WCMCW

5th Western Canadian Medicinal Chemistry Workshop

September 23-25, 2016
Saskatoon, Saskatchewan

Chair
Ed S. Krol

Vice Chair
David R. J. Palmer

Sponsors

Saskatchewan Health Research | GlaxoSmithKline | Foundation Biological and Medicinal Chemistry Division Canadian Society for Chemistry | K' Prime | PRISM | U of S AAPS Student Chapter | University of Saskatchewan Office of the Provost, College of Pharmacy and Nutrition, and Department of Chemistry

Contents

MESSAGE FROM THE ORGANIZERS 3
WCMCW SCHEDULE OF EVENTS 4
ABSTRACTS OF ORAL PRESENTATIONS 7
ABSTRACTS OF POSTER PRESENTATIONS 16
LIST OF ATTENDEES 25
CAMPUS MAP 27

Message from the organizers

We would like to welcome you to Saskatoon and the University of Saskatchewan for the fifth Western Canadian Medicinal Chemistry Workshop. The WCMCW was established 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.

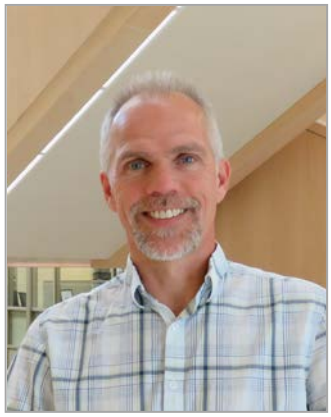
We hope that our efforts to put together the fifth WCMCW meeting will be both stimulating and productive. We are fortunate to have seven excellent invited speakers, each coming from different disciplinary backgrounds, but having common goals of improving the quality, efficacy and safety of pharmaceuticals,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

Finally, I don't think either of us knew if the WCMCW would last beyond the first offering in 2008, 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 23, 2016

TIME	EVENT	LOCATION
6:30 to 9 pm	**Registration**	Student lounge, Rm 1005 Education building, University of Saskatchewan
7 to 10 pm	**Opening reception and mixer**	

SATURDAY, SEPTEMBER 24, 2016

Registration and oral poster presentations to be held in Education Building, room 1004.
Poster presentations to be held in the student lounge of the Education Building, room 1005.

TIME	EVENT
8 am	**Registration and poster set up**
8:50 am	**Opening remarks**
9 am	**Chang-Chun Ling (University of Calgary)** *Cyclodextrin-based polyvalent systems: from design to applications.*
9:45 am	**Waleed Mohammed-Saeid (University of Saskatchewan)** *Design and evaluation of novel β–cyclodextrin cationic lipid based drug delivery systems: Efficient delivery of poorly soluble chemotherapy agents for the treatment of metastatic melanoma.*
10:05 am	**Ryan Kung (University of Lethbridge)** *Understanding the Structure of DNA Damaged by Aromatic Amines.*
10:25 am	Coffee break and poster viewing
10:50 am	**Hua Yang (TRIUMF)** *Oxidative stress imaging using [^{18}F] 5-fluoroaminosuberic acid.*
11:35 am	**Sarah Spreckelmeyer (University of British Columbia)** *Bifunctional acyclic chelator as potential therapeutic with Ac-225 and Bi-213.*
11:55 am	**Eric Price (University of Saskatchewan)** *Chelators in Radiochemistry: Attaching Radioactive Metals to Peptides, Antibodies, and Nanoparticles.*
12:15 pm	End of morning session. **WCMCW lunch in Education Building**
1:30 pm	**Carrie Haskell-Luevano (University of Minnesota)** *Chemical Neuroscience of the Melanocortin Pathway and Treatment for Genetic Obesity.*
2:15 pm	**Katie Wilson (University of Lethbridge)** *Understanding How Damaged DNA is Replicated: A Computational Investigation into the Bypass of O6-Benzylguanine.*

SATURDAY, SEPTEMBER 24, 2016 (cont'd)

TIME	EVENT
2:35 pm	**Kevin Allen (University of Saskatchewan)** *Chimeric agents targeting alpha-synuclein misfolding in vitro.*
2:55 pm	Coffee break and poster viewing
3:30 pm	**Frank Burczynski (University of Manitoba)** *MyoNovin: a novel skeletal muscle regenerator.*
4:15 pm	**Anas El-Aneed (University of Saskatchewan)** *Targeted strategies for the analysis of urine metabolites in asthma patients.*
4:35 pm	**Mays Al-Dulaymi (University of Saskatchewan)** *Establishment of Mass Spectrometric Fingerprints of Novel Peptide-Modified Gemini Surfactants Used as Gene Delivery Vectors.*
4:55 pm	End of afternoon session. **Poster viewing and judging.**
6:30 pm	Poster session ends.
7:30 pm	**WCMCW Banquet, St. Tropez, 238 2nd Avenue South**

SUNDAY, SEPTEMBER 25, 2016

Registration and oral poster presentations to be held in Education Building, room 1004

TIME	EVENT
8:00 am	**Breakfast workshop for trainees** Dale Cameron, West Coast Chemistry Services
8:55 am	**Introductory remarks**
9:00 am	**Charles Walsby (Simon Fraser University)** *Generating new activity in metal-based therapeutics through ligand design.*
9:45 am	**Mona Khamis (University of Saskatchewan)** *Expected vs. unexpected interferences in HPLC-MS/MS; validation of method selectivity in quantitative metabolomic analysis.*
10:05 am	**Arno Siraki (University of Alberta)** *The role of carbonate radical in drug metabolism and cytotoxicity: SOD peroxidase activity as a cytotoxic catalyst.*
10:25 am	Coffee break
10:50 am	**Ted Lakowski (University of Manitoba)** *Histone lysine demethylase 1 inhibitors and mocetinostat reduce HOXA9 expression, and increase global histone lysine dimethylation, acetylation, and arginine symmetric dimethylation.*
11:35 am	**Carlos Velazquez (University of Alberta)** *Targeting the FOXM1 transcription factor in cancer treatment & (possibly) cancer imaging.*
11:55 am	**Isaac Asiamah (University of Saskatchewan)** *Design and Synthesis of Inositol Derivatives as Imaging Probes*
12:15 pm	**Closing remarks, WCMCW lunch in Education building**
1:30 pm	**Conclusion of WCMCW**

Abstracts

Oral presentations / session 1

Cyclodextrin-Based Polyvalent Systems: From Design to Applications

Chang-Chun Ling

Alberta Glycomics Centre and Department of Chemistry, University of Calgary, Calgary, Alberta

Cyclodextrins (CDs) constitute a family of D-glucose-based host molecules with a hydrophobic cavity. They are known to form inclusion complexes with a wide range of organic molecules and have been used as excipients in drug formulations. The chemical modifications of CDs are challenging because of their polyhydroxy functionalities. Over recent years, we have developed several efficient methodologies to chemically functionalize CDs. This has allowed us to develop different families of polyvalent systems based on CD scaffolds for various applications. In this seminar, I will highlight our recent progress on methodology development to obtain multi-substituted CD hosts and some of the applications in biology such as inhibiting bacterial colonizations, studying weak carbohydrate-protein interactions and drug delivery.

Design and Evaluation of Novel β–Cyclodextrin Cationic Lipid Based Drug Delivery Systems: Efficient Delivery of Poorly Soluble Chemotherapy Agents for the Treatment of Metastatic Melanoma

Waleed Mohammed-Saeid[1#], Deborah Michel[1], Abdalla H. Karoyo[2], Ildiko Badea[1*]

1. Drug Discovery and Development Research Group, College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, Saskatchewan
2. Department of Chemistry, University of Saskatchewan, Saskatoon, Saskatchewan

Novel β-cyclodextrin modified drug delivery systems are developed to improve the biological behavior of poorly soluble drugs and improve therapeutic outcomes. Melphalan is clinically used for in-transit melanoma. The lipophilic nature of melphalan, the need to use an organic co-solvent, propylene glycol, and instability of injectable melphalan formulations limited their use to isolated limb perfusion. Thus, there is a need for formulation development to maximize the potential of the drug for melanoma therapy. The β-CD-modified cationic lipids (β-CD gemini) were specifically designed by our group to combine the solubilizing capacity of the β-CD moiety and the cell-penetrating ability of the gemini surfactant. Rapid and simple flow injection analysis-tandem mass spectrometric (FIA-MS/MS) methods were developed and validated to evaluate the solubilizing efficiency of the β-CD gemini and their ability to enhance the chemical stability of melphalan. FIA-MS/MS results showed a significant increase in the solubility of melphalan/ β-CD gemini complexes without the need for co-solvent (over three fold increase in aqueous solubility of melphalan). In addition, results illustrated that the delivery system significantly enhanced the chemical stability of the drug. The ability of the β-CD gemini delivery system to form host/guest complexes was evaluated using 1D/2D ROESY NMR methods. The results demonstrated that melphalan was self-included within the βCD internal cavity of the β-CD gemini. Physiochemical characterization of the novel lipid-based formulations showed optimal particle size in 200-250 nm range and overall positive zeta potential for endocytosis. *In vitro* evaluation showed that the drug/β-CD gemini complexes induced cell death in melanoma cells that were rendered resistant to melphalan and induced significantly higher cell death compared to the naked drug (3 – 6 folds decrease in melphalan IC_{50} values). In addition, the β-CD gemini delivery system did not alter the pathway of the cellular death triggered by melphalan and caused no intrinsic toxicity to the cells. These findings demonstrate the applicability of β-CD gemini delivery system in improving the anti-tumor activity of poorly soluble chemotherapeutic agents. The toxicity of the delivery system and the pharmacokinetic profile of the drug/β-CD gemini complexes will be evaluated in an animal model.

Abstracts

Oral presentations / session 1 (cont'd)

Understanding the Structure of DNA Damaged by Aromatic Amines

Ryan W. Kung[#] and Stacey D. Wetmore[*]
University of Lethbridge, Lethbridge, Alberta

DNA codes the genetic information for almost all life on earth. Unfortunately, exposure of DNA to carcinogenic species can result in the formation of bulky nucleobase addition products (adducts). The C8 position of guanine is particularly susceptible to adduct formation from exposure to aromatic amines (AAs). Several studies have shown that AA adducts can lead to numerous health effects including various types of cancer. A fundamental step in predicting the biological outcomes caused by this type of damage is understanding the structure that the damaged DNA adopts. Past experimental and/or computational work on select adducts has shown that various factors such as the size of the adduct, and the type of linkage between the damaging agent and nucleobase can affect the equilibrium between different damaged DNA conformations. However, a systematic investigation to understand the impact of these factors is missing from the literature. Computational techniques can provide molecular level details that are challenging to obtain from experiments. To this end, the current study uses molecular dynamics (MD) simulations to determine the most favourable conformations of N-linked guanine adducted DNA as a function of the size of the bulky moiety (e.g., single versus multiple aromatic rings).

Oxidative Stress Imaging Using [^{18}F] 5-fluoroaminosuberic Acid

Hua Yang[#1], Milena Colovic [1,2], Helen Merkens[2], Jack M. Webster[3], Francois Benard[2], Paul Schaffer[*1]

1. Life Sciences, TRIUMF, Vancouver, British Columbia
2. British Columbia Cancer Agency, Vancouver, British Columbia
3. GE Global Research, Niskayuna, NY, USA

Oxidative stress (OS) is the imbalance between the Reactive Oxygen Species (ROS) and the cellular antioxidants. OS is linked to inflammation, tumour cell/tumour stem cell survival, proliferation, and invasion, and chemoresistance and radioresistance. The predominant endogenous antioxidant is glutathione and its rate limiting precursor is cysteine (CysSH), which is brought into the cells by cystine (CysSSCys) transporter (system x_C^-). When the cellular ROS level is high, antioxidant response elements are triggered and system x_C^- is upregulated, making system x_C^- a potential biomarker for oxidative stress imaging. We have developed a PET tracer [^{18}F]5-fluoroaminosuberic acid (FASu) targeting system x_C^-. We have demonstrated the specificity of this tracer towards system x_C^- and its response to increased ROS. The *in vitro* and *in vivo* tumour uptake was studied in a broad range of tumour types: EL4 (lymphoma), U-87 (glioma), SKOV3 (ovarian), MCF-7 (breast) and MDA MB231 (triple negative breast cancer). [^{18}F]FASu had low background and modest to high tumour uptake in all cases studied, indicating its potential of a broad spectra oncology tracer. The chemistry study towards optically pure tracer will also be discussed. In conclusion, [^{18}F]FASu is a promising tracer to gauge system x_C^- and oxidative stress *in vitro* and *in vivo*.

Abstracts

Oral presentations / session 1 (cont'd)

Bifunctional Acyclic Chelator as Potential Therapeutic with Ac-225 and Bi-213

Spreckelmeyer, S. [#* 1,3]; Arane, K.[1]; Ramogida, C. F. [2]; Orvig,C.[1]

1. Medicinal Inorganic Chemistry Group, Department of Chemistry, University of British Columbia, Vancouver, British Columbia
2. TRIUMF, Vancouver, British Columbia
3. Dept. Pharmacokinetics, Toxicology and Targeting, Groningen Research Institute of Pharmacy, Netherlands

Ac-225 ($t_{1/2}$ = 10 days) and Bi-213 ($t_{1/2}$ = 45 min) are attractive radiometals for therapeutic applications. Our group has developed a series of "pa" (pa= picolinic acid) chelators with specific binding properties for a great number of radiometals. Herein, we report the synthesis and preliminary radio-labeling experiments of a bifunctional derivative of the previously reported H_5decapa. H_4neunpa-*p*-Bn-NCS was synthesized starting from the backbone diethylentriamine and the functionalization was placed on the middle nitrogen atom to keep its symmetry. Radio-labeling with Ac-225 of the precursor H_4neunpa-p-Bn-NO_2 in comparison to H_5decapa and the gold-standard DOTA was performed. Both chelators, H_4neunpa and H_5decapa, show labeling efficiencies >95 % within 15 minutes at room temperature at a chelator concentration of 10^{-5} M. DOTA showed only 1 % radio-labeling yield under the same conditions. To conclude, a new bifunctional chelator was successfully synthesized and shows promising preliminary radio-labeling abilities with big, highly charged radionuclides.

Chelators in Radiochemistry: Attaching Radioactive Metals to Peptides, Antibodies, and Nanoparticles

Eric W. Price[#*]

Chemistry Department, University of Saskatchewan, Saskatoon, Saskatchewan

Chelators are required to facilitate attachment of radioactive metal ions to drugs, which enables these drugs to be radiolabeled and used for nuclear imaging and therapy of cancer and other diseases. There has been a recent surge in interest for the development of new 89Zr chelators. The "gold standard" chelator desferrioxamine (DFO) is sufficient for 89Zr PET imaging, but some 89Zr is leached from the chelator and localizes in the bone. An improved chelator may decrease bone uptake and radiation burden to bone marrow in patients. 89Zr has a strong preference for oxygen donors, with hydroxamic acids being the most successful moiety used to date. With the goal of further enhancing 89Zr-chelate stability, we have been studying a new high denticity DFO derivative (potentially 12-coordinate) 'DFO2'. This presentation will outline work currently being done to make new and improved 89Zr chelators (DFO2). Previous work that has been done making chelators for 111In and 177Lu will be reviewed, as well as work done using 89Zr attached to the antibody rilotumumab (AMG102) for positron emission tomography (PET) imaging of cancer.

Abstracts
Oral presentations / session 2

Chemical Neuroscience of the Melanocortin Pathway and Treatment for Genetic Obesity

Carrie Haskell-Luevano[#*], Mark D. Ericson, Cody J. Lensing, Skye R. Doering, Katie T. Freeman, Sathya M. Schnell, Danielle N Adank, Stacey L. Wilber
University of Minnesota, Department of Medicinal Chemistry, Minneapolis MN USA

The melanocortin (MC) pathway consists of five G-Protein coupled receptor (GPCRs) isoforms and is stimulated by endogenous agonists derived from the proopiomelanocortin (POMC) gene transcript, and is antagonized by the endogenous agouti and agouti-related protein (AGRP) peptide hormones. The melanocortin-4 receptor (MC4R) has been demonstrated to play a critical role in the regulation of feeding behavior (desire to eat or feel full) and is one of the largest genetic GPCR single nucleotide polymorphism (SNP) modified protein targets in identified to date from morbidly obese children and adults. An overview of the MC pathway and its contributions to obesity will be presented along with different medicinal chemistry strategies towards the discovery of novel molecules and molecular probes for the development of weight loss and/or weight gain therapies.

Understanding How Damaged DNA is Replicated: A Computational Investigation into the Bypass of O6-Benzylguanine

Katie A. Wilson[#] and Stacey D. Wetmore*
University of Lethbridge, Lethbridge, Alberta

Our DNA is damaged over 60 000 times per cell per day by common environmental agents, such as tobacco, pesticides, and processed foods. DNA damage can affect many cellular processes, including standard DNA replication. Indeed, blocked DNA replication can have long-term harmful effects, such as cell death. To prevent these effects, cells have an alternative DNA replication method known as translesion synthesis (TLS). Since the discovery of TLS approximately 15 years ago, numerous studies have given insight into the replication of damaged DNA by TLS polymerases, including a proposal that the mechanism and efficiency of TLS is affected by the type of DNA damage. Unfortunately, the exact effects of DNA damage on TLS are currently unknown. In the present work, we gain indispensable structural insight about the replication of O6-benzylguanine. Specifically, MD simulations were performed on the DNA:polymerase complex prior to and during nucleotide insertion opposite the site of damage in order to understand the dynamic structure of the reactant complex for the mutagenic and non-mutagenic replication of O6-benzylguanine. Through these calculations, we unveil the varied and complex mutagenic profile of damage at the O6 position of guanine.

Abstracts

Oral presentations / session 2 (cont'd)

Chimeric Agents Targeting α-synuclein Misfolding In Vitro.

Kevin J. H. Allen,[1#] Joe Kakish,[2] Troy A. Harkness[3], Jeremy S. Lee[2] and Ed S. Krol.[1*]

1. Drug Discovery and Development Research Group, College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, SK.
2. Department of Biochemistry, University of Saskatchewan, Saskatoon, SK.
3. Department of Anatomy and Cell Biology, University of Saskatchewan, Saskatoon, SK.

The misfolding of α-synuclein is a critical event in the death of dopaminergic neurons and the progression of Parkinson's disease. We have previously shown that drugs which bind to α-synuclein and form a loop structure between the N- and C-termini tend to be neuroprotective, whereas compounds which cause a more compact structure tend to be neurotoxic. We have synthesized novel chimeric agents utilizing a caffeine scaffold linked to (R,S)-1-aminoindan, (R,S)-nicotine, caffeine and metformin, and their binding to α-synuclein determined through nanopore analysis and isothermal titration calorimetry. We also assessed the ability of the chimeric agents to interact with α-synuclein in a yeast model of PD which expresses an α-synuclein-Green Fluorescent Protein (AS-GFP) construct under the control of a galactose promoter. In 5 mM galactose, α-synuclein causes cell toxicity where the AS-GFP is observed as large cytoplasmic foci. Two of the chimeras, C_8-6-I and C_8-6-N, at a concentration of 0.1 μM allowed the yeast to grow normally in 5 mM galactose and the AS-GFP became localized to the periphery of the cell. Both chimeras were superior when compared to the monomeric compounds. The presence of these drugs could also cause the disappearance of preformed cytoplasmic foci. Nanopore analysis of C_8-6-I and C_8-6-N were consistent with simultaneous binding to both the N- and C-terminus of α-synuclein in spite of relatively lower binding constants (10^5 M^{-1}).

MyoNovin: a Novel Skeletal Muscle Regenerator

Frank Burczynski[#*1], Judy Anderson[2], Guqi Wang[3,4], Samaa Alrushaid[1]

1. College of Pharmacy, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Manitoba
2. Faculty of Science, University of Manitoba, Winnipeg, Manitoba
3. School of Medicine, Wake Forest University, Winston-Salem, NC, USA
4. Whole Pharm Biotechnology Corp., Matthews, NC, USA

MyoNovin (1,2-Di-O -nitro-3-(o -methoxyphenoxy)propanediol) is a novel skeletal muscle regenerator, capable of activating muscle satellite cells to initiate myogenesis. MyoNovin was synthesized using a two-step reaction involving the addition of guaiacol, allybromide and potassium carbonate in acetone followed by reacting the product with iodine and silver nitrate. The final product was concentrated by vacuum distillation and purified by column chromatography. HPLC and NMR were used to identify MyoNovin elution time and contaminants using different acetonitrile and methanol concentrations and injection-flow rates. Higher acetonitrile concentrations were associated with faster elution times. The MyoNovin peak appeared earlier in the solvent system using acetonitrile vs. methanol. Acetonitrile at 60% in water as the mobile phase, proved to be a good option for eluting MyoNovin. Bioactivity was assessed using muscle fibers were isolated from zebrafish grown in basal growth media with 200 ng BrdU/mL and treated with either MyoNovin or vehicle. Replicating darkly stained satellite cell (SC) nuclei were counted. Activation of SC was evaluated based on the mean number of BrdU+ SC per fiber. Fibers had a greater number of activated satellite cells when treated with MyoNovin compared to DMSO control group. SC on muscle fibers obtained from zebrafish demonstrated significant activation following MyoNovin treatment.

Abstracts

Oral presentations / session 2 (cont'd)

Targeted Strategies for the Analysis of Urine Metabolites in Asthma Patients

Mona Khamis, Hanan Awad, Kevin Allen, Darryl J. Adamko, and Anas El-Aneed[#*]
College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, Saskatchewan

Asthma is a complex syndrome with symptoms that can overlap with other respiratory conditions such as chronic obstructive pulmonary disease (COPD). The goal of this study is to develop a sensitive and selective liquid chromatography-tandem mass spectrometric (LC-MS/MS) method to analyze 35 urine metabolites that were differentially expressed in asthma patients. Based on their structural features, the metabolites were divided into two subgroups: amine/phenol bearing compounds and carboxylic acids. Light 12C and heavy 13C labeling agents were used for each subgroup. The 13C labeled derivatives were used as an internal standard. Derivatized metabolites were separated on a C18 column using LC-MS/MS. A qualitative and quantitative targeted LC-MS/MS methods are successfully developed for the analysis of the target metabolites. The methods achieved the desired selectivity and showed linearity within the range that will be applied for the analysis of patient urine samples. The methods are fully validated according to FDA guidelines. Clinical data analysis showed promising results into the use of some the target metabolites as diagnostic biomarkers. Asthma patients were successfully separated from other respiratory illness patients.

Establishment of Mass Spectrometric Fingerprints of Novel Peptide-Modified Gemini Surfactants Used as Gene Delivery Vectors

Mays Al-Dulaymi [1#,] Deborah Michel[1], Anas El-Aneed[1*]
College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, Saskatchewan

Diquaternary ammonium gemini surfactants have emerged as effective gene delivery vectors. A novel series of eleven peptide-modified gemini surfactants was synthesized, showing promising results in delivering genetic materials. The purpose of this work is to elucidate the tandem mass spectrometric (MS/MS) disccoaication behaviour of these novel molecules establishing a universal MS/MS fingerprints. Exact mass measurements were achieved using a hybrid quadrupole orthogonal time-of-flight mass spectrometer (QqToF-MS) and a multi-stage tandem mass spectrometric analysis was conducted using a triple quadrupole linear ion trap mass spectrometer (Q-LIT-MS). Both instruments were operated in the positive ionization mode and are equipped with electrospray ionization (ESI). Abundant triply charged [M+H]3+ ions were observed in the single stage analysis of all evaluated compounds with mass accuracies of less than 8 ppm. MS/MS experiments showed that gemini surfactants exhibited peptide-related dissociation characteristics due to the insertion of amino acids within the compounds spacer region. In particular, unique product ions were originated from the neutral loss of ammonia from the amino acids side chain resulting in the formation of pipecolic acid at the N-terminus part of the gemini surfactants. In addition, a charge directed amide bond cleavage was initiated by the amino acids side chain producing a protonated Î±-amino-Îµ-caprolactam ion and its complimentary c-terminus ion. MS/MS analysis revealed common fragmentation behaviour among all tested compounds, resulting in the production of a universal fragmentation pathway. The genesis of the observed product ions was established using MS3 analysis. The established MS/MS fragmentation pattern will be used for rapid and accurate identification of gemini surfactants within complex biological matrices.

Abstracts
Oral presentations / session 3

Generating New Activity in Metal-Based Therapeutics Through Ligand Design

Charles Walsby
Department of Chemistry, Simon Fraser University, Burnaby, British Columbia

Platinum anticancer drugs continue to inspire the development of metal-containing chemotherapeutics. In particular, the recent clinical successes of Ru(III) coordination complexes such as NKP-1339 and NAMI-A, and promising preclinical results from Ru(II) organometallic compounds, are leading the development of a variety of metal-based anticancer candidates with diverse metal centres and novel architectures. We are focused on the enhancement of the cytotoxic activity of metal complexes through the addition of medicinally functional ligands. This includes ligands that target protein interactions and passive intracellular transport by modulating hydrophobicity and coordination to amino acids. Furthermore, by linking redox-active metal centres to known therapeutics we show increased activity through mechanisms involving electron transfer and generation of reactive oxygen species. We have also demonstrated increased cytotoxicity by inclusion of nitric oxide donor ligands, and through ligands that promote DNA intercalation. Addition of fluorinated ligands has also enabled MRI studies of transport and redox processes, suggesting the potential for new theranostics. Overall, we are developing a tool kit of ligand design approaches to enable tuning of the properties of next-generation metallodrugs.

Expected vs. Unexpected Interferences in HPLC-MS/MS; Validation of Method Selectivity in Quantitative Metabolomic Analysis

Mona M. Khamis[1#], Hanan Awad[1], Kevin Allen[1], Darryl J. Adamko[2], Anas El-Aneed[1*]

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

Analytical methods designed for the quantification of drugs and/or metabolites in clinical and quality control settings need to be extensively validated before being used for their intended applications. One of the validation criteria set by the FDA and the EMA is the evaluation of method selectivity. The analytical method must correctly identify and quantify the analyte of interest in the presence of other components within the sample. HPLC with its recent advancements (UPLC, nano-HPLC) has dramatically improved the power of separation within complex real patient samples. However, other sources can also interfere in the response produced by the investigated analyte resulting in the compromisation of method selectivity. During the validation of two LC-MS/MS methods for the quantification of selected metabolites within urine, two possible causes of interference were identified. Dansylated compounds suffered from negative errors in their quantification. The reason was identified as the significant contribution of the second natural isotopic peak of each analyte on its corresponding internal standard, namely 13C2- dansylated derivative. On the other hand, lactic acid was found to be present in high concentrations within the blank samples. Upon extensive investigation, which involved different sources of chemicals and lab tools, the source of interference was identified to be the lactic acid contained within the breath of the analyst. Corrective measures were taken to address both issues.

Abstracts

Oral presentations / session 3 (cont'd)

The Role of Carbonate Radical in Drug Metabolism and Cytotoxicity: SOD Peroxidase Activity as a Cytotoxic Catalyst.

Naif Aljuhani, Lindsey Spruyt, Andrew Morgan, Arno G. Siraki

Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta

The carbonate radical ($E^{o\prime}$ = 1.8 V) can be produced from various sources, but a biologically relevant process involves the back-reaction of superoxide dismutase (SOD), where H_2O_2 produces a localized hydroxyl radical that can oxidize various substrates. One key substrate is the ubiquitously found bicarbonate anion, which subsequently forms carbonate radical. H_2O_2 is usually detoxified readily, but cancer cells have been noted to decrease mechanisms of H_2O_2 removal, and inflammatory conditions can lead to a prooxidant environment. The back-reaction described above is known as SOD peroxidase activity since it resembles peroxidase reactions. However, studies on the oxidation of drugs by this reaction has been limited. We have used two drugs (phenylbutazone and 6-mercaptopurine) to demonstrate the consequences of drug oxidation by carbonate radical generated from SOD peroxidase activity. Phenylbutazone is a potent NSAID used in horses (but no longer in humans due to various side-effects). This drug has a unique carbon (C4) that is acidic, and therefore, labile to oxidation. Phenylbutazone was shown to undergo one-electron oxidation at the C4-carbon to form a free radical using SOD/H_2O_2/bicarbonate. This reaction occurred through scavenging the carbonate radical and subsequently forming a phenylbutazone free radical. If 4-hydroxyphenylbutazone was used instead of phenylbutazone, there were no free radical species detected. The metabolites were characterized using ESR spin-trapping and LC/MS analysis. Using true peroxidase enzymes demonstrated that the products were identical to those produced from SOD peroxidase activity. To examine cytotoxicity of phenylbutazone, we used HepG2 (human hepatoma) cells treated with phenylbutazone in the presence or absence of SOD and H_2O_2. We found that extracellular SOD significantly catalyzed the cytotoxicity of phenylbutazone or H_2O_2 alone. The other drug used in this study was 6-mercaptopurine, a compound that contains an aromatic thiol. The latter is an anticancer/immunosuppressant drug that is also a metabolite of azathioprine. We found that 6-mercaptopurine, but not azathioprine (which has a thioether bond), was able to scavenge carbonate radical produced from SOD/H_2O_2/bicarbonate. Although we did not observe a thiyl radical as expected, LC/MS studies showed that a sulfoxide metabolite was produced from 6-mercaptopurine. In order to examine the biological relevance of 6-mercaptopurine-sulfoxide, we produced the metabolite and compared it with the cytotoxicity of 6-mercaptopurine in HepG2 and HEK293 (human embryonic kidney) cell lines. We found that the oxidized metabolite of 6-mercaptopurine was not cytotoxicity to both cell lines. In conclusion, we have shown that two different drugs are oxidized by carbonate radical generated from SOD peroxidase activity, which has differential cytotoxic consequences that depend on the species formed. These finding may have implications when drugs are used during inflammatory conditions.

Histone Lysine Demethylase 1 Inhibitors and Mocetinostat Reduce HOXA9 Expression, and Increase Global Histone Lysine Dimethylation, Acetylation, and Arginine Symmetric Dimethylation

Ryan Lillico, Courtney K. Lawrence and Ted M. Lakowski[*#]

The Rady Faculty of Health Sciences, College of Pharmacy, Pharmaceutical Analysis Laboratory, University of Manitoba, Winnipeg, Manitoba

Mixed lineage leukemia (MLL) is caused by changes in histone post-translational modifications (PTM) at the Homeobox A9 (HOXA9) promoter increasing its expression. Inhibitors of enzymes that add or remove PTM in the HOXA9 promoter are potential treatments for MLL. Accordingly, inhibitors of histone lysine demethylases (KDM), and methyltransferases (KMT) have been developed for this purpose. We previously found that the histone deacetylase (HDAC) inhibitor mocetinostat

decreased expression of KDM, thereby acting like a KDM inhibitor. We compared the KDM inhibitor GSK2879552, and mocetinostat measuring their effects on histone PTMs using LC-MS/MS, and HOXA9 expression using qPCR in an MLL cell line. GSK2879552 reduced HOXA9 expression (IC50=12nM) and increased histone lysine acetylation, mono- and dimethylation, and arginine symmetric dimethylation. Mocetinostat decreased HOXA9 expression (IC50=100nM) and we have previously shown that it increases histone lysine acetylation and methylation. Furthermore, we have shown that KMT inhibitors EPZ5676 and EPZ4777 paradoxically increased histone lysine dimethylation and acetylation while decreasing HOXA9 expression. These results indicate that inhibitors of histone PTM that increase lysine dimethylation, acetylation, and arginine symmetric dimethylation, also lower HOXA9 expression. Mocetinostat is a new example of such a drug that decreases HOXA9 expression and may be used to treat MLL.

Please Note: Withdrawn due to illness

Computational Study of the Substrate Specificity of RNA Nucleoside Hydrolases

Stefan A.P. Lenz[#], Stacey D. Wetmore*

University of Lethbridge, Lethbridge, Alberta

Parasitic protozoa are responsible for many tropical diseases, including African and American trypanosomiasis, and leishmaniasis**.** Parasitic protozoa lack the cellular pathways to synthesize DNA or RNA nucleotides that are essential for cell replication and are important cofactors in catalysis, and instead rely on the purine salvage pathway (PSP) to salvage nucleotides from the host organism. RNA nucleoside hydrolases are key enzymes in the PSP that function to hydrolytically break the *N*-glycosidic bond connecting nucleobases to ribose sugars. While X-ray crystallographic and kinetic experiments have identified critical amino acids for the deglycosylation reaction, key residues that play a role in nucleobase activation and the determinants of the broad substrate specificity exhibited by these enzymes are still unclear. My presentation reveals atomic level details related to the binding of the natural nucleosides to the active site of a variety of wild type and mutant nucleoside hydrolases using molecular dynamics (AMBER) and free energy methods. By examining the binding of multiple nucleosides to wild type and mutant active sites, my work reveals the active site residues that play an important role in the deglycosylation mechanism, and that play a role in determining substrate specificity. This work has several important implications, including the future development of drugs that treat trypanosomiasis and leishmaniasis by targeting nucleoside hydrolases and shutting down the PSP.

Design and Synthesis of Inositol Derivatives as Imaging Probes

Isaac Asiamah[1#], Rajendra Jagdhane[1], Christopher P. Phenix[1], David R.J. Palmer[1*]

Department of Chemistry, University of Saskatchewan, Saskatoon, Saskatchewan

Positron Emission Tomography (PET) is a molecular imaging technique that has wide-ranging applications in clinical oncology, cardiology, and neurology as well as basic biomedical research. Despite the great success of PET imaging in certain clinical and research domains, the development of new radiotracers remains a formidable challenge. PET radioisotopes have short half-lives and must typically be incorporated into tracer molecules at a late stage of the overall synthesis process. The most frequently used route of ^{18}F radiolabeling is based on the use of nucleophilic substitution reactions on suitable precursors carrying appropriate leaving groups. The most prominent PET radiotracer to date, [^{18}F]-2-Deoxy-2-fluoro-D-glucose (FDG), is non-specific. New imaging probes with higher selectivity and specificity will allow us to trace healthy processes and unhealthy ones, such as tumors, more precisely. We have designed and synthesized stable precursors to new imaging probes based on the natural products *myo*-inositol and *chiro*-inositol. Treatment of precursors with azeotropically dried potassium cryptand fluoride ([K_{222}][^{19}F]) using KF as F^- source, yielded after deprotection the ^{19}F versions. Thus, our activated precursors are expected to provide access to our desired radiotracers through nucleophilic ^{18}F substitutions.

Abstracts

Poster presentations

1.

Prodrug-Inspired Substrates Employing Lysosomotropic Reporter Groups for PET Imaging Cathepsins

Brady G Vigliarolo[#], Morshed A Chowdhury, Shusheng Wang, Ignace A Moya, Chris P Phenix*

University of Saskatchewan

Cathepsins B and L are lysosomal cysteine proteases with diagnostic and therapeutic implications in various diseases such as cancer, where they are involved in promoting tumor metastasis. Developing probes to help elucidate the pathogenic roles of cathepsins and quantify enzyme activity in vitro and in vivo is therefore of great importance. Substrate-based positron emission tomography (PET) imaging agents may offer the potential advantage of signal amplification compared to inhibitors which covalently bind to the enzyme active site with 1:1 stoichiometry. However, trapping the enzymatically released and radioactive products at the site of protease activation is a significant challenge to overcome for this approach. Weakly basic lipophilic compounds have been shown to accumulate within the acidic lysosomes of cells after becoming protonated and therefore membrane-impermeable – an effect known as lysosomotropism. Following enzymatic recognition and cleavage of a dipeptide specifier and the subsequent spontaneous decomposition of the prodrug-inspired linker PABA, released lysosomotropic reporter groups could foreseeably accumulate within the lysosomes of aggressive tumors with high cathepsin activity. We present here the synthesis, kinetic evaluation, and in vitro specificity of such model cathepsin substrates bearing potentially lysosomotropic reporter groups. In addition, the synthesis of labelling precursors and progress toward 19/18F labelling of those precursors will also be discussed. Such substrates and PET may have utility in defining the role that cathepsins play in tumor progression in vivo in animal models of aggressive cancer.

2.

MRM3 versus Differential Mobility Spectrometry (DMS) in Background Noise Reduction for Complex Matrices like Human Plasma

Deborah Michel[1#], Ahmed Almousa[1], Muath Helal[1], Shaokun Pang[2], Richard Tang-Wai[3], Richard Huntsman[4], Jane Alcorn[1*]

1. College of Pharmacy and Nutrition, University of Saskatchewan
2. Sciex, Redwood City, CA
3. Pediatrics, Edmonton Clinic Health Academy, University of Alberta
4. Pediatrics, College of Medicine, University of Saskatchewan

We wish to develop and validate a rapid and sensitive mass spectrometry (MS) method for quantitating cannabidiol (CBD) and tetrahydrocannabinol (THC) in small volume human plasma samples, but at present high background noise confounds the robustness of the method. We compared MRM3 with DMS for their ability to improve the signal to noise ratio in normal and hyperlipidemic human plasma.

Abstracts

Poster presentations (cont'd)

3.

Rapid and Simple Flow Injection Analysis-Tandem Mass Spectrometric (Fia-Ms/Ms) Methods for the Quantification of Melphalan in Lipid-Based Drug Delivery System

Waleed Mohammed-Saeid[#], Deborah Michel, Ildiko Badea, Anas El-Aneed[*]

College of Pharmacy and Nutrition, University of Saskatchewan

The use of the anticancer drug melphalan (Mel) is limited due to its poor water solubility. To address such limitation, it is incorporated within a novel delivery system using β-cyclodextrin-gemini surfactants (β-CD-gemini). Herein, two fast and simple FIA-MS/MS methods are developed for the quantification of Mel within the drug delivery system so that solubilization efficiency is assessed. Various formulation strategies were adopted to incorporate Mel within the delivery system. The use of 10% HCl ethanol was needed. FIA-MS/MS Methods are developed using a triple quad linear ion trap MS equipped with electrospray ionization (ESI) in positive mode. Deuterated form of Mel (Mel-d8) was used as an internal standard (IS). The methods were validated according to the FDA. Developed methods were fully validated according to the FDA bioanalytical validation criteria. Both methods were validated with linearity (r=0.9986, 0.9971 respectively) in the range of 1â€“200 ng/mL with accuracy and precision below 15% for all standard points and quality control samples. The intra- and inter-day variations, freeze-thaw stability and long term stability (1 month) were within the acceptable criteria according to the FDA guidelines. The developed methods were applied successfully in determining the encapsulation/solubilisation activity of [Mel:β-CD-gemini] delivery system and to evaluate the stability of the developed formulations. In addition, these new analytical methods will be applied to evaluated the influence of [Mel: β-CD-gemini] on the drug anti-neoplastic activity and pharmacokinetic profile in rat animal model. Simple FIA-MS/MS methods were developed without the need for tedious chromatographic separation. Solubilization of the target drug was enhanced.

4.

Development of Alpha Emitting Anti-GPNMB Antibody Targeted Gemini Nanoparticles for Melanoma

Amal Makhlouf [#], Istvan Hajdu, Kayla Wharton, Deborah Michel, Humphrey Fonge and Ildiko Badea*

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.

Abstracts
Poster presentations (cont'd)

5.

Synthetic Studies on [10]Annulenes

Christa Blaquiere#, Michel Gravel*
Dept. Of Chemistry, University of Saskatchewan

Hückel's rules predict that planar [10]annulenes are prototypical aromatic hydrocarbons. The all-cis isomer was synthesized over forty years ago and was found to adopt a non-planar tub-like conformation, thus making it non-aromatic. Later computational studies on [10]annulene predicted that the molecule could be forced into a planar conformation through the use of fused cyclopropanes, thus fulfilling Hückel's rules for aromaticity. A successful synthesis of this type of molecule would allow the study of its fundamental properties, including aromaticity, as well as potential medicinal applications as a molecular scaffold. Our efforts towards the synthesis of this type of product will be presented.

6.

A Newer Multi-Kinase Inhibitor Modulates Cellular Energy Metabolic Pathways within Breast Cancer to Impair

Franklyn De Silva [1], Shelley Yang *, and Jane Alcorn *
Drug Discovery & Development Research Group, College of Pharmacy and Nutrition, University of Saskatchewan

Tyrosine kinases (TKs) govern cancer progression and control. The human epidermal growth factor receptor I (HER1/EGFR) play a major role in tumor survival. The cellular energy sensory molecule, AMP-activated protein kinase (AMPK), is a potential therapeutic target in the treatment against breast (and other) cancers (BCs). Ibrutinib (Imbruvica™), a newer TKI, is an orally administered irreversible Bruton's tyrosine kinase inhibitor clinically approved for the treatment of several B-cell malignancies. Interestingly, Ibrutinib is a multi-kinase inhibitor with varying binding affinities to several receptor and non-receptor TKs, including EGFR. Therefore, its effects on different types of breast cancer cell lines are being evaluated in order to find translational evidence to support the idea of utilizing Ibrutinib (and several other newer TKIs) as candidates for the treatment of breast cancer. We plan to identify/confirm key targets within HER2 independent pathways primarily using a battery of *in vitro* assays. Although Ibrutinib alone is not sufficient to cause significant cytotoxicity in some cells at concentrations <10,000nM, addition of a second anti-cancer agent at lower concentrations may cause significant cell death involving synergistic effects. Therefore, Ibrutinib poses as a promising agent for the intervention (combinational therapy) of HER2 negative and HER2 non-overexpressing BCs.

Abstracts

Poster presentations (cont'd)

7.

Lanthanum Compounds for the Treatment of Bone Density Disorders

Jacqueline Cawthray[#1], David Weekes[2], Meghanne Rieder[1], Munawar Mohammed1, Jaweria Syeda[1], Ellen Wasan[1], Arash Panahifar[#1], David Cooper[3], Chris Orvig[2], Kishor Wasan[1]

1. College of Pharmacy and Nutrition, University of Saskatchewan
2. Department of Chemistry, University of British Columbia
3. Department of Anatomy and Cell Biology, University of Saskatchewan

Bone is constantly being remodelled through the process of bone formation and bone resorption by osteoblasts and osteoclasts, respectively. An imbalance in this remodelling process results in decreased bone mineral density, as seen with osteoporosis. Lanthanum (La) is known to preferentially accumulate in bone tissue, stimulate osteoblast proliferation and inhibits osteoclast activity. This is anticipated to be a more effective approach than currently available therapies like bisphosphonates. We have performed acute 1-month oral dosing studies of 2 La compounds, La(XT) and La(dpp)3 (Sprague-Dawley rats) to assess any differences the two chelating agents have on bone-uptake and biodistribution of the lanthanum ion. Further dosing and toxicity studies with La(XT) including liver and kidney toxicity studies have also been completed. The biodistribution of La^{3+} within plasma, organs, and the femur were determined via ICP-MS. Lanthanum is rapidly cleared from the blood with redistribution to bone with depot type of accumulation. Higher levels of La^{3+} were detected in the knee section of the femur versus the hip and middle sections indicating regions of higher bone turnover actively incorporate La^{3+} ions in the bone structure more rapidly. It was shown that La^{3+} accumulates in the bone after dosing, with La(XT) showing slightly higher uptake.

8.

A Multi-Enzyme Coupled Assay to Evaluate the Kinetics of Myo-Inositol Phosphate Synthase Catalysis and Inhibition

Josseline Ramos-Figueroa[#] and David R. J. Palmer*

Department of Chemistry, University of Saskatchewan

Myo-Inositol phosphate synthase (mIPS) is the first enzyme in the biosynthetic pathway of myo-inositol, which plays an important role as the structural basis for secondary messengers. Abnormalities in cellular myo-inositol levels have been related to bipolar disorder and depression; therefore, mIPS is a putative target for inhibitors that may combat these conditions. We have developed a coupled enzymatic assay to monitor the mIPS-catalyzed conversion of glucose 6-phosphate (G6P) to L-myo-inositol 1-phosphate conveniently. Also, phosphonate and monofluorinated phosphonate analogs of G6P were synthesized and evaluated as inhibitors of mIPS. The results of the synthetic and kinetics experiments will be discussed.

Abstracts

Poster presentations (cont'd)

9.

Dynamic Kinetic Asymmetric Transformations of Amino Aldehydes in Chemo- And Diastereoselective Cross-Benzoin Reactions Catalyzed by N-Heterocyclic Carbenes

Karn Parmar and Michel Gravel*
Department of Chemistry, University of Saskatchewan

Our group has recently demonstrated that protected amino aldehydes can undergo chemoselective N-heterocyclic carbene (NHC) catalyzed cross-benzoin reactions with aromatic and alkyl aldehydes to form α-hydroxy-β-amino ketones. Since certain derivatives of these aldehydes are known to racemize under basic conditions we hypothesized that a dynamic kinetic asymmetric transformation (DYKAT) of these substrates in a cross-benzoin reaction using a chiral NHC should be possible. This should allow for easy access to enantioenriched α-hydroxy-β-amino ketones starting from racemic substrates. This is a beneficial transformation as it permits the incorporation of non-natural amino acid residues which are easy to synthesize racemically.

10.

Probing the Allosteric Mechanism of Dihydrodipicolinate Synthase from *Campylobacter jejuni:* New Approach to Antibiotic Design

Mohadaseh Majdi Yazdi[#], Cheyanne Lehnert, and David R. J. Palmer*
Department of Chemistry, University of Saskatchewan

Antibiotic-resistant bacteria have raised a growing concern and it is vital to characterize new drug targets, as the existing antibiotics are not able to treat the infections caused by these bacteria. The Lysine/DAP biosynthetic pathway is an attractive target, because it plays an essential role in bacterial cell wall and amino acid biosynthesis. The aim of this research is to study the allosteric inhibition of dihydrodipicolinate synthase (DHDPS), an enzyme that catalyzes the first and rate-limiting step towards *meso*-DAP formation. In order to explore the role of allosteric amino acid residues, mutants of DHDPS were engineered and their properties were compared to the wild-type. High-affinity allosteric inhibitors were discovered via rational design, and the inhibitory activity and binding affinity were tested by kinetic and fluorescence assays.

Abstracts

Poster presentations (cont'd)

11.

Kinetic Analysis of Allosteric Site Mutants of UDP-Galactopyranose Mutase from M. Tuberculosis

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 analysis of some mutants have been carried out and the results will be described.

12.

Structure Activity Relationship of Novel Peptide-Modified Gemini Surfactants as Gene Delivery Vectors...

Mays Al-Dulaymi [1#], Jackson Chitanda[2], Ronald Verrall[3], Pawel Grochulski[4], Deborah Michel[1], Ildiko Badea[*1]

1. College of Pharmacy and Nutrition, UofS
2. Dept. of Chemical and Biological Engineering, UofS
3. Department of Chemistry, UofS
4. Canadian Light Source, Saskatoon, SK

Diquaternary ammonium gemini surfactants are a special category of drug carriers for DNA. Our aim is to elucidate the structure activity relationship of peptide-modified gemini surfactants and to develop an effective non-viral gene delivery system with a potential use as topical gene delivery to treat conditions like scleroderma or melanoma. Three families of peptide modified gemini surfactants, designated m-7N(GK)-m, m-7N(GK3)-m and m-7N(GK7)-m [G= glycine and K= lysine] where m is the alkyl chain, m= 12, 16 and 18:1 [18:1 chain= mono-unsaturated oleyl chain], were used to engineer DNA nanoparticles at phosphate to nitrogen charge ratios (N/P) of 2.5, 5 and 10. Transfection efficiencies and cell toxicity of gemini nanoparticles was evaluated in murine keratinocytes (PAM 212). Physiochemical properties of the nanopartides were examined by measuring the particle size and surface charge using dynamic light scattering and laser doppler velocimetry, respectively. Morphological characteristics and lipid organization were studied by using the small-angle X-ray scattering (SAXS) technique. Critical micellar concentration (CMC) was determined by using the specific conductivity method. The highest transfection efficiency was observed with the m-7N(GK)-m series having the 16-carbon tail compound at an N/P ratio 2.5. It showed a 2-3.5 fold increase in the level of reporter protein in PAM 212 cells compared to analogs in the m-7N(GK3)-m and m-7N(GK7)-m series. In addition, it exhibited the highest cell viability (85%) among the tested compounds. The nanopartides had an average particle size of 81Â±6 nm and surface charge of +21Â±1 mV. They adopted an inverted hexagonal phase which is known for its highest activity (Figure 2). Moreover, the 16-7N(GK)-16 compound exhibited the lowest CMC of 0.155 mM among the tested compounds which could indicate an increased lipoplexes stability during the delivery process. We conclude that the length of the alkyl tail and the number of lysine moieties in the spacer of gemini surfactants play an important role in determining the transgene efficiency of the delivery system.

Abstracts

Poster presentations (cont'd)

13.

Membrane Protein Clustering in Lymphocytes from Rats Repeatedly Treated with Corticosterone…

Raquel Romay-Tallon[1], Erin Y Fenton[2], Milann A Mitchell[3], Lisa E Kalynchuk[1], Hector J Caruncho[4]

1. Department of Medicine, UofS
2. Centre for Drug Research and Development. Vancouver
3. Department of Psychology, UofS
4. College of Pharmacy and Nutrition, UofS

Membrane protein clustering (MPC) in lymphocytes is altered in depression and it has been proposed as a putative biomarker of therapeutic efficacy in major depressive disorder (recently reviewed in Caruncho et al., Frontiers in Cellular Neuroscience, 2016,10:48). In the present experiment we analyzed the clustering patter of four transmembrane proteins in rats repeatedly treated with corticosterone (CORT), a well-characterized animal model of depressive-like behavior (see as review Sterner and Kalynchuk, 2010). Male rats received either 21 days of daily CORT injections (40mg/Kg s.c.) or vehicle injections. Behavioral analysis of the forced-swim test started in day 22, and was followed by collecting of blood samples and animal sacrificing by cardial puncture. After isolation of peripheral blood lymphocytes, samples were processed for immunocytochemistry for the serotonin transporter (SERT), serotonin 2A receptor (5-HT2A), beta-2 adrenergic receptor (β-2AR), or the cellular prion protein (PrPc). MPC of the labelled proteins was analyzed and quantified by using the ImageJ software. After repeated CORT treatment, animals showed a consistent depressive-like behavior as ascertained by measurement of immobility in the forcer-swim test. All four proteins studied showed a pattern of clustering immunolabeling in lymphocytes plasma membrane, and this pattern was altered upon CORT treatment: There is a significant increase of 13%, 6% and 12% in the average size of SERT, 5-HT2A and PrPc clusters, respectively. However, β-2AR clusters showed a 7% reduction in their size. No changes were found in the number of SERT, 5-HT2A and β-2AR clusters per lymphocyte, but there was a 20% increase in the number of PrPc clusters. We also found a positive correlation between the increase in size of SERT, 5-HT2A and PrPc clusters, and depressive behavior as ascertained by the forced-swim test. Interestingly, the pattern of changes induced by CORT treatment in SERT and 5-HT2A MPC in lymphocytes parallels those we previously observed in naïve depression patients (see reference from the first paragraph). As we identified specific alterations in MPC for the two additional proteins included in this study (β-2AR and PrPc), it would be of interest to analyze if similar changes would also be observed in naïve depression patients, and propose that the analysis of MPC in the CORT model of depression could be used to ascertain possible alterations in MPC in lymphocytes that could represent putative biomarkers to be further analyzed in depression patients.

14.

Comparison of protocols for quantitative analysis of membrane protein clustering in lymphocytes in relation to its use as a putative biomarker of depression

Tania Rivera-Baltanas[1], Raquel Romay-Tallon[2], Milann A Mitchell[3], Lisa E Kalynchuk[2], Jose Olivares[1], Hector J Caruncho[4]

1. Institute of Biomedial Research of Vigo, Spain
2. Department of Medicine, UofS
3. Dept. of Psychology, UofS
4. College of Pharmacy and Nutrition, UofS

Membrane protein clustering (MPC) in lymphocytes is altered in depression and it has been proposed as a putative biomarker of therapeutic efficacy in major depressive disorder (recently reviewed in Caruncho et al., Frontiers in Cellular Neuroscience, 2016,10:48). In the present experiment we compare different protocols to analyze the pattern of membrane protein clustering in human lymphocytes to ascertain the best and easiest manner of carrying out this analysis in a possible translation to a clinical setting. In our original protocol (protocol A), human blood samples were drawn by a registered nurse. Samples were centrifuged in a Ficoll-Paque-Plus gradient for isolation of lymphocytes, followed by immunolabeling with a specific anti-serotonin transporter (SERT) antibody. Immunolabeling was performed in small Eppendorf tubes. The subsequent analysis of the clustering was performed by using a confocal or an epifluorescence microscope. In protocol B, we obtained human blood smears by using a blood drop from pricking the forefinger tip. The smears were dried for an hour and stored at -80°C for unlimited time. Immunohistochemistry was per formed directly on the slide. Identification of lymphocytes in the smears was helped by nuclear counterstaining. We then collected pictures with an epifluorescence microscope. For both protocols we used the ImageJ software to quantitative MPC levels. The specific conditions of background and noise in ImageJ have to be adjusted for each protocol in order to procure a more accurate analysis.

Abstracts
Poster presentations (cont'd)

15.

Untying the Knot of Transcription Factors Druggability: FOXM1

S. A. Tabatabaei Dakhili[#] and C.A .Velázquez-Martínez[*]

Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta

Characterizing the FOXM1-DBD binding site and interacting residues might greatly aid in understanding and designing new drug moieties capable of suppressing the transcriptional activity of the oncogenic FOXM1. Accumulating evidence suggests that targeted FOXM1 inhibition may be a promising strategy to treat human malignancies, indicating that new FOXM1 inhibitors may become clinically useful drugs in the near future. It is reported that some compounds are capable of interacting directly with the FOXM1 to diminish its binding to its DNA binding domain (FDI-6, FDI-11 & Thiostrepton). To validate this, we performed a series of molecular modeling and molecular dynamic simulation studies to determine the structural requirements needed by a drug molecule to interfere with the FOXM1 DNA binding domain. Upon examining the binding modes of Troglitazone (a known FOXM1 downregulator), Thiostrepton, FDI-6 and FDI-11 to FOXM1-DBD, we observed that the π-sulfur interactions are mainly responsible for efficient binding of these drugs at the FOXM1-DBD interface.

16.

Synthesis of β-Glucocerebrosidase inhibitors for applications in diagnosing Parkinson's Disease and Gaucher's Disease

Zachary Huschi,[#] Isaac Asiamah, David R. J. Palmer[*]

Department of Chemistry, University of Saskatchewan

β-Glucocerebrosidase (GCase) is a glycosidase enzyme responsible for the metabolism and breakdown of glucosylceramide. While homozygous mutations in the gene encoding the GCase enzyme have been known to cause the lysosomal storage disorder known as Gauchers disease, it has been discovered that a deficiency in GCase could be a potential marker of Parkinson's disease. Previously, the Phenix group has shown that N-alkylated aziridine cyclitols, with the conduritol b geometry, are effective and irreversible inhibitors of GCase. Expanding on this work, we have created additional inhibitors, focusing on inhibitors that are amenable to radiolabelling with Fluorine-18. Additionally, we have explored the use of various protection groups that will allow the desired transformations to take place within the time frame allowed when working with radioactive fluorine. It is our hope to test these inhibitors in live cells in the future, and proceed to animal models with the most promising inhibitors found in order to see if we can observe the GCase concentrations in live subjects.

Abstracts
Poster presentations (cont'd)

17.

Pharmaceutical Strategy of Using Flaxseed Lignans as Adjuvant Therapy Against Prostate Cancer

Yunyun Di[1#], Shaoping Ji[2], Philipp Wolf[3], Ed S. Krol[1], Jane Alcorn[1,*]

1. Drug Discovery and Development Research Group, College of Pharmacy and Nutrition, UofS
2. Laboratory of Molecular Cell Biology, College of Pharmacy and Nutrition, UofS
3. Dept. of Urology, Medical Center, University of Freiburg, Germany.

Increasing evidence from preclinical and clinical studies are demonstrating that dietary flaxseed lignans may have chemopreventive and chemotherapeutic role against prostate cancer. Our in vitro combination study found that ENL, a major putative bioactive compound from flaxseed lignans, could synergy the anticancer effect of chemotherapeutic agents in cancer cells with different characteristics. However, the flaxseed lignans exist mainly as glucuronic conjugates systemically. Based upon the unfavourable pharmacokinetic profile of flaxseed lignans, we propose the using of antibody directed enzyme prodrug therapy (ADEPT) as adjuvant therapy could allow for within tumor activation of flaxseed lignans which can synergy the anticancer effect of drugs against prostate cancer. ADEPT is a two-step process by which the drug active enzyme is delivered to the tumor site by specific antibody, following administration of a nontoxic prodrug. Then the prodrug is activated by the localized enzyme, which will trigger cancer cell death. An anti-PSMA specific antibody conjugated with beta-glucuronidase was successfully generated. The fusion protein displayed excellent binding against cell surface PSMA in LNCap cells or purified PSMA, and favorable enzymatic activity in production of fluorescent product 4-methylumbelliferone form 4-methylumbelliferone glucuronide.

WCMCW Attendees

Dalia Ahmed
Department of Chemistry
University of Saskatchewan
dma750@mail.usask.ca

Kholud Algabbass
College of Pharmacy and Nutrition
University of Saskatchewan
kholud.algabbass@usask.ca

Akay Akohwarien
Dept. of Chemistry
University of Saskatchewan
aka060@mail.usask.ca

Mays Al-Dulaymi
College of Pharmacy and Nutrition
University of Saskatchewan
mays.al-dulaymi@usask.ca

Kevin Allen
College of Pharmacy and Nutrition
University of Saskatchewan
kja782@mail.usask.ca

Krishna Reddy Annadi
Dept. of Chemistry
University of Saskatchewan
kra534@mail.usask.ca

Isaac Asiamah
Dept. of Chemistry
University of Saskatchewan
isa356@mail.usask.ca

Ildiko Badea
College of Pharmacy and Nutrition
University of Saskatchewan
ildiko.badea@usask.ca

Christa Blaquiere
Dept. of Chemistry
University of Saskatchewan
csb188@mail.usask.ca

Frank Burczynski
Pharmacy
University of Manitoba
Frank.Burczynski@umanitoba.ca

Dale Cameron
West Coast Chemistry Services
dcameron@westcoastchemist.ca

Jacqueline Cawthray
College of Pharmacy and Nutrition
University of Saskatchewan
jcawth01@gmail.com

Frank De Silva
College of Pharmacy and Nutrition
University of Saskatchewan
f.desilva@usask.ca

Yunyun Di
College of Pharmacy and Nutrition
University of Saskatchewan
yunyun.di@usask.ca

Anas El-Aneed
College of Pharmacy and Nutrition
University of Saskatchewan
anas.el-aneed@usask.ca

Doug Fansher
Dept. of Chemistry
University of Saskatchewan
djf255@mail.usask.ca

Carrie Haskell-Luevano
Medicinal Chemistry
University of Minnesota
chaskell@umn.edu

Zachary Huschi
Department of Chemistry
University of Saskatchewan
zjh469@mail.usask.ca

Mona Khamis
College of Pharmacy and Nutrition
University of Saskatchewan
mmh882@mail.usask.ca

Ed Krol
College of Pharmacy and Nutrition
University of Saskatchewan
ed.krol@usask.ca

Ryan Kung
Chemistry
University of Lethbridge
ryan.kung@uleth.ca

Ted Lakowski
College of Pharmacy
University of Manitoba
Ted.Lakowski@umanitoba.ca

Stefan Lenz
Chemistry
University of Lethbridge
stefan.lenz@uleth.ca

Chang-Chun Ling
Chemistry
University of Calgary
ccling05@gmail.com

Mohadeseh Majdi Yazdi
Dept. of Chemistry
University of Saskatchewan
mom983@mail.usask.ca

Amal Makhlouf
College of Pharmacy and Nutrition
University of Saskatchewan
amal.makhlouf@usask.ca

Deborah Michel
College of Pharmacy and Nutrition
University of Saskatchewan
dlm137@mail.usask.ca

Waleed Mohammed-Saeid
College of Pharmacy and Nutrition
University of Saskatchewan
wam411@mail.usask.ca

Dave Palmer
Dept. of Chemistry
University of Saskatchewan
dave.palmer@usask.ca

Karn Parmar
Dept. of Chemistry
University of Saskatchewan
ksp325@mail.usask.ca

Christopher Phenix
Dept. of Chemistry
University of Saskatchewan
chris.phenix@usask.ca

Theerawat Prasertanan
Dept. of Chemistry
University of Saskatchewan
thp785@mail.usask.ca

Eric Price
Dept. of Chemistry
University of Saskatchewan
eric.price@usask.ca

Josseline Ramos-Figueroa
Dept. of Chemistry
University of Saskatchewan
jsr303@mail.usask.ca

Naheda Sahtout
Dept. of Chemistry
University of Saskatchewan
naheda.sahtout@usask.ca

Sagar Saran
Dept. of Chemistry
University of Saskatchewan
sas966@mail.usask.ca

Arno Siraki
Pharmacy and Pharmaceutical Sciences
University of Alberta
siraki@ualberta.ca

Sarah Spreckelmeyer
Medicinal Inorganic Chemistry
University of British Columbia
sarahspr@chem.ubc.ca

Seyed Amirhossein Tabatabaei Dakhili
Pharmacy and Pharmaceutical Sciences
University of Alberta
stabatab@ualberta.ca

Carlos Velazquez
Pharmaceutical Sciences
University of Alberta
velazque@ualberta.ca

Natasha Vetter
Dept. of Chemistry
University of Saskatchewan
ndv857@mail.usask.ca

Brady Vigliarolo
Dept. of Chemistry
University of Saskatchewan
bgv647@mail.usask.ca

Charles Walsby
Department of Chemistry
Simon Fraser University
cwalsby@sfu.ca

Kishor Wasan
College of Pharmacy and Nutrition
University of Saskatchewan
kishor.wasan@usask.ca

Katie Wilson
Chemistry and Biochemistry
University of Lethbridge
ka.wilson@uleth.ca

Hua Yang
Life Sciences
TRIUMF
hyang@triumf.ca

Nataliya Zalatar
Dept. of Chemistry
University of Saskatchewan
nataliyazalatar@gmail.com

Joshua Zimmerman
Dept. of Chemistry
University Of Saskatchewan
joshua.zimmerman@usask.ca

University of Saskatchewan map

Where to find us