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An Exploration of the Structure-activity Relationships of 4-Aminoquinolines: Novel Antimalarials with Activity In-vivo

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Abstract

The structure-activity relationships of bisquinolines, a potentially important group of novel antimalarial drugs, were studied.

The high-temperature (180–250°C) synthesis of 4-aminoquinolines, including bisquinolines, by nucleophilic displacement was both fast and efficient. Several bisquinolines including (±)-*trans*-N^1,N^2-bis(7-trifluoroquinolin-4-yl)cyclohexane-1,2-diamine and 1*R*,2*R*-(−)-, 1*S*,2*S*-(+)-, (±)-*trans*- and *cis*-N^1,N^2-bis(7-chloroquinolin-4-yl)cyclohexane-1,2-diamine exhibited potent activity against *Plasmodium berghei* in mice; (±)-trans-N^1,N^2-bis(7-chloroquinolin-4-yl)cyclohexane-1,2-diamine was orally active.

Our results indicate that these compounds conform to a putative receptor for quinoline antimalarials. In addition, a 7-haloquinoline linked by a heterocyclic bridge, at the 4-position, to another heterocycle (such as an acridine at the 9-position) maximally occupies the active site of our postulated target.

To facilitate the discovery of more effective antimalarials, the molecular features associated with antimalarial activity in-vivo have been collated, especially those involving quantitative structure-activity relationships. Correlation of published data relating to the in-vivo antimalarial activity of numerous chloroquine and mepacrine analogues with the spatial disposition of their individual atoms prompts several observations.

Certain dimeric quinoline structures have activity against drug-resistant strains of *Plasmodia*. Quinolines with potencies greater than chloroquine include a variety of bis(quinolyl)piperazines (Benazet 1965; Warhurst 1966; Peters 1970; LeBras et al 1983; Zhang et al 1987).

Antimalarials effective against drug-resistant *Plasmodia* tend to be insoluble in most common organic solvents and possess high melting points. The partition coefficients (expressed as log P values) of such compounds are ≥ 3 and lie within an optimum lipophilicity range (Hansch 1977).

Quinolines effective as antimalarials normally contain two or more nitrogen atoms capable of ionization in the range of physiological pH. Quaternization of the ring nitrogen can influence antimalarial activity. Mepacrine-10-methochloride has about one fifteenth the activity of mepacrine, whereas the *N*-oxide is as active as the parent compound (Mauss et al 1937).

A methyl substituent on the 1-position of the alkyl tail of the quinoline may reduce toxicity and increases the therapeutic index (Peters 1970).

Deductions from previous studies (Coatney et al 1953) indicated that several quinolines linked by a heterocyclic bridge at the 4-position to another heterocycle (especially an acridine at the 9-position) has antimalarial activity in-vivo against normal and drug-resistant forms of *Plasmodia*. Dimeric quinolines, unlike their monomeric counterparts, constitute a small but growing class which have a range of pharmacological actions. Schock (1957), for instance, synthesized a series of bisquinolines that have in-vivo activity against murine *Trypanosoma gambiense*, but these compounds have not, to our knowledge, been tested for antimalarial activity (Walls 1951).

Concurrently, the synthesis of a series of bisquinolines, each bridged by a homologous series of diamines, N^1,N^ω-bis (7-chloroquinolin-4-yl)-alkyl-1, ω-diamines (Fig. 1) purported to be amoebocides was reported (Pearson et al 1946; Jacob et al 1958). More recently, Vennerstrom et al (1992) have reported both the resynthesis and antimalarial activity of the aforementioned bisquinolines and others, most of which are bridged by a homologous series of diamines containing between two and fourteen methylene groups.

We have designed, constructed and tested a number of new 4-aminoquinolines which explore some of the aforementioned design criteria, including bisquinolines similar to (±)-N^1,N^2-bis(7-chloroquinolin-4-yl)cyclohexane-1,2-diamine, compound **1** (Table 1, see also Vennerstrom et al (1992)), with potential antimalarial activity. By determining the ability of molecular modifications (steric, electronic or lipophilic) to

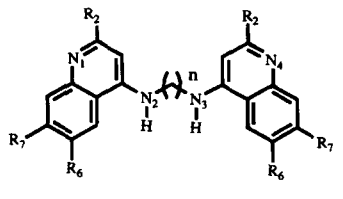

FIG. 1. Generalized structure of bisquinolines. R_2, R_6 and R_7 are H, Cl, or CH_2, or a combination of these, and n = 3–10.

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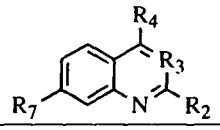
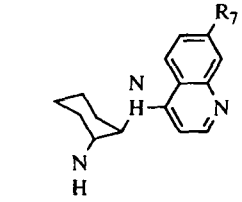
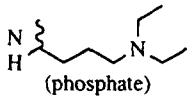
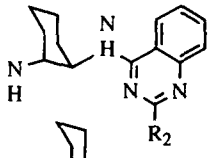
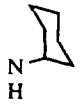
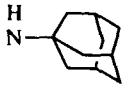
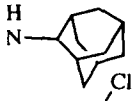
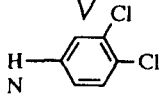
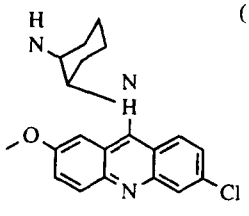
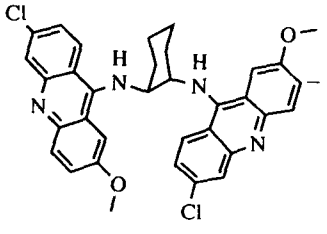
Table 1. The 4-aminoquinolines investigated.



No.	R_2	R_3	R_7	R_4	Stereochemistry	mp (°C)	Yield (%)	ID50‡	P.M.
1	H	CH	Cl		(±) *trans*	320	86	3·0	B
2	H	CH	Cl		*cis*	140	55	< 10	A
3	H	CH	Cl		*RR*	228	77	< 2·5	C
4	H	CH	Cl		*SS*	292	55	< 2·5	C
5	H	CH	CF_3		(±) *trans*	330	84	5·9	A
6	H	CH	Cl	 (phosphate)	(±)	–	–	4·3	–
7	Ph	N	H		(±) *trans*	274	57	> 50 (86)	B
8	H	CH	Cl		n/a†	82	63	> 50 (140)	A
9	H	CH	Cl		n/a	331	85	Inactive	A/B
10	H	CH	Cl		n/a	258	82	Inactive	A
11	H	CH	Cl		n/a	258	67	Inactive	A/B
12	H	CH	Cl		(±) *trans*	> 390	17	13	A/B
13					(±) *trans*	> 380	49	27	A/B

†n/a = not applicable. Yields have not been optimized. ‡ID50 values are the doses of compounds (mg kg^{-1}, s.c.) which in the three-day-suppression test inhibited *P. berghei* parasitaemia in mice by 50%; < and > indicate lowest and highest doses tested, respectively; values in parentheses are estimated ED50 values. inactive = not active in doses up to 50 mg kg^{-1}. P.M. = purification method; compounds were purified to constant mp.

increase or reduce the antimalarial activity of these compounds, the immediate microenvironment of the putative quinoline drug receptor has been postulated.

Materials and Methods

General

Glassware was cleaned with detergent, then by repeated rinsing with ordinary, then once-distilled water. All Pyrex glassware was cleaned further by baking at its annealing temperature, eliminating adsorbed organic residues. All reactions were treated as both moisture- and air-sensitive and the procedures outlined by Shriver & Drezdzon (1986) were used throughout. All materials used were of the highest purity commercially available (Lancaster Synthesis) Substances were repurified before use, using suitable procedures (Perrin & Armarego (1988)) or the original literature as appropriate, except chloroquine phosphate (Sigma Chemical Co.) which was used as received.

N-Methylpyrrolidin-2-one was dried with barium oxide then by addition and removal by distillation of one third its volume of anhydrous benzene using a Dean–Stark trap. The residue was then distilled and the fraction boiling between 204 and 206°C was collected (anhydrous *N*-methylpyrrolidin-2-one, bp 81–82°C, 10 mmHg). Triethylamine was purified according to the procedure described by Perrin & Armarego (1988).

Synthetic methods

All compounds were prepared by a modification of the method of Vennerstrom et al (1992). The condensation reactions between the 4-haloquinolines and amines were conducted in various anhydrous solvents, especially *N*-methylpyrrolidin-2-one. Although heating the mixture of starting materials under reflux for 1–3 days under argon ensured high conversion to the required products, quoted yields have not been optimized (Fig. 2). Crude reaction mixtures were processed using one of the following procedures.

Method A. The hot reaction mixture was left to cool in a Dewar flask for several days. Filtration of the mixture gave clear crystals (triethylamine hydrochloride) which were further washed with anhydrous *N*-methylpyrrolidin-2-one. The filtrate was then combined and concentrated under reduced pressure, poured on to ice and filtered or centrifuged. Unreacted starting materials in the resulting powder were removed by successive extraction with boiling ethyl acetate (or acetone) and distilled water (10 × 100 mL).

Method B. The crude reaction mixture was poured on to ice and triturated with ethyl acetate. The fine precipitate was collected by filtration and washed with distilled water (1 L). The solid was then cautiously dissolved in 2 M acetic acid, with cooling (liquid nitrogen) and the resulting solution was filtered. This was transferred to a separating funnel and extracted with ethyl acetate. The aqueous solution was tapped off, cooled and made alkaline (pH 12) with 2 M aqueous ammonia. In some cases, repeated purification was used to eliminate high-molecular-weight impurities. Failure to use dilute solutions of acetic acid and ammonia or letting the temperature rise during the purification process, caused decomposition of the 4-aminoquinolines into 4-hydroxyquinolines (mass spectral evidence). Quinolines containing trifluoromethyl groups on the 2 and 8 positions were especially susceptible to decomposition as were all compounds with a *cis*-diaminocycloalkyl bridging unit.

Method C. The crude reaction mixture was triturated with analytical grade acetone using a mortar and pestle. The resulting suspension was filtered and the solid was then cautiously dissolved in 2 M acetic acid, with cooling (liquid nitrogen), filtered and extracted with ethyl acetate. The aqueous solution was tapped off, cooled and made alkaline (pH 12) with 2 M aqueous ammonia. The procedure was repeated until the melting point of the compound was constant.

Compounds purified by the above methods were dried under vacuum before chromatography and biological testing. The yields quoted are averages of five experiments in the case of compound **1** but results from single experiments in other cases after final purification (Table 1).

Analysis of compounds

All substances were further purified by flash column chromatography before biological testing; separation was performed under pressurized argon (5 psi) on silica gel (Merck 60, 230–400 mesh) or alumina (Laporte Industries UG1, 100 mesh). Vacuum dry-column chromatography was performed in polythene tubing either on deactivated silica gel (grade 3) or basic alumina. Thin-layer chromatography (TLC) was performed on pre-coated silica gel 60 plates in tanks saturated with the vapour of the indicated solvent. Substances were detected by spraying with a 5% solution of phosphomolybdic acid in ethanol, observation at 254 nm under UV light, functional group tests, 5% sulphuric acid, 5% aqueous potassium permanganate, iodine vapour or by charring with a hot air gun, as appropriate. Most 4-aminoquinoline compounds are sensitive to the phosphomolybdic acid spray and develop intensely coloured blue spots.

Spectral and HPLC analyses were conducted using the facilities of the Chemistry Department, University of Hertfordshire. NMR was performed with a Bruker 250 MHz FT Aspect 2000 or a Varian VXR400; tetramethylsilane (TMS) was used as internal standard (chemical shifts in δ and coupling constants in Hz) and unless specified otherwise the solvent used was $CDCl_3$ or dimethylsulphoxide-d_6 $(CD_3)_2SO$. Substances such as the bis-(acridinyl-4-yl)ethane-1,2-diamines were sparingly soluble, even in CD_3SO. In such cases the ^{13}C NMR spectra were poorly resolved. IR spectra were acquired with a Mattson Galaxy series FTIR 5000; 1% compound-KBr mixture was compressed into discs under vacuum. Oils were dissolved in $CHCl_3$ and evaporated as a thin film on KBr discs. Mass spectrometry was performed with a VG 7070H with VG 2050 data system and heated probe. Spectra were recorded with an electron beam energy of 70 eV and a trap current of 200 μA. The ion source temperature was 200–240°C and the probe was operated at ambient temperature. Reversed-phase HPLC was performed with a Perkin–Elmer series 10 chromatograph (C_{18} column). The mobile phase (isocratic) was CH_3CN-THF-H_2O, 55 : 40 : 5 (v/v). UV was performed with a Philips PU8720 UV/VIS scanning spectrophotometer. A Gallenkamp capillary melting point apparatus was used to measure the melting points (mp) of each compound. The mp of compounds which sublimed were investigated in sealed tubes.

Although reported mp are uncorrected, verification was sought by differential scanning calorimetry (Perkin–Elmer DSC4).

Synthesis of bisquinolines

(±)-trans-N^1,N^2-bis*(7-Chloroquinolin-4-yl)cyclohexane-1,2-diamine,* **1**

4,7-Dichloroquinoline (3·962 g, 0·02 mol), the 1,2-diamine (1·142 g, 0·01 mol) and triethylamine (2·024 g, 0·02 mol) were heated under reflux in *N*-methylpyrrolidin-2-one (25 mL) for 3 days to give a dark brown liquid that was worked up using method B. Yields from three separate syntheses of **1** were 60%, 78% and 84%. The cream-coloured powder obtained after purification was recrystallized from aqueous ethanol to give greenish crystals (mp 331·4°C, determined by DSC; lit. 330–333°C, melting point apparatus, Vennerstrom et al (1992)). 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), 1083 (C-Cl stretch), 859 (C-H bend), 767, 643 (C-Cl stretch) 3096, 2938, 2766, 1631, 1584, 1550. ^{1}H NMR (Fig. 2a) (DMSO-d_6) δ (ppm): 1·34–1·69 (4H, m); 1·7–1·91 (2H, m); 2·0–2·2 (2H, m); 3·75–3·95 (2H, m); 6·74 (2H, d, $J_{2,3}=5·7$ Hz); 6·88–7·03 (2H, 16 Hz, NH); 7·31 (dd, $J_{5,6}=8·9$ Hz, $J_{6,8}=2·1$ Hz (the intensities are 1 : 3 : 3 : 1, ABX pattern, type I according to the classification used by Haigh et al (1965)); 7·58–7·68 (d, $J_{6,8}=2·1$ Hz); 8·02–8·18 (d, $J_{5,6}=8·9$ Hz); 8·22–8·35 (2H, d, $J_{2,3}=5·7$ Hz). The absolute positions of the quinoline ring protons are concentration-dependent and, therefore, differ from those reported by Vennerstrom et al (1992). The mass spectrum indicates that both compound **1** (Fig. 3a) with a relative molecular mass (RMM) of 440 atomic mass units, and the monoquinoline compound (Fig. 3b), RMM 275, are present in the crude mixture. Upon purification only the bisquinoline was detected. The purified compound shows molecular ions 436 and 438 and a base ion at 205. Accurate MS m/z: 437·13 confirming $C_{24}H_{22}N_4Cl_2$.

Attempts to synthesize the compound shown in Fig. 2b resulted solely in the formation of **1**, which was confirmed both by NMR and TLC analysis. Attempted purification of the monoquinoline (Fig. 3b) by dissolution in 2 M acetic acid and subsequent reprecipitation with 2 M ammonia, caused it to decompose to 4-hydroxy-7-chloroquinoline (MS). Similarly, the *1R,2R*-(−)-, *1S,2S*-(+)- compounds had identical spectra to that of **1** whereas the *cis* isomer differed significantly by the appearance of three broad singlets between 1 and 3 (ppm) (see below).

cis-N^1,N^2-bis*(7-Chloroquinolin-4-yl)cyclohexane-1,2-diamine,* **2**

4,7-Dichloroquinoline (3·962 g, 0·02 mol), the *cis*-1,2-diamine (1·142 g, 0·01 mol) and triethylamine (2·024 g, 0·02 mol) were heated under reflux in *N*-methylpyrrolidin-2-one (25 mL) for 3 days to give a dark brown oily liquid that was worked up using method A. The yield from a single synthesis of **2** was 55%. Purification of **2** was limited to trituration with analytical grade acetone and recovery of the insoluble product by filtration (method C). The presence of steric hindrance, arising from unfavourable interactions between the two quinoline rings, may contribute to the instability of this compound (mp 140°C). FTIR (cm^{-1}): 3446 (N-H stretch, quinoline). 400 MHz ^{1}H NMR (DMSO-d_6) δ (ppm): 1·56 (2H, broad m, H-3′ax); 1·79 (2H, broad m, H-3′eq); 1·89 (2H, broad m, H-2′ax); 2·52 (2H, broad m, H-2′eq); 4·55 (2H, broad m, H-1′ax); 7·00 (2H, d, 6·6 Hz, H-3); 7·67 (d, J = 9·2 Hz, H-6); 8·06 (2H, s, H-8); 8·55 (d, J = 6·6 Hz, H-2); 9·21 (2H, d, J = 9·2 Hz, H-5); 9·30 (2H, broad d, J = 5·2 Hz, N-H) The cycloalkyl peaks were broadened owing to slow ring inversion resulting in loss of fine couplings ($^2J_{H\text{-}H} < 3H_z$). ^{13}C NMR (DMSO-d_6) δ (ppm): 21·8 (C-3′, CH_2); 26·5 (C-2′, CH_2); 51·9 (C-1′, CH); 99·3 (C-3, CH); 116·12 (C-10, quaternary); 120·4 (C-8, CH); 125·9 (C-6, CH); 126·7 (C-5, CH); 137·0 (C-7, quaternary); 140·6 (C-9, quaternary); 144·6 (C-2, CH); 153·7 (C-4, quaternary). Accurate MS m/z: 437·13 confirming $C_{24}H_{22}N_4Cl_2$.

1R,2R-(−)-N^1,N^2-bis*(7-Chloroquinolin-4-yl)cyclohexane-1,2-diamine,* **3**, *and 1S,2S-(+)*-N^1,N^2-bis*(7-chloroquinolin-4-yl)cyclohexane-1,2-diamine,* **4**

4,7-Dichloroquinoline (3·962 g, 0·02 mol), the *1R,2R*-(−)-1,2-diaminocyclohexane (1·142 g, 0·01 mol) and triethylamine (2·024 g, 0·02 mol) were heated under reflux in *N*-methylpyrrolidin-2-one (25 mL) for 3 days to give a dark brown oily liquid that was worked up using method C. The yield from a single synthesis of **3** was 77%. The cream-coloured powder obtained after purification was recrystallized from aqueous ethanol to give crystals (mp 224–228°C). 250 MHz ^{1}H NMR (DMSO-d_6) δ (ppm): 1·19–1·28 (2H, broad m, H-3′ax); 1·44–1·48 (2H, broad m, H-3′eq); 1·81–1·83 (2H, broad m, H-2′ax); 2·06 (2H, broad s, H-2′eq); 3·90–3·93 (2H, broad m, H-1′ax); 6·80 (d, $J_{2,3}=5·7$ Hz, 2H); 7·11 (2H, broad, NH); 7·32 (dd, $J_{5,6}=8·9$ Hz, $J_{6,8}=2·1$ Hz); 7·67 (d, $J_{6,8}=2·1$ Hz); 8·14 (d, $J_{5,6}=8·9$ Hz); 8·28 (2H, d, $J_{2,3}=5·7$ Hz). ^{13}C NMR: (DMSO-d_6) δ (ppm): 24·6 (C-3′, CH_2); 31·5 (C-2′, CH_2); 55·6 (C-1′, CH); 99·0 (C-3, CH); 117·2 (C-10, quaternary); 123·5 (C-8, CH); 124·1 (C-5, CH); 127·0 (C-6, CH); 133·2 (C-7, quaternary); 148·7 (C-9, quaternary); 149·9 (C-4, quaternary); 153·7 (C-2, CH). Accurate MS m/z: 437·13 confirming $C_{24}H_{22}N_4Cl_2$. *1S,2S*-(−)-N^1,N^2-bis(7-chloroquinolin-4-yl) cyclohexane-1,2-diamine **4** was prepared in a similar fashion from *1S,2S*-(+)-1,2-diaminocyclohexane (55% yield); it had identical spectra when run under the same concentration and conditions but the mp of the compound was 292°C.

(±)-trans-N^1,N^2-bis*(7-(trifluoromethyl)-quinolin-4-yl)cyclohexane-1,2-diamine,* **5**

4-Chloro-7-(trifluoromethyl)quinoline (4·632 g, 0·02 mol), the (±)-1,2-diamine (1·142 g, 0·01 mol) and triethylamine (2·023 g, 0·02 mol) were heated under reflux in *N*-methylpyrrolidin-2-one (25 mL) for 3 days to give a dark brown viscous liquid that was worked up using method A (mp 330°C, 338°C by DSC). FTIR (cm^{-1}): 1092 (C-F stretch), 798 (C=C stretch). ^{1}H NMR (DMSO-d_6) δ (ppm): 1·34–1·69 (4H, m);

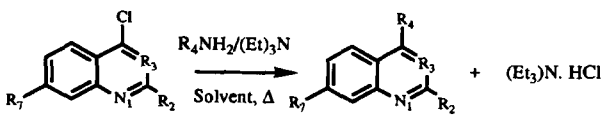


FIG. 2. General reaction depicting the synthesis of 4-aminoquinolines, where R_2 = H or phenyl, R_3 = N or CH_2, R_4 = secondary amine (aryl and alkyl).

1·7–1·91 (2H, m); 2·0–2·2 (2H, m); 3·75–3·95 (2H, m); 6·91 (2H, d, $J_{2,3}=5·7$ Hz); 7·17–7·23 (2H, NH); 7·50 (2H, dd, $J_{5,6}=7·1$ Hz $_{6,8}=2·1$ Hz); 7·88 (2H, d, $J_{6,8}=2·1$ Hz); 8·32 (2H, d, $J_{5,6}=8·9$ Hz); 8·35 (2H, d, $J_{2,3}=5·7$ Hz). ^{13}C NMR (DMSO-d_6) δ (ppm): 55·77 (C-1′, CH); 30·59 (C-2′, CH_2); 24·0 (C-3′, CH_2); 151·78 (C-2, CH); 100·12 (C-3, CH); 149·70 (C-4, quaternary); 117·5 (C-9, quaternary); 123·8 (C-5, CH); 117·0 (C-6, CH); 128·0 (C-7, quaternary); 126·5 (C-8, CH). MS m/z: 504 (100%), 484 (M-HF), FAB (M + H) 505.

(±)-trans-N^1,N^2-bis*(2-Phenylquinazolin-4-yl)cyclohexane-1,-2-diamine,* **7**

4-Chloro-2-phenylquinazoline (Am-ex-ol; 4·814 g, 0·02 mol), the (±)-1,2-diamine (1·142 g, 0·01 mol) and triethylamine (2·023 g, 0·02 mol) were heated under reflux in *N*-methylpyrrolidin-2-one (25 mL) for 3 days to give a black-brown liquid that was worked up using method B to give a white crystalline solid, mp 274°C. The yield from a single synthesis of **7** was 57%. NMR (DMSO-d_6) δ (ppm): 1·34–1·69 (4H, m); 1·7–1·91 (2H, m); 2·0–2·2 (2H, m); 3·75–3·95 (2H, m); 7·2 (2H, NH); 7·31 (dd, $J_{5,6}=8·9$ Hz, $J_{6,8}=2·1$ Hz); 7·5 (10H, m); 7·58–7·68 (d, $J_{6,8}=2·1$ Hz); 8·02–8·18 (d, $J_{5,6}=8·9$ Hz); 8·22–8·35 (2H, d, $J_{2,3}=5·7$ Hz). ^{13}C NMR ($CDCl_3$) δ (ppm): 56·50 (C-1′, CH); 32·63 (C-2′, CH_2); 24·98 (C-3′, CH_2); 100·12 (C-3, CH); 150·05 (C-4, quaternary); 113·40 (C-9, quaternary); 121·18 (C-5, CH); 125·43 (C-6, CH); 130·31 (C-7, CH); 132·62 (C-8, CH); 160·03 (C-2, quaternary); phenyl: (128·36, CH; 128·50, (C1′-quaternary)). MS m/z: 522·5 (M^+), 222 (100%), 301 (100%).

(±)-N^1*(7-Chloroquinolin-4-yl)cyclohexylamine,* **8**

4,7-Dichloroquinoline (1·981 g, 0·01 mol), the cyclohexylamine (0·992 g, 0·01 mol) and triethylamine (1·012 g, 0·01 mol) were heated under reflux in *N*-methylpyrrolidin-2-one (25 mL) for 3 days to give a black oil that was worked up using method A. The yield from a single synthesis of 8 was 63% (mp 82°C). FTIR (cm^{-1}): 3372, 1617. ^{1}H NMR (DMSO-d_6) δ (ppm): 1·05–2·0 (4H, m); 1·7–1·91 (m, 10H); 3·75–3·95 (1H, m); 6·91 (1H, d, $J_{2,3}=5·7$ Hz); 7·17–7·23 (1H, NH); 7·50 (1H, dd, $J_{5,6}=7·1$ Hz, $J_{6,8}=2·1$ Hz); 7·88 (1H, d, $J_{6,8}=2·1$ Hz); 8·32 (1H, d, $J_{5,6}=8·9$ Hz); 8.35 (1H, d, $J_{2,3}=5·7$ Hz). ^{13}C NMR (DMSO-d_6) δ (ppm): 55·77 (C-1′, CH); 30·59 (C-2′, CH_2); 24·0 (C-3′, CH_2); 151·78 (C-2, CH); 100·12 (C-3, CH); 149·70 (C-4, quaternary); 117·5 (C-9, quaternary); 123·8 (C-5, CH); 117·0 (C-6, CH); 128·0 (C-7, quaternary); 126·5 (C-8, CH).

N^1*-(7-Chloroquinolin-4-yl)-adamantyl-1-amine,* **9**

4,7-Dichloroquinoline (1·981 g, 0·01 mol), the 1-adamantanamine (1·513 g, 0·01 mol) and triethylamine (1·012 g, 0·01 mol) were heated under reflux in *N*-methylpyrrolidin-2-one (25 mL) for 3 days to give a black oil that was worked up by repeating method A three times. The yield from a single synthesis of **9** was 85% (mp 331°C). ^{1}H NMR (DMSO-d_6) δ (ppm): 1·5–2·2 (15H, m); 6·74 (2H, d, $J_{2,3}=5·7$ Hz); 6·88–7·03 (2H, NH); 7·31 (dd, $J_{5,6}=8·9$ Hz, $J_{6,8}=2·1$ Hz); 7·58–7·68 (d, $J_{6,8}=2·1$ Hz); 8·02–8·18 (d, $J_{5,6}=8·9$ Hz); 8·22–8·35 (2H, d, $J_{2,3}=5·7$ Hz). MS m/z: 312/314, 178/180.

N^1*-(7-Chloroquinolin-4-yl)-adamantyl-2-amine,* **10**

4,7-Dichloroquinoline (1·981 g, 0·01 mol), the 2-adamantanamine hydrochloride (1·877 g, 0·01 mol) and triethylamine (2·024 g, 0·02 mol) were heated under reflux in *N*-methylpyrrolidin-2-one (25 mL) for 3 days to give a black oil that was worked up by repeating method A two times. The yield from a single synthesis of **10** was 82% (mp 258°C). This compound decomposed in bright light over a period of one year. ^{1}H NMR (DMSO-d_6) δ (ppm): 1·5–2·2 (15H, m, complex); 6·74 (2H, d, $J_{2,3}=5·7$ Hz); 6·88–7·03 (2H, NH); 7·31 (dd, $J_{5,6}=8·9$ Hz, $J_{6,8}=2·1$ Hz); 7·58–7·68 (d, $J_{6,8}=2·1$ Hz); 8·02–8·18 (d, $J_{5,6}=8·9$ Hz); 8·22–8·35 (2H, d, $J_{2,3}=5·7$ Hz). MS m/z: 312/314, 178/180.

N^1*-(7-Chloroquinolin-4-yl)-2,4-dichlorophenyl-1-amine,* **11**

4,7-Dichloroquinoline (1·981 g, 0·01 mol), the 2,4-dichloroaniline (1·620 g, 0·01 mol) and triethylamine (1·012 g, 0·01 mol) were heated under reflux in *N*-methylpyrrolidin-2-one (25 mL) for 3 days to give a black-green oil that was worked up by repeating method A three times. The yield from a single synthesis of **11** was 85% (greenish solid, mp 258°C determined by DSC); FTIR (cm^{-1}): 1092 (C-F stretch), 798 (C=C stretch). FTIR (cm^{-1}): (C-H aromatic stretch), 1611 (N-bend, secondary amine), 1477 cm (CH bend, CH_2). 1238, 1212 (C-N stretch, aromatic C-N bond), 652 (C-Cl stretch). ^{1}H NMR (DMSO-d_6); Ù (ppm) 6.39 (2H, d, $J_{2,3}=5.7$ Hz); 7.67 (2H, H_5', H_6'; non-first order spectra); 7.86 (1H, dd, $J_{5,6}-7.1$ Hz, $J_{6,8}=2.1$ Hz); 7.95 (1H, H_3'; m, non-first order spectra); 8.27 (d, $J_{6,8}=2.1$ Hz); 8.5 (NH, s); 8.59 (d, 1H, $J_{2,3}=5.7$ Hz); 9.00 (1H, d, $J_{5,6}=7.1$ Hz). ^{13}C NMR (DMSO) δ (ppm): 100·91 (C-3, CH); 116·00 (C-9, quaternary); 126·2 (C-5, CH); 126·2 (C-6, CH); 129·7 (C-8, CH); 135·99 (C-7, quaternary); 154·63 (C-4, quaternary); 155·06 (C-2, CH); C11-C15-coincident peaks. MS m/z: 288, 290, 322/324/326 with a base ion m/z = 288.

(±)-trans-N^1*-(7-Chloroquinolin-4-yl),N^2-(9-chloro-2-methoxyacridin-6-yl)cyclohexane-1,2-diamine,* **12**

6,9-Dichloro-2-methoxyacridine (2·781 g, 0·01 mol), (±)-1,2-diamine (1·142 g, 0·01 mol) and triethylamine (1·012 g, 0·01 mol) were heated under reflux in *N*-methylpyrrolidin-2-one (25 mL) for 12 h. The reaction mixture was cooled to room temperature and 4,7-dichloroquinoline (1·981 g, 0·01 mol) and triethylamine (1·012 g, 0·01 mol) were added. This mixture was heated for 2 days, cooled to room temperature and worked up using method A and then method B to give a yellow solid in 85% yield. This yellow compound charred above 390°C (onset of charring: 258°C determined by DSC yield 17%) and because of its poor solubility precipitated before NMR spectra could be satisfactorily acquired. The expected molecular ion was seen at m/z = 486, 488 together with the base ion at m/z 259.

(±)-trans-N^1,N^2-bis-*(9-Chloro-2-methoxyacridin-6-yl)cyclohexane-1,2-diamine,* **13**

6,9-Dichloro-2-methoxyacridine (3·562 g, 0·02 mol), (±)-1,2-diamine (1·142 g, 0·01 mol) and triethylamine (2·024 g, 0·02 mol) were heated under reflux in *N*-methylpyrrolidin-2-one (25 mL) for 3 days. The mixture was worked up using method A and then method B to give a yellow solid. This

highly insoluble compound had an mp of approximately 380°C (yield 49%) FTIR (cm^{-1}): 3268·95, 3237·61, 3168, 3137, 3107 (N-H stretch, secondary amine), 3030·77 (C-H aromatic stretch), 1570 (N-H bend, secondary amine), 1223 (C-N stretch, aliphatic), 684·17 (C-Cl stretch). MS confirmed a molecular ion at m/z = 596, 598 together with the base ion at m/z = 258.

Screening for antimalarial activity

Plasmodium berghei (N/13/1A/4/203) was maintained by serial passage in MF1 mice and was injected intravenously into experimental male mice, 18–25 g, in doses of 2×10^7 parasitized erythrocytes/animal. Control mice were injected with 0·2 mL of normal mouse blood diluted to the same extent with 0·85% saline. All animals were treated topically with an alcoholic solution of monosulphiram (2·5%) to prevent infection by *Eperythrozoon coccoides*.

In a blind study, putative antimalarials were injected subcutaneously into groups of 5–6 mice using sterile, peroxide-free olive oil and dimethylsulphoxide as vehicle (24 : 1, dose volume 10 mL kg^{-1}). Some compounds required the addition of 5% water or Tween 80, or both, to facilitate carriage of the chemical; for all compounds tested a concurrent control malaria group received the appropriate vehicle. Mice were treated on the day of inoculation, approximately 3 h after being infected then twice daily for the next 2 days at 2–7 dose levels (range 2·5–200 mg kg^{-1}). Seventy-two hours 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 activities of the compounds were assessed as the reduction in parasitaemia compared with that in concurrent vehicle-treated mice (Student's *t*-test); their relative potencies were assessed by calculating 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 observation of the general autonomic and behavioural states of the mice was made throughout the experimental period.

Molecular modelling

Low-energy conformations were modelled on a Silicon Graphics Indigo workstation using a variety of force fields, including MM2*, AMBER* (MacroModel Version 4·0; Mohamadi et al (1990)) and CVFF (Insight II; Biosym (1993)). Systematic conformational searching was performed at 15–30° intervals.

Results and Discussion

Synthesis of bisquinolines

Classical syntheses of 4′-substituted quinoline antimalarial heterocycles exploit thermal condensations between 4-haloquinolines and a suitable amine or, less frequently, a 4-aminoquinoline with an alkyl or aryl halide. For example, (±)-chloroquine is commercially prepared by condensation of N^1,N^1-diethyl-1,4-pentanediamine with 4,7-dichloroquinoline in anhydrous phenol (Surrey & Cutler 1951). Although compound **1** was successfully synthesized in this study using phenol (bp 182°C) as the solvent, the yields were poor (0–20%). The required bisquinolines were prepared in adequate yield (4–84%) using *N*-methylpyrrolidin-2-one, which is the solvent preferred by Cain et al (1978) for the synthesis of various N^{α},N^{ω}-*bis*(quinolin-9-yl)alkyl-α,ω-diamines and N^{α}, N^{ω}-*bis*(acridinyl)alkyl-α,ω-diamines (Fig. 3).

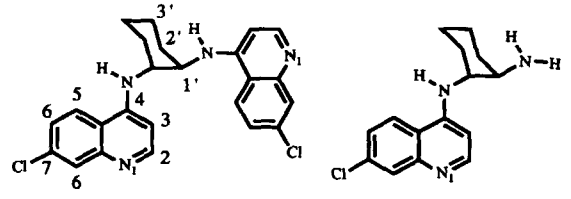


FIG. 3. Structural formulae of compound 1 and its monoquinoline precursor.

Displacement of chlorine from 4,7-dichloroquinoline, which is a solvent-dependent process, should be promoted approximately 1100-fold in protic solvents such as ethanol compared with aprotic ones such as toluene. Ethanol, methanol and DMSO were used as solvents in this study, but did not promote the formation of **1**. One compound, **9**, was synthesized using a published method (Barlin & Jiravinyu 1985) that uses HCl-MeOH (pH 4·7) as the solvent. Triethylamine was chosen as a readily available and cheap HCl scavenger because Na_2CO_3 and K_2CO_3 proved ineffective at high temperatures in these studies. Other tertiary amines, such as *N*-methylpiperidine could also be used, but offered no significant advantage over triethylamine in these studies. Table 1 illustrates some of the compounds that have been synthesized using *N*-methylpyrrolidin-2-one as the solvent.

The reaction time of 1–3 days limited both the numbers of compounds and the cost effectiveness of the study. The suitability of diphenyl ether, a high boiling solvent (bp 258–260°C) for accelerating the formation of **1** was, therefore, studied. Prolonged heating under reflux in diphenyl ether, however, encouraged the formation of novel compounds containing three molecules of quinoline to one of the diamine (fast atom bombardment mass (FAB) MS). We have so far been unable to establish the exact identity of these compounds by NMR because they are completely insoluble in common (and uncommon) deuterated solvents. Studies aimed at deducing their structure are progressing.

The optimum temperature range for bimolecular nucleophilic displacement of 4-haloquinolines in solution lies between 120 and 210°C. Reaction temperatures greater than 250°C always led to the formation both of the desired compounds and of the aforementioned high-molecular-weight products. Mono- or bis-4-aminoquinolines were not formed when 4,7-dichloroquinoline was heated with (±)-*trans*-1,2-diaminocyclohexane at temperatures below 170°C. This appears to be the lowest temperature at which a thermally assisted nucleophilic displacement will take place, in acceptable yield, in solution.

The quinolines shown in Table 1 were, in general, high-melting-point solids with poor solubility in common solvents. DMF, aqueous ethanol or methanol proved suitable for effecting recrystallization, but better solvents are required for NMR studies. NMR spectra were acquired in $(CD_3)_2SO$ and, where indicated, DCl/CD_3OD or CF_3CO_2H. Some compounds (especially **9–13**) could be dissolved in DCl/MeOH, but pre-

cipitated from solution before spectra could be acquired. NMR analysis of crude fractions isolated from the reaction mixture confirm the absence of 3-substituted quinolines, suggesting that a genuine bimolecular addition elimination process was operating. The sluggish nature of the reaction in the synthesis of some bisquinolines (1–3 days reaction time) may be because of unfavourable steric factors mitigating against the approach of the second haloquinoline.

Correlation of biological action with physiochemical properties of antimalarials

Preliminary molecular modelling studies using the COSMIC force field (Oxford Molecular Ltd 1993) indicated that there was a low-energy fit based on the coincidence of heteroatoms on both (±)-*trans*-N^1,N^2-bis-(7-chloroquinolin-4-yl)cyclohexane-1,2-diamine **1** and quinine. We decided, therefore, to construct a series of compounds that would explore possible connections between the position of heteroatoms in these quinolines and their biological activity in-vivo.

The antimalarial activity of **1** in this study (ID50 = 3·0 mg kg^{-1}) compared with chloroquine phosphate (Sigma; ID50 = 4·3 mg kg^{-1}) confirms our prediction that bisquinolines linked by a diamine bridge should be potent antimalarials. The 1*R*,2*R*-(−), 1*S*,2*S*-(+) isomers of **1** were synthesized (**3**,**4**) together with the isolipophilic *cis isomer*, **2**, in order to evaluate isomeric forms with the putative receptor. All three isomers had antimalarial activity comparable with that of **1**. Compound **5**, which should have a slightly higher lipophilicity than **1**, was slightly less active (ID50 = 5·9 mg kg^{-1}) possibly reflecting unfavourable steric interactions with the putative drug receptor. Similar observations have been observed with **12**; this compound, predicted to completely fill the putative active site of the postulated receptor, or to have the optimum lipophilicity whilst maintaining reasonable antimalarial potency, was also active (ID50 = 13 mg kg^{-1}).

In addition to their activity in the 3-day suppression test, compounds **1** and **5**, at dose levels of 5 and 10 mg kg^{-1} (s.c.), respectively, suppressed the development of malaria when treatment (twice daily) began 24 h after inoculation with *P. berghei*, when parasitaemia was already 3–5%. Compound (**1**) administered orally (3 doses beginning 24 h after infection, dose 5 or 25 mg kg^{-1}, reduced the parasitaemia measured on day 3 to 4 ± 1% and 5 ± 2% (n = 5), respectively, from 67 ± 3% in vehicle-treated mice. No adverse effects on behaviour, weight or body temperature were evident after the administration of **1** or its active isomers at dose levels effective in reducing parasitaemia (up to 200 mg kg^{-1}, s.c., on up to five occasions over 3 days).

The next compound evaluated in this study, in order of antimalarial potency, was **13** with an ID50 of 27 mg kg^{-1}. Further exploration of the interaction of compounds **1–4** with the putative receptor was sought by constructing an analogue, **11**, with an aromatic bridging unit (cf. amodiaquine); in this case antimalarial action was reduced. This also tests the approach made popular by Topliss (1972, 1977), because **11** is (+π, +σ), i.e. a highly lipophilic compound. This compound tested at doses of 10 mg kg^{-1} and 50 mg kg^{-1} (s.c.) had no significant antimalarial activity. Both planarity and the lack of additional nitrogen atoms in the bridging unit may account for the lack of antimalarial effect against *P. berghei* in-vivo. Similarly **7**, 10 mg kg^{-1} (s.c.), had no antimalarial effect, although 50 mg kg^{-1} reduced parasitaemia on day 3 from 58 ● 1% to 37 ± 4% ($P < 0.01$). **7** had poor solubility and was not tested at 100 mg kg^{-1}. Compound **8** also had no antimalarial effect at 10 mg kg^{-1} but 50 mg kg^{-1} reduced parasitaemia on day 3 from 61 ± 3% to 43 ± 4% (Table 1).

Two compounds, **9** and **10**, designed to test whether or not conjugation of an adamantyl moiety would enable the quinoline to cross lipid barriers, were both without antimalarial activity in-vivo in this study. These compounds lacked an additional amine positioned within 2-4 methylene groups distant from the secondary amine, indicating that the presence of at least two nitrogen atoms in the linking unit or bridgehead may be essential for activity. This indicates, furthermore, that (+π, −σ) parameters are important in the bridge or 4-amino substituent.

Modelling investigations

Dichloroquinazine (LeBras et al 1983) and 12,494 RP (Benazet 1965) both share some common features with quinoline methanols (n = 1), chloroquine (n = 3), and mepacrine (n = 3) which can be depicted as in Fig. 4a.

Superimposing the energy-minimized form of the structure shown above (molecular mechanics calculations, MM2), and 'matching' heteroatoms with similar calculations performed on quinine, haloquinolines and acridine antimalarials, and those synthesized in this study, suggested that the generalized structure (b) in Fig. 4 would fill the putative active site of the target receptor. The cyclic bridging unit serves to minimize the number of solution state conformers resulting in an increase in affinity with the receptor. R_2 can be methyl or trifluoromethyl; R_4 is methine or nitrogen. R_7 can be a variety of groups including NO_2, CN or halogen (preferably Cl) and R_8 is a methoxy grouping. This scenario assumes that the site of action of all these compounds is the same receptor, which may not be so. The assumptions made provide, nevertheless, an initial starting point to explore a common antimalarial pharmacophore.

The need to synthesize novel antimalarials arises from practical limitations on supplies of naturally occurring antimalarials and increased resistance to antimalarials in current use. Although numerous quinolines related to chloroquine have been prepared and screened for antimalarial activity by the Walter Reed Army Research Institute, there have been relatively few studies on bisquinolines. Benazet (1965) reported that 12,278 RP is active against some drug resistant strains of

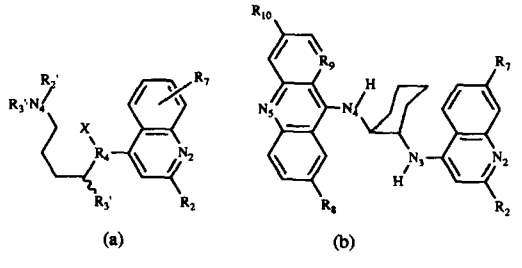


FIG. 4. General formula of antimalarial 4-aminoquinolines (a) and structure designed to fill the putative 4-aminoquinoline receptor site (b).

P. berghei, but it is, apparently, ineffective against other strains of *P. berghei* (Warhurst 1966). More recently, Basco et al (1994) have claimed that the bisquinoline 1 is active in-vitro against two different drug-resistant strains of *Plasmodia*, although further research by this group failed to substantiate this claim when a wider range of chloroquine-resistant *Plasmodia* were employed in-vitro (Basco et al 1994). In the experiments presented here the antimalarial activity of novel bisquinolines has been assessed in-vivo, as this procedure affords additional information on both the bioavailability and potential adverse effects of the compounds. A chloroquine sensitive *Plasmodium* was used in order for structural modifications to bisquinolines to register as changes in antimalarial potency (IC50) and for comparison with chloroquine as standard.

Previous studies (Berliner et al 1948; Coatney et al 1953) indicate that both heterocycles, either the quinolines or acridine, can be substituted at the 6, 7 or 8 position, preferably with a halogen (bromine or fluorine, but not iodine), without significant loss of activity. The halogen can be replaced with either a methyl or a nitrile group and the compounds maintain antimalarial activity in-vivo (Albert 1966). Methyl substitution in position 3 of the nucleus reduces activity, and additional methyl substitution in position 8 is claimed to abolish activity totally when tested against *P. galinaceum* (Berliner et al 1948; Coatney et al 1953). Trifluoromethyl substituents at the 2, 7 or 8 positions of the quinolinemethanols, at combinations of these positions results, however, in clinically effective antimalarials (Peters 1970). Methyl, trifluoromethyl or aryl substituents attached to the 2 position prevents metabolic inactivation of the drug (Williams 1959) whereas a phenyl substituent on the 2 position can result in phototoxic compounds (Boykin et al 1968). The aforementioned observations, together with the wide availability of 4-chloro-, 7-chloro- and 7-trifluoromethylquinoline, dictated our initial synthetic targets, namely **1** and **5** and the other compounds depicted in Table 1.

The antimalarial drugs chloroquine and mepacrine (quinacrine) have both been shown to intercalate between stacks of base pairs within strands of duplex DNA (O'Brien et al 1966, Waring 1979). It has also been suggested that the 1,4-diaminopentane side-chain of chloroquine (and mepacrine) protrudes from the central axis of the DNA base pairs and interacts ionically with phosphate strands of the complementary strands of DNA base pairs. The positioning of the drug may enable inhibition of the DNA polymerase with the starter (Cohen & Yielding 1965). Marquez et al (1974) have demonstrated that bisquinolyl derivatives of polyamines can significantly inhibit RNA polymerase activity of *E. coli* without increasing the melting temperature of DNA. It is not clear if the receptors previously proposed for chloroquine, namely DNA (O'Brien et al 1966), RNA polymerase (Marquez et al 1974) or haem polymerases (Chou et al 1980) are the targets for bisquinolines in-vivo.

The criteria for true bis-intercalation require that both heterocycles are linked by a bridging unit of certain critical distance, at which point the binding affinity of the compound with DNA becomes large (10^8–10^9 M^{-1}). Chen et al (1978) suggested that the minimum alkyl bridging unit required for bis-intercalation within DNA by acridines is a hexyl chain. Both molecular mechanics and solution-state NMR studies suggest that the quinoline rings in bis-intercalators can become orientated in a 'hair pin conformation' with the quinoline rings either in a parallel conformation or an extended form. Our molecular mechanics studies have shown that the axial-axial conformer of **1** can intercalate one of its quinoline rings within duplex DNA strand, whereas the second quinoline ring protrudes from the minor groove of DNA (McCarron et al 1994). As the bridging unit falls short of the minimum required bridging distance, our results indicate, furthermore, that bis-intercalation is discouraged. The mechanism of action of **1**, therefore, cannot rely on bis-intercalation of parasite DNA. An enzymic target, such as RNA polymerase or DNA topoisomerase for **1** or non-specific binding to DNA, or both of these, cannot be excluded at this stage.

Our results confirm the necessity for the presence of one or more side-chain nitrogen atoms in 4-aminoquinolines because the compounds (**8–11**) with no such atom showed minimal or no activity. To explore the three-dimensional requirements of the nitrogen atoms in active compounds, conformational searches were performed for all the active compounds listed in Table 1 and the distances between atoms N_1 and N_3 (Figs 1, 3 and 5 were computed.

Every sterically allowed conformation lies within the range 4·5 to 8 Å, with a common intersecting range of 6·5 to 7·5 Å. Such values may be compared with those of established antimalarials: chloroquine (6–9 Å, crystal structure distance = 8·4 Å); amodiaquine (6·3–9·9 Å, crystal structure = 7·5 Å), mefloquine (4·8–6·5 Å, crystal structure = 6·5 Å) and quinine (4·8–6·6 Å, crystal structure = 6·5 Å). Interestingly, in the compound 3-chloro-8-methoxy-9-(4-methyl-1-piperazinylmethyl)-11*H*-indolo[3,2-*c*]quinoline (Fig. 5), which has been reported (Go et al 1992) to be 10^4 times more active in-vitro than chloroquine against chloroquine resistant *Plasmodia*, the comparable distance is confined to the range 8·70–8·75 Å. Quinolines 'locked' into selected conformations might, therefore, bind strongly with the target receptors and be effective antimalarial drugs with clinical applications (Koh et al 1994).

In any event, our results concur with the putative pharmacophore model of Koh et al (1994) for amodiaquine. The different N-N distances of antimalarials in Table 1 (especially compounds **1–5**) suggest that there are two (or more) anionic sites in the putative pharmacophore, as well as a π-electron-rich, flat area positioned parallel to the quinoline ring. The restriction imposed on the substituent on position 7 may be steric in nature, as compounds with a CF_3 group (**5**) are less efficacious antimalarials than compound **1** with chlorine in this position. The steric requirements of the side-chain seem to be less exacting, especially given the differences in shapes and sizes of the side-chain in antimalarial compounds, even after allowance has been made for conformational flexibility. The

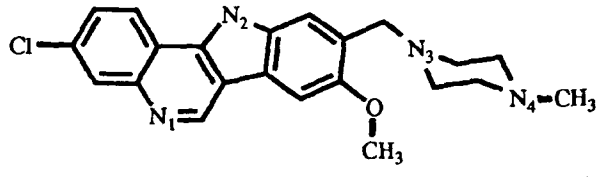


FIG. 5. 3-chloro-8-methoxy-9-(4-methyl-1-piperazinylmethyl)-11H-indolo[3,2-c]quinoline

equivalent activity for the 1*R*,2*R*-(−) and 1*S*,2*S*-(+) isomers of **1** illustrates this point. The flat dose-response curves found for several of the quinolines containing bulky substituents may, indeed, be a result of low bioavailability in the body but there is no evidence to substantiate this possibility at present. Antimalarial activity in-vivo may be moderated by several processes unrelated to the bisquinoline receptor binding site, e.g. drug absorption, distribution and metabolism.

These data, however, enable definition of a functional receptor, i.e. one that acts as if it had these properties, whilst evaluating bioavailability and potential toxicity. Elslager & Thompson (1964) consider that the ideal antimalarial would have the properties: activity when given in a single dose; administered parenterally; non-irritating; tolerated systemically; able to provide prolonged protection; active immediately; inexpensive; liable to produce minimum encapsulation; and effective against normal and drug-resistant strains of *Plasmodia*. The compounds described in Table 1 can be made efficiently in a one-pot reaction and compounds **1–5** fulfil many of the aforementioned criteria, especially the first seven. Clearly it is important that the activity of these compounds against drug-resistant strains of *Plasmodia*, in man, be determined.

In this study **1**, or its active isomers, have been shown to be active and well-tolerated antimalarial agents in-vivo, after systemic or oral administration to mice infected with *P. berghei* up to 24 h previously. These results and inferences made from previous studies, together with molecular modelling experiments, indicate, furthermore, that our putative pharmacophore model can be used to design novel antimalarials with in-vivo activity greater than or equal to that of chloroquine

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