Structure−Function Relationships in Aminoquinolines: Effect of Amino and Chloro Groups on Quinoline−Hematin Complex Formation, Inhibition of β-Hematin Formation, and Antiplasmodial Activity

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Received August 25, 1999

Comparison of 19 aminoquinolines supports the hypothesis that chloroquine and related antimalarials act by complexing ferriprotoporphyrin IX (Fe(III)PPIX), inhibiting its conversion to β-hematin (hemozoin) and hence its detoxification. The study suggests that a basic amino side chain is also essential for antiplasmodial activity. 2- And 4-aminoquinolines are unique in their strong affinity for Fe(III)PPIX, and attachment of side chains to the amino group has relatively little influence on the strength of complex formation. Association with Fe(III)PPIX is necessary, but not sufficient, for inhibiting β-hematin formation. Presence of a 7-chloro group in the 4-aminoquinoline ring is a requirement for β-hematin inhibitory activity, and this is also unaffected by side chains attached to the amino group. In turn, β-hematin inhibitory activity is necessary, but not sufficient, for antiplasmodial activity as the presence of an aminoalkyl group attached to the 4-amino-7-chloroquinoline template is essential for strong activity. We thus propose that the 4-aminoquinoline nucleus of chloroquine and related antimalarials is responsible for complexing Fe(III)PPIX, the 7-chloro group is required for inhibition of β-hematin formation, and the basic amino side chain is required for drug accumulation in the food vacuole of the parasite.

Introduction

Considerable evidence has been presented in recent years that antimalarial drugs such as chloroquine and amodiaquine (type-1 blood schizontocides) act by forming complexes with hematin, the hydroxo or aqua complex of ferriprotoporphyrin IX (Fe(III)PPIX), derived from parasite proteolysis of host hemoglobin.[1] Association constants for various antimalarials with hematin both in aqueous solution,[2] where hematin is expected to be aggregated,[3] and in 40% aqueous DMSO,[4] where it is monomeric,[5] have been reported recently. Ward and co-workers[6,7] have provided evidence that such interaction occurs within the food vacuole of the malaria parasite, the probable site of action of these drugs. Most compellingly, they have shown[6,7] that the activities of these drugs are Fe(III)PPIX-dependent, since inhibitors of plasmepsin (the enzyme responsible for host hemoglobin degradation in the food vacuole) are antagonists of drug activity. They have also shown this dependence in the case of mefloquine, a type-2 blood schizontocide, suggesting that drugs such as quinine, halofantrine, and mefloquine act by a similar mechanism.

It is generally believed[8] that detoxification of Fe(III)PPIX in the parasite food vacuole is brought about by polymerization to form malaria pigment, or hemozoin. Although this has recently been questioned by Ginsburg and co-workers[9] who have suggested that the major portion of Fe(III)PPIX is transported across the vacuolar membrane and degraded by glutathione-dependent redox processes involving reduced oxygen species,[10] no evidence has yet been presented for the large quantities of iron which would then have to be sequestered in the parasite. Indeed, to date hemozoin is the only known store of large quantities of iron in the parasite. Based on current evidence, it would then seem likely that type-1 and possibly also type-2 drugs inhibit hemozoin formation resulting in the buildup of significant quantities of toxic Fe(III)PPIX−drug complex which is probably then responsible for killing the parasite.

It is now known that hemozoin is chemically identical to β-hematin,[11] which is generally believed to be a polymer of hematin. In 1994 we showed[12] for the first time that several type-1 and type-2 blood schizontocides can specifically inhibit chemical formation of β-hematin by direct interaction between the drugs and Fe(III)PPIX. There is now substantial evidence that antimalarial drugs can inhibit both synthetic β-hematin formation and hemozoin formation in both chemical and biological systems by direct interaction between the drugs and Fe(III)PPIX.[2,12−20] The precise mechanism by which this occurs in the parasite food vacuole is still uncertain, but Goldberg and co-workers have shown that such hematin−drug complexes can bind to and inhibit further incorporation of Fe(III)PPIX into hemozoin.[19,20] They have thus suggested that the drugs cap the growing polymer and inhibit its further growth. Recently[9] direct evidence for decreased hemozoin formation in *Plasmo-*

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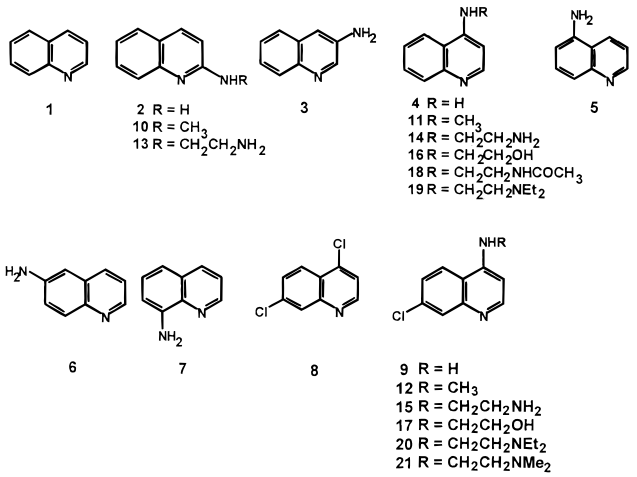
10.1021/jm990437l CCC: $19.00 © 2000 American Chemical Society
Published on Web 12/31/1999

Chart 1. Structures and Numbering of Quinolines Examined in This Study[a]



[a] Compounds **1, 3,** and **5**−**8** were obtained commercially.

dium falciparum in the presence of chloroquine has been provided.

In light of the above, a mechanism of action of type-1 antimalarials can be proposed in which the drug:[1,21,22] i. enters the food vacuole, possibly via diffusion of the free base across intervening membranes; ii. accumulates in the food vacuole, at least in part due to pH trapping of the protonated drug at the low pH of the vacuole[23] (this process involves diffusion of only the free base across the food vacuole membrane; increased protonation of the drug at the low pH of the vacuole results in inflow of more drug until free base concentrations are equal on both sides of the membrane); iii. forms a complex (which is predominantly a $\pi-\pi$ complex[24−26]) with Fe(III)PPIX, which may further enhance drug accumulation;[6,7] iv. inhibits formation of hemozoin via the formation of this complex; and v. exerts a toxic effect on the parasite in the form of the Fe(III)PPIX−drug complex.

The development of this detailed model of action provides an opportunity to test the activity of various quinolines in unprecedented detail. In this article we report the results of Fe(III)PPIX complexation, β-hematin inhibition, and antiplasmodial activity of 6 commercially available quinolines and 15 quinolines specifically synthesized for this study. The compounds were chosen to probe the effect of amino group position and of chlorination at the 7-position on the activities of these compounds with the aim of establishing a detailed structure−activity profile of chloroquine.

Chemistry

The compounds used in this investigation are presented in Chart 1.

Synthesis of 2-aminoquinoline, 4-aminoquinoline, and 4-amino-7-chloroquinoline and their derivatives was carried out by substitution of 2-chloroquinoline (Aldrich), 4-chloroquinoline (Aldrich), and 4,7-dichloroquinoline (Aldrich), respectively, with ammonia or the appropriate amine. Compound **18** was prepared by acetylation of **14** with acetic anhydride and triethylamine. Details are given in the Experimental Section.

Identity and purity of novel products were determined by elemental analysis, infrared spectroscopy, ^{1}H and ^{13}C NMR spectroscopy, mass spectrometry, and melting point determination. Previously known compounds were characterized on the basis of melting point determinations, ^{1}H NMR, and mass spectrometry. Elemental analyses were also performed in most cases, and ^{13}C NMR and infrared spectra were recorded where melting points differed by more than 3 °C from those previously reported.

All other quinolines were obtained commercially (Aldrich).

Determination of Fe(III)PPIX−Quinoline Association Constants. Association constants for Fe(III)PPIX−quinoline complexes were determined in 40% aqueous DMSO (pH 7.5) by spectrophotometric titration. The methods used have been described by us previously[4] when we reported association constants for various Fe(III)PPIX−antimalarial drug complexes. Typical spectral changes observed upon complexation are shown in Figure 1a. There is a substantial decrease in the intensity of the Fe(III)PPIX Soret band at 402 nm with no shift in the peak maximum. At longer wavelengths the Q-band of the metalloporphyrin (494 and 537 nm) also decreases in intensity, while the charge-transfer band at 620 nm increases in intensity and undergoes a substantial blue shift to 594 nm. These changes are quite distinct from those observed upon Fe(III)PPIX aggregation in pure aqueous solution (Figure 1b) which in turn results in a spectrum different from that reported[27] for the μ-oxo dimer. The spectrum also differs from that brought about by addition of a saturating concentration of NaCl to monomeric Fe(III)PPIX in 40% aqueous DMSO (Figure 1c) which is identical to that of the μ-oxo dimer.[27] As can be seen in Figure 1a, where the resulting complex involves a 1:1 stoichiometry, excellent isosbestic points are observed.

A typical titration curve is shown in Figure 2. The solid line is a best fit of the data to a 1:1 association model obtained by nonlinear least-squares analysis. Association constants for all of the compounds are reported in Table 1 (where these were measurable under our conditions).

Some of the quinolines form 2:1 complexes with Fe(III)PPIX (quinoline:Fe(III)PPIX) as can be seen in Table 1. In a few cases the second association constant could be extracted from the data, but in many instances where 2:1 complexation was observed values of K_2 were small and had excessively large errors and so these values are not reported. In some instances values of K_2 were so small that the decrease in absorbance with quinoline concentration at high concentrations was almost linear and values of K_1 were obtained by fitting data to a 1:1 model in the region of the titration where this complex dominates. In all instances the values of log K_1 have acceptably small errors and the data is well-fitted in the region of strong binding.

The conditions under which the association constants have been measured are of course quite different from those in the food vacuole of the parasite; however, comparison of the previously determined association constants of several well-known antimalarial drugs under identical conditions[4] to those employed here with those reported by other authors for the same drugs in acidic aqueous medium[2] (approximating to the conditions in the vacuole) using titration calorimetry indi-

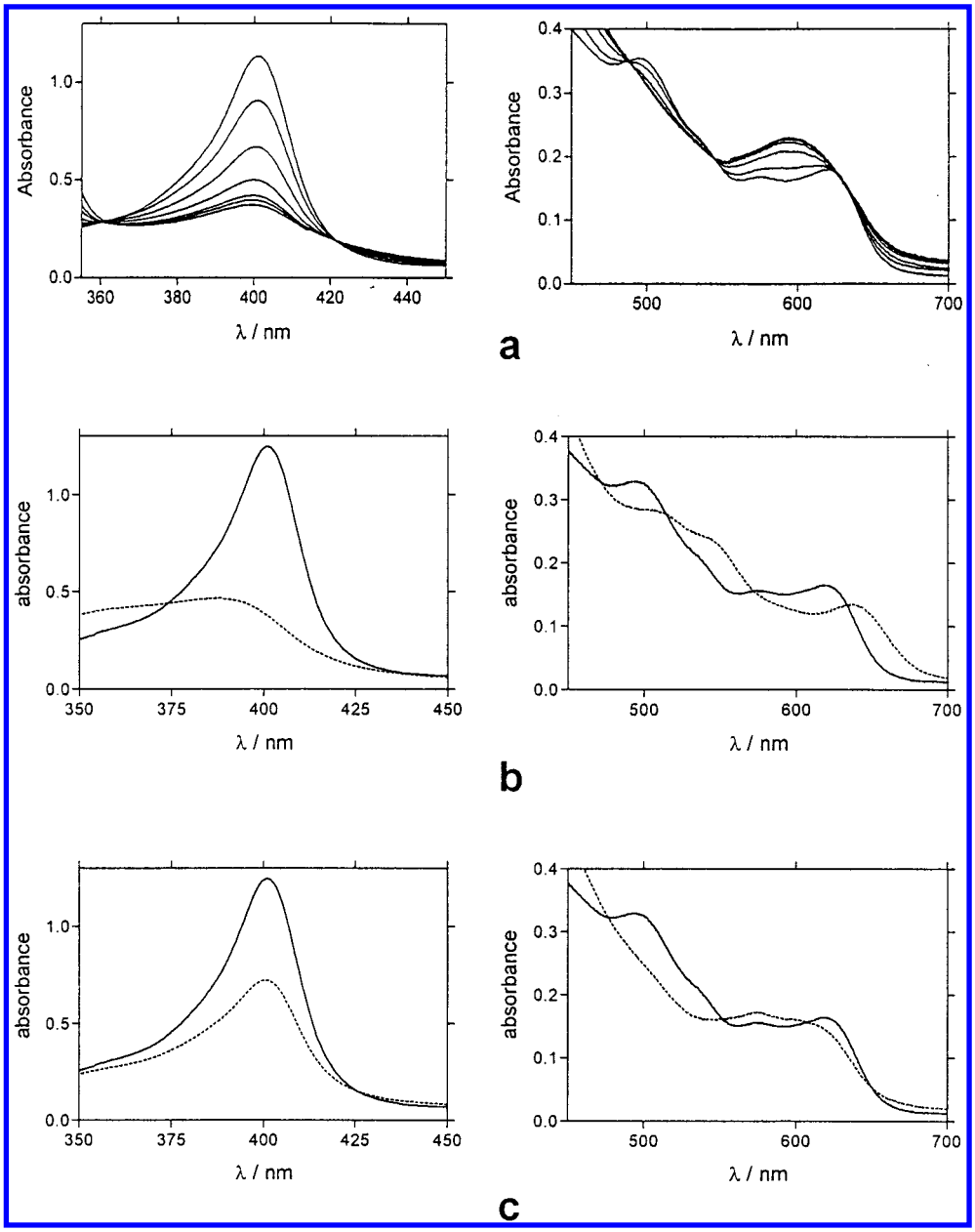


Figure 1. (a) Visible spectra of Fe(III)PPIX in 40% aqueous DMSO, pH 7.5, titrated with increasing concentrations of **9**. In the left panel the highest concentration of **9** corresponds to the weakest spectrum, while in the right panel the highest concentration of **9** corresponds to the spectrum which lies at the bottom of the series at 500 nm and at the top at 600 nm. (b) Visible spectra of Fe(III)PPIX in 40% aqueous DMSO, pH 7.5 (solid line), and in water (broken line). Note the large blue shift in the Soret region (left panel) and red shift of all the bands in the longer wavelength region (right panel). Fe(III)PPIX is aggregated in water. (c) Visible spectra of Fe(III)PPIX in 40% aqueous DMSO, pH 7.5 (solid line), and under the same conditions but with the addition of saturating NaCl (broken line). The spectrum of the latter is identical to that of the μ-oxo dimer reported by O'Keefe et al.[27] Note the peak maximum at 575 nm. Concentrations of Fe(III)PPIX are 8×10^{-6} M (left panels) and 2.4×10^{-5} M (right panels). All spectra corrected for dilution.

cates remarkably similar association constants and identical trends in their values.[1] We would thus argue that the association constants measured under our conditions are a good reflection of the interactions which would occur in the food vacuole. Utilizing a pH of 7.5, rather than more acidic conditions, improves the stability of Fe(III)PPIX solutions and hence data quality. The major advantage of 40% aqueous DMSO medium is that Fe(III)PPIX is strictly monomeric under these conditions and interpretation of results is not complicated by the need to consider Fe(III)PPIX disaggregation processes.

Inhibition of β-Hematin Formation. The ability of each of the compounds to inhibit β-hematin formation was determined by methods which we have reported previously[12,13] for antimalarial drugs and for a few commercially available quinolines. In the current work, 4 equiv of the quinoline was included in the reaction system for synthetic β-hematin formation. The β-hematin synthesis was carried out in the solid state as a slurry in 4.5 M acetate, pH 4.5 at 60 °C, for 30 min. The resulting product was filtered, washed extensively with water, and dried in a desiccator over phosphorus pentoxide. The identity of the product was then determined by infrared spectroscopy. Although questions have been raised as to whether the product of this synthesis is β-hematin,[28,29] we have recently used

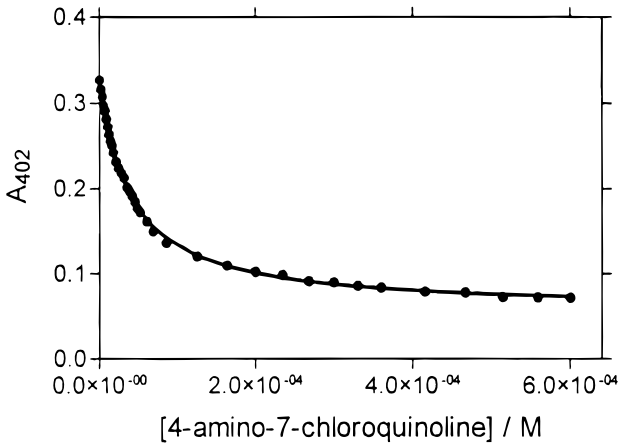


Figure 2. Typical spectrophotometric titration curve (in this case for **9**). The solid line is a best fit of the data to a 1:1 association model. Conditions: 40% aqueous DMSO, measured pH 7.5, 25 °C, 0.020 M HEPES buffer.

Table 1. Association Constants with Fe(III)PPIX, β-Hematin Inhibitory Activity, and in Vitro Antiplasmodial Activity against the D10 Strain of *P. falciparum* of the Quinolines Studied in This Work

compd[a]	log K^b ± SEM ($n = 3$)[c]	stoich[d]	β-hematin inhibition	IC_{50} (nM) ± SEM ($n = 3$)[c]
1	<2.5[e]	–	–	>10000
2	4.23 ± 0.02	2:1[f]	–	>10000
3	<2.5[e]	–	–	>10000
4	4.49 ± 0.01	2:1[g]	–	>10000
5	<2.5[e]	–	–	>10000
6	<2.5[e]	–	–	>10000
7	<2.5[e]	–	–	>10000
8	<2.5[e]	–	–	>10000
9	4.43 ± 0.01	1:1	+	3800 ± 500
10	4.26 ± 0.03	2:1[f]	–	>10000
11	4.38 ± 0.03	1:1[h]	–	>10000
12	4.48 ± 0.02	1:1	+	4000 ± 2200
13	4.34 ± 0.06	2:1[f]	–	>10000
14	4.59 ± 0.04	1:1	–	4700 ± 800
15	4.928 ± 0.003	1:1	+	92 ± 12
16	4.18 ± 0.03	1:1	–	>10000
17	4.66 ± 0.02	2:1[f]	+	5070 ± 80
18	4.28 ± 0.06	2:1[f]	–	>10000
19	4.75 ± 0.03 (3.2 ± 0.01)	2:1[i]	–	799 ± 404
20	5.81 ± 0.01 (4.47 ± 0.02)	2:1[i]	+	49 ± 14
21	4.96 ± 0.01	1:1	+	80.9 ± 6.2[j]
chloroquine	5.52 ± 0.03[k]	1:1[k]	+[l]	38 ± 14

[a] Refer to Chart 1 for structures. [b] In 40% aqueous DMSO, measured at pH 7.5, 25 °C, 0.020 M HEPES buffer. [c] Standard error of the mean, three determinations. [d] Quinoline:Fe(III)PPIX. [e] No association detected within the concentration range used in this study. [f] Second association constant too weak to obtain reliable constant (resulting in an almost linear decrease in absorbance with concentration); data fitted to strong association part of titration curve only, using 1:1 association model. [g] Fitted using a 2:1 model, but meaningful value not obtained for log K_2. [h] A sudden decrease in the entire visible spectrum at higher concentrations, probably due to adsorption onto the cuvette walls or precipitation, precluded measurement of log K_2 and the data fitted well to a 1:1 model up to the onset of this phenomenon. [i] log K_2 given in parentheses. [j] Two duplicate determinations. [k] From ref 4. [l] From ref 12.

elemental analysis, X-ray diffraction, and infrared spectroscopy to provide definitive evidence that this is indeed the product.[13] In addition we have previously provided evidence to this effect using Mössbauer spectroscopy.[30,31]

The major advantage of this infrared assay method for β-hematin inhibition is that it allows unequivocal identification of the reaction product. A disadvantage is that it does not permit quantitation of the inhibition process. However, we prefer this method to solubilization-based methods,[14,18] because of its ability to identify the product and because we have previously encountered problems with solubilization of the reaction products.[12,13] As a result we report all of the compounds as either active or inactive with respect to inhibition of β-hematin formation (see Table 1). No attempt is made to compare the relative strengths of inhibition, other than to note that all of those compounds which do inhibit are active at 4 equiv and can thus be considered as strongly inhibitory.

Biological Testing. All compounds were tested for antiplasmodial activity in vitro using a cultured chloroquine-sensitive strain of *P. falciparum*, D10. No attempt was made to test the compounds against chloroquine-resistant strains, as this would be likely to complicate interpretation of the data. Evidence presented in the literature to date suggests that resistance is compound-specific, rather than a result of changes in the drug target. For example, compound **20** has been reported to exhibit full activity against chloroquine-resistant strains of *P. falciparum*.[32]

No attempt was made to determine IC_{50} values in excess of 10 μM. It has been observed that chloroquine exhibits several effects at micromolar and millimolar concentrations which probably play no role in their therapeutic activity.[33] For example, at concentrations above 1 μM, it has been shown to inhibit protein synthesis.[34] At concentrations above 100 μM, chloroquine inhibits DNA and RNA replication,[35] and at concentrations in the millimolar range, it can inhibit proteolytic enzymes which occur in the food vacuole[36] as well as phospholipases which are believed to play an important role in the food vacuole.[37] None of these are likely to have any influence at therapeutic concentrations (about 100 nM outside the food vacuole and about 1 mM inside the food vacuole). It is likely that other quinolines may exhibit similar effects at high concentrations, and so IC_{50} values in excess of 10 μM were deemed to be of little relevance to the current study.

All IC_{50} values below 10 μM are reported in Table 1.

Discussion

Association between Quinolines and Fe(III)-PPIX. Although there have been many reports[1] of antimalarial drugs forming complexes with Fe(III)PPIX, to the best of our knowledge this is the first report to establish the minimal structural requirements for this association for a class of compounds. It is clear from Table 1 that of the quinolines studied here, only 2- and 4-aminoquinolines and their derivatives form strong complexes with Fe(III)PPIX. Under the conditions of the study, quinoline, 3-, 5-, 6-, and 8-aminoquinoline, and 4,7-dichloroquinoline (**1**, **3**, **5**−**8**) exhibited no evidence of complexation with Fe(III)PPIX. These compounds are under further investigation in our laboratories, and preliminary evidence suggests that **1** does in fact form a complex with Fe(III)PPIX, but the association is about a thousand times weaker than that found for **2** and **4** (log $K \sim 1.5$). Solubility limitations and strong UV tails extending into the visible region of the spectrum hamper studies on the interactions of the other weak-binding quinolines with Fe(III)PPIX (**3**, **5**−**8**).

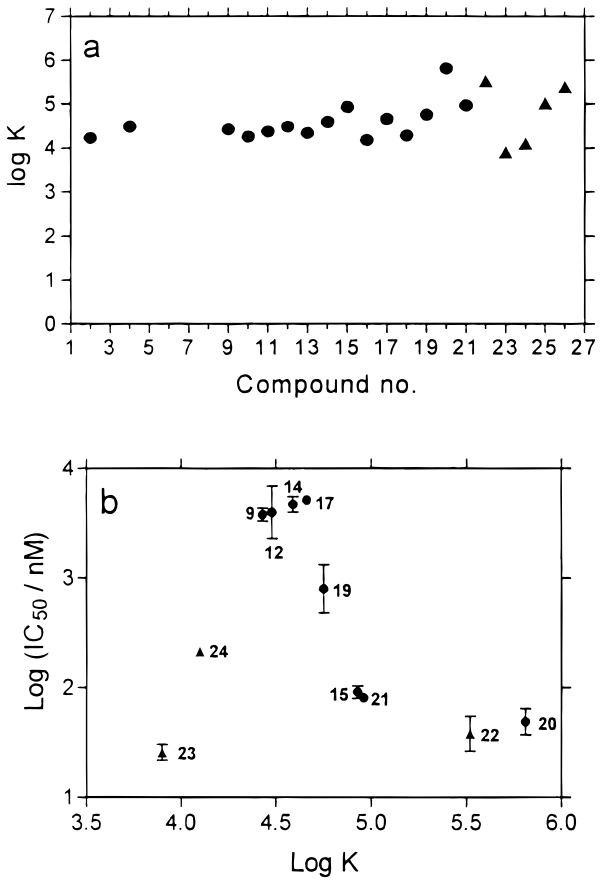


Figure 3. (a) Comparison of log *K* values for Fe(III)PPIX complexes of the quinolines investigated in this study (circles). Numbers refer to those given in Chart 1. Compounds for which no value is shown have association constants too weak to measure in the concentration range used in this study, corresponding to log $K \lesssim 2.5$. Triangles are the corresponding log *K* values for Fe(III)PPIX complexes of known antimalarial drugs and refer to chloroquine (**22**), mefloquine (**23**), quinine (**24**), and amodiaquine (**26**) (all from ref 4) and quinidine (**25**) (from ref 13). Conditions: as quoted in Figure 2 for all compounds. (b) Plot of log IC_{50} versus log *K* for Fe(III)PPIX association for those quinolines for which IC_{50} values are below 10 μM; numbers refer to those in Chart 1 except for chloroquine (**22**), mefloquine (**23**), and quinine (**24**). The plot illustrates the lack of a direct correlation between IC_{50} and log *K*.

Based on studies of amodiaquine, tebuquine, and analogues, it has been speculated[38] that interaction of the terminal tertiary amino group in the side chain of type-1 quinoline antimalarial drugs is essential for interaction with Fe(III)PPIX via intermolecular hydrogen bonding to the heme propionate groups. The current study demonstrates that this is not the case, as **4** itself forms a strong complex with Fe(III)PPIX. There is also remarkably little change in log *K* when an alkyl side chain is attached to the 2- or 4-amino group of aminoquinoline or indeed when a chlorine atom is placed at the 7-position of the quinoline ring. This suggests that in most cases the side chains do not interact strongly with Fe(III)PPIX. Exceptions appear to be chloroquine and **20** where there are more substantial increases in log *K* relative to **4** itself. Figure 3a graphically illustrates the insensitivity of the association constants to the nature of the quinoline side chains and to chlorination of the quinoline ring. It also shows that all 2- and 4-aminoquinolines in this study interact with Fe(III)-

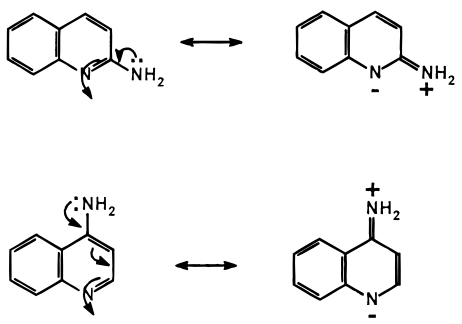


Figure 4. Unique resonance forms present in 2- and 4-aminoquinolines.

PPIX within the range of association constants observed for known antimalarial drugs (except for **20** which is marginally stronger). The log *K* values themselves do not thus distinguish active antiplasmodials from inactive compounds, and the association constants do not directly correlate with antimalarial activity as can be seen from Figure 3b.

Although the differences in log *K* between the various quinolines are relatively small, a number of interesting generalizations can be made on the strength of the data. Methylation of the amino group of the aminoquinolines causes almost no change in log *K*, while attachment of a hydroxyethyl group causes a modest decline in the case of 4-aminoquinoline but a small increase in the case of 4-amino-7-chloroquinoline. Attachment of an aminoethyl side chain to the aminoquinolines generally causes a small increase in log *K*, which is larger if there is a 7-chloro group present and is quite big if the terminal amine is ethylated. In the absence of structural data on the complexes these trends are difficult to rationalize.

The molecular basis for the specific interaction of **2** and **4** with Fe(III)PPIX is not clear at present; however, two properties of these compounds are striking. First they possess the resonance forms shown in Figure 4, and second their pK_a values are substantially higher than those of the other quinolines.[39] At the pH of the association experiment (7.5) **4** exists almost exclusively in the protonated form, while **2** is about 41% protonated. By contrast, the other quinolines are almost exclusively deprotonated. This might suggest that complex formation involves cation−π interactions; however, neither **1** nor **3** exhibit any observable association under similar conditions at pH 5.5, where significant (~22%) protonation occurs. Furthermore, association of **4** with Fe(III)PPIX was found to be essentially pH-insensitive. We have previously also reported this relative insensitivity to pH for association of both quinine and chloroquine with Fe(III)PPIX.[4]

At present the reasons for the differences between the various quinolines are obscure. We can only speculate that the differences may involve the ability of partial charges on the quinolines which form strong complexes with Fe(III)PPIX to match opposite partial charges on Fe(III)PPIX, while at the same time minimizing repulsive electrostatic interactions. Differences in the stoichiometries of the complexes, with some quinolines forming 2:1 (quinoline:Fe(III)PPIX) complexes and others forming 1:1 complexes, are also obscure. In many cases, however, the association of the second quinoline is very weak, and it may be that those quinolines which form 1:1 complexes would in fact form 2:1 complexes at

very much higher concentrations but show no evidence of this in the concentration range studied. In any event, the stoichiometry appears to be unrelated to either β-hematin inhibitory activity or antiplasmodial activity.

Chloroquine and **20** have substantially larger association constants with Fe(III)PPIX than the other quinolines in this study, indicating some additional interaction of the side chain of the quinoline with Fe(III)PPIX. The reason for this is unknown. There may be a hydrogen-bonding interaction between the tertiary amino group in the side chain of the quinoline and the heme propionate groups, although it seems unlikely that this would occur in the case of **20** where there is a bulky tertiary amino group if it does not occur with **15** which has a primary terminal amino group. More likely, there may be some direct van der Waals interaction between the side chain of the quinoline and the porphyrin ring. A third possibility, namely that the tertiary amino group coordinates directly to the iron center of Fe(III)PPIX, appears to be excluded as we could find no evidence of interaction between triethylamine and Fe(III)PPIX at comparable concentrations. In any case, these large increases in log *K* do not correspond to very profound increases in activity, as can be seen by comparing the antiplasmodial activities of **15** and **20**.

***β*-Hematin Inhibitory Activity.** There is a simple correlation between Fe(III)PPIX binding and β-hematin inhibitory activity inasmuch as those compounds which do not form measurable complexes fail to inhibit β-hematin formation. Perhaps more surprisingly, however, not all quinolines which do form strong complexes with Fe(III)PPIX inhibit β-hematin formation.

The minimal critical feature responsible for β-hematin inhibition appears to be the introduction of the chlorine atom at the 7-position on the quinoline ring. This is most simply illustrated by comparing **4** and **9**. Both have virtually identical log *K* values (4.49 ± 0.01 and 4.43 ± 0.01, respectively), yet only the latter appears to be able to inhibit β-hematin formation. Indeed all of the aminoquinolines which contain a 7-chloro group are inhibitory, while none of those which lack this group exhibit inhibitory activity under the conditions of the assay.

The question as to whether the role of the chlorine atom is predominantly steric, electronic, or a mixture of both cannot be answered on the basis of the current data. We are investigating this question further in our laboratories and will be examining the effects of other groups on β-hematin formation. A recent report by Krogstad and co-workers in which the 7-chloro group is replaced by a fluoro, bromo, or iodo group suggests that the latter two groups are likely to also cause β-hematin inhibition as these compounds have been reported to exhibit essentially undiminished antiplasmodial activity (the situation with fluorine is less clear as its activity is reduced).[40]

Relationship between Fe(III)PPIX Association, *β*-Hematin Inhibition, and Antiplasmodial Activity. Compounds **15**, **20**, and **21** show strong antiplasmodial activity, with IC_{50} values below 100 nM. These compounds share the following features: they all form complexes with Fe(III)PPIX; they all inhibit β-hematin formation; and they all contain basic amine side chains in addition to the basic quinoline ring nitrogen. In all of these features they resemble chloroquine. Compounds **9**, **12,** and **17** also form strong complexes with Fe(III)-PPIX and inhibit β-hematin formation but lack a terminal amino side chain. Their antiplasmodial activities lie between about 3.8 and 5 μM. These differences in activity strongly support the argument that drug accumulation through pH trapping is essential for antiplasmodial activity. Making the reasonable assumption that the K_a values for these nitrogen atoms are of the same order as the equivalent nitrogens in chloroquine, the former set of compounds would be expected to accumulate about 10000-fold (in the limit) in the food vacuole while the latter set would accumulate only about 100-fold through pH trapping. This difference alone can account for the approximately 100-fold difference in antiplasmodial activity between these two groups of quinolines. It has been argued by other authors[6,7] that additional accumulation occurs as a result of complex formation between chloroquine and Fe(III)PPIX, but this would not lead to increased free drug concentration in the food vacuole, whereas the pH trapping process would. Complex formation could, however, account for the observation that total drug accumulation in the food vacuole is greater than predicted from pH trapping alone and may indeed even account for the major drug species present in the food vacuole.

Compounds **14** and **19** exhibit limited antiplasmodial activity (micromolar level), despite showing no evidence of β-hematin inhibitory activity. At first sight this appears to contradict the model of activity proposed above (see Introduction); however, both of these compounds would be expected to accumulate strongly in the food vacuole due to the presence of terminal amino groups and may well reach concentrations in the region of 8−50 mM at their IC_{50}'s. At these concentrations it is quite likely that they may exhibit activity via alternative mechanisms. On the other hand, it is possible that they may be capable of inhibiting β-hematin formation at very high concentrations. The chloroquine analogue in which the 7-chloro group is replaced by a hydrogen atom is reported to be significantly more active than **19**.[32,41] The question as to whether this compound is active via a mechanism involving inhibition of β-hematin formation or some other mechanism is an open one.

All of the other compounds which fail to inhibit β-hematin formation (**1**−**8**, **10**, **11**, **13**, **16, 18**) show no significant antiplasmodial activity. Apart from **13** none of these are expected to accumulate more than about 100-fold in the food vacuole and are thus probably unlikely to be active via alternative mechanisms except at very high concentrations.

The relative antiplasmodial activities of **15**, **20**, and **21** may relate to differences in log *K* values, differences in lipophilicity, a combination of these, or other unknown factors. These differences are in any case quite small, spanning less than a 2-fold difference.

Conclusions

The evidence presented in this study supports the proposal that type-1 schizontocidal antimalarials: i. must be able to form relatively strong complexes with Fe(III)PPIX (log *K* in the range about 4−5.5), ii. must be able to inhibit β-hematin formation, and iii. must contain a basic side chain to assist accumulation at the site of action in the food vacuole.

Table 2. Melting Points for Previously Known Aminoquinolines Synthesized in This Work

compd	mp (°C)
2-aminoquinoline (**2**)	129 (lit.[45] 128−129)
4-aminoquinoline (**4**)	151−153 (lit.[45] 153−154)
4-amino-7-chloroquinoline (**9**)	134 (lit.[46] 105−110, lit.[47] 146−147, lit.[48] 152−154.4)[49]
2-methylaminoquinoline (**10**)	70−72 (lit.[50] 70.5−71.5)
4-methylaminoquinoline (**11**)	233[a] (lit.[51] 227−227.5, lit.[52] 238−240)
7-chloro-4-methylaminoquinoline (**12**)	245 sublimation (lit.[53] 245−246)
N-(7-chloro-4-quinolinyl)-1,2-ethanediamine (**15**)	137−139 (lit.[54] 137−139)
2-(7-chloro-4-quinolinyl)aminoethan-1-ol (**17**)	217−218[a] (lit.[55] 214)
N^2-(7-chloro-4-quinolinyl)-N^1,N^1-diethyl-1,2-ethanediamine (**20**)	106−108[a] (lit.[56] 94−97)
N^2-(7-chloro-4-quinolinyl)-N^1,N^1-dimethyl-1,2-ethanediamine (**21**)	122−124 (lit.[57] 121−122.8)

[a] Purity confirmed by elemental analysis.

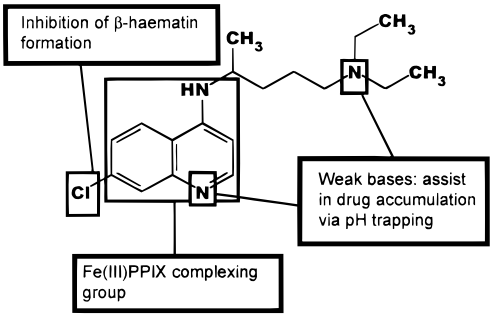


Figure 5. Proposed structure−function relationships in chloroquine based on findings in the current work.

We are now able to propose a detailed model of the structure−function relationships in chloroquine as follows (see Figure 5): i. the 4-aminoquinoline nucleus alone provides an Fe(III)PPIX complexing template but is not sufficient for inhibition of hemozoin formation; ii. introduction of the 7-chloro group is responsible for inhibition of hemozoin formation but probably has little influence on the strength of association with Fe(III)-PPIX; and iii. the aminoalkyl side chain is a requirement for strong antiplasmodial activity. It probably assists in drug accumulation in the food vacuole. It also appears to enhance the strength of association with Fe(III)PPIX in some cases, but this effect does not appear to be essential for its activity.

Based on these findings, it should be possible to elaborate other molecules which are found to form $\pi-\pi$ complexes with Fe(III)PPIX and which inhibit β-hematin formation so as to arrive at novel antimalarials by semirational design. These findings are also of considerable interest when combined with the recent structure−function investigations of Krogstad and co-workers[32,40] which have shown that changes in the length of the aminoalkyl side chain have little influence on activity against chloroquine-sensitive strains of *P. falciparum* (in agreement with our proposed structure−function model) but a profound influence on activity against chloroquine-resistant strains of the parasite. It would appear that sufficiently large changes in the side chain alone overcome chloroquine resistance without having to make changes to the 4-amino-7-haloquinoline template responsible for Fe(III)PPIX complexation and inhibition of β-hematin formation.

Experimental Section

Antiplasmodial Testing. All antiplasmodial experiments were carried out on the chloroquine-sensitive *P. falciparum* clone D10. The parasites were cultured continuously according to the method of Trager and Jensen,[42] with slight modifications. Cultures were maintained at a hematocrit of between 3% and 5% and at parasitemias of between 2% and 8%. Parasitized O+ human red blood cells were suspended in culture flasks containing RPMI culture medium supplemented with 10% A+ human serum. The flasks were gassed with a mixture of 3% O_2, 4% CO_2, and 93% N_2.

Chloroquine diphosphate was dissolved in water and all other compounds were dissolved in methanol and then diluted with medium to achieve the required concentrations (final concentrations all contained less than 1% methanol, which was found to be nontoxic to the parasites). Antiplasmodial activity was assessed by measuring the activity of the parasite lactate dehydrogenase enzyme, according to a modified version of the method of Makler.[43] All antiplasmodial testing was performed at 1% hematocrit and 2% parasitemia. Compounds were added at the trophozoite stage and applied for a period of 48 h before LDH measurement.

Dose−response curves were then constructed using nonlinear least-squares fitting with Graphpad Prism software[44] and the 50% inhibitory concentrations (IC_{50}) were calculated. Each IC_{50} value is the result of at least three separate experiments performed in duplicate.

Chemistry. Quinoline, 2-chloroquinoline, 4-chloroquinoline, 4,7-dichloroquinoline, 3-aminoquinoline, 5-aminoquinoline, 6-aminoquinoline, 8-aminoquinoline, ethylenediamine, *N*,*N*-diethylethylenediamine, *N*,*N*-dimethylethylenediamine, and ethanolamine were obtained from Sigma-Aldrich, Vorna Valley, South Africa. Methylamine (33% in methanol) was obtained from Merck, South Africa, and aqueous ammonia, acetic anhydride, triethylamine, and zinc chloride as well as common salts and solvents were obtained from Sarchem, Krugersdorp, South Africa. Precoated silica gel plates were obtained from Merck, South Africa.

Column chromatography was carried out on Merck silica gel 60. Infrared (IR) spectroscopy was carried out on a Perkin-Elmer 983 infrared spectrometer in the range 4000−600 cm^{-1}. Proton and carbon NMR spectra were recorded on a Varian 400 MHz spectrometer or a Varian VXR200 instrument at 200 MHz. Solvents are as indicated. Mass spectra were obtained on a VG Micromass 16F spectrometer operating at 70 eV with an accelerating voltage of 4 kV and a variable temperature source. Melting points were determined on a Reichert-Jung Thermovar hot stage microscope except compound **16** which was determined by differential scanning calorimetry (DSC). Microanalyses were performed by the University of Cape Town Microanalysis Service.

In the case of compounds which were synthesized, but are not novel, melting points are shown in Table 2 and are compared with those previously reported. These compounds were all further characterized by proton NMR, mass spectrometry, and in most cases elemental analysis. Where a discrepancy of 3 °C or more was observed in the melting point, the compound was further characterized by ^{13}C NMR. In all cases the identity of these products was confirmed and they were also found to be of acceptable purity for further study. In the case of novel compounds, full synthetic details and characterization are supplied below.

***N*-(2-Quinolinyl)-1,2-ethanediamine Monohydrochloride (13).** Reaction of 2-chloroquinoline (2.69 g, 16.4 mmol) with ethylenediamine (10 mL) in a cycloaddion tube at 105 °C for 19 h afforded the product after removal of excess ethylenediamine in vacuo. Column chromatography using methanol as eluent afforded **13** as a hydrochloride salt (2.67 g, 73%) which was recrystallized from ethanol: mp 174−178 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.20 (2H, brs, −NH_2), 7.90 (1H, d, J = 9.0 Hz, Ar−H), 7.65 (1H, dd, J = 8.0, 1.4 Hz, Ar−H), 7.54 (1H, dd, J = 8.0, 0.6 Hz, Ar−H), 7.50 (1H, m, Ar−H), 7.46 (1H, brs, −N*H*−), 7.18 (1H, m, Ar−H), 6.82 (1H, dd, J = 9.0, 1.2 Hz, Ar−H), 3.63 (2H, m, −CH_2−), 3.08 (2H, t, J = 6.2 Hz, −CH_2−); ^{13}C NMR (DMSO-d_6, 400 MHz) δ 156.82, 147.35, 136.53, 129.17, 127.54, 125.53, 123.04, 121.61, 113.27, 38.73, 38.59; IR (KBr) 3259, 3132, 3051, 2954, 2892, 2076, 1625, 1540, 1148, 813 cm^{-1}; MS (EI) *m*/*z*% 187 (M$^+$, 8.6), 157 (100). Anal. ($C_{11}H_{13}N_3$·HCl) C,H,N.

***N*-(4-Quinolinyl)-1,2-ethanediamine Monohydrochloride (14).** The product was prepared by reaction of 4-chloroquinoline (1.54 g, 9.23 mmol) with ethylenediamine (10 mL) in a cycloaddition tube at 130 °C for 4 h. After removal of excess ethylenediamine in vacuo the product was purified by column chromatography with ethanol elution and the crystalline product was obtained as a hydrochloride salt (1.14 g, 54%) and was recrystallized from ethanol: mp 211−212 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.43 (1H, d, J = 5.4 Hz, Ar−H), 8.33 (1H, d, J = 8.5 Hz, Ar−H), 7.80 (1H, dd, J = 8.4, 0.6 Hz, Ar−H), 7.63 (1H, m, Ar−H), 7.44 (1H, m, Ar−H), 6.56 (1H, d, J = 5.4 Hz, Ar−H), 3.2−4.2 (3H, brs, −N*H*− and −NH_2), 3.56 (2H, t, J = 6.3 Hz, −CH_2−), 3.10 (2H, t, J = 6.3 Hz, −CH_2−); ^{13}C NMR (DMSO-d_6, 400 MHz) δ 150.38, 149.91, 147.84, 129.04, 128.59, 124.05, 122.20, 118.88, 98.40, 40.32, 37.45; IR (KBr) 3498, 3396, 3035, 1610, 1562, 824 cm^{-1}; MS (EI) *m*/*z*% 187 (M$^+$, 39), 157 (100). Anal. ($C_{11}H_{13}N_3$·HCl) C,H,N.

2-(4-Quinolinyl)aminoethan-1-ol (16). Reaction of 4-chloroquinoline (202 mg, 1.24 mmol) with ethanolamine (3 mL) at 120 °C for 4.5 h afforded the product after extraction into ethyl acetate (three times) from aqueous sodium carbonate solution. The crystalline product (233 mg, 100%) was recrystallized from ethyl acetate/ethanol: mp 139 °C (DSC); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.37 (1H, d, J = 5.4 Hz, Ar−H), 8.19 (1H, dd, J = 8.4, 1.0 Hz, Ar−H), 7.76 (1H, dd, J = 8.4, 1.0 Hz, Ar−H), 7.59 (1H, m, Ar−H), 7.40 (1H, m, Ar−H), 7.06 (1H, t, J = 5.5 Hz, −N*H*−), 6.47 (1H, d, J = 5.4 Hz, Ar−H), 4.82 (1H, brs, −O*H*), 3.36 (4H, m, 2 × −CH_2−); ^{13}C NMR (DMSO-d_6, 400 MHz) δ 172.03, 150.65, 150.05, 148.28, 128.99, 128.69, 123.75, 121.60, 118.84, 98.19, 58.83; IR (KBr) 3372 (broad), 3322, 1585, 1547, 1343, 1070 cm^{-1}; MS (EI) *m*/*z*% 188 (M$^+$, 48), 157 (100). Anal. ($C_{11}H_{12}N_2O$) N; C: calcd, 70.19; found, 69.63; H: calcd, 6.43; found, 6.88.

***N*-2-(4-Quinolinyl)aminoethylethanamide (18).** Reaction of **14** (172 mg, 0.92 mmol) with acetic anhydride (112 mg, 1.1 mmol) in triethylamine (0.35 mL, 2.5 mmol) at 0 °C for 1 h and ambient temperature for 2.5 h under N_2 yielded the product which was then extracted into ethyl acetate (three times) from aqueous sodium carbonate. The product (164 mg, 78%) was then recrystallized from a mixture of ethyl acetate and ethanol: mp 200−202 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.39 (1H, d, J = 5.3 Hz, Ar−H), 8.11 (2H, m, −N*H*−CO− + Ar−H), 7.77 (1H, d, J = 8.4 Hz, Ar−H), 7.60 (1H, m, Ar−H), 7.41 (1H, m, Ar−H), 7.23 (1H, s, Ar−N*H*−R), 6.50 (1H, d, J = 5.3 Hz, Ar−H), 3.30−3.37 (4H, m, 2 × −CH_2−), 1.84 (3H, s, −CH_3); ^{13}C NMR (DMSO-d_6, 400 MHz) δ 170.03, 150.65, 149.82, 148.25, 129.01, 128.70, 123.86, 121.42, 118.75, 98.04, 42.43, 37.41, 22.62; IR (DMSO) 3463, 3300, 3062, 1665, 1583, 1542, 1285 cm^{-1}; MS (EI) *m*/*z*% 229 (M$^+$, 35), 157 (100). Anal. ($C_{13}H_{15}N_3O$) C,H,N.

***N*^{2}-(4-Quinolinyl)-*N*^{1},*N*^{1}-diethyl-1,2-ethanediamine (19).** Reaction of 4-chloroquinoline (130 mg, 0.8 mmol) with *N*,*N*-diethylethylenediamine (1.5 mL) at 130 °C for 18 h yielded the product as an oily solid after extraction into dichloromethane (four times) from aqueous potassium carbonate and evaporation of the solvent. Purification by column chromatography yielded pure product (116 mg, 60%): mp 82 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.55 (1H, d, J = 5.3 Hz, Ar−H), 7.97 (1H, dd, J = 8.5, 0.7 Hz, Ar−H), 7.74 (1H, dd, J = 8.3, 1.1 Hz, Ar−H), 7.62 (1H, m, Ar−H), 7.43 (1H, m, Ar−H), 6.38 (1H, d, J = 5.3 Hz, Ar−H), 6.07 (1H, brs, −N*H*−), 3.27 (2H, m, −CH_2−), 2.83 (2H, t, −CH_2−), 2.61 (4H, q, J = 7.1 Hz, 2 × −CH_2−), 1.08 (6H, t, J = 7.1 Hz, 2 × −CH_3); ^{13}C NMR (DMSO-d_6, 400 MHz) δ 151.26, 150.05, 148.55, 129.97, 129.06, 124.67, 119.71, 119.20, 99.14, 50.90, 46.70, 39.98, 12.26; IR (KBr) 3202, 2964, 2929, 1581, 1550, 1167, 1092 cm^{-1}; MS (EI) *m*/*z*% 243 (M$^+$, 33), 157 (100); HRMS 243.1735 (calcd 243.1735). Anal. ($C_{15}H_{21}N_3$) C,H,N.

Determination of association constants with Fe(III)PPIX and of β-hematin inhibitory activity was carried out by methods which we have described elsewhere.[4,12,13]

Acknowledgment. We thank the National Research Foundation of South Africa, the Medical Research Council of South Africa, the University of Cape Town, the University of the Witwatersrand, and the Drug Discovery Committee of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR) for financial support. We also thank Dr. Diego Monti, Department of Chemistry, University of Milan, Italy, for making facilities available for completion of parts of the work (C.H.K.).

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JM990437L