Synthesis and Activity of Some Antimalarial Bisquinolines

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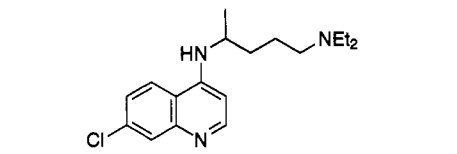
A new type of bisquinoline antimalarial, in which the basic side chain of chloroquine is retained, has been evaluated. Nine bisamides were prepared from aliphatic diacids with 6-amino- and 8-amino-((4-(4-(diethylamino)-1-methylbutyl)amino)quinoline, and screened against chloroquine-sensitive and -resistant strains of *Plasmodium falciparum* in vitro. The resistance indices for all compounds were lower than for chloroquine. The position of attachment and length of the linker chain markedly affected activity. The most active (IC_{50} = 120 nM against the chloroquine-resistant FAC8 strain) was the $-(O)C(CH_2)_4C(O)-$ linked 8-amino compound.

Chloroquine (CQ, 1) is, after 50 years, still a mainstream drug in the fight against malaria, but its efficacy is being steadily eroded by the development of resistant parasites.[1] While some other compounds such as verapamil can reverse CQ resistance in vitro, their toxicity prevents clinical use. An alternative approach is to modify the active drug in ways which retain the antimalarial activity of CQ but where the modified drugs are not recognized by the proteins involved in CQ resistance. There is interest in this regard in bisquinolines, using the rationale that the bulky bisquinoline structure may be less efficiently extruded by CQ-resistant *Plasmodium falciparum*.[2] A number of bisquinolines have been examined.[3] Typical of the class is piperaquine **2**, which has shown in vitro activity against resistant strains of *P. falciparum*[4] and activity against *P. berghei* in mice and against *P. falciparum* in humans.[5] Compounds **3** are recently synthesized bis-(4-amino-7-chloroquinolines), where R is a series of alkane bridges of varying length, which were shown to be very effective antimalarial agents, acting against *P. berghei* and against both CQ-sensitive and CQ-resistant strains of *P. falciparum*.[2] The bisquinolines to date have all retained the chloroquinoline part of CQ and linked the two parts through the basic side chain.

We have investigated a new series, where the aminoquinoline part of CQ is retained and the two units are joined by bisamide links from the carbocyclic ring. Compounds **6**–**14** have therefore been prepared, and their activities against sensitive and resistant strains of *P. falciparum* have been measured in vitro, in anticipation that this bisquinoline series might also circumvent the mechanism of CQ resistance.



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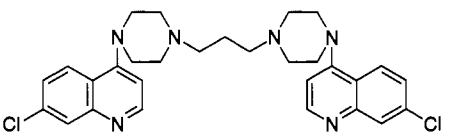

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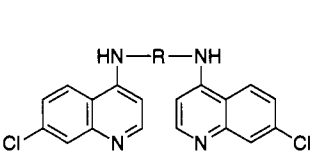

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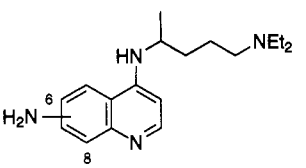

4: 8-NH_2
5: 6-NH_2

Chemistry

Two series of compounds, linked through the 6- and 8-quinoline positions were prepared. The 6-amino compound **5** was available from other work,[6] while the isomeric 8-amino compound **4** was prepared from a known 7-chloro derivative.[7] The bisamides were prepared by reacting **4** or **5** with appropriate diacid chlorides. The basic side chain complicated the reaction by reacting with the hydrogen chloride produced to precipitate the salt of product and/or reactant. The reaction stoichiometry prevented an excess of the quinoline being used. The problem was alleviated by adding 1 equiv of trifluoroacetic acid to the quinoline base solution—the resulting trifluoroacetate was soluble in the acetonitrile solvent. Reactions in the 8-series appeared to be faster than for the 6-analogs, and longer chain acid chlorides were more reactive than shorter ones. Repeat reactions were therefore necessary to get complete conversion for the least reactive combination, i.e., **11**. It appears that there was a preferred 1:1 reaction of **5** with succinyl chloride to give an imide (this was deduced from ^1H NMR analysis of the crude product), and we did not obtain the target bis compound. Unfortunately, attempts to acylate the 7-chloro derivative of **4** were also unsuccessful.

Antimalarial Activity

The capacity of CQ and of bisquinolines **6**–**14** to arrest the growth of CQ-sensitive and CQ-resistant strains of *P. falciparum* was determined (Table 1). As previously reported, CQ inhibited the growth of the three parasite strains examined with different efficiencies (Table 1 and ref 8). The marked difference in the potency of CQ against the CQ-sensitive (D10) and CQ-resistant strains (FAC8 and K1) is illustrated by the calculated resistance ratios of 9 and 13.5 respectively (Table 1). All of the

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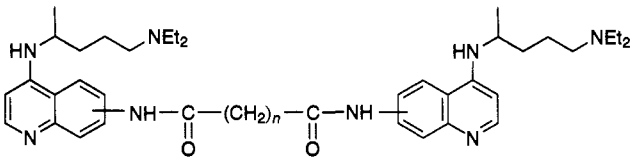
Table 1. IC_{50} Values and Resistance Index Values for Inhibition of Growth of *P. falciparum* in Vitro by Bisquinolines[a]



6–14

compd	series[b]	n	IC_{50} (μM) D10	IC_{50} (μM) FAC8	IC_{50} (μM) K1	resistance index FAC8[c]	resistance index K1[d]
1			0.04 ± 0.02 (3)	0.4 ± 0.2 (3)	0.54 ± 0.07 (2)	10	13.5
6	8	0	1.3 ± 0.7 (4)	1.9 ± 1.8 (3)	2.3 ± 0.6 (2)	1.4	1.7
7	8	2	0.5 ± 0.2 (3)	1.2 ± 0.3 (2)	2.5 (1)	2.2	5.0
8	8	4	0.13 ± 0.06 (4)	0.12 ± 0.07 (3)	0.23 ± 0.11 (2)	0.9	1.8
9	8	6	0.38 ± 0.13 (2)	0.35 ± 0.16 (2)	0.6 ± 0.3 (2)	0.9	1.6
10	8	8	0.26 ± 0.1 (4)	0.31 ± 0.16 (3)	0.29 ± 0.04 (2)	1.2	1.1
11	6	0	2.6 ± 1.6 (2)	1.8 ± 1.0 (2)	2.2 ± 1.1 (2)	0.7	0.8
12	6	4	9.6 ± 3.0 (3)	10.3 (1)	15.9 (1)	1.1	1.7
13	6	6	5.0 ± 0.63 (3)	5.2 ± 1.2 (2)	7.4 ± 3.1 (2)	1.0	1.5
14	6	8	5.7 ± 2.9 (4)	3.8 ± 1.0 (3)	8.4 ± 4.2 (2)	0.7	1.5

[a] Number of experiments in parentheses. [b] Position of linker attachment. [c] IC_{50} (FAC8)/IC_{50} (D10). [d] IC_{50} (K1)/IC_{50} (D10).

bisquinolines examined in this study showed some antimalarial activity, though members of the 8-series were substantially more effective than the 6-series. Interestingly, the relationship between activity and length of linking group is different in the two series. Within the 6-series, maximum activity occurred for 11, where the two relatively rigid amide groups are directly joined. By contrast, antimalarial activity in the 8-series was aided by a longer, more flexible link; maximum activity was observed for **8**, with four methylene groups in the linker.

Discussion

The reason for the greater activity of the 8- than the 6-series is not clear but may involve different uptake efficiencies for the different drugs. The dependence of activity on linker length is also different, and it is interesting to compare this dependence with that for series **3** where the linker length was also varied. Here, minimum activity was found when the linker comprised four carbons, with increased activity for shorter or longer chains.[2]

The bisquinolines examined in this study have higher IC_{50} values for inhibition of growth of *P. falciparum* than do those of series **3**.[2] The lower activity of the bisquinolines examined here may reflect the fact that these compounds lack a 7-chloro substituent. It has been demonstrated that the 7-H derivative of CQ has a 14-fold lower activity than CQ itself.[9] This report suggested that the chlorine group may be essential for efficient accumulation of 4-aminoquinolines by malaria parasites. Nevertheless, compound **8** was 2–3 times more effective than CQ as an inhibitor of growth of the CQ-resistant strains K1 and FAC8. Further modification of this structure may improve the antimalarial activity.

A comparison of the IC_{50} values for the inhibition of growth of the resistant and sensitive strains of *P. falciparum* suggests relatively low levels of cross-resistance. The resistance index values for members of the two series of bisquinolines were much lower than for CQ and were mostly in the range of 0.7–2 (Table 1). It has been suggested that the bulky bisquinoline structure may not be recognized by proteins involved in conferring CQ resistance.[2] Our data support this proposal.

We do not have information on the mechanism of action of these compounds, but studies on DNA binding and the inhibition of haem polymerase activity are in progress.

In conclusion, our results support the idea that bisquinolines are reagents which can circumvent parasite mechanisms of CQ resistance. While antimalarial activity among these first members of our series is generally disappointing, the enhanced action against resistant strains warrants further work. Alternate syntheses directed at obtaining analogs containing a ring-chloro substituent, and linking group other than $(CH_2)_n$ are planned.

Experimental Section

^1H NMR spectra were recorded on a Bruker AM-300 spectrometer in $CDCl_3$. Full details are given for the bisamides **6** and 11. Homologs showed the same patterns, with additional linker CH_2 signals as appropriate. Electrospray mass spectra were obtained on a VG Bio-Q triple quadrupole mass spectrometer using a water/methanol/acetic acid (50:50:1) mobile phase.

Chemistry. 8-Amino-4-((4-(diethylamino)-1-methylbutyl)amino)quinoline (4). A mixture of 8-amino-7-chloro-4-((4-(diethylamino)-1-methylbutyl)amino)quinoline[7] (0.5 g, 1.49 mmol), 10% palladium/charcoal (0.35 g), and ammonium formate (0.48 g, 7.5 mmol) in aqueous acetic acid (50%, 10 mL) was refluxed for 15 min. The solution was cooled, the catalyst was filtered off, and the filtrate was taken to pH 8 with 10 M sodium hydroxide and extracted with chloroform (2 × 20 mL). The combined extracts were dried ($MgSO_4$) and the solvent was removed to give the dechlorinated aminoquinoline **4** (0.32 g, 63%) as a light brown oil, sufficiently pure for the next reaction: ^1H NMR δ 1.1 (t, J = 7 Hz, 6H, NCH_2CH_3), 1.35 (d, J = 6 Hz, 3H, $NHCH(CH_3)$), 1.6–1.8 (m, 4H, $(CH_2)_2$), 2.4–2.55 (m, 6H, 3 × NCH_2), 3.7 (m, 1H, NHCH), 4.9 (br s, 2H, NH_2), 6.39 (d, J = 5 Hz, 1H, H-3), 6.83 (d, J = 7.6 Hz, 1H, H-7), 6.95 (d, J = 8.6 Hz, 1H, H-5), 7.19 (t, 1H, H-6), 8.39 (d, J = 5 Hz, 1H, H-2).

Synthesis of 6–14. A solution of acid chloride (0.7 mmol) in dried acetonitrile (2 mL) was added dropwise over 10 min to a solution of **4** or **5**[6] (0.33 mmol) and trifluoroacetic acid (0.35 mmol) in dried acetonitrile (5 mL). Stirring was continued for a further 24 h, and the solvent was evaporated. The

resulting salt was dissolved in water (40 mL), and the solution was basified with 5% sodium bicarbonate solution and extracted with chloroform (3 × 20 mL). The combined extracts were dried, and the solvent was evaporated to yield a light brown residual oil. This was extracted with hot petroleum ether (bp 90–110 °C) and the solvent evaporated to give the crude product.

***N,N'*-Bis[4-((4-(diethylamino)-1-methylbutyl)amino)-quinolin-8-yl]oxalamide (6)**: yellow solid (27%); mp 58 °C [from petroleum ether (bp 90–110 °C)]; ^{1}H NMR δ 1.1 (t, J = 7 Hz, 3H, NCH_2CH_3), 1.35 (d, J = 6 Hz, 3H, $NHCH(CH_3)$, 1.6–1.8 (m, 4H, $(CH_2)_2$), 2.4–2.6 (m, 6H 3 × NCH_2), 3.70 (m, 1H, $NHCH(CH_3)$), 5.1 (d, J = 7 Hz, 1H, NH), 6.43 (d, J = 5 Hz, 1H, H-3), 7.25–7.45 (m, 2H, H-5,6), 8.38 (d, J = 5 Hz, 1H, H-2), 8.78 (d, J = 7 Hz, 1H, H-7), 11.9 (s, 1H, NHC=O). Anal. ($C_{38}H_{54}N_8O_2 \cdot 0.5H_2O$) C, H, N.

***N,N'*-Bis[4-((4-(diethylamino)-1-methylbutyl)amino)-quinolin-8-yl]succinamide (7)**: bright yellow solid (67%); mp 50–52 °C [from petroleum ether (bp 90–110 °C)]. Anal. ($C_{40}H_{58}N_8O_2 \cdot 1.5H_2O$) C, N; H: calcd, 8.7; found, 8.0.

***N,N'*-Bis[4-((4-(diethylamino)-1-methylbutyl)amino)-quinolin-8-yl]adipamide (8)**: white solid (27%); mp 143–145 °C (from acetonitrile). Anal. ($C_{42}H_{62}N_8O_2 \cdot H_2O$) C, H, N.

***N,N'*-Bis[4-((4-(diethylamino)-1-methylbutyl)amino)-quinolin-8-yl]suberamide (9)**: the free base was a slightly impure oil and no crystalline salt could be formed; ESMS m/z 739.4 (M + 1), 370 ((M + 2)/2).

***N,N'*-Bis[4-((4-(dimethylamino)-1-methylbutyl)amino)-quinolin-8-yl]sebacamide (10)**: the oily free base (59%) was converted to a fawn oxalate salt; mp 123 °C (from ethanol). Anal. ($C_{46}H_{70}N_8O_2 \cdot 4H_2C_2O_4 \cdot 3H_2O$) C, N; H: calcd, 7.2; found, 6.4.

***N,N'*-Bis[4-((4-(diethylamino)-1-methylbutyl)amino)-quinolin-6-yl]oxalamide (11)**. The standard conditions gave only partial conversion so the reaction cycle was repeated three times on the crude product. The final product was an oil, still containing a trace of **5**, and a crystalline salt was not obtained: ESMS m/z 655.4 (M + 1), 328.2 ((M + 2)/2); ^{1}H NMR δ 0–5.1 as for **6**, 6.37 (d, J = 5 Hz, 1H, H-3), 7.58 (d, J = 8.7 Hz, 1H, H-7), 7.89 (d, J = 8.7 Hz, 1H, H-8), 8.32 (s, 1H, H-5), 8.42 (d, J = 5 Hz, 1H, H-2), 10.2 (s, 1H, NHC=O).

***N,N'*-Bis[4-((4-(diethylamino)-1-methylbutyl)amino)-quinolin-6-yl]adipamide (12)**: fawn solid (29%); mp 173–175 °C (from acetonitrile). Anal. ($C_{42}H_{62}N_8O_2 \cdot 2.5H_2O$), C, H, N.

***N,N'*-Bis[4-((4-(diethylamino)-1-methylbutyl)amino)-quinolin-6-yl]suberamide (13)**: this was an oil (25%), and a crystalline salt was not obtained; ESMS m/z 739.5 (M + 1), 370.3 ((M + 2)/2).

***N,N'*-Bis[4-((4-(diethylamino)-1-methylbutyl)amino)-quinolin-6-yl]sebacamide (14)**: the oily free base (50%) was converted to a fawn oxalate salt; mp 118 °C (from ethanol). Anal. ($C_{46}H_{70}N_8O_2 \cdot 4H_2C_2O_4 \cdot 3H_2O$) C, H, N.

Antimalarial Activity. Malaria parasites were continuously cultured as described.[10] *P. falciparum* (D10) is a CQ-sensitive cloned line of isolate FCQ27/PNG.[11] *P. falciparum* (FAC8)[12] is a CQ-resistant clone derived from another cloned line ITG2F6 (gift of L. Miller). The CQ-resistant isolate K1 (Thailand) was obtained from G. Knowles (Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea). Malaria parasites were plated at c. 1% parasitemia (2% hematocrit), in 96-well trays in the presence of different concentrations of CQ or the bisquinoline series. The latter were added from concentrated stocks (30 mM) in DMSO. The final concentration of DMSO was less than 0.1%. At this concentration, DMSO has no effect on the development of the parasites. Parasites were incubated for 4 days, with daily replacement of the drug-supplemented media. Growth curves were performed in duplicate as described,[13] and the concentration of drug required to produce 50% inhibition of growth (IC_{50}) was determined.

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