Malaria Libre
Project Meeting: 24th March 2022
# Status of action items

<table>
<thead>
<tr>
<th>Action item</th>
<th>Responsible group/scientist</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Design and synthesis of analogs</td>
<td>All medicinal chemists</td>
<td>ongoing</td>
</tr>
<tr>
<td>Generation of resistant mutants with MMV1804508</td>
<td>Akhil Vaidya</td>
<td>Repeat studies ongoing</td>
</tr>
<tr>
<td>Send exemplars from cyclopropyl amide scaffold for glu-gal assay</td>
<td>Kiran</td>
<td>MMV1804508&amp; MMV1899468 queued for screening at AZ</td>
</tr>
<tr>
<td>Send MMV1804508 to David Fidock for early MIR assay (actionable post meeting with Akhil)</td>
<td>Kiran to coordinate</td>
<td>Queued for profiling</td>
</tr>
<tr>
<td>Medicinal Chemistry brainstorming session around aryl imidazoles</td>
<td>Kiran to coordinate</td>
<td>Done, key discussion points on slide</td>
</tr>
<tr>
<td>Aryl imidazoles: antiplasmodial activity of selected compounds with verapamil Screening of MMV1901050 in Pf &amp; Pv clinical isolates</td>
<td>Caroline</td>
<td>Data by April</td>
</tr>
<tr>
<td>Aryl imidazole exemplar for generation of resistance</td>
<td>Caroline</td>
<td>Compound to be selected</td>
</tr>
</tbody>
</table>
General Update

Phenyl amide scaffold:
- Synthesis ongoing

Aryl imidazole scaffold:
- IKTos to explore the scaffold using their AI platform
- Compounds prioritised in brainstorming session, put in for synthesis at TCG
- Enantiomers of MMV1918856 were synthesised & submitted for screening

Compounds from cyclopropyl amide & aryl imidazole submitted for early & late-stage gametocyte assays

Exemplars from aryl imidazole submitted for albumax binding assay – results discussed
Agenda

Aryl imidazoles
- recent update
- snapshot of brainstorming session & next steps

Discussion around new scaffolds for lead optimization
- MMV1770436 analogs
  - Status & Medicinal Chemistry plans
Aryl imidazole scaffold

<table>
<thead>
<tr>
<th>Compound</th>
<th>MMV1899746</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pf potency (3D7, K1, NF54, Dd2 µM):</td>
<td>0.72/-/-/5.2</td>
</tr>
<tr>
<td>Cytotoxicity (HepG2, µM)</td>
<td>&gt;25</td>
</tr>
<tr>
<td>eLogD</td>
<td>1.81</td>
</tr>
<tr>
<td>Solubility (i.e. PBS), µM</td>
<td>187</td>
</tr>
<tr>
<td>CACO-2 (ER; AB; BA)</td>
<td>19.12; 0.67; 12.82</td>
</tr>
<tr>
<td>Binding (hPPB)</td>
<td>74.2</td>
</tr>
<tr>
<td>Metabolism; microsome (h) Clint</td>
<td>9.4</td>
</tr>
<tr>
<td>Metabolism; hepatocyte (r) Clint</td>
<td>4.8</td>
</tr>
</tbody>
</table>

Dd2 crossR most likely due to MoA switch

Pf CRT not involved

Limited modifications to increase lipophilicity

Improve activity?

Impact on crossR?

Next steps:
- Screen in Pf Dd2 LDH assay
- MMV1919542 for Tier1 ADME & CaCO2 assays
- Screen MMV1899746 or MMV1900786 in PRR & liver stage assay

Ongoing activities
Screening of MMV1900434 & MMV1901050 in Pf & Pv clinical isolates (Caroline)

(from Jan’22 presentation)
Update since January

MMV1901050
Pf3D7LDH : 0.13uM
eLogD : 4.14

MMV1902020
Pf3D7LDH : 0.27uM
clogD : 4.6

MMV1919466
Pf3D7LDH : 0.67uM
clogD : 1.4

MMV1919343
Pf3D7LDH : 0.39uM
clogD : 1.4

MMV1919570
Pf3D7LDH : 0.089uM
clogD : 2.6

MMV1919569
Pf3D7LDH : 0.13uM
clogD : 2.5

MMV1919570 submitted for Dd2 and Tier1 ADME assays

<table>
<thead>
<tr>
<th>Compound</th>
<th>MMV1918856</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pf (3D7, Dd2µM)</td>
<td>0.15/0.28</td>
</tr>
<tr>
<td>HepG2,µM</td>
<td>15.03</td>
</tr>
<tr>
<td>eLogD</td>
<td>2.98</td>
</tr>
<tr>
<td>Solubility (i.e. PBS), µM</td>
<td>15</td>
</tr>
<tr>
<td>CACO-2 (ER; AB; BA)</td>
<td>3;8.09;24.9</td>
</tr>
<tr>
<td>Microsome (h) Clint</td>
<td>70.4</td>
</tr>
<tr>
<td>Hepatocyte (r) Clint</td>
<td>41</td>
</tr>
</tbody>
</table>

Generate invivo Met ID
Summary of discussion in Med Chem brainstorming session

Aryl imidazoles

• Plateauing of potency at LogD~2.5. Why? likely to be the effect of binding to Albumax in the assay as LogD increases?

• If this is the case, then as LogD increases the free concentration of cpd in the assay is decreases. So, if compounds are getting more potent (say x10), but their free concentration may be reducing (say x10), then we would not measure better IC\textsubscript{50} and see an apparent plateauing of potency.

• Get % Albumax binding for compounds along the top of this plot. We can then correct the IC\textsubscript{50} for ‘free concentration’ of compound and get clearer SAR to use in optimisation.

• Data generated for exemplars (next slide) – compounds with logD<3 have low albumax binding

• MMV1900787 looks like a better ‘core’ to try and improve potency on.

• The new ‘amide’ derivatives such as MMV1918856 are also worth considering.

• Metabolic unstability could be due to relatively high LogD and structurally benzyl positions that may be metabolic soft spots?
Aryl imidazoles: Next set of compounds

Synthesis of highlighted compounds lined up at TCG
Albumax binding data

Compounds with logD >3 have high albumax binding.
Aryl Imidazoles: 3D7 pIC$_{50}$ corrected for Albumax binding

- We now see no plateauing of 3D7 activity with LogD.

- ‘Free’ IC$_{50}$ of MMV1919040 is 1 nM compared to 230 nM uncorrected (99.4% bound)!

- The more polar compounds, such as MMV1900787, ‘4346 & ‘8856 do not show a big shift in IC$_{50}$ when corrected for binding (ca. 2-fold, ca. 42-55% bound).

- Discussion: Do we focus on analogues of ‘9040 and improve ADME/PK or on analogues of ‘0787 and improve potency?

- Should we get Albumax on more of the key compounds?
Some SAR has become clearer:

- ‘Free’ IC₅₀ of MMV1919040 and MMV1918856 show a clear effect of amide linker vs. -CH₂O-linker

- Pyridyl vs. Phenyl ring in ‘0787 and ‘4346 has only small effect on potency.
<table>
<thead>
<tr>
<th>MMV ID</th>
<th>MMV1919040</th>
<th>MMV1900434*</th>
<th>MMV1918856</th>
<th>MMV1794346</th>
<th>MMV1900787</th>
</tr>
</thead>
<tbody>
<tr>
<td>MW</td>
<td>365.4</td>
<td>342.8</td>
<td>378.4</td>
<td>289.4</td>
<td>290.4</td>
</tr>
<tr>
<td>logD</td>
<td>3.215</td>
<td>5.182</td>
<td>2.453</td>
<td>2.699</td>
<td>2.05</td>
</tr>
<tr>
<td>eLogD-pH 7.4</td>
<td></td>
<td></td>
<td>2.98</td>
<td></td>
<td>2.99</td>
</tr>
<tr>
<td>IC50 3D7 LDH 72 hr uM</td>
<td>0.232</td>
<td>0.119</td>
<td>0.149</td>
<td>0.122</td>
<td>0.234</td>
</tr>
<tr>
<td>3D7 IC50 uM (corrected Albumax)</td>
<td>0.0013</td>
<td>0.0047</td>
<td>0.0663</td>
<td>0.0575</td>
<td>0.1353</td>
</tr>
<tr>
<td>Sol PBS pH 7.4/RT Kinetic uM</td>
<td></td>
<td>14.69</td>
<td></td>
<td>26.55</td>
<td></td>
</tr>
<tr>
<td>Hu Mics CLint uL/min/mg</td>
<td></td>
<td>70.44</td>
<td></td>
<td>79.64</td>
<td></td>
</tr>
<tr>
<td>Rat Heps CLint uL/min/10e6 cells</td>
<td></td>
<td>41.14</td>
<td></td>
<td>39.33</td>
<td></td>
</tr>
<tr>
<td>Albumax % bound</td>
<td>99.43</td>
<td>96.03</td>
<td>55.5</td>
<td>52.9</td>
<td>42.2</td>
</tr>
</tbody>
</table>

- Collect ADME data on this set to see what profile the very active compounds have.
- Consider getting more Albumax binding on wider range of compounds for clearer SAR.
Aryl imidazoles: next steps for discussion

Putative metabolic sites

Next steps:

- Volunteers for in vitro Met ID
- Retain lipophilicity in similar range and focus on improving metabolic stability
  - disubstituted phenyl ring
  - replacement of dimethyl imidazole with imidazopyridine
  - replacement of central phenyl ring with pyridyl?

Can methoxybenzyl be replaced with other groups?
Is the increase in potency based on increased lipophilicity without specific interactions?
What should be the first set of compounds to be synthesized?

To be taken up on confirming activity with R enantiomer
To be taken up after confirming the Dd2 potency
New hit from library screens – similarity to existing compounds

MW = 269
cLogP = 0.9
tPSA = 37

**MMV1831782-02** (purchased)

- 3D7 LDH IC$_{50}$: 0.38 μM (TCGLS)
- Dd2 LDH IC$_{50}$: 0.41 μM (TCGLS)
- Dd2 [SybrG] IC$_{50}$: 0.517 μM (Sanger)
- MXR – no resistant line (Sanger)
- No DHODH inhibition (UCSD)

- HepG2 IC$_{50}$: > 25 μM
- Sol (PBS pH 7.4): 180.5 μM
eLog D: 0.9
- HLM: < 3.5 μL/min/mg
- RHEPs: 23.6 μL/min/10${^6}$ cells

**MMV1900786**

- Pf 3D7/Dd2: 0.67/4.2 μM
eLogD: 1.4
- HLM: 3.5uL/min/mg
- rHeps: 33.8uL/min/10e6
- Solubility: 194uM

**MMV1919089**

- Pf 3D7: 8.4 μM
**MMV1770436 – profile (recap)**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pf 3D7/Dd2, IC₅₀, µM</td>
<td>0.43/0.54</td>
</tr>
<tr>
<td>HepG2, IC₅₀, µM</td>
<td>&gt;25</td>
</tr>
<tr>
<td>eLogD</td>
<td>2.44</td>
</tr>
<tr>
<td>HLM, µL/min/mg</td>
<td>44.6</td>
</tr>
<tr>
<td>R Heps, µL/min/10⁶ cells</td>
<td>111.8</td>
</tr>
<tr>
<td>Sol(PBS,pH7.4)</td>
<td>&gt;200</td>
</tr>
<tr>
<td>Caco-2 (B-A)/(A-B),ER</td>
<td>ND</td>
</tr>
<tr>
<td>PPB,h</td>
<td>ND</td>
</tr>
<tr>
<td>hERG % inhibition @1uM</td>
<td>&lt;30</td>
</tr>
<tr>
<td>MoA/resistance profile</td>
<td>No growth in barcode assay - unknown</td>
</tr>
<tr>
<td>PRR</td>
<td>4.3</td>
</tr>
<tr>
<td></td>
<td>Rate of kill like CQ, tested at 10xIC50</td>
</tr>
</tbody>
</table>

**Key questions to be answered**

- role of hydroxy and secondary amine
- minimum pharmacophore
- essentiality of THP

Only asexual blood stage activity
MMV1770436 – activity of recent compounds

Can some ionic interaction improve activity?

1919374 20
1919373 21
1919372 4.3
1919502 >25

Synthesis ongoing

Structural constraint

MMVID
Pf3D7LDH, IC50uM
MMV1770436 – SAR understanding so far

Secondary amine required for activity

Substituted phenyl ring required
Heteroaromatics not tolerated
What is optimum substitution/bulk?

Optimum substitution, Bulk?

Required for activity

Basic centre not tolerated
Site open for modulation, t-butyl amide is active
MMV1770436 analogs: property space vs activity

- High LipE (>5) for actives
- clogD of the compounds is in range of 1-2; increase of potency by building lipophilicity in the right regions is possible
- Replacement of THP with boc piperidine improves activity (explore this region)
- Modifications on the chlorophenyl ring & hydroxy pyridine ring

Sized by pIC$_{50}$
MMV1770436 analogs: Medicinal Chemistry

- Identify vectors to improve antiplasmodial activity by increasing log D
- Optimum substitution pattern on aryl rings

Representative examples

- logD in range of 1.8 - 2.2
- Synthesis ongoing at TCG & Carolyn lab

What should be the next set of compounds – Discussion in Med Chem brainstorming meeting
Send a list of compounds for prioritization before the meeting

Highlighted regions: regions for modification
General Chemistry for synthesis of analogs

Scheme-1

1. **Step 1**: Cl-CH=N-N-Cl
   - Reagents: NaH, DMF

2. **Step 2**: TFA, CH2Cl2
   - Reagents: Ei3N, CH2Cl2

3. **Step 3**: Pivoly chloride
   - Reagents: NH3, MeOH

4. **Step 4**: Raney Ni
   - Reagents: NaCNBH3, AcOH, MeOH

5. **Step 5**: TM10

6. **Step 4A**: NiCl2, NaBH4
   - Reagents: Boc anhydride

7. **Step 4B**: TFA, CH2Cl2

5A
- Brainstorming session in 2nd week of April
- Kick off meeting with IKTOS on 31st March
- Core strategy meeting in last week of April/1st week of May
● Back up
Phenyl group important for activity
- Both EWG & EDG are tolerated
- o & p- substituents are equipotent, m- substituted compound to be synthesized

3-hydroxy aryl in LHS required for activity
MMV1770436 – emerging SAR(2/2)

Evaluate role of secondary amine

Essentiality of pyran

- Secondary amine essential for activity
- Explore modifications of pyran ring

1919339 10.35
1919342 5.21
1919183 >25
1901686 5.96
1902024 0.18
1919341 1.73
ongoing
Aryl Imidazoles: Potency vs. LogD

- **Aryl Imidazoles**: There is an apparent plateauing of potency at LogD~2.5. Why? This is most likely to be the effect of binding to Albumax in the assay as LogD increases.

- If this is the case, then as LogD increases the free concentration of cpd in the assay is decreases. So if compounds are getting more potent (say x10), but their free concentration may be reducing (say x10), then we would not measure better IC\(_{50}\) and see an apparent plateauing of potency.

**Suggestion**

- Get % Albumax binding for compounds along the top of this plot. We can then correct the IC\(_{50}\) for ‘free concentration’ of compound and get clearer SAR to use in optimisation.
Aryl_Imidazoles: More detailed SAR vs. LogD

Some comparisons highlighted in next slide.
Aryl_Imidazoles: Some areas still to explore & discuss

- 5-Nitrogen here is acceptable and lowers LogD.
- May enable chemistry to add 6-substituents (by nucleophilic displacement).
- Explore 6-Substitution.
- Try a range of Sizes, Polarity and Electronics.
- Can the 'benzyl' groups be replaced, to see if metabolic stability improves?
- Nitrogen here is acceptable and lowers LogD
- -OMe, Cl were also acceptable
- Explore larger groups than –Ome.
- Remove one Me-group
- Replace one Me with larger alkyl, or small cycloalkyl
- Replace one Me with Phenyl or a Heterocycle
Aryl_Imidazoles: Some detailed SAR vs. LogD

Some direct comparisons from previous slide and ideas to consider:

1. MMV690095 vs MMV1794348 ca. 2 LogD unit difference but <0.5 Log difference in IC\textsubscript{50}
   - NH- compared to O-link has lower LogD. Explore more NH-linked analogues – with higher LogD.

2. MMV690095 vs MMV1901050 – constrained analogue. IC\textsubscript{50} increases only in line with LogD – no evidence of increase due to constraint
   - Make the meta Cl- isomer, as in combination with ‘constraint’ could improve IC\textsubscript{50} by more.

3. MMV1900787 vs MMV1901050 - constrained analogues. Cl vs Pyridyl >1 Log unit difference in LogD but only 2-fold difference in IC\textsubscript{50} – Is this Albumax effect or N-interaction? Get Albumax binding. Then consider follow-up analogues of ’0787.

4. MMV1901050 vs MMV1901659– both are constrained analogue, same IC\textsubscript{50} but ca. 2 LogD unit difference. Would 1901659 be more potent if we calculate Free IC\textsubscript{50}? Is Indole –NH or difference in geometry important?
   - Consider meta- and para- Cl analogues, to see if potency can be further improved.

5. MMV1901659 vs MMV1903501– both are constrained analogue, same LogD but IC\textsubscript{50} is 0.5 unit less for ’3501. Benzoxazole worse than indole – why? NH donor needed? Ring twist?
   - Try Benzimidazole.

6. MMV1794348 vs MMV1900434 – same LogD and same IC\textsubscript{50}. H- same as -OMe substituent – is potency driven just by LogD?
   - Should check Albumax binding for these two analogues to confirm relative IC\textsubscript{50}.

7. MMV1900434 vs MMV1900433 – Similar IC\textsubscript{50}, small difference in LogD. Suggests -H, -OMe, -Cl and possibly other substituents are possible at this position. Try some other substituents to find optimal for potency and properties.

8. MMV1900172 vs MMV1900640– same LogD but >10 fold different IC\textsubscript{50} – Me substitution in unacceptable position in MMV1900640.

9. MMV1918856 vs MMV1918815 – same LogD but ca. 4 x difference in IC\textsubscript{50}. F may be affecting the amide H-Bond?

10. MMV1918857 vs MMV1918815 – slight difference in potency between amide and the reserve amide – is this significant/useful?
Aryl Imidazoles: Potency vs. MW (sized by LogD)

- **Aryl Imidazoles:** Most molecules have MW <350, so there is ‘headroom’ to increase MW and make the molecules bigger – **but need to retain good drug properties** (e.g. Potency, Sol, Cl_ints etc).

- Some SAR shown by looking at the molecules with MW >350. Addition of certain groups leads to big drops in potency.
Aryl Imidazoles: Ligand Efficiency vs. Lipophilic Efficiency

- **Aryl Imidazoles**: Sized by pIC$_{50}$ vs. 3D7
- Should aim for LipE > 5
- None of the compounds have appropriate LipE for a Lead — but the IC$_{50}$ we are measuring is being affected by Albumax binding for LogD > 2.5.
- MMV '1050 and '4346 are more potent than '0787 but more lipophilic so LipE is lower.
- '0787 looks like a better ‘core’ to try and improve potency on.
- The new ‘amide’ derivatives such as MMV1918856 are also worth considering.

LipE=pIC$_{50}$-LogD
LE=pIC$_{50}$/NA*1.4
NA-number of heavy atoms
ArylImidazoles: Metabolic Stability is an issue

- **Aryl Imidazoles**: The most potent compounds are also the least stable metabolically (spots sized by potency)

- This could be due to relatively high LogD and structurally, the least stable also carry ‘benzyl’ positions that may be metabolic soft spots?
Core modification to benzoxazine led to retention of activity. Unsuccessful, led to benzoxazole formation via decarboxylation, led to mixture of products, led to regioisomeric mixture of products. No further work.
Future plan

- 3-aryl-1,4-benzoxazine and benzoxazole analogues

Under progress

Chemical stability?
Core modification to 2-arylindole led to improvement in activity.

C-3 Substitutions to improve stability

Cytotoxicity in the scaffold is presumed due to planarity of the 2-aryl indole scaffold.

Dearomatization functionalization of indole is planned to resolve the same.

Prem P. Yadav
Shubham
2-arylindole analogues

- Based on the similar activity of benzoxazole regioisomers

<table>
<thead>
<tr>
<th>Compound</th>
<th>3D7 LDH IC50</th>
<th>Dd2 LDH IC50</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMV1901658</td>
<td>0.32 µM</td>
<td>2.39 µM</td>
</tr>
<tr>
<td>S-021-0436</td>
<td>0.22 µM</td>
<td>0.99 µM</td>
</tr>
</tbody>
</table>

- Dearomatization of Indoles

Synthesis, chemical stability?
2 chiral centres – resolution may be challenging

Based on above two points - deprioritised