimal drug-like properties, and induce hypercalcemia *in vivo.* The limited numbers of VDR antagonists reported bear the same secosteroid scaffold and thus exhibit the same problems encountered with VDR agonists. Herein, we report the development of non-secosteroidal antagonists for VDR with better drug-like properties in order to target VDR *in vivo.* This will aid in the development of new treatments for allergies, Crohn's disease, and sarcoidosis. Furthermore, we expect to develop a new therapy for patient suffering from hypercalcemia. The compounds presented here were derived from GW0742, a known compound identified as a VDR antagonist during a screening campaign with the NIH chemical and genomics center (NCGC) and is currently used as scaffold to develop highly selective modulator for VDR.

MEDI 358

Synthesis of novel isoform selective histone deacetylase inhibitors containing chiral heterocyclic capping groups

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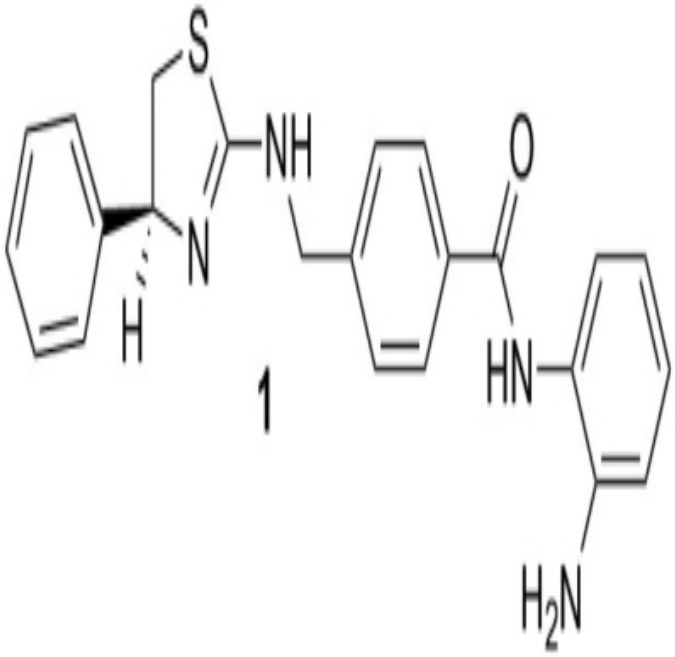
Histone deacetylase enzymes (HDACs) are one of the best characterised epigenetic targets and two HDAC inhibitors (Vorinostat and Romidepsin) are FDA approved for the treatment of cancer. Abnormal HDAC activity is associated with a variety of other diseases, but the therapeutic use of HDAC inhibitors is currently limited by high toxicity which is likely due to off target effects and a lack of isoform selectivity. We designed and synthesised a novel series of potent chiral HDAC inhibitors that contain a heterocyclic capping group and an *N*-(2-aminophenyl) benzamide unit that binds in the active site. *In vitro* assays for the inhibition of HDAC1, HDAC2, HDAC3-NCoR1, and HDAC8 by the *N*-(2-aminophenyl) benzamide **1** gave respective IC_{50} values of 930, 85, 12, and 4100 nM, exhibiting class I selectivity and potent inhibition of HDAC3-NCoR1.



1

MEDI 359

De novo microfluidic-assisted design and synthesis of 5-HT_{2B} receptor-selective leads

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Computer-assisted *de novo* design is currently seeing renewed interest in hit and lead finding programs as a complementary technology to mainstream approaches, *e.g.* high-throughput screening. *De novo* design methods have demonstrated their value for the tasks of scaffold-hopping, bioisosteric replacement, and even fine-tuning of a candidate compound. Here we present ligand-based *de novo* design studies aiming at the discovery of innovative lead molecules for high value targets, using our state of the art software tools DOGS (Design Of Genuine Structures) and MAntA (Molecular Ant Algorithm). We had previously reported on the discovery of new chemical entities targeting aurora A kinase, human polo-like kinase-1, and the most selective vascular endothelium growth factor receptor 2 (VEGFR-2) inhibitor known to date. We had also successfully repurposed *de novo* designed entities as G-protein coupled receptor (GPCR) ligands, and identified innovative scaffolds for adenosine and adrenergic receptors using an automated microfluidics-assisted synthesis platform. Furthermore, we designed new ligands for the dopamine D_4 receptor, presenting accurately predicted pK_i values against a target panel, through multi-objective optimization. Together with a brief overview over past efforts we disclose the automated synthesis of novel ligand efficient 5-HT_{2B} receptor-selective antagonists with predictable drug target panel affinities. The impact of *de novo* microfluidic-assisted design of NCEs for drug discovery will be discussed.

MEDI 360

Structure–activity relationships for ketamine esters as short-acting anaesthetics

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Racemic ketamine is an effective non-opioid anaesthetic/analgesic used in human anaesthesia and veterinary medicine. Its most clinically significant adverse effect is its hallucinogenic properties. Therefore it is normally administered together with sedatives like midazolam and/or propofol to control the prolonged period of post-anaesthesia hallucinations. To overcome this draw back we </del>prepared a series of aliphatic esters of ketamine as shorter-acting analogues and evaluated them in an infused rat model, measuring the time after infusion to recover from both the anaesthetic (righting reflex) and analgesic (response to stimulus) effects. For Me, Et and i-Pr esters recovery from anaesthesia was 10-15 fold faster and for n-Pr esters was20-25 fold faster, than from ketamine. There was no direct correlation between the potency of esters as sedatives and the ester chain length. Me, Et and i-Pr esters were the more dose potent (up to twofold less than ketamine), whereas n-Pr esters were less potent (from 2- to 6-fold less than ketamine). Pharmacokinetic studies in rabbits showed ultra-rapid metabolism to the corresponding (inactive) carboxylic acids in 1-2 min, yet an isopropyl ester analogue provided a duration of profound analgesia well beyond measured exposure of drug, and outlasting its sedative effect by more than three-fold in both rats and rabbits, suggesting alternative mechanisms downstream from the direct receptor effects. In summary, the data obtained from the study shows that it is indeed possible

with the esters to reduce the hallucinogenic effect of ketamine without significantly reducing the anaesthetic potential.

MEDI 361

Synthesis and pharmacology of 6TM/E11 opioid analgesics based on IBNtxA

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IBNtxA is a potent opiate which belongs to the group of 4,5-epoxymorphinans. IBNtxA labels the 6-transmembrane domain splice variants of the mu opioid receptor (6TM/E11 sites) and produces potent analgesia without respiratory depression, reward behavior, physical dependence and only causes little constipation. Early SAR studies on IBNtxA revealed that the iodo group on C-3' or C-4' of the phenyl ring attached at the C-6 of the opiate is critical to maintain 6TM/E11 activity. The nitrogen substituent at N-17 is also important: a N-17 cyclopropylmethyl or an allyl is necessary to maintain activity, which is lost in N-17 methyl derivatives. Similarly, the presence of a free phenolic OH is required.

In order to further understand the basic SAR of compounds with affinity for the 6TM/E11 sites in the brain, more analogs of IBNtxA were synthesized. IBNtxA analogs without the C-14 hydroxyl group, analogs with a double bond at the 7,8 position and without the C-14 hydroxyl group, analogs where the iodoaryl ring is separated from the opioid moiety by a spacer and analogs with the C-14 hydroxyl group alkylated were synthesized. Synthesis, *in vitro* radioligand binding and *in vivo* behavior on 19 analogs were carried out.

The synthesis of these new analogs allowed us to establish the optimum steric, lipophilic and electronic features of a compound required for affinity and selectivity for the 6TM/E11 sites over the traditional opioid receptors. Side-effect profile of 6TM/E11 analgesics remains superior over morphine reestablishing the 6TM/E11 target as a preferable drug target for discovery of novel opiates without addiction and abuse potential.

MEDI 362

Design, synthesis, and biological evaluation of selective FLT3 inhibitors for the treatment of acute myeloid leukemia

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FLT3 (FMS-Like Tyrosine kinase) is a member of the class III receptor tyrosine kinase family which plays an important role in the development of hematopoietic systems. Most FLT3 mutations arise from ITDs (internal tandem duplications) formed in the juxtamembrane domain which stimulates activation of FLT3. FLT3/ITD frequently occurs in AML (acute myeloid leukemia). Currently, several small molecule of FLT3 inhibitors acquired drug resistances during clinical trials caused by secondary mutations within the FLT3/ITD cells. Therefore, development of new FLT3 inhibitors are needed to ensure clinical applicability for AML.

We have designed and synthesized diverse thieno[2,3-d]pyrimidine compounds by introducing a variety of substituents at the 2, 4, 5 and 6-positions to discover selective FLT3 inhibitors. All 34 synthesized compounds were assayed for FLT3 kinase activity and further evaluated for growth inhibition on 2 leukemia cell lines (MV4-11, THP1) and 6 solid tumor cell lines (ACHN, HCT15, NCI-H23, PC-3, NUGC-3 and MDA-MB-231). The results of FLT3 inhibitory activities led to 4 compounds, some which exhibited better growth inhibition of FLT3-mutated MV4-11 cells than FLT3 inhibitor Quizartinib. Especially, **18a** which only showed significant growth inhibition against the MV4-11 cells, which indicates potent selectivity against FLT3/ITD AML cells.

FLT3 has been considered as a potential molecular target for the treatment of AML. Our study was focused on the discovery of selective FLT3 inhibitors, resulting in the development of **18a** which showed good FLT3 inhibitory activity as well as selectivity on the FLT/ITD AML cell lines.

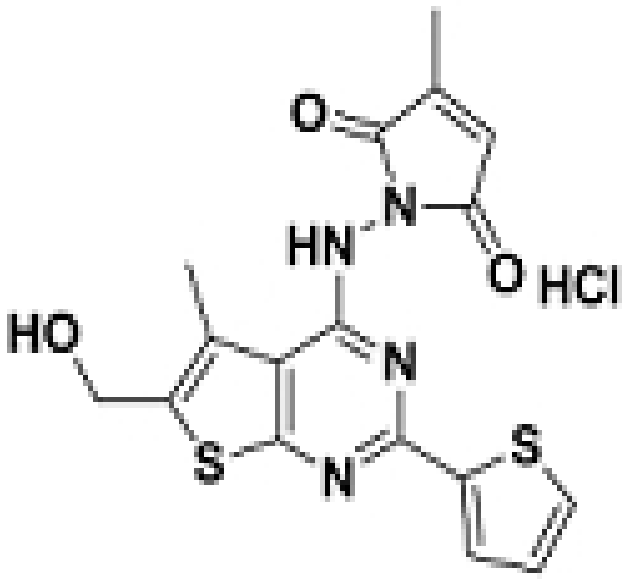

Figure 1. The structure of 18a

MEDI 363

Use of azamacrocycles to evaluate oxidative biotransformations

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Enzymes in the cytochrome P-450 superfamily are responsible for the biotransformation of over 90% of clinical drugs. Studying oxidative metabolites produced by these enzymes is necessary in the early stages of drug discovery and development.

In order to save time and money, in vitro methods have been developed to mimic this process to determine potentially toxic metabolites, as well as to examine potentially harmful drug-drug interactions. However, a number of problems are associated with current methods that use biological systems as in vitro models for drug metabolism, i.e euthanizing animals, cost, and varying metabolite potency.

Here we present a new method utilizing metalloporphyrin catalyst(s) that mimic the oxidative mechanism of cytochrome P-450 in order to synthetically produce these metabolites. This technology introduces a new avenue for drug discovery, and allows for a synthetic predictive model of drug-drug interactions for clinical diagnostics. A number of pharmaceuticals have been examined independently and in tandem, including but not limited to diabetes, hypertension, and hypercholesterolemia medications.

MEDI 364

Discovery of a benzimidazole lead class: Selective aldosterone synthase inhibitors for the treatment of hypertension

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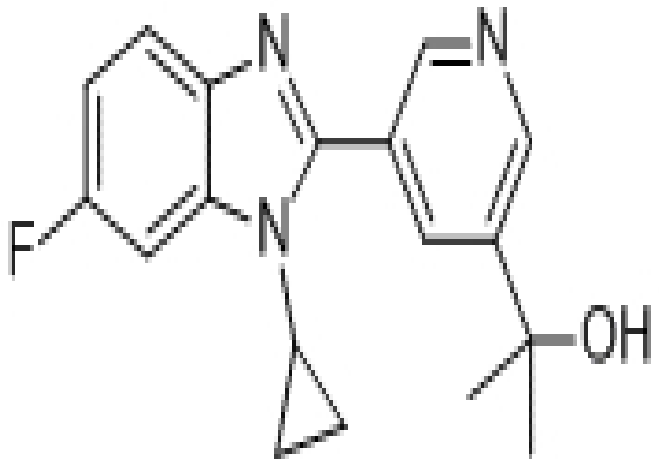
Aldosterone is a steroid hormone that promotes increased blood pressure, inflammation and fibrosis. The final three steps of its biosynthesis are catalyzed by aldosterone synthase (CYP11B2). A closely related enzyme, CYP11B1, catalyzes the biosynthesis of cortisol, an important regulator of glucose metabolism.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



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This presentation will outline the discovery of compound **1** , a potent CYP11B2 inhibitor that displays high selectivity vs. related CYPs, good pharmacokinetic properties in rat and rhesus, and good physical properties. Additionally, in a rhesus pharmacodynamic model, compound **1** displays robust, dose-dependent aldosterone lowering efficacy, with no apparent effect on cortisol levels.

MEDI 365

Development of a novel class αIIbβ3 receptor antagonist RUC-4 as anti-platelet aggregation agent for myocardial infarction

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Here we disclosed the campaign of developing a potent and selective αIIbβ3 receptor antagonist RUC-4 with suitable aqueous solubility and pharmacokinetic properties for intramuscular (IM) dosing. This platelet aggregation inhibitor has a novel mechanism of action by displacing the magnesium ion in the metal ion-dependent adhesion site (MIDAS) of the β3 subunit of the receptor. RUC-4 binding only causes minor conformational change of αIIbβ3 receptor judged by various techniques. This novel binding modality might explain why RUC-4 little or no priming of the αIIbβ3 receptor to bind its ligand fibrinogen. The antithrombotic effect of RUC-4 has been confirmed in animal models. Currently RUC-4 is undergoing preclinical development with the goal of administering it in the pre-hospital setting to reduce the mortality and heart failure caused by myocardial infarction.

MEDI 366

Role of ubiquitin proteasome system in atrial structural remodeling in sarcolipin T5A transgenic mice

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Sarcolipin (SLN) is a key regulator of cardiac sarcoplasmic reticulum Ca2+ ATPase (SERCA) and plays a critical role in maintaining cardiac Ca2+ homeostasis and atrial β-adrenergic response. Previous studies have shown that threonine 5 at the N-terminus of SLN modulates its function and mediates β-adrenergic responses.[1] To determine the importance of threonine 5 phosphorylation in modulating atrial SLN activity, we have generated a TG mouse model with cardiac specific expression of phosphorylation defective, gain-of-function mutant sarcolipin (SLNT5A). Transgenic expression of SLNT5A results in severe atrial structural remodeling accompanied by bi-atrial enlargement, slower AP propagation and diastolic dysfunction. To determine if the activity of ubiquitin-proteasome system (UPS) contributes to the atrial structural remodeling, we measured the UPS activity in atria and ventricles of TG mice. Our results show that the chymotrypsin-like activity of proteasomes was significantly increased in atria ($p<0.001$) and in the ventricles ($p<0.05$) of TG mice and was accompanied by increased protein expression of 20S and 19S subunits of UPS components. The amount of polyubiquitinated proteins was significantly increased in atria of SLNT5A TG mice. Gene expression analyses have shown altered expression of E2 and E3 components of UPS in the TG mice atria. Additionally, pharmacological inhibition of proteasome with epoxomicin restored atrial electrical conductivity in the TG mice by reversing structural remodeling of the atria. Together, our studies suggest that activation of UPS may contribute to the atrial structural remodeling and conduction defects in the SLNT5A TG mice. Our future studies will address if the activation of UPS contributes to structural remodeling in persistent atrial fibrillation (AF) in humans.

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MEDI 367

Building enzyme structure kinetic relationships in the discovery of neprilysin inhibitors

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The inhibition of the enzyme neprilysin, a Zn^{2+} endopeptidase protease which cleaves natriuretic peptides, is validated as a treatment for hypertension. We have developed inhibitors of neprilysin optimized for potency, selectivity and PK/PD. Herein, we report the discovery of a class of inhibitors in which *in vitro* potency and *in vivo* efficacy have

been optimized based on their structure kinetic properties. A medium throughput screening assay enabled the rapid determination of trends within a set of >700 molecules. Focus sets of inhibitors were then selected for discreet binding kinetic measurements and the resulting dissociation half-life values ($t_{1/2}$) were measured. Using the resulting enzyme structure kinetic relationships, we were able to design neprilysin inhibitors with extended enzyme residence times and study their effects in PK/PD *in vivo* models.

MEDI 368

Adipose derived stem cells for a vascular graft

***Julien Henri Arrizabalaga**, julien.arrizabalaga@ou.edu, Matthias Ulli Nollert. Bioengineering Center, University of Oklahoma, Norman, OK 73019, United States*

Coronary heart disease is the leading cause of death in the United States. An alternate therapy is needed to treat the 180,000 people each year who require coronary artery bypass surgery. Several different strategies have been explored to develop vascular graft substitutes, but so far all have proven unsatisfactory. Current engineered vessels lack sufficient strength or require many weeks to be fully functional. We propose an innovative solution using patient-derived stem cells obtained from fat tissue. Cells will be seeded onto a biological collagen sheet derived from the human placenta, and rolled into a tube with appropriate diameter. The tubular construct will be mounted in a perfused bioreactor to simulate the mechanical conditions of native vessels. This project has the potential to provide off-the-shelf availability of replacement blood vessels.

MEDI 369

Discovery of parenterally administered factor XIa inhibitors

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Genetic evidence demonstrates factor XIa (FXIa) is a potential anti-thrombotic target with net clinical benefit. Deficiency of human FXI is only associated with a mild bleeding diathesis. FXI is not required for normal hemostasis in mouse, and FXIa deficient mice do not exhibit prolonged provoked bleeding times. FXIa is positioned upstream in the coagulation cascade involved in the amplification of thrombin production. Inhibiting FXIa could provide a reduction in thrombin sufficient to impede occlusive thrombosis, yet allow enough thrombin generation to support hemostasis. We have reported on reversible, small molecule inhibitors of FXIa which demonstrated antithrombotic efficacy but did not prolong bleeding times in rabbit thrombosis models. We have also disclosed a series of potent FXIa inhibitors containing a phenylimidazole core. Herein we describe

the optimization at P1' and P2' regions with the phenylimidazole core, which led to the discovery of a potent and efficacious parenteral FXIa inhibitor.

MEDI 370

Selective MMP-3 inhibitors for the use in a PET imaging study for treating atherosclerosis

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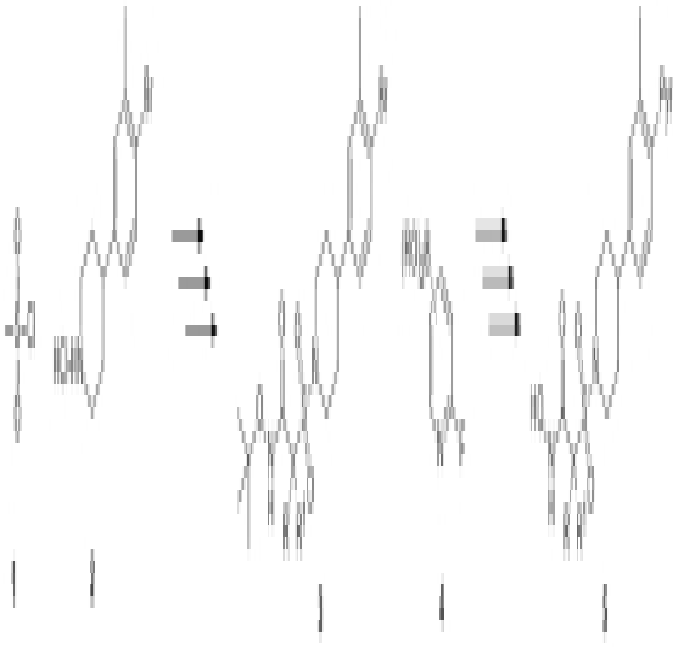
Matrix metalloproteinases (MMPs) are a group of zinc and calcium dependent endopeptidases that play a major role in tissue remodeling and extracellular matrix degradation.[1] Over expression of these proteases is an indication of pathological changes in a variety of conditions including cancer,[2] atherosclerosis,[3] arthritis,[4] and chronic non-healing wounds.[5] MMP-3 (stromelysin-1) is most heavily involved in chronic non-healing wounds and atherosclerosis. A series of hydroxamic acid targets were designed, synthesized, and tested for potency as well as selectivity against other MMPs. This SAR revolved around the incorporation of a 2-fluoro pyridine in the molecule through a Suzuki coupling reaction. The 2-fluoro pyridine is critical for efficient transition from ^{19}F to radio-labeled ^{18}F for the positron emission tomography (PET) study.



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MEDI 371

Progress toward a polyphenol releasing bare metal stent

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This undergraduate organic chemistry project seeks to attach polyphenols, natural products found in berries and grapes, to metal cardiovascular stents. Stents are devices that hold open narrow arteries in patients with coronary artery disease, but stents can fail due to thrombosis or renarrowing of the artery post implantation. Some polyphenols have been shown in cell studies to enhance the migration and growth of endothelial cells that line healthy arteries. These polyphenols have also been shown to increase the production of nitric oxide in endothelial cells, helping to improve cardiovascular health and healing. The structure of polyphenols can easily be modified, resulting in a collection of synthesis targets where small changes in structure could vastly change the behavior of endothelial cells. The overall hypothesis of this research is that a bare metal stent releasing polyphenols will stimulate rapid endothelial healing, leading to long-term device success with less risk of thrombosis or renarrowing of the artery. To accomplish this goal, we have synthesized a library of polyphenols, investigated the attachment of these molecules to metal surfaces, and characterized the endothelial cell response to these molecules *in vitro*.

MEDI 372

Discovery and optimization of pyrazole carboxamides as Jak1 selective inhibitors for rheumatoid arthritis

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This poster describes the identification and optimization of a novel class of pyrazole carboxamides as highly Jak1 selective inhibitors, which originated from historical Merck Jak2 preferring molecules. Guided by extensive in-vitro studies, Liabilities related to both oxidative metabolism and plasma stability were successfully addressed. Evidence suggested that active transport might be the main mechanism of clearance resulting in the observed poor in-vitro-in-vivo clearance correlation.

[figure]

MEDI 373

Design and synthesis of thienopyrimidine based FLT3 inhibitors from SPC-839, a known IKKβ inhibitor

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Inactivation of the NF-κB signaling pathway by inhibition of IKKβ is a well-known approach to treat inflammatory diseases such as rheumatoid arthritis and cancer. To discover new IKKβ inhibitors, known IKKβ inhibitor **SPC-839** was modified by introducing a thienopyrimidine core and then biologically evaluated. Resulting analogues had good inhibitory activities against both nitric oxide and TNF-α, which are well-known inflammatory responses generated by activated NF-κB. However no promising inhibitory activities against IKKβ were observed. To probe the target of thienopyrimidine-based analogues, we carried out a kinase panel assay and FLT3 was identified as the target. Thienopyrimidine-based analogues showed good inhibition profiles against FLT3 under 1μM as well as good cell growth inhibitory activities on leukemia cell lines. Overall, these compounds represent promising inhibitors for the future development of a treatment for acute myeloid leukemia (AML).

MEDI 374

Synthesis and characterization of radiometals-labelled apoptosis-targeting peptide

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Programmed cell death (apoptosis) and cell proliferation, though antagonistic, goes hand-in-hand while maintaining the normal physiology of any cell. Any kind of imbalance, in either of the processes, leads to severe consequences in terms of dreaded diseases. These facts have spiked up the interest of the research community and lots of effort have been made to develop probes for non-invasive detection of cell death.

Peptides are known to have properties of an ideal targeting probe. They can efficiently penetrate into tissue due to small size and low immunogenicity. Moreover, peptides are relatively easy to be chemically modified for subsequent conjugation with imaging agents.

Apoptosis targeting peptide, ApoPep-1, was identified recently and was used to image cell death *in vivo*. However, most studies were conducted by using fluorescent probes which are not suitable for *in vivo* applications, or by using radiohalogens, which are not so stable *in vivo*.

In order to overcome this problem, we conjugated ApoPep-1 with NOTA (1,4,7-triazacyclononane-triacetic acid) chelator, and further radiolabeled with ^{68}Ga and ^{64}Cu in high yield. The radiolabeled ApoPep-1-NOTA showed high stability both in PBS and FBS. Also, thisradiolabeled ApoPep-1 peptides showed good uptake in cell-death lesion in animal models.

MEDI 375

Potent and selective pyridone BTK inhibitors with an induced fit binding mode and activity vs. mutant forms of BTK

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Bruton's tyrosine kinase (BTK) plays a critical role in the development, differentiation and proliferation of B-lineage cells, making it an attractive target for the treatment of immunological disorders as well as B-cell lymphoma. There have been significant efforts from the pharmaceutical community with the goal of identifying BTK inhibitors for the treatment of immune disorders. Of these, the most advanced to date is Ibrutinib, which launched in 2013 for cancer indications. It has been reported that some patients have acquired resistance to ibrutinib therapy, and these have been shown to have mutations in BTK (C481S) and its direct substrate PLCγ2 (R665W). Herein we disclose BTK inhibitors that are active against a range of BTK-mutant enzymes, including C481S.

MEDI 376

RXR partial agonist NEt-4IB exerts therapeutic effects on inflammatory bowel disease without the side effects of RXR full agonists

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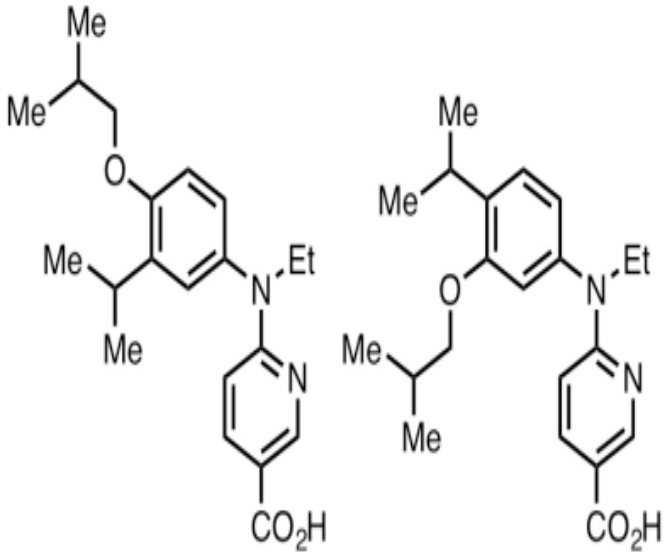
Exerting strong anti-inflammatory effects, steroidal anti-inflammatory drugs produce serious side effects including hyperglycemia, ulcer disorder by inhibition of prostaglandin production, infections by excessive immuno-suppression, and Cushing's syndrome. These back grounds prompted us to create new anti-inflammatory drugs which can be replaced for steroidal anti-inflammatory drugs. Retinoid X receptors (RXRs) form heterodimers with peroxisome proliferator-activated receptor (PPAR) and liver X receptors (LXRs) which are known to exert anti-inflammatory effects through the suppression of NF-kB by their activation. These receptors form heterodimers with retinoid x receptors (RXRs), and these heterodimers can be activated by RXR agonists alone (permissive effect). Since RXR anti-inflammatory effects are thought to be produced differently by glucocorticoid receptor (GR), RXR agonists are expected as new anti-inflammatory drugs. However previously reported RXR agonists are full agonists and produce blood triglyceride elevation, hypothyroidism, weight gain and hepatomegaly. We hypothesized that these side effects are caused by the excessive activation of RXRs and moderate activation of RXRs is enough for the desired medical effects. This time, 6-[ethyl(4-isobutoxy-3-isopropylphenyl)amino]-3-pyridinecarboxylic acid (**1** : NEt-4IB), which is a regioisomer of a RXR full agonist NEt-3IB (**2**), was found as a RXR partial agonist. Compound **1** inhibits nitric oxide (NO) production in LPS-stimulated RAW 264.7 cells at 10 mM by ca 50%. Moreover **1** showed anti-inflammatory effect in NBD-Cl induced inflammatory bowel disease model mice at 10 mg/kg oral administration, though prednisolone and **2** did not show the effects. And **1** did not produce hepatomegaly and hyperglicemia.



MEDI 377

Radiosynthesis and biological evaluation of a novel Enoyl-ACP reductase inhibitor for *Staphylococcus aureus*

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A novel and potent (K_i = 0.01 nM) *Staphylococcus aureus* enoyl-ACP reductase (saFabI) inhibitor (PT119), identified from a saFabI inhibitor library, showing favorable interactions with the target enzyme and a long residence time (750 min), was radiolabeled with carbon-11 to evaluate its biodistribution and pharmacokinetics in both healthy and *S. aureus* infected mice using positron emission tomography (PET). The biodistribution of [^{11}C]PT119 and/or its labeled metabolites does not differ significantly between the healthy group and the infected group. Furthermore, we evaluated the *in vivo* antibacterial efficacy of PT119. The drug showed promising efficacy in two different *S. aureus* infection models: it decreased 3 Log CFU in the thigh muscle infection model and increased the survival rate from 0% - 50% in the systemic infection model. This approach is important to determine biodistribution and pharmacokinetics of novel drugs as well as to screen tracers for bacterial infection imaging.

MEDI 378

Investigation of the mechanism of action of a species-selective antibacterial compound

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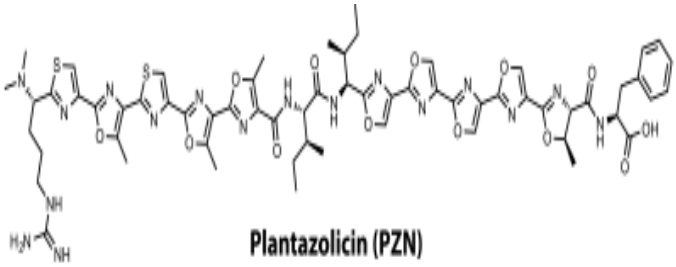
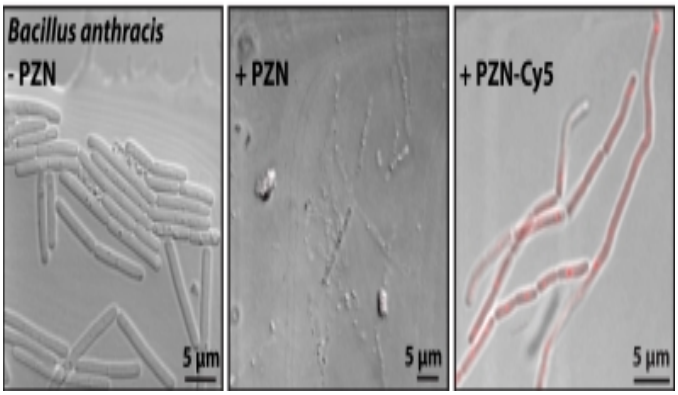
As drug-resistant pathogens pose an increasing threat to human health worldwide, there is a need for new antibacterial compounds with novel and selective targets. Plantazolicin (PZN) is a ribosomally produced peptide with extensive posttranslational modifications that exhibits exquisite antibacterial selectivity for *Bacillus anthracis*, the causative agent of anthrax, suggesting a species-specific and novel mechanism of action. In order to determine its molecular target, a structure-activity relationship study of PZN was initiated to identify moieties essential for bioactivity. Synthetically prepared truncations of the polyazole core of PZN displayed broader-spectrum antibacterial activity but were less potent than the full-length natural product, suggesting the cruciality of the polyheterocyclic core for bioactivity. Based on the ability to modify the C-terminus of PZN without significantly affecting bioactivity, biotinylated and fluorescently labeled derivatives of PZN were prepared. These probes are being employed in affinity purification and fluorescence microscopy to demonstrate the unique mechanism of action of PZN.



Plantazolicin (PZN)



MEDI 379

6,7-Dihydropyrrolo[3,4-*g*]indol-8-ones as selective inhibitors of CDC2-like kinases (CLKs)

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Alternative splicing is an essential process in eukaryotes often following the transcription of DNA into pre-mRNA. The removal or retention of exons from a single pre-mRNA during splicing gives rise to an array of different mRNAs and subsequently translated proteins. Among other regulatory mechanisms involved in this process, phosphorylation of splicing factors by kinases of the CLK family (CDC2-like kinases) plays an important role. [1] Hence, any dysregulation of these kinases may alter or abolish the original function of an affected protein thus contributing to the development of diseases such as Alzheimer's disease or the metastasis of ovarian cancer. [2, 3] The relevance of CLKs in the onset of the abovementioned diseases and the lack of potent and selective inhibitors shows an unmet need for novel small molecules as tools for biological experiments or as lead compounds in the drug discovery process. We here present the synthesis and biological evaluation of 6,7-dihydropyrrolo[3,4-*g*]indol-8-ones which exhibit IC_{50}-values for CLKs in the nanomolar range while being inactive on all members of a panel of related serine threonine kinases (e.g. DYRKs).

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MEDI 380

Viral Achilles heel: The nucleocapsid protein of FIV and related lentiviruses as a therapeutic target

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Therapeutic resistance to anti-viral drugs *via* mutation is a major challenge affecting both human and veterinary medicine. Since the discovery of the human immunodeficiency virus (HIV) in 1984 this single disease has caused 25 million deaths worldwide clearly highlighting the unique challenge in this research area. Amongst non-human vertebrates, feline immunodeficiency virus (FIV) infection is perhaps the closest biological model of HIV infection with an analogous late-stage AIDS-type progression. FIV infection primarily occurs through biting and during mating with about 11% of cats currently infected worldwide. FIV and HIV are closely related to other lentiviruses including, the simian (SIV) and equine (EIAV) varients, each of which is a species-specific virus using analogous proteins in the viral life cycle. Exploiting these overlaps by targeting the mutation resistant nucelocapsid protein (NCp) in FIV that performs the same role as the NCp7 protein in HIV and other lentiviruses, has led to the development of highly active small molecules which represent a new therapeutic approach. There is currently no crystal structure for the FIV nucleocapsid protein, so through the design of a strong homology model, we are able to dock existing and potential compounds in a form of *in-silco* screening using our results and the literature to train the model and improve the results. Active compounds are then synthesised and tested against chronically infected FIV cell lines, with pre-screen cytotoxic testing to assess the therapeutic window of activity. The validated anti-viral activity of the compounds puts us in a strong position for further development and the results of our approach will be described.

MEDI 381

N-Benzoyl-2-hydroxybenzamides displayed high activity against protozoan parasites

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N-(4-ethylbenzoyl)-2-hydroxybenzamide (**1**) was identified in a high-throughput screening of a small molecule collection satisfying the Lipinski's rule of five. Compound **1** was active *in vitro* against *Toxoplasma gondii* parasites in nanomolar range (IC_{50} = 32 nM) with no apparent toxicity against human foreskin fibroblasts (HFF) at the highest

(10 uM) compound's concentration.[1] The hit compound **1** was selected for further rounds of structure optimization in search of derivatives with improved potency and ADME/Tox properties.

In general, a wide range of *N*-benzoyl-2-hydroxybenzamides and related compounds was synthesized and evaluated for efficacy against five protozoan parasites: *Toxoplasma gondii, Trypanosoma brucei rhodesiense, Trypanosoma cruzi, Leishmania donovani,* and *Plasmodium falciparum*.[1,2]

Structure−activity analyses led to the identification of compounds with excellent activity, and in some cases selectivity, against *T. gondii*, the K1 isolate of *P. falciparum* and *L. donovani*. On the other hand, these analogs were only moderately active against *T. cruzi*. The lead compound **2** exhibited an IC_{50} of 0.005 *μ*g/mL against the K1 *P. falciparum* isolate, and showed excellent selectivity index, SI = 1640. The compound's potency against the K1 isolate was 21-fold superior to that of the standard anti-malarial drug, chloroquine (IC_{50} 0.108 *μ*g/mL). The activity of another compound in this series (**3** , IC_{50} 0.135 *μ*g/mL) against *L. donovani* was comparable to that of miltefosine (IC_{50} 0.188 *μ*g/mL), an orally administered drug currently in use to treat leishmanial infections.[2]

This research study characterizes *N*-benzoyl-2-hydroxybenzamides as highly effective anti-protozoal agents with the potential for further development.

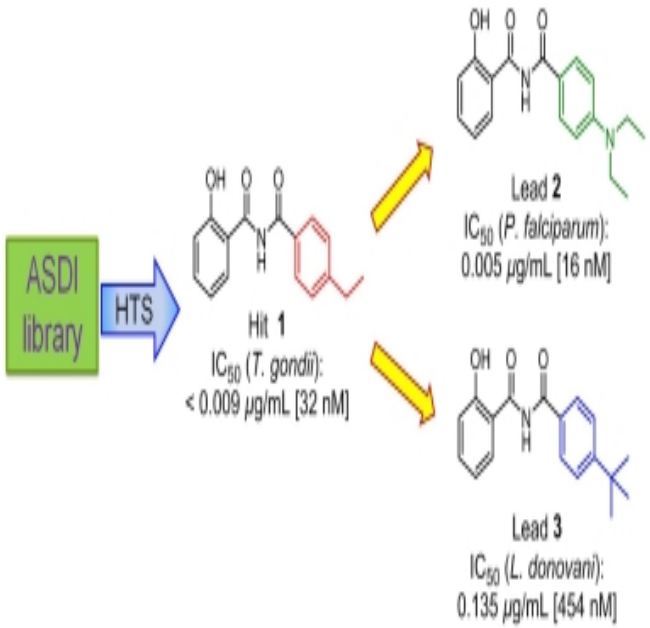

Figure 1. Structural optimization of hit **1** afforded highly active lead compounds **2** and **3**

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MEDI 382

Binding mode analysis of small molecule trypanothione reductase inhibitors and variability between parasitic species

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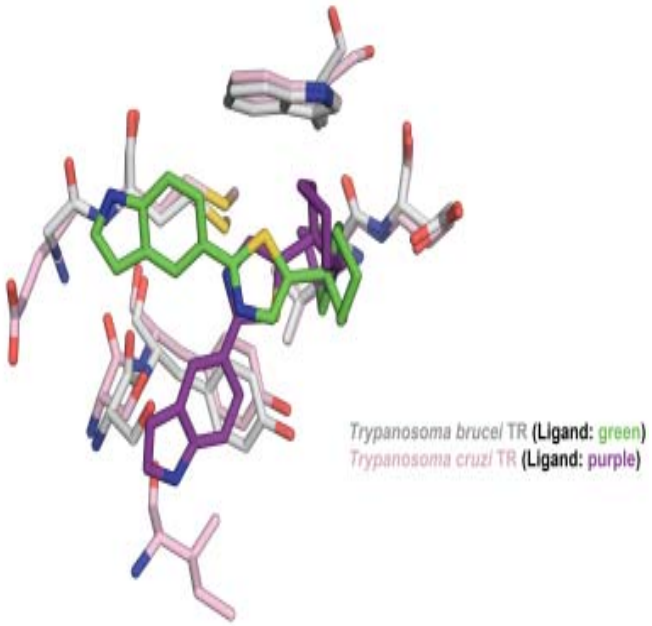
The flavoenzyme trypanothione reductase (TR) is a key player in the redox metabolism of the causative agents of human African trypanosomiasis, Chagas' disease, and leishmaniasis and has been identified as promising potential drug target.[1] Here, we report the design, synthesis, and biological activity of novel, small-molecule TR inhibitors and the analysis of their binding mode to the large active site of TR in a multidimensional approach.[2]



The conjunction of biological activities, mutation studies, and virtual ligand docking simulations led to the prediction of a binding mode that was confirmed by crystal structure analysis. Thereby, a detailed picture of the binding interaction is given showing the importance of electrostatic interactions in contrast to hydrogen bonding. Furthermore, for the first time co-crystal structures of one and the same inhibitor bound to TR from two different species were obtained and revealed that though the overall location of the ligand in the active site is maintained some significant differences are observed. These findings are supported by kinetic analysis and suggest that minor differences in both protein and inhibitor can largely affect the interactions.

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MEDI 383

Nitrofurazone bioisosters (semicarbazone, thiosemicarbazone and aminoguanidine derivatives) as antichagasic candidates

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Currently, 7 to 8 million people are affected by Chagas disease, a parasitosis caused by *T. cruzi*. Treatment of this disease is inadequate because there are only two drugs available and both can cause serious adverse effects and aren't active in the chronic phase of the disease. Semicarbazones and thiosemicarbazones derivatives showed inhibition against cruzain, the major cysteine protease in *T. cruzi*. In this work, analogues of nitrofurazone antichagasic candidates were designed by bioisosterism. In molecular modeling and docking studies the thiosemicarbazone derivatives showed a good complementarity with the active site of cruzain. Six semicarbazone, thiosemicarbazone and aminoguanidine derivatives were synthesized and tested in cruzain inhibitory assays and against *T. cruzi* epimastigote forms. Compounds presented significant cruzain inhibition between 70 and 75%. The IC_{50} values observed in epimastigotes forms of *T. cruzi* ranged from 19.8 to 139.45 μM. The compounds did not presented cytotoxicity at concentrations up to 50 μM and 250 μM in MTT tests. This study shows that this class of compounds can be used as a prototype in the search for new antichagasic drugs.

MEDI 384

Structure based inhibitor discovery against beta-lactamase in countering bacterial resistance

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Antibiotic resistance is a world wide epidemic that is growing in magnitude. One of the main resistance mechanisms against β-lactam antibiotics, the primary antibacterial chemotherapeutic agents, is the production of serine β-lactamases in Gram-negative bacterial pathogens. These enzymes hydrolyze β-lactam antibiotics such as penicillins and thereby render them unreactive with their original targets, the penicillin binding proteins essential for bacterial survival. There is an urgent need for inhibitors that can restore susceptibility to β-lactam antibiotics in multi-drug-resistant Gram-negative pathogens. My research project has consisted of cloning and mutating TEM-1, a β-lactamase commonly observed in clinic, crystalizing and solving the structure to aid in future complex structure determination, and using virtual screening to identify novel, non-covalent inhibitors against this protein. Using the computational program DOCK, I have screened the ZINC database of commercially available small molecules against the active site of TEM-1. From the 500,000 fragment (MW < 250 Dalton) and 5 million lead-like (250 < MW < 350 Dalton) compounds of ZINC. True inhibitors can potentially be developed into new antibiotics in the future to counter drug resistance caused by β-lactamases.

MEDI 385

Bis-sulfonamide inhibitors of 2-methylerythritol 2,4-cyclodiphosphate synthase (IspF) from *Arabidopsis thaliana* and *Plasmodium falciparum*

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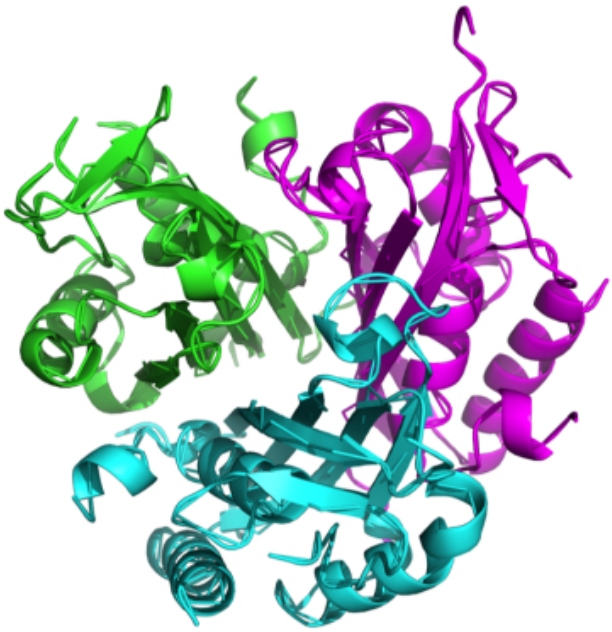
Malaria, caused by parasites of the genus *Plasmodium*, is responsible for over 600'000 deaths per year.[1] Emerging resistances against malaria drugs demand for the development of new antimalarials.

Humans utilize the mevalonate pathway for the biosynthesis of isoprenoids, whereas many pathogens, such as *P. falciparum* exclusively obtain isoprenoids via the non-mevalonate pathway, which is a validated target for treatment of malaria.[2]

In this pathway, the enzyme IspF catalyzes the cyclization of diphosphocytidyl-2-methylerythritol 2-phosphate into 2-methylerythritol 2,4-cyclodiphosphate. The IspF enzyme is active as a trimer with three active sites.



The inhibition of plant IspF enzyme from *A. thaliana* was screened against 40'000 compounds. A bis-sulfonamide derivative was identified as possible inhibitor. Several derivatives were synthesized, the most active showing one-digit micromolar IC_{50} values against *P. falciparum* IspF.

The dissociation constant of the most active bissulfonamide to the three active sites was determined by ESI-MS.

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MEDI 386

Rational design of inhibitors targeting drug resistant mutants of the influenza A virus M2 proton channel

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The influenza A virus M2 proton channel (A/M2) is the validated target of the antiviral drugs amantadine and rimantadine, however their use has been discontinued due to widespread drug resistance. The predominant drug-resistant mutants are S31N, V27A, and L26F, which show greatly decreased inhibition by amantadine. The discovery of inhibitors of these mutants has been hampered by the lack of high resolution structures and the limited size, polarity, and dynamic nature of their drug sites. Nevertheless, coupled with molecular dynamics simulations, solution- and solid-state NMR spectroscopy, we have designed small molecule drugs that inhibit each mutant with potencies greater than amantadine's potency against WT M2. The potent inhibitors we discovered also enabled the determination of the first solution NMR structure of the S31N mutant. Recently we have made progress in designing dual inhibitors targeting both WT and the S31N mutant. NMR NOESY measurements and MD simulations of drug-M2 interactions validated our design hypothesis: dual inhibitor binds in the WT M2 channel with its aromatic headgroup facing down towards the C-termini; while the same drug binds in the S31N M2 channel with its headgroup facing up towards the N-termini. The discovery of this flipping mode of binding also correlates with the structure-activity relationship we observed and has paved the way for the next round of rational design of antiviral drugs with broad specificity.

MEDI 387

Novel oxazolidinone hydroxamic acid derivatives: Synthesis and biological evaluation

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Oxazolidinones are antibacterial agents active against Gram-positive bacteria. Studies have shown that optionally varied C-5 substituted oxazolidinones resulted in derivatives with comparable or enhanced antibacterial activity in comparison to linezolid. Oxazolidinones are also plagued with unwanted monoamine oxidase (MAO) inhibition as side-effect, due to structural similarity with MAO inhibitors, namely, toloxatone. In this study, we synthesized optionally varied C-5 hydroxamic acid containing oxazolidinones and investigated their antibacterial and MAO inhibitory activities.

Susceptibility testing of the compounds was performed by agar dilution method with discs containing 30 µg/mL test compounds and the diameter zone of inhibition (mm) measured. Computer model interactions of representative oxazolidinones at the bacterial ribosomal receptor site was performed using Molecular Operating Environment (MOE v 2012.10) software. MAO-A or -B inhibition was determined in 96 well microtiter plates using tyramine as a mixed substrate for MAO-A and -B. Blanks containing buffer instead of tyramine and controls containing distilled water instead of test compounds, were run parallel to the samples in a SunriseTM microplate absorbance reader at 498nm every minute for 10 minutes, then every 10 minutes for 20 minutes and every 20 minutes until 90 minutes.

The oxazolidinone hydroxamates and their N-carbamate precursors were devoid of antibacterial activity. Computer modeling suggested that this lack of activity could be due to disruption of hydrogen bond formation between the hydroxamate N-OH moiety and the 5'-phosphate of G2540, as a result of steric overlap of the two oxygen atoms due to their proximity (~1.5 Å). However, the compounds showed weak to moderate MAO-A and -B inhibitions at 50 and 200 µM concentrations. MAO inhibitions ranged from 0 to 68.8% for both isoenzymes. This study showed that the presence of a hydroxamic acid functional group at the C-5 position of the oxazolidinone ring is detrimental to antibacterial and MAO inhibitory activities.

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MEDI 388

Structure-based design and optimization of dipeptidyl inhibitors of norovirus 3CL protease

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Human noroviruses are the primary cause of sporadic and epidemic acute gastroenteritis in the US and worldwide. Noroviruses constitute an important public health problem, as well as a potential bioterrorism threat. The problem is further compounded by the current dearth of effective vaccines and norovirus-specific antiviral therapeutics and/or prophylactics.

Human noroviruses are single-stranded, positive sense RNA viruses in the *Caliciviridae* family. Their ~7.5 kb genome encodes a polyprotein precursor that is processed by a virus-encoded 3CL protease (3CLpro) to generate mature non-structural proteins. Processing of the polyprotein is essential for virus replication, consequently NV 3CLpro has emerged as a potential druggable target for the discovery of anti-norovirus small molecule therapeutics and prophylactics. NV 3CLpro is a chymotrypsin-like cysteine protease with a Cys-His-Glu catalytic triad and an extended binding site. The primary substrate specificity of the protease is for P1 glutamine residue and a strong preference for a –D/E-F-X-L-Q-G-P- sequence, where X is H, Q or V, corresponding to the subsites S_5-S_4-S_3-S_2-S_1-S_1'-S_2'-. Cleavage is at the P_1-P_1' (Q-G) scissile bond.

We have recently reported the first high throughput FRET assay of 3CLpro from GI and GII noroviruses as a screening tool for identifying potential protease inhibitors and have determined the first high resolution X-ray crystal structures of NV 3CLpro in complex with peptidyl transition state inhibitors of the protease, as well as the first solution structure of the protease using high-field NMR. We report herein the structure-based optimization of a series of dipeptidyl inhibitors of NV 3CLpro using X-ray crystallography and an array of structure-activity relationship, biochemical, and cell-based studies.

MEDI 389

Design of New Delhi metallo-β-lactamase 1 inhibitors using a synthetic and computational approach

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The emergence of bacterial resistance against current antibiotics poses a global threat. New Delhi Metallo-β-Lactamase 1 (NDM-1) is a lactamase found in gram-negative bacteria whose function involves hydrolyzing the amide bond in nearly all β-lactam

drugs, rendering them inactive. The active site of NDM-1 consists of two zinc metal ions, which coordinate a nucleophilic hydroxide to mediate hydrolysis. Chelator fragment libraries (CFLs) were screened against the NDM-1 enzyme, resulting in >10 hits. Current synthetic aims involve building derivatives of the metal-binding fragments in order to generate focused libraries of compounds for inhibition studies. In addition, computational aims involve constructing in silico models to predict how those compounds bind to the NDM-1 active site.

MEDI 390

Design and synthesis of peptidomimetic inhibitors of *Porphyromonas gingivalis* biofilm formation

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Porphyromonas gingivalis is a periodontal pathogen that initially colonizes the oral cavity by adhering to *S. gordonii* via interaction of the minor fimbrial antigen Mfa1 with a specific motif of the streptococcal SspB protein designated BAR. Synthetic peptides which mimic BAR function are potent inhibitors of *P. gingivalis* adherence/biofilm formation, but are both costly to synthesize and active compounds are susceptible to proteolysis. Thus rationally-designed, stable small molecule peptidomimetics of BAR that inhibit *P. gingivalis* adherence to *S. gordonii* represents a potential therapy in maintaining oral health. A small-molecule click chemistry strategy is being developed whereby the azide-bearing and acetylenic partners constitute trisubstituted oxazole and diaminoaryl frameworks respectively. Seventeen azide-bearing compounds were synthesized as mimics of the NITVK motif of BAR. Two compounds partially inhibited planktonic growth of *P. gingivalis* and several were found active at varying levels in inhibition of adherence to streptococci. Our route to the azide-bearing trisubstituted oxazole scaffolds employs a unique strategy starting with an array of nonsymmetrical acyloins and culminates with the azido group being strategically positioned. Though synthetically more straightforward, the preparative routes to the acetylenic click partners employ an array of ethynyl diamino aryl components. The synthesis of the heterocyclic click partners and the evaluation of their inhibitory efficacy in the BAR assays will be presented.

MEDI 391

Effects of sidearm electron density in unsymmetrical cyclotriazadisulfonamide (CADA) analogs on anti-HIV and human CD4 down-modulating abilities

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Cyclotriazadisulfonamide (CADA) compounds prevent HIV entry and replication in cell culture by inhibiting co-translational translocation of human CD4. A series of unsymmetrical CADA analogs have been synthesized and evaluated for CD4 down-modulation. CK147, having a cyclohexylmethyl tail group and one 4-*N,N*-dimethylaminobenzenesulfonamide side arm, was found to have highest potency towards CD4 down-modulation in CHO cells transfected with a fluorescent CD4 fusion protein (IC_{50}= 60 nM) and in the T-lymphoid cell line MT-4 expressing human CD4 naturally (IC_{50}= 140 nM). In addition, the CK147-induced reduction in CD4 correlated with enhanced anti-HIV-1 NL4.3 activity (IC_{50}= 180 nM). In this study, we investigated the relationship between calculated dipole moment of one arylsulfonamide side arm of the CADA analog and CD4 down-modulation potency. Generally, it was found that CADA compounds having a larger dipole moment in one side arm and no hydrogen bond donor group exhibit higher potencies for inhibition of CD4 translocation and expression on the cell surface.

MEDI 392

ProTides of *N*–(3-(5-(2'-deoxyuridine))prop-2-ynyl)octanamide phosphate, a potent and selective inhibitor of thymidylate synthase X from Mycobacterium tuberculosis, as potential anti-tubercular and anti-viral agents

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Objectives: The flavin-dependent thymidylate synthase X (ThyX), rare in eukaryotes and completely absent in humans, is crucial in the metabolism of thymidine (a DNA precursor) in many microorganisms including several human pathogens. Conserved in mycobacteria, including *Mycobacterium leprae*, and essential for *Mycobacterium tuberculosis* growth, it represents a prospective anti-mycobacterial therapeutic target. In a *M. tuberculosis* ThyX-enzyme inhibition assay, *N*–(3-(5-(2'-deoxyuridine-5'-monophosphate))prop-2-ynyl)octanamide was reported to be the most potent and selective 5-substituted 2'-deoxyuridine monophosphate analogue. We aimed to synthesize lipophilic derivatives of the compound mentioned above and evaluate their anti-tubercular and anti-viral activity.

Methods: In this study, we masked the two negative charges at the phosphate moiety of the parent compound using our ProTide technology in order to increase its lipophilicity and therefore allow improved permeation through the complex mycobacterial cell wall barrier thus increasing its effectiveness. A series of *N*–(3-(5-(2'-deoxyuridine))prop-2-ynyl)octanamide phosphoroamidates were chemically synthesized and their biological activity as potential anti-tuberculars was evaluated. In addition to mycobacteria, several DNA viruses depend on ThyX for their DNA biosynthesis, thus these prodrugs were also screened for their antiviral properties.

Results: Twelve lipophilic phosphoramidate derivatives were successfully synthesized and evaluation of their biological activity helped ascertain potential lead molecules showing activity against *M. bovis* BCG, *M. tuberculosis* H37Rv and varicella zoster virus (VZV).

Conclusions: The increased bioactivity of some derivatives as compared to the parent molecule can be attributed to a better permeation through the mycobacterial cell wall due to well-optimized balance between their lipophilicity and molecular size, made possible by the ProTide motif.

MEDI 393

Development of curcumin-inspired compounds for the treatment of HIV and its comorbidities

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Curcumin, a naturally occurring *bis*-phenolic compound isolated from *Curcuma longa,* has displayed promising activity as an antiretroviral, anticancer, antidiabetic, and anti-inflammatory agent. Phase I and Phase II clinical trials indicate that curcumin is well-tolerated even at high doses of 12 g/day. However, curcumin is not considered as an attractive candidate, in spite of its potent activity, primarily due to its poor oral absorption. It has a short half-life and is extensively metabolized to its pharmacologically inactive glucuronide derivative. We have altered the core structure of curcumin iteratively and systematically to furnish novel curcumin analogs, termed as curcuminoids. They possess improved stability at the physiological pH. Our *in vitro* biological data indicates that the novel curcuminoids have retained their potent antiretroviral and anti-inflammatory properties. In addition, one of our analogs has shown promising neuroprotective activity indicating a potential for its use in the treatment of HIV-associated neurocognitive disorders (HAND). Our rationale for the development of a small, focused library of curcuminoids will be presented.

MEDI 394

6-Substituted pyrrolo[2,3-d]pyrimidines as dihydrofolate reductase inhibitors and potential anti-opportunistic agents

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Pneumocystis jirovecii, *Toxoplasma gondii*, *Mycobacterium avium* and *M. intracellular* are some of the most common organisms that cause life-threatening opportunistic infections in AIDS and other immunocompromised patients. Selective dihydrofolate reductase (DHFR) inhibitors in combination, represent a viable therapeutic approach for the treatment of these infectious diseases as well as in cancer chemotherapy. First line treatment of Pneumocystis pneumonia (PCP) requires a combination of trimethoprim (TMP)-sulfamethoxazole (SMX), due to weak inhibitory activity of TMP. The second line treatment involves potent, but non-selective DHFR inhibitors such as trimetrexate (TMQ) and piritrexim (PTX) which cause myelosuppression and require co-administration of leucovorin increasing the cost of therapy. Thus there is a significant unmet clinical need for agents that combine the potency of PTX and selectivity of TMP in single agents. We synthesized a series of 6-substituted pyrrolo[2,3-*d*]pyrimidines that are selective and potent inhibitors of DHFR derived from *Pneumocystis jirovecii*. The synthesis and biological evaluation of the further analogs designed in an attempt to optimize selectivity and potency for pjDHFR over hDHFR will be presented.

MEDI 395

Synthesis and antimicrobial evaluation of a new class of pyrido[1,2-a]thieno[3,2-e]pyrimidine

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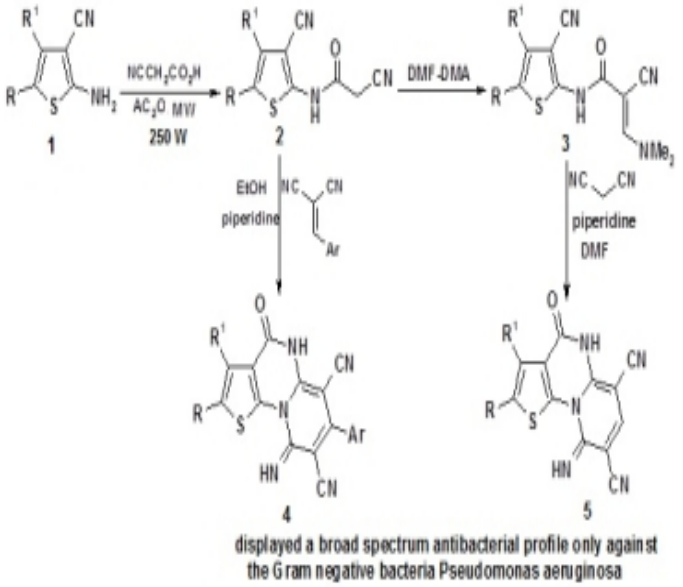
Multisubstituted 2-aminothiophenes **1a-c** can be readily cyanoacylated *via* reaction with cyanoacetic acid in presence of acetic anhydride under microwave irradiation to form the corresponding cyanoacetamides **2a-c** , which condensed with DMF-DMA to form the corresponding enamines **3** . Moreover the cyanoacetamides **2a-c** reacted with a variety of arylidenmalononitrileto afford novel pyrido[1,2-*a*]thieno[3,2-*e*]pyrimidine derivatives **4a-o** . In addition the enamines **3** reacted with malononitrile to afford the pyrido[1,2-*a*]thieno[3,2-*e*]pyrimidine derivatives **5a,b** . The X-ray crystallographic analyses of seven products could be obtained thus establishing with certainty the proposed structures in this work. Most of the synthesized compounds in this investigation were tested and evaluated as antimicrobial agents; the results of biological evaluations demonstrate that members from these compounds have promising antimicrobial activities against Gram negative bacteria, Gram positive bacteria.



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MEDI 396

Optimized quinoline-based disruptor with improved efficacy against biofilm formation in *Vibrio cholerae*

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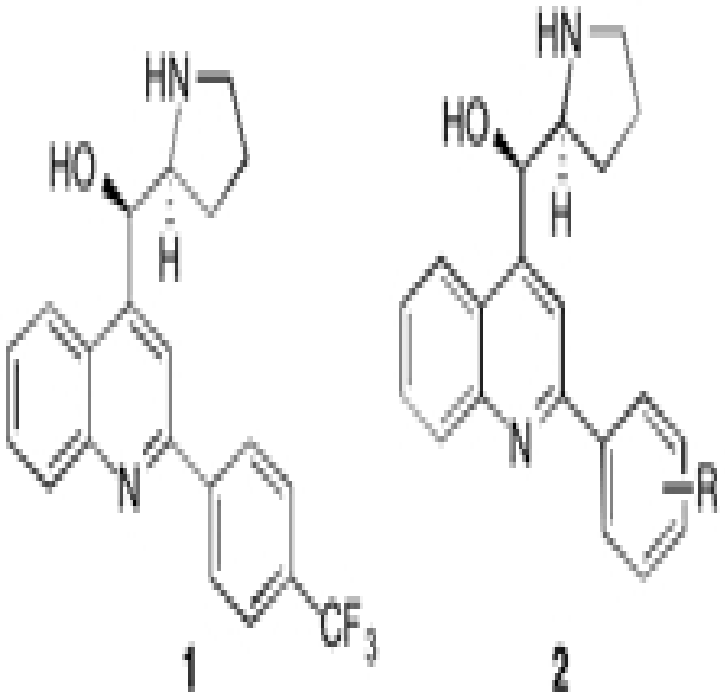
Bacterial biofilms are a major medical issue because they are up to 1000 times more resistant to traditional antibiotics. It is believed that antibiotic resistant bacteria cost up to $20 billion per year. This problem is not just financial, because the NIH estimates that about 75% of pathogenic microbial infections are biofilm mediated. This is supported by the fact that in aquatic environments bacteria remain in the biofilm state 99.9% of the time. The formation of bacterial biofilms is commonly described as the formation of a

community of microorganisms on a surface. However, it now appears that this description is inadequate for such a dynamic and multifaceted process. For this reason, our laboratory has begun a program to discover biofilm inhibitors/molecular probes that will allow us to study biofilm progression and physiology, as well as providing new avenues for therapeutic intervention. Initial studies began with the development of a first generation library, which identified lead compound **1** of simpler structure and increased potency relative to the original hits. A second generation library has now identified lead compound **2** with an improved window of efficacy and potency. These improvements in the biological profile will now allow us to further study the Mechanism of Action (MOA) of this class of compounds.



MEDI 397

Design, synthesis, and evaluation of novel hydrazide-based small-molecule inhibitors of plasminogen activator inhibitor-1

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Plasminogen activator inhibitor-1 (PAI-1) is a mammalian serine protease inhibitor and a key regulator of fibrinolysis. Individuals in certain disease states experience higher

levels of active PAI-1, resulting in an increased risk of thrombosis and embolism. Reduction of circulating active PAI-1 levels by use of an appropriate PAI-1 inhibitor may therefore decrease this risk. The focus of this research is to explore a novel class of PAI-1 inhibitors identified from a dual-reporter high-throughput screen. Here we present initial data related to the structure-activity relationships of these hydrazide-containing inhibitors in a plasma-based assay system.

MEDI 398

Evaluation of ^{64}Cu radiolabeled Ceragenin (cationic selective antimicrobial) in a biological system

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Endogenous antimicrobial peptides (AMPs) are a central aspect to innate immunity to bacteria, however, they are susceptible to degradation by proteases and are costly for large scale production. Ceragenins (CSAs) are non-peptide mimics of AMPs and have been shown to overcome these problems. The goal of the current study was to evaluate a lead CSA as a potential agent for non-invasive imaging of bacterial infections. This study evaluated CSA-150 that contains bacterial recognition sites and CSA-151, where the bacterial active sites have been blocked by amide groups. Both CSA-150 and 151 having a 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA) chelator in one end facilitated an excellent radiolabeling with ^{64}Cu (an imaging radionuclide) using mild conditions (pH 5.5, 37 ^{o}C, incubation time = 1 h).

Preliminary in vitro binding studies clearly demonstrated the specificity of CSA-150 to the E. Coli membranes compared to the control CSA-151. An in vivo biodistribution study was conducted in normal mice with three different time points (t= 30 min, 2 h and 4 h post injection). The results of the biodistribution and also ex vivo autoradiography imaging indicated a significant increase of uptake of ^{64}Cu-NOTA-CSA-150 in mice kidney compared to the tested control.

MEDI 399

Learning lessons from nature: Design and synthesis of DNA gyrase inhibitors based on the structure of Simocyclinone D8

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Antimicrobial resistance (AMR) is a global healthcare concern,[1] a shortage of new therapies has led to a race to develop new treatments to fight 'superbugs'. DNA gyrase, a prokaryotic topoisomerase enzyme responsible for regulating the topological state of bacterial DNA,[2] is only found in bacteria and thus is a rational drug target.[3] Ciprofloxacin, a quinolone antibiotic, stabilises a DNA-gyrase-drug intermediate resulting in cell death.[4] Ciprofloxacin's success is a paradigm for disrupting topoisomerase function; however enzyme mutations, alterations in efflux pumps and plasmid mediated resistance are diminishing the quinolones efficacy.[5] Simocyclinone D8, (SD8) a natural product from *Streptomyces*, also targets DNA gyrase but with a novel mode of action,[6] preventing binding of DNA with the gyrase enzyme. SD8 is bifunctional, containing a chlorinated dihydroxylated aminocoumarin, a tetraene linker, D-olivose sugar and angucyclic polyketide. Inspired by the structural diversity and distinct mechanism of action of SD8,[7] we describe herein the synthesis of small molecules designed to mimic its mechanism of action and move towards the synthetic construction of both the natural product and designed analogues. We will also present biological assays for the activity of the compounds against DNA gyrase.

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MEDI 400

Synthesis of Doxycycline-[$^{13}CD_3$]

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Doxycycline is a tetracycline antibiotic that is used to treat a wide variety of infections. A mass spectrometry (MS) analytical standard with M + 4 was prepared to support pharmacology and toxicology. Our approach was to first *N*-demethylate doxycycline and re-methylate using stable isotope labeled methyl-[$^{13}CD_3$] iodide to produce doxycycline-[$^{13}CD_3$]. An iron mediated Polonovsky reaction efficiently *N*-demethylated doxycycline. In this process, doxcycline was oxidized to the N-oxide using *m*-CPBA. The hydrochloride salt of the tertiary *N*-oxide was treated with Fe(0)/$FeCl_3$ catalyst yielding the *N*-demethylated product. Isolated pure demethyldoxycycline was re-methylated using diisopropyl azodicarboxylate (DIAD), polymer-supported triphenylphosphine and methyl-[$^{13}CD_3$] iodide. We have synthesized labeled doxycycline with an isotopic purity of 99% using this methodology.

MEDI 401

Identification of norstictic acid as an inhibitor of Med25-activator interactions

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Mediator subunit 25 (Med25) is a component of the megadalton Mediator complex, whose recruitment is required for the expression of most genes in eukaryotic organisms. The activator interaction domain (ACID) is a key component of the Med25 protein that has been recently shown to make critical contacts with several transcription factors, allowing for the recruitment of the full complex to target genes. These transcription factors include VP16, a component of the herpes simplex virus (HSV) responsible for the switch from latent to lytic infection; ATF6α, an endoplasmic reticulum stress response transcription factor; and ERM, a member of the Ets family of transcription factors that has been implicated in the progression of ovarian cancer. Given the functional importance of Med25 recruitment by various transcription factors, inhibitors of these interactions would be useful mechanistic probes for interrogating Med25 dependent gene expression.

Recently published solution structures of the Med25 ACID domain in complex with the VP16 transcriptional activation domain (TAD) have revealed two distinct binding sites capable of independently binding transcriptional activators. We are currently focused on the identification and development of small molecule inhibitors that selectively target each of these binding interfaces; as such compounds would be useful tools for elucidating the functional model of Med25 recruitment. Additionally, these molecules could also serve as potential lead compounds for the development of antiviral and anticancer therapeutics.

In order to accomplish this ambitious goal, we have developed a fluorescence polarization based assay that is well adapted to high-throughput screening. A pilot screen of known biologically active molecules revealed depsides and depsidones, natural products derived from lichens, as a compelling class of potential inhibitors. Compounds containing the depside or depsidone core were then selected and utilized in a dose response assay, revealing norstictic acid as the first *in vitro* inhibitor of Med25•activator interactions.

MEDI 402

Bicyclic 4-amino-1-hydroxy-2-oxo-1,8-naphthyridine-containing compounds having high potency against Raltegravir-resistant integrase mutants of HIV-1

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Integrase (IN) inhibitors are the newest class of antiretroviral agents developed for the treatment of HIV-1 infection. Merck's Raltegravir (RAL) (October 2007) and Gilead's Elvitegravir (EVG) (August 2012), which act as IN strand transfer inhibitors (INSTIs), were the first anti-IN drugs to be approved by the FDA. However, the virus develops resistance to both RAL and EVG and there is extensive cross-resistance to these two drugs. New “2nd-generation” INSTIs are needed to improve efficacy against RAL and EVG-resistant strains of IN. The FDA has recently approved the first 2nd generation INSTI, GSK's Dolutegravir (DTG) (August 2013). We report the design and synthesis series of 1-hydroxy-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamides, as well as the determination of their IN inhibitory potencies in biochemical assays and their antiviral efficacies (EC_{50} values) in cell-based assays employing viral constructs harboring wild-type (WT) and RAL-resistant IN. Further development led to 4-amino-1-hydroxy-2-oxo-1,8-naphthyridine 3-carboxamidesthat have improved antiviral efficacies against recombinant IN in biochemical assays. These new compounds show single-digit nanomolar antiviral potencies against HIV vectors that carry wild-type (WT) IN, and have improved potency against vectors harboring the major forms of resistant mutant IN. These compounds also have low cytotoxicity in cultured cells and reach selectivity indices (CC_{50}/EC_{50}) over 20,000. The compounds have the potential to yield new anti-HIV drugs that are broadly effective against the known resistant mutants.

MEDI 403

Repurposing the antimistamine terfenadine as an antibiotic

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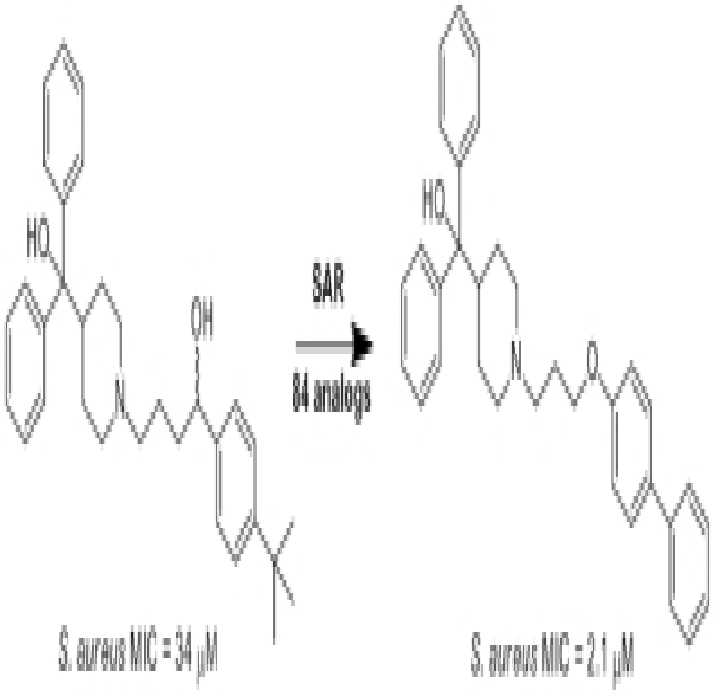
Staphylococcus aureus accounts for 16% of hospital-acquired infections in the U.S. annually leading to $9.5 billion in healthcare costs. Strains of *S. aureus* have become increasingly resistant to several currently available antibiotics including methicillin, vancomycin and fluoroquinolones. As resistance and virulence continues to increase

there is a concurrent decrease in the number of novel chemical scaffolds in the antibiotic drug discovery pipeline and according to the Infectious Disease Society of America the problem is only worsening. Utilizing a “drug repurposing” strategy our group has discovered the antihistamine terfenadine to possess previously unreported anti-staphylococcal properties. Given this information the mechanism of action was sought and a structure-activity relationship optimization campaign undertaken. The minimum inhibitory concentration of terfenadine analogs versus wild type and resistant strains of *S. aureus*was improved up to 16-fold and a mechanism of action identified. This data suggests terfenadine analogs have promise as potential novel chemical scaffolds possessing antimicrobial activity.



MEDI 404

Novel inhibitors of bacterial DNA gyrase identified by a high throughput phenotypic screen of bacterial SOS response

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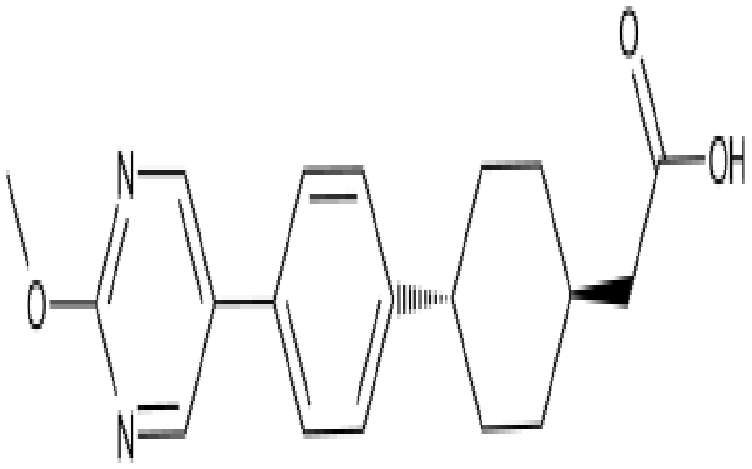
Novel classes of antibacterial agents are urgently needed due to increasing bacterial resistance against the current standards of care in both hospital and community

settings. Toward this end, a high throughput cell-based SOS reporter screen utilizing an *E. coli ΔtolC* cell line was developed to identify inhibitors of DNA metabolism and replication. Following the screening of AstraZeneca's corporate deck, a biarylcyclohexylacetic acid that triggered an SOS response was identified for additional work and profiling. Optimization of the hit and isolation of resistant mutants against this class of compounds in *H. influenzae* identified the target as DNA gyrase. This talk will cover the SAR and target identification work undertaken to identify the mode of action for the series.



E. coli SOSr EC_{50} = 4.7 μM

E. coli ΔtolC MIC = 1 μg/mL

MEDI 405

Design of antivirulants targeting the iron-regulated heme oxygenase (HemO) of *Pseudomonas aeruginosa*

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Iron is required for the survival of almost all forms of life. For pathogenic bacteria, including the opportunistic *Pseudomonas aeruginosa*, iron also regulates the production of virulence factors involved in establishing and prolonging an infection. In the host environment, this iron is most available in the form of heme, stored in hemoproteins. Acquisition of heme by Gram-negative pathogens is well studied, involving membrane-bound transporters as well as periplasmic and cytoplasmic heme transfer proteins. Iron is finally released upon enzymatic degradation of heme by heme oxygenase enzymes.

Previous computational studies of the *P. aeruginosa* iron-regulated heme oxygenase (HemO) identified inhibitors that were predicted to bind to the active heme-binding site. These inhibitors and the inhibitor-protein complex were characterized by NMR techniques. In addition, the compounds' activities were confirmed by fluorescence quenching, growth inhibition, and a *Caenorhabditis elegans* infection model. Synthesis of a variety of analogues allows for the determination of structure-activity relationships to optimize inhibition and antivirulant activity. Further, a novel *in cellulo* assay demonstrates direct inhibition of enzymatic activity. Future studies include analysis of pharmacokinetic parameters in animal models and demonstration of *in vivo* anti-infection activity.

MEDI 406

Antimicrobial surfaces for healthcare applications

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The high incidence of hospital-acquired infection (HAI) is an extremely topical problem, constantly piquing media interest. In the U.K. it has been approximated that the treatment of these infections annually cost NHS hospitals over £1 billion. One strategy to reduce the spread of infection in healthcare environments is the use of self-sterilising surfaces. This technology can be applied to a range of touch surfaces, to help disrupt the cycle of transmission of bacteria from surfaces-to-patients or surfaces-to-healthcare workers. The use of antimicrobial surfaces in medical devices such as urinary catheters can also potentially reduce the incidence of associated infection. A novel approach that can be utilised across a wide range of surfaces is the use of a modified form of photodynamic therapy.

A “swell-encapsulation-shrink” technique was used to incorporate the light-activated antimicrobial agents, crystal violet and methylene blue, in addition to 2 nm nanogold, into medical grade silicone. The samples were tested against *Staphylococcus epidermidis* and *Escherichia coli*. These surfaces induced the lethal photosensitisation of both bacteria upon activation with either a low power red laser for short illumination time periods (13.5 minutes), or a white hospital lighting source over a lengthier illumination time period. Under both irradiation regimes, when tested against *S. epidermidis*, bacterial numbers were reduced below the detection limit. When tested against *E.* coli, a 2.6 log reduction in bacterial numbers was noted after laser irradiation (635 nm) and bacterial numbers were reduced below the detection limit under white light illumination conditions within 6 hours. These samples also demonstrated significant antimicrobial activity under dark conditions, against both bacteria tested.

Ultimately, it is hopeful that this efficacious photo-activated antimicrobial activity can be harnessed in healthcare applications, for use in both hospital surfaces and in medical devices, to help combat and lower the rates of contraction of associated infections.

MEDI 407

Novel naphthalene derivatives as potent HCV NS5A inhibitors: Design, synthesis, SAR, and optimization studies

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We report the discovery of a novel naphthalene series of NS5A HCV inhibitors. Optimization of this lead series was carried out as five segments which led to compounds with greatly improved plasma exposure following oral dosing in multiple species while exhibiting excellent antiviral activity, especially on Gt-1a/Gt-1b genotype potencies and showing excellent mutant profiles. Further structure−activity relationship studies on this series resulted in the identification of compounds **A** and **B** that demonstrated potent inhibition of HCV in a genotype Gt-1a/1b replicon with an EC_{50} of 32/6 pM (**A**) and 9/3 pM (**B**) and a favorable pharmacokinetic profile across multiple species.

MEDI 408

Design and synthesis of inhibitors of the dimetalloprotease DapE as novel broad-spectrum antibiotics

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Lysine and meso-diaminopimelic acid are essential precursors in the production of bacterial cell walls and require the activity of the enzyme N-succinyl-L,L-diaminopimelic acid desuccinylase (DapE).[1] Inhibition of DapE thus provides an opportunity to develop new antibiotics with a new mechanism of action, and since mammals obtain lysine through their diet, there is no comparable enzymatic pathway in mammals, and inhibitors of DapE are expected to show toxicity towards bacteria without mechanism-related toxicity in humans.[2,3] A high-throughput screen of a random library of ca. 40,000 compounds against DapE was performed, which afforded new structures exhibiting significant inhibition of the enzyme, including indoline sulfonamide derivatives. Analogs have been synthesized to optimize inhibition of DapE, aided by molecular docking models alongside traditional medicinal chemistry methods to improve and guide inhibitor

design. Progress in the synthesis and SAR of new inhibitors of DapE within this structural class of inhibitors will be described.

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MEDI 409

On the way to fusion inhibitors for tick-borne flaviviruses: Identification of lead compounds and mechanism studies

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Ticks are small blood-sucking arthropods transmitting a number of viral and bacterial diseases. Despite their rarity, viral tick-borne diseases such as tick-borne encephalitis and Powassan encephalitis cause severe brain pathologies leading to serious disabilities or even death. The only current method to prevent these diseases is vaccination, availability of which is limited. No generally applicable post-infection treatment methods are avialable.

We performed a structure-based virtual screening study against detergent-binding pocket in the envelope E proteins of tick-borne flaviviruses. Two compounds were identified with submicromolar activity in the plaque formation inhibition assay against tick-borne encephalitis virus and micromolar activity against Powassan virus [1]. Inhibition of virus entry and haemagglutinating activity was shown for these compounds.

Possible binding modes for the replication inhibitors were analysed by means of molecular docking and pocket identification for the pre-fusion dimeric and post-fusion trimeric forms of the envelope proteins. Molecular dynamics simulations allowed us to

identify the most favourable conformations of the molecules, which can be utilised for further shape-based search of novel, more effective fusion inhibitors.

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MEDI 410

Molecular mechanism and ligand design of a PLP/GABA-dependent bacterial transcription regulator GabR

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Antibiotic development for multi-drug resistant bacterial strains that contribute to recurring infections in Cystic Fibrosis (CF) patients is a medically-relevant area of biochemistry and medicinal chemistry research. Interdisciplinary studies on potential activators and inhibitors of GabR and PLP-dependent GABA metabolism in the nonpathogenic species *Bacillus Subtilis* will enable us to modulate the GABA shunt pathway, which is under GabR-dependent transcription regulation, in the related pathogens *Burkholderia cenocepacia* and *Burkholderia multivorans*.[1,2] These two species belong to the *Burkholderia cepacia* complex (Bcc) often associated with infections in CF patients, and synthesized ligand modulators of GabR in *B. Subtilis* should have a deleterious effect on viability in the pathogens. Taking advantage of the existing PLP in GabR, we start by elucidating the ligand recognition mechanisms of agonistic or antagonistic ligands, which are derived from designed inactivators of PLP-dependent aminotransferases in GabR. In *B. Subtilis*, *B. Cenocepacia*, and *B. Multivorans*, through site-directed mutagenesis and X-ray crystallography, we aim to provide insights into the three-dimensional structure of the Gab-R/GABA/PLP complex formed in initiating transcription of the gabTD operon. Potentially more potent analogs of 4-amino-5-fluoropentanoic acid have been designed, synthesized, and characterized for their antibiotic potential against the bacterial strains of both the nonpathogenic model and those belonging to the Bcc.

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MEDI 411

General methodology for the radiosynthesis of FMAU and analogous positron emission tomography (PET) probes

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A new general approach for the rapid access to 2'-deoxy-2'-arabino-[18F]fluoro-5-substituted uridines is presented. Radiolabeled analogs of nucleosides have wide applications as Positron Emission Tomography (PET) tracers for imaging of cell proliferation and immune system organs. However, the problem of quite long syntheses and low radio-corrected yields has hampered their clinical use. In order to overcome this hurdle, a number of technical advances such as on-chip synthesis and robotic devices have been developed, but they all suffer from a significant loss of radioactivity due to short half-life of the 18F atom, namely110 minutes. Herein, we report a distinctly different route for accessing such PET probes. The high yielding fluorination under standard fluorination conditions was carried out in only 40 min starting from the precursor bearing the nuclear base in the *a*-anomeric position. The anomerisation of the base to the desired *b*-anomer was governed by its higher thermodynamic stability and achieved with TMSOTf in only 30 min. The subsequent global deprotection was complete in 10 min using TFA mediated hydrolysis. The first step required filtration through an alumina cartridge, while the second and third steps were better done in one pot with a single final purification through a silica gel cartridge. All manipulations can be finished in 110-120 min. Thus a new cold synthesis of FMAU has been successfully conducted. This approach is superior compared to other commonly used methods in terms of time and efficiency of radiolabeling.

MEDI 412

Combating drug resistance via multi-targeting

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Drug resistance is now becoming a major public health problem with acronyms such as *MRSA* entering the lexicon. The strategy we use here to combat resistance is to

develop drug leads that have multiple targets. It is well known that many of the more successful drugs used in mono-therapy have more than one target, while drugs that are used in combination therapies have single targets and are often quite susceptible to single-point mutations leading to resistance in mono-therapy. This multi-targeting will reduce the occurrence of spontaneous drug resistance since more than one target would have to mutate. With this strategy, we have identified multi-targeting drug leads which simultaneously inhibit two key enzymes involved in isoprenoid (and hence, cell wall) biosynthesis: farnesyl diphosphate synthase (FPPS) and undecaprenyl diphosphate synthase (UPPS). We also find that the most potent anti-bacterial compounds we have developed so far (active in mice) also bind strongly to AT-rich DNA, and we have obtained X-ray structures showing minor-groove binding. This multiple-targeting/combination therapy concept is particularly suitable for targeting cell wall biosynthesis in bacteria since FPPS makes the product (FPP) used by UPPS, and methicillin and vancomycin target more downstream targets, opening up great possibilities for synergistic interactions – reducing resistance as well as, perhaps, dosings of more toxic antibiotics like vancomycin.

MEDI 413

Potent inhibition of *t*RNA−guanine transglycosylase investigated by isothermal titration calorimetry

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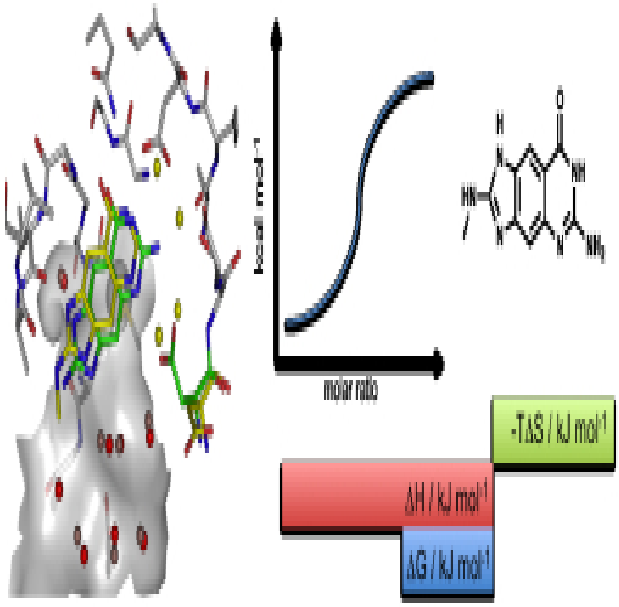
Shigellosis, caused by the *Shigella* bacterium, is an intestinal infection. The emergence of multidrug-resistant strains demands the development of novel drugs. *t*RNA−guanine transglycosylase (TGT), one enzyme involved in the virulence mechanism, has been identified as a drug target and inhibition was shown to reduce virulence dramatically. Due to the structural similarity of the active sites, the *Z. mobilis*TGT has been utilized as a model system.



A *lin*-benzoguanine scaffold was previously identified as the central core of a series of highly potent inhibitors.[1] The high affinity can be explained by a charge-assisted hydrogen bond, which could recently be investigated in more detail by isothermal titration calorimetry (ITC).[2] Comparison of a series of *lin*-benzoguanines and *lin*-benzohypoxanthines reveals significant changes in the binding mode, inhibition constant, and pK_a values. The binding modes were resolved by several X-ray crystal structures of TGT–ligand complexes.[3]

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MEDI 414

Towards chemoprevention of Aminoglycoside-induced hearing loss

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Aminoglycoside (AGA) antibiotics are potent and highly effective against a wide range of bacterial infections. However, deleterious side effects of moderate to severe ototoxicity and nephrotoxicity greatly limit their use to the treatment of life-threatening bacterial infections. AGAs activate cell death pathways leading to destruction of inner ear mechanosensory neuronal cells specialized for detecting and transducing sound vibrations. To address the need for a preventive medication for this irreversible drug-induced hearing loss, we previously screened 11,000 compound library-using zebrafish as the model system. Our most promising hit Proto-1, showed 50% hair cell protection (HC_{50}) of 3-6 μM. Structure–activity relationship studies led to the identification of candidates with improved safety profiles and potencies (*ca.*10 to 100 fold). Employing a Proto-1-based affinity pull-down probe, further studies are underway to identify and validate the cellular target of Proto-1 analogs that orchestrate auditory neuronal cell survival against aminoglycoside-induced ototoxicity.

MEDI 415

Effect of head group variation on human CD4 down-modulating and anti-HIV potencies of CADA analogs

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The small molecule cyclotriazadisulfonamide (CADA) is known to down-modulate expression of the cell-surface protein CD4 by blocking its co-translational translocation across the ER membrane. This way, CADA prevents entry of HIV into host cells. Previous studies of solid-state conformations and structure-activity relationships suggest that CADA compounds bind to the target in an unsymmetrical manner. The isobutylene head group of CADA is believed to favor an unsymmetrical conformation of the 12-membered ring. We propose that other head groups might also effectively pre-organize the proposed bioactive conformation. For this reason, a series of novel CADA analogs with either saturated or unsaturated head groups have been synthesized and tested for CD4 down-modulation and anti-HIV activity. In general, modification of the head group resulted in decreased activity of the analog as compared to the isobutylene head group analog. Compound RA003 with a 2-hydroxymethyl-1,3-propylene head group was found to have highest potency for CD4 down-modulation (IC_{50}= 380 nM).

MEDI 416

Silver–gold alloy bimetallic nanoparticles as a therapeutic strategy against anaerobic pathogens

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We synthesized silver-gold (AgAu) bimetallic nanoparticles (NPs) via the galvanic replacement reaction between maltose coated silver NPs and $HAuCl_4$ in 1-5% (w/v) triblock F127 copolymer solutions. This synthesis method is facile, economic, and environmentally benign. Silver NPs are commonly used as antimicrobials but their antimicrobial efficacy is limited typically to aerobic conditions that produce silver ions (Ag^+). Because of this, silver NPs are ineffective against anaerobes. In the case of AgAu alloy NPs, the difference in redox potentials between Ag (~0.8 V) and Au^{3+} (~1.5V) results in surface sequestration of Ag^+ ions that can be released into the solution under anaerobic conditions. We hypothesized that our synthesized AgAu NPs would exhibit antimicrobial activity against the anaerobic oral pathogen *P. gingivalis*. To test this hypothesis, we evaluated the effect of AgAu NPs on *P. gingivalis* planktonic growth rates, biofilm formation, and biofilm removal. AgAu NPs significantly inhibited *P. gingivalis* planktonic growth and biofilm formation. This inhibition was enhanced in the presence of hydrogen peroxide. Additionally, AgAu NPs significantly promoted *P. gingivalis* biofilm removal. These results suggest that AgAu NPs may be a promising therapeutic strategy against anaerobic pathogens.

MEDI 417

Antimicrobial metal oxide thin films for use in healthcare applications

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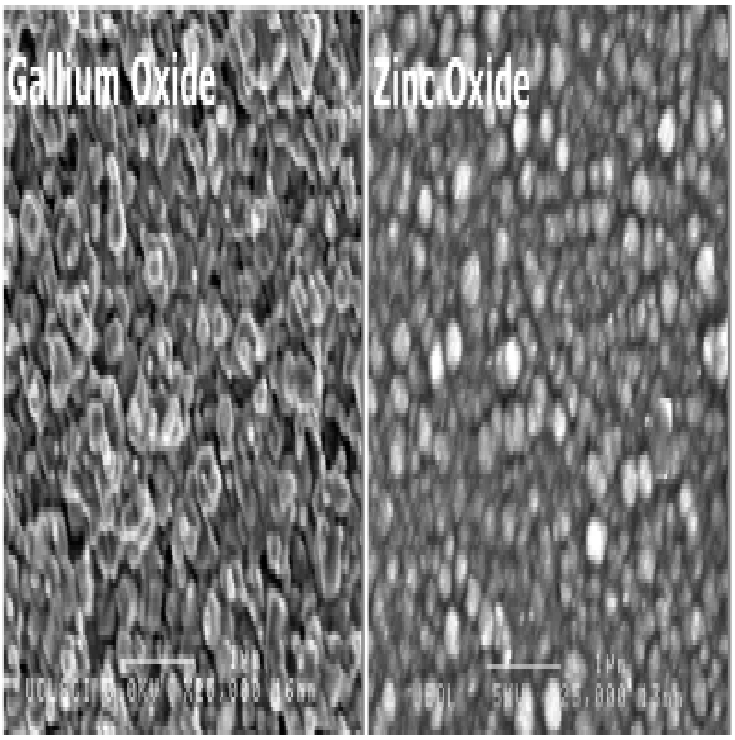
Hospital Acquired infections (HAI) have become a major problem in western hospitals. Statistics show 1 in 10 patients that are admitted to UK hospitals contract a HAI, costing the NHS approximately £1 billion annually. The spread of nosocomial infections is attributed to contact between healthcare personnel and infected patients. Consequently, the utilisation of antimicrobial surfaces is proposed to decrease HAI. This project investigates antimicrobial thin films using Aerosol-Assisted CVD (AACVD).

AACVD is a variation on conventional CVD commonly used to deposit metal oxides, since it depends on precursor solubility rather than volatility.[1] Gallium oxide is a semiconductor that can target the iron metabolism in bacteria. Since Ga^{3+} and Fe^{3+} have similarities in ionic radius, gallium has been explored as an antimicrobial agent, as it cannot go through redox reactions leaving redox-active enzymes non-functional.[2] Zinc

oxide, another effective antimicrobial active metal oxide, is thought to generate reactive oxygen species due to leaching of Zn ions.[3]

Deposition and antimicrobial activity of Ga_2O_3 and ZnO tested against *Escherichia coli* and *Staphylococcus aureus* using serial dilution and viable colony counts will be described as well as the incorporation of natural antimicrobial material such as copper into transparent films to enhance efficacy.



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MEDI 418

Characterization of *pftrxr* inhibitors using in silico methodology and antimalarial assays

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Plasmodium falciparum is the most predominant and fatal species of the human malaria parasites and its redox response to oxidative stress involves the enzyme thioredoxin reductase (TrxR). Essential for *P. falciparum* survival, the enzyme is a promising target for novel antimalarial drugs. A joint experimental and computational study has been carried out to identify *Pf*TrxR inhibitors using molecular modeling and mass spectrometry. The compounds 1,4-napthoquinone (1,4-NQ), bis-(2,4-dinitrophenyl)sulfide (2,4-DNPS), 4-nitrobenzothiadiazole (4-NBT), 3-dimethylaminopropiophenone (3-DAP), menadione (MD), curcumin and demethoxycurcumin (DMC) were tested for antimalarial activity against both chloroquine sensitive (D6) and chloroquine resistant (W2) strains of *P. falciparum* in cell cultures with a detailed analysis of the predicted binding poses revealing the non-covalent interactions of the molecules to *Pf*TrxR. *In silico* analysis showed that 2,4-DNPS, 4-NBT, MD, curcumin and DMC interact non-covalently with the inter-subunit region of the homodimeric enzyme. The differences in binding in the active sites of the human and parasite enzymes were assessed to explain the observed experimental selectivity which will be discussed in the presentation.

MEDI 419

SQ109 and analogs: Drug leads against tuberculosis and Chagas' disease have multiple targets

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SQ109, a promising TB drug lead, is in phase II clinical trials. We show here that it has multiple sites of action and in addition it has potent activity against Trypanosoma cruzi, the causative agent of Chagas disease. In addition to targeting the membrane transporter MmpL3 in TB bacteria, MenA and MenG are inhibited and SQ109 and its analogs are potent uncouplers. In trypanosomes, activities are in the 50-500 nM range found, and there is synergistic activity with posaconazole.

MEDI 420

Hologram QSAR studies of antiprotozoal activities of sesquiterpene lactones

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Infectious diseases as trypanosomiasis and leishmaniosis are considered neglected tropical diseases due the high indices of mortality and the lack of safety and effective drug therapy. Natural products as sesquiterpene lactones has been showed activity against parasites as *T. brucei* and *L. donovani* responsible for these neglected diseases. In this work, HQSAR models were constructed to relate a series of 40 sesquiterpene lactones with activity against *T. brucei*, *T. cruzi*, *L. donovani* and *P. falciparum* and also with its citotoxicity. All constructed model showed good internal (leave-one-out q^2 values ranging from 0.637 to 0.775) and external validations coefficients (r^2_{test} values ranging from 0.653 to 0.944). From HQSAR contribution maps, several differences between most and least potent compounds were found; and the fragment contribution of PLS generated model showed the presence of a-b-unsatured carbonyl groups are fundamental do biologic activity according previous QSAR works. Therefore, the constructed HQSAR models are suitable to predict activity of new natural products.

MEDI 421

HCV NS5A replication complex inhibitors: Effects of substituted pyrrolidines in balancing genotype 1a/1b potency

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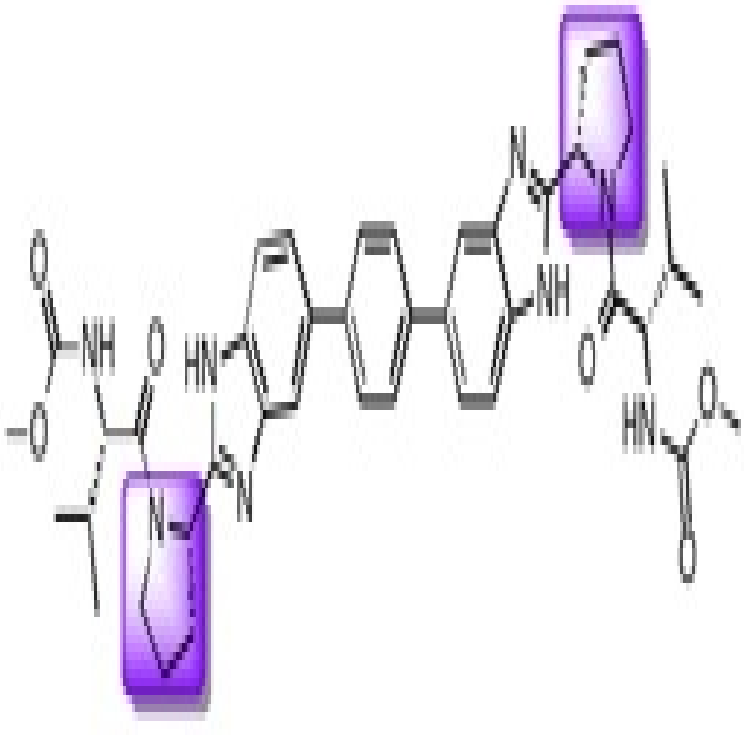
The treatment of HCV with highly efficacious, well-tolerated, interferon-free regimens has become a compelling clinical goal. Trials employing combinations of direct-acting antivirals that include NS5A inhibitors have shown significant promise in meeting this challenge. Herein, we describe the exploration of pyrrolidine substitution in a series of bis-benzimidazole NS5A inhibitors and highlight a subtle structural change that profoundly increases genotype 1a potency and can be effectively applied to other series.



MEDI 422

Ferrous ion/PfATP6 dual requirement for antimalarial activity: Making strides towards understanding the MOA of artemisinin

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Artemisinin and its derivatives have proven clinical efficacy against drug-resistant malaria. Although many decades have been spent researching this peroxide-containing sesquiterpene, its antimalarial mechanism of action (MOA) is still a topic of debate and requires further exploration. An understanding of its antimalarial MOA may drive the design of novel antimalarials with less structural complexity than artemisinin itself. Recent studies have suggested that both ferrous (Fe2+) ion and the *Plasmodium falciparum* sarco/endoplasmic reticulum calcium (Ca2+) ion transporter (SERCA) PfATP6 enzyme play roles in artemisinin's antimalarial activity. To find a likely MOA, these roles have been systematically explored by means of homology modeling, metal ion prediction and modeling using the fragment transformation method, and molecular docking studies. A PfATP6 hypothesis for artemisinin's antimalarial MOA will be presented demonstrating the ability of Fe2+ to react irreversibly with artemisinin and nearby residues within the transporter, further explaining the dual requirement of Fe2+ and PfATP6 for the antimalarial activity of artemisinin and its derivatives.

MEDI 423

3D quantitative structural activity relationship on 1,5-diaryl pyrazole containing peripherally acting cannabinoid 1 receptor antagonists as anti-obesity agents

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Peripherally acting cannabinoid 1 (CB1) receptor antagonists could be a potential target for the treatment of obesity. A dataset containing 72 compounds of 1,5-diaryl pyrazole having peripheral antagonistic activity of CB1 receptor was utilized for three dimensional quantitative structure–activity relationship studies. All the compounds used in the studies, exhibited a high variation in the biological activity and the chemical structures. Different types of alignment such as atom-based, data-based, centroid-based, centroid/atom-based have been performed to develop the best comparative molecular field analysis (CoMFA) model. The best CoMFA model was developed with database alignment which was followed with comparative similarity indices analysis (CoMSIA) on the same alignment. The best CoMFA model was obtained with cross-validated r^2 = 0.552 with six component, non-cross-validated r^2 = 0.973, standard error of estimates 0.162, F-value = 281.239 and the best CoMSIA model was obtained with cross-validated r^2 = 0.571 with six components, non-cross-validated r^2 = 0.960, standard error of estimates = 0.196 and F-value = 188.701. The predictive r^2 values of these models also showed best test set prediction of 0.528 and 0.670 for best CoMFA and CoMSIA model respectively. CoMSIA model with steric, hydrophobic and H-bond acceptor descriptors was found to be the best model on the basis of higher predictive r^2 value. The contour map of the best CoMFA and CoMSIA (SHA) would be further utilized for the designing of highly active CB1 receptor antagonists or to modify the existing compounds in our research work.

MEDI 424

Aminosteroid inhibitors of the inositol phosphatase SHIP1

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The inositol phosphatase SHIP1 is a key participant in the PI3K pathway, a major cellular signaling axis. Alteration of SHIP1 activity may provide a means to increase blood cell production and facilitate bone marrow transplantation. While gene knockouts can be used to explore the biochemical role of SHIP1, the enzyme also acts as a docking protein, which recruits other enzymes to the cell membrane. Deconvoluting the phosphatase activity from structural functions is therefore difficult utilizing only genetic tools. To gain more insight into the role of SHIP1, small molecule inhibitors of the enzymes phosphatase activity were found using high throughput screening. This method identified several aminosteroids as inhibitors of SHIP1. Both synthetic studies that have been undertaken to explore pharmacophoric relationships and improve water solubility as well as the preliminary biological evaluation of these molecules will be presented.

MEDI 425

TAAR1 agonists as anti-diabetic agents: Discovery and characterization of (S)-4-[(ethyl-phenyl-amino)-methyl]-4,5-dihydro-oxazol-2-ylamine (RO5166017)

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Trace amine-associated receptor 1 (TAAR1) was identified in 2001 as a receptor for endogenous trace amines including *p*-tyramine and β-phenylethylamine, which are metabolites of amino acids with structural similarity to biogenic amines. Initial research focused on TAAR1 function in the brain, in which TAAR1 is expressed in specific areas where it modulates monoaminergic neurotransmission. TAAR1 thus emerged as a novel target for treatment of psychiatric disorders such as schizophrenia. TAAR1 is also expressed in the periphery, especially in stomach, duodenum and pancreas, but little is known about the physiological effects of TAAR1 activation in these tissues. Trace amines have limited suitability as pharmacological tools for probing TAAR1 function, due to low selectivity against monoaminergic receptors and transporters and short *in vivo* half-lives due to degradation by monoamine oxidases (MAOs).

We sought novel TAAR1 agonists having good selectivity and favourable *in vivo* pharmacokinetic properties, which would be suitable for investigating the effects of TAAR1 activation in animal models of metabolic disease. Through screening a set of known adrenergic ligands, the alpha 2A adrenergic receptor agonist S18616 was found to have considerable activity at TAAR1 and was selected for medicinal chemistry optimization. Modifying the linker region of S18616 afforded good selectivity for TAAR1 over adrenergic receptors. A further series of iterative variations optimizing selectivity and ADME properties then led to (*S*)-4-[(ethyl-phenyl-amino)-methyl]-4,5-dihydro-oxazol-2-ylamine (RO5166017).

RO5166017 exhibits high affinity and functional activity at mouse, rat, cynomolgus and human TAAR1 *in vitro*, is selective against a panel of >100 pharmacological targets, and is not a substrate for MAOs. RO5166017 has drug-like physicochemical properties, low binding to plasma proteins, and is orally bioavailable in mice having a plasma half-life of several hours. RO5166017 normalized glucose excursion in an oral glucose tolerance test in mice, suggesting that TAAR1 agonists may be useful for treatment of type 2 diabetes.

MEDI 426

Design and synthesis of antidiabetic agents with agonistic activity on both PPARγ and FFAR1

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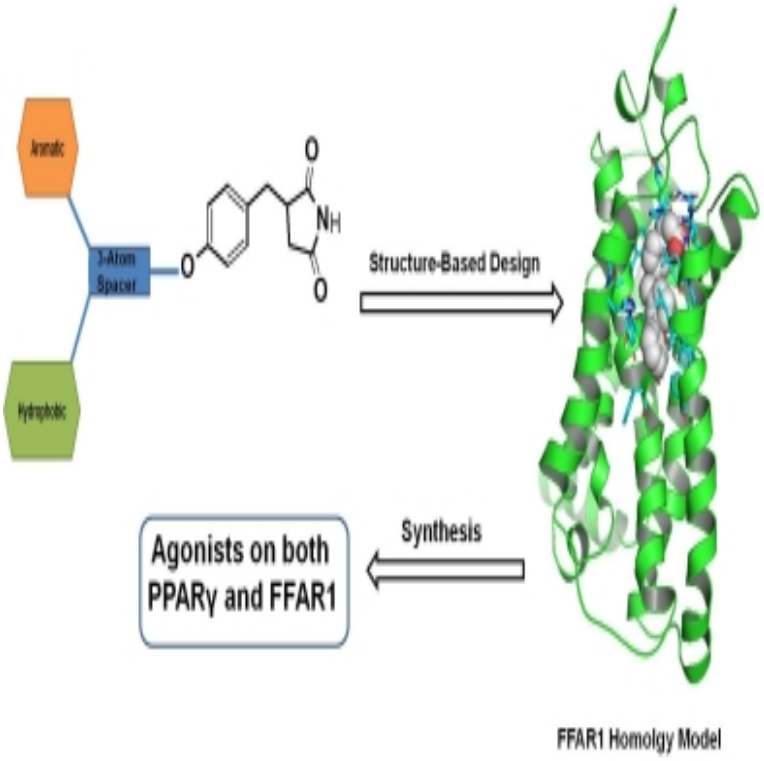
Diabetes mellitus is a chronic metabolic disorder that affects nearly 26 millions in the United States. Peroxisome proliferator activated receptors (PPARs) are a group of nuclear receptors that control cellular metabolism through the modulation of gene expression. Targeting PPARγ with thiazolidinediones (TZDs) has been proven successful for the management of type II diabetes. Another emerging target for the design of antidiabetic agents is the free fatty acid receptor 1 (FFAR1) expressed in the β-cells of the pancreas. It is believed that agonists of this receptor could be useful for enhancing insulin secretion in diabetic patients. Recently, it has been reported that some TZDs could activate FFAR1 with micromolar potencies. Moreover, the similarity between PPARγ and FFAR1 agonists is evident. In this study, drug-like molecules with agonistic activity on both receptors, FFAR1 and PPARγ, were designed, synthesized, and biologically evaluated. Our strategy depends on combining the 5-benzyl-thiazolidinedione head of TZDs with diverse hydrophobic fragments. Selection of the appropriate hydrophobic fragments relied on the "privileged structures" approach. Five diverse scaffolds were designed based on docking studies into the X-ray crystal structure of PPARγ as well as into a homology model of FFAR1 generated in our lab. Twenty compounds were prepared; three of them showed dual EC_{50} values of less than 10 μM. These molecules could represent the first antidiabetic agents acting as insulin sensitizers as well as insulin secretagogues.



MEDI 427

Homology modeling of Giardia intestinalis arginine deiminase: Insights into its structure and inhibition

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Giardia intestinalis arginine deiminase (ADI) is an important arginine metabolic enzyme involved in the energy production and defense in this protozoan and its absence in the human host makes ADI is an attractive target for drug design against *G. intestinalis*. However, the crystallographic structure of *G. intestinalis* ADI remains unresolved. Because of its relevance, in this work we generated a theoretical model for the ADI homodimer structure of *G. intestinalis* using computational technique homology modeling. The sequence alignment, secondary and three-dimensional structure generated shows amino acid conservation at the active site compared with ADI in other organisms. Also, in proposing possible active-site inhibitors, a set of 3196 commercial and 19 *in-house* benzimidazole derivatives were docked into the active site cavity. Molecular dynamics were carried out to evaluate the stability of the dimer enzyme and the ligand-enzyme complexes. The results allowed the identification of molecules with

theoretically high affinities for the catalytic site, also enabling the most relevant interactions that promote stabilization in it. These results constitute valuable information for the design and optimization of selective inhibitors.

MEDI 428

Development of a vaccine for the treatment of heroin addiction: Determination of the optimal heroin hapten-carrier density

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A treatment strategy for heroin addicts is to develop a vaccine against heroin that induces antibodies that bind heroin in the blood, preventing it from crossing the blood-brain barrier, and blocking the psychoactive effects of heroin. Heroin is too small of a molecule to induce antibodies and thus, a structural analog (hapten) must be coupled to a carrier protein to be used as a vaccine. The maximum number of haptens to attach to the carrier to induce the optimal antibody titer has not been reported. We coupled the heroin hapten (MorHap) to Tetanus toxoid (carrier) using maleimide-thiol coupling chemistry. The MorHap density of the Tetanus toxoid conjugates was measured using trinitrobenzenesulfonic acid (TNBS) assay, modified Ellman test and High Mass Matrix Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF). Analytical characterization of the conjugates showed that we were able to synthesize six Tetanus toxoid-MorHap conjugates with varying hapten densities.

Tetanus toxoid-MorHap conjugates were mixed with liposomes containing Monophosphoryl Lipid A (PHAD™) to yield the heroin vaccine formulation. Female Balb/c mice were immunized 3 times at 3 week intervals with the heroin vaccine. Three weeks after the last immunization, vaccinated mice were challenged with heroin. Our data showed that mouse group immunized with Tetanus toxoid conjugate saturated with MorHap was best protected against the pain-relieving effects of heroin. Our results suggest that Tetanus-toxoid MorHap conjugates could abrogate the effects of heroin and thus, has a potential use to treat heroin addiction.

MEDI 429

Elucidation of the pharmacokinetic difference of regioisomeric retinoid X receptor agonists having an alkoxy group by PET imaging

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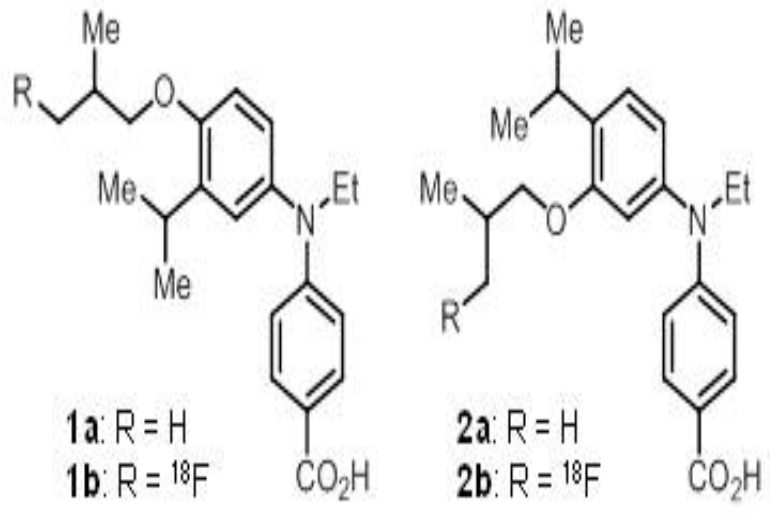
Retinoid X receptor (RXR) agonists exert therapeutic effects on type 2 diabetes and Alzheimer's diseaseand so on. However past RXR agonists were reported to produce significant adverse effects such as blood triglyceride elevation and hypothyroidism. Compound **1a** (NEt-4IB: E_{max} = 55%, EC_{50} = 169 nM), which was created as an RXR partial agonist in our laboratory, shows highly oral blood migration (30 mg/kg *p.o.*,AUC= 9.07 hr·mg/L) and showed exert therapeutic effects on type 2 diabetes without significant adverse effects. However, RXR full agonist **2a** ,which isregioisomer of **1a** (NEt-3IB: E_{max} = 114%, EC_{50} = 0.58 nM), showed lower oral blood migration (30 mg/kg *p.o.*,AUC= 0.48 hr·mg/L) than **1a** . In this study, we aimed to address the difference of blood migration between these regioisomers. To compare biodistribution, PET probes of **1b** and **2b** were synthesized by converting a hydrogen atom of isobutoxy group to ^{18}F. Administrating each compound to mice by *p.o.* or *i.v.*, **1b** showed higher blood concentration than **2b** and significant difference was observed when *p.o* administration. When each compound was administered by *i.v.*, in the gallbladder, **1b** showed lower accumulation than **2b** . These results indicate that higher oral blood migration of **1a** than **2a** is caused by higher intestinal absorption and lower gallbladder excretion of **1a** than **2a** .



MEDI 430

Structure-metabolism relationships of novel macrocyclic peptide opioid receptor ligands

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We are pursuing metabolically stable peptidic ligands for kappa opioid receptors (KOR) as potential treatments for drug abuse and pain. We synthesized the natural product macrocyclic tetrapeptide CJ-15,208 (*cyclo*[Phe-D-Pro-Phe-Trp]), which was reported to be a KOR antagonist *in vitro*,[1] and found that it displays mixed agonist/KOR antagonism *in vivo*.[2] Both CJ-15,208 and its D-Trp isomer are active after oral administration and appear to penetrate into the CNS[3,4,5]making these peptides promising agents for further development. In order to optimize their structures, we have examined the metabolic stabilities of the lead peptides in blood and in liver microsomes. Both peptides were completely stable in blood for at least 20 h. CJ-15,208 was substantially more stable in liver microsomes than its D-Trp isomer. The evaluation of metabolic stabilities in liver microsomes of a series of both L-Trp and D-Trp analogs revealed that the stereochemistry of the tryptophan residue is critical in determining the metabolic stability of these macrocyclic tetrapeptides. The results from these metabolism studies are guiding the design of additional analogs with improved pharmacokinetic properties. This research was supported by NIDA grants R01 DA023924 and R01 DA032928.

1. T. Saito *et al.*, *J. Antibiot*. **2002,** *55*, 847.
2. N. C. Ross *et al.*, *Br. J. Pharmacol.* **2012,** *165***,** 1097.
3. S. N. Senadheera *et al.*, *In Peptides: Building Bridges Lebl M. Ed. American Peptide Society: San Diego CA* **2011** , 346.
4. S. O. Eans *et al.*, *Br. J. Pharmacol.* **2013** , *169*, 426.
5. J. V. Aldrich *et al.*, *J. Nat. Prod*. **2013** , *76*, 433.

MEDI 431

Structure-activity relationship studies on *O*-[3-(heterocyclyl)phenyl] carbamate FAAH inhibitors

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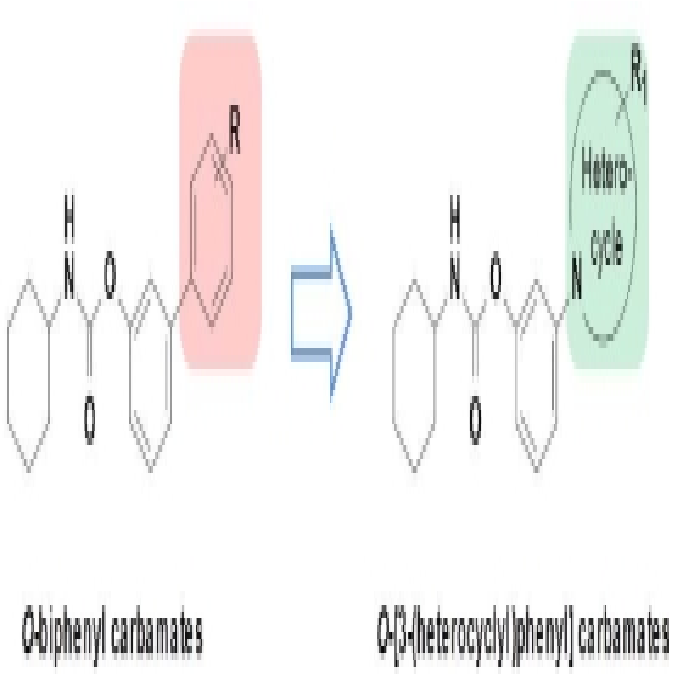
Fatty acid amide hydrolase (FAAH) is an intracellular serine hydrolase responsible for the deactivation of the endocannabinoid anandamide (AEA), which is involved in the regulation of several physiological functions and mammalian behaviors, including pain, inflammation and cognitive/emotional state.

These findings have triggered a growing interest in the discovery of several chemically distinct classes of FAAH inhibitors, such as the *O*-aryl carbamates and the piperidine/piperazine ureas.

As a part of our research program aimed at further exploring the class of *O*-biphenyl carbamates, represented by URB597 (R = 3'-$CONH_2$), we conducted a focused structure-activity relationship (SAR) study by exploring the replacement of the distal phenyl ring of URB597 with *N*-heterocyclic rings, such as pyrrolidynyl, piperidinyl, morpholinyl and piperazinyl derivatives.

Herein, the results of this SAR exploration will be presented which allowed the identification of novel and potent FAAH inhibitors with improved kinetic solubility and plasma stability.



MEDI 432

Synthetic study of acyclic terpenoid compounds for potent inhibitors of tyrosinase

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This paper represents a synthetic study of several citronellol type compounds and their derivatives with anti-tyrosinase activity.

MEDI 433

Stereospecific total synthesis of the indole alkaloid Ervincidine: Establishment of the C-6 hydroxyl stereochemistry

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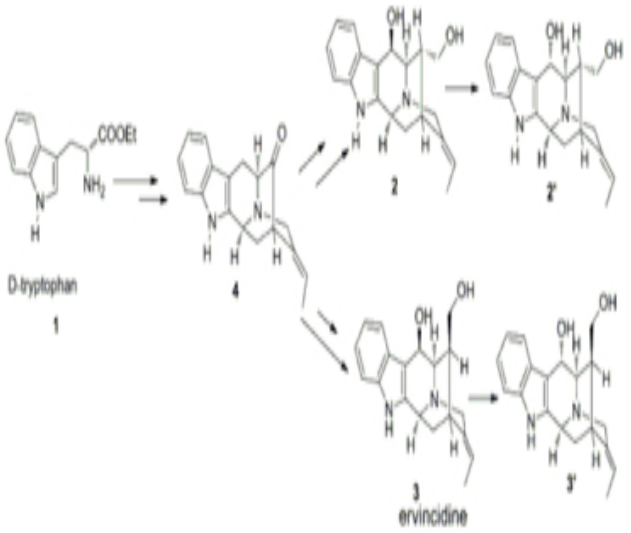

The total synthesis of the indole alkaloid ervincidine (3) is reported. This research provides a general entry into C-6 hydroxy substituted indole alkaloids with either alpha or beta configuration. This study corrects the errors in Glasby's book and Lounasmaa's review as well as clarifies the work of Yunusov. It establishes the correct absolute configuration of the C-6 hydroxyl function in ervincidine. This serves as a structural proof and corrects the mis-assigned structure reported in the literature.

MEDI 434

Development of fluorescent retinoid X receptor ligands and Fer resonance energy transfer (FRET)-based RXR ligand screening method

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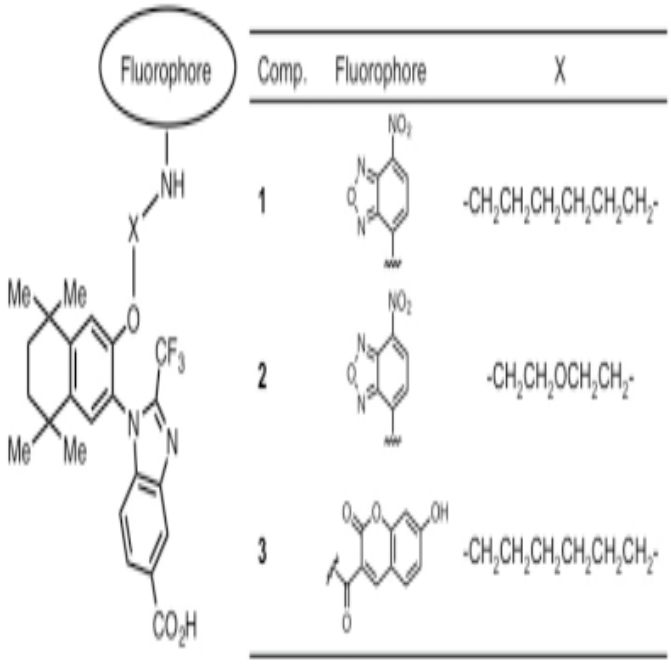
Retinoid X receptors (RXRs) are promising target for the treatment of type 2 diabetes and Alzheimer's disease. Though the binding assay using radiolabeled compounds and the transcriptional assay are performed for the screening of RXR ligands. these methods have time and cost consuming protocols. These backgrounds prompted us to create a simple and inexpensive RXR ligand screening system.

We have developed fluorescent RXR ligands, because fluorescence polarization (FP) assay is known as a simple and inexpensive ligand screening system. However, compound **1** , which possesses benzofurazan type fluorophore via an alkyl chain, was not suitable for the FP assay. Since the lipophilicity and flexibility of **1** were thought as causes of the disability, novel fluorescent RXR antagonists **2** and **3** , which possesses less-lopophilic spacer or fluorophore than **1** , were created. Unfortunately, these compounds were unsuitable for the FP assay. Based on the interaction between nuclear receptors and co-factors, we have developed RXR ligand screening method employing fluorescein labeled co-factor peptide (*J. Med. Chem.,* **2013** , *56*, 1865.). In addition, coumarinand fluorescein can produce the Förster resonance energy transfer (FRET). In the presence of RXR, **3** and fluorescein labeled co-repressor SMRT peptide produced the FRET phenomenon. Moreover, the addition of non-fluorescent RXR ligand reduced the FRET signal. This system can be carried out by using general fluorescent spectrometer, and is more simple and inexpensive than existing methods.



MEDI 435

Syntheses and in vitro evaluation of fluorine-containing inhibitors for vesicular acetylcholine transporter

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Vesicular acetylcholine transporter (VAChT) is a reliable biomarker for the cholinergic system. A potent and selective VAChT inhibitor containing fluorine atom can be radiolabeled with [^{18}F]/F^{-}, and potentially serves as a PET probe for imaging the cholinergic system *in vivo*. We report the design, syntheses of fluorine containing VAChT ligands, and *in vitro* binding affinities of these ligands toward VAChT and their selectivity for VAChT over sigma receptors.Fifteen novel benzovesamicol derivatives were synthesized and fully characterized. Structural-activity relationship (SAR) study suggested: a) 5-Substitution on the A-moiety increases VAChT potency, with 5-fluoroethoxy > 5-hydroxy > 5-amino > unsubstituted; b) PEGylation on the C-ring lead to higher selectivity to sigma receptors; c) VAChT binding is stereoselective, with the (-)-isomer having a higher potency than the (+)-counterpart. Utilizing *in vitro* screening, 6 compounds were identified possessing high potency for VAChT (< 5 nM) and high specificity over the sigma receptors (> 500-fold). These 6 compounds can be radiolabeled with F-18 to be evaluated as PET probes for imaging VAChT *in vivo*.

MEDI 436

Development of selective Grp94 inhibitors derived from radicicol

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The development of isoform-selective Hsp-90 inhibitors provides an opportunity to reduce the side effects that results from *pan*-inhibition of all four human isoforms: Hsp90α, Hsp90β, Grp94, TRAP-1. To date, several client Grp94 client proteins have been identified which involve the Toll-like receptors, integrins, IGF-I, IGF-II, and immunoglobulins. In addition, inhibition of Grp94 has been implicated for the treatment of various diseases, including arthritis, glaucoma, and multiple myeloma. Recently, Grp94 co-crystal structures were solved and shown to contain a secondary binding site that provides an opportunity to develop isoform-selective inhibitors. These crystal structures were used as a platform to develop Grp94 selective inhibitors. Results suggest that rational drug design can lead to the development of isoform-selective compounds for inhibition of closely related Hsp90 isoforms. In this work, Grp94 inhibitors were designed using the resorcinol ring of natural product radicicol as a starting point. Synthesis and biological evaluation of these Grp94-selctive inhibitors will be presented herein.

MEDI 437

Synthesis and evaluation of protein tyrosine phosphatase inhibitors by targeting a novel allosteric site

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Type II diabetes is a chronic disease in which glucose is unable to enter cells due to insulin resistance. Protein tyrosine phosphatase 1B (PTP1B) is a negative regulator of insulin-receptor activity. Therefore, deactivation of PTP1B is a promising therapeutic strategy for alleviating the symptoms of type II diabetes. Selective inhibitors targeting the active site of PTP1B are extremely difficult to produce due to the conserved catalytic site among the PTP family. Through crystallography, a novel allosteric site was identified. Using computer aided drug design (CADD) in conjunction with organic synthesis; a family of compounds targeting the novel allosteric site have been identified and synthesized.

MEDI 438

Novel sulfone compounds as potent and selective 5-HT_6 receptor antagonists

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Alzheimer's disease, the most common cause of dementia among older people,is characterized by behavioral disorders and a progressive decline in memory. 5-HT_6 receptor has received increasing attention of late, because of the setbacks observed in the development of a disease-modifying therapy and draw backs associated with existing therapies. 5-HT_6 antagonists improve memory performance by modulating cholinergic as well as the glutamatergic systems. 5-HT_6 receptors are localized wholly in the brain, thus decreasing chances of peripheral side effects.

A novel series of indolyl and phenyl sulfones were identified using classical medicinal chemistry and scaffold hopping approach. The compounds are highly potent and selective over closely related receptors. The lead compound is orally active in animal model of cognition. Details on the synthesis, SAR, *in-vitro,* Pharmacokinetic and *in vivo* data will be discussed in the poster.

MEDI 439

Synthesis, structure activity relationships of imidazopyridine derivatives as 5-HT_4 receptor partial agonists

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Alzheimer's disease (AD) is a neurodegenerative disease which has a higher prevalence and incidence in people older than 60 years. The need for improved AD therapies is unmet. 5-HT_4 receptor may play a role in memory and learning. 5-HT_4 receptor partial agonists may be of benefit for both the symptomatic and disease-modifying treatment of cognitive disorders associated with AD. Design, synthesis and SAR of imidazopyridine derivatives as 5-HT_4 receptor partial agonists was achieved by the combination of fragment based drug design and classical analogous discovery approach. The in-vitro potent compounds were further evaluated for their ADME properties and in-vivo efficacy. The detailed SAR, structure optimization to achieve the acceptable ADME and in-vivo efficacy in animal models of cognition will be disclosed.

MEDI 440

Conformationally constrained proline derivatives as a4b2 nicotinic acetylcholine receptor ligands and their efficacy in animal models of depression

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A series of 4, 5-fused tricyclic proline derivatives were synthesized and evaluated for their affinity at a4b2 nicotine acetylcholine receptor. The in vitro potent compounds were evaluated in in-vivo ADME assays for their metabolic stability and bio availability. The compound which had acceptable ADME properties was further evaluated in rat in-vivo efficacy models of depression. The introduction of rigidity at the 4-5 position of the proline ring exerted a profound influence on both receptor binding and antidepressant effects. In our SAR, it is observed that the position of the substituent on heteroaromatic group played a key role in translating in-vitro affinity into in-vivo efficacy. These results indicated that the structural requirements for receptor binding and functional activity are distinctively different. The design, synthesis, SAR and pharmacological profile of these novel compounds in animal models of depression will be presented.

MEDI 441

Structural manipulations of aporphines en route to new chemical probes for CNS receptors

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Aporphines are naturally occurring alkaloids and possess diverse pharmacological effects including various central nervous system (CNS) receptors such as dopamine, serotonin and adrenergic receptors. Thus the aporphine scaffold can be considered to be a “privileged scaffold” for the design of new, potent and selective CNS receptor ligands, which can serve as valuable chemical probes to study receptor function in a variety of neuropsychiatric disorders. However, unexplored chemical space around the aporphine template needs to be systematically evaluated and exploited in order to make this ultimate goal a reality.

Previous structure-activity relationship (SAR) studies with the aporphine alkaloid Nantenine, have resulted in the identification of a number of selective 5-HT_{2A} receptor ligands via structural modifications at the C1, C2 and C3 positions. Here we present work on continued SAR studies focusing on the C4 and N6 positions.

The design of the C4 analogs was predicated on the hypothesis that the addition of a phenyl moiety to the C4 position of the aporphine template would engender enhanced affinity for 5-HT_{2A} receptors (given the pharmacophoric similarity to known 5-HT_{2A} ligands). In case of the N6 analogs, the importance of the nitrogen atom for 5-HT_{2A} affinity was evaluated via isosteric replacement with an oxygen atom to yield a novel isochroman-containing scaffold.

The compounds were evaluated for affinity at 5-HT_2 receptor subtypes. Interestingly, the C4 analogs displayed a shift in selectivity towards the 5-HT_{2B} receptor. The isochroman derivatives were devoid of affinity at 5-HT_2 receptors, indicating that the nitrogen atom is required for affinity.

Our results provide further insights into the structural features of aporphines for tolerance at 5-HT_2 receptors. Furthermore, this work underscores the untapped potential of utilizing structurally diversified aporphines as novel, potent and selective 5-HT_2 receptor ligands. Details of our synthetic work and further extrapolations of SAR study will be presented.

MEDI 442

Discovery and optimization of selective mGluR3 PAMs towards novel neuroprotective agents for the treatment of Parkinson's disease

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It has recently been shown that the specific activation of mGluR3 induced the production of neurotrophic factors such as GDNF and TGFβ. Consequently, both in vitro and in vivo studies demonstrated that LY379268, a reference mGluR2/mGluR3 agonist, showed neuroprotection in Parkinson's disease preclinical models.

GPCR allosteric modulators constitute a novel class of small molecule able to modulate the endogenous ligand activity on its receptor. They bring two main advantages in terms of drug discovery as compared with orthosteric ligands: a. an access to a more tractable chemistry for peptide, lipid and class C GPCRs. b. an easier access to subtype selective agents.

At Domain Therapeutics, a novel family of submicromolar mGluR3 PAMs was discovered. These small molecules are heterocyclic derivatives showing no activity for the close mGluR2 subtype. Chemical optimization was performed using not only SAR on the receptor (compound dose-response or glutamate shift experiments) but also microsomal stability determination (HLM and RLM) and rapid in vivo B/P evaluation (p.o, rat) of a small set of compounds in order to identify potential candidates for in vivo evaluation in Parkinson's disease models.

Moreover, some of these mGluR3 PAMs have been characterized in vitro in neuroprotection models using cultures from wild-type and mGluR3-KO mice and in neurotrophic factor production. Our results show that mGluR3 PAMs have similar in vitro activities as LY379268.

MEDI 443

Opioid scaffolds obtained by the Ugi multi-component reaction

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Effective pain relief is a quintessential part of clinical care. Opioid analgesics are the most widely used drugs for the treatment of chronic and severe pain. Their pharmacological effects, including dangerous adverse effects of most clinically used opioids, are exerted through the mu opioid receptor (MOR). The primary goal of opioid research is to find a potent compound devoid of the hazardous side effects (respiratory depression, constipation, dependence and addiction) associated with traditional opioids.

Our approach utilizes the four-component Ugi reaction between *N*-phenethylpiperidone (a ketone), aniline (amine), propionic acid (carboxylic acid) and isocyanides to synthesize a small series of carfentanyl amides. The utilization of multicomponent reactions (MCR) to synthesize opioid scaffolds in one pot in a library friendly fashion can lead to the synthesis of a large pool of diversified compounds in minimal time. Our lead showed high affinity for MOR and DOR with poor affinity for KOR in radioligand binding assays. In GTPgammaS functional assays, our lead compound was found to be a mixed MOR / DOR agonist. It had moderate analgesic potency *in vivo* but showed a side-effect profile far superior than the clinically used mu analgesics.

Opioids with mixed MOR / DOR activity have been reported in the literature but most are peptides and thereby systemically inactive. This MCR approach thus has the promise to deliver more potent and efficacious small-molecule opioids after further optimization of the series.

This work was supported by research grant from the National Institute on Drug Abuse (DA034106) to SM and (DA06241) to GWP.

MEDI 444

Optimization of a D_4 antagonist as an in vivo tool to study L-DOPA-induced dyskinesia and cocaine addiction

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Dopamine receptors are involved in many important central nervous system processes and are indicated in diseases such as schizophrenia, attention deficit hyperactivity disorder, Parkinson's disease, and drug addiction. Since the discovery of the five subtypes of dopamine receptors, great effort has been taken to synthesize selective ligands in order to study each receptor's involvement in disease. The Lindsley laboratory has developed an enantioselective synthesis of a morpholine-based dopamine receptor 4 (D_4) antagonist. This compound binds D_4 with a K_i of 70 nM, has

an IC_{50} of 180 nM, and is highly selective over the remaining dopamine receptors and other GPCRs. Through iterative, parallel synthesis, a structure-activity relationship study was conducted around this lead to improve binding affinity and other pharmacological properties. We hope to develop a highly selective D_4 antagonist for use as a PET tracer and an *in vivo* tool to study two diseases that have shown evidence of treatment by D_4 antagonism, L-DOPA-induced dyskinesia and cocaine addiction.

MEDI 445

[^{18}F]Fluorinated amino acids prepared using spirocyclic iodonium ylides

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The application of [^{18}F]fluoride for radiofluorination of non-activated aromatic rings, such as alkyl-substituted benzene rings, phenols, and indoles, poses a significant challenge, and has slowed the development of fluorine-18 ($t_{1/2}$ = 109.7 min) labeled amino acids for positron-emission tomography (PET). Imaging of amino acid transport and metabolism (e.g., with [^{18}F]6-fluoro-L-DOPA, which remains a challenging synthetic target) has shown clinical utility for tumor characterization and for neurodegeneration associated with Parkinson's disease. We have recently discovered a method for one-step radiofluorination of non-activated arenes using [^{18}F]fluoride and spirocyclic iodonium ylide-based precursors. This approach, which takes advantage of a highly optimized iodonium ylide auxiliary, was first applied to the synthesis of model compounds, such as ^{18}F-phenyl ethers and ^{18}F-indolines. We have since developed our approach for fluorinated derivatives of the amino acids phenylalanine, tyrosine, and tryptophan through a two-step radiosynthesis that involves radiofluorination with [^{18}F]Et_4NF in DMF at 120 °C for 10 min, followed by a deprotection step. Precursors for radiolabeling were prepared from protected iodoaryl amino acids, by a one-pot procedure that involved selective oxidation to form a diacetoxyiodoarene followed by conjugation with a spirocyclic auxiliary. The radiolabeling proceeded in synthetically useful yields (55% for a phenylalanine derivative relative to [^{18}F]fluoride), with exquisite regioselectivity, and the products are easily purified using solid-phase extraction and/or semi-preparative HPLC. [^{18}F]Fluorinated amino acids prepared in our laboratories will be applied for in vivo PET imaging studies.

MEDI 446

Discovery of MK-4409, a novel oxazole FAAH inhibitor for the treatment of inflammatory and neuropathic pain

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Elliot Martel[2], Joseph Leone[2], Shane Krska[2], Lisa Frey[2], Bindhu Karanam[3], Maria Madeira[3], Raul Alvaro[3], Joyce Shuman[3], Gino Salituro[3], Catherine Abbadie[5], Erin McGowan[5], Shruti Mistry[5], Nina Jochnowitz[5], Richard Hajdu[5], Mark Rosenbach[5], Jenna Terebetski[4], Jessica Alexander[6], Lin-Lin Shiao[6], Kathleen Sullivan[6], Ravi P Nargund[1], Matthew J Wyvratt[1], Linus S Lin[1], Robert J Devita[1]. (1) Department of Medicinal Chemistry, Merck and Co. Inc., Kenilworth, NJ 07033, United States (2) Department of Process Chemistry, Merck and Co. Inc., Rahway, NJ 07065, United States (3) Department of Drug Metabolism and Pharmacokinetics, Merck and Co. Inc, Kenilworth, NJ 07033, United States (4) Department of Preclinical Development, Merck and Co. Inc, Kenilworth, NJ 07033, United States (5) Department of Pharmacology, Merck and Co. Inc, Kenilworth, NJ 07033, United States (6) Department of Immunology, Merck and Co. Inc, Kenilworth, NJ 07033, United States

We report herein the identification of MK-4409, a potent and selective fatty acid amide hydrolase (FAAH) inhibitor. Starting from a High Throughput Screening (HTS) hit, medicinal chemistry efforts were geared towards the optimization of FAAH inhibition in vitro potency, improvement of the pharmacokinetic (PK) profile, and achievement of efficacy in rodent inflammatory and neuropathic pain *in vivo* assays.

MEDI 447

Design and optimization of heterocyclic 4-azaxanthenes as potent and selective inhibitors of BACE1 for the treatment of Alzheimer's disease

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Alzheimer's disease (AD), a common form of dementia characterized by memory loss, cognitive impairment, and ultimately death, is a serious unmet medical need costing hundreds of billions of dollars annually. The pathogenesis of Alzheimer's is believed to involve the accumulation and aggregation of Ab peptide fragments to form insoluble plaques in the brain. The enzyme BACE1 is responsible for initiating the formation of Ab fragments; consequently, inhibition of BACE1 in the brain has emerged as an attractive target for treatment of AD within the pharmaceutical industry.

During our efforts to develop a series of aminooxazoline xanthenes as potent and selective BACE1 inhibitors, we utilized crystallographic data to develop a series of 4-azaxanthenes with increased BACE1 potency and reduced off-target hERG activity. In

order to further optimize BACE1 potency while maintaining the high permeability and low Pgp-mediated efflux required for adequate CNS exposure, extensive examination of the P2' and P3 substituents was conducted. This work identified several modifications that led to increased BACE1 potency, reduced hERG activity, and improved permeability and Pgp-efflux. Additionally, several novel heterocyclic warheads were designed as alternatives to the aminooxazoline moiety to modify the physiochemical profile of our inhibitors. These efforts resulted in potent, selective BACE1 inhibitors with low hERG activity, good CNS penetration, and robust reduction of brain Aβ levels in a preclinical rodent model.

MEDI 448

Discovery of novel 3-thiomethyl bicyclo[3,1,0]hexane analogs as highly potent and selective antagonists of metabotropic 2/3 receptors for the treatment of depression

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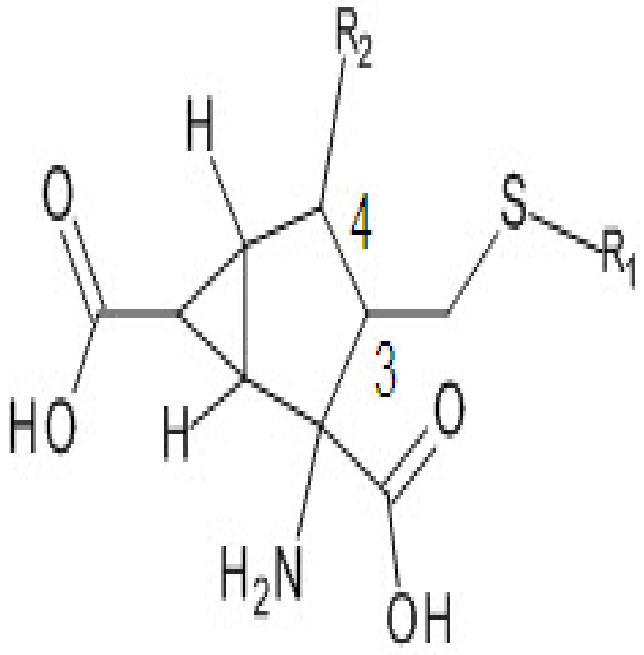
Metabotropic glutamate receptor subtypes 2 and 3 (mGlu2/3R) are members of a family of G-protein coupled receptors activated by the excitatory amino acid L-glutamate. These two receptors are grouped together based on their similar sequence homology, second messenger coupling, and pharmacological characteristics. Antagonists of mGlu2/3 receptors exhibit significant anti-depressant and wake promoting properties in animal models. For these reasons, they may have therapeutic potential for the treatment of depressive disorders. This poster will detail the chemistry developments, synthetic challenges and SAR development on a bicyclo[3,1,0]hexane scaffold leading to the discovery of highly potent and selective mGlu2/3R antagonists possessing a thioethermethyl functionality at the 3-position with an additional substituent at the 4-position of the bicyclic ring system.



MEDI 449

Dimeric two-ring scaffolds for selective targeting of human melatonin receptors MT_1 and MT_2

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Melatonin is the principle hormone of the pineal gland that regulates mammalian circadian system. Both melatonin receptors MT_1 and MT_2 receptors bind melatonin with high affinity. Luzindole is a competitive antagonist of melatonin that binds both MT_1 and MT_2 receptors. A key structural feature responsible for melatonin and luzindole binding is an indole ring and in the case of luzindole a cyclohexyl ring. Flexible linkage between these two moieties allows binding to dual hydrophobic patches on either receptors. A suitable starting point for developing receptor specific compounds is therefore to emulate the positioning of ring structures akin to these compounds while fine tuning interactions through ring size and linker rigidity. Alignment of MT_1 (human, hamster, mouse, sheep) or MT_2 (human, partial mouse) sequences with bovine rhodopsin revealed the residues conserved within the subfamily of melatonin receptors, which may be implicated in ligand binding and receptor activation. In an initial docking study, in which the native ligand melatonin was allowed to sample the entire receptor interior using Flexidock (Tripos inc.,), the generated conformations were checked/filtered with reported mutational data to select a reference docked pose. The comparison with

rhodopsin suggests binding site plasticity capable of accommodating a wider spectrum of ligands. We explored the possibility of generating more accurate and predictive models of melatonin receptors based on a reported x-ray crystal structure using an MD-based approach developed in our laboratory. The current findings suggest a dimeric, synthetically-amenable scaffold that can serve as a platform for selective targeting of MT_1.

MEDI 450

Discovery of novel bicyclo[3.1.0]hexane analogs as antagonists of metabotropic glutamate 2/3 receptors for the treatment of depression

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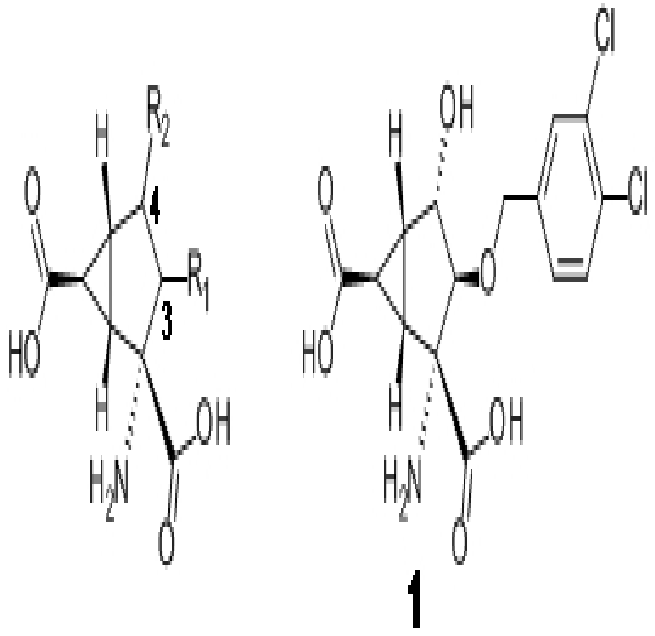
Metabotropic glutamate 2/3 receptors (mGlu2/3R) are members of the class C G-protein coupled receptor family. These receptors are highly expressed in forebrain regions (e.g. cortex, hippocampus, striatum & amygdala) where they act to regulate neuronal excitability. Negative modulators of mGlu 2/3 receptors demonstrate antidepressant-like activity in animal models and hold promise as novel therapeutic agents for the treatment of major depressive disorder. This poster will describe efforts to prepare and optimize a series of conformationally constrained 3,4-disubstituted bicyclo[3.1.0]hexane glutamic acid analogs as orthosteric (glutamate site) mGlu2/3R antagonists. This work led to the discovery of a highly potent and efficacious tool compound **1** (mGluR2 IC_{50} 43.3 nM (± 14.2, n = 6), mGluR3 IC_{50}= 27.2 nM (± 9.33, n = 5)) that exhibited wake promoting and antidepressant-like properties in rodents.



MEDI 451

Epoxysuccinyl prodrugs: Discovery of an overlooked prodrug breakdown pathway and its implications in drug development

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Epoxysuccinate containing peptidomimetic inhibitors of several cysteine-dependent proteins, including calpain and cathepsins, are candidate therapeutic agents, in particular for anti-neurodegenerative therapy. Epoxysuccinates have been administered as ethyl ester prodrugs to circumvent their poor pharmacokinetic properties; for example, E64d, a therapeutic candidate for Alzheimer's disease (AD) that has been recently entered clinical trials. The stability and reactivity of epoxysuccinate salts and their putative ethyl ester prodrugs was measured using LC-MS/MS analysis. The parent carboxylates were stable in the presence of excess thiols: reactivity with GSH was negligible after 24 h at physiological pH and temperature. In contrast, the ester prodrugs were reactive towards thiols. Importantly, formation of the desired parent drug by hydrolysis of the ester prodrug was observed to be a secondary reaction pathway. Activity directed probes bearing epoxysuccinate carboxylate and ester moieties were designed and studied in incubation with recombinant protein, and cells and their lysates. Covalently modified proteins were visualized in gels using clicking techniques and/or by LC-MS/MS. These methods confirmed that epoxysuccinate ester prodrugs react with cysteine residues of multiple off-target proteins. Assigning the *in vivo* efficacy of

epoxysuccinate ester prodrugs to on-target cysteine protease inhibition is therefore problematic. This previously unappreciated reactivity of epoxysuccinate esters creates challenges in drug design. In order to improve on the pharmacokinetic properties of epoxysuccinates while avoiding promiscuous reactivity, cysteine protease inhibitors with novel warheads were explored. A thiol-reactive functional group was developed that may effectively substitute for epoxysuccinate.

MEDI 452

Synthesis of a candidate ^{11}C-labeled brain histamine subtype 3 receptor radioligand for evaluation in monkey

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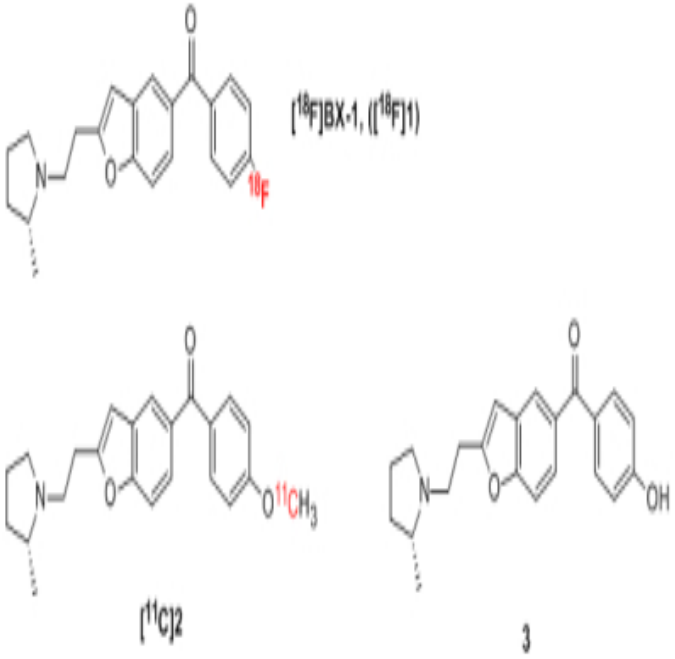
Histamine subtype 3 (H_3) receptors are implicated in several neuropsychiatric disorders including sleep disorders, schizophrenia, pain and obesity. Effective radioligands for measuring H_3 receptor density in human brain with positron emission tomography (PET) are keenly sought. We previously reported the discovery of the new ^{18}F-labeled ligand [^{18}F]BX-1 (**1** , *K*i = 0.09 nM, clogD = 2.90) that showed specific binding to H_3 receptors in monkey brain and we were interested in developing a ^{11}C-labeled PET radioligand from the same structural class. **2** was selected based on similar properties to those of **1** , namely high affinity (*K*i = 0.52 nM), and moderate lipophilicity (clogD = 2.91), plus amenity to ^{11}C-labeling with $^{11}CH_3OTf$. Labeling precursor **3** was prepared in 5 steps starting from commercially available 4-dianisyl ketone. [^{11}C]**2** was obtained by reaction of **3** with $^{11}CH_3OTf$ in the presence of NaH in MeCN in high radiochemical yield (7.5 % based on $^{11}CO_2$), high radiochemical purity (>98%) and specific activity (5.9 Ci/mmol). Radioligand evaluation is in progress.



MEDI 453

Structural modification on the 2,4-diarylaminopyrimidine scaffold and discovery of potent inhibitors targeting both wild and mutant ALK kinases

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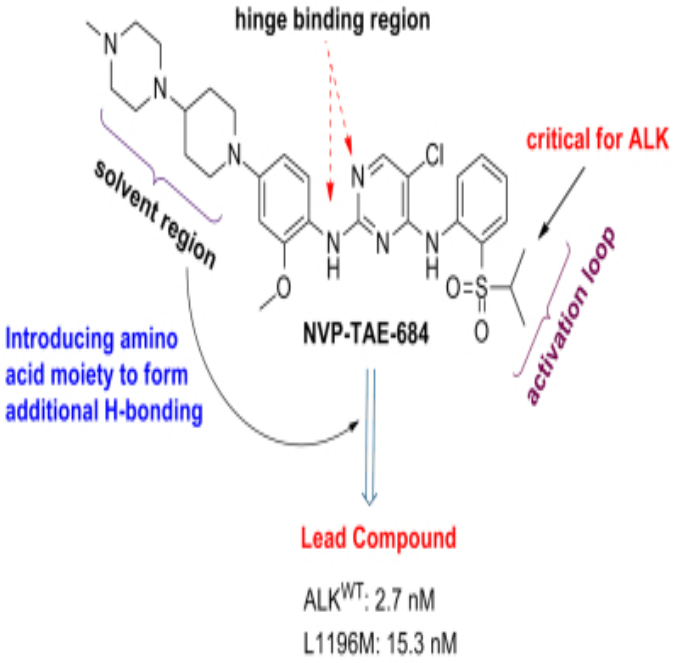
Anaplastic lymphoma kinase (ALK) is an orphan receptor tyrosine kinase (RTK), originally identified in 1994 in anaplastic large-cell lymphoma (ALCL) as a translocated form by fusing its intracellular domain to the N-terminal portion of nucleophorsmin (*NPM*), however, its normal function has not been fully understood. In 2007, the ALK fusion gene - *EML4-ALK* was identified specifically existing in lung cancer and recognized a distinct molecular subset of NSCLC populations. Since then, ALK has rapidly emerged as a novel and promising 'personalized' anticancer drug target. Crizotinib, one of the small molecule ALK inhibitors has been successfully launched in 2011; however, similar to other RTK inhibitors, resistance to crizotinib has been reported within a year of starting therapy. Studies disclosed that the resistance to crizotinib was much complex with more than twenty secondary mutations, not only including the gate-keeper L1196M, and the C1156Y near the α-C-helix. In the course of our drug discovery program toward the discovery of novel RTK inhibitors, we took advantage of the 2,4-diarylaminopyrimidine (DAAP) scaffold that has long been recognized as a classical kinase inhibitor motif by introducing an amino acid side chain to the solvent interaction region to bring in additional H-bondings. Therefore, a series of

new DAAP analogues (DAAPalogues) were developed (Figure 1) and one compound was identified with high potency against both wild type ALK and L1196M mutations with IC_{50}values of 14.5 nM and 15.3 nM, respectively. Further investigations both in vitro and in vivo were also conducted. The high potency of this novel compound allows it for further evaluation.



MEDI 454

Structure-activity relationships of novel curcumin-based anticancer agents

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To engineer curcumin analogues with an improved pharmacokinetic profile and a greater potential to treat aggressive cancers, five scaffolds of heteroaromatic curcumin analogs (over sixty analogues in total) have been designed and synthesized for the evaluation of their cytotoxicity against two hormone-independent prostate cancer cell lines and an aggressive cervical cancer cell line. The five scaffolds were designated according to the following linkers between two identical heteroaromatic rings: *N*-methylpiperidone, cyclohexanone, dienone, trienone, and 4,4-dimethyl-1,6-dien-3,5-dione. More than 90% of these analogues are more cytotoxic than parental curcumin towards these three human cancer cell lines in trypan blue dye exclusion assay. The effects of five different linkers and the effects of different heteroaromatic rings on the

cytotoxicity were explored. The design, syntheses, cytotoxic data, and structure-activity relationships will be presented.

MEDI 455

Identification of novel benzamide-pyridone-containing EZH2 inhibitors

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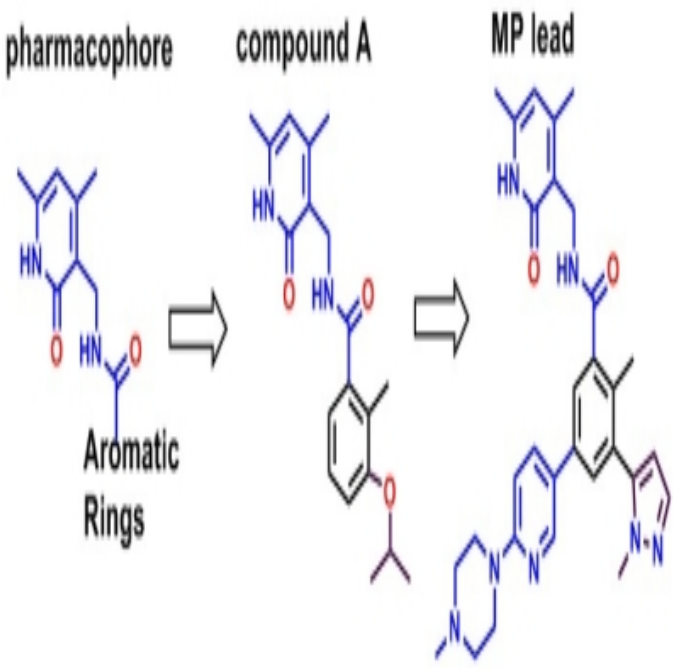
The EZH2 histone methyltransferase is a member of the Polycomb Repressor Complex 2 and initiates long-term gene silencing by methylating lysine 27 on histone H3 (H3K27). The high H3K27me3 levels in diffuse large B-cell lymphoma (DLBCL) cell lines harboring Y641 and A677 mutations suggest a high enzymatic activity for the PRC2 complex. This phenotype also suggests that inhibition of EZH2 methyltransferase activity using small molecules may represent a novel therapeutic strategy for treating cancer patients with DLBCL.

Our internal research program seeks small molecule inhibitors that can inhibit the EZH2 SET domain catalytic function and therefore reduce the formation of trimethylated H3K27. We developed a robust biochemical assay to measure blocking the transfer of the tritium-containing methyl group from the SAM EZH2 co-factor to the histone 3 lysine 27 residue of the nucleosome isolated from the Hela cells. The common EZH2 inhibitor pharmacophore reported in the literature contains a dimethylpyridone moiety coupled to a variety of aromatic residues. We therefore started our lead finding effort by incorporating this important pharmacophore into a molecule containing a simple phenyl carboxyamide (compound A). In this presentation, we report the SAR we developed in this benzamide series and the optimization process that was used to improve the potency and lipophilic efficiency (LipE) of the initial lead. This medchem optimization process rendered a promising compound (MP lead) which displayed improved potency and LipE compared with the initial lead (compound A). MP lead is a selective and SAM competitive EZH2 inhibitor. MP lead demonstrated a dose-dependent tumor growth inhibition in female scid-beige mice subcutaneously implanted with Karpas422 cells.

Further optimization of MP lead is warranted to further improve its potency and PK profile.



MEDI 456

Design, synthesis, and development of a novel PAMAM-based bow-tie dendrimer platform for tumor-targeting drug delivery

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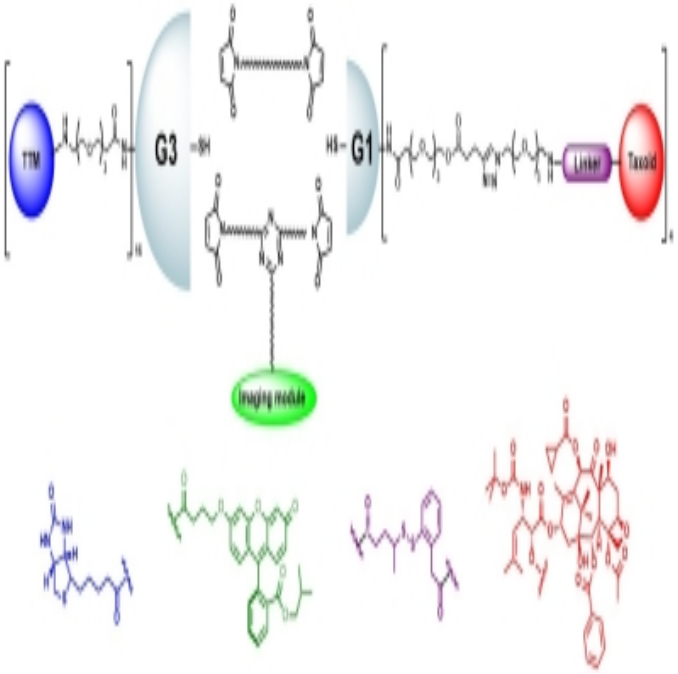
Poly-(amido amine) (PAMAM) dendrimers have been studied as macromolecular carriers to deliver drugs, resulting in selective accumulation in tumor tissues due to EPR effect. The use of PAMAM derivatives with a cleavable cystamine core enables the assembly of different generations of half dendrons modified with different functionalities into single molecule. Thus, a novel PAMAM-based asymmetric bow-tie dendrimer platform bearing a PEGylated bis-maleimido spacer was designed. Furthermore, this platform with a triazine module as the branching unit was designed to incorporate an imaging component to the drug delivery system (DDS). This platform has been applied to the construction of several tumor-targeting DDSs bearing a vitamin as the tumor-targeting module and a new-generation taxoid as the warhead, connected by a self-immolative disulfide linker, wherein a fluorescein derivative was incorporated as the imaging module. The synthesis and biological evaluation of novel dendrimer-based drug conjugates will be presented.



MEDI 457

Novel ROS-activated quinone prodrugs: A new strategy for tumor-specific damage

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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 these new agents upon oxidation, undergo addition reaction via Michael chemistry, thus reacting with anti-oxidants in the cell to cause its depletion leading to cell death. Synthesis of a new series of these agents with modifications at various locations in the parent molecule (SAR analysis) has revealed the importance of an amine that imparts selectivity towards specific cancer cell line. One such hit novel agent has shown >10 fold selectivity when compared to normal blood cells. Here I will be discussing the synthesis and strategies of ROS-activated molecules and how ROS-activation can be used for development of new pro-drugs for efficient and selective treatment of cancers.

MEDI 458

Synthesis of the differentially functionalized cyclopenta[*b*]benzofuran core of silvestrol through a CAN mediated oxidative cyclization

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Silvestrol is a novel rocaglamide derivative (flavagline) that has demonstrated potent cytotoxic activity (low nM IC_{50} values) *in vitro* against numerous human cancer cell lines. Moreover, silvestrol has displayed this potent cytotoxicity against both acute and chronic lymphoblastic leukemia while exhibiting selectivity for malignant B-cells. Structurally, silvestrol contains the densely functionalized cyclopenta[*b*]benzofuran core, which is characteristic of all flavaglines, but also possesses an unprecedented C6 dioxanyloxy side chain that makes silvestrol unique. In order to access the C6, C8 differentially functionalized cyclopenta[*b*]benzofuran core required to synthesize silvestrol, we have utilized a CAN mediated oxidative addition of 1,3-cyclohexadine derivatives into an allylic ether to construct the core ring system. This methodology has permitted the development of a rapid, versatile, and modular synthesis of this system, and provides access to the flavagline class of natural products as a whole. This ongoing effort has facilitated the synthesis of two classes of structurally distinct flavagline analogues designed to explore structure activity relationships, delineate the mechanism of action, and ultimately improve the pharmacological profile of these compounds.

MEDI 459

Cucurbitacins as novel inhibitors of Hsp90 chaperone function

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Heat shock protein 90 (Hsp90) is a molecular chaperone that facilitates the conformational maturation of Hsp90-dependent proteins (clients) via the Hsp90 chaperone cycle. Hsp90 functions as a homodimer and forms a large, multiprotein complex that relies upon co-chaperones, immunophilins, and partner proteins to fold nascent polypeptides, as well as the rematuration of denatured proteins. Hsp90 inhibition has emerged as a clinical strategy for the development of anticancer chemotherapeutics due to the involvement of Hsp90 clients in a variety of oncogenic signaling pathways that contribute to the six hallmarks of cancer (i. e. ErbB2, B-Raf, Akt, steroid hormone receptors, mutant p53, HIF-1, survivin, telomerase, etc.). Inhibition of this chaperone cycle results in client protein ubiquitinylation and subsequent degradation by the proteasome, resulting in tumor digression. Traditional small molecule Hsp90 inhibitors perturb the ATPase activity located at the N-terminus and include derivatives of geldanamycin, radicicol and purine. However, N-terminal inhibition

of Hsp90 also leads to displacement of the Hsp90-bound transcription factor, Heat Shock Factor-1 (HSF-1). Upon N-terminal inhibition, HSF-1 translocates to the nucleus and induces transcription of the heat shock proteins, including Hsp90. This is known as the pro-survival heat shock response (HSR). Therefore, inhibitors that do not induce the HSR are sought.

Alternative strategies for Hsp90 inhibition include disruption of the Hsp90 heteroprotein complex and disruption of the Hsp90 C-terminal dimerization domain. Disruption of the Hsp90 heteroprotein complex has emerged as an effective strategy to prevent client protein maturation without induction of the HSR. Disruptors of the heteroprotein complex inhibit cancer cell proliferation at concentrations similar to N-terminal inhibitors and results in client degradation. Herein, I will discuss cucurbitacins D and isoD as disruptors of client maturation and the mechanism by which these cucurbitacins induce the degradation of client proteins without induction of the HSR.

MEDI 460

Development of structure-activity relationships of BnIm for selective-Grp94 inhibition

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The 90 kDa heat shock protein (Hsp90) is a molecular chaperone that is responsible for the maturation and the rematuration of denatured proteins. Hsp90 is an attractive target for the treatment of cancer due to the presence of Hsp90-dependent client proteins in all six of the hallmarks of cancer. Inhibition of Hsp90 can simultaneously affect multiple oncogenic pathways. Many Hsp90 inhibitors have entered clinical trials, however some toxicities (ocular, cardio, and hepatotoxicities) were observed and have caused concerns. Hsp90 consists of four isoforms: cytosolic Hsp90α and Hsp90β, mitochondria-localized Trap-1, and the endoplasmic reticulum (ER) resident Grp94. Grp94 plays a role in the maturation of proteins associated with cell signaling and cell adhesion. Client proteins of Grp94 include IGF-II, Toll-like Receptors, and mutant myocilin. Multiple myeloma has been shown to be dependent upon Grp94 due to increased ER stress and inhibitors of Grp94 have been shown to decrease the proliferation of these cells. The N-terminal ATP-binding site of Grp94 possesses a unique hydrophobic binding pocket that is not present in the other isoforms of Hsp90. This pocket results from a five amino acid insertion into the primary sequence of Grp94, which allows small molecule access to this region of the pocket. Previously, inhibitors have been shown to utilize this hydrophobic region to gain selectivity for Grp94 over other Hsp90 isoforms, which was achieved through modification of the radamide scaffold via the incorporation of a *cis*-amide bioisostere. Structure-activity relationships have now been developed for the aryl moiety that projects into this pocket, which has resulted in both increased selectivity and potency for Grp94 inhibitors.

MEDI 461

Development of ring-constrained novobiocin analogs as Hsp90 C-terminal inhibitors

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Heat shock protein 90 (Hsp90) is the key component of a protein folding machinery that regulates the conformational maturation, activation, and stability of more than 200 client proteins. These client proteins perform several cellular functions, such as signal transduction, protein trafficking, cell proliferation, and survival. In cancer cells, many of these client proteins (e.g., Her2, Raf1, Akt, MET, Src, CDK4 etc.) are frequently mutated and/or over-expressed. Consequently, Hsp90 inhibition provides an attractive opportunity for the development of anti-cancer agents.

Novobiocin was the first natural product identified as an Hsp90 C-terminal inhibitor. Although it manifested poor anti-proliferative activity, it did not induce the pro-survival heat shock response, which is a major drawback of N-terminal inhibitors. Subsequent structural modifications to novobiocin led to the development of analogues that manifested improved anti-proliferative activity against various cancer cell lines. Based on previous inhibitory data and molecular docking studies, it was hypothesized that compounds containing lactams with various substituents in lieu of the sugar moiety would provide ring-constrained analogues with improved activity. Herein, the design, synthesis, and biological evaluation of ring-constrained novobiocin analogues as Hsp90 C-terminal inhibitors will be presented.

MEDI 462

Site-specific antibody-drug conjugates enable the exploration of structure-activity relationships at the conjugate level

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Antibody-drug conjugates (ADCs) hold the promise of delivering a highly toxic payload directly to diseased cells, reducing systemic toxicity and increasing the therapeutic index for the toxin. The two approved ADCs utilize native residues on the antibody to conjugate the toxin, leading to a distribution of both the number of drugs per antibody and the locations of those drugs. Our technology utilizes the formylglycine generating enzyme (FGE) to introduce a reactive aldehyde that can be specifically targeted for conjugation, allowing us to explore the structure activity relationship between toxin position and ADC activity.

We evaluated a library of aldehyde-tagged antibodies and chose three tag positions for further experiments. To varying degrees, the three ADCs were highly potent in vitro and in vivo. Results from pharmacokinetic studies with the three ADCs will also be presented.

MEDI 463

Analysis of ^{64}Cu-NOTA-cetuximab fragments for PET imaging of epidermal growth-factor receptor (EGFR) positive tumors

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The overexpression of the epidermal growth-factor receptor (EGFR) in a variety of tumors has been targeted in immunotherapy. Two monoclonal antibodies that have been approved for the treatment of metastatic colorectal carcinoma in patients (an EGFR positive tumor) are Cetuximab and Panitumumab. Our aim was to develop and evaluate the potential of Cetuximab fragments for positron emission tomography (PET) imaging of EGFR positive tumors. Delineation of tumors with the use of radiolabeled intact IgG (MW = 150kD) often require waiting 3 - 5 days post injection before scanning, while with Fab or F(ab')$_2$ fragments, blood clearance is faster and adequate tumor delineation can be achieved within 24 h. The cetuximab F(ab')$_2$ and Fab fragments were generated by enzymatic digestion of cetuximab IgG with Ficin, purified and analyzed by SDS-PAGE. The intact IgG (Cetmab), CetF(ab')$_2$ and CetFab fragments were conjugated with p-isothiocyanatobenzyl-NOTA and radiolabeled with ^{64}Cu ($T_{1/2}$ = 12.7 h) to give ≥97 % radiochemical purity. The number of NOTA on the Cetmab, CetF(ab')$_2$, and CetFab, was determined by a [^{64}Cu]$CuCl_2$ titration to give 7, 16, and 6 NOTA chelates per conjugate, respectively. Serum stability of the radioimmunoconjugates in human serum showed good stability up to 48 h. Internalization studies on EGFR expressing cancer cells showed that the radioimmunoconjugates were slightly to moderately taken up into the cells after 16 h–19.8 ± 5.6 % , 31.4 ± 6.2 % and 52.5 ± 1.7 % respectively for Cetmab, CetF(ab')$_2$ and CetFab. Excellent immunoreactivity (> 70%) of the radioimmunoconjugates on EGFR expressing cancer cells was observed. Specificity of ^{64}Cu-labeled Cetmab, CetF(ab')$_2$ and CetFab was also determined *in vitro* in the presence of excess Cetuximab IgG. Further studies are currently underway to assess the biodistribution and PET imaging of the radiolabeled Cetuximab fragments *in vivo*.

MEDI 464

Structure-based design of potent and selective tankyrase inhibitors to target the Wnt pathway

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Tankyrases are proteins in the poly-ADP-ribose polymerase (PARP) family. Their inhibition is emerging as a novel approach to the treatment of *APC*-mutant colorectal cancer. Herein we report the hit to lead efforts of two novel series of compounds, 'dual-pocket binders' and 'induced-pocket binders'. By utilizing unique structural information we obtained from two crystal structures, we conducted an expedited HTS of the Amgen compound collection using a minimal binding pharmacophore hypothesis. This led to identification of 'dual-pocket binders' . Upon further exploration of compounds with similar binding motifs, we discovered a novel second series, 'induced-pocket binders'. The potencies and pharmacokinetic properties of each series were investigated following the structural and metabolism identification guidance. The lead molecules identified could serve as excellent tool compounds for further rodent in-vivo validation studies.

MEDI 465

11a-N-Tosyl-5-deoxi-pterocarpan (1a), a promising prototype for targeting MDR leukemia cell lines and DNA topoisomerase I from Plasmodium falciparum (PfTopoI)

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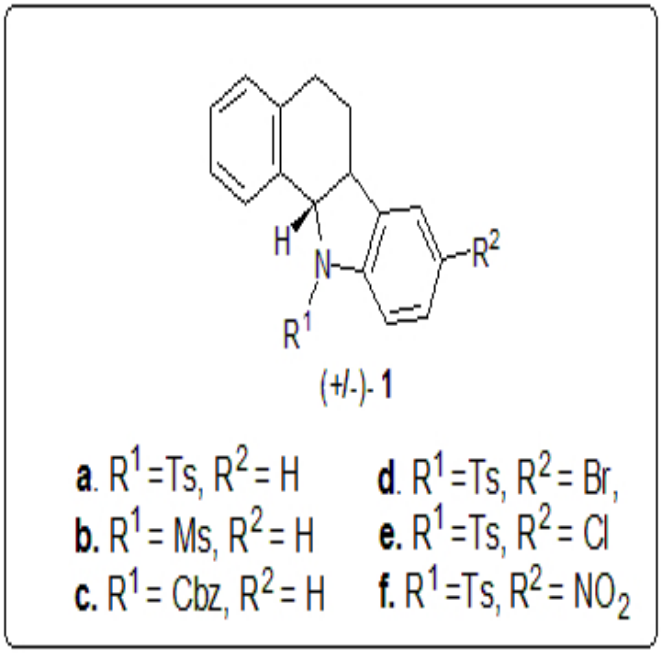
During the course of our studies aiming for the discovery of new pterocarpan-based antineoplastic and antiparasitary compound, we reported the synthesis of 11a-*N*-Tosyl-5-deoxi-pterocarpanand analogues **(1a-f)** through palladium-catalyzed aza-arylation of dihydronaphtalen or cyclohexadiene respectively, using palladium acetate or COF300@Pd(OAc)$_2$. These compounds showed antineoplastic effect on MDR leukemic cell lines K562, Lucena-1 and FEPS with IC_{50} 2.90 ± 0.65, 2.49 ± 0.14 and 2.12 ± 0.73 respectively. Compound **1a** is the most promising antileukemic agent since it was the most active on MDR cells without detectable toxicity to normal immune system cells. This compound promoted the inhibition of DNA proliferation and cell cycle arrest at G2 phase regardless of MDR phenotype.



These compounds showed antineoplastic effect on MDR leukemic cell lines (K562, IC50 = 2.90 Lucena-1 and FEPS). Compound **1a** is the most promising antileukemic agent since it was the most active on MDR cells without detectable toxicity to normal immune system cells. This compound promoted the inhibition of DNA proliferation and cell cycle arrest at G2 phase regardless of MDR phenotype. Compound **1a** is also a promising antimalarial. Theoretical results suggest that this compound docks into the binding site of camptothecin and topotecan inside both enzymes selectively in DNA topoisomerase I from Plasmodium falciparum (PfTopoI). PfTopoI is a potential selective target for chemotherapy and drug development against malaria, is used together with human Topo I (HssTopoI), for docking, molecular dynamics (MD) studies and experimental assays. Compound **1a** and the known PfTopoI inhibitors camptothecin and topotecan were evaluated in parallel. In vitro tests against P.falciparum blood parasites corroborated the theoretical findings since it presented the selectivity index (SI) of 98. In vivo experiments in mice infected with P. berghei showed that **1a** has an antimalarial activity similar to that of chloroquine.

MEDI 466

Synthesis and binding to telomeric DNA of novel curcumin analogs

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- In recent years the reverse transcriptase enzyme telomerase, has attracted a lot of attention due to this enzyme over-expression in 80-85% of cancer cell types. Inhibition of telomerase induces cell senescence and death and, for this reason, it has become a very important target for cancer therapeutics. Telomerase inhibition has been accomplished by inducing the folding of telomeric DNA into stable G-Quadruplex structures with small molecules. We here present the synthesis and preliminary telomerase inhibition studies of novel curcumin derivatives that could potentially pave the way to the development of novel anticancer drugs.

MEDI 467

Substituted 4-hydroxy-5,6-dihydropyran-2-one as potent inhibitors of human lactate dehydrogenase A

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A hallmark of cancer cells' altered metabolism is their ability to rapidly convert glucose to lactate in normoxic conditions via a process termed aerobic glycolysis. The enzyme LDH catalyzes the final step of glycolysis - the inter-conversion of pyruvate and lactate – and has been proposed to be a potential cancer target. However, the high abundance of cellular LDHA as well as the highly hydrophilic nature of its catalytic site presents significant challenges to the development of inhibitors useful for *in vivo* testing. Here we report on the structure-based discovery of substituted 4-hydroxy-5,6-dihydropyran-2-one inhibitors of LDHA with low nanomolar biochemical and single-digit micromolar cellular potencies. Crystal structures of these molecules bound to LDHA revealed distinct sub-pockets contributing to binding affinity as well as a close coupling between the binding mode and small molecule conformational preference. Inhibition of LDHA significantly enhanced the anti-proliferation activity of antimycin (a mitochondria inhibitor) when tested in non-glycolytically dependent cells.

MEDI 468

Small molecule probes for protein-protein interactions: Mimicking beta-sheet motifs

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Protein-Protein interactions (PPIs) are involved in many cellular processes such as gene expression, proliferation, intracellular communication, apoptosis and others.

Consequently the discovery of small molecule modulators has become an important goal in medicinal chemistry.[1,2]

Beta-sheet mediated PPIs have been implicated in numerous disease areas including cancer[3] and although a number of compounds have been developed to mimic beta-sheet motifs, few have made significant progress against a PPI in a disease relevant target.[4]

In order to obtain a molecular understanding we have developed a model system based upon a chromone peptidomimetic (**Chr**) that mimics the ADAD hydrogen-bonding motif of a short section of a beta-strand.

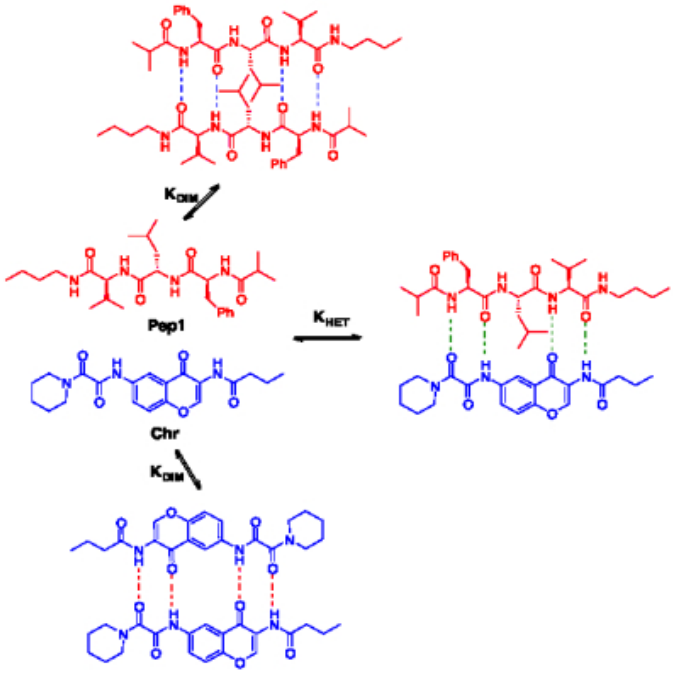

Figure 1: ***Model system involving peptidomimetic Chr and tri-peptide Pep1***

Presented are the synthesis and investigation of the hydrogen-bonding interactions of **Chr** . ^{1}H NMR dilution studies were carried out to determine $\mathbf{K_{DIM}}$ for a number of analogues of **Chr** .

As a comparison, $\mathbf{K_{DIM}}$ of tri-peptide **Pep1** was measured and found to be a stronger interaction; however, the hetero-dimerisation constant ($\mathbf{K_{Het}}$) between **Chr** and **Pep1** was larger than either $\mathbf{K_{DIM}}$ value.

A thermodynamic analysis of these interactions using variable temperature ^{1}H NMR showed that differences in equilibrium constant for dimerization are largely enthalpy driven.

This data provides fundamental insight into the nature of a simple model of a beta-sheet interaction and provides a platform to develop small molecule beta-sheet mimetics and assess their ability to disrupt a PPI.

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MEDI 469

Novel modified nucleobases that show cytotoxicity towards breast cancer cells

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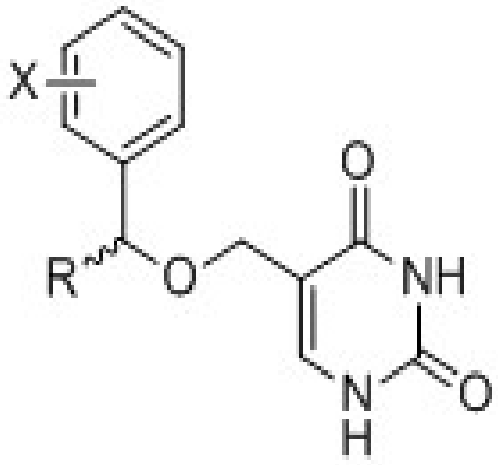
In the last fifty years, modified nucleosides and nucleobases, otherwise known as antimetabolites, have found use as therapeutic agents to treat various types of cancer. Current FDA-approved antimetabolite anti-cancer drugs, although effective, have numerous detrimental off target side effects. Our aim is to develop novel modified antimetabolites with improved properties, in particular with wider therapeutic window. Thus, a library of thymine derivatives bearing modifications at the 5-methyl position has been synthesized followed by cellular testing which has demonstrated their cytotoxicity towards MCF7 breast cancer cells. Structure-activity relationship study has revealed the lead compound, which has provided the opportunity to elucidate the mechanism of action of these novel modified nucleobases. With these in hand, we have the potential to develop novel anti-cancer agents with increased efficiency and minimized side effects.



R = Me, *i*-Pr, *t*-Bu, Ph, *neo*-Am

X = H, Me, MeO, CN, Cl, NO_2

MEDI 470

Investigating Grb7 inhibition by use of small molecules

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The over-expression of the Grb7 protein (human growth factor receptor bound protein 7) has been linked to various cancers including breast, ovarian, blood, and pancreatic. Grb7 elicits its function solely through protein-protein interactions (PPIs); inhibition of PPIs is a therapeutic modality that the pharmaceutical industry typically does not pursue. This novel approach requires drugs to bind to large surface areas as opposed to much smaller catalytic pockets, often rendering potential candidates too large and non-polar to ever make it to market. Excitingly, Grb7 inhibition was shown to decrease the viability of several breast cancer cell lines, reduce cell migration in pancreatic cancer cells, and delay the onset of tumors in nude mice. This established connection between abnormal Grb7 activity and cancer has rendered the protein a viable therapeutic target. Grb7 functions as an adaptor protein by binding its partners via its SH2 domain (Src homology 2); small molecules that bind the Grb7 SH2 domain have the potential to modulate protein function by rendering the protein ineffective in binding its signaling partners. To that end, previous studies have identified a benzopyrazine small molecule, which selectively binds the Grb7 SH2 domain. To further improve the efficacy of this lead molecule, we used rational design and recent advances in synthetic chemistry to develop new potential drugs. These compounds utilize a cyclized core with varied substitution to enhance binding interactions between the small molecule and the

Grb7 SH2 domain. The motivation, design and innovative synthesis of these Grb7 inhibitors along with preliminary efficacy data will be presented.

MEDI 471

Lead based development and evaluation of selecitve estrogen mimics in tamoxifen resistant breast cancer

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Tamoxifen is the standard of care for many patients with estrogen receptor positive ER+ breast cancer, antagonizing the actions of endogenous estrogen at ER. Tamoxifen increases the risk of endometrial cancer and thrombotic events, and most significantly, half of women develop resistance or do not respond to tamoxifen therapy, underlining the need for an alternate therapeutic option. Paradoxically, prior to tamoxifen therapy, estradiol (E_2) and the ER agonist, diethylstilbestrol, had been used in breast cancer therapy, though with serious side effects. Development of Selective Estrogen Mimics (SEMs) that cause regression of tamoxifen-resistant breast cancer, but without the side effects of E_2, represents a transformational therapeutic strategy. Based on the benzothiophene scaffold, a chemical library consisting of 30 SEMs was synthesized in order to obtain an ideal SEM. The SEMs were assayed in tamoxifen-resistant cell lines, MCF-7:5C and T47D:PKCα, with the objective of developing partial agonists at ERa, which do not fuel growth of estrogen-dependent T47D xenografts and do not cause uterine growth; which we postulate as ideal characteristics for therapy of breast cancer beyond tamoxifen-resistance. Thrombosis risk is adversely increased in breast cancer and with chemotherapy, therefore, we sought in parallel to address this cause of increased mortality. A prodrug strategy was developed for SEMs to add ancillary anticoagulant activity, whilst maintaining the anti-cancer activity towards breast tumors. This research paves the way for use of SEMs with partial agonist activity at ER, for use in breast cancer, with enhanced safety profiles through reduced risk of endometrial cancer and thrombotic events.

MEDI 472

Investigating the ototoxicity of gold nanoparticle conjugated cisplatin

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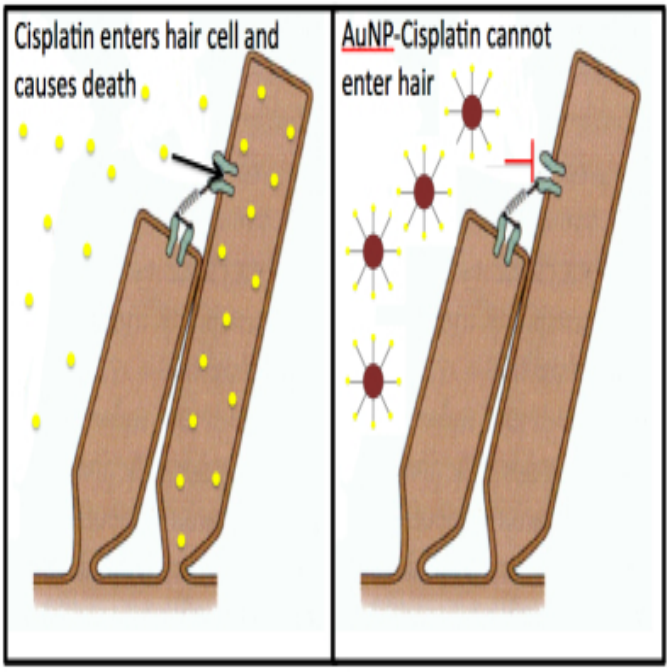
The chemotherapy drug cisplatin is used to treat a wide variety of cancers; however, it has a number of side effects, such as damage to hearing or balance functions of the ear defined as ototoxicity. The ototoxic effect of cisplatin has recently been shown to depend on entry into inner ear hair cells through the mechanotransduction channel, and we hypothesized that conjugation of the drug to gold nanoparticles (AuNP) larger than the diameter of the channel would reduce ototoxicity. Such drug-nanoparticles conjugates have been shown by others to effectively delivery the drug to tumor via the enhanced permeability and retention (EPR) effect. The goal of this study was to use larval zebrafish as an *in vivo* model to investigate whether conjugation to gold nanoparticles reduces cisplatin's ototoxicity. Gold nanoparticles were synthesized and linked to cisplatin via mercaptoundecanoic acid. The loading of cisplatin on the nanoparticles was measured by inductively coupled plasma optical emission spectroscopy. The ototoxic effect of the AuNP-cisplatin conjugates was tested in zebrafish larva and compared to unconjgated cisplatin. This poster will summarize the results of these experiments.



MEDI 473

Antioxidant dendrimers

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Oxidative stress caused by free radicals is a major underlying problem for many diseases including cancer, inflammation, asthma, neurodegenerative and

cardiovascular disease. The free radicals are formed by our body's normal metabolic activities. These free radicals can attack our genetic information and many biomolecules like protein or lipids, causing damages to our cells. Antioxidants scavenge free radicals and make these reactive radicals harmless. However, many naturally available antioxidants can also act as pro-oxidants by reducing transition metals which lead to formation of even more free radicals. In this presentation, we report unique dendritic antioxidants, made of naturally occurring antioxidants, syringaldehyde and vanillin. The surface consists of phenolic hydroxyl groups and electron donating ring substituents and the interior is composed of metal chelating groups. The antioxidants also demonstrated strong protective effects on human low-density lipoprotein and DNA. The novel antioxidants in the presence of physiological concentration of copper ions showed no DNA damage. On Chinese hamster ovary cells, cell viability at 50 μM was unaffected over 5 days.

MEDI 474

Targeted synthesis of novel ROS activated cancer pro-drugs resistant to CYP3A19 metabolism

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Current chemotherapy approaches for cancer treatment lack selective cytotoxicity, causing unwanted side effects. Recent literature studies have shown that cancer cells show elevated levels of reactive oxygen species (ROS). For example, acute myeloid leukemia (AML) cancer cells have shown a 100-fold increase in superoxide ROS compared to normal blood cells. We are translating this observation into a pro-drug design approach, allowing ROS to oxidize the pro-drug in order to improve selective cytotoxicity. Our current lead agent has shown 10-fold selectivity between AML cells over normal CD34+ blood cells in vitro, but has been found to rapidly metabolize in vivo. We hypothesize, based on calculations, that metabolism predominately occurs via enzymatic reaction with CYP3A19, one of the most common xenobiotic resistance enzymes. I have computationally designed a series of new molecules that impart the same mechanism of action but are resistant to CYP3A19 metabolism. I have synthesized 7 targets and evaluated their metabolism by CYP3A19 using a luminescence assay. Next, I evaluated their anti-AML activity and selectivity compared to CD34+ blood cells.

MEDI 475

Novel 2-benzylidene benzothiazolidinones as potent and selective inhibitors of protein kinase CK2 and PIM kinases

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Protein kinase CK2 (Casein Kinase 2) is a highly conserved protein serine/threonine kinase that is ubiquitously distributed in eukaryotes, constitutively active and has been implicated in multiple cellular functions, as well as in tumorigenesis and transformation. CK2 regulates multiple oncogenic pathways involved in cell cycle progression, suppression of apoptosis, hypoxia, angiogenesis, inflammation and DNA repair. Unlike other signaling molecules such as PI3K, PTEN, RAF, RAS, where genetic alterations lead to deregulated signaling pathways, in the case of CK2, only its high expression levels have been associated with a disease state and no mutations have been found to date.

Here we present the discovery and biological characterization of ON 108110, ON 108600, and water-soluble ON 1081050, ON 1081080 and ON 1081090 the potent ATP competitive inhibitors of CK2. To understand the mechanism of cytotoxicity of these compounds, we selected these three molecules from a library of >1000 compounds and screened against a panel of 355 functional recombinant kinases in *in vitro* and these three were found to be very potent inhibitors of CK2 along with PIM3 kinases. These are synthetically derived small molecule CK2 inhibitors which showed broad spectrum anti-proliferative and cytotoxic activity in multiple cancer cell lines while having little or no effect on normal cells. To understand the structural basis of CK2 inhibition we performed X-ray crystallographic studies of these compounds. The co-crystal structures of ON108110-CK2 and ON108600-CK2 revealed that these compounds bind in the active site pocket of CK2 wherein they mimics the binding of ATP and GTP in the CK2 active site. Recent studies have shown that CK2 has emerged as novel druggable target and selective inhibitors of CK2 such as ON 108110, ON 108600 and ON 1081050 may prove as potential anti-cancer therapy. Our ongoing studies are focused towards identifying novel combinations of these compounds with existing chemotherapeutic agents in multiple myeloma, prostate and breast cancer. Hence, herein we describe the synthesis, characterization, cytotoxicity, kinase profile of water-soluble ON 1081050, ON 1081080 and ON 1081090.

MEDI 476

Design synthesis, and biological evaluation of novel MEK-5/ERK-5 inhibitors

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Inhibition of MAPK (Mitogen Activated Protein Kinase) cascade has recently been shown to be a useful therapeutic target for the treatment of various cancers. Despite this success, selective inhibition of multiple isoforms of the singular parallel event, ERK phosphorylation by its corresponding MEK, has been understudied. We will present the results of modifying the proposed internal H-bonding geometries and aryl substitutions that are ubiquitous as MEK-1 inhibitor features to develop unique and selective MEK5 inhibitors. Design, synthesis and biological evaluation will be presented.

MEDI 477

Design and synthesis of novel sulfamoyl benzoic acid (SBA) analogs as specific non-lipid LPA2 receptor agonists with picomolar affinities

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Exposure to radiation through a nuclear accident or explosion of a “dirty bomb,” is now a major public health threat. To date, there are no FDA approved radiomitigators available for the recovery of immune, hematopoietic and gastrointestinal compartments following radiation injury. Radiomitigators also provide potential therapeutic applications in the attenuation of the side effects of radiation therapy and chemotherapy like diarrhea in cancer patients. In an effort to discover novel highly potent radiomitigators, we designed and synthesized sulfamoyl benzoic acid (SBA) analogs as the first non-lipid specific agonists of the type 2 GPCR for lysophosphatidic acid (LPA2). Our research group identified a picomolar agent 5-chloro-2-(*N*-(4-(1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)butyl)sulfamoyl)benzoic acid (**RP-10-83** ; EC_{50} (nM): $5.06x10^{-3}\pm3.73x10^{-3}$) as specific non-lipid LPA2 receptor agonist.

MEDI 478

Identification of a novel small-molecule inhibitor of plasminogen activator inhibitor-1

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Plasminogen activator inhibitor-1 (PAI-1) is an endogenous serine protease inhibitor, pathologic levels of which have been implicated in a variety of conditions, including atherosclerosis, myocardial infarction, type 2 diabetes, and cancer. The development of potent and selective inhibitors of PAI-1 has therefore become a priority. A small-molecule inhibitor of PAI-1 based on a novel scaffold was identified by high-throughput screening and subsequent spectroscopic analysis of the screening mixture that provided the screening hit. The method of identification of the active component will be discussed, as will initial screening of related molecules as inhibitors of PAI-1.

MEDI 479

Formyl chromones as privileged structures: Development of drug-like scaffolds with antimicrobial and cytotoxic properties

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The basic chromone structure has a variety of biological activities and has therefore been considered a privileged structure with regards to medicinal chemistry. Our research group recently developed new tin-free stereoselective additions to 3-formylchromones.More recently, however, we have been investigating a project that focuses on developing a variety new antimicrobial agents based on the chromone substructure. This is important due to the increasing problem of the formation of resistant strains of bacterial pathogens. This poster presents a convenient and versatile multisynthetic pathway to obtain small libraries of substituted 3-formylchromones. These products exhibit good to excellent cytotoxic activity against several human pathogenic bacteria, and have also shown cytotoxic effects against murine B16 melanoma cells.

MEDI 480

Sustainable, green chemistry, and medicine: Targeted cannabinoid-based chemotherapy utilizing Cell-in-a-Box cellular encapsulation technology

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Targeted cannabinoid-based chemotherapy utilizing Cell-in-a-Box® cellular encapsulation technology offers a "green" approach to treating solid-tumor malignancies worldwide. The *Cannabis* plant has provided a sustainable source of fiber, food, energy, and medicine for thousands of years. The plant's constituents such as Δ^9-tetrahydrocannabinol and cannabidiol have been well-documented to have broad anti-inflammatory, antioxidant, analgesic, nerve protecting, and antineoplastic abilities, among many other therapeutic properties. Cell-in-a-Box® cellular encapsulation is a

patented technology that encapsulates living cells to become sustainable bio-inert “factories” containing specific enzymes capable of generating therapeutic end-products from precursor pro-drugs. This technology has shown initial success in the targeted treatment of pancreatic cancer utilizing the conventional chemotherapeutic agent Ifosfamide as the pro-drug. An understanding of the chemical and biochemical processes involved in the interaction of substances derived from a sustainable plant source (i.e., *Cannabis*) with sustainable cellular encapsulations (i.e., Cell-in-a-Box®) provides the opportunity to develop "green" approaches to treating cancers (e.g., pancreatic, brain, breast, and prostate) that affect hundreds of thousands of individuals worldwide every year. Non-proprietary, preliminary results related to metabolic studies and screening of cells will be presented.

MEDI 481

Small molecule inhibitors of the FASN thioesterase domain in oncology

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Fatty acid synthase (FASN) is a multi-enzyme protein that catalyzes fatty acid synthesis. Recently, this enzyme has attracted attention as a key player in several major diseases. It is up-regulated in all the major solid tumors, and its expression is usually indicative of poor prognosis. Inhibition of FASN by small molecules and natural products, as well as its knock down with siRNA, have been shown to induce apoptosis in cancer cells and slow tumor growth in animal models. Further, inhibition of FASN has been shown to disrupt viral replication in a number of human viral infections, such as hepatitis C virus (HCV). Despite the extensive body of evidence demonstrating the role of FASN in these major diseases, there is a significant unmet need for drug-like small molecule inhibitors of FASN. We have targeted our efforts on the thioesterase domain (FASN-TE) for drug discovery in collaboration with a high-throughput scren (HTS) performed through the MLPCN initiative. Here we report our progress towards the development of potent and selective small molecule inhibitors of FASN-TE.

MEDI 482

Nucleic acid bioconjugates in anticancer applications

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Nucleic acid bioconjugates have been successfully applied in anti-cancer research programs aimed at fighting the cancer epidemic en route towards the development of effective and safe treatment methods. Their widespread use stems from the ability to accelerate the drug development process by conjugating chemical functionality that may improve the pharmacology of the nucleoside. This therapeutic strategy has been realized with small molecule nucleosides and those derived from lengthy oligonucleotides. Considering the utility of nucleic acid bioconjugates in anti-cancer applications, our research aims to explore the synthesis, characterization and therapeutic potential of novel nucleic acid bioconjugates. These are based on (a) aminoacyl nucleolipids, from which a simple and efficient synthesis strategy has been developed for this new class of DNA binding molecule. This nucleic acid based bioconjugate displayed GRP78 oncogene binding affinity (K_D: 0.25 mM) and selective anti-leukemic activity in a single dose (10 μM) screen against a panel of 60 cancer cell lines.[1] (b) The synthesis, bio-physical properties and oncogene photocleavage acitivty of a phthalocyanine-linked oligonucleotide for potential photodynamic therapy applications. This novel conjugate was synthesized and characterized using HPLC, mass spectrometry, UV-Vis spectroscopy and thermal denaturation. The ability of this conjugate to damage GRP78 DNA/mRNA was validated.[2] This presentation will highlight our most recent contributions to the flourishing field of bioorthogonal chemistry applied to the synthesis of nucleic acid bioconjugates for anti-cancer applications.

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Fluorophthalocyanine-Oligonucleotide Bioconjugates and their GRP78 Oncogene Photocleavage Activity.

ChemComm. **2014** ,*Accepted Manuscript.* (**DOI:** 10.1039/C4CC00703D)

MEDI 483

Synthesis, characterization, and cancer gene therapy applications of branch and hyperbranch siRNAs

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The efficacy of conventional chemotherapeutic agents is limited by poor selectivity and widespread toxicity which compromises the road towards recovery. Thus, the ability to specifically seek and destroy cancer would be an extraordinary feat in the treatment of the cancer epidemic. Towards this goal, we have designed, synthesized and evaluated the biological activity of potent oncogene therapeutics, namely short-interfering RNA, siRNA that may selectively silence GRP78 overexpression in HepG2 liver cancer cells leading to significant anti-cancer effects. Specifically, branch and hyperbranch siRNAs have been prepared by automated solid-phase RNA synthesis. Following isolation of the branch and hyperbranch siRNAs by RP-IP HPLC and denaturing PAGE, siRNAs were characterized by thermal denaturation and CD spectroscopy to assess their bio-physical and structural properties, respectively. Interestingly, branch and hyperbranch siRNAs retained the pre-requisite hybrid A-type helical structure for triggering potent RNAi effects. With this mission at heart, siRNAs were tested and proved potent silencing of GRP78 expression (~40-60%) in HepG2 cell lines, and the ability to solicit cancer cell death (~20%) to a greater extent relative to the linear siRNA controls (~5%). Thus, branch and hyperbranch siRNAs effectively extend the repertoire of potent oncogene therapeutics and may serve as interesting leads in the development of RNAi screening methods. This presentation will highlight our latest research efforts towards the development of an effective cancer gene therapy approach.[1]

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MEDI 484

Synthetic peptides alternatives to epothilone and taxol as inhibitors of the dynamics of tubulin polymerization

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Altering microtubule polymerization has been known to be the mechanism of action for a number of chemotherapeutically relevant drugs including taxanes and epothilones. Recently, high-content assay platforms aimed to monitor tubulin polymerization status by directly measuring the acute effects of drug candidates on the cellular tubulin network were developed and shown to distinguish in a quantitative manner between compounds that act as tubulin stabilizers versus those that are tubulin destabilizers. To take advantage of the low cost involved in the production of synthetic peptides we performed of structure-based drug design (SBDD) and discovered peptides structural mimics of taxol and epothilone. The 1tvk-B.pdb (tubulin beta chain) was used as target in docking experiments which were performed using the software SCULPT from Accelrys (originally licensed by MDL). We first generated a library of peptides with

different sequence space (including D-isomers and un-natural amino acids) and assessed the free energy of interaction between the protein target and the ligands using the MMFF94 (force-field) built-in the software SCULPT. Both the van der Waals and the electrostatics were used to predict the relative free energy of binding between beta tubulin and the peptides ligands during rigid docking experiments. Peptide1: 2HN-D-Trp-L-Tyr-D-Asp-Gly-L-Ser-L-Tyr-L-Gln-L-Ser-D-Ser-CONH2 and Peptide 2: 2HN-D-Tyr- L-Tyr-D-Ser-Gly- L-Ser-L-Tyr-L-Gln-L-Ser-D-Ser-CONH2 were shown to stimulate the paclitaxel activity as a tubulin stabilizer agent, by increasing the Vmax for the growth phase during tubulin polymerization in an vitro assay. Cyclic peptides were further design from the lead linear peptides and their preliminary SBDD is presented.

MEDI 485

Design, synthesis, and biological evaluation of substituted monocyclic pyrimidines with potential antitubulin activities as antitumor agents

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Tubulin binding agents are an important class of antitumor agents and the most extensively studied binding sites are those that accommodate taxanes, vinca alkaloids and colchicine. Antimicrotubule disrupting agents like paclitaxel have major limitations against multidrug resistance (MDR) tumors. Overexpression of P-glycoprotein (Pgp) and/or *β*III-tubulin can severely limit their clinical utility as cancer chemotherapeutic agents. Recently, we reported substituted monocyclic *N*-methyl-4-methoxyanilino pyrimidines that are structurally simplified, conformationally flexible analogs of bicyclic pyrrolo[3,2-*d*]pyrimidines that circumvent some of the drawbacks of taxanes and vinca akaloids. Compounds **1-5** were designed to evaluate further the SAR at the 6-position of the pyrimidine scaffold. The design, synthesis and structure activity relationship of these agents with respect to their antitubulin activity will be presented.

MEDI 486

1,5-Diheteroaryl-1,4-diene-3-ones: Promising, curcumin-inspired anticancer agents

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Turtumic, an Asian spice, has long been used as an Ayurvedic medicine for the treatment of various ailments. Its major chemical component is curcumin, which was

first isolated in 1815. Recently, the in vitro and in vivo studies indicated that curcumin has potential to prevent and treat various cancers, and the clinical study demonstrated that curcumin processes a high safety profile in human. The key obstacle for curcumin to advance to clinical use is its low bioavailability. The above-mentioned benefits and disadvantage of curcumin as a drug candidate triggered a large amount of research on searching for improved curcumin analogs for the potential clinical treatment of cancers. Recently, we have reported three scaffolds of curcumin analogs and identify that 1,5-diheteroaryl-1,4-diene-3-one is an optimum scaffold for development of curcumin-based anti-cancer agents.

As part of our continuing curcumin project, the present study is to explore the effect of the size of the heteroaromic rings on the cytotoxicity of 1,5-diheteroaryl-1,4-diene-3-ones. Eleven new, bulky, *N*-containing heteroaromatic curcumin analogs have been designed and synthesized through the Horner-Wadsworth-Emmons reaction of 1,3-bis(diethylphosphonato)acetone with appropriate aromatic aldehydes. Each of our new analogs features two identical bulky heteroaromatic rings and a dienone linker. The cytotoxicity of these synthesized analogs towards an aggressive cervical cell line (HeLa) and a hormone-independent prostate cancer cell line (PC-3) has been evaluated. This poster will present the design, synthesis, and cytotoxic evaluation of these synthetic analogs inspired by curcumin.

MEDI 487

Immunomodulatory activity of dehydroepiandrosterone and oxygenated and fluorinated androstenes

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The results of nearly 50 years of research have shown that DHEA (dehydroepiandrosterone) is a multi-functional steroid implicated in a broad range of biological processes. DHEA, an androstene hormone, has been shown to possess a wide range of beneficial biological effects mainly attributed to immune system modulation. DHEA is metabolized into more active metabolites, that is, 17beta-AED and 17beta-AET, as well as testosterone and estradiol [1]. 17beta-AED and 17beta-AET have been reported to prevent infections and counteract the immune suppressive action of hydrocortisone, thus leading to beneficial effects in diverse human diseases [2].

In this work we report the syntheses of 17beta-AED, 17beta-AET and six androstenes oxygen- and fluorinated in C-3 and C-7 derived from DHEA. The immune regulation of

DHEA and its related compounds were evaluated. The cytotoxic concentration, the modulation on TNF-α and IL-6 production by macrophages were determined for each compound.

The antiagiogenic activity was also studied by the effect in VEGF expression and in the migration of HUVEC cells. Finally, the major histocompatibility complex (class I and II) expression and the IL-12 production were measured in dendritic cells stimulated with M. tuberculosis.

The results showed that AED, AET and any of the synthetic compounds with less androgenic effect, can be relevant in immunomodulatory therapies.

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MEDI 488

Design of triazole-stapled TRF2 peptide inhibitors targeting NF-kB signaling pathway through direct binding to RAP1

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Repressor Activator Protein 1 (RAP1) and Telomeric Repeat Binding Factor 2 (TRF2) are physically interacting partners within the shelterin complex, which has telomere-related functions. Previous investigations suggest that RAP1 also binds to IκB kinases (IKK) in cytoplasm, where RAP1 recruits IKK to p65 of NF- κB and subsequently activates NF-κB signaling pathway via phosphorylation of p65 S536.

TRF2 and IKK share the same binding domain on RAP1. Potentially, a TRF2 peptide mimetic that binds to RAP1 with high affinity could block the protein–protein interactions of TRF2-RAP1 and IKK-RAP1.

In this study, we optimizated the binding affinity, helical propensity, charge distribution and water solubility of a TRF2 peptide. This resulted in a 16-residude, cell-permeable, triazole-stapled peptide molecule which potently (K_i = 10 nM) binds to RAP1, down-

regulates phosphorylated p65 of NF-κB and inhibits proliferation of MDA-MB-157 breast cancer cell.

MEDI 489

Variation of linker composition in ADCs generated from aldehyde-tagged antibodies impacts both efficacy and PK

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Linkers are key components of antibody-drug conjugates (ADCs) and their chemical and structural properties can be exploited in the design of these therapeutic agents. As a test of the contribution of linker chemistry to ADC activity, we generated a panel of anti-HER2 ADCs that vary at the linker portion. The ADCs were made using aldehyde-tagged anti-HER2 proteins conjugated using the hydrazinyl-iso-Pictet-Spengler (HIPS) ligation to a maytansine payload. The resulting ADCs were highly homogenous, with well-defined drug-to-antibody ratios (DARs) as assessed by hydrophobic interaction chromatography. Although absolute efficacy did vary according to the linker components, as a group, the aldehyde-tagged anti-HER2 ADCs demonstrated potent in vivo activity against subcutaneous solid tumors. Additionally, linker composition was found to affect ADC pharmacokinetics.

MEDI 490

Isosteric replacement of acyl piperizine with phenyl ether: Improving metabolic stability of GDC-0068 class of Akt inhibitors

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Bioisosterism has been a powerful tool in medicinal chemistry that affects compound potency, selectivity, and metabolism. Acyl piperizine and phenyl ether share similar shape, conformation, and hydrogen-bond acceptor but have dramatically different physiochemical properties. By replacing acyl piperizine with phenyl ether, we may alter a molecule's metabolism without disrupting its key interactions with receptor. Such isosteric replacement was applied to GDC-0068, a potent and selective Akt inhibitor currently in clinical development for treating cancer. A novel potent Akt inhibitor with clogD shifted by two units and improved metabolic stability was discovered based on the phenyl ether scaffold.

MEDI 491

Design, synthesis, and biological evaluation of pemetrexed homologs as multi-enzyme antifolates selectively targeting the folate receptor

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The two most important hurdles in cancer chemotherapy are toxicity due to inability of the therapeutic agent to selectively target tumor cells and chemotherapeutic resistance common to single agent therapy. Pemetrexed (PMX), the multi-enzyme targeted anticancer drug has dose-limiting toxicity due to its transport by the ubiquitously expressed reduced folate carrier (RFC). We recently reported a series of 5-substituted pyrrolo[2,3-*d*]pyrimidine classical antifolates that inhibit folate receptor (FR) expressing tumor cells (KB and IGROV1) at nanomolar IC_{50} values by selective uptake. The target enzymes were confirmed to be glycinamide ribonucleotide formyl transferase (GARFTase) and aminoimidazole carboxamide ribonucleotide formyl transferase (AICARFTase) having little to no DHFR (dihydrofolate reductase) or thymidylate synthase (TS) inhibitory activity. To determine the impact of the distance between the scaffold and the side chain phenyl on multi-target inhibition as well as selectivity, a series of classical 5-substituted pyrrolo[2,3-*d*]pyrimidines with varying bond lengths were designed and synthesized. The synthesis and *in vitro* evaluation of these compounds as substrates for folate transporters- RFC, FR and proton coupled folate transporter (PCFT) and as inhibitors of GARFTase, AICARFTase, TS and DHFR will be presented.

MEDI 492

Fluorinated N,N-dialkylaminostilbenes repress colon cancer by targeting methionine S-adenosyltransferase-2

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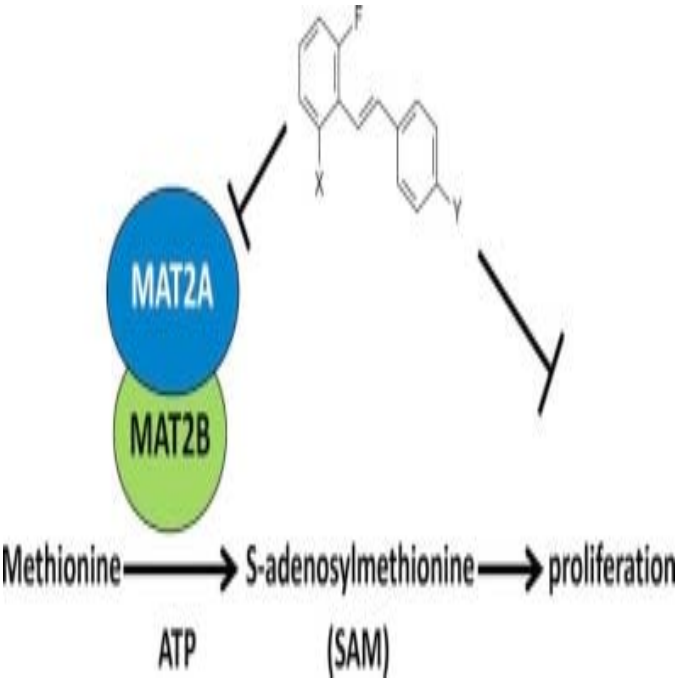
Colorectal cancer (CRC) is the second leading cause of cancer-related mortality in the United States. CRC is initiated by mutations of the tumor suppressor gene, adenomatous polyposis coli (APC), or the *β*-catenin gene. These mutations stabilize *β*-catenin and constitutively activate Wnt/*β* -catenin target genes, such as c-Myc and cyclin D1, ultimately leading to cancer. Fluorinated *N,N*-dialkylaminostilbene agents (**1**), called FIDAS agents, inhibit the proliferation of CRC cells *in vitro* at nanomolar levelsand cause a significant reduction in volume *in vivo* in human colorectal cancer xenografts in athymic nude mice at a dosage of 20 mg/kg. Heterocyclic analogs of these FIDAS agents possess similar potency and complete water solubility. These agents bind the catalytic subunit (MAT2A) of the heterodimeric methionine S-

adenosyltransferase-2 as the direct and exclusive binding target. Metabolites include the biologically active, N-desmethyl analog. FIDAS agents inhibit MAT2 activity in SAM synthesis, and thereby deplete a methyl source required for crucial histone methylations and proliferation events in cancer cells. FIDAS agents have no adverse cardiac properties (*i.e*., hERG inactive) and possess an appropriate pattern of interactions with CYP enzymes consistent with a drug candidate. These findings suggest that FIDAS analogs that target MAT2A represent a family of novel and potentially useful agents for cancer treatment.



MEDI 493

Targeting of PI3K-a and CDK4/6 kinases in mantle cell lymphomas with dual kinase inhibitor ON 123300-lactate

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In our earlier work, we have presented the development of a novel dual specificity kinase inhibitor, ON 123300, which exhibits potent activity against Mantle Cell Lymphomas (MCLs) both *in vitro* and *in vivo*. Mantle cell lymphoma is genetically characterized by the t(11;14)(q13;q32) chromosomal translocation which results in constitutive overexpression of cyclin D1. In addition, MCLs also activate other pathways, including aberrant B-Cell Receptor and PI3K/AKT/mTOR signaling. As a

result, MCL has a poor clinical outcome with a median survival of 4-5 years. We have shown that ON123300, which inhibits both CDK4/6 and PI3K-α (the predominant PI3K catalytic subunit expressed in MCL cells), was a superior inducer of apoptosis of MCL cells when compared to PD0332991, a selective inhibitor of CDK4/6 kinases.

In this study, we have made water-soluble analogs of ON 123300 (lactate, fumarate, citrate, sulfate, triflate and mesylate) and tested the effects of ON 123300-lactate in nude mouse xenograft assays using Z138 MCL cells. These studies revealed a strong inhibition of tumor growth when tumor-bearing mice were treated daily with 100 mg/kg of ON123300-lactate. In addition, there was little evidence of toxicity as measured by change in the body weight in ON123300-lactate treated mice.

MEDI 494

Targeting tumor stem cells by inhibiting BMI-1 expression: Discovery of substituted aminopyrimidines as potent and efficacious antitumor agents

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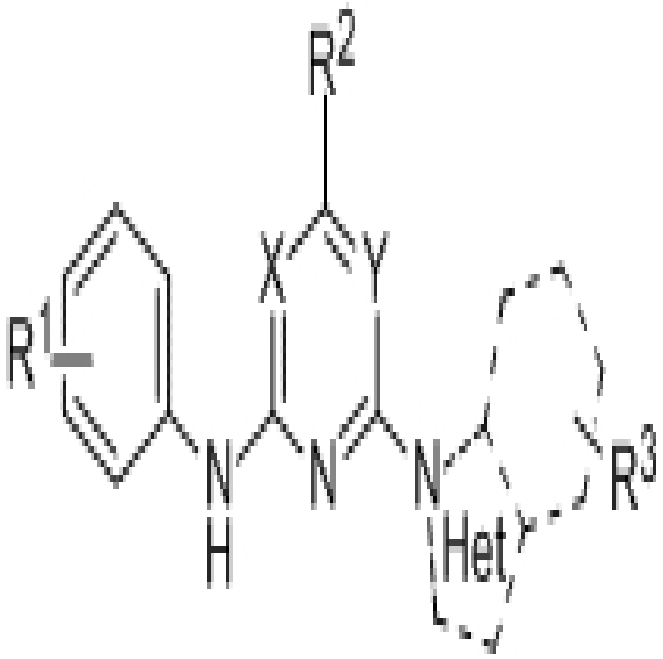

Recurrence of cancer cells is a major hurdle in cancer chemotherapy. It is believed that cancer stem cells are responsible for the tumor relapse that results in treatment failure.

BMI-1 is an oncogene that is over-expressed in tumor cells and is necessary for survival of cancer stem cells. Thus, inhibiting BMI-1 expression has become an emerging novel approach to treat drug-resistant tumors. In our earlier efforts, we discovered a series of anilinothiazoles as first-in-class small molecules that reduced BMI-1 levels and showed high anti-tumor activity. Further optimization on the central aromatic ring identified derivatives of 1,3-pyrimidine and 1,5-pyrimidine that showed excellent *in vitro* activity and improved pharmaceutical properties. The best analogs in this series were orally bioavailable and demonstrated dose-dependent efficacy in mouse xenograft models. During these studies we also discovered a highly selective reaction between anilines and 2-methylsulfonyl-4,6-dichloropyrimidine that facilitated the highly efficient and divergent synthesis of tri-substituted aminopyrimidines. The synthesis, structure-activity relationship (SAR), pharmacokinetic profiles and *in vivo* efficacies of these compounds will be presented.

MEDI 495

Synthesis and evaluation of triazine derivatives as novel antitumor agents that modulate BMI-1 expression in cancer stem cells

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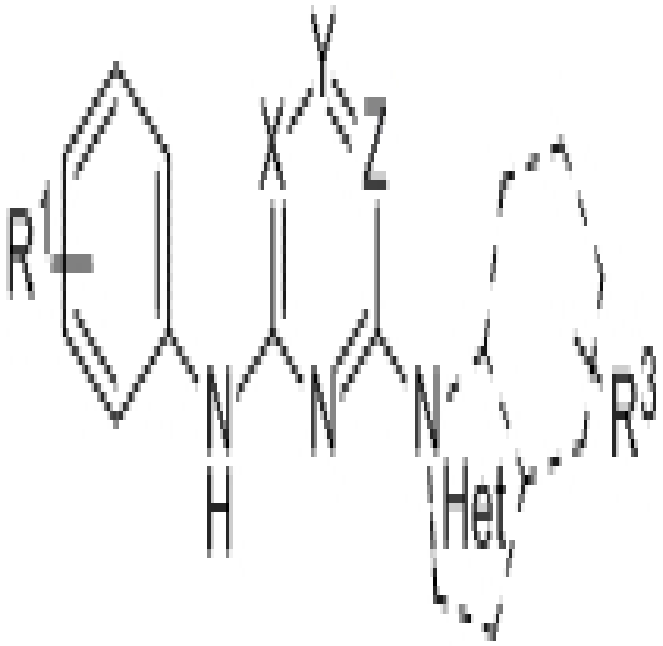

BMI-1 is an oncogene that is over-expressed in tumor cells and is required for the survival of cancer stem cells. It is believed that cancer stem cells are responsible for the tumor relapse that results in failure of chemotherapy treatment. Thus, inhibiting BMI-1 expression has become an emerging novel approach to treat drug-resistant tumors. In our earlier efforts, we discovered a series of anilinothiazoles as first-in-class small molecules that reduced BMI-1 levels and showed high anti-tumor activity. To optimize the central aromatic core, we synthesized and evaluated derivatives of 1,3,4-triazine, 1,3,5-triazine and 1,4,5-triazines. The best analogs from this series showed good *in vitro* activity and improved pharmaceutical properties. Some were further evaluated in mouse xenograft models. The synthesis, structure-activity relationships (SAR), pharmaceutical properties and *in vivo* efficacies of these compounds will be presented.

MEDI 496

Discovery of novel antagonists for proton-sensing receptor GPR4 and their pharmacological activities

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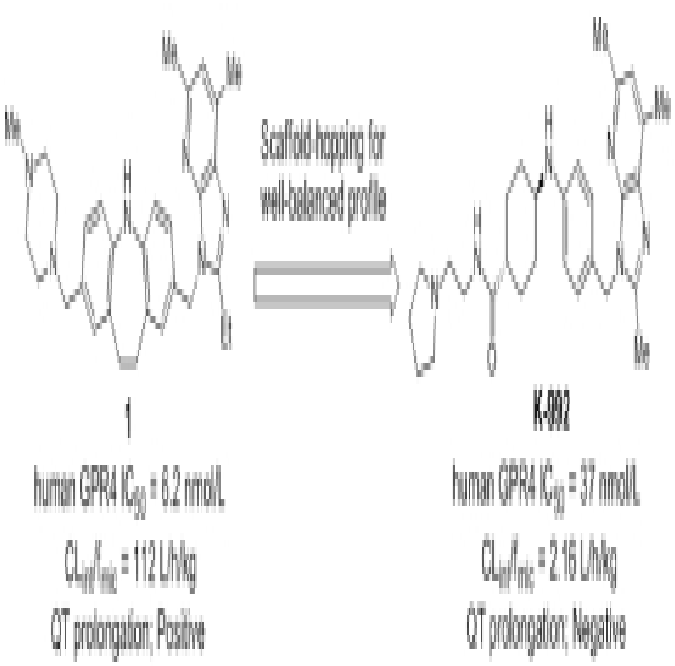
GPR4 was found as an orphan GPCR highly expressed in the lung tissues, and reported to be activated by acidification in 2003. The activation of GPR4 is thought to lead some inflammatory events such as the production of chemokines and the neutrophil migration. Using a luciferase-reporter assay system detecting the constitutive activity of GPR4, we discovered a small-molecule GPR4 antagonist **1** , which inhibited the neutrophilic chemokine production and the following neutrophil migration in mouse lung injury model.

However, compound **1** is metabolically unstable in human liver microsomes and induces QT prolongation, presumably due to high lipophilicity of its core structure: dibenzo[*b*,*f*]azepine. Therefore, we have explored scaffold-hopping to solve these issues and found a series of cyclohexylaniline-based novel GPR4 antagonists. Herein we will discuss the synthesis and SAR of these derivatives, leading to **K-992** with improved pharmacokinetics and promising pharmacological properties.



MEDI 497

Synthesis and in vivo assessment of arylsulfonyl acrylonitriles for the inhibition of peritoneal carcinomatosis

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Following surgical resection of malignant gastrointestinal and gynecological tumors, such as advanced pancreatic cancer and ovarian cancer, implantation of shed cancer cells into the peritoneal cavity can lead to peritoneal carcinomatosis, which has a low incidence of survival. We have previously disclosed a series of amide analogs of BAY 11-7085 [(E)-3-(4-tert-butylphenylsulfonyl)acrylonitrile], a tool compound which has been shown to induce apoptosis of colon and pancreatic cells during cell adhesion. Herein, we disclose additional analogs from the amide series as well as bioisosteric replacements and other derivatives aimed at achieving improved in vivo efficacy. Results from two in vivo metastatic models will be presented for which new analogs demonstrate reductions in local recurrence and distal metastases.

MEDI 498

Discovery and optimization of aminooxadiazoles as potent proviral integration site of Moloney murine leukemia virus kinases (Pim) inhibitors

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Pim-1 and -2 are constitutively active Ser/Thr protein kinases involved in cytokine signal transduction and the regulation of cell survival. Pim kinases have been found to be over-expressed in solid tumors, in multiple myeloma and in a variety of hematopoietic malignancies such as leukemia. High-throughput screening of Amgen's compound collection followed by an initial round of compound optimization led to 1,3,4-thiadiazole-based Pim-1/2 inhibitor **1** . Replacement of the aminothiadiazole motif with an aminooxadiazole led to improvements in the series pharmacokinetics (PK). X-ray crystallographic studies guided the replacement of the 6-isopropoxypyridinyl group with a cyclopropylpyrimidine and resulted in compound **2** with enhanced potency in Pim-1 and -2 enzymatic and cell assays.

MEDI 499

Application of structure based design techniques for targeting specific EGFR-kinase interactions

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The epidermal growth factor receptor (EGFR) is implicated in many cancers, and its kinase activity is the target of commercial anti-cancer agents such as Tarceva and Iressa. However, despite their effectiveness, EGFR kinase inhibitors often show only moderate antiproliferative activity against certain tumor types in the clinic. Resistance to EGFR inhibitors is mediated by mutation in the ATP site and often through activation of the MAPK pathways by other receptor tyrosine kinases. This inspired the investigation of agents directed not only at EGFR kinase but also at divergent targets such as Src kinase or DNA, with the purpose of producing single compounds, termed “combi-molecules,” with greater potency than the single EGFR inhibitor. A structure-based drug design modeling program, combined with PDB data-mining, protein structural fingerprints and pharmacophore searches, was used to help identify and characterize linkers for connecting EGFR-binding moieties to DNA and Src targeting functionalities. The resulting compounds showed EGFR inhibitory potency in the low micromolar to nM range and retained significant activity against their divergent targets.

MEDI 500

Development of classification models for the Aryl Hydrocarbon Receptor (AHR) activators using HTS big dataset: Utilizing applicability domain and addressing unbalance in the dataset

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The Aryl hydrocarbon receptor (AhR) is a ligand-dependent transcription factor. It regulates the expression of an array of genes in a wide range of species and tissues. Among the most characterized chemical classes that are known to be ligands for AhR are environmental toxins, such as the Halogenated Aromatic Hydrocarbons (HAHs) and Polycyclic aromatic hydrocarbons (PAHs).

We utilized a large highly unbalanced dataset from PubChem Bioassay database (aid: 2796) of 324751 tested compounds with an active/inactive ratio of 1:40. To evaluate the structural characteristics of small molecules responsible for AHR binding and toxicity,

cross-validated classification models were developed using the online tool OCHEM (http://ochem.eu). Multiple machine learning algorithms including ASNN, KNN, FSMLR, PLS, Random forests and J48 were compared. The descriptors packages used for modeling the dataset included Dragon v6, CDK, ALOGPS, ESTATE, ISIDA, ADRIANA.CODE as well as Chemaxon descriptors. Stratified Bootstrap aggregation was used to successfully handle the unbalance in the dataset. Distance-to-model applicability domain was estimated. Enrichments of 3-4 folds were observed for the most accurate predictions. Structural features contributing to the activation of AhR receptor were suggested. The most accurate models are made freely available online on http://ochem.eu.

MEDI 501

Structure-based modeling and direct coupled analysis for the prediction of dimeric macromolecular systems

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Structure-based models (SBM) have been a successful computational approach for simulating and studying structure, conformational changes and macromolecular function of proteins. It defines a specific energy potential that considers folded interactions from a particular experimental structure as parameters to guide molecular dynamics simulations. Direct-coupling analysis (DCA) is a co-variance computational method that enables the prediction of contacts between protein residues using only amino acid sequences of the target protein family. It is based on the hypothesis that mutations in residues that participate in interactions must be compensated by mutations in other interacting residues to preserve protein structure and functionality.

A promising and powerful computational methodology to assist the understanding of target proteins behavior and, consequently, the development of new drugs is the integration of SBM and DCA.These methods allow the identification of hidden conformational states that occur in the functional landscape of certain proteins but are not observed by crystallographic methods. The combination of SBM and DCA has proven to be successful in predicting folded structures and functional conformational changes of several protein systems.

Here we develop a procedure to predict the association of protein structures into homodimers using signals from DCA and SBM potentials to guide the simulations of dimerization process. Identification of dimerization contacts is more challenging than intradomain contacts since direct couplings can be confounded with internal contacts. Therefore a systematic way to extract dimerization signals has been elusive. We provide evidence that the prediction of homodimeric complexes is possible with high accuracy. To distinguish dimeric from intradomain contacts, a filter using solvent-

accessible surface area was applied followed by contact map analysis. The identification of dimeric complexes can provide interesting molecular insights about protein function and mechanisms and can also assist the design of novel compounds by elucidating new interesting inhibition mechanisms for protein interactions.

MEDI 502

Molecular modeling of subtype selective muscarinic antagonists

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G-protein coupled receptors (GPCRs) represent on of the most successful therapeutic target families, including approximately 30 % of all known drug targets covering a wide range of therapeutic fields [1]. GPCRs trigger multiple signal-switching mechanisms, not only G-protein activation but also binding of ß-arrestin proteins and activation of kinases. Surprisingly the understanding of the conformational changes resulting in these activations is very poor and remains a major challenge for the design of specific GPCR modulating drugs. The recently published crystal structure of the M2 muscarinic acetylcholine receptor (PDB: 3UON [2]) and mutational studies provides the possibility to rationalize and understand the binding of ligands to muscarinic acetylcholine receptors, explain their subtype preference and give insights into the flexibility of their binding pockets.

We present the results of extensive molecular dynamics (MD) simulations in combination with molecular docking and 3D-pharmacophore analysis of known ligands and related structures. The orientation of the tropane ring system plays an essential role for the arrangement of the whole ligand, resulting in subtype specific binding modes. These findings were in accordance with binding experiments and mutational data.

[1] Jacoby, E., et al.: The 7TM G-protein-coupled receptor target family. *Chemmedchem*, 2006, 1(8):760-782.

[2] Haga, K., et al.: Structure of the human M2 muscarinic acetylcholine receptor bound to an antagonist. *Nature*, 2012, 482(7386).

MEDI 503

Protein active site comparison tool for optimizing drug selectivity

***Ivana Uzelac**, ivana.uzelac@chem.gu.se, Thomas Olsson, Johan Gottfries. Department of Chemistry and Molecular Biology, University of Gothenburg, Gothenburg, Sweden*

Attaining selectivity of a drug towards one target is oftentimes a hard but nonetheless a crucial task when working in drug design. The challenge with selectivity partly depends on how unique the target protein itself is. If the target compared to other proteins has a

considerably large structural difference it may be easier to obtain selectivity rather than if there are structurally related or even homologues proteins existing. We have constructed an easily adaptable model for comparing and differentiating between closely related proteins. By combining PCA and OPLS discriminant methods the data could be described in an easily interpretable fashion. The present method can be applied to optimize lead structures, analyze target interaction, or simply as a protein comparison tool.

MEDI 504

Chelating-fragment libraries as a tool in novel metalloenzyme inhibitor discovery

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A fragment library consisting of metal-binding pharmacophores (MBPs) has been designed and employed in inhibition studies of medicinally relevant metalloenzymes. A second library of compounds has been combined with an earlier reported[1] 96 component chelator fragment library (CFL-1.1). This expanded library has been screened at micromolar concentrations (200-500 μM) against metalloenzyme targets, and hits from these screens are being elaborated to produce more potent inhibitory molecules by fragment-based lead discovery (FBLD). Enzymes evaluated include carbonic anhydrase, several matrix metalloproteases, histone deacetylases, HIV integrase, and influenza endonuclease. Even at fragment concentrations as low as 200 μM, this library produced hit rates >10%, with fragments exhibiting IC_{50}values as low as 2 μM. Current efforts involve the expansion of this library to include more chemically diverse MBPs and screenings against other relevant metalloenzymes, as well as lead-expansion to full length inhibitors with potential use as pharmaceuticals.

1. Jacobsen, J. A.; Fullagar, J. L.; Miller, M. T.; Cohen, S. M., Identifying Chelators for Metalloprotein Inhibitors Using a Fragment-Based Approach. *Journal of Medicinal Chemistry* **2011,** *54* (2), 591-602.

MEDI 505

Apply Ni(II) complexes to efficient asymmetric synthesis of chiral α- and β-amino acids

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Nonproteingenic α-, or β-amino acids have attracted tremendous attention, they are widely utilized for biological, biochemical, pharmaceutical, and asymmetric chemical investigations. The nickel(II) complex has been widely used to synthesize enantiopure amino acids via aldol, Michael addition, Mannich reaction, and C-alkylation reactions. Notable merits of the nickel(II) complex's methods include: (1) predictable

stereochemical outcome and high level of enantio- and/or diastereoselectivity; (2) inexpensive cost-structure and ready availability of nickel(II) complexes; (3) operationally convenient reaction procedures; (4) high overall reaction yields and reproducibility; and (5) easy and virtually complete recovery of chiral ligands, rivaling catalytic methods in terms of consumption of the stereocontrolling reagents. These unique features render this method an attractive strategy for practical synthesis of various α- and β-amino acids, in particular on relatively large scale. Recently, we developed a new strategy for preparing the chiral Ni(II) complex for the asymmetric synthesis of chiral amino acids. We applied this new method in our new progress of chiral Ni(II) complex for the asymmetric Mannich reaction to synthesis enantiopure α,β-diamino acids, the enantioselective tandem conjugate addition-elimination reaction to synthesis glutamic acid derivatives, the Suzuki coupling reaction to synthesis β^2-amino acid derivatives, the asymmetric Mannich reaction to synthesis of 3-aminoaspartate, the asymmetric Michael addition reaction to synthesis β-substituted-α,γ-diaminobutyric acid derivatives, the asymmetric alkylation reaction to synthesis linear ω-trifluoromethyl containing amino acids, the asymmetric Michael addition reaction to synthesis of *syn*-β-substituted tryptophans, the asymmetric Diels-Alder reaction to synthesis cyclic amino acid derivatives, the asymmetric oxidative heterocoupling reaction for 3-indolylglycines, and the asymmetric oxidative cross-dehydrogenative coupling reaction for tetrahydroisoquinolin-1-yl glycine derivatives.

MEDI 506

Utilizing a SMART approach to compound processing and management

***Christopher A LeClair**, leclairc@mail.nih.gov, C Thomas Louer, William Leister. Division of Pre-Clinical Innovation, National Center for Advancing Translational Sciences, National Institutes of Health, Rockville, MD 20850, United States*

The Division of Pre-Clinical Innovation (DPI) within the National Center for Advancing Translational Sciences (NCATS) is tasked with conducting collaborative research for enhancement of the pre-clinical phases of translational science. These efforts involve the development of new methods and technologies, as well as evaluation and improvement of existing methods, processes, and technologies. In fulfillment of this mission, medicinal chemists at DPI synthesize thousands of compounds annually for research focused on rare and neglected diseases. With the need for fast turnaround between small molecule synthesis and biological assays, an efficient transfer of compounds from Medicinal Chemistry to Compound Management was necessary. To fulfill this requirement, the Sample Management and Resource Tracking (SMART) system, a webpage-based management software, was created. Initially developed for analytical data tracking and sample management, SMART has evolved into an all-encompassing system for compound purification, compound processing, compound management, and data management, which is accessed through a web browser. Since implementation of SMART in 2009, productivity at DPI has increased as medicinal chemists can focus on compound synthesis. Furthermore, electronic data transfer and automation all contribute to maximized efficiency while maintaining high compound and

data integrity. SMART is a highly adaptable system that can and does change based on the needs of the various groups within DPI. As such, SMART has contributed significantly to the success of medicinal chemistry efforts at NCATS. While customized for DPI, the SMART system has the flexibility and robustness to fulfill the demands of any organization.

MEDI 507

Development of an ^{18}F radiotracer targeting GPR91

***Jeffrey D Klenc**, jklenc@emory.edu, Malgorzata Lipowska, Andrew T Taylor. Radiology and Imaging Sciences, Emory University, Atlanta, GA 30322, United States*

Objectives: Upon binding succinate, the G-protein coupled receptor GPR91 initiates a signal cascade leading to the activation of the renin-angiotensin system (RAS). This mechanism of RAS activation may contribute to the progression of renovascular hypertension and diabetic nephropathy. A radiotracer targeting GPR91 will allow investigators to probe these disease processes in vivo and potentially develop novel diagnostic or therapeutic strategies. In the presented work, our goal was to further the development of the first GPR91 radiotracer by designing and preparing a series of fluorine-containing compounds based on high-affinity GPR91 inhibitors (Bhuniya, et al.; *Bioorg Med Chem Lett*, **2011** , p. 3596).

Methods: The steric and electronic properties of high-affinity GPR91 ligands were examined (Avogadro 1.1.0) to assist the design of fluorine-containing compounds with comparable pharmacological properties. The development of an appropriate synthetic pathway for the target compounds focused on the inclusion of a final ^{18}F labeling step and the purification of the radioactive products.

Results: A small series of fluorine-containing compounds was designed. A 6-step synthetic pathway was developed to conclude with an ^{18}F radiolabeling reaction (nucleophilic aromatic substitution of a nitro group with tetrabutylammonium fluoride). An HPLC method for the purification and characterization of ^{18}F species was developed using a nonradioactive ^{19}F analog. Each prepared fluorine compound was obtained in high purity and fully characterized by NMR, HRMS, EA, and HPLC.

Conclusion: Candidates for the first GPR91 radiotracer have been designed and synthesized. Radiolabeling and purification methods required for ^{18}F radiotracer preparation for in vivo studies have been established. Results from consequent in vitro studies will identify the best candidate of this series for ^{18}F radiolabeling and in vivo evaluation.

MEDI 508

Intramolecular cyclizations of allyl- and vinyl-dihydropyridones under photolytic conditions

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The presented studies involve the intramolecular cyclizations of allyl- and vinyldihydropyridones under photolytic conditions. These substrates rapidly and efficiently generate [2+2] photocycloaddition products, which host structurally interesting and challenging tricyclic ring systems. These molecules show structural similarities to extremely useful tropane framework and will be further evaluated in the study of biology, medicine, agriculture and molecular imaging.

MEDI 509

High throughput screening, X-ray crystallography analysis, and structure-activity relationships (SAR) of CFTR inhibitory factor (Cif) inhibitors

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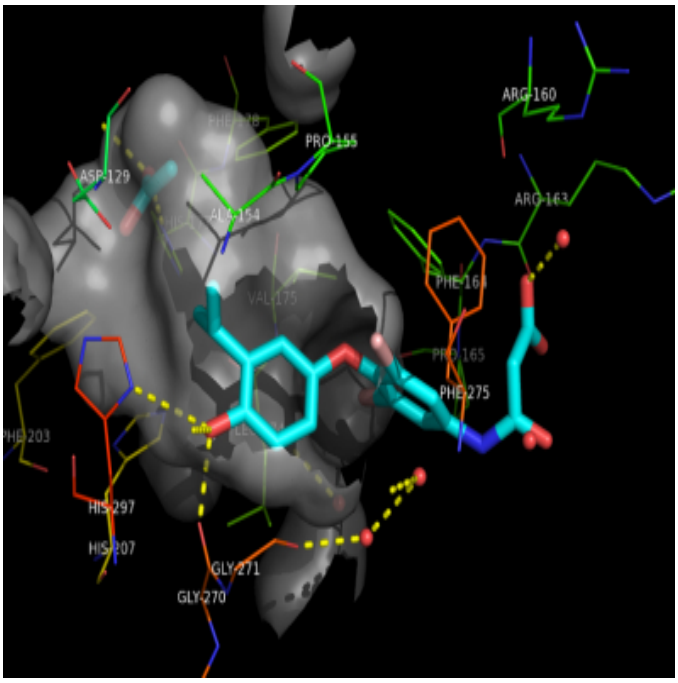
The cystic fibrosis transmembrane conductance regulator (CFTR) inhibitory factor (Cif) is a virulence factor protein secreted by an infectious bacterium *Pseudomonas aeruginosa*. Cif alters the trafficking of transporters such as CFTR, leading to the promotion of the ubiquitin-mediated degradation of these transporters. Cif is a member of the α/β hydrolase family with a conserved catalytic triad and has demonstrated epoxide hydrolase activity. However, the endogenous substrate and exact mechanism of Cif virulence are not known.

The availability of potent and selective Cif inhibitors is invaluable in order to better understand Cif pathobiology and its use for possible clinical applications. To meet this goal we screened a library of 1,600 known drugs from US and International Pharmacopeia using fluorescent based high throughput screening assay. Tiratricol and KB2115, thyroid hormone agonists, were found to be potent Cif inhibiters, with IC_{50}values of around 3 μM. The biochemical analysis revealed that these are the non-competitive inhibitors. The X-ray crystal structures of Cif complexed with inhibitors were solved, and these inhibitors were found to bind to the entrance of the catalytic pocket of the enzyme, which is consistent with the finding that they are non-competitive inhibitors. We further probed the interactions between the Cif and inhibitors by the structure-activity relationship approach. Structural optimization of the inhibitors are now in progress guided by the X-ray crystallography structures.



MEDI 510

α-Sulfonamidophosphonates and bis-α-aminophosphonates: New leads towards infectious disease chemotherapy

Srikant Bhagat[1]*, srikantbhagat@gmail.com, Methuka Supriya*[1]*, Tharun G. V. V. Kumar*[1]*, Shewta Mishra*[1]*, Jonnalagadda Padma Sridevi*[2]*, Perumal Yogeeswari*[2]*, Dharmarajan Sriram*[2]*, Asit Kumar Chakraborti*[1]*. (1) Department of Medicinal Chemistry, National Institute of Pharmaceutical Education and Research, S. A. S. Nagar (Mohali), Punjab 160062, India (2) Department of Pharmacy, Birla Institute of Technology & Science-Pilani (Hyderabad Campus), Hyderabad, Andhra Pradesh 500078, India*

Tuberculosis and leishmanisis have been prime concern for health-care establishments worldwide among the various infectious diseases especially in the low- and middle-income developing countries. As per WHO fact-sheet, 8.6 million people fell ill with TB and 1.3 million died from TB in 2012 whereas an estimated 1.3 million new cases and 20000 to 30000 deaths occur annually through leishmaniasis.[1] The increasing instances of resistance towards the available drugs for chemotherapy of these disease raised the necessity to search for new drug candidates. The remarkable potential of the α-aminophosphonate moiety in medicinal chemistry with diverse biological activities[2] have impressed us for biological evaluation of various structurally diverse α-aminophosphhonates (α-sulfonamidophosphonates, bis-α-aminophosphonates) as antileishmanial and anti-tuberculosis agent. The primary findings reveal α-aminophosphonates as novel antileishmanial chemotypes [3]and further extended result

of several new compounds for these infectious diseases have been obtained. These observations may provide new therapeutic lead(s) for these infectious diseases.

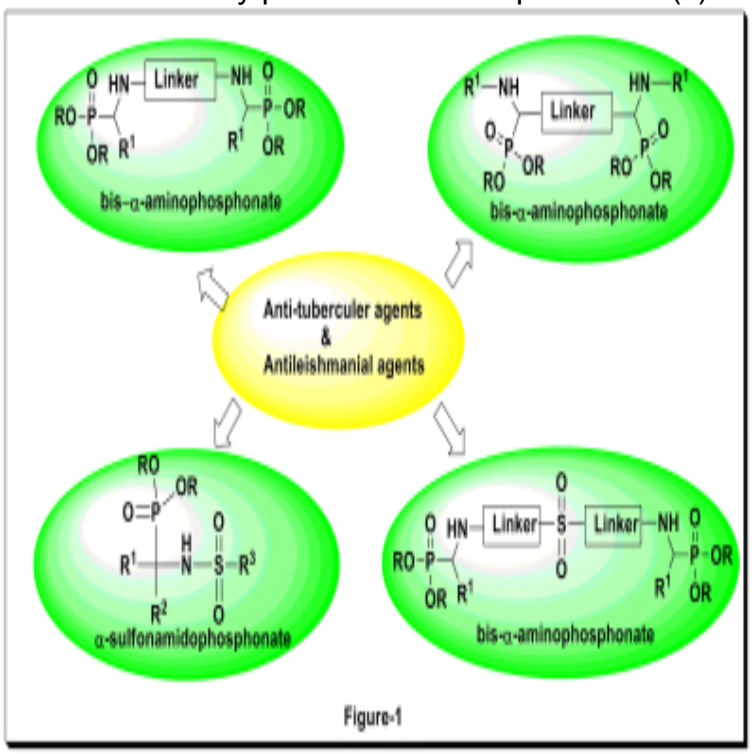


Figure-1

References:

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2. (a) Galezowska, J.; Gumienna-Kontecka, E. *Coord. Chem. Rev.* **2012** , *256*, 105–124 (b) Bhattacharya, A. K.; Rana, K. C.; Pannecouque, C.; De Clercq, E. *ChemMedChem,* **2012** , *7*, 1601-1611. (c) Mucha, A.; Kafarski, P.; Berlicki, L. *J. Med. Chem.* **2011** , *54*, 5955-5980.

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MEDI 511

Enantioselective synthesis of imidazolines and α,β-diamino amides derived from Ugi multicomponent high throughput screening libraries

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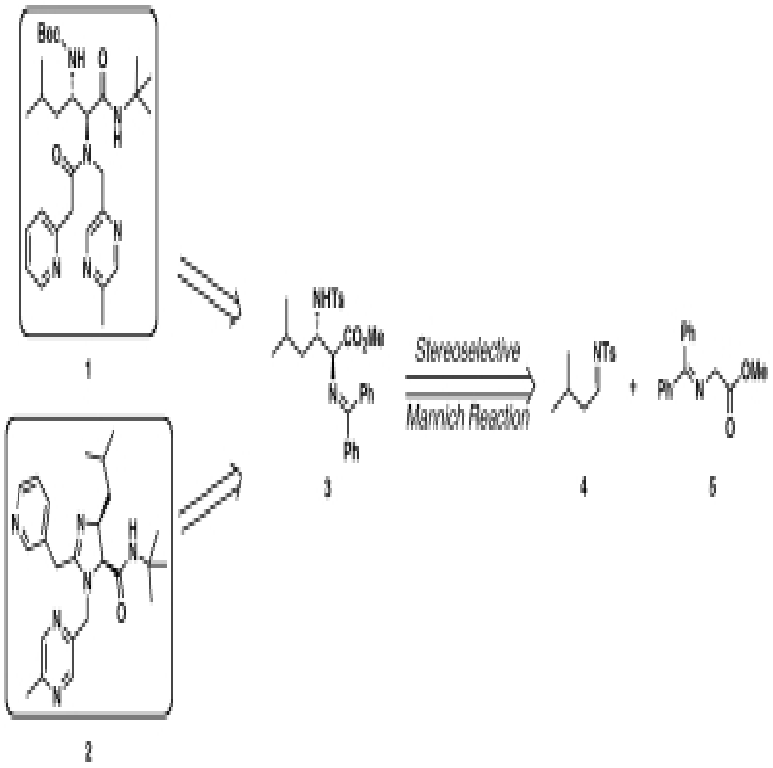

In drug discovery programs, Ugi multicomponent reactions have been valuable in generating large numbers of diverse compounds for use in chemical libraries for high throughput screening. In our high-throughput screening of small molecule libraries for new anti-fibrotic targets, two promising lead structures were discovered, acyclic alpha,beta diamino amide derivative **1** , and cyclization-derived imidazoline **2** . These structures can be accessed traditionally by the Ugi multicomponent reaction, which provides stereoisomeric mixtures of products as a statistical mixture of all 4 stereoisomers. In our hit-to-lead programs, we wished to prepare enantiomerically pure analogs for biological testing. Here, we describe a new synthesis that provides the stereoisomers selectively in enantiopure form. Our syntheses diverge late-stage from a common precursor **3** , whichisprepared in 7 steps respectively from commercially available compounds. Early in the synthesis, the stereocenters are set in a catalytic enantioselective Mannich reaction and retained throughout the rest of the synthesis. The enantioselective synthesis of these small molecules allows for their evaluation as anti-fibrotic treatments.

MEDI 512

Designed amphiphilic peptides toward fabrication of lipid-peptide nanoparticles

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Membrane proteins have received a lot of attentions as a target of drug development because of their crucial roles in various biological processes such as signal transduction, material transport and energy conversion. However, the functional and structural studies of membrane proteins are still challenging due to the technical difficulties in their handling in an aqueous solution. We have studied fabrication of well-defined nanoparticles encompassing a lipid bilayer as a novel platform for the analysis of membrane proteins. For the formation of lipid nanoparticles, we designed amphiphilic peptides, which can wrap the hydrophobic core of a lipid bilayer. In this presentation, we will discuss the effect of the peptide design on the interaction with lipid bilayer.

MEDI 513

Synthesis and magnetic resonance analyses of Melphalan-TEMPOL for site-targeted drug delivery

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A spin-labeled adduct of the therapeutic drug, Melphalan, was synthesized using a three-step synthetic route. Step one involves the protection of the amine group under basic conditions with Di-*tert*-butyl carbonate as the protecting group. Step two involves the addition of the spin label compound 4-hydroxy-2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPOL) to the carboxylate group of Melphalan via a Steglich esterification with the assistance of the coupling agent, *N,N'*-Dicyclohexylcarbodiimide, and a catalyst, 4-Dimethylaminopyridine, under the inert atmosphere of nitrogen gas. The final step comprises the restoration of the amine group by using a strong acid to remove the protecting group and obtaining the final product after purification of the Melphalan-TEMPOL adduct. To ensure that the spin label compound was successfully coupled to Melphalan, structural analyses of the intermediates and final product have been carried out using ^{1}H NMR and EPR (Electron Paramagnetic Resonance) for detecting free radical activity of the single electron spin of the nitroxyl group of TEMPOL. The main objectives of this project are: a) to improve the bioavailability, retention time and permeability of Melphalan during drug delivery with the assistance of nanoparticle drug delivery techniques; and b) to develop MRI techniques using the Melphalan-TEMPOL adduct enclosed polymeric nanoparticles.

MEDI 514

Novel synthesis of Isoquinoline derivatives by Rhodium(I) catalyzed between N-H ketimines and internal alkynes

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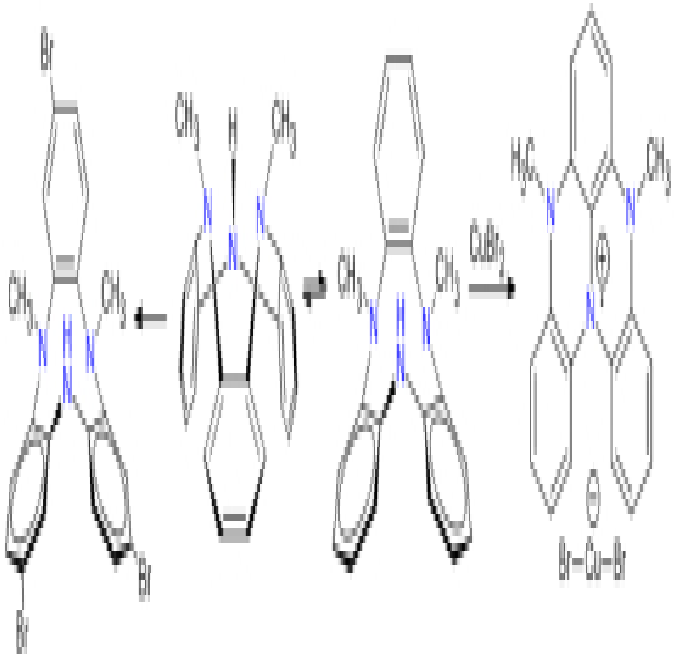
Nitrogen containing heterocyclic compounds such as Isoquinoline, Indole, and Pyridine derivatives are widely present in many natural products. Because the heteocyclic compounds provide structural backbone for pharmaceutical agents and organic materials, they have been in the center of attention in the organic synthetic community. In this project, Isoquinoline derivatives were produced via the coupling of benzophenone imines and alkynes in the presence of Rh(1) catalyst and DPEphos ligand. Working reactions were conditioned to occur in milder reaction condition and to provide improved yield. Significant ligand- and solvent effects on chemoselectivity and stereoselectivity have been observed. In this poster, preparation of Isoquinoline derivatives with different substituted functional groups will be presented.

MEDI 515

Peripheral functionalization and complexation of an ortho-triazacyclophane, N,N',N''-trimethyltribenzo-1,4,7-triazacyclononatriene, and Cu(II)-induced rearrangement to a stable cation radical

*Ross J. Beattie[2], David Upp[1], Trisha Tucker[1], Doug Steinman[1], Andrea Panagopoulos[1], Nick Nutile[2], Phillip E. Fanwick[3], Matthias Zeller[4], Thomas J. Smith[2], **Daniel P. Becker**[1], dbecke3@luc.edu. (1) Department of Chemistry and Biochemistry, Loyola University Chicago, Chicago, Illinois 60660, United States (2) Department of Chemistry, Kalamazoo College, Kalamazoo, Michigan 49006, United States (3) Department of Chemistry, Purdue University, West Lafayette, Indiana 47907, United States (4) Department of Chemistry, Youngstown State University, Youngstown, Ohio 44555, United States*

Cyclotriveratrylene (CTV) is a bowl-shaped molecular scaffold that is commonly employed in supramolecular chemistry for host-guest chemistry and biomimetic molecular recognition, and has been proposed as a vehicle for drug delivery. As part of our research program directed toward the synthesis and application of apex-modified CTV derivatives, we previously reported[1] the synthesis of a new *ortho*-triazaorthocyclophane, N,N',N''-Trimethyltribenzo-1,4,7-triazacyclononatriene via palladium-catalyzed Buchwald-Hartwig N-arylation reactions, and we recently reported mechanistic studies detailing unusual rearrangement chemistry.[2] With the goal of developing new medical imaging reagents, we wish to report our recent results of peripheral functionalization of the azacyclophane, including halogenation and subsequent Suzuki coupling chemistry, along with mass spectrometric evidence of metal complexation. In addition, we have observed a Cu(II)-induced oxidative rearrangement to a stable cation radical, as confirmed by X-ray crystallography and characterized by ESR spectroscopy.



MEDI 516

Sensing biological anions with luminescent pyridine/imine based lanthanide complexes

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The unique magnetic and luminescence properties of the lanthanide metals enable a variety of clinical applications including medical imaging and biosensor or assay development. For example, coordination complexes of the Gd(III) ion are often used in magnetic resonance imaging (MRI) due to the ability of such complexes to enhance the images produced from a scanner. Other lanthanide ions, such as Eu(III) and Tb(III) are often utilized in the form of luminescent sensors for a variety of biologically relevant targets. For sensing anions in particular, binding studies can be conducted in an aqueous environment due to the quenching of luminescence caused by water molecules bound to the lanthanide ion. Coordinating anions are capable of binding the metal and replacing coordinated water molecules. Once replaced, luminescence intensity of the metal complex increases and this effect can be used to assess anion binding efficiency.

In this study, a tripodal pyridine/Schiff base ligand for lanthanide complexation was synthesized and biosensor applications were explored. This complex was made from the so-called TRIPy (TREN-tris-imine-pyridine) ligand which effectively binds Eu(III) in a

hexadentate manner, leaving space for additional substrates to attach. Anion studies were performed in MES buffer with solutions of, for example, oxalate, carbonate, fluoride, phosphate, and citrate. It was determined that the metal complex exhibited a larger change in luminescence intensity when exposed to oxalate versus other anions studied suggesting selectivity for the oxalate anion. These results encourage further study of potential application in the development of clinical methods for diagnosis of oxalate-based kidney stones.

MEDI 517

Enthalpy-entropy compensation in protein-ligand binding: An adversary phenomenon that can lead to a novel strategy in drug design

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Enthalpy-Entropy compensation is a ubiquitous phenomenon in protein-ligand binding. In lead optimizations, enthalpically favorable structural modifications are commonly associated with entropic penalties that negate the improvements in the binding affinity which would otherwise be gained due to the enthalpic benefits. Enthalpy-entropy compensation, therefore, renders many lead optimization cycles futile, and consequently, increases the time and the cost of drug discovery (i.e. an adversary phenomenon). Can we get anything good out of this adversary phenomenon? In this study, binding thermodynamic data of a series of thermolysin inhibitors that demonstrate remarkable enthalpy-entropy compensation are presented. Analysis of these data, however, can unexpectedly lead to a novel approach that can be used to improve protein-ligand binding. We herein describe this approach and how to apply it in the practice of medicinal chemistry.

MEDI 518

From “brick dust” APIs to new solid phases: Biopharmaceutical improvement through cocrystallization

Irma A. Millán-Mendoza[2]*, Brenda C. Félix-Sonda*[2]*, Jesús Rivera-Islas*[2]*, Jorge G. Domínguez-Chávez*[1]*, Dea Herrera-Ruiz*[2]*,* ***Hugo Morales-Rojas***[1]*, hugom@uaem.mx, Herbert Höpfl*[1]*. (1) Centro de Investigaciones Químicas, Universidad Autónoma del Estado de Morelos, Cuernavaca, Morelos 62209, Mexico (2) Facultad de Farmacia, Universidad Autónoma del Estado de Morelos, Cuernavaca, Morelos 62209, Mexico*

Active Pharmaceutical Ingredients (APIs) such as praziquantel, nitazoxanide, and other imidazol-related APIs are among the most widely prescribed compounds to treat diseases caused by protozoa and parasitic worms (helmints). These active molecules are also found among the so-called “brick dust” type due to their low intrinsic solubility, limitation that generally leads to the high-dose employed during their administration in

oral formulations. Strategies to improve solubility and dissolution rates of pharmaceutical solids explore new polymorphs, solvates, or salts. Recently, cocrystals have found acceptance in both industry and academia as useful alternatives to overcome these limitations. Herein, we present the supramolecular design, screening and synthesis of several new cocrystals obtained from praziquantel (a potent anthelmintic that does not contain any salt-forming functional group), nitazoxanide (a nitro-imidazole) and other benzimidazol related APIs, in combination with carboxylic acid derivatives as cocrystal formers. These solid-state forms were synthesized mainly by three methodologies: crystallization from saturated solutions, solid-solid phase transformation, and grinding. Solids were characterized by means of X-ray diffraction (powder and single-crystal), solid state NMR (CP-MAS), FTIR and thermal analyses. Measurements of solubility and dissolution rates afforded from small (2x) to large (30x) increases in comparison to the parent reference compounds. Our studies demonstrate the variety of solid-state phases that can be obtained via cocrystallization, displaying in many instances a favorable biopharmaceutical performance.

MEDI 519

SAR by HDX: Optimizing ligands for the estrogen receptor using hydrogen deuterium exchange (HDX) mass spectrometry

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Measuring the exchange of hydrogen for deuterium in the backbone amides of a protein by mass spectrometry has become a recognized method to study protein structure and dynamics. HDX provides valuable structural insight into protein-protein and protein-ligand interactions. One area where HDX has been underutilized, to date, is the application to small molecule SAR. Thus, we have studied the use of HDX to optimize ligands for the estrogen receptor. Selective estrogen receptor modulators, or SERMs, represent a class of therapeutic agents that demonstrate tissue selective pharmacology, i. e, they can mimic the effects of estrogen in some tissues but block estrogen in other tissues. All actions of estrogen and SERMs were thought to be mediated by a single ER until a second isoform, ERbeta, was discovered in 1996. The identification of ERbeta has added further complexity to the molecular origin of tissue selectivity for SERMs. In order to better probe the biological roles of ERalpha and ERbeta, we sought to identify highly selective ligands for each subtype as chemical tools to probe pharmacological action. This presentation will describe the novel use of HDX mass spectrometry to optimize ligands for the estrogen receptors.

MEDI 520

Dual-mode HDAC prodrug induces an active site covalent modification with subsequent inhibitor release

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Prodrugs are effective tools in overcoming drawbacks typically associated with drug properties in vivo. Protecting group strategies are readily utilized in prodrug design wherein a stimulus-responsive trigger is appended to the drug, rendering it inactive until where desired. This work details the development of a prodrug of the canonical HDAC broad-spectrum inhibitor suberoylanilide hydroxamic acid, SAHA. Although hydroxamic acids are utilized universally as metal binding groups in the development of metalloenzyme inhibitors, they are considered poor pharmacophores due in part to poor cell permeability, hydrolytic instability and susceptibility to glucoronidation, leading to reduced activity in vivo. Alkylation of hydroxamates has been shown to enhance overall aqueous stability, cellular permeability and will likely prevent glucoronidation issues. We have developed a prodrug of SAHA by appending a promoiety sensitive only to nucleophilic thiols to the hydroxamic acid we term SAHA-TAP (Thiol Activated Prodrug). Upon the incubation of SAHA-TAP with HDAC, the thiol moiety of a conserved cysteine residue in the catalytic pocket of HDAC nucleophilically attacks the promoiety, initiating a cascade reaction that leads to the release of the inhibitor SAHA. Proteomic mass spectrometry validates that the cysteine residue of interest is covalently tagged with the TAP promoiety, which subsequently leads to the release of SAHA. This strategy represents an original prodrug design with a dual-mode of action for HDAC inhibition with enhanced therapeutic potential.

MEDI 521

Response selective fluorescence sensors: Insight from the DFT calculations

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Primarily fluorescence has many biological and medical applications over the other analytical techniques due to its high sensitivity, versatility,high speed of response, and high spatial resolution. Currently, fluorescence sensing has been used to detect anions, cations, neutral species, enzymes and even some biological processes.

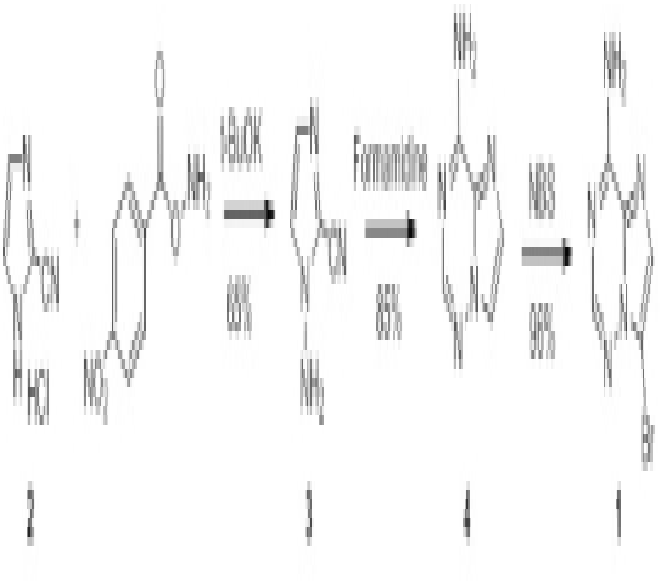
This poster will be focused on the detection of two analytes, $H^+$and Zn^{2+}, which are of interest in biology because the presence of abnormal quantities of these analytes may be indicative of disease. To study above two sensors (pH sensor and Zn^{2+} sensor) the calculations will be carried out by B3LYP functional and 6-31+G(d,p) basis set using Gaussian 09 software. Based on the DFT and TDDFT calculation results fluorescence “off-on” switching behavior of above systems will be determined. All the above mentioned systems will be compared with the experimental data.

MEDI 522

Process development of 4-amino-7-bromoimidazo[2,1-f][1,2,4]triazine

***Jingwei Zhu**, zhu_jingwei@pharmablock.com, Jun Mao, Qingning Shu, Minmin Yang, Xihan Wu. Department of Research, PharmaBlock (Nanjing) R&D Co. LTD., Nanjing, Jiang Su 210061, China*

A practical and efficient synthesis of 4-amino-7-bromoimidazo [2,1-*f*][1,2,4]triazine **1** was described. In the presence of potassium tert-butoxide, reaction of *O*-(4-nitrobenzoyl)hydroxylamine with 1*H*-imidazole-2-carbonitrile hydrochloride **2** yield aminoimdazole **3** , which reacted with formamidine to provide imidazo[2,1-*f*][1,2,4] triazin-4-amine **4 in high yield** . The following bromination of 4 afforded the title compound **1 in near quantatitive yield** . This new protocol was successfully applied to kilo scale production of compound **1** via three steps with 53% overall yield (**Fig. 1**).



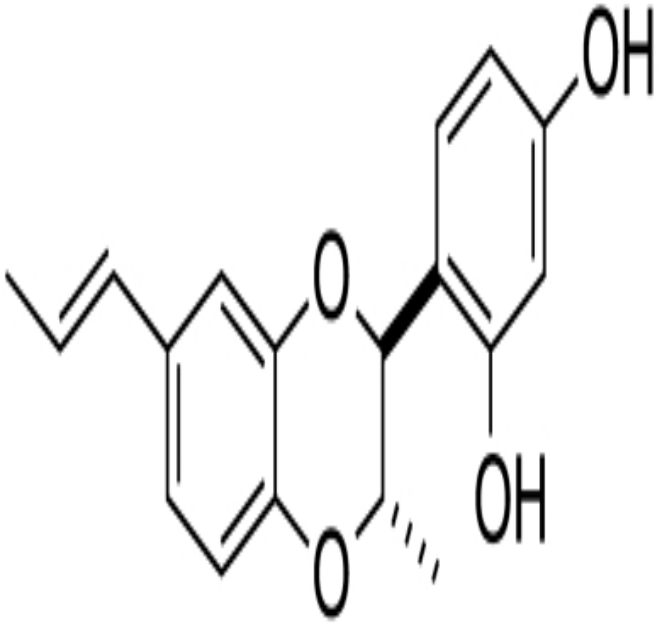
MEDI 523

Preparation of 1,4-benzodioxins via *hetero*-Diels-Alder reaction

***Guangming Xia**, jzhang2@csuchico.edu, Jinsong Zhang. Department of Chemistry and Biochemistry, California State University, Chico, Chico, CA 95929, United States*

1,4-Benzodioxin based natural products (see a sample structure below) possess various biological activities. An inverse electron demand *hetero*-Diels-Alder reaction

between *o*-quinones and enamines to prepare 1,4-benzodioxins has been developed. Characterization of different regioisomers was carried out with both X-ray diffraction chrystallography and density functional theory calculations. Biological screening and regioselective synthesis will also be reported.



MEDI 524

Flash chromatography gradient optimization to reduce solvent usage

Jack E Silver*, jsilver@teledyne.com, Paul C Bellinghausen, Nancy Fowler. Teledyne ISCO, Lincoln, NE 68504, United States*

Reducing the volume of solvent used during the purification of synthesized compounds likewise reduces both the direct cost of the solvents and that of solvent disposal. Moreover, the optimized gradients reduce the time required to purify the compounds. A synthetic chemist is generally interested in only a few compounds eluting from a column, so gradients can be optimized such that the valuable compounds are purified from other compounds rapidly using minimal solvent, while the impurities are minimally resolved from each other. Optimized linear gradients, step gradients, and isocratic runs are compared for resolution and sample load using a synthetic reaction.

MEDI 525

Reformulated transfection reagent with a single step protocol demonstrates high transfection efficiency with low cytotoxicity

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Transfection of nucleic acid in cells is a critical task for a variety of biological experiments. However, a protocol that requires no optimization, and consists of a simple single step transformation for transfection is lacking. We have reformulated the Xfect™ transfection reagent to prepare a single tube-single use format. This new format greatly simplifies the transfection protocol by allowing use of 1 to 10 ug of DNA with the same amount of transfection reagent which results in very high transfection efficiency in a variety of cell types.

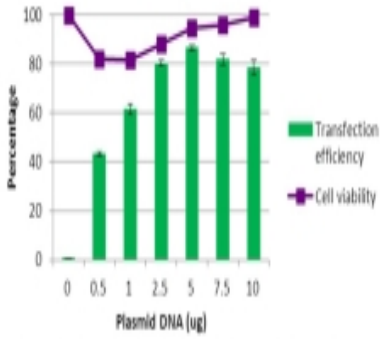

Figure 1. Hela cells plated on a 6-well plate were transfected with varying amounts of AcGFP expressing plasmid DNA. After 48 hours of incubation with the plasmid, cells were harvested and analyzed by flow cytometry to determine transfection efficiency. Cell viability was determined using dye exclusion assay (MTT assay)

We applied this high-efficiency transfection reagent to achieve single-step production of exosome-like nanoparticles in culture for the direct delivery of protein into mammalian cells. In addition, using this simplified protocol combined with an optimized lentiviral vector packaging mix resulted in a more robust and streamlined production of high-titer lentiviral particles for high efficiency gene delivery.

MEDI 526

Photoresponsive cross-linking oligodeoxyribonucleotides with a caged α-chloroaldehyde group

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Oligonucleotide analogues forming covalent bonds with complementary nucleotides in a sequence-specific manner under physiological conditions are of potential clinical and biological interest. In particular, photoresponsive oligonucleotide analogues which cross-link with complementary nucleotides using photo-irradiation as a trigger of the reaction have been developed to investigate and control gene functions without damaging living systems. In this study, we newly synthesized photo-cross-linking ODNs having a photoresponsive α-chloroaldehyde (PCA) group at the 5'-end of the ODN. The PCA group was comprised of an α-chloro-bis(2-nitrobenzyl)acetal group, which was converted to an α-chloroaldehyde group after 1 min of UV irradiation. Photo-cross-linking studies revealed that the oligonucleotide conjugates underwent sequence-selective cross-linking to target nucleotides in a time-dependent manner under physiological conditions.

N-Chlorosuccinimide was used for the chlorinationn of 6-oxohexanoyl benzoate. Because a-chloroaldehyde derivatives was inherently unstable and purification of the derivative from a mixture of the starting material and the bis-chlorinated derivative did not succeed, the crude product was used for introduction of the bis(2-nitrobenzyl)acetal group. After the protection of the carbonyl group using 2-nitrobenzyl alcohol, the benzoyl group was deprotected by methanolic ammonia. Under such basic conditions, the α-chloro-bis(2-nitrobenzyl)acetal group was stable. The phosphoramidite derivative was prepared using the standard procedures and was used for the synthesis of the α-chloroaldehyde-conjugated ODNs The specificity and efficiency of the cross-linking reaction of α-chloroaldehyde-conjugated ODNs were examined by gel electrophoresis and autoradiography. From the quantitative analysis of the cross-linking products, α-chloroaldehyde-conjugated ODNs can be seen to react with the target nucleotides having an adenine and a cytosine at the frontal position of the PCA.

MEDI 527

LC/MS analysis of choline and acetylcholine in living organisms using polymer-based cation IC column

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Choline is a water-soluble essential nutrient and is a component of phospholipids that are a major constituent of cell membranes. Choline is a precursor of the neurotransmitter acetylcholine found in living organisms. Their presence is related to several life phenomena and diseases, and it is important to be able to measure the ratio of these molecules.

Typically, reversed phase chromatography is used for analyzing choline and acetylcholine. Due to their hydrophilic nature, an ion-pair reagent is utilized for the mobile phase. A post-separation affinity column modified with choline oxidase / acetylcholine esterase, as well as, an electrochemical detector for monitoring hydrogen

peroxide are needed to enable highly sensitive analysis. In order to simplify the analysis, a new LC/MS method was studied.

An ion chromatography column, with polyvinyl alcohol base packing material modified with carboxyl groups, was used for the LC. The optimized eluent condition was 4 mM nitric acid / acetonitrile =70/30. The flow rate was 1.0 mL/min, and the column temperature was 30 °C. ESI-MS was used for detection.

The mixed standard solution of choline and acetyl choline (10 ng/mL of each) was analyzed. The retention times of choline and acetylcholine were 5.0 and 6.0 minutes, respectively. Both peak shapes were sharp with baseline separation, and the calibration curve was linear.

Our described LC/MS method should be more facile with higher selectivity than the previous method.

MEDI 528

Selective control of drug-to-antibody ratios (DAR) using continuous flow microreactor technology

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In the past decade, the field of antibody-drug conjugates (ADCs) has grown exponentially in terms of the synthesis, analysis, application, and efficacy of these molecules. ADCs are used to deliver a cytotoxic payload to an antigen-specific site in the human body. When the ADC arrives as its target site, the drug is cleaved from the linker and exposed to the cells of interest. It is crucial that an optimal drug-to-antibody ratio (DAR) is achieved due to formation of aggregates that often have differing pharmacokinetic, clearance, and efficacy profiles. Under conventional coupling conditions, this is often difficult to achieve; typically, a mixture of antibodies with a varied number of tags is generated. In this project, continuous flow microreactor technology is employed as a method to selectively control the number of tags on an antibody of interest. The superior heating and mixing capabilities of the microreactor help facilitate and expedite this process, which can be directly scalable and applicable to an industrial setting. This poster will present our initial conjugation results on batch and flow platforms, as well as linker strategies that can synergistically aid in the controllability of drug-to-antibody ratios in ADCs.

MEDI 529

Optimization of photolabeling and visualization of histone deacetylase 2 in cell lysates

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The ability to label specific proteins using photoactivatable small molecule probes with inherent affinity for explicit protein substrates, or photolabeling, has a vast array of applications. Examples range from target identification of bioactive small molecules to development of selective inhibitors for a particular protein. While these applications may be diverse in nature, they all require the ability for these probes to specifically crosslink to their targets and necessitate reliable detection of the resulting covalent protein-probe adduct. The aim of this research was to improve upon previous photolabeling studies of a group of hydroxamate-based photoreactive inhibitors/probes of histone deacetylases (HDACs) in HeLa cell lysates.

To improve upon the specificity and efficiency of photocrosslinking, we compared probes that used an aryl azide (ArN3) to probes that used a tetrafluoro aryl azide (4FArN3) for photoactivation and covalent addition to histone deacetylase. To improve upon the detection we compared probes that contained an alkyl azide substituent used to react with an alkyne conjugated tag, to probes that contained an alkyne substituent used to react with an azide conjugated tag. Several different tags were also explored: alkyne or azide conjugated biotin, IR dye, and His_6. We demonstrated that differences in the specificity and efficiency of covalent crosslinking of the ArN3 probes and the 4FArN3 probes to HDACs could be distinguished using SDS-PAGE coupled with fluorescent detection, and that these differences were affected by the wavelength of irradiation used to activate the probes. Furthermore, we found that incorporating an alkyne substituent on the probe to react with an azide detection tag decreased the amount of nonspecific photolabeling of proteins by the probes. Finally, we recognized that using an azide or alkyne conjugated biotin tag to react with the probes to be recognized by a streptavidin conjugated fluorophore can obscure interpretation of labeled proteins from cell lysates.

MEDI 530

Solution NMR investigations of weak interactions using peptidomimetic templates

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The thorough understanding of weak secondary interactions is of utmost importance for controlling molecular recognition processes, which play a pivotal role in chemistry and biology. Such weak chemical forces commonly act in a cooperative manner providing

an overall stability despite the weakness of each individual force involved. As part of the ongoing investigation we study the impact of an individual hydrogen bond using a β-hairpin peptide model system. This motif is common in proteins and is also interesting for drug discovery. β-Hairpins have been used as inhibitors of protein-protein interactions, for example [1]. By comparative investigation of strategically designed cyclic peptides, we describe the behavior of a specific weak interaction as part of a larger cooperative complex, in a biological-like context. Our combined computational and NMR spectroscopic ensemble analysis (NAMFIS) provides quantitative evidence for the influence of a specific, weak interaction force in a cooperatively folding β-hairpin[2].

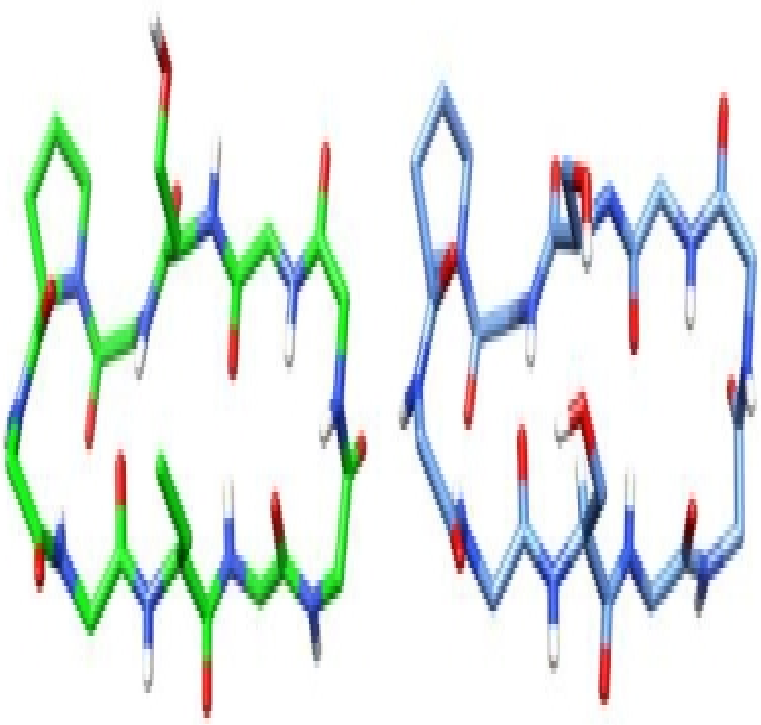

Figure 1. The folded conformations of reference peptide (green) and peptide capable of interstrand hydrogen bonding (blue). Amino acid side chains are shown for the interaction but are omitted for clarity in all other positions.

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MEDI 531

Retro–native chemical ligation rings in the changes for therapeutic peptides

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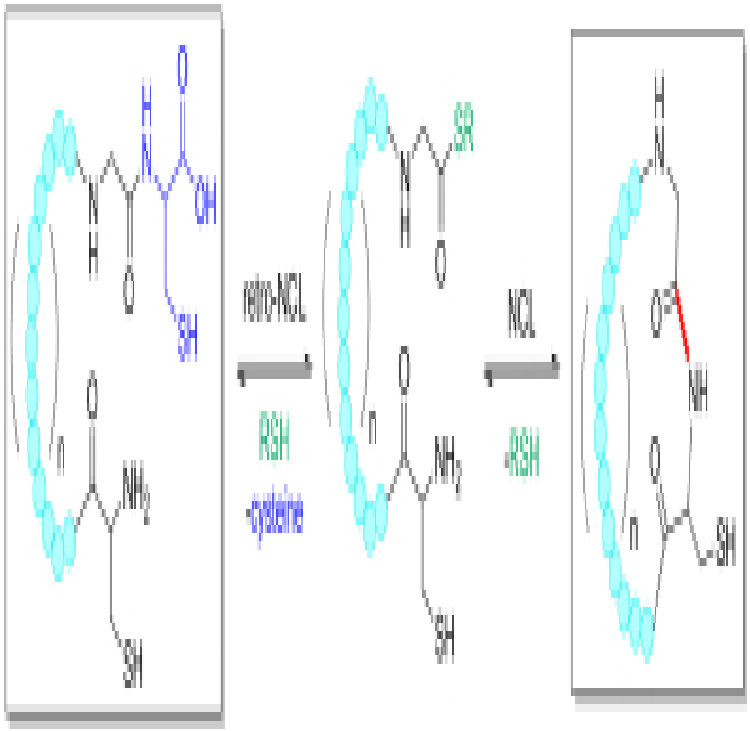
Circular versions of peptides with medicinal applications often exhibit improved biological activities and stability in comparison to their linear counterparts, but their synthesis can be costly, lengthy and complicated. Our group has developed a facile route for cyclising peptides using novel reactions based around native chemical ligation (NCL)[1]

NCL joins peptide fragments easily and under mild conditions when one contains a C-terminal thioester and the other an N-terminal free thiol (e.g. as cysteine). Using our chemistry, the required but otherwise problematic production of peptidyl thioesters is circumvented by adapting conditions for the *in situ* generation of intermediates that can be 'captured' with thiol additives — fragments terminating in Xaa–Cys (where Xaa = Gly, His or Cys) initially undergo retro–NCL to produce C–terminal thioesters. Thus a facile route to circularisation can be applied to linear peptides featuring cysteine at both their N– and C–termini (Figure 1).[1]



By applying our chemistry to several therapeutically interesting peptides we are investigating the mechanism, having produced unnatural amino acids, introduced various thiol additives and adjusted pH, and have demonstrated analogues of sunflower–trypsin inhibitor–1 inhibit kallikrein 5, an enzyme indicated in atopic dermatitis disease states like Netherton syndrome.[2]

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MEDI 532

Development of inhibitors of the Keap1/Nrf2 interactions

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The transcription factor nuclear factor-erythroid 2-related factor 2 (Nrf2) is one of the primary regulators of the adaptive stress response, because it regulates expression of phase II metabolic enzymes, such as glutathione *S*-transferases (GST), NADPH:quinone oxidoreductase 1 (NQO1), and UDP glucuronosyl transferases (UGTs). Disrupting the association of Nrf2 with its negative regulator Kelch-like ECH-associated protein-1 (Keap1) may aid in prevention or therapy of disease states made worse by electrophilic or oxidative stresses. Electrophilic small molecules are known to react with Keap1 Cys residues, which in turn leads to disruption of the Keap1/Nrf2 complex and activation of Nrf2; although some of these are clinically used therapeutics, many of these electrophiles are known to interact with other biological targets. It has been hypothesized that non-electrophilic activators of Nrf2 might be more selective and could be developed by directly inhibiting the Keap1/Nrf2 interaction. We have developed structure-activity relationships (SARs) of non-electrophilic Keap1/Nrf2 inhibitors to ascertain what functional groups are important for binding to Keap1 and, thus, activation of Nrf2. We have carried out both biochemical interaction assays, as well as cell-based assays of Nrf2 function. Using these SARs, we have enhanced the potency and physicochemical properties of known Keap1-Nrf2 inhibitors.

MEDI 533

Carvedilol lowers protein disulfides via the thioredoxin pathway in thiol oxidative stress

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Thiol oxidative stress has been implicated in a variety of cardiovascular diseases including heart failure. Thiol redox state (TRS) is expressed as the ratio of thiols to disulfides within the cell. Disulfides are reduced to their corresponding thiols by enzymes such as thioredoxin reductase (TrxR) and glutathione reducase (GR). Carvedilol is a β-blocker used to treat heart failure. It possesses antioxidant properties; however the exact mechanism of antioxidant effect is still unknown. H9c2 rat cardiomyocytes have been used as a model to demonstrate the protective effects of carvedilol against 1,3-bis-(2-chloroethyl)-1-nitrosourea (BCNU) induced thiol oxidative stress. BCNU significantly increased protein disulfide content; however, a combination of carvedilol and BCNU was able to lower protein disulfide content to control levels after a 2 hour treatment. This was correlated with an increase in TrxR activity. No change in GR activity was observed. Carvedilol may protect against thiol oxidative stress via the thioredoxin pathway.

MEDI 534

Synthesis of natural product FR33289 and analogs targeting inhibition of Mtb Dxr

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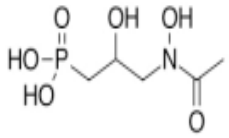
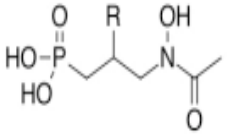
Tuberculosis (TB) is the second leading cause of death world wide due to an infectious agent. *Mycobacterium tuberculosis* (Mtb) is a rod-shaped bacillus, which causes this contagious disease. TB is estimated to infect one-third of the world's population. Due to increasing drug resistance and issues arising from HIV/TB co-infection, there is a significant need for new medications. One attractive target for drug design is the non-mevalonate pathway (NMP) of isoprene biosynthesis. The first enzyme of this pathway, 1-deoxy-D-xylulose-5-phosphate reductoisomerase (Dxr) has been the focus of recent efforts to find novel antitubercular agents. Natural product FR33289, an analog of antibiotic FR900098, inhibits Dxr from *Plasmodium falciparum*, the causative agent of malaria. We are interested in assessing the inhibition of Mtb Dxr by FR33289 and other β-substituted analogs. Here, we describe new synthetic routes to obtain FR33289 and related analogs as well as the evaluation of these compounds against Mtb and Mtb Dxr.



FR33289



β-substituted inhibitors