University of Dundee

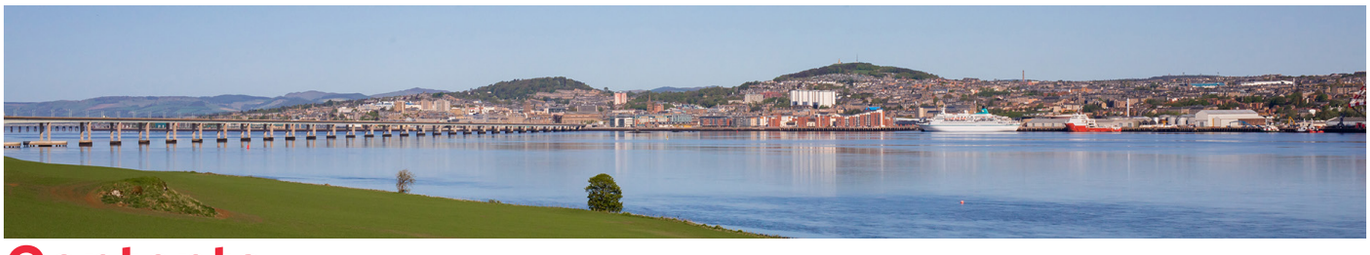
wellcome centre anti-infectives research

Setting our sights on **infectious diseases**

Innovating drug discovery and development
12–15 May 2019, University of Dundee, UK

University of Dundee

wellcome centre anti-infectives research



Contents

Drug Discovery Pathways

4. Discovery of novel falcipain 2 inhibitors by *in silic*o guided drug repositioning

Lucas Nicolás Alberca[1,2], S.R. Chuguransky[1,2], C.L. Álvarez[3], E. Salas-Surduy[4]. A. Talevi[1,2]
1.Laboratorio de Investigación y Desarrollo de Bioactivos (LIDeB), Facultad de Ciencias Exactas, Universidad Nacional de La Plata; 2. Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET); 3. Instituto de Química y Fisicoquímica Biológicas, Facultad de Farmacia y Bioquímica, Universidad Nacional de Buenos Aires; 4.Universidad Nacional de San Martin (UNSAM)

Malaria is a life-threatening condition that continues being one of global leading causes of death worldwide, being the main cause of death globally in the 5-14 year-old population. The disease is caused by parasites from the *Plasmodium* genre. The emergence of *Plasmodium falciparum* resistant strains with reduced sensitivity to the first line combination therapy have led to renewed interest in novel therapeutic options. In this work, our objective was the identification of novel Falcipan-2 (FP2) inhibitors through the application of an *in silico* approach and the subsequent *in vitro* evaluation of the emerging hits. FP2 is a key cysteine protease in the life cycle of *P. falciparum* constituting a promising target in the search for novel therapies due to their significant hemoglobinase capacity.
We have developed a ligand-based model ensemble capable of recognizing FP2 inhibitors from non-inhibitors. This ensemble has showed a powerful ability for the identification of FP2 inhibitors. The ensemble was applied in a virtual screening campaign of DrugBank database to identify approved drugs with FP2 inhibitory activity. 4 hits were acquired and tested against recombinant FP2 using a fluorogenic continuous enzymatic assay under balanced assay conditions ($[S]_o/K_M$=1). The reversibility of the interaction, mode of inhibition and K_i were further investigated for validated hits. Two drugs, Methacycline and Odanacatib, showed dose-dependent inhibition of FP2. Although both inhibitors showed reversible interactions with FP2, the compounds displayed different inhibition mechanisms. Dose-response analysis at growing substrate concentrations indicated that Odanacatib is a competitive inhibitor of FP2 (K_i=98.2 nM), whereas Methacycline displayed non-competitive behaviour (K_i=84.4 μM; α =1.42) being to our best knowledge the first report of the inhibition of plasmodial cysteine proteases by a tetracycline derivative in a non-competitive manner. To access the impact of Methacycline and Odanacatib on the intracellular parasite's development, parasite trophozoite forms were treated with the compounds for 48h and the parasitemia was analyzed by light microscopy. Treatment of infected erythrocytes with the compounds caused inhibition in a dose-dependent manner. These results demonstrate the utility of the *in silico* approach, since we could find two FP2 inhibitors with antiplasmodial activity with a minimal investment of time and money.

5. Development of novel combination chemotherapy targeting bacterial genome stability

V. Amarh, P. K. Arthur
West African Centre for Cell Biology of Infectious Pathogens, University of Ghana, Legon-Accra, Ghana.

Quinolones have been used for successful treatment of a wide variety of bacterial infections. The success of these drugs is attributed to the lethality of unrepaired DNA double-strand breaks (DSBs); quinolones form multiple and persistent DSBs in the genome of bacterial pathogens. Incidentally, bacterial strains that are resistant to quinolones have emerged, thereby obstructing efficient management of patients that relied on these drugs for chemotherapy. While it is imperative to develop novel drugs against the emerging resistant bacterial strains, it is equally prudent to ensure that pragmatic strategies are implemented during the initial phase of drug design to minimise the emergence of drug resistant strains. As a first step towards the development of novel combination chemotherapy targeting bacterial genome stability, a collection of organic extracts from diverse fungal sources were screened against a DSB-repair deficient cell line of *E. coli*. The initial screen identified 28 organic extracts that exhibit antibacterial activity via formation of DSBs. We reasoned that inhibition of DSB repair would exacerbate the sensitivity of *E. coli* to very small dose of the novel DSB-inducing antibiotics that we anticipate to develop. Thus, the collection of organic extracts were also screened against an *E. coli* cell line containing a system for inducing a site-specific DSB at the *lacZ* locus of the chromosome. A total of 25 of the organic extracts, which were different from the selected DSB-inducing extracts, exhibited antibacterial activity via inhibition of DSB repair. Large-scale fermentations of the individual fungi which produced the selected organic extracts are being utilized to generate sufficient yield for identification of the active components via bioactivity-guided fractionation. We also observed that the combination of streptomycin with specific phenotype-modifying compounds caused an increase in the sensitivity of *E. coli* to DSBs. The antibiotic-compound combination is currently being utilized as a tool for in-depth molecular analysis of the expression profile of DSB repair genes in *E. coli*. The study highlights formation of DSBs and inhibition of the concomitant repair as a suitable strategy for development of combination chemotherapy against infections caused by enterobacteriaceae, which are priority pathogens for which new antibiotics are urgently needed.