STRUCTURE-BASED DESIGN OF INHIBITORS OF CLASS 2 DIHYDROOROTATE DEHYDROGENASES

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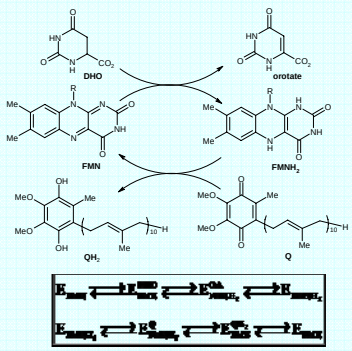
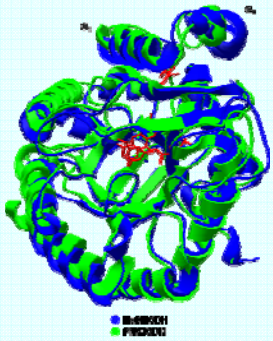
1. Introduction

- Pyrimidine biosynthesis is a validated target for chemotherapeutical intervention in various organisms, but perhaps most notably in humans, the malaria parasite *Plasmodium falciparum* and *Helicobacter pylori*.
- In humans the biosynthesis of pyrimidines is vital for only the most rapidly dividing cell lines and can therefore be used to tackle autoimmune diseases and cancer. On the other hand, the malaria parasites and *H. pylori* are completely dependent on synthesis of pyrimidines as the genes coding for the pyrimidine salvage pathway are missing from the genomes.
- Within the pyrimidine biosynthesis pathway, the rate-limiting enzyme dihydroorotate dehydrogenase (DHODH) presents a good target and is the site of action for rheumatoid arthritis drug Leflunomide® (active metabolite A771726). Additionally, X-ray crystal structures of both the human and *Plasmodium* DHODH are available which makes these attractive targets for structure-based inhibitor design applications.

2. Dihydroorotate dehydrogenase – the enzyme and a drug target

- The class 2 dihydroorotate dehydrogenase catalyses oxidation of dihydroorotate in two half-reactions by using FMN as a cofactor and ubiquinone as the terminal electron acceptor.
- The structures of class 2 DHODHs feature a α/β barrel core that forms a binding site for the substrate and cofactor, as well as two α-helices (α_1 and α_2) extending from the N-terminus to form a putative binding channel for the ubiquinone

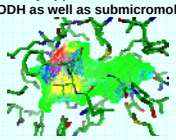




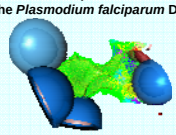
- Vast majority of known DHODH inhibitors bind to the N-terminal ubiquinone-binding cavity of the enzyme. Structural alignment of DHODHs indicates conserved residues where the quinone head of ubiquinone is thought to bind, and less conserved residues for the tail binding region. This is also reflected in the structures of the inhibitors as they typically feature a hydrophobic 'tail' and a polar 'hear' region

3. SPROUT and SPROUT LeadOpt: our structure-based approach

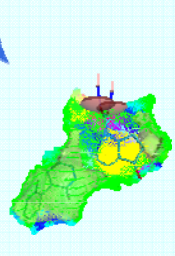
- SPROUT is divided into five modules, allowing the user to develop small molecule inhibitors *de novo* or alternatively dock existing inhibitors or fragments of these and design improvements that may result in increased binding affinities.
- We have successfully applied both of the strategies described above to produce low nanomolar inhibitors of the human DHODH as well as submicromolar inhibitors of the *Plasmodium falciparum* DHODH.



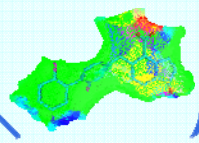
1. Cangaroo:
Identify possible target cavities from the protein structure



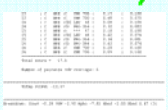
2. Hippo:
Identify hydrophobic pockets and sites of polar interactions



3. Elefant:
Dock molecular fragments to selected sites of interaction



4. Spider:
Put the puzzle together by joining the fragments



5. Alligator:
Scoring and clustering compounds based on predicted binding and complexity

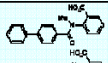
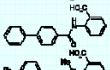
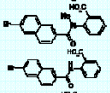
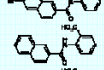
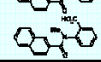
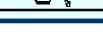
Abandon low-scoring and complex compounds

Synthesis and testing of selected compounds

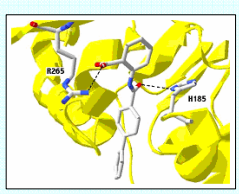
Feed compounds back for another round of SPROUT

4. DHODH inhibitors by *de novo* approach

- We have generated potent inhibitors of both HsDHODH and PfDHODH using the standard fragment libraries built into SPORUT
- Additionally, compounds 1 and 3 were shown to have good levels of specificity for the Plasmodium enzyme and offer possibilities for further optimisation

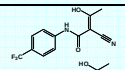
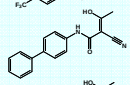
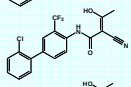
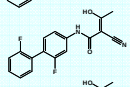
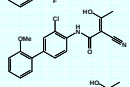
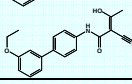
Compound ID	Structure	K_i for PfDHODH	K_i for HsDHODH
1		2.7 μM	>20 μM
2		>20 μM	1.5 μM
3		5.4 μM	>20 μM
4		>20 μM	1.5 μM
5		>20 μM	5.16 μM
6		>20 μM	>20 μM

Binding model of 1-PfDHODH complex

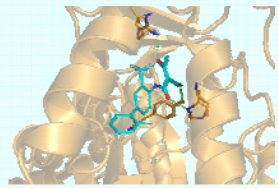


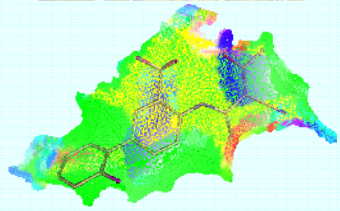
5. DHODH inhibitors by lead optimisation approach

- As an example of the inhibitor development, a series of highly potent DHODH inhibitors was designed by using SPROUT LeadOpt, starting with A771726 (the active metabolite of Leflunomide®) as the lead compound for the series.
  - The lead compound was grown within the confines of the target cavity by using a fragment library consisting mainly of hydrophobic elements, such as mono- and disubstituted benzene rings.

Compound ID	Structure	K_i for PfDHODH	K_i for HsDHODH
Lead A771726		22.2 μM	32.2 nM
5		3.0 μM	11.0 nM
6		730 nM	21.0 nM
7		20.5 μM	2.7 nM
8		1.0 μM	24.6 nM
9		21.5 μM	16.4 nM

Binding model of 6-PfDHODH complex





- Best HsDHODH inhibitors were also tested against a panel of cancer cell lines and found to be as active as a phase II clinical trial candidate Brequinar.

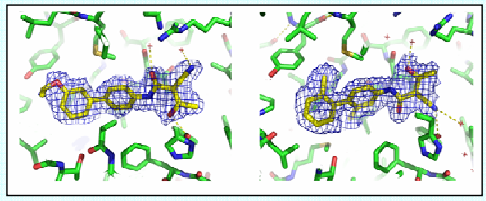
6. Structural validation of computational models

- The most potent inhibitors of the human DHODH were co-crystallised with the target enzyme in order to validate the computationally generated binding models

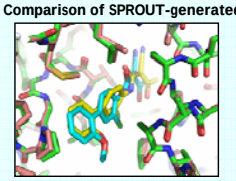
Protein-inhibitor co-crystals and data collection statistics

Crystal dataset	HsDHODH-5	HsDHODH-7	HsDHODH-8	HsDHODH-9
Space Group	$P\,3_2\,2\,1$	$P\,3_2\,2\,1$	$P\,3_2\,2\,1$	$P\,3_2\,2\,1$
Cell dimensions:				
a = b	90.696	90.752	91.013	90.888
c	122.950	122.799	123.207	122.915
Resolution (Å)	2.0	2.0	1.8	1.9
R-factor (%)	19.29	20.50	20.89	21.14
R_{free} (%)	21.44	23.27	23.12	24.20

Electron density maps contoured around the inhibitors (9 and 7)



Comparison of SPROUT-generated model and co-crystal structure for 8

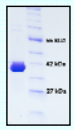


- In general, the SPROUT-generated binding models compare well with the cocrystal structures of the compounds in complex with HsDHODH
- In one of the structures (7-HsDHODH), the head group of the compounds is reversed with respect to the model, and also causes slight changes to the amino acid side chains lining the binding cavity

7. X-ray crystal structure of *Helicobacter pylori* DHODH

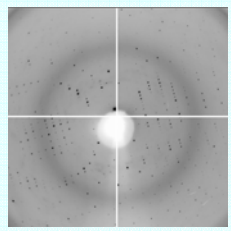
- In a recent collaboration we've started work on *H. pylori* DHODH, a known drug target for this bug causing stomach ulcers and cancers
- The gene was cloned and the protein expressed, purified and crystallised

HpDHODH and data collection statistics



HpDHODH	
Space Group	$P\,4_2\,2_1\,2$
Cell dimensions:	
a = b	106.587
c	77.024
Resolution (Å)	3.1
R-factor (%)	28.64
R_{free} (%)	33.34

HpDHODH diffraction pattern



- When completed, the high resolution structure of HpDHODH will allow structure-based development of potent and specific inhibitors

8. Summary

- We have successfully used SPROUT and SPROUT LeadOpt to develop inhibitors of both human and *Plasmodium falciparum* dihydroorotate dehydrogenases
- Additionally, we have obtained structural validation for the SPROUT-generated binding models by solving the co-crystal structures of inhibitor-target complexes
- We have also solved the structure of *Helicobacter pylori* DHODH which can be used for structure-based drug development

Acknowledgements

I would like to thank my supervisors Prof. Johnson and Dr. Parsons, as well as Dr. McConkey and Dr. Fishwick. Additionally, I'm grateful for all the students in Prof. Johnson's and Dr. McConkey's research groups who have contributed to this work. I would also like to acknowledge the collaborating groups of Dr. A. Boa (University of Hull) and Prof. J. Clardy (Harvard Medical School).

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