

Triazolopyrazines (TP Series)

Summary

We have made compounds in this series down to 16nM potency. The series also seems to have good in vitro HLM and hHEP stability $Cl_{int} < 8.1$ is compatible with 10nM potency. However, RLM remains stubbornly high, particularly for the more potent analogues translating to short half-lives in rat PK. The series also appears to have little polypharmacology or cytotoxicity.

The project has so far not challenged the hypothesis that rat metabolism may not be a great model for human metabolism for this series.

Concerns:

Although dofetilide binding looks weak or nil, the series has shown activity in a patch clamp assay at Essen (1-10uM) which is quite potent though with a window of >100 fold over Pfal potency. The series shows activity in Kieran Kirk's PfATP4 assay which goes away for Pfal inactives in the series. In Kip Guy's resistant mutants the picture is perhaps more mixed, but there is still support for the idea that some members of the series are weaker in the resistant strains. The series has no or weak >>1uM activity against gametocytes, no activity against Winzeler's Pb liver stage and may have weak activity against ookinetes but the dose-response data has not been completed.

Possible next step summary:

The series has good potency and in vivo efficacy with few toxicity concerns. The biggest issue is metabolic stability, as measured in rat in particular. Some possible future directions include:

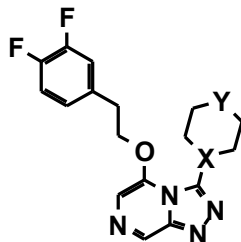
- Small scale changes around the side chains to attempt to balance potency and metabolism.
- Incorporation of a basic centre to increase volume as a potential fix for half-life. However, this might come at the expense of plasma concentration so would require high potency. In addition of the 29 compounds with a basic centre only one has a measured potency < 100nM.
- More significant structural changes. Of the changes made to the basic skeleton, the most successful might be the recent evaluation of the substitution position changes (eg MMV670945), possibly in combination with modifying the disposition of the N atoms in the core. Related compounds have been made by others and it would be wise to incorporate the learnings from these series into any plans to explore this substitution pattern further. The first few compounds look similar in terms of metabolic stability.

SAR summary

The objective of the current project was to improve the metabolic stability and the pharmacokinetic properties of this series in rat so as to meet the once-dosing criteria

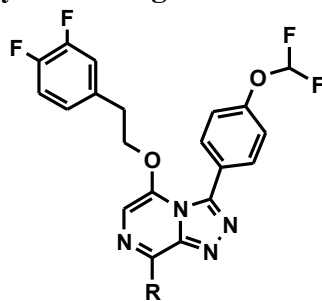
(TCP1) set by MMV. Towards this end, new chemistry directed towards blocking the putative metabolic sites was initiated.

1. Cycloaliphatic Triazole Substituent:



Attempts at lowering the lipophilicity of the TP compounds by replacing the triazole aryl substituent with a cyclo(hetero)aliphatic group, linked either by the heteroatom or otherwise (e.g. piperidine, tetrahydropyran, indoline or isoindoline) lowered the potency against PfNF54, as did an aniline substituent.

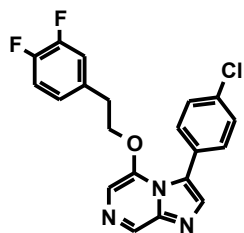
2. Core Modification of Pyrazine ring



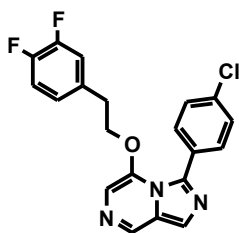
Based on an assumption that the pyrazine moiety of the TP could undergo AO metabolism at positions alpha- to the nitrogen a few compounds were made with different R groups (Cl, Me, NH₂, NEt₂). However, all these compounds lost potency against PfNF54.

3. Core Modification of Triazole ring

Two compounds based on imidazopyrazines were made. Both showed reduced potency against PfNF54 vis-à-vis the corresponding TP compound. The RLM stability of one was found to be poor.



MMV669846
Pfal IC₅₀ 0.105 uM
HLM CI = 55



MMV670250
Pfal IC₅₀ 0.831 uM
HLM CL/T_{1/2} 200/4.3
RLM CL/T_{1/2} >500/<3

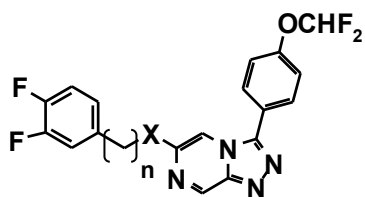
Approx 20 structures were made with variations to the 6,5 core system. MMV669846 was the most potent. As most of the analogues were > 1uM potency, fewer were tested in RLM (quite a few in HLM). Of the 4 tested in RLM, the greatest stability had a Clint of 109, (HLM 9.5), several had HLM Clint 8 or less, particularly after moving or removing the N from the pyrazine ring.

Variations to the TP core:

<p>MMV669846 - PCCBTAK-0192</p> <p>eryth_value_GM_nM: 105.4</p>	<p>MMV672942 - PCCBTAK-0399</p> <p>eryth_value_GM_nM: 509.6</p>	<p>MMV670250 - PCCBTAK-0222</p> <p>eryth_value_GM_nM: 830.9</p>	<p>MMV669025 - PCCBTAK-0100</p> <p>eryth_value_GM_nM: 4130</p>
<p>MMV672939 - PCCBTAK-0396</p> <p>eryth_value_GM_nM: 6779</p>	<p>MMV668955 - PCCBTAK-0072</p> <p>eryth_value_GM_nM: 8371</p>	<p>MMV669310 - PCCBTAK-0133</p> <p>eryth_value_GM_nM: 8714.88382022388</p>	<p>MMV668960 - PCCBTAK-0077</p> <p>eryth_value_GM_nM: 10000</p>
<p>MMV668962 - PCCBTAK-0079</p> <p>eryth_value_GM_nM: 10000</p>	<p>MMV672992 - PCCBTAK-0405</p> <p>eryth_value_GM_nM: 10000</p>	<p>MMV669846 - PCCBTAK-0273</p> <p>eryth_value_GM_nM: 1000000000</p>	

4. Transposing the Pyrazine side-chain

The side-chain on the pyrazine ring was shifted to the adjacent carbon. The chain length was varied (n = 0, 1, 2) and linked through either O or N.

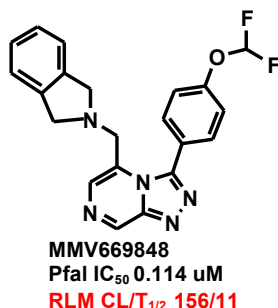


Among the ethers, the phenethyl ether (X=O, n=2) showed good potency (Pfal IC₅₀ 33nM) but a poor stability in RLM (Cl 100 ml/min/kg).

<p>MMV670945 - PCCBTAK-0276</p> <p>eryth_IC50_nM: 33.82 AlogP: 4.74</p>	<p>MMV672942 - PCCBTAK-0399</p> <p>eryth_IC50_nM: 509.6 AlogP: 5.05</p>	<p>MMV670946 - PCCBTAK-0277</p> <p>eryth_IC50_nM: 520.7 AlogP: 4.42</p>	<p>MMV671926 - PCCBTAK-0328</p> <p>eryth_IC50_nM: 3446 AlogP: 4.41</p>
<p>MMV672939 - PCCBTAK-0396</p> <p>eryth_IC50_nM: 6779 AlogP: 5.43</p>	<p>MMV670949 - PCCBTAK-0279</p> <p>eryth_IC50_nM: 10000 AlogP: 4.25</p>	<p>MMV671655 - PCCBTAK-0288</p> <p>eryth_IC50_nM: 10000 AlogP: 4.41</p>	<p>MMV671927 - PCCBTAK-0329</p> <p>eryth_IC50_nM: 10000 AlogP: 4.36</p>
<p>MMV672619 - PCCBTAK-0350</p> <p>eryth_IC50_nM: 10000 AlogP: 4.16</p>	<p>MMV672990 - PCCBTAK-0403</p> <p>eryth_IC50_nM: 10000 AlogP: 3.93</p>	<p>MMV672992 - PCCBTAK-0405</p> <p>eryth_IC50_nM: 10000 AlogP: 2.76</p>	

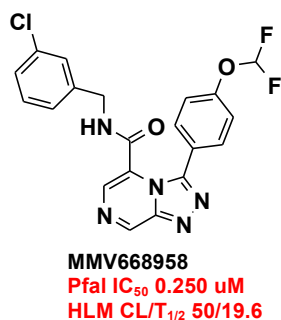
5. Pyrazine side-chain modifications

Suspecting that the phenethyl ether side-chain on the pyrazine ring is a potential metabolic hot-spot, several strategies were adopted to mitigate this risk. It soon became evident that changing the length of the side-chain to anything other than 3 atoms severely lowers potency against PfNF54. Moreover, the linker atom to the pyrazine ring is also crucial (O>>C>N). Heteroatoms within the side-chain also lowered potency, except for one compound (MMV669848) with an isoindolino-methyl group on the pyrazine ring. The latter however had poor RLM stability.

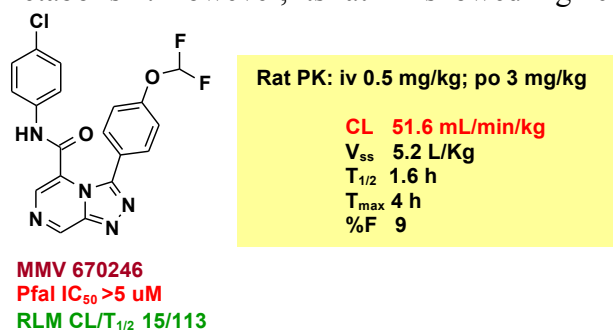


Constraining the linear side-chain into ring systems (azetidines, pyrrolidines, pyrazoles) severely reduced potency.

A small library of pyrazine amides was made of which the m-Cl-benzylamide (MMV 668958) showed promising potency. Other amides (derived from aliphatic or anilines) were either not active or had lower potency. The RLM stability of MMV668958 was poor, perhaps due to benzylic oxidation. Alpha-substitution at the benzylic position or constraining the benzylamine into an aminoindane did not improve potency.



The p-Cl benzanilide MMV670246, although not active against PfNF54, showed good RLM stability (the m-Cl analog had poor RLM stability) perhaps due to lack of benzylic metabolism. However, its rat PK showed high clearance.

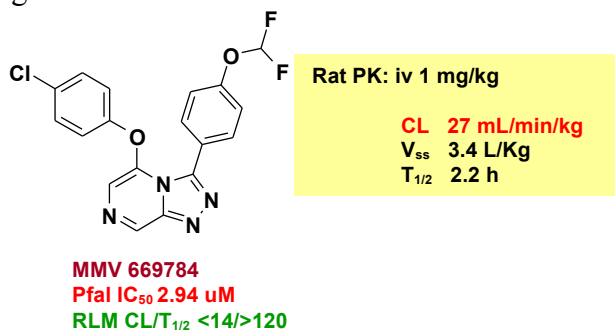


Several attempts to make aniline-amides with improved potency against Pfal failed. Loading the aniline ring with lipophilic substituents marginally improved potency but led to poor RLM stability.

Considering that the benzylic position in the phenethyl side-chain is prone to metabolic oxidation, several compounds having mono- and di-substitution in the benzylic position were made. Di-Substitution lowered the potency considerably whereas mono-substitution with OMe, OCHF₂, CH₂OH, NMe₂ groups retained good potency. Additional substitution alpha- to the ether oxygen led to complete loss of potency. The alpha-

OCHF2 compound MMV670652, with a p-CN-phenyl group on the triazole ring, showed better RLM stability (cLogP effect).

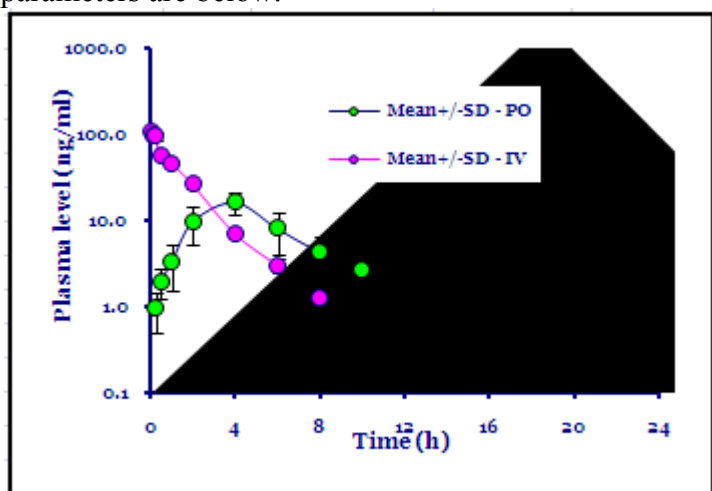
We also examined if the phenylethyl chain can be replaced by an aromatic group thus mitigating the potential metabolism of the ethyl chain. Several compounds with a phenol substituent on the pyrazine ring were made. Some substituted phenolates were metabolically more stable *in-vitro* as well as *in-vivo* in rat, although with reduced potency against Pfal.



A 2-naphthol substituent on the pyrazine ring showed reasonable potency against Pfal (IC₅₀ 114 nM) but suffered from poor RLM stability. Several hetero-analogs of 2-naphthol e.g. indole, indazole, quinoline, chroman, benzisoxazole, quinazoline, etc were made but all lost potency against the parasite.

Pharmacokinetics

Only one compound in this series first made at the CRO was measured in rat PK and that was the relatively weak amide MMV670246. The curve is shown for oral & IV legs & parameters are below.



Pharmacokinetics in Rats					
PK Analysis					
Project:	Malaria				
Project Code:	CR302 (TCGLS code)				
Compound:	MMV670246				
Batch:	PCCBTAK-0218				
Mwt Parent:	415.8				
Mwt Salt:	415.8	100.0%	Active		
Mol Formula:	C ₁₉ H ₁₂ ClF ₂ N ₅ O ₂				
Purity:	N/A	%			

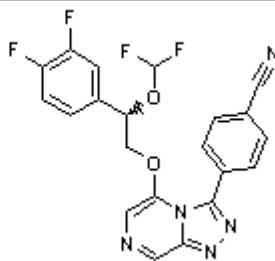
MMV670246					
	0.5mg/kg - IV (solution formulation)				
	R-4	R-5	R-6	Mean	SD
AUC _{0-t} (ng.h/ml)	149.9	153.9	167.0	156.9	8.9
AUC _{0-inf} (ng.h/ml)	153.0	156.8	169.9	159.9	8.9
Effective T _{1/2} (h)	1.2	1.1	1.2	1.2	0.06
Terminal T _{1/2} (h)	1.6	1.7	1.6	1.6	0.0
Cl (L/h/kg)	3.3	3.2	2.9	3.1	0.2
Vd initial (L/kg)	4.2	3.9	4.0	4.0	0.2
Vd _{ss} (L/kg)	5.7	5.0	4.9	5.2	0.4
MRT (h)	1.7	1.6	1.7	1.7	0.1
	3mg/kg - PO (suspension formulation)				
	R-1	R-2	R-3	Mean	SD
AUC _{0-t} (ng.h/ml)	103.1	63.0	72.1	79.4	21.0
AUC _{0-inf} (ng.h/ml)	116.1	70.2	78.9	88.4	24.4
C _{max} (ng/ml)	21.4	15.6	14.8	17.3	3.6
T _{max} (h)	4.0	4.0	4.0	4.0	0.0
Oral T _{1/2} (h)	2.4	2.2	2.3	2.3	0.1
BA (%)	12.1	7.3	8.2	9.2	2.5

ug.h/L	Mwt	415.79
ug.h/L		0.4 umol.h/L
		385 nM.h
~ 75% of rat liver blood flow (4.2 L/h/kg)		

ug.h/L	Mwt	415.79
ug.h/L		0.2 umol.h/L
		213 nM.h

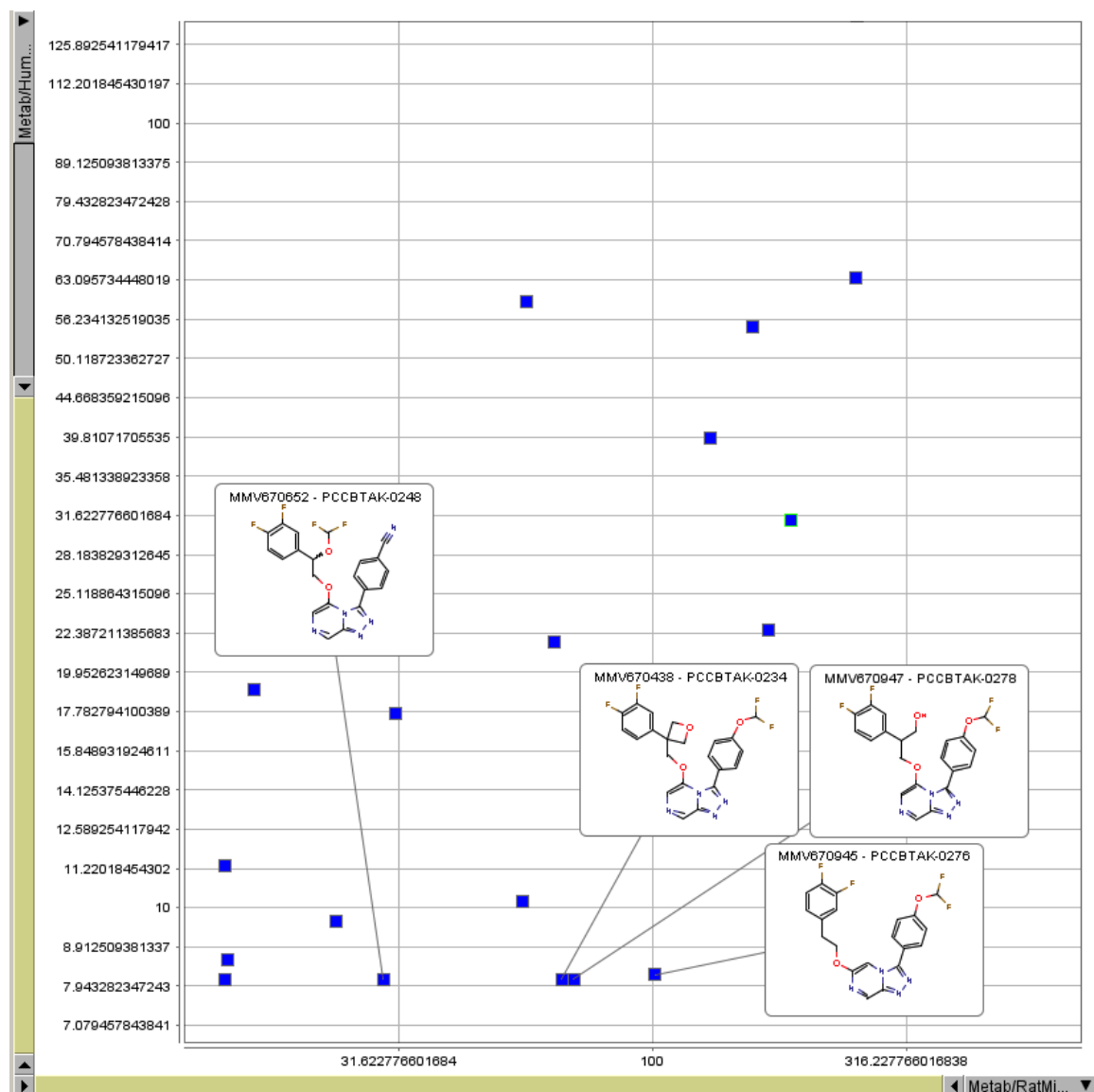
One of the compounds with better in vitro balance is MMV670652 with potency at 17nM, HLM Clint < 8 ul/min/mg and RLM Clint at 30 ul/min/mg. This compound has not been in rat PK. Additionally it may be possible to improve potency by synthesis of the more potent enantiomer.

**MMV670652 -
PCCBTAK-0248**



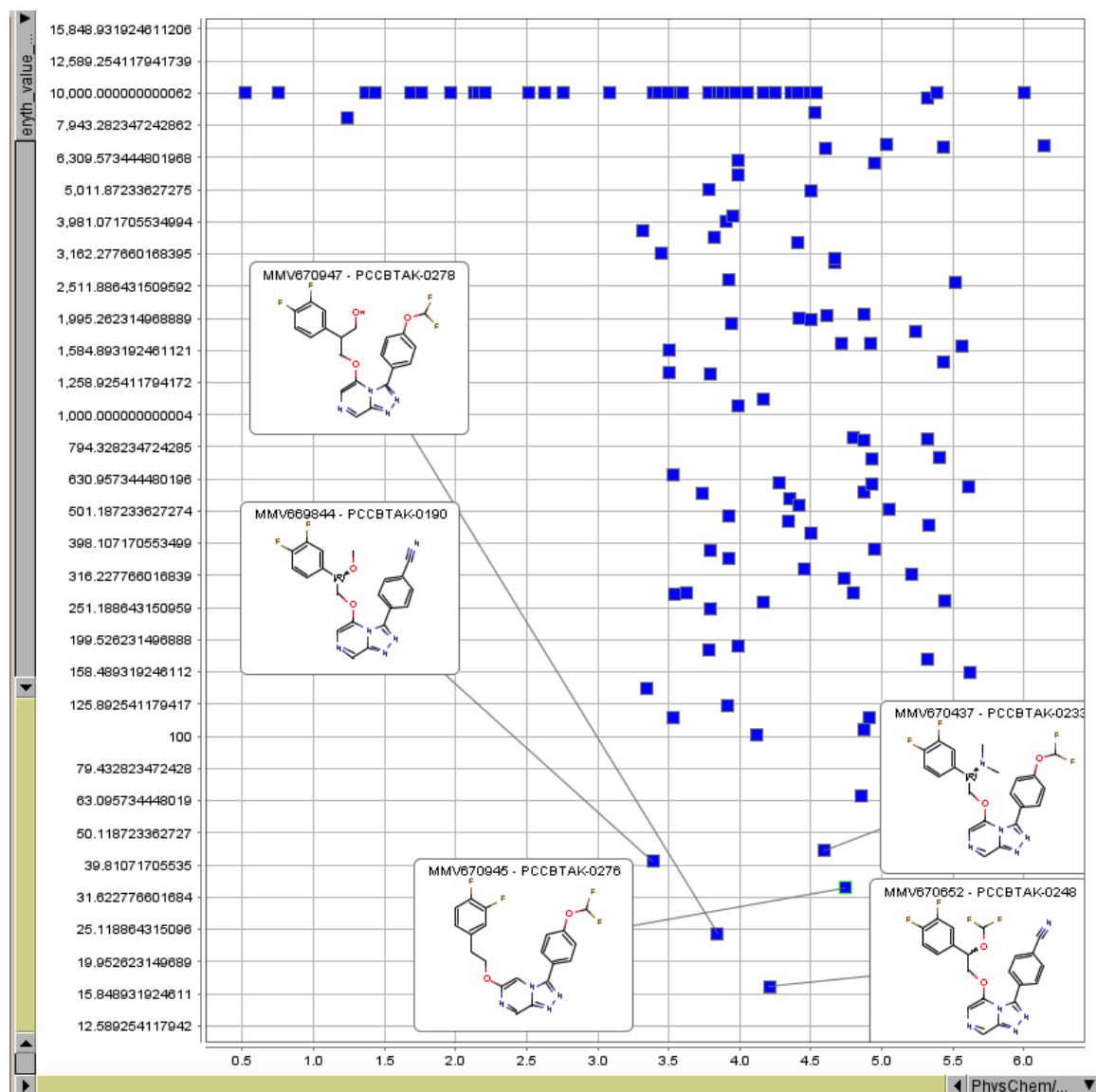
Other observations

As would be expected HLM vs RLM shows a general correlation with approx 4-fold shift on average. However, for most of the more potent analogues, this increases to over 10-fold. The figure below shows the 4 sub 30nM compounds with HLM & RLM measured.



Lipophilicity & lipophilic efficiency

Few compounds achieve a lipophilic efficiency (as measured by $\text{pIC}_{50} - \text{AlogP}$) of greater than 4.0

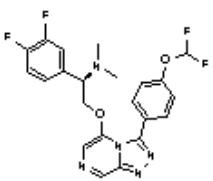


Potential next steps:

The series has good potency and in vivo efficacy with few toxicity concerns. The biggest issue is metabolic stability, as measured in rat in particular. Some possible future directions include:

- Small scale changes around the side chains, particularly phenethyl to attempt to balance potency and metabolism
 - N is tolerated in the ring, hasn't been explored much recently
 - Is 3,4-diF the best substitution pattern ?
 - Some evidence (eg MMV669848) that the phenethyl side chain can be rigidified, perhaps the iso-indoline of that compound could be improved on with other ring systems and by more optimal substitution of the aromatic benzene ring of the isoindoline.
 - The amide MMV670944 is interesting and shows good RLM stability, but many other amides failed to match its potency

- Incorporation of a basic centre to increase volume as a potential fix for half-life. However, this might come at the expense of plasma concentration so would require high potency. In addition of the 29 compounds with a basic centre only one has a measured potency < 100nM.

Id	Structure	eryth_value_GM_nM ▲
MMV670437 - PCCBTAK-0233	<div style="text-align: center;">Chiral</div> 	44.08

- More significant structural changes. Of the changes made to the basic skeleton, the most successful might be the recent evaluation of the substitution position changes (eg MMV670945), possibly in combination with modifying the disposition of the N atoms in the core. Related compounds have been made by others and it would be wise to incorporate the learnings from these series into any plans to explore this substitution pattern further. The first few compounds look similar in terms of metabolic stability.