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Synthesis and evaluation of phenylequine for antimalarial activity in vitro and in vivo

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ABSTRACT

Synthesis of the potent antiplasmodial 4-aminoquinoline, phenylequine (PQ), is reported for the first time. PQ and the two analogues show increased efficacy in moving from the chloroquine sensitive D10 to the chloroquine resistant K1 strain in vitro. The in vivo efficacy of PQ, and salts thereof, have been determined in *Plasmodium berghei* ANKA and *Plasmodium yoelii*. Phenylequine hydrochloride has shown an ED_{50} of 0.81 in *P. yoelii* (cf chloroquine ED_{50} = 1.31).

The search for new and effective antimalarial compounds continues. The facts of the devastating effect of this disease are well documented. Over 40% of the world's population is at risk of infection and over 1 million fatalities result from plasmodial infections annually.[1] Of the four strains of *Plasmodium* which cause malaria in humans, *Plasmodium falciparum* remains the most problematic. As resistance to known drugs such as chloroquine (CQ) **1** increases, the need for effective alternatives grows. Over the last several decades the modification of the side chain has proved to be a valuable trajectory of enquiry.[2] Ferroquine (FQ) **2**, a ferrocene containing chloroquine analogue,[3] continues to show promise as a potential new chemotherapeutic agent, as it makes its way through the complex and demanding process of clinical trials. Ferroquine shows a significant absence of cross resistance with chloroquine.[4–6] We reasoned that a simple phenyl analogue of ferroquine, termed phenylequine (PQ) **3**, might exhibit similar efficacy to FQ based on preliminary in vitro data (Fig. 1).[7] The phenyl analogue retains the necessary components for efficacy of a 4-aminoquinoline, that is, the 7-chloro group and weakly basic amino groups.[8] In a previous study we have shown that ferroquine analogues lacking the 7-chloro group on the aminoquinoline moiety have shown in vitro efficacy significantly higher than would be expected. This efficacy suggests that the ferrocene moiety may have an additive and/or synergistic effect on antiplasmodial activity.[9] In this brief Letter we disclose the four-step synthesis of PQ **3** and two analogues **5** and **6**, which were synthesised as a follow up to our earlier study to determine the role of the ferrocenyl moiety in ferroquine.[9] We have subsequently determined that a similar compound to **3** based on an acridine, **4**, rather than a quinoline has previously been synthesised and evaluated for antiplasmodial activity over 50 years

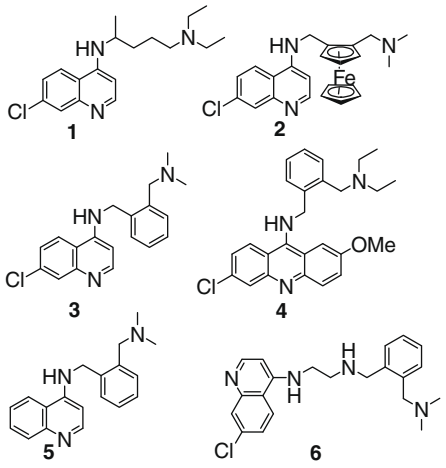

Figure 1. Structures of chloroquine (**1**), ferroquine (**2**), phenylequine (**3**), an acridine analogue of phenylequine (**4**), and two phenylequine analogues (**5** and **6**).

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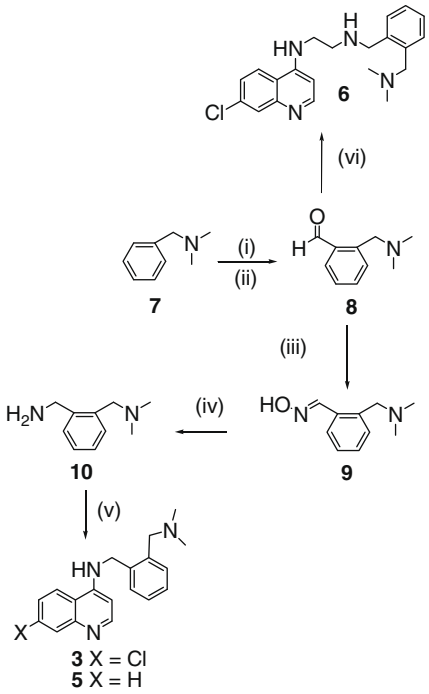
ago.[10] One of the significant reasons for pursuing this line of inquiry is to determine whether phenylequine and analogues could provide an economical alternative to ferroquine. In terms of the cost of goods, phenylequine would be significantly less expensive to manufacture than ferroquine. The starting material dimethylbenzylamine is approximately 70 times cheaper than the corresponding *N,N*-dimethylaminomethyl-ferrocene based on cost per mol from Sigma–Aldrich.

Compounds **3**[11] and **5**[12] were synthesised in an analogous manner to ferroquine,[1] Scheme 1, using known compounds 2-dimethylaminobenzaldehyde **8**,[13] 2-dimethylamino-benzaldehyde oxime **9**,[14] and 2-dimethylaminomethyl-benzylamine, **10**.[15] Compound **6**[16] was synthesized by a reductive amination of aldehyde **8**. Once again, this synthetic scheme is analogous to the route used to synthesize the ferrocenyl analogue.[17]

Compounds **3**–**6** were tested for in vitro antiplasmodial activity against the CQ sensitive D10 and CQ resistant K1 strain along with ferroquine for comparison purposes. From Table 1 it is evident that compounds **3**, **5** and **6** all showed improved efficacy in moving from the D10 to K1 strain. All compounds tested showed low cytotoxicity against mammalian KB cells, giving therapeutic indices of over 100 in all cases. It is also evident that **3** and **6** show comparable efficacy to ferroquine, **2**. The absence of the 7-chloro group significantly reduces the efficacy of compound **5** relative to compound **3**. It has previously been established that the presence of the 7-chloro group has a significant positive impact on the antiplasmodial efficacy in 4-aminoquinolines.[8] This result is consistent with those findings. Although it should be noted that as with the results reported for analogues of ferroquine,[9] the efficacy of compound **5** was higher than expected relative to the parent compound than has been found in compounds with an aliphatic side chain. The reason for this improvement anomaly has yet to be determined and is beyond the scope of this brief article. Although we have established that neither compound **5** nor its ferrocene analogue[9] are capable of inhibiting haemozoin formation, we have determined that the inhibition of haemozoin formation by PQ is comparable to that of chloroquine (data not shown). Given that compound **3** showed greatest efficacy in vitro it was selected for in vivo evaluation in two mouse models. In order to aid in vivo studies dihydrochloride and dicitrate salts of PQ were prepared.



Scheme 1. Reagents and conditions: (i) *t*-BuLi, Et_2O, 25 °C, 60%; (ii) DMF; (iii) NH_2OH, EtOH/H_2O, reflux, 91%; (iv) $LiAlH_4$, THF, reflux, 79%; (v) either 4-chloroquinoline (for **5**) or 4,7-dichloroquinoline (for **3**) K_2CO_3, Et_3N, NMP, reflux, 40%; (vi) diamine, MeOH, $NaBH_4$, 60%.

Table 1
Results from in vitro antiplasmodial testing conducted on chloroquine sensitive (D10), chloroquine resistant (K1) strains of *P. falciparum*, and cytotoxicity against human KB cell line

Compound	IC_{50}, nM D10	IC_{50}, nM K1	Cytotoxicity, μM
CQ	22.9	352.3	
2	17.7	13.5	nd[a]
3	13.2	6.9	70.0
5	106.2	70.3	8.5
6	19.5	9.8	14.0

[a] Not determined in this assay.

Table 2
In vivo activity against *P. berghei* ANKA

Compound	Schedule	% Mean parasitemia	% Inhibition
Control	Untreated	11.89	0
CQ·2PO_4	10 mg/kg ip × 4	0.00	100
3·2HCl	30 mg/kg ip × 4	0.00	100
3	30 mg/kg ip × 4	0.00	100

Table 3
In vivo efficacy of salts of compound **3** against chloroquine resistant *P. yoelii* NS

mg/kg ip × 4	**CQ**·2PO_4	**3**·2[HCl]	**3** Dicitrate
30		99.5	99.6
10	98.1	99.7	99.6
3	90.5	94.5	8.9
1	32.7		
ED_{50}	1.31	0.81	6.01
ED_{90}	2.96	2.18	6.01

The first in vivo assay carried out using the *P. berghei* mouse model showed that both the free base and the hydrochloride salt were efficacious when administered ip, Table 2. The second assay using the *P. yoelii* model, Table 3, showed that the hydrochloride salt of **3** to be active (>94.5% inhibition of parasitemia at concentrations as low as 3 mg/kg). On the other hand the dicitrate salt was less efficient at the lowest dose of 3 mg/kg while showing comparable activity to the dihydrochloride salt at the higher doses.

The phenyl analogues (**3** and **6**) of ferroquine both show comparable antiplasmodial activity to ferroquine. Given the diminished efficacy of compound **5** which lacks the 7-chloro group, it is likely that these compounds work in a similar manner to other 4-aminoquinolines. The fact that compounds **3** and **6**, which are similar to chloroquine exhibit significantly improved efficacy in the K1 strain suggests that these compounds are somehow able to circumvent the underlying mechanism of chloroquine resistance. The parent compound PQ shows efficacy in two in vivo models. This class of compounds is therefore worthy of further investigation.

Acknowledgements

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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.2009.12.030.

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11. 7-Chloro-*N*-(2-((dimethylamino)methyl)benzyl)-quinolin-4-amine **3**: $C_{19}H_{20}ClN_3$ requires C, 70.04; H, 6.19; N, 12.90. Found. C, 69.98; H, 6.25; N, 12.76; δ_H (400 MHz) 8.53 (1H, d, $^3J_{HH}$ = 5), 8.31 (1H, br s), 7.90 (1H, d, $^4J_{HH}$ = 2), 7.62 (1H, d, $^3J_{HH}$ = 9), 7.40–7.37 (1H, m), 7.31–7.22 (4H, m), 6.52 (1H, d, $^3J_{HH}$ = 5), 4.42 (2H, s), 3.46 (2H, s), 2.24 (6 H, s); $\delta_{C\{H\}}$ (100 MHz) 152.3, 150.7 (C^{IV}), 149.5 (C^{IV}), 137.5 (C^{IV}), 137.2 (C^{IV}), 134.7 (C^{IV}), 132.1, 130.1, 128.6, 128.1, 124.9, 122.3, 118.3 (C^{IV}), 99.0, 62.7, 47.4, 45.1 (2C); IR (KBr) ν_{max} 3426br m, 3212br m, 3065m, 3021m, 2968m, 2941m, 2858m, 2817m, 2790m, 2768m, 1662m, 1610m, 1575s, 1485m, 1449m, 1350m, 1326m, 1284m, 1237s, 1163m, 1147m, 1040w, 1014m, 967w, 901m, 882m, 840m, 811s, 771m, 746s, 645m, 590m, 541m; HRMS (EI) *m*/*z* 325.13380 [M^+, $C_{19}H_{20}N_3{}^{35}Cl$ requires 325.13458].
12. *N*-(2-((Dimethylamino)methyl)benzyl)quinolin-4-amine, **5**: mp 133 °C ; $C_{19}H_{21}N_3$ requires C, 78.32; H, 7.26; N, 14.42. Found C, 78.38; H, 7.21; N, 14.45; δ_H (300 MHz, solvent $CDCl_3$) 8.57 (1H, d $^3J_{HH}$ = 6 Hz), 8.14 (1H, br NH), 7.95 (1H, d $^3J_{HH}$ = 8 Hz), 7.69 (1H, dd, $^3J_{HH}$ = 8 Hz, $^4J_{HH}$ = 1 Hz), 7.52–7.60 (1H, m), 7.39–7.42 (1H, m), 7.20–7.36 (4H, m), 6.48 (1H, d $^3J_{HH}$ = 6 Hz), 4.46 (2H, s), 3.48 (2H, s), 2.26 (6H, s); $\delta_{C\{H\}}$ (75 MHz, solvent $CDCl_3$) 151.1, 150.5, 148.7, 137.6 (C^{IV}), 137.2, 131.8, 130.5, 129.6, 128.7, 124.0, 120.4, 119.6, 98.5 (C^{IV}), 62.5, 41.2, 44.9 (2C); *m*/*z* (FAB) 292 (M+H, 100), 290 (15), 247 (17), 148 (7), 129 (10).
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16. *N*-(7-Chloroquinolin-4-yl)-*N*′-(2-dimethylamino-methylbenzyl)-ethane-1,2-diamine, **6**: 1.88 g, 60%; $C_{21}H_{25}N_4Cl$ requires C, 68.37; H, 6.83; N, 15.19. Found C, 68.05; H, 6.75; N, 12.10; δ_H (400 MHz) 8.53 (1H, d, $^3J_{HH}$ = 5), 7.95 (1H, d, $^4J_{HH}$ = 2), 7.76 (1H, d, $^3J_{HH}$ = 9), 7.38 (1H, dd, $^4J_{HH}$ = 2 and $^3J_{HH}$ = 9), 7.33–7.18 (4H, m), 6.41 (1H, d, $^3J_{HH}$ = 5), 3.85 (2H, s), 3.43 (2H, s), 3.34–3.29 (2H, m), 3.00–2.95 (2H, m), 2.11 (6H, s) $\delta_{C\{H\}}$ (100 MHz) 152.0, 150.5 (C^{IV}), 149.2 (C^{IV}), 138.0 (C^{IV}), 137.5 (C^{IV}), 131.1 (2C), 130.8 (2C), 128.7, 127.8, 125.3, 121.2 (C^{IV}), 99.2, 62.5, 51.6, 45.0, 44.7 (2C), 40.2; IR (NaCl neat) ν_{max} 3265m, 3054m, 2944m, 2858m, 2820m, 1611s, 1582vs, 1535s, 1450s, 1428m, 1367s, 1331s, 1265s, 1140m, 1081m, 1041w, 1017m, 878m, 843m, 808m, 738s, 703m; HRMS (EI) *m*/*z* 368.17829 [M^+, $C_{21}H_{25}N_4{}^{35}Cl$ requires 368.17677].
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