Antimalarial Drug Discovery: Exploring the MEP Pathway

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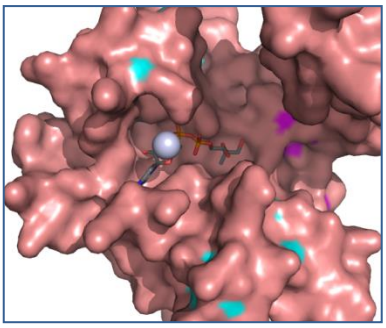
The development of effective antimalarial chemotherapeutics remains one of the major challenges within drug discovery. Malaria remains a major threat to global health, with ~198 million cases per year and 584,000 deaths annually, primarily in children under the age of five.[1] Despite a 30% decreased global incidence of malaria between 2000 and 2013 through upscaling of malaria interventions, it is the adept ability of the *Plasmodium* parasite to develop drug resistance which is so significantly hindering the fight against this entirely preventable disease. With 91 countries registering malaria transmission in 2014, the development of new antimalarial drugs and battle to overcome parasite resistance has never been more crucial.

The non-mevalonate (or MEP) pathway has been validated as a target for treatment of malaria and has the added advantage that it is absent in humans.[2] Therefore, small molecules that inhibit the enzymes of this pathway represent attractive targets for the development of novel antimalarial chemotherapy. Our objective is to deliver a lead candidate molecule suitable for clinical development, targeting *P.falciparum* IspD (*Pf*IspD).

Following a chemoinformatics led high throughput screen identifying the 1,2-benzoisothiazolone (BITZ) chemotype as a promising *Pf*IspD inhibitor (IC_{50} = 484nM),[3] organic synthesis has explored structural modifications to the side chain of the BITZ core. Resulting inhibitors have been used to develop structure-activity relationship (SAR) around the *Pf*IspD active site. Molecular modelling techniques have been used to propose a mechanism of enzyme inhibition and guide the selection of new inhibitor analogues through modelling predictions. Molecular and chemical biology techniques have been used to study the effect of the BITZ inhibitors on the IspD enzyme and MEP pathway. Enzymatic and whole cell assays have been performed to determine inhibitory activity at *Pf*IspD and the whole cell; site directed mutagenesis has assessed mutation effects on the behaviour and activity of inhibitors and fluorescent microscopy has evaluated localisation of the BITZ motif within the cell and its effects on morphology.



*Pf*IspD active site occupied by the native ligand, CDP-ME.

This work has resulted in the generation of the three most potent *Pf*IspD inhibitors to date, exhibiting enzymatic inhibition of 0.07-0.27µM, alongside impressive whole cell activity of 0.6-1.0µM. Site directed mutagenesis and structural manipulations have confirmed a covalent interaction between the BITZ core and cysteine residue in the *Pf*IspD active site essential in achieving enzyme inhibition. We seek to progress the BITZ inhibitor series into lead optimisation, enhancing pharmacokinetics and ultimately generate an optimised lead targeting *Pf*IspD, which conforms to MMV TCP2. Alongside this, we are also exploring a second chemical series of non-covalent *Pf*IspD inhibitors, based around the tetrahydro-β-carboline chemotype, identified in a phenotypic screen by GSK and made available to researchers by the Medicines for Malaria Venture (MMV). Early analogues of this series display low nM activity at *Pf*IspD and provides a contrasting chemical construct, SAR and mechanism of inhibition to the BITZ analogues explore to date.

1. World Health Organization, World Malaria Report, 2014.
2. A. R. Odom and W. C. Van Voorhis, *Mol Biochem Parasit*, 2010, **170**, 108-111.
3. K. E. Price, C. M. Armstrong, L. S. Imlay, D. M. Hodge, C. Pidathala, N. J. Roberts, J. Park, M. Mikati, R. Sharma, A. S. Lawrenson, N. H. Tolia, N. G. Berry, P. M. O'Neill, A. R. Odom