Malaria Libre
Project Meeting: 29th July 2021

Kirandeep Samby
Welcome to Dr. Takeshi Yura (new ESAC mentor)

Status of action items

General update

SAR update and next set of compounds
  aryl imidazole
  aryl piperazine (cyclopropyl amides, sub series 1a)
  aryl piperazine (sub series 1c)

Discussion points
  right time to screen in CEREP panel
  Medicinal Chemistry brain storming sessions – update
<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 in aryl imidazole and aryl piperazine(subseries, 1c)</td>
<td>CDRI, Clint, TCGLS</td>
<td>ongoing</td>
</tr>
<tr>
<td>Mechanism of action studies: metabolomics – concentration and time dependent changes in metabolite levels, CETSA</td>
<td>Carlo Giannangelo</td>
<td>ongoing</td>
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<tr>
<td>Screening of compounds in Hemozoin adsorption model - Determine β-hematin IC50 of a few compounds based on the final results of adsorption model - Cell fractionation assays to confirm MoA</td>
<td>Katherine D Villers</td>
<td>ongoing</td>
</tr>
<tr>
<td>Blood stage specificity assays: Repeat the assay and study the morphological changes</td>
<td>Shailja Singh</td>
<td>ongoing</td>
</tr>
<tr>
<td>Screen MMV1804508 and MMV1899468 in: Pf bc-1 assays Glu-gal cytotoxicity assays Resistant cell lines (other than atovaquone and ELQ 300 resistant cell lines) Generation of resistant mutants</td>
<td>Akhil Vaidya</td>
<td>ongoing</td>
</tr>
</tbody>
</table>
General updates

- MMV1804317 (aryl imidazole) was cross-resistant (crossR) in STPH screens – earlier in the Sanger multiplex assay, growth was observed in multiple cell lines – could be attributed to loss of activity in Dd2

- yDHODH data indicates that MMV1804508 and MMV1804743 (cyclopropyl amides) are bc-1 inhibitors

- Invitro ADME data for aryl imidazole compounds generated – to be discussed

- Work on evaluation of back up series is ongoing – parasitology profiling and hit confirmation

- Separate medicinal chemistry brainstorming sessions, where research scholars contributing to the project participate in the discussion and come up with ideas

- ESAC review in October
Transmission to Human

MMV1900434

Moderate rate of killing (MMV693239)

DGFA: in active

Parasitology – aryl imidazole scaffold

Pb (sporozoite): 0.26 (MMV690095); 1.58 (MMV1804317)

Pf (3D7, NF54, Dd2): 0.24, 0.18, 0.33; No cross resistance in UCSD panel of resistant strains (MMV690095)

Stages (Ring/Troph/ Schizont): ongoing

MMV1804317: growth in multiple cell lines in multiplex assay – probably due to loss in activity in Dd2 strain (cross R in STPH strains)

MMV1900434 with 1.5 fold shift in Dd2/3D7 sent for screening at Sanger

MMV1794348 caused no distinct metabolic profile and antimalarial activity is unlikely to involve perturbation of parasite metabolism

Negative in pH finger printing assay

Concentration/time dependent metabolomics & CETSA planned
Aryl imidazole - strategy

Fix dimethyl imidazole and imidazopyridine on LHS for SAR exploration on the core and RHS
- Dd2/3D7 shift: compound specific or scaffold dependent
- Improve potency and address metabolic stability issues

- Identify new chemical space with improved potency
- Improve metabolic stability by reducing lipophilicity
- Fixing the conformation to improve potency
Core hopping

Understand Dd2/3D7 shift is compound specific or series specific

Introduction of Me and replacement of pyridyl ring with substituted phenyl reduces DD2/3D7 ratio compounds for screening
Cyclisation has led to improvement in activity
- Substitution on the indane ring didn’t improve activity
- Introduction of pyridine led to a higher Dd2/3D7 shift than chloro

Compounds evaluated in ADME assays (slide 11)
Replacement of benzyl group

- Loss in activity observed with alicyclic amines
- Dd2/3D7 shift >5 fold for two compounds with fused bicyclics – depriortise, is it a MoA switch?
Dd2/3D7 shift – role of physico chemical properties

Based on the limited data set: no correlation with TPSA, difficult to determine the XlogP range.

Increasing the number of ionisable centre appear to increase the Dd2/3D7 shift.

Role of efflux pumps?
### Aryl imidazole – ADME

<table>
<thead>
<tr>
<th>MMV ID</th>
<th>1900786</th>
<th>1899746</th>
<th>1900787</th>
<th>1901050</th>
</tr>
</thead>
<tbody>
<tr>
<td>3D7/Dd2; uM</td>
<td>0.67/4.23</td>
<td>0.72/5.2</td>
<td>0.23/0.67</td>
<td>0.13/0.20</td>
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<tr>
<td>$\text{e} \log D$</td>
<td>1.39</td>
<td>1.8</td>
<td>2.99</td>
<td>4.1</td>
</tr>
<tr>
<td>HLM, $\text{Clint}, \text{mL/min/mg}$</td>
<td>3.5</td>
<td>9.4</td>
<td>79</td>
<td>92</td>
</tr>
<tr>
<td>r hep, $\text{Clint}, \text{uL/min}/10^6$</td>
<td>33.8</td>
<td>4.8</td>
<td>39</td>
<td>163</td>
</tr>
<tr>
<td>Solubility(PBS, uM)</td>
<td>194</td>
<td>187</td>
<td>27</td>
<td>4.52</td>
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</table>

Fused bicyclic amines have a better solubility and metabolic stability as compared to isoindoline derivatives - removal of benzyl moiety and lower lipophilicity
Ideas for next set of compounds

Impact on potency & Dd2/3D7 shift

Improve potency

Lower priority

MMV
Medicines for Malaria Venture
Aryl piperazine – cyclopropyl amides

yDHODH assay: MMV1804508 & MMV1804743 are bc-1 inhibitors

Ongoing activities: confirmation in Pf bc1 assays & mammalian selectivity; resistance generation

Discussion points:

Right time to reinitiate Medicinal Chemistry?

Insilico modelling to decipher the probable binding mode – Andre to take up

3-Cl Py to pyrimidine – 2 fold improvement in activity, reconfirm the activity

1899468
Pf 3D7/Dd2: 0.13/0.34
clogP:4.18

1901272
2.32
clogP:2.99
To improve 1) potency  2) metabolic stability 3) solubility

Few thoughts..

Share your suggestions by 14th August – prioritization discussion in next meeting or separate brainstorming session
Aryl piperazine – phenyl amides

Current status:

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<tbody>
<tr>
<td>No. of compounds synthesised</td>
<td>~100 compounds</td>
</tr>
<tr>
<td>Pf potency (3D7, µM) range</td>
<td>0.3 - &gt;10</td>
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<tr>
<td>SAR understanding</td>
<td>(details in the back up)</td>
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<tr>
<td>PRR</td>
<td>Fast kill in 2 point FACS and PRR</td>
</tr>
<tr>
<td>Blood stage specificity</td>
<td>ongoing</td>
</tr>
<tr>
<td>Profile in resistant strains</td>
<td>No crossR in Sanger&amp;STPH</td>
</tr>
<tr>
<td>MoA</td>
<td>unknown</td>
</tr>
<tr>
<td>γDHODH screening</td>
<td>Not a bc-1 or DHODH inhibitor(MMV024406)</td>
</tr>
<tr>
<td>Potency (liver stages)</td>
<td>MMV024406 showed activity, profiling of exemplars ongoing</td>
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<tr>
<td>Potency (transmission),DGFA</td>
<td>Inactive</td>
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<tr>
<td>eLogD</td>
<td>Lower log D compared to cyclopropyl amides</td>
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<tr>
<td>Solubility (i.e. PBS)</td>
<td>Can be modulated, some compounds have ~ 50uM</td>
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<tr>
<td>Metabolic stability</td>
<td>Needs to be worked upon</td>
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<tr>
<td>CYP Inhibition</td>
<td>Can be modulated through structural modifications</td>
</tr>
<tr>
<td>hERG (K+CHO) IC50, µM</td>
<td>&lt;1(MMV1803899)</td>
</tr>
<tr>
<td>In vivo PK</td>
<td>Identify potent compounds before selection</td>
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Current focus:

Can pyridine be replaced by other groups which can provide handle on dialling out hERG liability?

Explore other regions for modifications which can contribute to improvement in activity
Probable replacement of pyridine

Address hERG, improve potency

- replacement of py with other heteroaryls, substituted phenyls led to loss in activity
- can it be replaced with other alicyclic groups

**MMV024406**
- Pf 3D7 LDH : 0.42µM

**MMV1900956**
- Pf 3D7 LDH : 1.25µM

Based on the structural overlay with crizotinib

Synthesis on going

Screening on going

Are these modifications tolerated?
**Modifications in less explored regions**

**Improve potency, metabolic stability by modifications in amide region**

- Reverse amide and extension of linker led to 2-3 fold loss in activity
- 5-Py to 4-py shift not tolerated

- Des chloro synthesised
- Ongoing at TCG

- Ongoing at TCG

**Optimum spatial arrangement?**

- Pf 3D7IC_{50} : 2.2µM

**Other linkers (stability, additional interactions?)**

**Essentiality of amide**

**Is extended pocket available**

**MMV024406**

- Pf 3D7 LDH : 0.42µM
- eLog D: 3.7

**Synthesis at Epichem**

- Kishor
Discussion

Right time to screen exemplars in CEREP panel

Thoughts on how to improve engagement of chemists (Medicinal) in the project

- separate brainstorming sessions initiated
  - increase participation of researchers working on the bench
- group members to focus on specific regions of the scaffold to have better engagement

Are there additional Medicinal Chemistry groups interested to be part of the project?
Aryl imidazole scaffold – snapshot of SAR

Snap shot

Methyl subsituents required, NMe not tolerated
Fused pyridine core retains activity
fused piperidine ring detrimental for activity but
cyclohexyl tolerated

Oxadiazole, triazole, pyridine, pyrimidine, pyrazoles, thiazole
benzimidazole not tolerated

Indole, imidazopyridine are tolerated
replacement of imidazole with amides is not tolerated

Dimethyl imidazole and imidazopyridine used for further SAR expansion
Core & RHS amenable to modifications
Aryl piperazine scaffold (series1c): SAR

- Identify regions to improve potency and modulate ADME parameters
- Address CYP liability (log D < 3 and modifications at pyridyl ring)
- Modification of putative metabolic hot spots

hERG

- Shifting amide to 4th position led to loss in potency
- ~2 fold loss in activity for reverse amides

Phenyl is tolerated, Cl not essential for activity, pyridazine tolerated, loss of potency with pyrazine and pyrimidine

4-pyridyl necessary
3-Me, 4-py retains activity
loss of potency with 3-pyridyl and substituted phenyl

4-Pyridyl substituents are tolerated
Amide library

Identify vectors for improving activity, moving away from hidden aniline

Key understanding:
- Substituted pyridines are tolerated
- Other heteroaryls or polar substituents on phenyl not tolerated
- Cycloalkyls were detrimental
- Possibility of moving away from hidden aniline

Next steps – explore hydrophobic space?
substituted pyridyl modifications of benzyl piperidine

NF54 IC_{50}
3D7(LDH or SYBR)IC_{50}

MMV024406
Pf 3D7 LDH : 0.42µM
elog D: 3.7
Role of amide

- MMV024406
  Pf 3D7 LDH: 0.42µM
elog D: 3.7

3D7(LDH)IC50

Other linkers (stability, additional interactions?)

Essentiality of amide

Next steps – explore into the hydrophobic space?
Role of terminal pyridyl

MMV024406
Pf 3D7 LDH : 0.42µM

Previous data

Next set of compounds?
(Discussion point)

NF54/3D7 IC_{50}(µM)
Initial SAR investigations and chemistry plans

Modification of linker – impact on potency, metabolic stability, hERG, probable replacement of pyridine.

MMV024406
Pf 3D7 LDH : 0.42µM

Next steps
Core hopping – improvement in potency?

MMV024406
Pf 3D7 LDH : 0.42µM

Previous data

Modulating the steric bulk and electronics on the core

Is 3-Cl Py a linker?

Mimic 4-Aqs (Prem)

Low priority

NF54/3D7 IC₅₀(µM)

Medicines for Malaria Venture
Ideas from virtual screening hits

Crizotinib K1 IC$_{50}$: 0.78uM

Next steps

Violet: MMV024406

Low priority
Is it the optimum spatial arrangement?

Low priority - kinase inhibition?