Inhibitors of *Plasmodium Falciparum* Calcium-Dependent Protein Kinase 1 (*Pf*CDPK1), a Novel Target for the Potential Treatment of Malaria

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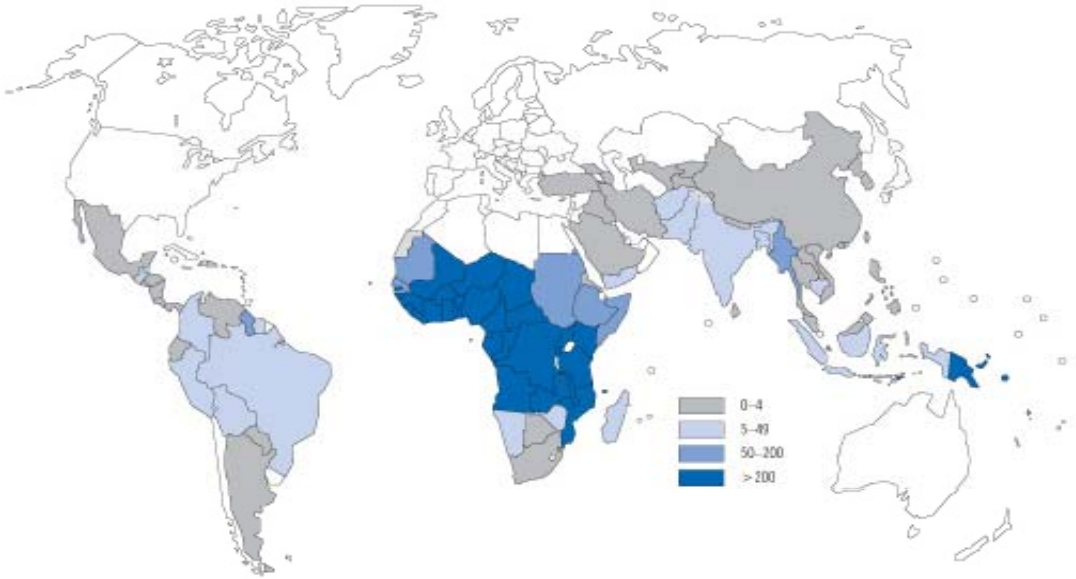
Young Chemist in Industry

8th May 2013

Contents

Why Malaria?

- A major public health problem in more than 90 countries with combined population 3.3 billion people (50% of the world's population)
- ~220 million new infections and an estimated 660,000 deaths per year
- Mainly in Africa but incidence is growing in Asia and Latin America

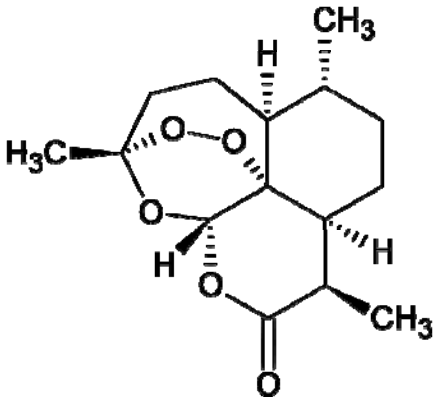


Estimated incidence of malaria per 1000 population (2006)

Why Malaria?

- Emergence of drug resistance has compromised the therapeutic efficacy of most anti-malarial drugs

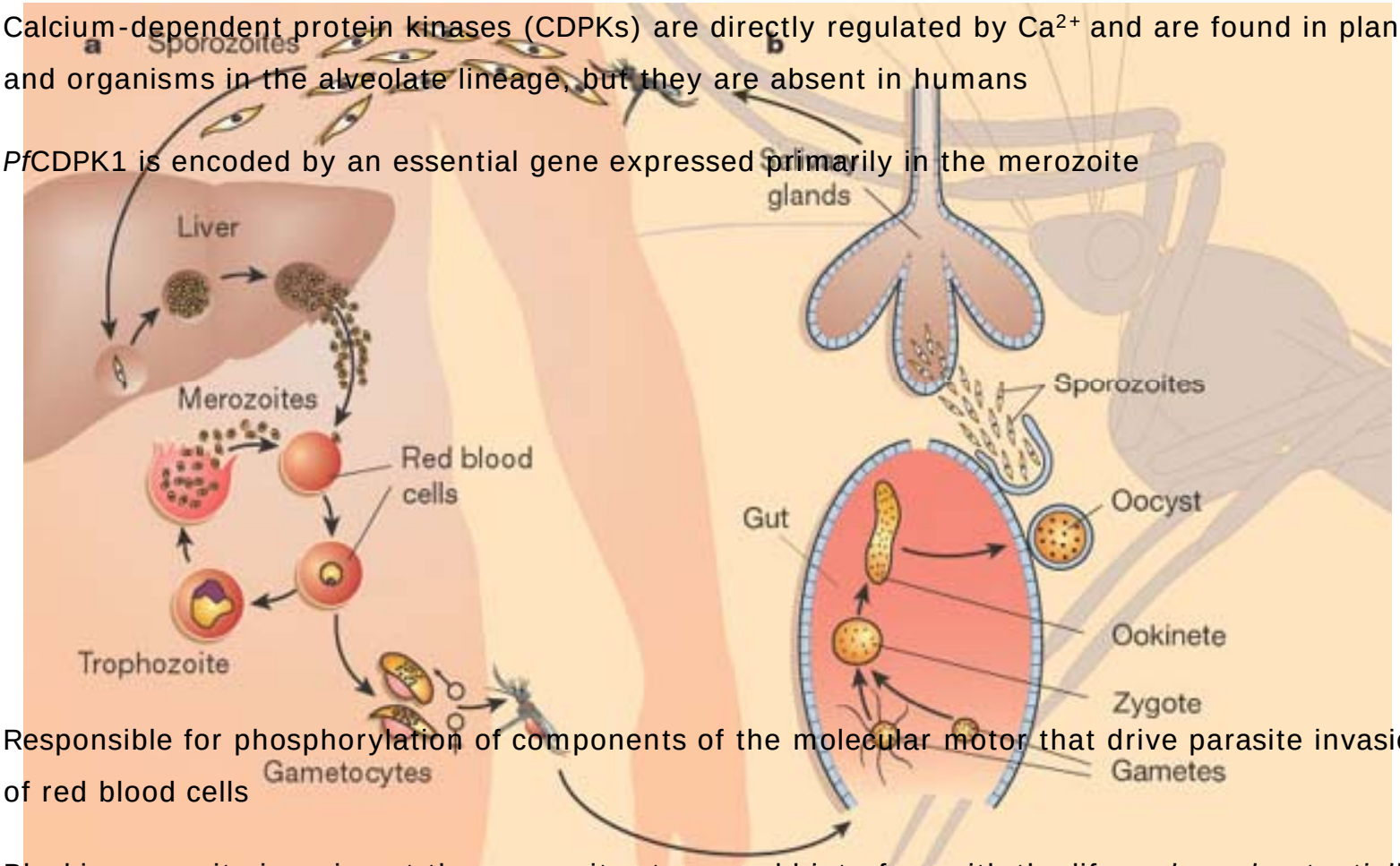
- Artemisinin and derivatives, used in combination therapy, are currently the main line of defence against drug-resistant malaria



- Humans can be infected by 11 species, but two account for vast majority of cases: *Falciparum* (~70%) & *Vivax* (~25%)

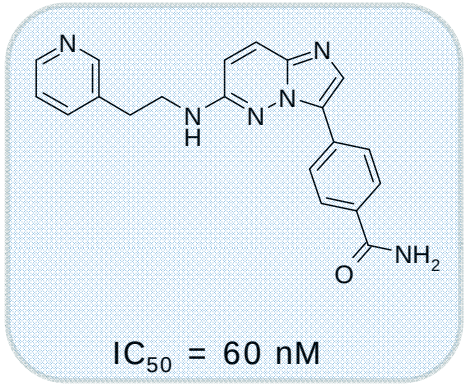
*Pf*CDPK1: A Novel Target

- Calcium-dependent protein kinases (CDPKs) are directly regulated by Ca^{2+} and are found in plants and organisms in the alveolate lineage, but they are absent in humans
- *Pf*CDPK1 is encoded by an essential gene expressed primarily in the merozoite



- Responsible for phosphorylation of components of the molecular motor that drive parasite invasion of red blood cells
- Blocking parasite invasion at the merozoite stage could interfere with the life cycle and potentially allow the infection to be cleared

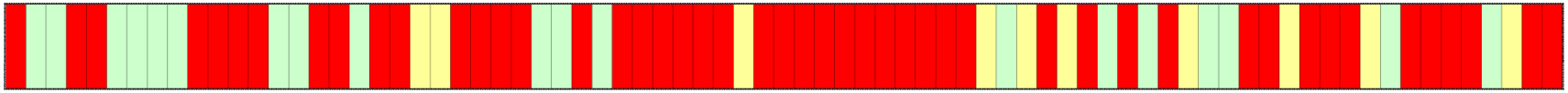
Hit Identification



IC_{50} = 60 nM

- Hit series of Imidazopyridazines showed sub 100 nM IC_{50} values against CDPK1 and strong inhibition of parasite growth
- Concerns over high log D values, poor microsome stability and selectivity over human kinases

Kinase Selectivity Panel:



Key: % inhibition >90: red, 70-90: yellow, <70: green

10 µM

Hit to Early Lead

*Pf*CDPK1 IC_{50} = 13 nM

P. *falciparum* EC_{50} = 400 nM

Cytotox IC_{50} = 9 µM

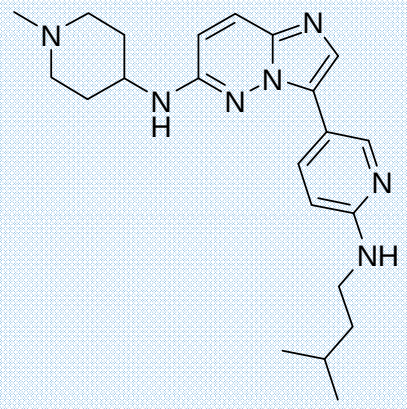
Log D = 3.4

MLM 85 % rem

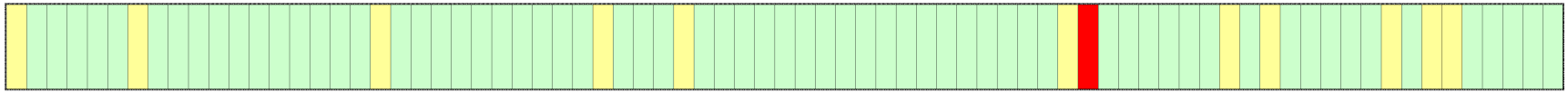
HLM 63 % rem

PAMPA = 81 nm/s

In vivo = 46 % reduction in parasitaemia (50 mg/kg dose orally)



Kinase Selectivity Panel:



Key: % inhibition >90: red, 70-90: yellow, <70: green

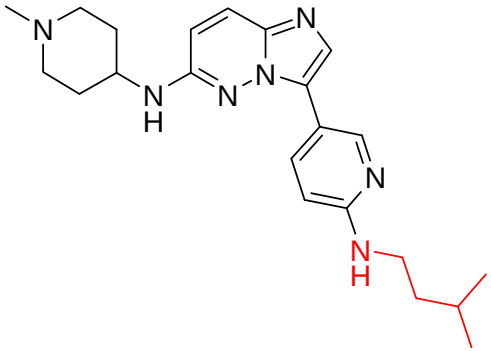
1 µM

- Left hand side less important to binding affinity
- Introduction of a more basic side chain to improve ADME properties
- Phenyl amide replaced with pyridyl isopentylamine which improved binding affinity and kinase selectivity

Bioorg. Med. Chem. Lett., **2013**, *23*, 3064

Optimisation of Imidazopyridazines

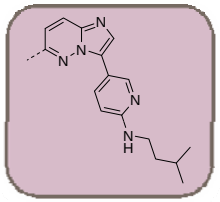
- A structure-based design approach using the homology model of *Pf*CDPK1 was used to attempt to gain increased binding affinity against the target and correspondingly increase the cellular potency

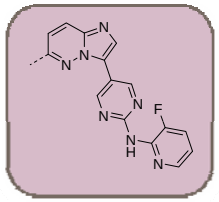


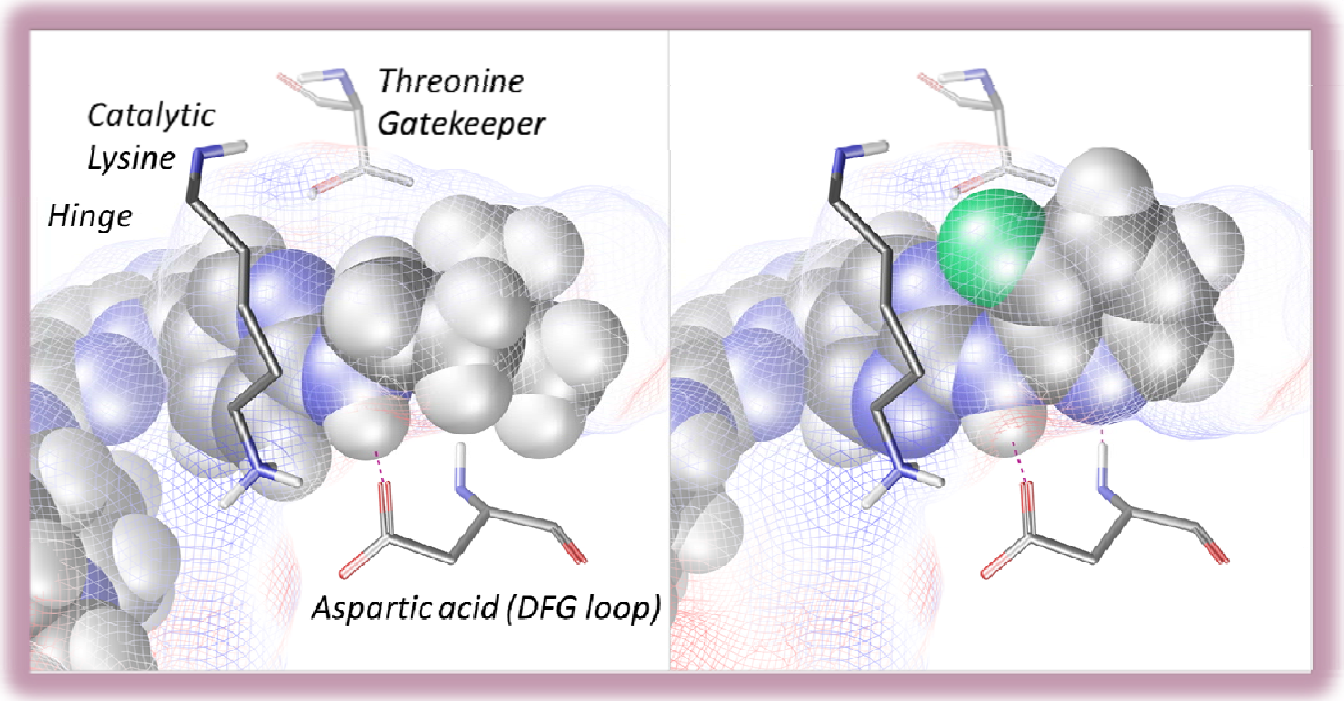
- The model suggested that the binding pocket occupied by the alkyl chain was not optimally filled

- Virtual libraries were enumerated with a range of groups at this position and examined through docking

Optimisation of Imidazopyridazines

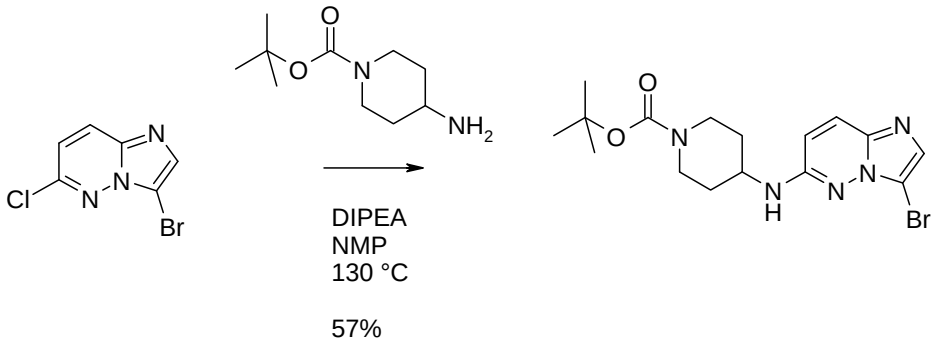






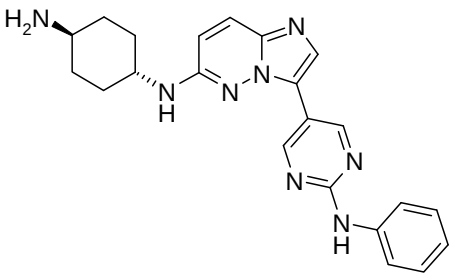
- Additional VDW interactions and fewer clashes
- Improved electrostatic complementarity and additional H-bond
- Reduced entropic penalty due to conformational focussing

Synthesis of Imidazopyridazines



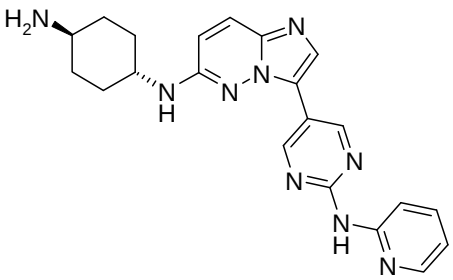
- <5 steps – A prerequisite for MMV collaboration

Testing the Model



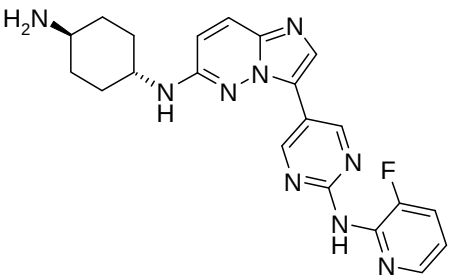
IC_{50} < 9 nM

EC_{50} = 465 nM



IC_{50} < 8 nM

EC_{50} = 83 nM

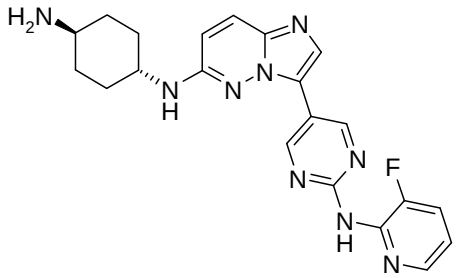


IC_{50} < 8 nM

EC_{50} = 12 nM

- Introduction of a hydrogen bond acceptor *ortho* to amine showed a significant cellular efficacy boost
- A further boost obtained by the introduction of a fluorine at the 3 position
- Poor permeability, could be due to diaminocyclohexane?

Optimisation of Left Hand Side



IC_{50} = < 8 nM

EC_{50} = 13 nM

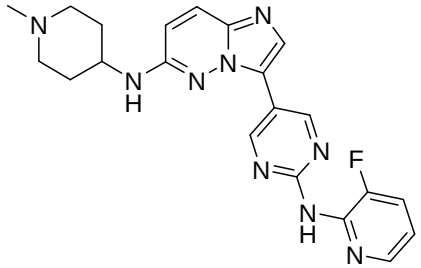
Cytotox IC_{50} = >20 µM

Log D = 0.2

MLM 85 % rem

HLM 93 % rem

Pampa = 3.9 nm/s



IC_{50} = 13 nM

EC_{50} = 40 nM

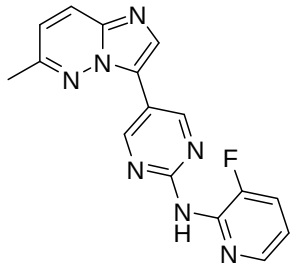
Cytotox IC_{50} = 5.9 µM

Log D = 1.4

MLM 85 % rem

HLM 98 % rem

Pampa = 1.0 nm/s



IC_{50} = 44 nM

EC_{50} = Inactive

Cytotox IC_{50} = 6.7 µM

Log D = 2.5

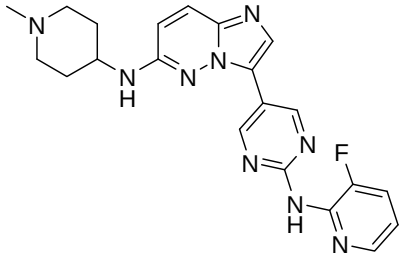
MLM 92 % rem

HLM 82 % rem

Pampa = 137 nm/s

- Variation of left hand side to improve ADME properties
- Basic side chain required on the left hand side
- Attempts to remove resulted in loss of cellular efficacy
- A range of basic groups with varying pKa explored (lower pKa improved permeability)
- Trade off between good ADME and good efficacy!

Optimisation of Linker



IC_{50} = 13 nM

EC_{50} = 40 nM

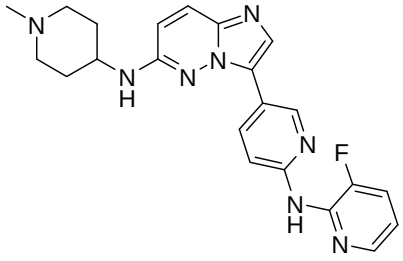
Cytotox IC_{50} = 5.9 µM

Log D = 1.4

MLM 85 % rem

HLM 98 % rem

Pampa = 1.0 nm/s



IC_{50} = 9 nM

EC_{50} = 388 nM

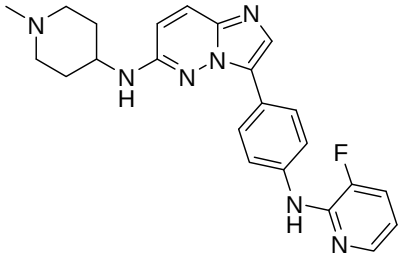
Cytotox IC_{50} = 0.5 µM

Log D = 3.1

MLM 57 % rem

HLM 77 % rem

Pampa = 63 nm/s



IC_{50} = 16 nM

EC_{50} = 406 nM

Cytotox IC_{50} = 3.2 µM

Log D = 3.2

MLM 47 % rem

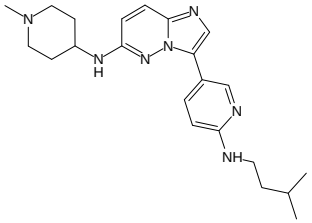
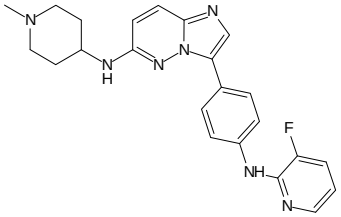
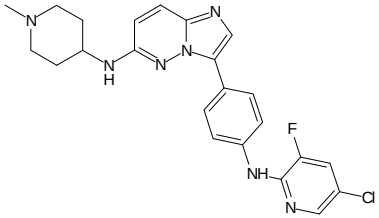
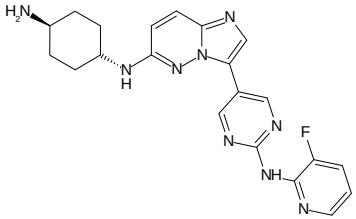
HLM 80 % rem

Pampa = 170 nm/s

Increasing Permeability

Decreasing Efficacy

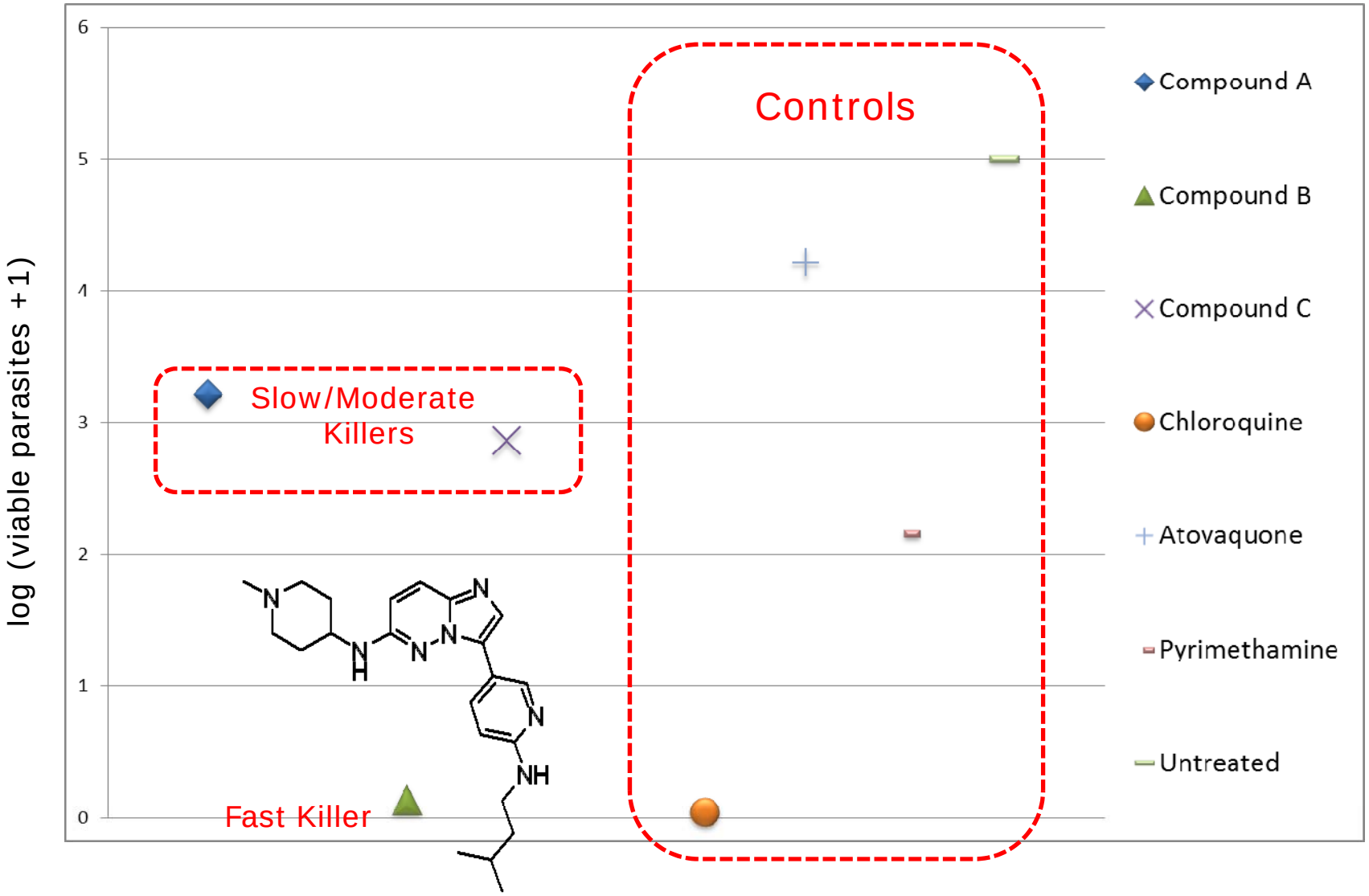
In Vivo Results

	IC50 (μM)	Cytotox v-50 (μM)	EC50 (μM)	HLM (% rem)	MLM (% rem)	LogD	PAMPA Papp (nm/s)	In vivo reduction in parasitaemia (%)
	0.013	9.0	0.400	63	85	3.4	81	46
	0.016	3.2	0.390	80	47	3.2	171	44
	0.012	2.0	0.078	86	61	3.7	48	51
	<0.008	>20	0.013	93	85	0.2	4	4

Parasite Rate of Reduction Assay

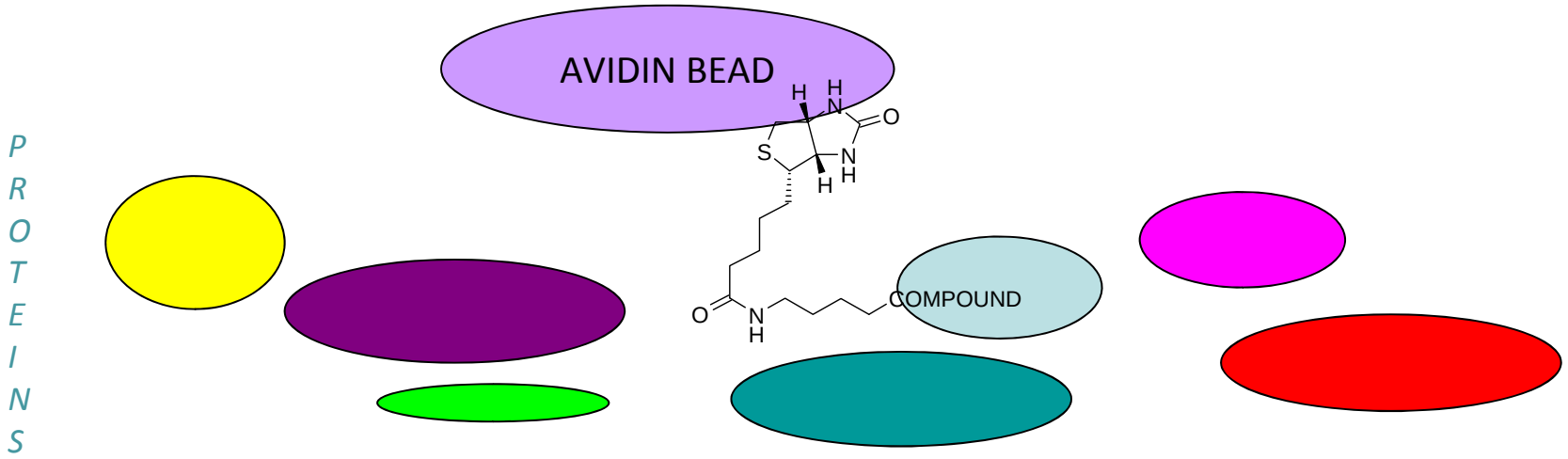
- Quantifies number of parasites that remain viable after drug treatment
- Parasites are treated with the selected drug at a concentration corresponding to 10 x IC_{50} over 48 hours
- Dilutions are made and cultured for 21 days
- Samples taken at 21 and 28 days to confirm number of viable parasites
- Can group according to control compounds
  - *Fast killers (chloroquine)*
  - *Moderate killers (pyrimethamine)*
  - *Slow killers (atovaquone)*

Parasite Rate of Reduction Assay



Biotinylation Experiments

- Used to determine other targets that a compound may interact with
- Biotin is tethered to compound by a short linker and exposed to potential target proteins
- (Strept)Avidin has a high affinity for the biotin portion and can be used to “pull-out” the proteins for which the compound has an affinity.



Biotinylation Experiments

- Assuming a similar binding mode biotin was tethered to the basic side chain
- However, a new target could mean a new binding mode

Conclusion

- A series of *Pf*CDPK1 inhibitors was identified from a high throughput screen
- Subsequent optimisation yielded potent, selective inhibitors with suitable ADME and pharmacokinetic profiles
- Testing in an *in vivo* efficacy model showed insufficient reduction in parasitaemia for the project to progress further
- On going investigation into off-target effects through biotinylation experiments

Acknowledgements

MRCT

Simon Osborne
Tim Chapman
Jon Large
Nathalie Bouloc
Ela Smiljanic-Hurley

Kris Birchall

David Tickle
Sadhia Mahmood

Keith Ansell
Hayley Jones
Alison Levy
Michelle Raynor
Debbie Taylor
David Whalley

NIMR

Tony Holder
Judith Green
Barbara Clough
Munira Grainger

MMV

Didier Leroy
Paul Willis
Simon Campbell

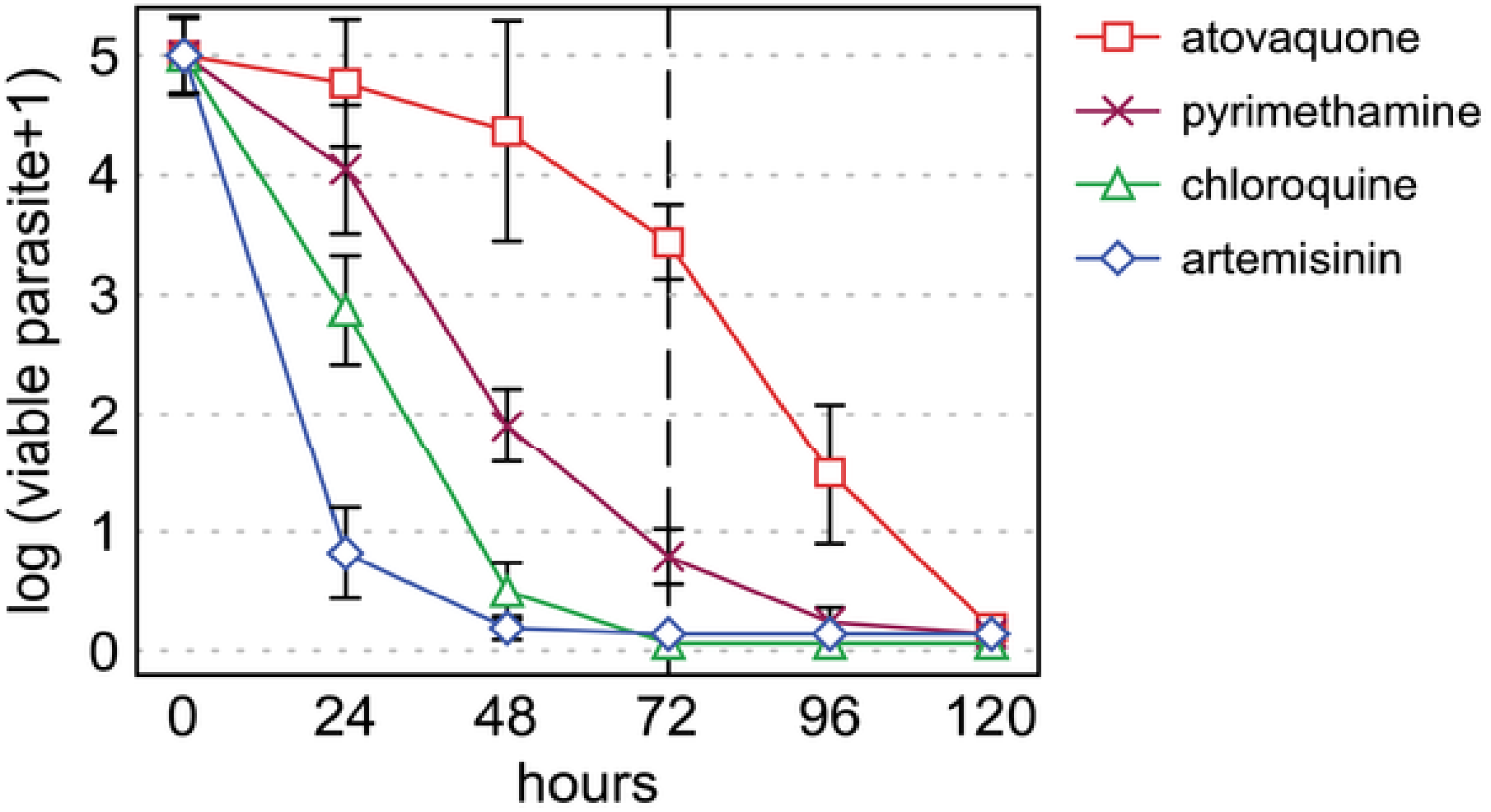
Swiss TPH Institute

Sergio Wittlin

Monash University

Sue Charman
Karen White

Figure 5. Classical antimalarial killing rate profiles.



Sanz LM, Crespo B, De-Cózar C, Ding XC, et al. (2012) P. falciparum In Vitro Killing Rates Allow to Discriminate between Different Antimalarial Mode-of-Action. PLoS ONE 7(2): e30949. doi:10.1371/journal.pone.0030949
http://www.plosone.org/article/info:doi/10.1371/journal.pone.0030949