

## The development of therapeutics for the treatment of Chagas disease and Leishmaniasis

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### Project Background Information/Introduction:

Neglected tropical diseases (NTDs) are a diverse group of infectious diseases that affect more than one billion people in 149 countries with millions of others at risk. The official list of NTDs prepared by the World Health Organization (WHO) currently consists of 17 infectious diseases that prevail in tropical and subtropical conditions. These diseases predominantly affect populations living in poverty where they live without adequate sanitation, nutrition, and healthcare and in close contact with infectious vectors of disease-causing agents.<sup>1</sup> NTDs impair physical and cognitive development, productive capacity as well as causing a substantial number of adverse outcomes of pregnancy, morbidity and mortality. The economic repercussions of these diseases can be as devastated as their health effects.<sup>2</sup>

Leishmaniasis is one of the vector-borne parasitic diseases categorized under NTDs that is endemic in large areas of tropics, subtropics, and the Mediterranean basin around the world. It presents a significant global health problem in approximately 98 countries, where there are a total of 350 million people at risk and 12 million cases of infections.<sup>3</sup> Human infections are caused by more than 20 species of obligate protozoan parasites from genus *Leishmania* (Trypanosomatida: Trypanosomatidae).<sup>4</sup> Leishmaniasis is transmitted through more than 30 species of infected female sandflies, (in old world-Phlebotomine and in new world-Leutzomia) whose hosts are animals such as canids, rodents, marsupials, hyraxes or human beings.<sup>5</sup>

Leishmaniasis has been classified into three main clinical forms namely: Visceral leishmaniasis (VL), Cutaneous Leishmaniasis (CL), Mucocutaneous leishmaniasis (MCL) which differ in immunopathologies and degree of morbidity and mortality. Changes of natural and man-made environments make leishmaniasis an emerging public health concern.<sup>5</sup> Current front-line treatments suffer from issues including high cost, teratogenicity, drug resistance, the requirement of non-oral administrations and prolonged treatment courses. Hence, there is a need for more effective, safer, and more convenient therapeutics. To reduce both the time and financial costs increased efforts are now being placed on drug repurposing.<sup>6</sup>

### **Research Aim/Objectives/Questions/Hypotheses:**

It has been shown that some FDA approved anti-fungal drug molecules can be repurposed to inhibit the growth of Leishmania species. The Cobb group has identified the anti-leishmanial activity of Pyrithione against axenic amastigotes and intracellular amastigotes of *L. mexicana* and the DNDi has published data for the activity of Ciclopirox Olamine against axenic amastigotes and intracellular amastigotes of *L. donovani*. This project will involve the synthesis of a modified compound library of Pyrithione derivatives and the subsequent determination of their anti-leishmanial activity, cytotoxicity, and mode of action.

### **Data/Methods/Analysis:**

Fundamental compounds in this part of the project were selected depending on previous work related to their anti-parasitic activity. Pyrithione was modified to make four novel compounds and their anti-parasitic activity was determined against *L. mexicana* parasites. Due to the light sensitivity of the product, reactions were carried out in the dark. Pyrithione (1 equiv.) was dissolved in DCM (1 mL) and the temperature was lowered to 0°C. Pyridine (1.31 equiv.) and the required acyl chloride (3.14 equiv.) were added to the reaction flask which was stirred at 0°C for 2 h. The product was purified by column chromatography (silica gel, hexane: EtOAc, 1:1).

As for Biological assays, Compounds to be tested were added to 96-well plates containing amastigotes  $1 \times 10^6$  cells/ml. After 44 hours incubation, the cell viability reagent AlamarBlue® was added to each well and incubated for 4 hours at the appropriate temperature for the parasites; 26 °C for promastigotes and 32 °C for amastigotes. AlamarBlue® assay uses the reducing activity of living cells to quantitatively determine cell viability via a fluorescent or colorimetric detection technique. The cell viability can then be calculated by measuring the intensity of fluorescence emitted from cells and GraphPad Prism software was used to calculate EC50 values and 95% Confidence interval (CI) level for each compound.

### **Contributions to the SDGs:**

Access to medicines for the treatment of leishmaniasis is problematic in the poverty-stricken countries that have the highest burden of cases. But there has been limited number of funding available to support the research and development of new antileishmanial treatments. Because an average income of a patient in an endemic area is less than 2 dollars per day this is not an economically attractive disease for new drug development for pharmaceutical companies.

Developing a drug from the start is a lengthy and expensive process. But a drug repurposing strategy guided by established target product profiles can be a fast-track approach. Hence, this project will contribute to increasing the public health which directly links to the SDG Goal 3; Health and well-being.

### Lessons learnt and key takes/reflections:

Pyrithione had the lowest  $EC_{50}$  value (0.04211  $\mu\text{M}$ ) against axenic amastigotes. And all the modified compounds had slightly decreased yet promising  $EC_{50}$  values compared to Pyrithione. As the next step, infection assays and cytotoxicity assays (RAW 264.7) were carried out. Compared to the  $EC_{50}$  recorded for axenic amastigotes and promastigotes, these compounds can categorize as non-toxic to mammalian/host cells. Pyrithione (4.418  $\mu\text{M}$ ) and 2-thioxopyridin-1(2H)-yl acetate (5.003  $\mu\text{M}$ ) retained their activity in the infection assay. Being able to retain their anti-parasitic activity and drug-like properties even after modifications, is a positive sign to carrying out further studies of these molecules towards drug development.

### Project Information:

- Supervisors/partners
  - Associate Professor Steven Cobb (Principal Supervisor)
  - Dr. Paul Denny
  - Professor Ariel Silber (USP, Brazil)
- Project Duration: 3 Years (01/05/2019- 31/04/2022)
- Project Resources (funded by): Durham University
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### References:

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