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Novel Aryl-bis-quinolines with Antimalarial Activity In-vivo

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Abstract

Three rationally designed isomeric aryl-bridged bis-quinolines, N^1,N^x-bis(7-chloroquinolin-4-yl)phenylene-1,x-diamines, where x = 2, 3 or 4, i.e. *o*-, *m*- and *p*-substituted analogues respectively, were synthesized and evaluated against *Plasmodium berghei* in-vivo.

The compound with x = 2 had an ID50 of 30 mg kg^{-1}, whereas the *p*-substituted analogue (x = 4) was not statistically schizonticidal at either of the two dose levels tested in olive oil–dimethylsulphoxide (5 and 25 mg kg^{-1}, ID50 = 60 mg kg^{-1} approx.). When the delivery vehicle was changed to saline–DMSO, antimalarial potency increased for the *p*-substituted compound (ID50 17 mg kg^{-1}). In contrast, the *m*-substituted analogue had marked antimalarial activity (ID50 1·2 mg kg^{-1}), which compares favourably with that of chloroquine diphosphate (ID50 = 4·3 mg kg^{-1}). The data presented show that the amino-methylene side chain in amodiaquine can be successfully replaced by a 7-halo-4-aminoquinoline, establishing that carbon bridges containing less than four contiguous carbon atoms can be present within highly active aryl-substituted 4-aminoquinoline antimalarials.

These results confirm that the presence of an OH group in the aryl bridge is not necessary for antimalarial activity and substantiate the view that, despite the appearance of resistant strains, new and existing aminoquinolines still have an important role in treating malaria.

There is a continuing need to develop inexpensive antimalarial drugs, because of the development of resistance and the cost of existing drugs. The lack of cross-resistance between amodiaquine and chloroquine against chloroquine-resistant forms of *Plasmodium falciparum* makes amodiaquine an attractive and cheap treatment for malaria (Muller et al 1996; Olliaro et al 1996), as substantiated in a number of clinical trials (Watkins et al 1984; Looareesuwan et al 1986; White et al 1987; Fadat et al 1991). However, prophylactic administration of amodiaquine can produce severe adverse effects including agranulocytosis and hepatitis (Neftel et al 1986; Cook 1995). These adverse effects might be because of oxidation of the 4-aminophenol group generating either a semiquinone-imine free radical or a quinone-imine. Irreversible binding of such intermediates to proteins, especially in liver microsomes in man might explain the observed toxicity (Maggs et al 1988; Bisby 1990; Harrison et al 1992; Jewell et al 1995). To reduce toxicity some workers have replaced the hydroxyl group with a halogen such as fluorine (O'Neill et al 1997). Here we report an alternative strategy, the replacement of the hydroxyl group by a bio-isosteric hydrogen. In addition, the aminomethylene side chain of amodiaquine, which is considered essential for antimalarial activity (Werbel et al 1986; Ruscoe et al 1995), has been replaced by a second 7-chloro-4-aminoquinoline ring. The rationale for this is threefold: introduction of symmetry into cycloalkyl-bridged compounds produces bisquinolines in which the 4-amino group corresponding to the aminomethylene group (Ismail et al 1996) is replaced by an aryl or heterocyclic amine, which could prove as effective as an alkylamine in drug-receptor interactions; the compounds do not contain any chiral centre, which facilitates synthesis and purification; and the overall synthetic route consists of a simple, cheap one-pot reaction using readily available materials.

Both (±)-*trans*- and *cis*-N^1,N^2-bis(7-chloroquinolin-4-yl)cyclohexane-1,2-diamine are active against *Plasmodium berghei* in mice (Ismail et al 1996; Ridley et al 1997). Replacing the cycloalkyl

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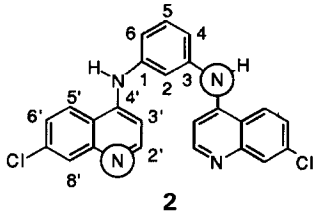
bridging unit with an aromatic ring could provide a lead compound, for example N^1,N^2-bis(7-chloroquinolin-4-yl)phenylene-1,2-diamine **1**, as a potential amodiaquine substitute (Marie 1993). The cyclohexane bridging unit in (±)-*trans*- and -*cis*-N^1,N^2-bis(7-chloroquinolin-4-yl)cyclohexane-1,2-diamine enables these molecules to adopt a number of solution-state conformers. The consequent introduction of aromaticity into the bridging unit should reduce flexibility and produce a more limited conformer set. Finally two theoretically isolipophilic regio-isomers of N^1,N^2-bis(7-chloroquinolin-4-yl)phenylene-1,2-diamine **1** were constructed, N^1,N^3-bis(7-chloroquinolin-4-yl)phenylene-1,3-diamine **2** and N^1,N^4-bis(7-chloroquinolin-4-yl)-phenylene-1,4-diamine **3** (Figure 1). The aryl-bridged bisquinolines reported here are new and have not, to our knowledge, been tested for antimalarial activity in-vivo.

Materials and Methods

General

All chemicals were purchased from Aldrich. Reactions were treated as light- and air-sensitive and special synthetic procedures detailed elsewhere were used throughout (Ismail et al 1996); reactions were performed using vacuum-line techniques under dry argon or nitrogen. *N*-Methylpyrrolidin-2-one, triethylamine, 4,7-dichloroquinoline, 1,2-phenylenediamine, 1,3-phenylenediamine and 1,4-phenylenediamine were purified before use (Perrin & Armarego 1988). All solvents were re-distilled under argon before use. Methanol was purified carefully by distillation from magnesium because reagent-grade methanol can contain measurable quantities of lead salts. Compound **3**, metaquine, was prepared using the method first described by Marie (1993).



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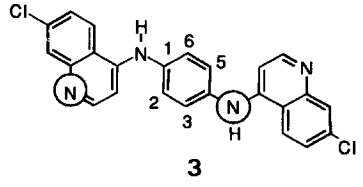

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Figure 1. Structural formulae of compounds **2** and **3** (the structure of compound **1** is given in Figure 2). Pairs of encircled nitrogens indicate the atoms used for calculation of N-N distances in molecular-modelling experiments.

Synthetic methods

Reactions between 4-haloquinolines and amines were conducted in various solvents; acidified aqueous methanol (pH 3·5) proved the cheapest and most convenient. Although heating the starting mixture under reflux for 24–48 h under argon ensured high conversion, the yields have not been optimized. Crude reaction mixtures were purified by leaving the hot reaction mixture to cool, washing with distilled water and then acetone, and vacuum filtration. Solid material was triturated with dry acetone and suction-filtered three times. Compounds were oven-dried (120°C) and then desiccated over KOH under dry argon and protected from light until analysis (Ismail et al 1996).

Synthesis of bisquinolines

Most previously reported syntheses of 4′-substituted quinoline antimalarial heterocycles use phenol or high boiling dipolar aprotic solvents to enhance thermal condensations between 4-haloquinolines and a suitable amine (Tyman et al 1989; Vennerstrom et al 1992; Ismail et al 1996). Our attempts to prepare **1** using this method generated products inconsistent with the postulated structure. Using phenol (bp 182°C) as the solvent, product yields were poor (0–20%) and compounds were of unsuitable quality for pharmacological evaluation. The desired bisquinolines were synthesized in good yields by heating the appropriate phenylenediamine under reflux with 4,7-dichloroquinoline in acidified aqueous methanol (pH 4·7 with 2 M HCl).

1,3- and 1,4-phenylenediamines reacted with 4,7-dichloroquinoline (Figure 3) at lower temperatures (90–110°C) than the bimolecular nucleophilic displacement of 4-haloquinolines with alkylamines (120–210°C; Ismail et al (1996)). To synthesize the *ortho* compound an oil bath heated to 180°C was used. Compounds **1–3** were solid dihydrochlorides which decomposed without melting. NMR spectra of **2** were acquired in $(CD_3)_2SO$, those of **1** and **3** in CF_3CO_2D.

N^1,N^2-Bis(7-chloroquinolin-4-yl)phenylene-1,2-diamine.2HCl (orthoquine; **1**)

4,7-Dichloroquinoline (1·981 g, 10 mmol) and freshly recrystallized 1,2-phenylenediamine (0·541 g, 5 mmol) were heated under reflux in methanol–water (3 : 1, v/v, 40 mL adjusted to pH 4·7 with 2 M HCl) for 48 h to give a cream precipitate; this was washed successively with water (3 × 500 mL) then acetone (3 × 500 mL, Analar). The solid was triturated with and filtered from acetone (3 × 50 mL) before drying (120°C). Yields from four syntheses of **1** were 45%, 50%, 54% and 60%; the product was a white powder (decom-

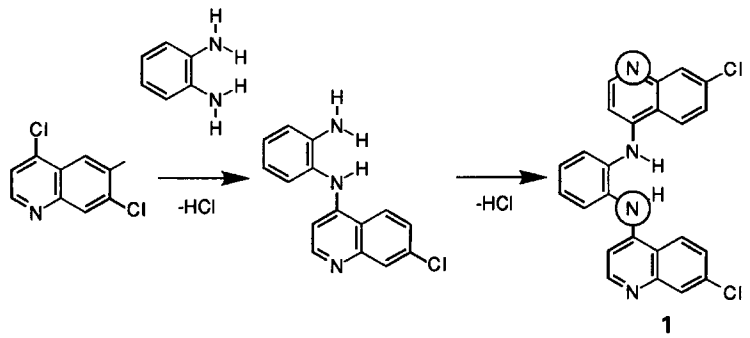

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Figure 2. Reaction scheme illustrating the two-step formation of compound **1**. Pairs of encircled nitrogens indicate the atoms used for calculation of N-N distances in molecular-modelling experiments.

position point 320°C determined by differential scanning calorimetry (DSC)). FTIR (cm^{-1}): 3446 (N–H stretch, quinoline), 3442, 3257 (N–H stretch, secondary amine), 3233 (secondary amine), 2930 (C–H aromatic stretch), 1571 (N–H bend, secondary amine), 1416 (C=C stretch), 859 (C–H bend), 767, 643 (C–Cl stretch). ^{1}H NMR (D_2O-TFA-d): δ ($\pm$0·305 Hz): 6·98 (d, 2H, $J_{2',3'}$(=7·11 Hz, H-3′), 7·76 (dd, 2H, $J_{5',6'}$ = 9·15 Hz, $J_{6',8'}$ = 1·69 Hz, H-6′), 7·85 (4H, singlet: non-first-order A_2X_2 system, H-3, H-4, H-5, H-6), 8·00 (d, 2H, $J_{6',8'}$ = 1·62 Hz, H-8′), 8·37 (d, 2H, $J_{5',6'}$ = 9·14 Hz, H-5′), 8·39 (d, 2H, $J_{2',3'}$ = 7·09 Hz, H-2′); ^{13}C NMR (TFA-d): 102·0 (CH, C-3′), 116·9 (quaternary C), 121·0 (CH, C-8′), 122·9 (quaternary C), 124·7 (CH, C-5′), 130·1 (CH, C-3/6 or C-4/5), 131·1 (CH, C-6′), 132·7 (CH, C-3/6 or C-4/5), 133·7 (quaternary C), 140·1 (quaternary C), 144·0 (quaternary C), 144·3 (CH, C-2′), 158·0 (quaternary C). The electron-impact (EI) mass spectrum of purified compound **1** contained the ions m/z = 430/432 (100%) confirming $C_{24}H_{16}N_4Cl_2$, 395 (12%) loss of HCl, 268 (18%) monomer, 252 (12%) - NH_2 from monomer, 215 (16%), 178 (4%), 163 (9%), 128 (6%)· FAB: 431 (100%) mass ion and parent ion, 523, mass ion + glycerol + H, 269 (35%), 253 (12%), 218 (6%) 397 (10%).

N^1,N^3-*Bis(7-chloroquinolin-4-yl)phenylene-1,3-diamine.2HCl (metaquine;* **2***)*

4,7-Dichloroquinoline (10·23 g, 52 mmol) and freshly sublimed 1,3-phenylenediamine (2·80 g, 26 mmol) were heated under reflux in acidified (pH 4·7) methanol–water (180 : 50, v/v). Yields from two syntheses were 79% and 87%. Purification of **2** was limited to trituration three times with Analar acetone and recovery of the insoluble green product by suction filtration (decomposition at 295–296°C determined by DSC). FTIR (cm^{-1}): 3446 (N–H stretch, quinoline), 3442, 3257 (N–H stretch, secondary amine), 3233 (secondary amine), 2930 (C–H aromatic stretch), 1571 (N–H bend, secondary amine), 1416 (C=C stretch), 859 (C–H bend), 767, 643 (C–Cl stretch). ^{1}H NMR (DMSO-d_6) assuming aromatic bridge is a first-order system: δ 7·09 (d, 2H, $J_{2',3'}$ = 5·59 Hz, H-3′), 7·22 (dd, 2H, $J_{4,5} = J_{5,6}$ = 7·94 Hz, $J_{2,6} = J_{2,4}$ = 1·70, H-4, H-6), 7·44 (d, 1H, $J_{2,6} = J_{2,4}$ = 1·70, H-2), 7·50 (t (apparent overlapping dd), 1H, $J_{4,5} = J_{5,6}$ = 8·01 Hz, H-5), 7·62 (dd, 2H, $J_{5',6'}$ = 9·05 Hz, $J_{6'8'}$ = 2·09 Hz, H-6′), 7·96 (d, 2H, $J_{6',8'}$ = 2·05 Hz, H-8′), 8·55 triplet (i.e. overlapping dd integrating to 4H; d, 2H, $J_{2',3'}$ = 4·84 Hz, H-2′; d, 2H, $J_{5',6'}$ = 8·67 Hz, H-5′); ^{13}C NMR: (DMSO-d_6) 102·3 (CH), 115·7 (CH), 117·8 (CH), 118·2 (quaternary C), 124·6 (CH), 125·2 (CH), 126·7 (CH), 130·3 (CH), 134·3 (quaternary C), 141·05 (quaternary C), 148·2 (quaternary C), 148·4 (quaternary C), 151·5 (CH). The chemical ionization (CI) mass spectrum of **2** contained the ions m/z = 431/433 confirming $C_{24}H_{16}N_4Cl_2$, 432 (64%), 430 (100%), 394 (7%), 252 (19%), 217 (6%), 215 (9%), 197/198 (10%), 43 (10%).

N^1,N^4-*Bis(7-chloroquinolin-4-yl)phenylene-1,4-diamine.2HCl (paraquine;* **3***)*

4,7-Dichloroquinoline (10·24 g, 52 mmol) and freshly recrystallized 1,4-phenylenediamine (2·79 g, 26 mmol) were heated under reflux in acidified (pH 4·7) methanol–water (180 : 50, v/v) for 10 h on a steam bath. Yields from three syntheses of **3** were 8·80 g (79%), 10·03 g (90%) and 10·13 g (91%). Purification of **3** was achieved by repeated trituration with Analar acetone and recovery of the insoluble pale yellow product by suction filtration (mp 295–296°C determined by DSC; thermometer > 330°C). FTIR (cm^{-1}): 3446 (N–H stretch, quinoline), 3257 (N–H stretch, secondary amine), 3233 (secondary amine), 2930 (C–H aromatic stretch), 1571 (N–H bend, secondary amine), 1416 (C=C stretch), 859 (C–H bend), 767, 643. ^{1}H NMR (DMSO-d_6) spectra run at 360 K with digital resolution $\pm$0·984 Hz: δ 6·98 (d, 1H, $J_{2',3'}$ = 5·92 Hz, H-3′), 7·57 (s, 4H, H-2, H-3, H-5, H-6, A_4 system), 7·62 (dd, 2H, $J_{5',6'}$ = 9·05 Hz, $J_{6',8'}$ = 2·09 Hz, H-6′), 8·06 (d, 2H, $J_{6',8'}$ = 1·99 Hz,

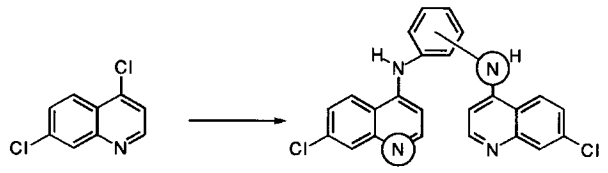

Figure 3. General reaction depicting the synthesis of bis-aryl-4-aminoquinolines; x = 2, 3 or 4 indicates *o*-, *m*- or *p*-substitution, respectively. 1,x-Ph$(NH_2)_2CH_3OH/H_2O$ (3 : 1, v/v, pH 4·7. Pairs of encircled nitrogens indicate the atoms used for calculation of N–N distances in molecular-modelling experiments.

H-8′), 8·55 (d, 2H, $J_{2',3'} = 6{\cdot}69$ Hz, H-2′), 8·66 (d, 2H, $J_{5',6'} = 9{\cdot}19$ Hz, H-5′). ^{1}H NMR: (TFA-D_2O) δ 7·04 (d, 1H, $J_{2',3'} = 7{\cdot}00$ Hz, H-3′), 7·72 (s, 4H, H-2, A_4 system), 7·85 (dd, 1H, $J_{5',6'} = 9{\cdot}06$ Hz, $J_{6',8'} = 1{\cdot}49$ Hz, H-6′), 8·02 (s, 1H, $J_{6',8'} = 1{\cdot}58$ Hz, H-8′), 8·33 (d, 1H, $J_{2',3'} = 6{\cdot}98$ Hz, H-3′), 8·43 (d, 1H, $J_{5',6'} = 9{\cdot}11$ Hz, H-5′). ^{13}C NMR: (DMSO-d_6) 102·3 (CH), 118·2 (quaternary C), 124·6 (CH), 125·2 (CH), 126·7 (CH), 130·3 (CH), 134·3 (quaternary C), 141·05 (quaternary C), 148·2 (quaternary C), 148·4 (quaternary C), 151·5 (CH). CIMS: m/z = 431/433 confirming $C_{24}H_{16}N_4Cl_2$, 432 (63%), 431 (30%), 430 (100%), 394 (5%), 268 (5%), 252 (12%), 215 (5%), 218 (10%).

Screening for antimalarial activity

Plasmodium berghei (N/13/1A/4/203) was maintained by serial passage in MF1 mice and injected intravenously into experimental male mice, 18–25 g, at a dose of 2×10^7 parasitized erythrocytes per animal. Control mice were injected with 0·2 mL normal mouse blood diluted to the same extent with 0·85% saline. In a blind study putative antimalarials were injected subcutaneously into 5–6 mice using sterile, peroxide-free olive oil and dimethylsulphoxide as vehicle (24 : 1, dose volume 10 mL kg^{-1}) unless indicated otherwise. Some compounds required the addition of Tween 80 (2 drops/10 mL) to facilitate carriage of the chemical, but all compounds tested had a concurrent control malaria group receiving the appropriate vehicle. Mice were treated on the day of inoculation, approximately 3 h after infection then twice daily for the next two days at 2–5 dose levels (range 1–33 mg kg^{-1}). Three days after inoculation body weights and colonic temperatures were measured and blood smears taken for the determination of parasitaemia, which was assessed as the percentage of erythrocytes containing Leishman-positive bodies. The antimalarial activity of compounds was assessed as the reduction in parasitaemia compared with that in concurrent vehicle-treated mice (Student's *t*-test). Relative potencies were calculated as the dose (mg kg^{-1}, s.c.), which would, in this three day suppression test, inhibit blood parasite counts to half of those in vehicle-treated mice (ID50). Visual observations on the general autonomic and behavioural states of the mice were made throughout the experimental period.

Molecular modelling

Calculations were performed on a Silicon Graphics Indy workstation. Energy calculations were performed using the AM1 Hamiltonian of MOPAC 6·0 (Stewart 1990) in addition to a variety of force fields: COSMIC of Oxford Molecular (1993), MM2*, amber* and opls* of MacroModel (1993) detailed by Mohamadi et al (1990) and CVFF of Discover 2·9 (Biosym 1993). Conformational searches were performed by step-wise variation of torsion angles using Search-Compare 2·03 (Biosym 1993) and also by Monte Carlo methods (MacroModel 1993). Electrostatic potential maps were calculated in-vacuo from partial charges generated by the Charge-2 method (Oxford Molecular 1993) and in aqua from MNDO-derived charges using Delphi 2·4 (Biosym 1993). Crystal-structure distances, including those for chloroquine (clquon01, Furuseth et al (1990)) and amodiaquine (votfit, Yennawar & Viswamitra (1991)), were obtained from the EPSRC Chemical Structure Data Bank at Daresbury (Fletcher et al 1996). This information was then used for rational design of compounds **1–3** using accepted strategies that combine both crystallography and molecular mechanics for designing drugs against uncharacterized receptors (Duchamp 1990; Holtje 1996).

Results

Synthesis of bisquinolines

The ideal solvent for the reactants used here should be one which acts as the catalyst for the two sequential SN_{ar} substitution reactions depicted in Figure 2, and be sufficiently volatile to facilitate purification of products. *N*-Methylpyrrolidin-2-one accelerates the formation of 4-alkyl-substituted aminoquinolines and has recently been used to synthesize alkyl-bridged dimers. However, in our hands this method resulted in variable yields (12–55%) of **1**, which was isolated absolutely pure only after three successive fractional crystallizations from several litres of acetone (Marie 1993). Mass spectrometric analysis of the crude mixture (mp 266–280°C) confirmed that the product was contaminated with N^1-(7-chloroquinolin-4-yl)phenylene-1,2-diamine (Figure 2).

CH_3OH–water–hydrochloric acid (3 : 1 : trace, pH 4·7) was successfully used as solvent for the synthesis of aryl-bridged bisquinolines. Reaction side-products, in addition to the corresponding monomers, seemed to be oxidation products of the

phenylenediamines. Exclusion of oxygen reduced the yields of these high molecular weight side-products, which were readily separated from the target compounds by washing with excess acetone. Accurate determination of the melting point transition of bisquinolines by visual observation proved troublesome as these compounds char or decompose. Investigation of **1** by differential scanning calorimetry (DSC) indicated the onset of the phase transition to charring at 299°C for crude material and 316°C for acetone triturated material.

Reaction mechanisms

The reaction mechanism involved in the formation of compounds **1–3** probably involves two sequential SN_{ar} substitutions (Figure 3). In aqueous acidic methanol the reaction of 4,7-dichloroquinoline with 1,2-phenylenediamine initially (2–7 h) produces the monomer as the major component and small quantities (10–15%) of the dimers (Figure 3). In solution, solvation or steric hindrance (or both) of the monomer hinders approach of the second 4,7-dichloroquinoline molecule, thereby reducing the probability of dimer formation. The unreacted primary amino group of the monomer decreases in nucleophilicity (compared with *o*-phenylenediamine), because it is protonated under these conditions and is also attached to the aryl quinoline ring, consequently the amino group is electronically deactivated towards substitution reactions. As the temperature of the mixture increases, solvents are driven off producing a semi-solid mass which evolves HCl; as a consequence these conditions favour the formation of aryl-bridged dimeric quinolines isolated as hydrochloride salts. The approach of the second quinoline during the formation of bisquinolines **2** and **3** is probably more favourable because of reduced steric hindrance. The increase in yield might be attributable to the phenomenon of acidity jump, i.e. when a strong acid is introduced into a weakly acidic solvent the overall Hammett acidity parameter (H_o) increases, partly because of the reaction generating HCl.

Mass spectrometry and NMR studies

Mass spectrometry of compounds **1–3** proved useful in assessing drug purity. Despite the decomposition points of the compounds, clear evidence for the suggested molecular formulae (strong $M–H^+$ ions) were obtained for all compounds. Solubility in common deuterated NMR solvents (D_3COD, DMSO-d_6, $CDCl_3$) was higher for **1** and **3** than for **2**. Although the solubilities of **1** and **2** were higher in deuterated trifluoroacetic acid (TFA) than in DMSO-d_6, the smaller (*meta*) coupling constant information was lost because of signal broadening. Despite doubling of the number of scans to 96 the ^1H spectra remained broad. Although addition of D_2O to the solution reduced the solubility of **3**, the smaller coupling constants ($J_{5,8}$) within the quinoline ring were calculated by extending the number of scans to 1000. Compounds precipitated from TFA within 48 h, which complicated the automated acquisition of 2-D NMR data. Although spectra of **3** were obtained in hot DMSO-d_6 in one experiment (360 K, 64 scans), the solubility of **3** was insufficient to enable acquisition of 2D HETCOR or COSY information.

Correlation of biological action with the physicochemical properties of the antimalarials

The antimalarial activity of compound **1** (ID50 30 mg kg^{-1}) compared with chloroquine diphosphate (ID50 4·3 mg kg^{-1}) confirms our prediction that bisquinolines linked by an aromatic diamine bridge have antimalarial activity. The isolipophilic regioisomer **3** was not statistically schizonticidal ($P > 0·05$) at either of the two doses (5 and 25 mg kg^{-1}) tested in oil–DMSO (control parasitaemia, 65 ± s.e.m. 10%; 5 mg kg^{-1}, 56 ± 13%; 25 mg kg^{-1}, 43 ± 12%; estimated ID50 ≈ 60 mg kg^{-1}). More marked antimalarial activity for **3** (ID50 17 mg kg^{-1}) was observed after administration in 0·9% NaCl–DMSO (24 : 1, v/v). In contrast with the relatively poor antimalarial activity of this *p*-substituted phenylenediamine, the *meta*-substituted compound **2** was more active. Tested at doses of 1, 2·5, 5 and 25 mg kg^{-1} in saline–DMSO (24 : 1, v/v), this compound had significant schizonticidal activity ($P < 0·05$) at all doses tested above 1 mg kg^{-1} (compared with vehicle-treated controls). When tested in oil-based vehicles the ID50 for **2** was 1·2 mg kg^{-1}, in 0·9% NaCl-based vehicles the ID50 was 1·7 mg kg^{-1}. No adverse effects on behaviour, weight or body temperature were evident after administration of **1–3** at dose levels effective in reducing parasitaemia.

Modelling investigations

Crystal structures of various 4-aminoquinolines indicate that the amino nitrogen atom is sp^2 hybridized, with a short C–N bond length consistent with π-conjugation of the amine nitrogen with the lone pair in the quinoline ring (Figure 4). In chloroquine the amino group and quinoline ring are nearly coplanar, indicating near maximum conjugation (compare with Furuseth et al (1990)). Our analysis of published crystal-structure data shows that if the alkyl side chain in chloroquine is replaced by a substituted benzene ring there is competition between the quinoline and phenyl rings for the lone-pair on the amino nitrogen atom, as

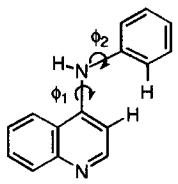

Figure 4. Generalized aryl-bridged quinoline structure.

observed in amodiaquine (Yennawar & Viswamitra 1991). Our modelling studies are in accordance with theirs and show that steric hindrance between the *ortho* positions causes twisting of the two rings out of the idealized molecular plane. In the crystal structures of such compounds the amounts of twist, ϕ_1 and ϕ_2, are different for the two rings, with the amino group tending towards near coplanarity with the quinoline ring rather than the phenyl ring. There is also correlation between the magnitudes of twist and the associated C–N bond-lengths. In molecules with the 4-amino group more nearly coplanar with the quinoline ring there is, for example, correlation with a short N–C^4 bond length and a correspondingly longer N–C^1 bond length together with a large amount of twist for ϕ_2. Such observations imply coupling between these two torsional barriers to rotation, as the extent of conjugation of the nitrogen atom with one ring is affected by the torsional angle of the other. Many normal force fields fail to reproduce this effect and tend to predict geometries in which both twist angles are approximately equal. Accordingly using a method of distance-searching independent of force-field calculations, the angles of twist ϕ_1 and ϕ_2 for each ring were varied systematically in steps of 30° and the distances between nitrogen atoms, a potentially important feature of antimalarial bisquinolines, were measured (Figure 2). Conformers with unduly close inter-atom contacts were removed from the data set. The resulting ranges of inter-atom distances were **1**, 4·7–7·2 Å; **2**, 7·2–9·2 Å and **3**, 9·0–9·4 Å and represent outer limits as no energy screening was performed. These ranges are not markedly affected by protonation because little extra steric hindrance is incurred if addition of protons occurs on the quinolinyl nitrogens at the periphery of the molecule (i.e. position 1′). Although these ranges are outer limits (as they take no account of the relative energies of conformations), they should be valid for a wide variety of situations.

Molecular electrostatic potential maps (not shown) of the antimalarials (as free bases) showed regions of high positive potential on hydrogens attached to electronegative elements such as nitrogen and oxygen. Somewhat surprisingly, the distribution of potential in the aryl bridging unit in bisquinolines **1–3** was found to be asymmetric. The hydrogen atoms numbered 2 in **2**, and 2 and 3 in **3** correspond to regions of high positive potential compared with the near neutral regions on the opposite side of the bridge. In addition, the calculated potentials varied from one face of the quinoline ring to the other, consistent with the potential on the obverse face of the quinoline ring being more negative.

Discussion

Bisquinolines, particularly aryl-bridged bisquinolines, are a relatively new class of antimalarial compound. We have previously established that in these compounds at least two nitrogen atoms in the linking unit or bridgehead (at the 4-position of the quinoline) are essential for in-vivo activity (Ismail et al 1996). Because the drugs most structurally similar to our novel, designed, compounds **1–3** are amodiaquine, amopyroquine and, perhaps, tebuquine, comparisons with these drugs seem justified. Although many interdependent physicochemical parameters influence the structure–activity profile, O'Neill et al (1997) state that inter-nitrogen distances cannot be correlated with antimalarial activity. But as classical Hansch-type analysis has not been performed (Kubinyi 1997), it is uncertain whether a correlation does exist between N–N distances and antimalarial activity. Results presented here show that the aliphatic side chain methylene amine in compounds such as amodiaquine can be successfully replaced by a quinoline ring without loss of antimalarial activity. Previously, the methylene amine has been considered an essential component of amodiaquine-like drugs. This observation indicates that lipophilic bridgehead substituents (especially $+\pi$, $-\sigma$ substituents) can replace alkyl bridges if the 4-aminoquinolinyl rings are separated by a three-carbon bridge, and argues against the proposal that C-4 substitution of two amine functions separated by a four-carbon bridge is necessary for activity (Werbel et al 1986; Ruscoe et al 1995). We believe that the charge on the aliphatic side chain nitrogen atom of amodiaquine and tebuquine can be emulated by that present on the 4-aminoquinoline ring. This additional site of charge might be involved in specific binding to critical amino acids (for example histidine) as well as π–π stack interactions, binding to our putative bisquinoline receptor, weak interactions within the minor groove of parasite DNA (Pilch et al 1997; Squire et al 1997), inhibition of polyamine transport (Singh et al 1997) or modulation of the haeme polymerization–haemazoin depolymerization process (Sullivan et al 1996).

Transport, metabolism and fate of aryl-bridged bisquinolines

Results indicate that bioavailability is vehicle-dependant, but more detailed studies of **1–3** are required. Assessment and interpretation of antimalarial activity in-vivo might be moderated by several processes unrelated to the bisquinoline-receptor binding site, for example drug absorption, distribution and metabolism. The enhanced efficacy of amopyroquine has been attributed to metabolic stability in-vivo (Pussard & Verdier 1994) compared with the pro-drug amodiaquine. Biliary excretion appears to be the main pathway for eliminating these drugs and this might promote their hepatotoxicity. Because compounds **1–3**, unlike amodiaquine and amopyroquine, lack an aminomethylene side chain that is extensively metabolized, the most likely metabolites of these novel compounds might result from *N*-oxidation or hydroxylation, or both, of the aromatic ring (Wermuth & Testa 1996). By analogy with 4′-dehydroxy-4′-fluoroamodiaquine, biliary excretion of **1–3** after adduct formation with intracellular glutathione seems unlikely (Harrison et al 1992); this might reduce potential hepatotoxicity. In compounds **1–3** replacement of the OH group by H (rather than F) was a rational decision, because hydrogen and fluorine are bio-isosteric and both can be considered group-7 elements. Because using fluorinated precursors increases the cost of production, our bio-isosteric modification is a cost-effective means of reducing the likelihood of iminoquinone formation.

Laboratory screening of novel compounds for antimalarial activity employs models of malaria in-vitro or in-vivo. In this study we used a well documented and widely used in-vivo model, *P. berghei* in mice (see Albert (1966) and Cox (1988) for reviews). This choice was determined by several factors, including: the comparatively small number of compounds, products of a rational drug-design exercise, to be evaluated; the low solubility of the compounds, which precludes the formation of solutions for use in-vitro but which can be negated by the use of suspensions in-vivo; and the opportunity to assess drug bioavailability and drug toxicity in-vivo in a mammalian host.

In addition, there are considerations of a more general nature, including the apparent differences in chloroquine sensitivity (Geary et al 1990) and enzymic antioxidant defence systems (Slomianny et al 1996) between malaria parasites in-vitro and in-vivo.

Correlation of modelling investigations with antimalarial activity

Bhattacharjee & Karl (1996) in a study of 4-quinolincarbinolamines noted that the electrostatic potential in certain regions close to the quinolinyl ring tended to be more positive for active compounds and negative for the non-curative or less active antimalarials. Our results agree with their observation in that **1–3**, as well as (±)-*trans* and *cis*-N^1,N^2-bis(7-chloroquinolin-4-yl)cyclohexane-1,2-diamine (Ismail et al 1996), have areas of positive potential within the quinoline ring.

Compounds **1–3** form a discrete set of almost non-overlapping ranges of inter-nitrogen distances (Figure 2). Maximum antimalarial activity was observed for **2**, a 3-carbon-bridged compound with inter-nitrogen distances ranging from 7·2 to 9·2 Å. This result is consistent with values for amodiaquine (6·6–10·2 Å) and amopyroquine (8·2–10·4 Å). However, there is no single range of inter-nitrogen distances common to all active quinoline antimalarials (Ismail et al 1996). In general, the steric requirements of flexible aliphatic side chains in antimalarial drugs seem to be less exacting, perhaps because of the large number of conformers available for binding to the target receptor. In contrast, relatively rigid antimalarial compounds (for example **1**), have more exacting requirements, because the aryl-bridged compounds (for example **3**) have much higher antimalarial activity. Receptor interactions of **1–3** might involve the second quinoline ring at a low-affinity binding site. Two or more possible binding sites for the terminal nitrogen atoms in known antimalarials might explain the variation observed in antimalarial activity (Vennerstrom et al 1992), because non-covalent bonding to adjacent residues within a putative binding site is not uncommon. However, it is important to construct and evaluate a larger set of closely related compounds to eliminate chance correlations and rigorously analyse the data using Hansch-type analysis before definite conclusions are made regarding the importance of N–N distances in clinically active antimalarials.

Importance of side chains and bridging elements in antimalarials

It has been argued that the function of the nitrogen in the side chain is to facilitate the accumulation of the antimalarial drug in the parasite (Albert 1966, 1985), perhaps at a receptor site containing a variety of anchorage points for this hetero atom. If the function of the side chain nitrogen is to act as a base then its exact location is less important than its capacity to attract a proton or to hydrogen-bond with one of several adjacent binding sites within the putative receptor. Current evidence indicates that a common receptor site would have to have some flexibility towards the nitrogen–nitrogen distances within antimalarial bisquinolines. A

similar observation has been made on the antimalarial activity of a homologous series of acridines against *Plasmodium lophurae* (Albert 1966).

O'Neill et al (1997) have shown that certain correlations can be obtained by plotting cell accumulation concentrations of various antimalarial compounds against activity in-vitro. Such univariant analyses imply either that drug accumulation in the cell is governed solely by binding to the receptor or that the chloroquinolinyl ring constitutes the pharmacophore, the side chain being responsible for transport. This observation is not unexpected as most antimalarial 4-aminoquinolines contain the highly conserved 4,7-dichloroquinolinyl group. Nevertheless, the nature of the side chain appears critically important for selective introduction of 4-alkylaminoquinolines into *Plasmodia* which become resistant to chemotherapy by reducing drug accumulation (Ward et al 1997). The nature of the side chain is critical when restoring activity against drug-resistant *Plasmodia* to alkyl-substituted 4-aminoquinolines (De et al 1996); this is in accord with the observations of Ridley et al (1996) on truncated analogues of chloroquine. One potential risk of altering aliphatic chain-lengths is the increased toxicity of the compounds to the vertebrate host. This consideration is prompted by the observations that led to the development of chloroquine as an antimalarial with the most acceptable therapeutic index (Albert 1966), although at present there are insufficient data to generalize on aromatic bisquinolines such as **3**.

Homology and differences between quinoline, bisquinoline and arylbisquinoline binding sites

The features of the ferriprotoporphyrin IX receptor, currently the most acceptable model of an antimalarial drug receptor, consists of a 30–40 Å planar ring system with an anionic site capable of binding a terminal nitrogen atom located in the alkyl side chain (Chou et al 1980). Chloroquine is currently thought by some workers to exert its antimalarial effect by preventing the polymerization of toxic haeme released during proteolysis of haemoglobin in the *Plasmodium* digestive vacuole (Sullivan et al 1996; Ridley et al 1997), although this theory has been criticized by Meshnick (1996). The same mode of action is suggested for (±)-*trans*-N^1, N^2-bis(7-chloroquinolyl)cyclohexyl-1,2-diamine in-vitro, implicating protoporphyrin IX as the receptor for this cycloalkyl bridged bisquinoline (Ridley et al 1997). However, the evidence that this mechanism is the only one for aryl-substituted quinolines, for example amodiaquine, is less well established. Although Warhurst (1987) has suggested that amodiaquine does not interact with ferriprotoporphyrin IX, which might suggest a different receptor than chloroquine, subsequent Mossbauer studies contradict this view (Blauer et al 1993). Subsequent studies on chloroquine-urohaemin(I) chloride complexes also indicate that Fe^{3+} species present in this complex do not co-ordinate to the quinoline ring nitrogen and, by analogy, cannot do so in ferriprotoporphyrin IX-chloroquine complexes (Constantinidis & Saterlee 1988). We have no evidence yet for the activity of aryl-bridged bisquinolines in this model of 4-aminoquinoline action.

Ginsburg & Krugliak (1992) and more recently Ridley et al (1997) have reviewed the possible modes of action of 4-aminoquinoline antimalarial drugs. At present the DNA intercalation theory seems discredited, although selective accumulation by *Plasmodia* might explain why parasite DNA could be preferentially affected (Krogstad et al 1992; Slater 1993; Martiney et al 1995). In addition, recent NMR evidence indicates that melting-point assays of DNA (used as evidence for determining intercalation) can provide misleading information in comparison with stronger NMR evidence. This evidence might invalidate objections against DNA being a putative receptor for 4-aminoquinoline-type antimalarials (Sartorius & Schneider 1997). The DNA processing enzyme topoisomerase 1 derived from *Trypanosoma cruzi* is inhibited by chloroquine (Douc-Rasy et al 1984), which can also enhance camptothecin-induced cytotoxicity and cleavable complex formation of mammalian topoisomerase 1 (Sorensen et al 1997). Such studies have been extended to *Plasmodia*, in which chloroquine induces transcription of the helicase-family genes (Thelu et al 1994). Further evidence for a DNA receptor site is provided by Gamage et al (1994), who have demonstrated that some *Plasmodial* topoisomersases are strongly inhibited by the acridine antimalarial pyronaridine.

In conclusion, we have designed and synthesized three novel bisquinolines, N^1,N^2-bis(7-chloroquinolin-4-yl)phenylene-1,2-diamine **1**, N^1,N^3-bis-(7-chloroquinolin-4-yl)phenylene-1,3-diamine **2** and N^1,N^4-bis(7-chloroquinolin-4-yl)phenylene-1,4-diamine **3**, which conform to suggested guidelines for effective receptor mapping (Duchamp 1990; Wermuth & Testa 1996). When tested against chloroquine-sensitive *P. berghei* in mice, **2** was found to be a potent antimalarial apparently well tolerated in-vivo. This novel bisquinoline, the result of rational drug design based on a putative antimalarial receptor model (Ismail et al 1996), was both inexpensive and simple to synthesize. The efficacy of **2** and related bisquinoline antimalarials

against drug-resistant malaria in man now needs to be determined to explore further their clinical potential.

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