EPSRC Centre for Doctoral Training (CDT) in Molecular Sciences for Medicine (MoSMed)

Identification of new molecular targets for Neglected Tropical Diseases (NTDs)

Durham University, Department of Chemistry

Supervisory Team	• **Lead Supervisor: Prof. Steven Cobb (Durham University)** • **Secondary Supervisor: Prof. Wyatt Yue (Newcastle University)** • **Collaborators: Prof. Ariel Silber (USP, Brazil) and Assis. Prof. Kalesh Karunakaran, Teesside University/ NHC, UK)**

Project overview/context

American Trypanosoma and Leishmania are the causative agents of two of 17 Neglected Tropical Diseases – a collection of infections characterized by a lack of control measures and major impacts on developing nations. These insect vector-borne protozoa are the cause of Chagas Disease and leishmaniasis respectively. Despite recent efforts in areas such as vector control, cases of Chagas Disease, caused by *Trypanosoma cruzi* and leishmaniasis, caused by *Leishmania spp*, remain high - up to 7M infected and 1.7M new cases per year respectively. There is an urgent need to identify and validate new drug targets and this is what the current project aims to do.

Research Project

As part of an ongoing program of work within the Cobb group to identify novel scaffolds with anti-parasitic activity, screening of a library of 72 fluorinated compounds identified 4-(benzene sulfonyl)-2,3,5,6-tetrafluoropyridine (**1**) (**Fig. 1A**) with promising activity against both *L. mexicana* (EC_{50} against promastigotes = 1.33 μM, EC_{50} against axenic amastigotes = 0.433 μM), and *T. cruzi* species (EC_{50} against epimastigotes = 1.55 μM). Subsequently, a second-generation library (25 compounds) was designed and synthesised from compound **1** and evaluation of this series identified further molecules with activity against both *L. mexicana* (Durham) and *T. cruzi* species (Prof. A. Silber, USP, Brazil). Initial efforts to carry out protein target identification in *Leishmania* (with **1**) using mass-spectrometry-based proteomics (**Fig. 1B** and **Fig. 1C**) have been undertaken in collaboration with the Karunakaran lab (Teesside, National Horizon Centre, UK). This project will continue on from these preliminary studies with the core aims of the work focused on – a) identifying the mode of action and biological targets for **1** (and related molecules identified), and b) designing and synthesising next-generation analogs of **1** with enhanced biological activity.

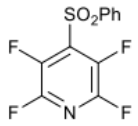

Fig. 1A. Chemical structure of compound **1**.

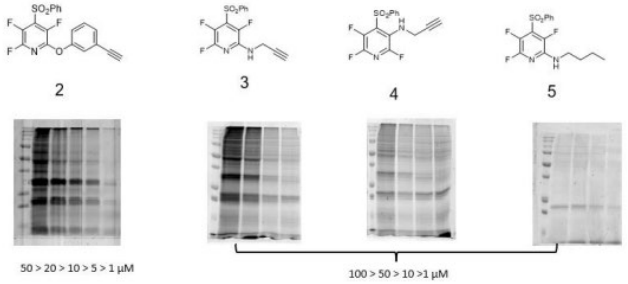

Fig 1B. Structures of selected compounds and their in-gel fluorescence scanning images

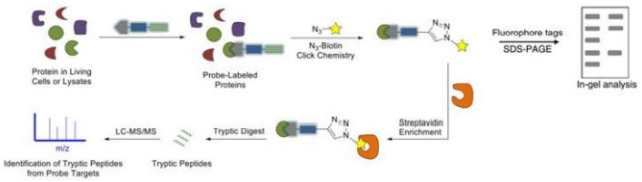

Fig. 1C. Workflow of the proteomics experiments to identify target proteins

Work package 1 (WP1) - Biological screening and mass-spectrometry-based proteomics
We will use mass-spectrometry-based proteomics to identify a series of proteins that the fluorinated compounds with anti-parasitic activity target in both *Leishmania* and *T. cruzi*. This approach will also be used to analyze protein production levels in the presence and absence of inhibitor. This will allow us to begin to build up a picture of possible molecular targets for the fluorinated compounds. Work in this WP will be supported via our collaborations with the Silber lab (USP, Brazil) and the Karunakaran lab (Teesside, UK).

WP2 – Mode of action studies
We will carry out mode of action studies in both *Leishmania* and *T. cruzi*, initially using hit compounds identified from the previous screen of 97 molecules. Preliminary work in this area has been carried out for **1** in *T. cruzi* (collaboration with the Silber lab at USP, Brazil). For example, with the purpose of investigating the ability of 1 to trigger Programmed Cell Death (PCD) in *T. cruzi* epimastigotes, the typical morphological, cellular, and biochemical PCD hallmarks such as variations in Ca2+ concentrations, mitochondrial inner membrane potential (ΔΨm), ATP level imbalance, exposure of phosphatidylserine residues from the inner to the outer leaflet of the plasma membrane and/or membrane permeabilization were all evaluated. Similar mode of action studies will be carried out with relevant compounds in *Leishmania* (in Durham and Teesside).

WP3 – Design and synthesis of a 3rd generation library of fluorinated molecules
We will use the activity data already gathered to design and prepare a 3rd generation library of molecules (upto 100) for biological evaluation (in WP1 and WP2). As the library uses a pentafluoropyridine (PFP) core scaffold it is highly amenable to modification and libraries with diverse chemical space profiles can be easily assembled (e.g. for applications of PFP see Cobb et al, JOC 2020, OBC 2022, OBC 2019). The fluorinated core also affords an opportunity to apply ^{19}F NMR ligand screening approaches in WP4.

WP4 – Characterization and biophysical analysis of target proteins
While an ambitious aim of a 4-year PhD project we hope to identify, using the data gathered in WP1 and WP2 specific protein targets that can be further investigated and validated using a range of biophysical and structural analysis techniques. Work in this area will be supported via a collaboration with the Yue lab at Newcastle and the Silber lab at USP.

Training & Skills

This project establishes a new research collaboration between the Cobb and Yue research groups. Cobb and Yue are researchers that have scientific skills that align with the aims of the project but that also complement one another e.g. Cobb – chemical biology/ organic synthesis/ fluorine chemistry, Yue – biophysics/ structural biology. The recruited PhD student will be based primarily in the Cobb group but they will carry out work within the Yue lab also, giving them a broad training experience in terms of scientific skills. The project benefits both in terms of enhanced scientific skills capacity and external training opportunities for the PhD student from the inclusion of two additional academic collaborators. Professor Ariel Silber (University of São Paulo (USP), Brazil) is an expert in *T. cruzi* biology and Assistant Professor Kalesh Karunakaran, (Teesside University/ NHC, UK) is an expert in chemical probe design and mass-spectrometry-based proteomics. Both academics have established collaborations with the Cobb group and will offer research placement opportunities to the recruited PhD.

Further Information
For any enquiries regarding this project, please contact
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How to Apply

To apply for this project please visit the Durham University application portal to be found at: Home · Application Portal (microsoftcrmportals.com)

Please select the course 'PhD in Molecular Sciences for Medicine (EPSRC CDT)', which is registered in the Chemistry Department and indicate the reference **mos23_05 i**n the 'Field of Study' section of the application form. Please note that there is no need to submit a Research Proposal with your application, however we do require a Covering Letter, CV, academic transcripts, the contact details of two referees and proof of English language proficiency if relevant.

Within the MoSMed CDT we are committed to building a diverse community based on excellence and commitment. To that end in our recruitment of Doctoral Researchers we welcome applications from outstanding candidates of all backgrounds regardless of ethnicity, disability, gender identity, sexual orientation and will consider all applications equally based on merit.

Should you have any queries regarding the application process at Durham University please contact the

Newcastle University · Durham University · UKRI Engineering and Physical Sciences Research Council

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