BIOLOGICAL & MEDICINAL CHEMISTRY

BMCS 7TH POSTGRADUATE SYMPOSIUM

FRIDAY 13TH DECEMBER 2013

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Wolfson Lecture Theatre
Department of Chemistry
University of Cambridge, CB2 1EW

Biological Chemistry & Medicinal Chemistry
Postgraduate Symposium

Chemistry Department, Lensfield Road, Cambridge

The organising committee, Dr Dave Alker, Dr Gordon Saxty and Dr Rebecca Myers, would like to thanks the following companies and organisations for their generous support and sponsorship for the 2013 symposium.

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Programme

0900-0950	Registration, tea and coffee
0950-1000	Welcome, opening remarks and house-keeping announcement Andreas Bender, University of Cambridge
1000-1200	**First session: Chairman, Martin Swarbrick (Cancer Research Technology)**
1000-1030	**Working in a CRO Wonderland!** Ashley Jarvis, Domainex, Cambridge
1030-1100	**SGC-CBP30: A Chemical Probe for CBP/p300 Bromodomains** Duncan Hay, University of Oxford
1100-1130	**Identification of In Vivo active BCATm Inhibitors Using Fragment-Based Drug Discovery** Sophie Bertrand, GlaxoSmithKline R&D and the University of Strathclyde
1130-1210	Student Flash Presentations prior to Lunch Time Poster Competition
1210-1330	Lunch and poster session
1330-1530	**Second session: Chairman, Stuart Conway (University of Oxford)**
1330-1400	**40 Years of Cancer Drug Development – Chemical Warfare to Patient Welfare** Herbie Newell, Newcastle Cancer Centre, Northern Institute for Cancer Research
1400-1430	**Antitumour Activity of an (E)-Styrylsulfonyl Methylpyridine Analogue of ON01910.Na: A Novel Kinase Inhibitor Targeting Mitotic Pathways** Tiangong Lu, University of Nottingham
1430-1500	**Modulation of Aurora-A Activity and Conformation by Chemical Modification** Fiona Rowan, Institute of Cancer Research
1500-1530	**The Design, Synthesis and Evaluation of Inhibitors of the HIF-1α/p300 Protein-Protein Interaction** George Burslem, University of Leeds
1530-1600	Tea and coffee

1600-1800	**Third session: Chairman, Dave Alker (David Alker Associates)**
1600-1630	**Cracking the Histone Code: Developing Inhibitors of Bromodomain-Acetyl-Lysine Interactions** Stuart Conway, Oxford University
1630-1700	**Alternative Strategies for Targeting HSP70** Lindsay Evans, Institute of Cancer Research
1700-1730	**Discovery of a Potent and Selective Small Molecule Inhibitor of FBXL11, a JmjC Histone Demethylase** Katherine England, University of Oxford
1730-1800	**A Functionalised Linker Strategy for the Diversification of Stapled Peptides** Yu Heng Lau, University of Cambridge
1800-1810	Closing remarks, Dave Alker
1810-2000	Wine mixer
1830	Presentation of prizes
2000	Meeting closes

Working in a CRO Wonderland!

Ashley Jarvis

Department of Medicinal Chemistry, Domainex Limited

Email: ashley.jarvis@domainex.co.uk

Domainex is a Cambridge-based Contract Research Organisation (CRO) with an excellent track history of supporting projects: from target identification to candidate drug. Domainex's range of drug discovery services includes medicinal chemistry and computer-aided drug design. This talk will outline the opportunities afforded by the CRO sector for a rewarding career in drug discovery. The changing role of this sector is also discussed, where collaborations with academic 'drug-hunters' are becoming more common-place alongside the more traditional biotech and 'big pharma' clients. Case studies will be used to illustrate the contribution Domainex has made to its clients' drug discovery programmes, and to further reinforce that a CRO can be both a scientifically-challenging and extremely enjoyable place to work.

Biography

Ashley is the Medicinal Chemistry Group Leader at Domainex and has held this position since 2007, having joined the company in 2004.

In addition to his line management responsibilities Ashley leads several medicinal chemistry projects; from target selection and hit identification through to lead optimisation and beyond. Several of the client-focused programmes have successfully led to pre-clinical candidate selection and further evaluation in clinical trials.

Ashley has supported research programmes directed towards molecular targets such as kinases, proteases, growth-factor receptors, and PARPs; and in fields as diverse as (for example) oncology, inflammation, virology, and cardiovascular medicine. He is a named inventor on the corresponding patents. In addition to his general small-molecule drug discovery know-how, Ashley provides specific expertise in carbohydrate chemistry, 'peptide to peptidomimetic' lead generation and identifying inhibitors of protein-protein interactions.

Ashley's prior experience covers both academic and private sectors within the UK and New Zealand; including The Wolfson Institute for Biomedical Research, Industrial Research Ltd. and Oxford Asymmetry International (became Evotec).

Ashley obtained his PhD from the University of Reading in 1998, where he researched the preparation and ring-opening reactions of *N*-diphenylphosphinyl vinylaziridines within Professor Joe Sweeney's group.

SGC-CBP30: A Chemical Probe for CBP/p300 Bromodomains

Duncan A. Hay[1,2]

Paul E. Brennan[2], *Stuart Conway*[1], *Oleg Fedorov*[2], *Panagis Filippakopoulos*[2], *Stefan Knapp*[2], *Susanne Muller-Knapp*[2], *Sarah Martin*[2], *Marin Philpott*[2], *Sarah Picaud*[2], *Chris Schofield*[1], *Tony Tumber*[2], *Chris Wells*[2], *Clarence Yapp*[2].

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[2]Structural Genomics Consortium, University of Oxford, Old Road Campus Research Building, Roosevelt Drive, Oxford, OX3 7DQ, UK.

In eukaryotic cells, DNA is packaged in the nucleus in the form of chromatin. Chromatin consists of DNA wrapped around sets of histone protein, in a beads-on-a-string structure. Reversible chemical modification of chromatin provides a mechanism by which transcription of particular genes can be switched on or off, and thus provides an additional layer of transcriptional information over what is contained in the genome. This *epigenetic* control is governed by the 'writing', 'reading' and 'erasing' of chemical marks on DNA and histones by specific chromatin-modifying protein domains. One such mark is histone lysine acetylation, which is 'read' by bromodomains. In humans, there are 61 known bromodomains found in 46 separate proteins, and these can be clustered into 8 families based on sequence similarity.

The misregulation of these proteins has been linked to many disease areas, but more work is needed to elucidate the cellular pathways involved and to investigate if these are suitable points for therapeutic intervention. One way to advance this is through the development of potent and selective small molecule chemical probes for the different bromodomain families. This work describes the discovery of a novel chemical probe selectively inhibits CBP (K_d = 21 nM) and p300 (K_d = 38 nM) bromodomains. CBP/p300 are ubiquitously expressed paralogous transcriptional coactivators whose misfunction is implicated in developmental disorders, learning and memory impairment, viral infection, and diseases including cancer and asthma. This chemical probe therefore represents an important tool to study these proteins and could pave the way for development of new therapeutic agents.

Identification of *In Vivo* Active BCATm Inhibitors Using Fragment-Based Drug Discovery

Sophie Bertrand[1,2]

Jennifer Borthwick[1,2], Nicolas Ancellin[1], Benjamin Beaufils[1], Paul Carter[1], Chun-Wa Chung[1], Ian Churcher[1], Nerina Dodic[1], Marie-Helene Fouchet[1],Charlene Fournier[1], Peter Francis[1],Laura Gummer[1], Andrew Hobbs[1], Craig Jamieson[2], Stephen Pickett[1], Iain Reid[1], Graham Simpson[1], Lisa Sloan[1], Sarah Smith[1], Don Somers[1], Claus Spitzfaden[1], Colin Suckling[2], Klara Valko-Slegel[1], Yoshiaki Washio[1], Rob Young[1].

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The mitochondrial form of branched-chain aminotransferase (BCATm) is a key metabolic enzyme, which plays an important role in protein synthesis and protein degradation. Recently, BCATm has been linked to obesity in knock-out animals.[1] To date, potent small molecule BCATm inhibitors have not been reported, although there are some reports of inhibitors of the related cytosolic form of the enzyme, BCATc. Thus, BCATm inhibitors represent a novel approach to understand the underlying pharmacology and its potential link with weight loss.

Fragment screening of BCATm using a combination of biochemical and biophysical assays (Thermal melt, STD-NMR) successfully identified a diverse set of compounds displaying a range of binding modes. Based on this, several series were progressed for optimisation. This talk will focus on the discovery and optimisation of one of these fragment hits into a novel series of potent, BCATm inhibitors with encouraging *in vivo* activity. Structure-based design combined with knowledge from an HTS series were used to rapidly optimise the initial hit. Focus on physicochemical properties delivered high quality molecules with good DMPK profiles, which showed a positive effect on branched chain amino acid levels in acute *in vivo* studies.

References

[1] She, P.; Reid, T. M.; Bronson, S. K.; Vary, T. C.; Hajnal, A.; Lynch, C. J.; Hutson, S. M. *Cell Metab.* **2007**, *6*, 181-194; [2]. Hu, L. Y.; Boxer, P. A.; Kesten, S. R.; Lei, H. J.; Wustrow, D. J.; Moreland, D. W.; Zhang, L.; Ahn, K.; Ryder, T. R.; Liu, X.; Rubin, J. R.; Fahnoe, K.; Carroll, R. T.; Dutta, S.; Fahnoe, D. C.; Probert, A. W.; Roof, R. L.; Rafferty, M. F.; Kostlan, C. R.; Scholten, J. D.; Hood, M.; Ren, X. D.; Schielke, G. P.; Su, T. Z.; Taylor, C. P.; Mistry, A.; McConnell, P.; Hasemann, C.; Ohren, J. *Bioorganic & Medicinal Chemistry Letters* **2006,** *16* (9), 2337-2340.

Flash Presentations of Posters

Notice to flash slide presenters: If you are presenting your Flash poster slide today please sit close to the edges of the theatre. We will run in order that is presented in the booklet. If you are the 1st speaker, please introduce yourself to the chair of session. The next speaker should approach the stage while the presentations are ongoing. Note the slides change automatically to the next slide on a 2 min timer. The chair of session will move the slide forward if you finish early.

1. Martin Bachman, University of Cambridge
'Tracking cytosine modifications and identifying active DNA demethylation mechanisms using stable isotope labelling approaches in cells and in vivo'

2. Chris Brown, Durham University
'Inhibitors of LmjIPCS – New Therapies for Leishmaniasis'

3. Ramatoulie Camara, University of Hertfordshire
'In search of S100P/RAGE inhibitors as therapeutic agents in pancreatic cancer using structure-based drug design (SBDD)'

4. Gerta Cami-Kobeci, University of Bath
'Naltrexone esters as a new therapeutic treatment for opiate abuse'

5. James Carter, University of Cambridge
'Engineering Folding Dynamics from Two-State to Downhill: Application to λ-Repressor'

6. James Clulow, Imperial College London
'Unravelling the targets of electrophilic natural products using quantitative activity-based chemical proteomics'

7. Hawa Diallo, GlaxoSmithKline R&D and University of Strathcyde
'Synthesis and optimisation of H3K27 demethylase inhibitors:- Investigations into the modulation of the pro-inflammatory macrophage response'

8. Mustafa Gabr, Mansoura University, Egypt
'EGFR tyrosine kinase targeted compounds: Synthesis, in vitro antitumor activity and molecular modeling studies of new series of benzothiazole and pyrimido[2,1-b]benzothiazole derivatives'

9. Natalie Griffiths, University of Bath
'From daffodils to drugs: Design, synthesis and biological evaluation of narciclasine analogues'

10. Sara Kramar, University College London
'Computer-Aided Molecular Design of Novel Keap1-Nrf2 Inhibitors'

11. Hitendra Lautre, Govt.V.Y.T.P.G.Autonomous College, Durg, India
'Discovery of potent inhibitors of breast cancer cell proliferation and thymidylate kinase of Mycobacterium tuberculosis'

12. Honorine Lebraud, Northern Institute of Cancer Research, University of Newcastle
'Anticancer agents targeted against cyclin-dependent kinase (CDK2): Structure-based design of irreversible and reversible inhibitors'

13. Jonathan Macdonald, Institute of Cancer Research
'Synthesis & kinome selectivity patterns of imidazo[4,5-b]pyridine-derived fragment libraries'

14. James Murray, Imperial College London
'Towards the development of DMAP-N-oxide derived organocatalytic kinase mimetics: reaction rate and selectivity studies'

15. Sarah Narramore, University of Leeds
'In silico design of bacterial Type II topoisomerase inhibitors: new "dual-target" antibiotics'

16. Jennifer Norcliffe, Durham University
'Inhibitors of Kinetoplastid Sphingolipid Synthases as Potential Therapeutic Agents'

17. Oran O'Doherty, University of Sussex
'Synthesis of novel trypanosomal alternative oxidase inhibitors for the potential treatment of African trypanosomiasis'

18. Elizabeth Osoba, University of Hertfordshire
'Design, synthesis and biological activity of novel stilbenesulfonamides'

19. Colin Robinson, Institute of Cancer Research
'Small Molecule Probes for Protein-Protein Interactions: Mimicking Beta-Sheet Motifs'

20. Lucy Smith, Imperial College London
'Antagonists of the IgE:FcεRI protein-protein interaction as potential anti-asthma therapeutics'

21. Hannah Straker, University of York
'Chemical Tools for Investigating Multiple Herbicide Resistance in Black Grass alopecurus myosuroides'

P1. Tracking Cytosine Modifications and Identifying Active DNA Demethylation Mechanisms Using Stable Isotope Labelling Approaches in Cells and *in vivo*

Martin Bachman[1,2]

Santiago Uribe-Lewis2, Xiaoping Yang2, Michael Williams2, Pieter Van Delft1, Adele Murrell2, Shankar Balasubramanian1,2

1 Department of Chemistry, University of Cambridge, UK, 2 CRUK Cambridge Institute, University of Cambridge, UK

It is now widely accepted that both genetic and epigenetic alterations are playing an important role in cancer progression. Unlike the former, epigenetic changes are reversible and therefore represent an interesting target for therapeutic intervention. Our aim is to understand the chemical mechanisms underlying the turnover of cytosine modifications, measure their lifetime, and shed light on the dynamics of DNA methylation and demethylation in various biological contexts. For this purpose, we have developed novel strategies based on isotopically labelled probes and a sensitive HPLC/MS/MS technique capable of accurate identification and quantification of the labelled nucleosides obtained from genomic DNA. Our systems are suitable for both cell culture and *in vivo* experiments and are able to distinguish between base excision repair-dependent and carbon-carbon bond cleavage-dependent mechanisms with a detection limit as low as 1 in 10 million bases. I will present our findings concerning the chemistry and timing of 5mC oxidation during the cell cycle and discuss potential implications on mammalian development and tumorigenesis.

P2. Inhibitors of *Lmj*IPCS – New Therapies for Leishmaniasis

Christopher Brown[1]

John G. M. Mina,[1] Andy T. Merritt,[2] Patrick G. Steel[1] and Paul W. Denny[1]

[1]Biophysical Science Institute, Durham University, Durham DH1 3LE, [2]Medical Research Council Technology, Mill Hill, London NW7 1AD

Leishmaniasis is an insect vector-borne Neglected Tropical Disease caused by the protozoan *Leishmania* spp. Over 350 million people are at risk of this disease which claims at least 50,000 lives each year. With no vaccine or prophylactic medication currently available, treatment typically requires long, expensive courses of exposure to toxic medicines *via* intravenous or intramuscular administration. Furthermore, resistance to some anti-leishmanials is a major threat. There is an urgent need for novel treatments that are inexpensive and free of side-effects.

Our group has previously identified the essential kinetoplastid inositol phosphorylceramide synthase (IPCS) enzyme as an attractive drug target. No orthologuous enzyme exists in mammals, enabling the development of selective anti-leishmanials. Moreover, the fungal IPCS has been previously characterised and successfully targeted in the development of anti-fungal molecules, serving as a proof of concept.

In order to identify and develop a new class of enzyme inhibitor, *Leishmania major* IPCS (*Lmj*IPCS) has been characterised and incorporated into a multiwell plate compatible assay. From a screen of 1200 pharmacologically active compounds (NINDS set – supplied by MRCT), 57 were found to exhibit >70% inhibition of *Lmj*IPCS. A secondary assay against *L.major* promastigote parasites highlighted 16 compounds with cytotoxic effects, of which 4 displayed ED_{50} values greater than that of pentamidine (2.05 μM), a second-line treatment for leishmaniasis. Chemical modification of these key compounds is on-going.

P3. In Search of S100P/RAGE Inhibitors as Therapeutic Agents in Pancreatic Cancer using Structure-Based Drug Design (SBDD)

Ramatoulie Camara[1]

Sharon Rossiter[1], Nasir Mahmoud[2], Tatjana Crnogorac-Jurcevic[2], Stewart B. Kirton[1]

[1] Department of Pharmacy, School of Life & Medical Sciences, College Lane, Hatfield, AL10 9AB, [2] Centre for Molecular Oncology, Barts Cancer Institute, Queen Mary University of London, London, UK

S100P is a 95-amino acid calcium-binding protein that belongs to the S100 family of proteins and exerts its effect both intracellularly through calcium ion modulation, and extracellularly through binding to the receptor for advanced glycation end-products, RAGE. Numerous studies have linked the protein to many cancers and it is its high expression in pancreatic cancer that has led to proposals for it to be used as a clinical marker in early diagnosis of the disease [1]. Pancreatic cancer is one of the leading causes of cancer deaths in the developed world with a high mortality rate and a less than 5% 5-year survival rate. There is therefore an urgent need to find an effective therapeutic agent for a timely intervention in this lethal disease.

Using the NMR ensemble of S100P (PDB ID 1OZO) and cromolyn – a ligand that has been shown to bind to and inhibit the protein's interaction with RAGE [2] – protein informatics was carried out to identify appropriate S100P conformers for drug discovery, and subsequently, small molecules from the MOE database with the potential to bind to and therefore stop S100P from interacting with its receptor.

Virtual screening of the MOE database of lead-like compounds resulted in a hit rate of 0.008%. Of the 52 hits identified, 15 were purchased and five synthesised in the lab. All were tested in an MTS cell migration assay and five compounds from different chemical classes showed inhibitory activity which was at least equivalent to that of cromolyn-S100P interaction. This corresponds to a hit rate of 25% which is a promising start in search of a potential therapeutic agent for pancreatic cancer. Compounds identified as hits are currently being explored in hit-to-lead studies.

References

[1] Deng, H., et al., *Usefulness of S100P in diagnosis of adenocarcinoma of pancreas on fine-needle aspiration biopsy specimens*. Am. J. Clin. Pathol., 2008. **129**(1): p. 81-8; [2] Arumugam, T., V. Ramachandran and C.D. Logsdon, *Effect of cromolyn on S100P interactions with RAGE and pancreatic cancer growth and invasion in mouse models*. J. Natl. Cancer Inst., (2006). **98**(24) 1806-18

P4. Naltrexone Esters as a New Therapeutic Treatment for Opiate Abuse

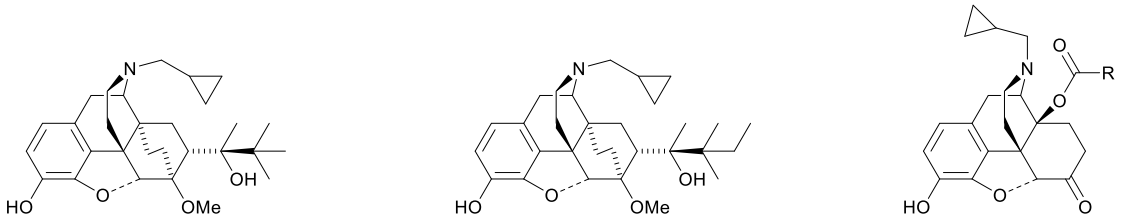
Gerta Cami-Kobeci[1]

Willma E. Polgar[2], Taline V Khroyan[2], Lawrence Toll[3], Stephen M. Husbands[1]

[1]Department of Pharmacy and Pharmacology, University of Bath, Bath, BA2 7AY, UK and [2]SRI International, Menlo Park, USA, and [3]Pines Institute for Molecular Studies, Port St. Lucie, Fl

Although morphine, is the main medication as a chemical analgesia, still possess the predominant unwanted effects as a mu (MOP) agonist (the reinforcing effects which lead to abuse and physical dependence). Since nociception (NOP) agonists block development of tolerance and physical dependence to MOP agonism, ligands having both NOP and MOP agonism should maintain analgesic activity but show less tolerance and addiction liability. Buprenorphine (1) is such a lead having (partial) MOP agonist activity together with KOP antagonism and, importantly, low

efficacy, modest potency NOP partial agonism. Structure-activity relationship studies have suggested that the region of space occupied by the *tert*-butyl group in buprenorphine is key to good NOP receptor activity.[1] However, buprenorphine itself has been shown to produce conditioned place preference (CPP), having an inverted U-shaped dose-response curve.[2]



(1) (2) (3)

Fig 1. Structures of Buprenorphine, BU0828, 14-O-naltrexone esters

Previously we reported that BU08028 (2), a close analogue of buprenorphine had the desired profile *in vitro*. However, despite its increased NOP activity, BU08028 itself has also been shown to produce CPP and was unable to attenuate cocaine-induced CPP.[3]

In order to improve the profile that we were looking for, we focused on the esterification of 14-OH position of naltrexone (3) a distinguished MOP antagonist. This esters show low MOP and NOP affinity and potency compare to buprenorphine, at the [^{35}S]GTPgammaS binding assays studies, compounds within this series were low efficacy agonist at MOP and NOP receptors. The further evaluation of these ligands, including initial *in vivo* evaluation will be presented.

This work was supported by NIDA grants DA020469 (SMH) and DA023281 (L. Toll)

References

[1] Cami-Kobeci, G.; Polgar, W. E.; Khroyan, T. V.; Toll, L.; Husbands, S. M.; *J. Med. Chem.*, 2011, 54, 6531-6537; [2] Rowlett, J.K.; Gibson, T.R.; Bardo, M.T.; *Pharmacol. Biochem. Behav.*, 49: 241-245, 1994; [3] Khroyan, T. V.; Polgar, W. E.; Cami-Kobeci, G.; Husbands, S. M.; Zaveri, N. T.; Toll, L.; *J. Pharmacol. Exp. Ther.*, 2011, 336, 952-961.

P5. Engineering Folding Dynamics from Two-State to Downhill: Application to λ-Repressor

James W. Carter[a]

Christopher M. Baker [a], *Robert B. Best* [b] and *David De Sancho* [a]

[a] University of Cambridge, Department of Chemistry, Lensfield Road, Cambridge, CB2 1EW and [b] Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892-0520

The energy landscape approach to protein folding predicts that the free energy barrier between the folded and denatured states, a consequence of the imperfect cancellation of entropic and enthalpic contributions to the free energy, can be reduced to the size of the residual roughness of the landscape ($\sim k_B T$) giving rise to downhill folding. This downhill folding limit allows direct experimental observation of the folding mechanisms of two-state proteins, which under normal conditions is not possible because structures close to the transition state are effectively hidden by the low population of states within the barrier region. One strategy used experimentally to reach

this downhill folding regime is mutation [1], but the choice of amino acid to mutate is often based on chemical intuition alone. We have developed a computational method to screen for protein engineering "hot spots" by analysing the effect of point mutations on folding cooperativity and then confirming these predictions within a Markov state model framework [2]. Using coarse-grained, topology-based Gō model simulations of the λ-repressor protein fragment and applying a novel metric for folding cooperativity with sampling from the energy landscape of the pseudo wild type protein, we assess the significance of different point mutations based on their effect on this energy landscape. To confirm the predictions of this approach, we run new simulations of selected mutants and then use Markov state models to obtain the kinetics of folding. We focus on one specific mutant, which is maximally affected by single point mutation according to our metric. The analysis confirms that free energy barriers are reduced to $\sim 1 k_B T$ and the kinetics deviate from a single exponential. These results shed light on the emergence of "strange kinetics" in protein folding.

References

[1] Yang W. Y. and Gruebele M., Folding at the Speed Limit, *Nature*, (2003) **423**, 193-197; [2] Carter J. W., Baker C. M., Best R. B. and De Sancho D., Engineering Folding Dynamics from Two-State to Downhill: Application to λ-Repressor, *J. Phys. Chem. B*, Submitted.

P6. Unravelling the Targets of Electrophilic Natural Products Using Quantitative Activity-Based Chemical Proteomics

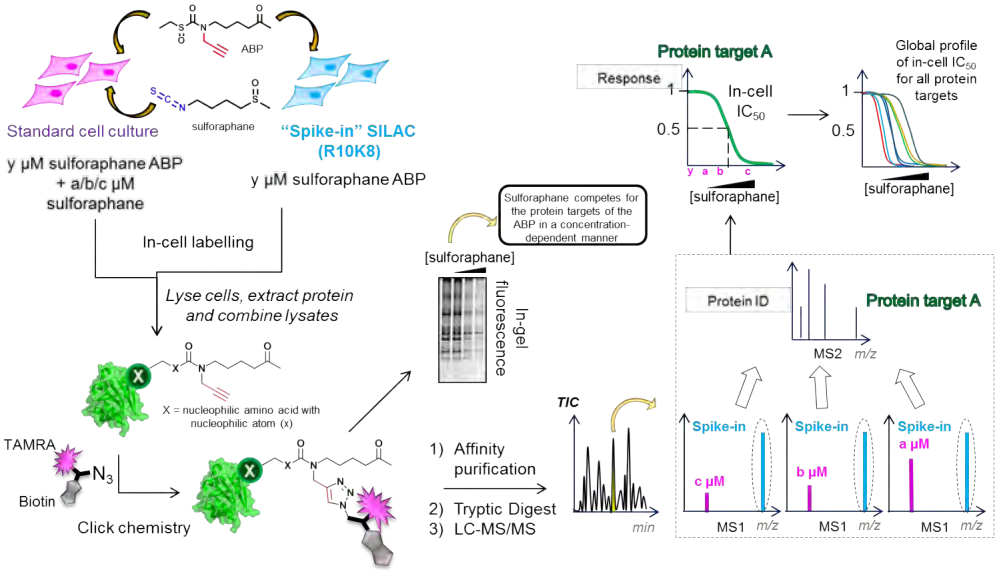
*James A. Clulow**

[a,b] Dr Lyn Jones [c] and Dr Edward W. Tate [a]

[a] *Department of Chemistry, Imperial College London, UK, SW7 2AZ;* [b] *Doctoral Training Centre, Institute of Chemical Biology, Imperial College London, UK, SW7 2AZ* [c] *WorldWide Medicinal Chemistry, Pfizer, 200 Cambridge Park Drive, Cambridge, MA 02140, USA.*

Electrophilic natural products that are found in dietary sources such as curcumin, piperlongumine and sulforaphane have attracted considerable interest on account of their broad range of biological activities, leading to their assessment as therapeutics for a number of diseases.[1] Despite extensive research (over 7,000 references in PubMed as of 2013), the mode of action and biological targets of these compounds remain poorly understood. These compounds are clearly not 'single target' molecules; dissecting their complex polypharmacology to determine the key targets and pathways presents a major challenge, and has limited progress in the clinic. A system-wide understanding of these targets would also enable hypothesis-driven development of more selective and drug-like analogues.

We have implemented a chemical proteomics strategy using so-called activity-based probes (ABPs) based on these small molecules that allows us to profile their molecular targets in disease-relevant models,[2] identifying the range and relative importance of targets that these molecules bind to covalently, across the entire system in an unbiased manner. Initial work identified a substantial number of potential targets of these natural products (>1000) in breast cancer cell lines and an in-house quantitative proteomics platform has been developed to generate in-cell IC_{50} values for sulforaphane targets that unravels the pharmacologically significant target set of sulforaphane for the very first time.[3] On-going work is applying a similar strategy to curcumin and piperlongumine which will provide deeper understanding of the mode of action of these small molecule electrophiles, with the potential to reveal new drug targets or pathways for therapeutic intervention.



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P7. Synthesis and Optimisation of H3K27 Demethylase Inhibitors:- Investigations into the Modulation of the Pro-Inflammatory Macrophage Response

Hawa Diallo

GlaxoSmithKline R&D and University of Strathcyde

Jumonji (Jmj) histone demethylases are Fe(II) and 2-oxoglutarate dependent dioxygenases that constitute essential components of regulatory transcriptional chromatin complexes. These enzymes demethylate lysine residues found in histones. Herein, I present a structure-based design and chemoproteomic approach to elucidate the functional role of JmjD3. High throughput screening identified a pyridyl-pyrimidine template that was confirmed to bind in the JmjD3 active site by X-ray crystallography. Optimisation of this series enabled the identification of a cell active, small molecule inhibitor of JmjD3,[1] which was shown to reduce lipopolysaccharide-induced pro-inflammatory cytokine production in human primary macrophages.

References

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P8. EGFR Tyrosine Kinase Targeted Compounds: Synthesis, *In Vitro* Antitumor Activity and Molecular Modeling Studies of New Series of Benzothiazole and Pyrimido[2,1-*b*]benzothiazole Derivatives

Moustafa T. Gabr

Nadia S. El-Gohary, Eman R. El-Bendary, Mohammed M. El-Kerdawy

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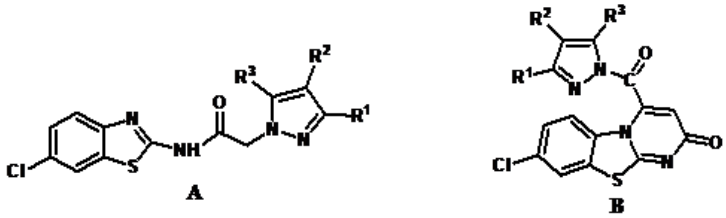
New series of benzothiazole and pyrimido[2,1-*b*]benzothiazole derivatives have been synthesized and characterized by analytical and spectrometrical methods (IR, HRMS, ^{1}H and ^{13}C NMR). All the newly synthesized compounds were selected by National Cancer Institute (NCI), USA for their antitumor evaluation at a single high dose (10 μM) against a panel of 60 cancer cell lines. The most active compounds were selected for further evaluation at five dose level screening. In addition, these active compounds were studied for their EGFR tyrosine kinase inhibitory activity. Virtual screening utilizing molecular modeling and QSAR techniques enabled the understanding of the pharmacophoric requirements for antitumor activity. Docking the designed compounds into the ATP binding site of epidermal growth factor receptor (EGFR) kinase domain was also studied.



P9. From Daffodils to Drugs: Design, Synthesis and Biological Evaluation of Narciclasine Analogues

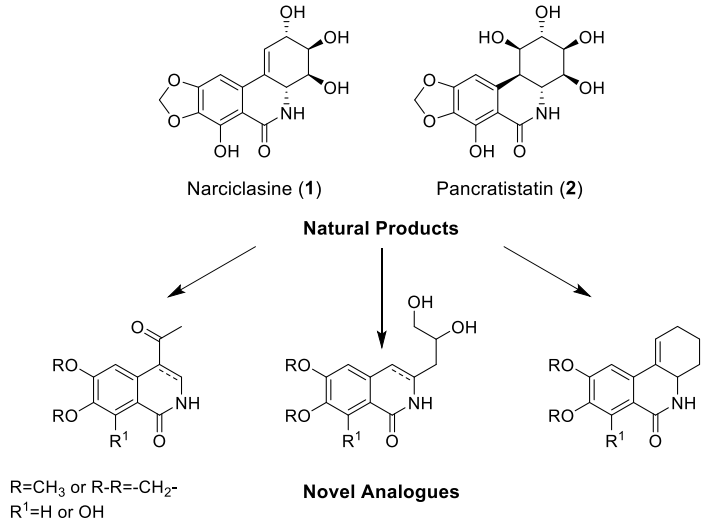
Natalie Griffiths

Dr Lorenzo Caggiano

Department of Pharmacy and Pharmacology, University of Bath, Bath, BA2 7AY

Narciclasine (**1**) and pancratistatin (**2**), isolated from the daffodil bulb, have been shown to possess potent anticancer activity. Direct inhibition of protein synthesis and the initiation of mitochondrial dependent and independent apoptosis have been attributed to their biological mode of action. Unfortunately these compounds have yet to fully exploited as therapeutic agents due to their complex total synthesis and scarce availability from natural sources.

Both compounds contain a dihydroisoquinolinone core which is retained in a series of simplified AB and ABC-ring analogues currently under investigation, using a one pot procedure for the conversion of a carboxylic acid to a lactam. The ensuing analogues are being subjected to biological evaluation by MTS cell proliferation assay using HT29 colon cancer cells.



P10. Computer-Aided Molecular Design of Novel Keap1-Nrf2 Inhibitors

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Despite considerable effort and money being spent on the discovery of new and optimized cancer treatments, so far success has been rather limited. In parallel, new approaches focusing on cancer prevention rather than treatment are being pursued. One of the possible approaches to prevent cancer involves inhibition of Kelch-like ECH-associated protein 1 (Keap1) - nuclear factor erythroid 2-related factor 2 (Nrf2) protein-protein interaction (PPI), leading to the release of Nrf2, a transcription factor which plays an important role in controlling xenobiotic and oxidative stress (Motohashi and Yamamoto, 2004). Direct inhibitors of the Keap1-Nrf2 PPI could therefore act as new drug candidates for cancer chemoprevention and inflammation. The aim of this project is to design such new ligands with improved Keap1 binding and biological activity.

Various computational chemistry approaches have been applied to understand the binding of the Nrf2 Neh2 domain to Keap1, as observed from the X-Ray crystal structures (PDB entries 1X2R and 2FLU). Rigid and flexible docking experiments (Glide, Hex, GOLD, GLUE) were carried out to reproduce the crystal structure of the Keap1-Nrf2 complex and provide a method to obtain the initial position of the ligand in the presence of Keap1 for molecular dynamics (MD) simulations of modified peptides, which were reported together with their binding activities (Hancock et al., 2012). This turned out to be challenging, since we found that the crystal packing of units in the PDB entries 1X2R and 2FLU results in intermolecular interactions of the ligand with other asymmetric units. We propose that these interactions in the crystal influence the conformation of the bound ligand and in turn contribute to a poor reproduction of the X-ray structure using a docking approach. Our extensive studies indicate that ligands can adopt several conformations within the binding site with favourable calculated interaction energies.

X-Ray crystal structure of a non-peptide molecule in complex with Keap1 (PDB entries 3VNG and 3VNH) was reported recently. Combining our understanding of the Nrf2 Neh2 domain interaction with Keap1 and the reported small molecule, we rationally designed a reference analogue for scaffold hopping. Spark software (Cresset) was used as a bioisostere and fragment replacement tool

and a library of new possible drug candidates was created. Virtual screening of these compounds will be followed by synthesis and biological testing in the future.

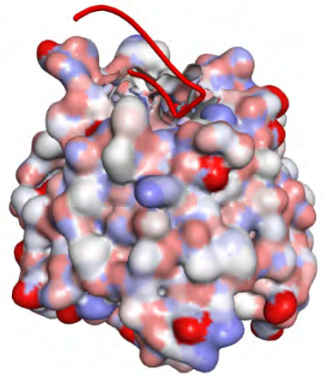

Figure 1: 3D representation of the Keap1-human Nrf2 Neh2 domain complex (PDB entry 2FLU). *Keap1 represented as interpolated charge surface and schematic representation of the Nrf2 Neh2 domain*

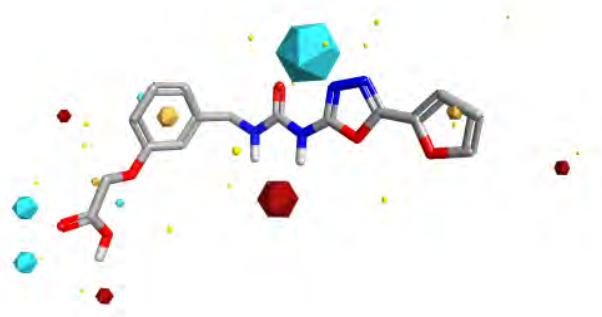

Figure 2: Ligand from PDB entry 3VNG represented with field points in Spark (Cresset)

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P11. Discovery of Potent Inhibitors of Breast Cancer Cell Proliferation and Thymidylate Kinase of *Mycobacterium tuberculosis*

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Thymidylate kinase (TMK) in tubercular bacteria plays a key role in the DNA synthesis and represents an attractive target for antitubercular drug development. In the present work, N',4-dihydroxybenzamidine and their Co(II), Zn(II) and Cr(II) complexes were synthesized and evaluated as inhibitors of 1GSI thymidylate kinase enzyme of *M.tuberculosis*, showing MIC (minimum inhibitory concentrations) of 1.25- 4.5 µg/mL and IC_{50} values of 0.91-3.5 µM. This compound exhibited high selectivity for TMK and was efficacious when dosed intravenously in mice. The apparent inhibition constants (K_i) for *M.tuberculosis* were found to range from 0.6-8.3 µM. The bioactivities of these compounds have also been evaluated on breast cancer MCF-7 cell lines preliminary. Among all the designed compounds, Co(II) complex exhibited more potent in vitro anticancer activities with IC_{50} value of 0.7 ± 0.2 µM, which was superior to the positive control. Dose response data were generated in vivo using mice. When combined with the high throughput in vitro through biochemical and molecular biological analyses showed that hydroxyamidine moiety upon coordination with metal regulates proliferation. We have also described the application of a ligand-based virtual screening, and were optimized to improve their ADME-Toxicity profile, following Lipinski's rule of five. In this way a pharmacophore model was

generated to explain the structural relationships of the compounds with respect to their antiproliferative and antibacterial activity. A systematic SAR study showed that the compounds under investigation fitted well in the ATP binding pocket of TMK enzyme with good docking scores and form nonbonding interactions with the crucial residues of the catalytic site. Further, we identify the microsomal metabolite and describe synthesized compounds to address the rapid metabolism. All the compounds were tested in vivo for its ADME screening process, pharmacokinetic profile, brain penetration, and efficacy in mice, found their potential to significantly accelerate the processes of lead identification and optimization. The Zn(II) and Co(II) complexes showed significant activity in the mouse model, as important novel therapeutic lead for the treatment of TB and breast cancer. Both in vitro potency and pharmacological properties have been dramatically improved via chemical modification to corresponding derivatives. Compounds may be appear useful for the treatment of Tuberculosis and breast cancer.

Keywords- Thymidylate kinase inhibitor, N',4-dihydroxybenzamidine metal complex, anticancer activity, antitubercular activity, ADME screening.

P12. Anticancer agents targeted against cyclin-dependent kinase 2 (cdk2): structure-based design of irreversible and reversible inhibitors

*H. Lebraud**

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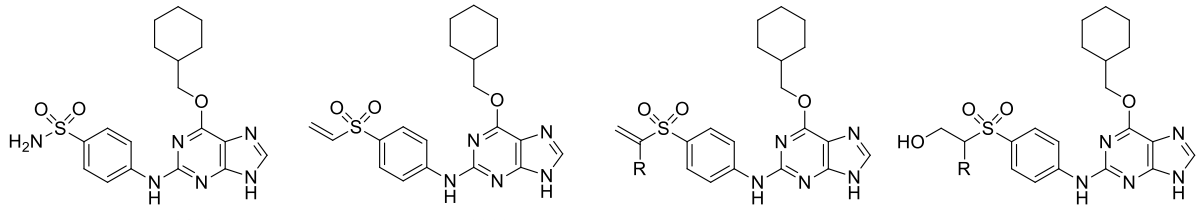
Inhibition of enzymes that participate in the cell cycle is a valuable approach to targeted cancer therapy.[1] This strategy aims to reduce side effects by improving selectivity for tumour over normal cells. The Cyclin-Dependent Kinase (CDK) family of enzymes plays a key role in the regulation of the mitotic cell cycle. Dysfunctional CDK activity has been observed in all cancers. CDK2, which facilitates transition through two phases of the cell cycle (G1/S and early S phase), is overexpressed in a range of tumours including breast[2] and colorectal cancer,[3] and melanoma.[4] Modulation of CDK2 activity with small-molecule inhibitors has been shown to produce selective antitumour activity, while recent evidence suggests a beneficial interaction between inhibitors of CDK2 and the kinase PI3-K.[5] The development of potent and selective CDK2 inhibitors is clearly an area of considerable interest.

We have previously identified potent and selective CDK2 inhibitors, exemplified by the purine NU6102.[6] Our approach uses information from crystal structures of the CDK2 protein to design inhibitors. Subsequently, the compound NU6300 was synthesised and found to inhibit CDK2 in a time-dependent manner. This purine appears to be the first irreversible inhibitor of CDK2.[7] The compound reacts covalently with the CDK2 protein, specifically by addition of a lysine residue (Lys89), located in the 'hinge region' of the ATP-binding domain, to the vinylsulfone of NU6300. However, the high reactivity of NU6300 was found to compromise selectivity and further optimisation was required. Using the crystal structure of the CDK2-NU6300 complex as a guide to inhibitor design, analogues were synthesised bearing substituents predicted to reduce the reactivity of the vinylsulfone group, whilst retaining sufficient potency against CDK2. The synthesis and biological activity of this new series of irreversible CDK2 inhibitors and their corresponding reversible analogues will be illustrated in the poster.



* IC_{50} is the concentration of a drug required for 50% inhibition of a biological function *in vitro*.

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P13. Synthesis and kinome selectivity patterns of imidazo[4,5-b]pyridine-derived fragment libraries

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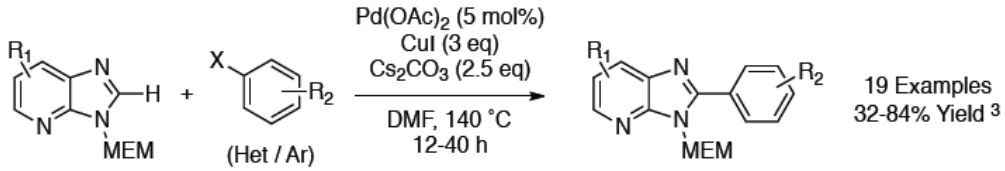
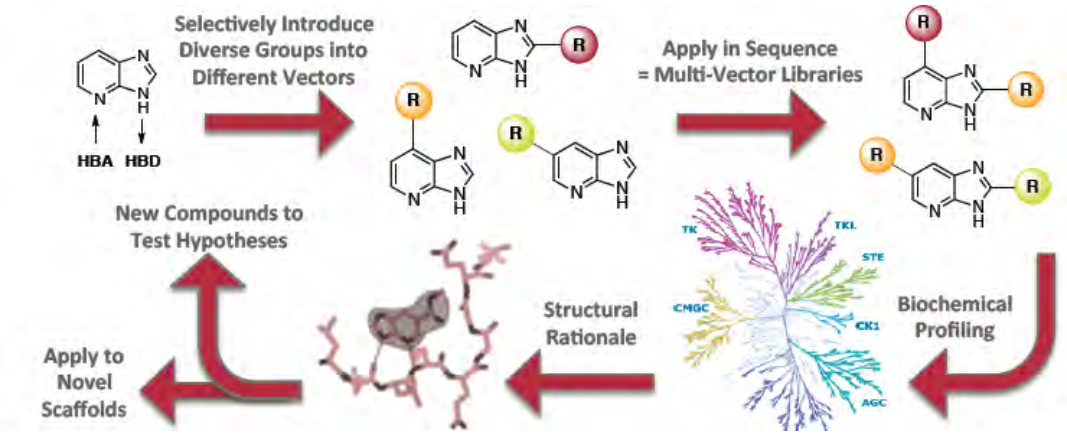
We are studying the influence of small structural changes to ATP-competitive kinase inhibitor scaffolds on binding mode and kinome-selectivity profile. To this end, we have developed synthetic routes to focused libraries of fragment-like molecules that comprise small structural modifications along multiple vectors of a single scaffold. We have investigated structure-selectivity profiles by biochemical screening (Caliper Profiler Pro® assay) and binding modes by X-ray crystallography. We anticipate that this knowledge can be applied to other kinase inhibitor scaffolds of interest, potentially accelerating the hit-to-lead phase of kinase chemical probe and drug discovery projects, and may contribute to the design of novel hinge-binding scaffolds.



We initially chose to investigate the imidazo[4,5-b]pyridine hinge-binding scaffold which has been elaborated in-house to give potent, orally bioavailable inhibitors of Aurora A kinase, and dual FLT3/Aurora kinase inhibitors for the treatment of Acute Myeloid Leukaemia.1,2 We will present synthetic methodology compatible with flexible elaboration along multiple vectors of the imidazo[4,5-b]pyridine scaffold, including the discovery of efficient direct arylation methods for C2 functionalisation and its incorporation into fragment-like multi-vector libraries.3 Our results demonstrate that small changes to kinase hinge-binding motifs can have dramatic effects on both selectivity and kinase binding mode. We will present our profiling results and analysis of kinome selectivity patterns with reference to both kinase primary sequence and to protein-structural

information. The extent to which the observed trends are replicated in alternative hinge-binding motifs is currently under study and application to the design of novel hinge-binding scaffolds is underway in our laboratories.



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P14. Towards the development of DMAP-N-oxide derived organocatalytic kinase mimetics: reaction rate and selectivity studies

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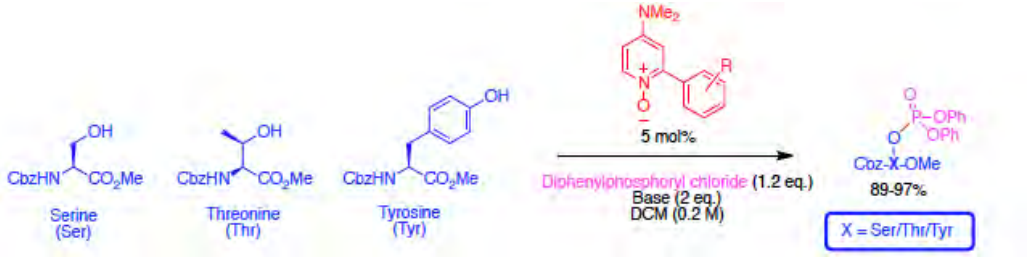
The development of novel 2-aryl-4-dimethylamino-**N**-oxide (DMAP-**N**-oxide) derived kinase mimetics for the selective phosphorylation of hydroxyl-containing amino acids is reported (Scheme 1). The reactions proceed in good to very good yields and good levels of selectivity for Ser **vs**. Thr **vs.** Tyr are achievable. Rate studies have been conducted on protected derivatives of these amino acids under various conditions and have revealed that reaction rates and thus substrate selectivity are highly dependent upon the choice of base and preliminary results indicate that optimal selectivity may be achieved through fine-tuning of the 2-aryl substituent of the catalyst.



Scheme 1 – Amino acid phosphorylation using 2-aryl-4-dimethylamino-**N** -oxide derived catalysts

Work towards demonstrating substrate selectivity is currently underway within our laboratory; we aim to demonstrate the utility of this methodology on a small peptide, selectively phosphorylating these amino acid resides, thus mimicking the action of protein kinase enzymes. This work provides a novel approach to phosphopeptide synthesis and is the first demonstration of a small molecule, organocatalytic kinase mimetic.

P15. In silico design of bacterial Type II topoisomerase inhibitors: new "dual-target" antibiotics

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University of Leeds

Bacterial resistance is a major threat to world health. With more multidrug resistant strains being reported each year, the development of new antibiotics is of vital importance. The identification of new antibiotics active against Gram-negative bacteria is of particular importance, as resistance is increasing in Gram negative bacteria and there are relatively few new drugs currently in development to treat infections of this type.[1]

The bacterial type II topoisomerase enzymes DNA gyrase and topoisomerase IV (topo IV) are responsible for manipulating the degree of supercoiling in bacterial DNA allowing DNA replication and transcription to occur. As there are no human counterparts, they are attractive drug targets. The fluoroquinolone antibiotics target the DNA binding regions of the two enzymes. Small molecule inhibitors targeting the ATP binding sites in these enzymes have been widely reported in the literature, but none have been successfully brought through into the clinic.

This project aims to develop dual-inhibitors of GyrB and ParE with broad spectrum antibiotic activity against both Gram negative and Gram positive bacteria.

In silico design of novel inhibitors was carried out using SPROUT HitOpt, and included studies involving modifications of an existing series discovered in Leeds[2] with the aim of improving enzyme potency and Gram negative activity. Based on structures suggested by the software, a small library of compounds was selected and a 6-step synthetic route was developed to produce these compounds for biological testing. Several of the compounds had IC_{50}s in the nanomolar range against the GyrB enzyme (10-46 nM) and the ParE enzyme (40-370 nM) respectively. These compounds were also active against *S. aureus*. Although these compounds showed no antimicrobial activity against Gram negative organisms, they are amongst the most potent GyrB/ParE inhibitors reported in the literature, showing that the combination of *in silico* design and synthetic chemistry can generate potent "dual-targeted" antibiotics.

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P16. Inhibitors of Kinetoplastid Sphingolipid Synthases as Potential Therapeutic Agents

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E. Alvarez-Ruiz,[2] S. Gonzalez-Del Valle,[2] J. J. Martin-Plaza,[2] P. G. Steel[1] and P. W. Denny[1]

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The protozoan kinetoplastid parasites *Leishmania* spp, *Trypanosoma brucei* and *Trypanosoma cruzi* are responsible for potentially fatal diseases that affect over 22 million people worldwide, with an

estimated 450 million at risk. Current therapies are expensive and not widely accessible. In addition, drug toxicity and emerging resistance are major concerns. Previous work has identified the essential kinetoplastid sphingolipid synthase (SLS) as an attractive pharmaceutical target due to the divergence of function compared with the mammalian orthologue. A high-throughput compatible screening assay was developed to test the 1.8 million compound library held at GlaxoSmithKline in Tres Cantos against the *Leishmania major* enzyme. The 19,669 compounds identified were subjected to further screening to identify those which were highly active yet selective for the parasite enzyme. 216 of these compounds were selected as being promising drug candidates and were subsequently tested on *Leishmania* promastigotes and amastigotes (the insect and mammalian stages of the parasite respectively), infected macrophages and HepG2 cells (a human liver cell line). The results were encouraging, with several compounds displaying high activity against the parasites whilst having a negligible effect on the human cells. A set of promising chemical scaffolds has subsequently been identified, and current and future work centres around redesign and resynthesis of these compounds in order to produce the best possible candidates for clinical trials.

P17. Synthesis of Novel Trypanosomal Alternative Oxidase Inhibitors for the Potential Treatment of *African trypanosomiasis*

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University of Sussex

Human African trypanosomiasis (HAT) or African sleeping sickness is a debilitating infection caused by the parasite Trypanosoma brucei that has a 100% mortality rate without chemotherapeutic intervention. An estimated 60 million people are at risk of HAT infection across 36 countries. Approximately 70,000 people are infected currently, with around15,000 new infections and deaths per annum.

Only five drugs exist for the treatment of HAT. The most effective must be given intravenously which is expensive and logistically challenging. The pressing lack of treatments results in the in the continued use of outdated and harmful therapeutics including an organoarsnide, melarsoprol, that kills 5% of patients.

A natural fungal metabolite, ascofuranone, has been shown to have picomolar activity against the HAT disease causing parasite in vitro and has been shown to clear the parasite in murine studies. This potent therapeutic effect has been shown to arise from the inhibition of a mitochondrial terminal oxidase that is specific to the parasite and not present in the mammalian cell, making it an excellent candidate for a specific small molecule therapeutic.

Unfortunately, the natural product has key functionalities that are susceptible to metabolic clearance and to undesirable or toxic secondary activities, which prevents its potential use in any clinical application. Furthermore, previous synthetic methodology towards the natural product and its derivatives has been very limited and so routes towards analogous compounds have been restricted.

The work undertaken in Prof Simon Ward's group has been to develop novel synthetic methods to access ascofuranone analogues and to direct synthesis towards compounds that address the metabolic clearance and toxicity issues whilst maintaining the exceptional potency of ascofuranone.

There has been excellent progress regarding synthetic tractability of analogues and in the discovery of new motifs that are less metabolically stable but which crucially retain potency across phenotypic and genotypic assays.

P18. Design, synthesis and biological activity of novel stilbenesulfonamides

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Natural peanut stilbenoids, such as resveratrol and their synthetic analogues are known for their various biological activities; including anti-inflammatory and chemopreventative properties. Recently, the cytotoxicities of a number of stilbenesulfonamides, against the National Cancer Institute's 60 human tumour cell lines have been reported[1]. Furthermore, anti-inflammatory effects via inhibition of COX-2 of novel heterocyclic methylsulfone and sulfonamide analogues have also been published[2]; however, both synthetic routes described are limited to primary sulfonamides.

A number of novel primary, secondary and tertiary stilbenesulfonamides and heterocyclic stilbenesulfonamides, synthesized via 3 steps, are currently being evaluated for their cytotoxicity against lung (A549) and ovarian (IGROV-1) cancer cell lines. Initial *in vitro* studies have shown these novel analogues to display increased activity, compared to those in the literature[1]. In this contribution, the viability studies of the analogues will be presented, we will also report progress on our current studies into the mechanism of cell death.

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P19. Small Molecule Probes for Protein-Protein Interactions: Mimicking Beta-Sheet Motifs

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Protein-protein interactions (PPIs) are ubiquitous in cells and have been linked to a number of disease pathways.[1] It has been shown that PPIs can be mediated by secondary protein structures such as alpha-helices and beta-sheets.[2,3] Beta-sheet mediated PPIs have been implicated in a number of disease areas including cancer and although a number of compounds have been developed to mimic beta-sheet motifs, few have made significant progress against a PPI in a disease relevant target.[4] In this work we have set out to gain further understanding of beta-sheet mediated PPIs by utilising small molecules as beta-sheet mimetics. We have developed a model system based upon a chromone peptidomimetic (**Chr**) that mimics the ADAD hydrogen-bonding motif of a short section of a beta-strand.

The first part of this work has focussed on the synthesis and investigation of the hydrogen-bonding interactions of **Chr**. ^{1}H NMR dilution studies were carried out to determine the homo-dimerisation constant ($\mathbf{K_{DIM}}$) of a number of analogues of **Chr**. As a comparison, the homo-dimerisation of the tri-peptide (**Pep1**) was measured and found to be a stronger interaction; however, the hetero-dimerisation constant ($\mathbf{K_{Het}}$) between **Chr** and **Pep1** was found to be larger than either homo-dimerisation constant. To understand the observed differences in dimerisation constant we have carried out a thermodynamic analysis of these interactions by variable temperature ^{1}H NMR. We have found that, in both the homo- and hetero-dimerisation, the hydrogen bond strength was consistent with the expected value for a typical hydrogen bond. However, large differences in entropy cost for dimerisation were observed between the interactions.

This data provides a fundamental insight into the nature of a simple model of a beta-sheet interaction and provides a platform to develop small molecule beta-sheet mimetics and assess their ability to interfere with PPIs.

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P20. Antagonists of the IgE:FcεRI Protein-Protein Interaction as Potential Anti-Asthma Therapeutics

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The protein-protein interaction (PPI) between the antibody immunoglobulin E (IgE) and its high affinity receptor (FcεRI), found on mast cell surfaces, is a key component of asthma and other allergic responses (Figure 1).1,2 Inhibiting the IgE:FcεRI PPI is an attractive strategy for therapeutic intervention and the development of allergy treatments.3

This work involves the design, synthesis and testing of two libraries of antagonists of this PPI; small molecules based on the natural product (+)-aspercyclide A4 and linear peptides based on a key epitope of FcεRI. An enzyme-linked immunosorbent assay (ELISA) and a time resolved fluorescence resonance energy transfer (TR-FRET) assay have been developed and used to test compounds. Biophysical and structural biology studies are being carried out, including surface plasmon resonance (SPR) experiments and protein crystallography, to try to gain further information and insights into how compounds disrupt the IgE:FcεRI complex. The long-term goal of this work is to develop potent, small-molecule inhibitors of this PPI.

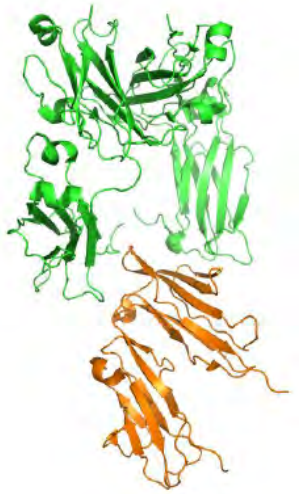

Figure 1: Crystal structure of Fc fragment of IgE (green) bound to extracellular domains of FcεRI (orange).2

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P21. Chemical Tools for Investigating Multiple Herbicide Resistance in Black Grass (alopecurus myosuroides)

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The increasing world population is placing an ever-growing demand on food production. However, the persistent use of herbicidal chemicals to sustain crop productivity has resulted in the emergence of resistance. The most serious form of resistance is that known as multiple herbicide resistance (MHR) in which plants become resistant to all herbicides regardless of their mode of action or metabolism. The focus of this project is concerned with developing novel chemical methods to investigate and combat MHR in the weed species *Alopecurus myosuroides*, commonly known as Black Grass.

When compared with wild type (WT) black grass, populations showing MHR exhibit a significant enhancement of the antioxidant response. Proteomic analysis revealed the expression of a specific Phi class GST, *Am*GSTF1. Cloning and expression of *Am*GSTF1 in the model plant *Arabidopsis thaliana*, resulted in an MHR phenotype. This suggested that *Am*GSTF1 played a significant role in MHR through enhanced oxidative stress tolerance and detoxification pathways.

Evaluation of a small set of known GST inhibitors against MHR black grass revealed the possibility for the reversal of the MHR response through treatment with small molecule chemical adjuvants. We have shown that by regulating *Am*GSTF1, plants that display MHR can become susceptible to such herbicides, with the most significant activity obtained with compounds containing the benzoxadiazole motif.

The design, chemical synthesis and evaluation of related small molecules, which contribute to a reversal of the MHR phenotype is currently on going. This involves the application of structure-based methods, e.g. x-ray crystallography and molecular modelling, to develop further chemical tools for investigating MHR in black grass. In addition to this, modification of the substituents of potential inhibitors is being carried out using a range of transformations including S_NAr chemistry, transition-metal mediated cross-couplings and various heterocyclic condensations reactions. Details of this and the subsequent generation of SAR will be presented.

40 Years of Cancer Drug Development – Chemical Warfare to Patient Welfare

Herbie Newell

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The genesis of cancer chemotherapy took place in the aftermath of World War II with the development of the nitrogen mustards, derivatives of the warfare agent mustard gas. In addition to the nitrogen mustard alkylating agents, notable contributions by UK scientists have been the platinum complexes, thymidylate synthase inhibitors, anti-oestrogens and anti-androgens, and the methylating agent temozolomide. From the outset cancer drugs were associated with significant toxicities, and the quest for enhanced selectivity through tumour-specific cytotoxic drug delivery led to innovative strategies that included enzyme-, antibody- and macromolecule-targeted approaches. Building on this track record the UK was ideally placed to embrace and exploit the field of molecular oncology as it has evolved. A substantial number of drug discovery groups are now active in the UK, building on the success of the more established centres that have already delivered first-in-class targeted agents such as HSP90, PI3K and PARP inhibitors. Examples of targeted drug discovery will be given, and associated challenges discussed.

Antitumour activity of an (*E*)-Styrylsulfonyl methylpyridine analogue of ON01910.Na: A novel kinase inhibitor targeting mitotic pathways

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ON01910.Na (*Rigosertib, Estybon*®), a styryl benzylsulfone, is a Phase III stage, non-ATP competitive anti-cancer agent. This kinase inhibitor has multi-targeted activity, promoting selective mitotic arrest and apoptosis in cancer cells. Extensive Phase I/II studies with ON01910.Na, conducted in patients with solid tumours and haematological cancers demonstrate excellent efficacy with an impressive safety profile.

By modifying the structure of ON01910.Na, TL-77, a novel (*E*)-styrylsulfonyl methylpyridine with improved oral bioavailability has been designed and synthesized in our laboratory. In this study, we have investigated the detailed cellular mechanism of TL-77 in comparison with ON01910.Na. Like ON01910.Na, TL-77 displays potent growth inhibitory activity *in vitro* (GI_{50} <1 mM against HCT 116 cells), and demonstrates ~ 3-fold greater potency in tumour cell lines over HMEC-1 human mammary endothelial cells. Cell cycle analyses reveal that TL-77 causes significant G2/M arrest ≥6 h, whereas ON01910 blocks the cells at G2/M phase ≥ 12 h at the GI_{50} values. Significantly, TL-77 suppresses tubulin polymerization, resulting in multi-polar spindles and misalignment of chromosomes. These are caused by effective inhibition of cdc25c phosphorylation and result in downstream inhibition of cyclin B1. Unlike ON01910.Na, non-significant effects on PI3K/AKT pathway are observed after TL-77 treatment. Analysis of apoptotic signaling pathways reveals that TL-77 alters expression of Bcl-2 family proteins and stimulates activation of caspases. Taken together, our data provide a rationale for the development of (*E*)-styrylsulfonyl methylpyridines as mitotic inhibitors and TL-77 represents a promising anti-tumour agent worthy of further evaluation.

Modulation of Aurora-A Activity and Conformation by Chemical Modification

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Protein kinases govern many cellular pathways and therefore need to be tightly regulated. Kinases can be controlled by changes to their conformation and many require phosphorylation to be fully active. In order to study the activation mechanisms of kinases, and for use in screening assays to identify potential inhibitors, it is desirable to prepare a homogeneous sample consisting of a fixed conformation or 'activation state'.

Attempts to produce homogeneous fully active kinases usually involve performing mutagenesis to introduce a residue to mimic phosphorylation. For a typical kinase, Aurora-A (AurA), introduction of glutamate or aspartate into the activation loop is not a suitable substitute for phosphorylation of serine or threonine residues. An alternative strategy is to chemically modify cysteine residues via a two-step reaction[1] to produce a phospho-cysteine bioisostere of phospho-serine or -threonine. We have used this approach to selectively determine the relative contributions to activity of two adjacent phospho-threonine residues in AurA, and revealed a novel inhibitory role for activation loop phosphorylation in this kinase.[2]

Furthermore, we now demonstrate that a range of chemical mimics of post-translational modification and other unnatural amino acids can be installed by this technique (Figure 1), and we have determined reactivity trends for a diverse range of thiol nucleophile addition reactions on AurA. We also show that chemical modification of cysteine residues is possible not only on a flexible surface-exposed loop, but also within a sterically-hindered active site pocket, which reveals the potential use of this method in exploring enzyme function through modification of catalytic site residues.[3] In conclusion, we show that chemical modification of cysteine residues is a valuable tool with which to modulate the activity or conformation of a recombinant protein.

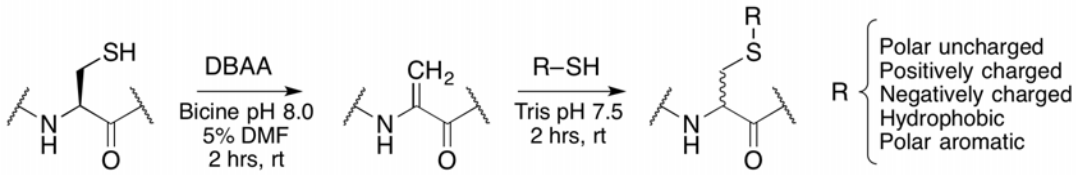

Figure 1: Chemical modification of cysteine residues on AurA

1) Chalker (2012) *Angew. Chem. Int. Ed.*, 51, 1835–1839; 2) Rowan (2013) *ACS Chem. Biol.*, DOI: 10.1021/cb400425t; 3) Manuscript in preparation

The Design, Synthesis and Evaluation of Inhibitors of the HIF-1α/p300 Protein-Protein Interaction

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Protein-protein interactions (PPIs) are implicated in virtually all biological pathways[1] and are emerging as new targets for medical intervention. Of particular interest are α-helix mediated PPIs where a key helical region of a protein, the helix donor, binds into a cleft on a partner protein, the helix acceptor.[2] This region of interaction has the well-defined structure of an α-helix and can therefore be inhibited by small molecules that bind tightly into the helix acceptor cleft. One example of a helix donor is Hypoxia Inducible Factor (HIF)-1α which forms a key protein-protein interaction with the protein p300, which leads to the hypoxic response cascade.[3]

Tumours rapidly deplete the oxygen supply to the tissue and cancerous cells exploit this hypoxic response pathway to resupply the tumour with oxygen. Inhibition of the HIF-1α/p300 interaction is therefore of interest in the development of new treatments for cancer by preventing the supply of additional oxygen to tumours.[4] We report an investigation into the interaction of the two binding partners, leading to the development of a biophysical assay capable of identifying inhibitors. This has allowed us to identify a series of low μM inhibitors based upon rational structure based based design. These compounds are the first small molecule HIF-1α/p300 inhibitors to be characterized biophysically and are selective for the HIF-1α/p300 interaction over other PPIs in the oncology field such as the eIF4e/eIF4g.

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Cracking the Histone Code: Developing Inhibitors of Bromodomain-Acetyl-Lysine Interactions

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Histones, the core proteins around which DNA is wound, are susceptible to multiple post-translational modifications, combinations of which are proposed to form a 'histone code' that is involved in regulating gene expression. One such PTM, lysine acetylation, influences recruitment of transcriptional regulators through interaction with bromodomain-containing proteins (BCPs), which 'read' lysine acetylation state. There are 61 bromodomains, found within 46 proteins; these modules are emerging important therapeutic targets and the protein-protein interactions they mediate are ligandable. To develop tools that enable elucidation of cellular bromodomain function, we have designed and synthesized small molecules capable of preventing the bromodomain-acetyl-lysine interaction. Herein, the design, synthesis and application of two different acetyl-lysine mimics will be described. The optimisation of these moieties to provide potent and selective ligands for the CREB binding protein (CREBBP) and the bromodomain and extra terminal domain (BET) family of BCPs will be discussed.

Alternative Strategies for Targeting HSP70

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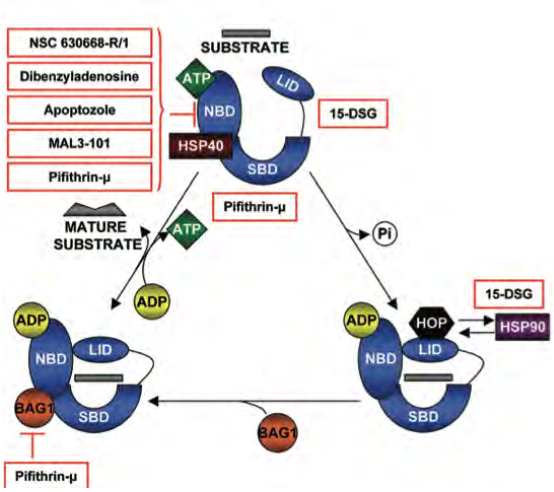
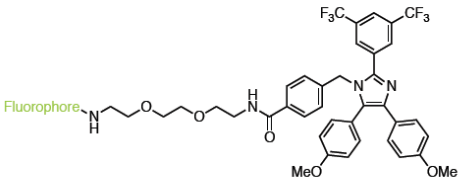
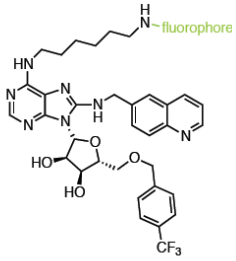
HSP70 is a stressed induced molecular chaperone, which stabilizes and refolds misfolded or aggregated proteins. Cancer cells typically experience high levels of stress and have been shown to overexpress HSP70 in order to remain viable. High HSP70 expression levels have been linked to poor prognosis in a variety of human cancers. However, targeting this protein has been challenging due to its large, flexible structure and complicated catalytic cycle. Despite huge efforts most published inhibitors of HSP70 have a poorly characterised mode of action and only moderate to weak potency.1,2





Probe derived from apoptozole



Probe derived from 1

Two previously reported inhibitors of HSP70, apoptozole3-°©-4 and nucleoside analogue 15, were investigated. Here we report work to investigate the mechanism of action of these inhibitors of HSP70 using fluorescence polarisation (FP) and SPR techniques. Fluorescent probes derived from apoptozole and nucleoside analogue 1 were designed and synthesised using new synthetic routes amenable to analogue synthesis and fluorophore attachment. An FP assay was established and used to interrogate the inhibitors interactions with HSP70's catalytic cycle. Finally a screen for small molecule inhibitors targeting a novel HSP70 complex was set up; the development, validation and screening will be discussed.

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Discovery of a Potent and Selective Small Molecule Inhibitor of FBXL11, a JmjC Histone Demethylase

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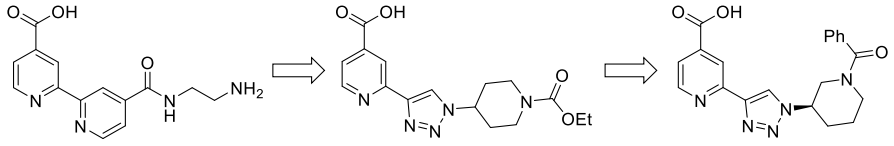
Eukaryotic DNA is packaged as a complex with histone proteins where 145 – 147 base pairs of DNA are wrapped around octamers of histone proteins. The *N*-terminal tails of these histone proteins can be post-translationally modified by epigenetic enzymes (for example through the introduction or removal of methyl, acetyl or phosphate groups). These post-translational modifications can cause both up- and down-regulation of the gene encoded by the surrounding DNA.[1] Two families of epigenetic enzymes can remove methyl groups from lysine residues on histone tails; the lysine specific demethylases (LSD1 and LSD2) and the Jumonji C (JmjC) demethylases (a much larger family of 30 proposed members).[2] Misregulation of JmjC demethylases has been associated with a variety of diseases including inflammation[3] and cancer proliferation.[4] Despite the JmjC domain being highly conserved, inhibitors that are sub-family selective have been discovered although their potency is typically only in the micromolar range.[5]

A new triazolylpyridine template for JmjC demethylase inhibition will be described, including its discovery from **1**, a potent inhibitor of the JmjC demethylase JMJD2E.[6] The synthesis of a series of triazolylpyridine compounds and the optimisation of potency and selectivity for FBXL11 over other enzymes in the JmjC family will also be described. These efforts have resulted in the potent and selective FBXL11 inhibitor **3**, which represents a 20-fold improvement in potency over daminozide, the most active and selective inhibitor of FBXL11 reported to-date.[7]



1
JMJD2E IC50 0.2 μM
FBXL11 IC50 2.1 μM

2
JMJD2E IC50 >100 μM
FBXL11 IC50 6.1 μM

3
JMJD2E IC50 >100 μM
FBXL11 IC50 0.07 μM

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A Functionalised Linker Strategy for the Diversification of Stapled Peptides

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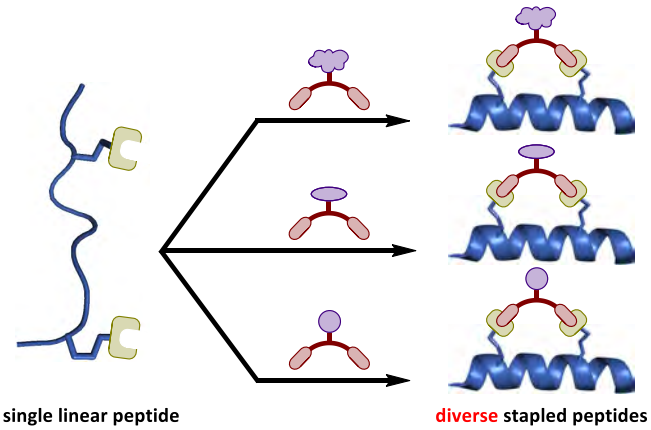
Stapled peptides are alpha-helix mimetics in which two amino acids with reactive side-chains are introduced into a native peptide sequence. Cyclisation of the two reactive groups constrains the peptide and reinforces an alpha-helical structure, providing a general method for designing inhibitors of protein-protein interactions.[1] An important feature of stapled peptides is that the staple component itself can greatly affect the overall properties of the molecule. Optimisation is needed to find the most effective linker length to match the peptide alpha helix, as well as the correct pose for maximising favourable interactions with the protein surface. However, many stapling methods require the synthesis of new amino acids and peptides each time a new optimisation variable is explored.[2]

We have designed a convergent two-component strategy for stapling diazido peptides with dialkynyl linkers. Using the Cu-catalysed azide-alkyne cycloaddition, a variety of linkers can be efficiently stapled onto a single peptide to yield a range of stapled peptides with different functionalities. As a proof of principle, we have designed functionalised stapled peptides for inhibiting the p53/MDM2 interaction, a well-established target for cancer therapy.[3] Using a p53 sequence previously reported as cell-impermeable using other stapling methods,[4] we are able to confer cell permeability by functionalising the linker with cationic motifs. The resulting stapled peptides have an increased binding affinity for MDM2 relative to the native sequence, are more stable under proteolytic conditions, and can increase cellular p53 levels in a T22-based gene reporter assay.



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