"One-Pot" Synthesis and Antimalarial Activity of Formamidine Derivatives of 4-Anilinoquinoline

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Amodiaquine (AQ) is an antimalarial which is effective against chloroquino-resistant strains of *Plasmodium falciparum* but whose clinical use is severely restricted because of associated hepatotoxicity and agranulocytosis. "One-pot" synthesis of formamidines likely to be transformed into AQ derivatives is reported. Compared with AQ, the new compounds were devoid of *in vitro* cytotoxicity upon human embryonic lung cells and mouse peritoneal macrophages. One showed a potent *in vivo* activity in mice infected with *P. berghei*. Transformation of this compound by reductive amination led to a new type of AQ derivatives that displayed an *in vitro* activity similar to that of AQ but did not lead to toxic quinone-imines.

Key words drug design; malaria; *Plasmodium falciparum*; chloroquine; amodiaquine; formamidine

Today, almost one half of the world's population is exposed to the threat of malaria and the disease was responsible for about 2 million deaths in 1997.[1)] Quinoline-containing antimalarials, such as chloroquine (CQ, Fig. 1), have long been used in the struggle against malaria. Since its discovery, CQ has proven to be a highly effective, safe, and well-tolerated drug for the treatment and prophylaxis of malaria. But the emergence of CQ-resistant strains has underlined the need for a synthetic alternative to CQ. The spread of resistance[2)] has prompted the re-examination of alternative antimalarials, such as amodiaquine (AQ, Fig. 1) which was found to be effective against both CQ-sensitive and CQ-resistant strains.[3)] However, the clinical use of AQ has been severely restricted because of associations with hepatotoxicity and agranulocytosis.[4,5)] As paracetamol, due to the presence of the 4′-hydroxyl substituent, AQ is known to undergo extensive bioactivation by hepatic cytochrome P-450 enzymes to a chemically reactive quinone–imine (AQQI, Chart 1), followed by nucleophilic addition of glutathione. The formation of these reactive species *in vivo* and their binding to cellular proteins and lipids could affect cell function either directly or by immune response.[6—8)] A 4′-dehydroxyfluoro AQ derivative has been reported[9)] and proved not to give a quinone-imine by simple oxidation. This was reflected in its high oxidation potential and no bioactivation. However, compared with AQ, it was found to be less potent against both CQ-resistant and CQ-sensitive parasites.

With the aim of avoiding bioactivation and toxicity, a series of formamidines likely to be transformed into 4′-dehydroxy AQ derivatives was prepared from the benzylic alcohol **1** (Fig. 2). Formamidines have been used extensively as pharmacological agents,[10—12)] and we have recently described their simple preparation in which bromo-tris-pyrrolidinophosphonium hexafluorophosphate (PyBroP) is used as a convenient activator of the formamide group.[13)] In the new series, antimalarial activity could be expected from the presence of the amidine side chain capable of interacting with a carboxylate group of heme. The synthesis and antimalarial activity of these compounds (**2**—**6**, Fig. 2) are reported as well as those of the AQ analogue (**7**, Fig. 2) corresponding to the most active among them.

Chemistry

The synthesis of compound **1** and that of the formamidine derivatives **2**—**7** were carried out as shown in Chart 2. Starting material **1** was obtained by condensation of 4,7-dichloroquinoline with 3,5-diaminobenzyl alcohol according to an

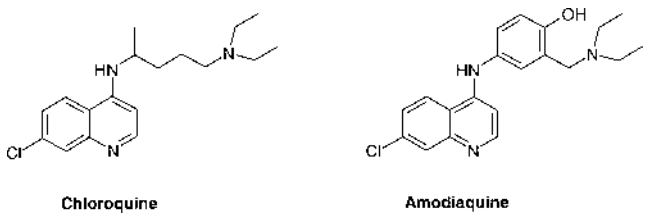

Fig. 1. Structure of Chloroquine and Amodiaquine

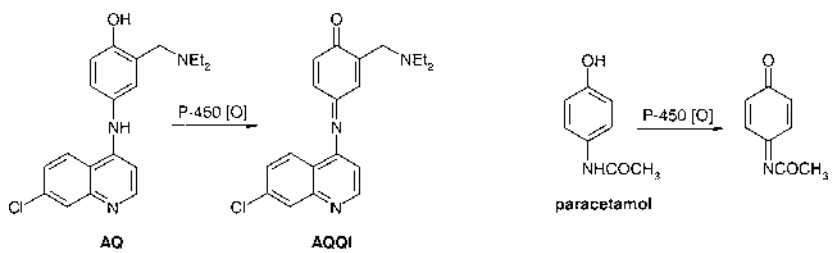

Chart 1. Oxidative Pathways of Amodiaquine and Paracetamol

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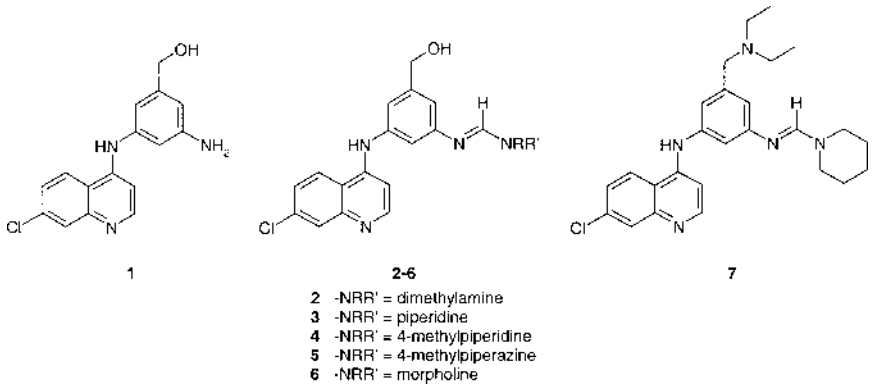

Fig. 2. Structure of Compounds **1**—**7**

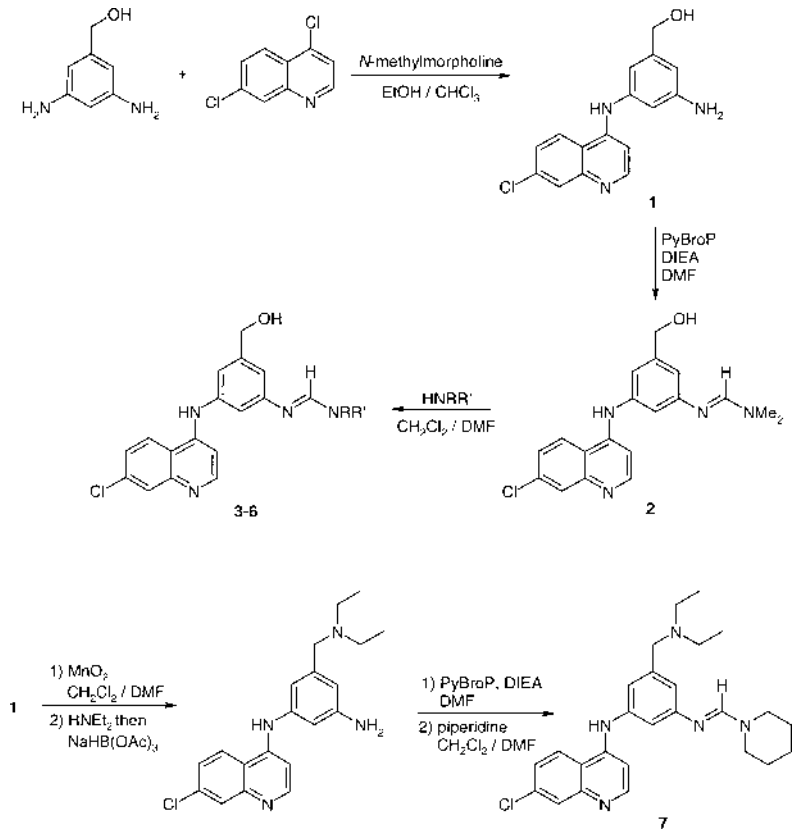

Chart 2. Synthesis of Compounds **1**—**7**

aromatic nucleophilic substitution mechanism.[14] Stirring of the reactive medium for 3 d in a EtOH/$CHCl_3$ 55 : 5 mixture, at room temperature, led to the precipitation of compound **1**, which was collected by filtration. Selective precipitation of compound **1** was due to its very poor solubility in EtOH and thus no further purification was necessary. The optimized procedure leads to yields as high as 88% (Table 1). The absence of formation of the corresponding bisquinoline can be explained both by the precipitation of compound **1** and by its capacity for steric hindrance.

Table 1. Optimization of the Synthesis of Compound **1**

Assay	Ratio amine/quinoline	Ratio EtOH/$CHCl_3$	Time (h)	Yield (%)
a	1	70 : 10	48	56
b	1	55 : 5	24	50
c	1	55 : 5	48	85
d	1	55 : 5	72	88

Fixation of the amino side chain was carried out in two steps using a "one-pot" procedure. During the first step, compound **2** was obtained by reaction of the PyBroP-activated DMF derivative with compound **1**.[13] During the second step, the dimethylamino moiety was substituted by various secondary amines HNRR′. Dichloromethane was used as a co-solvent to permit reduction at reflux temperature and thus avoid the degradation of the formamidine into formamide. Amine conversion rates were quantitative, but strong interactions between the final products and silica phase during chromatography purification led to moderate yields (27—43%).

Synthesis of compound **7** from compound **1** was carried

Table 2. *In Vitro* Antimalarial Activity against *P. falciparum* FcB1R Strain and *in Vitro* Cytotoxicity upon MRC-5 Cells and Mouse Peritoneal Macrophages of Compounds **1**—**7**

Compound	IC_{50} (nM) against FcB1R	Cytotoxicity (%) upon MRC-5 at (μM)				Cytotoxicity upon MPM No toxicity at (μM)
		25	12.5	6.3	3.1	
CQ	142	0	0	0	0	12.5
AQ	20	91	0	0	0	3.1
1	300	20	0	0	0	12.5
2	520	0	0	0	0	12.5
3	170	0	0	0	0	12.5
4	190	0	0	0	0	12.5
5	600	0	0	0	0	12.5
6	550	0	0	0	0	12.5
7	20	90	5	0	0	12.5

out in three steps. The benzylic alcohol group of compound **1** was first converted into an aldehyde by oxidation with MnO_2, followed by reductive amination with diethylamine using sodium triacetoxyborohydride as reducing agent (Chart 2).[15] A low yield was observed because of a side reaction during the oxidation step, since the amine moiety of the molecule can react with the aldehyde group of another molecule leading to a polymerization product. The previous protection of the amine moiety by a Fmoc or by a Boc group was found to be unsuccessful. In the final step, the procedure used for compounds **3**—**6** was applied to obtain the desired compound **7** in 45% yield.

Biological Results and Discussion

All compounds were tested for their antimalarial potency against a CQ-resistant FcB1R strain (IC_{50} CQ=142 nM) and for their cytotoxicity upon human MRC-5 cells (human diploid embryonic lung cell line) and mouse primary peritoneal macrophages (MPM) (Table 2).

Formamidines **2**—**6** were found to be less active than AQ *in vitro* against the CQ-resistant FcB1R strain but were non cytotoxic at a concentration of 25 μM upon MRC-5 cells as well as upon MPM. Two compounds, **3** and **4**, displayed an *in vitro* antimalarial effect against the FcB1R strain in the same range as that of CQ while the others were less active. Therefore the presence in the side chain of a cyclic tertiary amine without an additional hetero atom appears favourable for antimalarial activity. The most active, compound **3**, was then tested *in vitro* against two other strains of *P. falciparum*, F32a and PFB, which are respectively more CQ-sensitive (IC_{50} CQ=25 nM) and more CQ-resistant (IC_{50} CQ=340 nM) than the FcB1R strain. IC_{50} values of compound **3** were, respectively, 80 and 355 nM, thus revealing a correlation with CQ values and potentially a mode of action similar to both CQ and AQ, *i.e.* by inhibiting heme polymerization.[16] Compound **3** was also tested at 40 mg/kg in mice infected with *Plasmodium berghei*. While the control mice died 7 d following infection, animals receiving compound **3** displayed a 60% reduction in parasitemia at 7 d and mean survival time of about 19 d.

AQ derivative **7**, corresponding to the formamidine **3**, was synthesized and displayed an activity similar to that of AQ against the CQ-resistant FcB1R strain while its cytotoxicity was slightly inferior to that of AQ. Therefore it proved to be more active than 4′-dehydroxyfluoro AQ which displayed a 2-fold increase in IC_{50} values when compared with AQ against both CQ-sensitive and -resistant strains.[9] In the 4′-dehydroxyfluoro AQ, the lack of the hydrogen bond, which is present in AQ between the phenol group and the diethylamino moiety, was responsible for the decreased activity. In compound **7**, this phenomenon is balanced by an additional amino moiety. The increase in activity observed can be explained by the presence of a bis-cationic site, likely to interact simultaneously with the two carboxylate groups of heme. Moreover, its synthesis is simple, when compared with the five steps, including use of bromine, irradiation, and catalytical hydrogenation, required to prepare 4′-dehydroxyfluoro AQ.

In conclusion, a series of formamidines without the hydroxyl group of AQ, eliciting hepatotoxicity and aganulocytosis by bioactivation, was synthesized using a "one-pot" method from an aminoalcohol, easily obtained by precipitation. The new compounds are non cytotoxic upon a human embryonic lung cell line and upon MPM. The most active among them has an *in vitro* antimalarial activity in the same range as CQ against three strains varying in their resistance to CQ, while also demonstrating an activity against *P. berghei* in mice. These formamidines are intermediates for the facile synthesis of new AQ derivatives, have activity similar to that of AQ with less cytotoxicity, and do not lead to toxic quinone–imine formation.

Experimental

Chemistry All reactions were monitored using thin-layer chromatography carried out on 0.2 mm E. Merck silica gel plates (60F-254) using UV light as visualizing agent. ^{1}H-NMR spectra were obtained using a Brücker 300 MHz spectrometer. Mass spectra were recorded on a time-of-flight plasma desorption mass spectrometer (TOF-PDMS) using a Californium source. Chromatography was carried out using silica gel 60 (230—400 mesh ASTM) from Macherey-Nagel. Thick-layer chromatography (TLC) was performed using silica gel from Merck and the compounds were extracted from silica gel using the following solvent system: CH_2Cl_2/MeOH 80 : 20. The melting point (mp) of the chlorhydrate form for compounds **1**—**6** and of the basic form for compound **7** were determined on a Büchi 535 capillary mp apparatus and were uncorrected. The purity of final compounds was checked by high pressure liquid chromatography (P_{HPLC}) with a C18 Vydac column. Analytical HPLC was performed on a Shimadzu system equipped with a UV detector set at 254 nm. Compounds were dissolved in MeOH and injected through a 50 μl loop. The following eluent systems were used: A (H_2O/TFA, 100 : 0.05) and B (CH_3CN/H_2O/TFA, 80 : 20 : 0.05). HPLC retention times (HPLC t_R) were obtained at flow rates of 1 ml/min using a gradient run from 100% eluent A for 5 min and then to 100% eluent B over the next 25 min. CQ and AQ were obtained from Aldrich and Sigma, respectively. 4,7-Dichloroquinoline and 3,5-diaminobenzyl alcohol dihydrochloride were obtained from Aldrich.

Procedure for Chlorhydrate Salts Trimethylchlorosilane (1 eq) was

added to a solution of formamidine (1 eq) in MeOH. After stirring the mixture for 15 min at room temperature, the solvent was evaporated to yield the desired chlorhydrate.

{3-Amino-5-[(7-chloro-4-quinolyl)amino]phenyl}methanol (1) To a solution of 4,7-dichloroquinoline (1.88 g, 9.5 mmol, 1 eq), and *N*-methylmorpholine (1.3 ml, 11.8 mmol, 1.2 eq), in EtOH 50 ml was added a solution of 3,5-diaminobenzyl alcohol dihydrochloride (2 g, 9.5 mmol, 1 eq), and *N*-methylmorpholine (1.7 ml, 15.5 mmol, 1.6 eq), in a EtOH/$CHCl_3$ 50 : 50 mixture 10 ml, at 0 °C. After stirring for 72 h at room temperature, the mixture was filtered and the residual solid washed with ice cold EtOH and dried to yield compound **1** as a yellow solid. Yield: 88% (2.5 g). mp: 206 °C. *Rf*: 0.13 (CH_2Cl_2/MeOH, 9 : 1). ^{1}H-NMR (DMSO-d_6) δ: 2.70 (1H, s, OH), 3.60 (2H, br s, NH_2), 4.47 (2H, s, CH_2), 6.82 (1H, d, J=7.0 Hz, H quinoline), 6.85 (1H, s, Ar-H), 6.92 (2H, s, Ar-H), 7.84 (1H, dd, J=9.1, 2.1 Hz, H quinoline), 8.10 (1H, d, J=2.0 Hz, H quinoline), 8.52 (1H, d, J=7.0 Hz, H quinoline), 8.78 (1H, d, J=9.2 Hz, H quinoline), 11.06 (1H, s, NH). TOF-PDMS *m/z*: 299.4 (M^+). HPLC: P_{HPLC} 98%, t_R 11.85 min.

***N′*-{3-[(7-Chloro-4-quinolyl)amino]-5-[hydroxymethyl]phenyl}-*N*,*N*-dimethyliminoformamide (2)** To a solution of compound **1** (150 mg, 0.5 mmol, 1 eq), in DMF 10 ml were added DIEA (88 μl, 1 mmol, 2 eq), and PyBroP (235 mg, 0.5 mmol, 1 eq). After stirring the mixture for 7 h at room temperature, the solvent was evaporated, the solid residue washed with 1 M $NaHCO_3$ 15 ml and purified by TLC (CH_2Cl_2/MeOH, 60 : 40), to yield compound **2** as a yellow solid. Yield: 42% (50 mg). mp: 166 °C. *Rf*: 0.37 (CH_2Cl_2/MeOH, 6 : 4). ^{1}H-NMR (DMSO-d_6) δ: 2.87 (3H, br s, CH_3), 2.94 (3H, br s, CH_3), 4.42 (2H, s, C<u>H</u>$_2$-Ph), 5.11 (1H, br s, OH), 6.63 (1H, s, Ar-H), 6.71 (1H, s, Ar-H), 6.86 (1H, s, Ar-H), 6.89 (1H, d, J=5.4 Hz, H quinoline), 7.50 (1H, dd, J=9.0, 2.1 Hz, H quinoline), 7.71 (1H, s, N=C<u>H</u>–NRR′), 7.83 (1H, d, J=2.2 Hz, H quinoline), 8.38 (1H, d, J=8.8 Hz, H quinoline), 8.41 (1H, d, J=5.2 Hz, H quinoline), 8.94 (1H, s, NH). TOF-PDMS *m/z*: 354.8 (M^+). HPLC: P_{HPLC} 94%, t_R 11.00 min.

General Procedure for Synthesis of Compounds 3—6 To a solution of compound **1** (150 mg, 0.5 mmol, 1 eq), in DMF 10 ml were added DIEA (88 μl, 1 mmol, 2 eq), and PyBroP (235 mg, 0.5 mmol, 1 eq). After stirring the mixture for 12 h at room temperature, the appropriate amine (4 mmol, 8 eq) was added. Following reflux of the mixture for 5 h, the solvent was evaporated, the solid residue washed with 1 M $NaHCO_3$ 15 ml and purified by TLC (CH_2Cl_2/MeOH, 70 : 30), to yield the desired compound.

{3-[(7-Chloro-4-quinolyl)amino]-5-[(piperidinomethylidene)amino]-phenyl}methanol (3) Yellow solid. Yield: 40% (80 mg). mp: 231 °C. *Rf*: 0.66 (CH_2Cl_2/MeOH, 7 : 3). ^{1}H-NMR (DMSO-d_6) δ: 1.55—1.57 (2H, m, CH_2 piperidine), 1.66—1.70 (4H, m, CH_2 piperidine), 2.93—2.99 (4H, m, CH_2 piperidine), 4.42 (2H, s, C<u>H</u>$_2$-Ph), 5.10 (1H, br s, OH), 6.63 (1H, s, Ar-H), 6.70 (1H, s, Ar-H), 6.86 (1H, s, Ar-H), 6.89 (1H, d, J=5.4 Hz, H quinoline), 7.49 (1H, dd, J=9.0, 2.3 Hz, H quinoline), 7.69 (1H, s, N=C<u>H</u>–NRR′), 7.83 (1H, d, J=2.2 Hz, H quinoline), 8.38 (1H, d, J=9.1 Hz, H quinoline), 8.40 (1H, d, J=5.3 Hz, H quinoline), 8.95 (1H, s, NH). TOF-PDMS *m/z*: 394.8 (M^+). HPLC: P_{HPLC} 95%, t_R 12.12 min.

{3-[(7-Chloro-4-quinolyl)amino]-5-[({4-methylpiperidino}methylidene)amino]phenyl}methanol (4) Yellow solid. Yield: 37% (75 mg). mp: 188 °C. *Rf*: 0.70 (CH_2Cl_2/MeOH, 8 : 2). ^{1}H-NMR (DMSO-d_6) δ: 0.99 (3H, d, J=6.4 Hz, CH_3), 1.12 (2H, m, CH_2 piperidine), 1.29 (1H, s, CH piperidine), 1.65—1.80 (4H, m, CH_2 piperidine), 3.06 (2H, m, CH_2 piperidine), 4.52 (2H, d, J=5.8 Hz, C<u>H</u>$_2$-Ph), 5.19 (1H, t, J=5.8 Hz, OH), 6.73 (1H, s, Ar-H), 6.80 (1H, s, Ar-H), 6.97 (1H, s, Ar-H), 6.99 (1H, d, J=5.4 Hz, H quinoline), 7.60 (1H, dd, J=9.0, 2.2 Hz, H quinoline), 7.80 (1H, s, N=C<u>H</u>–NRR′), 7.93 (1H, d, J=2.2 Hz, H quinoline), 8.48 (1H, d, J=9.1 Hz, H quinoline), 8.51 (1H, d, J=5.3 Hz, H quinoline), 9.04 (1H, s, NH). TOF-PDMS *m/z*: 408.4 (M^+). HPLC: P_{HPLC} 95%, t_R 13.19 min.

{3-[(7-Chloro-4-quinolyl)amino]-5-[({4-methylpiperazino}methylidene)amino]phenyl}methanol (5) Yellow solid. Yield: 27% (55 mg). mp: 195 °C. *Rf*: 0.28 (CH_2Cl_2/MeOH, 7 : 3). ^{1}H-NMR (DMSO-d_6) δ: 2.16 (3H, s, CH_3), 2.27 (4H, t, J=5.1 Hz, CH_2 piperazine), 3.40—3.50 (4H, m, CH_2 piperazine), 4.42 (2H, s, C<u>H</u>$_2$-Ph), 5.10 (1H, br s, OH), 6.64 (1H, s, Ar-H), 6.72 (1H, s, Ar-H), 6.88 (1H, s, Ar-H), 6.89 (1H, d, J=5.4 Hz, H quinoline), 7.50 (1H, dd, J=9.0, 2.2 Hz, H quinoline), 7.73 (1H, s, N=C<u>H</u>–NRR′), 7.83 (1H, d, J=2.2 Hz, H quinoline), 8.38 (1H, d, J=8.9 Hz, H quinoline), 8.41 (1H, d, J=5.3 Hz, H quinoline), 8.95 (1H, s, NH). TOF-PDMS *m/z*: 409.9 (M^+). HPLC: P_{HPLC} 95%, t_R 12.09 min.

{3-[(7-Chloro-4-quinolyl)amino]-5-[(morpholinomethylidene)amino]-phenyl}methanol (6) Yellow solid. Yield: 43% (85 mg). mp>250 °C. *Rf*: 0.83 (CH_2Cl_2/MeOH, 7 : 3). ^{1}H-NMR (DMSO-d_6) δ: 3.37—3.49 (4H, m, CH_2 morpholine), 3.55—3.58 (4H, m, CH_2 morpholine), 4.43 (2H, d, J=5.5 Hz, C<u>H</u>$_2$-Ph), 5.13 (1H, t, J=5.5 Hz, OH), 6.65 (1H, s, Ar-H), 6.73 (1H, s, Ar-H), 6.88 (1H, s, Ar-H), 6.94 (1H, d, J=5.3 Hz, H quinoline), 7.50 (1H, dd, J=9.0, 2.2 Hz, H quinoline), 7.76 (1H, s, N=C<u>H</u>–NRR′), 7.85 (1H, d, J=2.2 Hz, H quinoline), 8.38 (1H, d, J=9.3 Hz, H quinoline), 8.41 (1H, d, J=5.3 Hz, H quinoline), 8.96 (1H, s, NH). TOF-PDMS *m/z*: 396.7 (M^+). HPLC: P_{HPLC} 99%, t_R 11.09 min.

***N*-{3-[(7-Chloro-4-quinolyl)amino]-5-[(piperidinomethylidene)-amino]phenyl}methyl-*N*,*N*-diethyl amine (7)** To a solution of compound **1** (600 mg, 2 mmol, 1 eq), in a CH_2Cl_2/DMF 60 : 1 mixture 35 ml were added DIEA (350 μl, 2 mmol, 2 eq), and MnO_2 (2.6 g, 30 mmol, 15 eq). After stirring the mixture for 18 h at room temperature, the mixture was filtrated on celite and the solid residue washed with CH_2Cl_2 30 ml. To the filtrate was then added diethylamine (1 ml, 10 mmol, 5 eq), and, after stirring at room temperature for 1 h, $NaHB(OAc)_3$ (1.2 g, 6 mmol, 3 eq) was added. Following further stirring at room temperature for 18 h, aqueous $NaHCO_3$ 1 M was introduced and the mixture was left to stir for 15 min. The organic layer was then separated, and the aqueous layer washed with CH_2Cl_2. The organic layers were combined, washed with brine, separated and dried over $MgSO_4$. The solvent was evaporated and the residue purified by TLC (CH_2Cl_2/MeOH, 80 : 20) to yield the desired intermediate in 30% yield (210 mg). To a solution of this intermediate (130 mg, 0.37 mmol, 1 eq) in DMF 10 ml were added DIEA (65 μl, 0.73 mmol, 2 eq), and PyBroP (180 mg, 0.39 mmol, 1.1 eq). After stirring the mixture for 12 h at room temperature, piperidine was added (145 μl, 1.47 mmol, 4 eq). Following reflux of the mixture for 5 h, the solvent was evaporated, the solid residue washed with 1 M $NaHCO_3$ 15 ml and purified by TLC (CH_2Cl_2/MeOH, 80 : 20), to yield compound **7** as a yellow solid. Yield: 45% (75 mg). mp: 79 °C. *Rf*: 0.30 (CH_2Cl_2/MeOH, 7 : 3). ^{1}H-NMR (DMSO-d_6) δ: 1.00 (6H, t, J=4.6 Hz, CH_2<u>CH_3</u>), 1.52—1.54 (2H, m, CH_2 piperidine), 1.57—1.66 (4H, m, CH_2 piperidine), 2.90—3.03 (8H, m, CH_2 piperidine and <u>CH_2</u>CH_3), 3.55 (2H, s, C<u>H</u>$_2$-Ph), 6.64 (1H, s, Ar-H), 6.72 (1H, s, Ar-H), 6.95 (1H, d, J=5.4 Hz, H quinoline), 7.00 (1H, s, Ar-H), 7.48 (1H, dd, J=9.0, 2.3 Hz, H quinoline), 7.85 (1H, d, J=2.2 Hz, H quinoline), 8.28 (1H, s, N=C<u>H</u>–NRR′), 8.42 (1H, d, J=9.1 Hz, H quinoline), 8.46 (1H, d, J=5.3 Hz, H quinoline), 9.02 (1H, s, NH). TOF-PDMS *m/z*: 450.6 (M^+). HPLC: P_{HPLC} 100%, t_R 12.68 min.

Biological Evaluation Compounds **1**—**6** were evaluated in their chlorhydrate form and compound **7** in its basic form.

***In Vitro P. falciparum* Culture and Drug Assays** *P. falciparum* strains were maintained continuously in culture on human erythrocytes as described by Trager and Jensen.[17] *In vitro* antiplasmodial activity was determined using a modification of the semi-automated microdilution technique of Desjardins *et al*.[18] *P. falciparum* CQ-sensitive (F32a/Tanzania) and CQ-resistant (FcB1R/Colombia, PFB/Brazil) strains were used in sensitivity testing. FcB1R, F32a, and PFB strains were obtained by limit dilution. Stock solutions of CQ diphosphate and test compounds were prepared in sterile, distilled water and DMSO, respectively. Drug solutions were serially diluted with culture medium and added to asynchronous parasite cultures (0.5% parasitemia and 1% final hematocrit) in 96-well plates for 24 h, at 37 °C, prior to the addition of [^{3}H]hypoxanthine 0.5 μCi (1 to 5 Ci/mmol; Amersham, Les Ulis, France) per well for 24 h. The growth inhibition for each drug concentration was determined by comparison of the radioactivity incorporated in the treated culture with that in the control culture (without drug) maintained on the same plate. The concentration causing 50% inhibition (IC_{50}) was obtained from the drug concentration–response curve and the results were expressed as the mean from two independent experiments. The DMSO concentration never exceeded 0.1% and did not inhibit parasite growth.

Cytotoxicity Evaluation upon Human MRC-5 Cells and MPM A human diploid embryonic lung cell line (MRC-5, Bio-Whittaker 72211D) and MPM were used to assess cytotoxicity in host cells. The MPM were collected from the peritoneal cavity 48 h after stimulation with potato starch and seeded in 96-well microplates at 30000 cells per well. MRC-5 cells were seeded at 5000 cells per well. After 24 h, the cells were washed and 2-fold dilutions of the drug were added in standard culture medium (RPMI+5% fetal calf serum) 200 μl. The final DMSO concentration in the culture remained below 0.5%. The cultures were incubated with four concentrations of compounds (25, 12.5, 6.3 and 3.1 μM) at 37 °C in 5% CO_2–95% air for 7 d. Untreated cultures were included as controls. For MRC-5 cells, the cytotoxicity was determined using the colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (thiazolyl blue) assay.[19] The concentration causing 50% reduction of viable cells (CC_{50}) was obtained from the drug concentration–response curve. For macrophages, scoring was performed microscopically.

***In Vivo* Drug Assays in *P. berghei*-Infected Mice** The *in vivo* antimalarial activities were determined in mice infected with *P. berghei* (ANKA 65 strain). Four-week-old female Swiss mice (CD-1, 20—25 g) were in-

traperitoneally infected with about 10^7 parasitized erythrocytes, collected from the blood of an acutely infected donor animal. At the same time, the animals (3 animals per group) were orally administrated the test compound at a dose of 40 mg/kg (drug formulation in 100% DMSO). The administration continued on the 4 subsequent days by the intraperitoneal route. Untreated control animals generally die between 7—10 d following infection. Drug activity was evaluated as the prolongation of the mean survival time observed in untreated controls and/or by the reduction in parasitemia at 7 d after infection. The suppression of parasitemia was calculated by the formula: (average % parasitemia in controls−average % parasitemia in treated mice)/average % parasitemia in controls. Three infected, DMSO-dosed mice were used as controls.

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References

1) World Health Organisation, "The World Health Report, Conquering, Suffering, Enriching Humanity," World Health Organisation Publishers, Geneva, 1997.
2) World Health Organisation, *Trans. R. Trop. Med. Hyg*., **80**, 1—50 (1986).
3) Watkins W. M., Sixsmith D. G., Spencer H. G., Boriga D. A., Karjuki D. M., Kipingor T., Koech D. K., *Lancet*, **1984-i**, 357—359.
4) Neftel K. A., Woodtly W., Schmid M., *Br. Med. J*., **292**, 721—723 (1986).
5) Lind D. E., Leir J. A., Vincent P. C., *Br. Med. J*., **1**, 458—460 (1973).
6) Maggs J. L., Tingle M. D., Kitteringham N. R., Parks B. K., *Biochem. Pharmacol*., **37**, 303—311 (1988).
7) Christie G., Brechenridge A. M., Park B. K., *Biochem. Pharmacol*., **38**, 1451—1458 (1989).
8) Harrison A. C., Kitteringham N. R., Clarke J. B., Park B. K., *Biochem. Pharmacol*., **43**, 1421—1430 (1992).
9) O'Neill P. M., Harrison A. C., Storr R. C., Hawley S. R., Ward S. A., Park B. K., *J. Med. Chem*., **37**, 1362—1370 (1994).
10) Aziz S. A., Knowles C. O., *Nature* (London), **242**, 417—418 (1973).
11) Beeman R. W., Matsumura F., *Nature* (London), **242**, 273—274 (1973).
12) Meyers A. I., Elworthy T. R., *J. Org. Chem*., **57**, 4732—4740 (1992).
13) Delarue S., Sergheraert C., *Tetrahedron Lett*., **40**, 5847—5890 (1999).
14) Su T. S., Chou T. C., Kim J. Y., Huang J. T., Ciszewska G., Rens W. Y., Otter G. M., Sirotnak F. M., Watanabe K. A., *J. Med. Chem*., **38**, 3226—3235 (1995).
15) Abdel Magid A. F., Carson K. G., Harris B. D., Maryanoff C. A., Shah R. D., *J. Org. Chem*., **61**, 3849—3862 (1996).
16) Ridley R. G., *J. Pharm. Pharmacol*., **49**, 43—48 (1997).
17) Trager W., Jensen J. B., *Science*, **193**, 673—677 (1976).
18) Desjardins R. E., Canfield C. J., Haynes J. D., Chulay J. D., *Antimicrob. Agents Chemother*., **16**, 710—718 (1979).
19) Carmichael J., DeGraff W. G., Gazdar A. F., Minna I. D., Mitchell J. B., *Cancer Res*., **47**, 936—942 (1987).