Articles

Structure−Activity Relationships for Antiplasmodial Activity among 7-Substituted 4-Aminoquinolines

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Aminoquinolines (AQs) with diaminoalkane side chains ($-HNRNEt_2$) shorter or longer than the isopentyl side chain [$-HNCHMe(CH_2)_3NEt_2$] of chloroquine are active against both chloroquine-susceptible and -resistant *Plasmodium falciparum*. (De, D.; et al. *Am. J. Trop. Med. Hyg.* **1996**, *55*, 579−583). In the studies reported here, we examined structure−activity relationships (SARs) among AQs with different *N,N*-diethyldiaminoalkane side chains and different substituents at the 7-position occupied by Cl in chloroquine. 7-Iodo- and 7-bromo-AQs with diaminoalkane side chains [$-HN(CH_2)_2NEt_2$, $-HN(CH_2)_3NEt_2$, or $-HNCHMeCH_2NEt_2$] were as active as the corresponding 7-chloro-AQs against both chloroquine-susceptible and -resistant *P. falciparum* (IC_{50}s of 3−12 nM). In contrast, with one exception, 7-fluoro-AQs and 7-trifluoromethyl-AQs were less active against chloroquine-susceptible *P. falciparum* (IC_{50}s of 15−50 nM) and substantially less active against chloroquine-resistant *P. falciparum* (IC_{50}s of 18−500 nM). Furthermore, most 7-OMe-AQs were inactive against both chloroquine-susceptible (IC_{50}s of 17−150 nM) and -resistant *P. falciparum* (IC_{50}s of 90−3000 nM).

Introduction

Malaria is a public health problem of overwhelming importance, with 300−400 million cases and 1−2 million deaths each year, especially among children with *Plasmodium falciparum* infection in Africa.[1] In addition, the available antimalarial drugs are losing their efficacy because the malaria parasite has become resistant to most existing antimalarials.[2] Chloroquine (Figure 1) is an exceptionally safe antimalarial and has been used widely for more than 40 years. However, its value has been compromised by the emergence of chloroquine-resistant *P. falciparum* in South America, Southeast Asia, and Africa.[3] Therefore, there is an urgent need for the development of alternative drugs that circumvent chloroquine resistance. Although the U.S. Army Antimalarial Drug Development Program and others have synthesized and tested more than 300 000 compounds as potential antimalarials (including 4-aminoquinolines),[4] relatively little information is available about the structure−activity relationships (SARs) responsible for their activity. In addition, chloroquine resistance was not recognized as a clinical problem until the mid-1960s. Thus, if the SARs responsible for aminoquinoline (AQ) activity against chloroquine-susceptible *P. falciparum* were different from those responsible for activity against chloroquine-resistant *P. falciparum*, then that would not have been apparent in those studies. On the basis of this rationale, it may be possible to develop AQs that are active against both chloroquine-susceptible and -resistant *P. falciparum*.

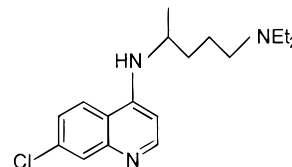

Figure 1. Structure of chloroquine.

We began by synthesizing a series of AQs and testing their activity against *P. falciparum* in vitro.[5] Those studies demonstrated that removal of the Cl at the 7-position abolished antiplasmodial activity and that most substitutions on the AQ ring markedly reduced antiplasmodial activity. We also found that AQs with short (2−3 carbon) and long (10−12 carbon) diaminoalkane side chains were active against chloroquine-susceptible, chloroquine-resistant, and multiply-resistant *P. falciparum* in vitro (IC_{50}s of 3−12 nM) (Table 1).[5a] In contrast, AQs with intermediate (tetra-, penta-, hexa-, and octa-methylene) diaminoalkane side chains were active against chloroquine-susceptible *P. falciparum* (IC_{50}s of 4−7 nM) but not against chloroquine-resistant *P. falciparum* (IC_{50}s of 41−90 nM) (Table 1).[5a] Furthermore, in vivo testing of AQs with short diaminoalkane side chains (**AQ-13**, **AQ-21**, **AQ-34**) demonstrated that they were active in monkey models of human *Plasmodium vivax* infection (the rhesus monkey/*Plasmodium cynomolgi* model of human *P. vivax* infection) and *P. falciparum* infection (the squirrel monkey/

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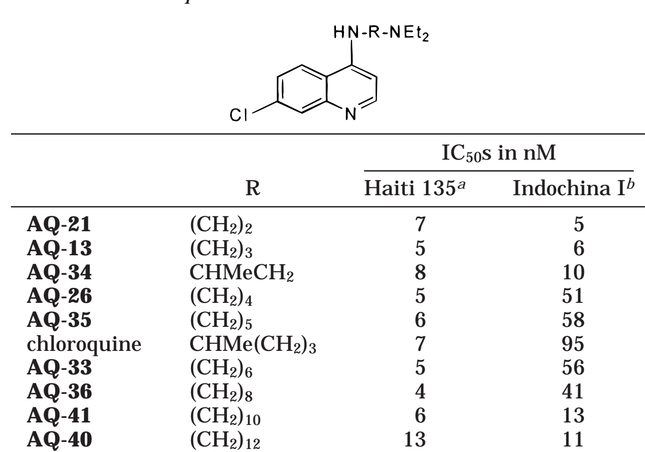
Table 1. Antiplasmodial Activity (IC_{50}) of Chloroquine Analogues with Varying Diaminoalkane Side Chain against *Plasmodium falciparum* in Vitro[5a]



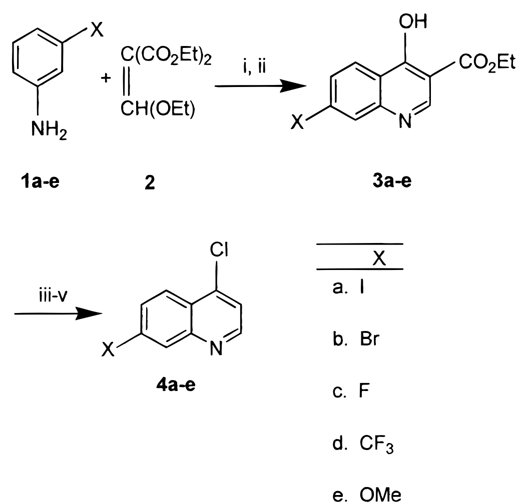
	R	IC_{50}s in nM	
		Haiti 135[a]	Indochina I[b]
AQ-21	$(CH_2)_2$	7	5
AQ-13	$(CH_2)_3$	5	6
AQ-34	$CHMeCH_2$	8	10
AQ-26	$(CH_2)_4$	5	51
AQ-35	$(CH_2)_5$	6	58
chloroquine	$CHMe(CH_2)_3$	7	95
AQ-33	$(CH_2)_6$	5	56
AQ-36	$(CH_2)_8$	4	41
AQ-41	$(CH_2)_{10}$	6	13
AQ-40	$(CH_2)_{12}$	13	11

[a] Haiti 135: chloroquine-susceptible *P. falciparum*. [b] Indochina I: chloroquine-resistant *P. falciparum*.

P. falciparum model of human *P. falciparum* infection).[5b] Thus, data available before these studies were performed suggested that one could circumvent chloroquine resistance in vitro and in vivo by modifying the diaminoalkane side chain.

After demonstrating that AQs with a variety of diaminoalkane side chains were active against chloroquine-resistant parasites, the next question was the importance of the Cl at the 7-position. Because each of the biologically active AQs studied initially had a Cl at the 7-position,[4] we have synthesized a series of AQs with different substituents at the 7-position and have tested their activity against chloroquine-susceptible and -resistant *P. falciparum* in vitro. In the studies reported here, we substituted F, Br, I, CF_3, or OMe for the Cl at the 7-position of the AQ ring. We were particularly interested in fluorine because of its distinct physical properties (high ionization potential, high electronegativity, small size, tightly held nonbonding electron pairs) in comparison with chlorine.[6] For these reasons, fluorocarbons often have different physicochemical properties from other halocarbons, and fluoro-substitution may alter the biological properties of drugs. For example, O'Neil et al. replaced the 4-hydroxyl group of amodiaquine with fluorine and found that the resulting AQ, like amodiaquine, was active against both chloroquine-susceptible and -resistant *P. falciparum* in vitro. However, unlike amodiaquine, it was not metabolized to a toxic quinone-imine.[7] They also reported several fluoro-substituted primaquine analogues, which had reduced hematologic toxicity because they were not metabolized to toxic quinone-imines, although their antimalarial efficacy was unaffected.[8] Other investigators have reported that fluoro-substituted artemisinin derivatives and 1,2,4-trioxanes are more active against *P. falciparum* in vitro than the nonfluorinated parent compounds.[9,10] Thus, the idea of replacing the 7-Cl with fluorine was attractive because it might produce chloroquine analogues active against chloroquine-resistant parasites. Trifluoromethyl (CF_3) analogues could also be active, although the trifluoromethyl group is more hydrophobic than fluorine.[6] In contrast, the size and electronegativity of bromine and iodine are quite different from those of fluorine or chlorine.[6] Because AQs

Scheme 1[a]



[a] Reagents: (i) 135−150 °C; (ii) Ph_2O, reflux; (iii) 2 N NaOH, reflux; (iv) Ph_2O, reflux; (v) $POCl_3$, 90−100 °C, NH_4OH.

with an OMe group at the 6-position such as primaquine [N^4-6-methoxy-8-quinolinyl-1,4-pentanediamine][11a,b] and WR238605[11c] are active against *P. vivax* and because OMe is more hydrophilic than Cl,[11d] we also replaced the 7-Cl with OMe to produce a series of 7-OMe-substituted AQs for in vitro testing.

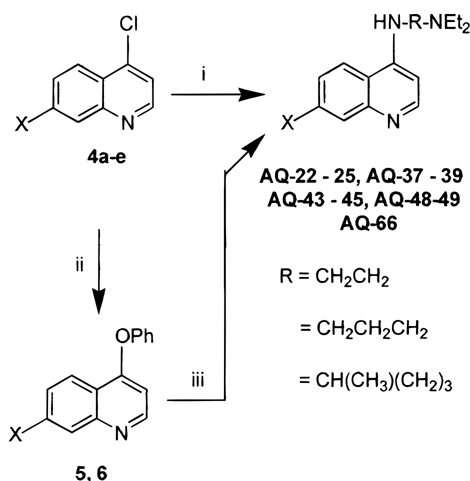
Chemistry

4-Chloro-7-substituted quinolines (CSQs) were prepared using a modification of methods reported previously (Scheme 1).[12,13] Condensation of *m*-substituted anilines (**1a**−**e**) with diethyl ethoxy methylenemalonate **2** at elevated temperatures (135−150 °C) produced *N*-substituted acrylates in quantitative yields. These acrylates were converted to 3-carbethoxy-4-hydroxy-7-substituted quinolines (quinoline esters) by thermal cyclization in boiling phenyl ether (Ph_2O). The yield of quinoline esters (**3a**−**e**) was optimized by using excess solvent (Ph_2O) and by adding the acrylates after the Ph_2O had begun refluxing to minimize formation of isomeric 3-carbethoxy-4-hydroxy-5-substituted quinolines. Subsequently, a single recrystallization from 70% EtOH−H_2O yielded pure **3a**−**e**. Alkaline hydrolysis of **3a**−**e**, followed by decarboxylation in refluxing Ph_2O and treatment with phosphorus oxychloride ($POCl_3$), produced **14a**−**e** in excellent yield.

After producing 4-chloro-7-substituted quinolines as outlined in Scheme 1, thirty AQs were synthesized using either (1) nucleophilic substitution at the C-4 chlorine with *N*,*N*-diethyl-α,ω-diaminoalkanes or (2) nucleophilic substitution with α,ω-diaminoalkanes, followed by regioselective reductive alkylation to convert the terminal primary amine on the side chain to diethylamine.

When appropriate *N*,*N*-diethyl-α,ω-diaminoalkanes were available commercially, they were used both as reagent and solvent to synthesize the desired AQs (Scheme 2, general method A). Although previous syntheses used phenol as a solvent,[14] we performed the nucleophilic substitution in neat amine to avoid the use of toxic phenol as a solvent and polymerization. The desired AQs were then purified using basic alumina column chromatography.[15] The synthesis of **AQ-17** and **AQ-27** by reacting **4c** or **4d** with 2-amino-5-diethylami-

Scheme 2[a]



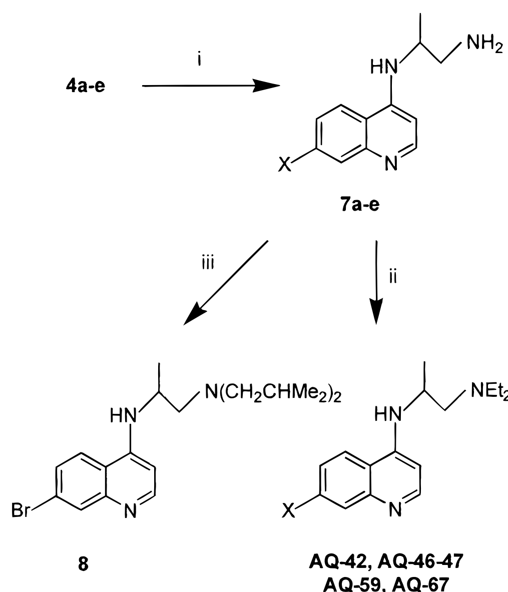
[a] Reagents: (i) H_2N-R-NEt_2, 135−150 °C; (ii) phenol; (iii) $H_2NCH(CH_3)(CH_2)_3NEt_2$, 145−155 °C.

nopentane in neat amine resulted in unacceptably poor yields. However, replacing the Cl at C-4 in **4c** and **4d** with a phenoxide produced intermediates **5** and **6**, and permitted the synthesis of **AQ-17** and **AQ-27** in yields of 57% and 65%, respectively.[14,16]

The structural assignments proposed for these AQs are consistent with their ^{1}H NMR spectra and are supported by the mass spectrometry data. For example, the ^{1}H NMR spectra of AQs with F at C-7 (e.g., **AQ-25**, **AQ-38**, **AQ-17**) revealed the coupling of aromatic protons (Ar−H,^{5} Ar−H,^{6} Ar−H^8) with the fluorine atom at C-7.[16] Likewise, the signal from the Ar−H^5 proton of **AQ-22** was recorded as a doublet (J = 9.0 Hz), whereas the same proton in **AQ-25** was recorded as a double doublet (J = 8.0, 1.5 Hz), consistent with *m*-coupling between that proton and the fluorine at C-7.[17,18] Moreover, the ^{19}F NMR spectrum of **AQ-17** [examined at 300 K in $CDCl_3$ with CCl_3F as the internal standard (δ 0.00 ppm) using a 500 MHz GE spectrometer[17]] revealed multiplates at δ −112.13 to −112.81 ppm from the coupling of aromatic protons (Ar−H,^{5} Ar−H,^{6} Ar−H^8) with the ^{19}F at C-7.

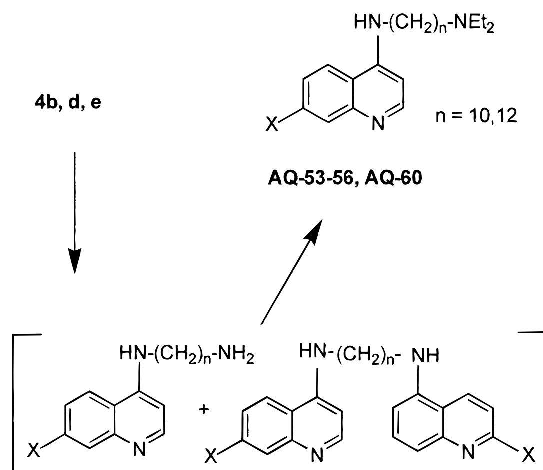
Because 2-amino-3-diethylaminopropane was not available commercially, we used a two-step synthesis to prepare the desired AQs (Scheme 3). In the first step (step I, general method B), equimolar amounts of **4a**−**e** were reacted with 1,2-diaminopropane to produce AQ intermediates (**7a**−**e)** with diaminoalkane side chains containing terminal primary amines in 80−90% yield. The monomeric structure of **7b** was confirmed by its ^{1}H NMR spectrum. In the second step (step II, general method B), regioselective reductive alkylation with sodium borohydride ($NaBH_4$)/acetic acid (AcOH) converted the terminal primary amines on the diaminoalkane side chain to diethylamines in 60−70% yield. To examine the mechanism of step II, reductive alkylation of **7b** was performed with $NaBH_4$ and 2-butyric acid. The ^{1}H NMR spectrum of the N-alkylated product **8** revealed a pair of doublets from *gem*-dimethyl groups (δ 0.92 and 0.98 ppm with J = 6.0 Hz), consistent with the addition of two *sec*-butyl species to the terminal amino nitrogen. These observations and previous studies[12a] suggest that this reductive alkylation is a three-step process involving: (1) in situ formation of an

Scheme 3[a]



[a] Reagents: (i) $H_2NCHMeNH_2$, 120−130 °C; (ii) $NaBH_4$, CHCOOH, 65−80 °C; (iii) $NaBH_4$, $Me_2CHCOOH$, 70−75 °C.

Scheme 4[a]

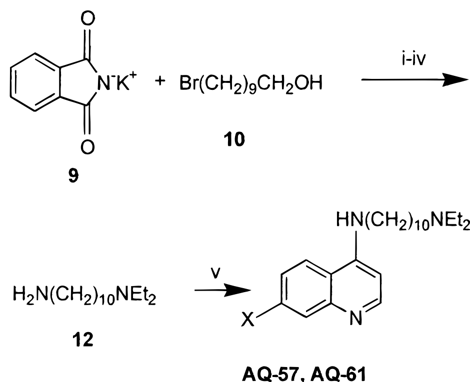


[a] Reagents: (i) H_2N-$(CH_2)_n$-NH_2, NMP, 120−140 °C; (ii) $NaBH_4$, CH_3COOH, 60−70 °C.

aldehyde from the acid by the reduction of Na[BH_3(OOCR)],[19,20] (2) reaction of the aldehyde with the primary amine under acidic conditions to form an imine, and (3) hydride reduction of the iminium ion by Na[BH_3(OOCR)][19a] or Na[$(RCOO)_3BH$].[21] Gribble et al. proposed a similar mechanism for the reductive alkylation of indoles because they identified acetaldehyde in gases liberated from $NaBH_4$ and AcOH using a 2,4-dinitrophenylhydrazone (2,4-DNP) derivative.[19] Although this reductive alkylation could have proceeded via a diamide intermediate,[12a] we were unable to isolate a diamide from the reaction mixture.

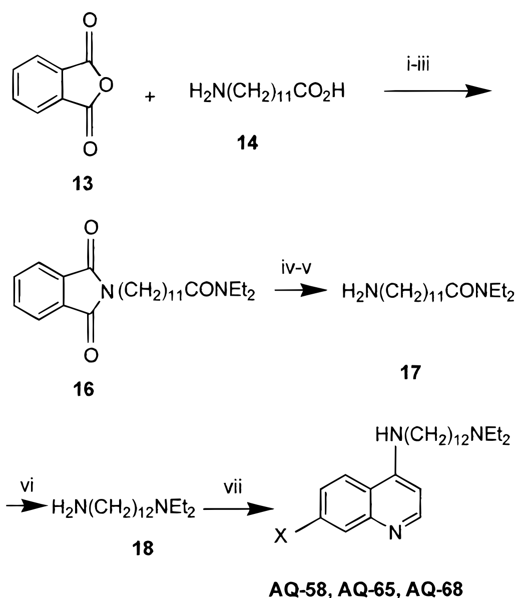
Because *N*,*N*-diethyl-1,10-diaminodecane and *N*,*N*-diethyl-1,12-diamino-dodecane were not available commercially, we initially used a two-step synthesis for AQs with 10 or 12 carbon side chains (Scheme 4). Although short (2−3 carbon) diaminoalkanes such as 1,3-diaminopropane reacted with CSQs to produce excellent yields of AQs, low yields and mixtures of products were limiting problems in the synthesis of long chain (10−

Scheme 5[a]



[a] Reagents: (i) Hexadecyltributylphosphonium bromide, toluene, 110 °C; (ii) MsCl, Et_3N, 0−10 °C; (iii) Et_2NH, 55 °C; (iv) $H_2NNH_2{\cdot}H_2O$, EtOH, 60 °C; (v) **4a**,**c**, 140−145 °C.

Scheme 6[a]



[a] Reagents: (i) toluene, 110 °C; (ii) PCl_5, CH_2Cl_2, 0−5 °C; (iii) Et_2NH, CH_3CN, rt; (iv) $H_2NNH_2{\cdot}H_2O$, EtOH, 45 °C; (v) (a) 2 N HCl, (b) NaOH; (vi) $BH_3{\cdot}THF$, 60 °C; (vii) **4a**,**c**,**e**, 140−145 °C.

12 carbon) AQs. This was because the primary amines at both ends of symmetrical long chain diaminoalkanes reacted with CSQs to produce bisquinolines as well as monomeric AQs[21,22] (Scheme 4). Attempts to prevent bisquinoline formation by changing the solvent, varying the reaction temperature and the molar concentrations of the reactants, and selective protection of one primary amine were unsuccessful. Despite these problems, AQs with diamino-decamethylene or -dodecamethylene side chains were prepared in small-scale preliminary syntheses (with overall yields 20−30%) (Scheme 4) (method B, steps I and II).

The problem of bisquinoline formation was overcome by preparing these AQs using other (alternative) techniques. Starting from potassium phthalimide **9** and 10-bromo-1-decanol **10**, we produced *N*,*N*-diethyl-1,10-diaminodecane **12** (Scheme 5, method C). Thereafter, nucleophilic substitution of the Cl at C-4 of **4a**,**c** with **12** followed by purification using basic alumina chromatography produced **AQ-57** and **AQ-61** in 65−70% yield (Scheme 5). Using this method **AQ-54**, **AQ-56**, and **AQ-60** were resynthesized in 60−70% yield (in contrast to the initial synthesis of these compounds as described in Scheme 4, which produced yields of only 20−30%). Similarly, starting from phthalic anhydride **13** and 12-aminododecanoic acid **14**, we prepared *N*,*N*-diethyl-1,12-diaminodecane **18** (Scheme 6, method D). Thereafter, nucleophilic substitution of the Cl at C-4 of **4a**,**c**,**e** with **18** followed by purification using basic alumina column chromatography produced **AQ-58**, **AQ-65** and **AQ-68**, respectively, in 50−70% yield (Scheme 6). Following this method **AQ-53** and **AQ-55** were also resynthesized in 60% yield.

Table 2. Antiplasmodial Activity (IC_{50}) of 7-Substituted 4-Aminoquinolines against *Plasmodium falciparum* in Vitro

(structure: HN-R-NEt2 at C-4, X at C-7 of quinoline)

compd	X	-R-	IC_{50} (nM)[a] Haiti 135	Indochina I
chloroquine[b]	Cl	$CHMe(CH_2)_3$	8	95
AQ-44	I	CH_2CH_2	6	6
AQ-45	I	$CH_2CH_2CH_3$	3	4
AQ-46	I	$CHMeCH_2$	7	7
AQ-43	I	$CHMe(CH_2)_3$	4	35
AQ-57	I	$(CH_2)_{10}$	6	16
AQ-58	I	$(CH_2)_{12}$	10	20
AQ-22	Br	CH_2CH_2	7	7
AQ-37	Br	$CH_2CH_2CH_3$	4	6
AQ-47	Br	$CHMeCH_2$	11	12
AQ-24	Br	$CHMe(CH_2)_3$	5	90
AQ-56	Br	$(CH_2)_{10}$	6	17
AQ-55	Br	$(CH_2)_{12}$	8	18
AQ-25	F	CH_2CH_2	35	60
AQ-38	F	$CH_2CH_2CH_3$	23	120
AQ-42	F	$CHMeCH_2$	30	60
AQ-17	F	$CHMe(CH_2)_3$	20	500
AQ-61	F	$(CH_2)_{10}$	7	40
AQ-65	F	$(CH_2)_{12}$	8	25
AQ-39	CF_3	CH_2CH_2	30	41
AQ-49	CF_3	$CH_2CH_2CH_2$	15	18
AQ-59	CF_3	$CHMeCH_2$	50	50
AQ-27	CF_3	$CHMe(CH_2)_3$	16	45
AQ-54	CF_3	$(CH_2)_{10}$	10	20
AQ-53	CF_3	$(CH_2)_{12}$	5	25
AQ-23	OMe	CH_2CH_2	48	155
AQ-66	OMe	$CH_2CH_2CH_2$	150	900
AQ-67	OMe	$CHMeCH_2$	94	180
AQ-48	OMe	$CHMe(CH_2)_3$	55	3000
AQ-60	OMe	$(CH_2)_{10}$	10	170
AQ-68	OMe	$(CH_2)_{12}$	17	90

[a] IC_{50} = 50% inhibitory concentration, which had standard deviations of ±10% (each AQ was tested in triplicate). [b] Chloroquine was used as a control.

Antiplasmodial Activity in Vitro. The antiplasmodial activities of these experimental AQs were measured by their ability to inhibit the incorporation of ^{3}H-hypoxanthine into nucleic acid via the parasite's purine salvage pathway.[23] To compare activities against chloroquine-susceptible and chloroquine-resistant *P. falciparum*, we used cloned chloroquine-susceptible Haiti-135[24] and chloroquine-resistant Indochina I *P. falciparum*[25] parasites. In each case, chloroquine was used as a control. Results are reported as the nanomolar AQ concentrations necessary to reduce ^{3}H-hypoxanthine incorporation by 50% (IC_{50}, Table 2).

Results and Discussion

7-Iodo- and 7-Bromo-Substituted AQs generally have excellent antiplasmodial activity against *P. falciparum* in vitro (Table 2). 7-Iodo-AQs with short side chains (**AQ-44**, **AQ-45**, **AQ-46**) were active against both chloroquine-susceptible and chloroquine-resistant *P. falciparum* in vitro (IC_{50}s of 3−7 nM). In fact, short chain 7-iodo-AQs were slightly (not significantly) more active than short chain 7-chloro-AQs against chloroquine-susceptible and -resistant *P. falciparum* (IC_{50}s of 3−7 vs 5−12 nM).[5a] 7-Iodo-AQs with long diaminoalkane side chains (10, 12 carbons) were active against both chloroquine-susceptible and -resistant *P. falciparum* (IC_{50}s of 6−10 and 16−20 nM). The 7-iodo-AQ with an isopentyl ($-HNCHMe(CH_2)_3NEt_2$) side chain like that of chloroquine (**AQ-43**) was active against chloroquine-susceptible (IC_{50} of 4 nM) but not chloroquine-resistant (IC_{50} of 35 nM) *P. falciparum*. As with the 7-iodo-AQs, 7-bromo-AQs with short and long diaminoalkane side chains were active against chloroquine-susceptible and chloroquine-resistant *P. falciparum*. IC_{50}s were 7−12 nM for AQs with 2 or 3 carbon side chains (**AQ-22**, **AQ-37**, **AQ-47**) and 6−22 nM for AQs with 10 or 12 carbon side chains (**AQ-56**, **AQ-55)** (Table 2).

Fluoro-, Trifluoromethyl-, and Methoxy-Substituted AQs. The 7-fluoro-AQs were less active than 7-chloro-, 7-iodo-, or 7-bromo-AQs. 7-Fluoro-AQs with short diaminoalkane side chains (**AQ-25**, **AQ-38**, **AQ-42)** were less active against both chloroquine-susceptible (IC_{50}s of 23−35 nM) and chloroquine-resistant *P. falciparum* parasites (IC_{50}s of 60−120 nM) (Table 2). Although 7-fluoro-AQs with long diaminoalkane side chains (**AQ-61**, **AQ-65**) were moderately active against chloroquine-susceptible parasites (IC_{50}s of 7−8 nM), they were less active against chloroquine-resistant parasites (IC_{50}s of 25−40 nM). 7-Trifluoromethyl AQs were less active against both chloroquine-susceptible and chloroquine-resistant *P. falciparum* (Table 2). One exception among the 7-trifluoromethyl-AQs was **AQ-53**, which was active against chloroquine-susceptible parasites (IC_{50} of 5 nM) and moderately active against chloroquine-resistant parasites (IC_{50} of 25 nM). In contrast, most of the 7-methoxy-AQs were inactive against both chloroquine-susceptible (IC_{50}s of 10−150 nM) and -resistant (IC_{50}s of 90−3000 nM) *P. falciparum*.

Comparison with Previous Studies. These results differ substantially from those in previous reports, which suggested that the antiplasmodial activity of 7-substituted AQs decreased progressively as follows: $Cl > I > Br = F = CF_3 > OMe$.[26] The major reason for these differences is that the earlier studies were based on (1) activity in the avian *Plasmodium gallinaceum*[27] and murine *P. berghei*[28] models and on (2) the hypothesis that AQs acted against plasmodia by intercalating within the DNA helix so the two amino nitrogens on the side chain spanned the minor groove of the DNA helix.[26]

SARs Potentially Responsible for the Antiplasmodial Activity of AQs in Vitro: Effects of Side Chain Length. The results reported here confirm the importance of side chain length for AQ activity against *P. falciparum*.[5] In general, the most active AQs were those with two or three carbon diaminoalkane side chains (**AQ-44−46, AQ-22**, **AQ-37**). In addition, AQs with 10 or 12 carbon side chains (**AQ-55** to **AQ-58**) were active. AQs with the isopentyl side chain of chloroquine [$-HNCHMe(CH_2)_3NEt_2$, **AQ-43** and **AQ-24**] were active against chloroquine-susceptible *P. falciparum*. These results suggest that the number of carbons between the two nitrogens in the diaminoalkane side chain is a major determinant of activity against chloroquine-resistant *P. falciparum*. The mechanism by which these AQs circumvent chloroquine-resistance is unknown but may involve resistance to transport (efflux) of AQs from the parasite, which may thus prevent their accumulation in the food vacuole (secondary lysosome).[29−31]

SARs Potentially Responsible for the Antiplasmodial Activity of AQs in Vitro: Effects of Substitution at the 7-Position. The results reported here are consistent with two hypotheses: (1) *Neither lipophilicity nor hydrophilicity at the 7-position are significant factors in antiplasmodial activity*. This hypothesis is consistent with the observation that 7-iodo-substituted AQs were substantially more active than 7-trifluoromethyl-substituted AQs, although both substituents are similarly lipophilic. Conversely, 7-methoxy-substituted AQs were not biologically active, although they are hydrophilic. (2) *Contrary to expectation, electronegativity at the 7-position did not correlate with antiplasmodial activity*. This hypothesis is consistent with the observation that 7-fluoro- and 7-trifluromethyl-substituted AQs were less active than 7-iodo-, 7-bromo-, or 7-chloro-substituted AQs.

Conclusion

The results reported here demonstrate that 7-iodo- and 7-bromo-AQs with short (2−3 carbon) or long (10−12 carbon) diaminoalkane side chains are active against chloroquine-susceptible and -resistant *P. falciparum* parasites. These AQs are active against chloroquine-resistant *P. falciparum* because they evade the mechanism(s) responsible for resistance to chloroquine.[5] These results suggest that the number of carbons between the two nitrogens in the diaminoalkane side chain of 7-bromo- and 7-iodo-AQs is a major determinant of activity against chloroquine-resistant *P. falciparum* as it is with the 7-chloro-AQs. These observations and the consistent pattern observed with diaminoalkane side chain length suggest that it may be possible to develop a series of 7-iodo and 7-bromo-substituted AQs active against chloroquine-resistant *P. falciparum* in vivo.

Experimental Section

Unless otherwise noted, reagents and solvents were obtained from Aldrich (Milwaukee, WI) and used without purification. Reactions were performed in oven-dried glassware. Reaction temperatures refer to silicon oil bath temperatures unless stated otherwise. Reactions were monitored by thin-layer chromatography (TLC) using silica gel (F254 polyethylene-backed) plates containing a fluorescent indicator (EM Separations−Gibbstown, NJ) or aluminum oxide on polyester plates (layer thickness 200 μm, particle size < 60 μm−Aldrich). Compounds were visualized with UV light by exposure to iodine vapors in an iodine chamber or by spraying the plate with 2% aqueous potassium permanganate ($KMnO_4$) containing 1% sulfuric acid. Standard workup refers to washing organic extracts with deionized water, followed by drying over anhydrous Na_2SO_4, and evaporating the solvent under reduced

pressure with a rotary evaporator. Chromatographic purification was performed with activated basic alumina (Brockman I, ~150 mesh, 58 Å—Aldrich) or silica gel (0.06−0.2 mm, Spectrum Chemical Mfg. Corp., NJ) columns. Melting points (T_m) were determined with a Thomas-Hoover melting point apparatus (model 6406-K, Arthur Thomas, Philadelphia, PA) and are uncorrected. ^{1}H NMR spectra were recorded with a General Electric Omega 500 MHz spectrometer (Fremont, CA) in $CDCl_3$. Chemical shifts are reported in parts per million (δ ppm) and coupling constants (J) in Hz. The following abbreviations were used to describe peak patterns: s = singlet, d = doublet, t = triplet, m = multiplet, b = broad, dd = double doublet. Mass spectra (MS) were determined by the electron-impact method (EIMS) using a Kratos profile mass spectrometer—Manchester, UK. Elemental analyses were performed by Atlantic Microlabs (Norcross, GA) and were within ±0.4% of the calculated values for carbon, hydrogen, and nitrogen.

General Method for the Synthesis of 4-Chloro-7-substituted Quinolines (CSQs). CSQs were prepared using the modifications of previously published procedures[12,13] as described below. Mixtures of *m*-substituted anilines (**1a**−**e**) (75 mmols) and diethyl ethoxy methylenemalonate (**2**) (100 mmols) were heated slowly to 90−110 °C for 60 min, and the liberated ethanol (EtOH) was allowed to evaporate under reduced pressure. The reaction mixture was cooled to room temperature, the dark brown anilide was added to boiling phenyl ether (Ph_2O, ~200 mL) with stirring, and the mixture was heated to reflux for 60−80 min and cooled to room temperature. The resulting 3-carbethoxy-4-hydroxy-7-substituted quinoline was filtered, washed several times with a 2:1 mixture of ethyl acetate (EtOAc) and petroleum ether, and recrystallized from 70% EtOH. The solid product was then suspended in hot 2 N sodium hydroxide (NaOH) (150 mL) with stirring and heated to reflux for 1−1.5 h. After the clear solution was charcoaled and filtered, the filtrate was neutralized with cold 2 N hydrochloric acid (HCl). The yellowish-white solid precipitate (3-carboxy-4-hydroxy-7-substituted quinoline) was filtered, washed with water, and dried in air. Small portions of this product were then added to a three-necked flask containing boiling Ph_2O with stirring and allowed to reflux for 1 h. [**Caution**: A violent reaction began soon after the quinoline carboxylic acid was added to the boiling Ph_2O because of effervescence from the liberated carbon dioxide]. After cooling to room temperature, petroleum ether (40 mL) was added to precipitate the solid product, which was then filtered, washed several times with a mixture of EtOAc and petroleum ether (2:1) to remove the Ph_2O, and recrystallized from EtOH. The resultant 4-hydroxy-7-substituted quinoline was added to phosphorus oxychloride ($POCl_3$) (15 mL) with stirring and heated to 100−110 °C for 70 min. After the evaporation of excess $POCl_3$ under reduced pressure at 65−70 °C and cooling to room temperature, the resultant dark brown liquid was added to ice-cooled 40% ammonium hydroxide. After trituration, the product solidified and was filtered, washed with water, and dried. The pure products (**4a**−**e**) were obtained by recrystallization from petroleum ether (yield 80−90%).

General Method A: Preparation of *N,N*-Diethyl-*N*-(7-substituted 4-quinolinyl)diaminoalkane. A mixture of 4-chloro-7-substituted quinoline (10 mmols) and the desired dialkyldiaminoalkane (15 mmols) was heated initially at 80−100 °C for 1 h with stirring and subsequently at 135−150 °C for 6−8 h with continued stirring to drive the reaction to completion. The reaction mixture was then cooled to 90 °C, and excess amine was evaporated under reduced pressure, followed by cooling to room temperature. After NaOH was added (1 N, 20 mL) and the organic product was extracted with CH_2Cl_2 (3 × 40 mL), standard workup yielded crude product as a brown oil. The crude product was then passed through a column packed with basic alumina, and eluted with $CHCl_3$−petroleum ether (1:3). Evaporation of the solvent in a rotary evaporator and recrystallization from petroleum ether or acetone yielded pure AQs such as **AQ-22**, for which data are provided below.

***N*²-(7-Bromo-4-quinolinyl)-*N*¹,*N*¹-diethyl-1,2-ethanediamine (AQ-22):** T_m 111−112 °C;^{1}H NMR ($CDCl_3$) δ 1.08 (t, J = 7.0 Hz, 6H), 2.60 (q, J = 7.0 Hz, 4H), 2.80 (t, J = 5.5 Hz, 2H), 3.26 (q, J = 5.5 Hz, 2H), 6.16 (bs, 1H), 6.38 (d, J = 5.5 Hz, 1H), 7.49 (dd, J = 9.0, 1.0 Hz, 1H), 7.58 (d, J = 9.0 Hz, 1H), 8.12 (d, J = 1.0 Hz, 1H), 8.51 (d, J = 5.5 Hz, 1H); EIMS *m*/*z* 321, 323. Anal. ($C_{15}H_{20}BrN_3$) C, H, N.

General Method B: Synthesis of *N*¹,*N*¹-Diethyl-*N*²-(7-substituted-4-quinolinyl)-1,2-diaminopropane. Step I. A mixture of 4-chloro-7-substituted quinoline (10 mmol) and the appropriate diaminoalkane (35−40 mmol) was heated slowly from room temperature to 80 °C over 1 h with stirring, subsequently heated at 130−140 °C for 4−6 h with continued stirring to drive the reaction to completion, and cooled to room temperature. NaOH was then added (1 N, 20 mL), followed by heating to 40 °C for 10 min. Thereafter, EtOAc was added (100−150 mL), followed by warming to 40−50 °C for 30−45 min, and cooling to room temperature. The organic layer was separated from the aqueous phase, washed with water, and the standard workup yielded semisolid crude product. After trituration of the crude product in acetone (20 mL), solid product was obtained by filtration. This semipure compound was used in the next step without further purification.

Step II. A solution of *N*²-(7-substituted-4-quinolinyl)-1,2-diaminopropane (2−3 mmol) in glacial acetic acid (AcOH) (30−50 mL) was cooled to 5−10 °C in a three-necked flask before the careful addition of sodium borohydride ($NaBH_4$) (50−60 mmol) with stirring in an atmosphere of N_2. After the addition of $NaBH_4$, the reaction mixture was stirred at room temperature for 1 h and heated to 55 °C with continued stirring for 10 h. After cooling to ambient temperature, additional $NaBH_4$ (10−15 mmol) and AcOH (5−10 mL) were added, followed by heating to 55 °C with stirring for 8−10 h to drive the reaction to completion, and cooling to room temperature. After addition of an ice-cold aqueous solution of NaOH (pH ~ 8−9) and extraction with CH_2Cl_2 (60 mL), standard workup yielded a yellowish crude product which was purified by basic alumina column chromatography and eluted with $CHCl_3$−petroleum ether (1:4−1:5). Evaporation of solvent yielded final products such as **AQ-47** (for which data are provided below).

***N*²-(7-Bromo-4-quinolinyl)-*N*¹,*N*¹-diethyl-1,2-diaminopropane (AQ-47):** liquid; ^{1}H NMR ($CDCl_3$) δ 1.08−1.14 (m, 9H), 2.40−2.45 (m, 2H), 2.65−2.70 (m, 2H), 2.93−2.97 (m, 1H), 3.21−3.28 (m, 2H), 6.39 (d, J = 5.0 Hz, 1H), 6.50 (b, 1H), 7.52 (dd, J = 9.0, 1.0 Hz, 1H), 7.61 (d, J = 9.0 Hz, 1H), 8.18 (d, J = 1.0 Hz, 1H), 8.53 (d, J = 5.0 Hz, 1H); EIMS *m*/*z* 335, 337. Anal. ($C_{16}H_{22}BrN_3 \cdot 2HCl \cdot {}^1/_4H_2O$) C, H, N.

General Method C (Scheme 5). Synthesis of *N*¹,*N*¹-Diethyl-*N*¹⁰-(7-substituted-4-quinolinyl)-1,10-diaminodecane. Potassium phthalimide **15** (4.45 g, 24 mmol) was added to a toluene solution (12 mL) of 10-bromo-1-decanol **16** (3.8 mL, 19 mmol) and hexadecyltributylphosphonium bromide [HDTPB] (1.2 g, 2.4 mmol) at room temperature, followed by heating to 110 °C with stirring for 1.5 h. After the reaction mixture was cooled to room temperature, the inorganic precipitate was removed by filtration and washed with 20% petroleum ether in ethyl acetate (EtOAc) (100 mL). The combined filtrates and washings were then passed through a silica gel column, which was subsequently eluted with 20% petroleum ether in EtOAc (200 mL). The combined eluates were treated with 10% aqueous NaOH and yielded a viscous liquid after standard workup. After cooling overnight in a refrigerator (at 8 °C), the liquid solidified. The desired 10-phthalimido-1-decanol was obtained by recrystallization from methanol (5.65 g, 98%). A solution of 10-phthalimido-1-decanol (2.42 g, 8.0 mmol) in CH_2Cl_2 (10 mL) was placed in a three-necked flask and cooled to 5 °C. Solutions of MsCl (0.7 mL, 9 mmol) in CH_2Cl_2 (5 mL) and Et_3N (1.35 mL, 10 mmol) in CH_2Cl_2 (5 mL) were then added to this solution via separate syringes under N_2. After the mixture was stirred for 2 h at 5−10 °C, the reaction was quenched with ice−water. Standard workup yielded the crude mesylate as a liquid, which was used in the subsequent reaction without purification. Thereafter, diethylamine (8 mL) was added to the flask containing crude

mesylate, and the reaction mixture was heated to 55 °C with stirring for 5 h. Standard workup yielded a yellowish liquid product (2.3 g, 80%), which was subsequently purified using a silica gel column and eluted with 15% MeOH−EtOAc. Evaporation of the solvent yielded the product as a colorless liquid. This liquid (2.1 g, 6.0 mmol) was then mixed with hydrazine hydrate (0.55 mL, 11.3 mmol) in EtOH (10 mL) at room temperature with stirring. The reaction mixture was then warmed to 45 °C and stirred for 1 h. Standard workup yielded the desired product **17** as a viscous oil (1.0 g, 80% yield). Compound **17** was purified using silica gel column chromatography and eluted with a mixture of $CHCl_3$−MeOH−NH_4OH (88%:10%:2%). Evaporation of the solvent yielded the desired product as a yellowish liquid. Subsequently, AQs such as **AQ-57** (for which data are provided below) were obtained by reacting **17** (10 mmol) with **10a** (5 mmol) following the procedure described in method A (yield 70%).

***N*^{1},*N*^{1}-Diethyl-*N*^{10}-(7-iodo-4-quinolinyl)-1,10-diaminodecane (AQ-57).** T_m 79−80 °C; ^{1}H NMR ($CDCl_3$) δ 1.05 (t, J = 7.5 Hz, 6H), 1.33−1.49 (m, 12H), 1.79−1.82 (m, 4H), 2.42 (t, J = 7.5 Hz, 2H), 2.57 (q, J = 7.5 Hz, 4H), 3.28−3.33 (m, 2H), 4.99 (bs, 1H), 6.46 (d, J = 5.0 Hz, 1H), 7.47 (d, J = 9.0 Hz, 1H), 7.71 (dd, J = 9.0, 1.0 Hz, 1H), 8.42 (d, J = 1.0 Hz, 1H), 8.55 (d, J = 5.0 Hz, 1H); EIMS *m*/*z* 481. Anal. ($C_{23}H_{36}IN_3 \cdot H_2O$) C, H, N.

General Method D (Scheme 6). Synthesis of *N*^{1},*N*^{1}-Diethyl-*N*^{12}-(7-substituted-4-quinolinyl)-1,12-diaminododecane. Finely powdered phthalic anhydride **18** (3.72 g, 25.05 mmol), 12-aminododecanoic acid **19** (5.0 g, 23.22 mmol), toluene (150 mL), and triethylamine (1.2 mL) were placed in a three-necked flask equipped with a reflux condenser, distillation head, and magnetic stirring bar. The reaction mixture was heated to reflux for 1.5 h with stirring to permit removal of the toluene and water (liberated during the reaction) from the reaction mixture. After the flask was cooled to room temperature, water (75 mL) and then concentrated HCl (2 mL) were added. After the crude reaction product solidified as a white cake, it was gently crushed with a spatula, filtered, washed with water, and dried in air. Pure 12-phthalimido-1-dodecanoic acid was obtained after serial recrystallization, first from methanol and second from $CHCl_3$ (7.9 g, 98%). Subsequently, a solution of 12-phthalimido-1-dodecanoic acid (8.2 g, 23.8 mmol) in CH_2Cl_2 (60 mL) was added slowly to an ice-cooled solution of anhydrous phosphorus pentachloride (5.05 g, 24.2 mmol) in CH_2Cl_2 (20 mL) under N_2. After this addition, the reaction mixture was stirred at 0−5 °C for 3 h before removing CH_2Cl_2 with a rotary evaporator at ambient temperature. The concentrated acid chloride was then diluted with anhydrous acetonitrile (10 mL) and cooled to 5 °C before adding a solution of diethylamine (8 mL) in acetonitrile (10 mL) dropwise with stirring. After the reaction mixture was stirred for 1 h at room temperature, the precipitated diethylamine hydrochloride was removed by filtration. After the solvent was evaporated from the filtrate, the crude liquid was diluted with CH_2Cl_2 and washed successively with 5% aqueous $NaHCO_3$ and 10% aqueous NaCl. The standard workup yielded the final product as an oil (7.5 g, 80%). The pure *N*^{1},*N*^{1}-diethyl-12-phthalimido-1-dodecanamide was prepared using silica gel column chromatography and eluted with 40−45% EtOAc in petroleum ether. Thereafter, a solution of *N*^{1},*N*^{1}-diethyl-12-phthalimido-1-dodecanamide (7.0 g, 17.5 mmol) in EtOH (20 mL) was mixed with hydrazine hydrate (1.3 mL, 27 mmol) in EtOH (5 mL) under stirring at room temperature. The reaction mixture was then warmed to 40 °C and stirred for 1 h. The standard workup yielded the desired 12-amino-*N*^{1},*N*^{1}-diethyl-1-dodecanamide as an oil (4.0 g, 85% yield). The pure 12-amino-*N*^{1},*N*^{1}-diethyl-1-dodecanamide was obtained as a yellowish liquid after purification through silica gel column chromatography and eluted with a mixture of $CHCl_3$−MeOH−NH_4OH (89%:10%:1%). A colorless powder was obtained after triturating the liquid product in hexane and filtration through a funnel. Subsequently, 12-amino-*N*^{1},*N*^{1}-diethyl-1-dodecanamide (6.0 g, 22.2 mmol) was placed in a two-necked round-bottom flask containing tetrahydrofuran (10 mL) and cooled to 5 °C before the addition of a 1 M solution of borane in tetrahydrofuran (50 mL, 50 mmol) with stirring (under N_2). After complete addition, the reaction flask was placed in an oil bath and the temperature was slowly raised to 60 °C, with stirring at that temperature for 3 h. The solution was then cooled to approximately 15 °C and additional borane in tetrahydrofuran (10 mL, 10 mmol) was added slowly to drive the reaction to completion. After being stirred for an additional 1.5 h at room temperature, the reaction mixture was added slowly to an ice-cooled solution of 6 N HCl (100 mL). The tetrahydrofuran was then removed from the aqueous mixture using a rotary evaporator. After the aqueous solution was alkalinized to pH ~ 9 by adding NaOH pellets and the liberated amine was extracted into CH_2Cl_2 (2 × 50 mL), the desired amine **20** was obtained as a yellowish liquid after the standard workup (4.8 g, 85%). Compound **20** was purified using silica gel column chromatography and eluted with a mixture of $CHCl_3$−MeOH−NH_4OH (88%:10%:2%). Subsequently, a mixture of **20** (4.0 g, 15.62 mmol) and 4-chloro-7-substituted quinolines (12 mmol) was heated slowly to 145 °C with stirring for 6 h. After cooling to room temperature, the mixture was diluted with 2 N HCl, and the aqueous solution was alkalinized by adding NaOH solution (pH ~ 9). The crude product was extracted with CH_2Cl_2, followed by the standard workup. The resulting reddish-brown, crude product was purified by basic alumina column chromatography and eluted with $CHCl_3$-petroleum ether (1:4), followed by removal of the solvent using a rotary evaporator to yield AQs such as **AQ-58** (for which data are provided below) as a white powder (4.2 g, 60% yield).

***N*^{1},*N*^{1}-Diethyl-*N*^{12}-(7-iodo-4-quinolinyl)-1,12-diaminododecane (AQ-58):** T_m 73−74 °C; ^{1}H NMR ($CDCl_3$) δ 1.07 (t, J = 7.5 Hz, 6H), 1.24−1.38 (m, 12H), 1.42−1.52 (m, 4H), 1.64−1.82 (m, 4H), 2.46 (t, J = 7.5 Hz, 2H), 2.61 (q, J = 7.5 Hz, 4H), 3.27−3.32 (m, 2H), 4.98 (bs, 1H), 6.42 (d, J = 5.0 Hz, 1H), 7.42 (d, J = 9.0 Hz, 1H), 7.66 (dd, J = 9.0, 1.0 Hz, 1H), 8.38 (d, J = 1.0 Hz, 1H), 8.52 (d, J = 5.0 Hz, 1H); EIMS *m*/*z* 509. Anal. ($C_{25}H_{40}IN_3$) C, H, N.

Measurement of in Vitro Antiplasmodial Activity. The effects of AQs on the growth of *P. falciparum* were evaluated as described previously.[4,23] In brief, synchronous cultures of early ring stage parasites (600 μL of a 2% red cell suspension with 0.2% parasitemia in 24-well tissue culture plates—Corning Cell Wells 25820, Corning, NY) were grown in the in vitro culture system with varying concentrations of the AQ being tested for 48 h and with the same AQ concentrations plus 0.5 μCi ^{3}H-hypoxanthine (New England Nuclear—Boston, MA) for an additional 18 h. They were then harvested on glass microfibers (Whatman 934 AH—Clifton, NJ) using a Skatron cell harvester (Sterling, VA), dried, placed in glass vials (Kimble—Toledo, OH) with 8 mL of liquid scintillation fluid (Cytoscint, ICN Radiochemicals—Costa Mesa, CA), and assayed in a β counter (Tri Carb 2100 TR, Packard—Meriden, CT). The IC_{50} was calculated by linear regression based on the percent inhibition of ^{3}H-hypoxanthine accumulation vs AQ concentration, using a logarithmic (exponential) scale for the AQ concentration because antiplasmodial activity is proportional to the logarithm of AQ concentration.[5,32] After initial screening to identify the relevant range of the dose−response curve, triplicate determinations were performed for each compound, and agreed within ±10% (Table 2).

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Supporting Information Available: Physical data and ^{1}H NMR for all compounds which do not appear in the Experimental Section and a copy of the ^{19}F spectrum of **AQ-17** (9 pages). Ordering information is given on any current masthead page.

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