The 56th Annual

MIKI Medicinal Chemistry

Meeting-in-Miniature

April 6th – 8th, 2018

University of Illinois at Chicago

Chicago, Illinois

THANK YOU TO OUR MIKI 2018 SPONSORS!!

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Welcome to MIKI 2018!

On behalf of the Department of Medicinal Chemistry & Pharmacognosy at the University of Illinois at Chicago, and this year's organizing committee, we would like to welcome you to Chicago and the 56th Annual MIKI meeting! This weekend is full of wonderful opportunities to renew friendships and network in a dynamic and collaborative atmosphere. We are excited to host such a brilliant and diverse group of talented scientists from the Midwest. We thank you for your scientific contributions, and we are looking forward to a great scientific program.

Since 1966, the MIKI meeting has a long standing tradition of being honored with a keynote lecture by a talented member of the medicinal chemistry community. This year, we are proud to welcome Dr. Michelle Arkin from University of California San Francisco as our keynote speaker.

This year, we are proud and excited to introduce a few additions to the MIKI meeting! ***We would first like to warmly welcome the University of Wisconsin at Madison to our meetings****. As one of the top Pharmacy schools in the nation, we are excited to form new relationships and develop even stronger networks of scientists in the Midwest. Secondly, we are* ***thrilled to provide a new segment of career development to the meetings****. By hosting career panels for students, we hope to formulate new networks and introduce fresh ideas of careers that MIKI scientists can pursue.*

Last, but not least, we wish to thank all members of the Organizing Committee, the Faculty Chairs, and our sponsors for the immense amount of hard work and support in making MIKI 2018 a huge success.

We hope this weekend's proceedings and events will be both an enjoyable and productive experience with an opportunity to meet and interact with scientists, researchers, friends, colleagues, sponsors, and exhibitors.

MIKI CHICAGO 2018

Sincerely,

Rachel Knopp, Sue Lee, Kyle Mangano, Taha Taha
2018 MIKI Executive Committee

Dr. Terry Moore and Dr. Laura Sanchez
2018 MIKI Faculty Sponsors

Rhea Bovee, Alanna Condren, Lauren Gutgesell, Tanja Florin, Vanessa Nepomuceno, Brian David, Emily Rue, Loruhama Delgado-Rivera
2018 MIKI Committee Chairs

BRIEF HISTORY OF MIKI

*The first Medicinal Chemistry Meeting-in-Miniature, informally known as "MIKI", was held at the University of Iowa in 1963 and was organized by the founders listed below. Since 1966, the Departments of Medicinal Chemistry & Pharmacognosy of the Universities of **M**innesota, **I**llinois, **K**ansas and **I**owa have alternately organized and hosted these annual regional meetings. Starting this year, we are pleased to welcome Wisconsin to our group. The MIKI meetings are organized by graduate students of the host university and have been one of the most successful regional meetings in the field of medicinal chemistry. Graduate students get a wonderful opportunity to present their work as well as share and exchange ideas with faculty and students from all four universities.*

Previous MIKI Keynote Speakers

Year	Host	Speaker	Affiliation
2017	Minnesota	Dr. Uttam Tambar	U. Texas
2016	Iowa	Dr. Amy H. Newman	NIH-NIDA
2015	Kansas	Dr. Bruce Roth	Genetech
2014	Illinois	Dr. Paul A. Wender	Stanford University
2013	Minnesota	Dr. Marvin J. Miller	University of Notre Dame
2012	Iowa	Dr. Heidi E. Hamm	Vanderbilt University
2011	Kansas	Dr. Dennis C. Liotta	Emory University
2010	Illinois	Dr. Thomas Hudlick	Brock University
2009	Minnesota	Dr. Dale Boger	Scripps Research Institute
2008	Iowa	Dr. Daniel Kahne	Harvard University
2007	Kansas	Dr. Albert Padwa	Emory University, Atlanta
2006	Illinois	Dr. William Fenical	U. California, San Diego
2005	Minnesota	Dr. Christopher Lipinski	Pfizer Pharmaceuticals
2004	Iowa	Dr. Kenner Rice	National Institutes of Health
2003	Kansas	Dr. C. Dale Poulter	U. Utah
2002	Illinois	Dr. Richard B. Silverman	Northwestern University
2001	Minnesota	Dr. Andrew Hamilton	Yale University
2000	Iowa	Dr. Michael Marletta	U. Michigan
1999	Kansas	Dr. Roger M. Friedinger	Merck Research Laboratories
1998	Illinois	Dr. Richard A. Lerner	Scripps Research Institute
1997	Minnesota	Dr. John Montgomery	Biocryst Pharmaceutical, Inc.
1996	Iowa	Dr. David Nichols	Purdue University
1995	Kansas	Dr. Paul Anderson	Dupont Merck Pharmaceutical
1994	Illinois	Dr. Arthur Patchett	Merck Research Laboratories
1993	Minnesota	Dr. Daniel Rich	U. Wisconsin, Madison
1992	Iowa	Dr. Laurence Hurley	U. Texas, Austin
1991	Kansas	Dr. Julius Rebek	Mass. Institute of Technology
1990	Illinois	Dr. Koji Nakanishi	Columbia University
1989	Minnesota	Dr. John Katzenellenbogen	U. Illinois, Urbana-Champaign
1988	Iowa	Dr. Carl Djerassi	Stanford University
1987	Kansas	Dr. William Roush	Indiana University
1986	Illinois	Dr. Joseph Fried	U. Chicago
1985	Minnesota	Dr. David Triggle	SUNY Buffalo
1984	Iowa	Dr. Alan Karitzky	U. Florida
1983	Wisconsin	Dr. Paul Bartlett	U. California, Berkeley
1982	Kansas	Dr. Henry Rapoport	U. California, Berkeley
1981	Illinois	Dr. Harry Wasserman	Yale University
1980	Minnesota	Dr. Eugene Jorgensen	U. California, San Francisco
1979	Iowa	Dr. Alan Sartorelli	Yale University
1978	Kansas	Dr. Albert Meyers	Colorado State University
1977	Illinois	Dr. Heinz Floss	Purdue University
1976	Minnesota	Dr. Donald Jerina	National Institutes of Health
1975	Iowa	Dr. Everett May	National Institutes of Health
1974	Kansas	Dr. Marjorie Horning	Baylor University
1973	Illinois	Dr. Arnold Brossi	Hoffmann-LaRoche
1972	Minnesota	Dr. Gertrude Ellion	Burroughs-Wellcome
1971	Iowa	Dr. Bernard Belleau	U. Ottawa
1970	Kansas	Dr. Corwin Hansch	Pomona College
1969	Illinois	Dr. Everett Maynert	U. Illinois
1968	Minnesota	Dr. Bernard Baker	U. California, Santa Barbara
1967	Iowa	Dr. Julius Axelrod	National Institutes of Health
1966	Kansas	Dr. Richard Schowen	U. Kansas

"Meeting in Miniature"

MIKI Medicinal Chemistry Conference 2018

FRIDAY, APRIL 6th, 2018

4:30-6:30 pm	Registration & Check-In *The Westin Chicago River North* *320 North Dearborn Street, Chicago, IL, 60654*
7:00-7:30 pm	Registration at River Roast *315 N. LaSalle Dr., Chicago, IL, 60654*
7:00-10:00 pm	Social Mixer at River Roast

SATURDAY, APRIL 7th, 2018

7:00-8:00 am	Continental Breakfast, Poster Setup, Registration *College of Pharmacy (COP) Lobby* *833 S. Wood St., Chicago, IL 60612* ***Poster Pick up (if ordered at MakeSigns Chicago)*
8:00-8:20 am	Opening Remarks/Welcome *Dr. Joanna Burdette, Associate Dean for Research, UIC College of Pharmacy* *COP, Rm 134-1*

Session 1 Moderator: Lauren Gutgesell

8:20-8:40 am	**Rhea Bovee**, Douglas Thomas lab, UIC *Nitric Oxide: An Epigenetic Signaling Molecule Mediates DNA Demethylation in Triple Negative Breast Cancer via Alterations to TET and 5hmC levels*
8:40-9:00 am	**Shaofei Ji**, Natalia Tretyakova lab,UMN *Reversible DNA-protein Cross-linking at 5-Formylcytosine and its Effects on Replication and Transcription*
9:00-9:20 am	**Kelsey E. Knewtson**, Blake Peterson lab, KU *Fluorescent Sensors of Cellular Peroxynitrite*
9:20-9:40 am	**Victoria Parker**, Michael Duffel lab, UI *The Potential for Disruption of Estrogen Sulfation and Adipocyte Differentiation by Hydroxylated Metabolites of Common Airborne Polychlorinated Biphenyls*
9:40-10:00 am	Coffee Break & Vendor Exposition *COP Lobby*
10:00-10:50am	Poster Session I (Even Posters) *COP Lobby*

Keynote Address

11:00-12:00 pm	Webster-Sibilsky Keynote Lecture *Dr. Michelle Arkin, Professor, UCSF* ***"Tackling Challenging Targets, a Biophysical Perspective"***
12:15–12:45 pm	MIKI Faculty Meeting *COP Room: 2 North Suites*
12:15-1:00 pm	Group I Lunch Group II: Industry Panel *(B32)*
1:00-1:45pm	Group I: Other Panels 1 *(Sci. Com.: B36, Govt.: 111, Recruiting: 231)* Group II: Lunch
1:45-2:30pm	Second Round of Panels *(Industry: B32, Sci Com: B36, Govt: 111 Recruiting: 231)*
2:45-3:35 pm	Poster Session 2 (Odd Posters)

Session 2 Moderator: Jose Colina

3:45-4:05 pm	**Ka Yang**, Weiping Tang lab, UW-Madison *Targeting Proprotein Convertase Subtilisin/kexin Type 9 (PCSK9) Related Lipid Regulation Pathways by Novel Small Molecules*
4:05-4:25 pm	**Ben Richardson**, Terry Moore lab, UIC *Replacement of a Napthalene Scaffold in Keap1/Nrf2 Inhibitors*
4:25-4:45 pm	**Joseph Buonomo**, Courtney Aldrich lab, UMN *Recent Advances in Redox Recycling Reaction Design*
4:45-5:05 pm	**Samuel E. Williamson**, Thomas Prisinzano lab, KU *Modular Total Synthesis Approach Toward Salvinorin A Inspired Opioids*
6:00 – 7:00 pm	Faculty Cocktail Hour at Crystal Gardens-Navy Pier *700 E. Grand Ave, Chicago, IL, 60611*
6:30-7:00 pm	Cocktail Hour at Crystal Gardens-Navy Pier
7:00-10:00 pm	Banquet at Crystal Gardens- Navy Pier

SUNDAY, APRIL 8th, 2018

8:00-9:00 am	Continental Breakfast *COP Lobby*

Session 3 Moderator: Rachel Knopp

9:05-9:25 am	**Ernane C. de Souza**, Robert Kerns lab, UI *Novel Direct Allosteric Inhibitors of Thrombin*
9:25-9:45 am	**Shaurya Chanana**, Tim Bugni lab, UW-Madison *Natural Product Discovery Using Planes of Principal Component Analysis in R*
9:45-10:05 am	**Camila M. Crnkovic**, Jimmy Orjala lab, UIC *Mass Spectrometry-Based Metabolomics for the Discovery of New Natural Products from Cyanobacteria*
10:05-10:25 am	**Clifford Csizmar**, Carston Wagner lab, UMN *Leveraging Affinity and Avidity to Control Intercellular Interactions*
10:25-10:45 am	Coffee Break & Vendor Exposition *COP Lobby*

Session 4 Moderator: Marianne Palczewski

10:45-11:05 am	**Sahishna Phaniraj**, Blake Peterson lab, KU *Nanodisc Technology for Studies of Small Molecule-Membrane Interactions*
11:05-11:25 am	**Jacob A. Poliskey**, Kevin Rice lab, UI *Double Stranded mRNA Polyplexes for In Vivo Gene Delivery*
11:25-11:45 am	**Mary Choules**, Guido Pauli lab, UIC *The Impact of Residual Complexity on Drug Discovery – The Case of Rufomyazine and Rufomycin*
11:45-12:05 pm	**Sara K. Coulup**, Gunda Georg lab, UMN *Total Synthesis of Metabolically Stabilized Analogs of Pironectin*
12:05-12:15 pm	Awards/Closing Remarks
12:15-12:30 pm	Pick up Boxed Lunches *COP Lobby*

Thank you for attending! We wish you a safe journey home.

From: 2018 MIKI organizing committee

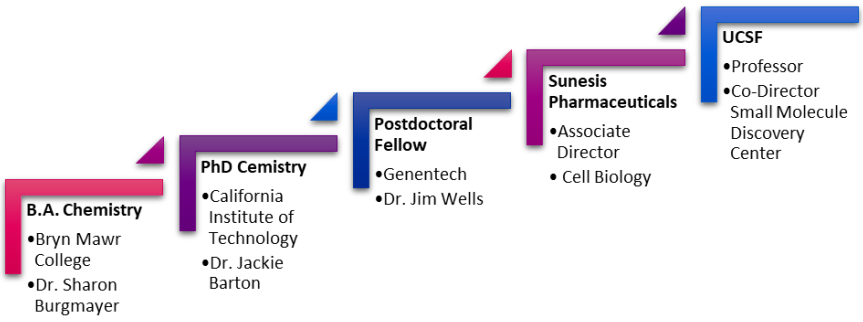
KEYNOTE LECTURE

The University of Illinois at Chicago: College of Pharmacy & Department of Medicinal Chemistry and Pharmacognosy present

The 16th ANNUAL WEBSTER-SIBILSKY LECTURESHIP

Dr. Michelle Arkin, Ph.D.

Dr. Arkin is an exceptional scientist, holding many achievements in the fields of medicinal chemistry and chemical biology: developing chemical probes to elucidate mechanisms of neurodegeneration, cancer, and parasitic diseases; advancing high-throughput screening modalities; pioneering the high-content imaging field; and extensive contributions to challenging targets and orphan diseases. Her research encompasses the realms of medicinal chemistry, chemical-biology, biophysics, biochemistry and spectroscopy. She currently is supported by 13 external funding sources, and she holds 10 patents. Dr. Arkin has co-authored over 90 publications (several of which hold over 1000 citations) and has given over 30 national and international talks.



"Tackling Challenging Targets, a Biophysical Perspective"

Protein-protein networks are critical regulators of health and disease, yet are widely considered "undruggable" or, at best, "challenging." Our long-term goal is to understand the features that lead to drug-like binding to protein interfaces and to develop small-molecule modulators of protein interaction networks. For example, the AAA ATPase p97/VCP is hypothesized to couple with more than two dozen 'adaptor' proteins to support protein degradation and trafficking in multiple organelles. We are developing tool compounds that modulate p97 activity through multiple mechanisms of action. We are also utilizing the disulfide-trapping technique to discover novel inhibitors and stabilizers of protein-protein interactions.

Scientific Program:
University of Illinois at Chicago: College of Pharmacy
833 S. Wood St.
Chicago, IL 60612

Lodging/Registration:
Westin River North Chicago
320 N. Dearborn St.
Chicago, IL 60654

THINGS TO KNOW

- *Public transportation can take you anywhere! Our College of Pharmacy is right off the Polk stop on the Pink Line*
- *Use ride share apps like Uber and Lyft to get around. Promotional codes available for new users!*
- *Use parking apps like ParkWhiz or SpotHero to find affordable parking*

Friday Night Reception at the River Roast

315 N. LaSalle Dr., Chicago, IL 60654

Our Friday night welcome reception is one block away from the MIKI hotel at River Roast. Located on the Chicago River, River Roast offers dramatic city and water views from every seat - inside and out. The contemporary American tavern fare is satisfying and soul-warming. With one of the best patios in the city, River Roast is a gathering place to connect with friends, colleagues and collaborators.

Saturday Night Banquet at the Navy Pier Crystal Gardens

700 E. Grand Ave., Chicago, IL 60611

The Crystal Gardens at Navy Pier is a beautiful indoor, one-acre botanical garden. This six-story glass atrium with a 50-foot arched ceiling holds over 80 live palm trees, lush foliage, hanging “twinkle lights” and dancing “leapfrog” fountains.
Located in Chicago’s Navy Pier, attendees will get a chance to enjoy the lake front and the magnificent Chicago skyline. The night starts with a cocktail hour at 6 PM followed by banquet dinner featuring full buffet at 7 PM.

ORAL PRESENTATIONS SESSION I

Nitric Oxide: an Epigenetic Signaling Molecule Mediates DNA Demethylation in Triple Negative Breast Cancer via Alterations to TET and 5hmC levels

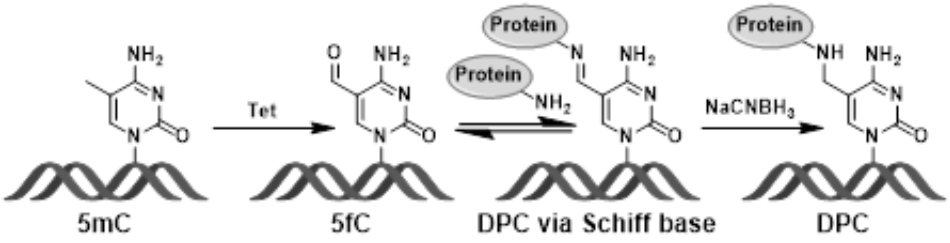
Rhea Bovee[1], Vy Pham[1], Jenna Fernadez[2], Chris Seiler[2], Natalia Tretyakova[2], Douglas D. Thomas[1]
[1]Department of Medicinal Chemistry, University of Illinois at Chicago, [2]Department of Medicinal Chemistry, University of Minnesota

The aim of this project was to identify the effect of NO signaling as a driver in triple negative breast cancer (TNBC) through alterations to several epigenetic mechanisms. Higher nitric oxide synthase 2 (NOS2) has been correlated as a negative prognostic indicator in several solid forming cancers including TNBC. However, the molecular mechanisms driven by higher nitric oxide levels is poorly understood. Previously, our lab discovered NO as an epigenetic regulator through inhibition of histone demethylases and globally alters 30+ histone post-translational modifications. The alterations to the epigenetic landscape caused by exposure to NO leads to changes in gene expression of several thousand genes, ultimately leading to the promotion of oncogenesis. To further investigate the impact NO has as an epigenetic regulator we observed the role of NO mediated alterations to active DNA demethylation. Ten Eleven Translocation (TET) is responsible for active DNA demethyaltion and is a mononuclear non-heme iron dioxygenase. A well studied role of NO signaling is its ability to inhibit the catalytic activity of mononuclear non-heme iron dioxygenases. Therefore we observed the role of NO to alter protein expression of all three TET isoforms and the three methyl-cytosine adducts TET produces 5-hydroxymethylcytosine (5hmC), 5-formlymethylcytosine (5fC), and 5-carboxymetylcytosine (5caC). Through mass spectrometry analysis of TET products we were able to observe global changes in TET products after exposure to NO. It was also observed that NO-mediated alterations of protein expression and was isoform specific. Enzyme activity analysis and electron paramagnetic resonance (EPR) provided key evidence that nitric oxide can inhibit TET activity. Based on these observations, further exploration of the role 5hmC has in gene regulation may provide TET as a therapeutic target or 5hmC as an early detection biomarker for TNBC.

Reversible DNA-protein Cross-Linking at 5-formylcytosine and its Effects on Replication and Transcription

Shaofei Ji, and Natalia Tretyakova
Department of Chemistry, Department of Medicinal Chemistry, University of Minnesota, Minneapolis, Minnesota 55455

5-Formylcytosine (5fC) is an endogenous DNA modification frequently found within regulatory elements of mammalian genes. Although 5fC is an oxidation product of 5-methylcytosine (5mC), the two epigenetic marks show distinct genome-wide distributions and protein affinities, suggesting that they have different roles in epigenetic signaling. In this work, we show that 5fC bases in DNA readily form Schiff base conjugates with Lys side chains of nuclear proteins (e.g. histones) in human cells. These covalent protein-DNA complexes are reversible ($t_{1/2}$, 1.8 h), suggesting that they may play a role in transcriptional regulation and chromatin remodeling. On the other hand, 5fC mediated DNA-protein cross-links, if present at replication forks or actively transcribed regions, may interfere with DNA replication and transcription. 5fC induced DNA-protein and DNA-peptide cross-links were stabilized by $NaCNBH_3$ treatment, and their effects on DNA replication and transcription was investigated using site-specifically modified substrates. We found that DNA-protein cross-links completely block DNA and RNA polymerase *in vitro*. In contract, short DNA-peptide cross-links were bypassed by human TLS polymerases and T7 RNA polymerase, although with reduced efficiency and inducing C→T and deletion mutations. We also employed RT-PCR and LC-MS/MS base strategy to investigate the transcriptional mutagenesis and efficiency of the DNA-peptide cross-links by RNAP II in human cells. We observed a moderate decrease of the relative transcription bypass efficiencies in the nucleotide excision repair (NER) deficient cells as compared with in the NER-complemented cells, suggesting that NER might be playing a role in removing these lesions in cells.



Fluorescent Sensors of Cellular Peroxnitrite

Kelsey E. Knewtson, Digamber Rane, and Blake R. Peterson
Department of Medicinal Chemistry, The University of Kansas Lawrence, KS 66045

Peroxynitrite is a highly reactive natural oxidant derived from superoxide and nitric oxide. This oxidant plays important roles in normal human physiology, and it is deployed by macrophages as a cytotoxin against foreign pathogens. This reactive nitrogen species can also contribute to disease when elevated levels manifest chronic cytotoxic effects. Although peroxynitrite can affect numerous biomolecules within cells, it is thought to be particularly deleterious to the extensive membrane network of the endoplasmic reticulum (ER). To probe the effects of this oxidant on this critical subcellular compartment, we synthesized fluorescent sensors comprising an ER-targeted profluorophore linked to phenols designed to undergo oxidative cleavage by peroxynitrite. Comparison with other oxidants demonstrated that these sensors can react selectively with peroxynitrite to release a highly fluorescent fluorinated rhodol product that associates with membranes of the ER. To optimize these sensors, we are measuring the kinetics of this reaction in buffer solutions and exploring structure-property relationships for detection of cellular peroxynitrite by flow cytometry and confocal microscopy. Optimized sensors can detect endogenous peroxynitrite in RAW264.7 macrophage cells after mild stimulation with synthetic beads. This approach provides highly sensitive tools for studies of this important inflammatory mediator in living mammalian cells.

The Potential for Disruption of Estrogen Sulfation and Adipocyte Differentiation by Hydroxylated Metabolites of Common Airborne Polychlorinated Biphenyls

Victoria S. Parker [1], Edwin Squirewell[1], Hans-Joachim Lehmler[2], Larry W. Robertson[2], Aloysius J. Klingelhutz[3], Michael W. Duffel[1]
[1]Department of Pharmaceutical Sciences and Experimental Therapeutics, College of Pharmacy and [2]Department of Occupational and Environmental Health, College of Public Health, and [3]Department of Microbiology, Carver College of Medicine, University of Iowa, Iowa City, IA 52242

Exposure to polychlorinated biphenyls (PCBs) has been linked to adverse health effects and diseases such as endocrine disruption, diabetes, cancer and others. PCBs have been found in indoor air of older buildings and as inadvertent byproducts in the manufacture of paints and pigments. The lower chlorinated PCBs, those with fewer than 5 chlorine atoms, are readily metabolized to form hydroxylated PCBs (OH-PCBs) that are further converted to PCB-sulfates in reactions catalyzed by cytosolic sulfotransferases (SULTs). Estrogen sulfotransferase (SULT1E1) helps to control intracellular levels of estradiol through formation of estradiol sulfate. SULT1E1 has also been shown to play a vital role in adipocyte differentiation. We hypothesized that lower chlorinated OH-PCBs inhibit human SULT1E1 and thereby inhibit human adipocyte differentiation. Using purified recombinant human SULT1E1, we found that 4'-OH-PCB 3, 4'-OH-PCB 8, 4-OH-PCB 11, 4'-OH-PCB 25, and 4-OH-PCB 52 were inhibitors of the sulfation of 7.0 nM estradiol. Moreover, 4-OH-PCB 11 and 4'-OH-PCB 25 were the most potent inhibitors with IC_{50} values of 7.2 nM and 7.3 nM, respectively. The least potent inhibitor was 4'-OH-PCB 3, with an IC_{50} of 1300 nM. 4-OH-PCB 11 inhibited the sulfation of estradiol in the cytosol of both pre-adipocytes and fully differentiated adipocytes. Using an immortalized human adipocyte model, pre-adipocytes were exposed for 72 hours to 1 and 10 □M concentrations of the above OH-PCBs and the known potent SULT1E1 inhibitor Triclosan. After an 11-day differentiation period, lipid accumulation (assessed by AdipoRed staining) was decreased at the 10 □M doses of Triclosan and all of the OH-PCBs with the exception of 4'-OH-PCB 3. Studies on OH-PCB-dependent changes in expression of key cellular protein markers of adipogenesis are in progress. [Supported by NIH P42 ES013661 and R25 GM058939, and by a William Townsend Porter Fellowship from the American Physiological Society].

ORAL PRESENTATIONS SESSION II

Targeting Proprotein Convertase Subtilisin/kexin Type 9 (PCSK9) Related Lipid Regulation Pathways by Novel Small Molecules

Ka Yang, Haibo Xie, Gabrielle Winston-McPherson, Qing Yu, Donnie Stapleton, Mark P Keller, Alan D. Attie and Weiping Tang

Pharmaceutical Sciences Division, School of Pharmacy, University of Wisconsin, Madison WI 53705

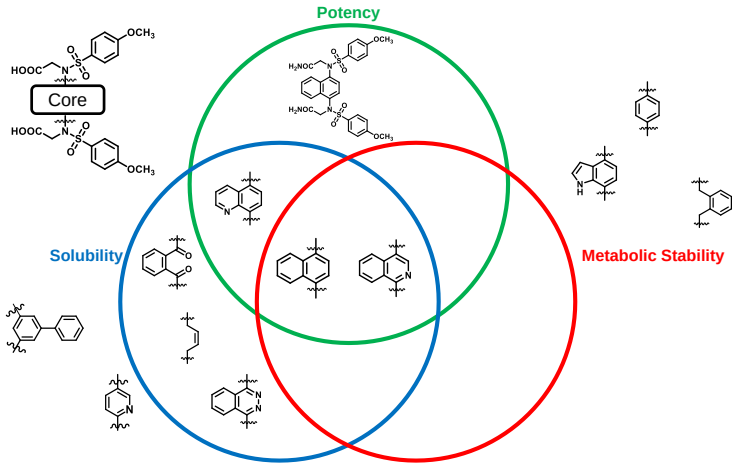
Cardiovascular diseases (CVDs) are one of the leading causes of death in the United States, and worldwide. High levels of low-density-lipoprotein-cholesterol (LDL-C) significantly increase the risk of CVDs. Statins are the major therapeutic approach to lower LDL-C, but >20% of patients are resistant to statin-based therapy. Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9), a circulating protein that binds to the LDL receptor (LDL-R), and targets it for degradation, is a novel therapeutic target. The combination of statin and anti-PCSK9 mAbs reduces LDL-C by 40-60% in statin-resist patients. However, this therapy is cost-prohibitive for all patients, thereby motivating development of small molecules inhibitors of PCSK9. We have identified a class of heterocyclic compounds that can selectively reduce PCSK9 protein levels in a cell-based screen, resulting in increased LDL-R. We have improved the potency and other pharmacological properties by structure-activity-relationship(SAR) studies, resulting in highly potent molecules (IC50 of 0.5 nM). We used RNA-seq based transcriptomics and an LC-MS based proteomics study to investigate the mechanism by which the compounds lead to a suppression of PCSK9. Our results suggest that the novel molecules act by inhibiting the transcriptional activation of the PCSK9 gene. Current efforts are aimed at discovering the direct molecular targets by functional probes based on our potent compound.

Replacement of a Napthalene Scaffold in Keap1/Nrf2 Inhibitors

Benjamin G. Richardson, Atul D. Jain, Phillip R. Lazzara, Brian P. David, Haranatha Potteti, Chandra Tamatam, Ewelina Choma, Kornelia Skowron, Katherine Dye, Yue-Ting Wang, Aleksej Krunic, Sekhar P. Reddy, Terry W. Moore

Department of Medicinal Chemistry, University of Illinois at Chicago, Chicago IL 60612

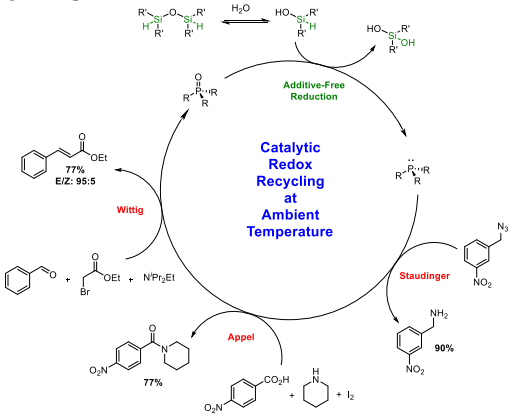
Small molecules that activate Nrf2 present potential therapeutics for prevention and treatment of chronic oxidative stress and inflammatory disorders. Nrf2 is negatively regulated by Keap1 through targeted polyubiquitination and degradation via the 26S proteosome. Competitive inhibition of the Keap1/Nrf2 interaction through targeting Keap1 has been shown to activate Nrf2. Previously, we and others have described a series of naphthalene-based Nrf2 activators, but the 1,4-diaminonaphthalene scaffold is not ideal as a drug-like scaffold. Paying particular attention to aqueous solubility, metabolic stability, and potency, we modified a naphthalene-based non-electrophilic Nrf2 activator to give a series of non-naphthalene and heterocyclic scaffolds. We found that a 1,4-isoquinoline scaffold provides similar potency, stability, and solubility to previously reported naphthalene-based compounds.



Recent Advances in Redox Recycling Reaction Design

Joseph A. Buonomo, Courtney C. Aldrich
Department of Chemistry, Department of Medicinal Chemistry, University of Minnesota, Minneapolis, Minnesota 55455

Silanes are well-known to reduce phosphine oxides chemoselectively in the presence of a variety of reactive functional groups. This impressive chemoselectivity has garnered their use in the design of catalytic reactions that utilize phosphines, such as the Wittig, Staudinger, Appel, and Mitsunobu reactions. Generally, the exploration of silane materials in these processes is limited in scope, and the reaction is often limited by the reactivity of the silane reagent, requiring the input of heat and/or the use of additives to facilitate catalysis. In order to improve these processes, we have begun exploring a new class of silane reducing agents containing a semi-stable hydrido-disiloxane moiety. These silanes are easy to access through a one-pot methodology we recently disclosed involving mono-chlorination followed subsequent hydrolysis from commercially-available reagents. Of these reagents, 1,3-diphenyl disiloxane is an ideal reagent that is highly chemoselective, unreactive with cyano, ester, nitro, alkynyl, and aldehyde moieties. This novel reactivity was then exploited in additive-free phosphorus redox recycling reactions to afford catalytic Wittig, Appel, and Staudinger reactions which undergo at ambient temperature, a first-in-class for phosphorus redox recycling.



Modular Total Synthesis Approach Toward Salvinorin A Inspired Opioids

Alexander M. Sherwood, Samuel E. Williamson, Rachel S. Crowley, Thomas E. Prisinzano
Department of Medicinal Chemistry, School of Pharmacy, the University of Kansas, Lawrence, KS, USA

The natural product salvinorin A isolated from the leaves of the *Salvia divinorum* plant is a unique non-nitrogenous opioid receptor ligand and has atypical pharmacology compared to classical morphine-derived opioids. Drugs inspired by and built upon this natural product scaffold yield valuable probes for understanding opioids and are potentially capable of circumventing some of the known abuse liabilities associated classical alkaloid opioids. As such, an adaptable total synthesis approach of designer opioids based upon the salvinorin A scaffold is desirable and potentially valuable for the development of analgesics with reduced abuse liability and drug abuse pharmacotherapies. Our total synthesis approach permits functionality to be introduced deliberately within the molecules with the goal of systematically exploring their activity by in vitro studies at opioid receptors and ultimately in animal models of pain and addiction. We have designed molecules able overcome potential shortcomings in salvinorin A, such as rapid metabolism, so that they may be useful for clinical pharmacotherapies. The desired chemical scaffolds have been accessed by a straightforward approach to bisenone 14-membered macrolides that are capable of undergoing a transannular Michael reaction cascade to assemble the tricyclic neoclerodane core representative of salvinorin A. The compounds produced provided access to otherwise unattainable molecular features on salvinorin A by semisynthesis on plant-derived material. The tricyclic neoclerodane core has been synthesized with manipulations targeting key features that are required for activity and an array of salvinorin A inspired structures was accessed.

ORAL PRESENTATIONS SESSION III

Novel Direct Allosteric Inhibitors of Thrombin

Ernane C. de Souza, Ioana Craciun, Chaitanya A. Kulkarni and Robert J. Kerns*
Department of Pharmaceutical Sciences and Experimental Therapeutics, Division of Medicinal & Natural Products Chemistry, College of Pharmacy, The University of Iowa, Iowa City, IA

Thrombin is a central enzyme involved in blood coagulation, being an important target for preventing thrombotic disorders such as myocardial infarction and stroke. Heparin, a widely used anticoagulant, catalyzes inhibition of thrombin indirectly by activating antithrombin. However, clinical utility of heparin is plagued by significant adverse reactions, most of which are associated with the chemical nature of heparin itself. Heparin is a heterogeneous polysulfated polysaccharide that nonselectively bind many glycosaminoglycan-binding proteins. Based on the molecular interactions observed in heparin-binding proteins, a series of charge-reduced modified heparin derivatives substituted with aromatic moieties in place of the N-sulfo groups were synthesized and each derivative was evaluated for activity towards direct thrombin inhibition. Modified-heparin derivatives possessing 3-(4-hydroxyphenyl)propionate moieties in place of N-sulfo groups were found to be potent, dose-dependent, direct inhibitors of thrombin. Tridimensional distribution of negative charges or degree of sulfation played a secondary role for inhibition. Taking these initial findings into consideration, it was postulated that similarly modified N-arylacyl O-sulfonated aminoglycosides might function as low molecular weight structural direct thrombin inhibitors. In an effort to N-acylate aminoglycosides, byproducts from the organic reactions were isolated and found to be potent, direct inhibitors of thrombin. One of these byproducts was characterized to be the first unsulfated small molecule to exhibit allosteric direct inhibition of thrombin. None of the N-arylacyl O-sulfonated aminoglycosides, designed to be heparin mimics, exhibited inhibition of thrombin. Overall, the results of this study indicate that heparin-binding proteins can be effectively targeted by introducing select N-arylacyl groups into modified-heparin derivatives. Moreover, the first unsulfated small molecules that are allosteric inhibitors of thrombin were discovered and characterized. Together this work will guide further investigations towards the design and synthesis of novel allosteric inhibitors of thrombin as anticoagulant agents.

Natural Product Discovery Using Planes of Principal Component Analysis in R

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Natural product discovery programs aimed at identifying new and interesting structural scaffolds for drug discovery and synthetic chemistry purposes are often limited by the rediscovery of known molecules. It is estimated that between 100,000 to 1,000,000 *Streptomyces* strains need to be screened for the discovery of one new antimicrobial compound. This has necessitated the development of highly efficient and effective methods for dereplication, defined as the process of determining if a compound is known. To date, most dereplication strategies have focused on the identification of known compounds versus unknowns. We posit that this emphasis on knowns, introduces an inadvertent bias into screening paradigms and that a potentially superior approach is to simply focus on what is different within a dataset; this logic can be applied not only to metabolites of interest but to their producing microbes as well. By employing statistics and the historical observation that common actinobacterial metabolites are likely known, focus can be increasingly shifted in favor of true discovery of unknowns. LC-MS-based principal component analysis (PCA) allows us to distinguish unique versus common metabolites. To simplify PCA analyses we have devised a script that identifies only those masses or molecules that are unique to each strain within a group; this dramatically reduces the number of data points requiring manual inspection. An important feature of this approach is that the script is written in R. As a result, our script is highly amenable to being integrated with other metabolomics workflows and also supports automated mass matching to databases like Antibase. In this way, computational chemistry has afforded a new tool for those seeking to identify new microbial-derived natural product scaffolds for chemically and therapeutically novel applications.

Mass Spectrometry-based Metabolomics for the Discovery of New Natural Products from Cyanobacteria

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Cyanobacteria (blue-green algae) produce chemically diverse metabolites of biotechnological importance, including but not limited to pharmaceuticals and molecular probes. As an addition to the classical bioassay-guided fractionation, omics-based approaches are expanding the field of natural product discovery. In this study, we applied mass spectrometry-based comparative metabolomics and *in situ* metabolomics to explore the chemical diversity of cultured cyanobacteria. In the first approach, strains growing under different culture conditions were analyzed by comparative metabolomics based on UPLC-HRMS profiling and statistical analysis. These experiments provided valuable insights into the effect of nitrate and phosphate on growth and metabolomic profiles of different strains. In addition, a novel metabolite (**1**) from strain *Scytonema* sp. UIC 10036 showed increased production under low nitrate and high phosphate conditions. In the second approach, cyanobacterial strains growing on solid media were analyzed *in situ* by droplet–liquid microjunction–surface sampling probe (droplet-LMJ-SSP) coupled with UPLC-UV-HRMS-MS/MS. *In situ* metabolomics identified two new compounds (**2**-**3**) in strain *Calothrix* sp. UIC 10520. Compounds **1**-**3** were isolated by HPLC and elucidated by a combination of HRMS, MS/MS, 1D and 2D NMR experiments. Their relative and absolute configurations were determined by *J*-based configurational analysis, chemical degradation reactions, and derivatization methods (Mosher's, Marfey's, and phenylglycine methyl ester). The structures of Scytoamide (**1**) and Calothrixamides A (**2**) and B (**3**) will be presented.

Leveraging Affinity and Avidity to Control Intercellular Interactions

Clifford M. Csizmar[1], Jacob R. Petersburg[1], Lawrence A. Stern[2], Benjamin J. Hackel[2], & Carston R. Wagner[1]
Departments of Medicinal Chemistry[1] and Chemical Engineering and Materials Science[2]
University of Minnesota, Minneapolis, MN 55455

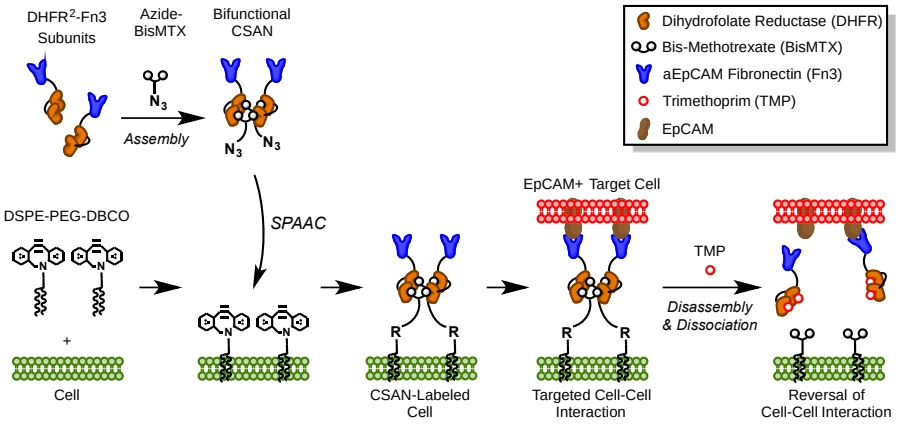
The ability to direct cell-cell interactions has tremendous value in several therapeutic fields. While genetically-encoded artificial receptors have proven efficacious, their scope is limited by the genetic-engineering that underlies the approach. To circumvent some of these limitations, our group has developed a non-genetic method to modify any cell surface with a targeted protein scaffold. As a recurring theme, we highlight how ligand affinity and scaffold avidity play several unique roles in our system.

First, we engineered a protein ligand based upon the human tenth type III fibronectin domain (Fn3) that binds to epithelial cell adhesion molecule (EpCAM), an overexpressed tumor antigen. Using yeast surface display, mammalian cell panning, and a novel titratable avidity-reduction selection technique, we evolved Fn3 clones exhibiting high affinity and robust selectivity for cellular EpCAM.[1]

We then incorporated these Fn3's into a multivalent chemically self-assembled nanoring (CSAN) for use as a cell-directing scaffold. EpCAM-targeted CSANs were anchored to cell membranes through the hydrophobic insertion of phospholipids into the lipid bilayer (**Figure 1**). The targeting elements were subsequently removed from the cell surface by disassembling the CSAN with the FDA-approved antibiotic, trimethoprim. Using this system, we successfully directed and reversed targeted intercellular interactions *in vitro*.[2]

Finally, the modular CSANs were used to study how avidity affects the apparent affinity of a multivalent targeting scaffold. By tuning the number of Fn3 domains on the CSAN, we quantitatively described how the apparent K_d changes as a function of ligand valency. These results are informing the development of a targeting scaffold capable of discriminating between cells expressing different quantities of an antigen.

In conclusion, we have developed a diverse tool-kit for directing and studying cell-cell interactions. The CSAN platform is applicable to several therapeutic arenas and, by balancing affinity and avidity, may offer distinct advantages over current cell-directing methods.



ORAL PRESENTATIONS SESSION IV

Nanodisc Technology for Studies of Small Molecule-Membrane Interactions

Sahishna Phaniraj; Blake R. Peterson
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Nanodiscs are engineered lipoparticles composed of a phospholipid bilayer surrounded by two membrane scaffold proteins. These particles can be used to mimic biomembranes, and they can be considered a state-of-the-art chemically-defined lipid bilayer. Nanodiscs are under investigation as tools for targeted drug delivery, for functional studies of membrane proteins by surface plasmon resonance and other biophysical techniques, and for structural studies of membrane proteins by nuclear magnetic resonance, among other methods. They comprise soluble nanoscale phospholipid bilayers that can self-assemble and incorporate integral membrane proteins. The ability to engineer the membrane scaffold protein to create structures of well-defined molecular size and shape, in addition to the interchangeability and accessibility of the lipid and scaffold components, makes nanodiscs a versatile platform for diverse studies of model membranes.

Standard nanodiscs made from the membrane scaffold protein MSP1D1 exhibit some variability in size, structure, and stability. Some of this variability results from non-covalent self-assembly of this protein as it wraps around the lipid bilayer. In our research, we are working to create novel covalently-linked membrane scaffold proteins that might provide better-defined and more stable nanodiscs. By modifying MSP1D1, we created a cyclic disulfide membrane scaffold protein that we termed MSPSS_M6. These nanodiscs are more thermally stable and more uniform than those derived from MSP1D1. We are currently using these nanodiscs to study interactions of membrane-disruptive peptides and small fluorescent molecules with lipid bilayers. We demonstrate that nanodiscs derived from MSPSS_M6 uniquely enable visualization of pore formation by short synthetic peptides using transmission electron microscopy. This approach is under investigation as a tool for elucidating mechanisms of action of biologically active peptides and small molecules.

Double Stranded mRNA Polyplexes for *in vivo* Gene Delivery

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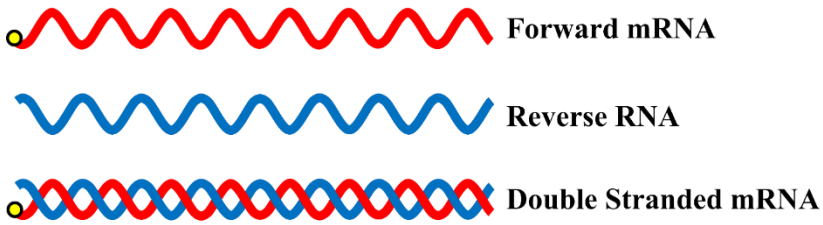
Gene therapy is a promising platform for treating acquired and genetic diseases. By delivering genes directly, dysfunctional proteins can be replaced. Attempts to efficiently deliver DNA *in vivo* by a non-viral method have failed because of the challenging barrier of crossing the nuclear envelope. Delivery of messenger RNA (mRNA) bypasses the need for nuclear translocation, but mRNA is much more susceptible to endogenous nucleases than DNA. Therefore, we sought to develop a metabolically stabilized form of mRNA that could be dosed i.v. in mice and express protein in hepatocytes. To this end, we have found that double stranded mRNA (ds mRNA) displayed greatly increased stability to nucleases than single stranded mRNA alone. Complexing ds mRNA with a cationic, PEGylated peptide (PEG-peptide) further increased the stability. Hydrodynamic dosing of luciferase-encoding ds mRNA in mice demonstrated translational competence equivalent to single stranded mRNA. Optimization of the length of the reverse strand of ds mRNA established an inverse relationship between length and translatability because of denaturation of the untranslated regions, but a direct relationship between length and circulatory stability. Progress toward development of a stable and persistent mRNA formulation will be discussed.



The Impact of Residual Complexity on Drug Discovery – The Case of Rufomyazine and Rufomycin

Mary P. Choules,[1,2] Larry L. Klein,[1] David C. Lankin,[1,2] James B. McAlpine,[1,2] Sang-Hyun Cho,[1] Jinhua Cheng,[3] Hanki Lee,[3] Joo-Won Suh,[3,4] Birgit U. Jaki,[1,2] Scott G. Franzblau,[1] and Guido F. Pauli[1,2]

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Residual Complexity (RC) is a concept that relates to the impact of unexplained, often minor chemical composition and properties that affect drug discovery and lead validation. Natural product (NP) drug discovery efforts are commonly plagued by the impact of RC due to the inherent complexity of NP source materials and the need for extensive purification. The new diketopiperazine, rufomyazine, and the previously known antibiotic, rufomycin, were found to represent a prototypical case of RC that initially resulted in the misassignment of biological activity. Originally, rufomyazine was isolated from an actinomycete strain as the bioactive principle against *Mycobacterium tuberculosis* (*M. tb*) with an MIC of 2 µg/mL. As a part of lead validation, the dipeptide was synthesized and surprisingly found to be inactive. After extensive analytical studies, the unexplained activity was eventually attributed to a very minor contamination (0.24% [m/m]) with the highly active cyclic peptide rufomycin (MIC ~0.02 µM). This study represents that impurities well below the natural abundance of ^{13}C (1.1%) can be highly relevant. This calls for advanced analytical characterization of drug leads with extended molar dynamic ranges of >1:1,000 using qNMR and LC-MS. The general implications of RC on drug discovery and what efforts are vital to improve lead validation, especially in NP related programs, will be presented.

Total Synthesis of Metabolically Stabilized Analogs of Pironetin

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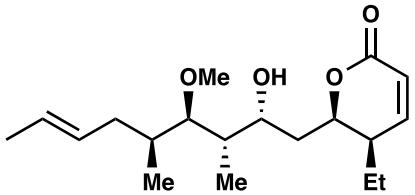
The natural product pironetin (**1**) displays potent cytotoxic activity against ovarian cancer cells both sensitive and resistant to first-line chemotherapeutics such as paclitaxel and cisplatin. Pironetin covalently binds α-tubulin, whereas all microtubule targeting agents currently approved by the FDA target β-tubulin. It has recently been shown that the gene encoding an isoform of α-tubulin (TUBA3C) is overexpressed in ovarian cancers and is associated with increased resistance to first line chemotherapeutics and shorter survival time, supporting α-tubulin as an attractive alternative target that would address the critical need for new treatments for drug-resistant ovarian cancers. Despite the potent in vitro activity, pironetin was only marginally effective at high doses in mice and resulted in severe weight loss, indicating poor pharmacokinetic/pharmacodynamic (PK/PD) properties as well as off target toxicities. In an effort to address these concerns, we found that pironetin has a short half-life (< 7 minutes) in liver microsomes, identified pironetin's major sites of metabolism, and confirmed the identity of the major metabolite through semi-synthesis. We are now engaged in the total synthesis of analogs which demonstrate improved PK/PD properties, highlighting the potential of metabolically stabilized pironetin analogs as novel anti-tubulin agents for resistant ovarian cancers.



Pironetin (1)

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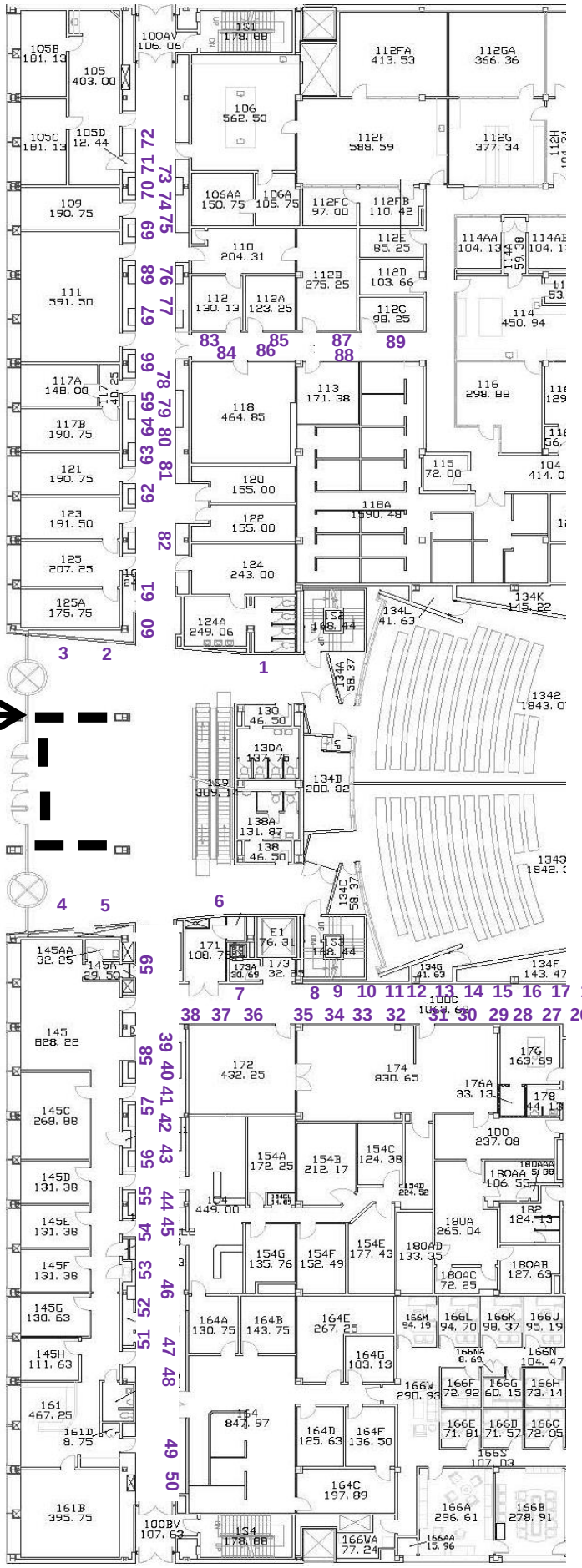

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POSTER ABSTRACTS

Poster #1

Determination of the in vitro effectiveness of novel Tipifarnib analogs via a fluorescence-based assay

Garrett Lee Schey, Feng Xu, and Mark D. Distefano
Department of Medicinal Chemistry, University of Minnesota

Protein prenylation is a post-translational modification where a C-15 or C-20 isoprenoid is appended to the C terminal end of a protein by either farnesyltransferase or geranylgeranyl transferase type 1, respectively. The isoprenoids are attached to the Cysteine residue of a four amino acid CaaX box sequence. Prenylated proteins have been implicated in many diseases, with the most infamous example being the mislocalization of RAS in cancer. In addition, changes in the expression of certain prenylated proteins has been shown to be important in many other illnesses including ALS, Alzheimer's Disease, and malaria. Recently, our lab has made analogs of the well-known farnesyltransferase inhibitor Tipifarnib, with the goal of gaining selectivity for the farnesyltransferase of pathogenic organisms including yeast and plasmodium falciparum. The IC50 values of these compounds can be assayed using a fluorescence based assay utilizing a substrate peptide with a fluorescent Dansyl functional group. This fluorophore is extremely sensitive to the environment, and the fluorescence intensity greatly increases when the substrate peptide is prenylated. Some of these compounds show high selectivity for the plasmodium farnesyltransferase enzyme and are promising potential drug candidates.

Keywords: prenylation, fluorophore, malaria

Poster #2

Engineering anti-EGFR fibronectin nanorings for cancer immunotherapy

Ozgun Kilic,
Department of Medicinal Chemistry,University of Minnesota

Engineered protein scaffolds have gained interest in recent years to overcome the challenges faced with antibody-based drugs. One of these promising scaffolds is the tenth type III domain of human fibronectin. Similar to antibodies, fibronectins can be engineered to provide target-specific binders with high affinity and specificity. One such target, epidermal growth factor receptor (EGFR) is a well-studied cancer biomarker and a promising target for cancer therapeutics. While some groups have already evolved high affinity EGFR-binding fibronectins using different methods, their use in cancer immunotherapy has not yet been explored.
Our lab has developed Chemically Self-Assembling Nanorings (CSANs) as a non-genetic approach for cell-directed immunotherapies. CSANs are formed when a bis-methotrexate dimerizer induces the oligomerization of dihydrofolate reductase-dihydrofolate reductase (DHFR2) fusion proteins. The CSAN platform can be easily expanded through fusion of additional protein domains and enable bispecific ring formats.
By fusing an EGFR-targeting fibronectin to the DHFR2 subunits, we formed fibronectin CSANs with both high affinity and high avidity for EGFR-overexpressing cancer cells. After investigating the biological outcomes of the fibronectin nanorings on cancer cells, we developed the bispecific nanorings that will target both the cancer cells and the T cells. We have formed bispecific CSANs by oligomerizing the fibronectin-DHFR2 subunits alongside anti-CD3 scFv-DHFR2 subunits that target T cells. These bispecific CSANs were able to recognize and activate T cells that would further kill the cancer cells. A unique advantage of this bispecific CSAN system is the ability to disassemble the CSANs in vitro and in vivo by introducing the FDA-approved antibiotic, trimethoprim, thus providing control over the T cell-directing activity of the CSANs. As the findings here suggest, fibronectin CSANs provide unique features for enhanced cancer immunotherapy.

Keywords: protein engineering, immunotherapy, fibronectins

Poster #3

Role of 3,4- dihydroxyphenylacetaldehyde in Pesticide Neurotoxicity

Brianna Suzanne Cagle, Derek Simonsem, Hans Lehmler, and Jonathan Doorn
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Pesticide exposure has been linked to Parkinson's disease (PD) and other neurodegenerative disorders. The "catechol-aldehyde" hypothesis that a buildup of intermediate aldehydes lead to neurotoxicity may underlie the neurotoxicity of pesticides. Dopamine (DA) is metabolized to a toxic catechol-aldehyde - 3,4-dihydroxyphenylacetaldehyde (DOPAL) - by monoamine oxidase (MAO) and then detoxified by aldehyde dehydrogenase (ALDH). Pesticides such as dieldrin and rotenone have been shown to affect ALDH activity and lead to an increase in DOPAL leading to toxicity. DOPAL toxicity can occur through protein modification and the formation of protein adducts. This work shows that treatment of dopaminergic N27 cells with DOPAL at non-toxic concentrations decreases the expression of the dopamine transporter (DAT). Such a modification of DAT could cause irregularities in DA cell trafficking. In addition, this study examined how DA metabolism is affected by organophosphate and pyrethroid pesticides, specifically chlorpyrifos and cypermethrin. These are insecticides that are neurotoxic to insects as well as humans. Cypermethrin has been shown to cause nigrostriatal degeneration with long-term exposure and may act synergistically with chlorpyrifos. Neither cypermethrin nor chlorpyrifos were found to be toxic at low micromolar concentrations using dopaminergic N27 cells. However, they could be acting together to cause increased toxicity. Understanding the mechanism of these insecticides and how they affect dopamine metabolism will further our understanding of PD and neurodegenerative diseases and be helpful in developing therapeutic strategies.

Keywords: Dopamine Metabolism, Neurotoxicity, Parkinson's Disease

Poster #4

Microwave-assisted, Asymmetric Synthesis of Flavonoid Derivatives from Chalcones

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The chalcone class of natural products displays a wide variety of biological activities and is a convenient starting point to access numerous natural-product like molecules, such as flavonoids. A route was developed utilizing microwave–assisted organic synthesis to access 3-amino-2,3-dihydrobenzofurans from chalcones to rapidly prepare analogues with increased stereochemical complexity and unique substitution patterns to explore the effect of these alterations on biological activity. The four step synthesis to access 3-amino-2,3-dihydrobenzofurans begins with utilizing commercially available acetophenones and benzaldehydes to synthesize chalcones through a microwave-assisted, acid-catalyzed aldol condensation. The chalcones then undergo a stereoselective Corey-Bakshi-Shibata reduction followed by a Sharpless asymmetric epoxidation to access the stereoisomeric epoxyalcohols. The final step is a one-pot, microwave-assisted, regioselective, europium-mediated epoxide opening with various amines followed by an intramolecular nucleophillic aromatic substitution reaction to afford the 3-amino-2,3-dihydrobenzofurans. Modifications to this route are currently being explored to access benzopyran flavonoid derivatives. A pilot library of chalcones and benzofurans was synthesized and evaluated in two human carcinoma cell lines to assess the biological activity diversity of the two compound classes, revealing that the benzofurans had a much more diverse biological response than the chalcones.

Keywords: benzofuran, chalcone, diversity oriented synthesis

Poster #5

Discovering Small Molecule Inhibitors of Early Autophagy

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Autophagy, a catabolic recycling process, has recently been implicated as a critical cellular pathway for cancer survival. Its role in maintaining cellular homeostasis helps to nourish hypoxic, nutrient-starved tumors and protects them from chemotherapy-induced death. While autophagy inhibition poses a tempting therapeutic target, no autophagy-specific inhibitors currently exist. To address this, two assays targeting critical protein-protein interactions in the autophagy pathway have been developed. The first, a fluorescent polarization assay, targets the interaction between Atg5 and the n-terminus of Atg16L1. The truncated fluorescently labeled Atg16L1 binds Atg5 specifically in solution, increasing the polarization of the emitted light. Small molecules will be screened to identify hits that disrupt this interaction and decrease the polarized signal. The second, a NanoBRET assay, targets the interaction between Beclin 1 and Atg14L. These two proteins are conjugated with bioluminescence energy transfer compatible protein tags which interact as a heterodimer in cells. This assay has been validated and used in an HTS screen which yielded several promising hits. These hits have been resynthesized and will be further evaluated for their effectiveness as autophagy inhibitors.

Keywords: autophagy, inhibitor, cancer

Poster #6

Exploring the Therapeutic Potential of H2S

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Hydrogen Sulfide (H2S) has gained increased interest as the third gaseous transmitter and is an enzyme product of cystathionine-γ-lyase (CSE) and cystathionine-β-synthase (CBS). H2S has been proposed to mediate a vast array of physiological processes, exerting its effects in multiple systems including cardiovascular, central and peripheral. Abnormal regulation of H2S production has been correlated with a number of diseases, including chronic heart failure, stroke, and endotoxemia, among others. L-propargylglycine (L-PAG), the inhibitor of choice for CSE suffers from poor potency and selectivity. The lack of selective inhibitors of CSE and CBS has greatly limited our ability to explore the therapeutic utility and on-target adverse effects of these enzymes and of H2S in disease. For this reason, this study focuses on understanding the mechanism of commonly used inhibitors in an effort to produce novel CSE inhibitors. By using three different CSE assays with several substrates (L-cysteine, L-homocysteine and L-cystathionine), a novel CSE inhibitor was identified. We have clarified observations on mechanisms via γ-elimination and β-elimination catalyzed by CSE using a Y114F mutant; and also identified substrate preferences, of which there is little awareness in the literature. Moreover, we have studied cystine as a substrate of CSE. Current research suggests that the range of physiological responses of H2S is largely mediated by covalent modification of proteins by H2S, a term called S-sulfhydration. Using cystine as a substrate produces cysteine hydropersulfide (Cys-SSH), which can serve as a model for S-sulfhydration in order to find ways to identify this modification in biological samples. Overall these studies have been able to clarify discrepancies of CSE inhibition found within the literature, as well as providing a better mechanistic view of this enzyme, which may aid in the discovery of potent, selective CSE inhibitors.

Keywords: Hydrogen Sulfide, Cystathionine-γ-lyase

Poster #7

Carnosine is a potent scavenger of biogenic aldehyde metabolites of neurotransmitters

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Parkinson's disease (PD) is a neurodegenerative disease that originates from insults such as oxidative stress within the neuron. Within these dopaminergic cells, the neurotransmitter dopamine (DA) is metabolized to 3,4-dihydroxyphenylacetaldehyde (DOPAL) via monoamine oxidase before oxidation to an acid metabolite. DOPAL is a highly protein-reactive aldehyde and toxic to neurons. Increased levels of this toxic aldehyde are hypothesized as chemical triggers for diseases such as PD. Therefore, a scavenger of these electrophiles is predicted to prevent or mitigate cell injury. Carnosine is a dipeptide comprised of the amino acids beta-alanine and histidine. Historically carnosine has been shown to act as an antioxidant, scavenger of reactive oxygen species (ROS), as well as scavenger of alpha-beta unsaturated aldehydes. The hypothesis presented in this study is that carnosine acts as a selective aldehyde scavenger towards toxic neurotransmitter metabolite DOPAL and the aldehyde metabolite of norepinephrine but not towards the primary lipid peroxidation product 4-hydroxy-2-nonenal(4-HNE) or other alpa,beta-unsaturated aldehydes. This reactive behavior is in direct contrast to another well-known ROS and aldehyde scavenger glutathione (GSH).

Keywords: Carnosine, Parkinson's, DOPA

Poster #8

Investigating the Structure Activity Relationship of Naphthalene Based Keap1-Nrf2 PPI Inhibitors

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The protein-protein interaction between the transcription factor, nuclear factor erythroid 2-related factor 2 (Nrf2), and Kelch-like ECH-associated protein 1 (Keap1) is a key regulator of the oxidative stress response. Nrf2 is negatively regulated by Keap1 via a polyubiquination mechanism that terminates in the proteosomal degradation of Nrf2. Inhibition of the Keap1-Nrf2 interaction allows for Nrf2 to promote transcription of detoxifying enzymes such as glutathione S-transferases (GST), NADPH:quinone oxidoreductase 1 (NQO1), and heme oxygenase (HO). Misregulation of this pathway is observed in many chronic inflammatory diseases; furthermore, activation of Nrf2 has been shown to enhance the healing of diabetic chronic wounds. Current therapies that target the Keap1-Nrf2 interaction are electrophilic in nature and behave as an electrophilic stressor to stimulate Nrf2's transcriptional ability; however, electrophilic compounds are prone to off-target effects which obscure their true mechanism of action. Non-electrophilic inhibitors of the Keap1-Nrf2 pathways have been reported, and our lab has focused on probing the structure activity relationship (SAR) of a known non-electrophilic inhibitor based on a 1,4-diaminonaphthalene scaffold. Previous work in our lab has shown that the naphthalene core can be replaced with other bicyclic heterocycles, such as isoquinoline, and retain potency. Recently, our attention has been turned to modifying the substituents of the bicyclic core to gain a more thorough SAR of these inhibitors. Development of a selective nonelectrophilic inhibitor of the Keap1-Nrf2 interaction will allow for a more reliable analysis of Nrf2's role in a number of disease states.

Poster #9

Comparison of Intrathecal and Intracerebroventricular Administration of Melanocortin Selective Ligands in Wildtype Mice

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It has been well established that intracerebroventricular (ICV) administration of compounds in animals allows direct access to the central nervous system (CNS); however, this technique is not without its flaws. ICV administration requires a minor surgery and recovery time, costly materials, and lengthens the experimental timeline. ICV administration is done by surgically implanting small metal cannulas into the lateral ventricles of the brain. In our laboratory, validation of cannula placement is done by giving human neuropeptide YY (hPYY3-36), a peptide known to increase food intake, and monitoring food intake post-injection. Successful validation is demonstrated by an animal consuming at least more than 0.8g of food four hours post-injection as compared to saline administration.
Intrathecal (IT) administration is an alternative administration technique that allows penetrance of the CNS via the spinal cord. While more time is spent initially learning and perfecting this technique, it can lower overall experimental costs and time. IT administration is performed by insertion of the injector into the intervertebral space between the L5 and L6 vertebra.
It is hypothesized that IT administration of melanocortin selective ligands will indicate differences in the physiological roles of centrally expressed melanocortin receptors accessibility (through the spinal cord versus through the lateral ventricles) in wildtype mice when compared to ICV administration.

Keywords: Melanocortins, Drug Delivery, Obesity

Poster #10

Structure-Activity Relationship Studies of a Macrocyclic AGRP-Mimetic Scaffold c[Pro-Arg-Phe-Phe-Asn-Ala-Phe-DPro] Yield Potent and Selective Melanocortin-4 Receptor Antagonists that Increase Food Intake in Mice

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The melanocortin system has five receptors and antagonists of the central melanocortin receptors (MC3R, MC4R) are postulated to be viable therapeutics for disorders of negative energy balance such as anorexia, cachexia, and failure to thrive. Agouti-related protein (AGRP) is an antagonist of the MC3R and an antagonist/inverse agonist of the MC4R. Structural studies have demonstrated that the active sequence of this hormone, Arg-Phe-Phe, is located on an exposed β-hairpin loop. It has previously been demonstrated that the macrocyclic octapeptide scaffold c[Pro1-Arg2-Phe3-Phe4-Asn5-Ala6-Phe7-DPro8] is 16-fold less potent than AGRP at the mMC4R. Herein, it was hypothesized that the Phe7 position may be substituted to produce more potent and/or selective melanocortin receptor antagonist ligands based on this template. A ten member library was synthesized that substituted small (Gly), polar (Ser), acidic (Asp), basic (Lys), aliphatic (Leu, Nle, and Cha), and aromatic (Trp, Tyr, hPhe) amino acids to explore potential modifications at the Phe7 position. The most potent mMC4R antagonist contained a Nle7 substitution, was equipotent to the lead ligand, was 200-fold selective for the mMC4R over the mMC3R, and caused a significant increase in food intake when injected intrathecally into male mice. These findings aid in the development of potent and selective probes to study the role of the melanocortin system in diseases of negative energy balance.

Keywords: obesity, peptides, melanocortin

Poster #11

Using Chiral Catalysts, Cation-Lone Pair Interactions, and Dispersion Interactions to Direct the Site-Selective Acylation of Carbohydrates

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Carbohydrates are ubiquitous in nature, yet general and efficient synthetic methods for their formation are rare. Furthermore, the site-selective manipulation of hydroxyl groups within carbohydrates adds another level of difficulty to their longstanding synthetic issues. Using a pair of chiral catalysts, we can predictably differentiate many trans-diols in O-glycosides and S-glycosides. Our working model is supported by DFT calculations, which indicate that site-selectivity hinges upon the presence or absence of a cation-lone pair interaction between the cation in the acylated catalyst and a suitable lone pair in the substrate in O-glycosides. Recently turning our attention to S-glycosides, we have further validated this model on a variety of substrates, but the origin of site-selectivity is now attributed to dispersion interactions between the catalyst and the C1 thioalkyl substituent. The predictive power of this method makes great strides towards streamlining the chemical synthesis of carbohydrates and will be useful for the investigation of more complex targets in the future.

Keywords: carbohydrate, site-selective, chiral catalyst

Poster #12

Hint1 regulated self-assembly of nucleoside phosphoramidate functionalized gelators

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To create a responsive and tunable self-assembly system, we have developed Histidine Triad Nucleotide Binding Protein 1 (HINT1) responsive nucleoside phosphoramidate pro-gelators (PPGs). HINT1 has been well characterized as a nucleoside phosphoramidase and acyl-adenylate hydrolase. At PPG concentrations above the critical micelle concentration and below the gelation point, these molecules assemble into highly regular nanofibers resulting in bulk viscous liquid formation. Utilizing HINT1, the self-assembling peptides may be released from the blocking effect of nucleoside phosphoramidate moieties which induces the soluble nanofibers to condense into highly associated nanofiber bundles observed by electron microscopy. The structural transition to nanofiber crosslinking at the nanoscale results in bulk material gelation. We have utilized chemical biological tools in conjunction with small amplitude oscillatory rheometry to further characterize the role of HINT1 in the observed gelation event. Small molecule inhibitors and catalytically dead HINT1 mutants were used to investigate the role of HINT1 active site and catalytic activity on the regulation of PPG assembly. Inhibitors have been shown to block HINT1 activity on PPG substrates, and Hint1 H112N catalytically dead mutant has been shown to be unable to activate self-assembly. Our goal is to develop an adaptable system for the construction of biologically responsive materials that may be assembled in situ in response to HINT1 activity.

Keywords: Hydrogel, Biomaterial, Self-Assembly

Poster #13

PAX2 Loss Recapitulates Secretory Cell Outgrowths (SCOUTs), Precursors to High-Grade Serous Ovarian Cancer

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Ovarian cancer is the most lethal gynecological malignancy and the 5th leading cause of cancer deaths among women. The deadliest subtype of the disease is high grade serous ovarian cancer (HGSOC) with an average 5 year survival rate of 29%. The fallopian tube epithelium (FTE) gives rise to pre-cancerous secretory cell outgrowths (SCOUTS) that can go on to become HGSOC. PAX2 is a transcription factor that is lost in HGSOC and in SCOUTs, indicating that loss of PAX2 is an early event in tumorigenesis. Additionally, it has been shown that re-expressing PAX2 in HGSOC models reduces cell survival and tumor burden. In the present study, we developed PAX2 deficient murine oviductal cell lines (MOE- murine equivalent of human FTE) to model SCOUTs and study how it potentiates the FTE for further transformation. We modeled PAX2 deficiency into MOE cells with either a stable PAX2 shRNA knock down to explore partial PAX2 loss or PAX2 CRISPR knockout to study cells with complete loss of PAX2. Loss of PAX2 in MOE cells, regardless of level of PAX2 deficiency, lead to no significant cancer specific phenotypic changes including adhesion, migration, and proliferation. However, RNA sequencing of PAX2 shRNA cells revealed a transcriptional overhaul that results in an mRNA expression pattern similar to human SCOUTs. Among these changes emerged potentiating alterations in key pathways in ovarian cancer such as AKT signaling. Furthermore, cross analysis with RNAseq of estrogen stimulated cells revealed remarkable overlap suggesting that loss of PAX2 regulates hormonal responses. Hormone responsiveness of these cells was investigated using ERE and PRE luciferase assays, which revealed higher basal hormone activity and sensitivity to hormone treatment. In summary, loss of PAX2 in early lesions does not manifest itself in transformative phenotypes, but rather in potentiating changes in the transcriptome, particularly in key pathways shown to be dysregulated in HGSOC.

Keywords: Ovarian Cancer, Pax2, Early tumorigenesis

Poster #14

Fully orthogonal translation system built on the dissociable ribosome

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The ribosome is the macromolecular RNA-based machine responsible for the protein biosynthesis. Mutational analysis is a powerful tool for understanding the functions of rRNA in translation. Unfortunately, mutations of the most conserved, and thus, most interesting nucleotides are often lethal and thus those mutations are hard to examine in the living cell. One solution to this problem is an orthogonal translation system, when a subpopulation of ribosomes, can translate a reporter protein but does not participate in housekeeping cellular translation. Recently, a fully-orthogonal ribosome, Ribo-T, was generated in our laboratory. Ribo-T is based on a hybrid 16S/23S rRNA molecule. In Ribo-T, the small ribosomal subunit, which is responsible for mRNA recognition, is covalently linked to the large ribosomal subunit, responsible for amino acid polymerization. By modifying the anti-Shine-Dalgarno sequence, it is possible to functionally isolate Ribo-T, which makes it possible to alter the critical 16S rRNA or 23S rRNA nucleotides and analyze their effect on the expression of the cognate reporter without disrupting cellular translation. Unfortunately, covalent linkage between the subunits impedes to some extent the assembly and functions of Ribo-T in comparison with the wild type ‘dissociable’ ribosome.
We explore a possibility of modifying the Ribo-T based system by creating a ‘flipped’ orthogonal system in which Ribo-T caries out the translation of cellular proteins, while dissociable ribosome functions as an orthogonal translation apparatus dedicated to the expression of a specific reporter. We demonstrated the general feasibility of this approach by being able to transform cells with the plasmid carrying mutations in the 23S rRNA gene, which would be lethal in the ‘non-orthogonal’ ribosome. We are currently optimizing the system in order to make it suitable for the broad analysis of the functional engagement of the critical rRNA nucleotides.

Poster #15

Application of a Phylogenetic-Molecular Networking Strategy to Dereplicate UIC Strain 10484 Resulting in the Identification of Trichormamide-like Secondary Metabolites

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Cyanobacteria have emerged as a prominent source of biomedically relevant natural products. The accumulation of compounds isolated from the phylum over the last few decades has resulted in the need to assess the novelty of a compound at the onset of the drug discovery process to prevent rediscovery of known chemistry. This process is known as dereplication. Components extracted from UIC strain 10484, found to be active against three cancer cell lines, were dereplicated by first identifying the taxonomic position of the strain using the 16S rRNA sequence. The strain was found to clade with trichormamide-producer UIC 10045. We then compared the MS/MS fragmentation profiles of the 10484 bioactive compounds to trichormamides using the Global Natural Products Social Molecular Networking (GNPS) platform. This confirmed the 10484 secondary metabolites were novel trichormamide analogs. Using 2D-NMR and MS/MS data, we were able to elucidate the structure of one of the compounds found in the active HPLC subfraction.

Keywords: Cyanobacterial Drug Discovery

Poster #16

Design and synthesis of peptidomimetics with attenuated reactivity in the treatment of neurodegenerative diseaseses

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It has been postulated that CNS diseases result from the dysregulation in either the expression and/or activity of cysteine proteases, and that inhibition of the respective enzymes should be an effective treatment. A well-known example of a CNS disease is Alzheimer's, and it has been suggested that its pathology could be linked to cysteine protease over-activity. More precisely, it has been posited that certain isoforms of cathepsins and calpains may precipitate such neuronal degeneration. Therefore, targeting these enzymes with small molecule inhibitors may present an effective strategy in impeding the progression of the illness. Our lead oxirane electrophilic compound, epoxysuccinate NYC-438, has been subjected to a battery of both in vitro and in vivo assays. Both NYC-438 and the commercial calpain inhibitor, E-64d, possess significant potency; however, both show limited brain bioavailability and poor selectivity. In addressing these issues, the objective now has been to design and synthesize reversible, selective inhibitors. More specifically, for calpain-1 and cathepsin B, since their inhibition is beneficial in neurodegeneration and traumatic brain injury (TBI), we hope to answer whether preferred selective inhibition of one enzyme alone matters. After modifying lead compound NYC-438, such endeavors so far have yielded compounds AJ1-35 and ING-108, which showed remarkable selectivity toward cathepsin K. In addition, recently synthesized novel series displayed similar selectivity, though they were less potent than either compound (i.e. AJ1-35 and ING-108). Other synthesized compounds showed neuroprotective effects using oxygen-glucose deprivation cell model assays as well as using neuroblastoma SH-SY5Y cell models.

Keywords: Cysteine Protease Inhibitors

Poster #17

CatSper Blockers as Male Contraceptive Agents

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The cation channel of sperm (CatSper) is the principal mode of entry for calcium in mature human sperm and mediates several important processes including hyperactivation and capacitation, both critical to successful fertilization. Mutations within CatSper, as confirmed by mouse knockout studies, led to complete infertility of males with no other observable phenotypes. As such, compounds that can block the function of this channel would be promising reversible, non-hormonal contraceptives capable of being taken by both male and female partners.

To this end, a calcium influx assay utilizing a FLIPR platform and freshly-collected human sperm was conducted on 35,000 compounds from the GPHR library. Screening hits showing both good potency for blocking calcium influx and well-behaved FLIPR traces were selected as initial hit compounds. Six hit compounds were selected for SAR by commerce and evaluation by patch clamp electrophysiology. Due to their promising activity in electrophysiology experiments, GPHR-00032750 and GPHR-00213869 were chosen for additional SAR studies. The elaboration of GPHR-00213869 by a substructure-based approach is described herein, as well as further evaluation of hits by sperm patch clamp electrophysiology.

Keywords: Contraception Ion Channel Drug Discovery

Poster #18

Kinetic and Structural Comparison of HINT1 and HINT2: Two Similar Enzymes with Separate Biological Roles?

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Histidine triad nucleotide-binding proteins (HINTs) have garnered attention for their role in activating the important class of antiviral and anticancer prodrugs nucleoside phosphoramidates, as well as their potential as a target for opioid tolerance reversal. While a body of literature has formed around the isozyme HINT1, revealing its enzymatic mechanism and potential endogenous functions, much less attention has been given to the mitochondrial localized variant HINT2.

With the aim of uncovering possible differences in the substrate specificity and thus the potential natural substrate of HINT2 from HINT1 (both of which still remain unknown), both steady state and transient burst phase analyses of HINT2 kinetics has been performed using a fluorescent substrate assay. Multiple nucleoside 5'-phosphoramidates with varied nucleobases have been analyzed and we've found the steady state parameters kcat and Km to both be approximately two fold higher when compared to HINT1, leaving the substrate specificity (kcat/Km) essentially the same. In studying the burst phase kinetics of HINT2, a strange behavior was observed in which the burst amplitude (which correlates to stoichiometry of substrate being consumed in the first turnover) was only one quarter of what was expected. Simultaneously, CPMG protein NMR dynamics experiments revealed that two hydrogen bonding residues distant from the HINT1 active site are extremely dynamic in microsecond to low millisecond time regime. Subsequent mutations of these residues in both HINT1 and HINT2 have allowed us to modulate the steady state rate of the enzymes as well as the burst amplitude. Interestingly, no mutations have affected the rates and binding constants of the burst phase however. These results indicate that HINTs contain a network of residues away from their active site which regulates burst phase substrate binding and steady state kinetics via the step of product release, but not the burst phase rate

Keywords: kinetics, CPMG-NMR, HINT

Poster #19

Investigation of β-Lactones as Selective Activity-Based Probes for Penicillin-Binding Proteins

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With the introduction of penicillin in the 1940s, β-lactam antibiotics became and have remained one of the most widely used classes of antibacterial agents. The targets of the β-lactams are penicillin-binding proteins (PBPs), which function to synthesize the peptidoglycan layer of the bacterial cell wall. PBPs synthesize peptidoglycan through transglycosylase activity to generate glycan strands, as well as transpeptidation activity to cross-link stem peptides of the glycan strands. All PBPs possess a catalytic active site serine in their peptidase domain that is essential for substrate turnover and is the site of inhibition by the β-lactams. Although these proteins have been exploited for decades, the individual function and regulation of each homolog is not well understood. A key issue in addressing this is a lack of compounds that specifically target individual homologs to promote their study. Previous work in our lab has exploited fluorophore conjugated-penicillin and cephalosporin as activity-based probes to study the function and spatial distribution of specific PBPs. Additionally, our investigation of 20 β-lactams from five drug classes showed that a number of the PBPs in Streptococcus pneumoniae and E. coli are poorly inhibited by these compounds, making development of probes for these proteins difficult. To overcome this issue, we have employed β-lactones in the design of PBP-selective activity-based probes and have identified a scaffold that possesses a unique PBP selectivity profile. This is a highly significant result, as β-lactones were not previously known to inhibit PBPs. Through the investigation of an initial β-lactone library, it was hypothesized that these compounds may be interacting with the PBP active site in a manner that differs from β-lactams. Our current work aims to develop new β-lactone analogues that target additional PBPs to understand structure-activity relationships between selective probes and the regulation of individual PBPs.

Keywords: Bacteria, Penicillin-Binding Proteins, Activity-Based Probes

Poster #20

Bacterial Histidine Kinase: Development of Novel Catalytic Domain Inhibitors

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Two-component systems (TCSs) are the main signal transduction pathways used by bacteria to regulate a variety of processes including bacterial development, metabolism, virulence mechanisms, and resistance to antibiotics. TCSs consist of a homodimeric membrane-bound sensor enzyme, a histidine kinase (HK), and a cognate effector, a response regulator (RR). A high degree of sequence conservation in the catalytic domain (CA) of HKs especially in the ATP-binding pocket and their essential role in bacterial signal transduction make an attractive target in broad-spectrum anti-virulence. It has been theorized that eliminating bacterial virulence would be a promising alternative to current antibiotic strategy. Detailed mechanistic and structural insights into ligand-domain binding in HKs is urgently needed as the development of potent inhibitors that modulate bacterial signal transduction could lead to a new mechanism for treatment of infectious diseases. Structure-activity relationship (SAR) studies have been performed with compounds that target the CA domain that our group identified through a small molecule high-throughput screening (HTS) campaign against HK853 (Thermotoga maritima). The most potent compounds discovered in these studies possess IC50 values in the low μM range, while also exhibiting activity against two additional HKs: VicK (Streptococcus pneumoniae) and CheA (Escherichia coli). These compounds have shown to be effective in whole cells, with anti-virulence activity against Pseudomonas aeruginosa, MRSA, Salmonella and Vibrio cholerae. Docking studies suggest the preference in potent of an exocyclic nitrogen for hydrogen bonding in the HK active site through a conserved aspartate residue confirmed by SAR studies. A recent screen of eukaryotic kinase inhibitors against HK853 showed minimal activity except for one inhibitor that presents hydrogen bonding to three conserved residues in the active site in a similar way than previously reported inhibitors.

Keywords: Histidine Kinase Virulence

Poster #21

Triflate-Catalysis Enables Selective Access to Alpha,Alpha-Difluoroalkylthioethers

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In the development of molecular probes, the substitution of hydrogen atoms with fluorine can greatly impact physicochemical, pharmacokinetic, and pharmacodynamic properties that influence drug metrics. However, current synthetic methods cannot access many fluorinated motifs, which impedes utilization of these groups. Thus, the development of new methods to access fluorinated substitutions is critical for developing the next generation of biological probes and therapeutic agents.
The synthesis of one such substructure, the alpha,alpha-difluoroalkylthioether, currently requires harsh methods that necessitates early-stage installation. Thus, many alpha,alpha-difluoroalkylthioethers remain underexplored, despite promising potential toward modifying the biophysical profile of thioether-derived chemical probes. To address this synthetic gap, we developed a simple and mild synthetic strategy to access this substructure that involves a base-catalyzed addition of alkyl thiols to readily available gem-difluoroalkenes. Early studies exploited our previously reported catalytic base system for this reaction using catalytic thiolate, which delivered an undesired monofluorinated side product. As this side product likely derived from an unstable anionic intermediate, we exploited additives to facilitate the protonation of the anion. Ultimately, the optimized milder conditions gave the desired alpha,alpha-difluoroalkylthioethers in high selectivity in moderate to excellent yields.

Keywords: Fluorine, Catalysis, Method

Poster #22

Quinazoline-2,4-diones: Probing the Quinolone-binding Pocket of Bacterial Type-II Topoisomerases for Overcoming Bacterial Resistance

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Fluoroquinolones are a class of antibiotics used clinically to treat a wide array of bacterial infections. Fluoroquinolones act by forming a ternary complex with bacterial type II topoisomerases (DNA gyrase or topoisomerase IV) and nicked DNA; religation of DNA is subsequently blocked. In ternary complex the keto-acid moiety of the fluoroquinolone is complexed with a divalent magnesium ion, forming a drug-magnesium-water bridge to a serine and/or an aspartate (or glutamate) residue on helix-4 of the topoisomerase enzyme. Mutation-mediated resistance arises through substitution of the serine or aspartate/glutamate residues, therefore preventing formation of the magnesium-water bridge and reducing stability of the cleaved complex/ dramatically diminishing the overall activity of the fluoroquinolone. Quinazoline-2,4-diones (diones) are structurally similar to fluoroquinolones; diones form ternary complex similar to fluoroquinolones, however, these complexes are less stable because the quinazoline-2,4-diones do not contain the keto-acid moiety and therefore do not form a magnesium-water bridge to helix-4. While diones are therefore less potent antibiotics, their non-reliance on the magnesium water bridge generally affords equipotent activity with wild-type and fluoroquinolone-resistant strains of bacteria. We hypothesized that the quinazoline-2,4-dione structural scaffold, and crystal structure, provide preliminary structural data to generate novel inhibitors of bacterial type-II topoisomerases that act on wild-type and fluoroquinolone resistant bacteria. In this presentation, the design and synthesis of novel quinazoline-2,4-dione derivatives expected to have additional binding contacts, and therefore increased potency, in ternary complex will be discussed.

Keywords: Fluoroquinolone, Antibiotic, Resistance

Poster #23

Identification of Specific Readers of Epigenetic Modifications in Human Bronchial Epithelial Cells Using a Quantitative Proteomics Approach

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5-Methylcytosine (mC) is a stable epigenetic modification of DNA that plays a key role in controlling gene expression. The removal of DNA methylation marks is mediated by ten eleven translocation (TET) dioxygenases which iteratively oxidize the methyl group to give 5-hydroxymethylcytosine (hmC), 5-formylcytosine (fC), and 5-carboxylcytosine (caC). Both fC and caC are removed by thymine DNA glycosylase (TDG) and replaced by cytosine via the base-excision repair mechanism (BER). Recent studies have identified proteins that specifically interact with hmC, fC, and caC in mESCs, suggesting that each of these epigenetic marks is recognized by a unique set of "readers" and elicit a distinct epigenetic response.
Lung tumors observe a significant loss in global levels of hmC. Furthermore, work in our lab has shown that exposure to inflammatory agents in cigarette smoke cause a decrease in hmC levels prior to tumor formation. By determining the specific interactions mC and its oxidized forms make in the lung, we hope to better understand hmC's role in normal lung function. Our current work employs affinity proteomics to identify the "readers" of oxidized forms of mC in human bronchial epithelial cells (HBEC). We selected the section of the WTH3 promoter and synthesized modified DNA duplexes containing mC, hmC, fC, or caC. The selectively binding proteins were TMT-labeled and analyzed by mass spectrometry.
We have identified unique binders to C, mC, hmC, fC, and caC respectively. Looking at the biological functions of the proteins preferentially binding to hmC, fC, and caC, we see a large number of proteins with biological functions including DNA repair, cellular response to stress, and response to DNA damage stimulus. Our results highlight how specific readers of DNA modifications such as hmC may interpret these signals and translate this modification into a functional output.

Keywords: Epigenetics, Proteomics, Cancer

Poster #24

Assay Development for High Throughput Screen of Selective TET Enzyme Inhibition

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Recently discovered Ten Eleven Translocation (TET) proteins 1-3 play a central role in epigenetic regulation of gene expression. These TET proteins employ an α-ketoglutarate cofactor and non-heme iron to oxidize the methyl group of 5-methylcytosine (5-mC) in DNA to 5-hydroxymethylcytosine (5-hmC), 5-formylcytosine (5-fC) and 5-carboxylcytosine (5-caC). fC and caC are excised by thymine DNA glycosylase and are replaced with C via base excision repair, thus mC oxidation can result in active demethylation and gene re-activation. TET expression and activity are deregulated in many diseases including systemic lupus erythematosus (SLE), chronic lymphocytic leukemia, autism spectrum disorders, and bronchial asthma. However, no small molecule inhibitors selective for TET are currently available. N-oxalylglycine, an α-ketoglutarate analogue, does inhibit the three proteins, but it also inhibits other Fe(II)/α-ketoglutarate-dependant enzymes. The lack of selective inhibitors impedes the exploration of using these proteins as potential therapeutic targets.
Current work has implemented computational screening coupled with a kinetic HPLC-ESI-MS/MS assay to identify direct and selective inhibitors of the TET proteins to elucidate their roles in human disease. Compounds showing high inhibitory activity will be used in the development of structurally diverse analogs. These compounds will also be utilized in screening human cells with TET overexpression. Furthermore, a fluorescence-based assay developed in our laboratory will be employed via high-throughput screen to further identify compounds that selectively inhibit TET proteins. This work will help achieve the long-term goal of developing novel epigenetic therapies that will employ selective TET inhibitors for the treatment of diseases such as SLE, autism, asthma, and cancer.

Keywords: TET Inhibition Screening

Poster #25

Software for the analysis of thermal shift assay data across a library of fragment molecules

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Differential Scanning Fluorimetry (DSF) is a method of determining the stability of a protein by monitoring changes in the fluorescence of a hydrophobic dye in the presence of a studied protein. Monitoring the fluorescence allows for the identification of the temperature at which a protein transitions from the folded to the unfolded state (Tm). Comparisons of these Tms in the presence and absence of particular compounds can lead to the identification of potential inhibitors due to the stabilizing or destabilizing ability of the compound. This makes DSF an ideal tool for conducting fragment based screening quickly and inexpensively.
Modern instrumentation permits DSF screening in 96 and 384 well plates using small sample volumes allowing for the efficient screening of large libraries, but the large amounts of data generated that can be difficult to manage. Current protocols have been described by Niesen et al (Nat Protocols, 2:2212-21; 2007) which employ Microsoft Excel to interface between data formatted by manufacturer control software, and a scientific package (such as GraphPad) that efficiently calculate Tms using non-linear regression. This method, while powerful and flexible, is limited by the fact that it can only analyze one plate at a time. This leads to difficulties with screens that span multiple or larger plates.
We have circumvented this limitation by creating an alternative data-processing protocol encoded in python. Our method has the capability to calculate Tms directly from instrument-provided spreadsheets via non-linear regression and output the data into a malleable Excel template. This template is infinitely more extensible as it allows for the analysis of data from an entire screen as well as compound behavior across multiple screens, which can lead to the identification of promiscuous binders or fluorescence interference compounds. Some of the capabilities of our data analysis protocol will be illustrated.

Keywords: DSF, data analysis

Poster #26

Development of Fluorescent Switch-On Probes for Hint1

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Histidine triad nucleotide binding protein 1 (Hint1) has emerged as a key protein involved in regulation of pain, opioid tolerance, and addiction properties. Specifically, Hint1 mediates the ability of the N-methyl-D-aspartate receptor to restrict the activity of the mu-opioid receptor (MOR). Hint1 is a part of the histidine triad (HIT) superfamily and possesses nucleoside phosphoramidase and acyl-AMP hydrolase activity. Inhibition of Hint1 enzymatic activity with a small molecule inhibitor has been shown to reduce recruitment of NMDAR to the MOR, resulting in increased morphine antinociception and decreased the development of tolerance in mice. To further investigate this interaction, we are developing a pair of two-photon activated switch-on fluorescent probes capable of monitoring Hint1 activity in living cells. These switch-on probes include a substrate of Hint1 in which the fluorescence relies on the phosphoramidase activity of the enzyme. The second probe is a Hint1 inhibitor that relies on binding to the Hint1 active site to activate its fluorescence. The probes should be valuable tools for interrogating the molecular mechanisms governing Hint1 function.

Keywords: Hint1, Fluorescence, Switch-On

Poster #27

Cephalosporin-Pyrazinoic Acid Conjugates: Novel Agents for Drug-Resistant Tuberculosis

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Tuberculosis (TB) is the leading source of infectious disease mortality globally, causing 1.4 million deaths worldwide in 2015. Combination therapy employing four first-line agents (rifampicin, isoniazid, ethambutol, and pyrazinamide) achieves greater than 95% treatment success against drug-susceptible strains, but less than 50% success against drug-resistant strains, which comprise an estimated 10% of new TB cases. Pyrazinamide (PZA), one of the first-line antituberculars, possesses unique activity against non-replicating Mycobacterium tuberculosis (Mtb) and shortens treatment duration by 3-6 months. PZA is a prodrug of pyrazinoic acid (POA) and is hydrolyzed intracellularly by pyrazinamidase, encoded by pncA in Mtb; resistance arises via point mutations in pncA which prevent this activation. PZA resistance poses an increasing threat to public health, as an estimated 60% of drug-resistant Mtb strains contain mutations in pncA. We have developed novel pyrazinoic acid prodrugs which exploit inherent tubercular β-lactamase activity to achieve selective, pncA-independent release of POA. Cleavage of the β-lactam promoiety furnishes elimination of the C-3' pyrazinoic acid in vivo; functionalization at C7 imparts selectivity for the tubercular β-lactamase BlaC over β-lactamases expressed by commensal organisms. Biochemical evaluations revealed favorable stability in serum and verified β-lactamase-dependent release of POA. Our conjugates demonstrate improved activity over POA in vitro against wild-type Mtb and PZA-resistant strains, and lack activity against other Gram-positive and negative organisms.

Keywords: Tuberculosis, Antibiotic resistance

Poster #28

Catalytic One-Step Deoxytrifluoromethylation of Alcohols

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The deoxyfluorination of readily available alcohols is a benchmark transformation in synthetic medicinal chemistry; however, the analogous deoxytrifluoromethylation is still limited to multi-step protocols. These protocols waste money, reagents, solvents, time, and typically involve one or more redox steps that limit functional group compatibility. To circumvent these limitations, we developed a new reagent and a copper-catalyzed transformation to trifluoromethylate readily available allylic, benzylic and propargylic alcohols using halodifluoroacetates as the source of CF3. This newly developed reaction provided yields of final compounds comparable to or better than previous multi-step synthetic sequences, while only using one simple, inexpensive and readily available CF3-source in a single-step reaction. Therefore, this method provides a straightforward tool for medicinal chemists tune molecular properties in drug-discovery campaigns.

Keywords: Fluorination, Catalysis, Alcohols

Poster #29

Evaluating Mediterranean herb extracts and selected phytochemicals for the potential to prevent development of inflammatory bowel disease

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The purpose of this study was to evaluate selected herb extracts and phytochemicals that have the potential for anti-oxidant and anti-inflammatory activity. HCT 116 colon carcinoma cells were treated for 24 hours with herb extracts and isolated phytochemicals. Cells were then lysed and the total protein content was isolated. Immunoblot analysis of proteins involved in anti-oxidant and anti-inflammatory pathways was conducted. The data suggest that the expression levels of various anti-oxidant and inflammatory proteins changed in response to extract and phytochemical treatment versus untreated cells. In an in vivo experiment, C57BL/6 mice were given either licorice or oregano extracts for 14 consecutive days. During the treatment, mice were given dextran sodium sulfate (DSS) through the drinking water to induce colitis. The diseases activity index (DAI) and intestinal permeability of the mice were measured to determine effectiveness of extract treatment in prevent colitis development. These data indicate that selected herb extracts and phytochemicals have the potential to reduce inflammatory signaling in the GI tract, particularly the colon. Through modulation of pathways such as the Nrf2-Keap1 and the unfolded protein response (UPR) pathways, cells can be relieved of oxidative and inflammatory stress. These compounds have the potential to prevent, slow, or treat chronic inflammatory diseases such as ulcerative colitis.

Poster #30

Towards synthetic cell surface receptors that activate pkc-mediated signal transduction

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Modern methods for the pharmacological treatment of disease predominantly focus on the identification of small molecules or proteins that block or activate specific biological receptors or enzymes. In some diseases, including specific types of diabetes and cancer, normal cell surface receptors are missing or are altered in ways that prevent them from being readily targeted by drugs, and few options generally exist to reactivate signaling pathways. As a new approach to intervene in these diseases, we seek to create the first synthetic molecules that have the potential to functionally mimic cell surface receptors that control specific signal transduction pathways. Towards this end, we are developing methods to synthesize transmembrane peptides that have the potential to become incorporated into plasma membranes of treated mammalian cells. To engage signaling pathways, these peptides will be linked to small molecules that are designed to project into the cytoplasm and bind intracellular signaling proteins. These peptides will additionally be linked to binding motifs that project from cell surfaces into the extracellular environment to allow dimerization by extracellular ligands. By linking small molecules that activate protein kinase C to the C-terminus of these peptides, and fluorophores that bind anti-fluorophore antibodies to the N-terminus, these agents have the potential to allow antibody-mediated dimerization of intracellular PKC and provide pharmacological control over activation of this signaling pathway. This approach could enable the discovery of therapeutics and new tools for elucidating detailed mechanisms of action of cell surface receptors and components of the signaling pathways that they regulate. Studies of model systems and progress towards these objectives will be described.

Poster #31

Rationally Designed Inhibitors of Tilivalline Biosynthesis in Whole Cell Klebsiella Oxytoca

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The gut microbiome is comprised of trillions of bacteria from hundreds of different species that has a profound impact on our health. Recent advances in genetics and metabolomics have enabled researchers to begin to dissect the innumerable interactions between the gut microbiome and the host. For example, the molecular basis for antibiotic associated hemorrhagic colitis (AAHC) was recently shown to be caused by tilivalline, a cytotoxic pyrrolobenzodiazepine produced by Klebsiella oxytoca, a commensal gram negative bacteria, which is normally a low abundant organism in a healthy person, but can rapidly overgrow in the gut during antibiotic treatment. Tilivalline is a secondary metabolite produced by a nonribosomal peptide synthetase encoded by three genes (npsA, thdA, and npsB), which sequentially condense 3-hydroxyanthranilic acid, L-proline, and indole to form the unique pyrrolobenzodiazepine scaffold. We will describe the reconstitution of the entire biosynthetic pathway in vitro with recombinant NpsA, ThdA, and NpsB to efficiently produce tilivalline, which was confirmed by LC-MS analysis with an authentic synthetic standard. Efforts are underway with the Gulick labs to solve the three-dimensional structures of each enzyme. In addition to these fundamental biochemical studies, we will also describe the design, synthesis, biochemical and biological characterization of a potent nanomolar small-molecule inhibitor of tilivalline biosynthesis that we hypothesize can be used to selectively combat antibiotic associated hemorrhagic colitis while sparing the gut microbiome.

Keywords: Microbiome, Natural Product, Analytical chemistry

Poster #32

Estrogenic activities of flavonoids in botanical dietary supplements used for women's health; importance of prenylation, C-ring unsaturation, and hydroxyl substituents on ER beta selectivity.

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Use of botanical dietary supplements have increasingly become a preferred option in women's health because of perceived efficacy and safety. Some of the compounds in these botanicals are estrogenic flavonoids and isoflavonoids that share a common structural backbone. Studies show that estrogen receptor (ERβ) ligands generally provide better safety and cytoprotective properties than estrogen receptor (ERα) ligands. A structure-activity relationship (SAR) on estrogenic potencies of the following flavonoids and isoflavonoids found in dietary supplements was conducted to determine which molecular structures favor ERβ potency and selectivity: 8-prenylnaringenin (hops), 8-prenylapigenin (licorice), apigenin (ubiquitous), naringenin (ubiquitous), icaritin (horny goat weed), desmethylicaritin (horny goat weed); isoflavonoids: genistein (soy), dihydrogenistein (soy; via gut microbiota), 8-prenylgenistein (licorice). Alkaline phosphatase assay was conducted using endometrial carcinoma cell lines (ERα+, Ishikawa) as in vitro biological endpoints to determine ERα potency. ERE-luciferase assays were used to quantify ERβ potency using breast carcinoma cells (ERβ+ transfected, MDA-MB-231:β41). Evaluations for these flavonoids showed A-ring prenylation at C8 position and C-ring saturation at C2-C3 position resulted in significant ERα potency, while A-ring prenylation at C8 position and unsaturation at C2-C3 position provided significant ERβ potency and selectivity. Methylation of 4' hydroxyl group at the B-ring robustly reduced overall estrogenic potency. In contrast, C8-prenylation and C-ring unsaturation at C2-C3 on isoflavonoids significantly decreased overall estrogenic potency. These results indicate that C8-prenylation, C2-C3 unsaturation (resulting in a more planar molecule), and 4' hydroxylation in these flavonoids is important for ERβ potency and selectivity, potentially providing favorable biological outcomes in vivo. Supported by NIH P50AT000155 and T32AT007533.

Keywords: Estrogenicity, flavonoids, chemoprevention

Poster #33

Development of Tissue-Selective ABCA1 Agonists as Potential Therapeutics for Alzheimer's Disease

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Apolipoprotein E (APOE) ε4 allele is the strongest risk factor for sporadic Alzheimer's disease (AD). Lipoproteins containing apoE4 have lower lipid content, which decreases stability and contributes to loss of lipoprotein function. To correct these deficits, we have developed tissue selective ABCA1 agonists (TSAAgs) that induce central nervous system expression of cholesterol transporter ABCA1, thereby increasing lipid content of apoE4 containing lipoproteins, with minimal impact on peripheral lipogenesis. High throughput screening (HTS) utilized luciferase reporter elements expressed by CCF STTG1 astrocytoma cells (primary screen) and HepG2 hepatocellular carcinoma cells (counterscreen) linked to ABCA1 and SREBP1c promoters, respectively, to identify several hits, which have since been validated by concentration response assay following repurchase. Priority hits, which showed antiinflammatory and insulin sensitizing properties in addition to TSAAg activity, served as scaffolds to synthesize a library of novel structural analogs. In vitro evaluation of this analog library via luciferase assay, PCR, and fluorescent cholesterol efflux measurements established structure activity relationships to identify compounds with improved TSAAg activity and guide further structural modification. The results demonstrate a proof of concept to develop TSAAgs with multifunctional therapeutic potential for Alzheimer's disease. Future in vivo experiments in healthy mice will establish pharmacokinetic profiles, determine magnitude of tissue selective ABCA1 induction, and monitor alterations in peripheral lipogenesis. Finally, treatment in EFAD mouse model will assess TSAAg effect on cognitive and pathological deficits. Our study represents a novel strategy to develop small molecule drug candidates that target multiple aspects of AD pathology, which would ultimately serve as leads for further pharmaceutical development and human clinical testing.

Keywords: Alzheimer's, ABCA1, cholesterol

Poster #34

Activity-associated Protein Target Profiling of the Androgen Receptor Antagonist EPI-002

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Constitutive activation of androgen receptor (AR) signaling in castration-resistant prostate cancer (CRPC) facilitates tumor growth in patients despite treatment with anti-androgen therapies that target AR C-terminal ligand-binding domain (LBD). EPI-001/EPI-002 (2R, 20S isomer) have been reported as AR antagonists by binding covalently to the N-terminal domain (NTD) through its chlorohydrin moiety and blocking protein-protein interactions that are required for transcriptional activity. Therefore, AR NTD antagonists may function as viable therapies for CRPC. However, recent studies have shown multilevel inhibitory effects of EPI-002 in prostate cancer cells through non-AR interactions. We have used unbiased activity-associated protein target profiling to annotate those proteins covalently bound by EPI-002 that likely confer AR inhibitory activity. Alkyne probe EPI-054 was synthesized and shown to have similar anti-proliferative activity compared to parent compound EPI-002. Using EPI-054 as a tool compound, our progress towards target profiling of this class of AR antagonists will be presented.

Keywords: EPI-002, Androgen Receptor, Activity Based Protein Profiling

Poster #35

Effect of Chemical Cross-Talk in Bacterial Co-Cultures on Differential Gene Expression and Antibiotic Production

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Marine Actinobacteria are an under-utilized resource for novel natural product drug discovery. Recent genomics studies of different Actinobacteria have shown that their genetic potential for producing therapeutically relevant compounds far exceeds previous estimates. Numerous 'cryptic' or 'silent' biosynthetic gene clusters (BGCs) remain inactive under standard laboratory growth conditions, limiting the discovery of potential new lead compounds. Research in our laboratory has shown that co-culturing different bacterial species can elicit the production of these silent novel secondary metabolites. Using this platform, we discovered a new antibiotic named Keyicin, produced by a Micromonospora sp. only when cultured with a Rhodococcus sp. A comprehensive 'omics' based approach has helped us not only identify the gene cluster responsible for the production of the antibiotic in the genome of the Micromonospora sp., but also given us promising results on differential gene and protein expression in co-culture versus mono-culture. Moreover, cell-free lysates of Rhodococcus sp. could activate the production of Keyicin, suggesting the use of small molecule chemical signaling as a means of communication between these species. Taken together, these studies will provide us a roadmap to potentially unlock other cryptic BGC's in Actinobacteria.

Keywords: antibiotic bacteria omics

Poster #36

Elucidating Rgg-mediated quorum sensing networks in Streptococcus pneumoniae and testing their contributions in pathogenesis

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Rgg proteins are a class of peptide pheromone-binding transcriptional regulators found in the cytoplasm of most genera of *Firmicutes*. In Group A Streptococcus (GAS), Rgg2 and Rgg3 proteins regulate the transcription of genes involved in moderating group behaviors such as biofilm formation, lysozyme resistance, and cell aggregation, in a process known as quorum sensing (QS). Addition of a short hydrophobic peptide (SHP) induces QS-related genes by altering the function of the individual Rgg transcriptional regulators. Interestingly, Rgg2 and Rgg3, though highly similar proteins, display opposing activities in transcriptional regulation; Rgg2 is a transcriptional activator, whereas Rgg3 is a repressor. The aim of this project is to investigate the properties of these Rgg proteins, including peptide binding, DNA binding, interactions with RNA polymerase, and oligomerization. Random and site-directed mutagenesis have been used to investigate functional regions of the proteins, by creating chimaeric Rgg2/Rgg3 proteins that can be assayed for transcriptional activity, DNA binding affinity, and oligomeric state. In addition, we will use *in vitro* transcription assays to explore Rgg2 interactions with RNA polymerase. Rgg2 contains a disulfide bond that potentially functions as a redox sensor; we intend to use transcriptional reporter assays to examine the role of the disulfide bond in oxidizing and reducing conditions. Understanding how Rgg proteins function will advance our understanding of QS in GAS and other pathogenic members of the phylum *Firmicutes*, and improve efforts to interfere with QS and therefore manipulate bacterial behavior for therapeutic purposes.

Poster #37

Assessing the efficiency of cultivation techniques to recover natural product biosynthetic gene populations from sediment

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Despite decades of cultivating microorganisms for use in drug discovery, few attempts have been made to measure the extent to which common cultivation techniques have accessed existing chemical space. Metagenomic studies have shown that cultivable bacteria represent only a fraction of those that exist in the environment, and that the uncultivated populations in sediment have genes that encode for a high diversity of novel natural product (NP) biosynthetic enzymes. Quantifying these genes in both sediment and cultivatable bacterial populations allows us to assess how much diversity is present on nutrient agar, and is critical to guiding the trajectory of future NP discovery platforms. Herein we employed next-generation amplicon sequencing to assess the NP biosynthetic gene populations present in two Lake Huron sediment samples, and compared these with populations from their corresponding cultivatable bacteria. After cultivation efforts, we recovered between 1.6% and 10% of three common types of NP biosynthetic genes from the original sediment population. We highlight two findings from our study: 1) between 75 and 89% of measured NP biosynthetic genes from nutrient agar have yet to be characterized or deposited into known biosynthetic gene cluster databases, indicating that readily cultivatable bacteria harbor potential to produce new NPs; 2) even though the predominant taxa present on nutrient media represented some of the major producers of bacterial NPs, the sediment harbored a significantly greater pool of NP biosynthetic genes that could be mined for structural novelty, and these likely belong to taxa that typically do not constitute modern microbial drug discovery libraries.

Keywords: biosynthetic gene clusters, microbial genomics, natural products

Poster #38

Humulus lupulus Activation of Aryl hydrocarbon Receptor Inhibits Estrogen Carcinogenic Pathways

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Many postmenopausal women use Botanical Dietary Supplements (BDS), such as Humulus lupulus (hops), instead of prescription estrogen drugs for relief of menopausal symptoms, because of fear of breast cancer. Estrogen carcinogenesis is in part mediated by the oxidative estrogen (E2) metabolite, estradiol-3,4-quinone, which is formed by P450 1B1 (CYP1B1). Conversely P450 1A1 (CYP1A1) converts E2 to a benign 2-hydroxylated product. E2 further enhances the genotoxic 1B1 pathway through epigenetic repression of CYP1A1. In contrast, activated Aryl hydrocarbon Receptor (AhR) induces degradation of estrogen receptor alpha (ER) and leads to preferential upregulation of the CYP1A1 mediated detoxification pathway in the presence of E2. The purpose of this ongoing study is to analyze how hops and its AhR agonist, 6-prenylnaringenin (6-PN), enhance the estrogen detoxification pathway through AhR. Using an In-Cell Western (ICW) assay in ER+ MCF-7 cells, a decrease in ER expression is seen for Hops and 6-PN in the presence of E2, potentiating ER degradation, which is reversed by proteasome inhibition and also by an AhR antagonist. Quantitative RT-PCR showed that 6-PN and hops preferentially upregulate CYP1A1 over CYP1B1 in MCF-7 cells. Additionally Hops and 6-PN were able to activate XRE in MCF-7 cells, the motif responsible for AhR-dependent transcription. Inhibition of E2-induced alkaline phosphatase activity in Ishikawa cells showed the potential for Hops and its compounds to exhibit antiestrogenic activity. This work describes a new chemopreventive pathway for hops and highlights the importance of elucidating bioactivities for individual phytochemicals and the standardization to these compounds for optimal resilience promoting properties in women's health BDS. NIH grant, P50 AT000155.

Keywords: botanical estrogen chemoprevention

Poster #39

Synthesis of quinazoline-2,4-dione dimers

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Bacterial type II topoisomerases are targets for broad spectrum antibiotics. There are typically two different type II topoisomerases in bacteria, DNA gyrase and topoisomerase IV, and these topoisomerases are responsible for relaxing positive supercoiling of DNA. Fluoroquinolones and other quinolone-class antibiotics bind to DNA and type II topoisomerase to form a drug-DNA-topoisomerase ternary complex. Formation of ternary complex blocks religation of nicked DNA ultimately leading to double strand DNA breaks and cell death. Target-mediated resistance to fluoroquinolones, that being amino acid substitution(s) in gyrase or topoisomerase IV that impedes formation of ternary complex, is a significant clinical problem. Quinazoline-2,4-diones (diones) bind bacterial type II topoisomerases and form a ternary complex similar to fluoroquinolones. Many diones are equipotent with wild-type and fluoroquinolone-resistant topoisomerases, and thus overcome target-mediated fluoroquinolone resistance. However, diones have lower absolute antibacterial potency than fluoroquinolones because diones are excellent substrates for bacterial efflux pumps. Past work in the Kerns lab has shown that C7-linked fluoroquinolone dimers overcome efflux-based fluoroquinolone resistance in Gram-positive bacteria while maintaining or having improved antibacterial activity with different Gram-positive organisms. The fluoroquinolone dimers also overcome target-mediated resistance to fluoroquinolones based on mutations in topoisomerase IV by having more potent inhibition of gyrase. Currently, I am exploring strategies for creating diones that can overcome or evade efflux-based resistance. Guided by the previous work showing fluoroquinolone dimers evade bacterial efflux pumps, I am synthesizing C7-linked dione dimers for similar evaluation The different routes and methods for synthesis of dione dimers will be presented.

Keywords: Synthesis, antimicrobials, topoisomerase inhibitors

Poster #40

Utilization of the Tetrazine Ligation in a Modular Liver-Targeting Strategy for Non-Viral Gene Delivery

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Non-viral gene delivery to the liver faces many challenges including maintaining DNA stability in circulation, avoiding macrophage uptake, and selectively inducing hepatocyte gene expression. Previously we developed a plasmid DNA carrier system utilizing a PEGylated polylysine/acridine peptide capable of condensing DNA into nanoparticles. These nanoparticles exhibit circulatory stability and hepatocyte gene expression in mice under hydrodynamic dosing, but lack intrinsic liver targeting. This study reports on the use of tetrazine click-chemistry to append NeutrAvidin to the surface of nanoparticles as a modular system for attachment of biotinylated targeting proteins. To furnish the reactive handle, a heterobifunctional 5-kDa PEG was synthesized containing the tetrazine moiety on one end and a maleimide on the other for attachment to the DNA-condensing peptide. The counterpart, trans-cyclooctene (TCO) labeled NeutrAvidin, was constructed using NHS ester coupling. SDS-PAGE, gel filtration chromatography, and dynamic light scattering verified the covalent linkage of NeutrAvidin-TCO to the DNA nanoparticles. The sizes of NeutrAvidin-labeled nanoparticles were found to be dependent on the mole percentage of tetrazine in the nanoparticles as well as the extent of TCO modification of NeutrAvidin. In a pilot study, NeutrAvidin nanoparticles were functionalized with biotinylated targeting proteins apolipoprotein E (LDL receptor-specific), Sambucus nigra lectin (sialic acid specific), and Erythrina cristagalli lectin (galactose specific). A HepG2-based binding assay with fluorescently labeled DNA revealed that the targeting proteins promoted cell binding over controls. Further work involves testing various other targeting proteins and applying the system to in vivo gene expression and pharmacokinetic experiments in mice. This novel strategy thus provides a modular platform for rapidly testing targeting ligands and optimizing targeted non-viral gene delivery systems.

Keywords: Non-viral gene delivery, tetrazine ligation, avidin-biotin

Poster #41

Design, synthesis and evaluation of novel antimalarials inhibiting apicoplast DNA polymerase (apPOL) from P. falciparum

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Plasmodium spp. are the causative agents of malaria, killing nearly 600,000 people each year. Resistance of Plasmodium to chloroquine and artemisinin-based therapies accentuates the need for new drugs that target novel aspects of the parasite's biology. Nearly all parasites in the phylum Apicocomplexa have an unusual organelle called the apicoplast, acquired through a secondary endosymbiotic event with algae. It participates in the biosynthesis of fatty acids, heme, iron-sulfur clusters, and isoprenoids and any defect in apicoplast metabolism or failure of the apicoplast to replicate and divide leads to the death of the organism. Additionally, lack of a human counterpart to the apicoplast makes apicoplast promising drug target. The 35-kb genome of apicoplast is replicated by select DNA replication enzymes of which the apicoplast DNA polymerase (apPOL) is unique to the parasite. The apPOLs from P. falciparum and P. vivax have 84% homology, while the most similar human DNA polymerases are the lesion bypass polymerases theta and nu (23 and 22% identity, respectively). This suggests that drugs targeted against the Pf-apPOL would also be effective in treating P. vivax infections with low human toxicity. Towards identifying inhibitors of apPOL, a high throughput screen of 400 compounds from the Open Malaria Box provided by MMV identified an inhibitor of apPOL with an IC50 of 0.8 ± 0.3 µM. Preliminary studies indicate that MMV666123 is specific for apPOL, with no inhibition of human DNA Pol or E. coli DNA Pol I. Also, MMV666123 inhibits the polymerase activity of apPOL but not its exonuclease activity, suggesting binding to the C-terminal polymerase domain of apPOL. Additionally, being from the malaria box substantiates anti-malarial activity of MMV666123. Presented here are our current design, synthesis and in vitro evaluation efforts toward understanding the structural requirements for inhibition of apPOL and identifying more potent and drug-like apPOL inhibitors.

Keywords: Antimalarial, polymerase, Inhibitor

Poster #42

Selective Calpain-1 versus Calpain/Cathepsin-B Dual Inhibition as a Therapeutic Approach to AD

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It has been hypothesized that the imbalance or over-activation of cysteine proteases (notably cathepsins and calpains) contributes to neurodegenerative progression. Specifically, hyper-activation of calpain-1 (CAPN1), a modulary cysteine protease, has been implicated in the early pathogenesis of Alzheimer's Disease (AD), traumatic brain injury (TBI), and ischemic stroke. Prolonged CAPN1 over-activation indirectly permeabilizes lysosomes, leading to release of cathepsin B (CTSB), a lysosomal cysteine-protease implicated in neurodegeneration. Several reports propose CAPN1 and CTSB as therapeutic targets in AD and TBI, but do not unambiguously provide evidence for a desired strategy, and selectivity for inhibition of CAPN1 over CTSB has been the goal of the most developed program in pharma. We hypothesize dual CAPN1/CTSB inhibition will afford superior efficacy in AD and TBI over selective inhibition. We have identified selective and dual inhibitors and established enzyme inhibition and neuroprotective profiles in neuronal cells using Oxygen Glucose Deprivation (OGD), an in vitro model simulating ischemia-reperfusion injury in stroke. All inhibitors were differentially neuroprotective against OGD-induced cell death, depending on the treatment paradigm (pretreatment, ischemia, and reperfusion). Monitoring spectrin breakdown products (CAPN1-specific) identified different pathways of neuronal death with varying neuro-insults. Additional in vitro models using chemical insults were utilized to monitor CAPN1/CTSB substrates with roles in neuroplasticity/neurodegeneration via immunoblots. After establishing the selectivity of inhibitors for CAPN1 and CTSB, monitoring of peptide substrate proteolysis confirmed inhibitory effects in neuronal cultures, and allowed selection of inhibitors for further study in vivo. Next we aim to test these in a mouse model of mTBI manifesting cognitive deficit and cytokine surge, monitoring behavioral and biochemical changes.

Keywords: Cysteine, Protease, Neurodegeneration

Poster #43

Genome mining of freshwater cyanobacteria for rare metabolites

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Freshwater cyanobacteria are a prolific group of natural product producers that are understudied. Among the natural product classes that cyanobacteria produce are polyketides biosynthesized by type III polyketide synthases (T3PKS). For example, T3PKS genes encode enzymes involved in the production of the cytotoxic molecules known as cyclophanes. The Orjala lab has built the world's largest freshwater cyanobacteria strain collection at UIC. Since freshwater cyanobacteria chemistry is understudied, we propose that genome mining for T3PKS genes will expand the knowledge of cyanobacterial chemistry and T3PKS products. We have sequenced cyanobacterium UIC 10110, a Nostoc sp. and merocyclophane producer. We then used the gene sequence coding for the merocyclophane T3PKS to find homologs using Basic Local Alignment Search Tool (BLAST), and designed a degenerate primer pair to probe the strain library for T3PKSs by Polymerase Chain Reaction (PCR). After the initial screen of 449 strains, 69 strains were identified as potential hits to contain type III polyketides. We are currently in the process of confirming the hits by repeating the PCR reactions, and gel-purifying and sequencing the PCR products, followed by BLAST search and phylogenetic analysis. We have sequenced eight hits thus far, two of which were false positives and six of which were confirmed as potentially encoding T3PKSs. Future work includes sequencing the genomes of selected hits, predicting the encoded metabolites using bioinformatics tools, and obtaining the type III polyketide natural products. This work will ultimately expand the current knowledge of cyanobacterial chemistry and has the potential to discover new molecules that may be of biomedical relevance.

Keywords: Genome mining

Poster #44

An improved dereplication strategy for novel active natural products discovery

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Drug resistant infectious diseases continue to threaten global health as well as contemporary medical practices. There is an urgent call for novel antibiotics discovery. Mining novel sources, such as the marine bacteria, will clear the way for chemical and biological novelties. Our lab employed LC/MS-principal component analysis (PCA) based strain selection followed by an automated, high-throughput LC/MS fractionation to generate marine bacterial natural product libraries for antimicrobial activities screening. Fractions with promising antimicrobial activities were analyzed by NMR and UHPLC/HRMS. The biological activity data and the spectroscopic data would provide information for dereplication efforts and further purification of active compounds. By utilizing this platform, a novel anti-MRSA lanthipeptide (m/z = 2232.8164 [M+Na]+) produced by Streptomyces sp. WMMC-911 was discovered rapidly. Overall, our result highlights the advantages of applying modern analytical techniques in marine natural product libraries to accelerate novel antibiotics discovery.

Keywords: antibiotic discovery, lanthipeptide, natural product library

Poster #45

Crystallographic Study of Mechanism Based Inhibition of Glutamate Racemase

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The rising number of multi-drug resistant bacteria has increased the need for novel antimicrobial agent development. A potentially powerful target is the enzyme glutamate racemase (GR), which is absent in humans but present in all bacterial cells. GR is integral for the synthesis of the of the peptidoglycan layer in virtually all bacterial cells. This enzyme is responsible for interconversion of L-glutamate and D-glutamate, which is incorporated into bacterial cell walls. GR, is able to catalyze this stereoinversion without the use of cofactors, such as metal cofactors or pyridoxial phosphate, for catalytic acidification of the Cα-H bond. The compounds β-chloro-D-alanine (BCDA) and L-serine O-sulfate (LSOS), which are structurally similar to D-glutamate, have been reported in the literature as mechainism based inhibitors. The proposed mechanism of inhibition suggests one of catalytic cysteines abstracts a proton causing the chlorine or sulfate to leave, while the other acts as a nucleophile forming an irreversibly modified cysteine. In order to confirm the mechanism of inhibition, thermus thermophilus GR was co-crystalized with BCDA, D-glutamate, LSOS, and without any ligand. The structures showed that BCDA and LSOS were binding to GR in a similar fashion to D-glutamate. In order to confirm the proposed mechanism of inhibition, mass spectrometry will be conducted for GR with BCDA and LSOS. With the elucidation of the mechanism of inhibition of GR, novel antimicrobial agents can be developed to combat the rising prevalence of multi-drug resistant bacteria.

Poster #46

Divergent Stereoselective Synthesis of Rare Amino-sugars

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The desire to study the biological roles played by carbohydrates, such as cellular recognition and protein folding, has made the synthesis of rare and unnatural carbohydrates an important area of research for decades. De novo synthesis of carbohydrates from simple feedstocks has emerged as a promising method for the production of these rare mono- and oligosaccharides. Dihydropyrans, especially those obtained from the Achmatowicz rearrangement, have proven to be particularly useful intermediates for the de novo synthesis of carbohydrates. Aminosugars are an exceptionally interesting class of carbohydrates as they appear in an abundance of natural products, anti-biotics, and other medicinally interesting molecules. We have recently developed a novel stereoselective route to access all possible stereoisomers of 2,3,4,6-tetradeoxy 4-amino sugars and their derivatives systematically from Achmatowicz rearrangement products. This strategy combines our dynamic kinetic diastereoselective acylation methodology, with a rhodium catalyzed asymmetric reduction and the Mitsunobu reaction to give these rare aminosugars in high yields and stereoselectivities.

Keywords: Carbohydrate, Stereoselective, Synthesis

Poster #47

Design and Synthesis of Bivalent Inhibitors for Bromodomain and Extra-Terminal (BET) family proteins

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Extra-Terminal (BET) family proteins (BRD2, BRD3, BRD4 and BRDT) are transcriptional coactivators that interact with acetylated lysine residues of histones. Each BET protein has two bromodomains. BET proteins are involved in multiple cell functions and processes. Among them, BRD4 was identified as a promising target for cancer therapy and BRDT emerged as a potential target for male contraception. Therefore, targeting BET family proteins is of interest, however high sequence homology between different BET family proteins is a significant challenge for the discovery of selective inhibitor for a specific family member.
Recent research revealed that bivalent inhibitors can either induce the dimerization of proteins intermolecularly or bind to both bromodomains intramolecularly. In light of the new mechanism of BET bivalent inhibitors, we prepared bivalent inhibitors based on a potent BET inhibitor SG3-179. Symmetric inhibitors with a PEG1 linker maintained potency and selectivity similar to the parent compound with activity against all BET proteins. Through iterative optimization of attachment and linker chemistry, a series of symmetric bivalent inhibitor with PEG-linkers (PEGn, n=2–5) exhibited up to 70-fold higher selectivity for BRDT-1 over BRD4-1 in the AlphaScreen assay. Compared to the parent compound, these compounds also showed improvements in potency (~10 fold) for BRDT-1. The selectivity profile of one of the compounds, GXH-II-052, was validated in a BROMOscan assay, which confirmed selectivity for BRDT-1. This compound was about two-fold more selective for tandem BRDT compared to BRD4. Notably, GXH-II-052 was also active in a multiple myeloma (MM1.S) cell-based assay. Investigations into the possible mechanism for the observed selectivity profile of bivalent BET inhibitors is underway.

Keywords: Bromodomain, bivalent inhibitor, selectivity profile

Poster #48

Toward the Discovery of Small Molecule Inhibitors of APOBEC3B

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Apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3B (A3B) catalyzes C to U deamination in ssDNA as a function of innate immune defense against foreign DNA. A3s cause hypermutation in pathogenic DNA causing genomic instability and clearance by the host immune system. However, A3B is overexpressed in various cancer types and at sublethal levels leads to evolution and progression of many cancer types [1]. A3B is shown to be an enzymatic source of mutation in breast cancer, lead to poor clinical outcomes in cancer patients, and promote tamoxifen resistance in ER+ breast cancer and is shown to be nonessential in humans making it a viable target for cancer therapy [2-6].
Efforts in the Harki and Harris labs to discover inhibitors of A3 enzymes have yielded several small molecule scaffolds, many of which were shown to inhibit through covalent mechanisms [7,8]. Recent proteomics studies in our lab have shown covalent adducts between known pan A3 inhibitor MN23 with Cys217 and Cys239 of A3B. A3B constructs with cysteine to alanine mutations at C217 and C239 have been expressed and are being evaluated for their deaminase activity inhibition propensity by MN23 to determine the essential cysteine for enzyme inhibition. This information may be used to rationally design a selective small molecule inhibitor of A3B.
Due to the closed conformation of the active site, a fragment-based discovery approach may also be appropriate for A3B [9]. A new approach for fragment screening called Protein Observed Fluorine (PrOF) NMR utilizing 19F NMR has been developed where the protein itself is fluorinated and perturbations in the resonance shift of the NMR spectrum are monitored to determine ligand binding. This change in shift may be used to both quantify and determine location of binding making this a powerful tool for inhibitor discovery. This poster will highlight recent efforts to develop new A3B inhibitors and unveil the mechanism of action by known covalent inhibitors.

Poster #49

Improving activity and microsomal stability of selective CHOP activators

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Cellular homeostasis is facilitated by the proper balance of synthesis and modification of essential proteins and effective clearance of misfolded proteins. Eukaryotic cells harbor a regulatory stress mechanism known as the unfolded protein response (UPR) by which the accumulation of defective proteins within the endoplasmic reticulum triggers a clearance pathway in an attempt to restore order. If this mechanism fails, transcription factors such as C/EBP-homologous protein (CHOP) are upregulated which lead to apoptosis. Cancer cells, which have dramatically higher levels of protein synthesis and folding events due to elevated growth and angiogenesis needs, also have an increased burden of misfolded and defective ER proteins that contribute to cellular stress compared to a normal cell. A screen and subsequent medicinal chemistry campaign identified a series of sulfonamidebenzamides that were capable of upregulating the apoptotic CHOP pathway in cancer cells which served as a critical proof-of-concept milestone. However, the compounds showed metabolic liabilities that limited in vivo use and development. To address the issue, new chemotypes were explored and have recently delivered a promising scaffold with a 40-fold improvement in microsomal stability while maintaining antiproliferative activity observed with the prototype. Optimization efforts are now in progress to explore this chemical series for its anti-cancer potential.

Poster #50

Design and Synthesis of Dihydropyridine Analogs as BRDT Selective Inhibitors for Male Contraception

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Bromodomains are essential protein recognition domains that bind to N-ε-acetylated lysine side chains of histones and recruit other transcription factors during posttranscriptional regulation processes. The BET (bromodomain and extra-terminal) proteins are a sub-family of bromodomain-containing proteins consisting of BRD2, 3, 4, and T, which have attracted attention due to their essential roles in the various diseases. Although the most extensively studied BRD4 protein has been recognized as a promising anti-cancer target, BRDT is selectively expressed in the testis and plays a crucial role in spermatogenesis. Knock-out study in mouse model suggested that BRDT-selective inhibitors could become a male contraceptive agent. A tricyclic, dihydropyridine scaffold was identified from virtual high throughput screening and demonstrated promising activities against BET family proteins. After a single-step multicomponent reaction, the preliminary SAR around the dihydropyridine core was investigated. The co-crystal structure with BRD4-1 revealed that modifications at the lactone ring could occupy the ZA channel and interact with surrounding residues, which might improve BET affinity as well as selectivity. Therefore the lactone was converted to a lactam, an acyclic ester or acyclic amide. Based on the AlphaScreen assay results, the lactam subset was prioritized over the corresponding ester or amide one. A convergent synthetic route was then developed to further explore the lactam side chain. Three lactam analogs with submicromolar affinities towards BRD4 and BRDT showed promising activities in cell-based assays. Current derivatizations focus on strengthening the interactions between the lactam side chain and unique Arg54 in BRDT so as to optimize selectivity.

Keywords: Male contraception, bromodomains, BRDT

Poster #51

The use of mammospheres as models for predicting P450 1A1/1B1 metabolism

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Since the Women's Health Initiative reported that hormone replacement therapy directly correlated with increased risk of breast cancer and heart disease, many American women have turned to botanical supplements to seek relief from menopausal symptoms. Unfortunately, little is known about how these extracts modulate the chemical carcinogenic effects of estrogens. The genotoxic pathway involves 4-hydroxylation of estrone/estradiol by P450 1B1 whereas detoxification of estrogen through 2-hydroxylation is catalyzed by P450 1A1. These pathways are classically regulated by the aryl hydrocarbon receptor (AhR) and epigenetically regulated by estrogen receptor alpha (ERα). Botanical supplements can affect both ERα and AhR, causing differential effects within the same supplement. The ethoxyresorufin-O-dealklase (EROD) assay is a relatively simple assay that measures the enzyme activity of P450 1 family of enzymes. Unfortunately, in 2D MCF-7 cells, EROD could not measure enzyme inhibition without pretreatment with a strong agonists or separate the AhR-mediated effect from the ERα-mediated effect. 3D-Mammospheres are considered to be better models of humans than 2D monolayers. qPCR was utilized to understand differences in gene expression of CYP1A1/1B1 in 2D monolayers and 3D mammospheres. EROD showed increased enzyme activity in the 3D models as compared to the 2D models, which allowed the detection of AhR antagonists without pretreatment with an AhR agonists. With the P450 1B1 selective inhibitor, 2,3',4,5'-tetramethoxystilbene (TMS), ERα-mediated effects can be separated from those mediated by AhR. These results indicate that MCF-7 mammospheres, not monolayers, can be utilized to screen for modulation of estrogen chemical carcinogenesis and should be investigated in other assays as a way to achieve in vitro results more similar to humans. Supported by NIH Grant P50AT000155.

Keywords: Mammospheres; estrogen carcinogenesis

Poster #52

Commensal gut bacteria metabolize tacrolimus

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Tacrolimus is a commonly prescribed immunosuppressive drug used after solid organ transplantation. Tacrolimus is a narrow therapeutic index drug, but maintaining blood drug concentrations within the therapeutic range has been difficult due to large inter- and intra-individual variability in tacrolimus disposition. The objective of this study was to examine the role of gut microbiota in tacrolimus disposition. To explore the possibility of gut bacteria directly metabolizing tacrolimus, tacrolimus was incubated with C57BL/6J mouse cecum content or human stool samples anaerobically for 24 hours, and the mixture was analyzed by HPLC-UV. The results showed that tacrolimus amount decreased by up to 80% upon incubation, and this was accompanied by appearance of two new peaks on the chromatogram. Such results were not observed when tacrolimus was incubated with boiled cecum content or hepatic microsomes. Based on a recent report showing a positive correlation between fecal abundance of Faecalibacterium prausnitzii (F. prausnitzii) and tacrolimus oral dose needed to maintain therapeutic concentration in kidney transplantation patients, the ability of F. prausnitzii to metabolize tacrolimus was also tested. Incubation of tacrolimus with F. prausnitzii led to production of the same metabolites. Results from mass spectrometer analysis of the metabolites suggest that the metabolites are likely reduction products of tacrolimus. Together, these results indicate that gut bacteria, specifically F. prausnitzii, can metabolize tacrolimus. The extent of its contribution to overall tacrolimus disposition remains to be defined.

Keywords: Gut microbiota; Tacrolimus; Drug metabolism

Poster #53

Improvement of Physicochemical and Metabolic Profiles of Novel Anti-Alphaviral Agents

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Venezuelan, Western, and Eastern Equine Encephalitis Virus (VEEV, WEEV, and EEEV) are alphaviruses transmitted to humans most often from the bite of an infected mosquito. There are no FDA-approved human vaccines or therapeutics for any of these viruses. This fact, compounded with the ease with which some of these viruses can be weaponized as biothreats, underscores the need for continued research into effective interventions for alphavirus infections. Hit optimization of a quinazolinone scaffold led to the discovery of novel amidines which inhibit VEEV in cell culture and in mice, affording impressive antiviral protection. More recently, our work has yielded another chemotype for exploration and development. To further improve the activity profile of these scaffolds for in vivo efficacy, bioisosteric replacements for an aryl nitro group were investigated and sites of potential metabolism were modified. Progress towards these goals will be discussed.

Poster #54

The Mechanistic Role of Metal Ions, Ca2+ and Mg2+, in RGS: G-Protein Interactions

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Regulator of G protein signaling (RGS) proteins are negative regulators of G protein-coupled receptor (GPCR) signaling through their ability to act as GTPase activating proteins (GAPs) for activated G subunits. The RZ subfamily, of which RGS17 is a member, binds to activated G o, G z, and G i1-3 proteins to modulate downstream pathways, including those involved in formation of cyclic AMP and Ca2+-dependent signaling. In contrast to other RGS proteins, less is known about the regulation of RZ family members. Both crystallization and 1H-15N 2D HSQC NMR experiments revealed a putative interaction of the metal ion Ca2+ with RGS17 at a defined binding site. Subsequent protein-protein interaction experiments, using AlphaScreen were used to assess the impact on the ions Ca2+ and Mg2+ on the RGS17 interaction with activated G o. The results indicate that both calcium and magnesium have an effect of promoting the RGS17 - G o interaction. These studies will extend to examining the selectivity and affinity of RGS17 for other physiologically relevant divalent metal cations, such as Zn2+, Cu2+, and Mn2+. In addition, the residues of RGS17 that bind Ca2+ are conserved in multiple RGS proteins. The functional impact of metal ion binding is likely not limited to RGS17 and a more in-depth evaluation of these proteins for metal binding deserves further attention.

Keywords: G-Protein, Binding, signaling

Poster #55

Synthesis of Leu-Enkephalin Peptidomimetics Containing Trifluromethylalkenes as Amide Isopolar Mimics

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Fluorinated peptidomimetics are valuable substrates for exploring peptide backbone conformations and for perturbing physiochemical properties of probe compounds. For instance, trifluoromethylalkenes have served as amide isopolar mimics, but are rarely utilized, because many standard peptide-coupling conditions promote alkene isomerization to thermodynamically favored compounds. To address this challenge, we report the conversion of a naturally occurring amino acid to a Tyr1-ψ[CF3=CH]-Gly2 dipeptide mimetic, and notably, successful peptide coupling reactions that avoid alkene isomerization. Using this strategy, we generated trifluoromethylalkene-containing Leu-enkephalin peptidomimetics in high purity and good yield. This sequence suggests that the trifluoromethylalkene peptidomimetics can be incorporated into other target molecules with appropriate optimization. Ongoing work in our laboratory focuses on deprotecting both the C- and N-termini to provide analogs of Leu-enkephalin, and on characterizing pharmacodynamic and physiochemical perturbations imparted by the trifluoromethyl alkene isopolar mimic.

Keywords: Leu-Enkephalin; Trifluoromethylalkene; Peptidomimetics

Poster #56

Fluorinated peptidomimetics for improving drug-like characteristics of opioid peptides

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Endogenous opioid peptides regulate activity within the central nervous system (CNS), and are particularly interesting for treating pain, depression, and anxiety. Unfortunately, clinical use of peptide-based agents is restricted by poor physicochemical and biophysical properties, which limit penetration into the CNS. Therefore, many peptide-based probes cannot be employed clinically for treating many disease states. To address this problem, the Altman group explores the use of fluorinated peptidomimetics (FPMs) to improve the drug-like properties of peptides, and to deliver peptides into the CNS.
In this field, recent efforts have involved the synthesis and characterization of rationally designed FPM-based analogs of opioid peptides. In this poster, we describe the design, synthesis, opioid activity, and stability of fluoroalkene {Tyr1-ψ[(Z)CF=CH]-Gly2} and trifluoroethylamine {Tyr1-ψ[(S)/(R)-CF3CH-NH]-Gly2} analogues of the endogenous opioid neuropeptide, Leu-enkephalin, with the fluoroalkene analogue emerging as an orally-active, stable and CNS-distributed in vivo probe. This fluoroalkene peptidomimetic exhibited low nanomolar functional activity, with a μ/δ-selectivity ratio that mimicked that of the natural peptide, as well as improved stability, BBB permeability, and drug-like characteristics relative to the parent. However, the trifluoroethylamine peptidomimetics, did not activate the opioid receptors, and were unexpectedly rapidly metabolized in plasma. Thus, the fluoroalkene peptidomimetic serves as the new lead for future studies to improve opioid potency, selectivity, and signaling bias, and physicochemical and biophysical drug-like characteristics. This overarching strategy should be amenable for modulating physicochemical and biophysical properties of a broad spectrum of neuropeptides, with the ultimate goal of converting small peptide-based probes into CNS-active clinical candidates.

Keywords: Fluorinated Peptidomimetics, Leu-enkephalin, and BBB permeability

Poster #57

Progress Towards the Development of Broad Spectrum Anti-Encephalitic Alphavirus Inhibitors

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Alphaviruses are predominantly mosquito-borne pathogens that can cause significant disease in humans and animals. Venezuelan, Western and Eastern Equine Encephalitis Viruses (VEEV, WEEV, and EEEV) are alphaviruses that can cause flu-like symptoms and lead to debilitating neuropsychological sequelae or fatal encephalitis in infected patients. The absence of FDA approved vaccines or therapeutics for any alphavirus, in combination with the threat of V/EEEV use as a bioweapon, fueled a medicinal chemistry campaign to develop lead compounds from a promising hit quinazolinone scaffold that emerged from a screening effort. Optimization led to the discovery of amidine ML336, a potent antiviral compound that resulted from a novel chemical transformation by which quinazolinones rearrange into benzamidines and which demonstrated impressive antiviral protection in mice. Recent work has been focused on improvement of the ADME and pharmacokinetic profile of these compounds which has evolved to the development of a non-amidine chemotype. Guided by detailed, systematic SAR and SPR studies, lead compounds have been identified that show improved activity in vivo compared to amidines and which translate to 100% protection in a VEEV-focused lethal mice infection model.

Keywords: Encephalitic Alphavirus, Inhibitors, ADME and pharmacokinetic profile, in vivo efficacy

Poster #58

Label-free Visualization of Peptides and Small Molecules in Tumor Explants Using Imaging Mass Spectrometry

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Matrix assisted laser desorption ionization mass spectrometry imaging, with a time-of-flight mass spectrometer (MALDI-TOF MSI), has become a powerful tool for monitoring compound distribution in tissues and 3D cell culture. Analysis using MALDI does not require the use of a chemical label, which represents an advantage compared to other methodologies, such as fluorescence microscopy. In this work, we have extended this concept to tumor explants, which are advantageous because a single tumor xenograft can be physically divide into many smaller explants of a desired size, with which we can carry out many different experiments.
We have used ex vivo explants derived from MCF-7 tumor xenografts to examine cellular uptake of several different drugs and probes, including the selective estrogen receptor modulator 4-hydroxytamoxifen, the macrocyclic peptide cyclosporin A, and peptides we have prepared to inhibit the estrogen receptor/coactivator interaction. Together, these data demonstrate that a 3D explant model can be used for visualization of not only small molecules, but also cell-penetrating peptides, and they provide a strong proof-of-concept for using mass spectrometry imaging to probe cellular penetration in tumor explants.

Keywords: imaging mass spectrometry; cancer; peptides

Poster #59

Progress Towards an Efficient Annulative Cascade Affording Natural Product Inspired 2-3-Dihydropyrrolo[3,4-b]quinolin-1,9-diones and Derivatives

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Natural products are a rich source of inspiration for methodology development and pharmacologically biased templates for medicinal chemistry optimization. Dihydropyrroloquinolinediones are one such core featured within the quinolactacins, a family of natural products with a diverse set of reported biological activities, including antibacterial, antifungal, antiproliferative, and insecticidal properties. To construct novel quinolactacin-like templates, a convergent annulation strategy was explored to assemble 2-3-dihydropyrrolo[3-4,b]quinolin-1,9-diones from isatoic anhydrides and tetramic acids. The method under development leverages the acidity of the tetramic acid motif to effect the transformation under mildly basic conditions with high atom economy and affording CO2 and water as the only reaction by-products. Avoidance of side reactions and promoting the intramolecular dehydrative annulation were instrumental to improving the efficiency of this reaction. To date, various substituted anhydrides have been employed, resulting in product yields up to 70%. Current efforts are focused on the rapid, modular generation of these unprecedented structures that will be evaluated for biological activity.

Keywords: Synthesis, Methodology, Organic

Poster #60

Synthetic mimics of cytochrome b5 as cell surface receptors

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Cell surface receptors are the most common targets of therapeutics. Unfortunately, in some diseases, such as specific types of cancer and diabetes, normal receptors that are targeted are either missing or altered in ways that prevent them from being modulated by drugs. To overcome this limitation, we seek to develop a novel class of therapeutics based on synthetic molecules that functionally mimic cell surface receptors that are missing or altered in ways that lead to disease. This unprecedented approach is challenging due to the inherent complexity of most cell surface receptors, which are generally capable of rapidly responding to specific extracellular ligands by transmitting extracellular signals across cellular membranes into the interior. For most receptors, these signals are propagated by interactions between receptors and intracellular signaling proteins. To attempt to overcome this challenge, and to learn more about fundamental cellular processes, we seek to create the first synthetic cell surface receptors that control specific signal transduction pathways. As an approach to develop these types of agents, we are synthesizing and evaluating peptides related to the tail-anchored protein cytochrome b5, which naturally inserts unassisted into the plasma membrane of targeted cells. In addition, we are linking these peptides to mimics of cholesterol to both facilitate insertion into the plasma membrane and promote endocytic recycling between the cell surface and early/recycling endosomes, similar to the trafficking of many natural cell surface receptors. Further coupling of these peptides to molecules capable of passing across the cellular plasma membrane and engaging specific intracellular proteins is proposed to provide a mechanism for activating signal transduction. We are particularly interested in compounds that have the potential to control pathways that affect cellular proliferation and differentiation. Studies of model systems will be described.

Keywords: receptors bioorthogonal signaling

Poster #61

Development of a Bacterial Host for Antibiotic Discovery and Production

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Microbial metabolites are remarkable resources for antibiotic discovery. The majority of FDA-approved antibiotics are microbial metabolites. Actinobacteria genomes in particular encode a large and mostly untapped capacity for antibiotic biosynthesis. However, challenges of using Actinobacteria as a native antibiotic producer are a) poor expression under laboratory conditions and low yields of most encoded natural products, and b) cumbersome genetics which are time-consuming and labor intensive. An attractive option to overcome these challenges is heterologous expression in a developed host. An ideal heterologous host should have 1) rapid growth, 2) genetic tractability, 3) capability of high metabolite production, and 4) similar metabolic capacity as the native producer. Burkholderia sp. FERM BP-3421, a Proteobacterium, is a non-pathogenic, industrial strain that offers the desired characteristics of a heterologous host. FERM BP-3421 has a doubling time of ~1 hour, is genetically tractable, has been optimized to produce a drug lead in high yields (up to 6 g/L), and has the potential to produce complex metabolites of >20 biosynthetic gene clusters (BGCs) encoded in its genome. We hypothesize that FERM BP-3421 can be used as a heterologous host for the discovery and production of antibiotics from Actinobacteria. To test our hypothesis, we aim to start by expressing known antibiotic BGCs from Actinobacteria. The antibiotic BGC of interest will be directly captured from Actinobacteria genomic DNA by transformation associated recombination (TAR) cloning in yeast. The cloned BGC will be transferred into FERM BP-3421 via an electroporation protocol established in our lab. Expression of antibiotics will be verified by RT-PCR and production of antibiotics can be detected by UV-HPLC and mass spectrometry. Upon affirmation that FERM BP-3421 can be used as a host for expression of Actinobacteria BGCs, expression of unknown BGCs will follow.

Keywords: antibiotic drug discovery

Poster #62

Cheese Rinds as a Model to Study the Chemistry of Complex Microbial Communities

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Recently, interest in microbiomes has exploded as they are increasingly recognized to play key roles in health and disease in humans and many other hosts. While many studies link microbial composition to biological outcomes, the molecular mechanisms that determine these outcomes are poorly understood. This is partially due to the difficulty of dealing with the large number of variables present in complex multi-domain microbial communities. Cheese rind biofilms have recently been described as a simplified model system with highly reproducible patterns of microbial community succession. As such, the cheese rind biofilm model can be used to establish patterns of underlying biochemical processes that drive bacterial-fungal interactions. For this study we investigate the molecules produced by bacteria and fungus in response to different growing partners. Based on RNAseq data from our collaborators we selected bacteria and fungus of natural cheese rind members to grow in pairs on cheese curd agar and using MALDI-TOF imaging mass spectrometry (IMS) we visualized differences in the presence and spatial distribution of molecules. E. coli and Pseudomonas sp. JB418 were grown with different Penicillium and Scopulariopsis fungal partners. IMS screening of these diverse interactions has shown some common molecular distributions as well as unique chemistry with putative identities based on tandem mass spectrometry data. In the future we will use HPLC purification and NMR of bacterial and fungal extracts to confirm suspected identity of molecules.

Keywords: Mass Spectrometry, microbiome

Poster #63

3D Treatment-Resistant Breast Cancer Spheroids as a Predictive Model of in vivo Response to Endocrine Therapy

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1 out of every 8 U.S. women will develop invasive breast cancer during her lifetime, making it the second most common form of cancer affecting women. Breast cancer is also a leading cause of cancer related deaths for women in the U.S., second only to lung cancer. Activation of estrogen receptor alpha (ERα) is the primary proliferative mechanism of breast cancer cells, making it a logical target for therapy. ER ligands with antiestrogenic activity, such as the selective estrogen receptor modulator (SERM), tamoxifen, and selective estrogen receptor degrader (SERD), fulvestrant, have proven clinically successful as treatments for breast cancer; however, resistance in up to 50% of patients provides a therapeutic challenge. Once resistant, breast cancer cells become endocrine-independent, because of this, there is an urgent need for both novel therapy and improved models of resistant breast cancer. Our lab has created a panel of various endocrine-independent cell lines to mimic SERM and SERD resistance. Along with traditional 2D cell culturing, 3D spheroids have also been utilized to gain a better understanding of resistance. Importantly, the response to therapeutic agents, of cell lines in 2D versus 3D cultures is not identical. We observe that 3D cultures better replicate observations in mouse xenograft models, demonstrating that elements of the spheroid microenvironment, such as cell-cell interactions and the presence of extracellular matrix (EM), mimic aspects of the tumor microenvironment in vivo. Cells cultured as spheroids are therefore a suitable in vitro model for drug discovery, predictive of response in preclinical animal models, in contrast to 2D monolayer cell cultures.

Keywords: Cancer Resistance Endocrine

Poster #64

Bile acid exposure alters specialized metabolism leading to biofilm inhibition in Pseudomonas aeruginosa

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To communicate and regulate infection pathogenic bacteria release a series of specialized metabolites to induce a cumulative response within the community. An example of this bacterial communication is transformation into a biofilm state. Bacterial biofilms are a contributory factor to the persistence and antibiotic resistance of up to 80% of microbial infections in the human body however previous studies have shown that bile acids, such as taurolithocholic acid (TLCA), have biofilm inhibition activity in vitro. Studies exploring the mechanism of action have shown that bioactivity observed from bile acid exposure does not hinder motility, c-di-GMP production, and virulence which are all necessary for biofilm formation. Therefore, we hypothesize that TLCA is triggering specific specialized metabolite production that leads to the observed biofilm inhibition. To identify the specialized metabolite(s) involved in biofilm inhibition from TLCA exposure Pseudomonas aeruginosa, a classified antibiotic resistant ESKAPE pathogen, was used as a model organism due to its previously characterized specialized metabolism. As a proof of principle for our technique, P. aeruginosa colonies were analyzed using matrix assisted laser desorption/ionization time-of-flight imaging mass spectrometry (MALDI-TOF IMS) to visualize the spatial distribution of specialized metabolites produced when exposed to TLCA. In this axenic culture study, we visualized a shift in specialized metabolism of known and unknown metabolites including an unknown metabolite with an m/z of 609 that is only produced when the P. aeruginosa colonies are exposed to TLCA. Further analytical techniques were used to work towards identifying m/z 609. Additional experiments have begun to investigate if specialized metabolism is altered in vivo by studying zebrafish infection with Vibrio cholerae and results have shown altered production between different disease states and genders.

Keywords: Pathogenic bacteria, biofilms, mass spectrometry

Poster #65

A streptomyces tendae specialized metabolite interferes with quorum sensing in group a streptococcus

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Quorum Sensing (QS) is a process where bacteria produce, secrete, and detect chemical signals that trigger specific phenotypic responses including competence, antibiotic production, biofilm formation, and secretion of virulence factors. In group A Streptococcus (GAS), this cross-talk between bacteria is believed to play a role in the regulation of virulence. Therefore, finding a natural product regulator of QS in GAS may aid in understanding and manipulating this "switch". A family of transcriptional regulators, the Rgg proteins, have been shown to exhibit regulatory activity on pathogenic behaviors in bacteria, such as lysozyme resistance and biofilm development. Thus, inhibition of Rgg may offer a way to regulate these behaviors. To discover small molecule modulators of QS, a high-throughput luciferase assay was used to screen our actinomycete specialized metabolite fraction library to identify natural product inhibitors of Rgg. A potential QS inhibitor has been identified from a Streptomyces tendae strain. The strain, D051, was cultivated in a 28 L fermentation that yielded 1.7 grams of crude extract. Consecutive rounds of chromatographic separation were used to isolate four milligrams of the bioactive molecule from the crude material. High resolution mass spectrometry (HRMS) along with nuclear magnetic resonance spectroscopy (NMR) is currently being employed to elucidate the structure of the compound. Successful structural elucidation allows the use of this molecule as a molecular probe to understand QS mechanisms within GAS.

Keywords: structural elucidation, natural products, chemistry

Poster #66

Small Molecule Interactions from the Cheese Microbiota: Pseudomonas vs. Candida

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Cheese rinds are often composed of relatively small microbial community populations, in terms of diversity, making them attractive for the study of a natural microbiome. These small communities allow us to more easily study interactions between specific species in a controlled laboratory environment. Specifically, growth inhibition of Candida sp. 135E as a direct result of its interaction with Pseudomonas sp. JB418 was observed during a phenotypic screen. We hypothesized that Pseudomonas is excreting an antifungal metabolite, which is contributing to the adverse effects shown in Candida when the two are grown in co-culture. To further investigate this interaction, we used matrix assisted laser desorption/ionization time-of-flight (MALDI-TOF) imaging mass spectrometry (IMS) to visualize the spatial distribution of metabolites in both a co-culture of the two microbial species and individually. Simultaneously, Pseudomonas was cultured at a 1L scale, and its metabolites were subsequently extracted and crudely fractionated using SPE. In order to narrow down the fraction containing the metabolite of interest, bioactivity guided fractionation was performed to determine if Candida exhibited growth inhibition upon exposure. Fraction F (100% methanol) was found to elicit the strongest growth response when spotted onto a lawn of Candida, supporting our hypothesis that the reaction is mediated by specialized metabolites. Further bioassay guided fractionation by reverse phase high performance liquid chromatography of fraction F was performed to identify specific compounds that directly impacted Candida growth. Currently, ten fractions have been found to exhibit antifungal properties and will be further rarefied to identify the metabolites responsible for growth inhibition of Candida. Future directions for this research include the incorporation of tandem mass spectrometry and NMR to carry out structure elucidation of the isolated antifungal metabolites.

Poster #67

Context-specific translation arrest by antibiotics targeting the small and large ribosomal subunits

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It has been assumed that antibiotics which target the ribosome universally block protein synthesis. However, due to the dynamic nature of translation and the variety of substrates the ribosome interacts with, modeling and simple biochemical experiments may not completely predict how protein synthesis is inhibited in vivo. Our lab has shown that the action of classic antibiotics such as erythromycin and chloramphenicol depends on the amino acid sequence of the protein being made. We therefore asked if antibiotics that bind to other sites on the bacterial ribosome also inhibit translation in a context specific manner. We chose two antibiotics which bind to unique sites on the ribosome, spectinomycin (Spc) and evernimicin (Evn). Spc inhibits small ribosomal subunit movement which should prevent the ribosome from moving along any transcript. We demonstrate that strength of Spc inhibition is gene dependent, and synthetic derivatization can modulate the mode of action. Evn binds the large ribosomal subunit and should inhibit all A-site tRNA accommodation. However, we discovered that Evn preferentially inhibits translation within specific motifs and with specific codon sequences for incoming tRNAs. Our findings can contribute to rational drug design of new antibiotics and help to understand ribosome dynamics during translation.

Keywords: Antibiotic, Ribosome, Translation

Poster #68

Exploring Great Lakes actinobacterial genomes for natural product discovery and development

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Actinomycetes are known for their ability to produce novel lead compounds of clinical and pharmaceutical importance. In contrast to their terrestrial and marine counterparts, little is known about the capacity of freshwater-derived actinomycete bacteria to produce novel secondary metabolites. The genome of the Great Lakes-derived actinomycete bacterium Micromonospora sp. B006 was sequenced using both Illumina technology and Oxford Nanopore. An analysis of the joint assembly with antiSMASH and BlastP revealed 16 biosynthetic gene clusters, including non-ribosomal peptide synthetases, polyketide synthases, and terpenes. Only five of the predicted gene clusters could be linked to their respective products: the carotenoid sioxanthin, the siderophore desferrioxamine B, the prenylated phenolic lipids alkyl-O-dihydrogeranylmethoxyhydroquinones, a type II polyketide spore pigment, and the antimicrobial alkaloid diazepinomicin that was in clinical trial to treat glioblastoma. We showed that the strain B006 is indeed an alternative producer of diazepinomicin. The identity of the natural products of the other elven gene clusters remains cryptic. We developed a genetics system for Micromonospora sp. B006 that shall contribute to activating silent BGCs and to assigning function to these orphan clusters in the future.

Keywords: natural product discovery, actinomycete, genome mining

Poster #69

Structure-Guided Targeting of the Hyaluronan Binding Domain of CD44

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CD44 is a cell surface hyaluronan (HA) binding protein implicated in a variety of different cancers by modulating tumor cell adhesion, growth and therapeutic resistance. HA is synthesized as a high molecular weight (HMW-HA; 10^7 Da) polymeric carbohydrate consisting of repeating disaccharides of glucuronic acid and N-acetyl glucosamine. HMW-HA is a major constituent of the extracellular matrix and is often present in elevated levels in cancer tissue. HMW-HA stimulates progression by binding to CD44 and activating oncogenic signals. These oncogenic signals can be inhibited using very small (<10^4 Da) HA fragments to disrupt HMW-HA/CD44 interactions. Therefore, the goal of this project is to limit cancer progression and improve response to therapies through the development and use of small molecules that selectively block binding of HMW-HA by CD44.

Previous work in the Finzel laboratory has confirmed that analogs containing an 8-amino tetrahydroisoquinoline (THIQ) pharmacophore can bind CD44 near the HA binding groove with moderate affinity [L.-K. Liu, B.C. Finzel. (2014) J. Med. Chem. 57:2714-25]. Visualization of THIQ analogs bound in the HA binding domain of CD44 by crystallography have substantiated the design of a series of THIQ-linked oligosaccharides that should extend into the binding site of HA. Computational modeling predicts increasing affinity with the addition of each oligosaccharide unit to the THIQ-derived molecule, while retention of the THIQ should impart selectivity for CD44 over other HA-binding proteins. The aim of our research is to prepare and evaluate some of these THIQ-saccharide conjugates in order to study their effect on cancer biology and assess their potential as possible therapeutic agents. Progress toward the preparation and characterization of the first of these conjugates will be presented.

Poster #70

Palladium-Catalyzed Carbonylation of Pyridine-Directing Vinylogous Amides to Generate Privileged Pyridopyrimidinediones

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A new method is described that permits assembly of novel pyridopyrimidinediones, a scaffold expected to demonstrate valuable, yet unknown, bioactivity based on diverse therapeutic relevance reported for related structures. To access these compounds, we explored a strategy that would use a pyridine-directed, metal-catalyzed carbonylation reaction to bridge an attached vinylogous amide. Carbon monoxide (CO) has been widely used as a carbonyl source in the transition-metal-catalyzed carbonylation reactions with high efficiency and atom economy. Over the past several decades, the use of directed C-H activation/carbonylation reactions has been advanced through the use of a variety of directing groups, including amides, anilines, hydroxyl groups and nitrogen-containing heterocycles. Here, the first synthesis of pyridopyrimidinediones has been achieved via our palladium-catalyzed, pyridine-directed, C-H activation/carbonylation procedure, in which the pyridine acts as both a directing group and an intramolecular nucleophile. Reactions proceeded successfully under mild conditions to afford products in up to 90% yield. Optimization studies and the scope of the reaction will be discussed.

Keywords: Carbonylation, C-H activation, Pyridopyrimidinediones

Poster #71

Expanding the Toolbox of Catch and Release DNA Decoys for Modulation of Transcription Factor Signaling

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Transcription factors (TFs) are DNA-binding proteins that regulate transcription, making them critical to a cell's biological response to internal and external stimuli. While necessary for normal cellular function, when TF activity or expression is abnormal, the resulting abnormal cellular gene expression can initiate or support carcinogenesis. Therefore, TFs are the target of therapeutic study as well as intense biochemical evaluation. One method used to inhibit and study TFs is through the use of DNA decoys: synthetic DNA that mimics genomic DNA binding sites of TFs. Additional utility can be imparted on these decoys by the addition of light sensitive nucleotides that allow for spatiotemporal control of the DNA and therefore TF activity. This can be accomplished through the addition of nucleotide analogs that depurinate upon exposure to UV light. So called Catch and Release DNA Decoys (CRDDs) are initially active and capable of binding TF targets (catch), but are inactivated upon UV irradiation (release).(1) Initial designs utilized a 7-nitroindole purine mimic, but subsequent generations of these CRDD probes would benefit from nucleoside analogues with additional H-bonding capacity to stabilize the decoys. Additionally, pyrimidine mimics would also be useful for advancing the repertoire of targetable sequences. Advances in both areas of CRDD design will be presented.

1. Struntz, N.B.; Harki, D.A. Catch and Release DNA Decoys: Capture and Photochemical Dissociation of NF-κB Transcription Factors. ACS Chem. Biol., 2016, 11, 1631-1638

Poster #72

Development of Allosteric Inhibitors against Cyclin-Dependent Kinase 2 (Cdk2)

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With the advent of specific kinase inhibitors to combat cancer, such as imatinib for Bcr-Abl in chronic myeloid leukemia, further development of small molecule kinase inhibitors has been a promising avenue to treat a variety of illnesses. In particular, cyclin-dependent kinase 2 (Cdk2) has been a promising anti-cancer target, as it is normally activated by cyclin E to proceed through the cell cycle at the G1/S phase transition, and is hyperactivated in diseases such as ovarian, breast, and colorectal cancers. Additionally, Cdk2 knock-out mice are viable, so healthy tissue would ideally be unaffected by selective treatment. Historically, targeting Cdk2 involved designing molecules that bind its active site, leading to many off-target effects due to the homology of the ATP-binding site among kinases, especially other Cdk enzymes. However, the recent discovery of an allosteric inhibitor of Cdk2, the dye 8-anilino-1-naphthalenesulfonic acid (ANS), has renewed efforts to target this kinase in a specific manner. We have developed a series of ANS analogs that show via HSQC NMR further stabilization of the inactive conformation of Cdk2 and bind to the kinase in a pocket other than the active site. Additionally, we have evaluated the affinities of these analogs to Cdk2 using surface plasmon resonance (SPR) and modeled their binding modality using docking protocols and molecular dynamic simulations. By using this combination of biophysical techniques, we can develop a structure-activity relationship (SAR) of our compounds to design a potent yet selective allosteric inhibitor of Cdk2.

Keywords: Allosteric Kinase Inhibitors, HSQC NMR, Surface Plasmon Resonance (SPR)

Poster #73

Isoquinoline Carboxylates as Traceless Leaving Groups for Copper Chelation Assisted Glycosylations

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Oligosaccharides have been shown to have utility in medicinal chemistry, including anti-cancer therapeutics, antibiotics and vaccines. However, the synthesis of oligosaccharides can be challenging and often requires the formation of unstable glycosyl donors and harsh conditions for the formation of glycosidic bonds. We envisioned that having a coordinating ligand on the leaving group might provide a relatively stable glycosyl donor that can be activated under mild conditions. We discovered that an isoquinolinic ester could be selectively activated by copper (II) salts and acted as a traceless leaving group for glycosylation reactions. Although previous research had shown that picolinic esters could similarly be activated by copper (II), a competition experiment showed that the isoquinolinic esters were at least two orders of magnitude more reactive than picolinic esters in the presence of copper (II) salts. In addition, the isoquinolinic esters could also avoid the competitive transesterification side reactions observed for some picolinic substrates. Finally, the isoquinolinic ester was shown to be compatible with ortho-alkynyl esters that can be activated by gold (I) complexes, allowing for an iterative synthesis of a tetrasaccharide from monosaccharide units in only three steps.

Keywords: Glycosylation, Carbohydrate, Oligosaccharide

Poster #74

Telescoped Multicomponent Synthesis of Novel Amidobenzamidines via an Intramolecular Rearrangement of Substituted Quinazolinones

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The regiospecific rearrangement of 2-alkylaminoquinazolinones to E-amidobenzamidines was a significant discovery in our development of a novel antiviral scaffold. To expand our use of this unique structural template, an alternate synthetic strategy was explored to permit amidine diversification that was otherwise difficult to implement. Here we describe the adaptation of a previously reported Ugi-Mumm multicomponent reaction, followed by an intramolecular aza-Wittig reaction to deliver differentially substituted quinazolinones suitable for subsequent rearrangement through the Golden protocol. The approach required optimization of stoichiometry, reagent choice, substrate concentration, solvent, temperature, and additives to yield new amidines efficiently and disfavor side reactions. As such, we disclose a four-reaction sequence involving six chemical transformations that was employed to generate 17 amidines in 11-53% overall yield. Subsequently, the four-reaction sequence was telescoped to negate the requirement for column purification between steps and has thus far produced 10 amidines in 20-70% overall yield. The method represents a practical means of constructing elusive amidines which will be profiled for biological activity.

Poster #75

Exploiting the reactivity of isatoic anhydrides to generate natural product inspired tricyclic and tetracyclic scaffolds

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The constant quest for new drug-like molecules necessitates the development of new chemical methodologies to access unprecedented scaffolds. We envisioned a library of compounds inspired by biologically active scaffolds and developed a chemical method which exploits the reactivity of isatoic anhydrides to generate 1,3,4,10-tetrahydroacridin-9(2H)-one cores. The relatively mild conditions developed allowed us to synthesize analogues with different functional groups/substituents around the scaffold. The present method was amenable to a late-stage diversification strategy and we used it to synthesize a series of secondary compounds containing this core. The same strategy when carried out intramolecularly gave us access to unprecedented tetracyclic cores whose biological activity is hitherto unknown and will be assessed in a broad screening effort.

Keywords: Methodology development, Library synthesis

Poster #76

Identification and Characterization of Synergistic Phytoprogestin Compounds in Herbal Supplements

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Progestins are used to treat many gynecological diseases that result from dysregulated progesterone. Detrimental side effects like breast cancer, cardiovascular disease, and stroke are associated with progestin therapy. To prevent these side effects, alternative progestins that are selective for the progesterone receptor are necessary. Herbal supplements are popular among patients and it is unclear what these supplements contain since they are not tightly regulated. Previous studies have shown that some herbal supplements contain phytoestrogens and there is evidence that herbal supplements contain phytoprogestins. The purpose of this study is to identify phytoprogestin compounds in the herbal supplements, dogwood and red clover, and to understand their effects on progesterone mediated signaling. Dogwood (Cornus officinalis) is a popular botanical in Traditional Chinese Medicine and red clover (Trifolium pratense) is a popular botanical for women's health. Phytoprogestin agonist compounds from dogwood and red clover were identified by bioassay guided fractionation using a luciferase reporter assay. To test if the compounds were antagonists, the compounds were combined with progesterone. No antagonistic activity was found but it was discovered the compounds in dogwood were synergistic. Irilone from red clover was also found to be synergistic. Furthermore, proliferation assays revealed that irilone significantly decreased ovarian cancer cell proliferation, which is characteristic of a progestin. To understand irilone's mechanistic activity, irilone was tested to see if it stabilized the progesterone receptor or caused progesterone receptor ubiquitination. It was found that irilone could potentially be stabilizing the progesterone receptor.
In summary, the herbal supplements, dogwood and red clover, contain phytoprogestin compounds that are synergistic with progesterone. This is a novel finding as synergistic compounds with progesterone have not yet been discovered.

Keywords: progesterone, herbal supplements, phytoprogestins

Poster #77

Modulating Pharmacokinetics for Radiotherapy of Prostate Cancer.

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There are currently limited treatment options for patients with metastatic prostate cancer. Prostate cancer affects 14% of all men over their lifetime, and is responsible for 26,000 deaths annually in the US. Recently, targeted radionuclide therapy (Lu-177-PSMA) has shown promise as a treatment for metastatic disease. This success has spurred development of targeted alpha therapy (TAT) to inhibit cancer growth, using an alpha particle emitting radioisotope such as 225Ac is one urgent and promising approach. Due to its high linear energy transfer (LET), and short path-length (70-100 μm), α-particles are particularly cytotoxic, thus, appropriately targeted, they may be particularly effective in the treatment of cancer if toxicity can be managed.
Prostate- specific membrane antigen (PSMA), which is a cell surface glycoprotein, is expressed in prostate cancer cells and also in other cancers such as those of the lung, colon and breast. However, PSMA is also expressed in normal tissues, including kidney, and parotid and lacrimal glands.
The powerful cytotoxicity of alpha particle emitting radionuclides makes TAT attractive for treatment of refractory cancers, but avoidance of normal tissue damage is required to provide effective treatment without causing a significant decline in quality of life. We hypothesize that targeting serum proteins in PSMA TAT can provide effective drug delivery to tumors while simultaneously protecting normal tissues that express PSMA. Herein we have developed a library of non-natural amino acids containing ornithine and lysine which appended to the amino acid side chain amine. This strategy enhances the circulatory longevity of the proteins and peptides for greater efficacy of targeted drug delivery via this small molecule serum albumin binding functionality.

Keywords: Prostate Cancer, Non-natural amino acids , Radionuclides

Poster #78

Development of Novel Small Molecule PCSK9 Modulators

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The ageing and growth of populations has led to an increase in the total number of cardiovascular deaths, accounting for almost a third death globally in 2013. A high blood cholesterol (LDL-c) level is a well-known risk factor for cardiovascular disease. The important role of proprotein convertase subtilisin/kexin type 9 (PCSK9) in cholesterol homeostasis has been confirmed by gain- and loss-of-function PCSK9 variations in human populations and two recently FDA approved mAbs drugs. In consideration of disadvantages of mAbs, small molecule inhibitor of PCSK9 naturally emerge as another promising strategy. We recently identified a small molecule that can reduce the PCSK9 level in cell-based phenotypic screening. We preliminary explored the molecule structure relationship with the potency and other pharmacological properties by synthesis and test a series of compounds with structural diversity but similar skeleton.

Keywords: PCSK9, LDLR, Cholesterol

Poster #79

Novel In Silico Strategies Towards Optimization of New Anthrax Antitoxin Leads

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Bacillus anthracis, a well-known bioterrorism agent, is capable of inducing cytotoxicity even after treatment with antibiotics via a secreted tripartite exotoxin. This toxin has therefore become a popular target in the development of post-exposure anthrax therapeutics, specifically the component known as the lethal factor (LF), a zinc metalloprotease that cleaves members of the mitogen-activated protein kinase kinase family. Unfortunately, no LF inhibitors have yet been approved to treat anthrax. Recently, we reported two promising hits that emerged from our large-scale high-throughput screen of small molecules against LF. Structural biology studies on these compounds, however, proved challenging due to low aqueous solubility. In an effort to increase both the biological activity and solubility of these hits, toward the goal of lead optimization, we employed novel computational strategies incorporating bioisosteric replacement, physicochemical property prediction, and a variety of virtual screening techniques. Here we report the results of these in silico approaches as well as experimental biological activity data on analogs that we have identified. We also present key results from a large-scale virtual screen of a unique library of analogs, not previously reported, that we generated using structure-based design. Finally, we report new X-ray crystallography and structural biology data crucial in elucidating the binding modes of our novel compounds.

Poster #80

Multiple endocrine resistant breast cancer cell lines retain ER and sensitivity to endocrine therapy

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Endocrine therapy is the standard of care for breast cancer expressing estrogen receptor (ER), which occurs in 70% of patients. Unfortunately, acquired or de novo resistance to endocrine therapy is observed in up to 50% of patients, leaving a significant portion of patients with insufficient treatment options. Endocrine-resistance, usually defined as resistance to tamoxifen and aromatase inhibitor (AI) therapy, can also include resistance to selective estrogen receptor degraders (SERDs), since these also target ER. Since multiple mechanisms contribute to resistance, development of multiple resistant cell lines is needed for drug discovery and to identify characteristics that may suggest susceptibility to alternative and combination therapies. We have developed 5 stable, endocrine-resistant cell lines from a parent MCF-7 cell line, which all retain ER: one of these cell lines, MCF-7:CFR is resistant to the SERD fulvestrant. Clinical metastatic breast cancers that have gained endocrine resistance are overwhelmingly ER+. In addition to ER, progesterone receptor (PR) and glucocorticoid receptor (GR) status of these lines was assessed, and correlated with the response of these cells in culture to four classes of endocrine therapeutics: SERDs, selective ER modulators (SERMs), and selective human ER partial agonists (ShERPAs). Growth of all 5 cell lines was endocrine independent, indicating resistance to AI therapy. Two of the ER+, PR- cell lines were most resistant to the spectrum of endocrine therapies, but these cell lines both showed sensitivity to ShERPAs, especially in combination with non-endocrine targeted therapies, such as the PI3K inhibitor, alpelisib. Paradoxically, all endocrine-resistant cell lines responded to at least one of the endocrine therapies tested, demonstrating that if ER is not lost in the metastatic state, it remains a vulnerability suitable for therapeutic targeting.

Keywords: cancer, resistance, profiling

Poster #81

Developing a novel imaging mass spectrometry method to detect chemical communication driving metastasis in ovarian cancer

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Ovarian cancer is the most lethal gynecological malignancy in the United States. A precancerous mutation in a fallopian tube epithelial (FTE) cell is hypothesized to be the origin of 60% of cases of high-grade serous ovarian cancer (HGSOC), and the primary metastatic event is characterized by the migration of the tumorigenic FTE cell into the ovary during ovulation. However, there is little known about the chemical processes that may be involved in this initial metastasis. Therefore, understanding the chemical exchange that occurs between the fallopian tube and the ovary is imperative to combatting HGSOC.

The spatial distribution of molecules within a system can be visualized using imaging mass spectrometry (IMS) to determine products of specific inter-tissue interaction. We have optimized a system where an ovary is co-cultured with cells that are the murine equivalent of FTE cells (murine oviductal epithelial cells) demonstrating a tumorigenic PTEN alteration (MOE PTEN shRNA). This experimental design provides spatial distinction between the ovary and MOE PTEN shRNA , and can determine the origin and relative abundance of the mass-to-charge ratios (m/z). This is the first development of an IMS platform that visualizes interactive chemical exchange between mammalian cell culture and tissue. Our platform has identified a molecule at m/z 170.6 whose upregulation in co-culture may be implicated in early-stage HGSOC. HPLC retention time matching and UPLC-MS/MS indicate the molecule is norepinephrine, a neurotransmitter. IMS reveals that upregulation of norepinephrine by the ovary is being induced by the MOE PTEN shRNA cells, recapitulating previous literature that has described a role for norepinephrine in ovarian cancer. The identification of a mammalian metabolite in this system has validated that our method of IMS can detect metabolites relevant to disease, so that we can continue to use this method to unravel the biological pathways causing or affected by HGSOC.

Poster #82

Identification and characterization of potential BRD4 inhibitors by fragment-based screening using differential scanning fluorimetry, PrOF-NMR, and protein crystallography

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Bromodomain (BRD) containing proteins are essential for acetylated lysine (Kac) recognition onhistone proteins during transcriptional activation. BRDT is a testis-specific BRD protein required formale germ cell differentiation has been validated as a target for non-hormonal male contraception.

Differential scanning fluorimetry (DSF), a thermal shift assay, was used to detect proteinbinding in a fragment library screen, with a hit identified as a fragment increasing the transitiontemperature (T_m) of the protein ≥ 0.5 °C as compared to the DMSO control. From this screen, 22 compounds were confirmed as BRDT stabilizers upon repurchasing and dose response testing. Aprotein-observed 19 F-NMR (PrOF-NMR) assay with ^{19}F-labeled BRDT was used to confirm proteinbinding. Fluorescence polarization was also used to determine K_i and IC_{50} values.

The sixteen PrOF-NMR confirmed hits were then taken into crystallographic studies to providedata on binding interactions. A number of crystal structures have been solved with BRD4 and BRDT, allowing for the prioritization of potential lead compounds. Structure-activity relationships will beestablished for selected hits. Fragment TL00757 is currently in hit-to- lead optimization to identify potent and selective BRDT inhibitors. A ligand growth strategy is underway to increase BRDT affinity and selectivity overthe closely related protein BRD4 by increasing molecular interactions in the ZA channel andparticularly with Arg154.

Poster #83

MtPPAT inhibitors development for the treatment of tuberculosis

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Finding new leads that explore new targets is of utmost importance to overcome drug resistant tuberculosis (TB). The enzyme phosphopantetheine adenylyltransferase (PPAT) generates scientific interest since it displays a regulatory role in the Mycobacterium tuberculosis (M. tb.) coenzyme A (CoA) biosynthesis and has no inhibitors reported. Regarding this, potential M. tb. PPAT (MtPPAT) inhibitors were designed through Structure-Based Drug Design and Ligand-Based Drug Design approaches. Docking simulations were performed with the potential inhibitors previously designed followed by semi-empirical calculations for model refining. The validation of the docking method was performed through docking simulation with E. coli's PPAT inhibitors. Docking simulations showed that inhibitors fit the active site similarly to phosphopantetheine (substrate of PPAT). The calculated enthalpies by semi-empirical method showed that the interaction is favorable for some of the designed compounds. These studies led to the selection of the five most promising compounds. These compounds were synthesized, and had their activity evaluated. It was shown that in cell culture (MIC) vs M. tb. H37Rv strains some compounds exhibited activity in micromolar range. Besides that, it was possible to identify one compound that inhibits MtPPAT at 150 µM.

Keywords: MtPPAT, inhibitors, tuberculosis

Poster #84

Dual catalytic human topoisomerase inhibitors with anticancer activities

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Fluoroquinolones selectively target the bacterial type IIA topoisomerases, DNA gyrase and topoisomerase IV with a few exceptions that are also capable of targeting eukaryotic topoisomerase II. Fluoroquinolones traditionally bind and stabilize type II topoisomerase-DNA covalent complexes that contain a DNA break. This unique mode of action is referred to as 'topoisomerase poisoning'. We discovered that two novel fluoroquinolones, UITT-III-217 (1) and UITT-III-227 (2), could inhibit the catalytic activity of human topoisomerase II without poisoning it. Surprisingly, these compounds are more effective in inhibiting the catalytic activities of human and bacterial topoisomerase I. The NCI-60 DTP human tumor cell lines screen demonstrated their significant anti-proliferative activities against the 60 cancer cell lines used in the screen. A proof of concept in vivo efficacy study using a HT-29 xenograft model of human colorectal cancer showed that UITT-III-217 (1) could inhibit the proliferation of human colorectal cancer cells as well as fluorouracil in mice. UITT-III-227 (2) also exhibited activity but was not as effective as UITT-III-217 (1) in this xenograft model. Thus, these compounds act as dual inhibitors of human topoisomerases I and II and exhibit considerable anti-proliferative activity both in vitro and in vivo. Human topoisomerase I and II are well established targets for several clinically used chemotherapeutics including epipodophyllotoxins, camptohecins, and anthracyclines. A common therapeutic issue with current topoisomerase targeting anticancer drugs is secondary malignancies and cardiotoxicity. One potential cause of secondary malignancy stems from the genomic damage that occurs due to the poisoning mechanism of these compounds. Thus, there is a need for development of topoisomerase inhibitors that act through a different mechanism, such as catalytic inhibition. Interrogation of structural requirements for activity, improvement of biophysical properties, the role of DNA binding and efficacy of N-1 aryl fluoroquinolone analogs developed as anticancer agents is presented here.

Keywords: drug development, cancer

Poster #85

Dual Kinase-Bromodomain Inhibition of Brd4 and p38α using Trisubstituted-Imidazoles

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As regulators of transcription, proteins that interpret post-translational modifications to N-terminal histone tails through molecular recognition are known to be essential for maintaining cellular homeostasis. When dysregulated, these 'reader' proteins become drivers of disease. In the case of bromodomains, which recognize N-ε-acetylated-lysine, developing isoform selective inhibitors has been a significant challenge to the field. Here we present the development of a tri-substituted imidazole scaffold with selectivity for the N-terminal Bromodomain and Extra Terminal (BET) bromodomains (Brd4(D1), K_d = 1.2 μM, Brd4(D2)>100 μM) and potent MAP kinase inhibition (p38α, K_d = 470 pM). Affinity for the BET family of bromodomains was characterized using a fluorescence anisotropy method to displace fluorescently labeled pan-BET inhibitor, BI-6727. Co-crystal structures of Brd4(D1) with our most potent molecules confirmed engagement with a conserved asparagine (N140) in the acetyl-lysine binding pocket. In a similar manner to (+)-JQ1, these molecules inhibited NF-κB signaling and IL-8 expression in A549 lung cancer cells. In cell target engagement of our lead molecule with Brd4 and p38α was verified using a CEllular Thermal Shift Assay (CETSA). In further support of bromodomain inhibition, this series of molecules suppressed production of the c-Myc oncoprotein in multiple myeloma cells. From these studies, we conclude a tri-substituted imidazole scaffold represents a valuable starting point for synergistic therapeutic inhibition of both Brd4 and p38 α, the aberrant functions of which play a key role in cancer and inflammatory signaling.

Keywords: Epigenetics, BET Bromodomains, ^{19}F-NMR

Poster #86

NMR-Based Standardization of an Isoflavone Aglycone-Enriched Soybean Botanical

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Soybean (Glycine max Merr., Fabaceae) contains the isoflavones daidzein (DA), genistein (GE), and glycitein, which are known for their in vitro and in vivo estrogenic effects. Especially DA and GE have shown beneficial effects through activation of chemopreventive properties. The objective of this study is to optimize soybean extraction to yield an extract that is enriched in isoflavone aglycones. First, the beans were extracted by maceration at room temperature (5 g/ 150 mL) with EtOH, MeOH, and hydro-alcoholic mixtures. Secondly, an auto-hydrolysis method was developed that utilizes the enzyme naturally present in the beans to increase the concentration of isoflavone aglycones. The auto-hydrolysis was performed by mixing bean powder (10 g) with water (150 mL) under slow rotation at 25 °C during 2, 4, and 6 days. After hydrolysis, the samples were defatted with hexanes, and extracted with neat EtOH, successively. The 1 H iterative full spin analysis (HiFSA) method was utilized for qHNMR analysis to identify and quantify the target compounds, along with HPLC-UV analysis. Although maceration with MeOH and EtOH yielded extracts with higher concentrations of total isoflavones, aglycones were not detectable (LOD 0.13%). Only the auto-hydrolyzed soy extracts contained isoflavone aglycones at levels around 10%, as determine by a 100% qHNMR method. As DA and GE are key standardization markers for estrogenic and chemopreventive properties, this enriched-isoflavone soy extract optimizes these beneficial soy bioactivities.

Keywords: Soybean, isoflavones, NMR

Poster #87

Overcoming Antibacterial Resistance by Decoupling Electron Transport and Proton Motive Force

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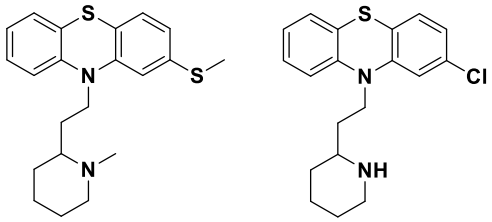
Antibiotic resistance is a rising global concern especially relating to resistant strains in *Mycobacterium tuberculosis* (*Mtb*) and *Staphylococcus aureus* (MRSA). This resistance has directly led to an increase in mortality and cost associated with treating these infections. Thioridazine (THZ), a classic tricyclic phenothiazine antipsychotic is bactericidal against both *Mtb* and MRSA. Notably, these compounds are able to inhibit type II NADH dehydrogenase (NDH-2) which is a non-proton transporting dehydrogenase that is found in pathogenic bacterium with no human isoforms. NDH-2 is also the preferred NADH dehydrogenase involved in electron transport to terminal oxidases in aerobic respiration in *Mtb* and other select obligate aerobes making NDH-2 essential in these organisms. Due to the limited structure activity relationships (SAR) of the phenothiazine antipsychotics as antibacterial agents we have begun a SAR campaign of novel phenothiazine analogues in an attempt to find more potent and selective antibacterial agents. One such analogue, **1**, achieved MIC_{90} values (8 μg/mL) against a virulent strain of *Mtb*, H37Rv. The analogues have also been characterized as efflux pump inhibitors which we have used to recover the antibacterial activity of chemotherapeutics that are principally effluxed such as fluoroquinolones. Photoaffinity probes are also being developed to study the mechanism(s) of action and to probe the binding pocket for this scaffold.



THZ **1**

Poster #88

Synthesis of NAMPT-targeted small molecule therapeutics for pulmonary arterial hypertension.

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Pulmonary arterial hypertension (PAH) is a fatal disease caused by vascular remodeling of lung arterial blood vessels which culminates in heart failure and death. There is no cure for PAH, and no treatment addresses its main pathologic process. There is a significant need to develop a selective and specific therapeutics for PAH. Our collaborator, Prof. Roberto Machado identified nicotinamide phosphoribosyltransferase (NAMPT) a therapeutic target for PAH, and a small molecule inhibitor of NAMPT (FK866) not only prevented the vascular remodeling but also reversed it in rat models. We have cooperated our group chemistry into developing novel analogues of NAMPT inhibitors to resolve the debilitating toxicity, solubility, and stability issues associated with current small molecule therapeutics.

On the basis of computer assisted molecular design and high throughput screening by Dr. Kiira Ratia, we have discovered and synthesized two new series of novel NAMPT inhibitors that exhibit comparable enzymatic activity relative to FK866. These compounds have shown very good *in vitro* NAMPT inhibition with IC_{50} values as low as 26 nM in CycLex colorimetric assay. The lead compound of the series was observed to prevent and reverse pulmonary arterial cell remodeling in the MCT-rat model and exhibits promising pharmacokinetics.

Pulmonary arterial hypertension (PAH), NAMPT inhibition

Poster #89

Activity-Based Labeling of IlvE: a PLP-Dependent Enzyme from *Mtb*

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Pyridoxal-5-phosphate (PLP)-dependent enzymes catalyze an extraordinary diversity of chemical reactions in both primary and secondary metabolic pathways. The human genome encodes more than 400 PLP-dependent enzymes while many pathogenic microorganisms contain dozens of essential PLP-dependent enzymes. A number of FDA-approved drugs are known that covalently modify PLP-dependent enzymes exemplified by vigabatrin, carbidopa, D-cycloserine, and eflornithine used to treat epilepsy, Parkinson's, tuberculosis, and African sleeping sickness, respectively. Moreover, drug discovery efforts are ongoing for numerous PLP-dependent enzymes in oncology, infectious disease, and neuroscience. In general, many of these aforementioned drugs are characterized by substantial side effects that are incompletely understood, but likely due to inhibition of other functionally related enzymes. Activity-based protein profiling (ABPP) is a powerful technique that has become popular in the field of chemical biology for characterizing enzyme selectivity and target identification of tool compounds; however, there is remarkably no described probe for PLP-dependent enzymes, one of the most important enzyme classes in biology and human medicine. We have successfully synthesized an activity-based probe capable of covalently labeling PLP-dependent enzymes. Preliminary data using purified IlvE from *Mycobacterium tuberculosis*, a PLP-dependent aminotransferase enzyme responsible for biosynthesis of the branched-chain amino acids, shows that our probe is fully capable of labeling. The native substrate for IlvE bears no structural resemblance to this probe suggesting it will promiscuously label other PLP-dependent enzymes. We currently seek to utilize this probe to label an *E. coli*strain that overexpresses *Mtb* IlvE. We anticipate that this work will allow for the profiling of enzymes in this class and the many FDA-approved drugs that target them to determine the mechanistic basis of their toxicity and potentially identify other unknown mechanisms of action of these multi-targeting inhibitors.

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