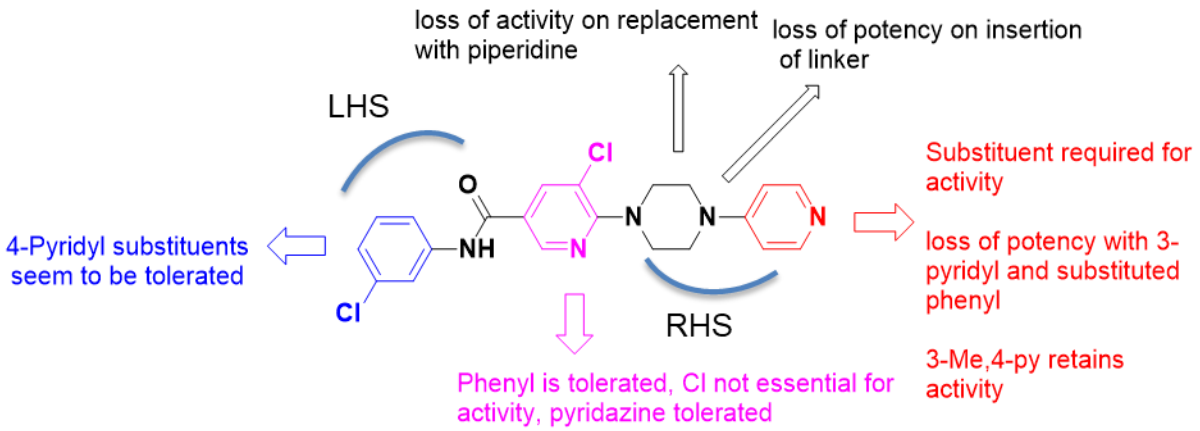
MMV024406 (aryl piperazine) scaffold

This template was identified from a GSK phentypic HTS screen (TCMDC-134600) and subsequently included in the Pathogen box. Around 40 analogs have been synthesied to date to draw a preliminary structure activity relationship (Pf ABS assays) and understand the challenges with this scaffold. Representative compounds were profiled across the plasmodium lifecycle - compounds are active against Pf and Pb liver stages but show little evidence of transmission blocking activity (weak stage V gametocyte activity, inactive in DGFA). Lifecycle profile will be further evaluated when more potent blood stage analogues are available. MMV024406 shows a fast rate of killing in *in vitro* two point FACS and two point PRR assays.

Fig 1: Snapshot of SAR



There are five regions for modification in this template, three of these regions have been broadly explored (synthesis of 40 analogues) to understand the SAR and effect on physicochemical and drug metabolism properties.

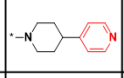
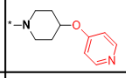
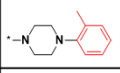
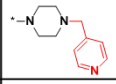
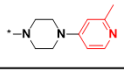
Profiling of MMV024406 in various biological assays showed that the compound has a log D of 3.7, borderline high human microsomal clearance (21µL/min/mg), low kinetic solubility (<2.5µM) in PBS and high CYP inhibition @10µM against the tested isoforms.

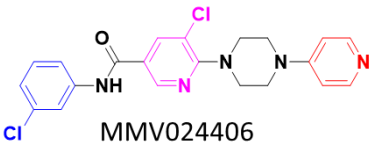
Poor kinetic solubility and CYP inhibition may be attributed to high lipophilicity and 4-Pyr respectively. Modifications in the central ring has led to reduction in lipophilicity - MMV690906 and MMV690872 have log D`s of 2.9 and 2.6 respectively but no improvement in solubility or CYP profile was observed. Insertion of a basic centre in the LHS of the molecule also led to a lowering of log D and improvement in solubility but no improvement in CYP profile.

Reduction in the number of aromatic rings by replacing the aryl amide with a cyclopropyl amide (MMV024408) retains activity and shows improvement in CYP profile. Cyclopropyl amides don't

appear to follow the same SAR as aryl amides and need to be explored further (MMV024408 vs MMV1803900 and MMV1803901 vs MMV024408).

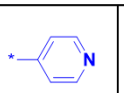
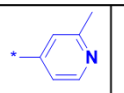
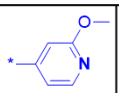
Fig 2: Representative modifications on RHS and LHS region

						
MMVID	1803905	1803904	1803902	1803900	1803903	1803899
Pf 3D7 (µM)	6.7	25	5.4	8.2	11.4	0.37



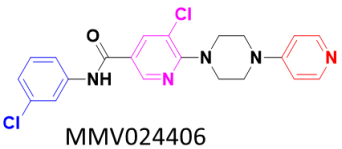
MMV024406

Pf NF54: 0.26 µM
Pb liver: 0.17 µM
HepG2: 4.8 µM
Gam stage V: 10.8 µM

				
MMVID	690089	693110	693244	1803901
Pf NF54/3D7 (µM)	0.68/-	0.34/-	0.77/-	-/23
Pb liver(µM)	7.78	1.25	ND	ND
HepG2 (µM)	23.8	8.43	ND	ND
Gam stage V (µM)	10	40	ND	ND

ND: not done

Fig 3: Modifications around central ring



MMV024406
Pf NF54: 0.26 µM
Pb liver: 0.17 µM
HepG2: 4.8 µM
Gam stage V: 10.8 µM

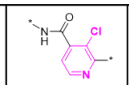
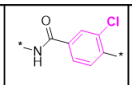
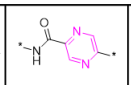
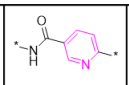
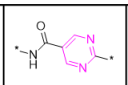
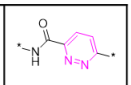
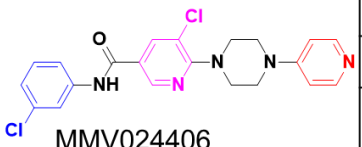
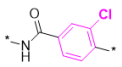
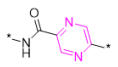
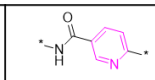
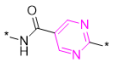
						
MMVID	1804112	692137	690872	690148	690149	1804208
Pf NF54/Pf 3D7 (µM)	-/2.27	0.38/0.53	0.55/-	0.91/-	2.4/-	-/3.5
Pb liver(µM)	ND	3.4	0.59	1.2	ND	ND
HepG2 (µM)	ND	5.2	17.1	17.1	ND	ND
Gam stage V (µM)	ND	0.99	4.7	5.8	ND	ND

Fig 4: Structure – Property Relationship: modifications in the central ring

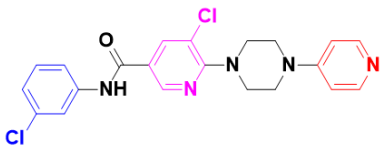


MMV024406
Pf NF54:0.26 µM
eLogD: 3.7
Clint(hmic): 20.6 µL/min/mg
Caco2: 2.1
Sol(PBS,pH7.4) <2.5 µM
CYP 1A2, 2C9, 2D6, 3A4
% I@10 µM: 45,69,72,84

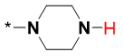
				
MMVID	692137	690872	690148	690906
Pf NF54(µM)	0.38	0.55	0.91	0.58
eLogD	4	2.6	2.7	2.9
Clint(hmic) : µL/min/mg	27	26	65.4	40.1
Caco2(A-B)	1.6	6.2	3.6	5.6
KS(PBS,pH7.4)	<2.5	5	8.4	<2.5
CYP 1A2, 2C9, 2D6, 3A4 % I@10 µM	47,48,74,77	47,73,53,66	35,62,54,58	39,65,62,67

Insertion of N in the central ring reduces lipophilicity but improvement in solubility with reduced logD is not observed

Fig 5: Structure – Property Relationship: modifications in the LHS and RHS

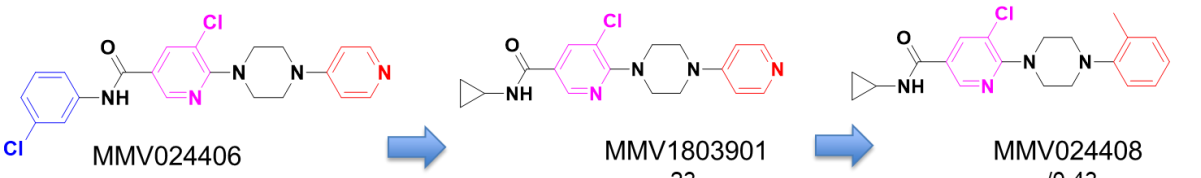


MMV024406
Pf NF54:0.26 µM
eLogD: 3.7
Clint(hmic): 20.6 µL/min/mg
Caco2: 2.1
Sol(PBS,pH7.4) <2.5 µM
CYP 1A2, 2C9, 2D6, 3A4
% I@10 µM: 45,69,72,84

			
MMVID	693110	693244	884933
Pf NF54 (µM)	0.34	0.77	>5
eLogD	2.3	2.6	2.1
Clint(hmic)/t1/2 : µL/min/mg; min	21,84	28,61	42,42
Caco2(A-B)	0.29	3.35	2.9
KS(PBS,pH7.4)	130	35	195
CYP 1A2, 2C9, 2D6, 3A4 % I@10 µM	42,52,73,88	0,76,81,91	30,25,35,27

Introduction of ionisable centre(basic) in LHS reduced log D and improved solubility.
Removal of 4-Py group(MMV884933) improved CYP profile and improved solubility but led to loss of activity

Fig 6: Structure – Property Relationship: miscellaneous modifications



MMV024406
Pf NF54:0.26 µM
eLogD: 3.7
Clint(hmic): 20.6 µL/min/mg
Caco2: 2.1
Sol(pH7.4,PBS) <2.5 µM
CYP 1A2, 2C9, 2D6, 3A4
% Inhibition@10 µM:
45,69,72,84

MMV1803901
23

MMV024408
-/0.43
4.3
18.1,77
7.2
CYP 1A2, 2C9, 2D6, 3A4
IC_{50}>30 µM

Pf NF54/3D7, µM
eLogD
Clint(hmic)/$t_{1/2}$: µL/min/mg; min
Sol(pH7.4,PBS),µM
CYP 1A2, 2C9, 2D6, 3A4
% Inhibition@10 µM

Challenges:

- High h microsomal clearance
- High CYP inhibition