The 59th Annual MIKIW Medicinal Chemistry Meeting-in-Miniature

April 29th – May 1th 2022

Hosted by the
Division of Medicinal and Natural Products Chemistry
Department of Pharmaceutical Sciences and Experimental Therapeutics
College of Pharmacy
The University of Iowa

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Welcome to the 59th Annual MIKIW Meeting!

On behalf of the Division of Medicinal and Natural Products Chemistry at The University of Iowa, and this year's student organizing committee, we welcome you to Iowa City for the 59th Annual MIKIW Midwest Medicinal Chemistry Meeting-in-Miniature! It is our pleasure to host the first in-person MIKIW meeting since the circumstances surrounding COVID-19. It is an especially distinctive conference for our first, second, and third year students, as it will mark their first ever in-person MIKIW meeting. Likewise, we are thrilled to host this year's meeting in our new College of Pharmacy Building! We look forward to a weekend of science, networking, collaboration, and fun between our universities. We also would like to thank all faculty, students, postdoctoral researchers, and staff for your scientific contributions to our meeting.

The MIKIW keynote address has historically been presented by world-class scientists, including multiple Nobel Prize winners and ACS Division of Medicinal Chemistry Hall of Fame Inductees. This year, we are delighted to welcome Dr. Kate Carroll, Professor from UF Scripps Biomedical Research, to present this year's Joseph G. Cannon Keynote Address. Dr. Carroll continues the tradition of esteemed speakers as she currently serves on the editorial board of *Cell Chemical Biology*, *Chemical Probes.org*, *Molecular Biosystems*, the *Journal of Biology Chemistry*, and is a contributing member to the *'Faculty of 1000'*. She is also the recipient of the *ACS Pfizer Award in Enzyme Chemistry* (2013), *Camille Dreyfus Teacher-Scholar Award* (2010), *Scientist Development Award* from American Heart Association (2008), and *Special Fellow Award* from the Leukemia and Lymphoma Society (2006).

Last, but not least, we would like to thank our organizing committee, faculty advisor, University of Iowa administrators, and our sponsors for all the hard work and support they have put into making MIKIW 2022 a huge success. We hope that you all enjoy this year's meeting and hope that you make new connections and learn something new!

Sincerely,

Rachel A. Crawford, Anna E. Bartman, and Moana E. Hala'ufia
MIKIW 2022 Student Organizing Committee

Zhendong Jin, Ph.D.
Faculty Advisor

MIKIW 2022 Sponsors

The MIKIW 2022 organizing committee would like to thank all of our generous sponsors! We would also like to specifically thank **Lynne Cannon** for her continuous donations to the MIKIW meeting. Without all of you, this meeting would not be possible.

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MIKIW History

The first Midwest Medicinal Chemistry Meeting-in-Miniature was held in 1963 at the University of Iowa. The meeting has often been informally referred to as 'MIKI' as its founders originated from the Departments of Medicinal Chemistry at the Universities of **M**innesota, **I**llinois-Chicago, **K**ansas, and **I**owa. Since then, these four universities have alternately hosted the MIKI meetings each year. In 2018, the School of Pharmacy at the University of **W**isconsin-Madison joined the original MIKI lineup, thus creating 'MIKIW'.

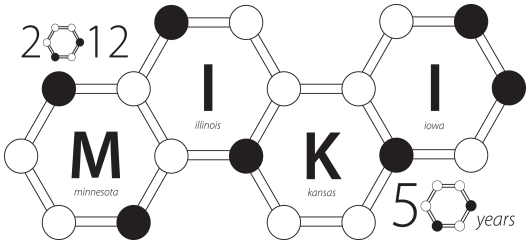
MIKIW is the longest-running and largest regional medicinal chemistry conference in the United States. The MIKIW meetings have historically been organized and led entirely by graduate students on an annual basis. The meeting integrates over 200 graduate students, post-doctoral fellows, and faculty members for a two-day, multidisciplinary conference that exposes students and faculty alike to a wide array of medicinal chemistry aspects. The conference provides an excellent environment in which research is presented as symposium talks and poster presentations given by graduate students.

The MIKIW meeting aims to promote scientific discussion and exchange of ideas that encompasses medicinal chemistry, drug discovery, chemical synthesis, bioanalytical chemistry, chemical biology, and drug metabolism between scientists across universities. Additionally, each year the MIKIW meetings showcase a keynote address given by an esteemed scientist in the field of medicinal chemistry and drug discovery. This year's Joseph G. Cannon keynote speaker is Dr. Kate Carroll – UF Scripps Biomedical Research. These keynote lectures provide students and faculty the valuable opportunity to interact with one of the top scientists in the field, both scientifically and socially.

List of Previous Keynote Speakers

Year	Host	Speaker	Association
2021	Wisconsin	Dr. Emma Parmee	Janssen Research & Development
2020			
2019	Kansas	Dr. Nicholas Meanwell	Bristol Myers Squibb
2018	Illinois	Dr. Michelle Arkin	University of California, San Francisco
2017	Minnesota	Dr. Uttam Tambar	University of Texas
2016	Iowa	Dr. Amy H. Newman	NIH-NIDA
2015	Kansas	Dr. Bruce D. Roth	Genentech Inc.
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. Tomáš Hudlický	Brock University
2009	Minnesota	Dr. Dale Boger	Scripps Research Institute
2008	Iowa	Dr. Daniel Kahne	Harvard University
2007	Kansas	Dr. Albert Padwa	Emory University
2006	Illinois	Dr. William Fenical	University of 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	University of Utah
2002	Illinois	Dr. Richard B. Silverman	Northwestern University
2001	Minnesota	Dr. Andrew	Hamilton Yale University
2000	Iowa	Dr. Michael Marletta	University of 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	University of Wisconsin, Madison
1992	Iowa	Dr. Laurence Hurley	University of Texas, Austin
1991	Kansas	Dr. Julius Rebek	Massachusetts Institute of Technology
1990	Illinois	Dr. Koji Nakanishi	Columbia University
1989	Minnesota	Dr. John Katzenellenbogen	University of Illinois, Urbana-Champaign
1988	Iowa	Dr. Carl Djerassi	Stanford University
1987	Kansas	Dr. William Roush	Indiana University
1986	Illinois	Dr. Joseph Fried	University of Chicago
1985	Minnesota	Dr. David	Triggle SUNY Buffalo
1984	Iowa	Dr. Alan Katritzky	University of Florida
1983	Wisconsin	Dr. Paul Bartlett	University of California, Berkeley
1982	Kansas	Dr. Henry Rapoport	University of California, Berkeley
1981	Illinois	Dr. Harry Wasserman	Yale University
1980	Minnesota	Dr. Eugene Jorgensen	University of 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	Hoffman-LaRoche
1972	Minnesota	Dr. Gertrude Ellion	Burroughs-Wellcome
1971	Iowa	Dr. Bernard Belleau	University of Ottawa
1970	Kansas	Dr. Corwin Hansch	Pomona College
1969	Illinois	Dr. Everett Maynert	University of Illinois
1968	Minnesota	Dr. Bernard Baker	University of California, Santa Barbara
1967	Iowa	Dr. Julius Axelrod	National Institutes of Health
1966	Kansas	Dr. Richard Schowen	University of Kansas



A Dedication to Joseph G. Cannon

Joseph G. Cannon (1926 – 2011) received his B.S. in Pharmacy (High Honors), his M.S. in Chemistry, and his Ph.D. in Chemistry from the University of Illinois in 1951, 1953, and 1956, respectively. Following his Ph.D. studies with G. L. Webster, Dr. Cannon was appointed as an Assistant Professor at the University of Wisconsin, reaching the status of an Associate Professor in 1960. In 1962, Dr. Cannon accepted a position to join the faculty at the University of Iowa in the College of Pharmacy where he was named a Professor of Medicinal Chemistry in 1965. Dr. Cannon remained on the faculty at Iowa for the remainder of his career fulfilling many roles including, Head of the Division of Medicinal and Natural Products Chemistry, Dean for Graduate Study and Research, Acting Dean of the College of Pharmacy, and achieving the status of Professor Emeritus in 1996.

Dr. Cannon served as a mentor to a total of 22 M.S. candidates, 55 Ph.D. candidates, and 20 postdoctoral fellows. He also served on the editorial board for various journals or book reviews including: *Journal of Medicinal Chemistry, Annual Reports in Medicinal Chemistry, Chirality, Indian Journal of Heterocyclic Chemistry*, and *Burger's Medicinal Chemistry*, Sixth Edition. Additionally, Dr. Cannon authored a book titled *Pharmacology for Chemists*. He also taught a short course with the same title at the American Chemical Society for 27 years and was bestowed with the *ACS Outstanding Teacher Award* in 2006. In addition to this honor, Dr. Cannon earned numerous awards over his career. In 1984 he was recognized as the *Dale E. Wurster Fellow* at the College of Pharmacy and went on to receive the *Iowa Regents Award for Faculty Excellence* in 1994. Dr. Cannon was the recipient of the *Smissman Bristol Myers-Squibb Award* in Medicinal Chemistry in 1997 and was inducted into the *ACS Division of Medicinal Chemistry Hall of Fame* in the same year.

Besides these contributions to the field of medicinal chemistry, Dr. Cannon was one of the founders of the MIKIW Medicinal Chemistry Meeting-in-Miniature. The first meeting was hosted by Dr. Cannon and his wife, Lynne, at their home in Iowa City in 1963. It aimed to provide for an in-person opportunity for scientific discussion and collaboration. Since its simple beginnings of a spaghetti dinner in Dr. Cannon's living room, MIKIW has progressed throughout the last 54 years to include formal presentations given by students, as well as a keynote address by a renowned individual in the field of medicinal chemistry. Despite the inclusion of these formal presentations, the MIKIW Meetings still retain the informal atmosphere envisioned by the founders. This historic meeting continues to act as a catalyst for the sharing and exchanging of ideas between students and faculty of the different universities.

To commemorate the immense contributions of Dr. Cannon to the successful tradition of MIKIW, and to the field of medicinal chemistry, the MIKIW keynote address at the University of Iowa has been named the *Joseph G. Cannon Keynote Address* since 2016.

MIKIW 2022 Program Agenda

Friday, April 29th		
3:00 – 6:00 pm	Hotel check-in	UI Students will meet at corresponding Hotels
7:00 – 10:00 pm	Welcome Reception	Celebration Farm
Saturday, April 30th		
8:00 – 8:50 am	Breakfast, poster set up, AV check	CPB 1st and 2nd Floors
8:50 – 9:00 am	Opening remarks: **Gary Milavetz**, BS, PharmD, RPh, FCCP, FAPhA *Executive Associate Dean*	CPB 210 Overflow Room: CPB 110 A/B
	SESSION 1	
9:00 – 9:25 am	**Md. Abdullah Al Noman**, Gunda I. Georg Lab, University of Minnesota *Toward the Discovery of a Male Birth Control Pill: RARα Antagonists*	
9:25 – 9:50 am	**Madeline Hennessy**, Andrew Riley Lab, University of Illinois-Chicago *Synthesis and Evaluation of Akuamma Alkaloid Analogues for Alternative Pain Therapies*	
9:50 – 10:50 am	Workshops and Vendor Session *Please see pg. 12 for details*	
10:50 – 11:00 am	Break	
11:00 – 12:00 pm	*Joseph G. Cannon Keynote Address* **Dr. Kate S. Carroll**, Professor, UF Scripps Biomedical Research *"Cysteine-Mediated Redox Signaling: Chemical Tools for Biological Discovery"*	
12:00 – 1:30 pm	Lunch	CPB Courtyard and 1st Floor
	POSTER SESSION	
1:30 – 2:15 pm	Odd numbered posters present	CPB 1st and 2nd Floors
2:15 – 3:00 pm	Even numbered posters present	CPB 1st and 2nd Floors
3:00 – 3:10 pm	Break (Preparation for Session 2)	
	SESSION 2	
3:10 – 3:35 pm	**Hannah K. Gruenwald**, Robert Kerns Lab, University of Iowa *Stability of Triphenylphosphonium Moieties in Biologically Relevant Conditions*	
3:35 – 4:00 pm	**Manvendra P. Singh**, Zarko Boskovic Lab, University of Kansas *Complexity Index-guided Exploration of New Chemical Space*	
4:00 – 4:25 pm	**Marianne B. Palczewski**, Douglas Thomas Lab, University of Illinois-Chicago *Nitric Oxide Reprograms DNA Methylation and Tumor-Associated Gene Expression by Inhibiting DNA Demethylases TET and ALKBH2 in Triple-Negative Breast Cancer*	
4:25 – 4:50 pm	**Grant T. Cooling**, M. Ashley Spies Lab, University of Iowa *Glutamate Racemase: A Shape-Based Inhibition Story*	
6:00 – 6:30 pm	Faculty mixer	The Heights Rooftop
6:30 – 7:00 pm	Open cocktail hour	
7:00 – 10:00 pm	Formal Dinner	The Heights Rooftop

Sunday, May 1st

Check out prior to breakfast		
8:00 – 8:45 am	Breakfast	CPB Lobby
	SESSION 3	CPB 210 Overflow Room: CPB 110 A/B
8:45 – 9:10 am	**Xiaolei Li**, Weiping Tang Lab, University of Wisconsin-Madison *A General Strategy for the Synthesis of Rare Sugars via Ru(II)-catalyzed and Boron-mediated Selective Epimerization of 1,2-trans-diols to 1,2-cis-diols*	
9:10 – 9:35 am	**Patrick Ross**, Mark Farrell Lab, University of Kansas *Covalent strategies for the study of protein-carbohydrate interactions*	
9:35 – 10:00 am	**Rachel A. Crawford**, Jonathan Doorn Lab, University of Iowa *Characterization of a 3,4-Dihydroxyphenylacetaldehyde and L-cysteine Adduct Formed In Vitro After Neurotoxicant Exposure Reveals Its Potential as a Biomarker of Catecholaminergic Stress*	
10:00 – 10:25 am	**Conrad A. Fihn**, Erin Carlson Lab, University of Minnesota *Development of Histidine Kinase Inhibitors as Anti-Virulence Agents in Pseudomonas aeruginosa*	
10:25 – 10:40 am	Break	
	SESSION 4	CPB 210
10:40 – 11:05 am	**Alexander K. Hurben**, Natalia Tretyakova Lab, University of Minnesota *Modulating DNA Methylation with Bifunctional Ten-Eleven Translocation Dioxygenases Inhibitors*	
11:05 – 11:30 am	**Tova M. Bergsten**, Joanna Burdette Lab, University of Illinois-Chicago *Investigating the Role of Soluble Metabolites in Primary Metastasis of High Grade Serous Ovarian Cancer*	
11:30 – 11:55 am	**Jacob R. Immel**, Steven Bloom Lab, University of Kansas *Photoredox Catalysis for Peptide SAR and Applications to HIV-1 Drug Development*	
11:55 – 12:00 pm	Concluding remarks, boxed lunches served (Faculty)	CPB Lobby
12:10 – 12:40 pm	Faculty meeting, boxed lunches served (Grad Students and Post Docs)	
~12:40 pm	Buses depart	

The University of Iowa: College of Pharmacy, Department of Pharmaceutical Sciences and Experimental Therapeutics, Division of Medicinal and Natural Products Chemistry presents:

The Joseph G. Cannon Keynote Address by Dr. Kate S. Carroll, Ph.D.

Dr. Kate Carroll received her B.A. in 1996 in biochemistry at Mills College followed by completion of her Ph.D. in biochemistry from Stanford University in 2003. From there she became a Postdoctoral Fellow under the mentorship of Dr. Carolyn Bertozzi at UC Berkeley from 2003-2006. Dr. Carroll has climbed the ranks from Assistant Professor of Chemistry at University of Michigan (2006-2010) to Associate Professor of Chemistry at Scripps Research (2010-2013) and finally as Professor of Chemistry at UF Scripps Biomedical Research.

The Carroll lab has a proven track record of attacking fundamental problems in redox biology through a powerful, interdisciplinary approach that integrates synthetic chemistry with proteomics, biochemistry, and cell biology. One of the overarching goals of her lab is to understand the biological chemistry and molecular mechanisms of redox-based cellular regulation and signal transduction. Specifically, Dr. Carroll places an emphasis on researching the role of cysteine oxidation, a ubiquitous and conserved mechanism for controlling protein function. Other interests are focused on developing a new class of inhibitors that targets oxidized cysteine residues of key proteins involved in human disease, such as kinases and phosphatases. Additionally, her lab also investigates sulfur metabolic pathways that are essential for infection and long-term survival of human pathogens.

Major goals of the Carroll research lab include:

- Discovery of key regulator nodes of redox-signaling networks
- Identification of profile changes in protein cysteine oxidation associated with disease
- Using the information discovered, development of new diagnostic and therapeutic approaches

Dr. Kate S. Carroll – Keynote Address Abstract

Cysteine-Mediated Redox Signaling: Chemical Tools for Biological Discovery

The exploration of thiol-based redox regulation and signaling offers a grand challenge for achieving a molecular-level understanding of its unique role in physiology and pathology. Redox biology also represents a frontier for developing new therapeutics for cancer, neurodegenerative, and metabolic diseases. We are developing novel small-molecule probes to identify and study the underlying chemistry that governs thiol-based redox systems in biology. This talk will present our latest results in the discovery and understanding of reactive oxygen species as emerging new chemical signals and their influence on protein function vis-à-vis oxidative post-translational modification of pivotal cysteine residues.

Workshops and Vendor Session

The MIKIW 2022 Organizing Committee would like to thank the University of Iowa Hardin Library for giving their time to present three informative workshops geared towards graduate student success. We would also like to thank our sponsor vendors who are present for the vendor session. Workshops will be presented concurrently, so we encourage you to attend the workshop that is most interesting to you. During this time, we will also have our sponsor vendors open for interaction. We hope that you find these workshops helpful in your pursuit of your research goals.

Available Workshops

Data Management Essentials **Room: 2-257**

Whatever the research you are doing, chances are, you're working with research data. Small changes to your data management practices can make a big difference during research, and when you are ready to share the results. In this workshop, you'll learn how to securely store and organize your data, keep track of changes to your files, create accompanying documentation, and submit your data for publication. Employing these practices will ensure that everyone, including you, can use and reuse your data in the weeks, months, and years to come.

NCBI Databases for Gene, Nucleotide Sequences & Protein Information **Room: 1-110A**

Overwhelmed by the number of databases that the National Center for Biotechnology Information (NCBI) has to offer on nucleotide sequences, genes and proteins? Wondering which database you should always start with? Would you like to learn how to set up an NCBI account to link articles in PubMed to records in other databases? Do you know about PubMed's Gene Sensor? Are you familiar with the concept of linear navigation? Learn all of these tips and more in this session that is designed for anyone who needs to search the NCBI databases for genetic information.

Open Access: Policies, Publishers, and Predators **Room: 1-110B**

Open Access to scientific literature is one of the most hotly debated topics in scholarly publishing. This workshop will provide an overview of what scientists need to know when making their research open access. We will cover the basics of what we mean by open access, how open access relates to the NIH Public Access policy, open access journals in the biomedical sciences, predatory publishing scams, and best practices for evaluating your open access options.

Available Vendors

Advion Interchim Scientific
Representative: Kelvin Hammond

Corteva Agriscience
Representatives: Erin Hancock
Tay Rosenthal

Teledyne ISCO
Representative: Steven Paeschke

Professional Headshots

We are very grateful to have Impact Photo at this year's MIKIW meeting. Impact Photo will be offering professional headshots to any and all MIKIW attendees. They will be taking headshots from **12:30 pm through the end of the poster session**. We encourage you all to take advantage of this opportunity to obtain a professional headshot for your personal portfolios. The MIKIW organizing committee will be in contact with you after the conference with details on how to access your photos.

Conference and Venue Locations

Scientific Program:
University of Iowa, College of Pharmacy Building (CPB)
180 S Grand Ave, Iowa City, IA 52242

Lodging:
Hilton Garden Inn Iowa City Downtown University | 328 S. Clinton St., Iowa City, IA 52240
Courtyard by Marriott Iowa City University Heights | 901 Melrose Ave., Iowa City, IA 52246

Friday Welcome Reception:
The Celebration Farm, Double Round Barn | 4696 Robin Woods Lane NE, Iowa City, IA 52240

Our Friday night Welcome Reception will be held outside of busy downtown Iowa City at the Celebration Farm. The Celebration Farm is a charming venue, nestled among the trees and fertile farm grounds of Johnson County. The double round barn features an extraordinary domed ceiling, functional limestone fireplace and covered patio that was all created by local craftsmen. This venue provides a welcoming and comforting environment for new connections.

Saturday Dinner Venue:
The Heights Rooftop, Ballroom | 901 Melrose Ave, Iowa City, IA 52246

Our Saturday night dinner venue will bring us back to the heart of Iowa City at the Heights Rooftop. This brand-new rooftop venue boasts 5,700 open square feet of dance floor, tables and chairs, a well-stocked bar, a state-of-the-art kitchen, and a rooftop patio with one of the best views in the city. With an impressive view over the Kinnick Football Stadium, this venue will allow for a memorable gathering for the final night of the MIKIW 2022 conference.

Oral Presentation Abstracts

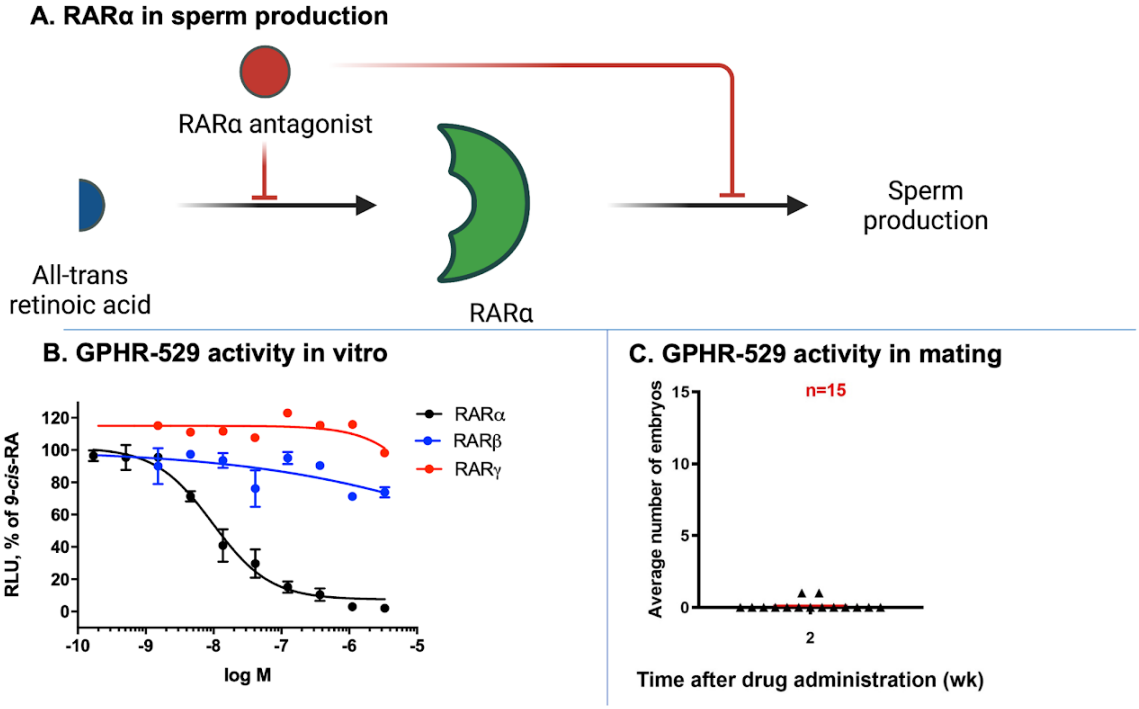
Toward the Discovery of a Male Birth Control Pill: RARα Antagonists

Md. Abdullah Al Noman[1], Soma Maitra[1], Tahmina Naqvi[1], Jon E. Hawkinson[1], **Gunda I. Georg[1]**
[1]*Department of Medicinal Chemistry and Institute for Therapeutics Discovery and Development University of Minnesota, Minneapolis, MN USA.*

~Talk #1, Saturday April 30th, 9:00 am – 9:25 am~

Despite much interest in the male birth control pill, none has been approved yet. Much of the earlier attempts to develop a "male pill" focused on the male sex hormone testosterone, which is an effective contraceptive agent but shows side effects such as weight gain, depression, etc. We are aiming at developing a non-hormonal male birth control pill to avoid the hormonal side effects. One such target is the vitamin A pathway. A vitamin-A-deficient diet causes mammalian male sterility due to reduced sperm production. Vitamin A is enzymatically oxidized to 9-cis and all-trans retinoic acid (9c-RA and ATRA) that bind to a plethora of receptors to exert their effects downstream. One receptor of ATRA, retinoic acid receptor α or RARα, is validated as the target for male contraception. Knocking out the RARα gene in mice reduced sperm production without any significant side effects. The goal of our research is to develop a selective RARα antagonist as a safe, effective, and reversible male contraceptive agent with no off-target effects on RARβ and RARγ. We envisioned exploiting the structural differences between RARα, β, and γ ligand-binding domains to achieve RARα selectivity. Also, the structural differences between RARα bound to the agonist and the antagonist facilitated the design of full antagonists. We designed and synthesized ~100 compounds and evaluated RARα antagonist activity and selectivity using a luciferase-reporter cell assay. We obtained several antagonists with high potency of antagonism for RARα and excellent selectivity over RARβ and RARγ. One RARα-selective antagonist showed good oral bioavailability and desired pharmacokinetic properties in mice, and upon oral administration, it showed a significant reduction in sperm count and complete inhibition of embryo formation in mating studies. This compound is now in preclinical development while the search for second-generation compounds is underway.

Keywords: male contraceptive, nonhormonal birth control pill, drug discovery, retinoic acid receptor α, vitamin A



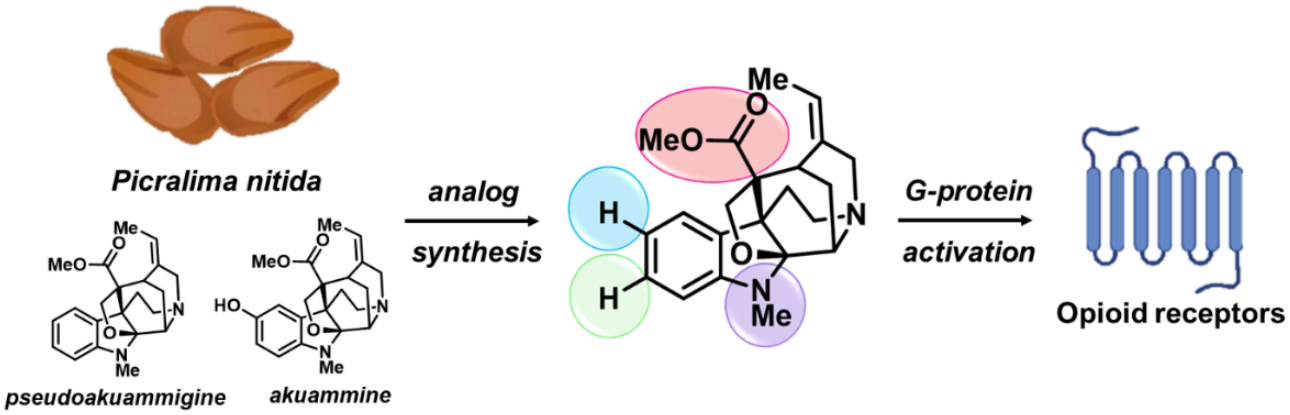
Synthesis and Evaluation of Akuamma Alkaloid Analogues for Alternative Pain Therapies

Madeline Hennessy[1], Anna Gutridge[2], Alexander French[2], Richard van Rijn[2-5], and **Andrew Riley[1]**
[1]*Department of Pharmaceutical Sciences, College of Pharmacy, University of Illinois at Chicago*
[2]*Department of Medicinal Chemistry and Molecular Pharmacology, College of Pharmacy, Purdue*
[3]*Purdue Institute for Drug Discovery, Purdue*
[4]*Purdue Institute for Integrative Neuroscience, Purdue*
[5]*Purdue Interdisciplinary Life Sciences Graduate Program, Purdue*

~Talk #2, Saturday April 30th, 9:25 am – 9:50 am~

Opioids produce potent analgesic effects through the activation of the mu-opioid receptor (μOR); however, this activation also produces adverse effects such as respiratory depression, tolerance, and dependence. Due to these deleterious side effects and the addictive nature of opioids, physicians have reduced the distribution of opioid prescriptions to patients. This has led some opioid users to turn to botanical supplements, such as akuamma, as alternative pain therapies. The bioactive compounds found in the seeds of the akuamma tree are a class of monoterpene indole alkaloids with moderate opioid receptor affinity. Specifically, the akuamma alkaloids akuammine and pseudoakuammigine can be isolated in high yields and are μOR agonists. Despite this activity, in our hands, pseudoakuammigine and akuammine produce minimal effects in animal models of antinociception, likely due to their modest potency at the μOR. Therefore, to aid in the design of more potent derivatives, we sought to establish structure-activity relationships that probe how these alkaloids interact with the opioid receptors. Toward this goal, here we report the synthesis of akuammine and pseudoakuammigine analogs utilizing highly chemoselective transformations to modify the C10, C11, C16, and N1 positions. The evaluation of these derivatives at the opioid receptors revealed key SAR trends and identified a compound with a 70-fold increase in potency at the μOR and a 7-fold increase in selectivity over the kappa opioid receptor. Ultimately, this new ligand class will be leveraged as chemical probes to study opioid receptor signaling and promising lead compounds for the development of safer analgesics.

Keywords: Natural products, structure-activity relationship, opioids



Stability of Triphenylphosphonium Moieties in Biologically Relevant Conditions

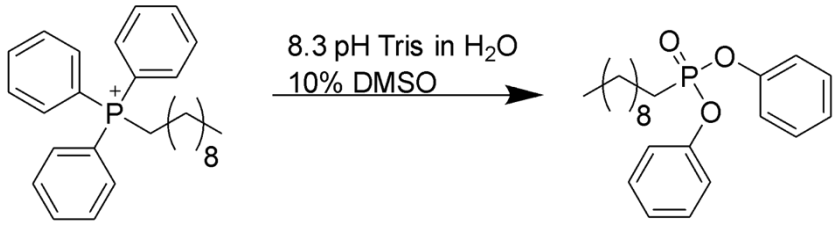
Hannah K. Gruenwald[1], and **Robert J. Kerns**[1]

[1]*Department of Pharmaceutical Sciences and Experimental Therapeutics, University of Iowa, Iowa City, Iowa, USA*

~Talk #3, Saturday April 30th, 3:10 pm – 3:35 pm~

Mitochondrial dysfunction has been implicated in a variety of diseases including neurodegeneration, aging, diabetes, radiation exposure, and cancer. The specific targeting of various molecular probes and therapeutic agents to mitochondria is an important strategy to study and treat these diseases as well as to develop new ways to probe and modulate mitochondrial function and cellular processes. Triphenylphosphonium (TPP^+) derivatives have been commonly used in commercially available mitochondria-targeted probes and medications including MitoQ, MitoSox and SKQ1. Chemical modifications to the para position of the TPP^+ phenyl rings have enabled modulation of mitochondrial toxicity of these moieties. However, observed chemical instability of TPP^+ with para-positioned electron withdrawing groups suggested potential for instability at biological conditions. With further testing, these molecules demonstrated instability in conditions commonly employed for laboratory use including various co-solvent conditions and ionic solutions. Observed degradation in these conditions may pose a risk for invalid experimental results. By use of multinuclear NMR, HPLC, and ESI-MS analysis, degradation of TPP^+ derivatives and mitochondrial probes in various conditions has been analyzed and degradation products identified.

Keywords: Triphenylphosphonium, Instability, Mitochondria, Degradation



Complexity Index-guided Exploration of New Chemical Space

Manvendra P. Singh[1], Bryce Gaskins[1], Daniel R. Johnson[2], Christopher G. Elles[2], and **Zarko Boskovic[1]**

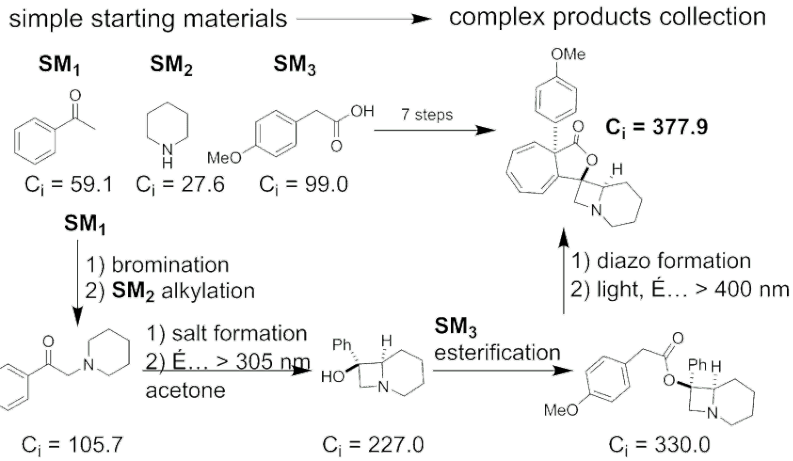
[1] *Department of Medicinal Chemistry, University of Kansas, Lawrence, 66044 Kansas*

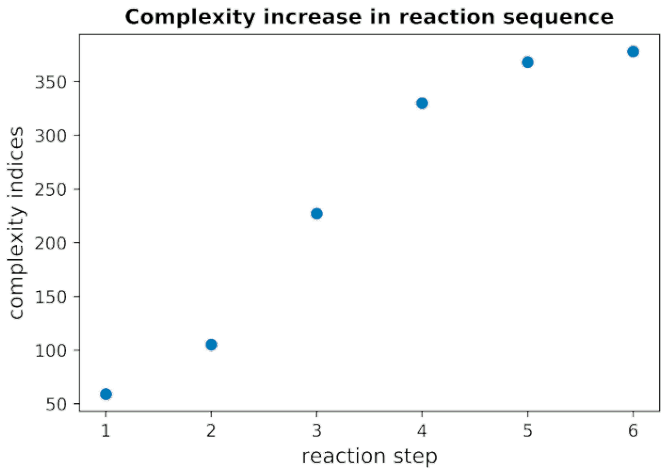
[2] *Department of Chemistry, University of Kansas, Lawrence, 66044 Kansas*

~Talk #4, Saturday April 30th, 3:35 pm – 4:00 pm~

Synthetic strategy for accessing a collection of structurally complex, "unnatural" compounds is presented. Key reactions in the 7-step synthetic sequence are an aza-variant of the Norrish-Yang cyclization and an insertion of carbene into a benzene ring (Buchner ring expansion). Critical advance in defining a new way of making azetidinols, relies on concealing the lone pair by protonation with a strong tosic acid. This obviates the need to introduce a protecting group for nitrogen (and remove it subsequently). Tosylate salts of several piperidines substituted with acetophenone derivatives were used in the first complexity-generating reaction. Interestingly, morpholine derivatives were photochemically inert; neither cyclization nor fragmentation was observed. Why this is so was revealed through X-ray diffraction of the starting salts: in morpholine derivatives tosylate anion is positioned parallel and proximal to the carbonyl group which undergoes pi*<-n excitation with light. We hypothesize that this arrangement is efficiently quenching the excited state through energy transfer to a neighboring pi-system of the tosylate. The geometry of tosylate in piperidine derivatives is perpendicular and away from the carbonyl. Products of aza-yang cyclization are formed as single diastereomers and contains a tertiary alcohol which we used for attachment of additional components. While the esterification of sterically congested, tertiary alcohols represents a challenge, we identified conditions under which we efficiently coupled various phenylacetic acids with the azetidinols. Converting activated methylene in these arylacetate esters to diazo compounds and photochemical expulsion of N_2 led to insertion of a carbene into proximal phenyl ring. Electrocyclic opening of the cyclopropyl diene leads to cyclohepatriene ring. Two diastereomers are possible in the carbene addition step, and we obtained both in 2:1 ratio. The compounds thus prepared contain 7-, 6-, 5-, and 4-membered rings, and 3 stereocenters, and lactone, azetidine, and cycloheptatriene functional moieties. These structural features are appealing for further biological experimentation with these compounds.

Keywords: Photochemistry, Azetidinols, Cycloheptatrienes, Complexity, Norrish-Yang





Nitric Oxide Reprograms DNA Methylation and Tumor-Associated Gene Expression by Inhibiting DNA Demethylases TET and ALKBH2 in Triple-Negative Breast Cancer

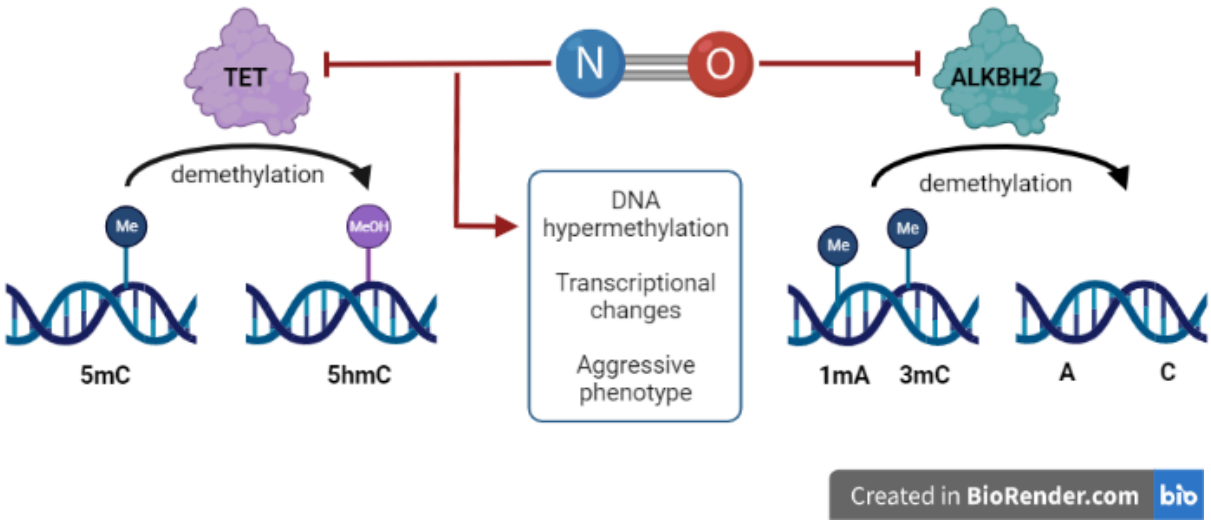
Marianne B. Palczewski[1], Hannah P. Kuschman[1], Sushma Sappa[2], Eric Kool[3], Kabirul Islam[2], **Douglas D. Thomas**[1]

[1]*Department of Pharmaceutical Sciences, University of Illinois at Chicago, Chicago, Illinois USA.*
[2]*Department of Chemistry, University of Pittsburgh, Pittsburgh, Pennsylvania USA.*
[3]*Department of Chemistry, Stanford University, Stanford, California USA.*

~Talk #5, Saturday April 30th, 4:00 pm – 4:25 pm~

The expression of inducible nitric oxide synthase (iNOS) predicts poor outcomes in triple-negative breast cancer (TNBC) patients, yet molecular mechanisms to explain this association are lacking. Epigenetics, and particularly DNA methylation, contributes significantly to the pathology of cancers including TNBC. DNA methylation at CpG sites in the human genome is a function of the concerted activities of DNA methyltransferases (DNMT) which catalyze methylation and ten-eleven translocation (TET) dioxygenases which demethylate cytosine residues. AlkB Homolog 2 (ALKBH2) is a DNA damage response demethylase that repairs 1-methyladenine and 3-methylcytosine on alkylated DNA. Both these DNA demethylases are in the family of 2-oxoglutarate dioxygenases which have been previously shown to be susceptible to inhibition by nitric oxide (NO). Although DNA methylation has been extensively studied over the past few decades in normal and disease settings, the discovery of endogenously produced regulatory molecules that control DNA methylation have not been described. Here, we report that the signaling molecule NO is a potent, dose-dependent, and reversible inhibitor of TET and ALKBH2 enzyme activity. Electron paramagnetic resonance (EPR) spectroscopy revealed that NO directly binds to ferrous iron in the TET catalytic pocket, forming a histidyl·iron·nitrosyl complex. Genome-wide oxidative bisulfite sequencing and ELISA showed that NO causes global increases in 5-methylcytosine (5mC) while global 5-hydroxymethylcytosine (5hmC) levels remain unchanged. The enrichment or absence of these adducts at gene-regulatory loci controls the expression of tumor-associated genes, and western blot verified that these changes are not due to altered TET or ALKBH2 protein expression. In summary, we have discovered a completely novel epigenetic signaling mechanism of NO by demonstrating for the first time how NO can regulate DNA methylation to control the expression of tumor-permissive genes. These studies provide a causal link between tumor NO production and more aggressive cancers and reveal potential new therapeutic pathways to target.

Keywords: Epigenetics, nitric oxide, cancer biology, triple-negative breast cancer, DNA methylation



Glutamate Racemase: A Shape-based Inhibition Story

Grant T. Cooling[1], Nick R. Vance[1], Katie Witkin[1], **M. Ashley Spies**[1]
[1]*Department of Medicinal and Natural Products Chemistry, University of Iowa, Iowa City*

~Talk #6, Saturday April 30th, 4:25 pm – 4:50 pm~

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 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 pyridoxal phosphate, for catalytic acidification of the C_α-H bond.[1] 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 mechanism based inhibitors.[1,2] The proposed mechanism of inhibition suggests one of the catalytic cysteines abstracts a proton causing the chlorine or sulfate to leave, while the other acts as a nucleophile forming an irreversibly modified cysteine.[1,2] 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.

Keywords: Glutamate Racemase

A General Strategy for the Synthesis of Rare Sugars via Ru(II)-catalyzed and Boron-mediated Selective Epimerization of 1,2-*trans*-diols to 1,2-*cis*-diols

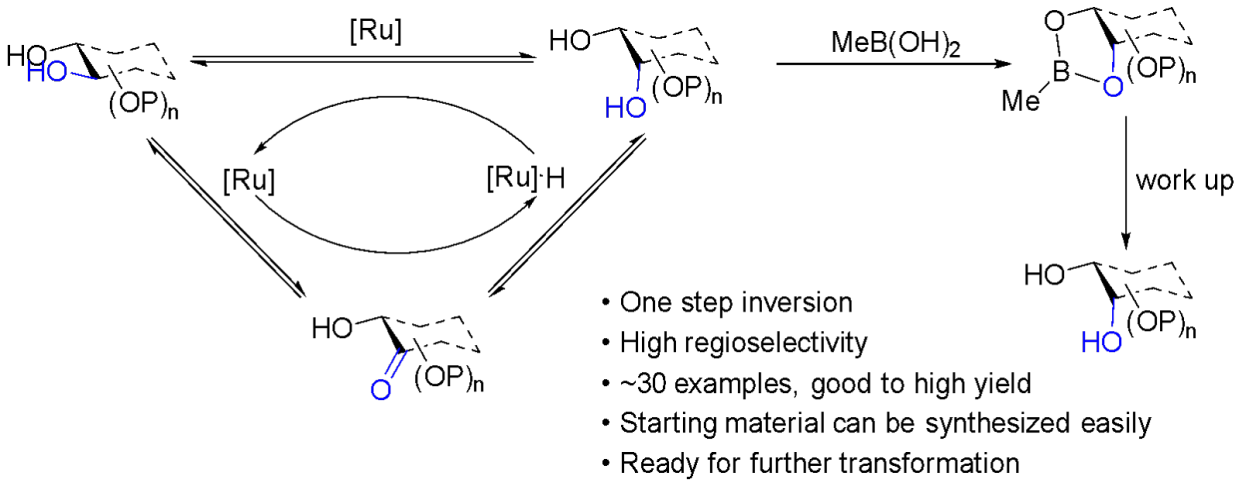
Xiaolei Li[1], Jicheng Wu[1], **Weiping Tang[1,2]**

[1]School of Pharmacy, University of Wisconsin-Madison, Madison, WI 53705, United States. [2]Department of Chemistry, University of Wisconsin-Madison, Madison, WI 53706, United States.

~Talk #7, Sunday May 1st, 8:45 am – 9:10 am~

Human glycans are primarily composed of nine common sugar building blocks. On the other hand, several hundred monosaccharides have been discovered in bacteria and most of them are not readily available. The ability to access these rare sugars and the corresponding glycoconjugates can facilitate the studies of various fundamentally important biological processes in bacteria, including interactions between microbiota and the human host. Many rare sugars also exist in a variety of natural products and pharmaceutical reagents with significant biological activities. Although methods have been developed for the synthesis of rare monosaccharides, most of them involve lengthy steps. Herein we report an efficient and general strategy that can provide access to rare sugars from commercially available common monosaccharides via a one-step Ru(II)-catalyzed and boron-mediated selective epimerization of 1,2-*trans*-diols to 1,2-*cis*-diols. The formation of boronate esters drives the equilibrium towards 1,2-*cis*-diol products, which can be immediately used for further selective functionalization and glycosylation. The utility of this strategy was demonstrated by the efficient construction of glycoside skeletons in natural products or bioactive compounds.

Keywords: rare sugars, regioselective, epimerization



Covalent strategies for the study of protein-carbohydrate interactions

Patrick Ross, **Mark P. Farrell**
Department of Medicinal Chemistry, University of Kansas, Lawrence, KS, USA

~Talk #8, Sunday May 1st, 9:10 am – 9:35 am~

Carbohydrate-protein interactions play key roles in many biological processes including cell division, viral and bacterial infection, and cell-cell interactions. Underpinning these interactions, protein-carbohydrate interactions rely on multivalency to induce biological effects. Therefore, the study these important biological interactions usually requires the synthesis of large glycans or polymeric constructs. To overcome this challenge, we reasoned that a covalent strategy could be developed to study protein-carbohydrate interactions. Toward this goal, we have prepared compounds with a glycan linked through a 2,5-dibromophenolic ester to a fluorescent label. With these compounds, we can preferentially label cells that express glycan specific lectins and label these lectins with a fluorescent tag. This work demonstrates a novel approach to use monovalent carbohydrate ligands to study protein-carbohydrate interactions.

Keywords: carbohydrates, protein-carbohydrate interactions, covalent modification

Characterization of a 3,4-Dihydroxyphenylacetaldehyde and L-cysteine Adduct Formed *In Vitro* After Neurotoxicant Exposure Reveals Its Potential as a Biomarker of Catecholaminergic Stress

Rachel A. Crawford[1], Ettore Gilardoni[1,2], Kate R. Bowman[1], T. Blake Monroe[1], Luca Regazzoni[2], Ethan J. Anderson[1], **Jonathan A. Doorn**[1]

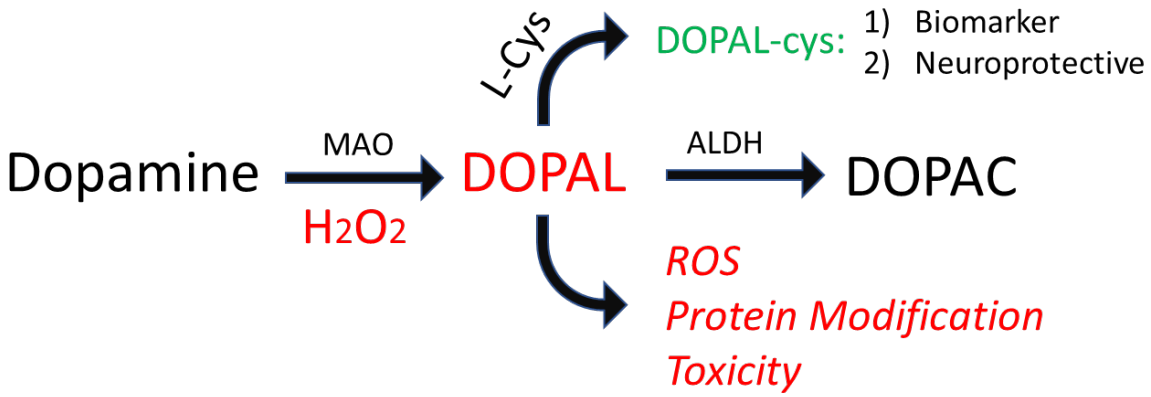
[1]*Department of Pharmaceutical Sciences and Experimental Therapeutics, University of Iowa, Iowa City, Iowa USA.*

[2]*Department of Pharmaceutical Sciences, University of Milan, Milan, Lombardy Italy.*

~Talk #9, Sunday May 1st, 9:35 am – 10:00 am~

Parkinson's disease (PD) is a neurodegenerative disorder that is characterized by death of dopaminergic neurons in the substantia nigra. While the etiopathology of PD is not well understood, it is thought that disruption of dopamine homeostasis plays an integral role. Dopamine is a neurotransmitter that is tightly controlled due to its reactivity and susceptibility to oxidation. Dopamine is metabolized to 3,4-dihydroxyphenylacetaldehyde (DOPAL), a potently cytotoxic and protein-reactive catecholaldehyde. Under normal physiological conditions DOPAL is further detoxified by aldehyde dehydrogenase (ALDH); in pathologic conditions of oxidative stress and aberrant DOPAL production, ALDH activity may be saturated. A variety of epidemiologically PD-linked environmental neurotoxicants are known to target the dopamine metabolic pathway and contribute to a global increase of DOPAL production and accumulation. Our interest in DOPAL's ability to modify proteins led to the discovery that L-cysteine protected glutathione S-transferase from DOPAL-mediated inhibition. We identified the mechanism of protection as a sequestration of DOPAL via the formation of a putative thiazolidine conjugate with L-cysteine (DOPAL-cys). This conjugate is identifiable, characterizable, and relatively quantifiable via mass spectrometric methods. Treatment of PC12 cells *in vitro* with dopamine and *N*-acetylcysteine, the precursors to DOPAL and L-cysteine, respectively, resulted in formation of the DOPAL-cys adduct which was exported to the extracellular media. Little to no DOPAL-cys was formed when only dopamine was supplemented. Interestingly, PC12 cells that were supplemented with dopamine and stressed with a non-acutely toxic dose of a PD-correlated neurotoxicant (i.e., benomyl, dieldrin, chlorpyrifos, or rotenone) also resulted in formation DOPAL-cys, suggesting that L-cysteine may sequester excess DOPAL under conditions of a disrupted metabolic pathway. We conclude that L-cysteine may represent an alternative catecholaldehyde detoxification pathway and that DOPAL-cys may be a biomarker of catecholaminergic and/or metabolic stress.

Keywords: Parkinson's disease, dopamine, DOPAL, biomarkers, neurotoxicity



Development of Histidine Kinase Inhibitors as Anti-Virulence Agents in Pseudomonas aeruginosa

Conrad A. Fihn[1], Hannah K. Lembke[2], and **Erin E. Carlson[1,2]**

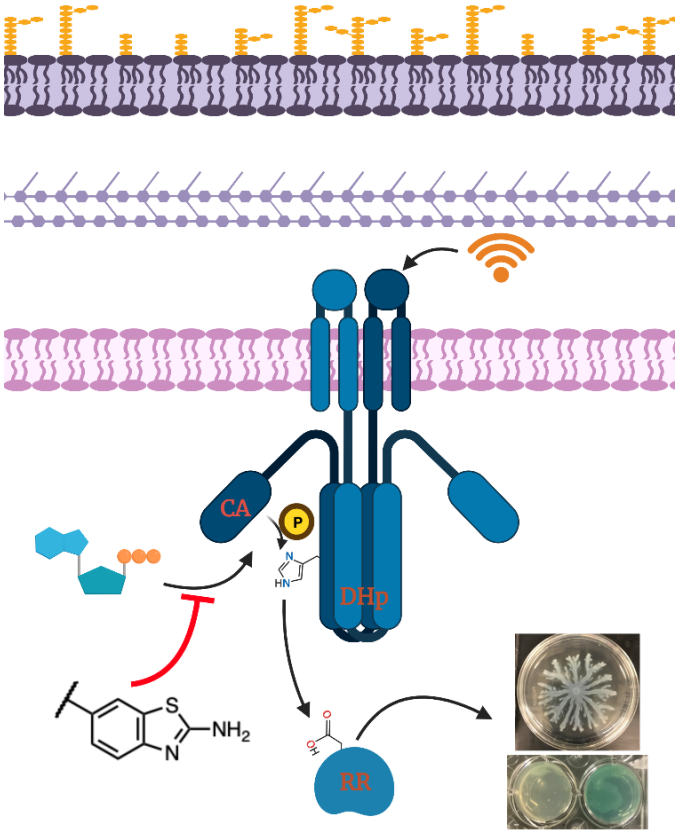
[1]*Department of Medicinal Chemistry, University of Minnesota, Minneapolis, Minnesota 55454, USA*

[2]*Department of Chemistry, University of Minnesota, Minneapolis, Minnesota 55455, USA*

~Talk #10, Sunday May 1st, 10:00 am – 10:25 am~

Bacterial resistance to antibiotics is rapidly increasing and is projected to cause more than ten million deaths per year by 2050. New strategies to combat resistant organisms are desperately needed. Two-component systems (TCSs) are the main signal transduction pathways used by bacteria to regulate a variety of processes including development, metabolism, virulence, and antibiotic resistance. TCSs consist of a homodimeric membrane-bound sensor histidine kinase (HK), and a cognate effector, the response regulator (RR). The high sequence conservation in the catalytic and ATP-binding (CA) domain of HKs and their essential role in bacterial signal transduction could enable broad-spectrum antibacterial activity. Targeting virulence, as opposed to bactericidal compounds, could reduce the evolutionary pressure for acquired resistance. Additionally, compounds targeting the CA domain have the potential to affect multiple two- component systems that regulate virulence in a single pathogen. We will present structure-activity relationship (SAR) studies of 2-aminobenzothiazole-based inhibitors of the CA domain of three distinct HKs. Recently, we have shown that these compounds are effective in whole cells with anti-virulence activities in *P. aeruginosa*. To identify the targets of these inhibitors, we have generated a covalent probe that contains both the 2-aminobenzothiazole scaffold and a handle for affinity isolation. Proteomic results indicate that these inhibitors target HKs in *P. aeruginosa*, labeling a residue in the active site of the CA domain. These probes will be utilized to map the HK inhibition events that are most responsible for the observed anti-virulence activities in *P. aeruginosa* and other gram-negative pathogens.

Keywords: Virulence, Two-Component Systems, Histidine Kinase, Pseudomonas aeruginosa



Modulating DNA Methylation with Bifunctional Ten-Eleven Translocation Dioxygenases Inhibitors

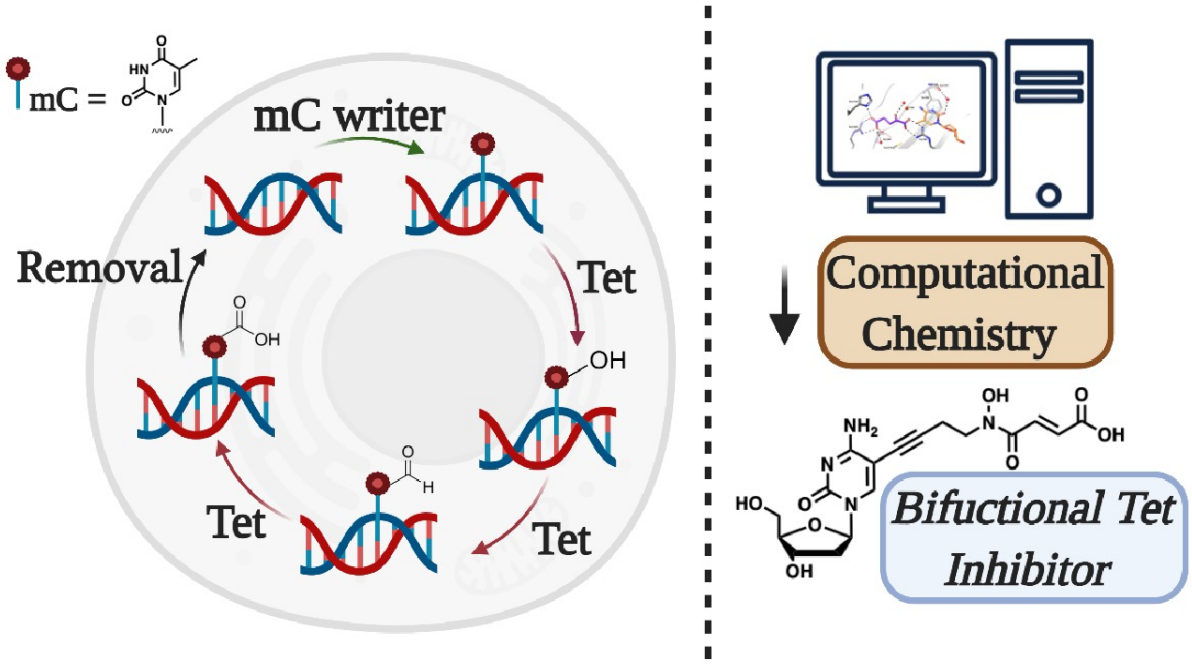
Alexander K. Hurben[1], Sang Le[1], Nicholas Weirath[1], Honnaiah Vijay Kumar[1], Christopher Chao[1], Makayla Brzycki[1], Jenna Fernandez[1], **Natalia Y. Tretyakova[1]**

Department of Medicinal Chemistry, University of Minnesota, Minneapolis, Minnesota USA.

~Talk #11, Sunday May 1st, 10:40 am – 11:05 am~

DNA methylation status is an important epigenetic regulator of gene expression, cell identity, and cellular function. Cells are equipped with various enzymes to carefully write and erase DNA methyl marks across the genome. One such family of DNA demethylases are ten-eleven translocation dioxygenases (Tet). Tet enzymes facilitate DNA demethylation through a Fe(II)/alpha-ketoglutarate dependent oxidation of methylcytosine (mC) to hydroxymethylcytosine (hmC), formalcytosine (fC), and carboxylcytosine (caC). However, the epigenetic equilibrium between methylation and demethylation is dysregulated in many pathologies, including lung cancer, chronic lymphocytic leukemia, and lung associated inflammatory diseases, due to aberrant Tet activity. To further investigate the role of dysregulated Tet enzymes, selective and potent small molecule inhibitors are needed. Currently there is a lack of such chemical tools, thus we utilized structure-based design to construct a set of novel Tet-specific inhibitors. We implemented a bifunctional strategy to simultaneously engage the enzyme's native substrate and cofactor binding sites. We utilized *in silico* docking results to guide our synthetic effort, where we successfully accessed an initial series of 15 potential inhibitors. These compounds Tet inhibitory activity were evaluated through a biochemical enzymatic Tet assay. The most potent molecule in our series had an IC50 value of ~ 1 μM. Additionally, this molecule lowered hmC levels in preliminary cellular assays at a concentration of 25 μM, indicating cellular inhibition of Tet. Collectively this work represents a critical first step in developing potent and selective Tet inhibitors to serve as epigenetic modulators to elucidate DNA methylation's role in disease etiology.

Keywords: Epigenetics, DNA methylation, TET



Investigating the Role of Soluble Metabolites in Primary Metastasis of High Grade Serous Ovarian Cancer

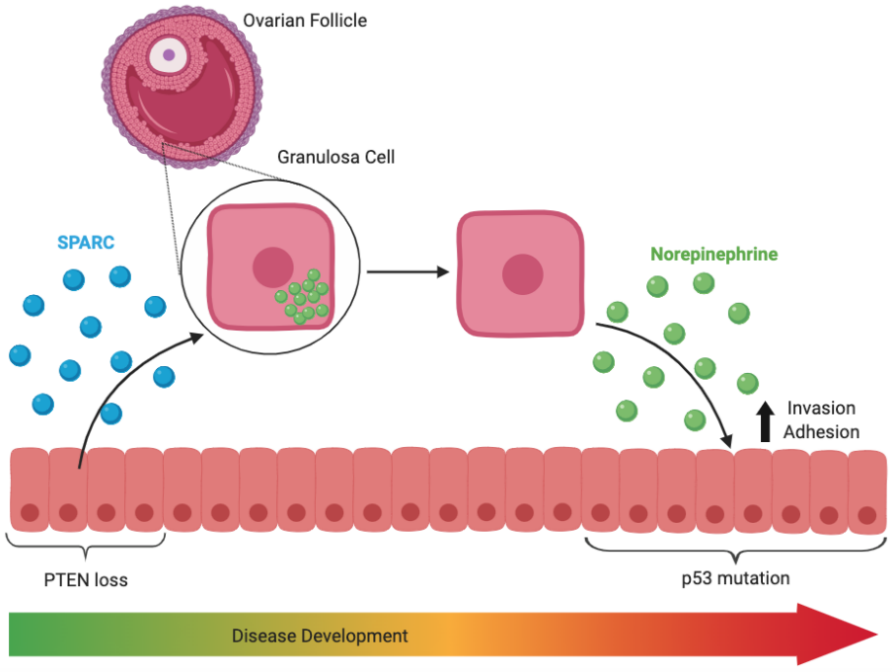
Tova M. Bergsten[1,2], Hannah Lusk[3], Laura Sanchez[3], **Joanna E. Burdette**[1]

[1]Department of Pharmaceutical Sciences, University of Illinois at Chicago College of Pharmacy, Chicago, Illinois USA. [2]Medical Scientist Training Program, University of Illinois College of Medicine, Chicago, Illinois USA. [3]Department of Chemistry and Biochemistry, University of California Santa Cruz, Santa Cruz, California USA.

~Talk #12, Sunday May 1st, 11:05 am – 11:30 am~

Ovarian cancer is a leading cause of cancer death in women. High grade serous ovarian cancer (HGSOC), the most common and lethal histotype, can originate from fallopian tube epithelium (FTE). However, little is known about metabolomics involved in the migration of transformed FTE from the fallopian tube to the ovary. Co-culture of FTE and ovaries enabled identification of several upregulated soluble metabolites via imaging mass spectrometry (IMS), including norepinephrine (NE). Treatment of tumorigenic MOE PTEN^{shRNA} p53 cells with NE (10 µM) increased their invasion in a Boyden chamber. NE enhanced pSRC expression in MOE PTEN^{shRNA} p53 cells, which has been linked to invasion in the literature. NE treatment also increased N-Cad and C-Myc expression in OVCAR4 cells. These data suggested that NE drives alterations in protein expression and oncogenic phenotypic changes. We are now conducting proteomics to identify other changes in response to NE. We have also attempted to identify the fallopian tube factors that drive ovarian NE release. We determined that conditioned media, specifically the 3-50kDa protein fraction thereof, derived from cultured MOE PTEN^{shRNA} cells induced ovarian NE release. Therefore, we conducted proteomic analysis of this media compared with conditioned media from non-tumorigenic tubal cells, MOE SCR^{shRNA}, identifying a uniquely abundant protein produced by the tumorigenic cells potentially responsible for the NE release: SPARC (secreted protein acidic and rich in cysteine). Using the IMS/co-culture paradigm, we determined that knockdown of SPARC in MOE PTEN^{shRNA} cells decreases levels of ovarian-derived NE. We are also investigating whether overexpressing SPARC in the wild-type MOE cell line, which does not inherently stimulate ovarian release of NE. Ultimately, we hope that our integration of mass spectrometry techniques with phenotypic and mechanistic readouts will elucidate the signals involved in early ovarian cancer progression to enable both earlier detection of and more effective treatments for HGSOC.

Keywords: ovarian cancer, small molecule signaling, IMS, metastasis



Photoredox Catalysis for Peptide SAR and Applications to HIV-1 Drug Development

Jacob R. Immel[1], Maheshwerreddy Chilamari[1], Anuradha Roy[2], **Steven Bloom**[1]

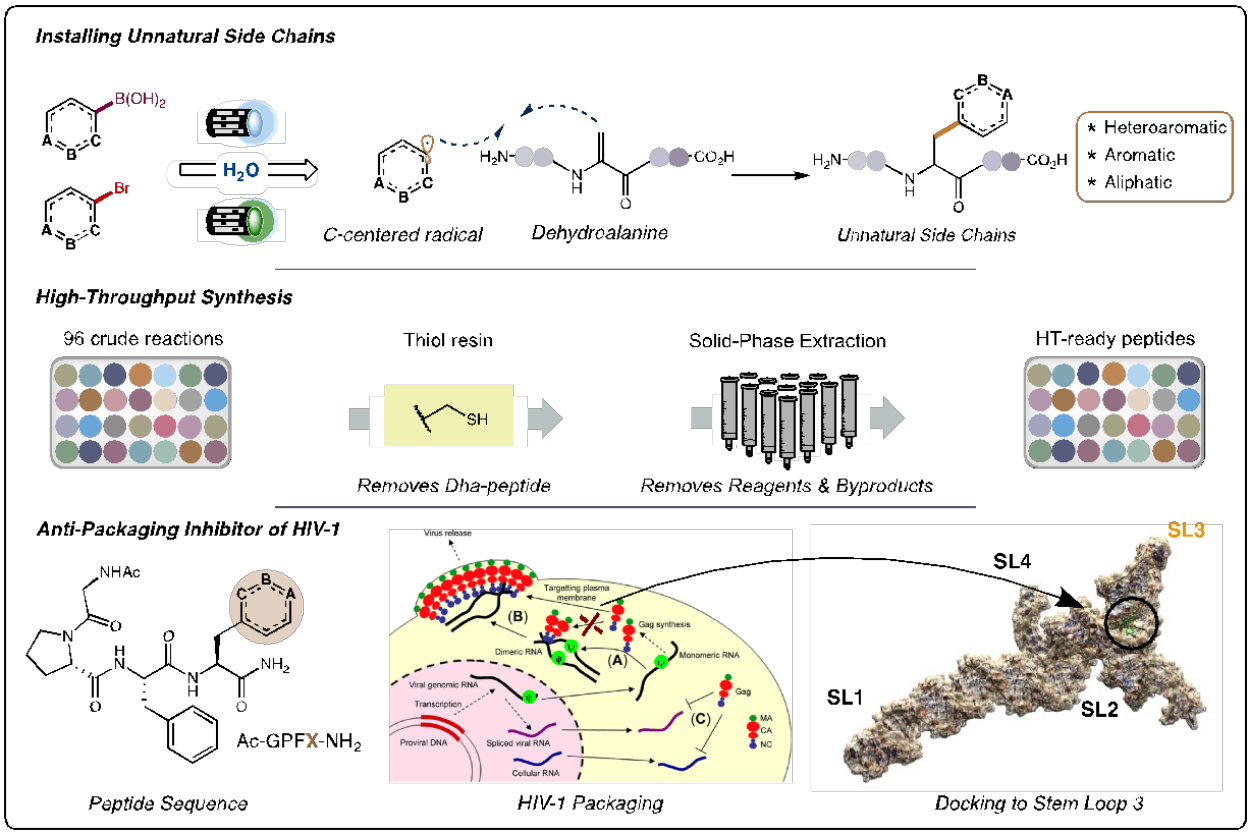
[1]*Department of Medicinal Chemistry, The University of Kansas, Lawrence, KS, US*

[2]*Director of COBRE CBID Infectious Disease Assay Development Core Facility, The University of Kansas, Lawrence, KS, USA*

~Talk #13, Sunday May 1st, 11:30 am – 11:55 am~

As alternatives to conventional small molecule-based therapeutics, peptides possess many attractive properties; being endogenous, highly selective, and relatively safe. As such, interest in peptides within the pharmaceutical industry has increased dramatically over the past two decades. While many peptides have already seen great success, skepticism continues to persist due to their intrinsic limitations, mainly related to their ease of proteolysis and poor membrane permeability. Replacing ordinary peptide side chains with unnatural amino acids can overcome the drawbacks posed by traditional peptides. However, limited access to unnatural amino acids continues to hamper their use in modern peptide drug discovery. By combining widely abundant boronic acids or bromides as "side chain donors" with peptides having the endogenous amino acid dehydroalanine as a "side chain acceptor", two novel photocatalytic methods were established to incorporate a diverse pool of unnatural side chain chemotypes-aliphatic, aromatic, heteroaromatic-into peptides. These methods were merged with modern high-throughput technologies to access a wide array of bioactive peptide analogs for direct biochemical testing. Using this platform, our lab evolved a brand-new RNA-specific tetrapeptide to abrogate viral genome packaging in HIV-1, preventing the spread of the infection. This tetrapeptide inhibits a conserved interaction between the HIV-1 nucleocapsid protein and stem loop 3 of viral Ψ RNA which directs genome selection for packaging. By disrupting this high-fidelity interaction, our peptide should not succumb to resistance-conferring mutations unlike most HIV-1 drugs.

Keywords: Photoredox, HIV-1, Peptide, SAR, and RNA

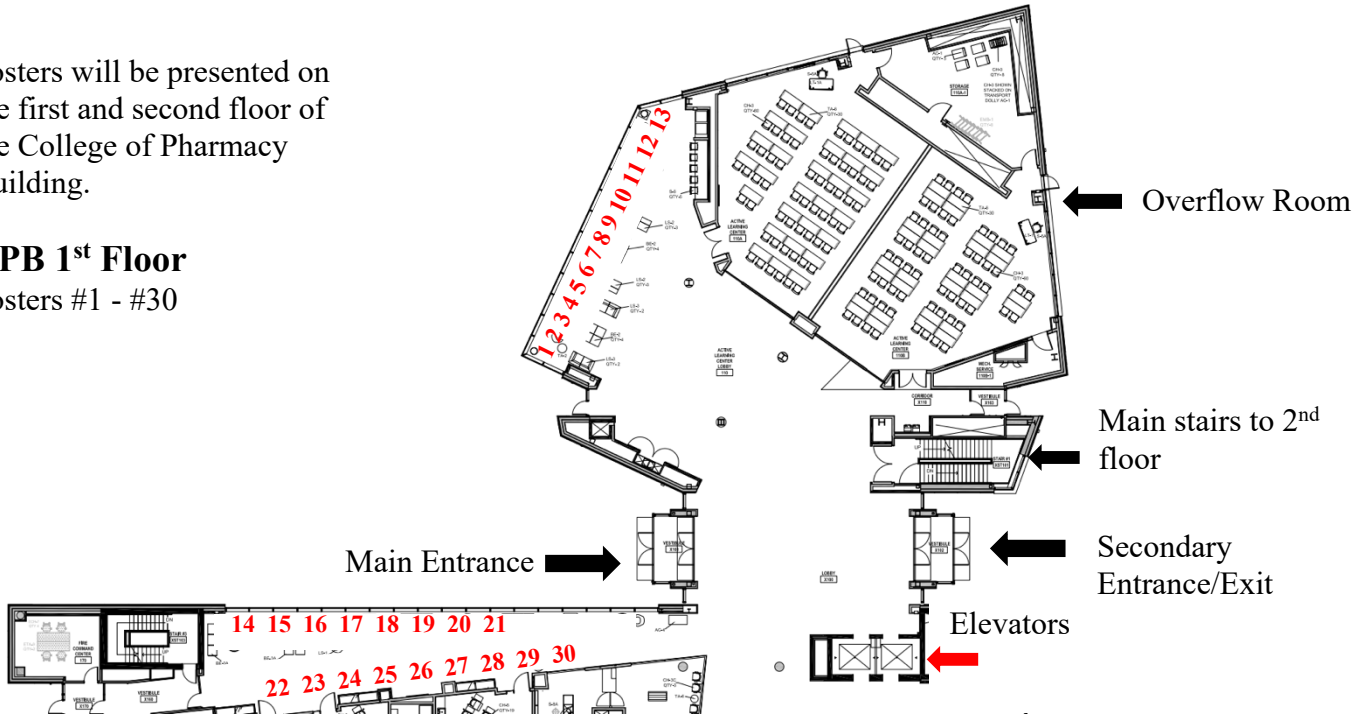


Poster Map

Posters will be presented on the first and second floor of the College of Pharmacy Building.

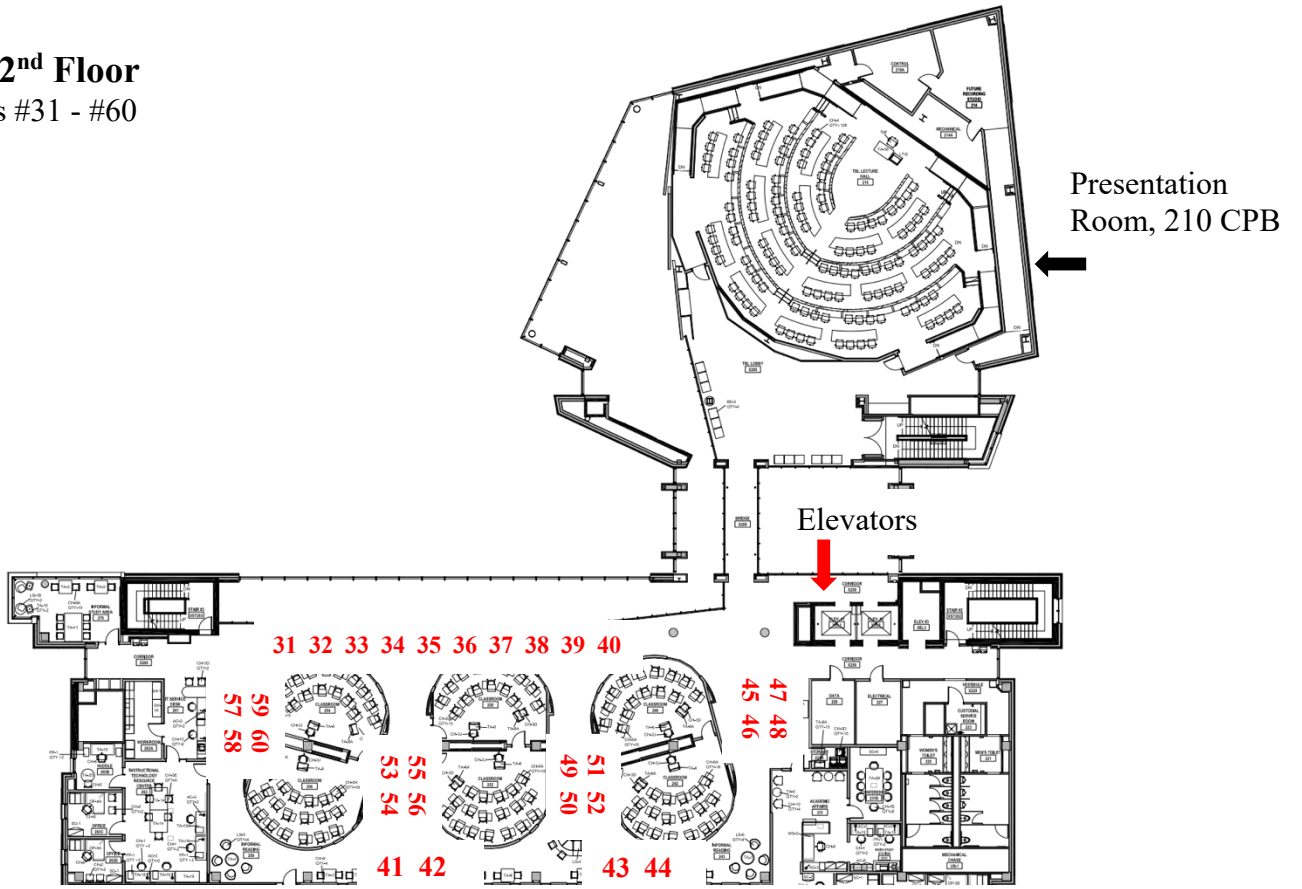
CPB 1st Floor

Posters #1 - #30



CPB 2nd Floor

Posters #31 - #60



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3	Pooja Hedge	UMN	Structure Activity Relationship of Pyrazinoic Acid Analogs as Potential Antimycobacterial Agents
4	Hannah K. Lembke	UMN	Elucidating Gene Expression Changes Following Treatment with 2-Aminobenzothizaole Inhibitors in Pseudomonas aeruginosa
5	Destiny I. Durante	UIC	Development of N-Substituted Furopyrroles as EBOV and MARV Antifiloviral Glycoprotein Inhibitors
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16	Kyle M. Kremiller	UIC	Modular Synthesis and Biological Assessment of Aristoquinoline, a Unique Inhibitor of α3β4 Nicotinic Acetylcholine Receptor
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30	Nan wang	UMN	Discovery of CDK2 Allosteric Inhibitors for Male Contraception and Anticancer Therapy
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Poster #	Presenter Name	School	Title
44	John A. Razidlo	UWM	The Effects of Ketamine and Psilocybin on Fear Acquisition and Extinction in Mice
45	Nicholas Weirath	UMN	Identification of potent and selective MC3R modulators from a mixture-based scaffold ranking library
46	Nathan T. Jones Laura C. Wagner	UWM	Glucocorticoid Release and Modification of Acute Threat-Associated Behaviors by Psilocybin
47	Erik J. Moran	UMN	Diet-Associated DNA Adducts: Determining the Origin of Endogenous N-7-(2,3,4-trihydroxybutane-1-yl)guanine in Tissue and Cell Culture
48	Brandi L. McKnight	UMN	Modulating the Avidity of Bispecific Chemically Self-Assembled Nanorings
49	Adam L. Worob	UWM	Development of Cross-Reactive Antibodies for the Identification and Treatment of Synthetic Cannabinoid Toxicity
50	Peijing Jia	UWM	Streamlined Iterative Assembly of Thio-oligosaccharides in Aqueous Solution and Development of Carbohydrate-based Antibiotics
51	Laura E. Hirsch	UMN	Targeting N-Myc Through Degradation Of Aurora Kinase A: Linker Design And Optimization
52	Mackenzie K. Wyllie	UMN	The Effect of Sugar Conformation on Molecular Recognition of DNA Substrates by APOBEC3A and APOBEC3B
53	Rocío Rivera Rodríguez	UIC	Fractionation of *Salvia rosmarinus* and Evaluation of Nrf2 and AhR Modulation for Ulcerative Colitis
54	Islam A. Berdaweel	UI	Glutathione Peroxidase 4 Gene Variants Association with Post Operative Atrial Fibrillation Risk and GPx4 Protein Phenotypic Characteristics in Patients Undergoing Elective Cardiac Surgery
55	Neha Paranjape	UI	Structure Activity Relationship and Gliotoxicity of Lower Chlorinated Biphenyls
56	Mohammad M. Hossain	UWM	Synthesis of Ketamine and 6-hydroxynorketamine haptens to develop the vaccination for the identification and mechanistic studies of specific immune response against ketamine and 6-hydroxynorketamine
57	Noah Z. Laird	UI	Development of a 3D Printed Gene-Activated Calcium Phosphate Cement Scaffolds for Application in Bone Regeneration
58	Ran Huo	UI	Cardiac-Specific Deletion of Prohibitin-1 Leads to Fetal Demise, Mitochondrial Abnormalities and Heart Failure
59	Joshua C. Wilkinson	UI	Identifying Selective Inhibitors of Adenylyl Cyclase 1 as Therapeutics for Chronic Pain
60	Kristopher E. Lukas	UI	Double-Stranded mRNA Lipid Nanoparticle Vaccines Display Superior Thermostability Compared to Current Single-Stranded mRNA Formulations

Poster Presentation Abstracts

Poster #1

Protein Structure Validation by limited proteolysis

Xinhao Shao[1], Rahul Narayanan[1], Chris Grams[1,2], **Yu Gao**[1]
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Protein structure determination has been one of the most challenging tasks due to its difficulties in crystallization. In 2021, breakthroughs in protein prediction using deep learning from both Google's AlphaFold and Baker lab have shown promising results for the elucidation of most human proteins. These predicted structures match well with the existing crystallography and Cryo-EM data since most of these are included in the training set. However, for proteins with unknown structures and domains, the accuracy of the prediction has not been fully validated. Here, by using a native limited proteolysis strategy and a 3D protein coverage visualization tool, we present a method to validate the result from prediction and possibly provide a new dataset for structure refinement. In total, we have collected tryptic native digestion data for more than 3,000 proteins. When each protein's digestion data is analyzed in a longitudinal manner, a time series could be generated for each protein. The average cleavage site distance from the centroid of the protein significantly decreases over time, showing the outer shell of the protein gets digested first. In contrast, the average number of atoms proximal to each cleaved Lysine and Arginine significantly increases over time, indicating enzyme digests at easy-to-access regions first. After statistical significance test, we were able to identify around 100 protein entries that lack consistency with the predicted structure, in which a large portion are membrane proteins or proteins with little knowledge in terms of known structures or domains. These results were then visualized by SCV (an inhouse-developed Sequence Coverage Visualization web application), in which the covered residues over time are highlighted, digestion progress for individual protein is visualized and proteins are differentiated by trends of cleavage site distance change. The preliminary data shows that the method we propose could identify proteins with predicted structures significantly different than the experimental data, also using proteomic data from limited proteolysis to compare between the predicted structure and existing PDB entries structurally could assist in refining current predictions further and represents an important step towards a complete and accurate protein structure database.
Keywords: shot-gun proteomics, limited proteolysis, deep-learning based protein structure validation

Poster #2

Targeting Orai1, Adenylyl Cyclase Type 8, and STIM1 in Triple Negative Breast Cancer

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Triple-negative breast cancer (TNBC) is one of the most aggressive forms of breast cancer that is not able to be treated by hormonal therapies. The current standard of care includes surgical removal and aggressive chemotherapy regimens. Because of this, there is an urgent need for new, molecular-based therapies to effectively treat these patients. Recently, it has been discovered that two proteins are consistently overexpressed in TNBC cells: adenylyl cyclase type 8 (AC8) and Orai1, a pore-forming calcium channel. In normal breast epithelial cells, the levels of AC8 and Orai1 exist in a ratio that allows for finely tuned calcium entry. When these proteins are overexpressed, excess calcium is produced within these cancerous cells, which causes negative cellular effects such as proliferation and migration. Additionally, our lab is interested in a third protein involved in this process, stromal interaction molecule 1 (STIM1). STIM1 is an endoplasmic reticulum-bound protein that directly binds to and activates the Orai1 channel. Currently, the unique interplay between AC8, Orai1, and STIM1 is not fully understood. Thus, our lab has utilized the NanoBiT protein interaction system to identify key attributes of each protein-protein interaction. Our data suggests that the interaction between Orai1 and AC8 is constitutive whereas the interaction between Orai1 and STIM1 is calcium dependent. Our lab aims to use these parameters to conduct a high throughput screen to discover small molecule inhibitors of each of these protein-protein interactions. Ultimately, we aim to observe how these three proteins act together in concert in a normal and cancerous environment in order to elucidate potential therapeutic targets for triple negative breast cancer.
Keywords: Orai1, Adenylyl Cyclase Type 8, STIM1, protein-protein interaction, triple negative breast cancer

Poster #3

Structure Activity Relationship of Pyrazinoic Acid Analogs as Potential Antimycobacterial Agents

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Tuberculosis (TB), an infectious disease caused by the pathogen Mycobacterium tuberculosis (Mtb), is a major cause of suffering worldwide. Pyrazinamide (PZA) is a critical component of the first-line TB treatment regimen because of its sterilizing activity against non-replicating bacteria, but its mechanism of action has remained enigmatic. PZA is a prodrug converted by pyrazinamidase encoded by pncA, within the bacteria, to the active moiety, pyrazinoic acid (POA) and PZA resistance is caused by loss-of-function mutations to pyrazinamidase. Dick, Grüber, Rubin and co-workers have recently shown POA induces targeted protein degradation of the enzyme PanD, a crucial component of the coenzyme A biosynthetic pathway essential for M. tuberculosis survival under both replicating and non-replicating conditions. Based on the newly identified mechanism of action of POA we designed several POA analogs using structure-based drug design to improve potency and overcome PZA resistance. We prepared and tested ring isosteres, carboxylic acid bioisosteres as well as ring substitutions to study the structure activity relationships of the POA scaffold. All the analogs were evaluated for their anti-tubercular activity and a few representative molecules were evaluated for their binding affinity, towards PanD, through isothermal titration calorimetry. We report that analogs with ring and carboxylic acid bioisosteres did not significantly enhance the antitubercular activity, whereas the ring substituted analogs showed some promise. The 3 and 5 position alkylamino-POA analogs were found to be 5 to 10–fold more potent anti- TB agents as compared to POA. Further development and mechanistic analysis of these analogs may lead to a next generation POA analog for treating TB.
Keywords: Tuberculosis, Pyrazinamide, structure-based drug design, SAR

Poster #4

Development of N-Substituted Furopyrroles as EBOV and MARV Antifiloviral Glycoprotein Inhibitors

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Ebola virus (EBOV) and Marburg virus (MARV), members of the *Filoviridae* family, are considered Category A biowarfare agents due to their stabilities in aerosolized form, high fatality rates, and lack of effective therapies for treatment in humans. The 2014-2016 Ebola epidemic in West Africa, which was the largest recorded outbreak; the recent 2018 epidemic in the Democratic Republic of Congo, which is the tenth outbreak since 1976 and the second-biggest Ebola epidemic; and a new outbreak in Guinea, reported in February of 2021, underscore the persistent threat of Ebola epidemics and the need for drug discovery and development efforts to produce effective treatments. Filoviral glycoprotein (GP), known to facilitate viral infection, has proven to be a drug-targetable site with recent FDA-approved drug Inmazeb, a cocktail of three anti-EBOV GP monoclonal antibodies. We have discovered a series of *N*-substituted furopyrroles that display excellent potency against both MARV and EBOV when tested in an HIV-based pseudovirus assay. Selectivity and potency of these viral fusion inhibitors were improved by structural modifications on the scaffold, which included optimization of the heterocyclic core and the substituents on the amide portion of the molecules. The best performing inhibitors resulted in nanomolar EC_{50} values with selectivity index values higher than 100. These compounds were validated in infectious assays and found to be potent inhibitors of EBOV and MARV. Our lead compounds display excellent *in vitro* metabolic stability and druglike properties and have potential to be developed as a new class of antiviral drugs.
Keywords: EBOV, MARV, pseudovirus, Ebola glycoprotein

Poster #5

Elucidating Gene Expression Changes Following Treatment with 2-Aminobenzothizaole Inhibitors in Pseudomonas aeruginosa

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Previous work has identified riluzole, an FDA approved drug, as a putative inhibitor of histidine kinases (HKs). HKs are sensor kinases that when activated autophosphorylate and undergo a phosphotransfer event to a cognate response regulator. Response regulators are often transcription factors and will then elicit a cellular response. Many HKs have been linked to virulence mechanisms in Pseudomonas aeruginosa, such as swarming, biofilm formation, quorum sensing, and toxin production. P. aeruginosa is a dangerous human pathogen well-known for its role in chronic infections of the lung in cystic fibrosis (CF) patients. P. aeruginosa is one of the ESKAPE pathogens, which are highly resistant to antibiotic treatment, making these infections increasingly difficult to clear. Therefore, inhibition of HKs is of great therapeutic interest to decrease the infectivity of this organism and increase clearance of chronic P. aeruginosa infections. Riluzole contains a 2-aminobenzothiazole scaffold of which further derivatives were tested to evaluate the phenotypic response of P. aeruginosa post treatment of this class of inhibitors. While phenotypic results show a promising decrease in swarming in addition to decreases in other virulence mechanisms, we set out to discover how these inhibitors alter transcription through ribonucleic acid-sequencing (RNA-seq) analysis. These data demonstrated significant decreases in the expression of genes associated with various motility pathways such as Type IV pilius production, flagellar proteins, and chemotaxis. They also showed a decrease in the expression of genes associated with nitrate respiration, which is important for anaerobic growth of P. aeruginosa as occurs in chronic CF lung infections. Further work is being done to understand which transcriptional promoters, which are cognate to the histidine kinases, the 2-aminobenzothiazole inhibitors are likely targeting. We are also performing confirmation studies with additional motility-associated phenotypic assays.

Keywords: Pseudomonas aeruginosa, histidine kinases, RNA-seq, virulence, inhibition

Poster #6

The Derivatization of *Aristotelia* Alkaloids by Biomimetic Synthesis

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The nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels that have been implicated in a variety of central nervous system disorders. Antagonists for the α3β4 nAChR subtype, specifically, have been shown to affect certain reward pathways in the brain, thereby reducing drug-seeking behavior and withdrawal symptoms in rodents. Unfortunately, existing α3β4 nAChR antagonists lack subtype selectivity, induce partial agonism of the receptor, or suffer from poor metabolic stability. Recently, alkaloids isolated from *Aristotelia chilensis* have been identified as α3β4 antagonists that are both moderately selective and potent, and could thus serve as a starting point to develop improved α3β4 nAChR antagonists. Furthermore, there are >30 *Aristotelia* alkaloids whose activity has remained largely unstudied. Herein, we report synthetic studies toward accessing the cores of several of these alkaloids and a preliminary evaluation of their functional activity at the α3β4 nAChRs. This work will expand our understanding of the structure-activity relationships between the *Aristotelia* scaffolds and the nAChRs, which in turn, will aid in developing highly potent and selective pharmacological probes to study the role of the α3β4 nAChR in substance use disorder.

Keywords: total synthesis, natural products, structure-activity relationships, substance use disorder

Poster #7

The Synthesis of Non-electrophilic, NRF2-activating Prodrugs and Bioisosteres

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Nuclear factor erythroid 2–related factor 2, or NRF2, is a key protein responsible for the regulation of numerous signaling pathways in the body, particularly those related to oxidative stress and cytokine release. As a result, NRF2 has been considered as a potential drug target in the treatment of inflammatory diseases and diseases that lead to malignant inflammatory responses such as multiple sclerosis, liver disease, diabetes, and chronic obstructive pulmonary disease. Studies show that electrophilic activators of NRF2 are promiscuous and may lead to potentially dangerous off-target effects when used in the body, prompting the development of non-electrophilic activators such as that being studied currently, PRL-295. Although testing in the chronic wound healing of diabetic mice has shown PRL-295 to be an effective NRF2 activator in-vivo, optimization of the compound's pharmacokinetic properties such as cellular permeability is essential. In an effort to increase cell permeability and lipophilicity of the compound, we have synthesized a series of PRL-295 prodrugs and bioisosteres, which will replace the carboxylic moiety of the original compound. With further testing, we hope to demonstrate improved pharmacological activity.

Keywords: NRF2, Inflammation, Diabetes, Wound Healing

Poster #8

SAR of Disulfides: Divergent Cyclization of Peptides

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Peptides are powerful therapeutic agents, characterized by high target specificity and strong binding affinity, yet remain largely underdeveloped due to their poor pharmacokinetic properties. One way to overcome these limitations is to cyclize the peptide. Cyclization enhances the plasma and metabolic stability of ordinary peptides and increases their cell permeability. While cyclization is a reliable strategy to better develop peptides as drugs, panning out the best peptide cycle (i.e., optimal ring size and cyclizing group) is synthetically laborious. Each cyclic variant is made as the unique product of a long, tedious, and chemically inefficient solid-phase peptide synthesis (SPPS) procedure. A better approach would be to make one peptide that can serve as the progenitor for any number of new cyclic products. Our lab has developed a convenient platform to construct libraries of cyclic peptides in parallel. In our approach, a single cyclic disulfide-containing peptide is made by conventional SPPS. The two cysteine residues of the disulfide bond are then converted into two distinct dehydroalanine (Dha) residues. By merging our newly minted bis-Dha peptide (essentially a dual-Michael acceptor) with an appropriate bis-nucleophile, we show that a wide array of new linking groups can be incorporated in place of the original disulfide bond. We show that many of our new cyclic peptides have enhanced biopharmaceutical properties.

Keywords: Peptide, macrocyclization, divergent synthesis

Poster #9

Synthesis and Evaluation of Aristoquinoline Analogs Using a Ritter-like Reaction

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Inhibitors of the α3β4 nicotinic acetylcholine receptor (nAChR) are reported to reduce psychostimulant-seeking and self-administration in rodent models, making the α3β4 nAChR a promising target for addiction therapy. However, existing ligands for the α3β4 nAChR suffer from suboptimal pharmacokinetics and poor target-selectivity. Aristoquinoline (ARQ), an alkaloid isolated from *Aristotelia chilensis*, was recently reported to inhibit human α3β4 nAChR preferentially over other neuronal nAChR subtypes, making it an attractive starting point in the development of a selective and potent α3β4 nAChR inhibitor. In pursuit of this goal, we have established the first synthesis of ARQ employing a unique Ritter-like reaction. Efforts are currently directed towards synthesizing structural analogs of ARQ employing this approach. Additionally, the mechanism and kinetics of the Ritter-like reaction are being explored to improve enantioselectivity and afford access to structurally diverse ARQ analogs. To complement our synthetic efforts, a cell-based receptor assay was employed to characterize the activity of ARQ and its derivatives at the α3β4 nAChR.

Keywords: alkaloid, Ritter, nAChR, addiction, synthesis

Poster #10

Novel Analogues of the Natural Product Fraxinellone Protect Against Glutamate-induced Toxicity in PC12 Cells

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Glutamate is the major excitatory neurotransmitter in the central nervous system (CNS) involved in synaptic plasticity, neuronal outgrowth and survival, and memory. Although intracellular glutamate concentration is often quantified at millimolar ranges, extracellular glutamate concentration must remain in the micromolar range. When extracellular levels of glutamate rise, aberrant synaptic signaling leads to excitotoxicity *in vitro* and *in vivo*. Chronic excitotoxicity is thought to contribute to neurodegenerative disease, including Parkinson's disease and Alzheimer's disease. In previous reports, limonoids isolated from *Dictamnus dasycarpus* showed significant neuroprotective activity against glutamate toxicity. Of the previously studied limonoid natural products, fraxinellone was one of four compounds that proved to be effective in protecting against glutamate excitotoxicity *in vitro*. With this information, our colleagues synthesized a library of analogues from the natural product fraxinellone that proved to be more effective at protecting against glutamate toxicity than natural fraxinellone. *In vitro* methods were used to measure the protective properties of the new fraxinellone analogues and to determine their mechanism of protection, which has not been achieved before. **Methods**. To measure the protective properties of the novel fraxinellone analogues, PC12 cells were first pre-treated with each compound at a range of 0.05 to 1.0 μM. The compounds were then washed from the cells and 100 μM glutamate was added for 24 hours. MTT analysis was then performed to measure cell viability to determine if the compounds were effective at protecting the cells against glutamate toxicity. **Results**. Of the ten analogues that were synthesized, four proved to be protective against glutamate toxicity, even more than natural fraxinellone. With this information, we are now investigating their mechanism of protection by considering potential targets of these compounds, including the Nrf2 pathway, glutamate receptors that control Ca^{2+} influx or if they reduce expression of Ca^{2+} -dependent enzymes and ROS production.

Keywords: Glutamate, excitotoxicity, fraxinellone, natural products, neurodegenerative disease

Poster #11

Self-Assembling Nucleoside Phosphoramidate Hydrogels:
A Novel Doxorubicin Drug Delivery System

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Self-assembling peptides and nucleobases are novel biomaterials that have been widely investigated for their self-assembling properties and ability to form supramolecular structures. These high-aspect ratio structures eventually form entangled matrices that result in supramolecular hydrogels. Previous work in our lab incorporated enzymatic activity in the regulation of peptide self-assembly, where we investigated Histidine Triad Nucleotide Binding Protein 1 (Hint1) as a modulator of supramolecular self-assembly. In this work, we developed a panel of self-assembling nucleoside phosphoramidates (SANPs) capable of Hint1 triggered hydrogelation.[1] SANPs are low molecular weight molecules that incorporate a self-assembling peptide conjugated to a nucleoside phosphoramidate group through a short PEG linker. A surprising observation in the development of these Hint1 responsive molecules was the spontaneous assembly of the SANPs into highly ordered nanofibers *without* enzymatic activity. Our current work focuses on characterizing the assembling properties of these SANPs and investigating how varying the molecular structure of the SANPs influences supramolecular assembly. Experimental data has concluded that the identity of the nucleobase defines the critical aggregation concentrations of the SANPs, as well as the ability of the SANP nanofibers to form hydrogels. Specifically, we demonstrate the ability of guanosine self-assembling nucleoside phosphoramidate molecule to form supramolecular hydrogels in PBS buffer due to ionic screening and G-tetrad formation. Further, we demonstrate the application of these novel biomaterials for controlled drug release with Doxorubicin, a potent chemotherapeutic agent.

Keywords: Biomaterials, Self-assembly, Peptides, Guanosine Tetrads, Drug Delivery

References:

1. West, H. T.; Csizmar, C. M.; Wagner, C. R. Tunable Supramolecular Assemblies from Amphiphilic Nucleoside Phosphoramidate Nanofibers by Enzyme Activation. *Biomacromolecules*. **2018**, 19, 7, 2650-2656.

Poster #12

Synthesis and Evaluation of Antagonist Activity of Tetrapeptide Ligands on the Melanocortin-4 Receptor (MC4R)

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The melanocortin pathway has been shown to regulate various physiological functions, including energy balance, sexual function, and eating behavior. Melanocortin receptors are members of the G protein-coupled receptor (GPCRs) family, have five receptor subtypes (MC1R, MC2R, MC3R, MC4R, and MC5R), with the melanocortin-4 receptor (MC4R) contributing to energy homeostasis. As a result, the MC4R is a pharmacological target for treating obesity and weight management. To explore the specific pharmacological features of the MC4R and to uncover new medicinal treatments, finding novel MC4R antagonists is important. A mixture-based positional scanning approach utilized by our laboratory identified a nanomolar potent MC4R antagonist scaffold with the structure of Ac-DPhe(pI)^{1}-Arg^{2}-Nal(2')^{3}-Arg^{4}-NH_2 (pA_2 = 9.0 ± 0.2, K_i = 1.0 nM). This tetrapeptide possesses equipotent antagonism as the MC4R endogenous AgRP(86-132) antagonist. The research presented here focused on continued structure-activity relationship studies on the identified tetrapeptides to boost the scaffold's potency. Natural and unnatural amino acids were substituted in the second position of the lead compound to investigate the pharmacological effects of these substitutions on the MC4R and other melanocortin receptors. These ligands were synthesized by standard solid phase peptide synthesis and examined by the AlphaScreen™ assay on mouse melanocortin receptors (mMCRs) to evaluate their antagonist potency. Our findings revealed that the positive charge at the second position was critical for the antagonist activity of the lead tetrapeptide at the mMC4R, and we identified several tetrapeptide ligands possessing antagonist activity at all assayed mouse melanocortin receptors (mMC1R, mMC3R, mMC4R, and mMC5R).

Keywords: melanocortin receptors, antagonist, homeostasis, tetrapeptide ligands

Poster #13

Optimization of antimicrobial peptide Api-137, a translation termination inhibitor

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Proline-rich antimicrobial peptides (PrAMPs), expressed by multicellular organisms as a part of their innate immune response, have been identified as a source of novel antibiotics. Previous studies indicate that Apidaecin, a PrAMP produced by honeybees, reaches the cytoplasm of Gram-negative bacteria via the SbmA transporter located in the inner membrane. In the cytoplasm, Apidaecin inhibits translation termination by binding in the nascent peptide exit tunnel after peptidyl-tRNA hydrolysis and trapping release factors on the ribosome. Structural analysis shows that the Apidaecin C-terminus interacts with the P-site t-RNA and the A-site bound release factor, while the N-terminus stretches down the length of the exit tunnel. Informed by structural and biochemical data, we have synthesized and tested synthetic Apidaecin variants in an effort to improve antimicrobial activity. We have incorporated unnatural amino acids and constraints into the Apidaecin sequence. We will present sequence modifications that enhance or diminish Apidaecin activity. These data provide compelling new research avenues for a novel antibiotic mechanism.

Keywords: antimicrobial, peptide, apidaecin

Poster #14

Novel Rifabutin Analogs Blocking Mycobacterial Drug Inactivation Exhibit Promising anti-*Mycobacterium abscessus* Activity

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Mycobacterium abscessus (*M. abscessus*) has become a growing health threat towards the public, especially to patients with impaired immune systems or preexisting pulmonary diseases. The intrinsic resistance of *M. abscessus* to virtually all classes of existing antibiotics seriously limits the treatment option, and thus safe and effective therapeutics are urgently needed. Rifamycins, one of the most powerful sterilizing antimicrobials for treating mycobacterial infections and a pillar component of antimycobacterial chemotherapeutics, are up to 500-fold less active against *M. abscessus* due to an unprecedented drug inactivation mechanism by an endogenous ADP-ribosyltransferase (Arr_{Mab}). Arr_{Mab} catalyzes site-specific ADP-ribosylation of rifamycins at C-23 position, which is crucial for binding to rifamycin's target RNA polymerase β subunit (RPoB), and thus produces rifamycin metabolites with no antimicrobial activity. In this work, rifabutin, one of the rifamycin antibiotics with potential anti-*M. abscessus* activity, was chemically derivatized at C-25 to produce an array of more than 30 analogs with diverse modifications. The synthetic analogs exhibited up to a 500-fold enhancement in their minimum inhibitory concentrations relative to rifampicin, with the best compounds demonstrating low nanomolar activity, commensurate with the activity of rifampicin against *M. tuberculosis*. Biochemical and structural studies confirmed that the most active analogs maintained their binding to the molecular target RPoB but were completely resistant to Arr-mediated ADP-ribosylation. The most potent compounds also demonstrated superior activity against a panel of *M. abscessus* clinical isolates. Finally, the selected lead compounds were shown to possess lowered CYP3A4 induction and promising pharmacokinetic properties. Evaluation of *in vivo* efficacy in an *M. abscessus*-infected mouse model and further lead optimization are ongoing in our lab.

Keywords: Mycobacterium abscessus, nontuberculous mycobacteria, rifabutin, rifamycin, antibiotics

Poster #15

Targeting the PAH2 Domain of Transcriptional Corepressors SIN3A/B for Inhibition of Proliferation in Triple-Negative Breast Cancer

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Triple Negative Breast Cancer (TNBC) accounts for 20% of all breast cancer cases and is one of the most clinically challenging subtypes of breast cancer due to its aggressive nature. Recently, Histone Deacetylase-associated transcriptional regulatory proteins SIN3A and SIN3B have emerged as potential molecular targets for TNBC therapeutics. Peptidic inhibitors and small molecules targeting the PAH2 domain, a well-characterized protein-interaction domain of the SIN3 homologs, have shown induction of epigenetic reprogramming along with upregulation of differentiation markers and downregulation of epithelial-to-mesenchymal transition markers in TNBC cell lines. In this work, we have developed a library of hydrocarbon-stapled peptidic inhibitors based on a known binding partner (MXD1) with various positions of cyclization. Fluorescence Anisotropy assays show varying binding affinity of these stapled peptides towards the PAH2 domain of SIN3A and SIN3B. Initial results show anti-proliferative effects when lead compounds are used as treatment with MDA-MB-231 and BT549 cell lines and acceptable blood plasma concentrations in DMPK studies. Here we present our methodology for the synthesis and binding affinity characterization for our peptidic inhibitors. These compounds will serve as potential chemical tools for elucidation of the mechanisms of action of SIN3A and SIN3B.

Keywords: Constrained Peptides, Triple Negative Breast Cancer, Cancer, Fluorescence Anisotropy, Peptidic Inhibitors

Poster #16

Modular Synthesis and Biological Assessment of Aristoquinoline, a Unique Inhibitor of α3β4 Nicotinic Acetylcholine Receptor

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The *Aristotelia* alkaloids are a class of natural products found in the leaves of the Maquai tree (*Aristotelia chilensis*) and other members of the *Aristotelia* genus of plants. As a class, these molecules are characterized by an unique azabicyclo core bound to an indole or quinoline nucleus. Recently, several of the *Aristotelia* alkaloids, were shown to selectively antagonize the α3β4 nicotinic acetylcholine receptor (nAChRs), a receptor implicated in drug addiction. Based on this promising activity, we have developed a modular synthetic route to one of these alkaloids, aristoquinoline, allowing us to interrogate its structure activity relationship (SAR). Aristoquinoline and its derivatives serve as a new tool to better understand the function of the α3β4 nAChR and new drug leads for drug addiction treatment.

Keywords: Nicotinic Acetylcholine Receptor a3B4, Addiction Treatment, Drug Development

Poster #17

Structural and Functional Characterization of Conformationally Discrete Amyloid-Beta Oligomers for Probe Development

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Alzheimer's disease (AD) is one of the most devastating neurodegenerative diseases and is currently the 6th leading cause of death in the United States.[1] While the exact cause of AD is unknown, soluble aggregates of the protein amyloid-beta (Ab), termed "oligomers", have been strongly implicated in disease pathology and increasing experimental evidence has demonstrated their toxic properties.[2] However; the study of these aggregates is difficult due to the inherent heterogeneity of oligomer formation and the tendency of Ab assemblies to dynamically interconvert. Consequently, previous studies have focused solely on understanding the disease properties of one or two aggregates or a heterogeneous mixture of oligomers. Therefore, efforts are needed to elucidate disease relevant structural isoforms.

We have developed methodology to generate stable, soluble oligomers of Ab from a recombinant protein expression system.[3,4] Through photo-crosslinking and size exclusion chromatography we were able to isolate discrete populations of oligomers for biophysical characterization and toxicity assessment.[4,5] Preliminary data show that SH-SY5Y cells treated with stable oligomers exhibit cellular dysfunction that resembles treatment with pooled oligomeric species, suggesting that these stabilized aggregates may possess similar attributes to that of disease state oligomers. Building off of these observations, a combinatorial library was designed and synthesized to screen stabilized oligomer species generated from a recombinant variant of Ab42. This screen identified hits that could serve as leads for the development of selective chemical probes.

Overall, this work highlights new tools for the study of Ab oligomers and their role in the pathophysiology of AD. These tools thus provide essential starting points for the development of diagnostics and potential therapeutics for this debilitating disease.

Keywords: Alzheimer's disease, oligomer, amyloid, recombinant Ab, quaternary structure

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Poster #18

Development of CatSper Inhibitors as Male Contraceptive Agents

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The cation channel of sperm (CatSper) is a calcium ion channel located on the flagellar membrane of mature sperm. CatSper has been validated as a target for nonhormonal male contraception. Genetic knockout of any pore-forming subunit of CatSper abrogates sperm Ca^{2+} current and hyperactivated motility, leading to infertility in male mice. Infertility of human males caused by CatSper mutations has also been reported. Since a selective CatSper blocker would only affect the activation of mature sperm, the treatment will be reversible, and the time of fertility recovery will be relatively short as compared to hormonal contraceptive agents. These observations underlie the rationale for the development of CatSper inhibitors as male contraceptive agents. To identify potential scaffolds as CatSper inhibitors, an HTS campaign with over 72,000 compounds was performed with our in-house library using a FLIPR Ca^{2+} influx assay. Four rounds of screening and triage provided us with 7 validated hits. Patch-clamp and FLIPR assays were performed to evaluate the selectivity of hit compounds over SLO3, hERG, hCav1.2, and hNav1.5. The cytotoxicity was tested in sperm and IMR-90 cells. Among the seven hit compounds, GPHR-00032750 and GPHR-00213869 were selected for further modifications due to a lack of toxicity in both cell lines. Previously we identified two analogs of GPHR-00032750 with submicromolar activities in the Ca^{2+} influx assay but also with significant hERG inhibition. To develop CatSper inhibitors with improved activity and selectivity, we recently studied the SAR of compound GPHR-00213869. Herein, we describe the design and synthesis of a series of GPHR-00213869 analogs.

Keywords: CatSper blockers

Poster #19

Development of Selective Small Molecule MDM2 Degraders for the Treatment of Cancer

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Tumor suppressor protein p53 plays a pivotal role in the regulation of cell processes and the prevention of cancer development. The function of p53 is tightly regulated by murine double minute 2 protein (MDM2). To restore p53 function, the inhibition or elimination of MDM2 is a potential therapeutic strategy. In this work, we aim to regulate the proteostasis of MDM2 by using PROteolysis TArgeting Chimera (PROTAC) technology. PROTACs are heterobifunctional molecules that co-opt the ubiquitin-proteasome system to induce the degradation of target proteins. PROTACs link one ligand that recruits the protein of interest (POI) to another ligand which recruits an E3 ligase to facilitate the polyubiquitination and subsequent proteasomal degradation of the POI. In this work, MDM2 PROTACs were designed and synthesized by tethering the ligands of MDM2 and CUL4-CRBN E3-ubiquitin ligase with varied linkers. A combination of NanoLuc luciferase assay and Western blotting were employed to quantify MDM2 depletion. The anti-proliferation effect of these molecules was evaluated in leukemia cells by MTT assay, and induced apoptosis was studied by means of flow cytometry. Selectivity profiling was done using global proteomic analysis. Compound WB156 was identified as a potent MDM2 degrader in RS4;11 leukemia cells. WB156 significantly inhibits cell growth (IC_{50} = 1.4 nM) and induces apoptosis at a concentration of 100 nM. Compound WB156 eliminates MDM2 in time-dependent and dose-dependent manners (DC_{50} = 23 nM). Proteomics analysis identified the degradation of other proteins including G1 to S Phase Transition 1 and 2 (GSPT1, GSPT2) induced by WB156. Future work will involve further evaluation of the therapeutic applications of WB156 as well as the development and characterization of additional MDM2 degraders in order to find more selective and potent compounds.

Keywords: PROTAC, leukemia, cancer, MDM2, p53

Poster #20

Highly Regio- and Diastereoselective Tethered Aza-Wacker Cyclizations of Alkenyl Phosphoramidates

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Tethered *aza*-Wacker cyclizations are a powerful class of intramolecular alkene functionalization reactions. In these types of reactions, a nitrogen-containing auxiliary is attached to the alkene substrate before oxidative cyclization. This frees the synthetic practitioner from requiring a pre-existing C–N bond in order to forge a new one. Tethered *aza*-Wacker cyclizations allow for the syntheses of a variety of heterocycles that are known to have important applications. Phosphoramidates are one such class of heterocycles and have applications ranging from asymmetric catalysis to medicinal chemistry. Oxidative cyclization for phosphoramidate construction is an underexplored strategy. Literature precedent has shown that phosphoryl azide decomposition into transient nitrene intermediates is a viable strategy for cyclic phosphoramidate synthesis, but these reactions generally furnish mixtures of diastereomers. We have developed a novel method for the diastereoselective construction of cyclic phosphoramidates using tethered *aza*-Wacker chemistry. We identified that a phosphoramidate tether with an unusual 5-chloro-8-quinolinol "arm" was essential for achieving >20:1 diastereoselectivity in these reactions, presumably through palladium chelation. The substrate scope with respect to alkenyl alcohols and the phosphoramidate tether was extensively explored. This cyclization reaction scaled well, and the product phosphoramidate heterocycles were valuable synthons, including for tether removal. From chiral alkenyl precursors, highly diastereoselective cyclization reactions furnished enantiopure cyclic phosphoramidate products.

Keywords: Cyclic Phosphoramidates, Tethered aza-Wacker reaction, Oxidative cyclization, Alkene functionalization, C-N bond formation.

Poster #21

Synthesis of Mannose-6-Phosphonate Conjugate for Targeted Protein Degradation through the Lysosome

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The binding and tagging of specific protein targets for degradation is a key area of medicinal chemistry and drug development that has become increasingly important as our knowledge of protein/ligand interactions improves. There are several different currently developed routes to targeted protein degradation, mainly through molecular glues, hydrophobic tagging of the target, and heterobifunctional proteolysis targeting chimeras. However, these strategies can only target intracellular proteins. About 40% of proteins in the proteome are outside the cells and many of them are associated with various diseases such as cancer. Lysosome targeting degraders are heterobifunctional molecules that contain a ligand for the protein target to be degraded and a ligand for cell internalization to a lysosomal compartment, which can complement existing strategies for intracellular proteins. One such target for cell internalization is the Mannose-6-Phosphate Receptor (M6PR). Current synthesis of the ligands for M6PR depend on large multivalences in Mannose-6-phosphate (M6P) that require long synthetic paths and are not optimized in their cell internalization. Our goal was to improve upon the synthesis of these lysosome targeting chimeras, and to probe the more exact binding requirements between M6P and its receptor. Our synthetic route for LYTACs streamlines those previously explored for M6P based lysosome targeting degraders and protein degradation studies have shown effective degradation of target proteins at sub micromolar concentrations of the chimera, which validates the refined models.

Keywords: Glycoconjugates, Lysosome, LYTAC, targeted degradation

Poster #22

New Insights from the Crystal Structure of the Active State of Fructose-1,6-Bisphosphatase Class II from *Francisella tularensis*

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Fructose-1,6-bisphosphatase class II (FBPaseII) catalyzes the hydrolysis of fructose-1,6-phosphate (FBP) into fructose-6-phosphate (F6P) and is one of the rate-determining enzymes in the gluconeogenic pathway. Previous biochemical analysis of the enzyme demonstrated complete dependency of enzymatic activity on Mn^{2+}. Presented here is the first analysis of the X-ray derived Mn^{2+} activated structure complexed with product F6P of *Ft*FBPaseII from the pathogenic bacteria *Francisella. tularensis*. Two structures were solved from triclinic crystal forms: form A (1 tetramer in the unit cell, diffracting to 1.9 Å) and form B (2 tetramers in the unit cell, diffracting to 2.4 Å). A third structure was solved from *Mycobacterium tuberculosis* FBPaseII containing the substrate FBP and metal cofactor Mg^{2+} and is discussed for further comparison among other homologous enzymes. Analysis of the interatomic distances of the substrate or product and divalent metal cations in the active center led to a revision of the catalytic mechanism suggested previously for class II FBPases. Instead of a metal cofactor stabilization of the transition state, we propose the positive dipole of the neighboring α-helix backbone (G88-T89-T90-I91-T92-S93-K94) oriented towards phosphate-1 is responsible for stabilization of the leaving phosphate. Hydrolysis would occur by addition of water coordinated by T89 and a proximal Mn^{2+}.

Keywords: Crystal structure, enzyme mechanism, metalloprotein, hydrolase, gluconeogenesis

Poster #23

Design and Development of Site Specific Null Allosteric Ligands for γ-Aminobutyric Acid Type A Receptor as Reversal Agents for General Anesthesia

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The γ-aminobutyric acid type A receptors (GABA(A)Rs) are the main inhibitory neurotransmitter receptors in the CNS. They control the synaptic and extrasynaptic GABA-mediated inhibitory signals in the brain. They are the target of general anesthetics, sedatives and anticonvulsants. Our overall goal is to design and develop site specific Null Allosteric Ligands (NALs) for GABA(A)Rs as reversal agents of these actions. For example, faster recovery from general anesthesia is associated with less postoperative anesthesia-related complications such as memory loss and confusion. Whereas flumazenil is a NAL that binds to the benzodiazepine site in the extracellular domain's (ECD) α+/γ- interface, there are no known NALs that bind to the transmembrane domain (TMD) sites. We adopted a strategy based on the structures of etomidate, flavanols and barbiturates as starting points, while utilizing computational docking, SAR studies and radioligand binding assays to assist in the design and development of the new agents. Here, we report the first NAL that binds to the TMD of GABA(A)Rs and that antagonizes the action of R-mTFD-MPAB (it binds to the TMD at the α+/β− and γ+/β− interfaces), while having a marginal effect on the binding of the agonist, [H]muscimol. The development of the first NAL(s) targeting the TMD of GABA(A)Rs will aid in understanding GABAergic actions in the CNS, support the mechanistic studies on GABA(A)Rs and potentially lead to reversal agents for general anesthesia and sedation.

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Keywords: GABA-A Receptors, Null Allosteric Ligands, Site Specific

Poster #24

Synthesis and Optimization of Imidazo[1,2-*a*]pyrimidines as Inhibitors of Influenza Viral Entry

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Influenza A virus (IAV) is a highly contagious virus that causes seasonal epidemics, which are a major public health issue. Previously, IAV caused many devastating pandemics, and it remains a potential threat for causing more pandemics. Current anti-influenza therapeutics, although useful, are not very effective due to the resistant strains of IAVs that are constantly emerging; thus, there is an unmet need to develop novel antiviral therapies. Here, we present a novel imidazo[1,2-*a*]pyrimidine scaffold that targets group 2 IAV. We have explored different regions of the lead compound, and we have developed a series of highly potent small molecules against group 2 IAV, leading to a well-developed structure-activity relationship (SAR). These small molecules likely target hemagglutinin (HA), a key component of the viral entry process.

Keywords: Influenza A, SARs, antiviral, pseudovirus. infectious virus

Poster #25

Quantitative proteomic analysis of exosomes in HR+/HER2- patients

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Hormone receptor-positive (HR+)/ HER2- breast cancer accounts for 73% of all diagnoses. The combination of tamoxifen with cyclin-dependent kinase (CDK) inhibitors has been proposed to treat late-stage HR+/HER2- patients. However, a significant portion of the patients involved in the clinical trial do not respond well and thus risk missing precious treatment window. Exosomes are small extracellular vesicles secreted by cells and participate in intercellular communication. The protein content of exosomes may serve as biomarkers for cancer development and patient status. Plasma samples (before and after each treatment cycle) were collected from the ongoing clinical trial (NCT02668666) by Pfizer and Big Ten Cancer Research Consortium, a combination of Tamoxifen and Palbociclib is evaluated for the treatment of late-stage metastatic breast cancer. Digested peptides were isotopically labeled with Tandem Mass Tag (TMT) 10plex for protein expression level quantitation. Patients' exosome proteomics data were classified by unsupervised clustering method. We have developed a sensitive and efficient exosome extraction method to obtain exosome protein from minimal patient plasma. Our optimized exosome isolation method could quantitatively identify 437 proteins from 100 µl patient plasma sample. A network model was developed to differentiate responder and non-responder from the exosome proteomics data even before the treatment. Our analysis for TMT labeled sample showed that proteins identified from the patient exosome before the treatment could provide crucial information for predicting response to this combination therapy with high specificity (95%) and sensitivity (89%). This finding could help classify and predict the prognosis of our patients involved in clinical trials and cancer treatments, providing an approach to avoid ineffective treatment. The underlying molecular mechanisms and a possible connection to the RNA expression are being investigated. Our results have the potential to provide new insights into biomarkers for prognosis prediction and precision medicine.

Keywords: breast cancer, exosome, proteomics, prognosis prediction, biomarker

Poster #26

Accelerated Peptide Diversification: Chemoselective Addition of Amino acid Side-chains into poly-Unsaturated Peptides.

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Most peptide drugs contain multiple non-proteinogenic amino acids (NPAAs). To optimize a peptide drug with many NPAAs requires one to find, by trial and error, which NPAAs to use and where to place them in the peptide. The factorial number of possibilities can thus become quite large and only a small fraction of these peptides will ever be made and tested by conventional solid-phase peptide synthesis (SPPS). New methods that enable a greater number of NPAAs and positions to be simultaneously sampled would accelerate hit-to-lead optimization of peptides with NPAAs. We have developed a novel and late-stage platform that uses photocatalyzed side chain addition to covert peptides containing several dehydroalanine (Dha) residues into NPAA-enriched products, incorporating one of a myriad of NPAAs at one specific Dha at-a-time. Through chemoselective library generation, a single poly-unsaturated peptide is strategically transformed into a functional poly-NPAA peptide.

Keywords: peptide diversification, non-proteinogenic amino acids, late-stage, poly-unsaturated peptides

Poster #27

Development of Broad-Spectrum Antiviral Nucleosides

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Nucleoside analogues have garnered a tremendous success as therapeutic molecules for viral infections. However, most nucleosides are not bioactive until they are metabolized to their 5′-triphosphate form through the activity of host cell enzymes. Despite the success of many nucleoside analogues, some compounds suffer from poor cellular permeability and metabolic activation. One strategy to address this issue is the utilization of 5′-phosphoramidate pronucleotides (or ProTides) that deliver the first 5′-monophosphate in masked form. This methodology has been successfully utilized in the blockbuster drug sofosbuvir, which is used for treatment of a hepatitis C. Our laboratory has utilized a similar strategy to afford the metabolic activation of 3′,4′-didehydro-cytidine (ddhC) to 3′,4′-didehydro-cytidine-5′-triphosphate (ddhCTP), which is a broad-spectrum antiviral nucleotide.[1] Our work to develop additional ddhC ProTides will be presented. In related work in response to the COVID-19 pandemic, the antiviral ProTide remdesivir has received emergency approval to treat SARS-CoV-2. This poster will highlight our efforts to synthesize RNA containing this antiviral nucleotide for mechanistic biochemical studies.[2] Finally, this poster will discuss our efforts to develop a novel chemical probe, 5′-bis(*m*-nitrophenyl) uridine monophosphate for use in studies of SARS-CoV-2 exonuclease activity. Collectivity, this work contributes to our understanding of the structure-activity relationship in nucleoside-based compounds, thus aiding the development of potent and effective antiviral agents.

Keywords: Nucleosides, ProTides, Antivirals

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Poster #28

Identification and Optimization of Novel Influenza A Entry Inhibitors Targeting Group 1 and Group 2 Hemagglutinin

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Seasonal influenza is an infectious disease generally caused by Influenza Group A viruses and is known to have a high morbidity and mortality rate. Current treatments are limited to vaccinations and some small molecule inhibitors such as baloxavir marboxil and oseltamivir. Their mechanisms of action are endonuclease and neuraminidase inhibition, respectively. However, due to rapid mutations in the virus, new vaccines need to be developed yearly, and many influenza A isolates have shown resistance to approved therapeutics. Additionally, the circulating strains of the virus are not always consistent with the developed vaccine; therefore, there is a need for new therapeutic treatments. Hemagglutinin (HA) is a glycoprotein located on the surface of the viral particle and is known to be responsible for viral entry into host cells. Using a high-throughput screen of a Chembridge Small Molecule Library (~19,200 compounds), our group discovered a novel small molecule entry inhibitor (M726-0025) for HA. The inhibitor displayed micromolar activity without cytotoxicity in representative Group 1 (H5N1) and Group 2 (H7N1) influenza A pseudoviruses. A high-resolution X-ray crystal structure of H5N1 HA in conjugation with an entry inhibitor (CBS1117) guided optimization of the scaffold. We synthesized a focused library of hit compound analogs to improve the potency of the hit compound. Moving forward, we will profile the library for ADMET properties with the goal of improving the potency and drug-like characteristics of the hit compound.
Keywords: Hemagglutinin, influenza, entry inhibitor, SAR, small molecule

Poster #29

Late-Stage Derivatisation of Akuammicine for Probing Kappa Opioid Receptor Activity

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Picralima nitida (akuamma) has been used for centuries in West African traditional medicine to treat multiple ailments including pain and fever. Akuammicine, an indole alkaloid isolated from the seeds of the akuamma tree, is an agonist at the kappa opioid receptor (KOR). KOR agonists are capable of eliciting an analgesic response without the adverse side effects of conventional mu opioid receptor (MOR) agonists such as tolerance, addiction and respiratory depression. KOR compounds may therefore be a viable alternative to current opioid therapeutics. Preliminary evaluation indicates that akuammicine has submicromolar affinity (K_i = 89 nM) and potency (EC_{50} = 240 nM) at the KOR. Previously, our lab has developed a robust method for isolating akuammicine from commercially available akuamma seeds. Herein we present the late-stage derivatisation of akuammicine for the purpose of studying its structure-activity relationships, and improving its potency and selectivity for the KOR.
Keywords: indole alkaloids, kappa opioid receptor, opioids, pain, analgesia

Poster #30

Discovery of CDK2 Allosteric Inhibitors for Male Contraception and Anticancer Therapy

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CDK2 is a crucial kinase in the cell cycle and binds to cyclins A/E to facilitate the transitions between different phases. It is a promising target for anti-cancer therapy. Overexpression of cyclin E causes hyperactivation of CDK2, which has been identified in diseases such as ovarian, breast, and colorectal cancers. Additionally, CDK2 activity is dispensable in somatic cells, and therefore selective inhibition of CDK2 is lethal for cancer cells but not harmful for normal cells. CDK2 is also an attractive target for male non-hormonal contraception because CDK2 knock-out mice are sterile and otherwise healthy. Historically, CDK2 inhibitors have targeted the ATP site of the enzyme, which is highly conserved across the kinome. Lack of selectivity of ATP-site inhibitors leads to significant off-target toxicity from inhibiting other CDKs. Our group previously discovered that 8-anilino-1-naphthalene sulfonic acid (ANS) inhibits CDK2 by binding to a previously unrecognized allosteric pocket of the enzyme. By targeting this ANS-allosteric site, we aim to achieve a high degree of affinity and selectivity against CDK2. We started virtual and high-throughput screens, targeting the ANS-allosteric pocket in order to discover new chemical entities. A new lead TW-8-67-2 (K_d = 5.2 M) with a novel and druggable scaffold was discovered. X-ray crystallography revealed that TW-8-67-2 binds to the allosteric pocket of CDK2 and causes the protein to adopt an inactive conformation. Optimizations and structure-activity relationships studies based on the TW-8-67-2 hit compound were performed and improved affinity to single digit nanomolar range. These inhibitors are the tightest-binding allosteric inhibitors of CDK2 discovered to date. We recently turned our attention to improving the physical chemical properties and metabolic stabilities of analogs for future cellular studies. Several new co-crystal structures of this series with CDK2 were generated and revealed opportunities of developing heterobifunctional inhibitors and covalent inhibitors.

Keywords: CDK2, allosteric inhibitors, contraception, anti-cancer therapy

Poster #31

Characterization of Circularly Permuted Caspase-2, a Structural and Enzymatic Equivalent of Caspase-2, and Use in Evaluation of Novel Caspase-2 Inhibitors

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The exact cause of Alzheimer's disease (AD) has yet to be completely described despite the disease being defined over 100 years ago. A potential approach to better understand the pathogenesis of AD could be the development of selective caspase-2 (Casp2) probes, as we have shown that a Casp2-mediated cleavage product of tau (Δtau314) reversibly impairs cognitive and synaptic function in animal models of tauopathies. We have developed multiple series' of peptide inhibitors which have been enzymatically and structurally characterized at Casp2 and caspase-3 (Casp3). Our most selective peptide to date, AcVDV(Dab)D-CHO, has been shown to have 27.7-fold selectivity for Casp2 over Casp3. Due to limitations with Casp2 protein production, we have characterized a recently published circularly permuted Casp2 (cpCasp2) to use as a surrogate for the wild type protein. cpCasp2 is both enzymatically and structurally similar to Casp2 and can be used in further activity and structure studies to characterize Casp2 inhibitors. Work from recent publications as well as a closer look at the structural biology data to date on this project will be presented.

Keywords: Alzheimer's disease, Structural Biology, Caspase-2, Protein Engineering

Poster #32

Investigating Extended Substrate Sequences of Farnesyltransferase via MALDI/MS Screening of Peptide Libraries

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Protein prenylation is a post-translational modification where a 15 carbon farnesyl or 20 carbon geranylgeranyl isoprenoid is appended to the C terminal end of a protein by either farnesyltransferase (FTase) or geranylgeranyl transferase type 1, respectively. Misregulation of prenylation has been implicated in many diseases such as cancer, Parkinson's, and Alzheimer's, making the enzyme or pathway a potential drug target. In the canonical understanding of FTase, the isoprenoids are attached to the Cysteine residue of a four amino acid CaaX box sequence. However, recent work has shown that five amino acid sequences can be recognized, such as the pentapeptide CMIIM. This new discovery greatly increases the number of potential FTase substrates, as the enzyme is already known to tolerate a wide variety of amino acids in the canonical CaaX box. With the goal of developing a more rapid and methodical method to evaluate potential substrates, we envisioned using MALDI to assay libraries of 10 peptides at a time, varying one amino acid in the CaaaX box to all 20 canonical amino acids over two libraries, utilizing both yeast and rat FTase. Through this method we observed over 30 hits in the mass spectrum and chose eleven for further evaluation. Nine of these sequences are novel substrates for FTase, with several meeting or surpassing the *in vitro* efficiency of the benchmark sequence CMIIM. Additionally, *in vivo* experiments in yeast demonstrate that proteins bearing these sequences can be efficiently prenylated in a biological context. Searching the human genome for pentapeptide CaaaX sequences found several hits that prenylated with similar efficiency to a native CaaX sequence, raising the possibility of relevance of these sequences in humans. In addition, it is likely that some of the tested sequences inhibit FTase and could be a starting point for a peptidomimetic inhibitor.

Keywords: prenylation, enzymology, MALDI, peptides

Poster #33

Construction and Characterization of Pro-Chemically Self-Assembled Nanorings (Pro-CSANs) for Cancer Immunotherapy

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The Wagner Lab has previously developed a non-genetic platform to re-direct human T cells to combat solid tumors. This approach is based on fusing a tumor-targeting protein or a T cell-targeting anti-CD3 protein to a dimer of *Escherichia coli* (*E. coli*) dihydrofolate reductase ($DHFR^2$). Incubating a mixture of tumor-targeting and T cell-targeting $DHFR^2$ fusion proteins with a chemical dimerizer, bis-methotrexate, leads to the spontaneous assembly of bispecific, multivalent nanorings, or CSANs. These bispecific CSANs bind to $CD3^+$ T cells and re-direct them to target and destroy cancer cells. Tumor-associated antigen targeting relies on differential antigen expression between cancer and healthy cells. The high affinity and avidity of the multivalent bispecific CSANs may potentially target healthy tissue with low antigen expression, thereby exhibiting "on-target, off-tumor" toxicity. Matrix metalloproteinases are overexpressed in a variety of solid tumors. Therefore, to improve tumor specificity, we hypothesized that masking the anti-CD3 moiety in our CSANs with a peptide or a protein, through a matrix metalloproteinase-2 (MMP-2) sensitive linker will prevent engagement and activation of T cells outside the tumor microenvironment (TME). Once the CSANs enter the TME, cleavage by MMP-2 will lead to unmasking of the anti-CD3 moiety, followed by engagement and activation of T cells, ultimately causing targeted cancer cell lysis. To this end, we generated an anti-CD3-$DHFR^2$ fusion protein containing an anti-CD3 nanobody. To mask the anti-CD3 nanobody, the first design consisted of genetically fusing a 27 amino acid polypeptide to the nanobody's N-terminus via an MMP-2 sensitive linker. In the second design, the polypeptide was replaced with human histidine triad nucleotide binding protein 1, which will potentially serve as a steric mask. The anti-CD3 and masked-anti-CD3-$DHFR^2$ fusion proteins were successfully expressed and purified from *E. coli*. The preliminary characterizations of these fusion proteins will be presented.

Keywords: Immunotherapy, T cells, nanorings, tumor microenvironment, matrix metalloproteinase

Poster #34

Development of Novel E3 Ubiquitin Ligase Ligands and Partial PROTAC Library for Targeted Protein Degradation

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Traditional drug discovery approaches have focused on using small molecules to modulate the function of a variety of proteins. However, almost 80% of the human proteome is considered "undruggable" due to the lack of proteins' defined active site to modulate the function. Proteolysis Targeting Chimeras (PROTACs) have emerged as a promising strategy to selectively promote the degradation of protein targets by using the ubiquitin-proteasome system. PROTACs are heterobifunctional small molecules consisting of two ligands connected by a short linker. One ligand binds to an E3 ubiquitin ligase, while the other binds to a protein of interest. Currently, only a small number of the E3 ubiquitin ligase ligands have been discovered for the PROTAC technology. These ligands have various limitations such as lack of potency, poor permeability, or low selectivity. This project focused on the design and synthesis of a small library of novel cereblon (CRBN) E3 ubiquitin ligase ligands. We then tested their binding affinity to the CRBN E3 ligase employing a cell-based target engagement assay and a biochemical fluorescence polarization assay. Some of them showed higher affinity and better selectivity than known CRBN ligand pomalidomide. We are currently employing these new CRBN E3 ligase ligands to create a partial PROTAC library for the degradation of various targets.
Keywords: PROTAC, CRBN, ligands, affinity, selectivity

Poster #35

Construction of Site-Specific DNA-Histone Conjugates in Chromatin Core Particles and Their Effects on DNA Transactions

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DNA-protein cross-links (DPCs) are bulky DNA lesions in which a protein becomes covalently bound to chromosomal DNA. Irreversible entrapment of cellular proteins on chromosomal DNA occurs following exposure to *bis*-electrophiles, transition metals, and free radical species. Subsequently, DPCs interfere with DNA-related processes, such as DNA replication, transcription, and repair. Recently, we discovered a novel mechanism of epigenetic regulation involving the formation of reversible DNA-histone cross-links between 5-formyl-2'-deoxycytidine residues (endogenously observed epigenetic marks) in DNA and lysine or arginine side chains of the proteins. The lysine side chains from histone proteins are known to undergo reversible acetylation, ubiquitylation, methylation, and other reversible posttranslational modifications that influence chromatin structure and control the levels of gene expression. There is currently poor understanding of how the DNA-histone cross-link affects chromatin structure, transcription and replication. To address this gap in knowledge, we are constructing site-specific DNA-histone conjugates in a nucleosome core particle with the unnatural amino acid amino-oxy lysine to form hydrolytically stable oxime linkages. Herein, we detail how this unnatural amino acid is incorporated in the histone H3 tail through solid phase peptide synthesis to the globular domain of histone H3 through Sortase-mediated ligation to afford a modified amino-oxy containing histone H3. These conjugates will serve as substrates in characterizing the biological function of DNA-histone cross-links and their potential role in epigenetic control of gene expression.
Keywords: DNA-Protein Crosslinks, DNA-Adducts

Poster #36

Developing potent and selective CDK11 degraders using PROTAC technology for anti-cancer therapy

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Treatment of cancers by targeted therapies have gained attention because they possess selectivity and specificity towards cancerous cells. However, because of the complexity of the disease, drug resistance and off-target toxicities, additional therapies are needed. Hence, it is of utmost importance to develop novel target specific treatments for cancer to overcome lack of efficacy, drug resistance and minimize toxicity and side effects associated with current cancer chemotherapies. Cyclin dependent kinases (CDKs) are serine/threonine protein kinase that are overexpressed in most cancers. CDKs are molecular targets for designing chemotherapeutic drugs since they play significant roles in regulating various cellular processes. CDK11 is a genetically and pharmacologically validated novel cancer target that binds to L-type cyclins and is involved in cell cycle progression, transcription, DNA repair, proliferation, and apoptosis. Recently it was found that OTS514, a thieno-quinolone, developed as an alleged TOPK serine/threonine protein kinase inhibitor, does not inhibit TOPK, but is a potent selective inhibitor of CDK11. The goal of this project is to develop Proteolysis Targeted Chimeras (PROTAC) degraders for CDK11. PROTACs have gained significant attention in recent years because they degrade the target protein instead of inhibiting the target. A PROTAC is composed of a ligand (targeting CDK11 in our case) and an E3-ligase ligand (to enable ubiquitination) connected by a linker. PROTACs can selectively degrade highly similar kinases even if the parent inhibitor is not selective. This work involves developing PROTACs using OTS514 as the CDK11 ligand, exploring various E3 ligase ligands and linkers of various nature/lengths, structure-based drug design, synthesis and performing various *in vitro* and *in vivo* studies to assess the potency and selectivity of PROTACs in CDK11 degradation.

Keywords: Cancer, CDK11, Targeted therapy, PROTACs, OTS514

Poster #37

How *Streptococcus pyogenes* Benefits from Rgg2/3 Quorum Sensing During Infection

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Streptococcus pyogenes is a Gram-positive bacterium and human pathogen that causes diseases ranging from pharyngitis to necrotizing fasciitis. The exact global disease burden caused by *S. pyogenes* is not known but is estimated that nearly 616 million pharyngitis cases and 111 million pyoderma cases exist. Our lab has characterized the ability of *S. pyogenes* to suppress proinflammatory innate immune responses through Rgg2/3 quorum sensing (QS) regulated genes. The Rgg2/3 QS system is highly conserved within *S. pyogenes* serotypes. We hypothesize that Rgg2/3-mediated immunosuppression aids *S. pyogenes* survival in its interaction with the host by diminishing the ability of innate immune cells to signal, recruit, and stimulate other cells and/or kill bacteria. Macrophages are critical in the containment of *S. pyogenes* during infection through effector and signaling roles. Using genetically engineered strains of *S. pyogenes* that place the bacteria in either a constitutively active or inactive state, I am testing whether macrophage effector and signaling activities are altered. Macrophage effector activity relies on destroying pathogens through phagocytosis and internally degrading them with reactive oxygen or reactive nitrogen species (ROS or RNS, respectively). Macrophage signaling activity uses chemokines and cytokines to recruit and stimulate other immune cells. I am measuring intracellular bacterial survival and host ROS/RNS production to determine altered effector activity. I will measure cytokine and chemokine profiles to determine altered signaling activity. This research would contribute to a better understanding of the *S. pyogenes* mechanism of infection using the Rgg2/3 QS system.

Keywords: Quorum Sensing, Streptococcus pyogenes, Immune Suppression, Innate Immunity, Rgg2/3

Poster #38

Colorimetric *β*-Galactosidase Assay Facilitates the In-Direct Measurement of cAMP Production via the activation of G_s-Pathways in The Melanocortin Receptors Subtypes.

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The Melanocortin Receptors are GPCRs that primarily activate the adenylate cyclase via Gs- signaling pathways. The melanocortin 3 and 4 receptor (MC3R and MC4R) subtypes are expressed in the central nervous system and are of interested due to their roles in maintaining energy homeostasis.[1] The Haskell-Luevano lab investigates and develop ligands targeting MC3R and MC4R,[2] and suitable tools are required for our investigations. The development of the *β*-Galactosidase assay, by Chen and colleagues[3], allows for the in-direct measurement of cAMP concentrations via the activation of Gs-signaling pathways. The colorimetric assay works by transfecting HEK293 cells with *β*-Galactosidase (lacZ) gene which fuses to five copies of the phosphorylated cAMP-response element (CREB) upon the increase production of cAMP. The lacZ gene can then produce *β*-Galactosidase, which can be used to indirectly measure cAMP production. This is measured by absorbance (405 nm) by introducing o-nitrophenyl-*β*-D-galactopyranoside (ONPG) substrate which is converted to ortho-nitrophenol via the cleavage of the sugar by *β*-Galactosidase. Herein, the 5-day β-galactosidase assay utilized in the Haskell-Luevano lab will be described.

Keywords: Melanocortin Receptors, Assays

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Poster #39

Anchimerically Activated ProTide Inhibitors of Eukaryotic Translation Initiation Factor 4E (eIF4E) as Host-Directed Antivirals Against SARS-CoV-2

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Despite the rapid development of highly efficacious vaccines and monoclonal antibody treatments, the COVID-19 pandemic continues to strain healthcare systems worldwide to their limits. Currently, only a few small molecule therapeutics are available to treat COVID-19, and those that exist have significant limitations. To date, remdesivir (Veklury®) is the only fully FDA-approved small molecule drug for the treatment of COVID-19. The clinical utility of remdesivir is severely limited by its cost, narrow therapeutic window, and the need for it to be administered intravenously. Molnupiravir and Paxlovid have been granted emergency use authorization but have limiting safety profiles. Additionally, these drugs target viral proteins directly which creates a risk for driving mutations that decrease the efficacy of the drugs or eliminate their utility entirely. Host-directed antivirals that target endogenous proteins essential to viral replication pose a far lower risk for driving mutations and are expected to retain their potency against emerging variants. Recently, eukaryotic translation inhibitors have emerged as exceptionally potent host-directed antivirals against SARS-CoV-2. Among these, plitidepsin and zotatifin have entered clinical trials for the treatment of moderate COVID-19. Plitidepsin inhibits eukaryotic translation elongation factor 1A (eEF1A) while zotatifin targets the RNA helicase eukaryotic translation initiation factor 4A (eIF4A). Inspired by the promising pre-clinical data of these translation inhibitors, we tested our previously developed eukaryotic translation initiation factor 4E (eIF4E) inhibitors, designated as 4Ei-10 and 4Ei-11, for their effect on SARS-CoV-2 replication. We observed significant replication inhibition at nanomolar concentrations in our initial *in vitro* viral plaque assays, as well as a significant reduction in viral spike protein expression. To further investigate the *in vitro* and *in vivo* activities of our compounds we have developed an efficient synthesis of 4Ei-10 and 4Ei-11. We anticipate that targeting eIF4E will represent a new avenue for therapeutic development in the treatment of COVID-19.

Keywords: COVID-19, SARS-CoV-2, antiviral, eIF4E, translation

Poster #40

Development of Bivalent Ligands Targeting GHSR/DR Oligomers for Substance Use Disorder

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At present, the lifetime prevalence of a stimulant use disorder diagnosis is approximately 4%, with annual new users of cocaine totaling almost 900,000 worldwide. Despite this prevalence, no pharmacologic treatments exist. Over the last several decades, the role of acyl-ghrelin and its receptor, the growth hormone secretagogue receptor (GHSR), has been shown to have a significant role in both food- and drug-reward. While GHSR is widely expressed, its role in reward has been shown to be strongly influenced by a population of receptors that form oligomers with dopamine receptors (DR). The goal of this project is to design and synthesize bivalent ligands targeting this population of heteromeric receptors in order to avoid adverse effects that were observed when targeting monomeric GHSR, including tachycardia, hypoglycemia, and anorexia.

The Liver Expressed Antimicrobial Peptide 2 (LEAP2) has been reported to be an endogenous antagonist of GHSR and studies have shown that the *N*-terminus is the active pharmacophore. Dopamine 1 Receptor (D1) is highly expressed in regions of the brain associated with drug reward, including the hippocampus, cortex, substantia nigra, and ventral tegmental area (VTA) and is likely the relevant binding partner for GHSR. Thus, we have targeted our synthetic campaign toward the generation of bivalent ligands containing the D1 antagonist SCH23390 and *N*-terminal region of LEAP2 and will utilize molecular modeling to determine optimal linker length. In addition to our lead compound, other moieties incorporating the D2 antagonist eticlopride, the *C*-terminus of LEAP2, and/or *O*-acyl-ghrelin will be generated as controls. Progress towards the synthesis of our probes will be discussed.

Keywords: substance use disorder, ghrelin, dopamine, bivalent ligands

Poster #41

Synthesis and Characterization of Small Molecules that Target UNC119 for the Treatment of Diabetes Mellitus

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Type II diabetes (T2D) is a disease that is characterized by insulin resistance. In skeletal muscle and adipose cells, T2D is induced by impaired GLUT4 translocation. As a result, insulin is unable to bind receptors that trigger a signaling cascade to allow GLUT4 in the vesicles to be pushed to the top of these cells for glucose storage. Currently, treatments for diabetes have largely failed to achieve glycemic control or restore insulin-stimulated GLUT4 translocation. As a result, a luciferase-based assay was developed to identify small molecules that could restore GLUT4 translocation in the presence of endogenous insulin in order to prevent hypoglycemia. Small molecule C3, and later C59, were identified as small molecule mediators of GLUT4 translocation in the presence of insulin. In knowing this, we are pursuing the synthesis and derivatization of novel compound C59 and anticipate that these compounds will increase GLUT4 translocation while in the presence of insulin.

Keywords: GLUT4, type II diabetes, insulin resistance

Poster #42

Chemical Probe Development for BPTF Reader Domains Utilizing Biophysical Assays

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In many disease states there is a dysregulation in gene expression, which can be driven by the aberrant levels or function of epigenetic protein complexes. One such epigenetic protein dysregulated in numerous cancers is the bromodomain and PHD-finger containing transcription factor (BPTF), which binds to chromatin via interactions with acetylated histone through its bromodomain and trimethylated histone through its C-terminal PHD finger to facilitate chromatin remodeling. This is in part via protein-protein interaction with the oncoprotein c-MYC. Together, this makes BPTF a promising anti-cancer therapeutic target. Although evidence supports the role of the BPTF protein in disease, the relevance of its individual domains is unclear, making the design of selective probes advantageous. Currently, there are several reported BPTF bromodomain inhibitors, but there is a significant need for potency and selectivity gains. A promising new class of BPTF inhibitors based on a pyridazinone scaffold will be discussed. We have utilized Protein-Observed ^{19}F (PrOF) NMR, AlphaScreen, and x-ray crystallography to rationally design and expand the pyridazinone scaffold into a potent lead against the BPTF bromodomain (K_d = 6.3 nM) with selectivity over the BET family of bromodomains (>350-fold). We have also shown preliminary on-target effects in synergistic cellular studies with chemotherapeutics. For the BPTF PHD finger, there are no reported small molecule binders. Recent progress in our lab towards assessing the ligandability of the PHD finger by computational analysis, as well as a NMR-based fragment screening will also briefly be discussed.

Keywords: chemical probe, FBDD, bromodomains, methyl lysine binding proteins, epigenetics

Poster #43

Nitric Oxide Promotes Expression of Tumorigenic Gene Signatures in Triple Negative Breast Cancer (TNBC) via Inhibition of m6A mRNA Demethylation.

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N6-methyladenosine (m6A) is the most abundant and conserved internal modification in eukaryotic messenger RNA (mRNA). m6A plays an essential role across multiple of stages RNA processing and metabolism activities such as localization, translation, stabilization, and decay. However, abnormalities in the enzymes that metabolize m6A and dysregulated m6A levels are associated with various pathologies including cancer. We demonstrated that the gasotransmitter nitric oxide (NO) inhibits the catalytic activity of m6A demethylases FTO and ALKBH5 by directly binding to the catalytic center. Since endogenous NO is closely associated with more aggressive cancers, we hypothesize that inhibition of m6A demethylation by NO leads to the expression of pro-tumorigenic genes that drive aggressive phenotypes. This establishes a mechanistic link between NO-associated tumor pathologies and epigenetic regulation. Our data demonstrated that TNBC cell lines chronically exposed NO exhibited a significant increase in global m6A levels as a result of inhibiting demethylation and not by promoting methylation. RNA- and m6A-Sequencing revealed that m6A was enriched in mRNA transcripts that are associated with invasion, inflammation, RNA metabolism, and metastasis. Taken together, we have elucidated a novel epigenetic mechanism such that endogenous NO inhibits demethylases FTO and ALKBH5 which increases cellular m6A levels to drive oncogenic signaling pathways. These results are the first ever to demonstrate that an endogenous signaling molecule (NO) can regulate the epitranscriptome.

Keywords: nitric oxide, epigenetics, breast cancer, mRNA, tumorigenic

Poster #44

The Effects of Ketamine and Psilocybin on Fear Acquisition and Extinction in Mice

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Major depressive disorder (MDD) has become an increasingly prevalent issue, compounded by the limited efficacy of current treatment options. Two promising treatments are the N-methyl-D-aspartate receptor (NMDAR) antagonist, ketamine, and the serotonergic $5HT_{2A}$ agonist and classical psychedelic, psilocybin. These compounds have been shown to exert rapid antidepressant effects through mechanisms that have yet to be fully understood. Both of these rapidly-acting antidepressants have been observed to induce acute stress, as measured by elevated glucocorticoid release after administration. We propose that the therapeutic effects of these drugs are due to a transient enhancement of neural plasticity in the ventral hippocampal medial-prefrontal-cortex (HPC-mPFC) pathway and subsequent memory formation produced by this acute stress. Impaired plasticity in the HPC-mPFC pathway is observed in patients with MDD, and individuals have reported decreased depressive symptoms after taking either psilocybin or ketamine. These improvements have been reported to last hours or even weeks, with psilocybin typically having longer lasting increases in mood. Despite this, there are no studies that assess the duration of this critical window. To study this window of plasticity at the behavioral level, we used male C57BL/6J mice and the associative learning task of fear conditioning. Mice were administered a single intraperitoneal (IP) injection of saline, ketamine (10-30 mg/kg), or psilocybin (3 mg/kg) at 4 or 24h prior to fear conditioning. Here we found that mice that received ketamine (30 mg/kg) 4h prior to initial testing showed enhanced fear acquisition compared to those that received ketamine 24h prior, psilocybin, or saline ($p<0.05$). This effect was even more pronounced in animals that received a lower dose of ketamine (10 mg/kg, $p<0.05$). Interestingly, psilocybin did not alter fear acquisition from saline controls when administered at either time point. Animals that received ketamine or psilocybin also displayed enhanced fear extinction, although these trends were non-significant.

Keywords: Ketamine, Psilocybin, Glutamate, Serotonin, Plasticity

Poster #45

Identification of potent and selective MC3R modulators from a mixture-based scaffold ranking library

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The melanocortin-3 and -4 receptors (MC3R, MC4R) are centrally expressed and responsible for maintaining energy homeostasis. Agonists of the MC3R and MC4R have been demonstrated to decrease food intake while antagonists have been demonstrated to increase intake. These properties thus offer therapeutic routes for the treatment of homeostatic feeding disorders like obesity, cachexia, and anorexia nervosa. Modulation of the MC4R has been successful, though ligands to selectively target the MC3R have remained elusive. MC4R modulators have been shown to exhibit undesirable side effects like increases in blood pressure, erectile activity, and skin darkening, shifting current efforts to the development of ligands for the MC3R.

Our goal is to identify novel MC3R peptidomimetic scaffolds that can selectively modulate MC3R activity with sub-micromolar activity in order to take advantage of the synergy between the MC3 and MC4 receptors in a novel therapeutic route. To do so, we report herein a mixture-based scaffold ranking library similar to those previously used to identify MC4R antagonists. We employ this method targeting the MC3R to identify potent and selective peptidomimetic ligand scaffolds using a cell-based gain-of-signal beta-galactosidase assay. Potent and selective scaffolds were further deconvoluted using a positional scanning screen which enabled us to identify a series of pyrrolidine bis-cyclic guanidines with nanomolar agonist activity at the MC3R and over 10-fold selectivity for the MC4R. A follow-up screen will confirm the observed activity of these ligands while simultaneously surveying new chemical space for additional agonists and antagonists of the MC3R. These scaffolds will be assessed for their therapeutic potential as lead candidates in novel interventions for homeostatic disorders.

Keywords: melanocortins, GPCRs, screening, libraries, peptidomimetics

Poster #46

Glucocorticoid Release and Modification of Acute Threat-Associated Behaviors by Psilocybin

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Clinical protocols for administering psilocybin-assisted psychotherapy have found a negative correlation between anxious responding and therapeutic outcomes. Despite this, few studies have directly assessed the role that corticosteroids have in altering psilocybin's acute or delayed behavioral effects. Here, psilocybin, lisuride, and ketamine were used to assess the role of glucocorticoid release in acute threat-associated behavioral responding. Using the open field test, psilocybin's anxiogenic effects at 15 min were found to be inversely and dose-dependently correlated with anxiolytic effects at 4 h in C57BL/6J mice. This corresponded with the observed ability of psilocybin to induce plasma corticosterone release at 15 min, which returned to baseline by 4 h. Lisuride and ketamine also showed this effect. Furthermore, psilocybin, lisuride, and ketamine reduced the latency to feed in the novelty suppressed feeding task at 4 h. Psilocybin and ketamine's effects in this task were lost following chronic oral corticosterone suppression of acute corticosterone release or following pretreatment with the glucocorticoid receptor antagonist mifepristone. When delayed effects were assessed in the open field test at 7 d following administration, anxiolytic effects were again observed for psilocybin, while lisuride and ketamine did not exhibit this long-term effect. Notably, this long-term behavioral effect of psilocybin was found to interact with corticosteroid status at the time of treatment, where chronic corticosterone exposure caused psilocybin to be anxiogenic at 7 d. Intriguingly, mifepristone pretreatment did not affect psilocybin's long-term behavior. These results suggest that drug-induced corticosteroid release is supportive of acute anxiolysis across drug classes, but this corticosterone release is neither sufficient nor necessary for long-term anxiolysis. Psilocybin's long-term anxiolysis is sensitive to elevated corticosterone concentrations at the time of drug administration. Delayed ketamine and psilocybin-enhanced fear extinction learning have also been observed and the corticosterone sensitivity of these observations is under study. Together, these observations highlight critical distinctions between drug-induced glucocorticoid release and exogenous factors causing elevated glucocorticoid concentrations on psilocybin's behavioral effects.
Keywords: Psilocybin, Ketamine, Corticosterone, Psychedelics, Behavioral

Poster #47

Diet-Associated DNA Adducts: Determining the Origin of Endogenous N-7-(2,3,4-trihydroxybutane-1-yl)guanine in Tissue and Cell Culture

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Adducts of DNA bases commonly occur through the reaction of an electrophilic species with a DNA nucleobase. These DNA lesions can result in deleterious gene misregulation and are known to arise from both exogenous and endogenous sources. Following exposure to 1,3-butadiene (BD), a common industrial carcinogen, genomic deoxyguanine can react with a metabolic intermediate of BD to form the DNA adduct N7-(2,3,4-trihydroxybut-1-yl) guanine (THB-Gua) in a dose-dependent fashion. The THB-Gua adduct has also been observed to form from a yet unknown endogenous source in BD-unexposed conditions. To examine endogenous THB-Gua formation, a sensitive nano-HPLC-NSI HRMS/MS methodology was developed and utilized to assess differences in THB-Gua adduct formation. Tissue samples of blood, heart, liver, lung, and pancreas were analyzed from Sprague-Dawley rats of different sex, weight, and age for organ- and biometric-based differences in endogenous THB-Gua adduct formation. Additionally, culture of various cell lines supplemented by particular nutrients (glutamine, erythrose, glucose, etc.) was performed to observe a dietary influence and potential metabolic input for endogenous production of THB-Gua. This study investigates a mechanistic route of formation for endogenous THB-Gua and its implication as a consequence of particular factors such as exercise and diet. It is the goal of this work that lifestyle change can be altered to affect the degree of endogenous DNA damage resulting from formation of N7-guanine adducts and thereby improve organismal health overall.
Keywords: Adduct, butadiene, exposome, endogenous, mass spectrometry

Poster #48

Modulating the Avidity of Bispecific Chemically Self-assembled Nanorings

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Our laboratory has developed an approach to non-genetically modify T cells using Chemically Self-assembled Nanorings (CSANs) as prosthetic antigen receptors (PARs). Bispecific PARs have been developed to selectively target the human CD3 receptor and a known Cancer antigen.[1] In recent work, we demonstrated that the distribution of each monomer in a bispecific CSAN could be quantitatively tuned by mixing different equivalences of each monomer during ring formation. Although the monomer distribution could be modified, bispecific αCD3/αEGFR rings either predominantly displayed the αCD3 single-chain variable fragment (scFv) or αEGFR fibronectin (FN3).[2,3] **Current work** focuses on the development of bispecific CSANs in which the valency of the αEGFR FN3 could be tuned without simultaneously decreasing the valency of the αCD3 scFv and *vice versa*. We **hypothesize** that higher αCD3 valency of the rings would enhance cytotoxicity due to preferred binding to T cells and modified valency for αEGFR would allow for discrimination between tissues displaying different amounts of EGFR. Consequently, we developed an αEGFR/αCD3 fusion protein which consisted of two targeting ligands fused to our dihydrofolate reductase ($DHFR^2$) molecules. As with our current CSANs, the αCD3 scFv is fused to the C-terminus of our $DHFR^2$-based scaffold while αEGFR is fused to the N-terminus of the $DHFR^2$-αCD3 scaffold. The results of ongoing studies to characterize the αEGFR/αCD3 fusion protein and activation properties of the fusion protein will be reported.

Keywords: CSANs, bispecific, immuno-oncology, T cells, cancer

Poster #49

Development of Cross-Reactive Antibodies for the Identification and Treatment of Synthetic Cannabinoid Toxicity

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Synthetic cannabinoid receptor agonists (SCRAs) are compounds which mimic the pharmacology of psychoactive components in cannabis. These compounds are inexpensive, commercially available and difficult to identify with modern analytical methods, making them highly accessible for recreational use. The diverse nature of SCRA structures contributes to their unique pharmacological profile, resulting in increased potency and toxicity compared to cannabis. Suspected SCRA toxicity, which can present with a breadth of cardiovascular, gastrointestinal and neurological disturbances, is currently addressed through symptom management followed by toxicological screening after patient discharge. We can address the dual need for improved diagnostic and therapeutic intervention against SCRA intoxication with antibodies generated in the presence of SCRA-resembling small molecule haptens conjugated to immune-activating proteins. Similar antibody strategies employed for opioids, nicotine and other commonly misused drugs have shown potential in both detecting these drugs *in vitro* and sequestering them to the periphery *in vivo*, blunting their psychotropic effects. As most SCRAs possess a generalized structure containing a tail, core, linker and head group, we generated antibodies in mice against haptens comprising several motifs observed within these groups from different SCRAs. Vaccinated animals were then subjected to structurally relevant SCRAs to determine if the vaccines could prevent SCRA-induced physiological changes in locomotion, body temperature and nociception. Lastly, these antibodies were tested against a panel of SCRAs with varying degrees of structural similarity through competitive ELISA methods to evaluate their diagnostic potential. We determined that haptens containing valine-like head groups linked to a heterocyclic core generated robust immune responses resulting in antibodies capable of binding SCRAs with bioisosteric changes to the core and tail groups compared to the hapten. Furthermore, our naphthyl head group haptens stimulated considerably weaker immune responses, resulting in antibodies with poor potency and minimal ability to bind structurally similar SCRAs.

Keywords: Cannabinoids, Vaccination, Antibodies

Poster #50

Streamlined Iterative Assembly of Thio-oligosaccharides in Aqueous Solution and Development of Carbohydrate-based Antibiotics

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The prevalence of carbohydrate molecules in all domains of life as well as their various function in signaling pathways made them attractive targets in biochemical and pharmaceutical research. Many synthetic strategies and glycosylation methods have been developed to access carbohydrate molecules. However, unlike enzymatic approaches which can take advantages of the chemical architectures of active sites to ensure preciseness, complex protection-deprotection steps become necessary in chemical synthesis. Traditional protection strategies inevitably increase synthetic length and decrease atom economy. In addition, increased lipophilicity of functionalized sugar molecules and water nucleophile competition make it almost impractical to run aqueous glycosylation. Glycoside hydrolases 76 (GH76) are endo-α-1,6-mannanases. This enzyme could participate in cleaving the α-1,6-mannan and forming new glycosidic bonds between glycoprotein-associated mannan and cell wall glucan in fungi and are involved in bacteria nutrition acquisition. GH 76 is thus regarded as a good target for the development of antibiotics. Development of GH 76 inhibitors could be potentially applied in antibacterial and antifungal drugs. However, reported GH 76 inhibitors have some limitations in synthesis difficulties and selectivity. In order to minimize the impediments brought by complex protecting groups and the anhydrous reaction condition required for most glycosylation, efforts were devoted for aqueous glycosylation using unprotected carbohydrate building blocks with limited success. There is still a lack of general method for quick assembly of structurally diverse unprotected monosaccharides under aqueous conditions. We herein report our progress toward the development of glycosylation in aqueous solution using glycosyl fluoride donors and positional deoxythio acceptors promoted by $Ca(OH)_2$. This strategy is further applied for the synthesis of GH76 inhibitors.

Keywords: Carbohydrate, Glycosylation, Inhibitors, Antibiotics

Poster #51

Targeting N-Myc Through Degradation Of Aurora Kinase A: Linker Design And Optimization

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The Myc family of transcription factors are dysregulated in a majority of cancers. In particular, N-Myc, encoded by the *MYCN* gene, is a driver of central nervous system cancers such as neuroblastoma. The structure of the oncoprotein is highly disordered and lacks suitable pockets for small molecule binding, which has contributed to its description as "undruggable." Although inaccessible for direct small molecule binding, N-Myc can be targeted through its interactions with Aurora kinase A (AurA). N-Myc is rapidly degraded in healthy cells, however it is stabilized by overexpressed AurA in *MYCN*-amplified neuroblastoma cells. In previous studies our laboratory has developed the chemical degrader, HLB-0532259, to indirectly decrease N-Myc levels through degradation of AurA. HLB-0532259, a Proteolysis Targeting Chimera (PROTAC), consists of an AurA targeting moiety, and an E3 ligase recruiter joined through a chemical linker. As AurA is degraded, N-Myc is polyubiquitinated and removed through its natural degradation pathway. In our current studies, we optimize the degradation efficiency (DC_{50}), selectivity and physiochemical properties of HLB-0532259 by varying the linker length, composition, and flexibility. We found that compounds with alkynes incorporated into the linker were poorer degraders than their fully reduced counterparts and linkers shorter than 7 atoms were weakly active. Through this work, we developed the further optimized compound HLB-0535051, which shows a 7-fold improvement in DC_{50} and 3.5-fold improvement in cytotoxicity against *MYCN*-amplified neuroblastoma cell line IMR-32. This poster highlights our efforts to optimize chemical degraders for N-Myc via degradation of AurA.

Keywords: N-Myc, Aurora Kinase A, Degrader, Neuroblastoma

Poster #52

The Effect of Sugar Conformation on Molecular Recognition of DNA Substrates by APOBEC3A and APOBEC3B

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The APOBEC3 (A3) proteins are cytidine deaminases that catalyze conversion of cytosine to uracil in single stranded (ss)DNA. Studies have shown that APOBEC3A (A3A) and APOBEC3B (A3B) activity are sources of mutation that promote drug resistance and tumor evolution in cancer patients. Although poor clinical outcomes have been associated with deaminase activity of A3A and A3B, whether these mutations are occurring via DNA or RNA editing is poorly understood. *In vitro* studies have shown that A3 proteins bind to RNA, however A3A and A3B selectively deaminate ssDNA over RNA. The discrete molecular features that dictate ssDNA binding and A3-mediated deamination over RNA binding are not well characterized on a molecular level. Molecular dynamics simulations suggest that the difference in sugar conformation between the ribose of RNA and 2′-deoxyribose of ssDNA, results in the selective deamination of ssDNA over RNA. The difference in sugar conformation arises from the various R groups present at the 2′-position of the sugar. The hydroxyl present at the 2′-position of ribose results in a 3′-endo conformation of the sugar, whereas the geminal hydrogens on the 2′-deoxyribose promote a preferred 2′-endo conformation. Key interactions that confer the molecular recognition capability of A3-substrate binding may be disrupted due to the difference in sugar orientation between DNA and RNA. To validate our molecular dynamics simulations, we have determined both the binding affinity and substrate turnover of oligonucleotides containing cytidine analogues that prefer either the 2′-endo (DNA) or 3′-endo (RNA) conformations. Oligonucleotide binding was assessed using fluorescence polarization and differential scanning fluorimetry, and substrate deamination was determined using a gel-based deamination assay. In this poster we provide critical insights into the importance of sugar conformation in the context of A3-substrate recognition.

Keywords: APOBEC, DNA, substrate recognition

Poster #53

Fractionation of *Salvia rosmarinus* and Evaluation of Nrf2 and AhR Modulation for Ulcerative Colitis

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Salvia rosmarinus (rosemary) is known to have anti-inflammatory and antioxidant properties. In an ulcerative colitis (UC) *in vivo* model, it protected against colitis damage by improving disease activity index and upregulating tight junction proteins. Diterpenes are prominent in rosemary with carnosic acid (CA) and carnosol (CL) being the most abundant in a diterpene-rich oil soluble extract. Data from our laboratory demonstrated that CL upregulates nuclear factor erythroid 2-related factor 2 (Nrf2), thus decreasing oxidative stress. Diterpenes are also known to have anti-inflammatory properties. Nonetheless, many of the other diterpenes in the extract are not well studied requiring further evaluation. Several semi-preparative chromatograms revealed significant peaks distinct from CA and CL. Additionally, preliminary transepithelial electrical resistance data in Caco-2 cells treated with 3 fractions from the oil soluble extract (excluding CA and CL) showed an increase in epithelial barrier integrity by all fractions. Therefore, we hypothesized that at least one of these fractions will be antioxidant and anti-inflammatory. To test our hypothesis, we evaluated the Nrf2 and aryl hydrocarbon receptor (AhR) pathways. We utilized luciferase reporter assays with either an antioxidant response element (ARE) or a xenobiotic response element (XRE) in stably transfected HepG2 cells. Activation of both pathways by all fractions suggested that additional compounds in each fraction have anti-inflammatory and antioxidant properties. Together, the data suggests that additional phytochemicals beyond CA and CL modulate Nrf2 and AhR and may be beneficial for UC.

Keywords: diterpenes, rosemary, inflammation, IBD

Poster #54

Glutathione Peroxidase 4 Gene Variants Association With Post Operative Atrial Fibrillation Risk and GPx4 Protein Phenotypic Characteristics in Patients Undergoing Elective Cardiac Surgery

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The incidence and prevalence of AF are increasing worldwide. Heterogeneity in the incidence of postoperative atrial fibrillation (POAF) following heart surgery suggests that underlying genetic and/or physiological factors impart a higher risk of this complication to certain patients. Glutathione peroxidase 4 (GPx4) is an antioxidant selenoenzyme that specifically neutralizes lipid hydroperoxides, and is master regulator of ferroptosis, a regulated form of cell death caused by lipid peroxidation which has recently been shown to play an important pathophysiological role in cardiovascular disease (CVD). Several clinical genetic studies have reported associations between gpx4 variants and CVDs such as coronary disease and heart failure. Furthermore, studies in experimental models have shown that lipid peroxidation is involved in arrhythmogenesis, although nothing is known regarding the potential role of GPx4 in this pathology. This study investigated the relationship between gpx4 variants and GPx4 content and activity in human atrial myocardium, and their association with POAF. Samples of atrial myocardium were dissected from the right atrial appendage obtained from 189 patients undergoing elective coronary artery bypass graft (CABG) surgery at a single tertiary-care hospital. DNA was extracted from these samples and sequencing analysis was performed across the gpx4 coding region on chromosome 19. GPx4 activity was determined in fresh sample's lysate using a coupled NADPH fluorometric assay, and GPx4 content was determined using ELISA. Incidence of POAF was 25% in this cohort. Five GPX4 variants were associated with POAF risk (permutated $P \leq 0.05$), and 8 variants associated with altered myocardial GPx4 content and activity ($P < 0.05$). One of these variants (rs713041) is a well-known modifier of CVD risk. Collectively, these findings illustrate a potential role for GPX4 in maintaining electro-mechanical function in the heart and provide unique insights regarding the effect of gpx4 gene variants on the phenotypic characteristics of GPx4 expression and activity.

Keywords: Post operative atrial fibrillation, glutathione peroxidase 4, lipid peroxidation, gene variants

Poster #55

Structure Activity Relationship and Gliotoxicity of Lower Chlorinated Biphenyls

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Exposure to polychlorinated biphenyls (PCBs) is associated with developmental neurotoxicity and, more recently, with neurodegenerative disorders. However, the underlying mechanisms yielding such adverse outcomes remain unknown. Because normal brain function is largely astrocyte-dependent, we hypothesize that astrocytes play an important role in PCB-mediated neurotoxicity. We assessed the toxicity of four lower chlorinated PCB congeners, 4-chlorobiphenyl (PCB3), 3,3'-dichlorobiphenyl (PCB11), 2,3',4-trichlorobiphenyl (PCB25) and 2,2',5,5'-tetrachlorobiphenyl (PCB52) and their corresponding human-relevant hydroxylated and sulfated metabolites, in C6 cells (rat glioma cell line) or primary glial cells (from C57BL/6 mice) exposed to test compounds at a concentration range of 0.5 to 50 μM for 24 h. The MTT assay was employed to determine cell viability. Reactive oxygen species (ROS) generation was analyzed using fluorescent probes, MitoSox Red and CellRox Green in C6 cells. PCB52 and both its metabolites were the most toxic in C6 cells at LC50 concentrations of 8.4 μM (PCB52), 2.2 μM (4-OH-PCB52), and 8.8 μM (4-PCB52-sulfate). Therefore, further studies focused on PCB52 and its metabolites. ROS generated in response to PCB52 metabolites was largely mitochondria-targeted. Further studies performed using a Seahorse analyzer demonstrate that C6 cells exposed to PCB52 metabolites cause proton leak in mitochondria, suggestive of the energetic crisis the cells may be undergoing. PCB52 and its metabolites were similarly cytotoxic to primary glia as observed in C6 cells. Current research is focused on assessing gene expression changes in metabolic pathways that have found to be relevant from previous work in C6 cells after exposure to PCB52 and its metabolites. These findings suggest that key cellular processes in astrocytes are impaired in a structure-dependent manner following PCB exposure. Future studies will assess the functional implications of these findings in PCB-mediated neurotoxicity. This work was funded by [ES005605, ES013661, ES 029035].

Keywords: Polychlorinated biphenyls, neurotoxicity, structure-activity relationship (SAR), astrocytes, apoptosis

Poster #56

Synthesis of Ketamine and 6-hydroxynorketamine haptens to develop the vaccination for the identification and mechanistic studies of specific immune response against ketamine and 6-hydroxynorketamine

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Ketamine is a hallucinogenic compound which is being used widely for the treatment-resistant depression (TRD) and different therapeutic use like anesthesia, adjunctive analgesia. Although ketamine is potentially NMDA receptor antagonist and it shows rapid and long-lasting antidepressant, it is also a popular substance of abuse.[1-3] For the intoxication or overdose of ketamine there is no suitable antidotes available. Overdose protection of ketamine can be studied through production of highly specific antibodies which can prevent the penetration across the blood-brain barrier, although antiketamine antibodies have not been fully studied for the therapeutic use. Apart from this, recent studies showed that the metabolites of ketamine [Norketamine and 6-hydroxy norketamine, 6-HNK] may have antidepressant potentials but it is still debated and remain to be fully explored. Generation of specific antibodies allow the opportunity to study the role of metabolites of ketamine's antidepressant effect through selective restriction of metabolites access and the selective immune recognition of small molecules to the central nervous system (CNS). To study this immune recognition of small molecules for the selective response, design of hapten is a crucial factor. Our study targeted to synthesize ketamine hapten and the 6-HNK hapten and to optimize the conditions for the bioconjugation of certain kinds of carrier proteins like BSA and CRM-197 for the purpose of vaccination to assess the immune response of such vaccination on behavioral change induced by ketamine by using dissociative-like doses (50 mg/kg). As an initial approach, generation of antiketamine, anti-6-hydroxynorketamine immune responses in mice will be measured by using Enzyme-Linked Immunosorbant Assay (ELISA) and competitive Surface Plasmon Resonance (SPR) techniques. Although, some preliminary results are being assessed[4] for the norketamine hapten, ketamine hapten and 6-hydroxy hapten, which shows immune response in vivo, we are focusing more depth study of those haptens selectivity and antibody response with more accurate and reproducible results are our ultimate target. Overall, we are optimizing the synthetic route to improve the yield of those haptens in large scales and assessing the immune response to generate the antibodies specific to the ketamine or its metabolites which will be a promising study to identify the individual and the combinatorial roles of ketamines and its potential metabolites for the rapid acting antidepressant effects.

Keywords: Ketamine, 6-hydroxynorketamine, DISSECTIV, Vaccine, Antibody

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Poster #57

Development of 3D Printed Gene-Activated Calcium Phosphate Cement Scaffolds for Application in Bone Regeneration.

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Bone tissue has a capacity to naturally heal after injury and fracture, however if the injury results in a defect that is too large this capacity is overcome. In some cases, the defect is so large that it will remain unhealed over the patient's remaining lifetime. Such defects are called "critically sized" defects and if left untreated they can impact the function of a patient's limbs. Current tissue engineering research is exploring the use of 3D printed scaffolds as alternatives to current therapies for regenerating bone within large defects. In this work we made our scaffolds out of calcium phosphate cement (CPC) which can be 3D printed at high resolution to form mesh-like scaffolds with precisely determined diameter, pore size, and morphology. Upon exposure to water, CPC can mineralize into calcium-deficient hydroxyapatite (the main mineral component of bone). Through modulation of the mineralization technique, the nanotexture of the resulting hydroxyapatite can also be controlled. In this project I am investigating methods of gene-activating CPC by producing plasmid DNA-polyethylenimine (PEI) nanosized complexes (nanoplexes) that encode the genes for bone-regenerative proteins and incorporating the nanoplexes into CPC via multiple methods. Thus far I have found quantifiable differences in the surfaces and mechanical properties of my scaffolds mineralized via different methods, have early indications that some mineralization methods result in enhanced osteoinductivity of the scaffolds, and have two gene-activation methods that are viable. Specifically, scaffolds mineralized by incubation in a humidified incubator followed by immersion in simulated body fluid have the best mechanical strength and osteoinductivity, and gene activation with nanoplexes through surface adsorption and surface coating with lyoprotectant both result in meaningful transfection of cells seeded onto the scaffolds. In future work I plan to assay the bone regenerative potential of my scaffolds in a rat model.

Keywords: bone, calcium phosphate, gene delivery, 3D printing

Poster #58

Cardiac-Specific Deletion of Prohibitin-1 Leads to Fetal Demise, Mitochondrial Abnormalities and Heart Failure

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Prohibitins (PHB1/2) are highly conserved lipid-raft associated proteins ubiquitously expressed in all cell types and localized to multiple compartments, including mitochondria, plasma membrane and the nucleus. PHBs are pleiotropic scaffold proteins involved in diverse cellular processes and disease pathogenesis. In mitochondria, PHBs are responsible for stabilizing electron transport chain and oxidative phosphorylation system (OXPHOS). However, the extent to which PHB1 regulates mitochondria energetics and electromechanical function of the heart, is currently not known. To determine the role that PHB1 plays in cardiac function, we first established a cardiac-specific PHB1-deficient mouse strain (cKO), but the vast majority of cKO mice died in utero during pregnancy (E12-E15). Uterine sonography of the expired cKO embryos showed significantly decreased crown-rump length and increased placenta thickness with large amount of fluids, restricted uterine artery flow and detachment between amnion and chorion compared with normal embryos. The few that were born develop severe dilated cardiomyopathy, resulting in mortality by 8-9 weeks of age. Heart weight/body weight ratio is ~2-fold greater in cKO mice vs. wild-type mice. Echocardiography reveals severely enlarged right atria and ventricles in cKO mice, corresponding to significantly decreased ejection faction (EF), increased end diastolic volume (EDV) and end systolic volume (ESV). Due to high rate of embryonic lethality, we generated tamoxifen-inducible cardiac deletion of PHB1 mice (cKO-MCM) which develop severe dilated cardiomyopathy accompanied by significantly decreased ejection fraction 12 weeks after 7-day tamoxifen injection. In mitochondria, cKO-MCM mice have disruptions in OXPHOS as evidenced by decreased pyruvate and palmitoylcarnitine-supported respiration and ATP production. Consistent with defective cardiac mitochondrial energetics, the rate of calcium uptake and reactive oxygen species (ROS) production were also altered in the KO mice. Taken together, these findings implicate PHB1 as a critical regulator of myocardial development and function, due in part to its role in maintaining OXPHOS and controlling metabolism.

Keywords: PHB1, knockout mice, embryonic lethality, mitochondria energetics, heart failure

Poster #59

Identifying Selective Inhibitors of Adenylyl Cyclase 1 as Therapeutics for Chronic Pain

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Chronic pain is among the most prevalent diseases to impact society abroad. In the United States alone, chronic pain is valued to debilitate tens of millions of lives while costing hundreds of billions of dollars per year. Unfortunately, available treatment options for chronic pain are limited, resulting in opioid prescriptions which are inadequate for managing patient relief and further contribute to an epidemic of their own. Many reports in literature suggest inhibiting the activity of adenylyl cyclase type 1 (AC1) mitigates chronic pain. These observations propose an alternative avenue of treatment. AC1 activity is dependent on the binding of calmodulin (CaM). Furthermore, it contains a unique CaM binding site, offering a protein-protein interaction that can be utilized as a tractable drug target. Consequently, several campaigns have identified agents that inhibit AC1 activity. While the initial success of these efforts was promising, they ultimately fell short primarily due to AC isoform selectivity and off-target effects. Our approach is innovative in its focus on discovering compounds that are both selective and potent towards the distinct AC1-CaM interaction. We developed a fluorescence polarization-based assay that probes the AC1-CaM interaction in a high-throughput, robust fashion. In our paradigm, validated hits are subjected to biochemical and cell-based assays to measure selectivity over the most relevant isoform, adenylyl cyclase 8 (AC8). Compounds that meet eligible criteria will be subject to optimization via medicinal chemistry and eventually to in-vivo experiments in mice. Here, we show the results of our screening campaign as we progress towards our goal of 100,000 compounds. Thus far, we have succeeded in identifying compounds that selectively inhibit AC1 and serve as novel chemotypes and drug scaffolds to aid in the management of chronic pain.
Keywords: Adenylyl cyclase type 1, calmodulin, fluorescence polarization, high-throughput screening, chronic pain

Poster #60

Double-Stranded mRNA Lipid Nanoparticle Vaccines Display Superior Thermostability Compared to Current Single-Stranded mRNA Formulations

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Most lipid nanoparticle (LNP) delivery systems have been utilized for the intravenous delivery of siRNA into the liver. Recently, significant advancements have been made to optimize this delivery system for intramuscular use, such as the Moderna COVID-19 vaccine used to deliver single-stranded spike protein mRNA. Despite its overwhelming success, concerns have arisen regarding the thermostability of this formulation during transportation. Therefore, this research aims to optimize the LNP gene delivery system to administer more stable double-stranded mRNA. By utilizing a small-scale PLEXER device, LNPs encapsulating single- and double-stranded Luciferase mRNA were assembled by turbulently mixing 4 lipid components with the mRNA: an ionizable cationic lipid (SM-102), a phospholipid (DSPC), cholesterol, and a pegylated lipid (DMG-PEG 2000). This apparatus allowed us to generate LNPs of comparable particle size (~100 nm) to those found in the existing vaccines as determined through dynamic light scattering measurements. After incubation at room temperature for 12 hours, the mRNA was extracted out of the LNPs. Band intensity from agarose gel analysis indicated that the RNA in the current Moderna COVID-19 vaccine and our single-stranded mRNA formulation degraded by 82% and 55% respectively, whereas our double-stranded mRNA formulation demonstrated no degradation. Furthermore, double-stranded mRNA remained intact after harsher incubation conditions at 37 °C for 12 hours whereas the other two formulations were destroyed. Indeed, single-stranded mRNA consistently degraded over multiple incubation experiments while double-stranded mRNA remained resilient. To discern the nature of this degradation, extractions were repeated in the presence of RNAse OUT, an RNAse inhibitor. Nearly all the RNA in each formulation was recovered, suggesting that the instability is caused by enzymatic degradation by RNAse when not stored under optimal conditions. This early data demonstrates the enhanced stability of double-stranded mRNA as a superior candidate for vaccines over single-stranded mRNA, and these findings could pave the way for significant enhancements in vaccine delivery and strengthen the global vaccine platform.
Keywords: Vaccines, COVID-19, mRNA, Thermostability

Conference Notes

MIKIW 2022 Conference Attendees

Minnesota

Abhishek Kulkarni
Adam Duerfeldt
Alex Hurben
Alexis Stoorza
Andrew Hunt
Bidisha Sarkar
Bill Howlett
Bo Hu
Brandi McKnight
Caroline Buchholz
Carston R. Wagner
Conrad A. Fihn
Courtney Aldrich
Daniel Harki
Ehfazul Haque
Elizabeth Ambrose
Erik Moran
Farhan M. Khan
Freddys Rodriguez
Garrett Schey
Hannah Lembke
Hira Khalid
Jacob Bouchard
Jacob Greenberg
Jacob Smith
Jerrett Holdaway
Jessica Fuller
Jigar P Sethiya
Julia Lee
Katelyn Stevens
Kelsey Holdaway
Laura Hirsch
Linh T. Tran
Makayla Brzycki
McKenzie Wyllie
Md Abdullah Al Noman
Melanie Nevins
Mikayla Twiggs
Muhammad Ilyas
Nan Wang
Nicholas Weirath
Nicole Bentz
Obaid Ullah
Pooja Hegde
Qiang Liu
Ricardo Rosas Jr.
Rui Shi
Ruiqin Wang
Samantha Kennelly
Siraj Khan
Taimeng Liang
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Jose Villegas
Karol S. Bruzik
Kasra Alizadeh
Kornelia Skowron
Kyle Kremiller
Laura Cooper
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