WCMCW

Sixth Western Canadian Medicinal Chemistry Workshop

September 21-23, 2018
Saskatoon, Saskatchewan

Chair
Ed S. Krol

Vice Chair
David R. J. Palmer

Sponsors

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Contents

Message from the organizers

We would like to welcome you to Saskatoon and the University of Saskatchewan for the sixth Western Canadian Medicinal Chemistry Workshop. 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; and
- 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 many sponsors for helping with this initiative. We have listed our sponsors on our website, in the program, and throughout the workshop; please join us in thanking these generous donors.

We hope that our efforts to put together the sixth 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 21, 2018

TIME	EVENT	LOCATION
6 to 9 pm	**Registration and mixer**	Student lounge, Rm 1005 Education building, University of Saskatchewan

SATURDAY, SEPTEMBER 22, 2018

Registration and sessions in room 1004
Poster presentations and meals in room 1005

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

Session 1 chaired by Ed Krol

9 am	**Rebecca Davis (University of Manitoba)** *Integrated computational strategies for the design of site selective bioactive compound*
9:40 am	**Francesco Gentile (University of Alberta)** *Computer-aided Drug Design of ERCC1-XPF Inhibitors for Combination Cancer Therapy*
10:00 am	**Christopher Rowley (Memorial University)** *Modeling Covalent Modifier Drugs*
10:20 am	Coffee break and poster viewing

Session 2 co-chaired by Chukwunonso Nwabufo and Sanyi Alwani

10:45 am	**Robert Laprairie (University of Saskatchewan)** *Structure-activity relationships of type 1cannabinoid receptor positive allosteric modulators*
11:20 am	**Mohadeseh Majdi Yazdi (University of Saskatchewan)** *Fluorescence-Based Binding Assay for Screening Inhibitors of Dihydrodipicolinate Synthase from Campylobacter jejuni*
11:40 am	**Michael Eze (University of Winnipeg)** *Indigenous Nutrition and Health: The Chemical Research Mandate*
12:00 am	**Asmita Poudel (University of Saskatchewan)** *Formulation of colloidal stable liposomes containing phytosterols and tocopherols in fortified orange juice for hypercholesterolemia*
12:20 pm	End of morning session. **WCMCW lunch**

SATURDAY, SEPTEMBER 22, 2018 (cont'd)

TIME	EVENT
Session 3 co-chaired by Stephanie Vuong and Brady Vigliarolo	
1:20 pm	**Tim Storr (Simon Fraser University)** *Designing Multifunctional Molecules to Treat Disease*
2:00 pm	**Akay Akohwarien (University of Saskatchewan)** *Design, synthesis and kinetic evaluation of conduritol aziridine derivatives as inhibitors of β-glucocerebrosidase; a potential biomarker for Parkinson's disease*
2:20 pm	**Chukwunonso K. Nwabufo (University of Saskatchewan)** *Preclinical Development of Novel Dimer Compounds for Parkinson's disease*
2:40 pm	Coffee break and poster viewing
Session 4 co-chaired by Franklyn DeSilva	
3:05 pm	**Darren Derksen (University of Calgary)** *Targeting Protein Function Using Natural Product Structure-Activity Relationship (SAR) Studies and Proteolysis-Targeting Chimeras (PROTACs)*
3:50 pm	**Douglas Fansher (University of Saskatchewan)** *Understanding the Substrate Scope of a Promiscuous Aldolase and its Application Towards Generating Substituted Quinolines*
4:10 pm	**Saniya Alwani (University of Saskatchewan)** *Amino acid functionalized nanodiamonds for gene delivery: Remodelling of functionalization design to improve gene transfection efficiency*
4:30 pm	End of afternoon session. **Poster viewing and judging**
6:00 pm	End of poster session
7:00 pm	**Banquet, St. Tropez Bistro, 238 2nd Avenue South**

SUNDAY, SEPTEMBER 23, 2018

Registration and oral presentations in room 1004

Meals in room 1005

TIME	EVENT
8:00 am	**Breakfast workshop for trainees** Katie Maloney, Northern Vine Labs
9:10 am	**Opening remarks by Vice-chair David Palmer**

Session 5 chaired by David Palmer

9:15 am	**Florence Williams (University of Alberta)** *Structure-Activity Relationship of Neurotrophic Phenylbutenoid Dimers and Progress Towards Uncovering Mechanism of Action*
9:35 am	**Seyed Amirhossein Tabatabaei Dakhili (University of Alberta)** *FOXM1 inhibitors: Emergence of a neglected binding interaction*
9:55 am	**Anjali Goel (University of Winnipeg)** *Effects of Gongronema latifolium methanolic leaf extracts on viability & pro-/anti-inflammatory properties of macrophages*
10:15 am	**Raj Rai (University of Saskatchewan)** *Engineered and functionalized nanodiamond particles for drug/gene delivery: biodistribution studies*
10:35 am	Coffee break

Session 6 co-chaired by Raj Rai and Omozojie Aigbogun

10:55 am	**Ekaterina Dadachova (University of Saskatchewan)** *Radioimmunotherapy of cancer and infections with alpha-particles emitting radionuclides*
11:35 am	**Wojciech Dawicki (University of Saskatchewan)** *Conjugation of Daratumumab with 225Actinium Greatly Increases its Antitumor Activity*
11:55 am	**Brady Vigliarolo (University of Saskatchewan)** *Fluorescent Cathepsin Substrates, their 18F-Labeled Equivalents for PET, and an Analogue Employing Doxorubicin*
12:15 pm	**Closing remarks and WCMCW lunch**

Abstracts

Oral presentations / Session 1

Integrated computational strategies for the design of site selective bioactive compound

Rebecca L. Davis[*#]
Department of Chemistry, University of Manitoba

Understanding molecular level interactions between biomolecules and ligands is essential for the design of effective small molecule inhibitors. This talk will discuss our efforts to develop small molecules inhibitors for an array of biomolecule targets using an unconventional mix of in silico methodologies. Our aim is to use molecular mechanics (MM) and QM/MM to understand the nature of the binding between the biomolecule and its native ligand. This is followed by the design of small molecules to exploit predicted key interactions and tightly bind to the sight of interest on the biomolecule. The site specificity of these compounds is evaluated through further using MM and molecular dynamics studies. The final molecular designs are synthesized and tested through kinetic and biophysical characterization studies to establish their viability as leads for drug candidates.

Computer-aided Drug Design of ERCC1-XPF Inhibitors for Combination Cancer Therapy

Francesco Gentile[1#*], Khaled H. Barakat[2], and Jack A. Tuszynski[1,3,4]
1. Department of Physics, University of Alberta

The ERCC1-XPF complex is a heterodimeric endonuclease active in nucleotide excision repair (NER) and inter-strand crosslink (ICL) DNA repair pathways. While its action is essential to maintain genome integrity in healthy cells, in cancer it is involved in the repair of DNA damages caused by platinum-based chemotherapy and radiotherapy, playing a critical role in the development of treatment resistance. Hence, developing small molecule inhibitors of the ERCC1-XPF activity is a new, promising way to enhance the effect of traditional cancer therapies. We applied computer-aided drug design to identify and optimize small molecules inhibiting the activity of ERCC1-XPF by targeting different sites of the complex involved in protein-protein interactions and catalytic activity. Our effort is part of the Alberta DNA Repair Consortium drug design pipeline, with the final aim to translate the compounds from the bench to the clinic. Once our top hits will be optimized to drug-like structures, they can be used in combination with DNA damaging cancer therapies on patients. It is our hope that this will turn to be a game-changing milestone for cancer treatment in Canada and worldwide.

Modeling Covalent Modifier Drugs

Christopher Rowley
Department of Chemistry, Memorial University

Many popular drugs, like aspirin and penicillin, act by forming a chemical bond with their target. This class of covalent-modifier drugs have received newfound interest as inhibitors of protein kinases due to their high affinity, improved selectivity, and long residence times. For example, chemotherapy drug ibrutinib acts through the addition of an acrylamide substituent to non-catalytic cysteine residue in the active site of Bruton's tyrosine kinase. Conventional computer modeling methods cannot describe the formation of chemical bonds, so our group has worked to develop new modeling methods to describe covalent modification. Our work has shown that electrophilic modification of cysteine likely occurs through a canonical Michael addition mechanism with the anionic thiolate form of the cysteine. As a result, the propensity of a given cysteine to undergo modification depends on its pKa. Our simulations indicate that most cysteine residues in kinase proteins that have been targeted for covalent modification have elevated pKa's due to electrostatic interactions with other residues and the desolvation of cysteine residues inside the active site.

Abstracts
Oral presentations / Session 2

Structure-activity relationships of type 1 cannabinoid receptor positive allosteric modulators

Mylyne Tham[1], Orhan Yilmaz[1], Sumanta Garai[2], Eileen M Denovan-Wright[3], Ganesh A Thakur[2], Robert B Laprairie[1,3#,*]

1. College of Pharmacy and Nutrition, University of Saskatchewan
2. Dept. of Pharmaceutical Science, Bouvé College of Health Science, Northeastern University, Boston MA
3. Dept. of Pharmacology, College of Medicine, Dalhousie University

The type 1 cannabinoid receptor (CB1R) is being extensively studied as a target for pain and epilepsy. Traditional agonists target CB1R's orthosteric site (i.e. the same site as the endogenous ligand). Agonists produce intoxicating and addictive effects, and lead to receptor desensitization and physiological tolerance. In contrast, positive allosteric modulators (PAMs) bind a distinct receptor site from orthosteric agonists and in doing so increase the receptor's binding affinity and signaling efficacy. We have previously developed and characterized the CB1R PAM GAT211 in cell culture assays. In an effort to obtain CB1R PAMs with greater in vitro and in vivo potency, a "fluorine walk" was conducted using the GAT211 scaffold. For this approach, a fluorine atom is substituted at various positions to improve the pharmacodynamic and pharmacokinetic aspects of the compound. Fluorine-substituted compounds were characterized for their ability to inhibit cAMP and recruit barrestin2 in CHO cells expressing human CB1R. Mono-substitution at 3 sites produced ligands more potent than the parent compound. Di-fluoro and tri-flouro-substituted analogs were superior to mono-fluoro-substituted analogs. These promising in vitro results demonstrate structure-activity improvements to PAM activity. These compounds are now being tested for their efficacy in vivo in the contexts of pain and paediatric epilepsy.

Acknowledgements: This work was supported by a partnership grant from the Canadian Institutes of Health Research (CIHR) and GlaxoSmithKline to RBL, and the National Institutes on Drug abuse (NIDA) at NIH to GAT. OY was supported by an Undergraduate Student Research Award from the Natural Sciences and Engineering Research Council of Canada (NSERC).

Fluorescence-Based Binding Assay for Screening Inhibitors of Dihydrodipicolinate Synthase from Campylobacter jejuni

Mohadeseh Majdi Yazdi[#], Krishna Annadi, Yulia Skovpen, David R. J. Palmer*

Department of Chemistry, University of Saskatchewan

Dihydrodipicolinate synthase (DHDPS) is an enzyme that mediates the first unique reaction of (S)-lysine biosynthesis in the diaminopimelate pathway. Lysine as the natural feedback inhibitor of DHDPS is a crucial component for bacterial cell wall peptidoglycan. DHDPS is the product of an essential gene that is absent in humans. This makes DHDPS a promising antimicrobial target. A series of allosteric inhibitors of DHDPS were designed and synthesized, and their detailed kinetic function was studied. Moreover, we report the generation and characterization of a specific DHDPS mutant that can be used for simple and informative screening of inhibitors on the basis of intrinsic tryptophan fluorescence change. The developed assay delivers valuable information on binding of inhibitors to allosteric site of DHDPS much more rapidly than kinetic studies. This also provides additional structural and activity relationships that are useful in designing further inhibitors.

Indigenous Nutrition and Health: The Chemical Research Mandate

Michael O. Eze
Department of Chemistry, University of Winnipeg

The Indigenous Holistic View of health and wellbeing demands that we remain in tune with the ecosystem. This is consistent with Hippocrates' ancient dictum: "Let food be thy medicine, and medicine be thy food." Since we are thermodynamic "open systems", avoiding polluting the ecosystem guarantees its sacrosanctity and ensures that we derive clean fresh foods, air and water therefrom; and establish homeostasis within us - keeping the body's chemistry optimized. Issues disparately plaguing Indigenous peoples' health may relate to (colonization-induced) violation of aspects of the Holistic View. To address these requires concerted interdisciplinary research: Commonalities in the chemical species participating in the operation and control of metabolic reactions in disease and healthy states. The new direction should involve investigations on reactive oxygen species (ROS), reactive nitrogen species (RNS), advanced glycation end products (AGEs), and others. These are as present in normal individuals, and in individuals suffering from diverse diseases, both infectious and chronic [diabetes and its complications, cancer, cardiovascular, and degenerative diseases (like Alzheimer's, Parkinson's)] etc. The role and mechanisms of traditional Indigenous resources as pro-health interventions should be verified. Ultimately, isolation of the active ingredients and formulation of efficacious, inexpensive and safe remedies therefrom, as per recent successes in the field, should be the goal.

Formulation of colloidal stable liposomes containing phytosterols and tocopherols in fortified orange juice for hypercholesterolemia

Asmita Poudel[1], George Gachumi[1], Zafer Dallal Bashi[2], Ildiko Badea[1] and Anas El-Aneed[1]

1. College of Pharmacy and Nutrition, University of Saskatchewan
2. Ministry of Agriculture, Government of Saskatchewan

Our purpose is to design phytosterols and tocopherols obtained from canola oil deodorizer distillate (i.e. waste stream) into functional food. Phytosterols have well-established cholesterol lowering effect while tocopherols are natural antioxidants. However, development of functional food containing these bioactives have been challenging due to their lipophilicity, heat and light sensitivity. The cholesterol-lowering effect of phytosterols is greatly affected by formulation approaches. Thus, the aim of this study is to design liposomal formulation of phytosterols and tocopherols in orange juice to solve formulation challenges. The long-term goal is to assess the cholesterol-lowering efficacy of phytosterol-containing juice in hypercholesteremic individuals. Three different techniques were adopted for the formulation of liposomes namely thin layer hydration homogenization, thin layer hydration ultrasonication and heating method. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) validated quantification method was developed to determine the incorporation efficiency of these bioactives into liposomes. The particle sizes were 192±1nm and 195±1nm for homogenization and ultrasonication, respectively. The particle size employing the heating method was significantly higher at 258±1nm. Liposomes formulated from all three techniques showed similar zeta potential (-15mv to – 18mv). LC-MS/MS analysis showed incorporation efficiency greater than 90 % for both homogenization and heating methods. Optimum liposomes incorporated in simulated orange juice showed adequate stability during storage time of 45 days after pasteurization. In sum, we formulated phytosterols and tocopherols obtained from canola oil waste stream into liposomes and prepared fortified orange juice. In future, we will conduct clinical trials to assess cholesterol-lowering efficacy in hypercholesteremic individuals.

Abstracts
Oral presentations / Session 3

Designing Multifunctional Molecules to Treat Disease
Tim Storr
Simon Fraser University

We are investigating protein aggregation and oxidative stress in the development of cancer and Alzheimer's disease (AD). AD is characterized by the formation of amyloid-beta plaques and neurofibrillary tangles in the brain. Amyloid plaques are insoluble extracellular deposits of the amyloid peptide, and peptide aggregation is accelerated in the presence of dysregulated metal ions (Cu, Fe, and Zn). These metal ions (Cu, Fe) have been shown to be redox-active and form toxic reactive oxygen species (ROS) in the presence of dioxygen. We are investigating bifunctional molecules that interact with the amyloid peptide and bind metal ions with the aim to limit peptide aggregation and minimize the production of toxic ROS. We are using a similar approach for reactivation of mutant p53 protein which plays a major role in cancer prevention, and over 50% of cancer diagnoses can be attributed to p53 malfunction. One destabilizing p53 mutation, Y220C, causes local protein unfolding and aggregation, and ultimately results in loss of Zn in the DNA-binding domain. We are developing new small molecule stabilizers of p53 Y220C that feature Zn-binding fragments to chaperone Zn to the metal depleted site and restore wild-type function.

Design, synthesis and kinetic evaluation of conduritol aziridine derivatives as inhibitors of β-glucocerebrosidase; a potential biomarker for Parkinson's disease.
Akay Akohwarien[1#], Isaac Asiamah[1], Christopher P. Phenix[1], and David R.J Palmer[1*]
Department of Chemistry, University of Saskatchewan

β-Glucocerebrosidase (GCase) is a lysosomal enzyme that hydrolyzes the glycosidic bond of β-glucosylceramide. There have been several works published that describes that the deficiency of this enzyme leads to Gaucher's disease, a lysosomal storage disorder. Also, a reduction of lysosomal GCase in neurons has been associated with Parkinson's disease. Therefore, traceable molecules, such as those bearing a radioisotope, which can selectively label GCase in vivo may be useful in the understanding, diagnosing and monitoring of both of these diseases. N-Octyl conduritol aziridine inactivates GCase at nanomolar concentrations and is a lead compound towards the development of molecular imaging probes. We have introduced new structural motifs that mimic the natural β-glucosylceramide substrate to tune the affinity of novel conduritol aziridines towards the inactivation of GCase. This has resulted in a novel, potent GCase inactivator that shows highly desirable selectivity for GCase over nonlysosomal β-glucosidase GBA2. The long-term goal is the development of optimal PET radiotracers for imaging GCase in animals and humans.

Preclinical Development of Novel Dimer Compounds for Parkinson's disease.
Chukwunonso K. Nwabufo[#] and Ed S Krol[*]
College of Pharmacy and Nutrition, University of Saskatchewan

The development of disease-modifying treatments and differential diagnostic agents is the focus of research in Parkinson's disease (PD). Recently, our translational research revealed that two dimer compounds comprising of a caffeine scaffold attached to nicotine (C8-6-N), and 1-aminoindan (C8-6-I) can prevent the pathogenic pathway that leads to PD. Additionally, caffeine linked to caffeine (C8-6-C8) can be developed as an imaging probe for diagnosis of PD. Given the therapeutic and diagnostic potentials of these novel dimer compounds, it is important to conduct further preclinical studies at this early stage of drug discovery and development. We performed in-vitro incubations of C8-6-N, C8-6-I, and C8-6-C8 using human, rat, and mouse liver microsomes (HLM, RLM, and MLM) in parallel with positive and negative controls. The metabolites were identified with accurate mass measurement using a quadrupole/time of flight mass spectrometer (QToF). Furthermore, a tandem mass spectrometric fingerprint was established using a triple quadrupole

linear ion trap mass spectrometer (QqQ). Two metabolites were identified for both C8-6-I and C8-6-N, whereas no metabolite was observed for C8-6-C8. The metabolites identified for C8-6-I were generated from de-alkylation (M1) and hydroxylation (M2); the metabolites for C8-6-N were also identified as being generated from de-alkylation (M3) and hydroxylation (M4). The tandem mass spectrometric analysis revealed diagnostic product ions and neutral losses for C8-6-I, C8-6-N, and C8-6-C8. C8-6-N and C8-6-I are metabolized in HLM, RLM, and MLM. Furthermore, the metabolism of C8-6-N and C8-6-I is dependent on NADPH and active liver microsomes. The same metabolic profile was observed for C8-6-N and C8-6-I in HLM, RLM, and MLM. Consequently, mouse and rat may be useful models for future animal studies of C8-6-I and C8-6-N. A mass spectrometric fingerprint was generated for C8-6-I, C8-6-N, and C8-6-C8. The established tandem mass spectrometric fingerprint will be used for the qualitative and quantitative analysis of these dimer compounds.

Abstracts

Oral presentations / Session 4

Targeting Protein Function Using Natural Product Structure-Activity Relationship (SAR) Studies and Proteolysis-Targeting Chimeras (PROTACs)

Darren Derksen[*#]

Alberta Children's Hospital Junior Chair in Medicinal Chemistry, Department of Chemistry, University of Calgary

Targeting protein function or protein-protein interactions is a central theme in medicinal chemistry and drug design. Modulation of TRP channels has been shown to impact pain signaling in animal models with examples now in clinical trials. This presentation will showcase recent work in our group exploring structure-activity relationships on natural products that interact with TRP channels, particularly TRPM8. In the course of this study, we have developed photo-rearrangement chemistry as a route to desired natural product targets. The traditional approach of developing protein inhibitors has been less successful when targeting proteins that lack a clear active site and are non-enzymatic. Recent work from pharmaceutical companies and research groups around the world, including the Derksen group, have demonstrated that PROTACs offer a potential solution through degradation of challenging protein targets. By synthesizing heterobifunctional PROTAC molecules capable of inducing close proximity of target proteins and the cellular ubiquitination machinery, we have shown that an important anti-apoptotic protein MCL1, can successfully be degraded. Our recent efforts in the design and synthesis of PROTACs capable of chemically inducing degradation of MCL1 will be described.

Understanding the Substrate Scope of a Promiscuous Aldolase and its Application Towards Generating Substituted Quinolines

Douglas Fansher[#], Richard Granger and David R. J. Palmer[*]

Department of Chemistry, University of Saskatchewan

Quinolines are a useful starting scaffold for organic synthesis and can be synthesized through many name reactions that all use different starting materials and approaches. However, most of these reactions require the use of high temperatures, strong acids and expensive starting materials to generate the corresponding quinolines. Enzymes can carry out reactions under mild conditions and allow a high degree of stereoselectivity and enantioselectivity that is often difficult to achieve with traditional synthesis. We have examined the substrate scope of an aldolase and observed that many substituted aromatic aldehydes could be accepted with either pyruvate or ß-fluoropyruvate as the nucleophile, resulting in either the a,ß-unsaturated ketone or the fluorinated aldol product, respectively. Aldolase-catalyzed reaction of pyruvate with o-aminobenzaldehydes gave products that spontaneously cyclized to generate substituted quinolines. The use of this promiscuous enzyme allows production of 3 different types of products depending on the starting materials used and demonstrates its application for chemoenzymatic production of substituted quinolines from easily-obtained starting materials.

Amino acid functionalized nanodiamonds for gene delivery: Remodelling of functionalization design to improve gene transfection efficiency

Saniya Alwani , Raj Rai, Jackson M. Chitanda, Larhonda Sobchishin, Deborah Michel, Chithra Karunakaran, Narayana Appathurai, Ronald E. Verrall and Ildiko Badea

College of Pharmacy and Nutrition, University of Saskatchewan, Canadian Light Source, Veterinary College Imaging Facility, Department of Chemistry

Purpose: This study focuses on improving the efficacy of amino acid functionalized nanodiamonds (NDs) as gene carriers. Lysine-NDs (lys-NDs) were designed to facilitate gene delivery. Remodelling on functionalization design was carried out by introducing histidine onto the system to facilitate intracellular endosomal escape of diamoplexes (functionalized NDs/siRNA complexes).
Methods: Covalent conjugation of lysyl-histidine amino acid moiety on the surface via an amine terminated 3 carbon chain linker. Lysyl-histidine NDs (lys-his-NDs) were characterized in comparison with lys-NDs via size and zeta potential measurements, infrared spectroscopy (IR) and thermogravimetry (TGA). Surface loading was calculated by thermogram. Formulation development was performed to determine the optimum ultrasonication time for producing a uniform dispersion. Gene binding capacity and biocompatibility were analysed by gel retardation and MTT assays respectively.
Results: Mechanistic studies revealed clathrin mediated endocytosis and macropinocytosis as the main pathways for the internalization of diamoplexes. Endo-lysosomal entrapment of diamoplexes was identified as a major limiting factor for effective gene transfection. Therefore, lys-his-NDs was synthesized and evidenced via IR spectra showing distinct amide peaks of N-H (stretch) at ~ 3500 cm-1 and C=O (stretch) at ~1690 cm-1. Surface loading was calculated to be 14.49 mmoles/gm conferring to the presence of a larger combinatorial moiety on the surface in comparison to lys-NDs (1.97 mmoles/gm). Lys-his-NDs also produced a relatively stable dispersion having highest volume % of particles sized ~68-78 nm and zeta potential of +30.5 mV. Lys-his-NDs showed concentration dependent binding with the genetic materials. They were also found biocompatible at a wide range of concentrations both in presence and absence of serum proteins.
Conclusion: Remodelling of NDs with lysyl histidine functionalization was capable in maintaining the dispersion stability, gene binding efficiency and biocompatibility of the carrier system. It is expected that histidine will facilitate the intracellular escape of diamoplexes from the endo-lysosomal system to enhance gene expression.

Breakfast Workshop for Trainees

Katie Maloney

Northern Vine Labs, Langley, BC

Katie Maloney is the Senior Scientist, Chemistry at Northern Vine Labs, a laboratory facility specialized in the chemistry and quality control testing of cannabis. On the eve of the legalization of cannabis in Canada, Ms. Maloney will discuss some of the key issues associated with quality control and the safe supply of legal cannabis, and what the general public should know about the quality of their cannabis products. A University of Saskatchewan graduate (B.Sc., Chemistry (2006); M.Sc., Pharmacy (2010)), Ms. Maloney has been working in industry in and around the Vancouver area for over 9 years. Having joined the work force in the midst of a global recession, Ms. Maloney found a way harness some unusual positions into an exciting role at Northern Vine Labs, and will share experiences and lessons learned along the way.

Abstracts
Oral presentations / Session 5

Structure-Activity Relationship of Neurotrophic Phenylbutenoid Dimers and Progress Towards Uncovering Mechanism of Action

Florence Williams*, Khyati Gohil, Mohd Zain Kazmi
University of Alberta

Neurodegenerative diseases (Alzheimer's, Parkinson's, etc) are characterized by unusually high levels of neuronal cell death in the brain. As such, a potential avenue to treat any neurodegenerative disease is to activate pro-survival and proliferative signaling pathways to counteract the stress elicited by the disease. Compounds or agents which trigger this type of anti-apoptotic pro-growth response are deemed "neurotrophic." Two phenylbutenoid dimers, hereafter referred to as PBD1 and PBD2, originating from a Javanese ginger (Zingiber purpureum), have shown promising neurotrophic responses in primary rat neuron cell culture. PBD1 and PBD2 increase both cell viability and neurite outgrowth. Moreover, oral administration to olfactory bulbectomized mice (a depression model characterized by neurodegeneration) resulted in increased neurogenesis. Despite these properties, PBD1 and PBD2 have no known binding target and there has been no investigation of mechanism of action. This talk will outline our synthetic access of PBD1 and PBD2, as well as derivatives, which have allowed us to test some structure-activity relationships in these molecules. We have also begun to examine potential mechanisms of activity.

FOXM1 inhibitors: Emergence of a neglected binding interaction

Seyed Amirhossein Tabatabaei Dakhili[#], David J.Perez, Keshav Gopal, John R. Ussher, Carlos A. Velazquez-Martinez*
Faculty of Pharmacy and Pharmaceutical Sciences, Katz Group-Rexall Centre for Pharmacy & Health Research, University of Alberta

The Forkhead boX M1 (FOXM1) is a transcription factor essential for normal activation of the cell cycle and replication. However, increasing evidence suggests that an overexpression of this protein is associated with cancer development and poor patient prognosis which makes it a promising drug target in medicinal chemistry. Based on a previous molecular modeling protocol reported by our group, in which we hypothesized that the FOXM1 inhibitor (FDI-6) binds to the FOXM1 DNA binding domain (DBD) mainly by (i) a pi-sulfur interaction with His287, and (ii) a halogen bonding with Arg297 within the FOXM1 DNA binding domain. To test this hypothesis, we used the structure of FDI-6 as a lead scaffold, and we synthesized a series of new derivatives by removing and replacing different groups at the 4-fluorophenyl position and swapping a sulfur atom with nitrogen and oxygen. Then, we determined the level of nuclear FOXM1 expression using a triple negative breast cancer cell line (MDA-MB-231), followed by a screening assay using recombinant FOXM1-DBD (ElectroMobility Shift Assay; EMSA). Next, using a site-directed mutagenesis technique, we confirmed the mechanism of action exerted by these molecules. The results presented in this investigation provide essential insights to elucidate the overall mechanism of action exerted by other FOXM1 inhibitors targeting this protein's DNA binding domain.

Effects of Gongronema latifolium methanolic leaf extracts on viability & pro-/anti-inflammatory properties of macrophages

Anjali Goel[1#], Ping Jia[2], Chukwuebuka Onyema[1], Jude Uzonna[2], Athar Ata[1], Michael Eze[1*]

1. Department of Chemistry, University of Winnipeg
2. Department of Immunology, Rady Faculty of Health Sciences, University of Manitoba

Gongronema latifolium, leaf extracts are reported to have antioxidant, anti-bacterial, pro-/anti-inflammatory and other health-promoting properties. It is used in Nigerian folk medicine and as a dietary vegetable. This study investigated the effects of methanolic crude leaf extracts of *G. latifolium* on viability, and pro-/anti-inflammatory properties of macrophages. Many inflammatory agents like nitric oxide (NO), and cytokines (TNF-, IL-6, IL-12p40), are agents of innate immunity and inflammation, and help to control infectious pathogens. The retrovirus immortalized bone marrow-derived murine macrophage cell lines (ANA-1 cells) were cultured for 24-48 hours in complete RPMI-10 medium with or without varying concentrations of methanolic extracts of *G. latifolium*, and cell viability was assessed by XTT assay and flow cytometry. Some cultures were also stimulated with lipopolysaccharide, a well-known innate activator of macrophages and inducer of NO, and proinflammatory cytokines (IL-6, IL-12 and TNF-) were determined by ELISA. The data revealed that *G. latifolium* leaf extracts are not toxic at concentrations ranging from 0.05 to 1µg/ml, after stimulation for 24 and 48 hr. However, 100 µg/ml after 48 hr was toxic. Furthermore, *G. latifolium* leaf extracts also showed pro-inflammatory properties as they augmented nitric oxide production.

Engineered and functionalized nanodiamond particles for drug/gene delivery: biodistribution studies

Raj Rai[#], Saniya Alwani, Ed Krol, Humphrey Fonge and Ildiko Badea.

College of Pharmacy and Nutrition, University of Saskatchewan

Purpose: To assess biodistribution of functionalized nanodiamonds, to elucidate their fate at organ levels and monitor their pharmacokinetics. A suitable chelating agent (deferoxamine, DFO) was attached to the functionalized nanodiamonds to assist with radio labelling with 89Zr to study the biodistribution in animal models.
Method: 1HNMR was used to confirm synthesis of amino acid conjugates at all steps. The lysyl-NDs were characterized for size and zeta potential measurements and thermogravimetric analysis. Lysine NDs were tagged with p-SCN- DFO and labeled with 89Zr for test labeling to confirm the DFO conjugation. The labeled lysine, lysyl-histidine NDs will be used for positron emission tomography in animal models to study biodistribution.
Results: Several batches of lys-NDs revealed a surface loading of 1.67 mmoles/gm of ND. The average particle size was 66 nm and zeta potential of +21 mV, showing consistency in all batches and validating the reproducibility of the designed protocol. 1HNMR revealed that the tagged NDs show all DFO protons of the aliphatic chain along with 4 protons of the benzene ring in the aromatic region, confirming the DFO-lysine-linker conjugation. Similarly, lysyl-histidine chemical moiety was synthesized and confirmed using 1HNMR through characteristic peaks at d 7.34, d 7.45 (t, 2H, imidazole side chain) as well as presence of 3 Boc protecting groups at d 1.4 (s 27H). The p-SCN-DFO tagged lysine NDs showed positive result in test labeling confirming the conjugation of DFO with lysine NDs.
Conclusion: This study establishes that lysyl- and lysyl-histidine functionalized NDs, as potential carriers for gene therapy, can be labeled with radiotracers for assessment of biodistribution and pharmacokinetic analyses. Understanding the in vivo behavior of the functionalized NDs is critical for their translation from benchtop to clinical applications.

Radioimmunotherapy of cancer and infections with alpha-particles emitting radionuclides

Ekaterina Dadachova
College of Pharmacy and Nutrition, University of Saskatchewan

The use of targeted therapy with a-particles emitters in oncology is burgeoning worldwide. This is driven by the advantages of a-emitters over ß-emitters, including very specific targeting of the diseased cells due to the a-particles' short 50-80 µm tissue range, and increased killing efficiency due to high linear energy transfer. This results in a controlled therapeutic modality with minimal normal tissue effects. Radioimmunotherapy (RIT) with a-emitters does not depend on the oxygenation status of the tumor, and a-therapy can break tumor resistance to chemotherapy, external beam radiation therapy, and even to ß-radiation therapy. In our laboratory we are investigating novel antigens and antibodies for using a-RIT to treat melanoma and pancreatic cancer. Several years ago we were the first to translate a-RIT approach into the field of infectious diseases for treatment of opportunistic fungal infections, multidrug resistant bacterial infections and HIV. The results of our pre-clinical and clinical work will be described.

Conjugation of Daratumumab with 225Actinium Greatly Increases its Antitumor Activity

Wojciech Dawicki[1#], Kevin Allen[1], Rubin Jiao[1], Mackenzie Malo[1], Keisha Thomas[2], Mark S. Burger[2], Dale L. Ludwig[2], and Dadachova Ekaterina[1*]
1. University of Saskatchewan
2. Actinium Pharmaceuticals Inc., New York, NY

Daratumumab is a human cytolytic antibody specific for CD38 that is used clinically for treatment of patients with multiple myeloma (MM). Current therapeutic regimens require multiple injections over months of treatment. Increasing the potency of daratumumab to shorten the length of treatment would be beneficial. 225Ac is an alpha-particle emitting radionuclide that has potent cytotoxic activities over short
distances, allowing for precise targeting of a lethal dose of radiation. Previously we have established that labeling daratumumab with 225Ac increased more than 10-fold its ability to kill MM cell lines in vitro. In this study we evaluated the ability of 225Ac-daratumumab to kill established tumors in mice. Mice deficient in T- and B-cells were injected subcutaneously with human MM tumor cells and once tumors reached an average volume of ~200 mm3, mice were treated with the 225Ac-daratumumab. To determine the localization of daratumumab within the tumor-bearing mice it was labeled with 111In, an γ-emitting radioisotope. 111In-daratumumab can be easily imaged and is used as a surrogate to estimate the localization of 225Ac-daratumumab. The distribution of the 111In-daratumumab was then followed for 10 days using a microSPECT/CT scanner. To evaluate the antitumor ability of the 225Ac-daratumumab, tumor-bearing mice were injected with 225Ac-daratumumab at a dose of of 400 nCi/0.3 µg of antibody, and 200 nCi/0.3 µg of antibody. As a control, mice were injected with either saline or an equivalent amount of unlabeled daratumumab. In addition, a group of mice was also treated with 30 times greater dose of unlabeled daratumumab (10 µg) - a dose which was previously shown to be effective against established tumors. 111Indaratumumab begun to accumulate in the tumor 24 hours after intraperitoneal injection and by 7 days was exclusively present in the tumor. The growth of the tumors in mice treated with 400 nCi/0.3 µg was significantly retarded compared to mice treated with equal concentration of unlabeled daratumumab or saline. Tumor growth was similar between mice treated with 400 nCi/0.3 µg of 225Ac-daratumumab and mice treated with 10 µg of unlabeled daratumumab. In conclusion, this study shows that labeling daratumumab with 225Ac increases its antitumor activity ~30-fold. This study suggests that approach to increase the potency of daratumumab via 225Ac labeling could greatly reduce the amount of daratumumab treatment needed in the clinic. This study also highlights the potential of targeting α-emitters to tumors as a viable therapeutic approach.

Fluorescent Cathepsin Substrates, their 18F-Labeled Equivalents for PET, and an Analogue Employing Doxorubicin

Brady G. Vigliarolo[#], Morshed A. Chowdhury, Shusheng Wang, Chris Phenix*
Department of Chemistry, University of Saskatchewan

Activity-based inhibitors remain dominant among PET tracers designed to image the activity of hydrolytic enzymes, and this is largely due to inherent immobilization at the site of enzyme activity. Alternatively, substrate-based PET tracers may offer the potential advantage of signal amplification compared to inhibitors which react with the target enzyme in 1:1 stoichiometry. However, trapping an enzymatically released and radiolabelled reporter group at the site of enzyme activity is a significant challenge to overcome when using a substrate-based approach. Toward the development of lysosomotropic reporter groups for PET imaging hydrolase activity using a substrate-based approach, we have designed novel fluorine-bearing quinolines which are highly fluorescent and sequester within lysosomes in live cell studies. Satisfyingly, peptide-based cathepsin B (CTB) probes bearing such reporter groups are extremely efficient substrates of our target protease. We present here the 18F radiosynthesis, kinetic efficiency/specificity, physiochemical properties, and live cell fluorescence imaging of such cathepsin substrates. Due to the roles of extracellular CTB in promoting tumor metastasis, we have also synthesized an analogous substrate bearing doxorubicin as a potential payload. Such probes and prodrugs would have valuable prognostic and therapeutic applications, while aminoquinolines may serve as versatile lysosomotropic reporter groups for PET imaging other hydrolase subclasses.

Abstracts

Poster presentations – Undergraduate Research

1.

Synthesis of diazirines to probe binding of dimer drugs to α-synuclein in vitro

Marissa Pirlot[#], Ed Krol*
College of Pharmacy and Nutrition, University of Saskatchewan

Alpha-synuclein is a protein found in the pre-synaptic terminal of nerve cells, and is ubiquitously expressed in the brain. Aggregates of alpha-synuclein have been implicated in the pathophysiology of Parkinson's disease. Previously, our Group (Kakish et al, 2016) designed dimer drugs linking caffeine to nicotine and 1-aminoindan, which bind to alpha-synuclein and prevent aggregation. However, specific binding sites on alpha-synuclein are unknown. Photoaffinity labelling, in which the ligand of interest possesses a photoreactive diazirine, can provide information about specific binding interactions between small molecules and proteins. Allowing the ligand-diazirine molecule to interact with alpha-synuclein then reacting the diazirine with UV light allows us to covalently trap the a-syn-dimer complex and determine the location of binding using protein mass spectrometry. We developed a general synthetic method for the tosylated diazirine, and we are currently working on incorporating the diazirine moiety on a theophylline molecule. Binding properties of the caffeine-diazirine will be investigated using ITC, ThioT aggregation and mass spec proteomics.

2.

Prospects for Novel Antibiotics from the Environment

Fatima Almira Feliciano Deuna[1,2#], Paul Holloway[1], Athar Ata[2]
1. Department of Biology, University of Winnipeg
2. Department of Chemistry, University of Winnipeg

Natural products isolated from microorganisms currently comprise the majority of clinical antibiotics, anti-fungal, and anti-cancer agents. Novel antibiotics are in great demand as gene transfer increase rates of antibiotic resistance among important bacterial pathogens. The discovery of new molecules with different chemical structures is integral for the continuous protection against evolved bacterial strains. Novel antibiotics are best found in novel microorganisms isolated from novel environments. In this research, we have isolated 390 bacterial strains from an under-explored environment, and tested these strains for growth inhibition of Gram-negative and Gram positive bacteria. Extractions and dilutions performed on samples were plated onto Tryptic Soy Agar (TSA), Actinomyces Isolation Agar, and Oat Bran Agar. Colonies were picked onto TSA before testing for growth inhibition against bacteria. Antimicrobial activities of novel isolates were tested by growing them on plate inoculated with either Gram-negative or Gram positive bacteria and observing zones of growth inhibition. We observed that 176 strains from a total of 390 strains tested inhibited the growth of one or more bacterial species. A total of 107 isolates inhibited the growth of Gram-positive bacteria and 75 isolates inhibited the growth of Gram-negative bacteria including 31 isolates which inhibited the growth of a Pseudomonas species. A number of isolates have been fermented in Trypticase Soy broth fermentations which were then extracted with XAD-7 and eluted with methanol. Several of the isolates produced an extractable and presumably small molecule inhibitor of bacteria growth. Thus it appears that under-explored environments are a good source of antibiotic producing microorganisms. Further investigation of the small molecule growth inhibitors will hopefully reveal antibiotics of novel structures.

Abstracts

Poster presentations – MSc. Research

3.

Alpha-synuclein Binding 18F Labelled Bifunctional Agents as Positron Emission Tomography Imaging Probes for Parkinson's disease

Omozojie P. Aigbogun[#], Christopher P. Phenix, Ed S. Krol.

Department of Chemistry, Drug Discovery and Development Research Group, College of Pharmacy and Nutrition, University of Saskatchewan

The misfolding, aggregation, and fibrillation of the intrinsically disordered protein alpha-synuclein is associated with the pathophysiology of Parkinson's disease. Recently we have developed novel compounds containing caffeine linked to nicotine or 1-aminoindan via a 6-carbon chain (C (8)-6-N and C (8)-6-I respectively) that bind to alpha-synuclein and protect yeast cells from alpha-synuclein mediated toxicity. The alpha-synuclein binding properties of these dimers may also be useful as a non-invasive diagnostic tool for Parkinson's disease. Our research objectives are to develop and optimize synthetic routes to incorporate 18F into several of the dimers (C(8)-6-N, C(8)-6-I) and use these 18F-labelled compounds as imaging probes for Positron Emission Tomography (PET) and to assess their biodistribution in vivo. Our initial proposed method for the synthesis of C (8)-6-N-18F involved adapting our method for preparing the unlabeled dimer, which required lithiation of 3-bromopyridine. We planned to carry out a fluoride-mediated nitro displacement from the pyridine ring of nicotine, which necessitated the presence of a nitro group on the pyridine ring. However, initial lithiation reactions using 3-bromo-2-nitro pyridine or 3-bromo-5-nitro pyridine and deprotonation of the ortho-proton on 2-nitro pyridine using LDA were unsuccessful. We hypothesize that the position and electron withdrawing properties of the nitro group might have some influence on the poor lithiation and deprotonation reactions. Our new approach for the C (8)-6-N-18F is to label the dimer with an alkyl fluoride on the caffeine moiety. In order to achieve this for C (8)-6-N-18F and C (8)-6-I-18F, we have set up a control reaction to label caffeine with an alkyl fluoride using Theophylline as the starting material. 19F-labelled analogues will be initially prepared and assessed for their binding to alpha-synuclein through nanopore and isothermal titration calorimetry methods to determine the influence of the fluorine on the labelled compounds.

4.

The establishments of tandem mass spectrometric fingerings of phytosterols and tocopherols and the development of targeted profiling strategies in canola seed oil deodorizer distillate

Kang Jiang[1#], George Gachumi[1], Bryn Shurmer[2], Zafer Dallal Bashi[3] and Anas El-Aneed[1*]

Drug Design and Discovery Research group, College of Pharmacy and Nutrition, University of Saskatchewan.

Melanoma is the deadliest form of skin cancer due to its high propensity of metastasis and limited treatment options. The aim of this work is to develop radiolabeled antibody-targeted gemini nanoparticles for imaging and treatment of melanoma. Gemini lipid nanoparticles were developed and characterised. The melanoma-specific anti-GPNMB antibody Fab fragment was chosen as the targeting moiety and the alpha emitter (111-Indium) was loaded to the nanoparticles utilizing DOTA as a chelator. The stability of the bifunctional nanoparticles was assessed and the cellular uptake was investigated in two melanoma cell lines. The size of gemini nanoparticles was in the optimal range for cellular uptake (50-200 nm). Confocal microscopy proved the internalization of the fluorescent dyed nanoparticles. Fab-binding study using flowcytometry exhibited the specific binding of the targeted nanoparticles to RPMI cells. The radiolabeled nanoparticles was stable over 168 hours with only 5% loss of 111-Indium at the end of the study. Radiometric measurements showed that the targeted and non-targeted nanoparticles have similar binding rates of 33.8% and 34.2%, respectively. However, internalization of the targeted nanoparticles was higher at 13.6%, compared to 7.2% for the non-targeted nanoparticles. These findings demonstrated that the radiolabeled gemini nanoparticles are promising for image-guided radiotherapy of melanoma.

5.

Phytochemical studies on *Curcuma longa*

Hadeel Alhazmi[1#] and Athar Ata[1*]
Department of Biology, University of Winnipeg

Plant natural products have played an important role in providing new lead bioactive compounds to the drug discovery program. Plants for discovering new bioactive agent is selected using different approaches including ethno-medicinal applications, reported bioactivities, random screenings and bioinformatics. *Curcuma longa* belongs to the family Zingiberaceae is found in India, Nepal, and Bangladesh. This plant is widely used in the health care system of this country. For instance, the powder of its roots is used to treat stomach disorders, inflammation, and treat wounds. It has also applications in treating joint pain, cough, diabetes, hepatitis, anorexia, to name just a few. Previous phytochemical reports on this plant have shown the presence of terpenoids, flavonoids, and curcuminoid. Our recent phytochemical investigation of the extract of this plant resulted in the isolation of a few natural products. Their isolation, structure elucidation with the aid of extensive NMR spectroscopic studies and their bioactivity data will be presented in this poster presentation.

Poster presentations – PhD Research

6.

Discovering Enzymes for Synthesis of Antibiotics Related to Kanosamine

Theerawat Prasertanan[#], David A.R. Sanders, and David R.J. Palmer*
Department of Chemistry, University of Saskatchewan

Our purpose is to design phytosterols and tocopherols obtained from canola oil deodorizer distillate (i.e. waste stream) into functional food. Phytosterols have well-established cholesterol lowering effect while tocopherols are natural antioxidants. However, development of functional food containing these bioactives have been challenging due to their lipophilicity, heat and light sensitivity. The cholesterol-lowering effect of phytosterols is greatly affected by formulation approaches. Thus, the aim of this study is to design liposomal formulation of phytosterols and tocopherols in orange juice to solve formulation challenges. The long-term goal is to assess the cholesterol-lowering efficacy of phytosterol-containing juice in hypercholesteremic individuals. Three different techniques were adopted for the formulation of liposomes namely thin layer hydration homogenization, thin layer hydration ultrasonication and heating method. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) validated quantification method was developed to determine the incorporation efficiency of these bioactives into liposomes. The particle sizes were 192±1nm and 195±1nm for homogenization and ultrasonication, respectively. The particle size employing the heating method was significantly higher at 258±1nm. Liposomes formulated from all three techniques showed similar zeta potential (-15mv to – 18mv). LC-MS/MS analysis showed incorporation efficiency greater than 90 % for both homogenization and heating methods. Optimum liposomes incorporated in simulated orange juice showed adequate stability during storage time of 45 days after pasteurization. In sum, we formulated phytosterols and tocopherols obtained from canola oil waste stream into liposomes and prepared fortified orange juice. In future, we will conduct clinical trials to assess cholesterol-lowering efficacy in hypercholesteremic individuals.

7.

Kinetic and computational evaluation of the allosteric site mutants of Mycobacterium tuberculosis UDP-Galactopyranose Mutase

Nataliya Zalatar# and David A. R. Sanders
Department of Chemistry, University of Saskatchewan

The enzyme UDP-galactopyranose (UGM) is critical for the biosynthesis of the cell wall of Mycobacterium tuberculosis, the causative agent for TB. Recently, an inhibitor (MS-208) proposed to bind to a novel allosteric site of MtUGM, has been identified. This project involves identification of the factors that influence the binding of MS-208 to MtUGM, and determination of the structure of UGM complexed with the inhibitor. However, MS-208 has a low solubility that may prevent determination of the crystal structure of MtUGM and the inhibitor. Therefore, some modifications of MS-208 for improving its binding and solubility will be proposed. Mutations have been designed to probe the binding of MS-208 to the allosteric site. Kinetic and computational analysis of the mutants have been carried out and the results will be described.

8.

α-Glucosidase Inhibiting Natural Products from *Chromolaena odorata*

Chukwuebuka ThankGod, Onyema [1,2#] Vincent I.E Ajiwe[2], Athar, Ata[1*]
1. Department of Chemistry, University of Winnipeg
2. Department of Pure and Industrial Chemistry, Nnamdi Azikiwe University, Awka, Anambra State Nigeria

Regardless of the alarming and persistent increase in the number of people globally affected by the Type II diabetes, there seems to be less natural curative measures except insulin and oral hypoglycaemic drugs (such as acarbose and magilitol) available to assist in tackling this issue. Both of these drugs work by inhibiting the activity of a-glucosidase, an enzyme involved in type II diabetes. *Chromolaena Odorata*, a medicinally important plant, is used to treat type II diabetes symptoms by traditional healers in Nigeria. The crude extract of this plant was active against a-glucosidase in our bioassay. Our recent phytochemical investigation of this plant resulted in the identification of flavonoids. In this presentation, isolation and structure elucidation of isolated compounds with the aid of detailed one - and two dimensional NMR spectroscopy will be discussed. Additionally, we will also discuss the bioactivity of these phytochemicals.

9.

Combining EXAFS Spectroscopy, DFT Modeling, and Radiochemical Assays to Improve the Design of New Radiometal Chelators

Elaheh Khozeimeh Sarbisheh, Akam Salih[#], Eric W. Price
Department of Chemistry, University of Saskatchewan

Positron Emission Tomography (PET) imaging is a critical tool for early diagnosis of disease. Zirconium-89 features a half-life matched to antibody biovectors (78.4 hours), favorable decay characteristics for PET imaging, and routine production at a growing number of cyclotron sites world-wide. However, successful use of zirconium-89 requires extremely stable chelation. Since the gold-standard chelator for zirconium-89 — desferrioxamine (DFO) — provides a sub-optimal six oxo-coordination sphere, new BFCs with eight oxo-donor sites have been of interest. We are designing new BFCs and more importantly investigating the use of EXAFS spectroscopy and DFT computational methods towards the elucidation of the possible coordination spheres of our metal-chelate complexes. A series of new high-denticity (CN=12) chelators have been synthesized and the complexation of these new chelators with non-radioactive metal ions including Zr(IV) and Ti(IV) are being investigated via DFT and EXAFS. Preliminary zirconium-89 radiolabeling and stability assays have demonstrated that zirconium-89 complexes of DFO2 and several of its derivatives are more stable than DFO. Synthesis of bifunctional derivatives of DFO2-type chelators are ongoing to facilitate antibody conjugation and in vivo stability evaluation, where we hope to find a predictive link between our spectroscopic and computational studies and in vivo performance.

10.

Synthesis and evaluation of enzyme-cleavable linker modifications to guide the design of Antibody Drug Conjugates

Moralba Dominguez Garcia[#], Brady Vigliarolo, Eric W. Price* and Christopher Phenix*
Department of Chemistry, University of Saskatchewan

Antibody drug conjugates (ADCs) have emerged as an effective cancer treatment due to the high specificity and affinity to recognize cancer-associated receptors, delivering the drug into the tumour microenvironment. Often, ADC linkers are cleaved by Cathepsin B (CTB), a protease that is overexpressed in metastatic cancers releasing the drug inside or adjacent to cancer cells. However, the effects of linker structure on cellular uptake and CTB hydrolytic rates are usually not evaluated, which could lead to side effects and inefficacy. We are currently synthesizing eight probes that mimic ADC behavior using a CTB sensitive fluorescent reporter instead of a drug to evaluate how linker length, attachment group, and substrate recognition element affects CTB hydrolysis and protease selectivity. Starting from prodrug inspired-probes developed in the Phenix laboratory selective to CTB, two novel probes have been synthesized and conjugated to a protected cysteine for initial enzymatic assays. Following these assays, the probes will be conjugated to Nimotuzumab, an epidermal growth factor receptor antibody, via cysteine attachment to test cellular uptake and hydrolysis rates in vitro. The more selective and stable probes will be radiolabeled and evaluated in vivo in murine models of aggressive cancers for biodistribution, PET imaging, and optical imaging studies.

11.

Inhibitors of Mycobacterium tuberculosis UDP-Galactopyranose Mutase (MtUGM), a potential antimicrobial drug target

Dalia M. Ahmed# ; Nataliya Zalatar ; David A. R. Sanders*
Department of Chemistry, University of Saskatchewan

Mycobacterium tuberculosis, the causative agent of tuberculosis, has developed multiple antibiotic resistance mechanisms and therefore requires novel therapeutic strategies. UDP-galactopyranose mutase (UGM) is an essential enzyme for M. tuberculosis, involved in bacterial cell wall synthesis; represents an attractive potential drug target. MS-208 was identified as an allosteric inhibitor of MtUGM with micromolar range inhibitory activity. A model to better understand the binding pattern of MS-208 within the allosteric site is needed for prospective structure-based drug design. Our research focuses on the development and testing of new MS-208 analogues. First generation of MS-208 analogues were designed based on in silico docking studies. Design, synthesis and kinetic analysis of these analogues will be presented. These results will help to establish structure-activity relationship, and enable us to design further generations of inhibitors.

12.

Possible Molecular Mechanism for the Regulation of Lipid Metabolism by Lignan Enterolactone: Link to its Anti-cancer Effects.

Franklyn De Silva#, Xiaolei Yang*, Ahmed Almousa*, Ahlam Hawsawai*, and Jane Alcorn*
Drug Discovery and Development Research Group, College of Pharmacy and Nutrition, University of Saskatchewan

Hypocholesterolemic agents, seems promising when used in combination with standard anticancer therapies. Interestingly, flaxseed (FLNs) lignans, can reduce serum and hepatic cholesterol. Lignans are capable of interfering with cancer cell survival and progression. Cancer cells exhibit elevated lipid metabolism. A link between FLNs anti-cholesterolemic and anti-cancer effects might exist through its ability to modulate lipid metabolism and ER stress. Therefore, identifying the molecular mechanism responsible for this is of value.
Hypothesis: Lignan, enterolactone (ENL) and its glucuronide metabolite (ENL-G) modulate cellular lipid metabolism and causes ER stress to sensitize cancer cells (dysregulated proliferation) to clinically relevant chemotherapeutic agents. ENL but not ENL-G caused significant cytotoxicity in cancer cell lines. Results revealed ENL as a PPARγ partial agonist. ENL modulated lipid metabolism targets, increased ER stress markers, reduced mitochondrial redox function, and caused mitochondrial toxicity. ENL sensitized anticancer drugs. Cells treated with ENL/ENL-G revealed reduced cholesterol uptake. Increased PPARγ activates INSIG1 a negative regulator of cholesterol synthesis. These findings warrant further investigations to support FLN's ability to modulate ER stress as the key mechanism involved in the disruption of dysregulated cellular signaling. ER stress and PPARγ mediated signaling can influence lipid metabolism and therefore are relevant targets in drug discovery.

13.

High-resolution X-ray Structures of an Allosteric Site Mutant of Campylobacter jejuni Dihydrodipicolinate Synthase in Complex with Natural and Synthetic Allosteric Inhibitors

Sagar Saran[#], Yulia Skovpen, David R. J. Palmer, and David A. R. Sanders*
University of Saskatchewan

Campylobacter jejuni (C. jejuni) is a gram-negative bacterium that is responsible for many of the gastroenteritis related human deaths each year. Potential antibiotics can be developed against this bacterium by targeting the cell wall. In C. jejuni, the dihydrodipicolinate synthase (DHDPS) tetrameric enzyme is the first committed step for the biosynthesis of lysine in the diaminopimelate pathway and is an antibiotic target. Biophysical studies have shown that C.jDHDPS is allosterically inhibited by lysine, and the synthetic inhibitor bislysine, but the structural determinants responsible for transmission of such allosteric inhibition signals are poorly understood. The crystal structures of C.jDHDPS bound with the inhibitors have revealed key residues required for binding of the inhibitor to the allosteric site, including a histidine residue that forms a direct hydrogen bonds to the inhibitor. Mutation of this residue to aspargine resulted in a mutant minimally inhibition by lysine but strongly inhibited by bislysine, despite these inhibitors binding in near-identical fashion. The research presented in my study shows the first high-resolution crystal structures of this mutant in the presence and absence of lysine and bislysine, revealing the precise effects of inhibitor binding on the protein structure.

14.

Phytochemical studies on *Carissa opaca*

Sania Izhar[1,2#], Dildar Ahmad[2], Athar Ata[1*]

1. Department of Chemistry, University of Winnipeg
2. Department of Chemistry, Forman Christian College, Lahore, Punjab, Pakistan

Emergence of new diseases and development of complications in the old ones, coupled with safety and efficacy factors associated with existing medicines, have necessitated continuous quest for new remedies. Plants being a virtually unending reservoir of potential bioactive natural products hold great hope for more effective, safer and affordable therapeutic agents. *Carissa opaca*, a medicinally important plant, is found in Pakistan. This plant is used to treat various ailments including jaundice, hepatitis, asthma, microbial infection, cough, diarrhea and fever. Our recent phytochemical investigation of the crude extract of this plant resulted in the isolation of coumarins, flavonoids and terpenoids. In this presentation, isolation and structure determination of these recently isolated natural products along with their bioactivity data will be presented.

15.

The development of targeted mass spectrometric method to assess the cellular uptake and distribution of gemini surfactants used as gene delivery agents

Wei Jin#[1], Mays A. Al-Dulaymi[1], Scot C. Leary[2], Ildiko Badea[1], and Anas El-need[1]*

1. Drug Design & Discovery Group, College of Pharmacy and Nutrition, University of Saskatchewan
2. Department of Biochemistry, University of Saskatchewan

Gemini surfactants are promising molecules used as non-viral gene delivery vectors. However, little is known about their biological fate (i.e., cellular uptake, distribution and metabolite formation). A simple and sensitive flow injection analysis-tandem mass spectrometric method (FIA-MS/MS) was developed to evaluate the cellular uptake and distribution of three lead gemini surfactants. The end goal is to possibly link the efficiency/toxicity to their cellular fate. Cultured PAM 212 epidermal keratinocytes were treated with three gemini surfactant/DNA nanoparticles. The treated cells were collected at various time points and the nuclear, mitochondrial, plasma membrane and cytosolic fractions were isolated by differential centrifugation. The gemini surfactants in each subcellular fraction were then extracted and quantified using the FIA-MS/MS method, which was fully validated according to the USFDA guidelines. The method was developed using positive electrospray ionization in multiple reaction monitoring mode on a triple quadrupole-linear ion trap (4000 QTRAP®) instrument. Deuterated internal standards were used to correct for matrix effects and variations in the ionization. Isotope dilution standard curves were established to calculate the concentrations of the gemini surfactants. The precision, accuracy, recovery and stability were all within the acceptable ranges as per the USFDA guidelines. The method was superior to existing liquid chromatographic (LC)-MS/MS methods in terms of sensitivity and time of analysis. The results showed variations in the cellular uptake among the various gemini surfactant structures, which explained the differences in their transfection efficiencies. The subcellular data showed variable accumulations in the nuclear fractions that were correlated with the measured toxicities of gemini surfactants.

Poster presentations – Postdoctoral/Associate Research

16.

Quantitative Analysis of Phytosterols and Tocopherols in Canola Oil Deodorizer Distillate (CODD) Using Liquid Chromatography-Atmospheric Pressure Chemical Ionization-Tandem Mass Spectrometry (LC-APCI-MS/MS).

George Gachumi[1#], Asmita Poudel[1], Zafer Dallal Bashi[2], Ildiko Badea[1] and Anas El-Aneed[1*]
1. College of Pharmacy and Nutrition, University of Saskatchewan
2. Ministry of Agriculture, Government of Saskatchewan

Canola is a major product cultivated in Canada and among the most consumed vegetable oils in the world. Saskatchewan accounts for more than 50% of the national production. Consumption of canola oil has been associated with various health benefits, due to the presence of bio-active metabolites namely, phytosterols and tocopherols (vitamin E). Despite their presence in oil, a significant portion of these bio-actives are lost during the refining process, particularly in the deodorization step where a deodorizer distillate is formed. Canola oil deodorizer distillate (CODD) is currently treated as waste or low value animal feed. However, CODD is highly pre-concentrated with these bio-actives and can be utilized as a natural source of phytosterols and tocopherols. Thus, effective extraction strategies should be developed. Quantitative analysis of these bio-actives is critical in assessing their content in CODD and in the extract. Therefore, a validated LC-APCI-MS/MS method was successfully developed for the simultaneous determination of these bio-actives in both hot- and cold-pressed CODD. The method was simple and fast (6.5 min), with a linear range of 0.05-10 µg/mL, and low detection limits of 5 ng/mL. Total phytosterols (brassicasterol, campesterol, and beta-sitosterol) and total tocopherols (gamma and alpha) were found to be 8.9% and 1.8% for hot-pressed, and 8.7% and 1.7% for cold-pressed CODD, respectively. A phytosterol extract from hot-pressed CODD had a yield of 7.6% at a purity of 81% and whose composition was determined to be; brassicasterol 22%, campesterol 25%, and beta-sitosterol 34%.

17.

Development of FOXM1 inhibitors as potential theranostic agents: initial steps in the validation of FOXM1 as a positron emission tomography (PET) probe for triple negative-breast cancer detection

David J. Perez[1#], Seyed Amirhossein Tabatabaei Dakhili[1], Cody Bergman[2], Stephanie Mattingly[2], Frank Wuest[1], Carlos A. Velazquez-Martinez[1*].
1. Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta
2. Department of Oncology, University of Alberta

The FOXM1 transcription factor controls the expression of essential genes related to cell cycle progression and cell replication; under normal physiological conditions its expression is significantly decreased in terminally differentiated cells, but it is abnormally activated in most (if not all) malignant cells. During the last three years, our research group has worked on the development of novel (still experimental) FOXM1 inhibitors. We hypothesize that binding interactions exerted by FOXM1 inhibitors could not only inhibit its transcriptional activity, but also serve as PET-based imaging probes, specifically, 18F-based imaging. With this purpose, we have synthesized a new series of FOXM1 inhibitors derived from FDI-6, a drug molecule reported back in 2014 by Gormally et al and selected the drug FDI-AF, which was able to dissociate the FOXM1-DNA complex using an electrophoretic mobility shift assay (EMSA; IC50 = 46.4 ± 1.19 uM, Ki = 22.2 ± 0.56 uM), and also exerted a time dependant downregulation of FOXM1 in a triple negative-breast cancer cell line (MDA-MB-231), as determined using a western blot. In summary, we submit a set of preliminary results suggesting that it might be possible to use transcription factors to develop dual acting therapy/diagnostic (theranostic). In this presentation we will describe the chemical synthesis using 18F-labeled groups, and the initial screening in vitro.

18.

Predicting protein-ligand binding modes and affinities using consensus docking and adaptive sampling strategies

Jordane Preto[#], Jack A. Tuszynski
University of Alberta

Computational methods to efficiently and accurately predict the binding mode and affinity of a molecular compound to its specific protein target are of paramount importance to drug discovery. By helping identify appropriate molecular candidates to inhibit or activate specific protein functions, such techniques may save an incredible amount of time and resources in the long process of new drug development. Methods like molecular docking or ligand-based drug design, including qualitative structure-activity relationship models (QSAR), have the benefit of having a low computational cost, making them suitable for virtual screening approaches. However, these methods usually lead to inaccurate estimates of the binding energies and poor information on the binding modes. Alternatively, advanced sampling techniques based on molecular dynamics (MD) simulations, can predict ligand affinities more precisely. Although such computational techniques look promising, they generally require a long computational time assuming the correct binding mode of the ligand or even a good reaction coordinate of the system is known beforehand. Here, different strategies based on consensus docking and adaptative sampling are suggested in order to rapidly predict the correct binding modes and binding energies characterizing protein-ligand complexes.

WCMCW Attendees

Dalia Ahmed
University of Saskatchewan

Omozojie Aigbogun
University of Saskatchewan

Akay Akohwarien
University of Saskatchewan

Hadeel Alhazmi
University of Winnipeg

Kevin Allen
University of Saskatchewan

Saniya Alwani
University of Saskatchewan

Ildiko Badea
University of Saskatchewan

Kate Dadachova
University of Saskatchewan

Gabriel Dalio Bernardes da Silva
University of Saskatchewan

Rebecca Davis
University of Manitoba

Wojciech Dawicki
University of Saskatchewan

Frank De Silva
University of Saskatchewan

Darren Derksen
University of Calgary

Fatima Almira Deuna
University of Winnipeg

Moralba Dominguez Garcia
University of Saskatchewan

Anas El-Aneed
University of Saskatchewan

Michael Eze
University of Winnipeg

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University of Saskatchewan

George Gachumi
University of Saskatchewan

Francesco Gentile
University of Alberta

Anjalia Goel
University of Winnipeg

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University of Winnipeg

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University of Saskatchewan

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University of Saskatchewan

Ed Krol
University of Saskatchewan

Robert Laprairie
University of Saskatchewan

Chang-Chun Ling
University of Calgary

Mohadeseh Majdi Yazdi
University of Saskatchewan

Katie Maloney
Northern Vine Labs

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University of Saskatchewan

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University of Saskatchewan

Chukwuebuka Onyema
University of Winnipeg

David Palmer
University of Saskatchewan

David Perez
University of Alberta

Christopher Phenix
University of Saskatchewan

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University of Saskatchewan

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University of Saskatchewan

Jordane Preto
University of Alberta

Eric Price
University of Saskatchewan

Shvan Raheem
University of Saskatchewan

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University of Saskatchewan

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Memorial University

Akam Salih
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University of Saskatchewan

Sagar Saran
University of Saskatchewan

Whitney Shannon
University of Saskatchewan

Tim Storr
Simon Fraser University

Seyed Amirhossein Tabatabaei Dakhili
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Kishor Wasan
University of Saskatchewan

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