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Synthesis, Antimalarial Activity and Inhibition of Haem Detoxification of Novel Bisquinolines

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Abstract—The synthesis of novel bisquinoline compounds comprising 4-(4-diethylamino-1-methylbutyl)aminoquinoline units joined through the 2-position by a $(CH_2)_n$ linker is described. Their ability to inhibit the growth of both chloroquine-sensitive (D10) and chloroquine-resistant (K1) strains of *Plasmodium falciparum*, the hydrogen peroxide-mediated pathway for decomposition of haem, and the conversion of haem to β-haematin have been measured. The activity was affected by the length of the linker and the most active (**6c**, $n=12$) showed effects similar to chloroquine in three of the assays. However, it was even more active against the resistant strain [IC_{50}, 17 nM (K1); 43 nM (D10)], much superior to chloroquine (IC_{50}, 540 nM) and slightly better than mefloquine (IC_{50}, 30 nM) in this regard.

Chloroquine (**1**) remains a main antimalarial drug but the efficacy of it and other chemotherapeutic agents is being steadily lessened by the spread of resistant parasites. Mefloquine (**2**) is a potent compound that has been extensively used against chloroquine-resistant malaria. But, as with chloroquine, resistance to mefloquine is increasing. Thus, the development of alternative drugs is a continuing and urgent requirement. In this regard there is current interest in 'bisquinolines' as novel antimalarials. This class of compounds and their potential role against chloroquine-resistant malaria has been reviewed.[1]

Most members of the class to date comprise two 4-aminoquinoline units linked at the 4-position and therefore can be regarded as related to chloroquine; compounds **3** (X = O, NH; $n=2$ and 3) are recent examples.[2] While excellent activities have been found, no compound of this general class has been suitable for ultimate development.

In previous work, we modified this approach to retain the side chain of existing antimalarials and used hydrocarbon linkers joined through amide functions at different positions to construct chloroquine-like (**4**)[3,4] and cinchonidine-like (**5**)[5] bisquinolines. The most active of these (**5a**) appeared to have overcome the chloroquine resistance mechanism (Table 1) but was toxic in animal studies.

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The malarial parasite produces haem by degradation of haemoglobin[6] and is thought to detoxify this by (a) crystallisation of the haem to form haemozoin and (b) destruction of haem by reaction with hydrogen peroxide or glutathione.[7–9] Chloroquine and a number of other quinoline drugs have been shown to inhibit haemozoin formation[4,7,10] and the destruction of haem by reaction with hydrogen peroxide.[9]

We have now developed new chemistry leading to bisquinolines **6a**–**c**, which retain the chloroquine side chain but with linkage at the 2-position through a hydrocarbon linker, in order to further study the effects of structure on antimalarial activity and haem detoxification within this general class of antimalarials.

The quinoline derivative **7**[11] was coupled through the active α-methylene group under basic conditions with a series of α,ω-dibromoalkanes to give the bis derivatives **8** (Scheme 1). Without isolation, **8** were subjected to alkaline hydrolysis of the ester functions and decarboxylation of the resultant acids in the same pot.[12] The intermediates **9** were then converted to the target **6** in two further standard reactions.[13,14] Compounds **6** were oils; samples were obtained by preparative TLC as salts were too hygroscopic to be used in purification.

Antimalarial activities, as IC_{50} values for inhibition of chloroquine sensitive (D10) and resistant (K1) parasite strains, are given in Table 1.[15] All three compounds **6a**–**c** displayed notably high activity against the resistant

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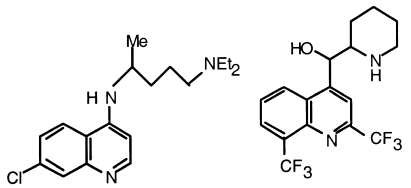

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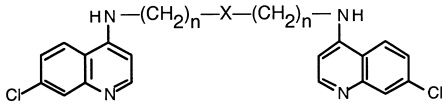

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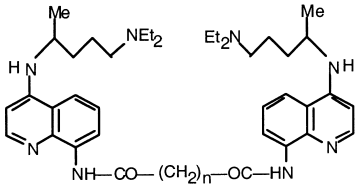

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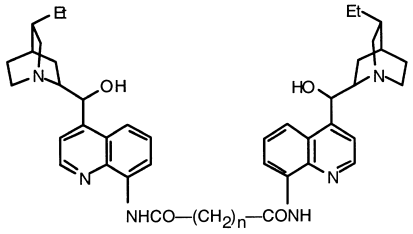


5 (a n = 8)

strain, while the activity against the sensitive (D10) strain showed a steady increase with increase in length of the linker. Compound **6c** was superior to mefloquine (**2**) in these assays. These bisquinolines may be able to circumvent the resistance mechanism by gaining access to the food vacuole by a different mechanism from that used by chloroquine.[16]

Table 1. IC_{50} values for inhibition of haem detoxification and growth of *P. falciparum* in vitro

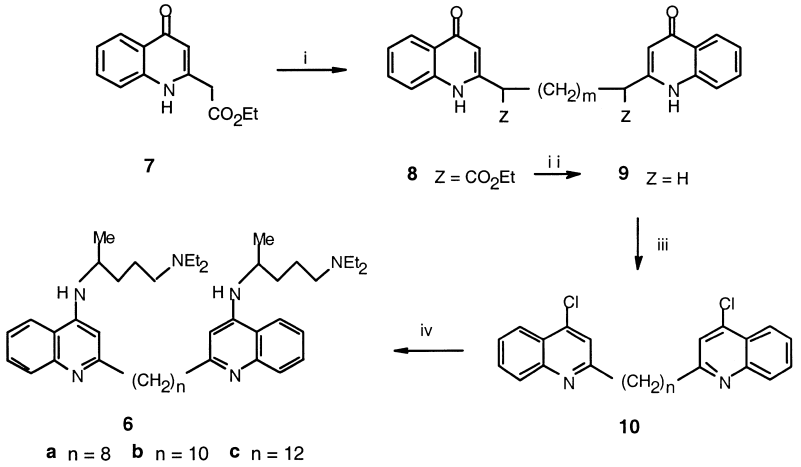
Compd	IC_{50} (μM)		IC_{50} (nM)	
	H_2O_2/FP decomp.	β-Haematin formation	Growth of D10 strain	Growth of K1 strain
1	37±6	75	40[a]	540[a]
2	30[b]		90[c]	30[c]
5a			50[c]	20[c]
6a	81±16	97±8	123±25	25±20
6b	80±17	94±9	154±4	16±12
6c	37±9	86±7	43±4	17±11

[a]Data from ref 3.
[b]Data from ref 9.
[c]Data from ref 5.

We have examined the formation of β-haematin (the synthetic equivalent of haemozoin) in the presence of dispersions of the neutral lipid, MOG, which provides a convenient system for monitoring the ability of the novel bisquinolines to inhibit haem crystallisation.[17] Chloroquine was an efficient inhibitor of β-haematin formation. The concentration needed to inhibit the reaction by 50% (IC_{50} value) was 75 μM (Table 1). Mefloquine (**2**) has previously been reported to inhibit β-haematin formation with an efficiency somewhat less than that of chloroquine.[4] Each of the bisquinolines (**6a**–**c**) inhibited β-haematin formation with an efficiency similar to that of chloroquine.

Chloroquine and mefloquine are also efficient inhibitors of haem degradation by the alternative hydrogen peroxide-mediated pathway that may be employed by the malaria parasite to detoxify haem molecules[9,18] (Table 1). Compound **6c** inhibited this peroxidative decomposition with a similar efficiency to chloroquine, while **6a**,**b** showed lower inhibitory activities.

There is a loose correlation between the activities of the different bisquinolines as inhibitors of parasite growth



Scheme 1. (i) EtO^-/DMSO/$Br(CH_2)_mBr$; (ii) 1% NaOH/reflux; (iii) $POCl_3$/115 °C/5 min; (iv) $NH_2CH(CH_3)CH_2CH_2CH_2NEt_2$/180 °C/24–48 h.

in vitro and their activities as inhibitors of β-haematin formation and haem degradation; the most active compound **6c** is also the most effective compound in each of these haem detoxification assays. Thus, these assays may be useful for initial high-throughput screening of novel compounds.

The data suggest that the bisquinolines exert their antimalarial activity in a manner similar to that of chloroquine, while the interesting antimalarial activity of **6c**, especially against the chloroquine-resistant strain of parasite, warrants further investigation of this compound.

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12. Preparation of 2,2′-octamethylenebis [4(1*H*)-quinolinone] (**9a**): Example of steps 1 and 2. To a solution of **7**[11] in dry EtOH (5 mL/0.1 g **7**) was added NaOEt in EtOH (0.5 M solution, 2 mol equiv). The mixture was stirred under nitrogen at room temperature for 20 min and the EtOH was then removed under reduced pressure. Dry DMSO (3 mL/0.1 g) was added followed by 0.5 mol equiv of 1,6-dibromohexane and the solution was stirred under nitrogen at room temperature for 40 min. Water (10 mL/0.1 g) was then added, followed by 10% NaOH (1 mL/0.1 g) and the mixture was refluxed for 3 h, cooled and acidified with 12% HCl. The solid bis-oxoquinoline product which separated was filtered off, and recrystallized from EtOH/MeCN to give 56% of **9a**, mp 150–155 °C. ^{1}H NMR (DMSO-d_6) δ 1.28 (m, 4H, $(CH_2)_2$), 1.65 (m, 2H, CH_2), 2.56 (t, J = 7.1 Hz, 2H, CH_2), 5.91 (s, H-3), 7.25 (t, J = 7.6 Hz, H-7), 7.51 (d, J = 7.1 Hz, H-8), 7.59 (t, J = 8.1 Hz, H-6), 8.02 (d, J = 8.0 Hz, H-5), 11.45 (s, NH). ES-MS: m/z 401 (M + 1). Compounds **9b**, mp 80–85 °C, and **9c**, mp 253–256 °C (from 2-butanone) were prepared similarly.
13. Preparation of 2,2′-octamethylenebis[4-chloroquinoline] (**10a**): Example of step 3. A mixture of **9a** (0.15 mmol) and dry $POCl_3$ (4 mL) was heated at 115 °C for 5 min. The excess $POCl_3$ was removed under reduced pressure, and cold water was added to the residue. The mixture was then basified with 12% NaOH and extracted with $CHCl_3$ (2×10 mL). The extract was washed with water, dried ($MgSO_4$), and the solvent was removed under reduced pressure to give the crude chlorinated product, as a brown oil (84%) sufficiently pure to be used in the next step. ^{1}H NMR ($CDCl_3$) δ 1.35 (m, 4H, $(CH_2)_2$), 1.76 (m, 2H, CH_2), 2.90 (t, J = 7.6 Hz, 2H, CH_2), 7.37 (s, H-3), 7.54 (t, J = 7.8 Hz, H-7), 7.70 (t, J = 7.3 Hz, H-6), 8.02 (d, J = 8.4 Hz, H-8), 8.15 (d, J = 8.2 Hz, H-5). ES-MS: m/z 437 (M + 1). Compounds **10b** and **10c** were similarly prepared.
14. Preparation of 2,2′-octamethylenebis[4-(4-diethylamino-1-methylbutyl)aminoquinoline] (**6a**): Example of step 4. A mixture of **10a** (0.23 mmol), and 2-amino-5-diethylaminopentane (1.5 mL) was heated under a nitrogen atmosphere at 180 °C for 24 h. The reaction mixture was then dissolved in ether (20 mL), washed five times with water, dried ($MgSO_4$), and the solvent was removed under reduced pressure to yield the crude product as a brown oil. This was extracted with hot hexane and the insoluble material was further purified by preparative TLC (alumina; ethyl acetate/hexane/triethylamine, 6:4:0.15) to give the product as an oil (40%, R_f 0.26). ^{1}H NMR ($CDCl_3$) δ 0.98 (t, J = 6.9 Hz, 6H, $N(CH_2C\mathbf{H}_3)_2$), 1.29 (d, J = 6.3 Hz, 3H, $HNCHC\mathbf{H}_3$), 1.36–2.1 (m, 10H, link CH_2×3 + side chain CH_2×2), 2.4–2.6 (m, 6H, 3×NCH_2), 2.79 (t, J = 7.6 Hz, 2H, $ArCH_2$), 3.72 (m, HNC**H**), 4.95 (d, J = 6.4 Hz, **H**NCH), 6.31 (s, H-3), 7.32 (t, J = 7.1 Hz, H-6), 7.56 (t, J = 7.1 Hz, H-7), 7.64 (d, J = 8.2 Hz, H-8), 7.90 (d, J = 8.3 Hz, H-5). ES-MS: m/z 681 (M + 1). Compounds **6b** [ES-MS: m/z 709 (M + 1)] and **6c** [ES-MS: m/z 737 (M + 1)] were similarly prepared.
15. Antimalarial activity was determined by a literature method.[19] CQ-sensitive (D10) and resistant (K1)[20] strains of *P. falciparum* were used and details are as described elsewhere.[5]
16. See ref 6 for a more detailed discussion.
17. Assays of the conversion of haem to β-haematin were performed as described.[21] A suspension of monooleoyl glycerol (MOG) in 90 mM sodium acetate, pH 5, was prepared by sonication and aliquots (0.5 mL) were mixed with haem from a stock in 50 mM NaOH to final concentrations of 100 μM haem and 0.5 mM MOG. Samples were incubated at 37 °C for 24 h with gentle rotation. Following incubation, the samples were centrifuged at 27,000*g*, 4 °C for 15 min. The β-haematin was washed four times by resuspending the pellet in 10 mM sodium phosphate, pH 7.4, containing 2.5% SDS and vortexing for 10 min at 20 °C, before repelleting. The remaining pellet was resuspended in 950 μL of 2.5% SDS in phosphate buffer and a 50 μL aliquot of 1 M NaOH was added to dissolve the β-haematin. The concentration of haem was determined by measuring the absorbance at 404 nm, assuming a molar extinction coefficient of 9.08×10^4 cm^{-1} M^{-1}.[22] Drugs were added from stock solutions in DMSO. Controls contained an equal amount of the solvent.
18. The H_2O_2-mediated pathway for decomposition of haem was monitored by measuring the decrease in absorption of haem at the Soret band (400 nm). Aliquots (0.2 mL) of 15 μM haem in 200 mM sodium acetate, pH 5.2, 1 mg/mL BSA, were equilibrated at 20 °C and the reaction was initiated by the addition of H_2O_2 to a final concentration of 1 mM. Drugs were added from stock solutions in 200 mM sodium acetate.
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