UNIVERSITY OF LEEDS

Protein structure based approaches to inhibit Plasmodium DHODH for malaria

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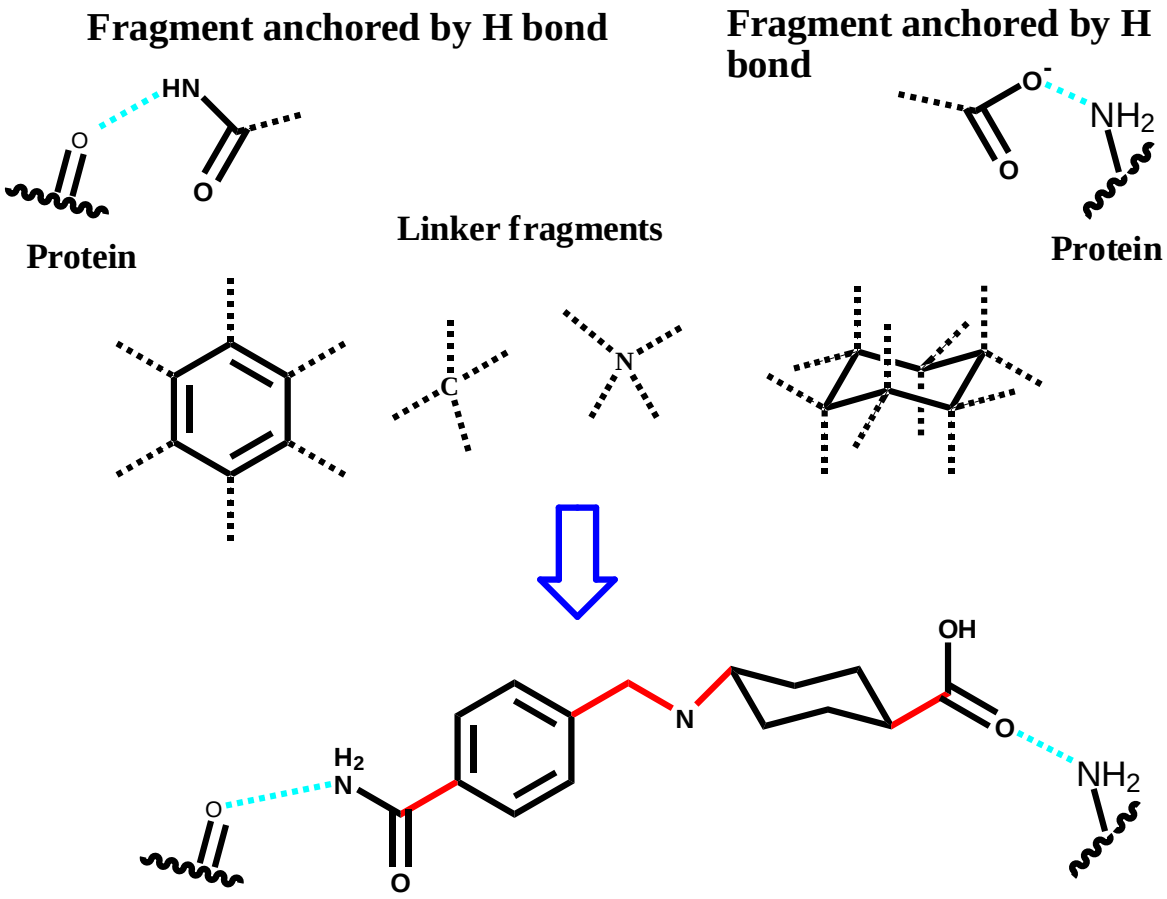
Tools for protein structure based approaches to hit finding

- *De Novo* Design – Builds hypothetical molecules in a protein cavity
  - The SPROUT program developed at Leeds
  - Can generate novel structural motifs whose preparation may require novel synthetic procedures

- Virtual screening of libraries of available compounds – uses docking programs such as eHITS, AUTODOCK and GLIDE

  Compounds corresponding to virtual hits may often be purchased

De Novo Design using Structure Construction by Fragment Joining



..... Dummies ie Hs or lone pair vectors - used for docking and joining - alternative growth points give different answers

— Rotatable Bonds - each one multiplies the number of possible conformations - could give a combinatorial explosion

Equivalent to using very big virtual library!

Starting Point – Protein Structure Based *De Novo* Ligand Design using various flavours of SPROUT

- SPROUT – fragment based de novo ligand design
- Suitable fragments are anchored to pharmacophore regions in correct orientation
- Growing operations join fragments together in pairwise connections (fragment linking)
- Can also induce growth from a single fragment if direction of growth is specified (fragment evolution)

Practical Applications in Leeds

Johnson/Fishwick group applies an array of structure based design techniques to potential drug targets focussed on

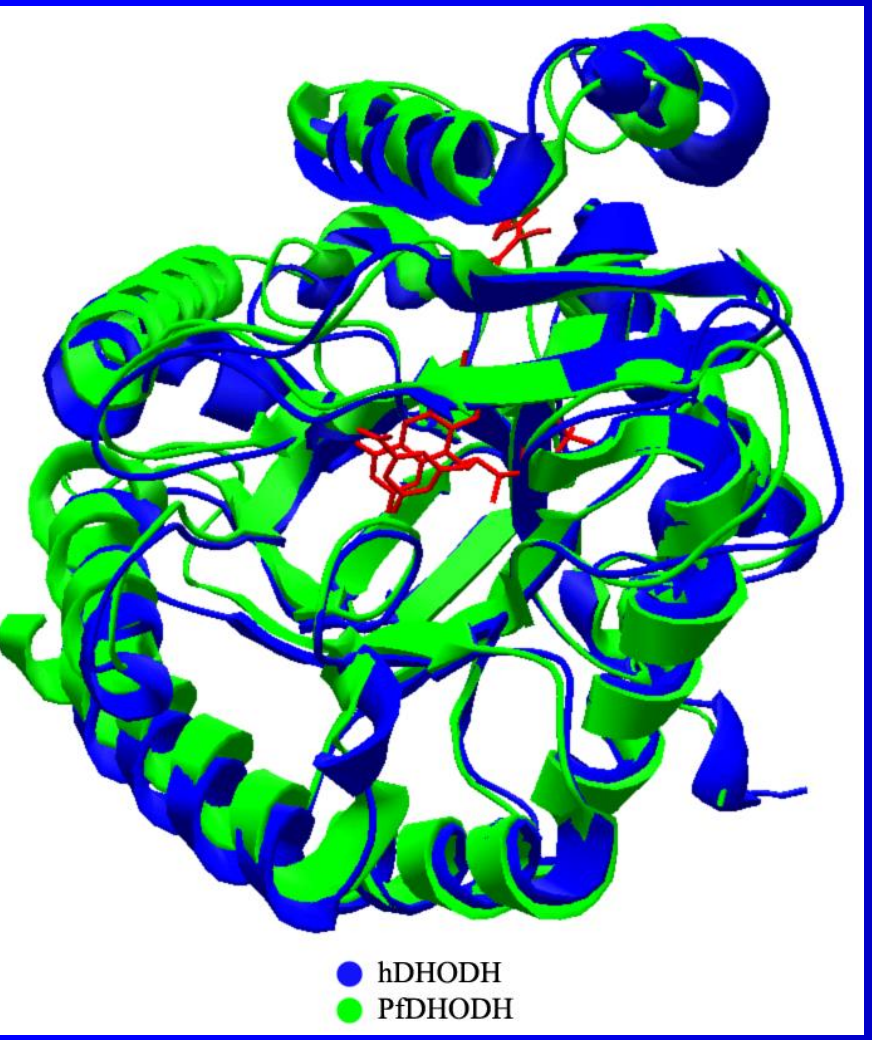
a) Antibacterials

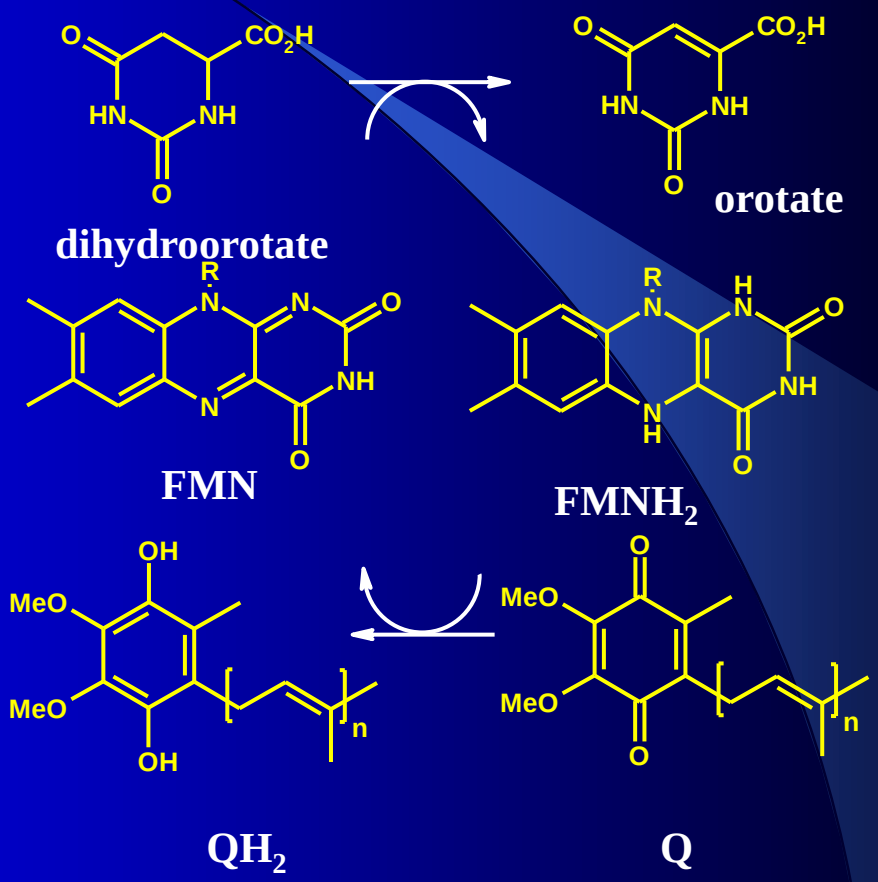
b) Antimalarials (Target : Dihydroorotate dehydrogenase – DHODH)

c) Cardiovascular

Design work is followed by synthesis, assays and structural studies

DHODH- an essential *plasmodium* enzyme

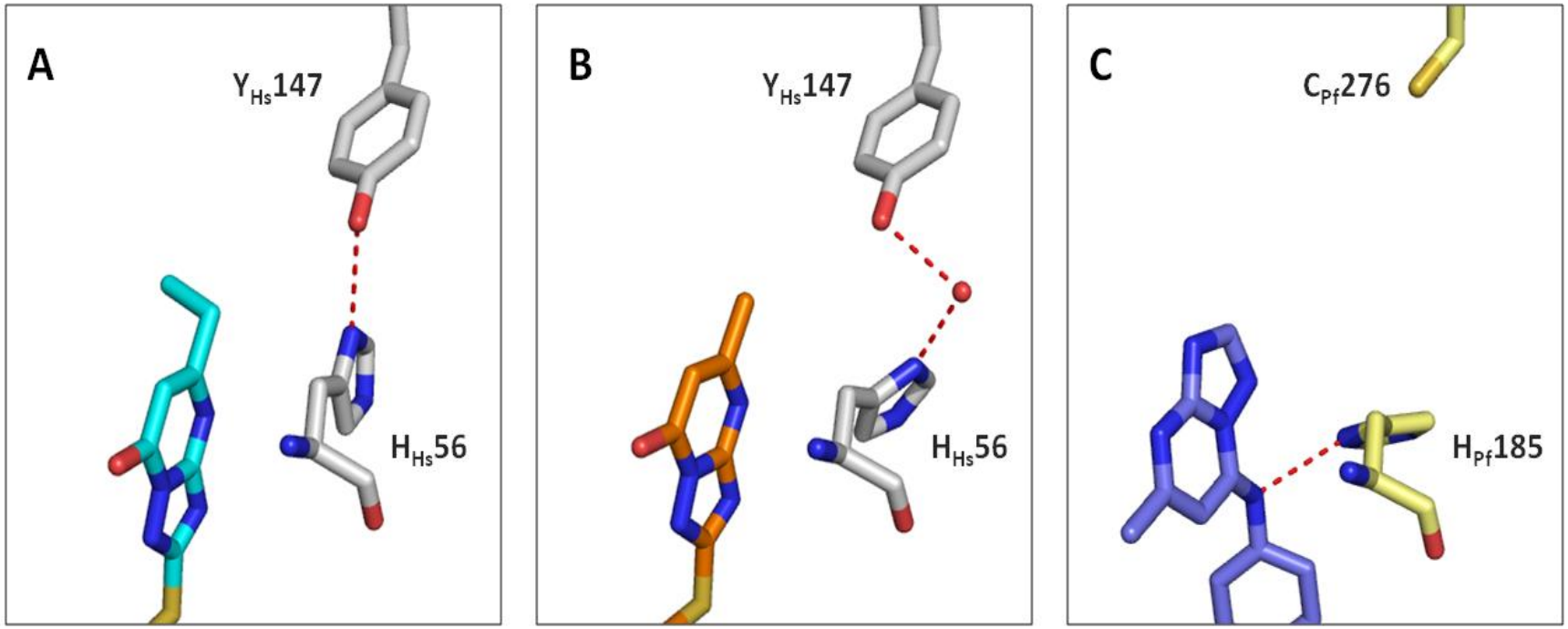




P Falciparum lacks the pyrimidine salvage pathway found in humans

Hurt, D. E.; Widom, J.; Clardy, J. *Acta Crystallogr. D Biol. Crystallogr.* 2006, ***D62***, 312-323.

For Hs DHODH Intramolecular H bond from H56 to Y147 in binding Site
Less available for binding to ligand



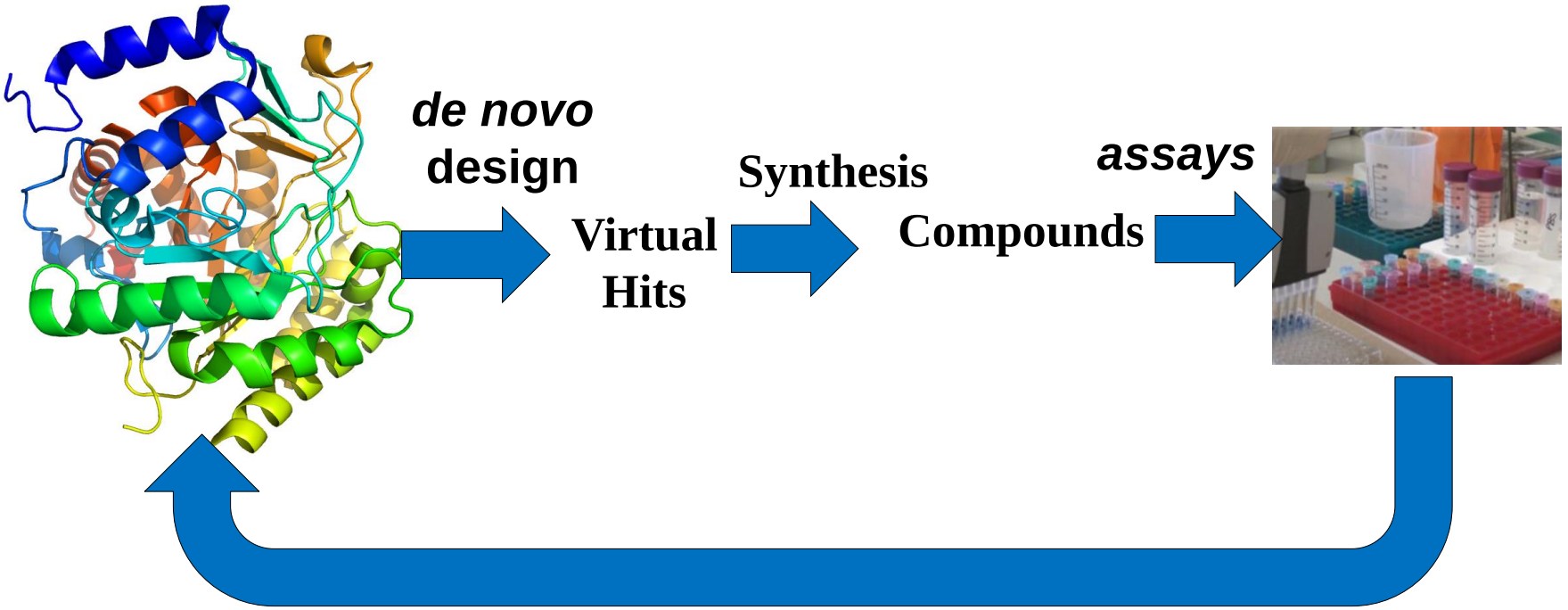
Hs DHODH
Direct H bond to Y147

Hs DHODH
Water mediated H bond

PfDHODH
no H bond to C276
H185 free to bind to ligand

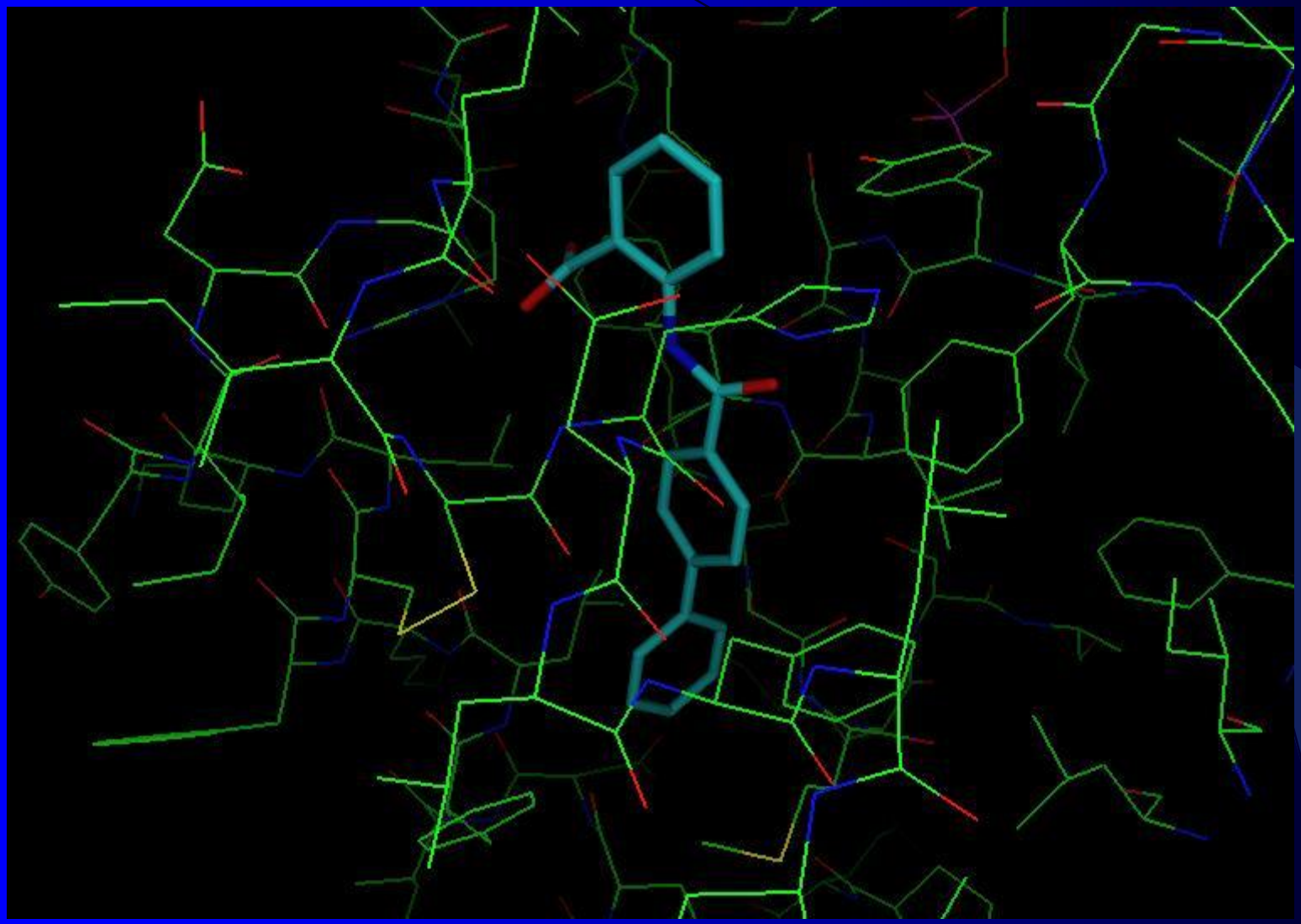
Could be a key determinant of selectivity!

DHODH Inhibitor Discovery at Leeds

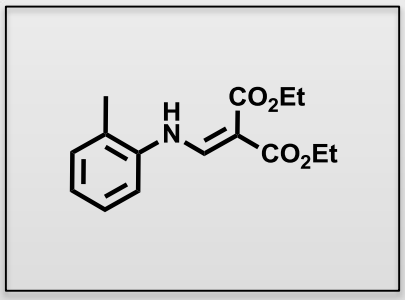


- **Our rational approach to hit finding is an alternative to high - throughput screening**
- **Our expertise in chemistry have allowed us to develop routes for hard to synthesise compounds not present in HTS libraries**

SPROUT analysis of binding site



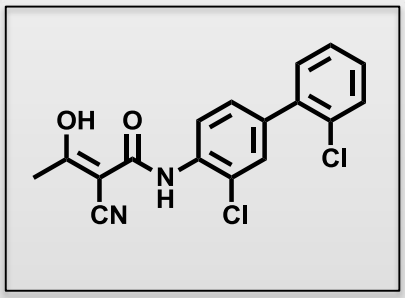
DHODH Inhibitor Discovery at Leeds



2003

- PhD thesis (Heikkila)
- IC_{50} 346 µM *Pf*DHODH

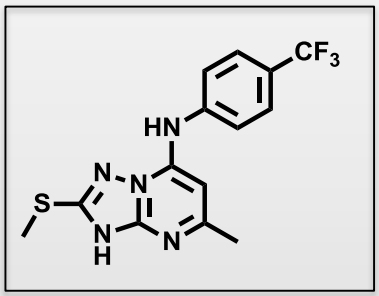
J. Med. Chem. 2007, 50, 186-191



2009

- PhD thesis (Davies)
- IC_{50} 4 µM *Pf*DHODH

J. Med. Chem. 2009, 52, 2683-2693



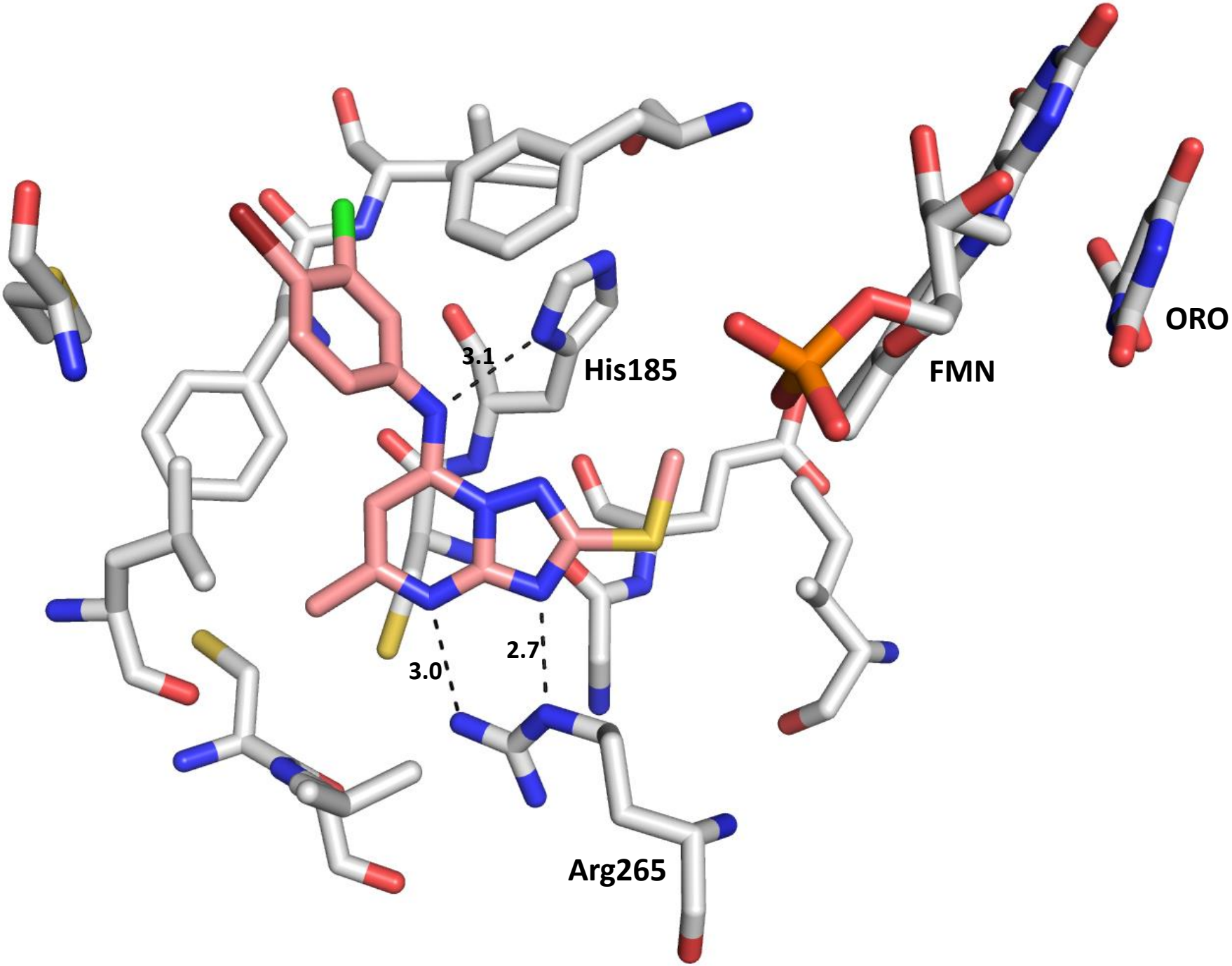
2010

- PhD thesis (Cowan)
- IC_{50} 52 nM *Pf*DHODH
- EC_{50} 9 nM *Pf*3D7

Latest scaffold

2013

- PhD thesis (Cunningham)
- IC_{50} 10 nM *Pf*DHODH
- EC_{50} 10 nM *Pf*3D7


3.1
His185
FMN
ORO
3.0
2.7
Arg265

Susceptibility to metabolism of S-Me series?

- SMe potential for metabolic degradation
- For 4-CF3 some indication of this

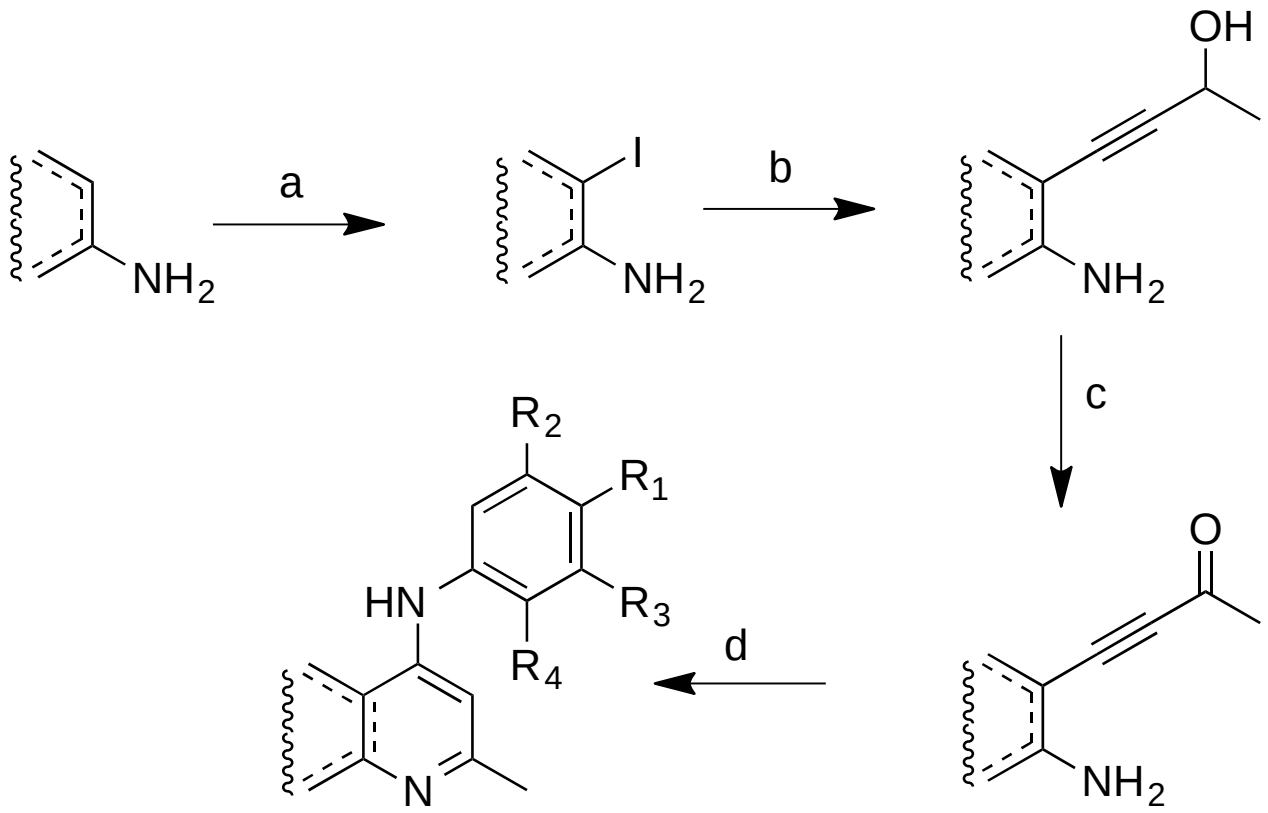
compd	log DpH 7.4	aqueous solubility pH 6.5 (µM)	human plasma protein binding[b] (%)	CLint human/mouse (µL/min/mg protein)	*Eh*[c] *human/mouse*
60	3.2	19–38	91.8 [b]	18.7/76.9	*0.51/0.77*

- Search for completely new scaffold lacking SMe

SPROUT used to scaffold hop to new scaffold which preserves same interactions

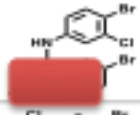
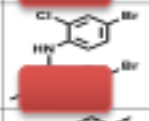
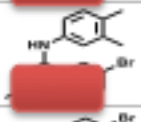
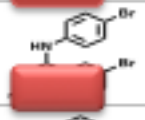
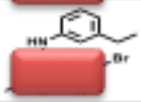
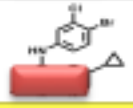
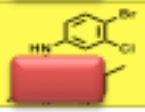
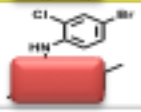
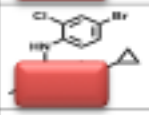
- New scaffold identified initial compounds show reasonable activity (ca 1μM against PfDHODH
- Structure based hit optimisation – 35 compounds synthesised by novel synthetic route

New Synthetic Route



Reagents and conditions: a) NIS, TFA, DMF, 50 ° C, 3h b) 3-butyn-2-ol, CuI, $Pd(PPh_3)_4$, NEt_3, toluene, 55° C, 48h,c) MnO_2, DCM, 72h d) arylamine, H_2SO_4, EtOH, 80° C

New Scaffold Assays – PfDHODH & Pf parasite inhibition

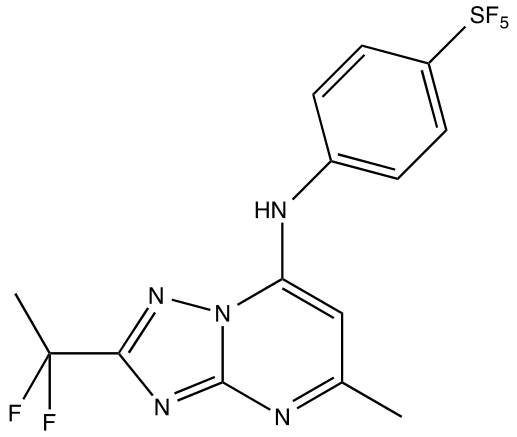
Compound ID	Structure	*Pf*DHODH IC_{50} (nM)	Average 3D7 Parasite EC_{50} (nM)
FC208		29 ± 8	20 ± 2
FC209		82 ± 9	>1000
FC210		29 ± 3	5.8 ± 0.5
FC211		19 ± 5	11 ± 2
FC245		22 ± 2	25.0±8.0
FC253		40 ± 35	160 ± 30
FC256		87 ± 9	2.6 ± 0.4
FC270		16 ± 3	3.3 ± 0.7
FC292		4901 ± 2347	710 ± 290
FC294		11744±4055	>1000
CAA-23		311 ± 16	340 ± 40

Substituents at 2-position of aniline moiety kill activity

Substituents at 3- and 4- positions enhance activity

Only small (Cl, Br, Me, CF3) substituents tolerated in nucleus

Current leading PfDHODH inhibitor – DSM265



IC_{50} *Pf*DHODH 33nM
EC_{50} *Pf* 3D7 cells 46nM
Excellent PK profile
Good in vivo efficacy (SCID mouse model)
Successfully completed phase 1 clinical trial

Coteron, J. M.; Marco, M.; Esquivias, J.; Deng, X.; White, K. L.; White, J.; Koltun, M.; El Mazouni, F.; Kokkonda, S.; Katneni, K.; Bhamidipati, R.; Shackleford, D. M.; Angulo-Barturen, I.; Ferrer, S. B.; Jimenez-Diaz, M. B.; Gamo, F. J.; Goldsmith, E. J.; Charman, W. N.; Bathurst, I.; Floyd, D.; Matthews, D.; Burrows, J. N.; Rathod, P. K.; Charman, S. A.; Phillips, M. A. Structure-guided lead optimization of triazolopyrimidine-ring substituents identifies potent Plasmodium falciparum dihydroorotate dehydrogenase inhibitors with clinical candidate potential. J Med Chem 2011, 54, 5540-5561.

Inhibitor testing against cultured Dd2 *Plasmodium falciparum* and resistant mutants (Cyber Green assay)

	IC50 (nM ± SEM, n=3) (fold increase)			
	Dd2	R1A	R2B	R3B
DSM265	240 ± 12 (1)	9600 ± 210 (40)	3400 ± 230 (14)	3700 ± 160 (16)
RJ65	3.8 ± 1.3 (1)	47 ± 17 (12)	84 ± 21 (22)	50 ± 18 (13)
RJ81	650 ± 57 (1)	>10000 (>15)	>10000 (>15)	>10000 (>15)
RJ89	900 ± 36 (1)	>10000 (>11)	8600 ± 1100 (9.6)	>10000 (>11)
MJM182	0.20 ± 0.03 (1)	46 ± 16 (230)	16 ± 3.7 (81)	12 ± 3.5 (60)
Artemisinin	2.0 ± 1.5 (1)	5.3 ± 10 (2.7)	6.9 ± 2.1 (3.5)	5.3 ± 3.5 (2.7)
Mutations	C276F	R265G	G181D	
		L531F	E182D	
				C276Y

Resistant strains from David Fidock (Columbia University)

Potent pfDHODH inhibitors which also show comparable activity against Pf parasite

What comes next?

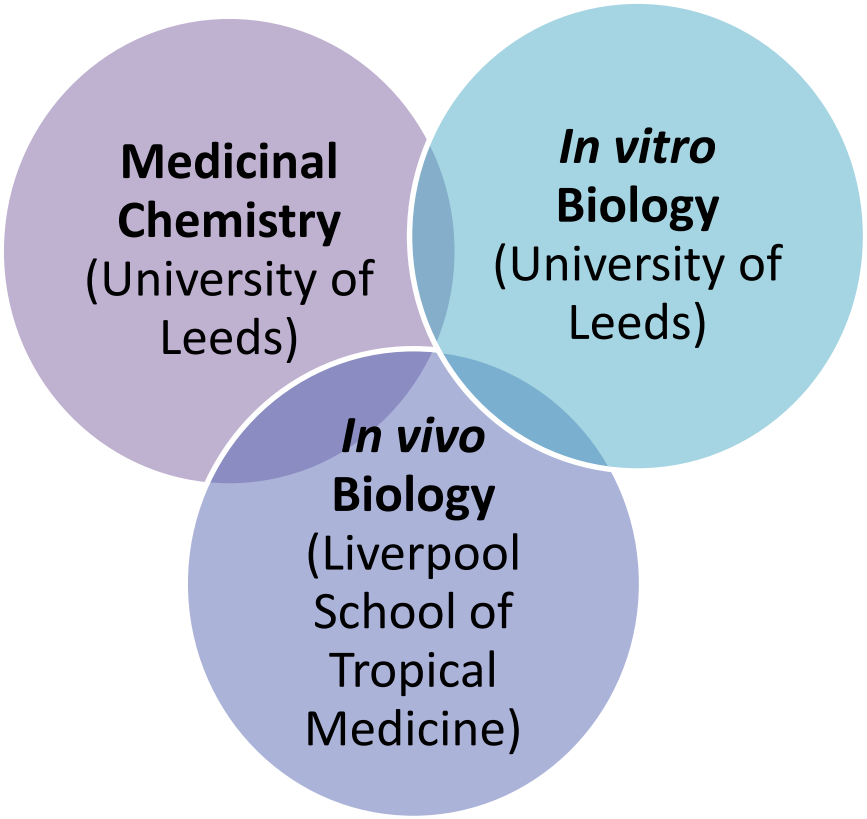
- Optimisation of PK etc
- Special attention to prolonging lifetime in body
- In vivo assays (P Berghei and SCID model)

Team Leaders

Prof Peter Johnson

Prof Colin Fishwick

Prof Philip Kocienski FRS



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