Malaria Libre

6th Project Meeting

18th December 2020
Agenda

• Feedback of ESAC review

• Status of action items from last meeting

• Project update

• Discussion – Medicinal Chemistry, Mechanism of action studies plan

• How to contribute- new members?
Feedback of ESAC review

Keep a check on the novelty of scaffolds being worked upon and incorporate any learnings specially related to off target pharmacology

There appears to be a potential difference in the MOA between aryl piperazine sub series, so investigate mode of action for both of them

Profile hits in MMV assays for complete biological characterisation and make an informed decision to prioritise series which is most likely to deliver an early lead
## Status of action items from last month

<table>
<thead>
<tr>
<th>Action Item</th>
<th>Responsible group/ scientist</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synthesis of compounds; TCGLS to provide key intermediates for amide coupling</td>
<td>TCGLS, CDRI, Clint, Gloria, Ram</td>
<td>Ongoing, details in the following slides</td>
</tr>
<tr>
<td>QSAR studies with aryl imidazole scaffolds to support design of compounds</td>
<td>Thiyaga to generate the QSAR model around aryl imidazole scaffold based on 3D7 studies</td>
<td>ongoing</td>
</tr>
<tr>
<td>Screening of MMV1804508 and MMV1803899 lab adapted clinical isolates (carried out at JNU)</td>
<td>Kiran to follow up with Shailja in an offline discussion. MMV692137, MMV690872, MMV892566, MMV893209 will be screened in Dd2 assay</td>
<td>Results from Dd2 screen in next slides</td>
</tr>
<tr>
<td>Mechanism of action studies stage specific assays to be performed to identify relevant concentration for metabolomics</td>
<td>Kiran to send compounds from aryl piperazine scaffold for metabolomics studies(MMV024406, MMV024384, MMV024408, MMV1804508, MMV1804743, MMV892566)</td>
<td>sent</td>
</tr>
<tr>
<td>Profile in hERG assay (patch clamp)</td>
<td>Profile diverse compounds from subseries 1c</td>
<td>Selected compounds(discussion)</td>
</tr>
<tr>
<td>Life cycle assays</td>
<td>Done by MMV partners</td>
<td>liver stage assays results awaited. DGFA results on slide 6</td>
</tr>
<tr>
<td>Screening in STPH resistant panel</td>
<td>STPH</td>
<td>Results on slide 11</td>
</tr>
</tbody>
</table>
Discussion points

Mechanism of action studies
Way forward based on PRR results
Challenges in synthesis of ongoing targets
New proposals
QSAR of aryl imidazole scaffold – update
Comments on data representation on MMV webpages

AOB
<table>
<thead>
<tr>
<th>Compound</th>
<th>MMV 1803899</th>
<th>MMV1804508</th>
<th>MMV892881</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pf potency (3D7, µM):</td>
<td>0.33</td>
<td>0.19</td>
<td>0.72</td>
</tr>
<tr>
<td>PRR</td>
<td>Fast kill in 2 point FACS</td>
<td>Slow kill(full PRR)</td>
<td>Moderate kill(full PRR of MMV1804317)</td>
</tr>
<tr>
<td>Cytotoxicity (HepG2, µM)</td>
<td>3.94</td>
<td>9.7</td>
<td>ND</td>
</tr>
<tr>
<td>Biochemical potency (Pf, human)</td>
<td>Target unknown</td>
<td>Target unknown</td>
<td>Target unknown</td>
</tr>
<tr>
<td>Potency (liver stages)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Potency (transmission), DGFA</td>
<td>inactive</td>
<td>Inactive(FGF); 0.87µM(MGF)</td>
<td>Inactive (MMV1804317)</td>
</tr>
<tr>
<td>eLogD</td>
<td>3.6</td>
<td>3.8</td>
<td>3.4</td>
</tr>
<tr>
<td>Solubility (i.e. PBS)</td>
<td>48.7</td>
<td>7.4</td>
<td>59</td>
</tr>
<tr>
<td>CACO-2 (ER; AB; BA)</td>
<td>ongoing</td>
<td>1.2,4,6,6,9</td>
<td>4.6,5,9,26.3</td>
</tr>
<tr>
<td>Binding (hPPB and Albumax)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Plasma Stability</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Metabolism; microsome (h) Clint</td>
<td>45.2</td>
<td>33</td>
<td>54.5</td>
</tr>
<tr>
<td>Metabolism; hepatocyte (r) Clint</td>
<td>2.9</td>
<td>138.6</td>
<td>ND</td>
</tr>
<tr>
<td>CYP Inhibition %I @10µM</td>
<td>9.6(1A2),12.3(2C19),15.5(2C9),30(2D6),37(3A4)</td>
<td>5.7(1A2),12.4(2C19),24(2C9),17.4(2D6),0(3A4)</td>
<td>91(1A2),11(2C9),53(2D6),39(3A4)</td>
</tr>
<tr>
<td>PK: Cl and V (m,r,d)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>PK: bioavailability (m,r,d)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>hERG (K+CHO) IC50</td>
<td>&lt;1uM</td>
<td>10.33µM</td>
<td>ongoing</td>
</tr>
</tbody>
</table>

ND: Not done
Next Steps defined in November Project meeting:
Confirm the difference in rate of kill between cyclopropyl and phenyl amides – MMV1848187, MMV1804743, MMV1803899(FACS) and MMV1804508(PRR)
Rate of killing profile (1/2)

2- time point *in vitro* FACS

Concentration: 10x $IC_{50}$

MMV1803899 shows a fast killing profile

Studies ongoing with MMV1848187, MMV1804743
PRR assay

MMV 1804508 shows slow killing profile

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose</th>
<th>Lag phase (h)</th>
<th>Log PRR</th>
<th>PCT_{99.9}% (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMV1804508</td>
<td>10xIC_{50}</td>
<td>72</td>
<td>3.82</td>
<td>94</td>
</tr>
<tr>
<td>Pyrimethamine</td>
<td>10xIC_{50}</td>
<td>24</td>
<td>3.55</td>
<td>61</td>
</tr>
</tbody>
</table>
Profiling in lab adapted resistant strains

<table>
<thead>
<tr>
<th>MMVID</th>
<th>3D7 (LDH, SYBR) IC50 (µM)</th>
<th>RKL-9 IC50 (µM)</th>
<th>Dd2 (LDH) IC50 (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMV1804508</td>
<td>0.19/0.033 (0.14)</td>
<td>0.046</td>
<td>0.07</td>
</tr>
<tr>
<td>MMV1803899</td>
<td>0.37/0.10</td>
<td>0.41</td>
<td>0.68</td>
</tr>
<tr>
<td>MMV024406</td>
<td>0.42/0.16</td>
<td>0.19</td>
<td>0.85</td>
</tr>
<tr>
<td>MMV024408</td>
<td>0.43/0.32 (0.29)</td>
<td>0.15</td>
<td>0.14</td>
</tr>
<tr>
<td>MMV892566a</td>
<td>0.27/0.053</td>
<td>1.8</td>
<td>0.52</td>
</tr>
<tr>
<td>MMV692137b</td>
<td>0.55/0.14</td>
<td>0.29</td>
<td>1.63</td>
</tr>
<tr>
<td>MMV690872</td>
<td>0.77/0.085</td>
<td>2.8</td>
<td>2.16</td>
</tr>
<tr>
<td>MMV893309</td>
<td>0.29/0.039 (0.29)</td>
<td>0.50</td>
<td>1.75</td>
</tr>
</tbody>
</table>

New data

NF54; ³H (IC₅₀: µM) a:0.15; b:0.54; LDH @TCG; SYBR @JNU; parenthesis: SYBR green 3D7 elsewhere
Profiling in resistant strains at STPH

<table>
<thead>
<tr>
<th>Mutated locus</th>
<th>Mutations (amino acid changes)</th>
<th>MMV643121 IC50 (nM)</th>
<th>MMV390048 IC50 (nM)</th>
<th>MMV018912 IC50 (nM)</th>
<th>MMV000130 IC50 (nM)</th>
<th>MMV034055 IC50 (nM)</th>
<th>MMV1803899 IC50 (nM)</th>
<th>MMV690095 IC50 (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dd2 wt</td>
<td></td>
<td>0.3436</td>
<td>14.97</td>
<td>10.05</td>
<td>8.990</td>
<td>24.51</td>
<td>462.3</td>
<td>385.4</td>
</tr>
<tr>
<td>Dd2 DDD107498</td>
<td>PfeEF2</td>
<td>1558 NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>379.5</td>
<td>455.5</td>
</tr>
<tr>
<td>Dd2 390048</td>
<td>PfPl4K</td>
<td>108.6 NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>439.0</td>
<td>395.0</td>
</tr>
<tr>
<td>Dd2 DSM265</td>
<td>Pfdhodh</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>481.0</td>
<td>481.8</td>
</tr>
<tr>
<td>Dd2 GNF156</td>
<td>Pfcarl</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>397.4</td>
<td>373.6</td>
</tr>
<tr>
<td>Dd2 ELQ300</td>
<td>PfcytB</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>418.9</td>
<td>423.9</td>
</tr>
</tbody>
</table>

| Compounds don’t show cross resistance in tested strains |

<table>
<thead>
<tr>
<th>MMV1803899</th>
<th>MMV690095</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC50 (nM)</td>
<td></td>
</tr>
<tr>
<td>NF54</td>
<td>186.1</td>
</tr>
<tr>
<td>K1</td>
<td>589.6</td>
</tr>
<tr>
<td>7G8</td>
<td>522.2</td>
</tr>
<tr>
<td>TM90C2B</td>
<td>384.6</td>
</tr>
</tbody>
</table>

RF12 (PH-1263-C; Ross et al. 2018 Nature Communications) | 774.3 | 592.0 |
Dd2          | 462.3 | 385.4 |
# Comparison of aryl piperazine sub series

<table>
<thead>
<tr>
<th>Compound</th>
<th>Aryl amides</th>
<th>Cyclopropyl amides</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of compounds synthesised</td>
<td>~45 (limited focus during past few months)</td>
<td>Mainly explored series in past few months</td>
</tr>
<tr>
<td>Pf potency (3D7, µM) range</td>
<td>0.33</td>
<td>0.15 - &gt;5</td>
</tr>
<tr>
<td>SAR understanding</td>
<td>Limited modifications explored</td>
<td>Diverse modifications explored</td>
</tr>
<tr>
<td>PRR</td>
<td>Fast kill in 2 point FACS and PRR</td>
<td>Slow kill(full PRR)</td>
</tr>
<tr>
<td>Blood stage specificity</td>
<td>To be taken up as a part of MoA studies</td>
<td></td>
</tr>
<tr>
<td>Profile in resistant strains</td>
<td>Discussed on slide 10</td>
<td>To be profiled</td>
</tr>
<tr>
<td>MoA</td>
<td>unknown</td>
<td>unknown</td>
</tr>
<tr>
<td>yDHODH screening</td>
<td>Not a bc-1 or DHODH inhibitor(MMV024406)</td>
<td>Exemplar to be sent</td>
</tr>
<tr>
<td>Potency (liver stages)</td>
<td>MMV024406 showed activity, profiling of exemplars ongoing</td>
<td>Profiling of exemplars ongoing</td>
</tr>
<tr>
<td>Potency (transmission),DGFA</td>
<td>inactive</td>
<td>Inactive(FGF); 0.87µM(MGF)</td>
</tr>
<tr>
<td>eLogD</td>
<td>Lower log D compared to cyclopropyl amides</td>
<td>-</td>
</tr>
<tr>
<td>Solubility (i.e. PBS)</td>
<td>Can be modulated, some compounds have ~50uM</td>
<td>screened compounds have sol. &lt;10uM</td>
</tr>
<tr>
<td>Metabolic stability</td>
<td>Needs to be worked upon</td>
<td>Improved stability observed with some compounds</td>
</tr>
<tr>
<td>CYP Inhibition</td>
<td>Can be modulated through structural modifications</td>
<td>No liability with current set of screened compounds</td>
</tr>
<tr>
<td>hERG (K+CHO) IC50 , µM</td>
<td>&lt;1 (MMV1803899)</td>
<td>10.33 (MMV1804508)</td>
</tr>
<tr>
<td>In vivo PK</td>
<td>Identify potent compounds before selection</td>
<td>Select one compound for PK</td>
</tr>
</tbody>
</table>
Proposed Strategy – Aryl piperazine

Discussion points:

Series 1a

Medicinal Chemistry efforts around on hold, revisit after generating more data?

Continue with the plan of target identification (metabolomics, screening in resistant strains, y DHODH and Pf ATP4 assays)

Continue screening of exemplars in liver stage assays

Series 1c

Focus on structural modifications in series 1c with an objective to:

- Improve potency 3D7 IC₅₀<100nM while reducing log D ≤ 3 (Most of the active compounds are in range of 3.5 – 4)
- Improve metabolic stability by reducing log D and/or blocking putative metabolic hot spots

Characterise stage specificity with exemplars and target identification (metabolomics, Pf ATP4 assays)

Screen more compounds in hERG assay to understand if it is compound specific/ scaffold specific
Series 1c: Ongoing/proposed modifications

Explore the region to improve potency
Reduction of log D: may improve metabolic stability and mitigate hERG liability

Evaluate role of linker

Improve in potency and metabolic stability?

MMV024406
\textit{clogP}: 3.6

Part of array synthesis planned at TCGLS
\textit{clogP}: 2.0 – 3.8

\textit{MMV024406}
\begin{align*}
\text{clogP} & : 3.6 \\
\text{Clinton} & : 2.8, 3.3, 2.5 \\
\text{MeO}_2\text{S} & : 2.1, 2.7, 2.1, 3.2 \\
\text{Sanjay} & : \\
\text{X} & : \text{no atom, O, NH} \\
\end{align*}
Series 1c: Proposed modifications

Core hopping

Check for improvement in potency

Terminal pyridyl ring

MMV024406 clogP: 3.6

Clint clogP: 3.1

2.9

2.3

2.3 - 3.3

2.3

2.9

2.3

X
Aryl imidazole – a snap shot of SAR

Challenge:
- Improve metabolic stability of compounds while improving potency
### Rate of killing profile

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose</th>
<th>Lag phase (h)</th>
<th>Log PRR</th>
<th>PCT&lt;sub&gt;99.9%&lt;/sub&gt; (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMV1804317</td>
<td>10xIC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>24</td>
<td>2.82</td>
<td>62</td>
</tr>
<tr>
<td>Pyrimethamine</td>
<td>10xIC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>24</td>
<td>3.55</td>
<td>61</td>
</tr>
</tbody>
</table>

**Graph:**
- **Artemisinin 10x IC<sub>50</sub>**
- **Atovaquone 10x IC<sub>50</sub>**
- **Pyrimethamine 10x IC<sub>50</sub>**
- **Chloroquine 10x IC<sub>50</sub>**
- **MMV1804317**
Aryl imidazole – SAR update

Results awaited

$X = \text{H, Cl}$

$R = \text{H, COPh}$, $3.5 \mu M$

$X = \text{O, S}$

$R = \text{H, COPh}$, $3.5 \mu M$

Pf3D7 LDH IC$_{50}$ uM >25
Addressing poor metabolic stability of MMV023327

Putative metabolites

- Replacement of dimethyl imidazole with imidazopyridine improved metabolic stability
- Replacement of phenyl with pyridyl as scaffold improved metabolic stability

<table>
<thead>
<tr>
<th>MMV ID</th>
<th>023227</th>
<th>692840</th>
<th>892881</th>
<th>1794034</th>
<th>893302</th>
<th>893303</th>
<th>1804344</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pf3D7(SYBR/LDH,IC50 µM)</td>
<td>0.46/1.1</td>
<td>0.33/-</td>
<td>0.72/-</td>
<td>0.86/-</td>
<td>0.92/-</td>
<td>0.7/2.4</td>
<td>-/1.34</td>
</tr>
<tr>
<td>elogD</td>
<td>3.4</td>
<td>4.4</td>
<td>3.4</td>
<td>4.7</td>
<td>4.8</td>
<td>4.8</td>
<td>1.36</td>
</tr>
<tr>
<td>HLM, Clint,mL/min/mg</td>
<td>205</td>
<td>173</td>
<td>54</td>
<td>246</td>
<td>98</td>
<td>70.3</td>
<td>11.6</td>
</tr>
<tr>
<td>r hep,Clint, uL/min/10^6</td>
<td>206</td>
<td>ND</td>
<td>ND</td>
<td>163</td>
<td>22.4</td>
<td>13.4</td>
<td>2.2</td>
</tr>
</tbody>
</table>

Pf3D7(SYBR/LDH,IC50 µM) values indicate the concentration of the compound required to inhibit parasite growth, with lower values indicating better activity. eLogD values reflect the octanol-water partition coefficient, with higher values indicating better hydrophobicity. HLM and r hep Clint values are measures of drug metabolism, with higher values indicating lower metabolism and thus better metabolic stability.
Proposed specific modifications

Expansion of SAR

MMV1848848

\[
\begin{align*}
\text{O} & \text{N} \\
\text{NH} & \text{Cl} \\
\text{C} & \text{H} \\
\end{align*}
\]

MMV1848849

\[
\begin{align*}
\text{N} & \text{O} \\
\text{NH} & \text{Cl} \\
\text{C} & \text{H} \\
\end{align*}
\]

MMV1804317

\[
\begin{align*}
\text{N} & \text{X} \\
\text{Y} & \text{CH} \\
\end{align*}
\]

X: N, CH; Y: NH, O

MMV1804344

\[
\begin{align*}
\text{N} & \text{O} \\
\text{NH} & \text{N} \\
\text{C} & \text{C} \\
\text{H} & \text{H} \\
\end{align*}
\]

\[Pf3D7 \text{ LDH: 1.39}\]

MMV1898909

\[
\begin{align*}
\text{N} & \text{NH} \\
\text{N} & \text{N} \\
\text{C} & \text{C} \\
\text{H} & \text{H} \\
\end{align*}
\]

\[n = 1, 2\]

Planned @ TCGLS

Ongoing synthesis

@CDRI

MMV1898909

RA
Proposed specific modifications

Expansion of SAR

Core hopping

MMV 892881
Update on MMV023227 analogues

Compounds being pursued..

Attempted synthesis of aminoalcohol

Proposed compounds
MMV 023227: Core modifications

- Chromatographic purification on silica led to degradation
- Will be tried again with resynthesized compounds

2-Arylindole analogue
3-phenyl-1,4-benzoxazine
Optimized
2-Arylquinoxaline

P. P. Yadav
Shubham/Kuldeep
Ethers to be prioritised over amines

Fluorination step.

**J. Org. Chem.** 2016, 81, 1269−1276

**Slide by Clint**
Proposed Strategy – Aryl imidazole

- Design and synthesis of compounds to
  - Improve potency 3D7 IC$_{50}$<100nM while reducing log D ≤ 4; fill in the SAR gaps
  - Improve metabolic stability by reducing log D and/or blocking putative metabolic hot spots

- Generate *in vitro* met ID of MMV892881 – volunteers?

- Evaluate exemplars from to understand TCP4 & TCP 5 potential – Q1 2021

- Initiate work on identifying MoA (generation of resistance, metabolomics)

- Characterise stage specificity

Identification of potent compounds with improved metabolic stability and take a go/no go decision by Q3 2021
Back up
MoA studies: Metabolomics, proteomics and CETSA of MMV Libre scaffolds on *P. falciparum* 3D7

**Aim:** Use untargeted metabolomics, proteomics and CETSA to determine the mode of action of the aryl piperazine and aryl imidazole scaffolds from the Malaria Libre program

**Metabolomics and proteomics methodological details:**
- Test compounds: active and inactive analogues
- Compound concentration: 1 µM
- Incubation time: 5 hours
- Metabolite extraction: Cold methanol
- Protein solubilisation: 5% SDC
- Metabolomics analysis: Untargeted
- Proteomics analysis: Untargeted (LFQ)
- Controls: Negative – DMSO, Positive – DHA, chloroquine, atovaquone and PfATP4 inhibitor

**CETSA methodological details:**
- Test compounds: active and inactive analogue
- Compound concentration: 10x and 40x IC50
- Incubation time: 3 mins
- Thermal challenge: 50°C and 60°C, 5 min
Series 1a: Snapshot of modifications explored

Replacement of piperazine

MMVID
Pf3D7 LDH IC₅₀ uM
RA: result awaited
Post meeting data

1848186
0.88
1848300
2.22
Synthesis ongoing
1848694
0.52
1848511
0.63
1848693
0.30

1848596
>25
1848594
>25
1848595
>25
1848597
2.8
New proposals for discussion

Post meeting comments: Proceed with these modifications
Series 1a: Possible structures

Post meeting comments: Head to Head compounds in both subseries for proposed structures 1, 2, 4 and 6. Structures 7 and 8 to be taken up after checking the activity of compounds in array synthesis.
Ways of contribution

- Design and synthesis of compounds to achieve the objectives
- Synthesis of compounds
- Carry out experimental in vitro Met ID
- Screen front runners in lab derived strains other than 3D7; asexual intraerythrocytic blood stage assays, mechanism of action

Details of objectives and plans: https://www.mmv.org/mmv-open/malaria-libre/malaria-libre-data-repository