Novel Inhibitors of Malarial Dihydrofolate Reductase (DHFR)

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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

Medicines for Malaria Venture
Substrate and inhibitor binding, PfDHFR

Asn108
DHF
WR99210
Pyr
Pyr30 (m-Cl)

S1
S2
S3
S4

CH3
Ala16
Asp54
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
Iterative Approach For Design-Based DHFR Inhibitors

- Assessment against Selection Matrix
- Exploratory rat toxicology
- Secondary activity evaluation (*in vivo*)
- Initial *in vivo* activity and ADME studies supporting lead optimisation
- Structural biology
- Identification of ADME limitations of inhibitors
- New chemistry concepts
- Primary *in vitro* activity screening (*K*_i* and *I*_C_{50}*)
- Computational chemistry, co-crystal structures and refinement of QSAR

*Medicines for Malaria Venture*
Activity Screening Approach

**Primary**

\[ K_i \text{ against WT and QM enzymes of } Pf\text{DHFR} \]

\[ IC_{50} \text{ against WT and QM of } Pf\text{DHFR in cultured parasites} \]

Mouse *in vivo* study

% parasite inhibition after 4 x 30 mg/kg PO in *P. chabaudi* AS

**Secondary**

Mouse *in vivo* study

ED\(_{50} / ED_{90}\) 4 daily PO doses in *P. berghei* ANKA

Compounds with >80% inhibition progressed

**Tertiary**

Mouse *in vivo* study

ED\(_{50} / ED_{90}\) 3 daily PO doses in *P. falciparum* SCID model

Mouse *in vivo* study

ED\(_{50} / ED_{90}\) 4 daily PO doses in *P. chabaudi* AS

Medicines for Malaria Venture
## Data Summary for Series 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>$K_i$ QM (nM)</th>
<th>$IC_{50}$ QM $P. falciparum$ (nM)</th>
<th>Oral $ED_{90}$ $P. chabaudi$ (mg/kg) (Pyr-sens)</th>
<th>Oral BA in rats (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WR99210</td>
<td>1.9</td>
<td>18</td>
<td>74.2</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Pyrimethamine</td>
<td>385</td>
<td>&gt;100,000</td>
<td>1.37</td>
<td>100</td>
</tr>
<tr>
<td>65 (series 1)</td>
<td>5.0</td>
<td>3,492</td>
<td>1.5</td>
<td>83</td>
</tr>
</tbody>
</table>

- 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
Data Summary for Series 2

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC$_{50}$ WT (nM)</th>
<th>IC$_{50}$ QM (nM)</th>
<th>Oral ED$_{90}$ P. chabaudi (mg/kg) (Pyr-sens)</th>
<th>Oral BA in rats (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrimethamine</td>
<td>79</td>
<td>&gt;100,000</td>
<td>1.37</td>
<td>100</td>
</tr>
<tr>
<td>65 (series 1)</td>
<td>229</td>
<td>3,492</td>
<td>1.5</td>
<td>83</td>
</tr>
<tr>
<td>111 (series 2)</td>
<td>150</td>
<td>4830</td>
<td>7.3</td>
<td>19</td>
</tr>
<tr>
<td>135 (series 2)</td>
<td>1.6</td>
<td>39</td>
<td>5.2</td>
<td>7</td>
</tr>
</tbody>
</table>

- 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
## Data Summary for Series 3

<table>
<thead>
<tr>
<th>Compound</th>
<th>$IC_{50}$ WT (nM)</th>
<th>$IC_{50}$ QM (nM)</th>
<th>Oral ED$_{90}$ $P. chabaudi$ (mg/kg) (Pyr-sens)</th>
<th>Oral BA in rats (%)</th>
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<tr>
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<tr>
<td>135 (series 2)</td>
<td>1.6</td>
<td>39</td>
<td>5.2</td>
<td>7</td>
</tr>
<tr>
<td>113 (series 3)</td>
<td><strong>4.1</strong></td>
<td><strong>50</strong></td>
<td><strong>0.01</strong></td>
<td>2</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>In Vivo oral ED\textsubscript{90} (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>\textit{P. chabaudi AS} (Pyr sensitive)</td>
</tr>
<tr>
<td>Pyrimethamine</td>
<td>0.9</td>
</tr>
<tr>
<td>65 (series 1)</td>
<td>1.5</td>
</tr>
<tr>
<td>113 (series 3)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

- 113 is highly active in all strains, and considerably more active than 65 against \textit{P. berghei ANKA}
- 113 is also more active than pyrimethamine against \textit{P. falciparum} in SCID mice
### Data Summary for Series 3

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<tr>
<th>Compound</th>
<th>IC$_{50}$ WT (nM)</th>
<th>IC$_{50}$ QM (nM)</th>
<th>Oral ED$_{90}$\n$P. chabaudi$ AS (mg/kg)</th>
<th>Oral BA in rats (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>113 (series 3)</td>
<td>4.1</td>
<td>50</td>
<td>0.01</td>
<td>2</td>
</tr>
<tr>
<td>149 (series 3)</td>
<td>3</td>
<td>18</td>
<td>0.02</td>
<td>16</td>
</tr>
<tr>
<td>153 (series 3)</td>
<td>0.2</td>
<td>5</td>
<td>0.013</td>
<td>28</td>
</tr>
<tr>
<td>154 (series 3)</td>
<td>3</td>
<td>23</td>
<td>0.043</td>
<td>8.4</td>
</tr>
<tr>
<td>157 (series 3)</td>
<td>2</td>
<td>36</td>
<td>0.012</td>
<td>26</td>
</tr>
</tbody>
</table>

- Highly potent *in vivo* activity and markedly improved oral bioavailability across the series
**In vivo Oral Efficacy against *P. chabaudi* AS:**
Series 3 Comparison with Conventional Drugs

<table>
<thead>
<tr>
<th>Compound</th>
<th>ED$_{50}$ (mg/kg)</th>
<th>ED$_{90}$ (mg/kg)</th>
<th>ED$_{99}$ (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>113 (series 3)</td>
<td>0.014</td>
<td>0.023</td>
<td>0.042</td>
</tr>
<tr>
<td>149 (series 3)</td>
<td>0.016</td>
<td>0.034</td>
<td>0.078</td>
</tr>
<tr>
<td>153 (series 3)</td>
<td>0.007</td>
<td>0.017</td>
<td>0.045</td>
</tr>
<tr>
<td>154 (series 3)</td>
<td>0.030</td>
<td>0.052</td>
<td>0.248</td>
</tr>
<tr>
<td>157 (series 3)</td>
<td>0.013</td>
<td>0.026</td>
<td>0.054</td>
</tr>
<tr>
<td>Artesunate</td>
<td>3.80</td>
<td>8.74</td>
<td>21.68</td>
</tr>
<tr>
<td>Mefloquine</td>
<td>2.48</td>
<td>5.26</td>
<td>11.97</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>4.02</td>
<td>6.01</td>
<td>9.33</td>
</tr>
<tr>
<td>Atovaquone</td>
<td><strong>0.044</strong></td>
<td><strong>0.067</strong></td>
<td><strong>0.11</strong></td>
</tr>
<tr>
<td>Pyrimethamine</td>
<td>0.34</td>
<td>1.28</td>
<td>5.33</td>
</tr>
</tbody>
</table>
Project Milestones

2005
- Prodrug approach not required for oral efficacy
- Structural features limiting oral efficacy of WR99210 identified
- Change in chemistry focus and identify key leads

2006
- Candidate nomination
- Pre-clinical studies
- Backup program initiated

2007
- Toxicology studies
  - Dog PK
  - Comparison to selection matrix

2008+
- Enhancement of potency and ADME of new series
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
The DHFR Project Team

• Chemical synthesis, structural biology, compound design, \textit{in vitro} testing
  BIOTEC; Chulalongkorn University
  (Sumalee Kamchonwongpaisan, Tirayut Vilaivan, Bongkoch Tarnchompoo, Chawanee Thongphanchang, Penchit Chitnumsub, Chairat Uthaipibull Yongyuth Yuthavong)

• \textit{In vitro} and \textit{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)