WCMCW

Seventh Western Canadian Medicinal Chemistry Workshop

September 20-22, 2024, University of Saskatchewan, Saskatoon

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Contents

Message from the organizers

We would like to welcome you to Saskatoon and the University of Saskatchewan for the seventh Western Canadian Medicinal Chemistry Workshop and are excited to be back after a hiatus of several years due to the restrictions arising from the Covid-19 pandemic. We established the WCMCW in 2008 to be an accessible, regional meeting that connects researchers from a variety of disciplines who are interested in different aspects of pharmaceutical sciences. The major goals of the WCMCW are:

- To foster research connections between researchers who would otherwise never meet at more traditional discipline-specific meetings.
- To make research at Western Canadian institutions the focal point.
- To provide exposure for students and postdoctoral researchers to faculty from other institutions and the pharmaceutical industry.

It is our hope that we have been successful in achieving these goals.

Anyone who has organized a scientific meeting knows that these events would be impossible without financial support and we are grateful to our sponsors for helping with this initiative. We have listed our sponsors on our website, in this program book and throughout the workshop, please join us in thanking these generous donors.

We hope that our efforts to put together the seventh WCMCW meeting will be both stimulating and productive. We are fortunate to have seven excellent invited speakers, each coming from different disciplinary backgrounds, but having common goals of improving the quality, efficacy and safety of pharmaceuticals, which ultimately gets to the heart of this meeting.

Finally, we would like to thank you for attending and helping to make this meeting a success.

Sincerely,

Ed S. Krol, WCMCW Chair

David R. J. Palmer, WCMCW Vice-Chair

Schedule of Events

FRIDAY, SEPTEMBER 20, 2024

TIME	EVENT	LOCATION
6:30 to 8:30 pm	**Registration, Opening Reception, and Mixer**	**Student Lounge, room 1005 Education building**

SATURDAY, SEPTEMBER 21, 2024

Registration and oral poster presentations to be held in room 1004
Poster presentations to be held in the Student Lounge, room 1005

TIME	EVENT
8:00 am	**Registration**
8:50 am	**Opening Remarks by Chair Ed Krol**

Session chair: Ed Krol

9:00 am	**Kirsten Wolthers (University of British Columbia Okanagan)** *Discovery of new therapeutic targets from the oncomicrobe, Fusobacterium nucleatum.*
9:40 am	**Kalindi Morgan (University of Northern British Columbia)** *Analysis of the Chemistry of Northern Canadian Bark Beetle-associated Bacteria utilizing Bioassays, Nitrogen NMR and Gene Cluster-encoded Chemistry for the Discovery of Antimycotic Agents.*
10:00 am	**Haixia Zhang (University of Saskatchewan)** *Faba bean, a possible food source of levodopa for treatment of Parkinson's Disease.*
10:20 am	**Coffee Break and Poster Viewing**

Session chair: Amir Khajavinia

10:50 am	**Jinqiang Hou (Lakehead University)** *Treating cancer without killing cancer cells.*
11:30 am	**Samira Poorsadeghi (University of Saskatchewan)** *LC-MS-Based Metabolism Study of Dimethylxanthine Derivatives as Potential Radiotracers Targeting α-Synuclein Fibrils in Parkinson's Disease.*
11:50 am	**Dominique X. Rwizinkindi (University of Saskatchewan)** *Zwitterionic Derivatives of the Bombesin Ligand RM2 for Improved Radiometal Delivery and Tissue Distribution for GRPR-Positive Tumors.*
12:10 pm	**End of Morning Session** **WCMCW Lunch in Education Building**

SATURDAY, SEPTEMBER 21, 2024 (cont'd)

TIME	EVENT

Session chair: David Palmer

1:20 pm **Jessica Willi (Lethbridge University)**
Beyond peptides: Expanding ribosome function through synthetic biology.

2:00 pm **Agnes Truc Nguyen (University of Saskatchewan)**
Characterizing substrate binding proteins of a putative maltose uptake system in Gardnerella swidsinskii.

2:20 pm **Sedigheh Barzegar (University of Saskatchewan)**
Rapid Analytical Strategies for Detection of Protein Bound-Metabolites of Nitrofurazone by Nucleophilic Substitution.

2:40 pm **Maryam Alyari (University of Saskatchewan)**
Advancing metabolite quantification for newborn HIE diagnosis by improving chemical isotope labeling for carboxylic acid metabolite profiling.

3:00 pm **Coffee Break and Poster Viewing**

Session chair: Melissa Mejia Gutierrez

3:30 pm **Antonio Ruzzini (University of Saskatchewan)**
Discovering and improving drugs for veterinary pathogens.

4:10 pm **Kathyana Deeyagahage (University of Saskatchewan)**
Increased soluble peptidoglycan levels drive vancomycin cross-resistance in antimicrobial peptide-resistant MRSA.

4:30 pm **Amir Khajavinia (University of Saskatchewan)**
Addressing a Major Interference in the Quantification of Psilocin in Mouse Plasma: Development of a Validated Liquid Chromatography Tandem Mass Spectrometry Method.

4:50 pm **End of Afternoon Session**

Poster Viewing and Judging

6:30 pm **End of Poster Session**

7:30 pm **WCMCW Banquet at St. Tropez Bistro, 238 2nd Avenue South**

SUNDAY, SEPTEMBER 22, 2024

Registration and oral presentations in room 1004

TIME	EVENT
8:00 am	**Breakfast Workshop for Trainees** **Curtis Rieder, Gilead Sciences, Edmonton, AB**
8:55 am	**Introductory Remarks**
Session Chair: Emma Finch	
9:00 am	**Mathew Macauley (University of Alberta)** *Advancing new Chemical Probes to Study Sialyltransferases*
9:40 am	**Dhanraj Kumawat (University of Alberta)** *Synthesis of chemically modified sialic acids using kinetic trapping strategy to probe the mechanism and function of sialyltransferase*
10:00 am	**Leonie O'Sullivan (University of Calgary)** *Harnessing pomalidomide's intrinsic fluorescence for the rapid assessment of cellular penetration*
10:20 am	**Coffee Break**
Session Chair: Ed Krol	
10:40 am	**Zev Ripstein (University of Manitoba)** *Targeting proteins for degradation with de novo designed synthetic protein tags*
11:20 am	**Melissa Mejia Gutierrez (University of Saskatchewan)** *Design and Synthesis of a Diazirine Photoaffinity Probe of the CB1 Cannabinoid Receptor.*
11:40 am	**Dave Palmer (University of Saskatchewan)** *How did that guy get hired? Demystifying the faculty search process*
12:00 pm	**Closing Remarks**
WCMCW Lunch in Marquis Hall	
1:30 pm	**Conclusion of WCMCW**

Abstracts
Oral presentations / Session 1

Discovery of new therapeutic targets from the oncomicrobe, Fusobacterium nucleatum.

Kirsten Wolthers
Department of Chemistry, University of British Columbia-Okanagan, Kelowna, BC

Fusobacterium nucleatum - a common member of the oral microbiota - is considered an opportunistic pathogen as it is associated with colorectal cancer. In this presentation, I describe two potential therapeutic targets to treat F. nucleatum infections. The first is lanthionine synthase, an enzyme that catalyzes the Î²-replacement of L-cysteine with D-cysteine to produce meso-lanthionine. Gene knock studies show that lanthionine synthase is essential for F. nucleatum survival, an expected result given that the bacterium uniquely contains meso-lanthionine in the pentapeptide crosslink of the peptidoglycan layer. The second therapeutic target is HmuF, which plays a vital role in acquisition of iron from heme (the most abundant source of iron in the human host), by fulfilling three functions. First, HmuF binds tightly to intracellular heme, minimizing the cellular toxicity of labile heme. Second, HmuF traffics heme to a radical S-adenosylmethionine transfera se for decyclization of the protoporphyrin ring, which liberates iron and generates a cytotoxic linear tetrapyrrole called anaerobilin. Third, HmuF reduces the cytotoxicity of anaerobilin through a four-electron reduction. Hence, HmuF offers F. nucleatum a competitive advantage in the colonization of anoxic sites of the human body and offers researchers new avenues for therapeutic drug design.

Analysis of the Chemistry of Northern Canadian Bark Beetle-associated Bacteria utilizing Bioassays, Nitrogen NMR and Gene Cluster-encoded Chemistry for the Discovery of Antimycotic Agents.

Nirasha Atapattu, Nicolas Justus, Daniel Coronado, Hariniha Selvarajan, Mitz Baylosis, Kalindi D. Morgan*
Department of Chemistry and Biochemistry, University of Northern British Columbia, 3333 University Way, Prince George, BC, V2N 4Z9

Northern Canada, particularly British Columbia and the Yukon, is home to numerous bark beetle species that have symbiotic relationships with key fungal species and specific tree hosts. Bacteria within the bark beetle holobiont have been shown to influence the ecological dynamics between bark beetles, fungi, and trees. Despite this, only one natural product, mycangimycin, has been isolated from bark beetle-associated bacteria in the literature. Mycangimycin possesses antibiotic and antifungal activity. In contrast, the well-studied termite-fungi-bacteria symbiosis has yielded hundreds of bacterial natural products with antibiotic or antimycotic activity. The chemistry of bacteria associated with Northern Canadian bark beetles, however, remains largely unexplored.
Our research seeks to fill this gap by analyzing the chemistry of bark beetle-associated bacteria, focusing on their potential to produce antimycotic agents for agrochemical and antifungal drug discovery. We utilize bioassays, nitrogen nuclear magnetic resonance (NMR) spectroscopy, and gene cluster-encoded chemistry to prioritize discovery and avoid compound rediscovery. This integrated approach aims to streamline the identification of novel bioactive compounds.

Faba bean, a possible food source of levodopa for treatment of Parkinson's Disease.

Savanna Spendiff, Haixia Zhang*
Department of Food and Bioproduct Sciences, University of Saskatchewan, Saskatoon, SK

Faba bean is a legume that has been grown in Canada as a minor crop for the last five decades. Faba bean seeds have high contents of protein and dietary fiber, and are also rich in minerals, B vitamins and levodopa (L-DOPA). B vitamins, especially nicotinamide (B3), has recently been considered as the foundation for treating metabolic disorder and neurodegenerative diseases. L-DOPA is a non-protein amino acid, which is frequently used for the treatment of Parkinson's Disease. A limiting factor for the widespread consumption of faba bean is the presence of vicine and convicine (VC), which could cause favism in people with glucose-6-phosphate dehydrogenase deficiency. To examine the levels and distribution of VC and beneficial compounds in the faba plant, we developed accurate quantitation approaches for VC, L-DOPA, and B vitamins using UHPLC-ESI MS. We measured levels in developing and mature seeds, and in various vegetative tissues. L-DOPA was found in all plant tissues, with young leaves, flowers, and young pods having significantly higher L-DOPA and lower VC than mature seeds. Faba bean, especially the green shoots, due to their low VC, high L-DOPA and good B vitamin content, could be a potential food-based treatment for PD patients.

Oral presentations / Session 2

Treating Cancer Without Killing Cancer Cells.

Jinqiang Hou
Department of Chemistry, Lakehead University/ Thunder Bay Regional Health Research Institute, Thunder Bay, ON

Given that tumor growth and tumor spread involve two distinct mechanisms, it is imperative that cancer drug discovery efforts are divided into two strategies: antiproliferative and anti-metastatic. Metastasis accounts for approximately 90% of cancer-related deaths, and anti-metastatic drugs, termed Migrastatics, offer a unique therapeutic approach to combat cancer migration and invasion. Migrastatics work by restricting the movement of cancer cells into surrounding tissues, unlike traditional cytotoxic drugs, which employ a direct cell-killing approach. While cytotoxic drugs inevitably induce drug resistance and promote the emergence of more aggressive cancer cells through the Darwinian selection of resistant clones that become the predominant cancer cell population, Migrastatics are less likely to induce drug resistance because cancer cells are not directly stressed during treatment. The Lysophosphatidic acid receptor 1 (LPA1) has been shown to support the progression of metastasis in many types of cancer. We will discuss our ongoing efforts to develop novel LPA1 antagonists as Migrastatic drugs, and present preliminary data demonstrating that LPA1 antagonists suppress the migration and invasion of triple-negative breast cancer cells without inducing apoptosis.

LC-MS-Based Metabolism Study of Dimethylxanthine Derivatives as Potential Radiotracers Targeting α-Synuclein Fibrils in Parkinson's Disease.

Samira Poorsadeghi,[1] Christopher P. Phenix,*[1] Ed. S. Krol[2]*
1. Department of Chemistry, University of Saskatchewan, Saskatoon, SK.
2. Pharmaceutical and Nutrition Sciences Research Group, College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, SK.

Various studies suggest that the formation of misfolded α-synuclein protein might be a cause of dopamine-secreting neurons impairment in the nervous system of patients with Parkinson's disease. One possible approach to detect α-synuclein aggregates and diagnose PD, is using a radiolabeled probe for positron emission tomography (PET) imaging.

We have developed a pharmacophore model to screen for compounds that could serve as potential radiotracers. We designed two groups of compounds containing caffeine analogues, one with a two-carbon alkyl linker and the other with a three-carbon alkyl linker. One caffeine analogue in each scaffold contains an N7-linked 3-fluoropropyl; the second analogue is linked to one of three distinct groups, nornicotine, 1-aminoindan or theophylline- *via* an alkane chain.

This study involves *in vitro* hepatic microsomal metabolism, conducted on some of these compounds, to ensure minimal degradation of the diagnostic probe and retention of the fluorine atom. Based on the results acquired from LC-MS and HPLC analyses, we have identified that Phase 1 metabolic processes predominantly occur on the nornicotine and 1-aminoindan moieties rather than fluorine loss. While the scaffold of caffeine analogues remains intact, the major metabolic reactions have been mono oxidation, dihydroxylation and N-dealkylation.

Zwitterionic Derivatives of the Bombesin Ligand RM2 for Improved Radiometal Delivery and Tissue Distribution for GRPR-Positive Tumors.

Dominique X. Rwizinkindi[1], Shvan J. Raheem[1], Behzad M. Toosi[2], Ronald C. Geyer[3], and Eric W. Price[1]*

1. Department of Chemistry, University of Saskatchewan, Saskatoon, SK, Canada S7N 5C9 2. 2. Department of Small-Animal Clinical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, Canada S7N 5B4
3. Department of Pathology and Laboratory Medicine, University of Saskatchewan, Saskatoon, SK, Canada S7N 5E5

Bombesin peptide derivatives have been explored for decades because of their known high affinity to gastrin-releasing peptide receptor, which is overexpressed on several tumors such as prostate, pancreatic, breast, and small cell lung cancer. Bombesin statin-based antagonist RM2, one of the most promising agents, has shown superior affinity to GRPR with high tumor accumulation but also high accumulation in other healthy GRPR-positive organs such pancreas and kidneys, in addition to some metabolic instability. This study describes the design and synthesis of DOTA-RM2 and a derivative DOTA-ZW-RM2 containing only a single additional unnatural amino acid bearing a permanent zwitterion. Another two versions were synthesized bearing azides instead of DOTA. Building on our previous success incorporating zwitterionic amino acids into octreotate to improve stability and reduced kidney and healthy tissue uptake, we are exploring the effects of incorporating these permanent zwitterions into RM2. Modulating polarity by incorporating zwitterion linkers into RM2 derivatives should impart drastic changes to their pharmacokinetics and tissue clearance, which would result in less non-specific uptake of the radiopharmaceuticals and less radiation exposure to healthy organs. Four RM2 derivatives have been successfully synthesized and their radiopharmacological profiles are being evaluated. Successful radiolabeling with gallium-68 and PET-CT imaging in healthy mice has been performed. Blood serum stability studies, shake-flask LogD, biodistribution experiments and dynamic small-animal PET studies in tumor-bearing mice is ongoing.

Oral presentations / Session 3

Engineering the ribosome for synthetic biology and peptidomimetic medicines.

Jessica A. Willi
Department of Chemistry & Biochemistry, University of Lethbridge, 4401 University Drive, Lethbridge, Alberta T1K 3M4.

Synthetic biology is a 21st century field that involves engineering the genetic material of organisms to have new characteristics. Our vision is to alter the machinery of the ribosome to expand beyond "normal" protein translation it evolved to do and unlock entirely new modes of polymer synthesis.
In engineering the ribosome, we focus on it's two active sites: 1). Altering the decoding mechanism to expand coding space beyond the standard triplet base codons and 2). Engineering the peptidyl transferase center to accommodate elongated peptide backbones from β- and γ-amino acids, enabling synthesis of protease-resistant peptidomimetic drugs. Furthermore, substrates with functionalized, bulky side chains would benefit from a dedicated ribosomal active site. As the catalytic center has evolved to accommodate α-substrates with side chains of modest size, we present strategies to redesign and engineer ribosomal RNA and to retro-evolve function.
The creation of custom specialized ribosomes is a transformative technology for enhanced protein production and creating novel bioactive drugs and contributes to ongoing efforts in molecular medicine and protein technologies.

Characterizing substrate binding proteins of a putative maltose uptake system in Gardnerella swidsinskii.

Agnes Truc Nguyen and Janet E. Hill*.
Department of Veterinary Microbiology, University of Saskatchewan, Saskatoon, SK

Glycogen breakdown products (maltose and malto-oligosaccharides) are key carbon sources that can affect population dynamics of the human vaginal microbiome, including *Gardnerella* species. Differences in uptake systems for glycogen breakdown products among *Gardnerella* spp. may explain their different fitness levels. All *Gardnerella* possess MusE operon encoding for maltose and malto-oligosaccharides transporter system. While it encodes two substrate binding proteins (SBP) in *G. swidsinskii* (MusE 1345 and 1346, 62% identical), in other *Gardnerella* the operon encodes one SBP that is ~90% identical to MusE 1346. We hypothesize that the two *G. swidsinskii* MusE SBPs bind glycogen breakdown products differently, providing competitive advantages by broadening the range of ligands. *G. swidsinskii* SBPs 1345 and 1346 were expressed in *Escherichia coli*. Purified monomeric SBPs were assessed for specificity and affinity to ligands using isothermal titration calorimetry (ITC). MusE 1346 SBP has a high affinity for maltose (3.23×10^6 M^{-1}), maltotriose (5.43×10^6 M^{-1}) and maltotetraose (1.09×10^6 M^{-1}), with no quantifiable binding to glucose, isomaltose, and lactose. Analysis of MusE 1345 is in progress. Results of this study will inform future investigations of the competition among *Gardnerella* species and other vaginal microbiota for the products of glycogen degradation.

Rapid Analytical Strategies for Detection of Protein Bound-Metabolites of Nitrofurazone by Nucleophilic Substitution.

Sedigheh Barzegar[1], Anas El-Aneed [1], Bryn O. Shurmer[2], Randy W. Purves *[1,2]
1. College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, SK, Canada S7N 5E5
2. Centre for Veterinary Drug Residues, Canadian Food Inspection Agency, Saskatoon, SK, Canada S7N 2R3

The presence of prohibited veterinary drug residues in animal products can be harmful to consumers. Among these veterinary drugs, nitrofurazone was previously used in animal feed to treat infections and promote growth. Nitrofurazone is rapidly metabolized, with most metabolites binding covalently to cellular proteins. Current detection methods for nitrofurazone residues focus on identifying semicarbazide (SEM), a side chain of the protein-bound metabolite released via acidic hydrolysis. However, SEM can originate from other sources. Besides, SEM detection process involves time-consuming overnight hydrolysis and derivatization steps. Therefore, more specific nitrofurazone VDRs and a faster and more reliable method for their detection is needed.
In this study, we used bovine liver S9 fractions to metabolize both nitrofurazone and 13C15N3-labeled nitrofurazone. Various buffers and nucleophiles were tested for their effectiveness in protein substitution reactions. We monitored the protein substitution process using liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). In some cases, we were able to identify cyano-metabolite conjugates with nucleophiles within 15 minutes. The stability of these metabolite-nucleophile conjugations is still under investigation, and preliminary results will be presented.

Advancing Metabolite Quantification for Newborn HIE Diagnosis by Improving Chemical Isotope Labeling for Carboxylic Acid Metabolite Profiling.

Maryam Alyari[1], Darryl Adamko[2], and Anas El-Aneed[1]*
1. College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, SK, Canada.
2. Department of Pediatrics, College of Medicine, University of Saskatchewan, Saskatoon, SK, Canada.

We adopted the chemical isotope labeling (CIL) approach to develop a quantification method for carboxylic acid metabolites using 4-(Dimethylamino) phenacyl bromide (12C2/13C2-DmPA). However, we encountered several challenges with DmPA in metabolome profiling, one of which was retention time shifts. Specifically, we noticed these shifts in two metabolites when increasing the concentration of the standards. To address the issue, we optimized and adjusted various analytical conditions. Although we improved the derivatization method, we still faced additional challenges. DmPA labeling was most effective in a nonaqueous solution, necessitating an extra step to extract the sample from a biological matrix. Another complication with DmPA was the relatively high noise background, which could potentially cause ion suppression of the labeled analytes. To address these issues, we are now testing Dansylhydrazine (DnsHz) labeling for profiling carboxylic acid metaboli tes. This method was applied to patients with hypoxic ischemic encephalopathy (HIE). The birth of an infant is a stressful event, and some deliveries lead to hypoxemia, which can cause brain damage (i.e., HIE). Without treatment, HIE can result in cerebral palsy (CP), but cooling the infant in the NICU can prevent this. Identifying which infants require cooling therapy remains challenging. Our research utilized the optimized quantification method along with two other validated methods to analyze various metabolites, establishing a metabolic profile for neonatal HIE. The goal is to improve the identification of infants in need of neuroprotective cooling.

Oral presentations / Session 4

Discovering and improving drugs for veterinary pathogens.

Poonam Dhindwal[1], Nishad Thamban Chandrika[2], Sylvia Garneau-Tsodikova[2], and Antonio Ruzzini*[1,3]

Departments of [1]Veterinary Microbiology and [3]Biochemistry, Microbiology and Immunology, University of Saskatchewan, Saskatoon, SK, Canada, and the [2]Pharmaceutical Sciences Department, University of Kentucky, Lexington, KY, USA

The need for new therapeutics to treat bacterial disease has been accelerated by the rise of antimicrobial resistance. Our group has recently focused on antibiotics that inhibit *Mycoplasmopsis bovis*, an organism that has been neglected in the context of small molecule drug discovery. *M. bovis* is a common cause of multidrug resistant pneumonia in beef feedlot cattle and contributes to bovine respiratory disease (BRD). The majority of cattle receive metaphylactic antibiotic treatment to manage BRD, known colloquially as “shipping fever,” and as the major cause of morbidity and mortality during beef production. As the same antibiotic classes are used for metaphylaxis as well as the treatment of BRD and other *M. bovis*-driven sequelae, there is an urgent need to develop alternatives. To identify potential therapeutics and *M. bovis*-specific drug targets, we completed a high-throughput screen of FDA-approved compounds. Potential anti-mycoplasmal agents included ebselen and elaiophylin. An approach relying on chemical ecology is being pursued to search for and study the elaiophylins. In contrast, a collaborative medicinal chemistry effort has allowed for structure-activity relationship studies of ebselen and ebsulfur analogues. Biotinylated ebselen has and will continue to support proteomic efforts to establish *M. bovis* drug targets. Insight into these potential targets and future target-specific efforts will be discussed.

Increased soluble peptidoglycan levels drive vancomycin cross-resistance in antimicrobial peptide-resistant MRSA.

Kathyana Deeyagahage, Tony Ruzzini*

Department of Veterinary Microbiology, University of Saskatchewan, Saskatoon, SK.

Cross-resistance between antimicrobial peptides (AMPs) and conventional antibiotics is generally limited, but exceptions exist. We recently selected methicillin-resistant Staphylococcus aureus (MRSA) mutants resistant to phenol-soluble modulins (PSMs)-derived AMPs, which exhibited intermediate resistance to vancomycin. This study investigates the relationship between intermediate vancomycin resistance and mutations occurring alongside AMP resistance. The mutant S. aureus not only had a mutation in the PSM export system but also a frameshift mutation causing a premature stop codon in the major autolysin gene (atl). The atl gene encodes two enzymes involved in cell wall remodeling: AmiA and NAGase. In these mutants, the catalytic domain of AmiA, an N-acetylmuramyl-L-alanine amidase, remains intact, while the Î²-N-acetylglucosaminidase (NAGase) is not. Interestingly, spent cultivation media from vancomycin intermediate-resistant mutants protected wild-type and other bacteria from vancomycin. Using vancomycin affinity chromatography and high-performance liquid chromatography-mass spectrometry (HPLC/MS), we identified peptidoglycan-derived fragments as major constituents in the active fractions. Increased soluble peptidoglycan in the spent media likely competes with cell wall peptidoglycan for vancomycin binding, contributing to observed vancomycin cross-resistance. The implications of the PSM export mutation, truncated AmiA enzyme, and peptidoglycan release will be discussed.

Addressing a Major Interference in the Quantification of Psilocin in Mouse Plasma: Development of a Validated Liquid Chromatography Tandem Mass Spectrometry Method.

Amir Khajavinia[1], Deborah Michel[1], Udoka C. Ezeaka[1], Randy W. Purves[1,2], Robert B. Laprairie[1,3], Anas El-Aneed[1]*

1. College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, SK S7N 5E5, Canada
2. Canadian Food Inspection Agency, Saskatoon, Saskatchewan S7N 2R3, Canada
3. Department of Pharmacology, College of Medicine, Dalhousie University, Halifax, NS B3H 4R2, Canada

Psilocybin is a psychedelic compound found in hallucinogenic “magic mushrooms”. Its active metabolite is psilocin, which has been the subject of several studies for the treatment of psychological disorders, such as anxiety, depression, and post-traumatic stress disorder. As such, the pharmacokinetic properties of psilocin should be evaluated to ensure its safety and efficacy as part of the drug development process. LC-MS/MS analysis, based on the published reports, showed a major interference in mouse plasma that was not, to the best of our knowledge, reported previously. We, therefore, aimed to identify and separate the interference. Various chromatographic columns, mobile phase conditions, and high-resolution mass spectrometers were tested. Exact mass measurement and MS/MS analysis confirmed the structure of the interfering compound to be tryptophan. After the identification of the interference, a fast and reliable hydrophilic interaction liquid chromatography (HILIC)-MS/MS method using a quadrupole-linear ion trap equipped with electrospray ionization was developed and validated as per the FDA guidelines. Psilocin was successfully separated from tryptophan in a 1.8 min run time while achieving a 0.5 ng/ml lower limit of quantification. The validated method was applied to plasma samples of mice receiving psilocin orally and psilocin concentration in 114 plasma samples was successfully determined.

Oral presentations / Session 5

Advancing new Chemical Probes to Study Sialyltransferases.

Dhanraj Kumawat[1], Taylor E. Gray[1], Kristin Labasan[1], Matthew S. Macauley [1,2]*

1. Department of Chemistry, University of Alberta, Edmonton, AB, Canada
2. Department of Medical Microbiology and Immunology, University of Alberta, Edmonton, AB, Canada

Sialyltransferases catalyze the transfer of sialic acid (Neu5Ac) onto the tips of complex carbohydrates (glycans) that decorate the cell surface. The many of biological roles played by sialic acid in cell-cell and host-pathogen interactions motivate us to develop tools to probe the function of sialyltransferases and their sialoglycan products. There is only one class of cell-activate sialyltransferase inhibitor, yet it inhibits all 20 members of the sialyltransferase family. Hence, a long-term goal of the lab is to develop selective inhibitors of sialyltranferases. To advance this goal, we developed a new chemoenzymatic synthetic route that greatly accelerates access to small molecule sialyltransferase inhibitors. We have initially used this new route to synthesize a photo-crosslinkable version of the inhibitor, with results demonstrating that we are able to selectively photo-crosslink one family member over others. We are further advancing this inhibito r by exploring chemical modifications at different locations around the sialic acid scaffold and will present progress towards their testing within in vitro assays and in cells.

Synthesis of chemically modified sialic acids using kinetic trapping strategy to probe the mechanism and function of sialyltransferase.

Dhanraj Kumawat[1] , Taylor E. Gray[1] , Cole R. Garnier[2] , Duong T. Bui[1] , Zhixiong Li[1] , Zeinab Jame-Chenarboo[1] , Jeremy Jerasi[1] , Warren O. Wong[1] , John S. Klassen[1] , Chantelle J. Capicciotti[2-4] , and Matthew S. Macauley[1,5] *

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5. Department of Medical Microbiology and Immunology, University of Alberta, Edmonton, Canada T6G 2E1

Sialic acid, also known as Neu5Ac, is added to glycoconjugates by enzymes called sialyltransferases (STs). These enzymes use cytidine monophosphate-Neu5Ac (CMP-Neu5Ac) as their donor for this reaction. Motivated by the lack of available tools to study STs, we embarked on developing new chemistry to access inhibitors of STs with the long-term goal of developing selective inhibitors. A common ST inhibitor scaffold is 3FaxNeu5Ac, which undergoes metabolic conversion into CMP-3FaxNeu5Ac within cells to act as a global ST inhibitor through acting as a competitive inhibitor. Selectively installing the fluorine in the axial configuration is challenging and our goal was to develop a strategy that avoid a challenging separation of the two 3F epimers. Different routes to install the fluorine atom have been developed but none are stereoselective. We demonstrate that the fluorine can be exclusively installed in the axial configuration by the actions of two enzymes, the second of which traps the kinetic product with the fluorine in the axial position, leading to the formation of CMP-3FaxNeu5Ac. This new route has a significantly lower number of steps and does not require a challenging purification. To demonstrate the utility of our new route, we used this synthetic approach to develop versions of CMP-3FaxNeu5Ac with modifications at the C5, C9, or both positions. Specifically, our kinetic trapping method allowed for the chemoenzymatic synthesis of a photo-crosslinkable forms of CMP-3FaxNeu5Ac that we demonstrate can be used to selectively photo-crosslink to ST6GAL1 over two other STs. This new synthetic route will be important in futures plans to further explore select ST inhibitors.

Harnessing pomalidomide's intrinsic fluorescence for the rapid assessment of cellular penetration.

Leonie O'Sullivan[1-3], Duncan K. Brownsey[1-3], Christopher J. Gafuik[2-5], Dae-Sun Kim[2-5], Evgueni Gorobets[1-3], Samuel Krukowski[1-3], Madison Turk[5,6], Craig N. Jenne[5,6], Douglas J. Mahoney[2-5] and Darren J. Derksen[1-3]*

1. Department of Chemistry, University of Calgary, **2.** Alberta Children's Health Research Institute (ACHRI), University of Calgary, 3. Arnie Charbonneau Cancer Institute, University of Calgary, 4. Department of Biochemistry and Molecular Biology, University of Calgary, 5. Microbiology, Immunology and Infectious Diseases, University of Calgary, 6. Calvin, Phoebe, and Joan Snyder Institute for Chronic Diseases, University of Calgary.

Optimizing protein degraders requires balancing multiple factors including potency, cell permeability and solubility. Pomalidomide is an important building block for protein degraders, facilitating the ubiquitin transfer essential for targeted protein degradation. Before this process can occur, the degrader must first penetrate the target cell. This talk will explore how we leveraged the intrinsic fluorescence of pomalidomide to evaluate the cellular penetration of degrader candidates. Our approach enables rapid evaluation of cellular uptake in high-throughput screening assays. In addition, this technique can be paired with endocytosis inhibitors to investigate the mechanisms by which candidates enter target cells. A model library of pomalidomide conjugates was synthesized and evaluated using fluorescence microscopy. This intrinsic fluorescence-based approach supports the rational design of pomalidomide conjugates without the need for additional labels or tags.

Oral presentations / Session 6

Targeting proteins for degradation with *de novo* designed synthetic protein tags.

Zev Ripstein

Department of Chemistry, University of Manitoba, Winnipeg, MB

Design and Synthesis of a Diazirine Photoaffinity Probe of the CB1 Cannabinoid Receptor.

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The endocannabinoid system (ECS)1 has two primary receptors subtypes, CB1 and CB2. Laprairie et. al. are investigating ligands which are allosteric activators of CB1 which may have the ability to reduce dosing of CB1 orthosteric ligands. The synthetic phenyl-indole GAT11022 was reported to have a significant positive allosterism with CBl. Garai et. al. shown that GAT2113 (a fluorinated analog of GAT1102) had greater effectiveness in activating CBl. Based on these observations, our research group designed the diazirine labelled compound GAT1102-2F-Dz (a fluorinated analog of GAT1102) to study CB1 allosterism through photoaffinity labeling. Photoaffinity labeling is a method in which a compound containing a photoreactive diazirine (Dz) group is converted into a carbene after irradiation with UV light. The carbene containing probe molecule covalently binds the protein target. Analysis of the photo-crosslinked complex helps us to elucidate the specific binding region with which the compound was interacting. Compound GAT1102-2F-Dz is being synthesized over a twelve-reaction step protocol. In this talk, preliminary results of this intricate synthetic route will be shown as well as discussion on the challenges of incorporating a diazirine moiety into a preexisting synthetic molecule.

How did that guy get hired? Demystifying the faculty search process.

David R.J. Palmer

Department of Chemistry, University of Saskatchewan, Saskatoon, SK

This presentation will describe the faculty hiring process from the perspective of the department seeking to hire, based on my nine years of experience serving as Department Head of a Canadian Chemistry department. The goal is to help job-seekers understand how hiring decisions are made—from how a position becomes available in the first place to finalizing the terms of hiring with a successful candidate

Abstracts

Poster presentations

1.

Comparing Random vs. Site-Specific Conjugation of Chelating Agents for anti-CD33 Antibodies in Alpha-Radioimmunotherapy.

Kevin JH Allen, Connor Frank, Rubin Jiao, Mackenzie E. Malo, Ekaterina Dadachova*.
University of Saskatchewan, College of Pharmacy and Nutrition, Saskatoon, SK.

Chelator conjugation plays a key role in radioimmunotherapy (RIT). Recently, a simple site-specific method for ^{89}Zr chelation was introduced that can be readily adapted to other radiometal chelators. To evaluate its benefits and limitations, we compared it with a standard non-site-specific method that is currently in use in clinical settings. Our study assessed the novel method using commercial reagents, where we focused on antigen binding, radioisotope chelation, antibody-conjugate stability, and in vivo distribution, to generate a thorough pre-clinical data set.

2.

Development of albumin-binding D-lysine derivatives for use in neuroendocrine-targeting radiopharmaceutical peptides

Nathan Dyck, Eric Price.*
Department of Chemistry, University of Saskatchewan, Saskatoon, SK.

Albumin is a transport protein within human blood plasma, accounting for ~50% of total plasma protein content. Recent development of ^{177}Lu-bearing drugs modified with unnatural para-chlorophenyl substituted side chains displayed optimized reversible binding to albumin via hydrophobic interactions, resulting in an improved therapeutic index and increased blood circulation half-life. These compounds were designed for prostate-specific membrane antigen (PSMA) targets, whereas the aim of this investigation is to harness the albumin-binding effect to target neuroendocrine tumors (NETs). Three novel amino acid derivatives based on D-Lys and D-Orn were synthesized, bearing p-chlorophenyl-functionalized side chains and Fmoc-protected N-termini for compatibility with ultrasonic benchtop solid-state peptide synthesis (SPPS) protocols. A single candidate was selected from the synthesized amino acid derivatives to be incorporated as part of a peptide-chelator linker alongside a zwitterionic pyridyl alanine moiety produced in the Price lab within several novel DOTA-TATE derivatives. Optimized derivatives of peptide-based drugs are difficult to predict in silico due to their complexity in structure and biological interactions, as such, the completed peptide derivatives and a control will be labelled with ^{68}Ga/^{177}Lu and compared, examining the effects of the albumin-binding group on hydrophobicity, binding affinities, blood circulation half-life, and tumor-to-kidney uptake ratios using murine in vivo assays.

3.

Continuous-flow synthesis of natural products: Synthesis of Ascorbic acid.

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The continuous flow synthesis of natural products has emerged as a transformative approach in organic chemistry, offering unprecedented opportunities for the efficient and sustainable production of complex bioactive compounds. This innovative methodology leverages the advantages of continuous flow processing, including enhanced mixing, reduced waste generation, precise temperature, and pressure control, to overcome the limitations of traditional batch processes. By enabling the seamless integration of multiple reaction steps and providing a platform for rapid optimization and scale-up, continuous flow synthesis has revolutionized the preparation of natural products, paving the way for streamlined and cost-effective manufacturing. This poster presentation provides a comprehensive overview of the recent advancements in the application of continuous flow chemistry for the multi-step synthesis of natural products. Through a detailed analysis of successful case studies and the exploration of new technological opportunities, this presentation aims to showcase the remarkable potential of continuous flow synthesis as a driving force in the sustainable production or synthesis of bioactive compounds. The focus will be on the synthesis of Ascorbic acid, highlighting the significant impact of continuous flow chemistry on the modern healthcare and pharmaceutical industries. Keywords: flow chemistry; natural products; drug discovery; multi step synthesis.

4.

Development of Novel Synthetic Methods for the Synthesis of Guanidine Containing Compounds.

Connor M.J. Brandow, Evgueni Gorobets, Darren J. Derksen*
Department of Chemistry, University of Calgary, Calgary, AB

Guanidine containing structures are of interest in the field of medicinal chemistry due to the range of biological activities they present. The guanidine moiety is found in some modern therapeutics, making the development of new synthetic methods of great interest. The synthesis and purification of guanidine containing compounds continues to be a challenge for synthetic chemists due to the high polarity, stability and basicity of the guanidine motif. For these reasons the Derksen group is interested in working toward new methodology for the synthesis of guanidine containing products. In this work we aim to devise new methodology for guanidine synthesis via the synthesis of the natural product (+)-guadinomic acid. There are few methods in the literature for the functionalization of protected guanidines, thus our current focus is the functionalization of protected guanidines to synthesize structural starting points that will facilitate the synthesis of (+) -guadinomic acid.

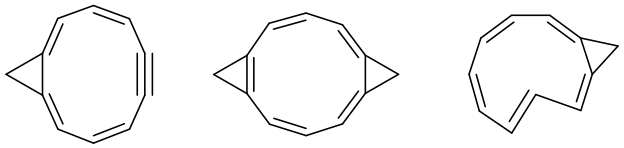
5.

Towards an Improved Synthetic Route for Planar [10]Annulenes.

Mehrdad Torabi, Karnjit Parmar, Michel Gravel*

Medium and large rings have been shown in many cases to display enhanced affinity to proteins and enzymes. We are interested in developing an efficient route to functionalized 10-membered rings via the electrocyclic ring opening of dihydro-naphthalene derivatives. A feature and requirement of this approach is the aromatic character and planarity of the product. Our primary goal is to demonstrate this strategy, with further derivatization of the resulting 10-

membered ring products being our long-term objective. According to Hückel's rule it is expected that after benzene, [10]annulene should be the next aromatic annulene. It was first synthesized in 1969 by Masamune.[1] NMR studies showed that the ring prefers to adopt a non-planar conformation, because of the angle strain. It is necessary for monocyclic polyenes with 4n+2 π-electrons to reach sufficient planarity to have π-delocalization. However, planarity is not easily achieved in medium-sized rings due to ring strain. It has been predicted computationally that this ring strain can be alleviated through the fusion of two or more cyclopropane rings and/or triple bonds to the annulene, thereby favouring planar structures.[2]
The objectives of this project are to develop a new synthetic route to 10-membered rings containing cyclopropanes and/or alkynes, to transform these 10-membered rings into aromatic [10]-annulenes, to characterize them, and to study the potential for their use in functional materials. Our efforts toward a more efficient and divergent route to [10]annulenes, including structures 1, 2, and 3, will be discussed.[3]



1 2 3

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6.

Too hot to handle: Development and validation of a 4°C rapid equilibrium dialysis (RED) method to assess plasma protein binding by heat sensitive compounds in early drug development.

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In drug development, lead compounds with favourable pharmacology under physiological conditions are selected for optimization. Phosphopyricin (PPR) is a novel compound with high antibacterial activity but poor thermal stability. To assess the fraction unbound in plasma (f_u) of PPR and its analogs, a 4 °C rapid equilibrium dialysis (RED) method was developed.
RED plates were loaded with 350 µL buffer and 100 µL spiked plasma. Propranolol (PL) and timolol (TL) served as highly and poorly bound controls, respectively. Plates were incubated at 37 °C for 4 hours (PL, TL) and 4°C for 24 hours (PL, TL, PPR and analogs). Analyte concentrations in buffer and plasma were determined by LC-MS/MS.
Experimental f_u for PL and TL were consistent across incubation conditions: at 37 °C, PL f_u = 0.21 and TL f_u = 0.70; at 4°C, PL f_u = 0.25 and TL f_u = 0.76. The f_u of PPR and its analogs could not be determined due to analyte precipitation in buffer; however, their relative plasma protein binding can be estimated.
The 4 °C RED method represents a quick, easy approach to assess f_u of heat sensitive compounds in early development. This permits the characterization and optimization of thermally unstable compounds that possess favourable pharmacological activity.

7.

Investigating a putative bacterial L-amino acid oxidase.

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L-Amino acid oxidases (EC 1.4.3.2) are flavoenzymes commonly found in snake venom that use L-amino acids as substrates, catalyzing their stereospecific oxidative deamination to produce 2-oxo acids along with generating ammonia and hydrogen peroxide. These widespread enzymes have promising applications in biomedical sciences and industries due to their potential as biocatalysts to obtain ketoacids and perform deracemization procedures. However, the main hindrance to using L-AAO is the lack of robust systems for heterologous expression of the active form of the enzyme. A putative L-amino acid oxidase is encoded by the Bacillus subtilis yobN gene, but its function has not been demonstrated experimentally. We are using a vector encoding an N-terminal hexahistidine-tagged YobN for expression in E. coli Rosetta D3 cells. The L-AAO activity of the protein expressed will be compared with that of a known L-AAO from the western rattlesnake (Crotalus atrox) using a peroxidase-coupled spectrophotometric assay of hydrogen peroxide produced.

8.

A Comparison of Normalization Techniques for Urinary Biomarkers Analysis in Clinical Respiratory Research.

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2. College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, SK

One prominent application of metabolomics is the identification of biomarkers for clinical diagnosis. A preliminary diagnostic model of 43 urinary metabolite biomarkers for separation between respiratory diseases, asthma and Chronic Obstructive Pulmonary Disease (COPD), was developed by previous lab members. Urine was chosen for its high concentration of metabolites and because its collection is non-invasive; however, it requires a method of normalization to account for the hydration status. Traditional methods of normalization are affected by patient characteristics, such as kidney function and BMI affecting their accuracy. Development of a total metabolome method of normalization is based on past research from the University of Alberta. In this work the total metabolome method is evaluated to determine if it can allow for increased patient inclusion and confirmation of proposed diagnostic biomarkers. A sample set of N=19 was chosen and both traditional and total metabolome normalization methods were applied to normalize metabolite concentration. The methods of normalization resulted in different metabolites being selected as biomarkers of significance and indicated the importance of appropriate normalization in development of a diagnostic model from urinary biomarkers. We are currently analyzing the identified metabolites and their relationship to the biochemistry of the diseases.

9.

Microbubble assisted drug delivery for glioblastoma.

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2 Department of Medicine Imaging, College of Medicine, University of Saskatchewan, Saskatoon, SK

Glioblastoma is the most common and malignant brain tumor in adults; moreover, the blood-brain barrier (BBB) is a significant obstacle in the effective delivery of anti-cancer therapies. Microbubble (MB) is a colloidal particle that consists of a gas core surrounded by a protective shell composed of biocompatible materials, used as contrast agents and, when combined with low-intensity focused ultrasound, is a promising technique to cross physiological barriers. Gemini lipid is a new class of surfactants, presenting a unique structure and properties compared to conventional surfactants. Besides a lower critical micellization concentration, it is also a versatile non-viral gene delivery system that, if incorporated into MB could bring beneficial advantages to the formulation. Throughout mechanical vibrations, High-intensity focused ultrasound (HIFU) forms an ultrasound wave which propagates across tissues, causing alternating cycles of increased and reduce d pressure. MBs loaded with drug or gene therapy vectors, if applied with HIFU, can be used to deliver and release the transport of substances into tissues of hard access. This project focuses on synthesizing MB combined with Gemini Lipids to increase chemotherapeutic cross BBB into glioblastoma, maintaining low toxicity and reducing side effects.

10.

Liquid Chromatography-Mass Spectrometry Strategy for The Assessment of A Novel Cyclodextrin-modified Gemini Surfactants Lipid-based Drug Delivery System.

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College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, SK

Neutrophilic asthma is considered severe refractory asthma, which does not respond well to conventional treatment such as steroid therapy. An anti-inflammatory patented peptide reduces neutrophilic inflammation and is highly effective to treat neutrophilic asthma. However, injectable administration of the peptide is not practical, thus an alternative, non-invasive intranasal drug delivery system based on cationic gemini surfactant-conjugated Î²-cyclodextrin (CD-Gem) is being developed and tested. Gemini surfactants are versatile lipid-based drug delivery agents because of their ability to encapsulate and protect biotechnology products (proteins, peptides, DNA and RNA drugs) and the CD-Gem has a unique cyclodextrin cavity, allowing it to encapsulate therapeutic peptides. A lung epithelial cell model will be used to test the delivery efficacy of the novel formulation and to assess trans-membrane delivery of the peptide/CD-Gem complex. To achieve this, a no vel liquid chromatography-tandem mass spectrometry (LC-MS/MS) method should be developed. Preliminary MS analysis of CD-Gem showed a precursor ion as doubly charged species [M]2+. MS/MS analysis showed the fragmentation behaviour of the compound, allowing for the identification of multiple ions to be used in quantification. LC-MS/MS using a C18 column showed promising results that require additional optimization to attain optimal peak shape.

11.

Structural Analysis of Thioredoxin1 Mimicking Mutants: Estimating Comparative Binding Affinities to Thioredoxin Reductase.

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The thioredoxin (Trx) system, composed of NADPH, thioredoxin reductase (TrxR), and thioredoxin, is a key antioxidant defense against oxidative stress. It transfers electrons from NADPH to FAD, then to TrxR, which reduces thioredoxin's active site disulfide, allowing the reduction of intracellular disulfide bonds. Despite structural similarities between oxidized (Ox.Trx1) and reduced (Red.Trx1) thioredoxin, it is unclear why Red.Trx1 does not disrupt the Ox.Trx1-TrxR interaction. We hypothesize that subtle differences in their active sites explain this and will test their binding affinities to TrxR. In the TrxR kinetic assay, maintaining Trx1 in its oxidized form is essential to evaluate its role as a substrate, complicating the assessment of Red.Trx1's affinity. To address this, we will use Ec-Trx1 mutants with altered active-site cysteines. Ser mutations are expected to mimic the reduced form, while Ala mutations will approximate the disulfide form. Varying concentrations of each mutant will be introduced into the TrxR assay to assess inhibitory potential. We expect Ox-mimicking mutants to show stronger inhibition. X-ray crystallography will validate these predictions, ultimately allowing comparison of Ki values for Ox.Trx1 and Red.Trx1 by integrating inhibitory and structural data.

12.

Formulating an aldolase for more convenient catalysis.

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Aldolases catalyze useful carbon-carbon bond forming reactions. One such aldolase, NahE, converts pyruvate and a variety of aldehydes into benzylidenepyruvates, useful precursors for many products of interest. Purifying enough enzyme for large scale application is very time-consuming. Lyophilization of lysate containing NahE led to an increase in the enzyme's stability in ambient conditions. However, due to the presence of various cell components in the crude mixture, the aldol condensation reactions catalyzed by the lyophilized enzyme are difficult to work up with acid-base extraction, resulting in the loss of isolated yield. When granular agro-waste-based adsorbent pellets containing chitosan, biogenic calcium carbonate, and torrefied wheat straw were added to the lysate, it was observed that the pellets could catalyze the aldol condensation reactions between an aldehyde and pyruvate and the workup and isolate yields are the same as the reactions catalyzed with the pure enzyme. The practicality and re-usability of this method will be discussed.

13.

Development of DISC1-Targeted Chemical Probes for Modulating Wnt Signaling Pathway and Neural Progenitor Proliferation.

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2. Chemical Neurobiology Laboratory, Center for Genomic Medicine, Department of Neurology, Massachusetts General Hospital, Harvard Medical School, 185 Cambridge Street, Boston, MA 02114, USA

Disrupted in Schizophrenia (DISC1) functions as an intracellular scaffold protein integral to neurodevelopmental processes, making it a promising target for psychiatric diseases. Previous research highlights DISC1 as a Wnt signalling pathway regulator that mediates neuronal proliferation. We aimed to design chemical probes targeting DISC1 to modulate these processes. A small molecule microarray of 20,000 small molecules was screened against cell lysates overexpressing several DISC1 variants and a purified MBP-tagged DISC1. We selected the hydrazide structures to build a small library for further testing. Surface plasmon resonance (SPR) was used to validate DISC1 binding. Next, we evaluated the probes' ability to activate the Wnt signalling pathway using a TCF/LEF reporter cell-based assay. Finally, the probes' effect on neural progenitor proliferation was evaluated using the Cell Titer-Glo Assay. SPR analysis revealed that our lead compound, JB87, exhibited micromolar binding affinity to DISC1. However, it did not activate Wnt signalling from the TCF/LEF reporter assay. Despite this, JB87 promoted neural progenitor proliferation at concentrations below 1 Î¼M. JB87 is a strong DISC1 binder that promotes neural progenitor proliferation, likely independent of the Wnt signalling pathway. Future studies will focus on clarifying its mechanism of action.

14.

Investigating mutations in the active site of the aldolase NahE altering substrate recognition.

Irene Nguyen and Dr. David R. J. Palmer*

Department of Chemistry, University of Saskatchewan, Saskatoon, SK

One of today's leading research agendas in Chemistry encompasses moving to a greener approach, decreasing the use of hazardous substances and minimizing chemical waste. Bond-forming reactions are of immense importance in organic synthesis and carrying out such reactions using biocatalysts like enzymes, with high selectivity for different substrates under mild conditions, is an attractive approach. The Palmer Lab has been studying the trans-o-hydroxybenzylidenepyruvate hydratase-aldolase (NahE), a type I aldolase found in bacterial naphthalene degradation. The wild-type of this enzyme catalyzes aldol reactions producing alpha,beta-unsaturated 2-keto acids from a wide range of aldehydes. Moreover, it can catalyze the Michael addition of pyruvate to various beta-nitrostyrenes in high yield and enantioselectivity. To further our understanding of NahE, we have been experimenting with the two mutants, F277W and F274W which alter two phenylalanine residues that form a hydrophobic pocket for the aldehyde/nitrostyrene acceptor. A series of kinetic UV-Vis assays have been run for p-dimethylaminobenzaldehyde and pyruvate, showing that F277W activity on this substrate has been decreased significantly, while F274W activity has not.

15.

Development of LC-MS/MS Methods for the Quantification of Metabolites in the TCA Cycle of Breast Cancer Cells.

Marziyeh Ghanbarian[1], Deborah Michel[2], Maryam Alyari[2], Oleg Dmitriev[1]*, Anas El-Aneed[2]* A

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MEMO1 (Mediator of ERBB2-driven Cell Motility 1) is a highly conserved protein that promotes the motility and invasion of cancer cells into surrounding tissues, contributing to cancer metastasis. Our latest research suggests that MEMO1 is an iron-containing dioxygenase involved in iron homeostasis. We have experimentally confirmed its genetic interactions with many iron-transport proteins, emphasizing its role in iron supply to mitochondria. Our gene set enrichment analysis revealed that MEMO1 interacts with multiple iron-containing proteins related to mitochondrial energy metabolism, the tricarboxylic acid (TCA) cycle, and respiratory electron transport. We hypothesized that MEMO1 knockout-induced disruptions in iron homeostasis would affect energy metabolism in mitochondria, in particular the TCA cycle. To test this hypothesis, we focused on the quantification of metabolites associated with the TCA cycle in high-MEMO1 and low-MEMO1 triple-negative breast cancer (TNBC) cells. We developed and validated two targeted LC-MS/MS methods. Specifically, glutamic acid, Î±-ketoglutaric acid, citric acid, malic acid, and fumaric acid were derivatized using 4-(dimethylamino) phenacyl bromide (12C2/13C2-DmPA), while pyruvate and succinate were quantified using a hydrophilic interaction liquid chromatography (HILIC)-MS/MS method. This research will reveal the role of MEMO1 in the iron-dependent regulation of TCA cycle and related metabolic pathways in the cell.

16.

Biocatalytic synthesis of chiral building blocks using aldolase and oxidoreductase system.

Negin Neissari, and David R. J. Palmer*

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The stereoselectivity of chiral molecules is crucial for pharmaceutical applications, as different stereoisomers can yield varying effects; some may be therapeutic, while others may be inactive or harmful. Biocatalysis has been used as an efficient method for synthesizing stereoselective chiral building blocks. Previously our group synthesized GABA analog, 4-nitro-3-phenylbutanoic acid with high enantioselectivity, and alpha,beta-unsaturated 2-oxo acids using an aldolase, NahE. Here, we have synthesized benzylidenepyruvate (1) and 5-nitro-2-oxo-4-phenylpentanoic acid (2) with NahE and report them as substrates of alcohol dehydrogenase (ADH) to generate chiral building blocks. The compounds (1 and 2) were characterized by ^{1}H-NMR and ^{13}C-NMR and the ADH reactions were monitored by UV-Vis spectrophotometry. As part of future work, our goal is to conduct a biocatalytic cascade reaction and assess the stereochemical outcome using chiral HPLC. 2-Hydroxy-5-nitro-4-phenylpentanoic acid could be used as an intermediate for synthesizing proline compounds and as a precursor for pharmaceuticals.

17.

Synergistic Approaches to Neurodegeneration: Enhancing Astrocyte Reprogramming and Targeted Nucleic Acid Delivery with Gemini Lipid Nanoparticles.

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1. College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, SK
2. Department of Chemistry, University of Saskatchewan, Saskatoon, SK

Neuronal reprogramming and lipid nanoparticles (LNPs) offer innovative strategies for addressing incurable neurological diseases and enhancing gene therapy delivery. Reprogramming astrocytes into induced neurons (iNeurons) provides a promising approach to replenish lost neuronal populations and combat neurodegeneration. The addition of gemini lipids in LNPs significantly improves the delivery of nucleic acid payloads to specific cell types, such as astrocytes, which play a crucial role in brain health and disease. Our study synergizes direct neuronal reprogramming with advanced LNP formulations, focusing on gemini lipids and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) for targeted astrocyte delivery. Using Small Angle X-ray Scattering (SAXS), we uncovered critical nanostructural details, revealing that the inclusion of DOPE in gemini lipid-based LNPs leads to the formation of a hexagonal phase, which enhances nucleic acid delivery efficiency by 8-fold while maintaining minimal toxicity. This hexagonal configuration optimizes the encapsulation, protection, and release of nucleic acids into targeted cells, significantly improving delivery to astrocytes. These structural insights are crucial for refining nanoparticle design and advancing hybrid approaches that combine cellular reprogramming with nanotechnology, paving the way for novel therapeutic modalities in the treatment of neurodegenerative diseases and brain injuries.

18.

Finding novel drugs to treat *Mycoplasmopsis bovis*: A common bovine respiratory disease pathogen.

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2. Department of Biochemistry, Microbiology and Immunology, College of Medicine, University of Saskatchewan, Saskatoon, SK, Canada, S7N 5E5
3. Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, Lexington, KY, USA, 40536, USA.

There are a lack of effective antibiotics and vaccines to treat and manage *Mycoplasmopsis bovis* (formely *Mycoplasma bovis*) infections. The impact of *M. bovis* is profound, contributing to bovine respiratory disease (BRD), pneumonia as well as chronic pneumonia and polyarthritis syndrome (CPPS). These are serious animal wellness and production limiting issues. In fact, BRD is the major cause of mortality in beef cattle feedlots, and antimicrobial resistance (AMR) is nearly synonymous with respect to *M. bovis* and conventional therapies such as tetracyclines and macrolides in western Canada. In an effort to discover novel therapies for *M. bovis*, we screened a library of ~3,000 FDA-approved compounds and ~400 known antibiotics for two potential anti-mycoplasmal activities: cell-membrane permeation and growth inhibition. The results of these two screens, including the discovery of drugs that cause non-antibiotic *M. bovis* cell membrane disruption and a small collection of antibiotics that have yet to be explored as anti-mycoplasmal agents will be presented. Our efforts to better understand the function of one antibiotic, ebselen, a synthetic selenium-containing drug, will be further discussed, including a medicinal chemistry effort as well as biochemical and proteomic approaches to define mechanism of action.

19.

Synthesis of C4-modified sialic acids as metabolic labeling agents and sialyltransferase inhibitors.

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N-Acetylneuraminic acid (Neu5Ac; sialic acid) is a nine-carbon terminal monosaccharide of the glycan on cell surface glycoconjugates of vertebrates. Although Neu5Ac-terminated glycoconjugates play numerous roles in biological and pathogenic processes, their participation in many biological pathways is not fully understood. Expanding the toolbox of chemical probes to study sialic acid biology is needed. One of the developed tools are azide derivatives of Neu5Ac, which have been made at the C5, C7, or C9 positions for use in metabolic oligosaccharide engineering (MOE). Modifications at the C4 position of sialic acid are relatively unexplored, in part, due to synthetic challenges associated with assessing this position. In this study, azide-modified sialic acid was synthesized through an efficient synthetic route and was tested in MOE. 4-Azido Neu5Ac was fed to cells in its peracetylated form, entered the sialic acid biosynthetic pathway, and was successfully incorporated on the cell surface. Furthermore, the azide modifications on the core $3F_{ax}$- Neu5Ac scaffold can be exploited by reducing it into an amine through *N*-acyl derivatizations. Therefore, a library of C4-modified sialic acids was synthesized in a facile manner from CMP-4-amino-Neu5Ac to screen for better inhibitors. Specifically, we hypothesized that any modifications at C4 position could result in changes in the affinity and selectivity of the Neu5Ac donor towards the sialyltransferases (STs), which can lead to a discovery of a more effective or selective ST inhibitor. Overall, our study provides an
efficient synthetic and chemoenzymatic routes to C4-modified sialic acids as metabolic labeling reagents and sialyltransferase inhibitors.

20.

Investigating the importance of an active-site asparagine residue in the aldolase reaction of NahE, a useful biocatalyst.

Farah Mustafa, David Palmer*

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Trans-o-Hydroxybenzylidenepyruvate hydratase-aldolase (NahE), is a type 1 aldolase from the naphthalene degradation pathway of Pseudomonas species that reversibly converts hydroxybenzylidenepyruvate into salicylaldehyde and pyruvate. NahE is known to catalyze carbon-carbon bond formation between pyruvate and a broad spectrum of aldehydes, and catalyze the nitro-Michael addition of pyruvate to beta-nitrostyrene, making it a useful biocatalyst. Much is known about the structure of the enzyme, including the identity of key active site residues such as lysine-183 and tyrosine-155, which are responsible for the Schiff base formation in the active site. Crystallography shows that asparagine-157 is positioned to form a hydrogen bond with the carbonyl oxygen of the acceptor aldehyde, and may assist Tyr 155 in the addition/elimination of water in the condensation reaction. We are investigating the effect of mutating Asn157 to serine, using the chromogenic aldol condensation of 4-dimethyaminobenzaldehyde, and comparing the kinetic constants with the wild-type NahE. Understanding the role of N157 will help optimize NahE for aldol condensation and nitro-Michael addition reactions.

21.

Synthesis of Derivatives of Conduritol Aziridines towards Imaging GCase.

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Parkinson's disease (PD) is caused by the loss of dopaminergic neurons in the substantia nigra pars compacta region of the brain. The lysosomal enzyme β-Glucocerebrosidase (GCase), encoded by the GBA1 gene, is responsible for hydrolyzing glucosylsphingolipids in dopaminergic neurons and is considered a high priority biomarker and therapeutic target for PD. However, the mechanistic link between GCase activity and PD progression is not well understood. Recently, mutations in the GBA1 gene have been established as the most serious genetic risk factor for developing PD. In patients with GBA1 mutations, a misfolded enzyme is produced that is degraded by the cell's quality control, prior to lysosomal delivery. This GCase deficiency results in accumulation of the lipid substrates in neurons leading to an aggressive early onset form of PD. Interestingly, recent studies have also shown that PD patients with wild-type enzyme also have reduced lysosomal GCase activity. Mechanistic studies have revealed that high levels of glucosylsphingolipids, resulting from lysosomal GCase deficiency, induces the aggregation of α-synuclein resulting in the formation of Lewy neurites and Lewy bodies, the pathological hallmark of PD. As a result, GCase-boosting therapies are currently under intense development as a novel treatment for PD. Chemical tools that can inhibit or report on lysosomal GCase activity in cells, animals and humans are urgently needed to help unravel the complex link between GCase activity and PD progression and guide the development of GCase-boosting therapies. The Phenix lab has discovered novel conduritol aziridines (CAz) that irreversibly and selectively inhibit GCase with extraordinary high affinity in cells and animals including the brain. To help identify the most promising inhibitors, we have developed a robust biological screening platform in cells and animals and established methods for labeling CAz's with ^{18}F; the most common isotope used for positron emission tomography (PET). In this presentation, we will describe the synthesis and biological characterization of fluorinated derivatives as PET tracer candidates for GCase. If successful, a PET tracer capable of imaging GCase would be valuable for guiding the development of GCase-targeted therapies and as a diagnostic aid for identifying aggressive forms of PD.

22.

Inactivating Dihydrodipicolinate Synthase for Stable Complex Formation: A Strategy for ASA-Bound Crystallography.

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Dihydrodipicolinate Synthase (DHDPS) is essential in the lysine biosynthesis pathway, but the structural details of its interaction with S-aspartate-β-semialdehyde (ASA) remain elusive. This project aims to stabilize DHDPS in a conformation that allows for the eventual crystallization and structural determination of the enzyme with ASA bound in the active site. To achieve this, the enzyme is first incubated with ASA to clear pyruvate from the active site, followed by the introduction of trifluoropyruvate, which is expected to form a stable, non-reactive complex. This complex will serve as a precursor to obtaining the DHDPS structure with ASA bound. Preliminary work has demonstrated the successful clearance of pyruvate and the stabilization of the enzyme for crystallization. While stable crystallization conditions have already been
established and multiple solved structures available, reported attempts have been few and far between for the dead-end complex DHDPS with ASA bound in the unreactive site (inactivated 'active'site), which will provide critical insights into its catalytic mechanism and pave the way for future inhibitor design.

University of Saskatchewan map

Where to find us

- **Rooms 1004 & 1005 Education Bldg.** Friday night mixer, scientific sessions, and meals.
- **Lot F** - Parking
- **St. Tropez Bistro, 238 2nd Ave. South** Saturday banquet