Abstract Book

QMB Drug Discovery Satellite

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DD1: Targeting BET bromodomains for the treatment of cancer

Chris Burns

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The BET family of bromodomains have recently emerged as promising drug targets for the treatment of cancer. These epigenetic reader proteins can regulate the transcription of multiple oncogenes, most notably c-MYC. Whilst a number of BET bromodomain inhibitors have been described in the patent and published literature, and a number are now in early phase clinical trial, there are drawbacks with these 'first generation' inhibitors. Thus, the compounds can be difficult to prepare and often suffer from poor pharmacokinetics and physicochemical properties. Furthermore, they show limited selectivity across the BET family. We will present our work towards improved second generation compounds that address a number of these shortcomings.

DD2: New TB drugs work by a common mechanism: uncouplers of the respiratory chain

Gregory M. Cook[1,2] and Kiel Hards[1]

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The poorly defined molecular mechanisms of many antimicrobials are a hindrance to rational, mechanism-based drug discovery. Bedaquiline (BDQ), the first anti-tubercular to be FDA approved in > 40 years, targets the mycobacterial F_1F_o-ATP synthase and inhibits ATP synthesis leading to slow time-dependent cell killing. The mechanism of cell death was proposed to be due to ATP depletion. We hypothesized that mechanisms alternative to ATP depletion may be important in BDQ's bactericidal mode of action. Importantly, we proposed that the F_1F_o-ATP synthase is critical for homeostatic control of the protonmotive force and inhibition of the complex may lead to uncoupling of the respiratory chain. We report that BDQ addition to resting mycobacterial cell suspensions activates oxygen consumption and dissipates the transmembrane pH gradient suggesting an uncoupler-like mode of action for BDQ. Activation of respiration was dependent on the terminal oxidase cytochrome *bd* (*cydAB*) and *cydAB* mutants were hypersusceptible to BDQ. Characterization of the BDQ mode of action in lipid-only (no ATP synthase target) vesicles demonstrated that bedaquiline could shuttle protons across lipid-bilayers with high affinity. It's relationship to the current modes of action in mycobacteria, or its effect on the mitochondrial inner membrane, will be discussed.

DD3: NO-Donor Cephalosporins as New Agents Against Bacterial Biofilms

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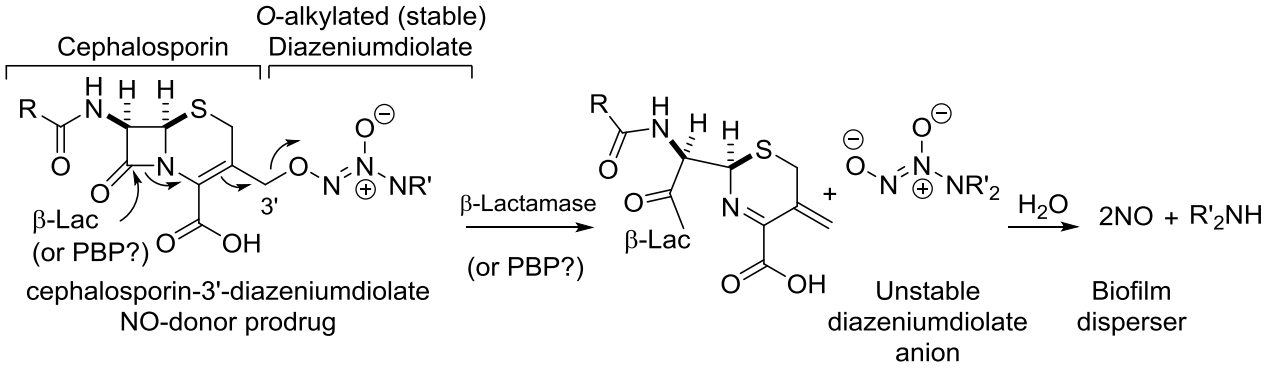
Low concentrations of nitric oxide (NO) have been shown to act as a signal that induces biofilm bacteria to disperse and revert to the free-swimming (planktonic) form.[1] This finding has unveiled an exciting new anti-biofilm paradigm; i.e. use of NO-donor compounds in combination with antibiotics to clear chronic biofilm infections, as it is well-known that planktonic bacteria are up to 1000x more susceptible to antibiotics and host immune defences than their better-protected biofilm counterparts.[2] Based on this discovery, we have designed, synthesized and provided *in vitro* proof-of-concept validation for a novel class of cephalosporin-based NO-donor prodrugs (cephalosporin-3'-diazeniumdiolates, DEA-CPs) that can provide biofilm-targeted NO delivery.[3,4] The targeted NO signal from DEA-CPs (activated by β-lactamases) induces biofilms to disperse and when used in combination with antibiotics the compounds are able to clear *in vitro* biofilms. The seminar will describe key results and highlight our latest efforts to create "all-in-one" bactericidal/anti-biofilm cephalosporins activated by penicillin-binding proteins (PBPs, transpeptidases), the normal target of β-lactam antibiotics.



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DD4: Regulation of protein production with pateamine

Paul Teesdale-Spittle, Richard Little, Claire Cuyamendous, Xu Tao, Sarah Brown, Hemi Cumming, Peter Northcote, Lifeng Peng, Gary Evans, Joanne Harvey

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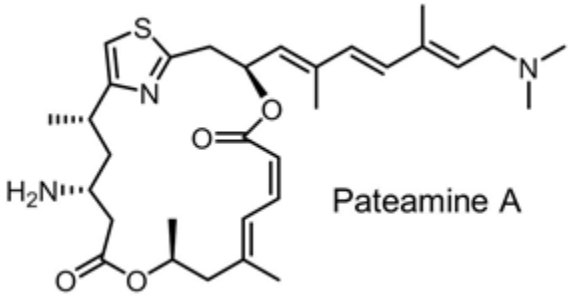
Sometimes the exploration of the mode of action of a natural product can lead to entirely new paradigms for disease treatment. One such case is the New Zealand marine natural product, pateamine A. First reported in 1991, pateamine was later shown to inhibit protein synthesis. However careful investigation of its target, a translation initiation factor known as eIF4A, revealed unexpected subtleties in the effect of pateamine at low concentrations. Whilst originally considered as an antifungal or anticancer compound with selectivity towards cells undergoing uncontrolled division, the potential therapeutic repertoire of pateamine at sub-toxic doses has rapidly expanded. Recent patents and publications for pateamine now include treatments for muscle wasting from cancer or age, viral infection, inflammatory bowel disease, and increasing lifespan. We are developing analogues of this remarkable natural product and undertaking detailed analysis of its effect on the proteome at sub-toxic concentrations. Our work shows how an apparent inhibitor of protein synthesis can lead to both decreases and increases in abundance of specific proteins in cells.



Pateamine A

DD5: From duocarmycins to nitroCBIs: converting toxic DNA alkylators into hypoxia-selective antitumour agents

Moana Tercel

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Duocarmycins comprise a small family of natural products isolated from soil bacteria that are notable for their extreme cytotoxicity. Despite some recent controversy it is generally accepted that this toxicity is a consequence of alkylation of DNA at adenine in the minor groove. Four synthetic and semisynthetic analogues proceeded to clinical trial as antitumour agents but all proved myelotoxic and lacking in activity at the low doses that could be administered systemically.

We have prepared and investigated aminoCBI analogues of the duocarmycins. Despite a fundamental change from phenol to aniline at the heart of the alkylating subunit these analogues share many of the properties of the natural products, including sequence-selective reaction with DNA via a cyclopropyl intermediate and similar cytotoxic potency. Importantly, aminoCBIs can be formed by enzymatic reduction of nitroCBIs in a process that is inhibited by oxygen. Since nitroCBIs cannot alkylate DNA, and since hypoxic regions are found in many solid tumours but not in healthy tissue, nitroCBIs represent a prodrug approach for selective tumour treatment. Moreover there is increasing awareness that hypoxic tumour cells are a significant problem, often harbouring a more malignant phenotype, and being resistant to many conventional and emerging therapies, including immunotherapy. This talk will describe the development and properties of advanced nitroCBI analogues with remarkable antitumour activity in preclinical models.

DD6: Semisynthesis-Based Structure-Activity Exploration of Portimine

Darby Brooke

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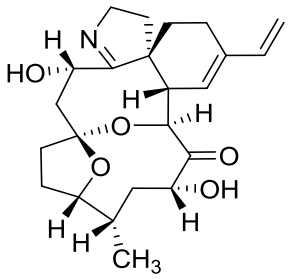
Portimine is a novel marine microalgal-derived cyclic imine toxin which was recently isolated and described by a Cawthron Institute-led team. The toxicity profile of portimine is distinct from other cyclic imines, suggestive of a novel mode of action, and it has also demonstrated activity against several marine biofouling organisms, and against a set of cancer cell lines.



portimine

We are targeting semisynthetic analogues of portimine as a means of probing its structure-activity relationships, and our current findings from these efforts will be discussed.

DD7:Nature's medicine chest – opportunities for drug discovery

Margaret A. Brimble

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Despite the ability of synthetic chemists to prepare libraries containing thousands of compounds efficiently, the ability to make discoveries pertinent to disease remains slow and arguably, serendipitous.[1] Commercial compound libraries suffer from low hit rates partly because their members possess low structural diversity and poor physicochemical properties (often combined with reactive and undesirable functional groups) since they are produced with an eye towards overall quantity rather quality.

Natural products have long been regarded as "Nature's medicine chest" providing invaluable platforms for developing front-line drugs. 65% of the 1211 small-molecule new drugs approved between 1981-2014 were "inspired by" natural products.[2] The chemical structures of natural products have evolved over several millennia for a specific biochemical purpose and their molecular frameworks can be considered "privileged scaffolds" that inspire the synthesis of focused natural product based libraries to advance biomedical research. Small libraries of compounds based upon natural products leads have several key benefits that do not apply to combinatorially-derived systems: they will have benefited from the optimisation of bioactivity for a given receptor as a result of natural selection; they will be expected to provide an enhanced rate of positive hits for a given library size; they will likely provide novel structural chemotypes not currently in use in existing therapeutic regimes; they would not be immediately susceptible to resistance-conferring genes in the bacterial and DNA pools; and they are likely to provide novel new target proteins and receptors. Natural product scaffolds also provide novel tractable heterocyclic systems of increasing molecular complexity by moving away from planar systems. The neglect of chirality has been recognized as a key deficiency of contemporary drug discovery methodology.[3]

This lecture will showcase some of our research on the synthesis of bioactive natural products and peptides for the development of novel anticancer, antibacterial and antithrombolytic agents.

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2. D. J. Newman, G. M. Cragg, *Natural Products as Sources of New Drugs from 1981 to 2014*. *J. Nat. Prod*., **2016**, *79*, 629-661.
3. F. Lovering, J. Bikker and C. Humblet, Escape from Flatland: Increasing Saturation as an Approach to Improving Clinical Success. *J. Med. Chem*., **2009**, *52*, 6752-6756.

DD8: Fun with arabinose? Looking for new small molecule anti-mycobacterial agents

Antony Fairbanks

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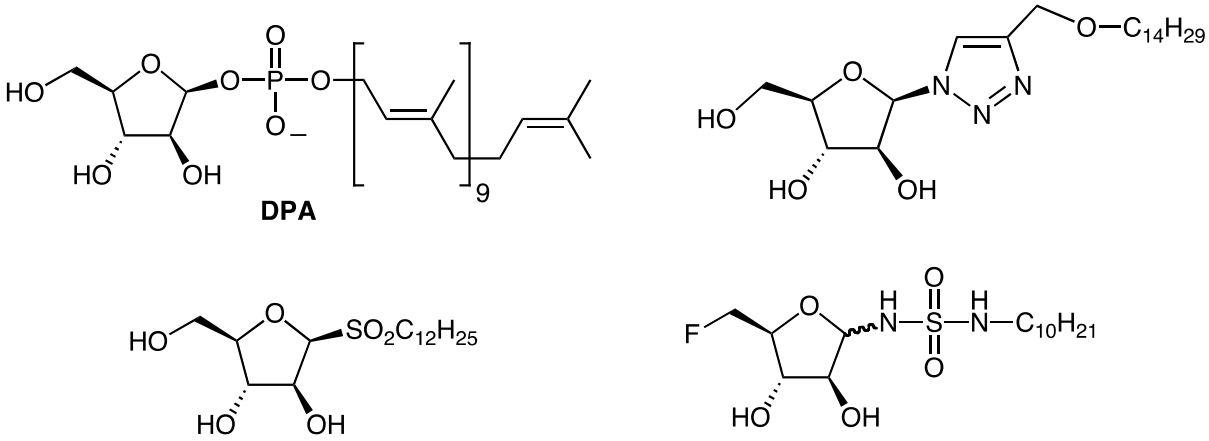
Inhibition of the biosynthesis of the mycobacterial cell wall represents an exciting therapeutic opportunity for the development of new drugs to combat TB. In particular assembly of the carbohydrate sections of the cell wall, many of the structures of which are unique to mycobacteria, has been a field of intense interest. We have been involved in an ongoing program over a number of years aimed at disrupting the assembly of the arabinose-containing mycobacterial polysaccharides arabinogalactan (AG) and lipoarabinomanan (LAM). Both of these are constructed in a stepwise fashion by a series of very poorly characterized arabinosyl transferases, which all use decaprenolphosphoarabinose (DPA) as the donor. A variety of approaches, including chain termination,[1] and the synthesis and testing of a series of mimics of DPA in which anomeric phosphate was replaced by sulfones,[2] triazoles,[3] and sulfamides[4] will be reported. Optimal MIC values of 31 μgmL^{-1} vs *M. smegmatis* and *M. Bovis BCG* were obtained in Alamar Blue assays.



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2. B. Ayers, H. Long, E. Sim, I. A. Smellie, B. L. Wilkinson, A. J. Fairbanks, *Carbohydr. Res.* **2009**, *344*, 739-746.
3. B. L. Wilkinson, H. Long, E. Sim, A. J. Fairbanks, *Bioorg. Med. Chem. Lett.* **2008**, *18*, 6265-6267.
4. a) K. Suthagar, A. J. Fairbanks, *Org. Biomol. Chem.* **2016**, *14*, 1748-1754; b) K. Suthagar, A. J. A. Watson, B. L. Wilkinson, A. J. Fairbanks, *Eur. J. Med. Chem.* **2015**, 102, 153-166; c) K. Suthagar, M. I. J. Polson, A. J. Fairbanks, *Org. Biomol. Chem.* **2015**, *13*, 6573 - 6579.

DD9: *Psychotria insularum*, a Samoan medicinal plant, is therapeutic via disruption of iron availability

Molimau-Samasoni, S.[1], Andreassend, S.[2], Patel, V.[1], La Flamme, A.C.[1], Atkinson, P.A.[1], Keyzers, R.A.[2], Munkacsi, A.B.[1]

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Natural products are a robust source of drug leads, and medicinal plants have been the source of natural products that are important pharmaceuticals in modern medicine. *Psychotoria insularum* is widely used in Samoan traditional medicine for the treatment of various ailments such as inflammation, wounds, abdominal distress and maternity difficulties. However, despite its extensive use, the underlying mechanism of its activity is not known. Given its multifaceted range of applications, we predicted the plant will have a mechanism of action affecting a fundamental cellular function that impacts a wide array of molecular processes. We report herein our genome-wide analysis of the aqueous extract of *P. insularum* leaves conducted in the genetic model *Saccharomyces cerevisiae* (Baker's yeast). This analysis implicated the involvement of iron transport in the extract mechanism of action. Quantification of total intracellular iron showed an increase in cells grown in the presence of *P. insularum*. Further investigation showed *P. insularum* treatment increased expression of iron transporter proteins and reduced biologically available heme. To translate results from yeast to mammalian cells, we treated primary murine macrophages with *P. insularum* extracts and detected an anti-inflammatory response. Here we will present our chemical genetic model that reduced iron bioavailability is responsible for the anti-inflammatory effects of *P. insularum*, correlating the elucidated mechanism of action and Samoan medicinal application.

DD10: One Ring To Rule Them All: The synthesis of biologically relevant compounds from cyclopropanes

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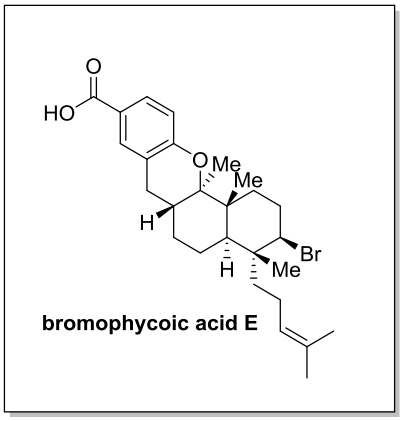
The rich chemical diversity of natural products and their often useful biological activity has provided a wealth of structures that lend themselves to the discovery of potential medicines. In fact, nearly 50% of all clinically used drugs are natural products or directly derived therefrom.[1]

Our research program is focussed on the development of new synthetic methods and their application to the synthesis of medicinally relevant scaffolds including natural and non-natural compounds.

Recently we described a method to generate highly functionalised oxazinones from various *in situ* generated *N*-acyl cyclopropyl iminium ions.[2] This seminar will discuss our efforts to extend this chemistry to provide robust and rapid entry into highly functionalised chromones. Furthermore, our efforts to apply this chemistry to a series of natural products including bromophycoic acid E, which possesses significant activity against vancomycin resistant enterococcus faecium, will be detailed.



bromophycoic acid E

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2. a) Craig, A.J.; van der Salm, L.; Stevens-Cullinane, L.; Lucas, N.T.; Tan, E.W.; Hawkins, B.C. *Org. Lett.* **2015**, *17*, 234. b) Smith, R.J.; Mills, D.A.; Nhu, D.; Tan, E.W.; Lucas, N.T.; Hawkins, B.C. *J. Org. Chem.* ***2016****, 81*, 2099.

DD11: Metagenome mining for the discovery of new bioactive natural products

Jeremy Owen

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Microbial secondary metabolites have been incredibly valuable as a source of antibiotics, anticancer agents, immunosuppressants and many other compounds used in both medicine and basic research. Traditionally, microbial secondary metabolites have been examined via the isolation and laboratory culture of microbes, however it is estimated that less than one percent of the microbes present in a given environment are currently able to be cultured in the laboratory. It is now possible to access the biosynthetic potential of the remaining 99% of bacterial species using a cultivation independent approach to discovery. This entails direct extraction of microbial genomic DNA from environmental samples and archiving this DNA as libraries. Fragments encoding small molecule biosynthesis can then be identified and transferred to a cultivable host that can read the new instructions and build the compounds they specify. In this talk I will discuss experimental and computational methods that allow rapid identification of genome fragments encoding biologically active small molecules in metagenomic samples. I will also present the structures and biological activities of new antibiotic and cytostatic natural products that were discovered using metagenomic methods.

DD12/CB13: Helping cancer vaccines

Ian F. Hermans[1,2,3]

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The concept that the immune system can target and eliminate cancer tissue has been validated clinically with the success of therapies that unleash the activities of T cells, such as bone marrow transplantation, immune checkpoint blockade and adoptive T cell therapy. Vaccines that specifically induce antitumour T cell responses have not yet had the same level of clinical impact, but this may change as we learn more about how immune responses are generated, and how to exploit this new knowledge in vaccine design. In this context, a brief overview will be presented on how populations of “innate-like” T cells in the lymphoid tissues can help initiate responses to vaccination, and how this knowledge is being used to develop a new series of powerful cancer vaccines.

DD13/CB14: Bioorthogonal synthesis and antitumour activity of CD1d dependent glycolipid-peptide vaccines

Painter, G.F.,[1] Tang, C.,[2] Anderson, R.J.,[1] Compton, B.J., Osmond, T.,[2] Farrand, K.J.,[2] Authier-Hall, A.,[2] Gasser, O.,[2] Hayman, C.M.,[1] Johnston, K.A.,[1] Larsen, D.S.,[3] Weinkove, R.[2] and Hermans, I.F.[2]

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Despite cancer vaccines having the potential to induce T cell responses with high specificity for defined tumour-associated antigens issues remain around lack of clinical efficacy, manufacturing and cost.[1] Synthetic vaccines based on antigenic peptides are highly defined and easily manufactured, however, in order to generate a robust anti-tumour response it is important not only that peptide fragments are acquired by antigen presenting cells (APCs) and presented to T cells in the context of MHC molecules, but also that the APCs are in the correct activation state.[2] This can be achieved by using adjuvants that target pattern-recognition receptors on APCs, such as the Toll-like receptors (TLRs). A less explored approach to activating APCs is to specifically stimulate innate-like T cells in the local environment. The most studied innate-like T cells are the invariant natural killer T (NKT) cells, which recognise glycolipid antigens that bind to the lipid antigen-presenting molecule CD1d.[3] In this paper we will describe the bioorthogonal synthesis of CD1d dependent glycolipid-peptide conjugate vaccines and their antitumor activity in preclinical models. The synthetic strategy includes the use of strain-promoted azide alkyne cycloaddition (SPAAC) and self-immolative linker technologies.

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2. Reed, S. G.; Orr, M. T.; Fox, C. B., *Key roles of adjuvants in modern vaccines*. *Nature medicine* **2013,** *19* (12), 1597-608.
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DD14/CB15: Synthesis and Activity of 6”-Deoxy-6”-Thio-α-GalCer and Peptide Conjugates

Larsen, D. S.,[1] Compton, B. J.,[2] Tang, C-W., [3] Johnston, K.A.,[2], Osmond T. L.,[3] Hayman C.M.,[2] Hermans, I.F.,[3] Painter, G.F.[2]

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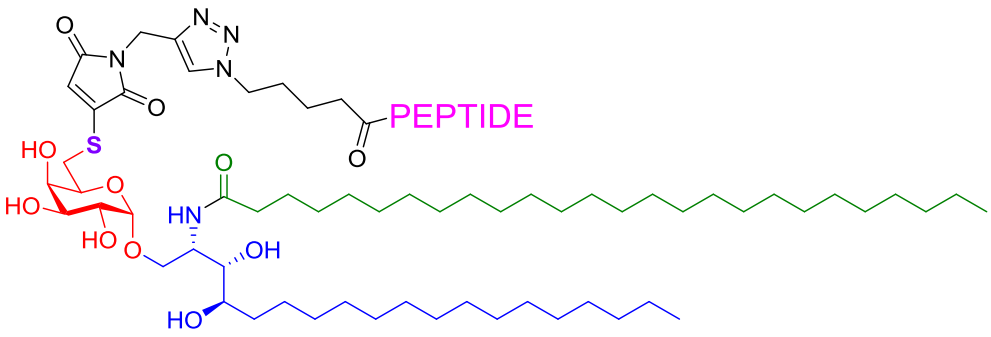
A major challenge in the development of highly defined synthetic vaccines is the codelivery of vaccine components (i.e., antigen and adjuvant) to secondary lymphoid tissue to induce optimal immune responses. This problem can be addressed by synthesizing vaccines that comprise peptide antigens covalently attached to glycolipid adjuvants through biologically cleavable linkers. Toward this, a strategy utilizing previously unreported 6”-deoxy-6”-thio analogues of α-GalCer that can undergo chemoselective conjugation with peptide antigens is described in this presentation.[1] Administration of these conjugate vaccines leads to enhanced priming of antigen specific T cells. This simple vaccine design is broadly applicable to multiple disease indications such as cancer and infectious disease.



6-thio-α-GalCer-Peptide conjugate

1. Compton, B. J.; Tang, C.-w.; Johnston, K. A.; Osmond, T. L.; Hayman, C. M.; Larsen, D. S.; Hermans, I. F.; Painter, G. F., *Synthesis and Activity of 6''-Deoxy-6''-thio-α-GalCer and Peptide Conjugates*. *Org. Lett. 17* (24), 5954-5957 (**2015**)

DD15/CB16: Enhancing immune therapies for cancer

Sarah Young

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Virus-like particles (VLP) are empty viral protein shells formed from capsid proteins of some viruses. They are a proven platform for immune stimulation and the delivery of subunit vaccines. The current vaccines against Hepatitis B virus and Human Papilloma Virus (HPV) are VLPs, for example. We have generated VLP from *Rabbit hemorrhagic disease virus (RHDV).* We have shown that unlike most VLP, which induce antibody production, RHDV VLP are particularly effective at promoting anti-tumour immunity. We have used these VLP to treat several tumours, including melanoma and colorectal cancer. We have also enhanced the immunogenicity of the VLP by linking immune adjuvants to the particle surface.

Our laboratory is also developing adoptive cell therapies (ACT) for cancer treatment. The majority of the current ACT are focused on using CD8 T cells, however some studies have shown that CD4 T cells can also be effective in tumour rejection, but the methodology of expanding these cells has not been well characterised. We have used the antigen-specificity of CD4 T cells from OTII transgenic mice in order to optimize the methodology for antigen-specific expansion these T cells in tissue culture. These T cells displayed an effector phenotype associated with an anti-tumour response. When these cells were adoptively transferred they demonstrated direct lysis of antigen-specific target cells *in vivo*. However in a mouse model of melanoma, CD4 T cells did not control tumour growth as well as CD8 T cells but a combination therapy of both CD4 and CD8 T cells induced an enhanced level tumour regression, leading to 85% tumour-free survival. Finally we have assessed the ability of the VLP to boost adoptively transferred T cells and shown that the vaccine can act to enhance T cell responses.

DD16/CB17: Trehalose Glycolipids: 'Upping the Ante'

Stocker, B.L.[1,2], Kodar, K.[1,2], Timmer, M.S.M.[1,2]

[1]School of Chemical and Physical Sciences, Victoria University of Wellington, [2]Centre for Biodiscovery, Victoria University of Wellington, NZ. Email: Bridget.Stocker@vuw.ac.nz

Vaccines have made a major contribution to the control and eradication of disease, yet there are still many pathogens or ailments for which effective vaccines are not available. There are two general classes of vaccine: prophylactic vaccines, which provide protective immunity against pathogens; and therapeutic vaccines, which assist in treating an existing disease. Both classes of vaccine require an antigen and an adjuvant to be effective, whereby the role of the adjuvant is to enhance the antigen-specific immune response.[1]

To address the need for new and enhanced vaccines there has been much interest in harnessing the potential of bacterial glycolipids as adjuvants. In particular, we are interested in the role of trehalose glycolipids as vaccine adjuvants.[2] This family of glycolipids was first isolated from the cell wall of *M. tuberculosis* and has since been found to activate the innate immune response via binding to the macrophage inducible C-type lectin (Mincle).[3] Using a combination of chemical synthesis and innate immunology, we have been exploring how trehalose glycolipids interact with macrophages so as to determine how the glycolipids may best be optimised for use as vaccine adjuvants.

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DD17/CB18: Structural adaptations of Class III PI3K and a PIKK to regulate cellular homeostasis

Roger L. Williams[1], Ksenia Rostislavleva[1], Domagoj Baretić[1], Nicolas Soler[1], Yohei Ohashi[1], Alex Berndt[1], Lufei Zhang[1], Els Pardon[3, 4], John E. Burke[1, 2], Glenn R. Masson[1], Olga Perisic[1], Chris Johnson[1], Jan Steyaert[3, 4], Nicholas T. Ktistakis[5]

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Many anti-cancer therapeutics induce cellular stress, and induction of autophagy is a pro-survival response to this stress. An early event in macroautophagy is production of phosphatidylinositol 3-phosphate (PtdIns3P) at the site of autophagosome induction. Our structural work has focused on two enzymes important for regulating autophagy, Vps34, the class III phosphoinositide 3-kinase that synthesizes PtdIns3P to promote autophagy and TOR, a S/T protein kinase that inhibits autophagy. The kinase domains of Vps34 and mTOR share a common ancestor and are present in all eukaryotic cells. When cells are starved of amino acids, Vps34 becomes upregulated and macroautophagy is initiated. In contrast, when amino acids are abundant in lysosomes, mTOR can become activated on the lysosomal membrane and it suppresses Vps34 activity. Both mTOR and Vps34 are present in large multi-subunit complexes whose structures we have determined.

Vps34 associates with a Vps15 regulatory S/T protein kinase, and this heterodimer can associate with another heterodimer, consisting of either Vps30 (Beclin1) with Vps38 (UVRAG) or Vps30 (Beclin1) with Atg14 (Atg14L). The complex containing Atg14 is known as complex I and activated in autophagy, while the Vps38 (UVRAG)-containing complex is known as complex II, which is involved in endocytic sorting.

Our crystal structure of the 385 kDa endosomal complex II (PIK3C3-CII) shows that the subunits form a Y-shaped complex, centered on the Vps34 C2 domain. The catalytic arm of the Y is made up of Vps34 and Vps15 where the Vps15 kinase domain binds to the activation loop of Vps34. Vps30 and Vps38 form the adaptor arm of the Y. Hydrogen-Deuterium Exchange Mass Spectrometry (HDX-MS) suggests that the adaptor arm of the complex interacts with membranes. Complex II is active on low-curvature membranes made of brain lipids, while complex I prefers highly curved membranes. Activities of complex I and II are exquisitely sensitive to the membrane packing density. HDX-MS shows that a fifth subunit known as Atg38/NRBF2 binds the Vps30/Atg14 arm of complex I, using its N-terminal MIT domain to bridge the coiled-coil I regions of Atg14 and Vps30 in the base of the Y of complex I. The Atg38 C-terminal domain is important for localization to the pre-autophagosomal structure (PAS) and homodimerization.

TOR regulates a range of cellular processes, including inhibiting Vps34 complex I. Our cryo-EM structure of the core of the two types of TOR complexes in cells shows a symmetric dimer. As with Vps34, the C-terminal kinase domain of Tor is preceded by a helical solenoid region, however, this region is greatly expanded in Tor and other PIKKs. The N-terminal residues of Tor form an α-helical solenoid with a complex topology. Our experimentally determined topology traces the course of the polypeptide for most of the 1300 residues of the α-helical solenoid in a direction that is opposite to what was recently proposed for mTORC1. Our topology implies that in mTORC1, RAPTOR binds to the dimer interface of mTOR, consistent with the ability of RAPTOR to stabilise the complex.

DD18: Cubane: a Benzene Isostere!

Craig M. Williams

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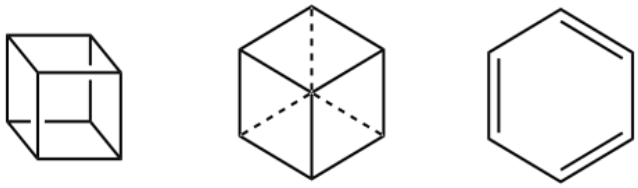
The synthesis of cubane (**1**), achieved in 1964 by Eaton, was long predicted to be impossible, due to the immense strain of the molecular structure. Since then a large array of chemical transformations have been performed on the cubane ring system demonstrating the framework to be both a stable and robust building block. Studies into the physical properties of cubane gave further insight, showing that the distance across the cube (the body diagonal) is 2.72 Å, which is almost equivalent to the distance across the benzene ring i.e. 2.79 Å. This similarity is best viewed from one of the 8 apexes (see apex representation **2**). Eaton observed that a number of other similarities exist between cubane (**1**) and benzene (**3**), for example the enhanced *s* orbital character of the C-H bond and the similar spatial relationships to *ortho* and *para* substituents (i.e. 1,2- and 1,4-disubstitution). However, whilst the physical, or spatial, appearance of cubane is similar to benzene, spectroscopically, cubane has both proton and carbon peaks much further up-field in the nuclear magnetic resonance (NMR) spectrum (^{1}H ~4ppm ^{13}C ~50ppm) suggesting obvious electronic differences. This lecture will disclose our recent efforts to explore,[1] using known drugs, whether cubane can act as a benzene ring surrogate, based on the above similarities and differences profile displayed by both ring systems.



1 **2** **3**

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DD19: Discovery and Development of PI3-Kinase Delta Inhibitors

Gordon W. Rewcastle

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Phosphoinositide-3-kinase (PI3K) is an important mediator of tumor cell growth, survival and proliferation. We have previously reported on the design, discovery and characterization of PWT33597 (now known as VDC-597), a dual inhibitor of PI3K alpha and mTOR, which entered human clinical trials in 2011. The discovery and development of PWT33597 was performed in collaboration with Pathway Therapeutics, and following on from that work, we began a joint investigation into PI3K delta inhibitors.

PI3K delta inhibitors have recently shown very promising results against a range of leukemias (both lymphomas and myeloid leukemias) and one of these, Idelalisib (Zydelig/CAL101/GS-1101) became the first PI3K inhibitor to be approved by the US FDA in 2014. Unfortunately serious toxicity issues have subsequently arisen, and several clinical trials with Idelalisib have now been terminated, due to an increased rate of adverse events, including deaths. Thus there is a proven need for less toxic PI3K delta inhibitors.

Unfortunately our PI3K delta collaboration with Pathway Therapeutics did not last beyond 2010, when they chose to withdraw their funding from us, and perform their synthetic chemistry in China. This led to the development of PWT143 (now known as ME-401), an orally bioavailable, potent and selective inhibitor of phosphatidylinositol 3 kinase (PI3K) delta which entered human clinical trials in 2015. The development of PWT143, which contains many elements derived from our work in Auckland, will be discussed.

DD20: Oxyamine linker methodology for the synthesis of glycoconjugates

Timmer, M.S.M., Stocker, B.L.S, Painter, G., Munneke, S., Hill, J.

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Glycoconjugates, such as glycolipids and glycoproteins, play an important role in health and disease. The synthesis and biological evaluation of these glycoconjugates allows for the development of novel carbohydrate-based therapeutics and analytical tools. Traditionally, the conjugation of glycans to other substrates required the installation of an anomeric linker during the total synthesis of the glycan, however, this strategy does not allow for the conjugation of naturally isolated glycans. To address this concern, we have developed glycan conjugation methodology for the protecting group free synthesis of glycoconjugates (Figure 1).[1] In particular, the synthesis of a variety of novel bi-functional oxyamine linkers enabled the rapid assembly of various types of glycoconjugates, including fluorescent- and biotinylated-glycans, glycoproteins and multivalent glycodendrons. The utility of our strategy will be illustrated through the rapid assembly of a fluorescent multivalent LewisX glycodendron,[2] and its biological evaluation for the detection of C-type lectins on human macrophages using flow cytometry.

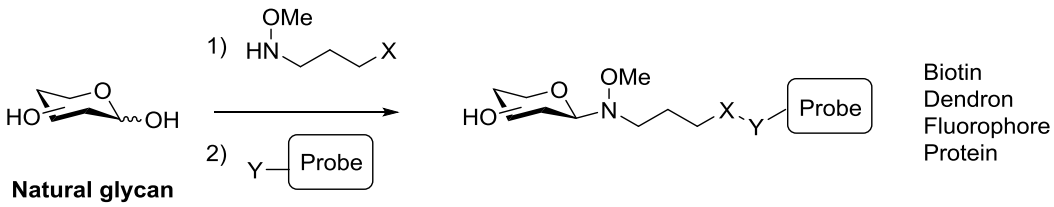

Figure 1: Synthesis of glycoconjugates using chemoselective bifunctional linkers.

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DD21: Second-generation analogues of the "new" TB drug bedaquiline

William A. Denny,[1,2] Brian D. Palmer,[1,2] Adrian Blaser,[1] Peter Choi,[1] Daniel Conole,[1] Hamish S. Sutherland,[1] Andrew A. Thompson,[1,2] Amy Tong,[1] Chris Cooper,[3] Anna Upton[3]

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Tuberculosis (TB), caused by the bacterium *Mycobacterium tuberculosis* (M.tb), is again a disease of major concern, due to the rapid increase of both multi-drug resistant TB (MDR; ~500,000 cases in 2014 [3.3% of total new cases], 50% treated successfully) and extremely drug-resistant TB (XDR; ~50,000 cases in 2014, 26% treated successfully). There is therefore a great need for new drugs with novel modes of action and activity in drug-resistant TB.

The drug bedaquiline (Sirturo; Janssen), registered in 2012, inhibits the c subunit of the M.tb ATP synthase and shows promise in drug-resistant TB, but has a number of limitations, including cardiovascular effects (due to its hERG blockade) and phospholipidosis (due to its very high lipophilicity).

Despite this promise, and the limitations observed, there has been relatively little work reported on the development of improved analogues of bedaquiline, partly because of the necessity of separating the active (R),(S) stereoisomer from the mixture of four produced. We present the results of an extensive structure-activity relationship study of more than 600 analogues of bedaquiline, designed and synthesized to ameliorate these limitations, and compare and contrast with bedaquiline six potential lead compounds from this study. These (and others) are currently in advanced evaluation with the aim of selecting a clinical candidate in early 2017.

DD22: Targeting a multimeric protease for new Chlamydia therapeutics

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Chlamydia (C.) trachomatis is an obligate intracellular pathogen and is responsible for the most common sexually transmitted bacterial infection world-wide. It was the most commonly diagnosed STI in New Zealand. It has a high rate of asymptomatic infection (approximately 70% in women and up to 50% in men). Infection in women without treatment can result in infertility, pelvic inflammatory disease, or ectopic pregnancy. The pathogen is the leading cause for preventable blindness. HtrA (high temperature requirement A) has been shown to be at high levels in Chlamydia. HtrA acts a serine protease with additional chaperone activity in a multmeric form with relatively broad specificity. It is essential for survival in a variety of bacteria and an important virulence factor in a number of pathogens including *Helicobacter pylori*, *Bordetella pertussis* and *Bacillus anthracis.*

Initial investigations of casein digests revealed elastase-like substrate specificity which could be inhibited by an isocoumarin inhibitor.[1] A subsequent screen of 1090 serine protease inhibitors revealed JO146 [Boc-Val-Pro-ValP(OPh_2); IC_{50} = 12 μM] a peptidic diphenylphosphonate inhibitor as the best hit compound.[2] This formed the starting point for inhibitor optimisation with the idea of developing inhibitors with more druglike characteristics. We aimed to optimise inhibition through variation of the transition state analogue with covalent and non-covalent bioisostere (e.g. N-methyl amide, boronic acid, α-ketoheterocycles) along with expanding our understanding of the SAR at the P1 site. We have been able to produce compounds with submicromolar activity against the enzyme that show promising results against chlamydial cells, in a human host environment. This paves the way for further optimisation with a view to pre-clinical studies.

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DD23: Virtual screening for novel Atg5-Atg16 complex inhibitors for autophagy modulation

Elizabeth Robinson[1], Euphemia Leung[2], Anna M. Matuszek[3], Niels Krogsgaard-Larsen[3], Daniel P. Furkert[3], Margaret A. Brimble[3], Alan Richardson[1], Jóhannes Reynisson[3]

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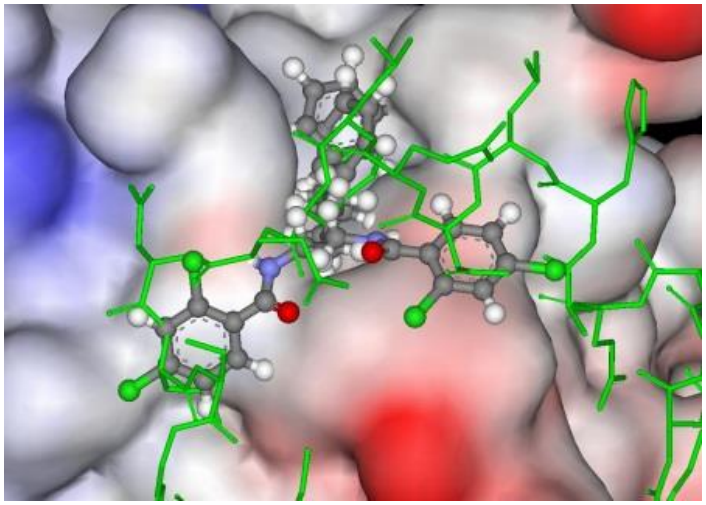
Two hit compounds (**14** and **62**) were identified using virtual high throughput screening (vHTS) inhibiting the autophagy process[1, 2] in A2780 ovarian cancer cells. The expression levels of the LC3-II and p62 autophagy marker proteins were monitored using Western blotting. Preliminary structure activity relationship (SAR) study of close structural analogues revealed another active compound **38**. The three active compounds were tested in the MCF-7 human breast cancer cells and severe reduction of autophagosomes formation was observed confirming the activity of the inhibitors. The docking scaffold used for the vHTS was a lipophilic cleft on the Atg5 protein, which is occupied by a phenylalanine residue in the Atg16 polypeptide. To the best of our knowledge this is the first report on inhibitors that specifically modulate autophagy by directly inhibiting autophagy specific proteins, which is significant due the role autophagy plays in a number of morbid diseases such as cancer.[3]



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DD24: Transition state analysis for ATP-PRT enzymes

Gert-Jan Moggré,[1] Peter Tyler,[2] Vern Schramm,[3] Emily Parker[1]

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Adenosine triphosphate phosphoribosyltransferase (ATP-PRT) catalyses the first step in histidine biosynthesis. The biosynthetic pathway is essential to microorganisms, but absent in humans making it an interesting target for novel drug design. Understanding the nature of the transition state can provide essential information on the reaction mechanism of this enzyme. Highly specific and potent inhibitors may also be designed based on this transition state.

The kinetic isotope effects (KIEs) for ATP-PRT from *Campylobacter jejuni* and *Mycobacterium tuberculosis* were determined. To overcome commitment to catalysis the reaction was studied in reverse with phosphonoacetic acid (PA), an alternative to the natural substrate pyrophosphate. The transition state structures were modelled with density functional theory at the B3LYP/6-31+G(d,p) level by closely matching the calculated KIEs to the experimental KIEs. Based on this a dissociated $D_N{*}A_N^{\ddagger}$ mechanism was predicted for the reaction.

This presentation discusses work towards measuring the KIE values for ATP-PRT enzymes and the transition state structure predictions.

DD25: Designing a Novel Series of p110α-Selective Phosphoinositide 3-Kinase Inhibitors: A Scaffold Hopping Approach

Jackie D. Kendall

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The phosphoinositide-3-kinase (PI3 kinase) pathway is important in cell proliferation, growth and survival. PI3 kinases are over-expressed in many cancer cell types, and the p110α isoform is often mutated in cancer cells. Many of these cancer-specific mutations lead to a gain in function which results in oncogenicity.

Hence there is a great deal of interest in the p110α isoform of PI3 kinase as a cancer target. Many groups are trying to exploit PI3 kinase inhibitors as an approach to cancer therapy, as exemplified by the number of small molecule PI3 kinase inhibitors currently in clinical trials, however few of these are selective for p110α.

This presentation will discuss our efforts to make novel p110α-selective PI3 kinase inhibitors derived from a scaffold-hopping approach from an earlier series. It will focus on the chemical synthesis, biological evaluation, structure-activity relationships (SAR) and molecular modelling of a novel series of inhibitors.

DD26: Features that influence inhibitor preference between different PI-3 kinases

Gong, G.Q. Kendall JD, Rewcastle GW, Denny WA, Shepherd PR, Flanagan JU

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Visualising a ligand bound to its target protein identifies the binding site, defines the amino acids, the atoms and the types of interactions involved in binding. This is a major enabling factor in directing the development and discovery of new compounds. While X-ray crystallography is the most powerful method for looking at these interactions, not all proteins or drug and target complexes are immediately amenable to this method. In these cases, computer based methods like molecular docking can improve the reach of existing atomic level structural information into the discovery of new compounds. Here I will give an overview of how we have used protein structure information to guide the design of new inhibitors that explore some of the molecular features that influence selectivity for PI3K isoforms.

DD27: Design and synthesis of SN36506: An optimised second generation hypoxia-selective cytotoxin under clinical development

Jeff B Smaill

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The presence of hypoxia (low oxygen) represents a physiological target that distinguishes solid tumours from normal tissues and is arguably the most validated target in clinical oncology yet to be addressed. The design of hypoxia-activated prodrugs (HAPs) that selectively 'unveil' a DNA-reactive cytotoxin at high dose-intensity within hypoxic tumours attempts to exploit this target. Thus far none have succeeded; the high profile examples tirapazamine and evofosfamide failed to meet their primary endpoints in pivotal Phase III registration trials.

PR-104 is a water-soluble phosphate 'pre-prodrug' of the hypoxia-activated prodrug PR-104A, a dinitrobenzamide nitrogen mustard prodrug discovered in the mid-2000's at the Auckland Cancer Society Research Centre that entered clinical development as a once weekly (Qw) or once every three week (Q3w) intravenous infusion. The subsequent finding that PR-104A is a substrate for human aldo-keto reductase 1C3 (AKR1C3), an off-mechanism source of activation that is oxygen independent and the likely cause of dose-limiting myelotoxicity, has led to the clinical re-deployment of this agent in high AKR1C3-expressing leukaemia.

We recently initiated a discovery program seeking to design a second generation hypoxia-selective analogue of PR-104 that is resistant to metabolism by AKR1C3. As an additional selection criterion a preferred candidate was also required to demonstrate acceptable aqueous solubility without relying on a phosphate pre-prodrug strategy. We reasoned this would eliminate the requirement for a primary alcohol substituent in the prodrug moiety, as this is a source of UGT2B7-mediated glucuronidation leading to poor pharmacokinetics (rapid clearance) of PR-104A in humans. We have now identified SN36506 as a water soluble, hypoxia-selective cytotoxin that is free of AKR1C3 metabolism. SN36506 is a 4-methylsulfonyl-2-nitrobenzamide prodrug readily synthesised in 8 steps from commercially available starting materials (36% overall yield). In the H460 multidrug resistant non-small cell lung cancer model, SN36506 had significant single agent activity and was particularly active against radiation-resistant (hypoxic) tumour cells in excision assays using a clonogenic endpoint. SN36506 is currently in IND-enabling studies in preparation for its clinical development.

DD28: Drug metabolism:- is it simply an esoteric academic exercise?

Nuala Helsby

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Understanding drug metabolism and pharmacokinetic parameters early in the drug discovery process is important as it can allow optimisation of Absorption, Distribution, Metabolism and Excretion (ADME) parameters. During the discovery period relatively high throughput strategies to assess microsomal (metabolic) stability and cytochrome P450 (CYP) inhibition are often used in the process of lead selection. Whilst useful the metabolic stability approach does have limitations. For example it doesn't take into account the role of non-CYP catalysed biotransformation reactions, the production of major metabolites with intrinsic pharmacological activity and the production of minor but potentially toxic metabolites. My talk will discuss some of these issues and highlight why consideration of the metabolite profile of lead candidates is not simply an esoteric exercise of interest only to drug metabolism scientists.

DD29: Development of a functional and biophysical screening pipeline to identify inhibitors of the growth hormone receptor

Flanagan J.U. [1], Hay M.P.[1], Langley R.[2], Shucksmith A.[3], Clow F.[2], Lu M.[3], Tan E.[3], Perry, J.K.[3]

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Localised expression of growth hormone (GH) is detectable in a variety of different human cancers, including breast cancer, and this is associated with poor survival outcomes for breast cancer patients. In contrast, humans with congenital deficiency in the cell surface receptor for GH have a dramatically reduced risk of developing cancer. Current results obtained from preclinical studies in different cancer models including breast cancer have been promising and provide evidence in favour of further testing the hypothesis that GH receptor antagonism could be effective in treating tumours that are GH or insulin like growth factor-1 (IGF-1) dependent. Consistent with this we have recently demonstrated that GH receptor antagonism suppresses tumour regrowth after radiotherapy in an endometrial cancer model.

In order to discover and develop small molecule inhibitors of the GH receptor we have established a discovery pipeline comprised of virtual screening, cell-bioassays and biophysical methods including Biacore that can identify functional, selective GH receptor blockers. To exemplify this algorithm, we have designed and screened a pilot library of 2045 compounds extracted from the Chembridge collection, based partly on the structure of the GH receptor and partly on testing a diverse range of random compounds.

DD30: Multifunctional ligands of the CB1 Cannabinoid GPCR, an intriguing challenge in drug design

Furkert, D. P.,[1] Kahlcke, N.,[1] Grant, P.,[1] Brimble, M. A.,[1] Hurst, D. P., Reggio, P. H. and Glass, M.[2]

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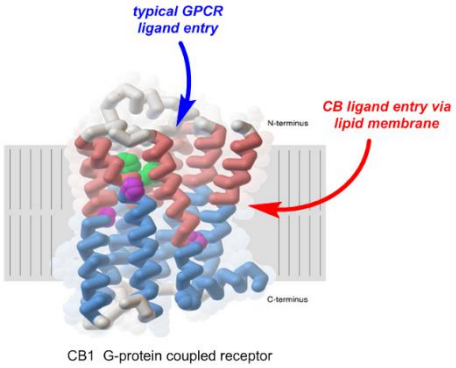

CB1 G-protein coupled receptor

Cannabinoid CB1 G-protein coupled receptors (GPCRs) are potentially useful therapeutic targets, involved in the regulation of important processes through the action of endogenous cannabinoid ligands. Development of clinical agents has proven challenging, due to the complex SAR and undesirable psychiatric side effects of the single antagonist approved to date (Rimonabant; later withdrawn). Complicating the issue, the CB1 receptor has been demonstrated to associate in homo- and hetero-oligomeric complexes and exhibits complex ligand-biased agonism and allosterically modulated signalling profiles.[1]

Fundamental understanding of ligand-CB1 receptor interactions for drug development will be increased through use of effective molecular probes. Several types of functional probes would be of high value, including (*i*) fluorescent tags, (*ii*) covalent ligands, (*iii*) neutral antagonists, and (*iv*) divalent ligands that bind CB1 and other GPCRs simultaneously (*e.g.* dopamine D2).[2] A final hurdle hampering development of these tools is the growing evidence that CB1 ligands enter the receptor via the lipid membrane (*inset*),[3] placing strict constraints on probe composition. Beginning from a validated CB1 ligand pharmacophore, attachment points for linkers to span the lipid membrane have been explored through synthesis of probe candidate libraries. These studies indicated that only one of the three feasible attachment points tolerates linkers while retaining high affinity at CB1. The nature of the linker itself proved an important determinant in retaining affinity and desirable physical properties. In combination with *in silico* docking studies, further synthetic manipulation led to the identification of a novel covalent CB1 ligand and has enabled significant progress towards both divalent (CB1-D2) and fluorescent-labelled high affinity ligands. An account of our current understanding of the molecular determinants for probe design, and ongoing studies towards related neutral antagonists will be presented.

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DD31: Collaboration, Translation and Commercialisation; Finding a Path to Success

Warwick Tong

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Successful commercialisation of preclinical small molecule drugs requires being at the front of the pack in a novel area of science supported by a comprehensive data package. Getting to the point when the sales package is compelling presents many challenges including scientific, IP and funding and also requires a degree of luck.

Cancer Therapeutics Cooperative Research Centre (CTx) is a public/private oncology drug discovery collaboration supported by the Australian Government Cooperative Research Centre Programme. A partnership of some of Australia's major research institutes and universities it recently licensed an epigenetic inhibitor program to a major pharmaceutical company. The five year history of the project will be discussed with a focus on key challenges and the solutions and tradeoffs that supported the path to successful commercialisation.

DD32: Commercialising Intellectual Property Arising from Drug Discovery

Melissa Yiannoutsos

PowerHouse Ventures, Wellington, NZ. Email: melissa.yiannoutsos@powerhouse-ventures.co.nz

The investment landscape in NZ is changing, allowing for more drug discovery and commercialisation to occur locally before international partnering. Hear how Powerhouse Ventures and others are addressing a capital market gap in the medical and healthcare sector. Avalia Immunotherapies, a preclinical stage vaccine company, is used as a case study to illustrate how research institutions can partner with New Zealand investors and utilize government initiatives. This approach is helping to accelerate the drug development process and IP portfolios, ultimately, capturing more value in New Zealand.

DD33: An Intellectual Property Horror Story – The Shade of Teixobactin and the Ghost of Patents Past

Jon Ashen

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Follow the harrowing intellectual property (IP) adventures of a group of scientists out to save the world from microbial disaster. Our Story begins in early late 2012 when our heroes file a US provisional patent application disclosing a novel depsipeptide useful for treating microbial infections. From this point forward, the spectre of the US government looms large, forcing the team to react quickly to new and unexpected IP challenges. Can our heroes overcome the significant hurdles that rise before them? Can they to obtain the robust intellectual property protection they may well deserve, or will all be lost? Don't miss the thrilling season finale where answers to these and other pressing questions may be revealed!

DD34: In vitro drug sensitivity testing of cancer patient cells for "individualized" treatments

Greg M. Arndt

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The identification of patients that will or will not respond to a specific chemotherapy, targeted therapy or drug combination has the potential to assist in the treatment and management of childhood and adult cancer patients. In vitro laboratory-based assays for determining the sensitivity of patient-derived cells to specific anticancer agents would provide a means of optimising the chemotherapeutic regimen for a specific patient and potentially improve response rates. As part of a larger Precision Medicine Program being developed by Children's Cancer Institute and Sydney Children's Hospital (Randwick), the ACRF Drug Discovery Centre for Childhood Cancer is implementing an in vitro drug sensitivity testing platform, using highly sensitive cell viability and high content imaging assays, to assess the sensitivity or resistance of childhood cancer patient cells to a range of chemotherapeutic and targeted drugs. This presentation will review the in vitro drug sensitivity testing platform, highlight examples of cell testing and discuss the potential of this platform for rapidly finding the right treatment for the right patient, especially for high risk patients that will not respond to current standard-of-care and those patients that undergo relapse and require new treatment plans.

DD35: Commercialisation – spending money to make money!

Paul Benjes

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GlycoSyn is a chemistry development and pharmaceutical manufacturing business unit of Callaghan Innovation. In its 13 year life, GlycoSyn has been involved with numerous clients commercialising innovative new drug candidates to Proof of Clinical Concept and successful exits. A case study will be presented with the aim of helping prospective developers better understand the role and importance of Chemistry, Manufacturing and Control (CM&C) in the commercialisation pathway.

DD36: War stories from protecting inventions

Richard Furneaux

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From the late 80's, I have been keen to see our research lead to patented inventions as the basis for private sector investment then wealth creation. Our science has now been the basis of 55 patent families. A high proportion have been licensed or are used commercially. But the path to granted patents and licenses has not always been smooth. Some of the more dramatic twists and turns will be described.

Summary of Abstracts for the Poster Session

No.	Title	Presenter	Institutions
DD37	Stereochemical Basis of the Anti-Chlamydial Activity of JO146	Ayodeji Agbowuro	University of Otago
DD38	Coumarin antifungal lead compounds from *millettia thonningii* and their predicted mechanism of action	Daniel Ayine-Tora	University of Auckland
DD39	Evaluating the use of plasma anti-GAGA4 IgM levels as a biomarker for multiple sclerosis	Chriselle Braganza	Victoria University of Wellington
DD40	Zampanolide as an anti-cancer lead: towards the synthesis of analogues	Sophie Geyrhofer	Victoria University of Wellington
DD41	Side-Chain Analogues of TAN-2483B as Kinase Inhibitors	Joanne Harvey	Victoria University of Wellington
DD42	Small molecule modulators of the adrenomedullin 1 receptor	Lydia Liew	University of Auckland
DD43	Novel phage-like nanorods as diagnostic tools for ultra-sensitive assays	Marina Rajic	Massey University
DD44	Development of selective fluorescent ligands for A_1 adenosine receptor as pharmacological tools	Sameek Singh	University of Otago
DD45	Metagenomics approach to natural product discovery and biosynthesis	Luke Stevenson	Victoria University of Wellington
DD46	A metagenomic approach to discover biosynthetic gene clusters from the marine sponge *Mycale hentscheli*	Matt Storey	Victoria University of Wellington
DD47	The synthesis of α-D-glucosyl steroids for glycolipidomic profiling	Thomas Teunissen	Victoria University of Wellington
DD48	Bedaquiline analogues with improved efficacy and safety profiles for tuberculosis treatment	Amy Tong	University of Auckland
DD49	Computational characterisation of protein-membrane interactions	William Irvine	Massey University

Poster DD37: Stereochemical Basis of the Anti-Chlamydial Activity of JO146

Agbowuro, A. A.[1], Sagatova, A.A.[1, 2], Wilbanks, S.M.[2], Huston, W.M.[3], Gamble, A.B.[1], Tyndall, J.D.A.[1]

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JO146 (Boc-Val-Pro-Val^{P}(OPh)$_2$ is a peptidic diphenylphosphonate inhibitor of *Chlamydia trachomatis* high temperature requirement protease A (CtHtrA) with reported activity against *Chlamydia* species in humans and Koalas. The selective toxicity displayed by the molecule presents it as a viable lead towards the development of drug candidates for the treatment of chlamydiosis.[1] As a result, current efforts are aimed at optimizing its safety and increasing its potency. The synthesis of JO146 produces a mixture of two diastereomeric forms (JO146-D1 and JO146-D2), suggesting potential variation in pharmacological and/or toxicological profiles. This study separated the diastereomers, assessed their biological activity and rationalized any observed difference that could assist in the design of clinically applicable analogues.

JO146 was synthesised using standard solution phase peptide synthesis along with published methods for producing diphenylphosphonate.[2] JO146-D1 and JO146-D2 were obtained from the racemic mixture using column chromatography ($CHCl_3$/EtOAc, 4:1, v/v). The impact of the difference in stereochemistry between both molecules on biological activity was assessed through an *in vitro* CtHtrA and human elastase inhibition studies. Similar assays for trypsin and chymotrypsin were conducted to measure off-target selectivity. The (*R*)-diastereomer (JO146-D2) was found to have significantly better *in vitro* CtHtrA inhibition potency than the (*S*)-diastereomer (JO146-D1). Both compounds had little or no activity against the digestive serine proteases, trypsin and chymotrypsin (IC_{50} > 500 µM). The superior *in vitro* activity exhibited by JO146-D2 which is the isomer with the physiologically relevant L-amino acid at the P_1 position is a result of better enzyme recognition and binding compared with the non-natural D-amino acid containing isomer. This finding was supported by results from docking studies which revealed more hydrogen bonding between the enzyme and JO146-D2. It is thus pertinent to develop asymmetric synthetic methods directed in favour of the (*R*)-diastereomer for further analogues of JO146 in the diphenylphosphonate and other series.

1. Gloeckl, S.; Ong, V. A.; Patel, P.; Tyndall, J. D.; Timms, P.; Beagley, K. W.; Allan, J. A.; Armitage, C. W.; Turnbull, L.; Whitchurch, C. B. *Mol. Microbiol*. **2013**, *89*, 676.
2. Winiarski, Ł.; Oleksyszyn, J. z.; Sieńczyk, M. *J. Med. Chem.* **2012**, *55*, 6541.

Poster DD38: Coumarin antifungal lead compounds from *millettia thonningii* and their predicted mechanism of action

Daniel M. Ayine-Tora and Jóhannes Reynisson

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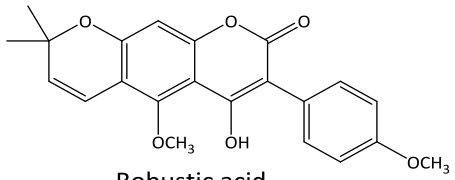
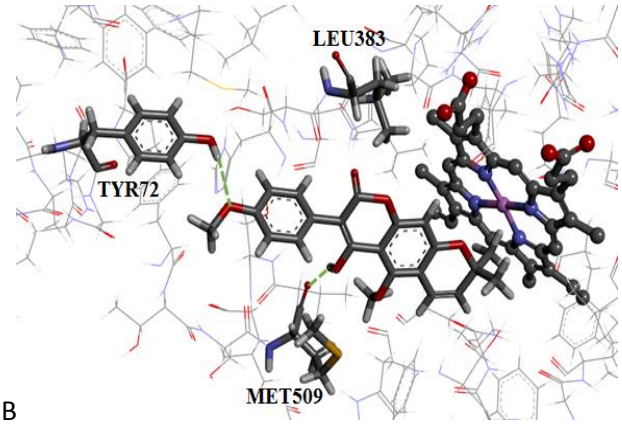


A Robustic acid



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Fungal pathogens continue to pose challenge to humans and plants despite efforts to control them. There are a number of drugs such as nystatin and terbinafine to address fungal infections. However, drug resistance, restricted systemic usage due to dose-related toxicity and emergence of new fungal strains, undermine their efficacy.[1] Hence, it is necessary to develop new antifungal treatments to address these emerging challenges. *Milletia thonningii* is a deciduous tree which is indigenous to West and Central Africa.[2]

Two compounds, robustic acid and thonningine-C isolated from *Millettia thonningii* show strong activity against the fungus *candida albicans* with minimum fungicidal concentration of 1.0 and 0.5 mg/ml respectively. Sterol 14a-demethylase (CYP51) catalyses the conversion of lanosterol to ergosterol. Disruption of this sterol results in structural changes of plasma membranes with cytotoxic consequences. Molecular modelling against this putative bio-molecular target, [3] revealed a plausible binding mode for the active compounds. Robustic acid is predicted to bind to the side chain of tyrosine 72 and the backbone of methionine 509 thereby disrupting the synthesis of several important sterols which are needed for the growth and survival of fungi.

1. Cowen, L. E.; Sanglard, D.; Calabrese, D.; Sirjusingh, C.; Anderson, J. B. and Kohn, L. M (2000), *Evolution of drug resistance in experimental populations of Candida albicans*. J. Bacteriol. 182: 1515-1522.
2. Asomaning, W. A.; Otoo, E.; Akoto, O.; Oppong, I. V.; Addae-Mensah, I.; Waibel, R. and Achenbach, H, (1999).*Isoflavones and coumarins from Milletia thonningii*. Phytochemistry. 51: 937-941.
3. Sagatova, A. A.; Keniya, M. V.; Wilson, R. K.; Sabherwal, M.; Tyndall, J. D. and Monk, B. C (2016).*Triazole resistance mediated by mutations of a conserved active site tyrosine in fungal lanosterol 14α-demethylase*. Scientific reports. 6: 26213.

Poster DD39: Evaluating the use of plasma anti-GAGA4 IgM levels as a biomarker for multiple sclerosis

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The correct diagnosis of multiple sclerosis (MS) remains challenging due to the complex pathophysiological and clinical characteristics of the disease. Current methods include invasive techniques such as cerebrospinal fluid analysis, which is used in combination with magnetic resonance imaging to deliver a diagnosis. Consequently, there has been interest in the development of non-invasive diagnostic tests for MS. Serum IgM anti-Glc-α-(1→4)-Glc-α (GAGA4) antibodies have recently been described as a potential specific biomarker for MS.[1,2] In our study, we evaluated the use and reliability of these antibodies as biomarkers for the diagnosis of MS. To this end, different carbohydrate antigens were synthesised and assessed for their ability to bind IgM in plasma from healthy and MS patients. Our results show that anti-GAGA4 titers in both groups are similar to other carbohydrate antigens. Moreover, no difference was seen in anti-GAGA4 IgM levels between the control and diseased groups. Our findings indicate that plasma anti-GAGA4 IgM levels may not represent a reliable and specific biomarker for MS.

1. Schwarz, M. et al. (2006). *Serum anti-Glc(α1,4)Glc(α) antibodies as a biomarker for relapsing–remitting multiple sclerosis*. Journal of the Neurological Sciences. 244: 59–68.
2. Freedman, M.S., Laks, J., Dotan, N., Altstock, R.T., Dukler, A. and Sindic, C.J.M. (2009). *Anti-α-glucose-based glycan IgM antibodies predict relapse activity in multiple sclerosis after the first neurological event*. Multiple Sclerosis.15: 422–430

Poster DD40: Zampanolide as an anti-cancer lead: towards the synthesis of analogues

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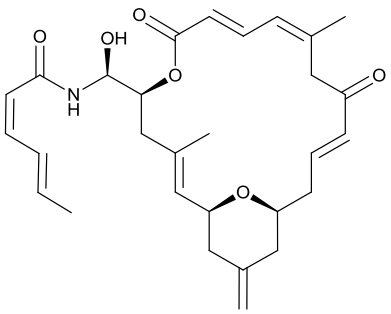

(-)-Zampanolide shows activity in the nanomolar range against various cancer cell lines in a similar manner to the chemotherapeutic agent Taxol. However, (-)-zampanolide exhibits potent activity compared to Taxol in cells overexpressing the P-gp multidrug resistance (MDR) pump,[1,2] which makes it a valuable anti-cancer lead and a potent alternative to Taxol. Our current synthetic approach includes a Bestmann ylide cascade, stereoselective alkynylation and ring-closing metathesis as key steps and allows for ready generation of analogues. The analogues will be submitted to structure-activity relationship studies to further understand the mode of action of (-)-zampanolide and to improve the biological properties of the lead. Proposed modifications of the structure include side chain modifications such as aromatic and alkyl variations, sequential deletion of methyl groups located on the macrocycle and alterations to the pyran and exo-methylene group.

1. Field, J. J. *et al.* Microtubule-Stabilizing Activity of Zampanolide, a Potent Macrolide Isolated from the Tongan Marine Sponge Cacospongia mycofijiensis. *J. Med. Chem.* **52,** 7328–7332 (2009).

2. Ghosh, A. K., Cheng, X., Bai, R. & Hamel, E. Total Synthesis of Potent Antitumor Macrolide (-)-Zampanolide: An Oxidative Intramolecular Cyclization-Based Strategy. *Eur. J. Org. Chem.* **2012,** 4130–4139 (2012).

Poster DD41: Side-Chain Analogues of TAN-2483B as Kinase Inhibitors

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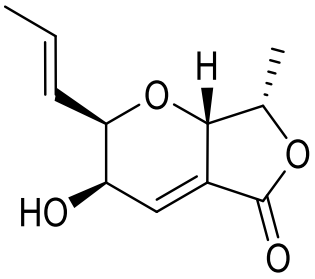
(-)-TAN-2483B was isolated from a Japanese filamentous fungus extract that inhibits c-src kinase and PTH-induced bone resorption. It is a member of the furo[3,4-b]pyran-5-one class of natural products, which includes TAN-2483A, waol A, the massarilactones and fusidilactones. Structurally, TAN-2483B differs from the other furo[3,4-b]pyran-5-one natural products in the relative stereochemistry across the pyran ring and this has circumvented its synthesis through the existing methodology. We have developed a route to (-)-TAN-2483B analogues with varied side-chain groups, which relies on the ring expansion of a D-mannose-derived cyclopropane and introduces the correct stereochemical configurations about the furopyran core. Inhibition of therapeutically relevant kinases, including BTK, BMX and PLK1, has been observed for an analogue, providing a new scaffold with drug discovery potential. The synthetic challenges associated with the TAN-2483B scaffold, together with results from cell growth and kinase inhibition assays, will be presented here.



(-)-TAN-2483B

Poster DD42: Small molecule modulators of the adrenomedullin 1 receptor

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Adrenomedullin (AM) is widely expressed in a range of tissue types with particularly interesting effects on vasculature and cellular growth. AM mediates contrasting responses in a pathology dependent manner. For example, increased AM levels have protective effects on the cardiovascular system in hypertension and heart failure. Yet in cancer, AM promotes angiogenesis to increase oxygen and nutrient delivery and so support tumour growth. Consequently, there is considerable interest in the identification of modulators of the AM pathway.

The effects of AM are predominantly mediated through the AM_1 receptor, which consists of two subunits: the calcitonin receptor-like receptor (CLR) (a G protein-coupled receptor) and receptor activity-modifying protein (RAMP2). A closely related receptor, the calcitonin gene-related peptide (CGRP) receptor has been the focus of extensive drug discovery efforts, through which a family of CGRP receptor antagonists (e.g., telcagepant, olcegepant) with clinical activity against migraine has been identified. We used structural information from the crystal structures of AM_1 and CGRP extra-cellular domain (ECD) complex to design small molecules that are selective for the AM_1 receptor.

We mimicked interactions of the antagonist telcagepant at the CGRP receptor through the use of a benzimidazolone motif to bind to THR122 of the CLR subunit. This motif was then elaborated with a variety of chemotypes to impart selectivity towards the AM_1 receptor. Activity at the AM_1 and CGRP receptors were evaluated using a combination of cAMP and β-arrestin recruitment assays. All compounds were independently screened ($\geq$ n=2) in the absence and presence of AM or CGRP to check for modulator activity or peptide-independent activity. We have identified a series of AM_1 receptor selective small molecules which appear to act as positive allosteric modulators of AM activity, rather than antagonists. We report our exploration of this class of AM_1 modulators, which represent first-in-class molecules.

Poster DD43: Novel phage-like nanorods as diagnostic tools for ultra-sensitive assays

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This proof-of-concept study establishes a safe and easy-to-produce biological particle for ultrasensitive diagnostic assays.

Ff Filamentous phages (M13, f1 and fd) are filament-like (800-1000 nm long and 6 nm in diameter) ssDNA viruses that infect F+ Escherichia coli. These phages have been adapted by genetic modification and used extensively in (phage) display technology and nanotechnology. However, the Ff-derived particle use outside the laboratory containment is hindered because they are live viruses. Furthermore, the Ff-derived functionalized particles are genetically modified organisms that carry antibiotic resistance genes packaged inside the virion-particle.

Here we overcome these limitations by developing a novel system for efficient production of Ff derived non-replicating short protein-DNA nanorods with no antibiotic resistance genes or other coding sequences. The length of these nanorods can be controlled, the shortest possible length being 80 nm. The particles can be functionalized in the same manner as the full-length filamentous phage for a variety of applications, including diagnostics, tissue targeting and as vaccine carriers. An added advantage of these particles is that they are about tenfold shorter than Ff, hence much less likely to form entangled filaments that interfere in diffusion-based applications than full-length Ff phages. We further demonstrate the optimized production and purification system for the short particles in the absence of live viruses, and their functionalization for use as diagnostic probes/particles in ultra-sensitive immuno-PCR assays.

The novel nanorod production system developed here is the second generation Ff (M13, f1 or fd) particle platform that eliminates risks intrinsic to Ff as GMO viruses. As biological particles, our nanorods eliminate the toxicity concerns intrinsic to synthesis and applications of metal-based nanoparticles.

Poster DD44: Development of selective fluorescent ligands for A_1 adenosine receptor as pharmacological tools

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G protein-coupled receptors (GPCRs) constitute the largest family of transmembrane receptors and are the target of at least 30% of all marketed drugs. A_1 adenosine receptor (A1AR) is a class A GPCR and selective A1AR modulators are under development to treat various pathological conditions such as atrial fibrillation, diabetes, angina pectoris, congestive heart failure, renal and immunological disorders. However development has been hampered by a lack of detailed knowledge of the role A1AR plays in various pathophysiological processes.

Development of tools which can be used to selectively visualise real-time and live cell dynamic processes of A1AR will prove invaluable in improving our understanding of the fundamental pharmacology of A1AR. Fluorescent ligands are attractive tools for studying GPCRs at the molecular level in live cells[1]. Fluorescent small-molecule based ligands have been developed for other adenosine receptors however none exist that are selective for A1AR. Recently, benzimidazolylisoquinoline derivatives were reported as highly selective and potent antagonists of human A1AR. The present work involves the development of BODIPY conjugates of these benzimidazolylisoquinolines as fluorescent ligands for studying the pharmacology of A1AR.

1. Vernall, A. J.; Hill, S. J.; Kellam, B., The evolving small-molecule fluorescent-conjugate toolbox for Class A GPCRs. *Br. J. Pharmacol.* **2014,** *171* (5), 1073-1084.

Poster DD45: Metagenomics approach to natural product discovery and biosynthesis

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Microbial natural products are an incredibly important source of drug lead compounds, making up the majority of FDA approved anti-infective agents, as well as a significant proportion of anti-tumour agents. Many of our most effective natural product drugs are produced in microbes by two biosynthetic mechanisms; non-ribosomal peptide synthetase (NRPS) and polyketide synthase (PKS) enzymes. Traditional methods for discovery of the natural products generated by these mechanisms rely on culturing bacteria from environmental samples and extracting compounds from the culture. This approach misses the >99% of bacteria that cannot be cultured in laboratory conditions. Metagenomics avoids these pitfalls by directly analysing the complete genetic complement of microbial life from an environmental sample for biosynthesis genes. We are employing these approaches to recover NRPS and PKS gene clusters, providing access to a far greater pool of unexplored biosynthetic diversity than has hitherto been accessible. Once recovered, the gene clusters can be cloned into cultivable bacterial lab strains for cheap and scalable expression of the biosynthetic machinery, and ultimately, natural product synthesis.

We have constructed large metagenome libraries from New Zealand soil samples, screened these using a variety of techniques, and recovered a large collection of natural product synthesis gene clusters. We have obtained and annotated sequence data for these clusters, and identified promising biosynthetic clones for further study. Our results to date show that New Zealand soil microbiomes are a rich source of novel biosynthesis that can potentially be used to generate new drug like small molecules.

Poster DD46: A metagenomic approach to discover biosynthetic gene clusters from the marine sponge *Mycale hentscheli*

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The marine sponge *Mycale hentscheli* is a known producer of bioactive secondary metabolites, including Peloruside A, a potent microtubule-stabilizing polyketide. So far, chemical synthesis and aquaculture have proved to be impracticable as means to produce this compound in significant quantities. Here, we propose a metagenomic approach involving a hybrid sequencing strategy to elucidate and recover the biosynthetic gene clusters responsible for the secondary metabolites produced by marine sponges with Peloruside A as a target of interest.

Poster DD47: The synthesis of α-D-glucosyl steroids for glycolipidomic profiling

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α-D-Glucosyl steroids [*e.g.*, 3-*O*-(α-d-glucopyranosyl)-cholesterol, Figure 1] are well known cell wall constituents of several bacterial pathogens like *B. burgdorferi* and *H. pylori*, and have been shown to activate the human immune system. As such, the establishment of efficient synthetic pathways for the production of glucosyl steroids would facilitate the glycolipidomic profiling of bacterial species, as well as provide insight into the structure activity relationships of the immune activation of this class of compounds. The establishment of an expeditious synthetic route and compounds produced thus far will be presented.

A general route using a trimethylsilyl-protected glucosyl iodide[1] has been exploited for the installation of the required α-selective glycosidic linkage, followed by deprotection of the TMS-groups to form the monosaccharide final products. These products could then be used for the installation of a second lipophilic moiety using a chemoselective enzymatic trans-esterification step. This mild-reaction pathway enabled the synthesis of a library of glycosyl steroids ready for the evaluation of their immunological activities.

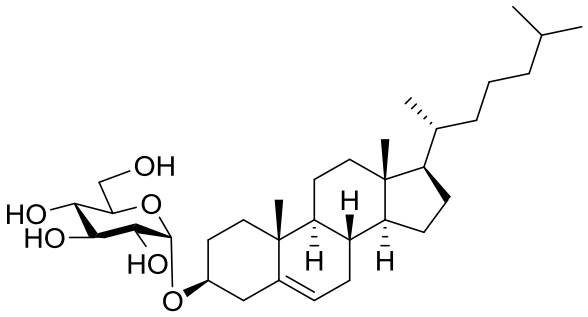

Figure 1. α-D-Glucosyl cholesterol.

1. Davis, R.A., Fettinger, J.C., Gervay-Hague, J. Tandem glycosyl iodide glycosylation and regioselective enzymatic acylation affords 6-*O*-tetradecanoyl-α-d-cholesterylglycosides. *JOC* **2014**, *79*, 8447

Poster DD48: Bedaquiline analogues with improved efficacy and safety profiles for tuberculosis treatment

Tong, S.T.[1], Denny, W.A.[1,2], Palmer, B.D.[1,2], Blaser, A.[1], Choi, P.[1], Conole, D.[1], Sutherland, H.S.[1], Tsang, K.Y.[1], Cooper, B.C.[3], Upton, A.M.[3], Franzblau, S.G.[4]

[1] Auckland Cancer Society Research Centre and [2]Maurice Wilkins Centre, University of Auckland, NZ, [3]Global Alliance for TB Drug Development, New York, USA, [4]Institute for Tuberculosis Research, College of Pharmacy, University of Illinois at Chicago, USA. Email: amy.tong@auckland.ac.nz

The current standard treatment for the potentially lethal tuberculosis (TB) infection is suboptimal, resulting in looming global health issues of multi-drug resistant tuberculosis (MDR-TB) and extensively drug resistant tuberculosis (XDR-TB) infections that no longer respond to existing drugs. In 2014 alone, about 500,000 new cases of TB worldwide were MDR-TB[1].

Bedaquiline is a new anti-TB agent with a novel mode of action that was approved by the US Food and Drug Administration in December 2012 for use in pulmonary MDR-TB that had failed standard treatment. While Bedaquiline clearly demonstrates superior outcomes when added to the standard regimen, there are several concerns to be addressed. Bedaquiline shows significant inhibition of the hERG potassium channel which causes QT prolongation and therefore cardiovascular risks. Bedaquiline is also very lipophilic rendering patients susceptible to phospholipidosis, and its long terminal half-life increases risks of tissue accumulation. These raise the need for a more efficacious analogue of Bedaquiline with improved ADMET properties.

Despite the challenging synthetic chemistry and extensive work required for the isolation of the active enantiomer from a mixture of 4 isomers, our medicinal chemistry program has produced over 700 analogues of Bedaquiline, many of which demonstrated significantly improved potency and pharmacokinetic properties. In particular, we have discovered that incorporation of di-substituted pyridine subunits significantly improves cardiac safety, and that replacement of bromine by cyano in the quinoline ring reduces lipophilicity while retaining potency. The chemistry and improved properties of a selection of analogues will be presented, highlighting the top six leads that have emerged for advanced preclinical studies.

1. WHO Global tuberculosis report 2015.
2. Diacon, A.H., Donald, P.R. , Pym, A. , Grobusch, M. , Patientia, R.F. , Mahanyele, R. , Bantubani, N. , Narasimooloo, T. , De Marez, RT. , van Heeswijk, R. , Lounis, N. , Meyvisch, K. , Andries, K. , McNeeley, D.F. (2012). *Randomized pilot trial of eight weeks of bedaquiline (TMC207) treatment for multidrug-resistant tuberculosis: long-term outcome, tolerability, and effect on emergence of drug resistance* Antimicrob. Agents Chemother. 56: 3271-3276.

Poster DD48: Computational characterisation of protein-membrane interactions

William A. Irvine[1], Jack U. Flanagan[2] and Jane R. Allison[1]

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As much as 30% of the human genome encodes membrane proteins, many of which act as drug targets whether they be signalling proteins or enzymes. Characterising the behaviour of these proteins more often than not requires an understanding of the interaction between the protein and the cellular membrane interface.

This research uses a series of molecular dynamics and analytical techniques to pinpoint the driving factors behind the PI3K enzyme's interactions with a bilayer representative of a brain cellular membrane.