Synthesis and in Vitro and in Vivo Antimalarial Activity of New 4-Anilinoquinolines†

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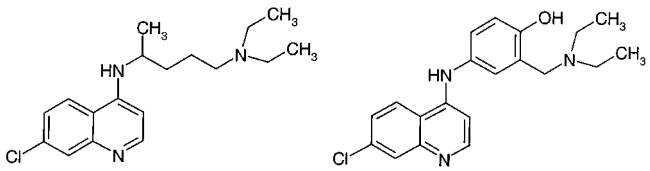
A new series of 4-anilinoquinolines with two proton-accepting side chains has been synthesized. Antimalarial activity and levels of cytotoxicity upon both MRC-5 cells and macrophages were found to be highly dependent upon the features of these side chains. Several compounds were found to be active in the low nanomolar range, against both chloroquine-sensitive and -resistant strains of *Plasmodium falciparum* in vitro. From among them, a morpholino derivative cured mice infected by *Plasmodium berghei* and displayed a lower toxicity than amodiaquine upon mouse macrophages.

Introduction

The last 40 years have seen the proliferation of multidrug-resistant *Plasmodium falciparum*, which has highlighted more than ever the need to develop new antimalarial drugs, by preference inexpensive, that is, affordable to the developing countries where the disease is prevalent.[1] This resistance has prompted a reexamination of the pharmacology of new and alternative antimalarials that may be effective against resistant strains. Early studies have revealed that chloroquine-resistant parasites are not necessarily cross-resistant to amodiaquine (AQ; Chart 1), another 4-aminoquinoline.[2] This was confirmed by a study in Kenya which demonstrated the effectiveness of AQ in the treatment of CQ-resistant *P. falciparum* malaria.[3] Recently, in a comparative trial of chloroquine (CQ; Chart 1) and AQ, for the treatment of uncomplicated *P. falciparum* infections in Gambia and in west and central Africa, AQ was found to be superior to CQ with lower parasitological and clinical failure rates.[4,5] Although AQ can no longer be recommended for prophylaxis because of reports of agranulocytosis and hepatitis,[6,7] no serious toxicity has been reported for its use in the treatment of acute malaria.[8,9] AQ toxicity has been explained by the presence of its 4-hydroxyanilino moiety, which is believed to undergo enzyme-catalyzed oxidation to its quinoneimine variant followed by nucleophilic addition of proteins.[10,11] Formation of this reactive species in vivo and subsequent binding to cytosol macromolecules could affect cellular function either directly or by immunological responses which initiate hypersensitivity reactions.[12,13] This bioactivation was found to be accompanied by the expression of a drug-related antigen on the cell surface, suggesting a type II hypersensitivity reaction[14] and causing the myelotoxicity of AQ.[15] Recent studies have demonstrated that the introduction of fluorine to the aromatic nucleus of AQ can influence the critical balance between drug activation and detoxification by reducing the process of oxidative bioactivation.[16] In addition, it has been confirmed that such modifications can be achieved without any significant loss of activity against CQ-resistant parasites and that deOH-4′-fluoro-AQ, which does not undergo bioactivation, displays an antimalarial activity which is similar to that of AQ. AQ has been reported as binding to heme,[17,18] and as inhibiting heme polymerization in vitro, with an efficiency similar to that of CQ,[19,20] supporting that they exert their antimalarial activity via similar mechanisms. AQ can adopt a bioactive conformation which includes an inter-nitrogen separation (N(quin)−N(diethyl)) of 8.3 Å, similar to that found in CQ.[21] The same distance has been measured by X-ray crystallography between the central iron atom and the oxygens in the carboxylate groups of heme,[18] confirming ferriprotoporphyrin as the "receptor target" of these 4-aminoquinolines. AQ competitively inhibits CQ accumulation, suggesting that these compounds operate via similar mechanisms of accumulation.[22] However, AQ is accumulated more efficiently in the parasite food vacuole than CQ although its pK_a values are lower, which indicates that uptake is enhanced by an additional mechanism.[23]

In view of this information we have synthesized a new series of 4-aminoquinolines whose design retains the aromatic ring of AQ, while lacking the hydroxyl group

Chart 1. Structure of Chloroquine and Amodiaquine



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† Abbreviations: AQ, amodiaquine; CQ, chloroquine; DIEA, diisopropylethylamine; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (thiazolyl blue); PyBroP, bromotrispyrrolidinophosphonium hexafluorophosphate.

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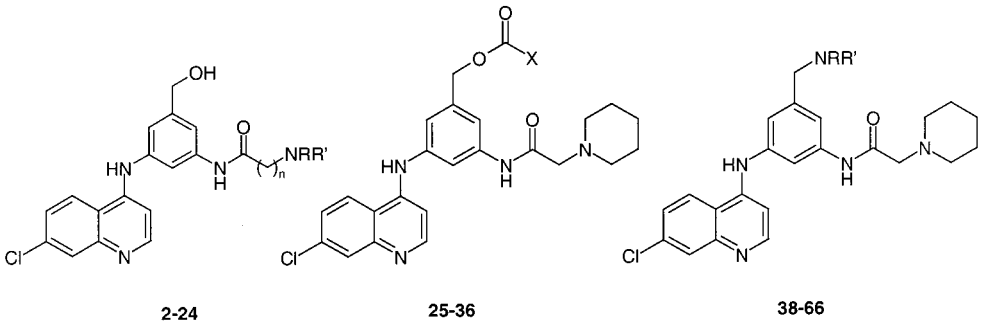
Chart 2. General Structures of Compounds **2**−**66**



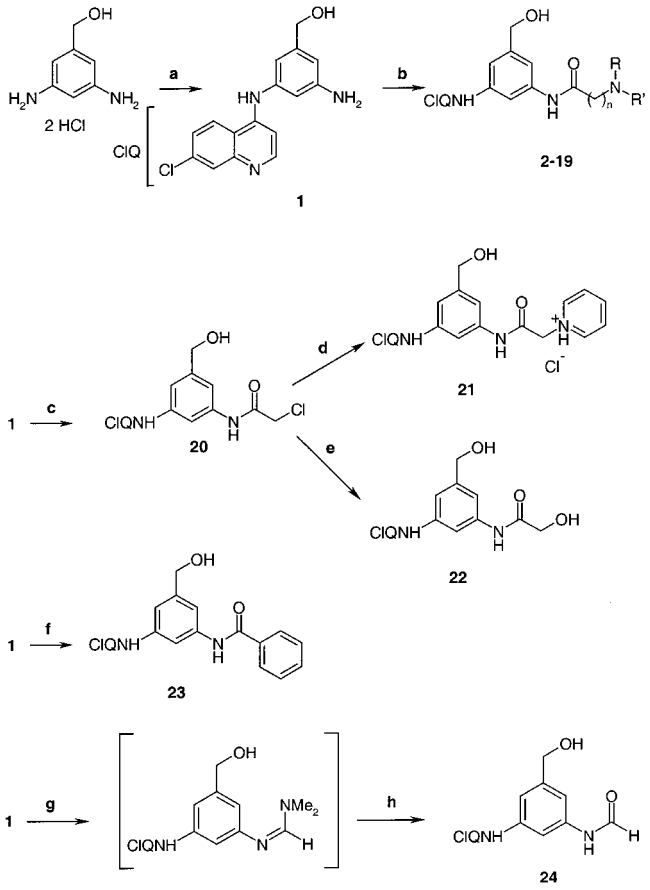
responsible for toxicity. The series equally possesses two proton-accepting side chains of varying length (Chart 2). This "dicationic" moiety is likely to interact with carboxylate groups of heme. A special feature of one of the chains is its linkage to the aromatic ring via a nitrogen atom. This attachment was optimized initially before the second chain was introduced by esterification of a benzylic alcohol or reductive amination of the corresponding benzaldehyde. The comparative ability of each compound to inhibit the growth of CQ-sensitive and -resistant strains of *P. falciparum* in vitro has been measured, the most potent among them being subsequently tested against *Plasmodium berghei* in mice.

Chemistry

The different compounds **2**−**66** are derivatives of compound **1**. The key intermediate **1** was obtained by condensation of 4,7-dichloroquinoline with 3,5-diaminobenzyl alcohol dihydrochloride, according to an aromatic nucleophilic substitution mechanism, in the presence of *N*-methylmorpholine as base and in a EtOH/$CHCl_3$ (55:5) mixture (Scheme 1).[24] Attachment of the lateral amino side chain of compounds **2**−**19** was accomplished in two steps: reaction of compound **1** with various halogenocarboxylic acids in DMF, using PyBroP as coupling reagent and 1-ethylpiperidine as base, followed by substitution of the remaining halogeno group by the appropriate amine (Scheme 1). The relatively low yields observed for compounds **3**−**7** were due to a side reaction yielding formamidines.[25] This was most notable for compounds corresponding to $n > 1$. Only the intermediate **20** (X = Cl, $n = 1$) was purified for the synthesis of compounds **21** and **22** (Scheme 1). The pyridinium salt **21** was obtained by substitution of the chloro group on compound **20** by pyridine (Scheme 1). The synthesis of hydroxyl derivative **22** was carried out in two steps: first, substitution of the chloro atom by potassium acetate, followed by the hydrolysis of the acetate group (Scheme 1). The low yield resulted from a dimerization during the first step. Aromatic derivative **23** was synthesized by acylation of compound **1** with benzoic acid, using PyBroP as coupling reagent, DIEA as base, and DMF as solvent (Scheme 1). The formamide **24** was obtained by heating at high temperature, in DMF, the formamidine obtained from the reaction of compound **1** with the "PyBroP-activated DMF" derivative (Scheme 1).[25]

Compounds **25**−**66** were derivatized from compound **8** ($n = 1$, NRR′ = piperidine). In the case of compounds **25**−**36**, the second side chain was introduced via an ester link, as shown in Scheme 2. The synthesis of esters

Scheme 1. Synthesis of Compounds **1**−**24**[a,b]

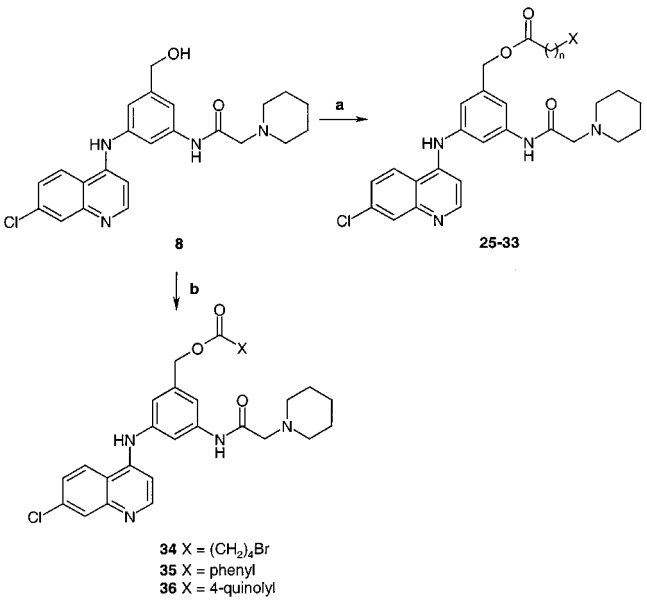


[a] Reagents: (a) 4,7-dichloroquinoline, *N*-methylmorpholine, EtOH/$CHCl_3$ (55:5); (b) $X(CH_2)_nCOOH$, PyBroP, 1-ethylpiperidine, DMF and then HNRR′, DMF; (c) chloroacetic acid, PyBrop, DIEA, DMF; (d) pyridine; (e) KOAc, DMF and then Na_2CO_3, MeOH; (f) benzoic acid, PyBroP, DIEA, DMF; (g) PyBroP, DMF; (h) DMF, Δ. [b] ClQ = 7-chloroquinolin-4-yl.

25−**33** was accomplished in two steps: reaction of compound **8** with various halogenocarboxylic acids in CH_2Cl_2, using DCC as coupling reagent and DMAP as base, followed by substitution of the remaining halogeno group by the appropriate amine. For compounds **34**−**36**, only the first step was carried out to obtain the desired product.

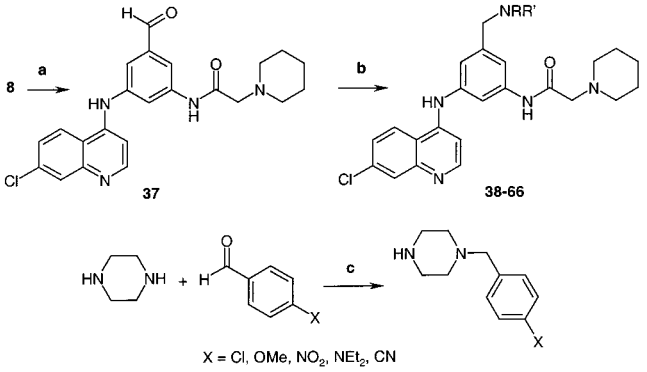
In the case of compounds **38**−**66**, the second side chain was introduced via an amino group, as described in Scheme 3. The first step was the oxidation of the alcohol to the corresponding aldehyde **37**, using MnO_2 as reagent. In the second step, a reductive amination between compound **37** and the appropriate amine, using sodium triacetoxyborohydride as reducing agent,[26] led

Scheme 2. Synthesis of Compounds **25**−**36**[a]



[a] Reagents: (a) $HOOC(CH_2)_nBr$, DCC, DMAP, CH_2Cl_2 and then HNRR′, 1-ethylpiperidine, CH_2Cl_2; (b) XCOOH, DCC, DMAP, CH_2Cl_2.

Scheme 3. Synthesis of Compounds **37**−**66**[a]



[a] Reagents: (a) MnO_2, CH_2Cl_2; (b) HNRR′, $NaHB(OAc)_3$, CH_2Cl_2; (c) $NaHB(OAc)_3$, CH_2Cl_2.

to the desired amino derivative. For benzylpiperazine derivatives **55**−**59**, the amines, not available commercially, were synthesized in advance via the same procedure from piperazine and the appropriate benzaldehyde (Scheme 3).

Biological Results

Initially the acylated derivatives of alcohol **1**, presenting a terminal 4-methylpiperidine, were tested for their activity against the CQ-resistant strain FcB1R (IC_{50}(CQ) = 126 nM, IC_{50}(AQ) = 7.4 nM). Activity was found to be dependent upon the number of methylene groups, and the best result (IC_{50} = 78 nM) was observed for compound **2** (n = 1, Table 1). This compound was devoid of cytotoxicity upon human MRC-5 cells (diploid embryonic lung cell line) at 6.3 μM and upon mouse peritoneal macrophages (MPMs) at 3.1 μM (Table 4). Replacement of the 4-methylpiperidine moiety by a variety of amines led to important differences of activity (IC_{50} values from 40 nM to the micromolar range). One of the three most potent derivatives, piperidino compound **8** (IC_{50} = 44.5 nM), was found to induce a decrease of 60% of parasitaemia on day 7 in mice infected by *P. berghei* (intraperitoneal route, 4-day test, 40 mg/kg) (Table 8) and was therefore selected for the derivatization steps of the benzylic alcohol group. For the ester series, in the optimization of the length of the basic side chain, the best activity profiles were attained for n = 4 (compound **27**) and n = 5 (compound **28**) (Table 2, IC_{50} = 15.5 and 27.4 nM, respectively), though cytotoxic effects upon MRC-5 cells were observed at 6.3 μM (Table 5). The replacement of the piperidino ring of compound **27** by a variety of groups, basic or not (compounds **30**−**36**), induced variations in the cytotoxicity upon MRC-5 cells (Table 5). Introduction of a morpholino group (compound **31**) was particularly interesting since no cytotoxic effects were observed while antimalarial activity was preserved (IC_{50} = 14.1 nM). In the series of amines (Table 3), prepared by reductive amination of the aldehyde **37**, all the IC_{50} values were below 20 nM, except for that of compound **39**, and the antimalarial activity was found to be independent of the hydrophobic character of the compounds (see compounds

Table 1. In Vitro Sensitivity of *P. falciparum* FcB1R Strain to Compounds **1**−**24**

compd	n	NRR′	IC_{50} (nM)
CQ			126 ± 26[b]
AQ			7.4 ± 2.7[b]
1			278.9 ± 83.0[b]
2	1	4-methylpiperidine	77.7 ± 52.3[b]
3	2	4-methylpiperidine	135.5 ± 23.5[a]
4	4	4-methylpiperidine	465.0 ± 47.0[a]
5	5	4-methylpiperidine	304.5 ± 21.5[a]
6	7	4-methylpiperidine	308.5 ± 43.5[a]
7	11	4-methylpiperidine	86.0 ± 5.0[a]
8	1	piperidine	44.5 ± 9.7[b]
9	1	*N*-methylpiperazine	344 ± 32[a]
10	1	morpholine	>1000
11	1	4-hydroxypiperidine	1050 ± 510[a]
12	1	pyrrolidine	44.0 ± 5.8[b]
13	1	thiazolidine	588.0 ± 41.0[a]
14	1	$NHC(CH_3)_3$	50.9 ± 10.6[b]
15	1	NEt_2	41.6 ± 6.1[b]
16	1	$NHCH_2C_6H_5$	192.7 ± 57.6[b]
17	1	$NHCH_2C_6H_4Cl$ (*para*)	855 ± 225[a]
18	1	1,2,3,4-tetrahydroisoquinoline	1450 ±350[a]
19	1	3-aminopyrazole	806.5 ± 86.5[a]
20	1	Cl	>1000
21	1	$NC_5H_5^+$, Cl^-	927 ± 37[a]
22	1	OH	>1000
23	0	phenyl	>1000
24	0	H	359.8 ± 7.8[a]

[a] n_e (the number of experiments) = 2. [b] n_e = 3.

Table 2. In Vitro Sensitivity of *P. falciparum* FcB1R Strain to Compounds **8** and **25**−**36**

compd	n	X	IC_{50} (nM)
CQ			126 ± 26[a]
AQ			7.4 ± 2.7[a]
8			44.5 ± 9.7[a]
25	1	piperidine	54.0 ± 12.6[a]
26	2	piperidine	78.3 ± 38.3[b]
27	4	piperidine	15.5 ± 4.2[b]
28	5	piperidine	27.4 ± 10.3[a]
29	7	piperidine	165.0 ± 59.0[a]
30	4	pyrrolidine	23.0 ± 3.5[a]
31	4	morpholine	14.1 ± 1.5[a]
32	4	*N*-methylpiperazine	65.1 ± 7.2[a]
33	4	NEt_2	141.1 ± 5.7[a]
34	4	Br	151.1 ± 22.7[a]
35	0	phenyl	75.5 ± 14.1[a]
36	0	quinol-4-yl	25.6 ± 5.2[c]

[a] n_e = 3. [b] n_e = 4. [c] n_e = 6.

Table 3. In Vitro Sensitivity of *P. falciparum* FcB1R Strain to Compounds **8** and **37**−**66**

compd	NRR′	IC_{50} (nM)
CQ		126 ± 26[b]
AQ		7.4 ± 2.7[b]
8		44.5 ± 9.7[b]
37		281.7 ± 35.3[b]
38	$NHCH_2CH_2NMe_2$	15.5 ± 2.9[b]
39	$NHCH_2CH_2CH_2NMe_2$	134 ± 2[a]
40	$NMeCH_2CH_2NMe_2$	5.0 ± 0.8[b]
41	$NHCH_2CH_2$-pyrrolidine	10.2 ± 1.3[a]
42	$NHCH_2CH_2$-pyrrolidine	6.6 ± 1.6[a]
43	$NHCH_2CH_2$-pyrrolidine	7.9 ± 0.7[a]
44	NEt_2	8.2 ± 1.4[a]
45	NH*t*Bu	3.7 ± 1.8[b]
46	piperidine	10.2 ± 3.6[c]
47	pyrrolidine	6.9 ± 3.3[b]
48	*N*-methylpiperazine	7.5 ± 3.1[d]
49	morpholine	9.5 ± 3.4[b]
50	4-hydroxypiperidine	6.5 ± 2.0[b]
51	*N*-(2-hydroxyethyl)piperazine	11.5 ± 0.6[a]
52	*N*-phenylpiperazine	5.5 ± 0.6[b]
53	*N*-benzylpiperazine	13.0 ± 4.7[b]
54	*N*-(diphenylmethyl)piperazine	12.5 ± 3.2[b]
55	*N*-(4-chlorobenzyl)piperazine	11.5 ± 0.3[a]
56	*N*-(4-methoxybenzyl)piperazine	11.0 ± 1.4[a]
57	*N*-(4-nitrobenzyl)piperazine	13.2 ± 0.5[a]
58	*N*-(4-diethylaminobenzyl)piperazine	11.9 ± 0.6[a]
59	*N*-(4-cyanobenzyl)piperazine	13.3 ± 0.5[a]
60	*N*-piperonylpiperazine	6.9 ± 2.6[b]
61	NHC_6H_5	21.2 ± 1.5[a]
62	$NHCH_2C_6H_5$	8.5 ± 5.7[b]
63	$NHCH(C_6H_5)_2$	9.1 ± 1.2[a]
64	$NHCH_2C_6H_4Cl$ (*para*)	4.6 ± 0.2[a]
65	$NHCH_2C_6H_4OMe$ (*para*)	4.4 ± 0.1[a]
66	$NHCH_2C_6H_4CF_3$ (*para*)	5.5 ± 0.1[a]

[a] $n_e = 2$. [b] $n_e = 3$. [c] $n_e = 6$. [d] $n_e = 7$.

Table 4. In Vitro Cytotoxicity of Compounds **1**−**24** upon MRC-5 Cells and MPMs

	cytotoxicity upon MRC-5 cells (%)			cytotoxicity upon MPMs[a]		
compd	12.5 μM	6.3 μM	3.1 μM	12.5 μM	6.3 μM	3.1 μM
CQ	0	0	0	–	–	–
AQ	0	0	0	T	T	–
1	0	0	0	–	–	–
2	100	0	0	T	T	–
3	100	0	0	T	–	–
4	0	0	0	–	–	–
5	00	0	0	–	–	–
6	0	0	0	–	–	–
7	100	0	0	T	–	–
8	0	0	0	T	–	–
9	0	0	0	–	–	–
10	0	0	0	–	–	–
11	0	0	0	nd	nd	nd
12	0	0	0	T	–	–
13	0	0	0	–	–	–
14	0	0	0	–	–	–
15	0	0	0	T	–	–
16	0	0	0	–	–	–
17	0	0	0	nd	nd	nd
18	0	0	0	nd	nd	nd
19	0	0	0	–	–	–
20	92	0	0	T	–	–
21	0	0	0	–	–	–
22	0	0	0	–	–	–
23	0	0	0	–	–	–
24	0	0	0	–	–	–

[a] The letter T means that the compound is toxic at this concentration, the minus sign denotes no toxicity, and nd means not determined.

52−**60**). Among them, several were more efficient than AQ (IC_{50} = 7.4 nM). However, cytotoxicities were generally enhanced (Table 6) with the exception of that of the morpholino derivative (compound **49**), which displayed a unique and interesting behavior as did its counterpart in the ester series. Therefore, morpholino ester **31** and morpholino amine **49** as well as their piperidino counterparts **27** and **46** were tested against the CQ-sensitive THAI strain and CQ-resistant K1

Table 5. In Vitro Cytotoxicity of Compounds **8** and **25**−**36** upon MRC-5 Cells and MPMs

	cytotoxicity upon MRC-5 cells (%)			cytotoxicity upon MPMs[a]		
compd	25 μM	6.3 μM	1.6 μM	12.5 μM	6.3 μM	2 μM
CQ	0	0	0	–	–	–
AQ	91	0	0	T	T	–
8	98	0	0	T	–	–
25	93	0	0	–	–	–
26	90	0	0	–	–	–
27	92	90	0	–	–	–
28	92	81	0	–	–	–
29	83	84	0	T	T	–
30	82	83	0	–	–	–
31	0	0	0	–	–	–
32	97	0	0	–	–	–
33	22	13	0	–	–	–
34	0	0	0	–	–	–
35	66	38	20	T	–	–
36	100	100	62	T	T	–

[a] The letter T means that the compound is toxic at this concentration, and the minus sign denotes no toxicity.

Table 6. In Vitro Cytotoxicity of Compounds **8** and **37**−**66** upon MRC-5 Cells and MPMs

	cytotoxicity upon MRC-5 cells (%)			cytotoxicity upon MPMs[a]		
compd	32 μM	8 μM	1 μM	32 μM	8 μM	2 μM
CQ	0	0	0	–	–	–
AQ	91	0	0	T	T	–
8	99	0	0	T	–	–
37	0	0	0	nd	–	–
38	100	15	0	T	–	–
39	78	0	0	T	–	–
40	100	100	43	T	T	–
41	100	100	6	T	T	–
42	100	100	27	T	T	–
43	100	100	0	T	T	–
44	100	100	0	T	T	T
45	100	100	0	T	T	–
46	100	100	43	T	T	T
47	100	100	0	T	T	–
48	83	80	0	T	T	–
49	100	0	0	T	–	–
50	100	100	0	T	T	–
51	100	100	0	T	T	–
52	100	100	0	T	T	–
53	100	100	0	T	T	–
54	98	97	87	T	T	–
55	100	100	0	T	T	–
56	100	100	50	T	T	–
57	100	99	0	T	T	–
58	100	100	100	T	T	T
59	100	100	0	T	–	–
60	100	99	86	T	T	–
61	100	100	0	T	T	–
62	100	100	96	T	T	–
63	100	100	0	T	T	–
64	100	100	100	T	T	T
65	100	100	100	T	T	T
66	100	100	0	T	T	–

[a] The letter T means that the compound is toxic at this concentration, the minus sign denotes no toxicity, and nd means not determined.

Table 7. Efficiency of Compounds **8**, **27**, **31**, **46**, and **49** To Inhibit Growth of Parasites Expressing Different Degrees of Resistance to CQ

	IC_{50} (nM)[a]		
compd	THAI	FcB1	K1
CQ	14.3 ± 2.4^b	126 ± 26^b	183 ± 35^b
AQ	7.3 ± 0.2^b	7.4 ± 2.7^b	14.5 ± 0.5^b
8	89.6 ± 13.1^b	44.5 ± 9.7^b	151.6 ± 48^d
27	17.3 ± 5.5^b	21.2 ± 8.4^d	19.1 ± 4.3^d
31	15.2 ± 1.6^b	14.1 ± 1.3^b	22.5 ± 6.5^b
46	6.7 ± 0.9^b	10.0 ± 3.8^c	8.1 ± 0.5^b
49	12.8 ± 1.1^b	9.6 ± 3.4^b	12.4 ± 1.1^b

[a] Parasites were considered resistant to CQ for $IC_{50} > 100$ nM. [b] $n_e = 3$. [c] $n_e = 4$. [d] $n_e = 6$.

Table 8. Antimalarial Activity of Compounds **8**, **27**, **31**, and **49** on *P. berghei* in Mice

compd	dose	reduction (%) in parasitemia at day 4	excess MST (%)[a]
8	40	60[b]	110
27	40	99.99	0[c]
31	40	97.9	100
49	40	100	C

[a] Excess MST is the change in the mean survival time of the treated mice, calculated by comparing the mean survival time of the control mice with the mean survival time of the treated mice. C indicates mice surviving the infection and which can be termed cured definitively. Each entry represents results with a group comprising three animals. [b] Reduction of parasitemia measured on day 7. [c] Mice which died on the seventh day owing to toxicity.

strain. IC_{50} values were found to be quite similar, whatever the CQ resistance of the strain (Table 7). In vivo activities of compounds **27**, **31**, and **49** (Table 8) were then compared with that of compound **8** in a series of assays with the murine *P. berghei* model. Piperidino compound **27** showed high toxicity against mice at 40 mg/kg. All the mice were dead by day 7, while an almost 100% reduction of parasitaemia was observed at day 4. The mean survival time was doubled at 20 mg/kg (data not shown). Comparatively, at 40 mk/kg, the morpholino amine compound **49** completely cured the mice from *P. berghei* infection, while the ester analogue **31** only increased the mean survival time by 100%. At 20 mg/kg, an absence of antimalarial activity and a weak activity were observed for these two latter compounds, respectively (data not shown).

Discussion

The metabolism of AQ has been widely investigated.[11,27,28] Its protein conjugates are involved in both toxicity and clearance,[28] and their formation has been so far prevented either by fluorine substitution at the 4′-position of the aromatic nucleus[16] or by introduction of an alkyl substituent at the 5′-position.[29] The special feature of the new family reported here is the presence of a basic side chain at both the 3′- and 5′-positions and therefore the impossibility of nucleophilic addition of proteins even in the case of a metabolic hydroxylation at the hindered 4′-site. The starting alcohol **1** was chosen because of its in vitro activity ($IC_{50} = 280$ nM against the CQ-resistant strain FcB1R), its possible use as a scaffold, and its easy synthesis in a large amount by simple precipitation. Acylation of its anilino group by side chains of varying length, bearing a terminal methylpiperidine, leads to a range of activities with optima for both the lowest and highest values of n (Table 1), the latter ($n = 11$) being explained by the folding of the chain, which may replace the cationic headgroup in the right position. In both cases, the decrease of activity (IC_{50} values of, respectively, 78 and 86 nM), compared with that of AQ ($IC_{50} = 7.4$ nM), can be attributed to a less efficient two-point attachment to the heme target at least partially dependent upon the loss of the intramolecular hydrogen bond responsible for the bioactive conformation of AQ. The same absence of an intramolecular hydrogen bond has been reported to induce a similar decrease for deOH-4′-fluoro-AQ.[16] As expected, the loss of the terminal basic site corresponding to the replacement of the methylpiperidine ring by a chlorine atom, a hydroxyl group, or an aromatic moiety led to a decrease of activity (Table 1). However, the lack of efficiency of the morpholino and thiazolidino analogues **10** and **13** was surprising. In the ester series, addition of a second basic site likely to increase intracellular accumulation within the food vacuole of the parasite and to interact with the second heme carboxylate permits the observation, at optimal distances from the phenyl ring ($n = 4$ and 5), of IC_{50} values similar to that of AQ (Table 2). Alternately, in the amine series, the efficient two-point attachment of AQ seems to be definitively restored since compounds are generally found to be as active as or more active than AQ. When it occurs, such an enhancement of activity can be explained in terms of lipophilicity since *tert*-butyl, phenyl, and polyphenyl side chains are among the most potent derivatives (Table 3). This increase is in accord with the effect described for an alkyl substituent in the 5′-position of the 3′-*tert*-butylamino analogue of AQ.[29] When compared with AQ, morpholino ester **31**, morpholino amine **49**, and their piperidino counterparts **27** and **46**, which all possess an additional proton-accepting site, displayed a similar efficiency against THAI, FcB1R, and K1 strains, revealing different degrees of resistance to CQ. The absence of cytotoxicity or low cytotoxicity of morpholino derivatives upon MRC-5 cells and murine macrophages, compared with other amino analogues such as the piperidino variants, remains unexplained. This difference in toxicity is recovered in vivo when mice are treated at 40 mg/kg, either with morpholino ester **31** or with the piperidino counterpart **27**. Although the latter induces a greater reduction in parasitaemia on day 4, the mice died on day 7 because of its toxicity (Table 8). Compound **49**, which cures mice at 40 mg/kg, shows a tendency toward less cytotoxic effects than AQ, expressing a similar level upon MRC-5 cells, while expressing a comparatively more modest toxicity upon mouse macrophages.

In conclusion, these families of derivatives constitute a new contribution in the establishment of the structural features of the 4-anilinoquinolines which can induce the absence of cross resistance with CQ and a decrease of cytotoxicity. Some of these features can be deduced from compound **49**, which displays a consistent activity irrespective of the degree of CQ resistance of the strains used and, unlike AQ, is devoid of toxicity upon mouse macrophages at 8 μM. Work is now in progress to optimize the nature and the length of the second side chain within the amine series which appears to be promising.

Materials and Methods

Biological Evaluation. Compounds **1**−**21** were evaluated in their chlorohydrate 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.[30] In vitro antiplasmodial activity was determined using a modification of the semiautomated microdilution technique of Desjardins et al.[31] *P. falciparum* CQ-sensitive (THAI/Thailand) and CQ-resistant (FcB1R/Colombia and K1/Thailand) strains were used in sensitivity testing. Stock solutions of chloroquine diphosphate and test compounds were prepared in sterile distilled water and DMSO, respectively. Drug solutions were serially diluted with culture medium and introduced to asynchronous parasite cultures (0.5% parasitemia and 1% final hematocrite) on 96-well plates for 24 h at 37 °C prior to the addition of 0.5 μCi of [^{3}H]hypoxanthine (1−5 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 into 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 ± standard deviation determined from several independent experiments. The DMSO concentration never exceeded 0.1% and did not inhibit the parasite growth.

Cytotoxicity Test upon MRC-5 Cells and Mouse Peritoneal Macrophages. A human diploid embryonic lung cell line (MRC-5, Bio-Whittaker 72211D) and mouse primary peritoneal macrophages were used to assess the cytotoxic effects toward host cells. The peritoneal macrophages were collected from the peritoneal cavity 48 h after stimulation with potato starch, and they were 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 200 μL of standard culture medium (RPMI + 5% FCS). The final DMSO concentration in the culture remained below 0.5%. The cultures were incubated with several concentrations of compounds (between 32 and 1.6 μM) at 37 °C in 5% CO_2−95% air for 7 days. Untreated cultures were included as controls. For MRC-5 cells, the cytotoxicity was determined using the colorimetric MTT assay[31] and scored as a percent reduction of absorption at 540 nm of treated cultures versus untreated control cultures. For macrophages, scoring was performed microscopically.

In Vivo Drug Assays upon *P. berghei*. The 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 intraperitoneally infected with about 10^7 parasitized erythrocytes, collected from the blood of an acutely infected donor animal. At the same time, the animals (three animals per group) were orally treated with the test compound at 40 mg/kg (drug formulation in 100% DMSO). The treatment was continued during the following 4 days by the intraperitoneal route. Untreated control animals generally die between 7 and 10 days following infection. Drug activity was evaluated by the reduction of parasitaemia at day 4 and by the prolongation of the mean survival time compared to that of untreated controls. Three infected, DMSO-dosed mice were used as controls.

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Supporting Information Available: Details of chemical procedures and spectral data. This material is available free of charge via the Internet at http://pubs.acs.org.

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