Novel Inhibitors of Malarial Dihydrofolate Reductase (DHFR)

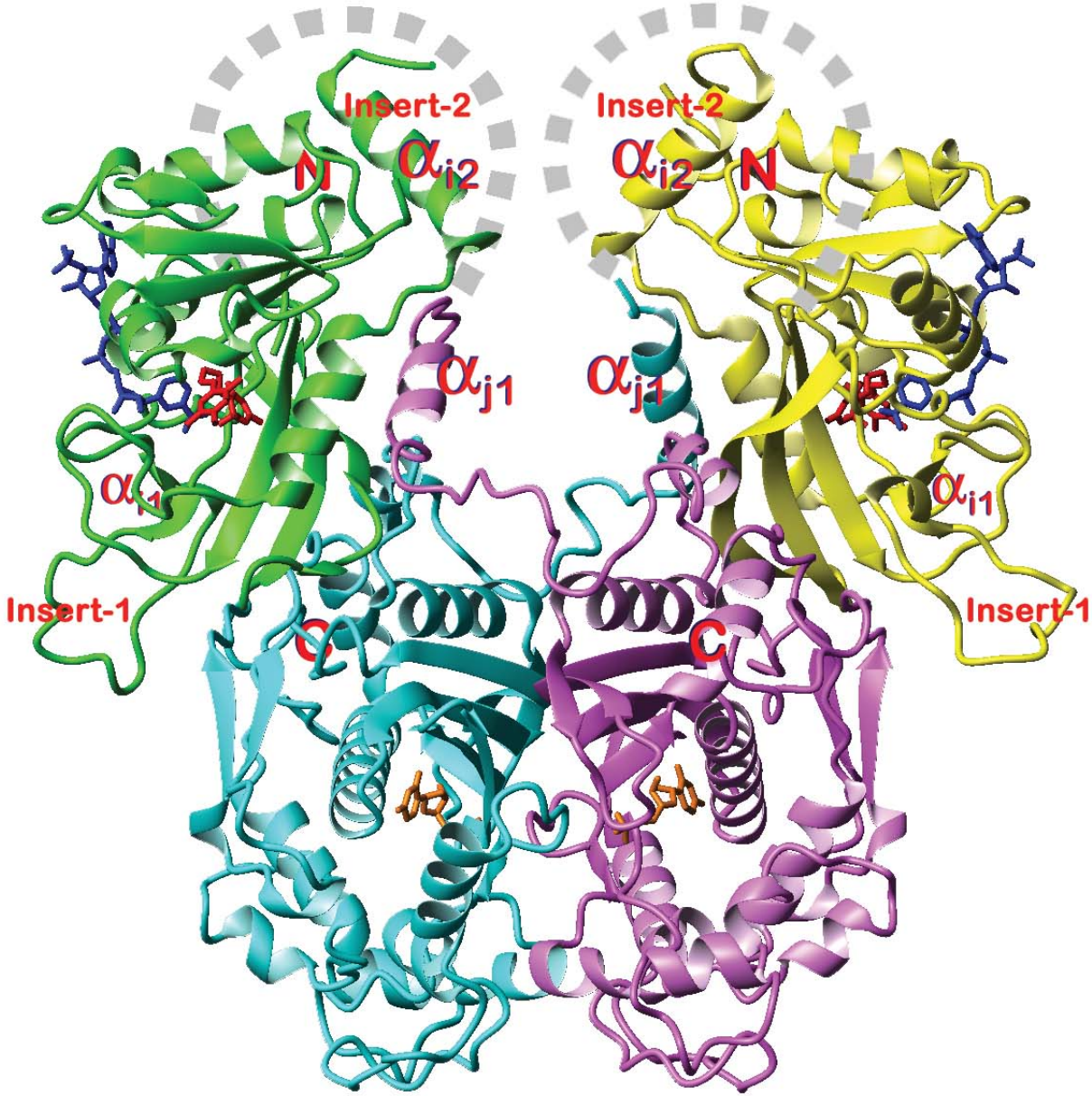
ASTMH Conference, November 5, 2007

Yongyuth Yuthavong
BIOTEC, Thailand

Medicines for Malaria Venture

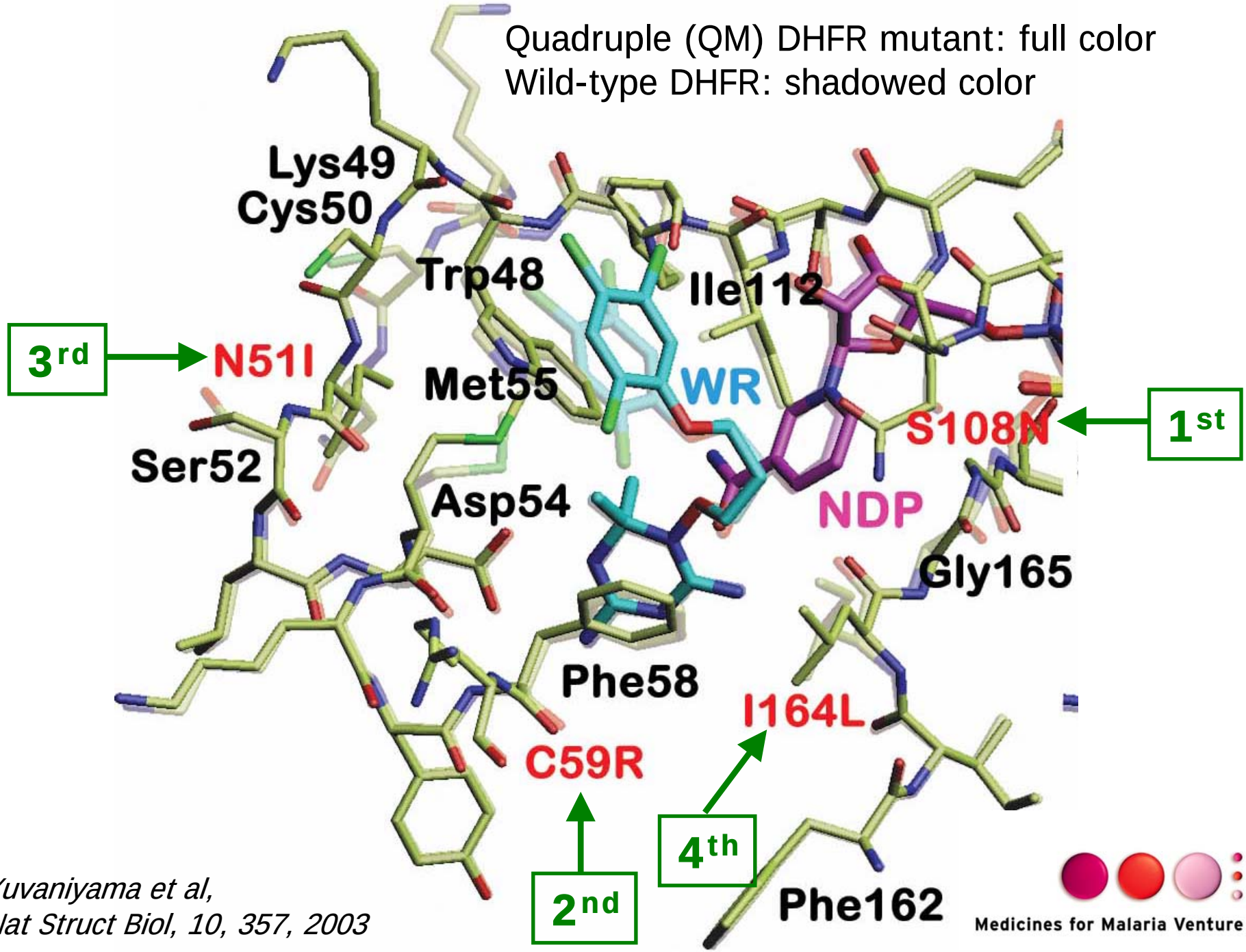
Project Rationale

- Malarial DHFR is a validated target for antimalarials
- Resistance to DHFR inhibitors due to a limited number of known single point mutations within the active site of the plasmodial DHFR enzyme
- Project Aims:
  - Design, synthesize and develop new antifolates exploiting structural knowledge of inhibitor binding to mutant enzyme
  - Identify activity and ADME limitations of inhibitor scaffolds
  - Optimize new scaffolds and leads for oral delivery and pre-clinical development

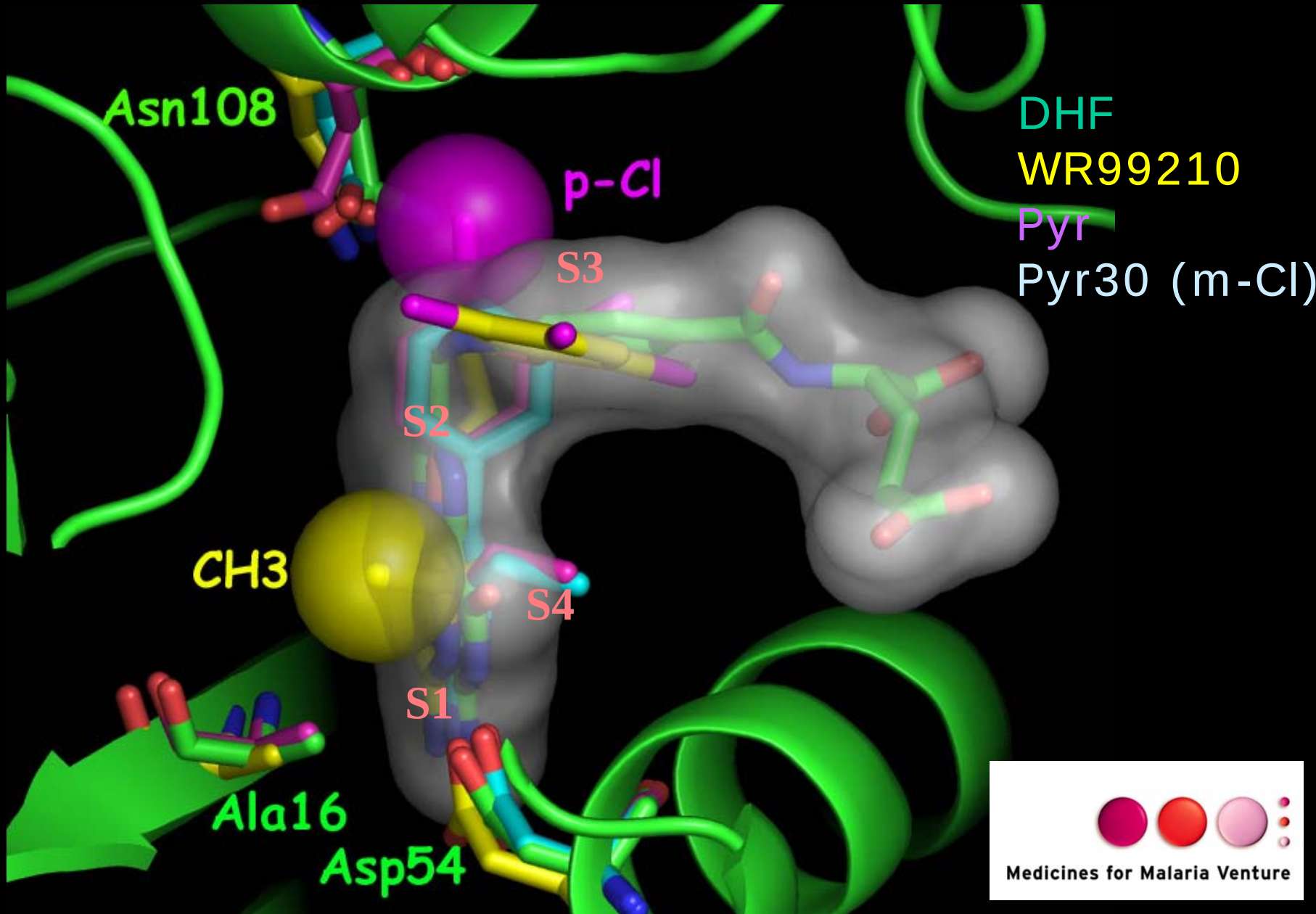


P. falciparum DHFR-TS
Yuvaniyama *et al., Nature Struct. Biol.* 2003, **10**, 357-365

Medicines for Malaria Venture

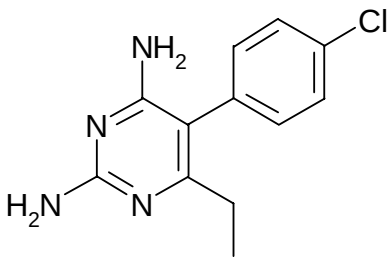

Quadruple (QM) DHFR mutant: full color
Wild-type DHFR: shadowed color
Lys49
Cys50
Trp48
Ile112
3rd
N51I
Met55
WR
S108N
1st
Ser52
Asp54
NDP
Gly165
Phe58
I164L
C59R
4th
2nd
Phe162
Yuvaniyama et al,
Nat Struct Biol, 10, 357, 2003
Medicines for Malaria Venture

Substrate and inhibitor binding, PfDHFR

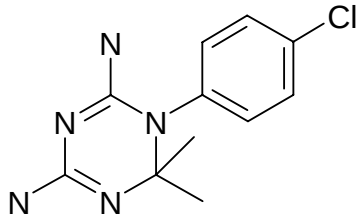


Medicines for Malaria Venture

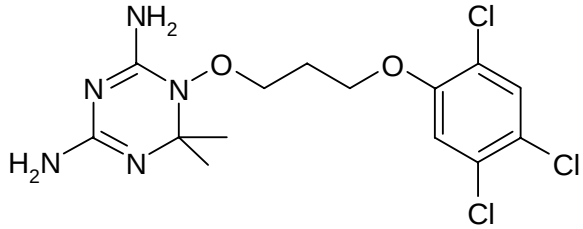
Structural Design Approaches



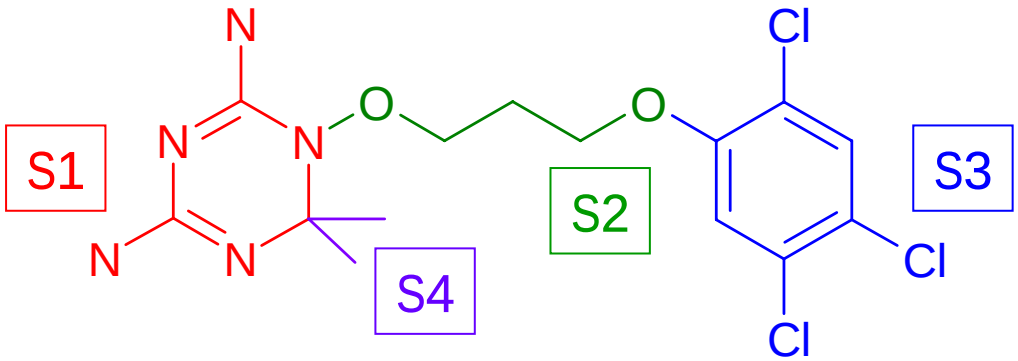
Pyrimethamine
well known DHFR inhibitor, but issues of resistance and toxicity to sulfa component



Cycloguanil
rigid triazine not active against mutant form of the enzyme



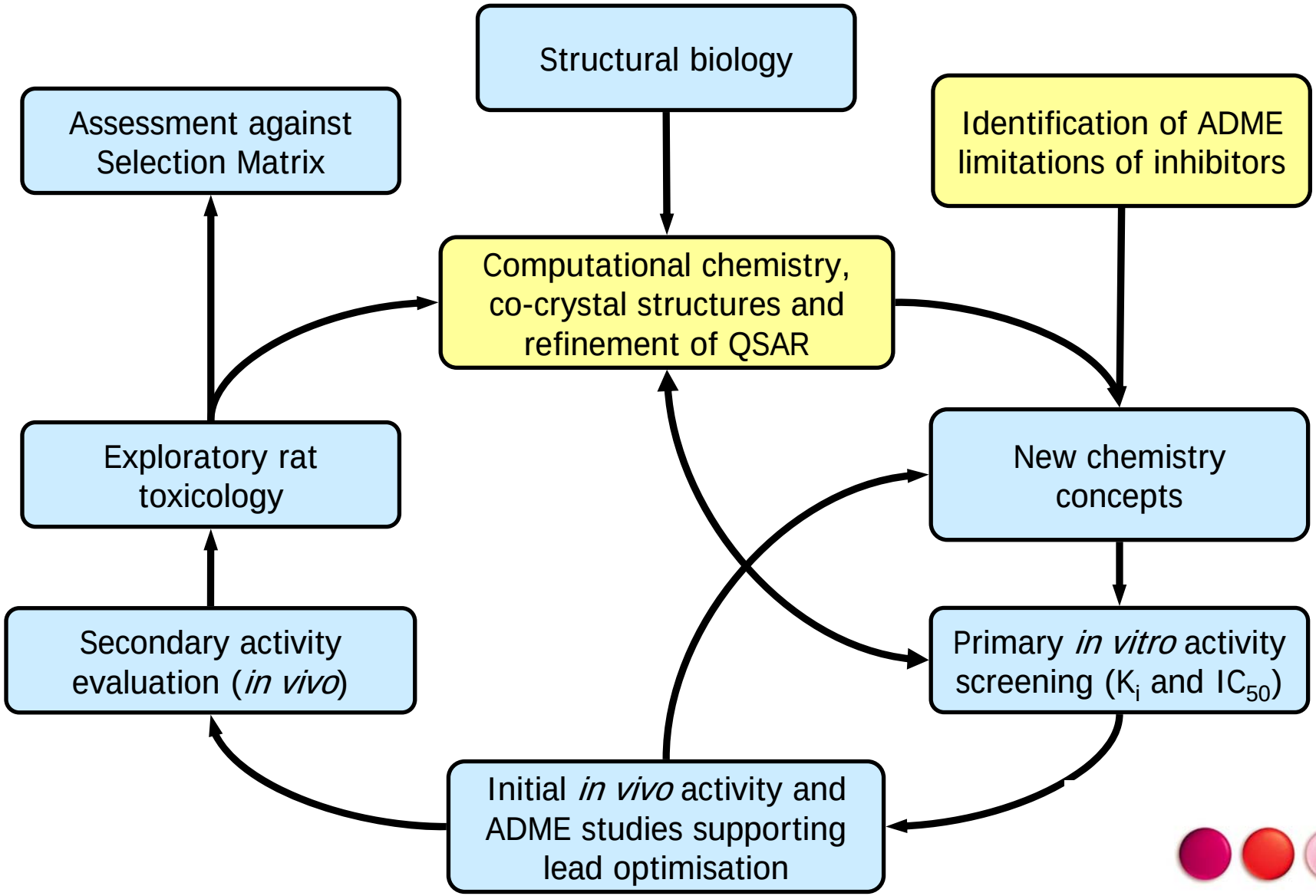
WR99210
flexible triazine required for activity against mutant enzyme, but low oral BA



Beginning with WR99210, optimise four sub-sites to enhance binding/activity and oral bioavailability

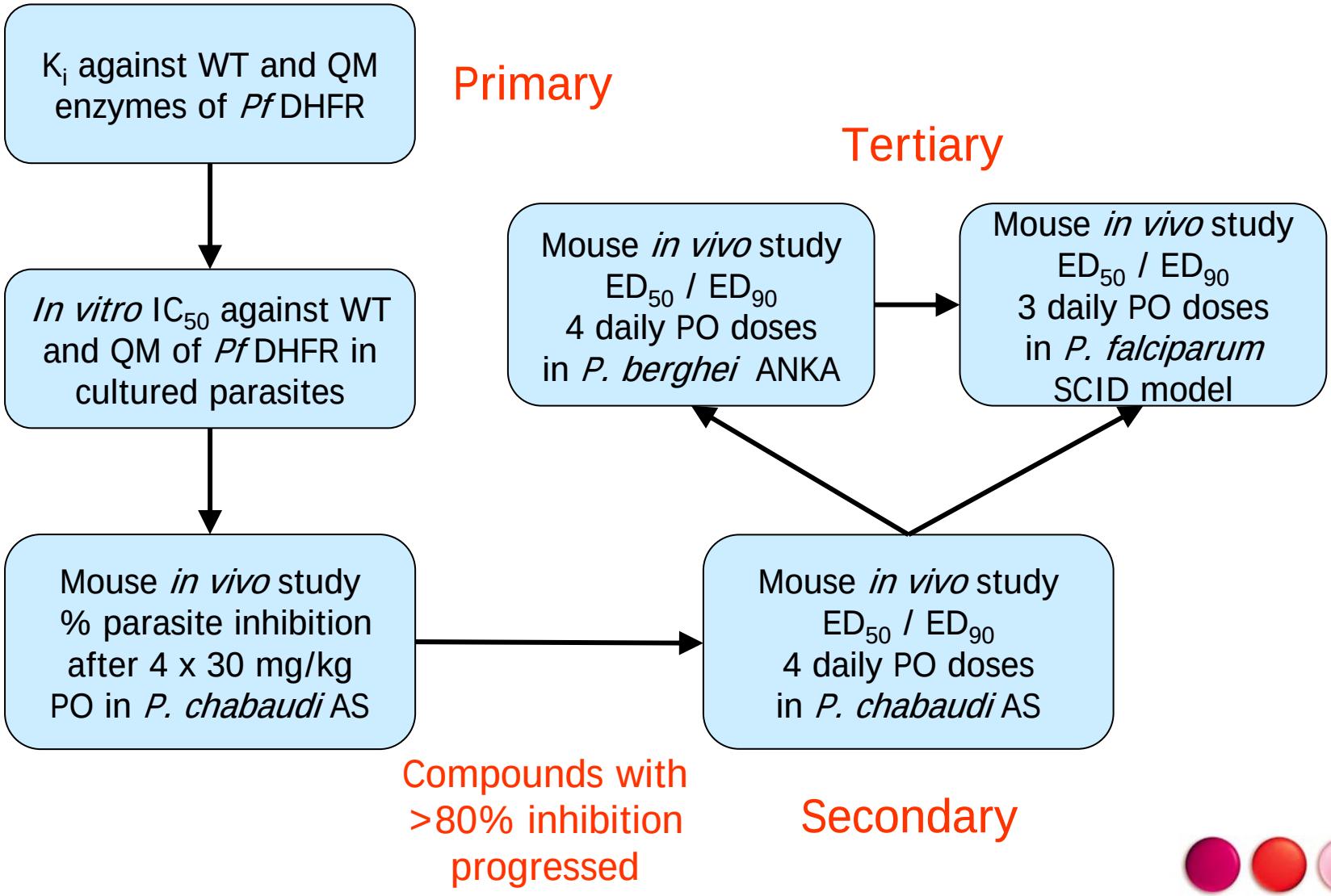
Medicines for Malaria Venture

Iterative Approach For Design-Based DHFR Inhibitors



Medicines for Malaria Venture

Activity Screening Approach



Medicines for Malaria Venture

Data Summary for Series 1

Compound	K_i QM (nM)	IC_{50} QM *P. falciparum* (nM)	Oral ED_{90} *P. chabaudi* (mg/kg) (Pyr-sens)	Oral BA in rats (%)
WR99210	1.9	18	74.2	<1
Pyrimethamine	385	>100,000	1.37	100
65 (series 1)	5.0	3,492	1.5 1.5 (Pyr-res)	83

- Good K_i, but reduced *in vitro* potency relative to WR99210
- Good *in vivo* efficacy in *P. chabaudi* infected mice
- Substantial increase in oral bioavailability relative to WR99210
- But ..... issues with synthesis and starting material

Medicines for Malaria Venture

Data Summary for Series 2

Compound	IC_{50} WT (nM)	IC_{50} QM (nM)	Oral ED_{90} *P. chabaudi* (mg/kg) (Pyr-sens)	Oral BA in rats (%)
Pyrimethamine	79	>100,000	1.37	100
65 (series 1)	229	3,492	1.5	83
111 (series 2)	150	4830	7.3	19
135 (series 2)	1.6	39	5.2	7

- Series 2 launched to improve binding and address issues in series 1
- Reduced *in vivo* potency and reduced oral bioavailability for series 2 compounds relative to series 1

Medicines for Malaria Venture

Data Summary for Series 3

Compound	IC_{50} WT (nM)	IC_{50} QM (nM)	Oral ED_{90} *P. chabaudi* (mg/kg) (Pyr-sens)	Oral BA in rats (%)
Pyrimethamine	79	>100,000	1.37	100
65 (series 1)	229	3,492	1.5	83
111 (series 2)	150	4830	7.3	19
135 (series 2)	1.6	39	5.2	7
113 (series 3)	4.1	50	0.01	2

- Significant increase in *in vitro* and *in vivo* potency for series 3
- High clearance, low oral bioavailability still an issue

Medicines for Malaria Venture

Data Summary for Series 3

Compound	*In Vivo* oral ED_{90} (mg/kg)			
	P. chabaudi AS (Pyr sensitive)	*P. chabaudi ASP* (Pyr resistant)	*P. berghei ANKA* (Pyr sensitive)	*P. falciparum* SCID (Pyr sensitive)
Pyrimethamine	0.9	13.5	3.2	0.85
65 (series 1)	1.5	1.5	25.4	n.t.
113 (series 3)	0.01	0.01	0.03	0.07

- 113 is highly active in all strains, and considerably more active than 65 against *P. berghei* ANKA
- 113 is also more active than pyrimethamine against *P. falciparum* in SCID mice

Medicines for Malaria Venture

Data Summary for Series 3

Compound	IC_{50} WT (nM)	IC_{50} QM (nM)	Oral ED_{90} *P. chabaudi* AS (mg/kg)	Oral BA in rats (%)
113 (series 3)	4.1	50	0.01	2
149 (series 3)	3	18	0.02	16
153 (series 3)	0.2	5	0.013	28
154 (series 3)	3	23	0.043	8.4
157 (series 3)	2	36	0.012	26

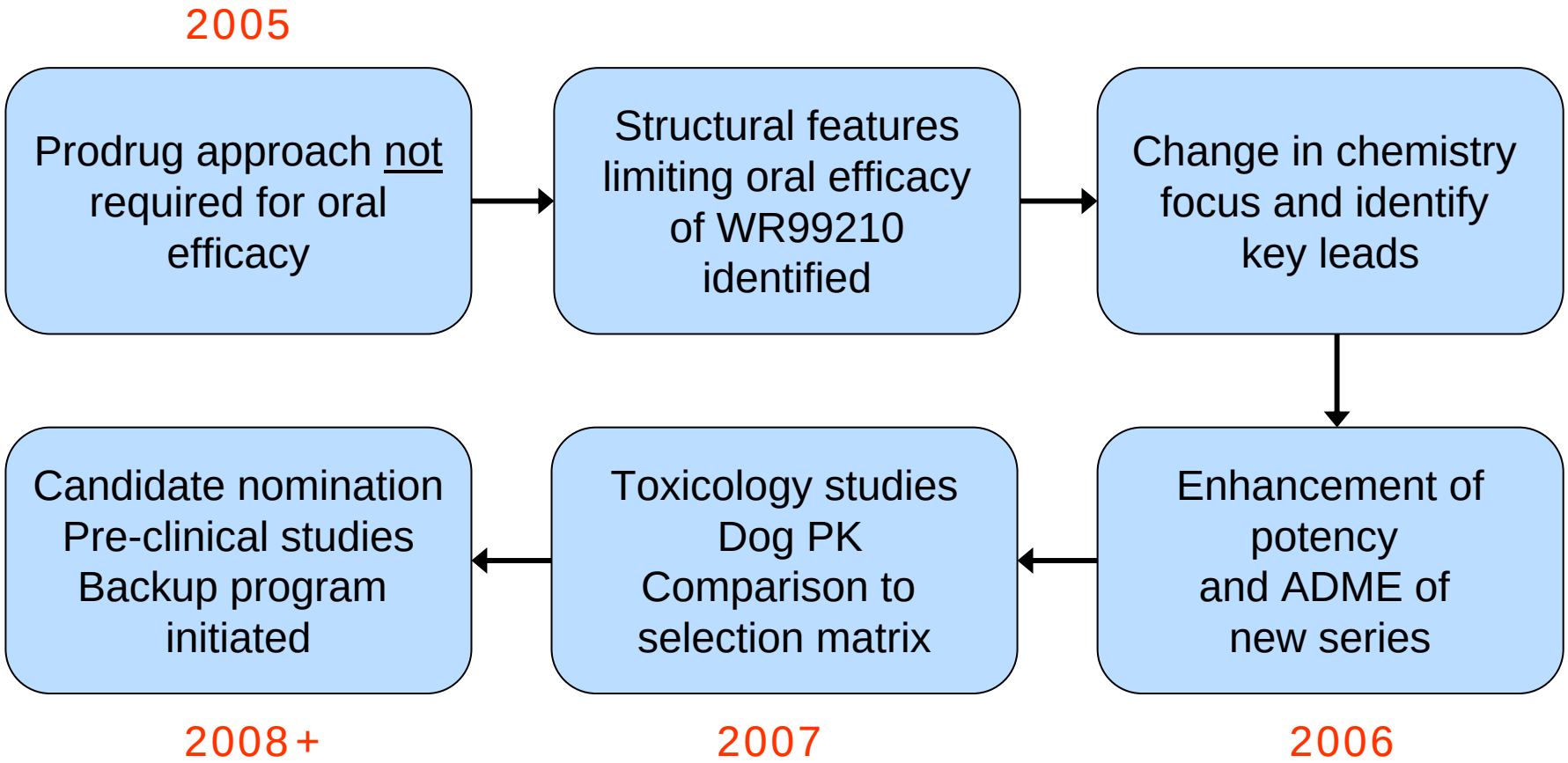
- Highly potent *in vivo* activity and markedly improved oral bioavailability across the series

Medicines for Malaria Venture

In vivo Oral Efficacy against *P. chabaudi* AS: Series 3 Comparison with Conventional Drugs

Compound	ED_{50} (mg/kg)	ED_{90} (mg/kg)	ED_{99} (mg/kg)
113 (series 3)	0.014	0.023	0.042
149 (series 3)	0.016	0.034	0.078
153 (series 3)	0.007	0.017	0.045
154 (series 3)	0.030	0.052	0.248
157 (series 3)	0.013	0.026	0.054
Artesunate	3.80	8.74	21.68
Mefloquine	2.48	5.26	11.97
Chloroquine	4.02	6.01	9.33
Atovaquone	0.044	0.067	0.11
Pyrimethamine	0.34	1.28	5.33

Project Milestones



Medicines for Malaria Venture

Overall Summary

- Validated target supported by strong structural biology
- Chemistry is tractable, relatively straightforward, low COGs
- Highly potent enzyme inhibitors; inhibition of parasite growth at nM concentrations, against wild-type and multiple mutants
- Prototype for drug development from a validated target: unique workaround to preserve a validated target for design of new therapeutic agents

Medicines for Malaria Venture

The DHFR Project Team

- Chemical synthesis, structural biology, compound design, *in vitro* testing

  BIOTEC; Chulalongkorn University
  (Sumalee Kamchonwongpaisan, Tirayut Vilaivan, Bongkoch Tarnchompoo, Chawanee Thongphanchang, Penchit Chitnumsub, Chairat Uthaipibull Yongyuth Yuthavong)

- *In vitro* and *in vivo* biological evaluation and mechanistic studies

  London School of Hygiene and Tropical Medicine
  (Livia Vivas, Emily Bongard)

- ADME, lead optimisation and compound profiling

  Monash University (Susan Charman, Danielle McLennan, Karen White, Bill Charman)

- Program advice and management

  David Matthews (ESAC member, discovery support)
  Sarah Arbe-Barnes (Fulcrum Pharma, project support)

Medicines for Malaria Venture