Inhibitors of *Plasmodium falciparum* calcium-dependent protein kinase 1 (*PfCDPK1*), a novel target for the potential treatment of malaria

Tim Chapman

11th September 2013
Contents

- Background to Malaria
- PfCDPK1: a novel target
- Hit series identification
- Progression to an early lead
- Optimisation
- Pharmacokinetics
- *In Vivo* efficacy
- Conclusion
A major public health problem in more than 90 countries with combined population 3.3 billion people at risk (50% of the world’s population)

~220 million new infections and an estimated 655,000 deaths per year

Mainly in sub-Saharan Africa but incidence is growing in Asia and Latin America, potential for increased endemicity in India & China

Confirmed malaria cases per 1000 population in 2009
Malaria therapy and resistance

The natural product artemisinin and its semi-synthetic derivatives, used in combination therapy, is the first line treatment for uncomplicated malaria.

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

- Resistance to artemisinin therapies has emerged in patients on Thai-Cambodian border
- Urgent need for new therapies

Humans can be infected by five species, but two account for vast majority of cases: *Plasmodium falciparum* (~70%) & *Plasmodium vivax* (~25%)
The life cycle of *Plasmodium*
Parasite invasion of red blood cells (RBCs) and subsequent multiplication and release from RBCs is the stage of the parasite’s life cycle that is responsible for manifestation of the disease.

If RBC invasion is prevented, the parasites die and the infection is cleared.

Invasive half life of merozoites is very short (minutes).

Parasite invasion of red blood cells is driven by an actomyosin motor complex.

- Kinase activity and protein phosphorylation play a key role in this process.
Structure of the Motor

Merozoite movement

Inner membrane complex

Force

Merozoite

Erythrocyte

Moving junction

GAP50
MyoA
Profilin

GAP45
MTIP
Formin

Aldolase
Parasite receptors
Sub 2
Erythrocyte receptors

Actin
PfCDPK1: a novel target

- One of five calcium-dependent Ser/Thr kinases of *Plasmodium*
- Calcium-dependent kinases unique to plants and alveolates, absent in humans
- PfCDPK1 is encoded by an essential gene
  - Expressed primarily in the merozoite (asexual blood stage)
  - Located at inner interface of parasite plasma membrane, phosphorylates two components (MTIP & GAP45) of the motor complex which drives invasion
  - Attempts to knock out the cdpk1 gene have failed in both *Plasmodium falciparum* and the rodent parasite *Plasmodium berghei*
- Inhibitors would be expected to be active against current resistant strains
- High homology between PfCDPK1 and PvCDPK1 – can target both species

*Cell Host Microbe* 2010, 8, 377
*Nat. Chem. Biol.* 2008, 4, 347
*J. Biol. Chem.* 2008, 283, 30980
Project aims

- Identify inhibitors of PfCDPK1 and show that they can block parasite growth
- Provide tool compounds for further evaluation of role of PfCDPK1 and parasite life cycle
- Achieve *in vivo* proof of concept (mouse model) with an orally active compound
- Collaborate with Medicines for Malaria Venture (MMV) to facilitate project progression
High throughput screen

35,422 compounds

Primary Screen (10 μM single point, Kinase Glo assay measuring inhibition of phosphorylation of MTIP by PfCDPK1)

Reconfirmed hits at 10 μM unique to screen (<50% control activity cut-off)

Structure triage → “hitlist” for IC₅₀ determinations

Compounds with IC₅₀ < 5 μM

Compounds with IC₅₀ < 1 μM
Most potent compound ~60 nM
5 series identified from HTS

Imidazopyridazines selected as primary series for further exploration

Examples with sub-100 nM IC₅₀ values against PfCDPK1 and strong inhibition of *P. falciparum* parasite growth *in vitro*

![Chemical structure](image)

PfCDPK1 IC₅₀ = 60 nM

Early work around this series revealed a number of issues:

- High log D values
- Poor stability in microsomes
- Poor selectivity over human kinases
Hit to Early Lead

**LHS:** Introduction of a more basic side chain gave an improved ADME profile

**RHS:** Replacement of phenyl carboxamide with isopentylaminopyridine led to improved binding affinity and kinase selectivity

<table>
<thead>
<tr>
<th></th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PfCDPK1 $IC_{50}$</strong></td>
<td>13 nM</td>
</tr>
<tr>
<td>$P. falciparum EC_{50}$</td>
<td>400 nM</td>
</tr>
<tr>
<td>Cytotox v-50</td>
<td>9 µM</td>
</tr>
<tr>
<td>Log D</td>
<td>3.4</td>
</tr>
<tr>
<td>MLM % rem @30 mins</td>
<td>85</td>
</tr>
<tr>
<td>HLM % rem @30 mins</td>
<td>63</td>
</tr>
<tr>
<td>PAMPA $P_{app}$</td>
<td>81 nm/s</td>
</tr>
<tr>
<td>Mouse iv $t_{1/2}$</td>
<td>2.0 h</td>
</tr>
<tr>
<td>m ppb</td>
<td>86%</td>
</tr>
<tr>
<td>Mouse oral BA</td>
<td>86%</td>
</tr>
<tr>
<td><strong>In vivo reduction in parasitaemia</strong></td>
<td>46% po qd @ 50mg/kg ($P. berghei$)</td>
</tr>
</tbody>
</table>

**Key:**
- % inhibition – red: >80, amber: 50-80, green: <50
- @1 µM inhibitor conc.

Optimisation of imidazopyridazines

- Improvement sought in binding affinity with corresponding increase in cellular potency
- Homology model of PfCDPK1 (based on TgCDPK1, PDB: 3I7C) had proved effective at explaining SAR up to this point
- A structure-guided design approach was employed to gain potency

The model suggested that the binding pocket occupied by the alkyl chain was not optimally filled
Virtual libraries were enumerated with diverse selection of R2 groups

- Examined through docking approach using Glide SP (Schrödinger), ~1200 compounds

Identified number of high scoring molecules with superior predicted binding energies to compound 2

Common feature among best R2 groups was potential to gain an additional H-bond with DFG-loop
Predicted binding mode: 2-pyridyl

- 2-Pyridyl variants were predicted to be high potency
  - Better complementarity with protein for shape fit and electrostatics
  - Additional H-bond with backbone N-H of Asp-212
Rapidly accessible through a short synthetic sequence from commercial SM

- Cost of goods is a key consideration for potential malaria treatments
Testing the model

<table>
<thead>
<tr>
<th>Compound number</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PfCDPK1 IC50 (µM)</strong></td>
<td>0.009*</td>
<td>0.008*</td>
<td>0.008*</td>
</tr>
<tr>
<td><strong>P. falciparum EC50 (µM)</strong></td>
<td>0.465</td>
<td>0.083</td>
<td>0.012</td>
</tr>
<tr>
<td><strong>Cytotox Hep-G2 ν50 (µM)</strong></td>
<td>6.6</td>
<td>6.8</td>
<td>&gt;20</td>
</tr>
<tr>
<td><strong>HLM (% rem)</strong></td>
<td>89</td>
<td>89</td>
<td>93</td>
</tr>
<tr>
<td><strong>MLM (% rem)</strong></td>
<td>92</td>
<td>93</td>
<td>85</td>
</tr>
<tr>
<td><strong>m logD (pH 7.4)</strong></td>
<td>1.6</td>
<td>1.1</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>PAMPA P_app (nm/s)</strong></td>
<td>2</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

- Introduction of a hydrogen bond acceptor at the 2-position gave a significant boost in anti-parasite effect.
- Introduction of a fluorine at the 3-position gave a further improvement.

Key: % inhibition – red: >80, amber: 50-80, green: <50

Compound 5 @ 1 µM inhibitor conc.
ADME issues

- Excellent PfCDPK1 binding affinity and anti-parasite potency with 5

- Poor permeability an issue
  - Basicity of diaminocyclohexane (ACD calc. pKa 10.4)
  - Low log D
  - 4 HBDs

- Compound 5 tested in standard P. berghei mouse model (4-day Peters test, with 50 mg/kg po qd and 25 mg/kg ip bid dosing)

- No significant in vivo efficacy observed, consistent with poor exposure
  - High stability in microsomes
  - Permeability apparently limiting factor
Addressing ADME issues: strategy

Optimisation of ADMET/PK required to achieve suitable balance in profile w.r.t. combination of potency/exposure:

- Modify basic group, attenuate pKₐ
- Increase log D
- Reduce HBD count & PSA

Change identity of linker ring, reduce HBA count & TPSA

Modify distal pyridyl ring, add additional substituents

Collaboration initiated
<table>
<thead>
<tr>
<th>Compound</th>
<th>R1</th>
<th>PfCDPK1 IC_{50} µM</th>
<th>P. falciparum EC_{50} µM</th>
<th>ACD calc. pK_{a}</th>
<th>m log D</th>
<th>HBD</th>
<th>PAMPA P_{app}/nms^{-1}</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>H₂C⁻⁻</td>
<td>0.044</td>
<td>inactive</td>
<td>3.0</td>
<td>2.5</td>
<td>1</td>
<td>138</td>
</tr>
<tr>
<td>7</td>
<td>N</td>
<td>0.013</td>
<td>0.070</td>
<td>9.4</td>
<td>1.4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>N</td>
<td>0.021</td>
<td>0.27</td>
<td>8.4</td>
<td>0.7</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>9</td>
<td>N</td>
<td>0.022</td>
<td>0.80</td>
<td>7.1</td>
<td>2.2</td>
<td>1</td>
<td>61</td>
</tr>
<tr>
<td>10</td>
<td>N</td>
<td>0.009</td>
<td>0.26</td>
<td>9.4</td>
<td>1.5</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>11</td>
<td>O</td>
<td>0.040</td>
<td>&gt;1</td>
<td>6.7</td>
<td>2.9</td>
<td>2</td>
<td>27</td>
</tr>
<tr>
<td>12</td>
<td>N</td>
<td>0.036</td>
<td>0.22</td>
<td>7.6</td>
<td>1.6</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>13</td>
<td>O</td>
<td>0.011</td>
<td>0.034</td>
<td>7.8</td>
<td>3.2</td>
<td>2</td>
<td>17</td>
</tr>
<tr>
<td>14</td>
<td>N</td>
<td>&lt;0.009</td>
<td>0.036</td>
<td>10.4</td>
<td>1.9</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>15</td>
<td>N</td>
<td>&lt;0.009</td>
<td>&gt;1</td>
<td>7.7</td>
<td>1.5</td>
<td>1</td>
<td>27</td>
</tr>
<tr>
<td>16</td>
<td>N</td>
<td>0.039</td>
<td>&gt;1</td>
<td>7.4</td>
<td>1.4</td>
<td>1</td>
<td>25</td>
</tr>
</tbody>
</table>
Basic side-chain essential for anti-parasite effect

Attenuating basicity tends to improve permeability

Trade-off between permeability and anti-parasite activity: highly potent compounds all low permeability

Leading compounds from basic group variation were tested for *in vivo* efficacy, but none showed a significant effect

Unable to obtain suitable balance in compound profile through modification of this group
### Addressing ADME – linker ring modification

Maintain N-methylpiperidine basic group for potency – modify RHS to balance profile

- Steep SAR w.r.t. permeability and anti-parasite activity

<table>
<thead>
<tr>
<th>Compound number</th>
<th>7</th>
<th>17</th>
<th>18</th>
</tr>
</thead>
<tbody>
<tr>
<td>PfCDPK1 IC50 (µM)</td>
<td>0.013</td>
<td>0.009</td>
<td>0.016</td>
</tr>
<tr>
<td>P. falciparum EC50 (µM)</td>
<td>0.070</td>
<td>0.39</td>
<td>0.41</td>
</tr>
<tr>
<td>Cytotox Hep-G2 v50 (µM)</td>
<td>5.9</td>
<td>0.5</td>
<td>3.2</td>
</tr>
<tr>
<td>HLM (% rem)</td>
<td>85</td>
<td>77</td>
<td>80</td>
</tr>
<tr>
<td>MLM (% rem)</td>
<td>98</td>
<td>57</td>
<td>47</td>
</tr>
<tr>
<td>m logD (pH 7.4)</td>
<td>1.4</td>
<td>3.1</td>
<td>3.2</td>
</tr>
<tr>
<td>PAMPA Papp (nm/s)</td>
<td>1</td>
<td>63</td>
<td>170</td>
</tr>
</tbody>
</table>

**Increasing Permeability**

**Decreasing Anti-parasite effect**
Addressing ADME – linker ring modification

- Trade-off again observed between permeability and anti-parasite effect

- Explored additional linker ring variants:
  - Fluorinated phenyl rings to mimic dipole of pyridyl or pyrimidine ring but allowing lower HBA count to be maintained
  - 5-membered heterocycles e.g. pyrazoles (BMCL, accepted for publication)

- Unable to achieve desired balance in profile through modifying this group alone
  - Improved permeability sufficiently in compound 18 to anticipate improved oral exposure
Pharmacokinetics

- Profiled small panel of compounds for PK in rat in collaboration with CDCO, Monash University
- Confirmed that *in vitro* ADME was a good predictor of *in vivo* PK for this series
- Compound 18 best PK profile
  - Advanced to efficacy model

### Compound 18

- Blood Cl (mL/min/kg): 14
- $V_{ss}$ (L/kg): 8
- iv $t_{1/2}$ (h): 4
- po $C_{max}$, $C_{av}$ (µM): 1.7, 0.8
- Bioavailability (%): 70
In vivo efficacy protocol (P. berghei mouse model)

4-day Standard Peters’ test

- Model uses rodent parasite P. berghei rather than human parasite P. falciparum
- Mice are infected intravenously with infected red blood cells from a donor mouse (day 0)
- Treatment is carried out with a solution of the test compounds 4 hours post-infection (first dose), and continued daily for a further 3 days
- 24 h after final drug treatment, blood sample is taken and parasitaemia is determined with a FACScan by counting red blood cells
- Results are expressed as a percent reduction in parasitaemia, calculated from the difference of the mean infection rate of the control group to the test group
- Chloroquine as a positive control (~99.9% reduction in parasitaemia @ 10mg/kg)
**In vivo efficacy: P. berghei mouse model**

- Advanced compound 18 to *P. berghei* in vivo efficacy test (50 mg/kg oral dosing, once daily)
- Limited effect observed, 44% reduction in parasitaemia
- Analysis of blood samples showed that compound levels of 10x in vitro anti-parasite EC$_{50}$ were achieved

<table>
<thead>
<tr>
<th>Compound number</th>
<th>18</th>
</tr>
</thead>
<tbody>
<tr>
<td>In vivo reduction in parasitaemia</td>
<td>44%</td>
</tr>
<tr>
<td>In vitro <em>P. falciparum</em> EC$_{50}$ (µM)</td>
<td>0.41</td>
</tr>
<tr>
<td>Compound plasma level at 1h</td>
<td>10x in vitro EC$_{50}$</td>
</tr>
<tr>
<td>Compound plasma level at 4 h</td>
<td>8.5x in vitro EC$_{50}$</td>
</tr>
</tbody>
</table>

- No improvement on early lead compound 2
  - More potent compounds against parasite do not possess suitable PK
Addressing species differences: *falciparum* vs *berghei*

Concerns that lack of efficacy may be due to species differences between *P. falciparum* and rodent parasite *P. berghei*

Sequence homology is high (~90%): based on the residues within 10Å of any atom of ATP in the PbCDPK1 crystal structure (PDB:3Q5I), there is 100% identity.

*In silico*: docking compounds to PbCDPK1 predicts binding with affinity close to PfCDPK1.

*In vitro*: expressed PbCDPK1 enzyme, inhibitors were equipotent between *P. berghei* and *P. falciparum* CDPK1 enzymes – confirmed *in silico* prediction.

*In vivo*: GSK Tres Cantos have developed a *P. falciparum* mouse model

- **Severe combined immunodeficient** (SCID) mouse can be injected with human erythrocytes infected with suitable strain of *P. falciparum* rather than using rodent parasite *P. berghei*
- **Opportunity to test representatives in this model through collaboration with MMV**
- **Compound 18 tested alongside early lead compound 2**

*PLoS One, 2008, 3, e2252*
**In vivo efficacy: P. falciparum (SCID mouse) model**

- Antimalarial efficacy of compound 18 and compound 2 was assessed at 50 mg/kg using a 4-day test, once daily with oral dosing.

- Both had shown ~45% reduction in parasitaemia in *P. berghei*.

- *Plasmodium falciparum* growing in peripheral blood of NODscidIL2Rγnull mice engrafted with human erythrocytes.

**Antimalarial efficacy of compounds 18 and 2**

- Compounds formulated at 50mg/kg in 70:30 Tween/ethanol with 10-fold dilution in water.

- No efficacy observed.
Exposure levels in *P. falciparum* mouse model

![Graph showing concentration over time for Compound 18 and Compound 2](image)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Target dose (mg/kg)</th>
<th>$C_{\text{max}}$ (µg/mL)</th>
<th>$t_{\text{max}}$ (h)</th>
<th>AUC$_{(0-23)}$ (µg.h/mL)</th>
<th>DNAUC$_{(0-23)}$ (µg.h/mL per mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound 2</td>
<td>50</td>
<td>1.76±0.502</td>
<td>1.50±0.707</td>
<td>18.7±3.28</td>
<td>0.374±0.0657</td>
</tr>
<tr>
<td>Compound 18</td>
<td>50</td>
<td>1.88±0.077</td>
<td>5.00±1.41</td>
<td>27.8±2.62</td>
<td>0.556±0.0523</td>
</tr>
</tbody>
</table>

DNAUC. dose normalized value of AUC$_{(0-23)}$

- **Lack of efficacy despite achieving good exposure**
  - **Compound 18**: ~11x in vitro anti-parasite EC$_{50}$ at 5h ($C_{\text{max}}$) and ~6x at 24h
Distal pyridyl ring modification

In parallel with in vivo PK/efficacy testing, continued to search for new compounds with improved balance in profile

- Retain phenyl as linker ring
- Adding substituents on distal pyridyl ring led to improved anti-parasite activity
- Good in vitro ADME profiles

<table>
<thead>
<tr>
<th>Compound number</th>
<th>19</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>PfCDPK1 IC₅₀ (µM)</td>
<td>0.019</td>
<td>0.014</td>
</tr>
<tr>
<td>P. falciparum EC₅₀ (µM)</td>
<td>0.080</td>
<td>0.080</td>
</tr>
<tr>
<td>HLM (% rem)</td>
<td>86</td>
<td>83</td>
</tr>
<tr>
<td>MLM (% rem)</td>
<td>61</td>
<td>90</td>
</tr>
<tr>
<td>m logD (pH 7.4)</td>
<td>3.7</td>
<td>2.2</td>
</tr>
<tr>
<td>PAMPA Pₐₚₚ (nm/s)</td>
<td>48</td>
<td>54</td>
</tr>
</tbody>
</table>
In vivo efficacy: *P. berghei*

- Advanced both compounds to *P. berghei* in vivo efficacy test
  - 50 mg/kg oral dosing, once per day
- Limited effect observed, only 51% reduction for compound 19 despite achieving excellent exposure

<table>
<thead>
<tr>
<th>Compound number</th>
<th>19</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>In vivo reduction in parasitaemia</td>
<td>51%</td>
<td>18%</td>
</tr>
<tr>
<td>In vitro <em>P. falciparum</em> EC(_{50}) (µM)</td>
<td>0.080</td>
<td>0.080</td>
</tr>
<tr>
<td>Compound plasma level at 4h</td>
<td>45x in vitro EC(_{50})</td>
<td>18x in vitro EC(_{50})</td>
</tr>
<tr>
<td>Compound plasma level at 24 h</td>
<td>35x in vitro EC(_{50})</td>
<td>0.2x in vitro EC(_{50})</td>
</tr>
</tbody>
</table>
An imidazopyridazine series of PfCDPK1 inhibitors was identified from a high throughput screen.

Optimisation yielded highly potent, selective inhibitors with suitable ADME and pharmacokinetic profiles for in vivo studies.

Despite achieving good coverage of in vitro anti-parasite EC\textsubscript{50}, leading compounds showed insufficient in vivo efficacy in mouse models (P. berghei & P. falciparum) for the project to progress further.

Further investigations into the role of CDPK1 and the mechanism of action of these compounds are ongoing with our academic collaborators.
Acknowledgements

MRCT
Simon Osborne
Claire Wallace
Nathalie Bouloc
Jon Large
Ela Smiljanic-Hurley
Kris Birchall
David Tickle
Sadhia Mahmood
Keith Ansell
Hayley Jones
Alison Levy
Michelle Raynor
Debbie Taylor
David Whalley

MRC NIMR
Tony Holder
Judith Green
Barbara Clough
Munira Grainger

GSK Tres Cantos
Iñigo Angulo-Barturen
Elena Fernández-Alvaro
María Belén Jiménez-Díaz
Maria Santos Martínez

MMV
Didier Leroy
Paul Willis
Simon Campbell

Swiss TPH
Sergio Wittlin
Matthias Rottmann

Monash University
Sue Charman
Karen White