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The first de novo designed inhibitors of *Plasmodium falciparum* dihydroorotate dehydrogenase

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Abstract—The de novo molecular design program SPROUT has been applied to the X-ray crystal structures of *Plasmodium* and human dihydroorotate dehydrogenase, respectively. The resulting design templates were used to prepare a series of molecules which, in keeping with predictions, showed useful levels of species-selective enzyme inhibition.

Malaria continues to represent a major threat to world health infecting between 300 and 500 million people annually and causing up to two million deaths.[1] The disease results from infection by parasites belonging to the *Plasmodium* species and is transmitted by the female mosquitoes of the *Anopheles* genus. Of the four species of parasite that infect humans, *Plasmodium falciparum* is responsible for the majority of fatalities. Although prophylactic treatments are available, widespread resistance to commonly employed anti-malarial drugs (e.g., chloroquine,[2] pyrimethamine[3] and atovaquone[4]) is widespread. There is, therefore, an urgent need for the development of new drugs that can control infection which can also act at previously unexploited biological targets.

De novo pyrimidine biosynthesis represents 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) is the fourth enzyme in the pyrimidine biosynthetic pathway and catalyses the oxidation of dihydroorotate (DHO) to orotate in the presence of the co-factors flavin mononucleotide (FMN) and ubiquinone (CoQ) (Scheme 1). The human version of this enzyme (hDHODH) is the target of a number of inhibitors with proven efficacy in the treatment of arthritis and leflunomide, a pro-drug that is metabolised to the active DHODH inhibitor, A77-1726, is approved for clinical use.[5–9] Additionally, random high-throughput screening of chemical libraries has been used to identify selective inhibitors of *Escherichia coli*,[10] *Helicobacter pylori*[11] and *P. falciparum*[12] PfDHODH, respectively. Additionally, Boa et al., have recently shown that selective inhibitors of PfDHODH can be developed from existing inhibitors.[13] The PfDHODH enzyme is thus an attractive target for the development of new anti-malarial drugs.

As part of a continuing structure-based synthesis and biological evaluation program for the discovery of new enzyme inhibitors and receptor antagonists, respectively, we have previously described the computer-aided molecular design, synthesis and biological evaluation of novel inhibitors of the bacterial enzyme MurD,[14] and of the human NK_2 receptor,[15], respectively. Here, we report the application of our de novo molecular design program SPROUT[16] to the design of novel inhibitors of PfDHODH.

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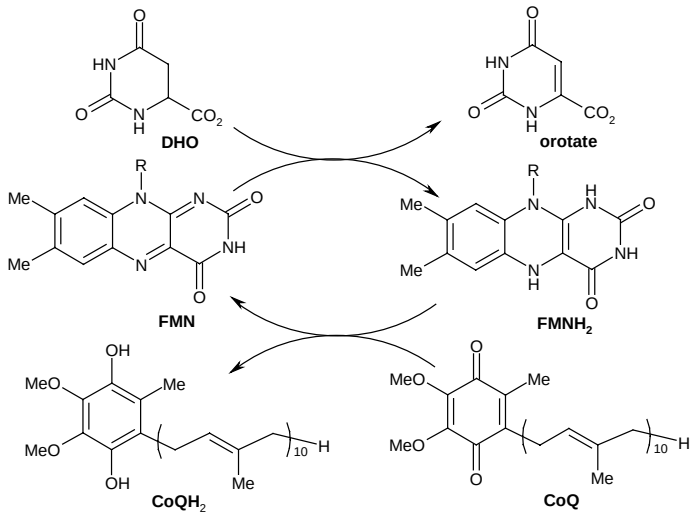


Scheme 1. Reactions catalysed by DHODH.

SPROUT[16] is a powerful suite of software modules for de novo structure-based molecular design. SPROUT has modules for (i) characterising regions within a protein, (ii) detecting 'hotspots' where ligand atoms would form favourable interactions with the protein, (iii) docking molecular fragments to these sites, (iv) joining these fragments with a molecular backbone to give a complete ligand and (v) ranking the set of predicted ligands by criteria including predicted binding energy, structural complexity and synthetic accessibility.

In order to use a de novo design approach for the discovery of novel PfDHODH inhibitors, we have applied SPROUT to the recently solved X-ray crystal structure of PfDHODH the details of which are published elsewhere.[17] The human and *Plasmodium* enzymes share a very similar structural morphology. The previously reported[18] X-ray crystal structure of hDHODH, co-crystallised with the inhibitor A77-1726, revealed the presence of an N-terminal α-helical domain that forms a channel leading to the active site (which contains both FMN and orotate) and that is thought to facilitate binding of CoQ. This structure also features a molecule of A77-1726 bound within this putative 'ubiquinone channel' which makes H-bonding contacts to R136 and Y356, respectively (Fig. 1).

The X-ray crystal structure of PfDHODH, also containing a molecule of A77-1726 in the ubiquinone channel, features analogous positioning of the FMN and orotate molecules to those found in the human enzyme (Fig. 2).

A detailed comparison of the two structures however reveals subtle differences, particularly in the dimensions and topography of the hydrophobic ubiquinone channels. In particular, the channel within the human enzyme is considerably 'flattened' by the protrusion of a methyl group from A59 in the region occupied by the aromatic ring of the bound inhibitor (Fig. 3).

Therefore, the shape of this cavity appears to require inhibitors of this type to be essentially planar. In contrast,

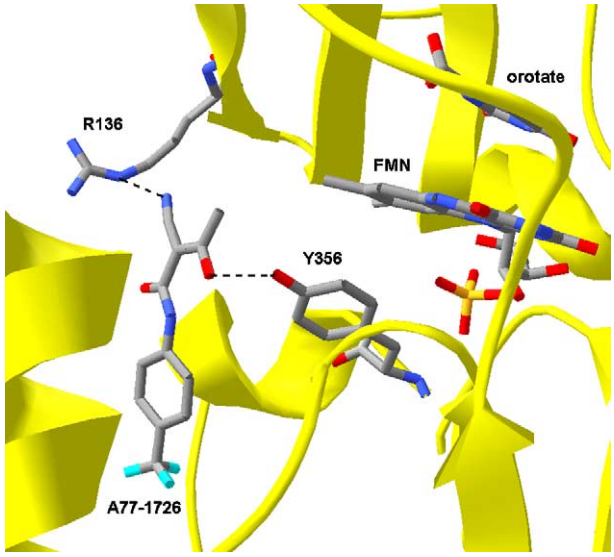


Figure 1. X-ray structure of human DHODH.

the same region within the *Plasmodium*-derived enzyme is much less congested and, unlike that in the human enzyme, appears to be able to accommodate inhibitors that are somewhat cylindrical in overall shape. With this difference in mind, we wished to explore the possibility of using SPROUT to design species-selective inhibitors targeted at this channel.

In order to design structurally simple inhibitors, for which good affinity for PfDHODH was predicted, we specified that in addition to use of the hydrophobicity within the channel, just two residues, histidine 185 and arginine 265, were to make direct H-bonding contacts to the designed inhibitors. Both of these residues are highly conserved in all type II dihydroorotate dehydrogenases of eukaryotic organisms studied to date.

SPROUT-based de novo ligand design produced some 20 different small molecule templates. The most

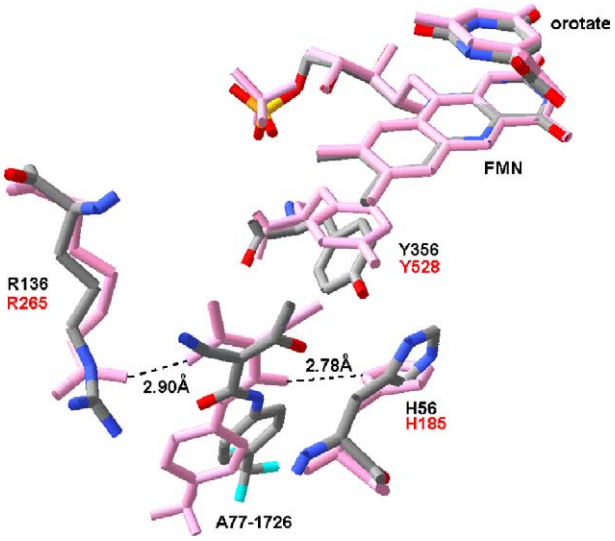


Figure 2. Overlay of X-ray structures of human and *Plasmodium* DHODH (*Plasmodium* structure and contacting residues numbered and coloured in red; distances of ligand to R265 and H185 indicated).

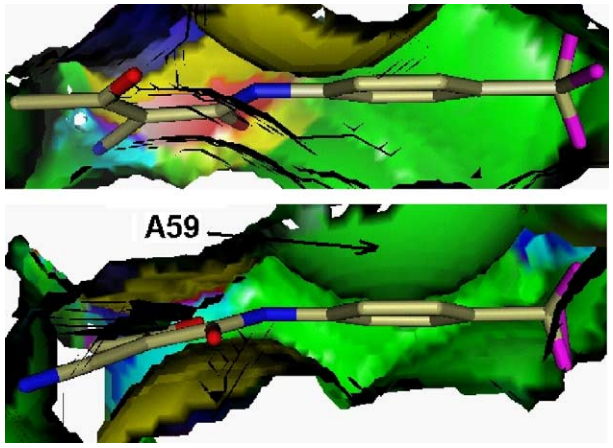


Figure 3. Surfaces of ubiquinone cavities within *Plasmodium* DHODH (top) and human DHODH (bottom), coloured according to electrostatic potential.

attractive of these, for which predicted binding affinities were in the micromolar range or better, consisted of simple and readily prepared amides of anthranilic acid, an example of which is shown in Figure 4. As noted previously, the differing shapes of the ubiquinone cavities within the *Plasmodium* and human enzymes suggest that inhibitors of this type would fit better within the *Plasmodium*—as opposed to the human enzyme, if they adopt a non-planar arrangement between the amide unit, attached aryl groups and the carboxylic acid moiety (**2**, Fig. 5). This twisting appears to maximise the interaction of the aromatic rings of the designed inhibitors with the walls of the hydrophobic cavity. In contrast, planar versions of this type of inhibitor (**1**, Fig. 5) are predicted to interact best with the human enzyme, where the somewhat flattened nature of the cavity allows close contacting with both faces of the aromatic portions of these inhibitors.

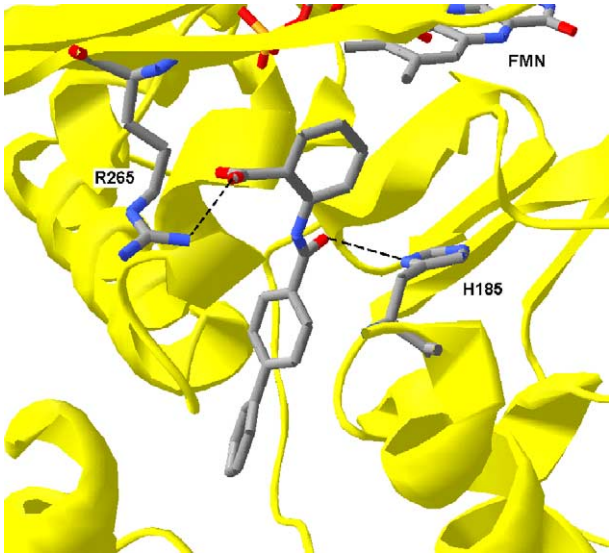


Figure 4. A SPROUT-designed template within the 'ubiquinone channel' of *Plasmodium* DHODH.

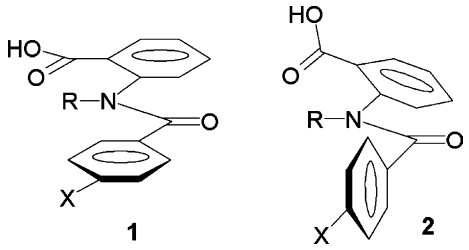


Figure 5. Conformations of designed inhibitor template.

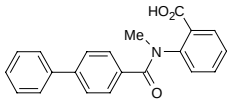
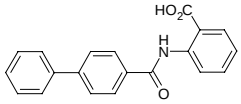
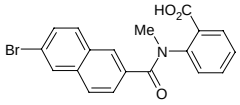
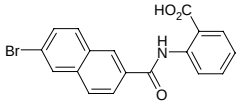
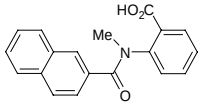
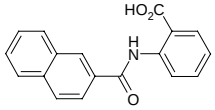
In order to test this hypothesis, we prepared[19] a small number of amides based upon this general design, which were either N-unsubstituted (R = H) to provide systems predominantly populating conformation **1** (via intramolecular H-bonding between the amide NH and carboxylic acid group), or N-methylated (R = Me) and therefore, preferring to exist predominantly as conformation **2**.

The binding affinities of these for both *Plasmodium* and human DHODH were then established from IC_{50} values as described[20,21] and are summarised below (Table 1). As is evident from the data in Table 1, four of the six inhibitors are active against the *Plasmodium* enzyme whereas only the N-unsubstituted systems (entries 2, 4 and 6) show affinity to hDHODH.

Additionally, in keeping with our design criteria, *N*-methyl systems (entries 1 and 3) exhibit a higher affinity for the *Plasmodium* enzyme than those found for the N-unsubstituted cases (entries 2 and 4). Curiously, whereas the bromonaphthyl-derived *N*-methyl amide (entry 3) was active towards PfDHODH, the simpler naphthyl-derived *N*-methyl amide (entry 5) was found to be inactive towards both enzymes.

In conclusion, the de novo design program SPROUT has been used to produce simple molecular templates for the selective inhibition of *Plasmodium* and human

Table 1. Structures, IC_{50} and apparent binding affinities[21] (μM) of designed inhibitors with PfDHODH and hDHODH

Entry	Structure	IC_{50} (Pf)	IC_{50} (h)	K_i^{app} (Pf)	K_i^{app} (h)
1		42.6 (4.6)	>200	4.9	n.a.
2		153.5 (13.2)	5.0 (1.6)	17.7	0.7
3		93.4 (6.4)	>200	10.8	n.a.
4		142.6 (14.2)	8.4 (2.7)	16.4	1.1
5		>200	>200	n.a.	n.a.
6		>200	13.8 (3.3)	n.a.	1.8

Values in brackets refer to ± error limits; n.a. = not attempted.

DHODH, respectively. Subtle differences in the structures within the targeted binding regions of these enzymes, which might provide the basis of this selectivity, have been identified.

In these proof-of-principal studies, the designed inhibitors show only modest enzyme affinities, but provide an excellent starting point for further optimisation. We believe that these results further illustrate the tremendous potential of a de novo design-based approach in inhibitor discovery. Such an approach is complementary to the use of high-throughput screening and is particularly attractive where such screening methodology is not available or where access to large collections of library compounds of sufficient molecular diversity is limited.

Acknowledgments

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References and notes

1. WHO report, 2002.
2. Wellens, T.; Plowe, C. *J. Infect. Dis.* **2001**, *184*, 770.
3. Wu, Y. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 1130.
4. Srivastava, S. *Mol. Microbiol.* **1999**, *33*, 704.
5. Fox, R. I.; Hermann, M. L.; Frangou, C. G.; Wahl, G. M.; Morris, R. E.; Strand, V.; Kirschbaum, B. J. *Clin. Immunol.* **1999**, *93*, 198.
6. Hermann, M. L.; Schleyerbach, R.; Kirschbaum, B. J. *Immunopharmacology* **2000**, *47*, 273.
7. Williamson, R. A.; Yea, C. M.; Robson, P. A.; Curnock, A. P.; Gadher, S.; Hambleton, A. B.; Woodward, K.; Bruneau, J.-M.; Hambleton, P.; Moss, D.; Thompson, T. A.; Spinella-Jaegle, S.; Morand, P.; Courtin, O.; Sautes, C.; Westwood, R.; Hercend, T.; Kuo, E. A.; Ruuth, E. *J. Biol. Chem.* **1995**, *270*, 22467.
8. Greene, S.; Watanabe, S.; Braatz-Trulson, J.; Lou, L. *Biochem. Pharmacol.* **1995**, *50*, 861.
9. Davis, J. P.; Cain, G. A.; Pitts, W. J.; Magolda, R. L.; Copeland, R. A. *Biochemistry* **1996**, *35*, 1270.
10. Marcinkeviciene, J.; Rogers, M. J.; Kopcho, L.; Jiang, W.; Wang, K.; Murphy, D. J.; Lippy, J.; Link, S.; Chung, T. D. Y.; Hobbs, F.; Haque, T.; Trainor, G. L.; Slee, A.; Stern, A. M.; Copeland, R. A. *Biochem. Pharmacol.* **2000**, *60*, 339.
11. Copeland, R. A.; Marcinkeviciene, J.; Haque, T. S.; Kopcho, L. M.; Jiang, W.; Wang, K.; Ecret, L. D.; Sizemore, C.; Amsler, K. A.; Foster, L.; Tadesse, S.; Combs, A. P.; Stern, A. M.; Trainor, G. L.; Slee, A.; Rogers, M. J.; Hobbs, F. *J. Biol. Chem.* **2000**, *275*, 33373.
12. Baldwin, J.; Michnoff, C. H.; Malmquist, N. A.; White, J.; Roth, M. G.; Rathod, P. K.; Phillips, M. A. *J. Biol. Chem.* **2005**, *280*, 21847.
13. Boa, A. N.; Canavan, S. P.; Hirst, P. R.; Ramsey, C.; Stead, A. M. W.; McConkey, G. A. *Bioorg. Med. Chem.* **2005**, *13*, 1945.
14. Horton, J. R.; Bostock, J. M.; Chopra, I.; Hesse, L.; Phillips, S. E. V.; Adams, D. J.; Johnson, A. P.; Fishwick, C. W. G. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1557.

15. Ali, M. A.; Bhogal, N.; Fishwick, C. W. G.; Findlay, J. B. C. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 819.
16. Gillet, V. J.; Newell, W.; Mata, P.; Myatt, G.; Sike, S.; Zsoldos, Z.; Johnson, A. P. *J. Chem. Inf. Comput. Sci.* **1994**, *34*, 207.
17. Hurt, D. E.; Widom, J.; Clardy, J. *Acta Crystallogr.*, submitted for publication.
18. Liu, S.; Neidhardt, E. A.; Grossman, T. H.; Ocain, T.; Clardy, J. *Structure* **2000**, *8*, 25.
19. Prepared via coupling of methyl 2-amino benzoate with the appropriate aryl carboxylic acid chlorides followed by ester hydrolysis.
20. The IC_{50} values were determined as described (Copeland, R. A.; Davis, J. P.; Dowling, R. L.; Lombardo, D.; Murphy, K. B.; Patterson, T. A. *Arch. Biochem. Biophys.* **1995**, *323*, 79) by the standard DCIP assay, using saturating concentrations of DHO (200 μM) and decylubiquinone (CoQd), (100 μM) and a range of inhibitor concentrations (1 nM to 200 mM). IC_{50} values were derived from the data by using Graphpad Prism v. 4.0 (Graphpad Inc.).
21. The IC_{50} values were converted into K_i values by using the Cheng–Prusoff equation (Cheng, Y.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099) assuming competitive inhibition with CoQd (K_m values used for CoQd were 13 μM for PfDHODH and 15 μM for HsDHODH, respectively).