Emerging Paradigms in Anti-Infective Drug Design

Issues and progress in designing treatments for neglected and tropical diseases

17th and 18th September 2012
"Welcome to the first joint British Society for Parasitology and Royal Society for Chemistry (Biological and Medicinal Chemistry sector) Symposium.

All of us involved in the discovery and development of drugs for the treatment of infections caused by viruses, bacteria, fungi or parasites share common challenges. We hope this Symposium gives us the opportunity to share these challenges in infectious disease research. It is also an opportunity to focus on the biology-chemistry-pharmacology interfaces where new understanding is essential for advances in drug R & D.

At the end of the Symposium on Tuesday we hope you will have time to complete the meeting evaluation form. We believe that bringing scientists across the disciplines together is an essential part of the drug discovery and development process and we will learn lessons for the organisation of future meetings."

Dave Alker, Michael Barrett, Simon Croft, Andrew Stachulski
Emerging Paradigms in Anti-Infective Drug Design
17 – 18 September 2012

Monday 17th September

10:00-10:45  Registration
10:45-11:00  Introduction and welcome

Session one - Target diseases and targets for drugs - Chair: Dave Alker

11:00  How many more antimalarials do we need?
       Jeremy Burrows, Medicines for Malaria Venture (MMV), Switzerland

11:30  Targets and strategies for drug discovery for kinetoplastid infections –
       Chagas’ disease, African trypanosomiasis and Leishmaniasis
       Rob Don, Drugs for Neglected Diseases initiative (DNDi), Switzerland

12:00  Any chance to find a new anthelmintic for humans?
       Ronald Kaminsky, Novartis AH, Switzerland

12:30  Discussion
12:45-13:45  Lunch

Session two - Target based medicinal chemistry - Chair: David Selwood

13:45  Fragment-based in silico design approach to anti-infectives
       Colin Fishwick, University of Leeds

14:15  Drug discovery for human African trypanosomiasis
       Ian Gilbert, University of Dundee

14:45  Development of a new generation of antimalarial lead quinolones targeting
       the mitochondrial respiratory chain for the treatment and prophylaxis of
       uncomplicated malaria
       Paul O’Neill, University of Liverpool

15:15  Developing peptide-inspired therapeutics for the neglected tropical diseases
       Steven Cobb, University of Durham

15:30  Discussion
15:45-16:15  Tea and coffee break

Session three - Target identification - Chair: Mike Barrett

16:15  Combining chemical probes for target identification and validation in anti-infective
       drug discovery
       Ed Tate, Imperial College, London

16:45  High-throughput decoding of drug targets and drug-resistance mechanisms in
       African trypanosomes
       David Horn, London School of Hygiene & Tropical Medicine

17:15  Unbiased determination of anti-parasitic drug mechanisms by metabolomics
       Darren Creek, University of Melbourne and University of Glasgow

17:30  From genome to anti-parasitic drug target
       Pascal Mäser, Swiss Tropical and Public Health Institute

17:45  Discussion
18:00-20:30  Posters and Reception
Tuesday 18th September

08:30  Registration

Session 4 - Intracellular pathogens - Chair: Jeremy Mottram

09:00  Malaria - finding new ways to fight an old foe
       Paul Smith, NITD, Singapore

09:30  ATP-dependent Mur ligases in the biogenesis of cell wall peptidoglycan in Mycobacterium tuberculosis: novel targets for anti-TB drug discovery
       Sanjib Bhakta, Birkbeck, University of London

09:45  Screening for inhibitors of Leishmania inositol phosphorylceramide synthase
       Paul Denny/ Patrick Steel, Durham University

10:00  Understanding and optimising iminosugars as antivirals against dengue virus
       Nicole Zitzmann, University of Oxford

10:15  Discussion

10:30-11:00  Tea and coffee break

Session 5 - Predictive models for drug evaluation - Chair: Steve Ward

11:00  Animal models to speed up drug discovery: A new approach
       Iñigo Angulo-Bartuen, GSK Tres Cantos, Spain

11:30  More predictive animal models for tuberculosis drug discovery: quantitative tools for functional and structural tools
       Clifton Barry, NIAID, National Institutes of Health (NIH), USA

12:00  Disposition- rationale for lead optimization and prediction of clinical efficacy
       Steve Wring, SCYNEXIS, North Carolina, USA

12:30  Discussion

12:45-13:30  Lunch

Session 6 - New anti-infectives – how do we get there? - Chair: Simon Croft

13:30  Open source drug discovery
       Matt Todd, University of Sydney, Australia

14:00  A-WOL drug discovery and development: Targeting the essential symbiont, Wolbachia to deliver safe macrofilaricidal therapy for onchocerciasis and lymphatic filariasis
       Mark Taylor, Liverpool School of Tropical Medicine

14:30  Selective inhibitors of protozoan protein N-myristoyltransferases
       Andrew Bell, Pfizer

14:45  TMC207 – a clinical development success story
       Wim Parys, Janssen Infectious Diseases (J&J), Belgium

15:15  Discussion

15:30  Closing remarks
How many more antimalarials do we need?

Jeremy Burrows

Head of Discovery, Medicines for Malaria Venture (MMV), Geneva

The global antimalarial pipeline has been strengthened in recent years with the delivery of new artemisinin combination therapies, promising new clinical candidates and early stage discovery projects. This talk will focus on the challenges that remain and the strategy adopted by Medicines for Malaria Venture (MMV) in targeting eradication.

New target product profiles and target candidate profiles defining the specific characteristics of individual molecules for asexual blood stage cures, transmission blocking, vivax and chemoprotection will be described. Finally these will be illustrated with several case studies: molecules progressing from early Hit Identification to Preclinical Development and beyond from within the MMV portfolio.
Targets and strategies for drug discovery for kinetoplastid infections – Chagas’ disease, African trypanosomiasis and Leishmaniasis

Rob Don

Drugs for Neglected Diseases initiative (DNDi), Geneva

There is an urgent need for modern drugs to treat the kinetoplastid infections: Chagas disease, Human African trypanosomiasis and Leishmaniasis. DNDi has implemented a screening and drug discovery programme to contribute to the research addressing this need. Because of the paucity of validated leads and the low success rate for translating hits against biochemical targets into leads against the whole organism, we have adopted a strategy of phenotypic screening using several different approaches. These include screening for low hanging fruit, diversity screening and screening focused series (mechanism of action and/or chemical series). This presentation will highlight some of the successes and failures of these approaches to expand the chemical diversity available for drug discovery in this area of neglected tropical diseases.
Any chance to find a new anthelmintic for humans?

Ronald Kaminsky

Head of Parasitology, Novartis Centre de Recherche Santé Animale, CH-1566 St-Aubin, Switzerland

Do we need a new anthelmintic for humans? And if, how could we find it? Is anybody looking? Is anybody looking seriously? Do we have an appropriate product profile? Who should search for a new human anthelmintic? What would be the best approach to search? Who should fund this? Can we learn from veterinary anthelmintic drug discovery successes? Can we salvage veterinary drug discovery programs or even veterinary anthelmintics? Expect the answers! But not from the presenter!
The fragment-based in silico design approach to anti-infectives

Colin W. G. Fishwick

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In silico molecular docking techniques such as virtual high-throughput screening (VHTS), is a powerful approach to the discovery of new enzyme inhibitors. However, this technique is limited by the size and diversity of the small-molecule databases used which, even for databases consisting of millions of compounds, can only sample a fraction of the available ‘diversity space’. De novo fragment-based design is a powerful complementary strategy for inhibitor discovery. Here, by using the structural features present within the enzyme only, new inhibitor designs are built-up sequentially according to the requirements of the targeted binding site. Therefore, de novo design is an important technique to use in parallel with VHTS in a particular hit identification campaign, as a good de novo design program will examine structure space larger by many orders of magnitude than that of most virtual libraries currently used for this purpose.

We have recently applied the de novo molecular design computer program SPROUT, developed at Leeds, to the rational design of inhibitors of essential enzymes from a number of different infective organisms including dihydroorotate dehydrogenase (DHODH) from Plasmodium falciparum and DNA gyrase/topoisomerase from S. aureus. A particular feature of this work has been to develop inhibitors that are not only enzyme selective, but are also highly effective at killing the targeted organism.
There is an urgent need for the development of new drugs for the treatment of tropical diseases. At the University of Dundee we have set up an integrated Drug Discovery Unit, with one of the key aims being early phase drug discovery for neglected tropical diseases such as malaria, the kinetoplastid diseases and TB. We have investigated both target-based and phenotypic (cell-based) approaches to drug discovery. In this presentation, some of our work in this area will be summarised, focusing on human African trypanosomiasis. For hit discovery we have used high throughput screening with diverse and focused libraries. This has been followed by medicinal chemistry and DMPK to optimise hits. In our experience, we have found a high attrition rate in target-based approaches, with many genetically validated targets not showing chemical validation. However, we have had particular success with one target, N-myristoyltransferase, for human African trypanosomiasis, where we have strong genetic and chemical validation that this is a good drug target for this disease. We have also had success with rapidly finding and optimising chemical start points from phenotypic screening. From our findings, an appropriate mixture of both target-based and phenotypic approaches should be applied for drug discovery in neglected tropical diseases. However, careful selection of drug targets is required; in particular we have had success with a target with pleiotropic effects.
Development of a new generation of antimalarial lead quinolones targeting the mitochondrial respiratory chain for the treatment and prophylaxis of uncomplicated malaria

Paul M. O'Neill

Department of Chemistry, The Robert Robinson Laboratories, The University of Liverpool, Liverpool L69 3BX  P.M.oneill01@liv.ac.uk

A failure to generate new antimalarials with novel mechanisms of action that circumvent current resistance challenges will contribute to a resurgence in the disease which would represent a global health emergency. Here we present a new generation of quinolone lead antimalarials with a dual mechanism of action against two respiratory enzymes, NADH:ubiquinone oxidoreductase (PfNDH2) and cytochrome bc1. Inhibitor specificity for the two enzymes can be subtly controlled by manipulation of the quinolones at the 2 or 3 position. Inhibitors display potent (nM) activity against both the enzymes and against multidrug-resistant Plasmodium falciparum parasites, via rapid and selective depolarisation of the parasite mitochondrial membrane potential leading to a disruption of pyrimidine metabolism and parasite death. Lead antimalarials also display activity against liver-stage parasites as well as transmission blocking-properties. Potent oral antimalarial activity is observed in murine models of malaria (Plasmodium berghei) and favourable pharmacokinetic features do not rule out a single dose treatment. The ease and low cost of synthesis of these inhibitors fulfil the Target Product Profile for the generation of a novel, potent, safe and inexpensive drug with the potential for eventual clinical deployment in control and eradication of falciparum malaria.
Developing peptide-inspired therapeutics for the neglected tropical diseases

S. L. Cobb 1, Frances Chadbourne, 1 P. W. Denny 2

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In recent years antimicrobial peptides (AMPs) have been proposed as potential compounds for the development of new treatments for cutaneous Leishmaniasis (CL). 1 A major advantage that they offer in this area is that their mode of action is believed to be different to all of the current drugs used to treat CL. Unfortunately, the majority of studies carried out in this area have focused on screening AMPs against the promastigote (insect) form of the parasite. We have shown that the data gathered in such studies cannot be used to accurately predict activity against the clinically relevant amastigote form of the parasite. To address the paucity of data available we have recently carried out the largest study into the activity of AMPs against the amastigote form of Leishmania mexicana, a causative agent of CL. 2

We have now screened over 100 peptides and identified several promising leads. The experimental data set generated also allows for the first time computational approaches and predictive tools to be developed, to aid in the discovery of AMP based anti-parasitics. In collaboration with researchers at Durham (Beth Bromley, Physics) and the MRC (Marc Torrent, Molecular and Systems Biology) we aim to translate the benefits that in silico screening methods and predictive models have offered in small molecule drug discovery to the development of anti-infective peptides for the neglected tropical diseases.

Combining chemical probes for target identification and validation in anti-infective drug discovery

Edward W. Tate

Department of Chemistry and Institute of Chemical Biology, Imperial College London, London SW72AZ

Rising drug resistance is a major concern across a wide range of infectious diseases, particularly those caused by pathogenic bacteria and protozoan parasites; there is a clear and urgent need for new classes of drugs against these infections, above all for neglected or poorly-controlled diseases. However, in a number of important pathogens, for example the causative agents of malaria and leishmaniasis, genetic validation of new drug targets is complicated by challenging molecular genetics and a highly complex life cycle. Although phenotypic screening against these organisms can be a very tractable route to hit material, target identification is often rather intractable, limiting both insights into the relevant biology and the potential to apply modern target-oriented drug discovery. An alternative and complementary approach is to identify highly selective tool inhibitors against a putative drug target that can then be used to explore the underlying biology, provide ‘chemical genetic’ target validation, and perform proof-of-concept studies to initiate a broader target-based drug discovery campaign. In our lab, we combine discovery and/or validation of such tool inhibitors together with chemical proteomic analytical tools to understand the biology and druggability of enzymes implicated in essential pathways. In this presentation I will outline our approach to drug target identification and validation for enzymes involved in posttranslational lipidation of proteins, focusing on our recent progress in anti-infective drug discovery.
High-throughput decoding of drug targets and drug resistance mechanisms in African trypanosomes

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The availability of genome sequence data has facilitated the development of high-throughput approaches for the identification of virulence determinants in microbial pathogens. In the African trypanosomes, *Trypanosoma brucei*, RNA interference, coupled to deep sequencing, has proven to be particularly effective in this regard. This RNA Interference Target sequencing, or RIT-seq approach, was developed for genome-scale phenotype screening and for the generation of genome-scale data-sets. In the first screening mode, RIT-seq was used to assess loss-of-fitness phenotypes, revealing genes and proteins that are required for parasite viability and growth, including cohorts of potential drug targets. In a second screening mode, RIT-seq was used to reveal gain-of-fitness phenotypes in the presence of antitrypanosomal drugs. This approach revealed the genes and proteins that facilitate drug action and those that are likely to be mutated in clinically relevant cases of drug resistance. In particular, an aquaporin (water channel) was linked to the long-sought mechanism of melarsoprol-pentamidine cross-resistance. In addition, suramin uptake was found to be via receptor-mediated endocytosis. The examples to be presented illustrate the power of the genome-scale genetic screen. The findings also immediately reveal opportunities for the development of new therapies and diagnostic tools. Other RIT-seq screens will allow for high-throughput decoding of virulence mechanisms in *T. brucei* and the approach should also be adaptable to other microbial pathogens.
Unbiased determination of anti-parasitic drug mechanisms by metabolomics

Darren J. Creek\textsuperscript{1,2} and Michael P. Barrett\textsuperscript{1}

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The discovery, development and optimal utilisation of pharmaceuticals can be greatly enhanced by knowledge of the modes of action, yet many drugs currently on the market act by unknown mechanisms. Untargeted metabolomics offers the potential to discover modes of action for drugs that perturb cellular metabolism. We studied the metabolomic profile of in vitro cultures of \textit{Trypanosoma brucei}, the causative parasite of human African trypanosomiasis, following drug treatment. Development of high resolution LC-MS methods and the IDEOM data analysis software allowed rapid detection of drug-induced changes to cellular metabolism in an untargeted manner. In a proof-of-concept study, eflornithine, a known inhibitor of ornithine decarboxylase, induced significant accumulation of the enzyme substrate, ornithine, and depletion of product, putrescine, while metabolites in other pathways were unaffected. The metabolic profiles induced by other clinically used trypanocides showed remarkably few changes, indicating that the modes of action for these drugs are not due to inhibition of metabolic enzyme targets. Nevertheless, detection of drug-derived metabolites for nifurtimox and melarsen oxide provides important mechanistic information for these drugs. We have also applied this method to experimental anti-parasitic compounds, with detected metabolic changes demonstrating modes of action for compounds with previously unknown targets. Untargeted metabolomic analysis of drug action allows detection and semi-quantitative analysis of endogenous, and drug-derived, metabolites, providing unbiased target discovery and validation to assist with optimisation of new drug candidates.
From genome to antiparasitic drug target

Pascal Mäser

Swiss Tropical and Public Health Institute, Basel, Switzerland

There are several possible mechanisms of how a drug can selectively kill a parasite but not its host. Only one is straightforward to predict in silico, the absence of an orthologue of the antiparasitic drug target in the host. I would like to discuss the potential and limitations of post-genomic target prediction using the malaria parasite *Plasmodium falciparum* as an example. We propose an in silico pipeline that is based on phylogenomics among the Plasmodium spp. and comparative genomics to *H. sapiens*, but largely independent of experimental data. Consecutive filters narrow down the potential target space of *P. falciparum* to proteins that are likely to be essential, matchless in the human proteome, expressed in the blood stages, and amenable to small molecule inhibition. A final set of 40 candidate drug targets was significantly enriched in essential proteins and comprised proven targets as well as new candidates. The potential targets were prioritized based on druggability scores and on the availability of in vitro assays. They provide insights into biochemical peculiarities and vulnerable points of the malaria parasite, and might serve as starting points for rational drug discovery.
Malaria: Finding new ways to fight an old foe

Paul W. Smith

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Despite the successful widespread use of artemisinin based combination therapies in recent years, fighting malaria continues to present a huge global challenge and there is a continued need to discover and develop new medicines for this devastating disease. NITD, together with our partners in the NGBS consortium have embarked on a long term research programme aimed towards the identification of new anti-malarial drugs working through novel mechanisms of action and targeting different stages of the parasite lifecycle. Over the past 6 years, substantial progress has been achieved with 2 compounds already in clinical development. This presentation will briefly review these achievements and then describe in more detail ongoing approaches that are specifically aimed at persistent liver stage forms (hypnozoites) of the parasite Plasmodium vivax.

The unmet medical need in Plasmodium vivax malaria is particularly high. Globally there is a high incidence of this form of malaria, and the formation of liver hypnozoites leads to a chronic, latent disease where re-activation can occur many months or years following initial infection. The only approved treatment effective against Plasmodium vivax hypnozoites is primaquine. However this drug produces severe side effects (hemolytic anemia) in G6PD-deficient patients and there is an urgent need to provide alternative treatment options.

Until recently the discovery of new anti-hypnozoite drugs was limited to in vivo evaluation in the P. cynomolgi rhesus monkey model. However, newly developed assays have enabled testing of new chemical entities on parasite liver-stages and compound classes are now emerging with potential anti-hypnozoite activity.

(NGBS: Novartis Institute for Tropical Diseases (NITD), Singapore, Genomic Institute of the Novartis Research Foundation (GNF), Biomedical Primates Research Center (BPRC), Rijswijk, Swiss Tropical Public Health Institute (Swiss TPH), Basel)
ATP-dependent Mur ligases in the biogenesis of cell wall peptidoglycan in *Mycobacterium tuberculosis*: novel targets for anti-TB drug discovery

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Cell-wall is a well-established target for antibacterial chemotherapy; however our knowledge on structure, function and regulation of cell wall peptidoglycan biogenesis in *Mycobacterium tuberculosis* is limited. ATP-dependent Mur ligases (Mur synthetases) play a pivotal role in the biosynthesis of cell wall peptidoglycan (PG) as they catalyse the ligation of key amino acid residues to the stem peptide at the expense of ATP hydrolysis; thus representing potential targets for antibacterial drug discovery. In this study, we characterised the division/cell wall (dcw) operon and identified a promoter driving the co-translation of Mur synthetases along with key cell division proteins such as FtsQ and FtsW. Furthermore, we have extended our previous investigations on MurE (¹, ²) to MurC, MurD and MurF synthetases from *Mycobacterium tuberculosis*. Functional analyses on the pure recombinant enzymes revealed that the presence of divalent cations is an absolute requirement for their activities. We also observed that higher concentrations of ATP and UDP-sugar substrates were inhibitory for the activities of all Mur synthetases suggesting a stringent control on the peptidoglycan biogenesis pathway. In line with the previous findings on the regulation of mycobacterial MurD and corynebacterial MurC synthetases via phosphorylation, we found that all the Mur synthetases were interacting with Ser/Thr protein kinases, PknA and PknB. In addition, we critically analysed the interaction network of all of the Mur synthetases with proteins involved in cell division and cell wall PG biosynthesis to re-evaluate the importance of these key enzymes as novel therapeutic targets in anti-tubercular drug discovery.

Screening for Inhibitors of Leishmania Inositol Phosphorylceramide Synthase

Jennifer L. Norcliffe, Christopher Brown, John G. M. Mina, Emilio Alvarez-Ruiz, Gonzalo Colmenarejo-Sanchez, Jose J. Martin-Plaza, Jose M. Fiandor, Francisco de Dios, Andy T. Merritt, Paul W. Denny and Patrick G. Steel

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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. We have identified the essential kinetoplastid sphingolipid synthase (SLS) as a particularly attractive pharmaceutical target due to the divergence of function compared with the mammalian orthologue. In order to better understand this membrane bound enzyme and develop structure-activity relationships we have synthesised a library of analogues of the natural ceramide substrate and assessed these for activity against a microsomal preparation of the Leishmania major SLS, an inositol phosphorylceramide synthase, in a 96-well plate assay. More recently, in work supported by the Tres Cantos Open Lab Foundation, a high throughput (1536-well plate format) yeast based assay has been developed. This has been used to screen the GSK 1.8 million compound collection for new selective inhibitors. This presentation will describe these assays and discuss the results as well as current work focused towards the chemical synthesis of further analogues.
Understanding and optimising iminosugars as antivirals against dengue virus

Joanna L Miller¹, Ruben Lachica², Andrew C Sayce¹, James P Williams¹, Manisha Bapat¹, Raymond Dwek¹, P. Robert Beatty³, Eva Harris², Nicole Zitzmann¹

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In the absence of a licensed vaccine, the need for antivirals against dengue virus is clear and increasing; however, there are currently no specific drugs to treat dengue virus infection. A key challenge faced by many promising antiviral drugs, including iminosugars, is in vivo delivery to achieve effective levels of drug without toxicity. Iminosugars are antiviral against dengue virus both in vitro and in vivo. They act by inhibiting ER alpha-glucosidases, host enzymes required by many viruses to help fold the proteins they need to become infectious. Four iminosugars, all deoxynojirimycin (DNJ) derivatives, potently inhibit both the percentage of cells infected with dengue virus and release of infectious virus from primary human monocyte-derived macrophages, demonstrating their efficacy in primary cells. In a lethal antibody-dependent enhancement mouse model of dengue pathogenesis, free NB-DNJ significantly enhanced survival and lowered viral load in organs and serum. To increase their potential we evaluated whether packaging iminosugars within liposomes, which can target them to their intracellular site of action, enhances their antiviral effects. Liposome-mediated delivery of NB-DNJ, in comparison with free NB-DNJ, resulted in a 3-log₁₀ reduction in the dose of drug sufficient to enhance animal survival. Optimising the effective dose in this way could liberate the therapeutic potential of many cytotoxic antivirals against dengue and a wide array of other viruses.
Animal models to speed up drug discovery: A new approach

Iñigo Angulo-Barturen

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Animal models are recognized as key tools in anti-infective drug discovery. They provide integrated systems in which the disposition, toxicity and anti-microbial activity of new drugs is assessed in a relevant physiological context. These characteristics make animal models ideal tools to select and characterise drugs that could become new medicines. Nevertheless, traditional experimental design tends to demand large numbers of animals in order to obtain significant results. This traditional paradigm has put strong practical limitations to the use of animals in drug discovery for ethical and technical reasons. Here we show that designing pre-clinical in vivo studies according to principles of design used in clinical trials can dramatically reduce the number of animals necessary in drug evaluation\(^1\,2\). This new strategy of experimental design in animal models allows a) performing powerful high content/high throughput in vivo screening of drugs at any stage of drug discovery and, b) providing parameters of efficacy useful to inform the design of clinical trials. The use of new experimental designs in vivo has significantly improved decision-making in malaria and tuberculosis drug discovery and might be useful to accelerate the development of new medicines.

\(^1\) All animal studies were ethically reviewed and carried out in accordance with European Directive 86/609/EEC and the GSK Policy on the Care, Welfare and Treatment of Animals.

\(^2\) The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents
More predictive animal models for tuberculosis drug discovery: quantitative tools for functional and structural imaging

Clifton E. Barry, 3rd

Tuberculosis Research Section, NIAID, NIH, USA

Animal models that accurately recapitulate the pathology of human disease are necessary to prioritize candidate drug regimens for expensive clinical trials. Rabbits and a small new world primate (the marmoset) develop tuberculosis disease with all the pathological characteristics of human disease, including cavities which are strongly associated with treatment failure in humans. We have developed experimental models of tuberculosis chemotherapy using positron emission tomography (PET) and computed tomography (CT) that allow for real time quantitative therapeutic response monitoring. These tools have been benchmarked against paired human clinical trials. In addition these models have allowed an assessment of the penetration ability of various TB drugs into tuberculous granulomas and cavities through application of imaging mass spectrometry of treated animals. These tools enable incorporation of lesion penetration and cavity resolving ability into late lead optimization programs and should improve prediction of clinical efficacy for new tuberculosis drug regimens.
A Disposition-Driven Rationale for Lead Optimization and Prediction of Clinical Efficacy


* SCYNEXIS, RTP, NC, USA; * Anacor Pharmaceuticals Inc, Palo Alto, CA, USA; § DNDi, Geneva, Switzerland.

Realizing therapeutically relevant tissue disposition is often a pivotal challenge in drug discovery. Many factors impact penetration into target tissues such as protein binding, perm-selective barriers, and uptake or efflux transporters at the blood-tissue barrier. A thorough understanding of these hurdles is required to guide lead optimization, predict the human clinical dose, and to identify any species-dependent variables that may impact the efficacy of a clinical candidate.

Fortunately in vitro ADME assays, and particularly disposition models such as MDCK-MDR1 transport assay, cellular uptake assays, or the skin penetration models provide tools to predict likely tissue exposure, and to identify potentially undesirable interactions that may lead to adverse events later in development.

In vitro data, both disposition and potency, may also complement tissue exposure data obtained as part of pre-clinical efficacy studies to provide a rationale for prediction of tissue exposure in humans and hence the potential human therapeutic dose.

In this presentation we will describe how a disposition-driven rationale employing in vitro and in vivo data was employed across several infectious disease projects in drug discovery to either:

- Predict the human therapeutic dose for a novel oxaborole in the treatment of human African trypanosomiasis, including a pharmacokinetic model to predict brain exposure from plasma concentration data,
- Demonstrate how in vitro macrophage uptake can be used to predict outcome in a murine model of visceral leishmaniasis,
- Introduce a reconstructed human skin epidermal model as a potential tool for evaluating skin deposition and hydrolysis of pro-drugs during lead optimization for cutaneous leishmaniasis.
Open Source Drug Discovery

Matthew Todd

School of Chemistry, The University of Sydney

Praziquantel is the only available drug for the treatment of schistosomiasis. WHO/TDR recommends its use as a single enantiomer, in order to reduce side effects, reduce pill size and remove the bitter taste. The challenge of producing PZQ in this way, without increasing the cost of the drug, was so severe that we abandoned traditional mechanisms of academic research, and carried out the project in an open source mode, where all data and ideas were freely available on the internet, and anyone could participate.\(^1\) The extensive contributions from scientists around the world, including many from industry, led us to an efficient preliminary solution to this problem faster than if we had conducted the research in a traditional way.\(^2\) We are continuing to use the open source mechanism to improve and change our synthetic route, including as part of a global educational project.

We have recently begun to extend this idea to open source drug discovery in collaboration with the Medicines for Malaria Venture. Several highly promising hits were released into the public domain by GSK Tres Cantos in 2010.\(^3\) Using these compounds as a starting point we are prosecuting a hit-to-lead campaign that is completely open and coordinated on the internet. Anyone can participate, and there will be no patents. The process has already identified several new nanomolar compounds with interesting late-stage gametocyte activity.\(^4\)

\(^4\) See [http://openwetware.org/wiki/OSDDMalaria:GSK_Arylpyrrole_Series:Story_so_far](http://openwetware.org/wiki/OSDDMalaria:GSK_Arylpyrrole_Series:Story_so_far)
Selective Inhibitors of Protozoan Protein N-myristoyltransferases as Starting Points for Tropical Disease Medicinal Chemistry Programs

Andrew S Bell1,5*, James E. Mills1, Gareth P. Williams2, James A. Brannigan3, Anthony J. Wilkinson3, Tanya Parkinson4, Robin J. Leatherbarrow5, Edward W. Tate5, Anthony A. Holder6, and Deborah F. Smith7

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Inhibition of the enzyme protein N-myristoyltransferase (NMT) has been validated pre-clinically as a target for the treatment of fungal and trypanosome infections. Although the target has been validated in other protozoa through genetic studies, there have been limited reports of chemical intervention against either Plasmodium or Leishmania NMTs. We created an academic/industrial collaboration to address this deficiency and have recently completed a high-throughput screen of a diverse subset of the Pfizer corporate collection against P. falciparum and L. donovani NMTs. Primary screening hits against either enzyme were tested for selectivity over both human NMT isoforms (Hs1 and Hs2) and for broad-spectrum anti-protozoan activity against the NMT from T. brucei. Analysis of the screening results has shown that structure-activity relationships (SAR) for Leishmania NMT are divergent from all other NMTs tested, a finding not predicted by sequence similarity calculations, resulting in the identification of four novel series of Leishmania-selective NMT inhibitors. We found a strong overlap between the SARs for Plasmodium NMT and both human NMTs, suggesting that achieving an appropriate selectivity profile will be more challenging. However, we did discover two novel series with selectivity for Plasmodium NMT over the other NMT orthologues in this study, and an additional two structurally distinct series with selectivity over Leishmania NMT. The observed selectivity profiles have been investigated through co-crystallisation of lead inhibitors with their target NMT, with the objective of aiding future design efforts towards clinical candidates.
A-WOL Drug Discovery and Development: Targeting the essential symbiont, Wolbachia to deliver safe macrofilaricidal therapy for onchocercasis and lymphatic filariasis.

Mark J. Taylor

Liverpool School of Tropical Medicine

Wolbachia bacterial have evolved a mutualistic symbiosis with filarial nematodes essential for parasite development, fertility and survival. Their mutualistic symbiosis has been exploited in a new approach to the treatment of lymphatic filariasis and onchocercasis with antibiotics. Anti-Wolbachia therapy delivers a safe macrofilaricidal treatment with superior therapeutic outcomes compared to all standard anti-filarial treatments, with the added benefit of substantial improvements in clinical pathology. A-WOL, an international consortium of academic and industrial partners funded by the Bill & Melinda Gates Foundation was formed to discover and develop new anti-Wolbachia drugs and regimes against onchocercasis and lymphatic filariasis, with the goal of delivering an alternative and complimentary strategy for their treatment, control and elimination. Key outcomes from A-WOL include the development of a portfolio of drug discovery projects with the potential to generate at least one new anti-wolbachial chemotype for eventual deployment as a macrofilaricide. To date 300+ “hits” have been identified and confirmed from screening of focused libraries from pharma and large diversity-based libraries. Registered drug screening has identified 69 hits, with the most advanced lead, showing a 50% increase in potency, reducing treatment time by half in in vivo disease models and has entered into human trials. Combinations of anti-wolbachial drugs can reduce the course of treatment from weeks to a few days in in vivo models, providing proof-of-concept that combination of A-WOL drugs can be delivered in timeframes compatible with MDA and that there is no biological barrier to a short-term regime. A-WOL aims to take the most tractable hit chemotypes from our screening campaigns and progress these through a lead optimization and candidate selection process to generate at least one pre-clinical candidate and a distinct back up to deliver a safe macrofilaricidal treatment suitable for MDA deployment.
TMC207 Clinical Development Success Story


*Janssen Infectious Diseases BVBA, Turnhout, Belgium; ΔJanssen Infectious diseases Inc., Titusville, USA.

TMC207 (bedaquiline) is the first in a new class (diarylquinoline) of anti-tuberculosis (TB) drugs to inhibit mycobacterial ATP synthase with potent late bactericidal and sterilizing properties in the established murine TB model.

The Phase IIb program consisted of a placebo-controlled, double blind trial (C208) in 2 stages, and a single arm, multicenter open label trial (C209). In C208 Stage-2, 161 patients with newly diagnosed MDR-TB were randomized to receive 24 weeks of either placebo or TMC207 added a 5-drug standardized background regimen (BR). The primary efficacy parameter was time to culture conversion at 24 weeks. Culture conversion was significantly faster and the culture conversion rate was significantly higher in the TMC207 group than in the placebo group (79% vs 58% respectively (p=0.008)). In C209, 233 patients with treatment experienced MDR-TB received 24 weeks of TMC207 added to an individualized BR. The primary efficacy parameter was time to sputum culture conversion in MGIT during the first 24 weeks. The proportions of subjects categorized as MDR-TB, pre-XDR-TB and XDR-TB were 54%, 25% and 21% respectively. The median time to confirmed culture conversion for patients in the primary study population was 57 days. The culture conversion rates by level of resistance were 87% for MDR-TB, 77% for Pre-XDR TB, and 56% for XDR-TB at 24 weeks. TMC207 administered orally for 24 weeks was found to be generally safe and well tolerated.

The successful clinical development program culminated in an NDA filing on June 29, 2012. A phase III trial will commence in QIV-2012.
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Stable and water soluble amphotericin B-poly(glutamic acid) complexes for the treatment of visceral leishmaniasis

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Introduction: Leishmaniases are a complex of diseases caused by obligate intracellular protozoa of the genus *Leishmania*. Visceral leishmaniasis (VL) is fatal if untreated. Amphotericin B (AmB) is a polyene antibiotic used for the treatment of systemic fungal infections and it is also highly effective against leishmaniasis. The main drawback of its clinical use is its toxic side effects especially nephrotoxicity, the need of hospitalisation and a complicated dosing regimen. Several lipid-based formulations have been developed and used clinically to overcome the limitation of AmB use. The liposomal formulation of AmB (AmBisome®) is safe and active against VL. Unfortunately the clinical use of AmBisome® is limited by its high cost and the need to use a cold chain.

Aim: To develop a water soluble non-covalent complex between amphotericin B (AmB) and poly(α-glutamic acid) (PGA) which is non-toxic to mammalian cells and active against *Leishmania donovani* in vitro and in vivo.

Methods: The AmB-PGA complex was prepared by dissolving a defined amount of PGA (50-70 kDa) and AmB in dry DMSO followed by slow addition sodium hydroxide (2 equivalents, 1N) and water. The reaction mixture was left to stir at room temperature for 1 h then purified by dialysis and then freeze dried. AmB loading was determined by UV spectroscopy (409 nm) in aqueous methanol (50%).

Results: Water soluble non-covalent complexes of AmB and PGA (AmB-PGA) were prepared with loadings in the range of ~20-50%. The size of the complex was in the range of 100-130 nm with a negative surface charge (~54.97±6.00 mV). These complexes were non-toxic to human red blood cells (RBCs) and KB cells at an AmB concentration of 50 µg/ml (24 h incubation period at 37°C) and 200 µg/ml (72 h incubation period at 37°C) respectively. These AmB-PGA complexes were stable and non-haemolytic with retention of anti-leishmanial activity against *L. donovani* amastigotes in differentiated THP-1 cells in vitro (EC50 0.27±0.03–0.35±0.04 µg/ml which is similar to Fungizone® 0.22±0.04 µg/ml) after storage at 37°C for 7 days as an aqueous solution. This anti-leishmanial activity of AmB-PGA complex (ED50 values of 0.24±0.03 mg/kg) was similar to AmBisome® (liposomal formulation of AmB) (ED50 values of 0.24±0.06 mg/kg) against *L. donovani* in BALB/c mice. A biodistribution and pharmacokinetic study indicated that the AmB-PGA complex cleared more rapidly from plasma than liposomal AmB with a comparable low level of distribution to the kidney.

Conclusion. Non-toxic AmB-PGA complexes were reproducibly prepared in a range of AmB loadings from ~20.0-50.0% that were active against *L. donovani* amastigotes in vitro and in vivo.
Antitrypanosomal activity of Fexinidazole metabolites, a potential new drug candidate for Chagas disease

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Fexinidazole, a 2-substituted 5-nitroimidazole drug candidate “rediscovered” following extensive compound mining by the Drugs for Neglected Diseases initiative, is an effective oral treatment of acute and chronic experimental Chagas disease. Fexinidazole acts as a pro-drug, being oxidized in vivo to more therapeutically relevant species - sulfoxide and sulfone metabolites. This study describes the in vivo activity of fexinidazole metabolites in murine model of Chagas disease. Female Swiss mice infected with Y Trypanosoma cruzi strain (10 animals/group) were treated with doses of 10, 25, 50 and 100mg/kg/day of the metabolites. Oral treatment was administered at the time of parasitemia detection, which occurred at day 4 post-inoculation for 20 consecutive days. The results were compared to those achieved with the benznidazole-treatment at the same doses. Our results showed that fexinidazole metabolites was effective in reducing the number of circulating parasites and protected against mortality compared with untreated mice, but without providing a cure at the doses of 10 and 25mg/kg/day. In addition, assessment of definitive parasite clearance (cure) through parasitological and qPCR assays, showed that both metabolites were effective in curing experimental Chagas disease in mice at 50mg/kg/day(30 to 40%) and 100mg/kg/day(100%). In the benznidazole-treated group, parasitologic cure was detected only in animals treated with the higher dose of 100mg/kg/day(80%). The in vivo antitrypanosomal activity of sulfoxide and sulfone fexinidazole metabolites provides evidence that these compounds have the potential to be an effective oral treatment for human Chagas disease.

Supported by DNDi, FAPEMIG, CNPq, Capes and UFOP
Generation of quinolone antimalarials targeting the Plasmodium falciparum mitochondrial respiratory chain for the treatment and prophylaxis of malaria


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There is an urgent need for new antimalarial drugs with novel mechanisms of action to deliver effective control and eradication programs. Parasite resistance to all existing antimalarial classes, including the artemisinins, has been reported during their clinical use. A failure to generate new antimalarials with novel mechanisms of action that circumvent the current resistance challenges will contribute to a resurgence in the disease which would represent a global health emergency. Here we present a unique generation of quinolone lead antimalarials with a dual mechanism of action against two respiratory enzymes, NADH:ubiquinone oxidoreductase (Plasmodium falciparum NDH2) and cytochrome bc(1). Inhibitor specificity for the two enzymes can be controlled subtly by manipulation of the privileged quinolone core at the 2 or 3 position. Inhibitors display potent (nanomolar) activity against both parasite enzymes and against multidrug-resistant P. falciparum parasites as evidenced by rapid and selective depolarization of the parasite mitochondrial membrane potential, leading to a disruption of pyrimidine metabolism and parasite death. Several analogs also display activity against liver-stage parasites (Plasmodium cynomolgi) as well as transmission-blocking properties. Lead optimized molecules also display potent oral antimalarial activity in the Plasmodium berghei mouse malaria model associated with favorable pharmacokinetic features that are aligned with a single-dose treatment. The ease and low cost of synthesis of these inhibitors fulfill the target product profile for the generation of a potent, safe, and inexpensive drug with the potential for eventual clinical deployment in the control and eradication of falciparum malaria.
Inhibitors of LmjIPCS – New Therapies for Leishmaniasis

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The Leishmaniases, caused by the protozoan kinetoplastid parasites Leishmania spp, are responsible for a range of potentially fatal diseases that are endemic in 88 countries, with 1.6 million new cases emerging annually. Existing therapies are expensive and not widely accessible. Furthermore, drug toxicity and emerging resistance are major concerns, highlighting the need for novel drugs. Previous work has identified the essential inositol phosphorylceramide synthase (IPCS) as an attractive pharmaceutical target by virtue of its divergent function compared to the mammalian orthologue. A high-throughput compatible screening assay was developed to test a library of potential inhibitors against the Leishmania major enzyme. Current work is ongoing towards the synthesis of analogues of identified hits. This will allow the elucidation of structure-activity relationships and facilitate future synthesis directed towards highly active, target specific compounds. Extension of this work to include the IPCS enzyme of Trypanosoma cruzi, causative agent of Chagas disease, is also being conducted.
Identification of novel inhibitors of *Acanthamoeba* species.

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*Acanthamoeba* species can cause severe disease in the eye (*Acanthamoeba* keratitis) in immunocompetent individuals, normally through incorrect contact lens usage. However, to date contact lens solutions do not eliminate *Acanthamoeba* contamination and current treatments for AK are limited and not completely effective. We have been able to examine a number antimicrobial agent targets in *Acanthamoeba* species. Potential targets were confirmed through PCR-assisted amplification of their genes from trophozoites in combination with information from the *Acanthamoeba* genome projects. Known inhibitors of those targets confirmed were tested for their ability to arrest *Acanthamoeba* growth with the use of a colorimetric assay. We now know that *Acanthamoeba* possess a number of biochemical pathways that are not present in the human host and therefore provide a significant rationale target identification in *Acanthamoeba* species.
Fragment-based approaches to targeting the CYP enzymes of *Mycobacterium tuberculosis*

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Tuberculosis remains one of the most prevalent diseases in developing countries. Many frontline drugs for the treatment of tuberculosis have significant shortfalls especially with the emergence of multidrug resistant and extensively drug resistant (MDR and XDR) strains of TB. Because of this there is the need for the development of new drugs that can treat TB. We are interested in targeting the cytochrome P450 (CYP) enzymes of *M. tuberculosis*. There are twenty CYP enzymes encoded by the *M. tuberculosis* genome (H37Rv) and we are involved in targeting CYP121 and CYP125 using the fragment based approach. The CYP121 isoform is of particular interest as it appears exclusive to Mycobacteria and is found to catalyse a unique intramolecular C-C bond forming reaction between the two tyrosines of the cyclo(LTyr-L-Tyr)dipeptide (cYY). CYP125 is involved in the oxidation of the side-chain of cholesterol to the carboxylic acid. We will report the results of our fragment screening work against both CYP121 and CYP125. We will show that a combination of synthetic chemistry, structural biology and biophysical techniques, as part of the fragment based approach, can be used to target these enzymes. Our future focus on CYP126, 141, 142 and 144 will be discussed and how what we learnt on CYP121 and 125 can be applied to these CYP enzymes.

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In a recent study, we have shown that phosphonium salt derivatives holding a diphenyl core were potent antitrypanosomal agents against wild-type and resistant lines of African trypanosomes. These phosphonium salts are lipophilic cations that can diffuse into cells driven by the plasma membrane potential. Because of the large membrane potential across the mitochondrial inner membrane this kind of compounds tends to accumulate into mitochondria. In leishmania parasites, compounds of this class inhibited complex II of the mitochondrial respiratory chain.

In the present work, we have synthesized a set of 14 ammonium, pyridinium and quinolinium salt analogues as possible surrogates for the phosphonium cations. The compounds were tested in vitro against wild type and resistant lines of Trypanosoma brucei (T. b. brucei strain 427, TbAT1-KO, and TbB48). Preliminary SAR studies confirmed some findings observed with the phosphonium series. That is: 1) the trypanocidal activity is increased when the cationic charge is shielded with substantial lipophilicity, 2) the uptake of these compounds does not depend critically on either of the two diamidine transporters (TbAT1/P2, HAPT1) of T. brucei. Interestingly, this work showed that charge delocalization is important but not mandatory to get active compounds. Hence, this class of cationic compounds is worth investigating as antitrypanosomal agents.

References
Fragment-based design, synthesis and pharmacological evaluation of potent Inhibitors of 
trypanosomal phosphodiesterase B1

Kristina M Orrling,1 Chimed Jansen,1 Anitha Shanmugham,2 Paul England,2 David Bailey,2 Vreni Balmer,3 Patrick Bregy,4 Xuan Lan Vu,5 Paul Cos,6 Louis Maes,6 Emily Adams,7 Erika van den Bogaart,7 Eric Chatelain,8 Jean-Robert Ioset,8 Andrea van de Stolpe,1 Johan Veerman,9 Thomas Seebeck,2 Geert Jan Sterk,9 Rob Leurs1, Iwan de Esch1,2 *

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Trypanosomal phosphodiesterases B1 and B2 (TbrPDEB1 and TbrPDEB2) play an important role in the life cycle of Trypanosoma brucei, the causative parasite of human African trypanosomiasis (HAT), also known as African sleeping sickness. By merging fragments of known inhibitors of human PDE4, a low-activity catechol pyrazolinone-based TbrPDEB1 inhibitor was generated. To improve antitrypanosomal activity, a set of analogues was synthesised following a structure-based design strategy that focussed on the targeting of a parasite-specific P-pocket in the enzyme binding site. These efforts were guided by Xray analysis, biophysical screening and biochemical evaluation. The resulting catechol pyrazolinones act as potent TbrPDEB1 inhibitors with IC50 values down to 42 nM. These new TbrPDEB1 inhibitors also block parasite proliferation at low micromolar or nanomolar concentrations (e.g. VUF13525 (20b): T. brucei rhodesiense IC50 = 60 nM, T. brucei brucei IC50 = 520 nM, T. cruzi = 7.57 µM), inducing a typical multiple nuclei- and kinetoplast phenotype. The mode of action of VUF13525 was investigated with recombinantly engineered trypanosomes expressing a cAMP-sensitive FRET sensor. Administration of TbrPDEB family inhibitor 20b resulted in an instant increase of intracellular cAMP levels in trypanosomes in dose-response related fashion. Our findings further validate the TbrPDEB family as antitrypanosomal target and suggest that the reported pyrazolinones can be regarded as an interesting lead series for the development of a new, less toxic treatment of HAT.

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Identification of transmission-blocking antimalarials

Michael J. Delves, Andrea Ruecker, Didier Leroy and Robert E. Sinden

Imperial College, London

Malaria is a disease with devastating impact affecting 216 million people each year and causing 655,000 deaths, most of which are children under 5 years old. Recent appreciation that malaria eradication will require novel interventions to target the parasite during transmission from the human host to the mosquito has lead to an exciting surge in activity to develop transmission-blocking drugs.

We have developed and/or standardised a series of assays interrogating a broad range of transmission stage cell biology, from gamete formation, ookinete production and in vivo mosquito transmission. The aim has been to both evaluate and validate the transmission-blocking efficacy of prospective antimalarials, to identify new starting points for transmission-stage drug development, and wherever possible increase the throughput thus reducing the cost of transmission stage assays. By using a combination of assays it is possible to pinpoint the where specific drugs act during transmission, and it is hoped that this information will inform design of future transmission-blocking drugs.
Factors affecting success in HTS across 4 global health diseases

Mark Gardner
Salvensis, Kent, UK

This poster examines the results from four neglected disease HTS of a portion of the Pfizer compound file against malaria, T. cruzi, T. brucei and Leishmania. It compares the physicochemical properties affecting hit rate for these four HTS with those affecting hit rate in a further 96 non neglected disease HTS.

The poster builds on some work comparing the hit rates of compound libraries synthesised as part of Pfizer’s file enrichment project and compounds accrued over the years by Pfizer, largely as a result of internal drug discovery programmes.

The poster gives some insight into how to design compound files for successful lead finding for global health diseases.
Discovering narrow-spectrum anti-mycobacterial activity of ibuprofen and related arylpropionic acids using HT-SPOTi whole-cell phenotypic assay

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The emergence of multidrug and extensively drug-resistant Mycobacterium tuberculosis strains is a major source of concern in the worldwide battle against tuberculosis (TB). Lead molecules with novel mechanisms of action are urgently required to fuel the anti-TB drug discovery pipeline. We report the miniaturisation of the spot culture growth inhibition assay (HT-SPOTi), an agar-based method which allows rapid but gold-standard determination of minimum inhibitory concentrations. Libraries of anti-TB entities along with known drugs were screened by HT-SPOTi. The antitubercular effect of 47 active hits selected from a focused library from the ChEMBL database was corroborated using this high-throughput methodology. A very small set of widely-available pain-killers was also screened and ibuprofen and related arylpropionic acids were found to display TB growth inhibitory properties. They were active against MDR strains and possessed intracellular killing ability. This chemical class exhibited narrow specificity against the M. tuberculosis complex, displaying encouraging selectivity in comparison with mammalian cells. The sequences of the human biological targets of the arylpropionic class were extracted from the biomolecular databases and homologue proteins were identified in the M. tuberculosis orfeome, which could shed some light on the potential mycobacterial targets of this class of compounds. In addition synthetic modifications of ibuprofen yielded derivatives from which preliminary structure-activity observations were extracted. These hits constitute starting points for medicinal chemistry optimization and biological characterisation of their mycobacterial-specific mechanism of action.
Heterocyclic betulin derivatives against protozoan leishmania parasites

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The (chemo)therapy of leishmaniasis is a neglected area of research and drug development. Current treatments of leishmaniasis are unsatisfactory in terms of safety and efficacy. Our group has previously shown that heterocyclic betulin derivatives inhibit growth of Leishmania donovani.\textsuperscript{1} Betulin is the principle extractive substance of the outer birch (Betula sp.) bark, which is a low-value waste product of forest industry. Betulin was converted to betulonic acid, which is a versatile intermediate, by the Jones procedure. Indole derivatives of betulonic acid were synthesized by the classic Fisher indole synthesis, and pyrazine derivative was synthesized by sulfur catalysis in morpholine. Pyrazine derivative of betulonic aldehyde was synthesized, and its formyl group was converted to an oxime group. An isoxazole derivative of betulonic acid was synthesized via α-hydroxymethylene ketone. The products have been tested against \textit{Leishmania donovani} at Department of Microbiology and Molecular Genetics, IMRIC, The Hebrew University of Jerusalem, Israel. Of these compounds the pyrazine derivative of betulonic acid was the most active one. When carboxyl group was changed to oxime group, while keeping the pyrazine ring, activity was lost. On the other hand isoxazole derivative of betulonic acid was inactive. Based on these results synthesis will be continued towards more active and more soluble compounds. More different kind of fused heterocycles will be synthesized including pyridine, pyrazole, and thiophene derivatives. Also carboxyl group of the heterocyclic derivatives will be modified.
Screening focused compound libraries against *Plasmodium falciparum*: Early to late lead


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Resistance of *Plasmodium falciparum* to existing therapies is emerging rapidly and therefore much effort is being devoted to discover, develop and deliver new treatments for malaria. We have developed a robust 384-well plate assay with a fluorescent read-out to phenotypically screen *Plasmodium falciparum* against potential inhibitors. The Drug Discovery Unit at the University of Dundee has assembled a number of Focused Compound Libraries tailored to certain target classes. With funding support from Medicines for Malaria Venture, two of these sets have been screened in this assay. A total of 9873 library compounds have been screened since project initiation, of which 160 have shown an inhibition of >70% in a single point assay; these compounds were progressed to determine potency. Compounds were then clustered into different chemical series based on structural similarities. Chemists have synthesised and purchased an additional 650 compounds, building on structure activity relationships (SAR) learned from screening data and increased the potency and selectivity of subsequent compounds. Further development of lead compounds, using in-house DMPK facilities and collaborations with other facilities via MMV, led to the progression of one of our compounds to Late Lead Status in February 2012.
Evaluation of antischistosomal properties of β-dicarbonyl compounds and their heteroanalogs: bridged 1,2,4,5-tetraoxanes, alphaperoxides and β,δ-triketones: tricyclic monoperoxides

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In recent years various peroxidic drugs were studied for their antischistosomal properties. Nonetheless, only very little is known about structural requirements of these compounds to elicit antischistosomal activity. We studied 64 peroxides belonging to three chemical classes, namely bridged 1,2,4,5-tetraoxanes, alphaperoxides or β,δ-triketones on newly transformed schistosomula (NTS) and adult worms of Schistosoma mansoni followed by in vivo efficacy studies. High in vitro activity was observed on NTS with 39 compounds. Seven bridged 1,2,4,5-tetraoxanes showed the highest activity, displaying IC50 values < 1 µM. Active drugs (IC50 <15 µM) presented either phenyl, adamantane or alkyl residues at the methylene bridge. A lower susceptibility was documented on adult schistosomes. On this parasite stage most hit compounds originated from the class of tricyclic monoperoxides (IC50: 7.7-13.4 µM). The highest activity (IC50: 0.3µM) on adult worms was observed for a bridged 1,2,4,5-tetraoxane characterized by an adamantane residue. Supplementation of the medium with haemin or haem, did not significantly alter in vitro activity of drugs against adult worms, indicating that an antischistosomal activation of peroxides is not necessarily triggered by hemin or heme. Two compounds, a tricyclic monoperoxide and a bridged 1,2,4,5-tetraoxane showed high worm burden reductions of 82.8 and 75.4%, respectively in the chronic S. mansoni mouse model whereas only moderate efficacy (WBR:18.9-43.1%) was observed in the juvenile S. mansoni mouse model. Our results might serve as starting point for the preparation and evaluation of related derivatives.
2-arylbenzimidazole derivatives as antileishmanial agents

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Leishmaniasis is listed as a neglected disease by WHO. It is a tropical disease transmitted by sandflies, and caused by protozoan parasites belonging to the genus *Leishmania*.¹ *Leishmania donovani* and *L. infantum* are primarily responsible for visceral leishmaniasis that is fatal if untreated. Existing drugs suffer from poor compliance, toxicity, cost and parasite resistance. New treatments are urgently needed for this disease that affects millions of people mostly in developing countries.

Benzimidazole derivatives are known to possess a wide variety of biological activities, in particular antibacterial and antiviral activities, and as a privileged scaffold benzimidazole structure is a potential starting point to drug discovery. Now, we have found that 2-arylbenzimidazoles show promising activity against Leishmania. Design and synthesis of small library of 2-arylbenzimidazoles² are described, and the activity results of the compounds against L. donovani and structure-activity relationships are reviewed. Best compounds show good inhibition activity at micromolar concentrations.

The role of transporters in protozoan drug targeting and resistance

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The development of genomic, proteomic and transcriptomic platforms has led to a surge in target identification for new anti-infective agents and some of these targets are follow up by high throughput screens, usually with purified recombinant protein targets, identifying and optimising high affinity inhibitors. However, it has recently become clear that the great majority of drugs rely on transporters to cross the plasma membranes of their target cells, rather than simply diffuse through them. In addition, transporters and/or diffusion rates determine the distribution of active compounds within the host and the uptake into host cells, thereby determining many aspects of ADME and toxicity. These issues are of vital importance to the success of lead compounds going into preclinical and clinical development. Conversely, the loss of transporters has often been linked to drug resistance and this too highlights the crucial role of transport proteins in drug action. These issues will be addressed, and paradigms for studying transport-related issues in drug development and resistance will be discussed.
Mefloquine – a novel treatment for Alveolar Echinococcosis

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Alveolar echinococcosis is caused by the metacestode stage of the fox tapeworm Echinococcus multilocularis and causes severe disease in the human liver and occasionally other organs, which is fatal if treatment is unsuccessful. The present chemotherapy is based on benzimidazoles and it has been found to be parasitostatic rather than parasiticidal usually consisting of lifelong uptake of large doses of drug. New treatment options are urgently needed but seldom developed due to the rare occurrence of the disease.[1] A screening of drugs currently used for the treatment of other parasitic diseases was performed. The selected candidate, mefloquine, applied in the prevention and treatment of malaria showed promising results both in vitro and in vivo. The damage to E. multilocularis metacestodes could be accessed in vitro by the release of phosphoglucose isomerase in damaged parasites.[2] An IC$_{50}$ of 42µM could be estimated for mefloquine. Intraperitoneally infected Balb/c mice were treated with mefloquine orally (25mg/Kg) or intraperitoneally for a period of eight weeks. Treatment with mefloquine administered intraperitoneally presented a reduction in total parasite burden similar to that of albendazole oral treatment.[3] Further investigations were made with intraperitoneally infected mice which were orally treated with different doses of mefloquine (25mg/Kg, 50mg/Kg and 100mg/Kg) for a period of 12 weeks. Animals treated orally with the highest dose of mefloquine showed a reduction in parasite burden similar to that of albendazole treated mice. Whether the action of the drug is indeed parasitocidal remains to be investigated. The mechanism of action of mefloquine against the malaria causing parasite Plasmodium falciparum is not yet clear. Mefloquine is believed to prevent the heme polymerization to hemozoin in the host red blood cells, a process that is essential to the survival of the parasite.[4] Such a mechanism would not explain the action of the drug against metacestodes, as these are not intracellular parasites. Affinity chromatography with a mefloquine-bound matrix followed by mass spectrometry of binding proteins resulted in the possible identification of the E. multilocularis ferritin. Ferritins are well conserved intracellular proteins responsible for the storage of iron in a non-toxic form, as well as controlled iron transport. In order to validate ferritin as the possible target from mefloquine experiments with RNAi are currently being performed.

Approximately 40% of the world’s population are exposed to the risks of malaria, resulting in roughly one million deaths annually. Previous successes in attempting to eradicate the disease were only short lived due to increased resistance of the parasite to established drugs such as chloroquine. Computer aided-drug design is an essential part of modern drug discovery, not least in the field of antimalarial chemotherapy. Our research has been to find novel structural chemotypes which are active against the *Plasmodium falciparum* cytochrome bc\(_1\) complex, which has been confirmed as an antimalarial target. Many ligand based virtual screening techniques were used to identify potential lead like structures that are active against *Pf*bc\(_1\), based on a number of known inhibitors. Techniques include Fingerprint Similarity searching, TurboSimilarity searching, Substructure searching, Principle Component Analysis, Bayesian Classification and Decision Tree models. These methods were used to screen the Zinc lead like library of compounds, with hits being scored based on factors such as physicochemical properties, and their similarity to known actives. Filters were applied to remove compounds which contained structural motifs that may produce unwanted biological interactions, with a number of diverse molecules selected. Nineteen compounds were purchased and biological testing performed. Initial testing was against the whole cell growth inhibition assay for the 3D7 strain of the parasite, with five compounds reporting IC\(_{50}\) values ranging from 4.5 µM to 8 µM. Close inspection of these chemotypes has allowed for their incorporation into the next phase of the molecular design loop, to develop more potent inhibitors of the parasite cytochrome bc\(_1\) complex.
Bioluminescent Trypanosoma cruzi murine infection models for monitoring drug efficacy using real-time in vivo imaging

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Various aspects of Chagas disease make small animal models experimentally challenging, including pleiotropism of the causative agent (Trypanosoma cruzi), and relatively low and transient parasitaemia, particularly during the chronic stage of disease. Bioluminescence imaging methods therefore represent a potentially valuable tool for the study of disease pathogenesis and for evaluating novel therapeutic compounds. A transgenic T. cruzi cell line, constitutively expressing the ‘red-shifted’ firefly luciferase variant Ppy RE9, was generated by integration of a construct into the ribosomal DNA locus. Luciferase expression levels were similar in trypomastigote and amastigote forms and were tightly correlated with parasite number. The in vivo limit of detection was <1000 parasites per animal and therefore vastly more sensitive than peripheral blood parasitaemia counts. Bioluminescence imaging allowed infections to be monitored for >250 days. Two models were developed as the basis for an improved strategy to test the efficacy of chemotherapeutic compounds: an acute fulminant disease model in immuno-compromised SCID mice and a chronic disease model in immuno-competent BALB/c mice. As proof of concept we observed significant loss of bioluminescent signal in both models after oral treatment with benznidazole compared with untreated controls.
Experimental induction of miltefosine and paromomycin resistance on intracellular *leishmania* amastigotes

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The limited number of treatment options linked to the increasing rate of treatment failures related to antimony (Sb) resistance is the major challenge in current anti-leishmania therapy. Miltefosine (MIL) has already moved to first-line status while the use potential of paromomycin (PMM) is currently under investigation. Once routinely used in the field, both will also become at risk for resistance. The present laboratory study addressed the dynamics of *in vitro* PMM and MIL resistance induction in *Leishmania donovani* and *L. infantum* isolates/clones, adopting a novel approach of exerting drug pressure only at the intracellular amastigote level. Briefly, intracellular amastigotes surviving the highest drug pressure were collected for promastigote expansion in the absence of drug pressure and used to start a next selection cycle on intracellular amastigotes. Compared to ‘standard’ induction protocols on promastigotes, this procedure adequately mimics the natural situation in the field. Regardless of the used species/strain, PMM-resistant parasites could be selected within 2-3 cycles. There was no cross-resistance with MIL and the Sb-resistance background (if present) remained unchanged. Subsequent cloning of one of the strains revealed the polyclonal nature of the induced strain, with some clones still fully susceptible to PMM, while others were tolerating 10x higher levels of PMM compared to the parent clone. It is difficult to judge whether such a ‘rapid’ selection for MIL would indeed occur in the field, but these laboratory observations at least stress the need for close epidemiological monitoring and the implementation of strong treatment policies to ensure long term efficacy of PMM. Surprisingly, selection for MIL-resistance using the same selection protocol did not lead to resistant *L. donovani* parasites. Although recovery of promastigotes from exposed amastigotes was possible at increasing drug concentrations, no decrease in susceptibility could be observed after 8 successive selection cycles, both as amastigote or promastigote. These results in some way reflect the current field situation where isolates of MIL-treated relapse patients still prove to be susceptible for MIL in *in vitro* drug-susceptibility assays. Relevant to note is that *L. infantum* on the other hand showed increasing levels of MIL-resistance during successive selection cycles, suggesting that MIL-resistance selection mechanisms may at least include some species-specific factors.
In vivo imaging models for rapid screening studies in African trypanosomiasis

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Human African Trypanosomiasis (HAT) remains highly prevalent in sub-saharan Africa. Chemotherapy for HAT is associated with toxicity, complex dosing regimens and emerging drug resistance. Current models for assessing drugs against second (CNS) stage disease are cumbersome and time consuming. This project aims to improve drug screening approaches by using fast and robust \textit{in vivo} imaging systems, and enhance our understanding of how and when trypanosomes cross the blood-brain barrier to become established in the brain. The GVR35 strain of \textit{Trypanosoma brucei} was modified to express luciferase for non-invasive \textit{in vivo} imaging of whole mice using a sensitive camera (IVIS). This allows longitudinal monitoring of parasite distribution and burden in infected mice through the full course of infection. Fluorescently-labelled \textit{T. brucei} were imaged through the thinned skull \textit{in vivo} with two-photon microscopy to localise and examine trypanosomes in the brain in high resolution. Using these imaging modalities we monitored disease progression \textit{in vivo} in a second stage GVR35 model. Trypanosomes were detected in various organs including the brain. Two-photon microscopy showed early invasion of the superficial meninges, and that this site was accessible to first stage trypanocidal drugs, such as diminazine aceturate (Berenil). We showed effects of known trypanocidal drugs on regional parasitaemia, demonstrating the value of our imaging approaches for future drug discovery.
Lead series development & optimisation of a new drug against mycobacterium tuberculosis targeting the electron transport chain


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A major failure of current tuberculosis (TB) therapies is that they predominantly target replicating Mycobacterium tuberculosis (Mtb) but are unable to sterilize slow growing (dormant) Mtb, leading to protracted treatment regimes and the development of drug resistance. Our strategy to overcome these shortcomings is to target the Mtb respiratory chain, specifically NADH:menaquinone oxidoreductase (ndh). This target is essential for the survival of replicating, dormant and drug resistant Mtb, and it is absent in humans. Over the past 2 years we have successfully progressed from target validation to hit identification from a targeted High Throughput Screen of over 11,000 compounds leading to the discovery of novel quinolones with potent (nM) in vitro sterilization activity against replicating and dormant Mtb. These compounds also have some cytochrome bd oxidase activity. The medicinal chemistry strategy is focused around two main templates optimising antituberculosis activity and minimising toxicity and metabolic alerts. The current lead compound MTD403 has an IC50 of 307 nM (replicating TB) and 360 nM (Wayne model). All compounds are synthesisable in 3-6 high yielding steps from inexpensive, commercially available starting materials. These early leads are being progressed through to late lead development with a full medicinal chemistry and biological testing programme in place.
A High-Throughput Screen for Inhibitors of *Leishmania* Inositol Phosphorylceramide Synthase

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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. We have identified the essential kinetoplastid sphingolipid synthase (SLS) as a particularly attractive pharmaceutical target due to the divergence of function compared with the mammalian orthologue. In order to identify new selective inhibitors of the *Leishmania major* SLS, an inositol phosphorylceramide synthase, a yeast based assay suitable for high throughput screening was developed with support from the Tres Cantos Open Lab Foundation. This was subsequently used to screen the GSK 1.8 million compound collection. The resulting 19,669 compounds were subjected to further screening in order to identify those selective for the *L. major* enzyme over the yeast native enzyme. From this, 216 compounds have been selected for further investigation.
Design and Synthesis of N-Myristoyltransferase Inhibitors: A Novel Drug Target for Malaria

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Diseases caused by parasitic infections such as malaria represent a huge global health burden, and new therapies against these diseases are in great demand.\textsuperscript{1} N-Myristoyltransferase (NMT) is an essential eukaryotic enzyme, important for multiple functions \textit{in vivo}.\textsuperscript{2} The implication of NMT in many biological processes indicates for the potential of NMT inhibition to display pleotropic effects, and as such represents an attractive drug target to combat \textit{Plasmodium falciparum} (Pf) infections.

The initial hit compound I displayed excellent selectivity over \textit{Homo sapiens} NMT1 (HsNMT) but had poor ligand efficiency (LE). Utilisation of a lead-hopping approach by modulation of scaffold aromaticity and substitution pattern yielded highly selective and ligand efficient 2.

In order to obtain a late-lead compound for candidate development, further drug design focused on bioisosteric replacement of the potentially labile ester moiety, followed by matched molecular pairs analysis to maximize the lipophilic ligand efficiency (LLE) of the lead series. Further structure-based drug design and optimisation of polar contacts within the active site yielded compound 3, displaying excellent LLE and enzyme affinity against PfNMT.\textsuperscript{3}

Further work shall focus on improvement of the enzyme to cell activity translational as well as the structural basis behind PfNMT/HsNMT selectivity, with the aim of producing a clinical candidate for the treatment of malaria.

High-Content Screening for Neglected Diseases Drug Discovery

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The kinetoplastids *Trypanosoma cruzi* and *Leishmania donovani* pose a major challenge for drug discovery programmes. Their intracellular localisation makes it difficult to set up straightforward high-throughput screening assays. Instead, many primary screening campaigns are conducted using either the free-living insect stage of the parasite or an axenic form of the intracellular stage. The consequence of simplifying the assay in this manner is a significant reduction in physiological relevance of the assay with the potential of high false-positive and false-negative rates compared to the physiologically relevant life-stage. The intracellular life-stages are usually assayed as a follow-up to the primary screen, using a labour-intensive low-throughput microscopy based assay. Here we present the development and use of 384-well imaging-based screening platforms for intracellular *Trypanosoma cruzi* and *Leishmania donovani* which have allowed us to conduct primary small-molecule screens at throughputs of up to 15,000 compounds / week. Parallel screening campaigns using an axenic growth assay were carried out and highlighted the benefits of using the high-content assay as primary platform. The results of a 100,000 compound screening campaign will be presented as well as the lessons learned from our approach.
Screening for inhibitors of pore formation by bacterial cytolysin using a cell based assay

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Listeriolysin (LLO) is a major virulence factor of \textit{Listeria monocytogenes}. The toxin belongs to the family of cholesterol-dependent, pore forming cytolysins (CDCs). Other members of this family include streptolysin, perfringolysin, pneumolysin and alveolysin, all of which are secreted proteins of Gram-positive pathogens. CDCs attack cholesterol rich membranes and form large oligomeric ring-shaped transmembrane pores that mediate cell death. The pores are exceptionally large, assuming an internal diameter of up to 30 nm and comprising around 50 monomer subunits. These large pores allow ions to pass through, and thereby induce an influx of Ca\textsuperscript{2+}. We used this Ca\textsuperscript{2+} influx to monitor pore formation and to establish a screening assay for pore formation inhibitors. Before treating the cells with cytolysins they were loaded with Fluo-4-AM. This Ca\textsuperscript{2+} insensitive acetoxymethyl ester is easily taken up by the cells and hydrolyzed by ubiquitous intracellular esterases, releasing the Ca\textsuperscript{2+} sensitive fluorescent indicator. After addition of a cytolysin, Ca\textsuperscript{2+} influx can be monitored in a fluorescence plate reader. We established an assay with U-937 lymphoma cells in a 384-well format using recombinant LLO as model cytolysin. The test volume was 35 \( \mu \)l. Using this assay in combination with a robotic pipetting device we screened a collection of natural products isolated at the Helmholtz Centre in Braunschweig from Myxobacteria. Active compounds were checked in a second assay that measures the hemolysis of sheep erythrocytes. First results showed that compounds which reduced Ca\textsuperscript{2+} influx in our screening assay with U-937 cells also inhibited hemolysis. We therefore believe that these compounds interfere with pore formation of cytolysins. Such compounds could be leads for a novel kind of anti-infective that is not inhibiting propagation of a pathogen but interfere with its pathogenicity. It should have less selection pressure for resistance development.
Phosphodiesterase inhibitors for the treatment of leishmaniasis and/or trypanosomiasis

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Neglected diseases, have a severe impact on human health and represent a major economic burden on developing countries. An estimated 70,000 new cases of human African trypanosomiasis occur each year, resulting in the death of approximately 40,000 people. Leishmaniasis has a much higher incidence with approximately 2 million new cases per year. The clinical presentation of leishmaniasis can vary from self-healing lesions, affliction of mucosal membranes and infection of the internal organs. The latter manifestation, visceral leishmaniasis, is fatal with 100,000 victims each year. Treatment is with toxic drugs (having serious side effects) and often failing due to emerging drug resistance and new drugs are not in the pipeline. A consortium comprising academia, industrial partners and the Drugs for Neglected Diseases initiative are searching for new compounds to fight these diseases. The project aims to develop solutions to these diseases by targeting parasite specific forms of the enzyme phosphodiesterase (PDE) through the development of specific phosphodiesterases inhibitors that block one or more of the five enzyme subtypes, therefore preventing the inactivation of the intracellular second messengers cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) by the respective PDE subtype(s). At present >120 compounds have been screened in parasite specific assays with varying rates of inhibitory activity.
The identification and characterization of chemical inhibitors of the chaperone–usher pilus assembly

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Uropathogenic *Escherichia coli* (UPEC) are the primary causative agent of urinary tract infections (UTIs) and account for ~8 million physician visits and ~ 100,000 hospital admissions per year in the US alone and represent 20-40% of nosocomial infections in the US and Europe. Type 1 pili are key in the adherence, invasion and establishment of biofilm in the bladder epithelium. Chemical inactivation of type 1 pili represents a promising chemical strategy for treatment and prevention of UPEC – caused urinary tract infections. In this poster we will show that in silico screening followed by biochemical screening can identify inhibitors of biofilm formation in human bladder epithelial cells.
Discovery based metabolomics reveals novel host and parasite mechanisms in *Plasmodium*.

Anubhav Srivastava

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An untargeted discovery based metabolomics study to compare metabolomes of host erythrocytes and *Plasmodium berghei* asexual and sexual stage parasites showed that reticulocytes offer a richer environment than normocytes for parasites to grow and may offer redundancy to some parasite genes in intracellular stages owing to species specific compensation in *Plasmodium* species preferentially invading reticulocytes. Also, a potential new source of energy in exflagellating male gametocytes was identified during the metabolomics study indicating that glycolysis may be supplemented by other metabolic reserves during gamete formation.
P30

Investigating NMT as a Potential Drug target for Leishmaniasis

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N-Myristoyltransferase (NMT), which catalyses the transfer of myristic acid from myristoyl-CoA to the amino group of amino-terminal glycine residues, is required for the membrane targeting and biological activity of many important proteins. Whilst NMT has been validated as a potential therapeutic target in human African trypanosomiasis, there is limited evidence for its validation in leishmaniasis. To this end, a number of known NMT inhibitors were screened against Leishmania major NMT and potent compounds identified. Initial compounds showed poor selectivity and also a large drop in activity when tested against the Leishmania parasite.

In order to address these issues, X-Ray crystallography of key compounds with human and leishmanial NMT was utilised to improve selectivity. Also, a strategy of pKa modulation was used in an attempt to improve cellular activity.

One compound was selected for an in vivo efficacy study in a balb/c mouse model of infection, and showed a reduction in parasite burden.

This work suggests NMT is a potential target for anti-Leishmanial therapy, although compounds with improved selectivity and in vitro activity are required to further validate the target.
Flow cytometry has become a powerful tool for the measurement of parasitemia in peripheral blood of murine models of malaria. In this work, we validate the unspecific nucleic acid dye YOYO-1 to analyze Plasmodium spp. blood stage population distribution in murine models of malaria. To address this point, we stained samples of peripheral blood of mice infected with *P. yoelii*, *P. berghei*, *P. chabaudi*, *P. vinckei* or *P. falciparum* with YOYO-1. The samples were obtained at different time-points during one cycle of each parasite. The fluorescence of infected erythrocytes was measured in bidimensional 530/585 dot plots and the pattern of staining was analyzed. The results obtained indicate that the pattern of staining provides information of the population distribution of parasites as a function of parasite differentiation (measured at 585 nm) and DNA replication (measured at 530 nm). The YOYO-1\textsubscript{530/585} method accurately detected parasite differences in preference for infection of different erythrocyte subpopulations and degree of synchronicity. Moreover, the YOYO-1\textsubscript{530/585} flow cytometry method was able to distinguish specific patterns of staining for parasites treated with a set of standard antimalarials of different mechanism of action. Overall, the YOYO-1\textsubscript{530/585} flow cytometry method might be useful for the early assessment of susceptibility and mechanism of action of new drugs targeting erythrocytic stages of Plasmodium spp. *in vivo*. This technology may contribute to accelerate the development of new antimalarials.
Target identification and mechanism of action studies in folate metabolism in *trypanosoma brucei*

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*Trypanosoma brucei* is the aetiological agent of Sleeping Sickness or human African trypanosomiasis. The highly adaptive parasite is able to evade both the innate and acquired immune response of its human host to render this disease fatal if left untreated. The essential folate pathway is a well-studied area for cancer, malaria and antibacterial diseases, but for such a promising druggable pathway little is understood about folate drug targeting and its possible applicability in drug development in *T. brucei*. In this poster, we describe the use of chemical proteomics to study folate metabolism in *T. brucei*. Utilizing chemical proteomics, the immobilisation of antifolate inhibitor/drug molecules onto beads, allows for the capturing of a subproteome. The proteins captured are identified and quantified by mass spectrometry, aided by isobaric tagging for relative and absolute quantification (iTRAQ). This novel technique, when used in conjunction with competition experiments, can identify the binding of drug molecules in cell lysates. This will allow us to study which protein(s) in the folateome are inhibited by anti-folate compounds. This work could be used to gain a better understanding of the mode of action of current antifolates and help in the design of new agents.
Microcalorimetry, a new tool to monitor drug action on African trypanosomes

Tanja Wenzler* (1,2), Marcel Kaiser (1,2), Donald A. Patrick (3), Michael Zhuo Wang (4), Sihyung Yang (4), Richard R. Tidwell (3), Reto Brun (1,2)

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Objectives:
African sleeping sickness, also known as human African trypanosomiasis (HAT) is a lethal disease caused by two subspecies of Trypanosoma brucei. Chemotherapy is the only possibility for a cure. However, current drugs in use have strong liabilities. Better drugs are needed especially for the 2nd stage, which involves an infection of the central nervous system.

Microcalorimetry is a novel method in biology to determine the kinetics of drug action on African trypanosomes. It is a nonspecific technique that allows direct measurements of heat generated by biological processes in living cells. As the heat flow signals correlate with the number of viable cells, we are able to display growth of the parasite culture on a real-time basis. The continuous measurement enables us also to monitor effects of drugs such as the onset of drug action, the degree of inhibition and the time to kill of the parasite population at various drug concentrations. So far, this was not possible as accurately with conventional drug assays as inhibition could only be assessed at single time points.

We will visualize the potential of microcalorimetry in the field of drug discovery by monitoring inhibition over time of drugs in use for African sleeping sickness. Additionally, we will monitor the time of drug action of the 5-nitroimidazole Fexinidazole, the oxaborole SCYX-7158 and the two novel diamidines DB829/CPD0801 and 28DAP010/CPD0905. These four molecules are currently the best compounds in the drug discovery pipeline for 2nd stage African sleeping sickness.

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Novel diamidines for 2nd stage African sleeping sickness: new preclinical drug candidates

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Objectives:
African sleeping sickness, also known as Human African trypanosomiasis, is a neglected tropical disease threatening millions of people living in sub-Saharan Africa. The disease is transmitted by infected tsetse flies and caused by two subspecies of Trypanosoma brucei. The availability of effective drugs is crucial, as African sleeping sickness is a fatal disease and chemotherapy is the only option for cure. However, most currently available drugs are old, must be given parenterally and have severe adverse effects. Better drugs are needed especially for the 2nd stage of the disease, which involves cerebral infections. The number of cases has declined during the last decade due to various interventions to control the disease. If these interventions stop, African sleeping sickness will reemerge. Elimination of the disease would now be feasible, but for this, also better drugs are needed.

In collaboration with the Consortium for Parasitic Drug Development (CPDD), we were working on the discovery of novel diamidines for sleeping sickness. This consortium identified an oral drug (pafuramidine) which passed Phase III clinical trials for 1st stage disease but unexpected renal toxicity stopped further development. However, during the backup program, we identified compounds with superior activity especially regarding the 2nd stage of the disease. Interestingly, the prodrug approach was not absolutely essential to achieve blood brain barrier passage, and thus therapeutic drug levels in the brain. Two novel diamidines that have not masked their cationic amidine moiety (CPD-0801/DB829 and CPD-0905/28DAP010), were identified as 2nd stage active compounds. Both compounds attained cure in mice with cerebral infections (GVR35 mouse model) by a short treatment of only 5 days.

We will present in vitro and in vivo data (mouse and monkey) of diamidine drug candidates for the treatment of African sleeping sickness.

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Chemical tools for probing N-myristoyltransferase as a drug target in protozoan parasites

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Protein N-myristoylation is the attachment of a 14-carbon fatty acid, myristate, onto the N-terminal residue of specific proteins. This co- and post-translational modification is catalysed by myristoyl CoA:protein N-myristoyltransferase (NMT), an essential enzyme in eukaryotes. N-Myristoylated proteins have diverse roles, but typically localise to cellular membranes, where many are thought to be involved in signalling or trafficking processes. NMT is a potential therapeutic target in diseases caused by parasitic protozoa, such as malaria, leishmaniasis and African sleeping sickness (trypanosomiasis). N-Myristoylation is difficult to study by conventional biochemical methods. Recently, chemical proteomics has emerged as a powerful technology for probing protein lipidation. In our approach, tagged myristic acid substrate analogues are incorporated metabolically into N-myristoylated proteins. Tagged proteins are then selectively ‘captured’ after cell lysis via a bioorthogonal ligation reaction using multifunctional probes, and analysed by a combination of gel-based methods and mass spectrometry. We are applying this technique to analyse myristoylated proteins in several parasitic organisms, including trypanosomes, Leishmania and Plasmodium species, where NMT is a promising drug target. By profiling lipidation in the presence of putative NMT inhibitors we can monitor efficacy and assess on-target effects of these compounds in live parasites. Correlating enzyme potency, efficacy against multiple parasite life-stages and on-target activity will provide strong evidence for NMT as a drug target in these organisms.
Searching for antitrypanosomal and antiplasmodial natural products from plants and fungi

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Introduction: For the last six years we have cooperated in screening plant and fungal extracts against protozoan parasites and identifying their active compounds.

Aim: With our work we contribute to the discovery of new leads for the development of drugs to treat these neglected tropical diseases, and to gain a better understanding of antiprotozoal natural products.

Methods: Promising extracts were analysed by HPLC based-activity profiling to identify active constituents which were subsequently isolated and tested against Trypanosoma brucei rhodesiense and Plasmodium falciparum¹.

Results: So far libraries containing more than 2500 plant and fungal extracts have been screened. Some of these extracts were traditionally used in South African, in Renaissance European, or in Iranian folk medicine to treat malaria.

The compounds with highest in vitro activity against P. falciparum were the alkaloids carpaine (IC₅₀ 0.8 μM) from Carica papaya, berberine (IC₅₀ 0.1 μM) from Coptis chinensis, and the triterpenoid perovskone B (IC₅₀ 0.18 μM) from Salvia hydrangea². In vivo tests with carpaine in the P. berghei model, however, showed no decrease in parasitaemia. Selected compounds with a sesquiterpene lactone or a miltirone-type diterpene scaffold showed high in vitro activity against T. b. rhodesiense (IC₅₀ 0.3 – 0.8 μM). Cynaropicrin (IC₅₀ 0.3 μM) was tested in vivo and reduced the parasitaemia in the acute sleeping sickness mouse model³.

Conclusion: Our lead discovery project against protozoan parasites resulted so far in 55 natural products with in vitro activity. Of these, 8 were new compounds. Most compounds showed moderate activity in the 1-10 μM range, with little selectivity. Two compounds were tested in vivo, and one of them was able to reduce parasitaemia. Cynaropicrin is the first plant derived natural product with in vivo activity against African sleeping sickness.

INVITED SPEAKERS

**Jeremy Burrows, Medicines for Malaria Venture (MMV), Switzerland**

Jeremy obtained a MA in chemistry and a D.Phil. in synthetic organic chemistry at Oxford University (1989-1996).

In 1997 he joined ZENECA/ AstraZeneca as a medicinal chemist working in Infection, Cardiovascular and Inflammation research in the UK. He led Lead Generation chemistry delivering new lead series for inflammatory diseases. In 2005 he was seconded to Sweden in CNS/Pain where he led a section focused on Alzheimer’s disease.

In 2008, he moved to Geneva to join MMV where he is Head of Discovery overseeing a growing portfolio of enabling technology, screening, Hit-to-lead and Lead Optimisation projects both with academic and industrial partners.

He has so far contributed to the delivery of nine candidate drugs and has published over 70 papers, patent applications and book chapters.

**Robert Don, Drugs for Neglected Diseases initiative (DNDi), Switzerland**

Dr. Robert Don holds overall responsibility for DNDi’s discovery research and preclinical development.

Prior to joining DNDi, he was Senior Vice President for Research and Clinical Development at the Australian based biotechnology company, Progen Pharmaceuticals for 10 years. In this position he was responsible the company’s research program to develop new therapeutics for treatment of cancer and guided the development novel anti-cancer agents from basic research to clinical trials in several countries including USA, United Kingdom, Taiwan and Australia.

He was awarded his PhD by the University of Queensland in Australia and completed postdoctoral fellowships at the Medical University in Geneva and the New South Wales Cancer Council in Australia followed by a faculty position at the University of Queensland.
Ronald Kaminsky, Novartis KH, Switzerland

Dr. Ronald Kaminsky works at Novartis Animal Health in Switzerland and heads parasitology research for the global business. He previously worked in malaria research at the University of Göttingen in Germany, then on cultivation techniques for African trypanosomes in the USA (University of Amherst) and for 6 years in Africa (ILRAD) before he moved to the Swiss Tropical Institute (now STPH) in Basel where he worked on in vitro screening technologies for various parasites and on mechanisms of drug resistance in trypanosomes. He received two Animal Welfare Awards for the innovation of in vitro techniques. Dr. Kaminsky has published approximately 90 scientific papers and is currently guest lecturer at the University in Bern, Switzerland. In his time with Novartis Animal Health, Dr. Kaminsky has been deeply involved in the discovery and development of the anthelmintic monepantel (Zolvix).

Colin Fishwick, University of Leeds, UK

Colin Fishwick joined the staff at University of Leeds, School of Chemistry in 1985, being appointed to a Chair in Medicinal Chemistry in 2009. Other roles include; Honorary Visiting Professor, University of Bradford, Institute for Cancer Therapeutics; Director of the Medicinal Chemistry and Chemical Biology technology group of the University of Leeds Biomedical and Health Research Centre (2008 – present); Consultant to UK Wellcome Trust Seeding Drug Discovery Programme (2006 – present); Consultant to AstraZeneca (India) on aspects of anti-TB drug discovery (2005 – 2008); Full Member, Astbury Centre for Structural Molecular Biology, University of Leeds, UK; Member of Executive Committee, Multidisciplinary Centre for Cardiovascular Research, University of Leeds. Additionally.

His research interests focus on the application of structure-based molecular design to the identification of new therapeutic drug leads, particularly for anti-infective agents. He has authored more than 100 publications.

Ian Gilbert, University of Dundee, UK

I obtained my PhD in synthetic organic chemistry at the University of Cambridge with Andrew Holmes. Following a post-doctoral position with Parke-Davis, I spent a year lecturing chemistry at the University of Zambia in Lusaka. On returning to the UK, I carried out post-doctoral research with Jim Staunton at University of Cambridge in chemical biology. I then obtained a lectureship at the Welsh School of Pharmacy in 1994, when I started a medicinal chemistry research group, with a major focus on neglected diseases. I moved to the University of Dundee in 2005, where I am Head of Chemistry in the Drug Discovery Unit.
Paul O'Neill, University of Liverpool, UK

Paul M. O’Neill carried out his degree in Chemistry and Pharmacology at the University of Liverpool in 1990 and subsequently carried out a Wellcome Trust funded Ph.D degree under the guidance of Dr. Richard C. Storr and Professor B. Kevin Park. In 1997 he carried out postdoctoral research with Professor Gary H. Posner at the Johns Hopkins University, USA before returning to Liverpool in 1998 where he was appointed to a joint lectureship between the Departments of Chemistry and Pharmacology. He was promoted to Senior Lecturer in 2003, Reader in 2005 and Professor of Medicinal Chemistry in 2006. Professor O’Neill has published over 100 papers and twelve patents. His research has led to a drug candidate (Isoquine) entering clinical trials in 2008 and his group have also recently candidate selected two additional antimalarials selected for full preclinical testing on route to Phase 1 clinical trials in humans. In 2011 he was joint recipient of the RSC BMCS Malcolm Campbell Memorial Award.

Research: Paul works in several areas of synthetic organic chemistry and pharmacology with a strong emphasis on drug-design, chemical biology and medicinal chemistry of antimalarial and anticancer drugs.

Edward Tate, Imperial College, London, UK

Dr. Ed Tate is currently Reader in Chemical Biology at Imperial College. Following a PhD in organic chemistry at the University of Cambridge, he worked as an 1851 Research Fellow at Ecole Polytechnique and Institut Pasteur in Paris. On his return to the UK he started a research program at Imperial College developing multidisciplinary approaches to understanding protein modification, and in 2006 was awarded the BBSRC David Phillips Research Fellowship. His subsequent achievements were recognised by appointment to Senior Lecturer in 2010, and to Reader in 2012. Ed leads a multidisciplinary group of more than 20 researchers aiming to understand the functional chemical biology of protein posttranslational modification (PTM) and validate or de-risk potential drug targets within PTM pathways through research projects driven by both biological hypotheses and chemical technology development. His group has contributed to the field of protein modification in multiple biological contexts, from protozoan parasites to mammalian cells, and in bacteria. He is the Chemical Genetics Research Theme Leader in the British Heart Foundation Centre of Research Excellence, on the management board of the EU COST Action CM1004 “Synthetic Probes for Chemical Proteomics”, an active member of the Institute of Chemical Biology at Imperial College, and director of Imperial College’s MRes course in Drug Discovery and Development, designed to train the next generation of multidisciplinary medicinal chemists and biologists.
David Horn, London School of Hygiene & Tropical Medicine, UK

David Horn’s group investigates antigenic variation and drug resistance in African trypanosomes, with a long-term goal of aiding the development of improved chemotherapies. Recent developments include the application of RNA-interference libraries to genome-scale phenotyping, the elucidation of mechanisms of gene silencing and the elucidation of mechanisms of drug action and resistance. Dr. Horn received his Ph.D. from University College London in 1993 and then held a postdoc at Rockefeller University. He joined the London School in 1997 and was a Wellcome Trust Research Career Development Fellow.

Paul W. Smith, Novartis Institute for Tropical Diseases, Singapore

Paul joined NITD in September 2010 having previously worked for over 20 years in small molecule drug discovery at GSK in the UK. Prior to his career in the pharmaceutical industry he obtained his D.Phil in organic chemistry at Oxford University (1986) and spent 2 years at Columbia University as an SERC-NATO post-doctoral fellow. He has drug discovery experience in many disease areas (anti-bacterial, anti-viral, sexual dysfunction, cardiovascular, CNS) and led multi-disciplinary research projects that have successfully advanced molecules into early development.

Iñigo Angulo-Barturen, GSK Tres Cantos, Spain

Dr. Iñigo Angulo-Barturen is PhD in Biology (Universidad Complutense de Madrid, Spain) and Specialist in Clinical Immunology (residency at Hospital Universitario San Carlos, Madrid, Spain). After 2 years as postdoctoral fellow at the Centro de Biología Molecular (Universidad Autónoma de Madrid, Spain) he joined GlaxoWellcome. During the last 9 years, Dr. Angulo-Barturen has been Head of the Therapeutic Efficacy Unit (GlaxoSmithKline, Diseases of the Developing World- DDW, Tres Cantos), which is in charge of the development of new animal models and the evaluation of the efficacy of new drugs against malaria and tuberculosis. During his career Dr. Angulo-Barturen has gained experience in basic immunology, immunology of cancer, fungal diseases, malaria and tuberculosis. He has also led drug discovery projects of malaria (pyridones) and tuberculosis (pleuromutilins, mycobacterial gyrase inhibitors).
Clifton E. Barry, 3rd, NIAID, National Institutes of Health (NIH), USA

Dr. Clifton E. Barry received his Ph.D. degree in organic and bioorganic chemistry in 1989 from Cornell University, and then was a postdoctoral fellow in the department of chemistry at the Johns Hopkins University. In 1991, he joined the NIH intramural research program as an investigator at the Rocky Mountain Laboratories, studying DNA-protein interactions during chlamydial development. In 1993 he established the Mycobacterial Research Unit which initially studied mycolic acid biosynthesis in *Mycobacterium tuberculosis*. In 1998, he was tenured as a Senior Investigator of the Tuberculosis Research Section (TRS) and relocated his laboratory to the Bethesda area.

The TRS is a multidisciplinary group of research scientists comprised of biologists, chemists and clinical researchers who share a common interest in TB. TRS projects focus on understanding the scientific issues that facilitate the development of drugs that will make a genuine difference in the outcome for TB patients globally. TRS scientists are highly interactive worldwide in this endeavor and as a result of our outstanding collaborations TRS is the most widely cited TB research group in the world. In addition to current TRS laboratories in Bethesda TRS works closely with the International Tuberculosis Research Center located in Masan, South Korea; with Chinese colleagues at the Henan Provincial Chest Hospital in Zhengzhou, China; and with colleagues at Stellenbosch University and the University of Cape Town in South Africa.

Stephen Wring, SCYNEXIS Inc., USA

Dr. Steve Wring is the Director of Drug Metabolism and Pharmacokinetics for SCYNEXIS inc. with responsibility for advancing new drug-like molecules from early hits through lead optimization to candidate selection for human and animal health indications. Currently Steve is focusing on Neglected Disease research projects including the human African trypanosomiasis and visceral leishmaniasis programs sponsored by the Drugs for Neglected Diseases initiative (DNDi). Prior to joining SCYNEXIS in 2007, Steve was Principal Investigator of DMPK at Trimeris Inc., where he led the DMPK optimization of synthetic peptide and small molecule fusion inhibitors of HIV infection. Before joining Trimeris in 2002, Dr. Wring gained 11 years discovery and development experience with GSK and predecessor companies based in both the UK and USA. Steve received his PhD in Analytical Chemistry from the University of the West of England (Bristol, England) and has professional qualifications in Clinical Biochemistry and an MSc in Instrumental Analysis.
Matthew Todd, School of Chemistry, The University of Sydney

Mat Todd was born in Manchester, England. He obtained his PhD in organic chemistry from Cambridge University in 1999, was a Wellcome Trust postdoc at The University of California, Berkeley, a college fellow back at Cambridge University, a lecturer at Queen Mary, University of London and since 2005 has been at the School of Chemistry, The University of Sydney where he is currently Senior Lecturer.

His research interests include the development of new ways to make molecules, particularly how to make chiral molecules with new catalysts. He is also interested in making metal complexes that do unusual things when they meet biological molecules or metal ions. His lab motto is “To make the right molecule in the right place at the right time”, and his students are currently trying to work out what this means.

He has a significant interest in open science, and how it may be used to accelerate research, with particular emphasis on open source discovery of drugs and catalysts. He is Chair of The Synaptic Leap, a nonprofit dedicated to open biomedical research. In 2011 he was awarded a NSW Scientist of the Year award in the Emerging Research category for his work in open science. He is on the Editorial Boards of PLoS One, Chemistry Central Journal and ChemistryOpen.

Wim Parys, Janssen Infectious Diseases (J&J), Belgium

Wim Parys obtained a MD degree from the Katholieke Universiteit Leuven, Belgium. He was in private practice for 9 years before joining the Janssen Research Foundation in Beerse, Belgium where he held several R&D positions and developed Galantamine for Alzheimer’s Disease (marketed as Reminyl™ / Razadyne™ by Johnson&Johnson).

In 2000 he became the Head of Development at the biotech company Tibotec and relocated to the US to establish Tibotec Inc., the US based subsidiary. Under his tenure, Tibotec (then acquired by J&J) developed and launched Prezista™ and Intelex™two innovative HIV drugs. In 2011, Edurant was approved for HIV and Incivo for Hepatitis. Currently he leads the development of other medicines for HIV, Hepatitis C and TB.

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Cipla Limited is an Indian pharmaceutical company, best-known outside its home country for manufacturing low-cost anti-AIDS drugs for HIV-positive patients in developing countries. It has played a similarly prominent role in expanding access to drugs to fight influenza, respiratory disease and cancer. Founded by nationalist Indian scientist Khwaja Abdul Hamied as The Chemical, Industrial & Pharmaceutical Laboratories in 1935, Cipla makes drugs to treat cardiovascular disease, arthritis, diabetes, weight control, depression and many other health conditions, and its products are distributed in virtually every country of the world.

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Biological & Medicinal Chemistry Sector

As part of the Royal Society of Chemistry, Industry & Technology Division, the Biological and Medicinal Chemistry Section (BMCS) aims to further the interests of all members of the RSC, both industrial and academic, involved in the pursuit and understanding of biologically active molecules. It also acts to promote public awareness of the crucial role played by chemistry in the modern industrial environment.

The BMCS seeks to achieve these objectives by the organisation of scientific meetings and symposia, support for educational activities in the UK, and advising the RSC on policies that directly affect the BMCS. The BMCS currently has more than 1200 members and welcomes the participation of anyone who share our aims.

The British Society for Parasitology

The British Society for Parasitology celebrated its 50th Anniversary in 2012. The Society is the central networking and meeting point for many professional and amateur parasitologists throughout the UK and across the world and includes more than 1500 members, over a third of whom reside outside the UK. It promotes and supports the academic study of Parasitology, from experimental to theoretical approaches as applied to infection biology and disease research or from ecological to medical & veterinary studies in global health and international aid.
London School of Hygiene & Tropical Medicine

The London School of Hygiene & Tropical Medicine is a world-leading centre for research and postgraduate education in public and global health, with 4000 students and more than 1300 staff working in over 100 countries. The School is one of the highest-rated research institutions in the UK, and was recently cited as one of the world’s top universities for collaborative research.

The School’s multidisciplinary expertise includes clinicians, epidemiologists, statisticians, social scientists, molecular biologists and immunologists. We work with our partners worldwide to support the development of teaching and research capacity, and our alumni work in more than 180 countries.

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The RSC is the largest organisation in Europe for advancing the chemical sciences. Supported by a worldwide network of members and an international publishing business, our activities span education, conferences, science policy and the promotion of chemistry to the public. The current membership is about 47,500.