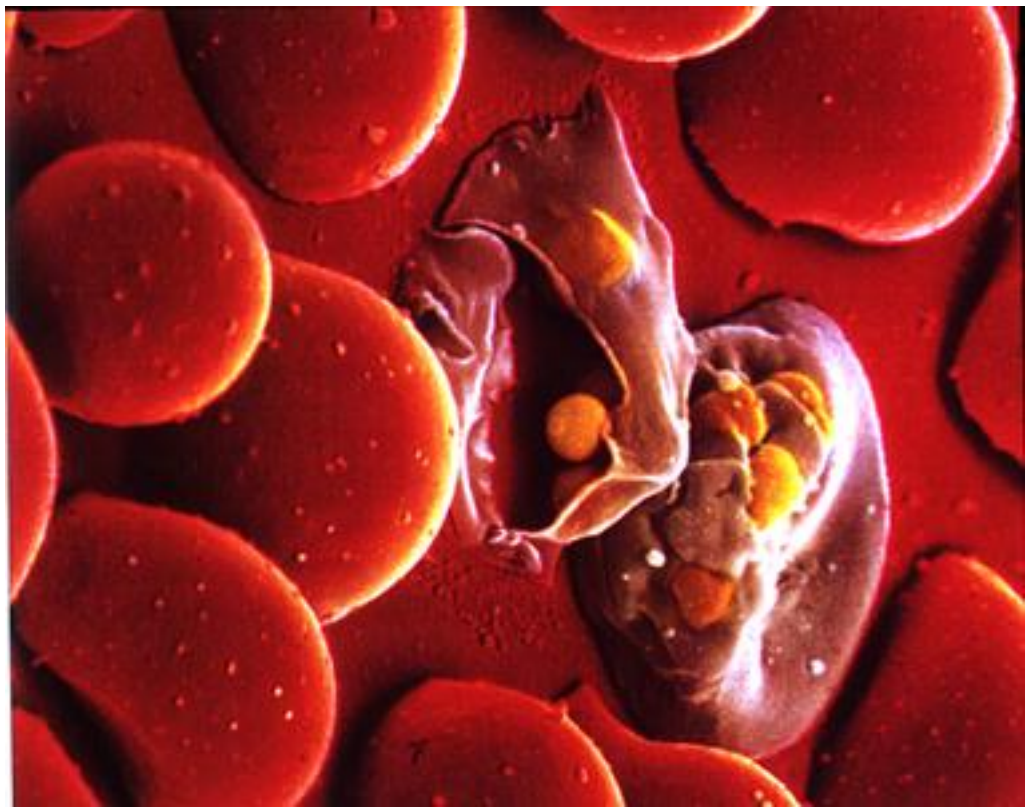
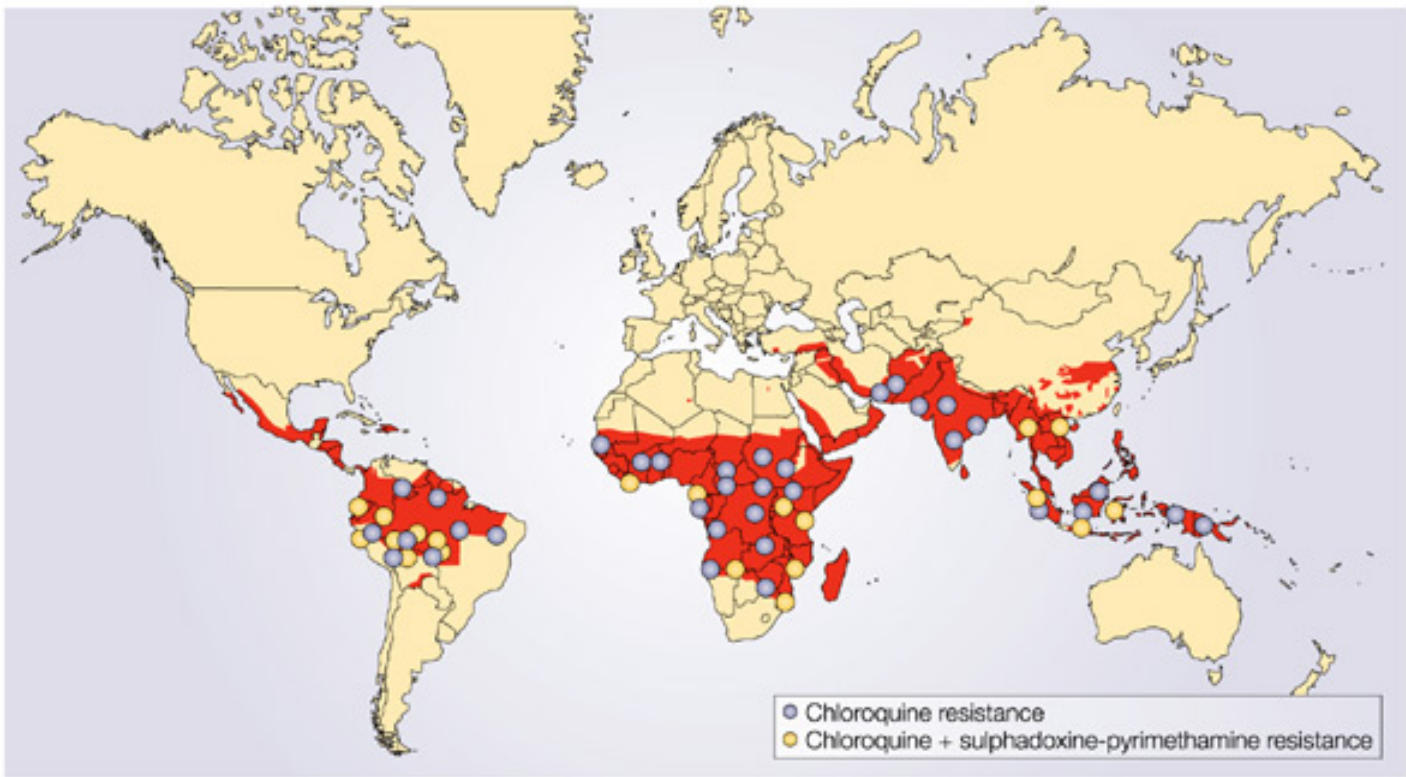
Anti-malarial Drug Discovery

Jeffrey Baldwin, PhD
UT Southwestern Medical Center
Department of Pharmacology



Malaria Burden



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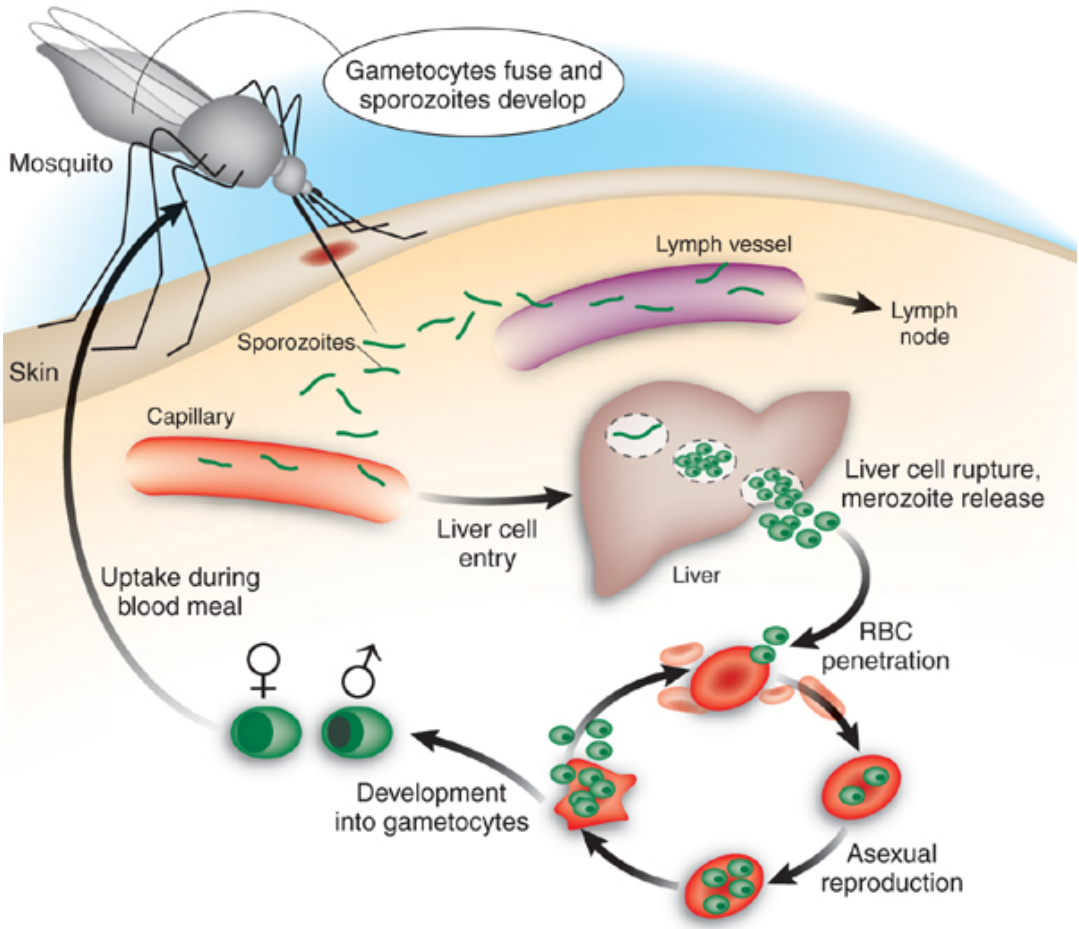
Life Cycle

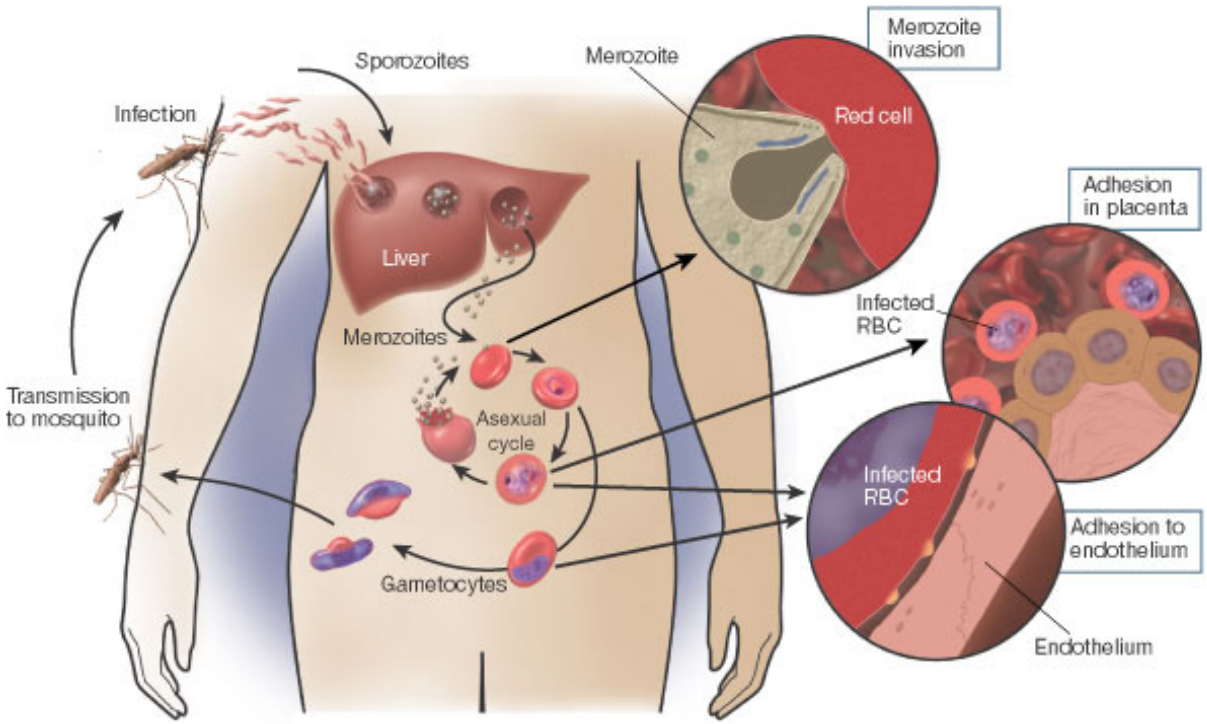


Jones & Good, Nature Medicine (Feb. 2006)

Complex life cycle

Intracellular parasite

Pathogenesis



Miller *et al*, Nature (7 Feb, 2002)

4 *Plasmodium* species are human pathogens:

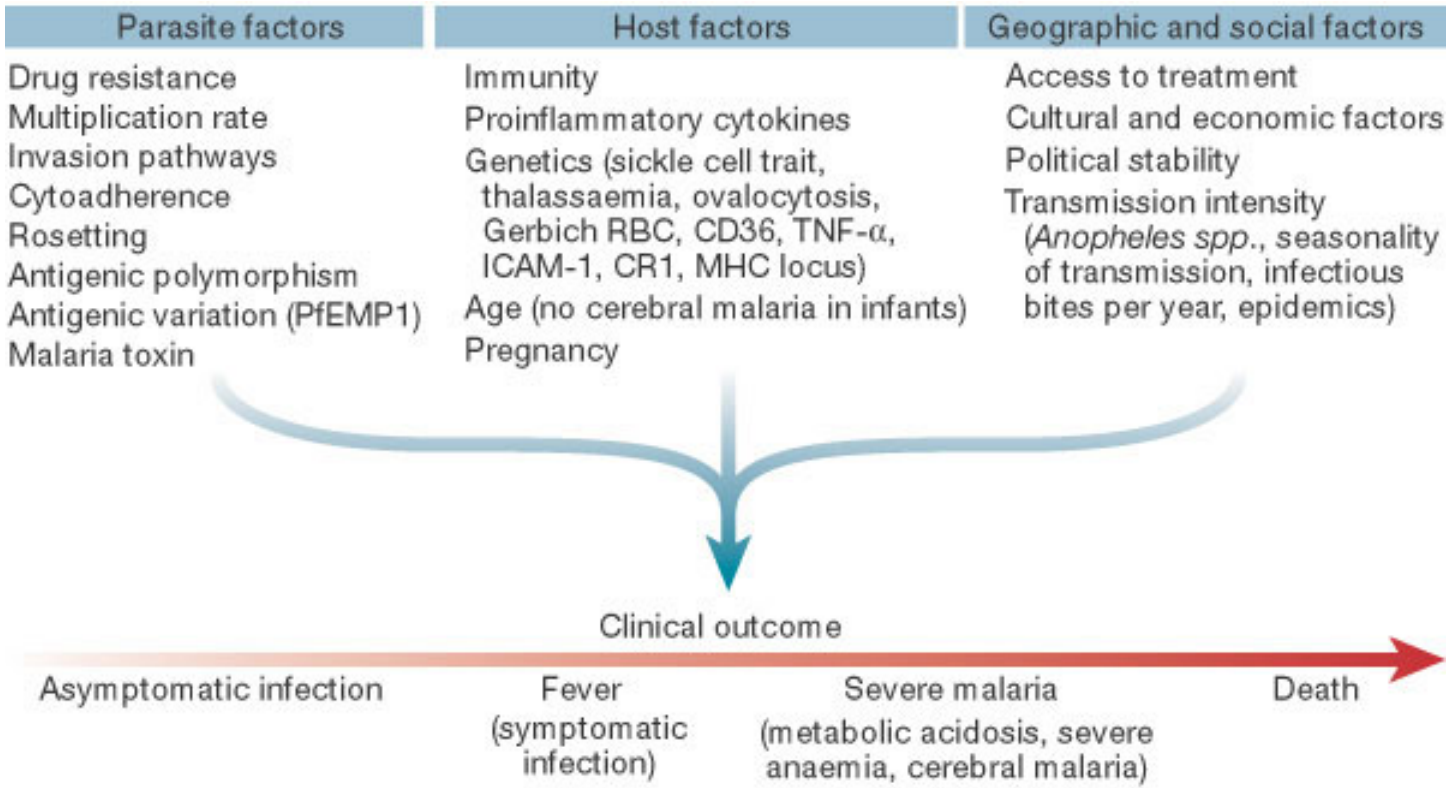
Falciparum

Vivax

Ovale

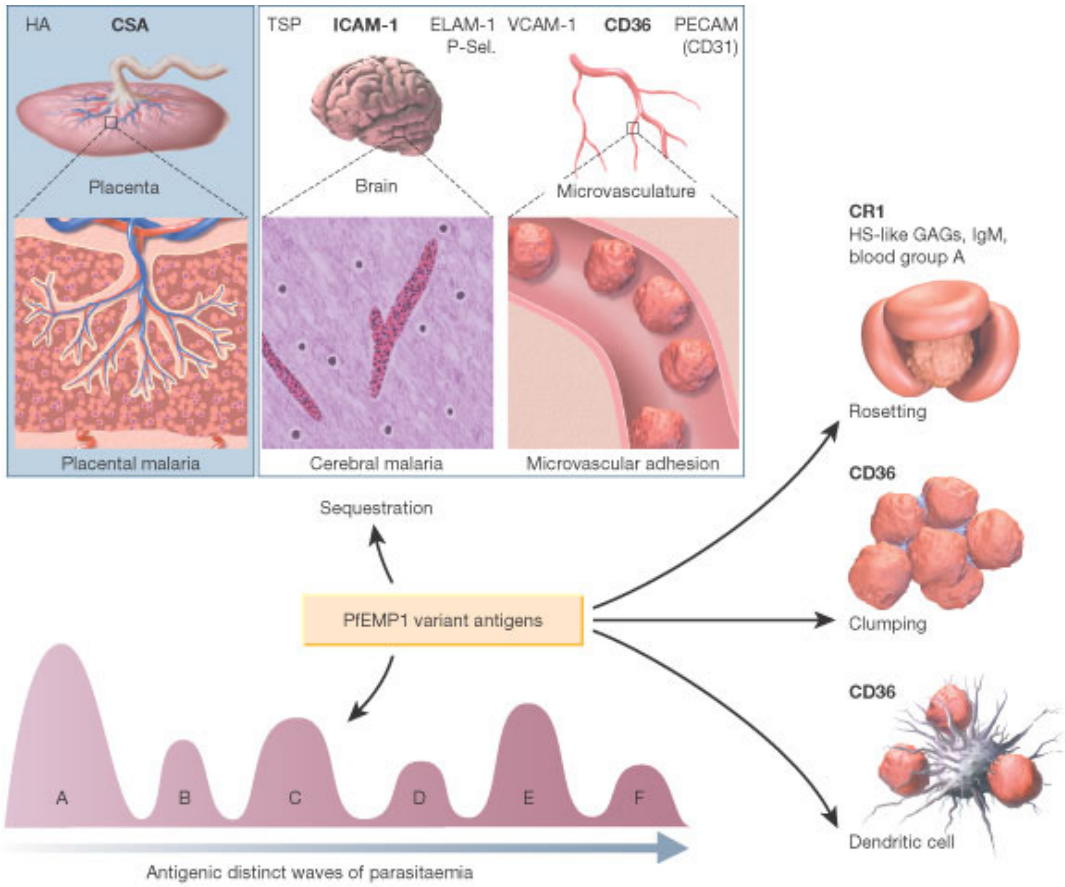
Malariae

Clinical Outcomes



Miller *et al*, Nature (7 Feb, 2002)

Immune Evasion

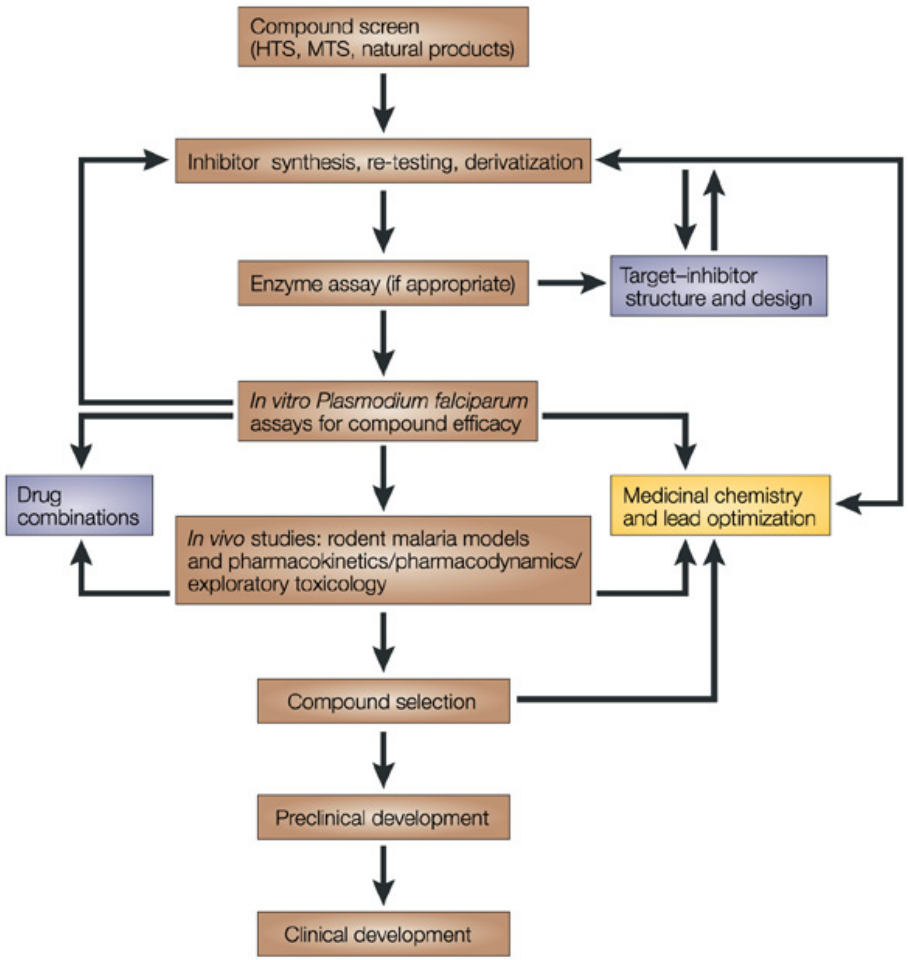


*Pf*EMP1 is key to lethality of *P. falciparum*

Sticky molecules evade immune response and clog capillaries

Miller *et al*, Nature (7 Feb, 2002)

Discovery Process



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I. Rational Drug Design

requires detailed structural information

II. Combinatorial Chemistry

compound diversity is based on a core structural template

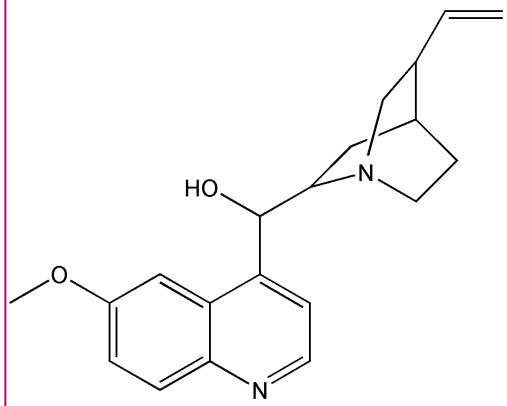
III. Compound Libraries

screen compounds with diverse chemical properties

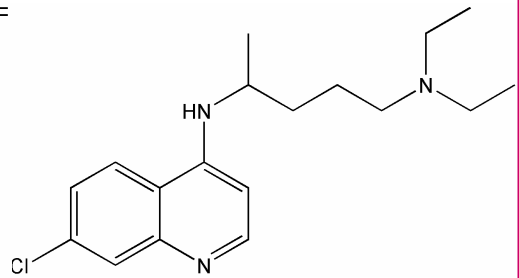
identification of novel "scaffolds"

Why more anti-malarials?

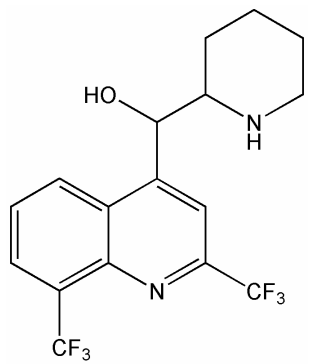
Quinoline and related antimalarials



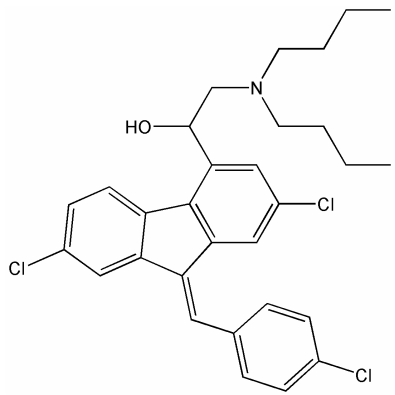
Quinine



Chloroquine

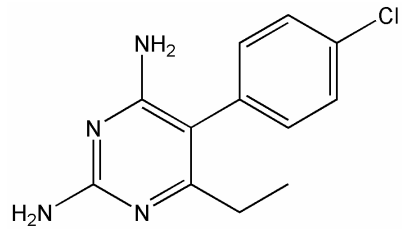


Mefloquine

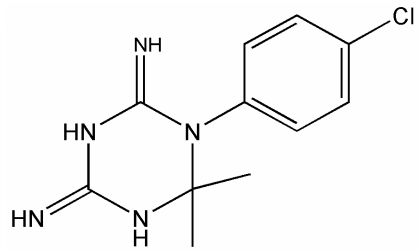


Lumefantrine

Antifolates

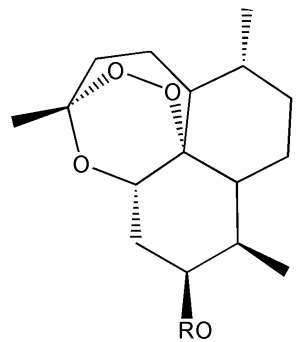


Pyrimethamine

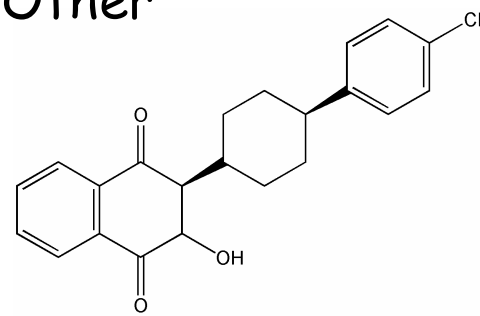


Proguanil

Other

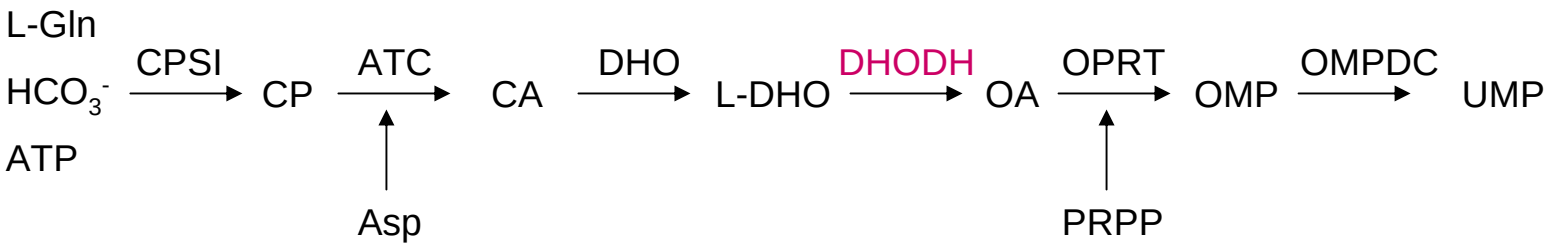


Artemisinin



Atovaquone

Pyrimidine Biosynthesis in *Plasmodium falciparum*

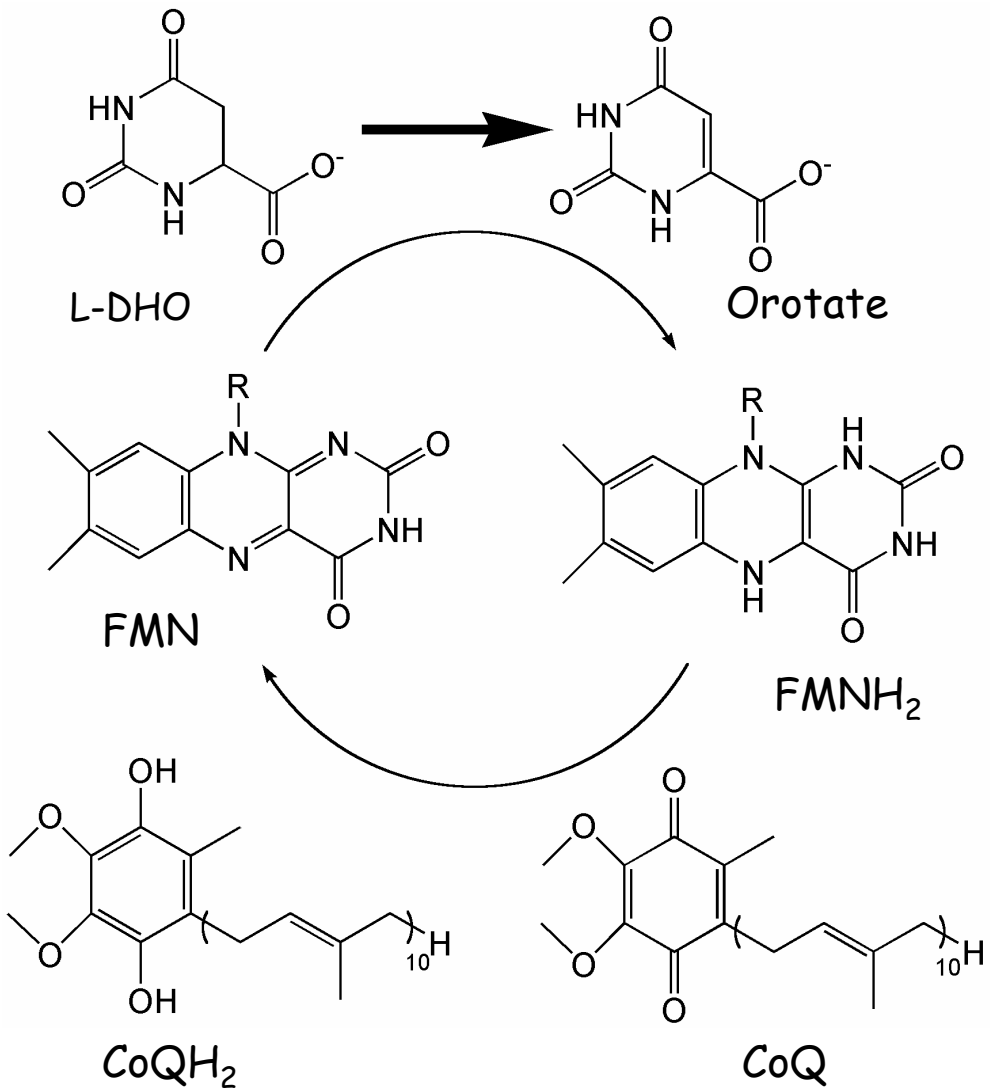


Malaria parasites rely exclusively on *de novo* pathway whereas the human host is also capable of salvage

Inhibitors of pyrimidine biosynthesis are proven drugs, eg. TS and DHFR

These data suggest other enzymes in the pathway are also essential and therefore represent potential drug targets

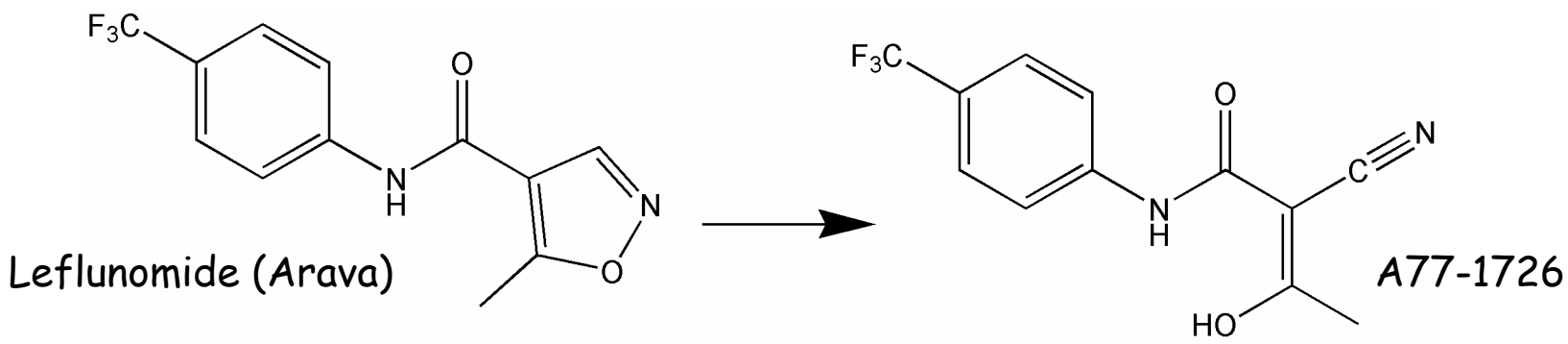
DHODH as a drug target against Malaria



DHODH catalyzes the FMN-dependent oxidation of L-dihydroorotic acid

Malarial DHODH is mitochondrial and is rate limiting in the synthesis of UMP

Human DHODH is a validated target



DMARD approved for treatment of rheumatoid arthritis

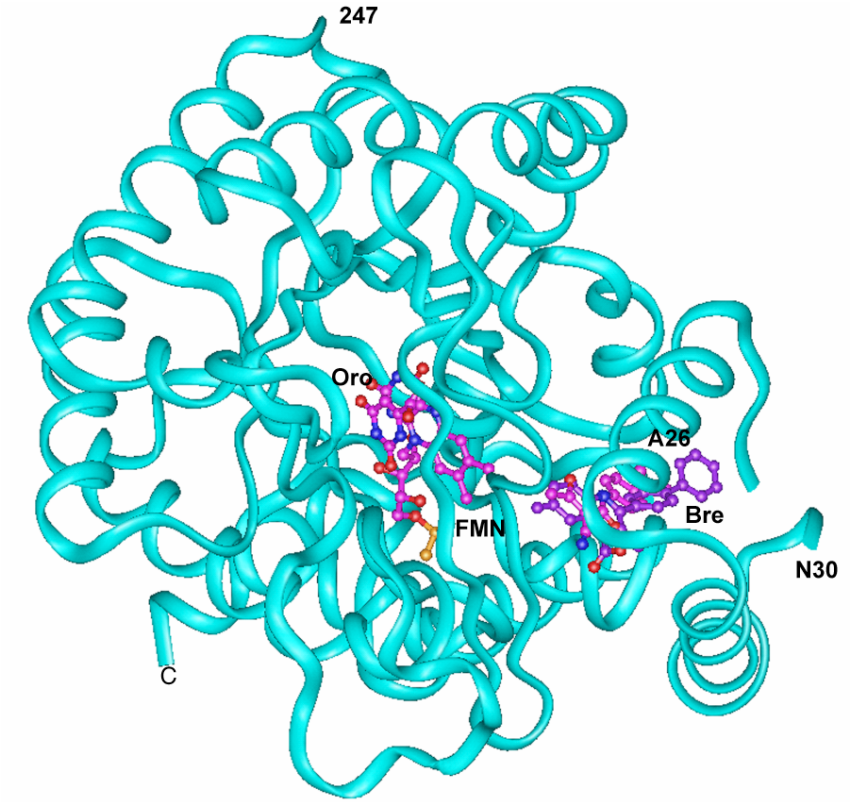
Mode of Action: Inhibition of DHODH

-DHODH is major binding protein of A77-1726

-uridine reverses growth toxicity effects

Selectivity based on differential pyrimidine requirements in resting versus activated T/B cells

X-ray structure of human DHODH



Truncated human DHODH (Δ30)

Two domains:

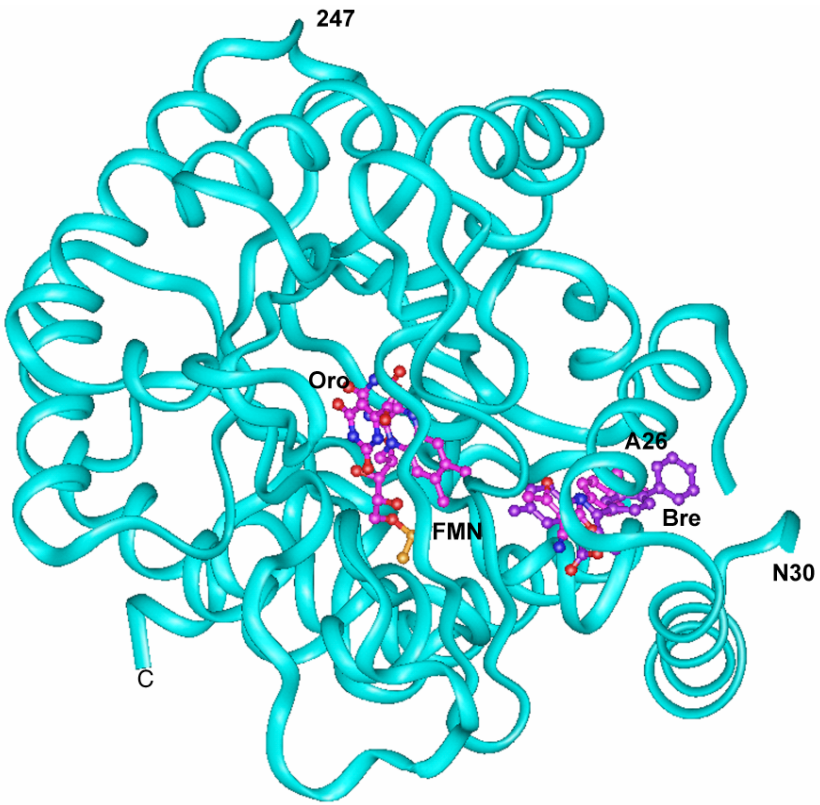
1. TIM barrel

   -orotate and FMN binding sites

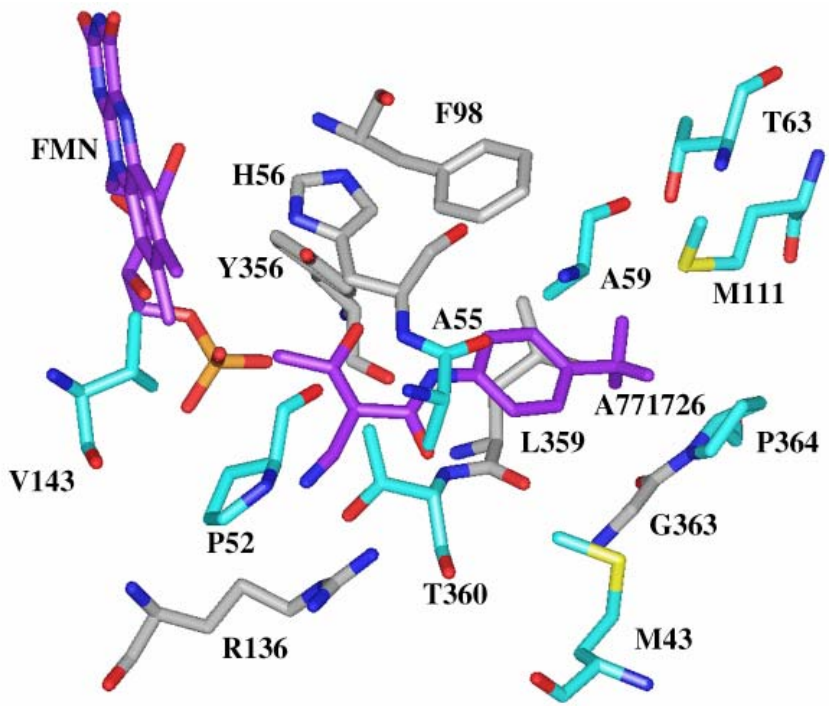
2. α-helical domain forms tunnel opening leading to the active site

The A77-1726 and brequinar binding site is in a channel formed by the helix region and is the site of binding for the CoQ substrate

Active site of DHODH is variable



Inhibitor binding site is not conserved in malarial enzyme—selective inhibition is feasible



Grey residues are conserved between human and malarial enzymes

Validation of active site selectivity

Confirm species selective inhibition between human and malarial enzymes

Test derivatives of existing scaffolds that inhibit DHODH from other species,

- A77-1726 analogs
- Redoxal, DCL

Strategies to identify malaria DHODH specific inhibitors

Strong species selectivity as predicted from structure and sequence alignments

Existing inhibitor scaffolds will require a significant chemistry effort to improve activity on the malarial enzyme

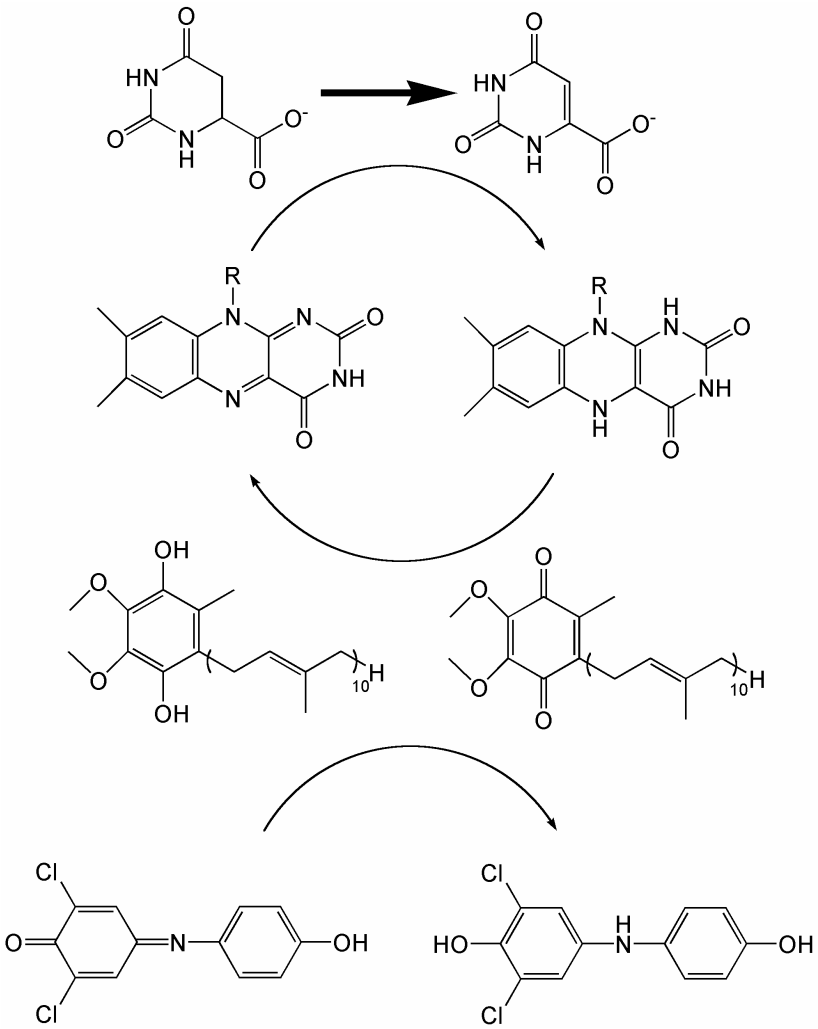
Search for novel scaffolds

High-throughput screening of a small molecule library

High-throughput Screening (HTS)

- Small molecule library consisting drug-like compounds
- Automated screening of molecules for inhibition of enzyme activity
- Assay Requirements
  - Simple, robust, and reproducible
  - End-point
  - Reliable method of detection

DHODH HTS Assay



DCIP
λ_{max}=600nm

$DCIPH_2$
(colorless)

Endpoint colorimetric assay

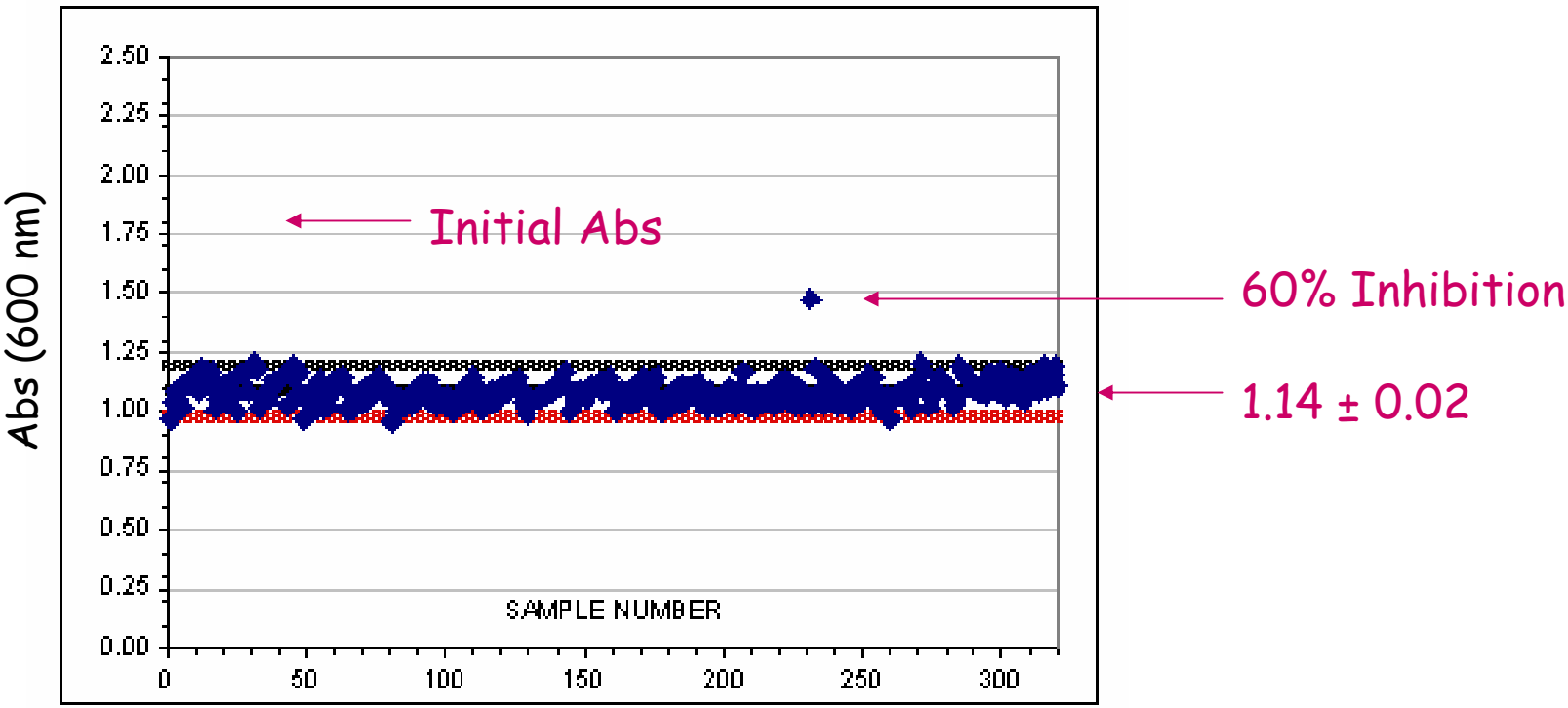
Initial HTS of malaria DHODH at 3 μM for compound collection > 200,000 small molecules

Hit was defined at > 4 SD from the mean

Screen 12,800 per day

DHODH HTS Results

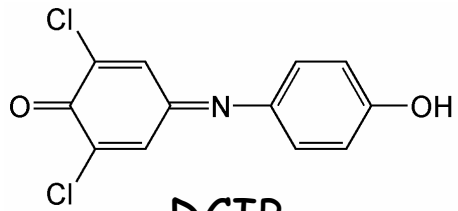
Representative 384-well plate from HTS



1350 compounds were identified as 'hits' from the primary HTS

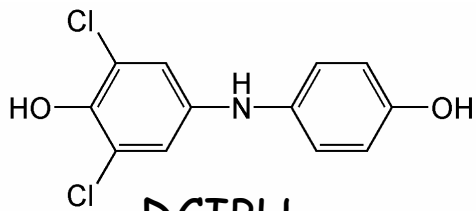
DHODH HTS Strategy

End-point calorimetric assay



DCIP

λ_{max}=600nm



$DCIPH_2$
(colorless)

1350 hits from initial screen were tested at 0.12, 0.6, and 3 μM for both malaria and human DHODH enzymes

DHODH HTS Results

63 compounds were identified with IC_{50} values less than 600 nM for pfDHODH

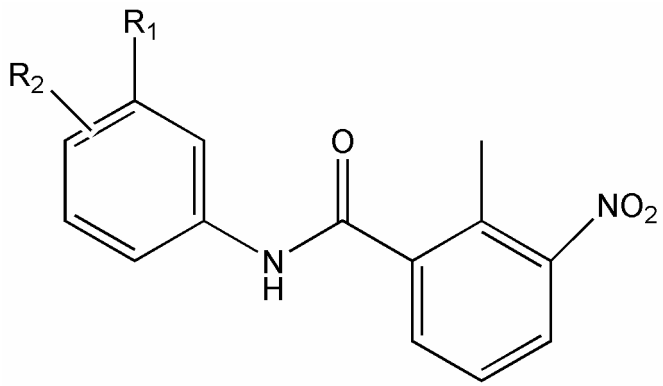
-all but one displayed selective binding to the malarial enzyme

~30 compounds fall into related structural classes

1. Halogenated phenyl benzamide/naphthamides
2. Urea-based naphthyl or quinolinyl compounds

-remaining compounds identified with novel scaffolds

General classes of HTS hits



#	R_1	R_2	IC_{50} μM	fold
4	Br	H	.06	1800
5	Cl	F	.10	1200
6	Cl	Cl	.016	12500
7	F	F	.26	770
9	Cl	Cl	.08	900
10	CF_3	H	.08	1900

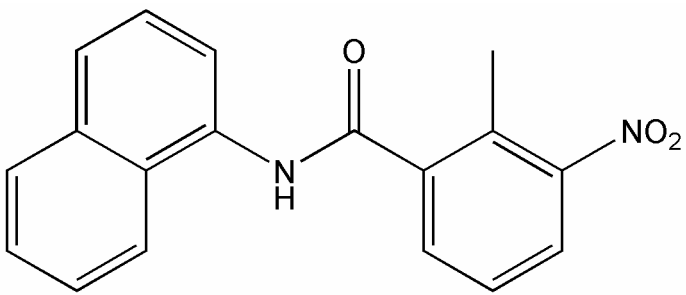
Biphenyl amides:

-Reversible enzyme inhibitors

-IC_{50} = 20 – 300 nM pfDHODH

-900 - 20,000 fold selective

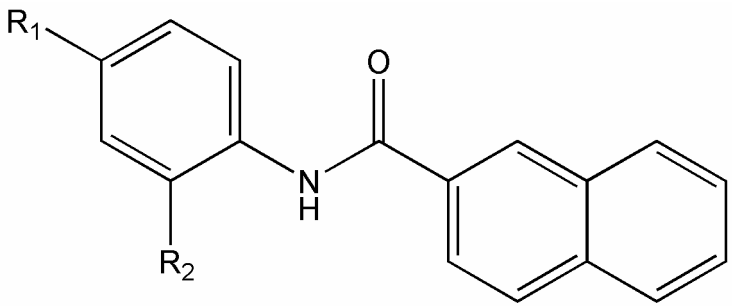
General classes of HTS hits



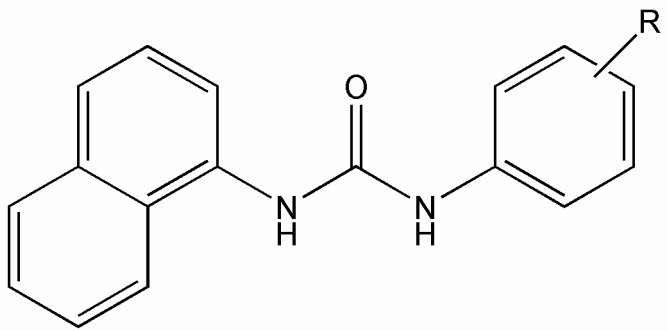
Naphthyl phenyl amides:

-IC_{50} = 50 – 500 nM *pf*DHODH

-70 – 4,000 fold selective



R_1, R_2 = halogen, H, or CH_3



Napthyl phenyl ureas:

-IC_{50} = 200 – 800 nM *pf*DHODH

-150 – 2,000 fold selective

R = halogen, CF_3, OCH_3

SAR analysis of pfDHODH Inhibitors

-aromatic rings

-prefers amide bonds

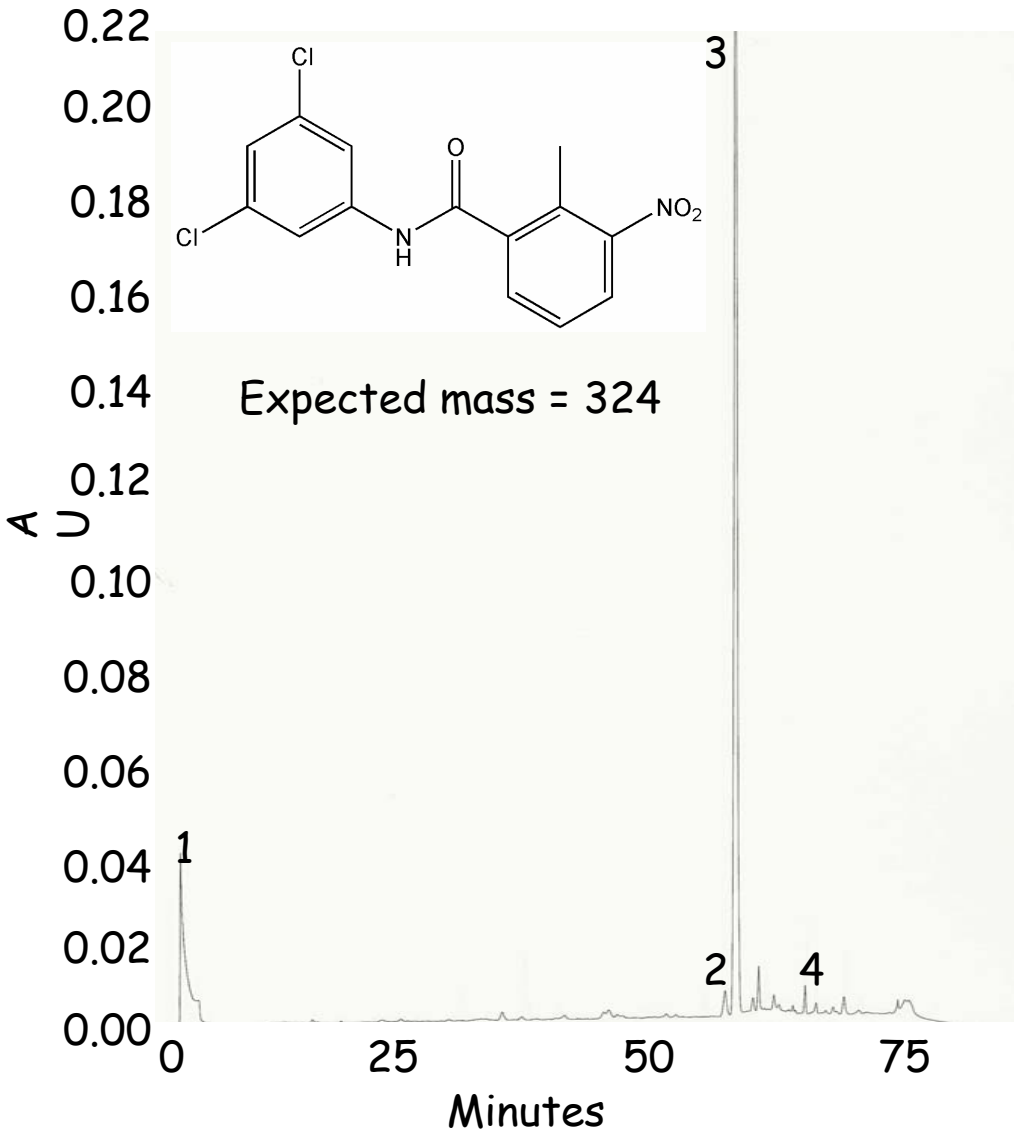
-tolerates variable size and substituents on one ring

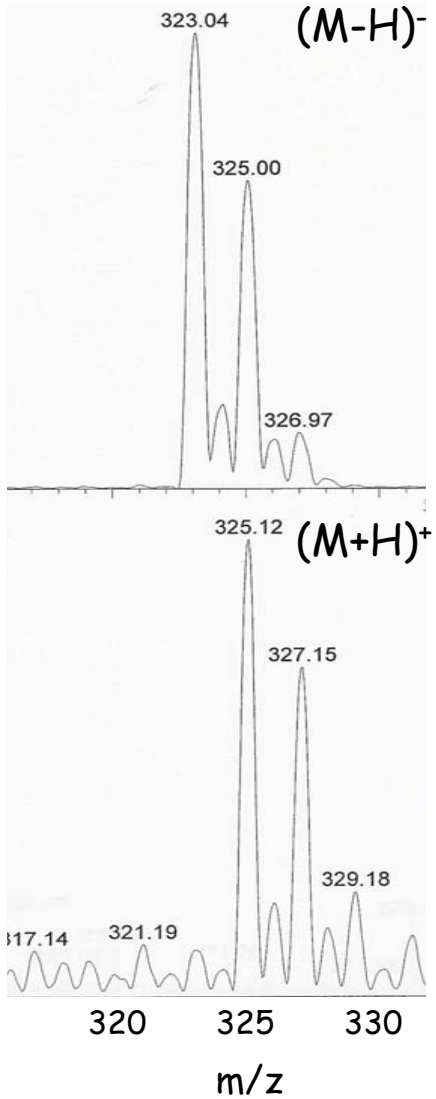
-has a strong preference for 2,3-methyl-nitro substituents

-selectivity increases with potency

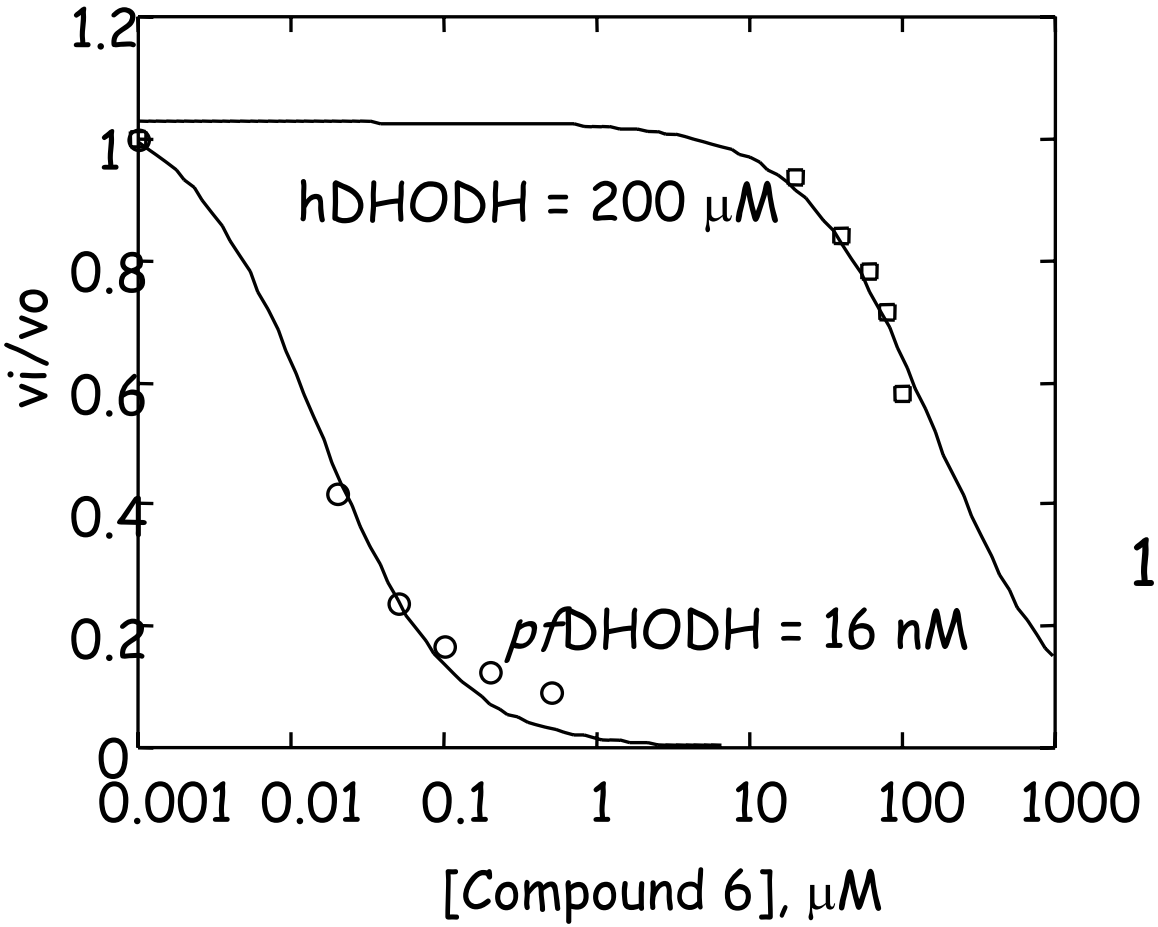
Reconfirm and validate hits

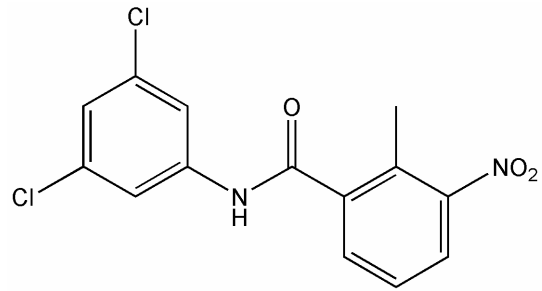
HPLC purification and MS analysis





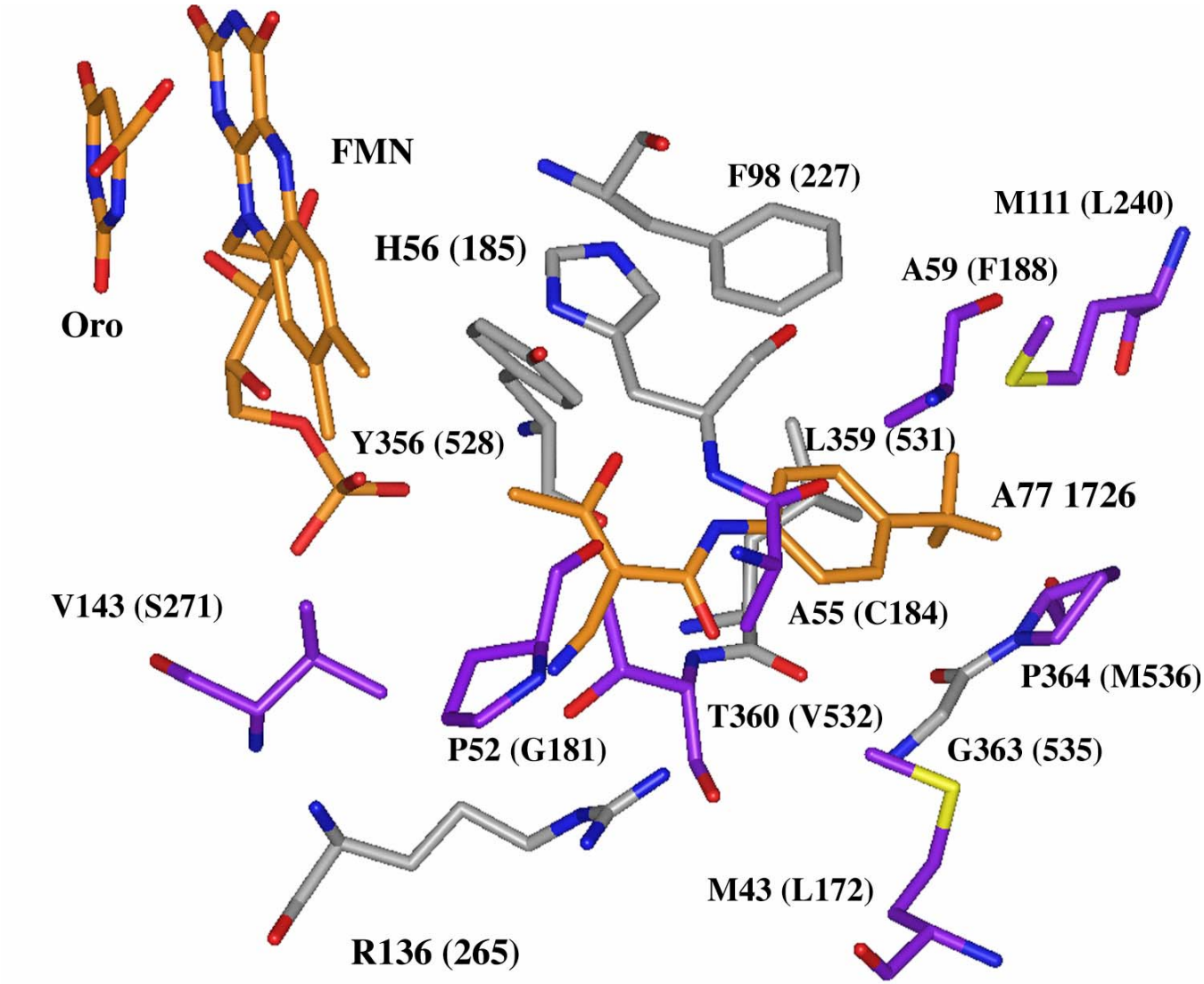
Selective inhibition of *pf*DHODH





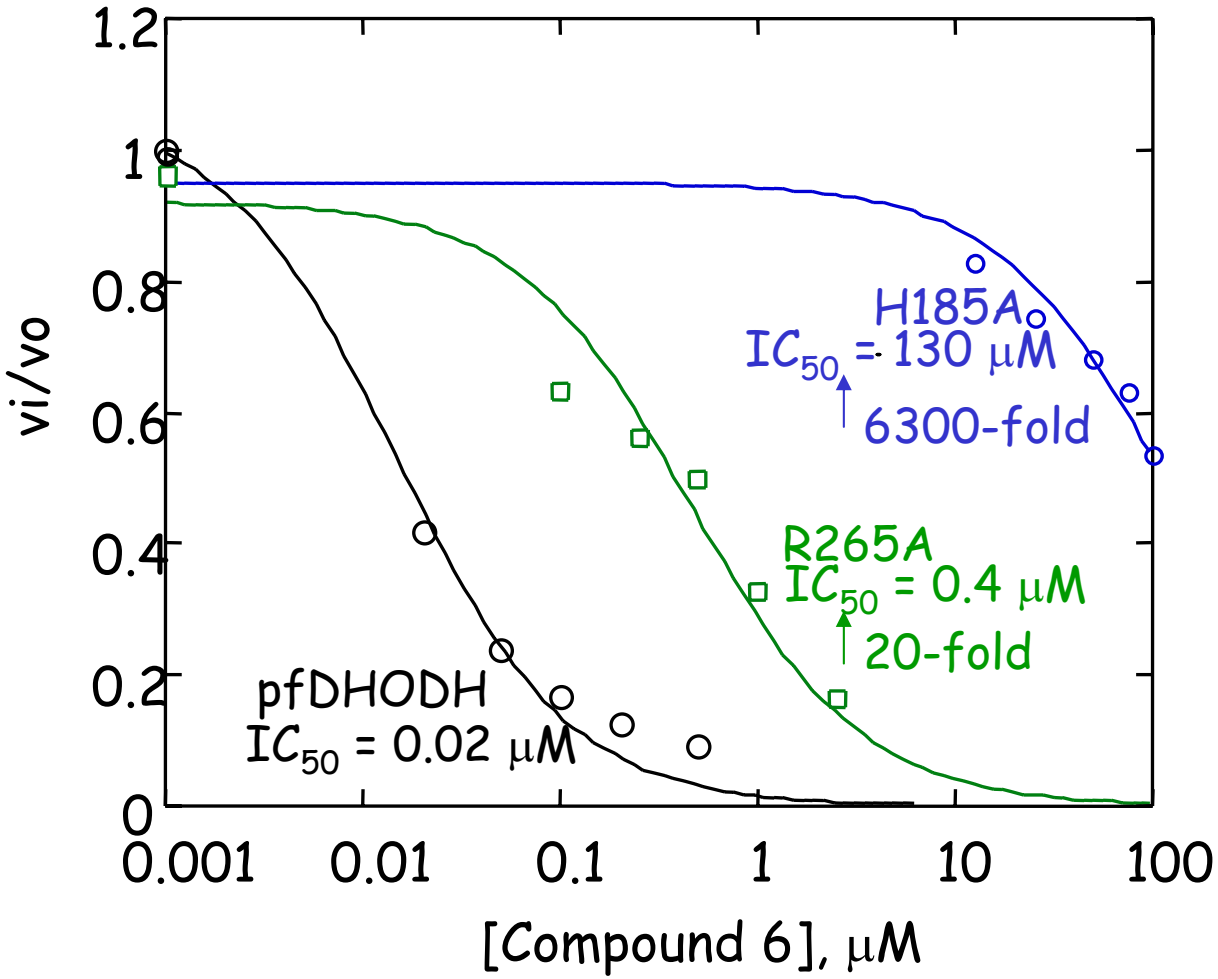
12,500 fold selective

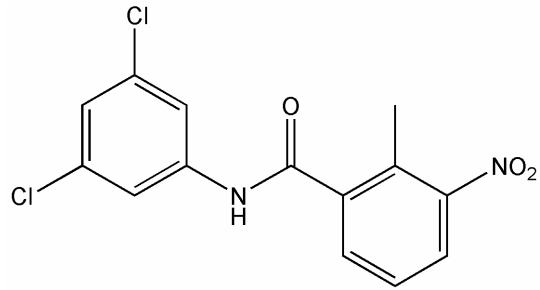
Analysis of inhibitor binding site



Grey = conserved between human and malaria enzymes

Analysis of inhibitor binding site

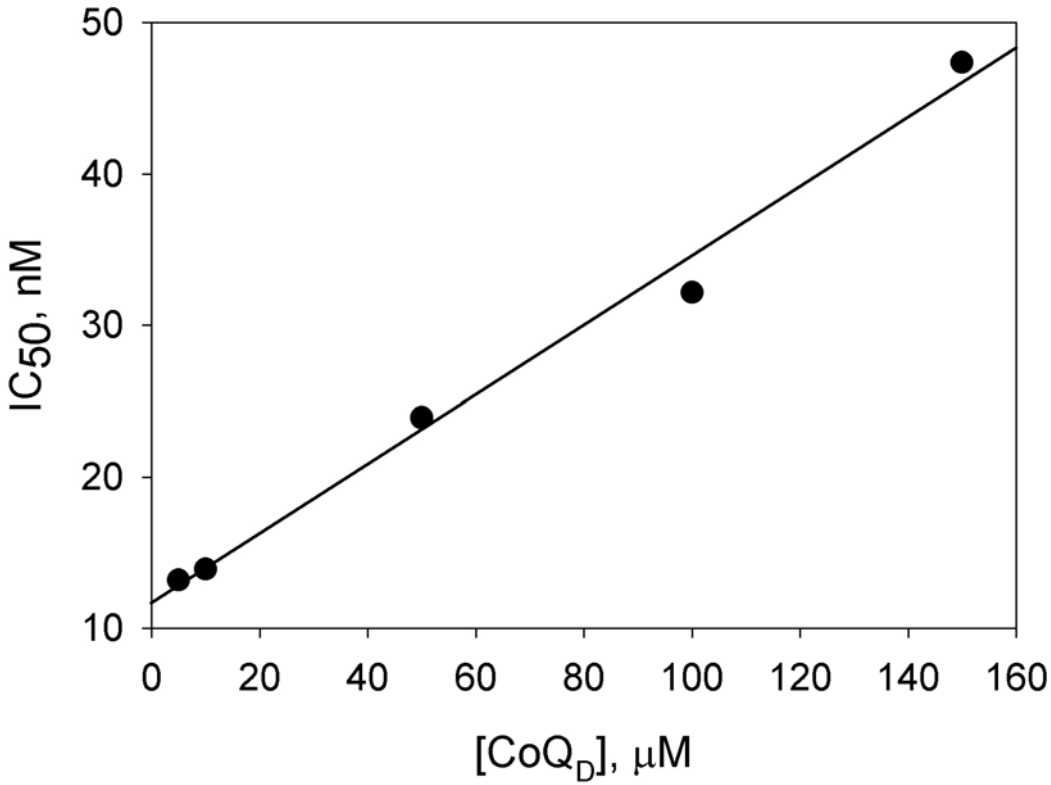


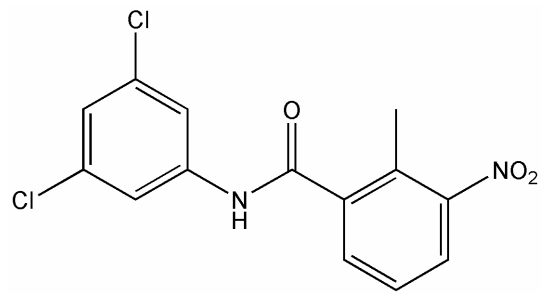


Mutation of conserved residues in the CoQ site reduces inhibitor potency

The effect is larger for H185 (20 vs. 6300 fold)

Analysis of inhibitor binding site





Compound 6 is a competitive inhibitor of CoQ

Analysis of inhibitor binding site

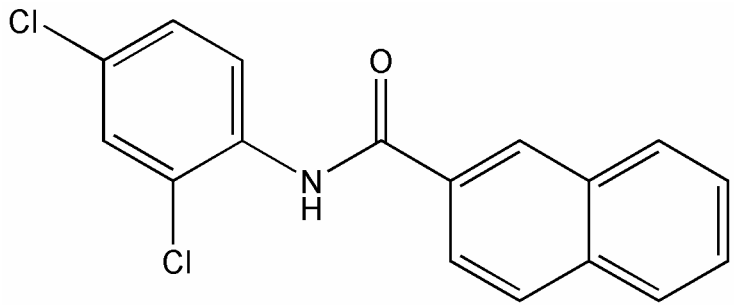
Mutagenesis data strongly suggests that these newly identified *pf*DHODH inhibitors bind the same site as the established inhibitors of hDHODH (e.g. A77-1726 and brequinar)

Kinetic analysis suggests this is also the CoQ site

Species differences in the amino acid composition of this site explain the structural basis for selective binding

The more conserved orotate site does not appear to be targeted in screen

Activity of biphenyl amides and ureas on *P. falciparum* cultures

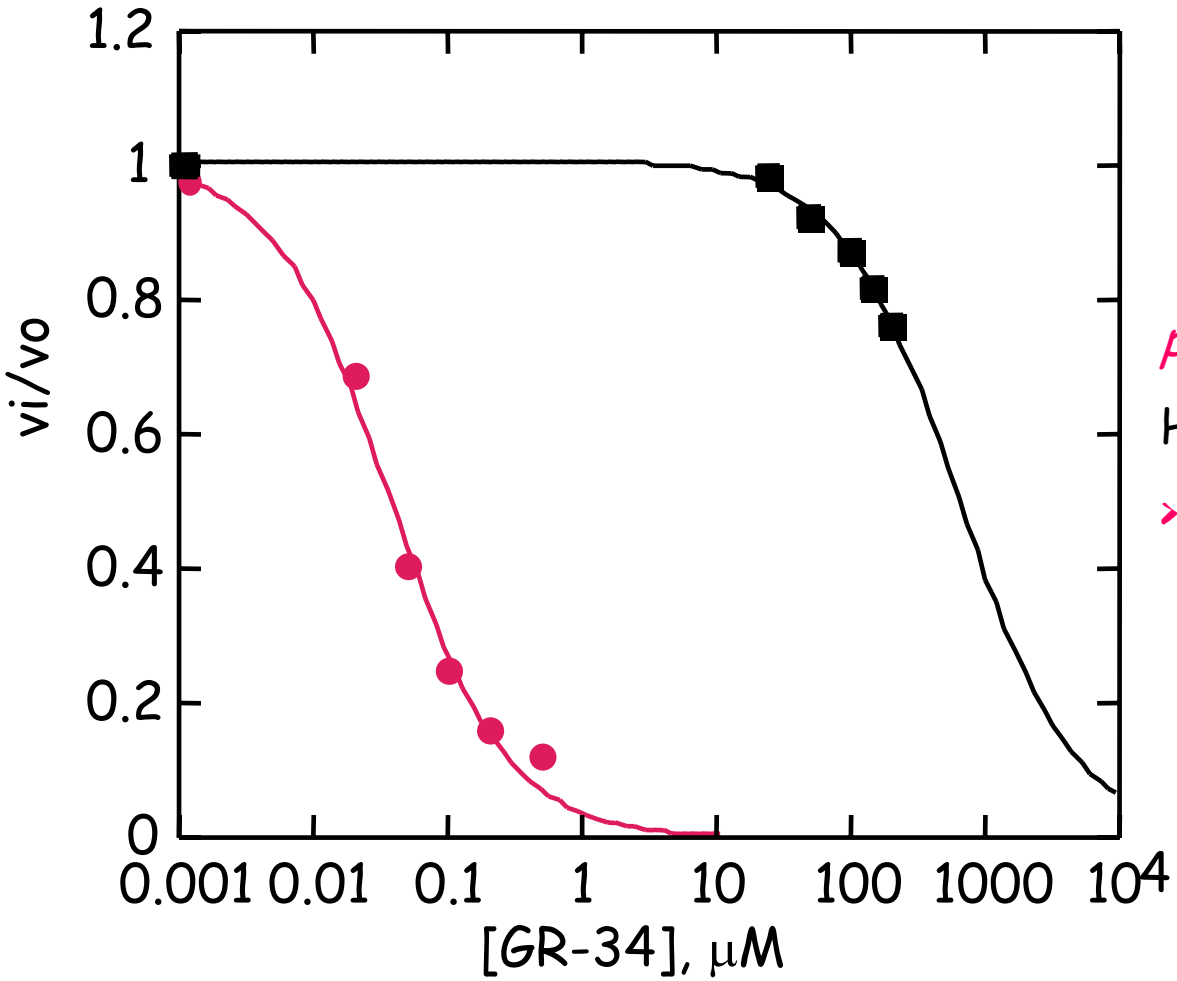


Compound 1; 20% at 10 μM

No growth inhibition observed for others up to 10 - 100 μM

Selective and potent inhibitors of the malarial enzyme

DHODH HTS Results—Selective inhibition by GR-34

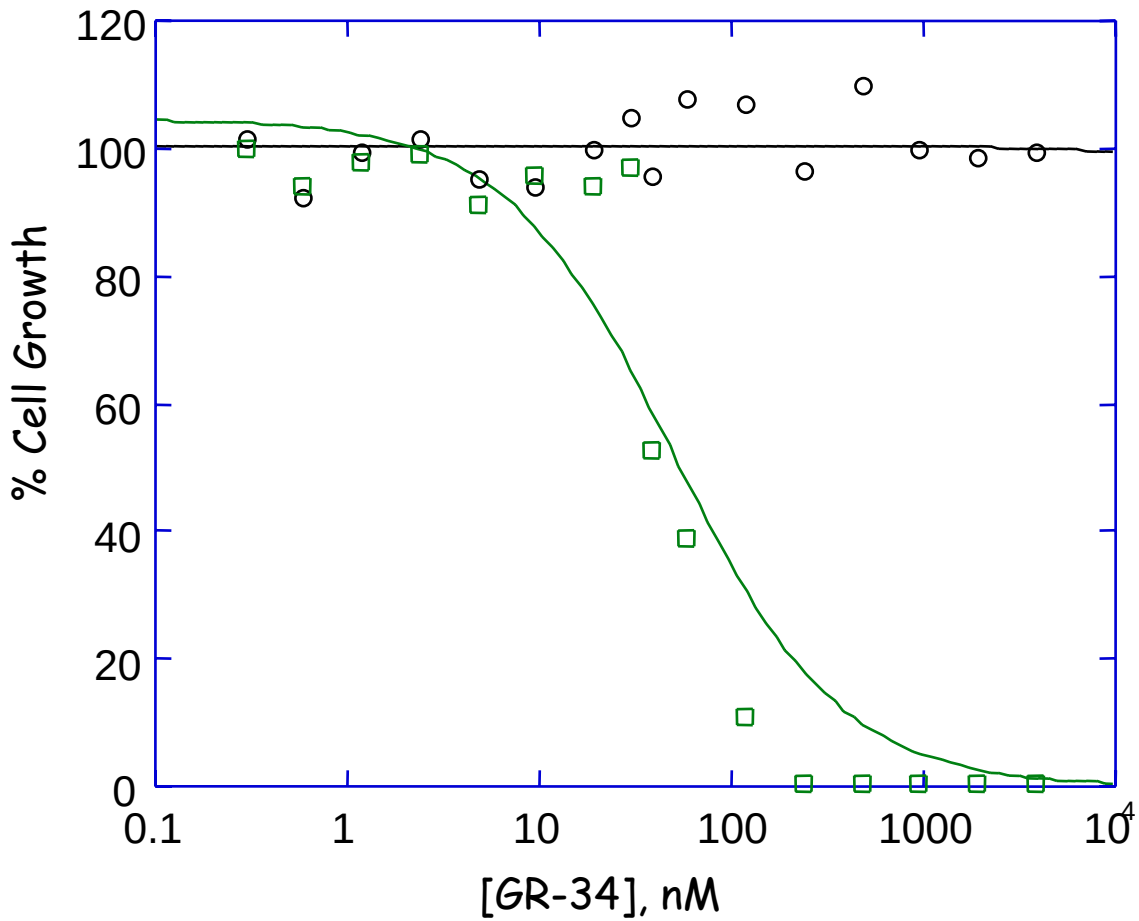


*pf*DHODH IC_{50} = 38 nM

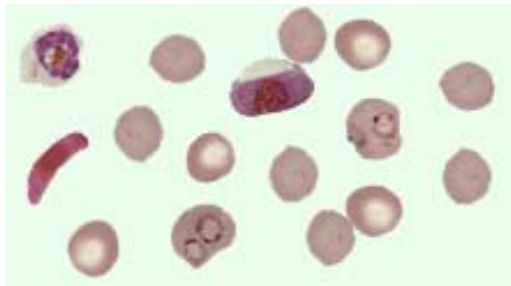
Human DHODH IC_{50} = 640 μM

> 17,000 fold selectivity

DHODH HTS Results—Activity of GR-34 on *P. falciparum* cultures



Human L1210

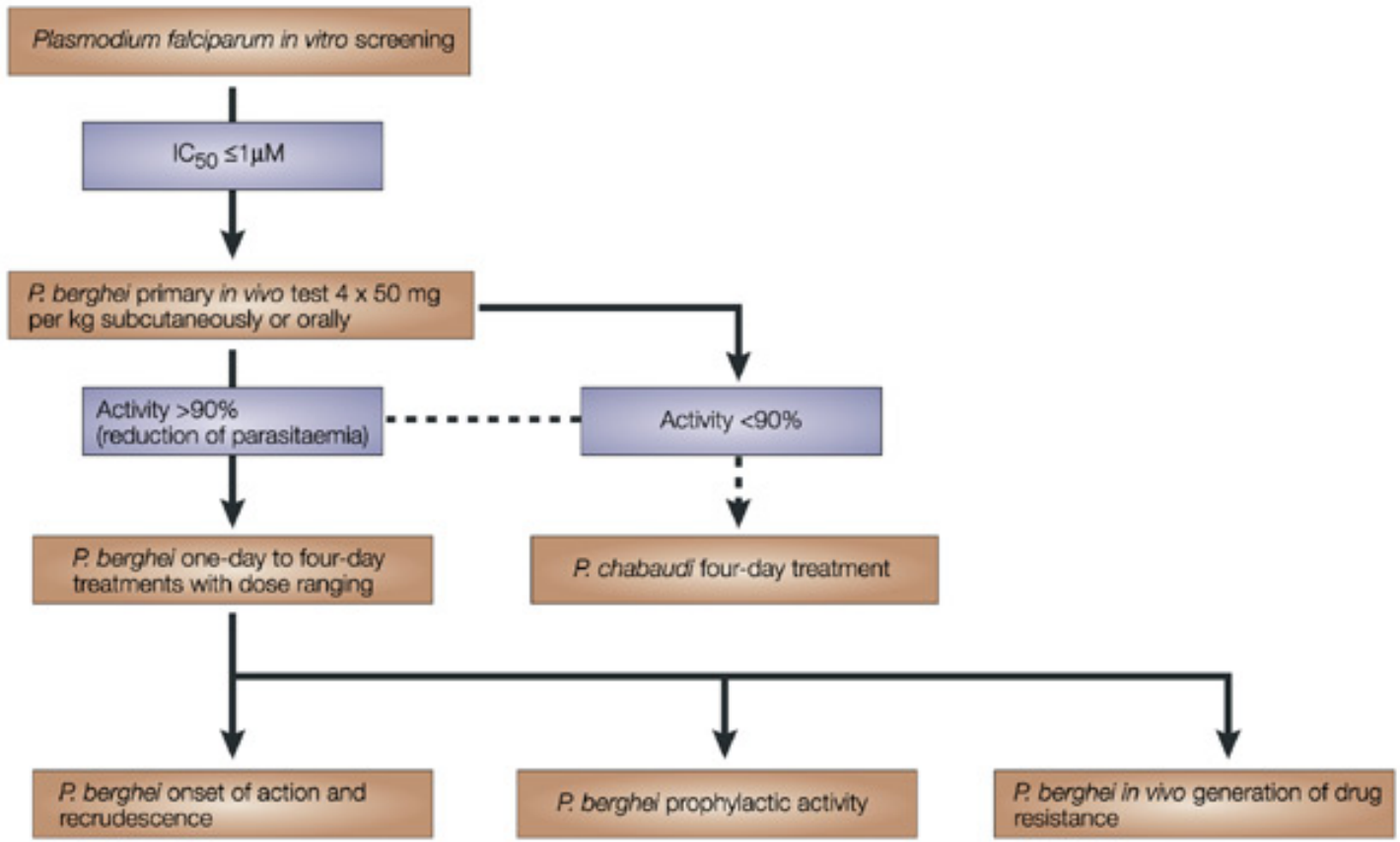


P. falciparum 3D7

EC_{50} = 60 nM

Measured by ^{3}H-hypoxanthine incorporation

In vivo Screening



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Conclusions

Identified potent and selective inhibitors of malarial DHODH

- –Inhibitors likely bind the CoQ binding site
- –Structural basis for selectivity is large sequence variations in this site between species

Identified a *pf*DHODH inhibitor that kills malarial parasites with specificity

Acknowledgments

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-Meg Phillips

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