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Benzoheterocyclic amodiaquine analogues with potent antiplasmodial activity: Synthesis and pharmacological evaluation

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ABSTRACT

The synthesis and evaluation of antiplasmodial activity of benzothiazole, benzimidazole, benzoxazole and pyridine analogues of amodiaquine is hereby reported. Benzothiazole and benzoxazole analogues with a protonatable tertiary nitrogen atom possessed excellent activity against the W2 and K1 chloroquine resistant strains of *Plasmodium falciparum*, with IC_{50}s ranging from 7 to 22 nM.

About half the world's population is at risk of malaria. In 2010 alone, malaria afflicted an estimated 216 million people worldwide, resulting in 655,000 deaths. Most fatalities occur in sub-Saharan Africa, where a child dies from the disease every minute.[1] The rapid development of resistance against first line antimalarials underscores the imperative for the discovery of new antimalarial drugs.[2] The use of existing antimalarials as starting leads remains a useful approach in drug discovery.[3] In this regard, the 4-aminoquinoline antimalarial amodiaquine (**1**) has many favorable aspects including efficacy against most *Plasmodium falciparum* strains that are resistant to chloroquine (**2**), a reasonable costing, relatively easy synthesis, and ease of administration, with daily dosing over three days.[4] These factors make amodiaquine a good lead compound for the synthesis of newer antimalarials.

Amodiaquine appears to act by the same mechanism as chloroquine, exerting its intra-erythrocytic antimalarial action by preventing heme detoxification to the pigment hemozoin.[5] Amodiaquine is rapidly absorbed and extensively metabolized after oral administration in humans, which leads to a low plasma exposure of the parent drug. Desethylamodiaquine is the main metabolite of amodiaquine. Other reported metabolites are bisdesethylamodiaquine and 2-hydroxydesethylamodiaquine.[6] The elimination of desethylamodiaquine is very slow, unlike that of amodiaquine, giving this metabolite a long terminal half-life. Both amodiaquine and desethylamodiaquine have antimalarial activity.[7] The main enzyme responsible for amodiaquine deethylation is CYP2C8.[8]

It was observed through pharmacovigilance programs that weekly prophylactic use of amodiaquine rarely caused severe side effects including hepatitis, myelotoxicity and agranulocytosis.[9] Consequently, its use is restricted to the treatment of acute malaria.[10] Currently, amodiaquine monotherapy is discouraged, and the drug is principally used in combination with artesunate in one of the artemisinin-based combination therapies currently recommended by the World Health Organization for the treatment of falciparum malaria.[11]

The 4-aminophenol moiety of amodiaquine has been identified as a 'structural alert' for toxicity. Bioactivation of amodiaquine to reactive quinone imine (**3**) and aldehyde (**4**) metabolites has been identified as the likely cause of amodiaquine induced hepatotoxicity and agranulocytosis, respectively.[12,13]

Several analogues such as **5**–**8** (Fig. 1) have been designed that block the formation of the quinone imine but not the aldehyde metabolite. The strategy adopted involves designing out the 4-aminophenol moiety or introducing bulky groups *ortho* to the phenolic hydroxyl group.[12,14–16]

In an effort to discover new approaches to block the formation of both the quinone imine and aldehyde metabolites, we hypothesized that analogues of amodiaquine in which the hydroxyl group

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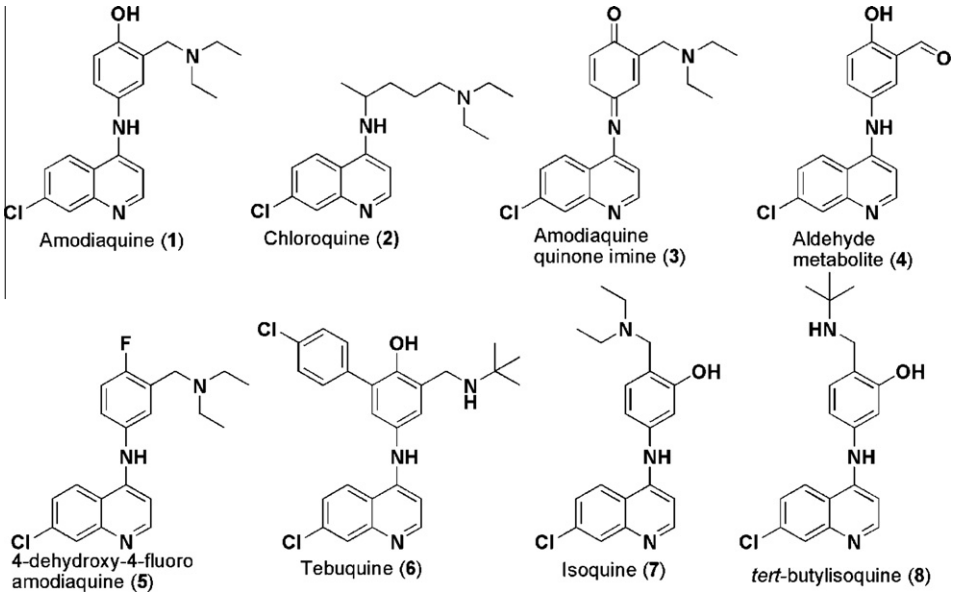


Figure 1. Chemical structures of amodiaquine (**1**), chloroquine (**2**), reactive amodiaquine metabolites (**3**–**4**) and analogues designed to prevent quinone imine formation (**5**–**8**).

and the Mannich side chain are cyclized into a benzothiazole (**9**–**13**), benzoxazole (**14**, **15**) or benzimidazole **(16–18)** ring system will not form reactive metabolites. In addition, analogues (**19** and **20**) in which the aromatic ring is converted to a pyridyl ring bearing an aminoalkyl side chain but without a hydroxyl group were envisaged as lacking an essential functional group for bioactivation. Accordingly, these analogues were designed, synthesized and evaluated for antiplasmodial activity and cytotoxicty.

In the synthesis of the benzothiazole series, commercially available **21** was converted to the bromo intermediate, **22**, by the Sandmeyer reaction. This was coupled with the appropriate primary amines by nucleophilic substitution to give **9a**–**13a**. The nitro group was reduced via Palladium catalyzed hydrogenation to aniline derivatives **9b**–**13b** which were coupled with 4,7-dichloroquinoline to give target compounds **9**–**13**. Similarly, the synthesis of the benzimidazole and pyridine series proceeded via the bromo intermediates (**27** and **29**), amination under microwave conditions (**16a**–**20a**), reduction to aniline derivatives (**16b**–**20b**) and coupling with 4,7-dichloroquinoline to obtain target molecules **16**–**20**. The benzoxazole series was realized via the cyclization of 2-amino-4-nitrophenol **23** with potassium ethyl xanthate to give benzoxazole thione, **24**. This was converted to the methylthio ether **25** prior to amination under microwave conditions (**14a**–**15a**). Reduction of the aromatic nitro group to **14b** and **15b** followed by coupling with 4,7-dichloroquinoline afforded target compounds **14** and **15**. All the target compounds **9**–**20** gave ^{1}H-, ^{13}C NMR and

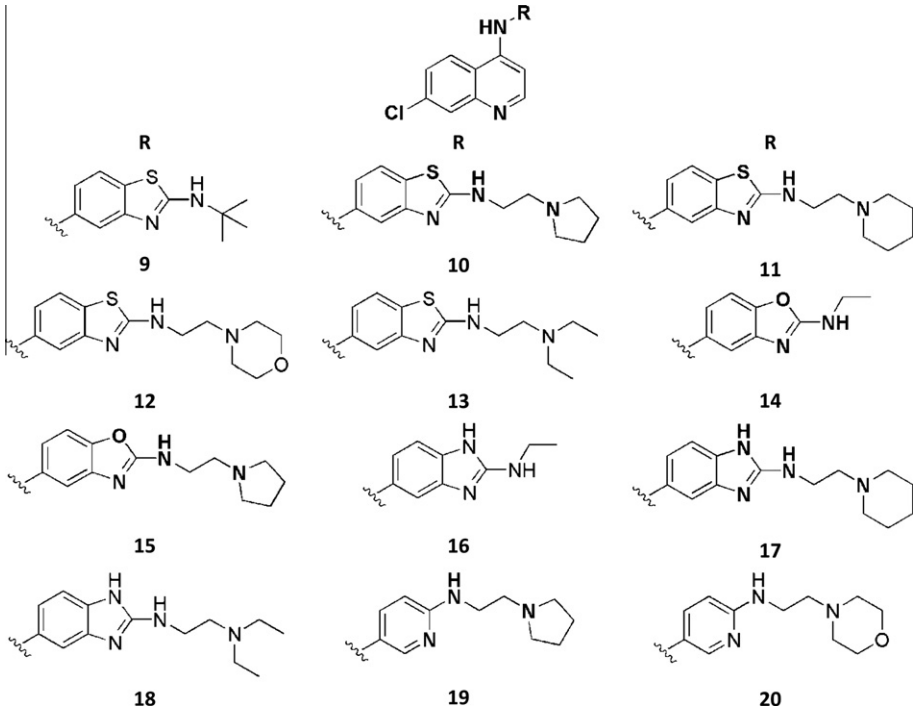
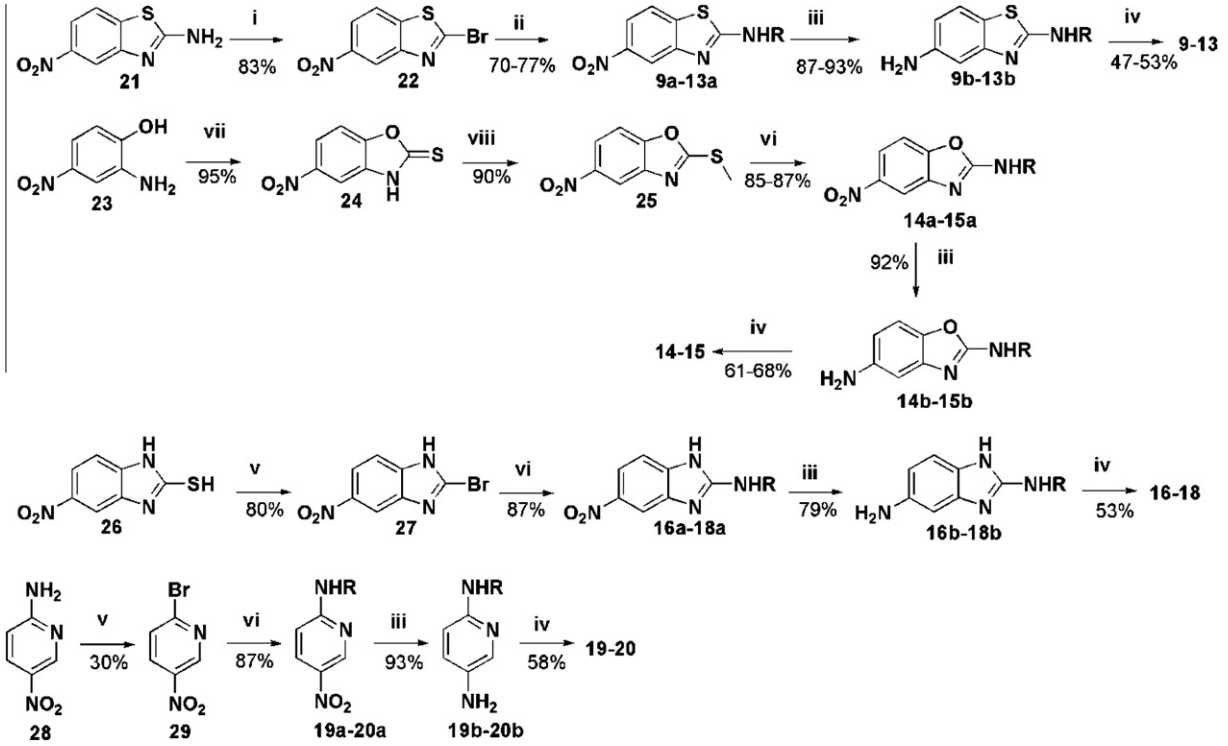
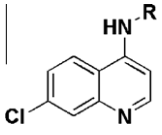


Figure 2. Chemical structures of target benzoheterocyclic (**9**–**18**) and pyridyl (**19**–**20**) analogues.



Scheme 1. Reagents and conditions: (i) HBr, $NaNO_2$, CuBr, H_2O, 4 h, rt; (ii) Amine, THF, 40 °C, 15 h; (iii) H_2, 10% Pd/C, rt, 8 h; (iv) 4,7-dichloroquinoline, acetonitrile, HCl, reflux, 24 h; (v) Br_2, 48% HBr, AcOH, 5 °C-rt, 4.5 h, rt −0 °C, H_2O, NaOH, pH 4; (vi) Amine, EtOH, MW 100 °C, 60 min; (vii) Potassium ethyl xanthate, EtOH, reflux, 4.5 h, AcOH, pH 5; (viii) MeI, DMF, 25 °C, 4 h.

Table 1
In vitro antiplasmodial activity against chloroquine-resistant *P. falciparum* K1 and W2 strains and cytotoxicity in rat L6 myoblasts



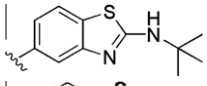
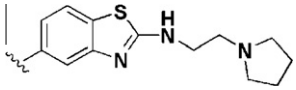
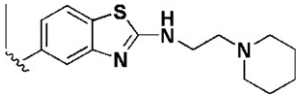
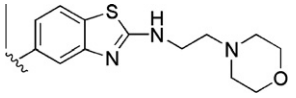
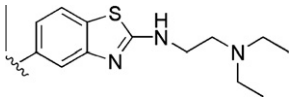
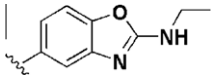
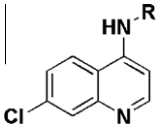
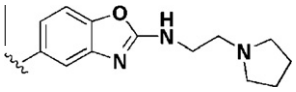
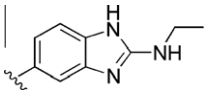
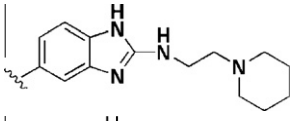
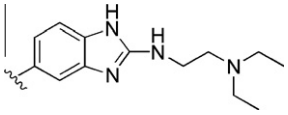
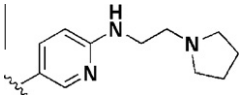
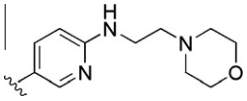
	R	IC_{50} (μM)			Selectivity index
		W2	K1	L6	(L6/W2)
Amodiaquine		0.005	0.008		
Chloroquine		0.049	0.344		
9		1.158	0.917	4.126	3.563
10		0.012	0.014	5.118	426.500
11		0.013	0.007	3.539	272.231
12		0.040	0.025	4.67	116.750
13		0.011	nt[a]	nt[a]	–
14		0.491	0.921	16.352	33.303

Table 1 (*continued*)



	R	IC_{50} (μM)			Selectivity index
		W2	K1	L6	(L6/W2)
15		0.008	0.022	8.703	1087.875
16		0.501	0.249	5.713	11.403
17		4.580	0.090	5.25	1.146
18		0.200	0.827	15.284	76.420
19		0.021	0.092	18.593	885.381
20		0.041	0.073	47.412	1156.390

[a] Not tested.

HRMS data consistent with the proposed structures (see Fig. 2 and Scheme 1).

Antiplasmodial activity was determined against two chloroquine resistant *Plasmodium falciparum* strains, K1 and W2. Cytotoxicity against L6 (rat myoblast) cells was also determined. For studies with the W2 and K1 strains as well as cytotoxicity, assays were conducted as previously reported.[17,18] Table 1 shows antiplasmodial activity, cytotoxicity and selectivity indices for compounds **9**–**20**. The results suggest that the presence of a protonatable amine is essential for high potency, for example as illustrated by the low potency of compound **14** (W2 IC_{50} 491 nM) compared to **15** (W2 IC_{50} 8 nM). Additionally, the benzimidazole series represented by compounds **16**–**18** (W2 IC_{50} 200–4580 nM) was less potent compared to the other series. The pyridine analogues **19**–**20** exhibited moderate potency against the W2 strain (IC_{50} 21–41 nM) but lower activity against the K1 strain (IC_{50} 73–92 nM). Benzothiazole analogues **10**, **11** and **13** as well as the benzoxazole analogue **15** had excellent activity against both strains, with IC_{50} values ranging from 7 to 22 nM. Among the most potent compounds, the benzoxazole analogue, **15**, had the highest selectivity index (1088). The Resistance Index (IC_{50} of the resistant strain/IC_{50} of the sensitive strain) is an indicator of the likelihood of cross resistance. Although the two strains tested were both chloroquine resistant, the W2 strain displayed greater sensitivity than K1. The resistance index for chloroquine and the pyrollidine analogues of the benzothiazole (**10**), benzoxazole (**15**) and pyridine (**19**) series were 7.0, 1.2, 2.8 and 4.4, respectively. These results suggest that all the analogues have low levels of cross resistance with the benzothiazole analogue (**10**) exhibiting the lowest risk.

In conclusion, we have demonstrated a new class of amodiaquine analogs that low levels potentially block the formation of the toxic quinone imine and aldehyde metabolites using a benzoheterocyclic ring system attached to the 4-amino-7-chloroquinoline ring, without compromising antiplasmodial activity. The benzothiazole and benzoxazole analogues possessed excellent activity against chloroquine resistant *P. falciparum* strains. Further studies will focus on determining whether these analogues generate other reactive metabolites.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.06.010.

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