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Synthesis and evaluation of 2-pyridyl pyrimidines with in vitro antiplasmodial and antileishmanial activity

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ABSTRACT

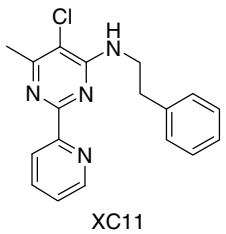
A series of 2-pyridyl pyrimidines, reported inhibitors of *Plasmodium falciparum* methionine aminopeptidase 1b were synthesized and evaluated for their antiplasmodial activities. An analysis of physicochemical properties demonstrated a link between lipophilicity and antiparasitic activity. Cross screening of the library against cultured *Leishmania donovani* parasites revealed this class of compounds as potent inhibitors of parasite development in vitro.

Annual global clinical cases of malaria infection exceed 300 million, resulting in over 1 million deaths.[1] Among the four species of the parasite that cause malaria in man, *Plasmodium falciparum* is the most virulent and is responsible for 90% of reported malaria cases. Many first line defenses against malaria, such as chloroquine (CQ) have been rendered ineffective due to development of drug resistance in the parasite. Although the discovery of new treatments such as artemisinin has been a significant achievement in the fight against malaria,[2] there is still an urgent need for the discovery, development and delivery of new chemotherapies to combat the disease.

Other tropical diseases also cause significant morbidity and mortality in the developing world. For example, leishmaniasis causes debilitating effects, severely attacking visceral, cutaneous and mucocutaneous organs.[3] Caused by more than 20 different species of *Leishmania*, leishmaniasis has a prevalence of an estimated 12 million cases annually, the majority of which are reported in the tropics and subtropics.[4] The disease is further compounded by low efficacies of current therapies as well as resistance and toxicity. As with malaria, new chemotherapeutic agents are urgently needed for the treatment of leishmaniasis.

It has recently been disclosed that 2-pyridyl pyrimidines act as selective inhibitors of *P. falciparum* methionine aminopeptidase (PfMetAP) 1b and arrest parasite development in vitro.[5] For example, XC11 (Fig. 1) was found to be a potent inhibitor of PfMetAP 1b enzyme activity and XC11 also inhibited *P. falciparum* proliferation of both the chloroquine-sensitive 3D7 and chloroquine-resistant Dd2 strains. XC11 was also shown to possess in vivo activity in two mouse models of malaria, and was shown to have a synergistic effect when dosed in vivo with CQ.

As part of a collaboration between Pfizer and WHO-TDR to discover new hits and leads to treat neglected tropical diseases,[6] we sought to expand the SAR governing the activity of the 2-pyridyl pyrimidine scaffold reported by Chen et al.[5] In addition to exploring antiplasmodial SAR, we also determined the activity for this series against other parasites where MetAP enzymes have been shown to be important in parasite survival. For example it has



XC11

P. falciparum MetAP1b IC_{50} 0.11μM
P. falciparum Dd2 IC_{50} 3.1μM
Active in *in vivo* plasmodia models
Synergisitc *in vivo* activity with CQ

Figure 1. Structure and biological activity of XC11.

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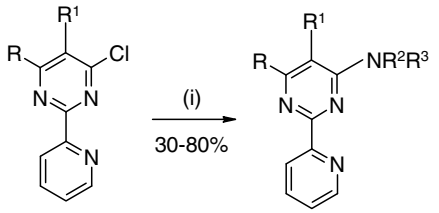


1 R = Me R^1 = Cl
2 R = Me R^1 = H
3 R = CF_3 R^1 = H

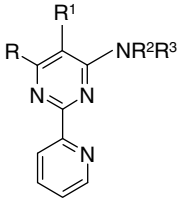
4 - 33

Scheme 1. Reagent and condition: (i) R^2R^3NH, Et_3N, EtOH, reflux.

recently been demonstrated that MetAP2 inhibitors such as fumagillin and TNP-470 arrest parasite growth in *Leishmania donovani* parasites.[7] Herein, we present the results of a synthesis and screening programme of 2-pyridyl pyrimidines against malaria and leishmania parasites.

The medicinal chemistry design strategy focused on understanding which parts of the XC11 structure were important for antiparasitic activity (the 2-pyridine had previously been shown to be crucial for Pf MetAP 1b and antiparasitic activity).[5] A series of compounds was then designed to test where polarity could be tolerated, the effect of conformational constraint and to inform on the relationship between activity and lipophilicity (as estimated by calculated Log*P* values).

Table 1
Chemical structures and in vitro *P. falciparum* K1, *L. donovani* activity and rat myoblast L6 cytotoxicity for compounds **4**–**33**



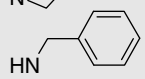
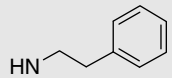
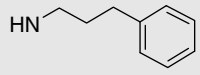
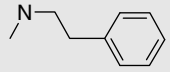
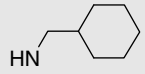
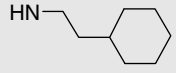
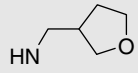
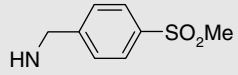
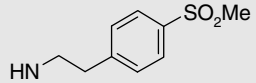
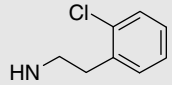
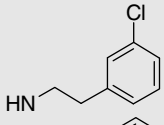
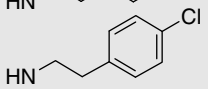
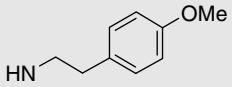
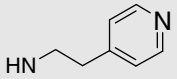
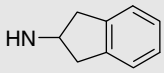
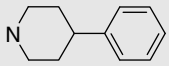
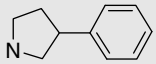
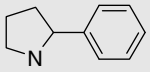
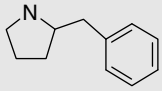
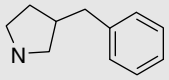
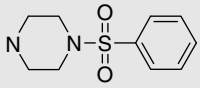
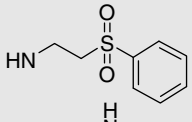
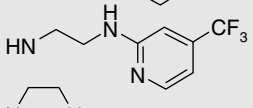
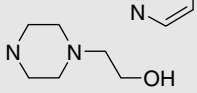
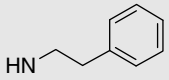
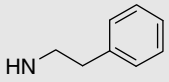
Compound	R	R^1	NR^2R^3	*c*Log*P*	*P. falciparum* IC_{50} (μM)	*L. donovani* IC_{50} (μM)	Cytotoxicity IC_{50} (μM)
Chloroquine	—	—	—		0.25	nd[a]	70.1
Artemisinin	—	—	—		0.005	nd[a]	>170
Miltefosine	—	—	—		nd[a]	0.7	nd[a]
4	Me	Cl	NH_2	1.8	20.8	3.3	85.3
5	Me	Cl	NMe_2	2.7	15.1	1.1	115
6	Me	Cl	NEt_2	3.8	9.0	1.22	148
7	Me	Cl		2.9	8.1	0.53	80
8	Me	Cl		4.1	3.2	0.47	70.1
9	Me	Cl		4.8	9.8	0.5	25.5
10	Me	Cl		5.1	1.9	0.48	73.5
11	Me	Cl		4.8	3.5	0.32	62.5
12	Me	Cl		5.3	2.4	0.31	42.4
13	Me	Cl		5.8l	2.3	0.26	49.5
14	Me	Cl		2.5	15.3	10.5	125
15	Me	Cl		2.5	10.1	10.7	93.6
16	Me	Cl		3.1	8.2	7.4	103.5
17	Me	Cl		5.5	6.3	1.2	91.6

Table 1 (*continued*)

Compound	R	R^1	NR^2R^3	*c*Log*P*	*P. falciparum* IC_{50} (μM)	*L. donovani* IC_{50} (μM)	Cytotoxicity IC_{50} (μM)
18	Me	Cl		5.5	2.5	0.44	32.6
19	Me	Cl		5.5	2.0	0.45	40.4
20	Me	Cl		4.7	2.5	0.43	8.7
21	Me	Cl		3.3	9.5	14.1	110
22	Me	Cl		4.7	3.7	0.42	33.2
23	Me	Cl		4.8	8.9	1.1	183
24	Me	Cl		4.4	4.7	0.39	133
25	Me	Cl		4.4	4.1	0.70	76.5
26	Me	Cl		4.8	4.3	0.63	92.3
27	Me	Cl		4.9	3.1	0.38	88
28	Me	Cl		3.7	11.6	54.4	210
29	Me	Cl		3.3	10.4	11.4	72.8
30	Me	Cl		4.7	2.74	0.28	5.2
31	Me	Cl		1.9	7.5	156	270
32	Me	H		4.0	3.7	3.0	18.3
33	CF_3	H		4.5	8.2	2.0	74.2

[a] nd, not determined.

The target compounds were synthesized according to the general route in Scheme 1. The commercially available chloro-pyrimidines **1**–**3** were aminated with the desired amines in EtOH in presence of Et_3N to afford compounds **4–33** (Table 1).[8]

The antiplasmodial activity of compounds **4–33** was determined in a CQ-resistant K1 *P. falciparum* strain using chloroquine as standard (Table 1).[9] Simple N-alkylated analogues **4**–**7** had weak to moderate *P. falciparum* activity. Extension of the side chain and incorporation of an aryl group (**8**–**11**) gave a trend towards increasing activity, although with a rise in lipophilicity also. In our hands, XC11 (**9**) had similar activity in a CQ-resistant *P. falciparum* strain when compared to data reported in the literature.[5] Saturation of the aryl ring in **12**–**13** was well tolerated. However, incorporation of a more polar aliphatic heterocycle (**14**) reduced activity significantly. Polar substitution on the aryl ring (**15**–**16**) also reduced activity, whereas chloro and methoxy analogues **17**–**20** were more active. Swapping the aryl for a 4-pyridyl ring (**21**) led to a drop in *P. falciparum* potency. Conformational constraints on the side chain were then investigated. Analogues **22**–**27** all had similar activity, despite locking the aryl ring in a number of different orientations.

More polar linker systems were then tested (**28**–**31**), and all showed reduced *P. falciparum* activity. Having spanned many changes on the 4-N side chain, the SAR of other substituents was briefly explored. Deletion of the 5-Cl (**32**), and swapping R to a trifluoromethyl group (**33**) retained activity when compared to XC11 (**9**).

Overall the in vitro plasmodia screening results demonstrated a very flat SAR, with all analogues spanning only one order of magnitude in activity. Additionally all the activity appeared to be driven by increases in lipophilicity (Fig. 2), and none of the analogues containing polar groups possessed good in vitro activity. This was a cause for concern, due to the potential for more lipophilic compounds to have higher attrition risks associated to polypharmacology, pharmacokinetics and toxicology.[10] The calculated Log*P*/potency relationship for *P. falciparum* K1 indicated that a very high *c*Log*P* (>9) would be required to achieve CQ levels of in vitro *P. falciparum* K1 activity in this series.

The antileishmanial activity of compounds **4–33** was then determined in *L. donovani* using miltefosine as standard (Table 1).[9] Activity was still linked to lipophilicity (Fig. 3), however activity spanned three orders of magnitude and a significant number of highly active compounds were identified. In particular, analogues **7**–**13, 18**–**20, 22, 24**–**27** and **30** were as active or more active than the standard agent miltefosine, and most had excellent selectivity over cytotoxic effects in rat myoblast L6 cells.[9]

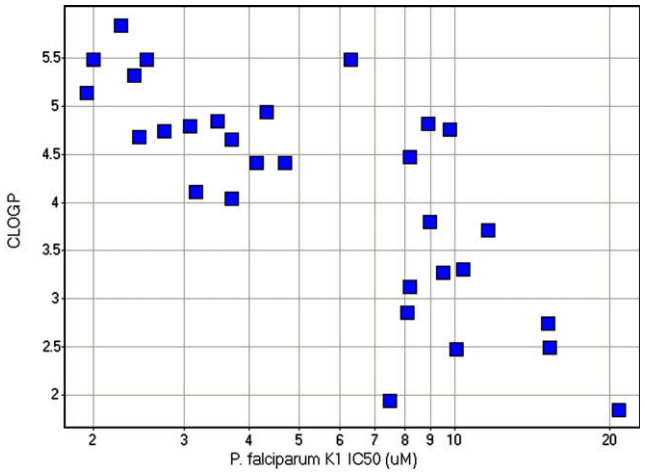


Figure 2. Plot showing relationship between *c*Log*P* and *P. falciparum* activity.

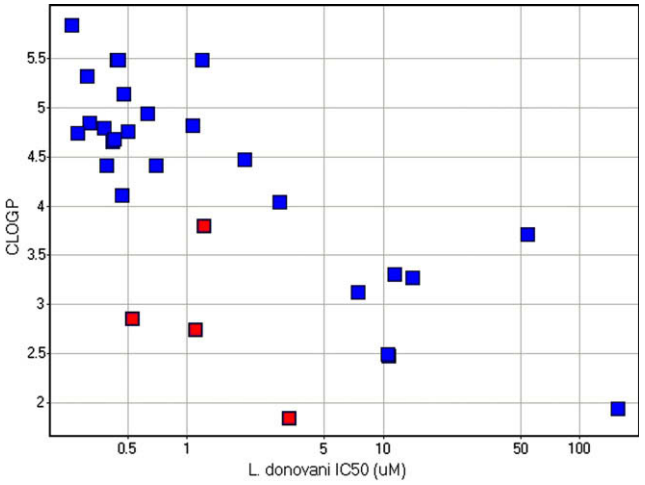


Figure 3. Plot showing relationship between *c*Log*P* and *L. donovani* activity.

Of particular interest were analogues **4**–**7**, which did not possess a large 4-N substituent (highlighted in red in Fig. 3). This result indicated that a large lipophilic group in this position was not required for good *L. donovani* activity. Analogues **5** and **7** had the best combination of low *c*Log*P* and *L. donovani* activity, and could form the basis for a hit-to-lead program to identify additional compounds with increased *L. donovani* potency.

The relationship between antiparasitic activity and cytotoxicity was then explored, to determine if such a relationship existed (Figs. 4 and 5). There was a general trend towards the most potent antiparasitic compounds also exhibiting more cell-based cytotoxicity. However, within a given potency range (for example *L. donovani* $IC_{50} < 0.5\ \mu M$) the cytotoxicity value varied >10-fold, indicating that antiparasitic activity was not intimately linked to cell-based cytotoxicity.

In conclusion, we have extended the SAR of the 2-pyridine pyrimidines with regard to their antiplasmodial activity, giving an insight into the structural features that drive potency. To this end, we have shown that lipophilicity is correlated with antiplasmodial activity.

We have further demonstrated the efficacy of the 2-pyridyl pyrimidines against *L. donovani* parasite development in vitro.

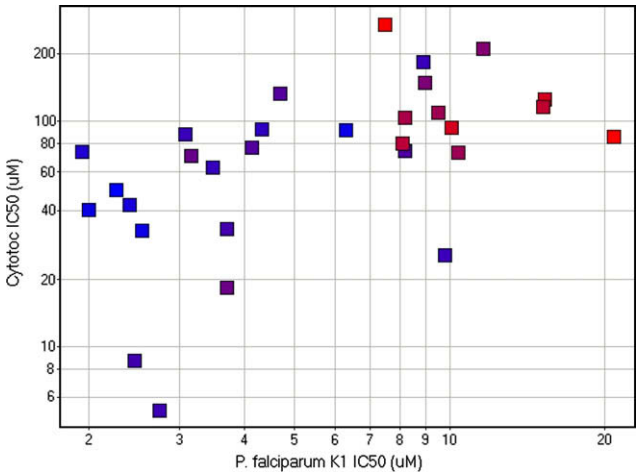


Figure 4. Plot showing relationship between cytotoxicity and *P. falciparum* activity. Coloured by *c*Log*P* (red = polar to blue = lipophilic).

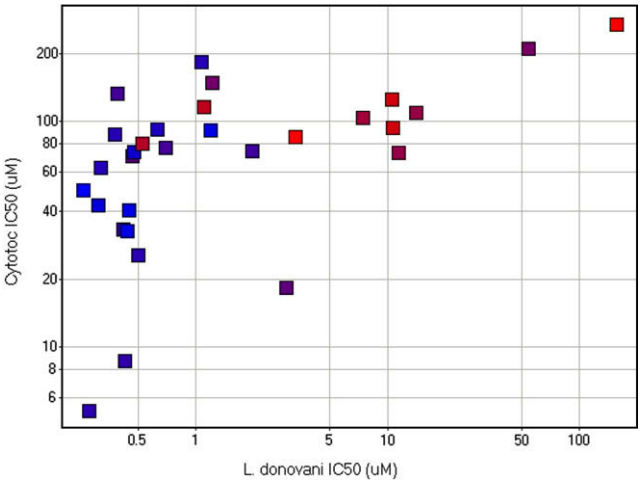


Figure 5. Plot showing relationship between cytotoxicity and *L. donovani* activity. Coloured by *c*Log*P* (red = polar to blue = lipophilic).

The low molecular weight, ease of synthesis and low cLog*P* of compounds **5** and **7** lend themselves to being interesting starting points for a hit-to-lead generation project against *L. donovani*.

Acknowledgements

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8. Final compounds were characterized by ^{1}H NMR and LC–MS analysis, and all data was in agreement with the assigned structures. Representative ^{1}H NMR data is outlined below:
   Compound **5**: ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.68 (s, 3H), 3.27 (s, 6H), 7.28–7.32 (m, 1H), 7.72–7.77 (m, 1H), 8.36 (d, J = 7.8 Hz, 1H), 8.75 (d, J = 3.9 Hz, 1H).
   Compound **7**: ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.92–2.11 (m, 4H), 2.64 (s, 3H), 3.57–3.66 (m, 4H), 7.28–7.34 (m, 1H), 7.76–7.81 (m, 1H), 8.41 (d, J = 7.8 Hz, 1H), 8.77 (d, J = 4.7 Hz, 1H).
   Compound **10**: ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.03–2.10 (m, 2H), 2.62 (s, 3H), 2.78 (t, J = 7.4 Hz, 2H), 3.67–3.71 (m, 2H), 5.44 (br s, 1H), 7.20–7.24 (m, 3H), 7.29–7.37 (m, 3H), 7.76–7.82 (m, 1H), 8.33 (d, J = 8.2 Hz, 1H), 8.81 (d, J = 4.7 Hz, 1H).
   Compound **21**: ^{1}H NMR (400 MHz, CD_3OD) δ ppm 2.52 (s, 3H), 3.05 (t, J = 7.0 Hz, 2H), 3.94 (t, J = 7.0 Hz, 2H), 7.35 (d, J = 6.2 Hz, 2H), 7.48–7.52 (m, 1H), 7.93–7.98 (m, 1H), 8.35 (d, J = 7.9 Hz, 1H) 8.37 (d, J = 6.2 Hz, 2H) 8.68 (d, J = 3.9 Hz, 1H).
   Compound **25**: ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.90–2.02 (m, 2H), 2.03–2.10 (m, 1H), 2.37–2.46 (m, 1H), 2.59 (s, 3H), 4.01–4.06 (m, 1H), 4.29–4.35 (m, 1H), 5.61 (t, J = 6.6 Hz, 1H), 7.16–7.19 (m, 1H), 7.24–7.30 (m, 5H), 7.62–7.67 (m, 1H), 7.87 (d, J = 7.8 Hz, 1H), 8.72 (d, J = 3.9 Hz, 1H).
   Compound **26**: ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.76–1.86 (m, 2H), 1.92–2.01 (m, 2H), 2.62–2.69 (m, 1H), 2.68 (s, 3H), 3.34 (dd, J = 13.3, 3.5 Hz, 1H), 3.50 (d, J = 5.1 Hz, 1H), 3.81–3.87 (m, 1H), 4.01–4.08 (m, 1H), 7.23 (m, 1H), 7.28–7.32 (m, 4H), 7.35–7.38 (m, 1H), 7.81 (m, 1H), 8.41 (d, J = 7.8 Hz, 1H) 8.83 (d, J = 3.9 Hz, 1H).
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