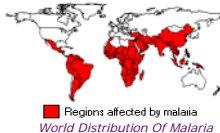


1. Malaria

Malaria infects between 300-500 million people annually and causes up to 2 million deaths¹. The following map indicates the distribution of malaria according to the World Health Organisation (WHO)¹.



The disease results from infection by parasites belonging to the *Plasmodium* species and is transmitted by the female mosquitoes of the *Anopheles* genus (see left). Of the four species of parasite that infect humans, *Plasmodium falciparum* (Pf) is responsible for the majority of fatalities.

However, resistance to commonly employed antimalarial drugs is widespread.

Artemisinin 1 and the more active OZ277 2. Both contain the endoperoxide bridge, key to their antimalarial activity.

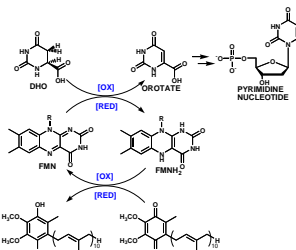
The redesign and synthetic optimization of existing antimalarials extends their lifetime as useful drugs but still there is a

NEED TO IDENTIFY NOVEL CHEMICAL SCAFFOLDS THAT INHIBIT PREVIOUSLY UNEXPLOITED TARGETS !!

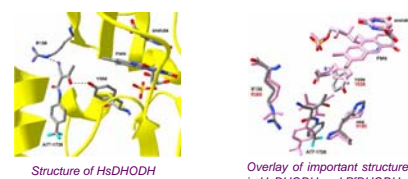
2. A Target for New Drugs

De novo pyrimidine biosynthesis is an attractive and potentially selective target for the development of new therapeutics against *P. falciparum*. Unlike human cells, which can both synthesise and salvage pyrimidine bases, *P. falciparum* lacks any pathway for the salvage of preformed pyrimidine bases or nucleosides and relies completely on a *de novo* biosynthesis pathway.

Dihydroorotate dehydrogenase (DHODH), the 4th enzyme in the pathway, catalyses the oxidation of dihydroorotate (DHO) to orotate in the presence of the co-factors flavin mononucleotide (FMN) and ubiquinone (Q):



3. Structure of DHODH



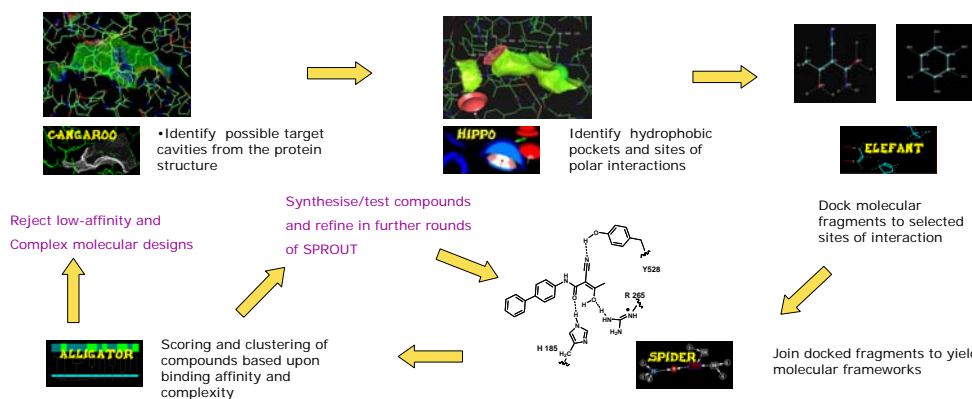
The X-ray crystal structure of human (Hs) DHODH³ is an α/β barrel with orotate and FMN stacked in the active site, elaborated by 2 helices thought to form the channel for CoQ. The inhibitor **A77-1726** is bound within this putative 'ubiquinone channel' and makes H-bonding contacts to R136 and Y356 respectively.

The X-ray crystal structure of PfDHODH, with **A77-1726** in the ubiquinone channel, features analogous positioning of FMN and orotate molecules to those found in HsDHODH but there are subtle differences⁴.

Inhibitors will be designed that target the ubiquinone binding site. Differences between the human and *Plasmodium* enzymes will be exploited to produce novel inhibitors that selectively target PfDHODH.

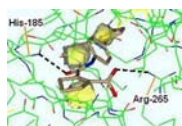
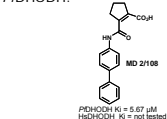
4. SPROUT: Drug Design Software

SPROUT⁵ is divided into five modules, allowing the user to develop small molecule inhibitors *de novo* or alternatively dock existing inhibitors or fragments of these and design improvements that may result in increased binding affinities



6. A New Inhibitor

In keeping with the design criteria a range of analogues were designed and synthesised. Compound **MD 2/108** was found to be active in PfDHODH:



The active compound (left) and its predicted mode of binding in the ubiquinone channel of PfDHODH (right-with hydrophobic regions in yellow).

The carboxylic acid H-bonds with arginine-265 and the amide carbonyl interacts with histidine-185.

A non-planar arrangement between the amide, attached aryl groups, and the carboxylic acid is apparent.

Optimisation.

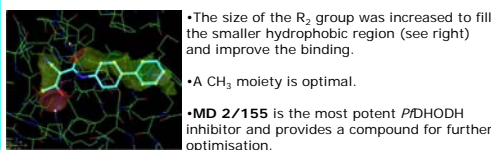
Using **MD 2/108** as the lead compound, we intend to synthesise molecules that maintain the biphenyl structure of this active compound but incorporate **head groups** that are similar to that of **A77-1726**.



7. Developing our Lead Compound.

The following analogues were synthesised and tested:

Compound	R ₁	R ₂	Ki (μ M)	Pf	Ki (μ M)	Hs
MD 2/155	CN	CH ₃	3.0	0.011		
MD 3/170	CN		26.1	0.007		
MD 3/173	CN		49.2	12.0		
MD 3/156	CN	OEt	90.3	2.9		



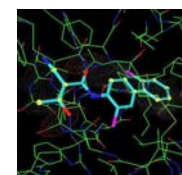
These compounds were more selective for HsDHODH. However we have identified some of the first potent PfDHODH inhibitors and some extremely potent inhibitors of the human enzyme!

8. Next Generation of Inhibitors

SPROUT was used for lead optimisation, starting with **MD 2/155** as the lead and modifying the biphenyl 'tail'.

Compound ID	Structure	PfDHODH Ki	HsDHODH Ki
MD 3/204		0.73 μ M	0.021 μ M
MD 3/209		1.0 μ M	0.025 μ M
MD 3/213		0.48 μ M	0.022 μ M
MD 4/264		1.2 μ M	0.026 μ M

Binding model of MD 3/213



The binding affinity to PfDHODH has improved almost 100-fold!

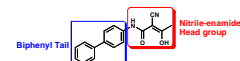
The binding affinity for HsDHODH has decreased.

Selectivity for PfDHODH may be achieved by modifying the biphenyl substituents. Although selectivity is desirable, it is not essential – the human salvage route would replace *de novo* pyrimidine biosynthesis during the expected short period of malaria treatment.

9. What makes a Good Inhibitor?

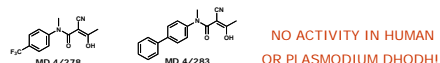
We have established that a BIPHENYL TAIL is required for an active inhibitor.

What about the head group?



The nitrile-enamide head group is clearly superior.

The *N*-Me analogues of **A77-1726** and **MD 2/155** were designed to pick up an additional hydrophobic interaction with a local Leucine residue but:



What determines the activity?

The X-ray crystal structure of **A77-1726** bound to Hs and PfDHODH has a planar head group, the result of an INTRAMOLECULAR H-BOND (shown in red).

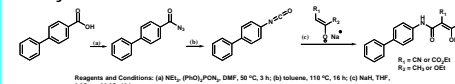
The *N*-Me removes this intramolecular H-bond and skews the planarity, disrupting the ligand-enzyme H-bonds. The observed activities tell us much about the binding of this head group:

NO PLANARITY, NO ACTIVITY!

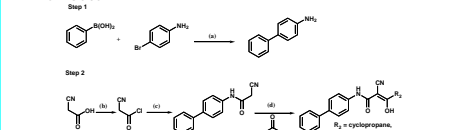
10. Synthesis of Inhibitors

All of the inhibitors were synthesised according to a CARBOXYLIC ACID ROUTE or an AMINE ROUTE.

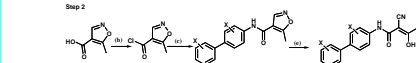
Carboxylic Acid Route



Amine Route



Or for the substituted biphenyls:



Reagents and Conditions: (a) K₂CO₃ (5.5 eq.), Pd(OAc)₂ (5 mol %), PPh₃ (15 mol %), Toluene, 160 °C, 24h; (b) Thionyl chloride (5.5 eq.), reflux, 2 h; (c) polyphosphazene (1 eq.), N-methylpyrrolidine (1.5 eq.), DCM, RT, 24 h; (d) (e) NaH (1.2 eq.), THF, 0 °C to RT, 1 h; (f) (g) RT, 24 h; (h) NaOH (2 eq.), MeOH, reflux, 16 h.

The simplicity of the syntheses makes these inhibitors extremely attractive as drugs!!

11. Conclusions and Future Work

We have successfully applied SPROUT to design potent inhibitors of both human and *Plasmodium falciparum* dihydroorotate dehydrogenase.

In this series, an active inhibitor has:

> a non-planar, substituted biphenyl 'tail'. The substituents may be modified to exploit the shape differences between the hydrophobic region in both human and *Plasmodium* DHODH.

> a planar nitrile-enamide head group. This H-bonds to histidine-185 and arginine-265 residues.

The ease of synthesis of these inhibitors makes them extremely attractive as drugs.

We aim to produce X-ray co-crystal structures of the inhibitors bound to PfDHODH and use the information to improve our inhibitors.

12. References

(1) WHO report, 2002; (2) P. M. O'Neill, *Nature*, 2004, **430**, 838; (3) S. Liu et al., *Structure*, 2000, **8**, 25-33; (4) D. E. Hurt et al., *Acta Cryst. D*, 2006, **62**, 312-23; (5) V. Gillet et al., *Perspect. Drug Discovery Des.*, 1995, **3**, 34; (6) T. Heikkilä et al., *Bioorg. Med. Chem. Lett.*, 2006, **16**, 88-92.

13. Acknowledgements

I would like to thank Prof. A. P. Johnson, Dr. C. W. G. Fishwick, Dr. S. Thirumalaiahari and T. Heikkilä. Thanks also go to the UK Engineering and Physical Sciences Research Council for financial support.