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Synthesis and in vitro evaluation of imidazopyridazines as novel inhibitors of the malarial kinase PfPK7

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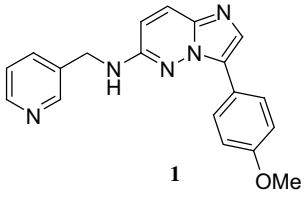
ABSTRACT

A high-throughput screening campaign identified a number of imidazopyridazines as novel inhibitors of the malarial kinase PfPK7. Further synthetic chemistry efforts enabled the preparation of a number of analogues with promising in vitro potencies. Although these compounds show likely broad spectrum inhibitory activity, they represent a useful starting point for further chemical optimisation.

Plasmodium falciparum, the most virulent species of human malaria parasites, is responsible for the most severe forms of the disease. Malaria causes over two million deaths annually, predominantly among children from sub-Saharan Africa. *P. falciparum* is becoming resistant to currently available antimalarial treatments, which has led to an urgent and continuing search for new methods of control.[1,2] Within malaria parasites, inhibition of protein kinases can modulate intracellular protein phosphorylation events, just as in other eukaryotes. Many *P. falciparum* kinases have been assigned to ePK families whose involvement in key cellular processes, such as cell growth and division, is well known.[3,4] Hence some show potential as targets for drug design, because they may be implicated in the regulation of the *P. falciparum* life cycle. Equally, some *P. falciparum* kinases are not related to any ePK family, and these may be validated as potential *P. falciparum*-specific drug targets.[5,6] One such emerging target is PfPK7, an 'orphan' plasmodial kinase which is distantly related to the MAPKK family of kinases (mitogen-activated protein kinase kinase).[7,8] It is expressed at several stages of the parasite life cycle, including both the asexual and sexual stages in man and also in the mosquito, but has no human homologue.[3] It thus represents an interesting target for small molecule intervention in the context of antimalarial therapy.

A recent in-house high-throughput screening campaign identified a number of imidazopyridazines such as **1** (IC_{50} 11.6 μM) as weak inhibitors of PfPK7. The 3D structures of several PfPK7–ligand complexes have recently been disclosed,[9] including compounds based on the same imidazopyridazine scaffold. This prompts us to disclose some initial synthetic and SAR investigations, which have significantly improved the in vitro potency of **1**, and provide compounds with useful levels of activity in a functional antiparasitic hypoxanthine incorporation assay.



1

PfPK7 IC_{50} 11.6 M

The key intermediate **3** was prepared by condensation of 6-chloropyridazine **2** with chloroacetaldehyde and subsequent bromination at the 2-position with NBS (Scheme 1).[10] Suzuki coupling with aryl boronic acids was selective for the 2-bromo position and provided advanced intermediates of type **4**. Then reaction with primary or secondary amines under microwave heating conditions afforded the initial target compounds **5**–**11**.[11] The flexibility of this approach allowed the order of the two diversification steps to be reversed where necessary, incorporating the 7-amino substituent first (to give **12**) before subsequent Suzuki coupling.[12]

Initial replacement of the 4-methoxyphenyl ring in **1** with 4-pyridyl (in **5**) delivered a modest increase in potency (Table 1).

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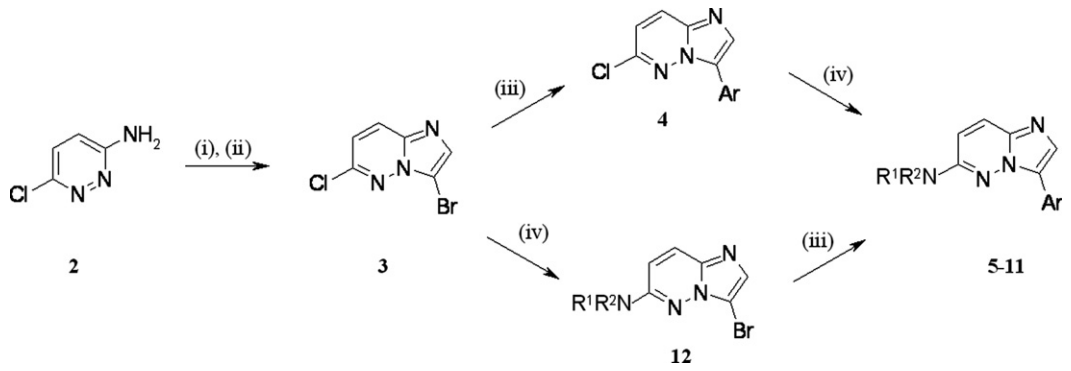
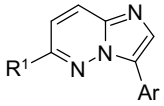
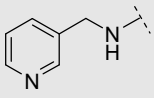
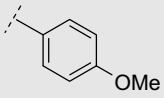
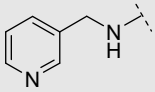
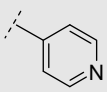
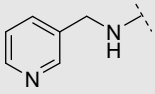
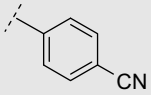
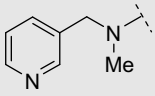
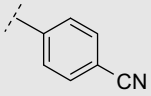
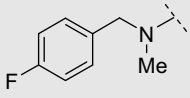
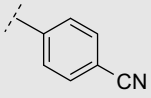
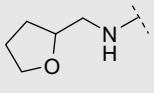
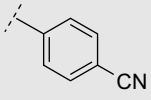
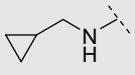
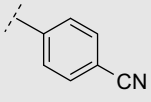
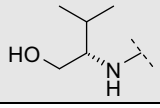
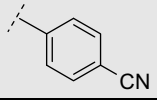


Scheme 1. Reagents and conditions: (i) a—$ClCH_2CH(OMe)_2$, HCl (aq), 100 °C, 1 h; b—**2**, EtOH, reflux 18 h; (ii) NBS, MeCN, CH_2Cl_2, rt, 2 h; (iii) $PdCl_2$(dppf), $ArB(OH)_2$, Na_2CO_3 (aq), MeCN, microwave, 150 °C, 20 min; (iv) R^1R^2NH, NMP, microwave, 190 °C, 30 min.

Table 1
Initial optimisation of aryl and amine substituents



Compound	R^1	Ar	$PfPK_7$ IC_{50}[a] (μM)
1			11.6
5			7.9
6			0.81
7			1.26
8			1.07
9			0.75
10			0.58
11			0.28

[a] Mean of at least two separate measurements with typical variability <20%.

We were pleased to find that the 4-cyanophenyl congener **6** was significantly more active at 0.81 μM. Hence the amine portion was re-examined whilst retaining this aryl motif. Methylation of the secondary amine did not appear to be critical for good activity (see **7**). Other aryl and (hetero)cyclic analogues were slightly more potent (**8**–**10**) than **1**. Encouragingly, the introduction of a chiral alcohol side chain[13] in **11** resulted in a 40-fold improvement in potency relative to **1**. Compound **11** has recently been found to show an activity of 6.5 μM in an alternative assay format, and its co-crystal structure with PfPK7 (pdb number 2pnm) has been reported.[9]

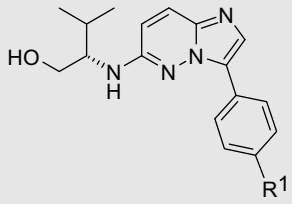
Crystal structure data[9] indicated that modification of the 4-nitrile aryl substituent might facilitate a salt bridge interaction through a proximal water molecule to the Lys55 residue. Thus we first prepared compounds containing the chiral amino alcohol side chain present in **11** (Table 2). Primary amide **13** retained a good level of potency, but one carbon homologations to alcohol **14** or nitrile **15** resulted in approximately 25-fold and 100-fold decreases in activity, respectively. Amine **16**, its homologue **17** and acid **18** were also significantly less active.

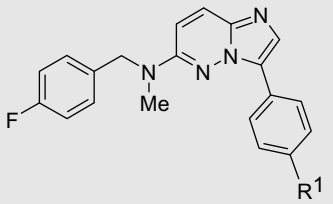
In an attempt to further probe the SAR at the 4-position of the aryl ring, additional new amine targets were prepared. Synthetically, these required conversion of **20** to the corresponding bromide and nucleophilic displacement with amines (Scheme 2). Oxidation and reductive amination of aldehyde **25** offered a complimentary approach. Here we maintained the 4-fluorobenzylmethyl side chain present in **8**, which had previously been associated with reasonable levels of potency. Again, the primary amide **19** retained potency, but alcohol and amine congeners **20** and **21** were less active. The incorporation of larger amine substituents in **22**–**24** resulted in significantly poorer activities (see Table 2).

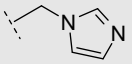
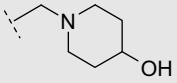
Finally, the role of the isopropyl amino alcohol was examined in an effort to elucidate the importance of the proposed interaction with the Ser176 residue.[9] Hence, a set of amino alcohol analogues bearing the 4-cyanophenyl motif were prepared. A more reliable route to these targets involved a one-pot hydroboration and cross-coupling of alkyne **26**[14] with 4-iodobenzonitrile to give **27** (Scheme 3). Bromination and condensation with **2** then provided intermediate **28**, which underwent smooth reaction with amines as before. In addition, 3D modelling data suggested that larger amines bearing solubilising groups might be accommodated in this region. Chiral variants were prepared by means of organometallic additions to chiral sulfinimines[15] derived from **44** or **47** (Scheme 3).

Table 3 shows the activities of a selection of these analogues. The stereochemistry of aminoalcohol motif did not appear to be critical for maintaining potency (see **29**–**31**). Further, the importance of the free alcohol seemed to be negligible, as the *O*-methyl congeners **32** and **33** also broadly retained activity. Pleasingly, conformationally constrained aminocyclohexanol **34** delivered the first improvement on the potency of **11**, showing good in vitro activity of 0.13 μM. Other variants of this substituent (**35** and **36**) possessed similar activities, as did the unsubstituted cyclohexane **37**. Among the larger side chain analogues **39**–**43**, only **42** was reasonably active and the stereochemistry of the side chain was not influential.

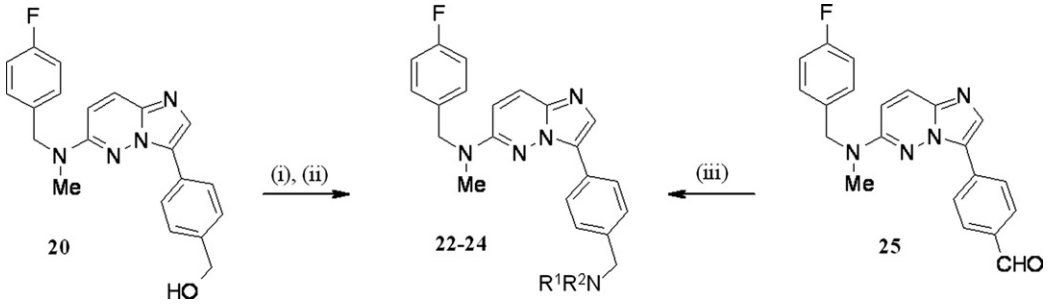
Table 2
Replacements for the 4-cyanophenyl motif



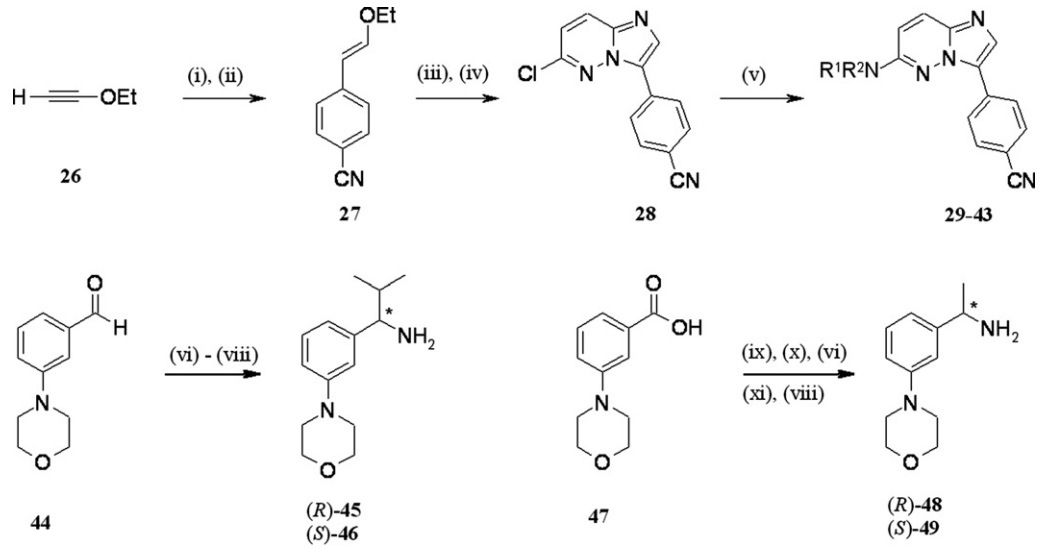


Compound	R^1	PfPK$_7$ IC_{50}[a] (μM)	Compound	R^1	PfPK$_7$ IC_{50}[a] (μM)
11	–CN	0.28	**8**	–CN	1.0
13	$-CONH_2$	0.77	**19**	$-CONH_2$	1.1
14	CH_2OH	7.4	**20**	$-CH_2OH$	21
15	$-CH_2CN$	25	**21**	$-NH_2$	42
16	$-NH_2$	34	**22**	$-CH_2NH(CH_2)OH$	34
17	$-CH_2NH_2$	104	**23**		56
18	$-CH_2CO_2H$	111	**24**		103

[a] Mean of at least two separate measurements with typical variability <20%.



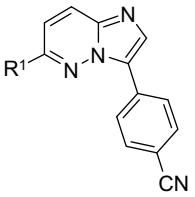
Scheme 2. Reagents and conditions: (i) HBr, AcOH, reflux, 18 h; (ii) R^1R^2NH, THF, reflux, 18 h; (iii) R^1R^2NH, $NaBH(OAc)_3$, CH_2Cl_2, rt, 18 h.

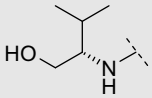
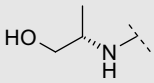
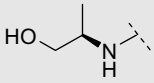
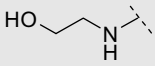
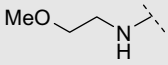
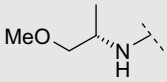
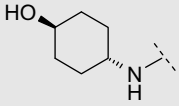
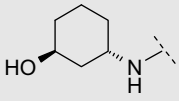
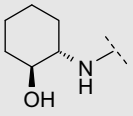
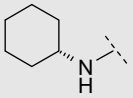
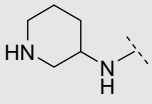
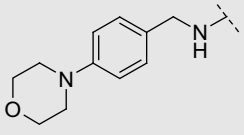


Scheme 3. Reagents and conditions: (i) BH_3·THF, THF, rt, 2 h; (ii) 4-CNC_6H_4I, $Pd(OAc)_2$, PPh_3, NaOH, THF, reflux, 18 h; (iii) NBS, EtOH, rt, 1 h; (iv) **2**, EtOH, reflux, 18 h; (v) R^1R^2NH, NMP, mw, 190 °C, 30 min; (vi) (*R*)- or (*S*)-^{t}BuS(O)NH_2, $Ti(O^iPr)_4$, THF, rt, 18 h; (vii) ^{i}PrLi, THF, −70 °C, 1.5 h; (viii) 4 M HCl, dioxane, MeOH, rt, 18 h; (ix) Me(OMe)NH·HCl, TBTU, Et_3N, CH_2Cl_2, rt, 2 h; (x) MeMgBr, THF, −78 °C–rt, 18 h; (xi) LiBH(*sec*-Bu)$_3$, THF, 0 °C–rt, 3 h.

Six analogues were tested (Table 4) against two different strains of *P. falciparum*, namely 3D7 strain, which is a standard drug-sensitive laboratory clone of the NF54 isolate, and K1 strain (Thailand), which is a chloroquine, pyrimethamine and cycloguanil resistant strain. The analogues were also tested in a cytotoxicity assay against KB cells and a selectivity index relative to the 3D7 strain

Table 3
Amino alcohol replacements



Compound	R^1	$PfPK_7$ IC_{50}[c] (μM)
11		0.28
29		0.44
30		1.0
31		1.0
32		1.4
33		0.82
34[a]		0.13
35[a,b]		0.26
36[a,b]		0.22
37		0.34
38[b]		2.1
39		8.0
40	(*R*)-**45**	No inhibition
41	(*S*)-**46**	43
42	(*R*)-**48**	4.8
43	(*S*)-**49**	25

[a] Relative stereochemistry shown.
[b] Racemate.
[c] Mean of at least two separate measurements with typical variability <20%.

Table 4
Antiparasitic activity and cytotoxicity of key analogues

Compound	3D7 IC_{50}[a] (μM)	K1 IC_{50}[a] (μM)	KB IC_{50}[b] (μM)	SI[c]
Chloroquine	0.04	0.80	97.48	2437
10	7.15	12.73	21.20	2.96
11	2.60	6.86	52.17	20.10
13	2.46	3.89	12.98	5.27
29	5.26	9.93	21.21	4.03
33	6.06	10.47	25.78	4.25
34	1.03	2.65	24.02	23.32

[a] IC_{50} assessed via 3H hypoxanthine incorporation using chloroquine as standard (μM).
[b] Cytotoxicity against human KB cells (IC_{50} μM).
[c] Selectivity index IC_{50} KB/IC_{50} 3D7.

was determined. All six examples showed evidence of antimalarial activity in this hypoxanthine incorporation assay,[16] albeit at least an order of magnitude less potently than chloroquine. The weak activity could reflect the fact that inhibition of PfPK7 is expected to slow and not arrest parasite growth,[17] and also that several examples from this series have been shown to be relatively promiscuous inhibitors of a panel of ~80 kinases at the Dundee Protein Phosphorylation Unit. Hence they could be targeting a number of *P. falciparum* kinases. The Gini coefficient (value between 0 and 1) has recently been used to express the selectivity profile of compounds against large numbers of kinases[18]; less selective compounds give scores closer to 0. Here, compounds **11** and **34** gave Gini scores of 0.32 and 0.21, respectively, at 10 μM.

In summary, we have demonstrated the optimisation of a series of imidazopyridazine derivatives from a 11.6 μM hit to a 0.131 μM inhibitor of PfPK7. Encouragingly, several compounds show modest inhibitory activity in a 3H-hypoxanthine incorporation assay against two strains of *P. falciparum* without appreciable cytotoxicity. Future efforts will focus on addressing the kinase selectivity of this series of compounds and will be the subject of a future publication.

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References and notes

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11. The preparation of compound **37** is as follows: a solution of **28** (50 mg, 0.20 mmol) and cyclohexylamine (5 equiv, 0.98 mmol, 97 mg) in *N*-

methylpyrrolidinone (1 mL) was stirred with microwave heating at 180 °C for 30 min. Further amine (97 mg) was added and the reaction mixture was again stirred at 180 °C for 30 min. The solvents were removed in vacuo and the residue was partitioned between water and EtOAc. The aqueous layer was extracted with EtOAc and the combined organic extracts were washed with brine, dried ($MgSO_4$) and concentrated in vacuo. Column chromatography (EtOAc–MeOH, 19:1) gave **37** (29 mg, 47%) as a pale brown solid: δ_H (400 MHz, MeOD) 8.45 (d, J = 8.2 Hz, 2H), 7.97 (s, 1H), 7.81 (d, J = 8.7 Hz, 2H), 7.66 (d, J = 9.6 Hz, 1H), 6.78 (d, J = 9.6 Hz, 1H), 3.79–3.72 (m, 1H), 2.23–2.18 (m, 2H), 1.91–1.85 (m, 2H), 1.80–1.74 (m, 1H), 1.57–1.46 (m, 2H), 1.41–1.30 (m, 4H); LC–MS (loop): 318 ($[M+H]^+$, 100%).

12. The preparation of compound **8** (see Table 2) is as follows: a mixture of the intermediate of type **12** (80 mg, 0.24 mmol), 4-cyanophenylboronic acid (52 mg, 0.36 mmol), $PdCl_2$(dppf) (0.0075 mmol, 8 mg) and 2 M aqueous Na_2CO_3 (0.72 mmol, 0.360 mL) in CH_3CN (1.5 mL) was stirred with microwave heating at 150 °C for 10 min. The reaction mixture was diluted with EtOAc, filtered, and the filtrate was concentrated in vacuo. The residue was purified by chromatography (EtOAc–MeOH, 98:2) to give **8** (60 mg, 70%) as a pale brown solid: δ_H (400 MHz, DMSO-d_6) 8.31 (d, J = 8.7 Hz, 2H), 8.17 (s, 1H), 7.95 (d, J = 10.0 Hz, 1H), 7.85 (d, J = 8.7 Hz, 2H), 7.32 (dd, J = 8.7, 5.5 Hz, 2H), 7.21 (d, J = 1.0 Hz, 1H), 7.17 (t, J = 8.7 Hz, 2H), 4.81 (s, 2H), 3.23 (s, 3H); LC–MS (loop): 358 ($[M+H]^+$, 100%).
13. For example, the CDK inhibitor purvalanol A bears such an amino alcohol substituent Gray, N. S.; Wodicka, L.; Thunnissen, A.-M. W. H.; Norman, T. C.; Kwon, S.; Espinoza, F. H.; Morgan, D. O.; Barnes, G.; LeClerc, S.; Meijer, L.; Kim, S.-H.; Lockhart, D. J.; Schultz, P. G. *Science* **1998**, *281*, 533.
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