Design and Synthesis of New Dihydroorotate Dehydrogenase Inhibitors as Potent Antimalarials

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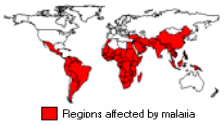
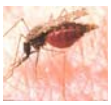
1. Malaria

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



World Distribution Of Malaria

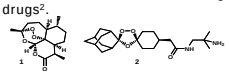


• The disease results from infection by parasites belonging to the *Plasmodium* species and is transmitted by the female mosquitoes of the *Anopheles* genus (see left). Of the four species of parasite that infect humans, *Plasmodium falciparum* (*Pf*) is responsible for the majority of fatalities.

• However, resistance to commonly employed antimalarial drugs is widespread.

•Artemisinin **1** is the basis for synthesis of very modern antimalarial drugs (e.g. compound **2**). So far there is no evidence of drug resistance to the artemisinin family of drugs[2].

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



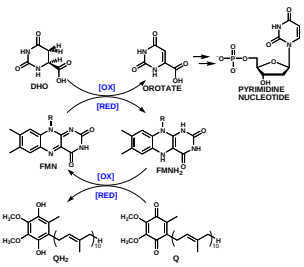
•The redesign and synthetic optimization of existing antimalarials extends their lifetime as useful drugs but still there is a

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

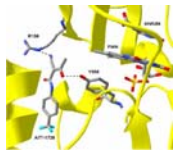
2. A Target for New Drugs

• *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.

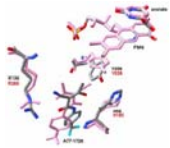
• Dihydroorotate dehydrogenase (DHODH), the 4th enzyme in the pathway, catalyses the oxidation of dihydroorotate (DHO) to orotate in the presence of the co-factors flavin mononucleotide (FMN) and ubiquinone (Q):



3. Structure of DHODH

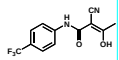


Structure of HsDHODH



Overlay of important structures in HsDHODH and PfDHODH

• The X-ray crystal structure of human (Hs) DHODH[3] 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.



Inhibitor A77 1726

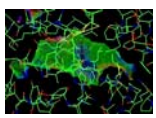
• The X-ray crystal structure of *Pf*DHODH, with **A77-1726** in the ubiquinone channel, features analogous positioning of FMN and orotate molecules to those found in HsDHODH but there are subtle differences[4].

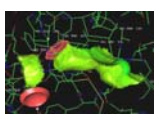
•Inhibitors will be designed that target the ubiquinone binding site.

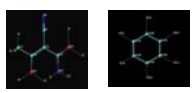
•Differences between the human and *Plasmodium* enzymes will be exploited to produce novel inhibitors that selectively target *Pf*DHODH.

4. SPROUT: Drug Design Software

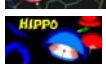
SPROUT[5] is divided into five modules, allowing the user to develop small molecule inhibitors *de novo* or alternatively dock existing inhibitors or fragments of these and design improvements that may result in increased binding affinities







•Identify possible target cavities from the protein structure

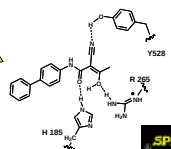


Identify hydrophobic pockets and sites of polar interactions

Reject low-affinity and Complex molecular designs

Synthesise/test compounds and refine in further rounds of SPROUT

Dock molecular fragments to selected sites of interaction

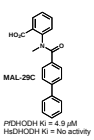


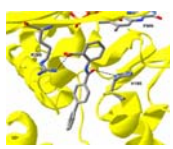
Scoring and clustering of compounds based upon binding affinity and complexity

Join docked fragments to yield molecular frameworks

5. Targeting the Ubiquinone Site

•Previous work in the group has used SPROUT to design simple inhibitors that target the ubiquinone channel. They make H-bonds to histidine-185 and arginine-265 residues and satisfy the hydrophobic central portion of the ubiquinone channel[5]. One such active compound is:

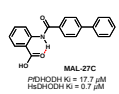




The structure of the active compound (left) and the predicted model of binding in the ubiquinone channel of *Pf*DHODH (right).

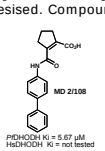
•The optimal conformation of **MAL-29C** in the ubiquinone channel requires a non-planar arrangement between the amide unit, attached aryl groups, and the carboxylic acid moiety. This appears to optimize the fit of the biphenyl within the hydrophobic region.

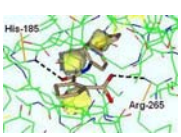
•The ubiquinone channel in HsDHODH has a hydrophobic region of differing shape to that of *Pf*DHODH such that the corresponding NH compound **MAL-27C** (right), populating a more planar conformation, was predicted to show better binding to the human enzyme. Biological testing confirmed this hypothesis.



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 *Pf*DHODH:





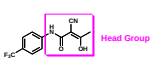
The active compound (left) and its predicted mode of binding in the ubiquinone channel of *Pf*DHODH (right-with hydrophobic regions in yellow).

✓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**.



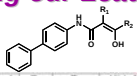
A77-1726

*Pf*DHODH Ki = 22.2 μM

HsDHODH Ki = 0.03 μM

7. Developing our Lead Compound.

The following analogues were synthesised and tested:



Compound	R_1	R_2	Ki (μM) *Pf*	Ki (μM) 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

•The size of the R_2 group was increased to fill the smaller hydrophobic region (see right) and improve the binding.

•A CH_3 moiety is optimal.

•**MD 2/155** is the most potent *Pf*DHODH inhibitor and provides a compound for further optimisation.

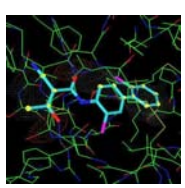
These compounds were more selective for HsDHODH. However we have identified some of the first potent *Pf*DHODH inhibitors and some extremely potent inhibitors of the human enzyme!

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	*Pf*DHODH Ki	HsDHODH Ki
MD 3/204		0.73 μM	0.021 μM
MD 3/209		1.0 μM	0.025 μM
MD 3/213		0.48 μM	0.022 μM
MD 4/264		1.2 μM	0.026 μM

Binding model of **MD 3/213**



•The binding affinity to *Pf*DHODH has improved almost 100-fold!

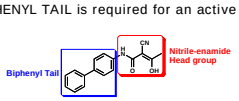
•The binding affinity for HsDHODH has decreased.

•Selectivity for *Pf*DHODH 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?

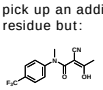
•We have established that a BIPHENYL TAIL is required for an active inhibitor.

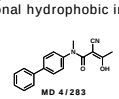
What about the head group?



•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:

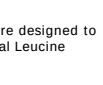




NO ACTIVITY IN HUMAN OR PLASMODIUM DHODH!!

What determines the activity?

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



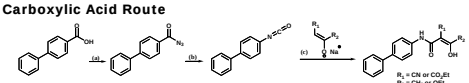
•The *N*-Me removes this intramolecular H-bond and skews the planarity, disrupting the ligand-enzyme H-bonds. The observed activities tell us much about the binding of this head group:

NO PLANARITY, NO ACTIVITY!

10. Synthesis of Inhibitors

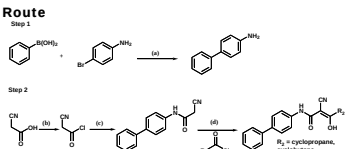
•All of the inhibitors were synthesised according to a CARBOXYLIC ACID ROUTE or an AMINE ROUTE.

Carboxylic Acid Route

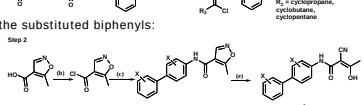


Reagents and Conditions: (a) NEt_3, $(PhO)_2PON_3$, DMF, 50 °C, 3 h; (b) toluene, 110 °C, 16 h; (c) NaH, THF, 0 °C → 60 °C, 48 h.

Amine Route



Or for the substituted biphenyls:



Reagents and Conditions: (a) K_2CO_3 (3.5 eq.), $Pd(OAc)_2$ (5 mol %), PPh_3 (15 mol %), Toluene, 160 °C, 24h; (b) Thionyl chloride (50 eq.), reflux, 2 h; (c) *p*-biphenylaniline (1 eq), triethylamine (1.5 eq.), DCM, RT, 24 h; (d) (i) NaH (1.2 eq.), THF, 0 °C → RT, 1 h, (ii) RT, 24 h; (e) NaOH (2 eq.), MeOH, Reflux, 1h.

•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 *Pf*DHODH 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

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