Structure−Activity Relationships in 4-Aminoquinoline Antiplasmodials. The Role of the Group at the 7-Position

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Antiplasmodial activities versus the chloroquine sensitive D10 strain of *Plasmodium falciparum* of a series of N^1,N^1-diethyl-N^2-(4-quinolinyl)-1,2-ethanediamines with 11 different substituents at the 7-position on the quinoline ring have been investigated in vitro. Electron-withdrawing groups at the 7-position have been shown to lower the pK_a of both the quinoline ring nitrogen atom and the tertiary amino nitrogen in the alkyl side chain. The quinoline nitrogen pK_a ranges from 6.28 in the nitro derivative to 8.36 in the amino derivative, while the tertiary amino nitrogen has a pK_a ranging between 7.65 in the trifluoromethyl derivative and 10.02 in the amino derivative. Calculation suggests that the resulting pH trapping of these compounds in the parasite food vacuole ranges between about 7% of that observed in chloroquine for the NO_2 derivative and 97% in the amino derivative. A direct proportionality between antiplasmodial activity normalized for pH trapping and β-hematin inhibitory activity was observed. Activity could not be correlated with any other observed physical parameter. The β-hematin inhibitory activity of these derivatives appears to correlate with both the hematin−quinoline association constant and the electron-withdrawing capacity of the group at the 7-position (Hammett constant). For the compounds under investigation, the hematin association constant is in turn influenced by the lipophilicity of the group at the 7-position.

Introduction

Historically, chloroquine has been by far the most successful antimalarial, being cheap, highly efficacious, and of relatively low toxicity,[1] but owing to the spread of chloroquine resistance, its use is now very limited.[2] Nonetheless, several reports have shown that close analogues of chloroquine and certain chloroquine derivatives maintain activity against chloroquine-resistant parasite strains,[3−7] strongly suggesting that the resistance mechanism does not involve any change to the target of this class of drug but rather involves a compound-specific resistance. As a result, it may be possible to develop new antimalarials based on the same mechanism of action as chloroquine. In this endeavor, a detailed knowledge of the mechanism of action of chloroquine could provide the basis for the rational design of novel antimalarials.

In a previous study,[8] we developed a detailed structure−activity relationship (SAR) for chloroquine and closely related 4-aminoquinoline antiplasmodial compounds based on the following model: (i) accumulation of the quinoline derivative at the site of action in the acidic food vacuole of the parasite, (ii) association of the quinoline with hematin, (iii) inhibition of hemozoin formation, (iv) resulting buildup of hematin or drug hematin complex, which exerts a toxic effect on the parasite. The study showed that 2- and 4-aminoquinolines have a unique affinity for hematin, with association constants approximately 3 orders of magnitude stronger than quinoline, 3-, 5-, 6-, 8-aminoquinoline, or 4,7-dichloroquinoline. Attachment of an alkyl side chain to the amino group of 2- or 4-aminoquinoline was found to have much smaller effects on the association constant. Similarly, introduction of a Cl atom at the 7-position of the quinoline ring was found to have only a small effect on the association constant. The 4-aminoquinoline nucleus of chloroquine was thus identified as the hematin-associating nucleus and was proposed to be a necessary, but not sufficient, requirement for antiplasmodial activity.

Inhibition of β-hematin (synthetic hemozoin) formation was demonstrated in all 4-aminoquinoline derivatives with a Cl atom attached to the 7-position of the quinoline ring but in none of the derivatives in which a hydrogen atom replaced the Cl atom. The 7-chloro group was thus identified as the critical feature needed for inhibition of β-hematin formation. Once again, however, this feature was proposed to be necessary, but not sufficient, for strong antiplasmodial activity.

The findings suggested the further requirement, in terms of antiplasmodial activity, of a basic nitrogen atom attached to the aminoalkyl side chain of the quinoline (hereafter referred to as the lateral side chain). This feature was related to the need for pH trapping of the compound in the acidic food vacuole of the parasite.

Despite the development of this SAR for chloroquine, the study left two important questions unanswered.

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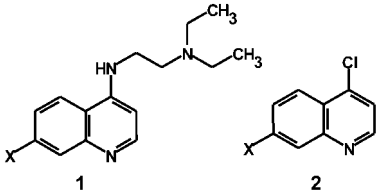
Table 1. Experimental pK_a Values, Hematin Association Constants, β-Hematin Inhibitory Activities, and Antiplasmodial Activities of the Aminoquinolines under Investigation[a]

	pK_{a1}[b]	pK_{a2}[b]	log K[c]	βHI[d]	BHIA$_{50}$[e]	IC$_{50}$,[f] nM
1a	8.36 ± 0.02	10.02 ± 0.01	4.35 ± 0.02	−	>20	>5000
1b	6.82 ± 0.01	8.953 ± 0.007	4.48 ± 0.02	−	>20	3071 ± 112
1c	8.094 ± 0.002	9.495 ± 0.002	4.83 ± 0.01	−	>20	448 ± 40
1d	7.964 ± 0.003	9.422 ± 0.002	4.75 ± 0.03[g]	−	>20	1060 ± 103
1e	8.118 ± 0.006	9.508 ± 0.004	4.86 ± 0.01	−	17 ± 4	82 ± 6
1f	7.62 ± 0.01	9.241 ± 0.009	4.60 ± 0.02	−	9.7 ± 0.8	138 ± 26
1g	7.48 ± 0.02	7.79 ± 0.06	5.02 ± 0.02	+	2.8 ± 0.2	35 ± 3
1h	7.93 ± 0.02	7.97 ± 0.04	4.99 ± 0.01	+	4.4 ± 0.7	36 ± 1
1i	7.56 ± 0.02	8.83 ± 0.01	5.81 ± 0.01[g]	+	2.2 ± 0.2	37 ± 3
1j	7.50 ± 0.02	7.65 ± 0.03	4.67 ± 0.02	+	8 ± 2	99 ± 29
1k	6.28 ± 0.03	8.80 ± 0.02	4.65 ± 0.02	+	3.1 ± 0.6	325 ± 170

[a] All data are the average of three independent measurements; errors represent 1 SD for pK_a determinations and 1 SEM for the remaining data. [b] At 25 °C; 0.15 M NaCl background electrolyte. [c] 1:1 complex formation at 25 °C, pH 7.5, in 40% aqueous DMSO, 20 mM HEPES buffer. [d] Inhibition of β-hematin formation as determined by infrared spectroscopy; 4 equiv of aminoquinoline relative to hematin. [e] β-Hematin inhibitory activity in equivalents of aminoquinoline relative to hematin causing 50% inhibition. [f] Versus chloroquine sensitive D10 strain of *P. falciparum*. [g] From ref 8.

Chart 1. Quinolines Used in This Study



X = a) NH_2, b) OH, c) OCH_3, d) H, e) CH_3, f) F, g) I, h) Br, i) Cl, j) CF_3, k) NO_2

First, why is chlorine required at the 7-position for β-hematin inhibition and what is the effect of replacing it with other groups? Second, is there a quantitative relationship between the strength of β-hematin inhibition and antiplasmodial activity?

To attempt to address these questions, we have synthesized a series of 11 derivatives of N^1,N^1-diethyl-N^2-(4-quinolinyl)-1,2-ethanediamine with various substituents at the 7 position (**1a**−**k**), shown in Chart 1. Synthesis of all but three of these compounds has been reported previously, and antiplasmodial activities of many of them have also been reported.[3,5,8] However, systematic data on their pK_a values, association constants with hematin, and β-hematin inhibitory activities have not been reported. As a result, literature data are not suitable for constructing a detailed SAR for these compounds.

Chemistry

Compounds **1c**−**k** were prepared by reaction of *N*,*N*-diethylethanediamine with compounds **2c**−**k**. The reaction was carried out using literature procedures[5,9,10] with only one minor modification: reaction of **2k** with *N*,*N*-diethylethanediamine was carried out under milder conditions (85 °C for 5 h) than previously reported for the analogue with a chloroquine lateral chain[10] in order to avoid decomposition of the compound. Compounds **2i** and **2j** are commercially available. Compounds **2d** and **2e** were prepared from quinoline and 7-methylquinoline, respectively, by oxidation to the corresponding *N*-oxide with peracetic acid,[11] followed by nitration at the 4-position with potassium nitrate in trifluoroacetic acid[12] and then reaction with phosphorus trichloride. Compounds **2c**,**f**−**h**,**k** were prepared from the appropriately meta-substituted aniline using the method of Price and Roberts[13] followed by treatment with $POCl_3$. The decarboxylation step in the preparation of **2k** was carried out via the formation of a silver salt to facilitate the process.[10] Compounds **1a** and **1b** were prepared from compounds **1k** and **1c**, respectively. In the case of **1a**, the nitro group of **1k** was reduced under hydrogen over a Pd−C catalyst,[10] while **1b** was prepared by demethylation of **1c** with BBr_3.

Identity and purity of all of the compounds that were isolated as solids were determined by melting point analysis, elemental analysis, ^{1}H NMR, and mass spectrometry. Oils (**1a** and **1b**) were characterized by ^{1}H and ^{13}C NMR and high-resolution mass spectrometry. Syntheses and characterization of **1d** and **1i** were reported in our earlier publication.[8] Three of the compounds prepared for this study (**1c**, **1f**, and **1h**) exhibited melting points that differed by more than 3 °C from literature values,[3,5] but elemental analyses were in close agreement with calculated values and their identity was further demonstrated by ^{1}H NMR and mass spectrometry.

Determination of Hematin−Aminoquinoline Association Constants. Association constants for hematin−aminoquinoline complex formation were determined by spectrophotometric titration in aqueous dimethyl sulfoxide (DMSO) at pH 7.5 (Table 1, log *K*). The method used was identical to that reported in our previous publications.[8,14,15] We have reported details of the spectroscopic changes observed[8,14] and justification for the use of this nonphysiological medium in previous publications.[8] Suffice it to reiterate that where identical compounds have been studied trends observed in the association constants are the same[16] as those reported in acidic, purely aqueous medium by other laboratories using titration calorimetry.[17] Spectrophotometric titration of hematin with aminoquinolines in purely aqueous media is not feasible because there is little difference between the Soret band of the porphyrin in the resulting complexes and that of the starting hematin dimers present under these conditions. In the absence of a titration calorimeter, spectrophotometric titration of monomeric hematin with aminoquinolines in aqueous DMSO provides the best alternative method of investigation. We believe that a good indication of the trends in association constant that are likely to occur in the food vacuole is obtained using this method, and indeed, the free energies of association of hematin with known

quinoline antimalarials have been shown to be very similar using both methods.[16]

Inhibition of β-Hematin Formation. The ability of the aminoquinolines to inhibit β-hematin formation was evaluated both qualitatively using infrared spectroscopy and quantitatively by means of the so-called BHIA (β-hematin inhibitory activity) assay that we recently reported.[18] The infrared assay was performed exactly as described by us in earlier publications.[8,15,19] In each case, the effect of 4 equiv of aminoquinoline relative to hematin was investigated. Absence of the strong and characteristic infrared bands of β-hematin at about 1660 and 1210 cm^{-1} was taken as evidence of inhibition. The quantitative BHIA assay that we recently reported in detail[18] is based on differential solubilization of hematin and β-hematin in DMSO and NaOH solution, respectively. The method determines a 50% inhibitory concentration of aminoquinoline for β-hematin inhibition in equivalents of aminoquinoline to hematin ($BHIA_{50}$) under the prevailing experimental conditions. For four of the compounds (**1a**−**d**), no β-hematin inhibition could be detected at the highest practicable concentrations (20 equiv relative to hematin) in the BHIA assay. Results of both assays are reported in Table 1. Both methods give essentially consistent results. The infrared assay gives a negative result for all compounds, showing a $BHIA_{50}$ in excess of 8 equiv, while all compounds with a $BHIA_{50}$ of 8 equiv or less gave a positive infrared result for β-hematin inhibition. Given that a total of 4 equiv of aminoquinoline was used in the infrared assay, this suggests that the compounds may be somewhat more inhibitory under the conditions used in this assay or that the β-hematin infrared peaks are masked by hematin under conditions of partial inhibition.

Determination of pK_a Values. The pK_a values of each of the aminoquinolines used in this study were determined by glass electrode potentiometric titration under nitrogen at 25 °C. The study was performed using 0.15 M NaCl as a constant background electrolyte and was conducted according to methods described in detail by Jackson et al.[20] The program ESTA[21,22] was used to analyze the data. In each case, two pK_a values were obtained. The pK_a values are reported in Table 1.

Biological Testing

Compounds **1a**−**k** were all tested for antiplasmodial activity against the chloroquine-sensitive D10 strain of *Plasmodium falciparum* in vitro. No attempt was made to investigate activities against chloroquine-resistant strains because the inherent mechanism of antiplasmodial activity could then potentially be masked or complicated by mechanisms responsible for resistance. The IC_{50} values are reported in Table 1. The trends in activities in the case of the six compounds previously reported by De et al.[5] (**1c**,**f**−**j**) are in close agreement with results of that report (although the IC_{50} values differ because of differences in experimental conditions and parasite strain).

Discussion

Effect of the Group at the 7-Position on pK_a Values. There is strong evidence that pH trapping plays a role in the activity of aminoquinoline antimalarials such as chloroquine.[8,23−26] Most convincing is the fact that a decrease in pH of the external medium decreases chloroquine activity.[27] This behavior implies that the pK_a values of compounds under investigation are critical for determining the activities of these compounds. Despite this, pK_a determination seems to have been largely neglected in the study of aminoquinoline antiplasmodials. The data presented in Table 1 show that the pK_a of the quinoline N (the acid dissociation constant of the quinolinium cation and referred to below as pK_{a1}) is strongly dependent on the nature of the group at the 7-position on the quinoline ring. Groups that are electron donors, such as NH_2 and OCH_3, raise pK_{a1} relative to that of the 7-H derivative. By contrast, strongly electron-withdrawing groups such as NO_2 cause a considerable decrease in pK_{a1}. Overall, pK_{a1} ranges over more than 2 log units, from a minimum of 6.28 in the NO_2 derivative to 8.36 in the NH_2 derivative. As can be seen in Figure 1a, with the exception of the OH derivative, there is a good quantitative correlation between the pK_{a1} value and the Hammett constant for the meta position (σ_m, an empirical measure of electron-withdrawing capacity; Table 2[28]) of the group at the 7-position ($r^2 = 0.77$, $P = 0.0009$). The apparently anomalous behavior of the OH derivative may be an indication that pK_{a1} represents ionization of the OH group in **1b** rather than deprotonation of the quinolinium nitrogen.

The strong dependence of the quinoline ring pK_a on the nature of the group attached to the 7-position of the

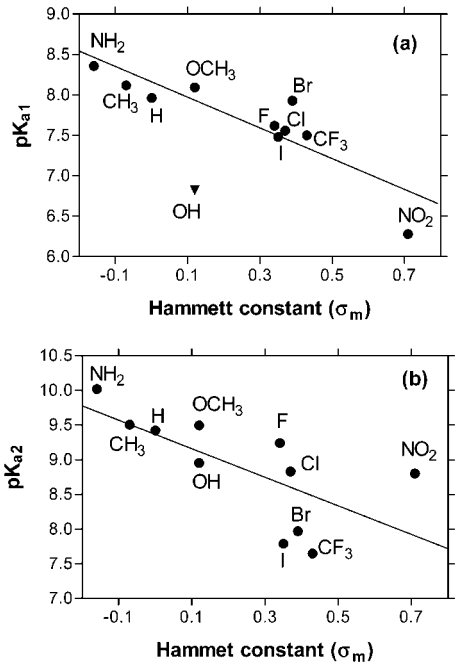


Figure 1. (a) Correlation between the quinoline nitrogen pK_a (pK_{a1}) and the Hammett constant for the meta position (σ_m) of the group at the 7-position of the quinoline ring in compounds **1a**−**k**. Without the OH derivative (**1b**), the data conform to the empirical equation $pK_{a1} = -1.90\sigma_m + 8.2$ ($r^2 = 0.77$, $P = 0.0009$). (b) Correlation between the pK_a of the lateral chain terminal amino group (pK_{a2}) and Hammett constant for the meta position of the group at the 7-position of the quinoline ring. The data conform to the empirical equation $pK_{a2} = -2.06\sigma_m + 9.4$ ($r^2 = 0.46$, $P = 0.021$). The NO_2 derivative (**1k**) appears to conform poorly to this correlation. Its omission leads to a significantly better correlation: $pK_{a2} = -3.19\sigma_m + 9.5$ ($r^2 = 0.70$, $P = 0.0026$).

Table 2. Vacuolar Accumulation Ratio, Relative Vacuolar Accumulation Ratio (versus Chloroquine), and Normalized IC_{50} for the Aminoquinolines under Investigation and Hammett Constants and Lipophilicity Constants for the Group at the 7-Position on the Quinoline Ring

	group[a]	acc ratio[b]	α[c]	(IC_{50})(α),[d] nM	σ_m[e]	π[e]
1a	NH_2	5693	0.966	>4830	−0.16	−1.23
1b	OH	1347	0.228	700 ± 26	0.12	−0.67
1c	OCH_3	5253	0.891	399 ± 36	0.12	−0.02
1d	H	4964	0.842	892 ± 87	0	0
1e	CH_3	5302	0.899	74 ± 5	−0.07	0.56
1f	F	3846	0.652	92 ± 17	0.34	0.14
1g	I	2937	0.498	17 ± 1	0.35	1.12
1h	Br	4607	0.781	28 ± 1	0.39	0.86
1i	Cl	3706	0.629	23 ± 2	0.37	0.71
1j	CF_3	2844	0.482	48 ± 14	0.43	0.88
1k	NO_2	500	0.085	28 ± 14	0.71	−0.28

[a] At the 7-position. [b] Vacuolar accumulation ratio calculated using eq 1 and assuming a vacuolar pH of 5.5 and external pH of 7.4. [c] Relative vacuolar accumulation ratio (relative to a chloroquine acc ratio of 5896 based on measured pK_a values of 8.55 ± 0.05 and 9.81 ± 0.04). [d] Normalized IC_{50}. [e] From ref 28.

quinoline ring is to be expected on resonance grounds. What is more surprising is the strong variation of the pK_a of the tertiary amino group in the lateral chain (the acid dissociation constant of the tertiary ammonium group in the lateral side chain is referred to as pK_{a2}). The value of pK_{a2} ranges over about 2.5 log units from 7.65 for the CF_3 derivative to 10.02 for the NH_2 derivative. Evidently in these short-chain chloroquine analogues, there is significant through-space interaction between the amino terminal end of the lateral chain and the quinoline ring. Presumably the interaction would diminish as the chain length increases, and the effect could thus be smaller in longer-chain analogues (such as chloroquine). Although pK_{a2} significantly correlates with the Hammett constant of the group attached to the 7-position (Figure 1b), the correlation is far poorer than that exhibited by pK_{a1} ($r^2 = 0.46$, $P = 0.021$).

Interestingly, both pK_a values of the chloro derivative **1i** are considerably lower than in chloroquine (7.56 and 8.83 versus 8.55 and 9.11). The increase in both pK_{a1} and pK_{a2} can possibly be ascribed to the greater σ-electron-donating strength of the more substituted α-position (position α to the quinoline 4-amino group) of the chloroquine lateral chain.

The extent of pH trapping of each of the compounds under investigation can be estimated from the pK_a values using

$$\frac{[Q]_v}{[Q]_e} = \frac{\left\{1 + \frac{[H^+]_v}{K_{a2}} + \frac{[H^+]_v^2}{K_{a1}K_{a2}}\right\}}{\left\{1 + \frac{[H^+]_e}{K_{a2}} + \frac{[H^+]_e^2}{K_{a1}K_{a2}}\right\}} \quad (1)$$

Here, $[Q]_v$ and $[Q]_e$ represent the vacuolar and external concentrations of the aminoquinoline, respectively, and $[H^+]_v$ and $[H^+]_e$ represent the vacuolar and external hydrogen ion concentrations. The calculation presupposes that equilibrium is achieved and assumes that the cellular membranes are completely impervious to the protonated aminoquinoline species. It is quite likely that neither of these assumptions is entirely correct and furthermore the precise vacuolar pH is not known. Nevertheless, the equation probably gives at least a good indication of relative vacuolar accumulations of the different derivatives, even if the absolute value is not totally reliable. Predicted vacuolar accumulation ratios based on eq 1 and assuming a vacuolar pH of 5.5 are given in Table 2. To facilitate easy comparison, we also define a relative accumulation ratio with respect to chloroquine, α (the calculated accumulation ratio of the derivative of interest divided by the calculated accumulation ratio of chloroquine). These values are also reported in Table 2. Vacuolar accumulation is of course strongly dependent on the pH of the vacuole, and this value is not known with certainty. As can be seen from Figure 2 however, the relative accumulation ratios are virtually independent of pH below about pH 6. Because all studies reported to date agree that the vacuolar pH is below this figure,[24,29−31] we can be confident that the α value is essentially unaffected by our somewhat arbitrary choice of a vacuolar pH of 5.5.

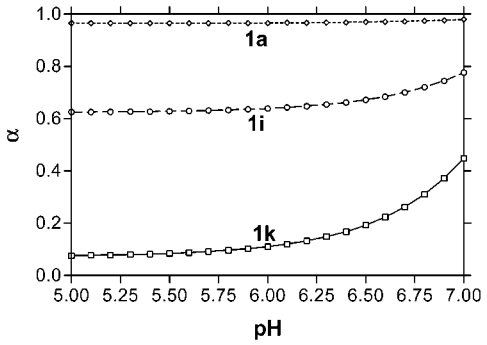


Figure 2. Plot showing the dependence of the relative vacuolar accumulation ratio (α) on pH for compounds **1a**, **1i**, and **1k**. The value of α is obtained by calculating the vacuolar accumulation ratio for the compound of interest using eq 1 and dividing it by the ratio calculated for chloroquine using the same equation. Note that α is essentially insensitive to pH below pH 6.

Correlation between IC_{50} and β-Hematin Inhibition. As can be seen from Figure 3a, there is no significant correlation between antiplasmodial IC_{50} and the association constant for hematin−aminoquinoline binding ($r^2 = 0.39$, $P = 0.053$ for the log−log plot). Thus, despite evidence that the activity of quinoline antiplasmodials is dependent on the presence of hematin,[32−34] this activity does not arise as a mere result of hematin association. No correlation is observed between IC_{50} and $BHIA_{50}$ either ($r^2 = 0.08$, $P = 0.55$ for the log−log plot; see Figure 3b). Both of these results are to be expected if pH trapping is important for the activities of these compounds, since the differing pK_a values of the various derivatives mean that their concentrations at the site of action in the parasite food vacuole will differ markedly at a given external concentration. Multiplying the observed IC_{50} by the relative vacuolar accumulation ratio (α) compensates for these differences, in effect normalizing the activity data. These normalized IC_{50} values are reported in Table 2. There is no correlation between the normalized IC_{50} value and log K ($r^2 = 0.24$, $P = 0.15$ for the log−log plot) as shown in Figure 3c. Thus, the activity of these aminoquinolines does not arise as a direct result of their mere association with hematin. On the other hand, the correlation between the normalized IC_{50} and $BHIA_{50}$ is impressive, as shown in Figure 3d ($r^2 = 0.83$, $P = 0.0043$). Indeed, in this case, deviations from linearity can be ascribed almost entirely

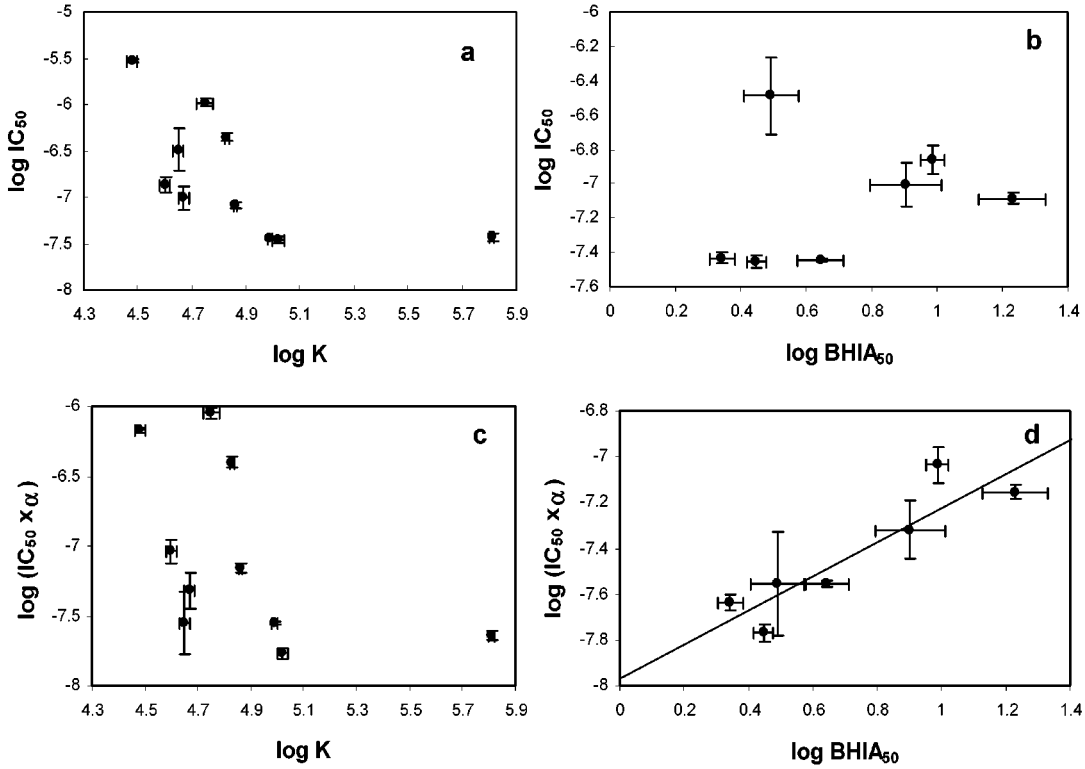


Figure 3. (a) Plot of log IC_{50} versus log *K* shows that there is no correlation between antiplasmodial activity against the chloroquine sensitive D10 strain of *P. falciparum* and the association constants for **1b**−**k** with hematin ($r^2 = 0.39$, $P = 0.053$). (b) Plot of log IC_{50} versus log $BHIA_{50}$ shows that there is also no correlation between uncorrected antiplasmodial activity and β-hematin inhibitory activity ($r^2 = 0.08$, $P = 0.55$). (c) Even when the IC_{50} is normalized for different extents of pH trapping in the food vacuole ((IC_{50})(α)), there is no correlation between the log of the normalized antiplasmodial activity and log *K* ($r^2 = 0.24$, $P = 0.15$). (d) By contrast, there is an excellent linear correlation between the log of the normalized antiplasmodial activity and the log of the β-hematin inhibitory activity that conforms to the empirical equation $\log[(IC_{50})(\alpha)] = (0.741)\log BHIA_{50} - 7.97$ ($r^2 = 0.83$, $P = 0.0043$). Error bars represent 1 SEM ($n = 3$).

to experimental error, since all but one of the data points deviate from the best-fit straight line by less than 3 standard errors in either the measured IC_{50} or $BHIA_{50}$ value. The points are well-spaced on the line, and the correlation is not a result of either clustering of points or weighting by any single point. Furthermore, the four compounds that have $BHIA_{50}$ values too high to measure (>20 equiv, **1a**−**d**) all have normalized IC_{50} values well above those for the compounds with measurable $BHIA_{50}$ values as predicted (see Table 2). Of course, it is not possible to say whether they conform to the quantitative correlation.

For the set of compounds in this study, the data strongly indicate that both pH trapping and β-hematin inhibition are the basis of antiplasmodial activity. Other groups have also observed a relationship between antiplasmodial activity and strength of β-hematin inhibition,[10,17,35] but only in one instance has a correction been made for accumulation.[35] When no correction is made, a relationship that is substantially linear has been reported,[17] but the evidence from Figure 3b seems to suggest that such a correlation may be partly fortuitous. Vippagunta et al.[10] reported a linear correlation between antiplasmodial activity and β-hematin inhibitory activity that has been divided by the association constant for the corresponding hematin−quinoline complex. We find no equivalent correlation for our compounds ($r^2 = 0.32$, $P = 0.19$), and indeed, despite the good statistical correlation reported in that study, closer scrutiny indicates that the data appear to be strongly weighted by a single outlying point. A report by Hawley et al.,[35] on the other hand, has previously shown a correlation between accumulation-normalized IC_{50} and β-hematin inhibition. In that case, total cellular quinoline accumulation was measured from the magnitude of the inoculum effect. There is considerable evidence that about 90% of the total observed accumulation of quinoline drugs within parasitized red cells is due to association with hematin.[33,34] It may thus at first sight seem unexpected that antiplasmodial activity would depend on pH trapping, which probably only accounts for about 10% of the overall accumulation.[33,36] As we have pointed out elsewhere however,[26] at concentrations in the region of the IC_{50} of chloroquine, in the absence of accumulation, negligible association of aminoquinolines with hematin would occur. Accumulation of the free aminoquinoline through pH trapping is therefore essential to drive the equilibrium toward complex formation through Le Chatelier's principle.

Correlation of β-Hematin Inhibitory Activity with Other Physical Parameters. The structures of hematin−aminoquinoline complexes are unknown, and so the steric and electronic requirements for inhibition of β-hematin formation are likely to be difficult to establish. To attempt to gain some insight into the origin of the β-hematin inhibitory activity of these compounds, we have looked for correlations with both observed parameters and tabulated empirical substituent constants[28] using multiple correlation analysis. It should be noted, however, that the interaction of ami-

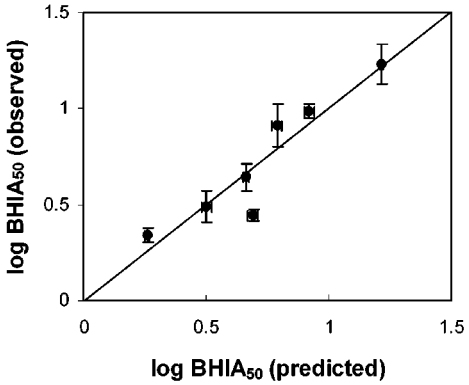


Figure 4. β-Hematin inhibitory activity of these aminoquinolines is significantly correlated with both the association constant for hematin−aminoquinoline association and the Hammett constant for the meta position (σ_m) according to the empirical equation $\log BHIA_{50} = -(0.519)(\log K) - 1.06\sigma_m + 3.67$. For these data, $F = 12.8$ ($F_{crit} = 5.79$ at the 95% confidence level), $r^2 = 0.87$, and for both of the individual parameters, $\log K$ and σ_m, P lies in the range 0.01−0.02. This correlation is illustrated by a plot of observed versus predicted $\log BHIA_{50}$. Error bars represent 1 SEM ($n = 3$).

noquinolines with hematin involves neither formation of chemical bonds nor docking with structurally well-defined binding pockets. The complexes are thus likely to be highly flexible and capable of assuming many different conformations. Correlations between $BHIA_{50}$ values on one hand and substituent and association constants on the other are likely only in cases where complexes are structurally similar, so the utility of these correlations is likely to be limited.

The observed $\log BHIA_{50}$ values are significantly correlated with the log of the hematin−aminoquinoline association constants and Hammett constants (Table 2) for the meta position (σ_m) as shown in Figure 4. Together, these two parameters provide an apparent empirical prediction of β-hematin inhibitory activity ($r^2 = 0.87$, $P = 0.0024$). The result indicates that inhibition is partly controlled by the strength of association of the aminoquinoline with hematin. The stronger the association, the stronger the inhibition of β-hematin formation. The second factor suggests that the more strongly electron-withdrawing the group is (as measured by the Hammett constant), the more strongly it inhibits β-hematin formation. In particular, it appears that withdrawal of electron density from the positions meta to the 7-position is crucial. This may indicate that a reduction of electron density from one or both of the 5 and 8′ positions on the quinoline ring is key to this activity. The reason for this requirement is not clear, but it is possible that it permits the quinoline to assume or maintain a particular conformational alignment with respect to hematin that is especially productive in preventing β-hematin formation.

Despite this good correlation, the lack of β-hematin inhibitory activity of **1b**, **1c**, and **1d** is unexplained. This suggests that the correlation is fortuitous or that the inactive compounds interact with hematin in a manner that is structurally quite distinct from the remaining compounds. In the cases of **1b** and **1c**, this could involve coordination of the O atom at the 7-position to the Fe(III) center, but the case of **1d** is less clear.

Correlation of Association Constants with Lipophilicity. With the exception of the chloro derivative,

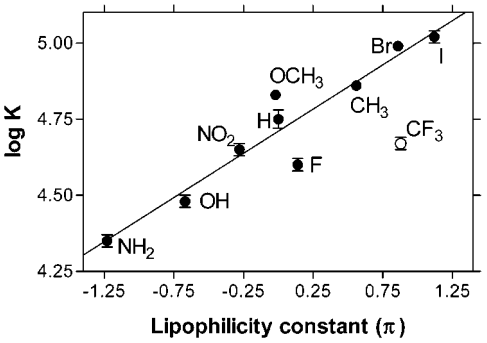


Figure 5. Correlation between hematin−aminoquinoline association constant ($\log K$) and the lipophilicity constant (π) for the substituent at the 7-position on the quinoline ring. The data for the Cl derivative (**1i**, $\log K = 5.81$) is not shown because it lies far above the remaining data. The data for the CF_3 derivative (**1j**) was also not used in the correlation. The remaining points conform to the empirical equation $\log K = 0.290\pi + 4.71$ ($r^2 = 0.89$, $P = 0.0001$). Error bars represent 1 SEM ($n = 3$).

the association constants for these compounds fall within a fairly narrow range, exhibiting a little less than a 5-fold difference between the weakest (NH_2, **1a**) and the strongest (I, **1g**). By contrast, the Cl derivative (**1i**) has an association constant 6-fold higher than the iodo derivative. The origin of the exceptionally strong association of the chloro derivative cannot be rationalized on the basis of currently available knowledge about these complexes, but chloroquine itself also has a similarly strong association constant[14] that is in agreement with values reported by titration calorimetry,[17,37] so the effect does not appear to be artifactual. Once again, this effect is probably a reflection of the exceptional flexibility of these complexes. It is quite possible that the hematin complex formed with the Cl derivative assumes a unique comformation.

For the remaining derivatives, a significant correlation is seen between $\log K$ and the lipophilicity constant π (Table 2) ($r^2 = 0.73$, $P = 0.0017$). The CF_3 derivative (**1j**) lies somewhat below the line (Figure 5), possibly because of its relatively large steric bulk. Omission of this point results in an even better correlation ($r^2 = 0.89$, $P = 0.0001$). The increase in $\log K$ with increasing lipophilicity seems to accord well with previous evidence that we have reported showing that the association of chloroquine with hematin involves a major hydrophobic component.[14] It therefore stands to reason that attachment of more hydrophobic groups to the quinoline ring enhances the association.

Conclusions

The current study has confirmed and considerably extended our earlier proposed SAR for chloroquine and related aminoquinoline antiplasmodials.[8] The linear dependence of the IC_{50}, corrected for pH trapping, on β-hematin inhibitory activity (and not merely on the strength of association with hematin) in this family of compounds strongly supports the idea that activity results from inhibition of hemozoin formation in the parasite. It further supports the proposal that pH trapping is essential for drug activity by ensuring that the compound is concentrated at the site of action. Of course, we cannot rule out the possibility that β-hematin inhibitory activity may fortuitously be quantitatively

Table 3. Melting Points of Previously Known Aminoquinolines Synthesized in This Work

compound[a]	mp, °C
N^1,N^1-diethyl-N^2-(7-methoxy-4-quinolinyl)-1,2-ethanediamine, **1c**	98−99 (lit. 94−95)[5]
N^1,N^1-diethyl-N^2-(7-methyl-4-quinolinyl)-1,2-ethanediamine, **1e**	102−105 (lit. 99−103.6)[42]
N^1,N^1-diethyl-N^2-(7-fluoro-4-quinolinyl)-1,2-ethanediamine, **1f**	81−82 (lit. 72−73)[5]
N^1,N^1-diethyl-N^2-(7-iodo-4-quinolinyl)-1,2-ethanediamine, **1g**	133−136 (lit. 131−132)[5]
N^2-(7-bromo-4-quinolinyl)-N^1,N^1-diethyl-1,2-ethanediamine, **1h**	120−121 (lit. 111−112)[5]
N^1,N^1-diethyl-N^2-(7-trifluoromethyl-4-quinolinyl)-1,2-ethanediamine, **1j**	121−123 (lit. 122−123)[5]
4-chloro-7-nitroquinoline, **2k**	169−172 (lit. 171−173,[43] 156−160[10])

[a] The purities and identities of all of these compounds were confirmed by ^{1}H and ^{13}C NMR, by mass spectrometry, and (except for **2k**) by elemental analysis. All elemental analyses were within 0.2% of the calculated C and N values and within 0.3% of the calculated H values.

correlated with one or more other activities[38,39] that could be the true basis of antiplasmodial activity, but inhibition of hemozoin formation appears to be the simplest explanation for the current findings.

Overall, the results appear to confirm the hypothesis that antiplasmodial aminoquinolines accumulate at the site of action through pH trapping because of the basic quinoline and lateral chain nitrogen atoms. The accumulated free aminoquinolinium ion then associates with hematin in the acidic food vacuole (according to our earlier study, as a result of the strong hematin-associating properties of the 4-aminoquinoline nucleus[8]). It is then the influence of the group at the 7-position on the quinoline ring that determines the ability of the complex to resist incorporation of the hematin molecule into hemozoin.

The ideal properties of this group are tentatively identified as the following.

(i) Moderately strong electron-withdrawing capacity. If the group is too strongly electron-withdrawing, it causes too large a decrease in the pK_a of the quinoline nitrogen and too little compound accumulates in the parasite food vacuole, reducing activity. On the other hand, if the group is not sufficiently electron-withdrawing, it does not appear to inhibit hemozoin formation strongly enough, and antiplasmodial activity is consequently also reduced.

(ii) Strong lipophilicity. The more lipophilic the group, the more strongly the compound associates with hematin and the more strongly it thus appears to inhibit hemozoin formation, thus enhancing antiplasmodial activity.

Interestingly, this combination of properties is best exhibited by the halogens chlorine, bromine, and iodine, probably accounting for the fact that these appear to show the strongest antiplasmodial activity.

The findings also suggest a small modification to the conclusions of our previous study.[8] Although the nature of the group at the 7-position has only a small influence on the association constant with hematin compared to the effect of the presence and position of the amino group, its influence on log K is nonetheless not entirely insignificant. Thus, in addition to its previously noted[8] direct effect on β-hematin formation, the group at the 7-position also appears to have important effects on the strength of association with hematin (which in turn seems to affect the strength of inhibition of hemozoin formation) and on accumulation at the site of action (through its effects on pK_a values).

Although these findings provide an apparently quantitative, empirical prediction of antiplasmodial activity, it must be emphasized that it is confined to this particular family of aminoquinolines. It cannot be generalized to aminoquinolines with other lateral chains, other groups attached to different positions on the quinoline ring, or to other aromatic nuclei. Such generalization would require that a deeper theoretical understanding of the interactions be attained using both computational and physical methods. Given the fact that the interactions between iron porphyrins and aminoquinolines involve formation of no chemical bonds[40,41] and that the solvent appears to play an important role in the interactions,[14] this is likely to be a formidable challenge. Despite these limitations, the study on this closely related family of aminoquinolines appears to have provided unique insights into the SARs in chloroquine and to have thrown more light on its mechanism of action.

Experimental Section

Antiplasmodial Testing. All antiplasmodial experiments were carried out on the chloroquine-sensitive *P. falciparum* clone D10 using methods and conditions identical to those that we described in an earlier report.[8]

Chemistry. All starting materials were obtained from Sigma-Aldrich, Vorna Valley, South Africa, except for solvents, acids, and common salts. These were obtained from Sarchem, Krugersdorp, South Africa. Precoated silica gel plates were obtained from Merck, South Africa, as were silica and alumina for column chromatography.

Melting points were determined using a Reichert-Jung Thermovar hot stage microscope or by differential scanning calorimetry. Infrared spectra were recorded on a Perkin-Elmer Paragon 1000 FT-IR spectrophotometer and a Perkin-Elmer 983 IR spectrophotometer in the range 3600−800 cm^{-1}. ^{1}H and ^{13}C NMR spectra were recorded on a Varian VXR-200 spectrometer at 200 MHz, a Varian Mercury spectrometer at 300 MHz, and a Varian Unity spectrometer at 400 MHz. All spectra were recorded in deuteriochloroform or d_6-dimethyl sulfoxide. Elemental analyses were performed using a Fisons Instruments elemental analyzer EA1108. Mass spectra were recorded on a VG Micromass 16F spectrometer operating at 70 eV with an accelerating voltage of 4 kV and a variable-temperature source. Accurate mass determinations were performed on a Kratos Limited MS9/50 spectrometer. All mass spectra were obtained using electron-impact techniques.

Compounds **1d** and **1i** were synthesized for a previous study, and details have been described elsewhere.[8] The remaining compounds used in the structure−activity study were synthesized. For those that are not novel, melting points are shown in Table 3 and are compared with previously reported values.[5,10,42,43] For compounds **1c**, **1f**, and **1h**, the melting points that we observed were more than 3 °C higher than previously reported. Elemental analysis, ^{1}H NMR, and mass spectrometry nonetheless confirmed the purity and identity of these compounds. In particular, the percentages of C, H, and N differed from calculated values by no more than 0.2%, 0.3%, and 0.2%, respectively, well within acceptable limits. Melting points of compounds **1e**, **1g**, and **1j** agreed with reported values to better

than 3 °C, and their identities and purities were further confirmed by elemental analysis, ^{1}H NMR, and mass spectrometry. Elemental analyses were all within accepted limits. Three novel compounds were prepared. Compound **1k** was prepared from **2k**, the synthesis of which has been previously reported. The melting point of **2k** agrees well with a previously reported value (Table 3), and ^{1}H NMR, ^{13}C NMR, and mass spectrometry further confirmed its identity. Compound **1a** was prepared from **1k**, and **1b** was prepared from **1c**. Full details of the synthesis and characterization of these three compounds are supplied. Compounds **1a** and **1b** were obtained as oils and were characterized by ^{1}H and ^{13}C NMR, IR, and HRMS. Elemental analyses on these two compounds were unsuccessful because of the hydrated nature of these hydrophilic oils.

***N*^{1},*N*^{1}-Diethyl-*N*^{2}-(7-Nitro-4-quinolinyl)-1,2-ethanediamine (1k).** A mixture of **2k** (0.160 g, 0.767 mmol) and *N*,*N*-diethylethanediamine (7 mL) was heated at 85 °C under N_2 for 5 h. The reaction mixture was then cooled to room temperature and Na_2CO_3(aq) added. The organic material was extracted into ethyl acetate (three times) and dried over $MgSO_4$, and the solvent was removed under reduced pressure. The residue was chromatographed first on silica gel (150:1) using ethyl acetate as the eluent to remove the excess reagent and then on silica gel (200:1) using mixtures of triethylamine/ethyl acetate (0.5:99.5 to 3:97) as the eluent to isolate **1k** (0.140 g, 63%): mp 110−113 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.09 (6H, t, J = 7.2 Hz, CH_3), 2.63 (4H, q, J = 7.2 Hz, CH_2), 2.85 (2H, t, J = 5.9 Hz, β CH_2), 3.28 (2H, m, α CH_2), 6.28 (1H, s, NH), 6.49 (1H, d, J = 5.4 Hz, Ar−H3), 7.85 (1H, d, J = 9.0 Hz, Ar−H5), 8.17 (1H, dd, J = 9.0 Hz, 2.3 Hz, Ar−H6), 8.65 (1H, d, J = 5.4 Hz, Ar−H2), 8.84 (1H, d, J = 2.3 Hz, Ar−H8); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 12.1, 39.8, 46.5, 50.5, 101.1, 117.7, 121.5, 122.5, 125.9, 147.9, 147.9, 149.7, 153.2; IR ($CHCl_3$) 3018, 2972, 1754, 1590, 1546, 1479, 1374, 1352 cm^{-1}; MS (EI) *m*/*z* 288 (M^+, 0.5), 155 (3), 86 (100), 58 (21), 30 (29). Anal. ($C_{15}H_{20}N_4O_2$) C, H, N.

***N*^{2}-(7-Amino-4-quinolinyl)-*N*^{1},*N*^{1}-diethyl-1,2-ethanediamine (1a).** A mixture of **1k** (0.108 g, 0.375 mmol) and 10% Pd−C (0.012 g, 0.011 mmol) in ethanol (15 mL) was stirred under H_2 for 22 h. The mixture was then filtered through Celite and washed with large volumes of ethanol. The filtrate was evaporated under reduced pressure, and the residue was adsorbed onto silica gel (80:1) and was eluted using mixtures of methanol/triethylamine/ethyl acetate (0.5:10:89.5 to 1.5:10:88.5). Purification was achieved by neutral alumina chromatography (70:1), eluting first with large volumes of ethyl acetate and then methanol/25% NH_3(aq)/ethyl acetate (10:2:88) to obtain **1a** (0.076 g, 78%) in the form of a brown oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.06 (6H, t, J = 7.2 Hz, CH_3), 2.58 (4H, q, J = 7.2 Hz, CH_2), 2.78 (2H, t, J = 6.0 Hz, β CH_2), 3.23 (2H, m, α CH_2), 3.96 (2H, bs, NH_2), 5.86 (1H, s, NH), 6.20 (1H, d, J = 5.2 Hz, Ar−H3), 6.84 (1H, dd J = 9.0 Hz, 2.4 Hz, Ar−H6), 7.11 (1H, d, J = 2.4 Hz, Ar−H8), 7.54 (1H, d, J = 9.0 Hz, Ar−H5), 8.40 (1H, d, J = 5.2 Hz, Ar−H2); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 12.0, 39.8, 46.5, 50.9, 97.1, 110.4, 112.3, 116.0, 120.9, 147.2, 150.0, 150.0, 151.3; IR ($CHCl_3$) 3402, 3382, 3020, 1631, 1586, 1538, 1471, 1329, 1224 cm^{-1}; HRMS 258.1843 (calcd 258.1845).

***N*^{1},*N*^{1}-Diethyl-*N*^{2}-(7-hydroxy-4-quinolinyl)-1,2-ethanediamine (1b).** A solution of **1c** (1.10 g, 4.02 mmol) in dry dichloromethane (15 mL) was cooled under N_2 to −78 °C. BBr_3 (4.02 g, 16.1 mmol) was added to this solution, and the mixture was stirred at −78 °C for 1 h. The reaction mixture was then placed in the freezer at −13 °C for 40 h. Workup involved recooling the mixture to −78 °C and basification by dropwise addition of K_2CO_3(aq). When the mixture was heated to room temperature, a white solid precipitate formed. This was filtered, washed with water, and dried in vacuo over P_2O_5. The dried organic material was extracted into methanol, and the insoluble material was filtered off. The product was then chromatographed on silica gel (three times) (20:1) using ethyl acetate to pack the column and methanol to load the material. Elution using triethylamine/methanol/ethyl acetate (2:20:78) afforded **1b** (0.685 g, 61.6%) as a luminous, yellow-green, thick oil: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 0.95 (6H, t, J = 7.2 Hz, CH_3), 2.53 (4H, q, J = 7.0 Hz, CH_2), 2.66 (2H, t, J = 7.0 Hz, β CH_2), 3.35 (2H, t, α CH_2), 6.38 (1H, d, J = 6.2 Hz, Ar−H3), 6.99 (1H, dd, J = 9.1 Hz, 2.5 Hz, Ar−H6), 7.04 (1H, d, J = 2.5 Hz, Ar−H8), 7.10 (1H, bs, NH), 8.02 (1H, d, J = 9.1 Hz, Ar−H5), 8.25 (1H, d, J = 6.2 Hz, Ar−H2); ^{13}C NMR (DMSO-d_6, 100.6 MHz) δ 12.5, 41.5, 47.4, 51.4, 97.3, 108.5, 112.3, 117.3, 124.0, 147.6, 148.5, 152.3, 159.9; IR (DMSO) 3257, 1617, 1584, 1545, 1458, 1346, 1301, 1235 cm^{-1}; HRMS 259.1677 (calcd 259.1685).

Determination of association constants of compounds **1a**−**k** with hematin was carried out exactly as we have described elsewhere.[14] Inhibition of β-hematin formation by these compounds was carried out by both an infrared-based assay and a solubilization-based assay, the β-hematin inhibitory activity (BHIA) assay. The former assay was performed exactly as we have described previously[19] except that 4 equiv of the quinoline compounds relative to hematin was used rather than 3. The full details of the BHIA assay have recently been published,[18] and the assay was performed exactly as described therein. The pK_a values of the quinolines were determined exactly as described by Jackson et al.[20]

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