4(1H)-Pyridones as putative antimalarials

Domingo Gargallo
Diseases of Developing World R&D Center, GlaxoSmithKline
4(1H)-Pyridones as putative antimalarials

- **Project Background**
  - Mode of Action
  - Critical Pathway
  - Chemical approach

- **Reference compound**
  - Biology
  - DMPK
  - Safety Assessment
  - Resistance
  - Chem. Dev & Pharm. Dev

- **Summary and upcoming steps**
4(1H)-Pyridones Mode of Action

Inhibition of *P. falciparum* mitochondrial Electron Transport Chain.

**Reasons for selectivity:**

- *Plasmodium* uses little oxygen. ATP synthesis mainly through fermentation
- *Plasmodium* mitochondria is essential for pyrimidine and haem synthesis
- Mitochondrial electron transport in *Plasmodium* acts as a sink for reduced equivalent (i.e. succinate or orotate)

(Electron micrograph kindly provided by Dr. Peter David, Unité d’Immunologie Moléculaire des Parasites, I.P.)
http://www.pasteur.fr/recherche/unites/ImmStr/en/projects/malaria.html
4(1H)-Pyridones Mode of Action

Inhibition of *P. falciparum* mitochondrial Electron Transport Chain.

Succinate:Ubiquinone Reductase

IC$_{50}$ (µM) > 10

Cytochrome c Reductase

IC$_{50}$ (µM) = 0.00X

Cytochrome C Oxidase

IC$_{50}$ (µM) > 10

Mitochondrial Matrix Side
4(1H)-Pyridones as putative antimalarials

Target Product Profile: Treatment of uncomplicated malaria

- Efficacy against *Plasmodium spp.* including multi-drug resistant strains
- No antagonism against potential combination drugs
- Maximum 3-days treatment, orally administered (1-day is optimal)
- Safe and well tolerated
- Low generation of resistance
- Inexpensive, easy to manufacture, transport and store
4(1H)-Pyridones Critical Pathway

Medicinal Chemistry

- **P. f. in vitro (II)**
  - IC\textsubscript{50} Pf isolates (R&S)
  - Time Course
  - Synchronous cultures

- **P. falciparum in vitro**
  - IC\textsubscript{50}
  - Rate of action (fast)

- **In vitro target selectivity**
  - Mammalian vs. Pf & Py Mit bc1 IC\textsubscript{50}

**Cytotoxicity (II)**
- Cell lines from target organs
- Primary hepatocytes

- **In vitro Metabolism**
  - (mouse, rat, dog, monkey, human)
  - Microsomes
  - P-450
  - Hepatocytes

- **Pharmacokinetics (II)**
  - Linearity Studies.
  - (Therapeutic & Toxicity doses)
  - Different Species PK
    (Allometric scaling)

- **Toxicity**
  - AMES & MLS (genotox)
  - hERG
  - 7-day dog tox
    (1 significative species tox)

**In vitro**
- Protein Binding
- Solubility -Stability
- Particle Size

**Candidate**

- **IC\textsubscript{50} vs. Tox**

**Therapeutic efficacy (II)**
- 1 day test P. yoelii model
- P. falciparum mice model

**Pharmacokinetics**
- Exposure: i.v. / p.o. (%F)

**Mice Tolerability**
- 4 Days MTD

**Resistance**

- **P. vivax**

**Pf gametocytes**
- **Pf hepatic stages**
Hydrophilic moiety (Pyridone): Responsible for the antimalarial activity

Lipophilic tail: Modulation of antiparasitic potency, PK
4(1H)-Pyridones: Chemical approach

![Chemical structure diagram]

<table>
<thead>
<tr>
<th>R</th>
<th>IC$_{50}$ (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-Cl</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Cl</td>
<td>0.9</td>
</tr>
<tr>
<td>O</td>
<td>0.06</td>
</tr>
</tbody>
</table>

GSK GlaxoSmithKline
Medicines for Malaria Venture
4(1H)-Pyridones: Reference compound (RC)
4(1H)-Pyridones: RC Biology

Target Activity and Selectivity

Inhibition of Mitochondrial electron transport chain Complexes IC50 (µM)

<table>
<thead>
<tr>
<th></th>
<th>Complex II</th>
<th>Complex III</th>
<th>Complex IV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>P. falciparum 3D7A</strong></td>
<td>&gt; 3</td>
<td>0.002</td>
<td>&gt; 3</td>
</tr>
<tr>
<td><strong>Plasmodium yoelii 17X</strong></td>
<td></td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>Human HEK293 cells</td>
<td>&gt; 3</td>
<td>0.51</td>
<td>&gt; 3</td>
</tr>
<tr>
<td>Dog MDCK1 cells</td>
<td></td>
<td>0.61</td>
<td></td>
</tr>
<tr>
<td>Mouse L1210 cells</td>
<td></td>
<td>0.69</td>
<td></td>
</tr>
</tbody>
</table>

Target Selectivity Ratio $\times 255$
Whole cell activity and selectivity

Cytotoxicity (human cell lines)

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC₅₀</th>
<th>IC₉₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. falciparum liver stages</td>
<td>&gt; 2.5 µg/ml</td>
<td></td>
</tr>
<tr>
<td>P. falciparum blood stages</td>
<td>0.020 µg/ml</td>
<td>0.050 µg/ml</td>
</tr>
<tr>
<td>P. vivax blood stages</td>
<td>&lt; 0.002 µg/ml</td>
<td></td>
</tr>
</tbody>
</table>

The selectivity index, cytotoxicity vs activity against *P. falciparum* x1000
## Activity against *P. falciparum* resistant strains

<table>
<thead>
<tr>
<th><em>P. falciparum</em> strains</th>
<th><em>P. falciparum</em> whole-cell inhibition IC$_{50}$ (µg ml$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RC</td>
</tr>
<tr>
<td>3D7A</td>
<td>0.002</td>
</tr>
<tr>
<td>FCR3</td>
<td>0.001</td>
</tr>
<tr>
<td>K1</td>
<td>0.002</td>
</tr>
<tr>
<td>Dd2</td>
<td>0.002</td>
</tr>
<tr>
<td>Hb3</td>
<td>0.003</td>
</tr>
<tr>
<td>W2</td>
<td>0.002</td>
</tr>
</tbody>
</table>

RC was active against *P. falciparum* resistant strains to the most affordable and widely-used drugs.
- 90% inhibition of growth was achieved after 15-24hrs exposure
- The anti-plasmodium effect seems to be time-dependent
### 4(1H)-Pyridones: RC Biology

#### In vitro Combinations with Antimalarial Drugs

<table>
<thead>
<tr>
<th>Partner Drug</th>
<th>Strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>atovaquone</td>
<td>3D7 (standard sensitive strain)</td>
</tr>
<tr>
<td>artemisinin</td>
<td>K1 (CQR, PyrR)</td>
</tr>
<tr>
<td>chloroquine</td>
<td>FCR3 (ATVR, CQR)</td>
</tr>
<tr>
<td>pyrimethamine</td>
<td>Dd2 (CQR, PyrR)</td>
</tr>
<tr>
<td>proguanil</td>
<td>HB3 (PyrR)</td>
</tr>
</tbody>
</table>

- All the combinations investigated were additive or indifferent.
- Antagonistic effects were not observed with any of the combinations tested.
**4(1H)-Pyridones: RC Biology**

**Therapeutic Efficacy against *P. falciparum***

- **Treatment (u.i.d. x 4d)**

<table>
<thead>
<tr>
<th>% parasitemia</th>
<th>Days after infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>0</td>
</tr>
<tr>
<td>0.1</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>3</td>
</tr>
</tbody>
</table>

- **Vehicle**
- **0.18 mg/Kg**
- **0.5 mg/Kg**
- **1.7 mg/Kg**
- **5 mg/Kg**
- **15 mg/Kg**

- Good correlation *P. yoelii* and *P. falciparum*
- Non Recrudescence Dose (NRD) *P.yoelii* 4 mg/Kg

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>0.18 mg/Kg</th>
<th>0.5 mg/Kg</th>
<th>1.7 mg/Kg</th>
<th>5 mg/Kg</th>
<th>15 mg/Kg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ED50</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mg/Kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ED90</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mg/Kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **P. yoelii**
- **P. falciparum**
4(1H)-Pyridones: RC Safety Assessment

Biochemical Serum Analysis - Toxicity markers

- Total Protein (g/dl)
- Albumin (g/dl)
- Cholesterol (mg/ml)
- Triglycerides (mg/ml)
- Glucose (mg/ml)
- Calcium (mg/ml)
- Alanine-Aminotransferase (ALT) (UI/L)
- Aspartate-Aminotransferase (AST) (UI/L)
- Alkaline Phosphatase (ALP) (UI/L)
- Bilirubine (mg/ml)

Haematological Analysis

- Red cells (x10^9/ml)
- White cells (x10^6/ml)
- Platelets (x10^6/ml)
- Hemoglobin (g/dl)
- (MCH, MCHC)
- Hematocrit (%)
- Medium corpuscular volume (fl)
- Reticulocyte (%) 

Histopathology

- Heart
- Brain
- Liver
- Kidneys
- Spleen

Mouse 4-day oral toxicity study up to 1000 mg/kg/day

No dose-limiting toxicity up to 1000 mg/kg/day.

Dog 7-day oral toxicity study up to 300 mg/kg/day

No dose-limiting toxicity up to 300 mg/kg/day. Only emesis observed and slight body weight decrease (7-8%)

No Genotox alerts (AMES, MLS, Micronucleus)
**4(1H)-Pyridones: RC DMPK**

## Linearity studies in mice

<table>
<thead>
<tr>
<th>Dose² (mg/Kg)</th>
<th>$C_{\text{max}}$ (µg/ml)</th>
<th>$T_{\text{max}}$ (h)</th>
<th>AUC(0-t) (µg h/ml)</th>
<th>AUC(0-∞) (µg h/ml)</th>
<th>DNAUC (0-∞) b (µg h/ml per mg/Kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 ED90</td>
<td>0.05</td>
<td>6</td>
<td>0.74</td>
<td>0.74</td>
<td>1.60</td>
</tr>
<tr>
<td>1</td>
<td>0.18</td>
<td>6</td>
<td>2.75</td>
<td>2.82</td>
<td>2.43</td>
</tr>
<tr>
<td>4 NRD</td>
<td>0.48</td>
<td>3</td>
<td>7.17</td>
<td>7.52</td>
<td>1.95</td>
</tr>
<tr>
<td>10</td>
<td>0.81</td>
<td>3</td>
<td>12.65</td>
<td>12.75</td>
<td>1.29</td>
</tr>
<tr>
<td>50</td>
<td>1.33</td>
<td>10</td>
<td>20.97</td>
<td>21.38</td>
<td>0.35</td>
</tr>
<tr>
<td>100</td>
<td>2.18</td>
<td>3</td>
<td>33.37</td>
<td>33.29</td>
<td>0.32</td>
</tr>
<tr>
<td>500</td>
<td>3.56</td>
<td>3</td>
<td>51.98</td>
<td>52.51</td>
<td>0.103</td>
</tr>
</tbody>
</table>

### Graphs

- **Graph A:**
  - AUC and $C_{\text{max}}$ vs. Dose
  - Doses: 0.5 mg/Kg, 10 mg/Kg, 50 mg/Kg, 100 mg/Kg, 500 mg/Kg
  - R² values: 0.9393, 0.9432

- **Graph B:**
  - AUC and $C_{\text{max}}$ vs. Dose
  - Dose: 10 mg/Kg
  - R² values: 0.9595, 0.9505

### Notes

- **ED90** indicates the effective dose for 90% of the population.
- **NRD** signifies non-representative data.
- **DNAUC (0-∞)** refers to the dose-normalized area under the curve from 0 to infinity.

---

[Source](#)
Pharmacokinetics in infected mice

The comparison of the pharmacokinetic profile of RC in healthy and infected mice showed no statistically significant differences in absorption, distribution or clearance

<table>
<thead>
<tr>
<th>Experimental conditions</th>
<th>Dose (mg/Kg)</th>
<th>Cmax (µg/ml)</th>
<th>Tmax (hours)</th>
<th>AUC(0-t) (µg.h/ml)</th>
<th>AUC(0-inf) (µg.h/ml)</th>
<th>DNAUC(0-inf) (µg.h/ml per mg/Kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected</td>
<td>10</td>
<td>1.06</td>
<td>6</td>
<td>16.46</td>
<td>18.03</td>
<td>1.7</td>
</tr>
<tr>
<td>Non-Infected</td>
<td>10</td>
<td>0.81</td>
<td>3</td>
<td>12.65</td>
<td>12.75</td>
<td>1.3</td>
</tr>
</tbody>
</table>
4(1H)-Pyridones: RC Therapeutic Index in mice

Therapeutic Efficacy
According to ‘4-days test’

- $ED_{50}$
- $ED_{90}$
- Recrudescence

Safety Assessment
- MDWF
- > 500 mg/Kg (51.98)
- > 1900x
- > 900x
- > 125x
- > 140x
- > 70x
- > 7x
- Dose based
- Exposure based

- 0.26 (0.35)
- 0.54 (0.74)
- 4 (7.17)
## 4(1H)-Pyridones: RC DMPK

### Intravenous Pharmacokinetics in pre-clinical species (Mouse, Rat, Dog and Monkey)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mouse</th>
<th>Rat</th>
<th>Monkey</th>
<th>Dog</th>
</tr>
</thead>
<tbody>
<tr>
<td>$CL_b$ (mL/min/kg)</td>
<td>4.0</td>
<td>12.5 ± 1.8</td>
<td>6.8 ± 1.1</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>Vdss (L/kg)</td>
<td>1.3</td>
<td>1.4 ± 0.1</td>
<td>1.5 ± 0.2</td>
<td>2.6 ± 0.2</td>
</tr>
<tr>
<td>$T_{1/2}$ (h)</td>
<td>4.0</td>
<td>1.5 ± 0.3</td>
<td>2.8 ± 0.3</td>
<td>61.4 ± 18.4</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>5.5</td>
<td>1.9 ± 0.3</td>
<td>3.7 ± 0.2</td>
<td>67.0 ± 20.6</td>
</tr>
</tbody>
</table>

Low clearance in all species
- Intrinsic clearance was low in all species (moderate in rat and monkey hepatocytes)

- Metabolites detected in human were also detected in all preclinical species

- PB and blood partitioning conserved across species
4(1H)-Pyridones: RC Resistances \textit{in vivo}

\textbf{P. yoelii} infection in CD-1 mice

No Resistant strains were isolated with this model
- Five stages involving readily available materials
- Robust, scalable and straightforward until hundreds of gram scale
## 4(1H)-Pyridones: RC Pharmaceutical Develop.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alternative versions (salts)</td>
<td>Not available (weak acid/base character)</td>
</tr>
<tr>
<td>Solid state form</td>
<td>Crystalline</td>
</tr>
<tr>
<td>Hydration</td>
<td>Anhydride</td>
</tr>
<tr>
<td>Polymorphic forms</td>
<td>2 identified (one dominant polymorph)</td>
</tr>
<tr>
<td>Hygroscopicity</td>
<td>Non-hygroscopic</td>
</tr>
<tr>
<td>Moisture pick-up (5-90% RH)</td>
<td>&lt; 0.2%, No form change</td>
</tr>
<tr>
<td>Solid state stability</td>
<td>Stable at 1 month, Chemically &amp; Physically</td>
</tr>
<tr>
<td>Solubility</td>
<td>&lt;0.1 µg/mL pH 2-10</td>
</tr>
<tr>
<td></td>
<td>&lt;0.1 µg/mL in SGF</td>
</tr>
<tr>
<td></td>
<td>0.9 µg/mL in Fasted SIF</td>
</tr>
<tr>
<td></td>
<td>4.8 µg/mL in Fed SIF</td>
</tr>
</tbody>
</table>
Efficacy against *Plasmodium* spp. including multi-drug resistant strains

No antagonism against potential combination drugs

Maximum 3-days treatment, orally administered (1-day is optimal)

Safe and well tolerated

Low generation of resistance

Inexpensive, easy to manufacture, transport and store
4(1H)-Pyridones: upcoming steps

FTIH: October 2008