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Antiplasmodial activity of piperazine sulfonamides

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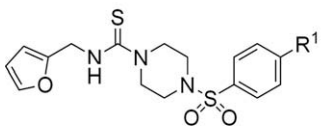
ABSTRACT

A high-throughput screening program identified two piperazine sulfonamides with activity against *Plasmodium falciparum*. Both screening positives had three structural features with potential liabilities: furanyl, thiourea and nitrophenyl groups. The furan could be replaced with no loss of activity, replacement of the nitrophenyl led to some loss of activity, and any attempt to replace the thiourea led to a significant decrease in activity, which implicates this reactive functional group's role in the antiplasmodial activity of this compound class.

Malaria is a major parasitic disease in many areas of Africa, Asia and South America. *Plasmodium falciparum* causes the majority of cases, and its resistance to many contemporary antimalarials throughout Africa and parts of Asia has compounded the problem of disease control.[1] To address the discovery of novel chemotherapeutic strategies, the Broad Institute's Infectious Diseases Initiative, the Genzyme Corporation and the Medicines for Malaria Venture have formed the Malaria Drug Discovery Initiative to create a collaborative pipeline for candidate therapeutics. Our primary means of identifying small molecule candidates utilizes a high-throughput screen to uncover scaffolds amenable to the development of novel antimalarials.[2] Two closely related screening positives are displayed below (**1a**–**b**, Fig. 1). While these piperazine sulfonamide-based compounds displayed excellent antiplasmodial activity, the furanyl, thiourea and nitrophenyl functionalities have noted metabolic liabilities, and we undertook a stepwise strategy to determine whether potency was maintained when they were replaced with more benign functional groups.

We initially addressed replacement of the furanyl group using readily available compounds[3] (**2a**–**h**, Table 1). The furanyl group can be metabolically transformed to α,β-unsaturated dicarbonyls or γ-ketocarboxylic acids via a furan-2,3-epoxide intermediate that is capable of reacting with a range of biomolecules.[4] The carcinogenicity of aflatoxin B1, which results from a furan-derived epoxide reacting with N7 of guanine,[5] provides one well-known example. The antiplasmodial activity of the commercial compounds was measured by an in vitro DAPI staining-based method for determining *P. falciparum* cell viability after exposure to the compound for 72 h.[2] Compounds were tested against the laboratory strain 3D7 and the multidrug resistant southeast Asian isolate Dd2,[6] using chloroquine as a control to validate the assay results. Replacement of the furanyl with tetrahydrofuranyl or methoxymethyl groups resulted in some decrease in activity, whereas replacement with a phenyl group (**2e**) resulted in a compound equipotent to screening positive **1a**. An analog of **2e** with an additional methylene at R^1 (**2g**) displayed a two-fold decrease in activity against Dd2. Compounds **2a** and **2h** revealed that bulky groups at R^1 and R^2 are required for activity, as methyl groups at either position eliminated activity.

Replacement of the nitrophenyl group was investigated with compounds **3a**–**i** (Table 2). The toxicological properties of nitroarenes have been studied intensively.[7] While several marketed drugs possess nitroarenes, many suffer from a range of side effects.[8] Compounds **3a**–**i** displayed decreased antiplasmodial activity compared to **1a**. Only two compounds displayed submicromolar activity (**3c** and **3g**), and both of these compounds



1a $R^1 = NO_2$
Pf 3D7 EC_{50} = 0.064 μM
Pf Dd2 EC_{50} = 0.117 μM
1b R^1 = Cl
Pf 3D7 EC_{50} = 0.615 μM
Pf Dd2 EC_{50} = 0.544 μM

Figure 1. Structure and antiplasmodial activity of screening positives **1a**–**b**.

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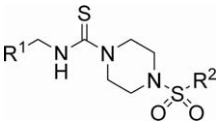
had R^1 at the 4-position (cf. **1a**–**b**). However, the only other analog with R^1 at C4 (**3i**) was >10-fold less active than **1a**, demonstrating that not all functionalities at this position are well tolerated. The bicyclic compound (**3a**) had the most significant decrease in activity compared to **1a**, suggesting that bulkier groups are not tolerated at this position.

We then focused on the replacement of the thiourea. This functionality has a range of known toxicological effects, such as thyroid depression, pulmonary edema, and liver necrosis.[9] Teramoto et al. demonstrated that a range of structurally diverse thioureas were teratogenic in rats, whereas the corresponding ureas were not.[10] The adverse effects of thioureas are attributed to the formation of *S*-oxide intermediates catalyzed primarily by CYP450 enzymes and FAD-containing monooxygenases.[11] The resulting *S*-oxide can be hydrolyzed to the corresponding urea, or react with a range of biomolecules to induce toxicity.[12] Two compounds that lacked a thiourea but retained the distal phenyl and 4-nitrophenyl groups of **2e** were tested against the Dd2 strain (**4a**–**b**, Table 3). Both compounds were significantly less active than **2e**, which revealed that heteroatom-containing groups needed to be investigated as a replacement for the thiourea.

The synthesis of analogs of **2e** was undertaken to determine if a suitable replacement for the thiourea could be found. Our initial effort focused on replacement of the thiourea with the more benign cyanoguanidine moiety, an approach that has been used previously to give compounds with more favorable pharmacokinetic properties.[13] To this end, *N*-Boc piperazine (**5**) was reacted with 4-nitrophenylsulfonyl chloride to afford the desired sulfonamide in 92% yield, which was deprotected and isolated as the free amine **6** in 99% yield (Scheme 1). Reaction with diphenyl cyanocarbonimidate gave intermediate **7**, which was converted by reaction with benzylamine to afford **8**, the cyanoguanidine analog of **2e**. Purification by mass-directed reverse phase HPLC[14] gave this material in 12% yield over the final two steps. Testing of **8** in the *P. falciparum* live-dead assay revealed that it had no activity against both the 3D7 and Dd2 strains (EC_{50}s >20 μM), revealing that a cyanoguanidine was not a suitable replacement for the thiourea.

We next investigated whether the thiourea could be replaced by a urea. The synthesis of two ureas with the furanyl and aryl functionalities possessed by **1a**–**b** is displayed in Scheme 2. Piperazine **5** was reacted with furfuryl isocyanate to afford urea **9**,[15] which was then deprotected and reacted separately with two sulfonyl chlorides to give **10a**–**b**. While these compounds displayed greater antiplasmodial activity than cyanoguanidine **8**, they were significantly less potent than screening positives **1a**–**b** (see Table 4).

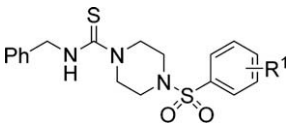
Table 1
Antiplasmodial activity of **2a**–**h**



Compd	R^1	R^2	*Pf* EC_{50} (μM)	
			3D7	Dd2
2a	Furan-2-yl	Me	>10	>10
2b	4-ClPh	4-ClPh	ND[a]	0.400
2c	Tetrahydrofuran-2-yl	4-ClPh	0.667	0.860
2d	$MeOCH_2$	$4\text{-}NO_2Ph$	0.310	0.354
2e	Ph	$4\text{-}NO_2Ph$	0.056	0.103
2f	Tetrahydrofuran-2-yl	$4\text{-}NO_2Ph$	0.313	0.337
2g	Bn	$4\text{-}NO_2Ph$	ND	0.211
2h	Me	$2\text{-}CF_3Ph$	>10	>10

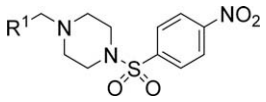
[a] ND = not determined.

Table 2
Antiplasmodial activity of **3a**–**i**

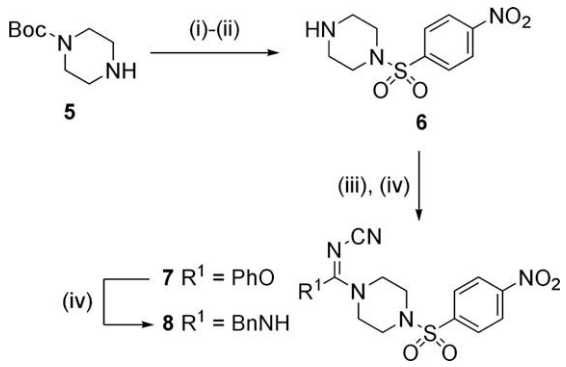


Compd	R^1	*Pf* EC_{50} (μM)	
		3D7	Dd2
3a	$3,4\text{-}(OCH_2CH_2O)$	6.49	>10
3b	4-Br-2,6-Cl	2.67	4.41
3c	4-F	0.447	0.492
3d	2,3,4-F	1.52	1.61
3e	3-F	1.47	1.97
3f	H	1.86	1.92
3g	4-Br-2-Cl	0.830	0.873
3h	2-F	2.41	3.13
3i	4-OEt	1.23	1.96

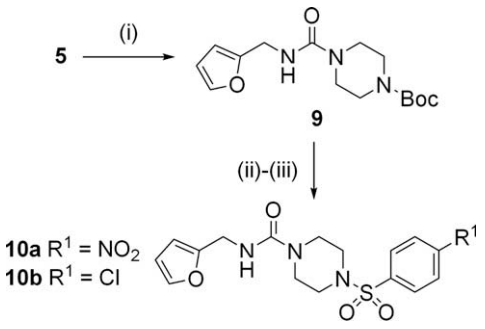
Table 3
Antiplasmodial activity of **4a**–**b**



Compd	R^1	*Pf* Dd2 EC_{50} (μM)
4a	Ph	>10
4b	PhCH=CH	6.05



Scheme 1. Reagents and conditions: (i) 4-Nitrophenylsulfonyl chloride, C_5H_5N, rt, 2 h (92%); (ii) HCl/dioxane, 0 °C→rt, 4 h (99%); (iii) diphenyl cyanocarbonimidate, C_5H_5N, reflux, 18 h. (iv) $BnNH_2$, C_5H_5N, reflux, 18 h (12% over two steps).



Scheme 2. Reagents and conditions: (i) Furfuryl isocyanate, hexanes, 60 °C, 2 h; (ii) HCl/dioxane, 0 °C→rt, 4 h; (iii) C_5H_5N, rt, 2 h and either 4-nitrophenylsulfonyl chloride (**10a**, 31% over three steps) or 4-chlorophenylsulfonyl chloride (**10b**, 20% over three steps).

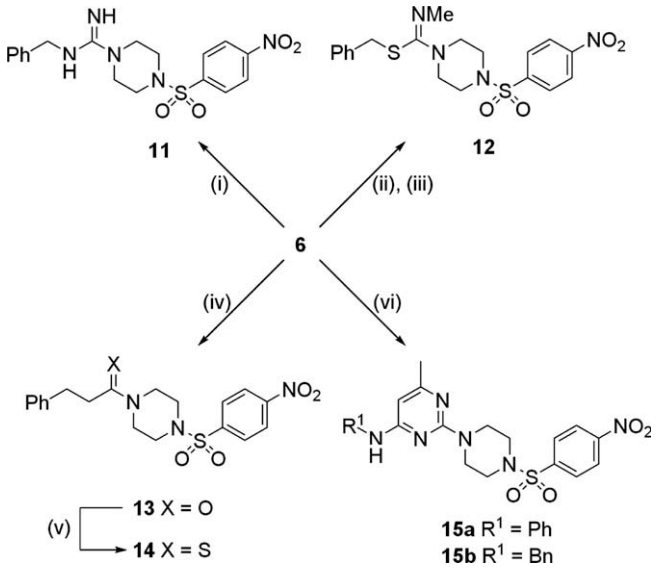
With the cyanoguanidine and urea functionalities proven to be unsuitable replacements for the thiourea, we decided to synthesize a selection of compounds with a range of moieties in place of the thiourea to determine whether they would have activity approaching that of **1a**. Amine **6** was subjected to a range of conditions to achieve this goal (Scheme 3). First, **6** was reacted with benzylcyanamide in $(CF_3)_2CHOH$[16] to afford guanidine **11** in 18% yield. Amine **6** was also reacted with methyl isothiocyanate,[17] then benzyl bromide to give **12** in 8% yield over two steps after purification by reverse phase HPLC. Reaction of **6** with hydrocinnamoyl chloride afforded tertiary amide **13** in 75% yield, which was subsequently reacted with Lawesson's reagent[18] to give thioamide **14** in 74% yield. Finally, **6** was irradiated under microwave conditions with two chloropyrimidines in separate reactions to afford **15a–b** in 92% and 88% yield respectively.

Two additional compounds were prepared to afford analogs with a different amide/thioamide backbone to the tertiary amide/thioamide **13** and **14** (Scheme 4). To this end, carboxylic acid **16** was coupled to benzylamine, and subsequent deprotection gave amide **17** as the free amine in 66% yield over two steps. Exposure to 4-nitrophenylsulfonyl chloride afforded the secondary amide **18** in 65% yield, which was converted to thioamide **19** in 67% yield with Lawesson's reagent.

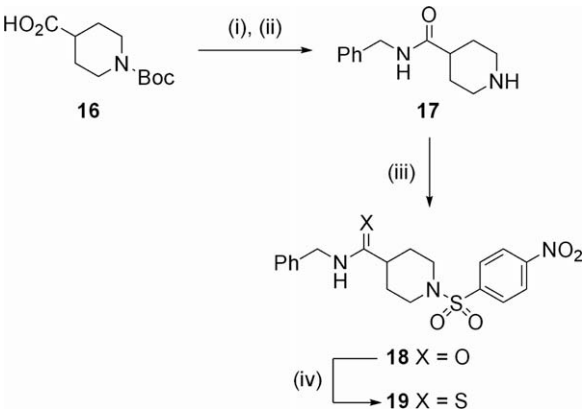
None of the compounds tested (**11–14**, **15a–b** and **18–19**, Table 5) displayed activity near that of thioureas **1a** or **2e**. Replacement of the sulfur with NH, as in guanidine **8**, completely abrogated activity. It is interesting to note that two of the four compounds with EC_{50}s < 10 μM (thioamides **14** and **19**) possessed a sulfur atom at the same position as **1a–b** and **2e**.

Table 4
Antiplasmodial activity of **10a–b**

Compd	R^1	*Pf* EC_{50} (μM)	
		3D7	Dd2
10a	NO_2	6.41	4.48
10b	Cl	2.55	2.30



Scheme 3. Reagents and conditions: (i) BnNHCN, $(CF_3)_2CHOH$, 110 °C, 16 h (18%); (ii) MeNCS, MeCN, reflux, 24 h; (iii) BnBr, DIEA, DMF, 100 °C, 3 h (8% over 2 steps); (iv) $Ph(CH_2)_2COCl$, Et_3N, DMAP, CH_2Cl_2, 0 °C→rt, 18 h (75%); (v) Lawesson's reagent, THF, reflux, 2 h (74%); (vi) ^{i}PrOH, μwave, 185 °C, 1 h and either 2-chloro-6-methyl-*N*-phenylpyrimidin-4-amine (**15a**, 92%) or 2-chloro-6-methyl-*N*-benzylpyrimidin-4-amine (**15b**, 88%).



Scheme 4. Reagents and conditions: (i) $BnNH_2$, PyBOP, DIEA, CH_2Cl_2, rt, 18 h; (ii) HCl/dioxane, 0 °C→rt, 4 h (66% over 2 steps); (iii) 4-nitrophenylsulfonyl chloride, C_5H_5N, rt, 2 h (65%); (iv) Lawesson's reagent, THF, reflux, 2 h (67%).

Table 5
Antiplasmodial activity of **11–14**, **15a–b**, **18–19**

Compd	*Pf* EC_{50} (μM)	
	3D7	Dd2
11	>25	>25
12	11.4	13.0
13	13.5	13.6
14	1.25	1.69
15a	11.1	10.0
15b	3.97	5.81
18	5.95	3.74
19	6.06	6.19

In summary, a high-throughput screening program to identify compounds amenable to the development of novel antimalarials discovered piperazine sulfonamides **1a–b**. These compounds displayed potent activity against *P. falciparum* in a live-dead assay, however they possessed furanyl, thiourea and nitrophenyl groups, all of which have metabolic liabilities. The furan could be replaced with no loss of activity, and the nitrophenyl could be replaced with some loss of activity. A number of analogs were synthesized that replaced the thiourea with a range of functional groups or heterocycles, but in all cases there was a significant decrease in activity, highlighting the thiourea's importance for antiplasmodial activity.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.10.130.

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