Relationship between NMR Shielding and Heme Binding Strength for a Series of 7-Substituted Quinolines

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Chemical shielding tensors are calculated for the carbons in a series of 4-aminoquinolines with different substituents at the 7-position. The σ_{11} component is used as a measure of the relative π-electron density at each carbon. By comparing the π-electron density at each carbon with the log K of binding to heme (Kaschula et al. *J. Med. Chem.* **2002**, *45*, 3531), the drug-heme association is found to increase with increasing π-electron density at the carbons meta to the substituent and with decreasing π-electron density at the carbons ortho and para to the substituent. The greatest change in π-electron density is at the ortho carbons, and log K increases with a decrease in π-electron density on the ring containing the substituent, which corresponds to an increase in the π-dipole between the two rings. An examination of the solution structures of the $\pi-\pi$ complexes formed by amodiaquine and quinine with heme (Leed et al. *Biochemistry* **2002**, *41*, 10245. de Dios et al. *Inorg. Chem.* **2004**, *43*, 8078) shows that the π-dipoles in each drug and in the porphyrin ring of heme may be paired. The chloro-substituted compound has an association constant that is an order of magnitude higher than the other compounds in the series, but the π-electron density at the ring containing the substituent is not correspondingly low. This lack of correlation indicates that the Cl-substituted compound may be binding to heme in a manner that differs from the other compounds in the series.

Introduction

Malaria remains as one of the most serious health concerns throughout the world. The molecular mechanism that describes how quinoline-based antimalarial drugs function is not yet precisely known. During the erythrocytic stage of its life cycle, the parasite digests red blood cell hemoglobin as a source of amino acids.[1,2] A byproduct of this hemoglobin digestion is the heme group (ferriprotoporphyrin IX, FPIX), which is toxic to the parasite. The toxic heme is sequestered in the form of hemozoin, a crystalline Fe−O41 dimer that is structurally identical to synthetic β-hematin.[3] Hemozoin is also known as "malaria pigment" and appears as a dark spot in the digestive vacuole of the parasite, the organelle in which hemoglobin digestion and hemozoin formation occur. Quinoline antimalarial drugs such as chloroquine (Figure 1), amodiaquine, quinine, and mefloquine are believed to function by inhibiting the formation of hemozoin from heme. This allows toxic heme to remain in the digestive vacuole, eventually killing the parasite.

One major problem in the fight against malaria is the emergence of resistance to known treatments. Chloroquine-resistant strains of malaria are now found throughout Africa, South America, and Southeast Asia.[4] Likely candidates for circumventing resistance are structurally similar to existing drugs. Hence, an understanding of the different functional groups responsible for both antimalarial activity and resistance are important to the discovery of new drugs. Toward this end, much work has been done in developing quantitative structure−activity relationships (QSARs) for quinoline-based antimalarial drugs.[5]

Several aspects of antimalarial drug activity are relevant to these studies. First, quinoline antimalarials are thought to prevent hemozoin formation by binding to heme. In addition to binding heme, successful drugs must also inhibit hemozoin formation. The β-hematin inhibition can be measured in vitro. This parameter has likewise been used in QSAR studies. To come into contact with heme at all, the drugs must be present in the digestive vacuole of the parasite. The concentration of drug that accumulates in the digestive vacuole has also been measured and compared in QSAR studies. Last, drug IC_{50}'s against both sensitive and resistant strains can also be measured as a test of antiparasitic activity.

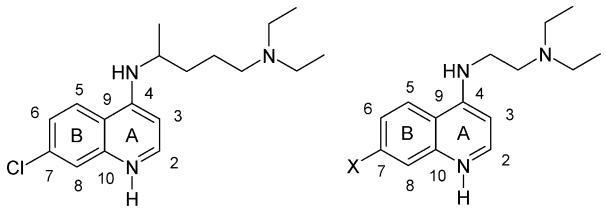

Figure 1. Chemical structures of the antimalarial drug chloroquine and the 7-substituted 4-aminoquinoline compounds synthesized by Kaschula et al. (*J. Med. Chem.* **2002**, *45*, 3531) and considered theoretically in this study. Quinoline carbons are numbered for reference, and the A and B rings of quinoline are indicated.

These four quantitative responses to antimalarial drugs, association constant for heme binding, β-hematin inhibitory concentration, cellular accumulation ratio, and inhibition of parasite growth (IC_{50}), are related to one another. The ability to bind to heme has been shown to be a necessary, but not a sufficient, condition for inhibition of β-hematin formation, and inhibition of β-hematin formation has been shown to be necessary, but not sufficient, for antimalarial activity.[6] In a study

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of 4-aminoquinolines with different substituents at the 7-position, a correlation was found between β-hematin inhibition and antimalarial activity normalized for vacuolar accumulation.[7] Relationships have been found between drug-heme binding K and inhibition of hemozoin formation as well as between inhibition of hemozoin formation and drug IC_{50}.[8] A correlation was also found between drug IC_{50}'s and β-hematin formation inhibition normalized for heme binding K's.[9]

Hawley et al.[10] found a relationship between drug accumulation ratios and IC_{50} values in a series of amodiaquine derivatives. This relationship holds in four different *P. falciparum* isolates. Although drug-heme binding constants were not measured directly, they argued that these findings could be rationalized, in part, by the assumption that cellular accumulation is presumably related to strength of binding to heme, so increased accumulation could be due to stronger binding to heme, which could lead to stronger antiparasitic activity.[10] In a series of quinolines and nonquinolines, the accumulation-normalized IC_{50}'s (drug accumulation normalized to the highest cellular accumulation ratio multiplied by IC_{50}) were found to correlate with inhibition of hemozoin formation in the case of a chloroquine-sensitive (CQS) isolate but not for a chloroquine-resistant (CQR) isolate.[11] This indicates that both cellular accumulation and inhibition of β-hematin formation are important to antimalarial activity and that the mechanism of chloroquine resistance could be related to preventing accumulation of the drug in the digestive vacuole of the parasite.

Previous studies relating the structure of aminoquinoline antimalarial drugs to their function have focused on two areas: changes to the aminoalkyl side chain and changes to substituents on the quinoline ring. In a study by De et al., 7-chloroquinolines with *N*,*N*′-diethyldiaminoalkane side chains with lengths between 2 and 12 carbons were found to be as effective as chloroquine against CQS strains.[12] The compounds with ethyl, propyl, isopropyl, decyl, and dodecyl side chains were also effective against CQR strains. Analogues of chloroquine with branched and unbranched side chains containing 2 and 3 carbons between amino nitrogens were also found to have both in vitro and in vivo antiparasitic activity similar to that of chloroquine in CQS strains of *P. falciparum* and were more effective than chloroquine against CQR strains.[13] Madrid et al.[14] synthesized a series of quinolines with two different amino side chains and various substituents at the 5-, 6-, 7-, and 8-positions of the quinoline ring. One side chain was the (*N*, *N*-diethyl)-1,3-diaminopentane side chain of chloroquine and the other was (*N*, *N*-diethyl)-1,3-diaminopropane. They found that for all ring substitutions, the compound with the shorter side chain was the more potent of the pair against both CQR and CQS strains.

QSAR studies of quinoline antimalarials have also examined the effect of different substituents on the quinoline ring. De et al.[12] found that chloroquine analogues with a methyl group at the 2-position, without the chloro substituent, or with the chloro substituent at the 6- or 8-position had reduced activity against both CQS and CQR strains, as compared to chloroquine. Vippagunta et al.[9] studied a series of aminoquinolines with different substituents on the quinoline ring and concluded that effective antimalarials must have an electron-withdrawing group at the 7-position, preferably a chloro substituent. Several other studies have focused on the substituent at the 7-position in particular. A series of 4-aminoquinolines of various side-chain lengths with different substituents at the 7-position were tested against CQS and CQR strains.[15] While the 7-iodo and 7-bromo compounds showed IC_{50} values comparable to those of chloroquine against both CQR and CQS strains, there was no relationship found between lipophilicity, hydrophilicity, or electronegativity of the substituent and the IC_{50} of the aminoquinoline compounds. Kaschula et al.[7] synthesized a series of 4-aminoquinolines with different substituents at the 7-position. A QSAR developed from these compounds gave good predictions of the β-hematin inhibitory activity. Two parameters were included in this QSAR: log K for drug-heme binding and the Hammett constant for the meta position. Additionally, log K was found to be correlated with the published lipophilicity constant of each substituent.

Egan and co-workers[6] have recently proposed a structure−activity relationship for the chloroquine functional groups based on measurements of heme-binding ability, β-hematin formation inhibition, and IC_{50}'s of a series of substituted quinolines. They proposed that the 4-aminoquinoline ring is responsible for chloroquine's ability to bind strongly to heme, the 7-chloro substituent is responsible for inhibition of β-hematin formation, and that the aminoalkyl side chain aids in accumulation of the drug in the digestive vacuole through pH trapping. They found that all three were necessary for strong antimalarial activity; not all compounds that bound to heme inhibited hemozoin formation, and compounds without an aminoalkyl side chain were able to bind to heme and inhibit β-hemozoin formation but did not show antimalarial activity.

In the area of using quantum-mechanical parameters in QSAR studies of antimalarial drugs, several studies by Rode and coworkers appear in the literature.[16−19] They have developed multiparameter QSAR models for series of antimalarial drugs similar to primaquine,[16] mefloquine,[17] and chloroquine.[18] These models were based on Mulliken charges calculated by semiempirical CNDO/2 methods at various carbon sites. The carbons that were found to be least important, based on the parameter size, were gradually eliminated, and the "active center" of the drug was determined to be the remaining carbons. The response data that they used were antimalarial activities in laboratory animals[16,17] and human patients.[18] Recently, they have proposed a nonlinear predictive model to explain this data set for the mefloquine derivatives.[19]

The nuclear magnetic resonance (NMR) chemical shift is a unique parameter to use in QSAR studies, because of its incredible sensitivity to the local environment.[20−22] As has been known for some time, chemical shifts are sensitive to the electron density surrounding a particular nucleus.[23−29] In the case of aromatic carbons, Strub et al.[30] have shown that as the π-electron density increases, going from the tropylium cation to benzene and the cyclopentadienide ion, the carbon chemical shift decreases. The δ_{11} and δ_{22} components of the chemical shift tensor were found to be particularly sensitive to the change in π-electron density, while the δ_{33} component was found to be relatively unaffected by the increase in π-electron density. In aromatic systems, the δ_{11} and δ_{22} components are in the plane of the aromatic ring (δ_{11} is radial to the ring and δ_{22} is tangential), while the δ_{33} component is perpendicular to the ring. Thus δ_{11} and δ_{22} would be expected to give information about the relative π-electron density at an aromatic carbon, with δ_{11} being less sensitive to the radial bond. On the other hand, δ_{33} would be expected to give information related to the σ-electron density.

Only the isotropic component (the average of δ_{11}, δ_{22}, and δ_{33}) is observed in solution NMR, due to rotational averaging. A solid-state powder pattern gives the magnitude of the three principal components, but their orientation can only be determined experimentally through single-crystal studies. However, the entire chemical shielding tensor can be calculated from first

TABLE 1: Absolute σ_{11} Values (ppm) for Quinoline Carbons of 7-Substituted Quinoline Compounds Listed in Figure 1

substituent	C2	C3	C4	C5	C6	C8	C9	C10
NH2	−64.722	−3.780	−55.252	−52.363	−35.881	18.295	5.488	−54.071
OH	−65.144	−5.903	−57.370	−51.393	−40.306	8.697	1.406	−51.390
F	−66.207	−6.400	−59.106	−50.469	−37.260	−2.424	−4.315	−48.868
NO2	−66.757	−7.195	−59.774	−46.401	−44.576	−15.020	−14.693	−45.012
CF3	−65.621	−6.938	−59.668	−47.968	−60.884	−27.665	−10.710	−45.261
H	−64.451	−6.479	−60.617	−48.137	−68.890	−38.371	−10.977	−48.369
OCH3	−64.470	−5.736	−56.588	−49.542	−43.103	10.733	2.539	−52.492
CH3	−63.788	−5.764	−59.258	−45.973	−65.453	−30.065	−5.147	−47.916
Br	−64.841	−5.999	−59.046	−43.817	−61.913	−32.444	−6.243	−44.671
I	−64.030	−5.583	−58.659	−40.841	−69.816	−43.059	−6.353	−42.977
Cl	−65.341	−6.270	−59.140	−45.679	−56.976	−26.621	−6.187	−45.847

principles. Based on the results of Strub et al.,[30] the calculated shielding tensor components can be used to gain information on the relative σ- and π-electron density at aromatic carbon sites. Previously, we have used shielding tensors calculated by ab initio methods to examine the change in π-electron density upon N-oxidation of the quinoline ring and to compare the π-electron density among a series of antimalarial drugs.[31] The drugs amodiaquine, chloroquine, and quinine were shown to have increasing and decreasing π-electron density on alternating carbons. In addition, for quinine, which is a 6-methoxy quinoline, carbons on the ring containing the methoxy substituent have increased π-electron density compared to carbons on the opposite ring. Conversely, for the 7-chloro quinolines amodiaquine and chloroquine, carbons in the ring containing the amino substituent have increased π-electron density relative to carbons in the ring containing the chloro substituent.

In the present work, we explore the validity of using calculated chemical shielding tensor components as structural parameters in QSAR studies of antimalarial drugs. The π-electron density on the quinoline ring is presumably related to the ability of these antimalarials to bind to heme, another aromatic system. Based on the above discussion, heme-binding ability is required for a compound to be an effective antimalarial agent, and thus it is an important parameter to study. Other activity parameters such as IC_{50} are not expected to be dependent solely on drug-heme binding strength and may be complicated by other factors such as cellular accumulation. A series of chloroquine-like compounds with different substituents on the quinoline ring were recently synthesized.[7] The heme-binding association constant for each compound was also measured.[7] These compounds are shown in Figure 1. This series of compounds, along with their measured log K values, present an ideal data set from which to construct a chemical shielding QSAR. Thus, we have calculated the chemical shielding tensor at each carbon for the compounds shown in Figure 1 and related these to the drug-heme binding strength. The results are expected to provide a rationale for the underlying electronic effects responsible for drug-heme binding, and this information may be useful to consider in the rational design of future antimalarial quinolines.

Computational Details

Structures of the 7-substituted quinolines listed in Figure 1 were geometry optimized using the B3LYP functional[32,33] and a 6-31G basis set.[34] For the iodo-substituted quinoline, a triple-ζ basis set was used for the iodine atom,[35] and a 6-31G basis set[34] was used for all other atoms. The quinoline nitrogen was protonated in all calculations. In the case of heme, the iron was replaced by a diamagnetic Mg^{2+} ion and geometry optimization was performed for a monomer of Mg (protoporphyrin IX) at the HF/6-31G level.[34]

Chemical shift tensors were calculated using GIAO[36] with the B3LYP functional[32,33] and a 6-311G(2d,2p) basis set.[37] Chemical shift calculations for the iodo-substituted compound used a triple-ζ basis set for the iodine atom[35] and a 6-311G-(2d,2p) basis set[37] for all other atoms. All calculations were performed using the Gaussian 98 program[38] on an SGI Origin 2000 workstation (Silicon Graphics, Inc.; Mountain View, CA) with 4 processors.

Results and Discussion

Listed in Table 1 are the σ_{11} components of the calculated chemical shielding tensor for C2−C10 of the various 7-substituted quinoline derivatives. The carbon numbering refers to the scheme shown in Figure 1. C2−C4 and the quinoline nitrogen belong to the A ring, C5−C8 comprise the B ring, and C9 and C10 belong to both rings. The carbon bearing the substituent, C7, is not included in the table, as calculated shielding values for carbons directly bound to heavy atoms are not expected to follow the trends experienced by the other quinoline carbons since relativistic corrections were not included in the calculations. In general, for compounds with electron-donating substituents (NH_2, OH, OCH_3, CH_3), the quinoline carbons have more shielded σ_{11} values, and for compounds with electron-withdrawing substituents (NO_2, CF_3, halogens), the quinoline carbons have more deshielded σ_{11} values. C6 and C8, which are both ortho to the substituent, experience the greatest range of σ_{11} values, followed by C9 (para) and the meta carbons C5 and C10. Carbons on the A ring, opposite from the substituent (C2, C3, and C4), are least influenced by the presence of the different substituents.

Plots of log K vs σ_{11} for C5, C6, and C8 are shown in Figure 2. These are the carbons on the B ring of the quinoline and are expected to experience a larger change in π-electron density with a change in the substituent at position 7. The bridging carbons, C9 and C10, are para and meta to the substituent, respectively, but are also part of the A ring as well. Thus, the π-electron density at these carbons will be influenced by both the presence of the substituent at position 7, and by the amino substituent at position 4. Experimental log K values are taken from ref 7. For each carbon, the point for the Cl-substituted quinoline (shown as an open square) lies far above the best-fit line determined by the other substituents. Excluding the compound with the chloro substituent, the relationship between σ_{11} and log K for binding to heme is linear with R^2 values between 0.55 and 0.77 for C5, C6, and C8. The log K decreases with increasing π-electron density at C6 and C8, which are both ortho to the substituted carbon and C9 (not shown), which is para to the substituted carbon. On the other hand, the strength of binding to heme increases with increasing π-electron density at C5 and C10 (not shown), which are both meta to the substituted carbon.

Of the compounds studied, the Cl-substituted compound was found to bind to heme more strongly than any of the other quinolines, by an order of magnitude.[7] Chloroquine, which also

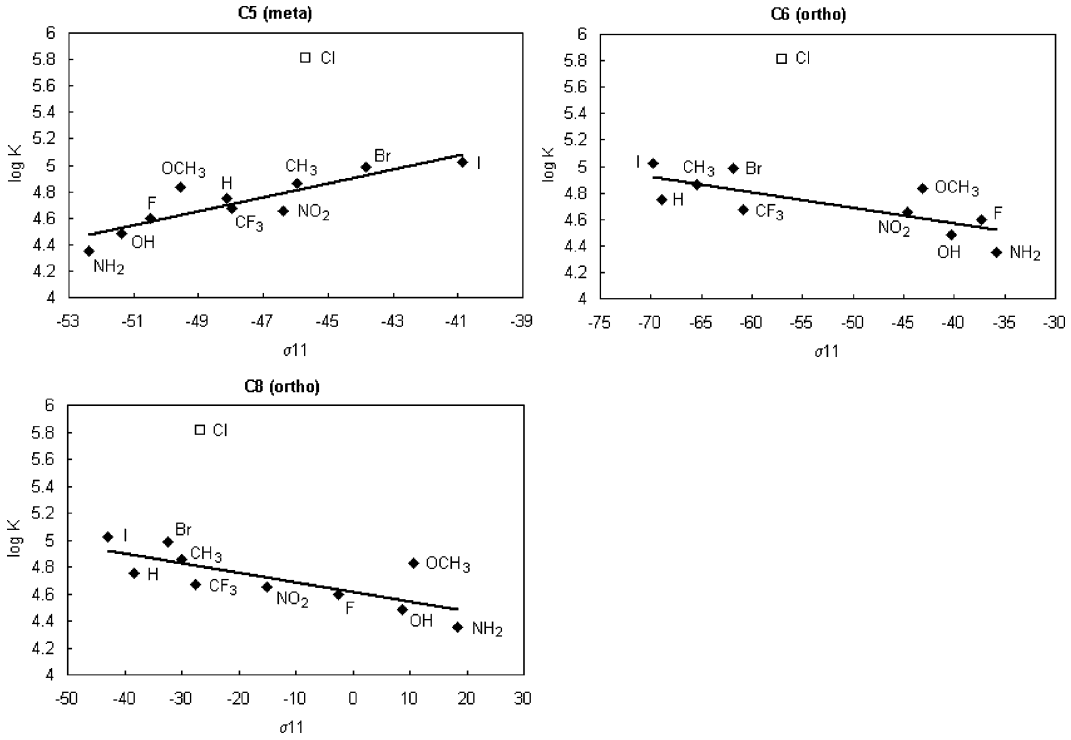

Figure 2. Plots of log K vs σ_{11} for C5, C6, and C8. K is the experimental binding constant between 7-substituted 4-aminoquinolines and heme, taken from Kaschula et al. *J. Med. Chem.* **2002**, *45*, 3531. C5 (meta to the carbon with the substituent) $R^2 = 0.77$; C6 (ortho) $R^2 = 0.57$; C8 (ortho) $R^2 = 0.55$. In each case, the point for the Cl-substituted compound (shown as open square) was not included in the linear regression analysis.

has a chloro substituent at the 7-position, has a similarly high drug-heme association constant.[6] However, in the present study, a corresponding difference in chemical shielding, and thus π-electron density, between the Cl-substituted compound and the other substituted quinolines is not evident. This indicates that the compound with the chloro substituent may be binding to heme via a different or additional mechanism than the other 7-substituted compounds. This additional mechanism appears to be uncorrelated with the π-electron density at particular carbon sites. Previous solid-state NMR measurements[39] indicate evidence for a covalent complex with an Fe−N bond between heme and chloroquine. Evidence has also been found for a covalent complex between heme and the drug metaquine, which contains two 7-chloroquinoline rings.[40] Thus, an explanation for the increased drug-heme binding strength of the Cl-substituted compound is that this drug may be forming a covalent complex with heme in solution.

As shown in Figure 2, an increase in π-electron density on the meta carbons and a decrease in π-electron density on the ortho and para carbons may be important to the binding strength between antimalarial drugs and heme. In our previous paper,[31] we found that alternating increasing and decreasing π-electron densities, as suggested by σ_{11}, were present on adjacent carbons in the antimalarial drugs amodiaquine, chloroquine, and quinine. In addition, there was a π-dipole between the two rings, with the amino-substituted ring being more π-electron rich in amodiaquine and chloroquine and the methoxy-substituted ring being more π-electron rich in quinine. We have performed the same calculation on a monomer of heme, to see if a corresponding π-dipole is present in the porphyrin structure of heme as well, and if matching of the π-dipoles could be responsible for drug-heme binding. Shown in Figure 3 is a plot of σ_{11} for each porphyrin carbon of heme. Also shown in Figure 3 is a visual representation of the π-electron density at each of these carbons, as indicated by σ_{11}. In this visual representation, larger spheres represent more shielded carbons, and thus, carbons with

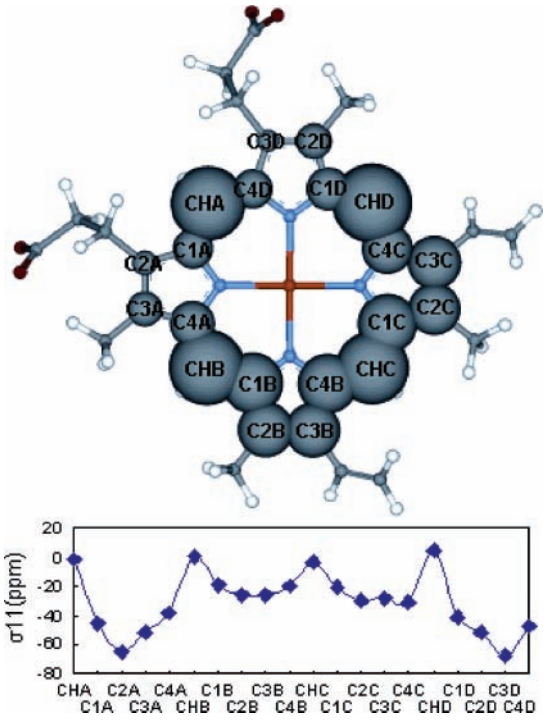

Figure 3. Visual representation of the relative π-electron density, as indicated by σ_{11}, for each porphyrin carbon of a heme monomer. Larger spheres represent more shielded carbons and thus carbons with more π-electron density. Note that data are shown for porphyrin carbons only and not for nitrogen or iron atoms.

increased π-electron density.[30] As can be seen in Figure 3, the heme monomer has alternating increasing and decreasing π-electron density, similar to the antimalarial drugs, but in heme the period of this alternating π-electron density is greater than every other carbon. This indicates that it may be the π-dipole between the A and B rings of quinoline in the drugs, rather

than the alternating π-electron density on individual carbons, which is responsible for the $\pi-\pi$ interactions between antimalarial drugs and heme.

The plots in Figure 2 also indicate that the π-dipole of these drugs is more important to the binding between drugs and heme than is the degree of alternating π-electron density on adjacent carbons. In most cases, C8 and C9 are more shielded than C5 and C10, so that the combination of increasing the shielding at the meta carbons and decreasing the shielding at the ortho and para carbons actually leads to a decrease in the amplitude of the oscillating π-electron density. Furthermore, the degree to which the shielding at C5 and C10 change (-41 to -54 ppm) is much smaller than the degree to which the shielding at C6 (-36 to -70 ppm) and C8 (18 to -43 ppm) change. Thus the factor that is primarily responsible for the increasing strength of binding to heme in these compounds is the decrease in π-electron density at carbons C6 and C8. The compounds considered in the present study have an amino group at the 4-position of the quinoline ring, similar to the drugs amodiaquine and chloroquine. Thus it would be expected that the A ring should be π-electron-rich and the B ring should be π-electron poor for optimal interactions with heme. Because the shieldings of C2, C3, and C4 do not change considerably with the different substituents (Table 1), a decrease in shielding at C6 and C8 corresponds to a further decrease in π-electron density of the B ring compared to the A ring.

Vippagunta et al.[9] have proposed that $\pi-\pi$ interactions between drugs and heme could be reduced to electrostatic effects. Calculated electrostatic potential maps indicate a region of decreased electron density surrounding the iron at the center of the porphyrin molecule.[9] Their proposed structure of the drug-heme complex is similar to that of Moreau,[41] in which the quinoline ring of the drug is positioned over the heme Fe. Chloroquine analogues that showed strong heme-binding affinities were found to have greater electron density around the quinoline ring than compounds that did not bind to heme.[9] Thus they concluded that favorable $\pi-\pi$ interactions arise from the electron-rich quinoline ring of the drug being positioned over the electron-poor region in heme.

Recently, atomic-level solution structures of several antimalarial drugs with heme were solved by a combination of experimental distance restraints determined by NMR relaxation measurements and molecular dynamics simulated annealing.[42,43] These structures indicate that the quinoline ring of the drugs do not lie above the heme Fe but instead are positioned above the aromatic carbons of the porphyrin ring. However, the explanation of electrostatic effects dictating $\pi-\pi$ interactions still applies. Structures for the complexes formed between amodiaquine and heme and quinine and heme are shown in Figure 4. The xyz coordinates were taken from low-energy solution structures of the complexes formed between antimalarial drugs and heme.[42,43] In Figure 4A, the amodiaquine molecule is positioned such that the A ring, which is electron-rich (shown in red), is positioned over electron-poor carbons of the heme porphyrin. The B ring, which is electron-poor (shown in yellow), is positioned above heme carbons that have increased π-electron density. In Figure 4B, the quinine molecule is positioned in the opposite orientation, with the A ring above the electron-rich carbons of heme and the B ring positioned above electron-poor heme carbons. The A ring of quinine is electron poor, compared to the B ring, which has increased π-electron density.

Although these pictures provide a simple explanation for drug-heme binding, it is important to highlight some of the limitations of these proposed structures. The structures used in these figures were determined from distance restraints between drug protons and a single point (heme Fe), and the FPIX monomer is presumed to be rotating. Also, the NMR chemical shieldings were calculated for the heme carbons only and not for the nitrogen or iron atoms. Nevertheless, drawings of these structures do indicate that the pairing between a π-electron-

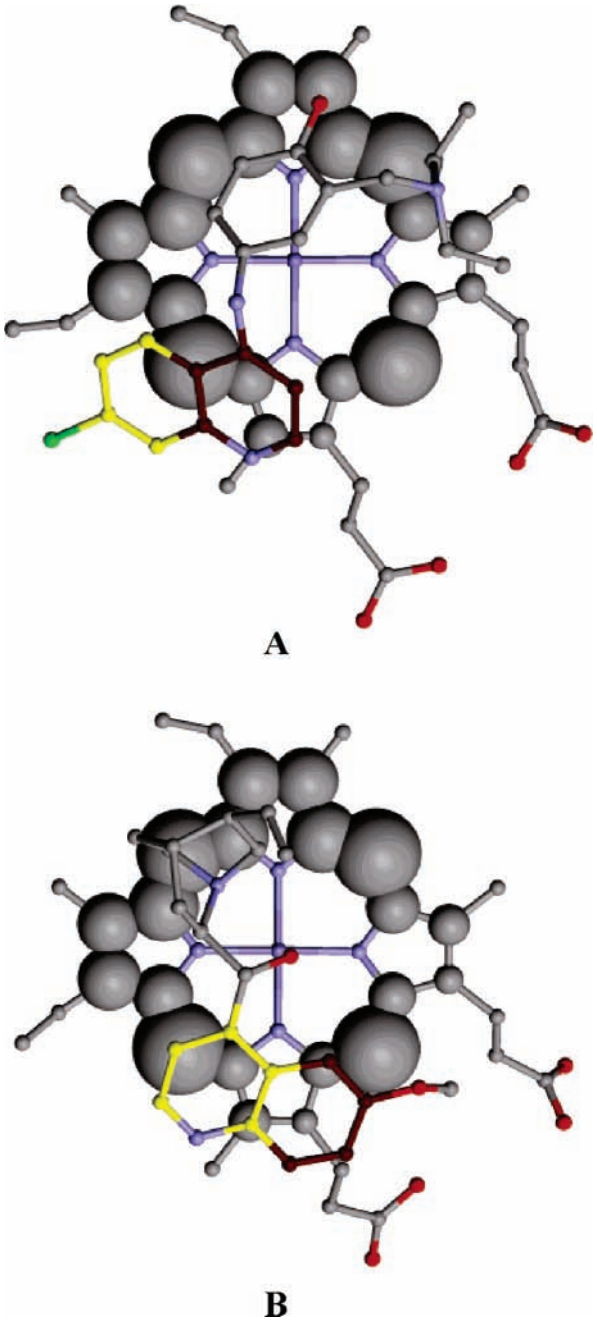

Figure 4. Low-energy solution structures of the (A) amodiaquine-heme complex and the (B) quinine-heme complex. For clarity, hydrogen atoms and the second monomer of the heme μ-oxo dimer are not shown. The more electron-rich ring of each drug is shown in red, and the electron-poor ring is shown in yellow. The porphyrin carbons in heme are shown with the size of the carbon proportional to the π-electron density at that carbon, as indicated by σ_{11}. Larger spheres represent carbons with increased π-electron density. In (A), the electron-rich A ring of amodiaquine is positioned above an electron-poor pyrrole ring in heme. The electron-poor B ring of amodiaquine is positioned over electron-rich carbons of heme. In (B), the electron-rich B ring of quinine is positioned above an electron-poor pyrrole ring in heme, and the electron-poor A ring of quinine is positioned over electron-rich carbons of heme.

poor pyrrole-like ring in the heme porphyrin and a π-electron rich ring of the drug is possible in these complexes. In our analysis we have considered only π-electron density, as indicated by the σ_{11} component of the chemical shielding tensor. The distance between the drug and heme in these structures (4−8 Å)[42,43] is great enough that σ-electron density is not expected to play a role in drug-heme binding. Thus an important contribution to the $\pi-\pi$ interactions between drugs and heme may be an electrostatic effect due to a nonuniform π-electron distribution.

Conclusions

The results presented in this work provide an explanation for the measured drug-heme association constants[7] for a series of 4-aminoquinolines with different substituents at the 7-position. Ab initio calculations of the σ_{11} component of the chemical shielding tensor indicate that the drug-heme association constant increases with increasing π-electron density at the carbons meta to the substituent and with decreasing π-electron density at the carbons ortho and para to the substituent. Because the ortho carbons C6 and C8 experience the greatest change in π-electron density, this increasing strength of binding to heme is attributed to a decrease in electron density of the B ring relative to the A ring. Indeed, an examination of the solution structures formed by amodiaquine and quinine with heme shows that it is possible to orient and pair the π-dipoles in each drug and in the porphyrin ring of heme. Thus the $\pi-\pi$ interactions involved in drug-heme binding are due in part to electrostatic effects.

The association constant between the chloro-substituted compound and heme was found to be an order of magnitude higher than the other compounds.[7] This increase in heme-binding ability was not accompanied by a corresponding decrease in π-electron density at C6 and C8. This indicates that this compound may be binding to heme in a different manner than the other compounds, possibly through formation of a covalent complex. These results give insight into the mechanism by which these substituted quinolines bind to heme and may be useful in the design of future antimalarial drugs.

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References and Notes

(1) Zarchin, S.; Krugliak, M.; Ginsburg, H. *Biochem. Pharmacol.* **1986**, *35*, 2435.
(2) Sherman, I. W. *Bull. W. H. O.* **1977**, *55*, 265.
(3) Pagola, S.; Stephens, P. W.; Bohle, D. S.; Kosar, A. D.; Madsen, S. K. *Nature* **2000**, *404*, 307.
(4) Bloland, P. 2001. Drug resistance in malaria. WHO monograph WHO/CDS/CSR/DRS/2001.4. World Health Organization, Geneva, Switzerland.
(5) Egan, T. J. *Mini-Rev. Med. Chem.* **2001**, *1*, 113.
(6) Egan, T. J.; Hunter, R.; Kaschula, C. H.; Marques, H. M.; Misplon, A.; Walden, J. *J. Med. Chem.* **2000**, *43*, 283.
(7) Kaschula, C. H.; Egan, T. J.; Hunter, R.; Basilico, N.; Parapini, S.; Taramelli, D.; Pasini, E.; Monti, D. *J. Med. Chem.* **2002**, *45*, 3531.
(8) Dorn, A.; Vippagunta, S. R.; Matile, H.; Jaquet, C.; Vennerstrom, J. L.; Ridley, R. G. *Biochem. Pharmacol.* **1998**, *55*, 727.
(9) Vippagunta, S. R.; Dorn, A.; Matile, H.; Bhattacharjee, A. K.; Karle, J. M.; Ellis, W. Y.; Ridley, R. G.; Vennerstrom, J. L. *J. Med. Chem.* **1999**, *42*, 4630.
(10) Hawley, S. R.; Bray, P. G, O'Neill, P. M.; Park, B. K.; Ward, S. A. *Biochem. Pharmacol.* **1996**, *52*, 723.
(11) Hawley, S. R.; Bray, P. G.; Mungthin, M.; Atkinson, J. D.; O'Neill, P. M.; Ward, S. A. *Antimicrob. Agents Chemother.* **1998**, *42*, 682.
(12) De, D.; Krogstad, F. M.; Cogswell, F. B.; Krogstad, D. J. *Am. J. Trop. Med. Hyg.* **1996**, *55*, 579.
(13) Ridley, R. G.; Hofheinz, W.; Matile, H.; Jaquet, C.; Dorn, A.; Masciardi, R.; Jolidon, S.; Richter, W. F.; Guenzi, A.; Girometta, M.; Urwyler, H.; Huber, W.; Thaithong, S.; Peters, W. *Antimicrob. Agents Chemother.* **1996**, *40*, 1846.
(14) Madrid, P. B.; Sherrill, J.; Liou, A. P.; Weisman, J. L.; DeRisi, J. L.; Guy, R. K. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1015.
(15) De, D.; Krogstad, F. M.; Byers, L. D.; Krogstad, D. J. *J. Med. Chem.* **1998**, *41*, 4918.
(16) Kokpol, S. K.; Hannongbua, S. V.; Thongrit, N.; Polman, S.; Rode, B. M.; Schwendinger, M. G. *Anal. Sci.* **1988**, *4*, 565.
(17) Polman, S.; Kokpol, S.; Hannongbua, S.; Rode, B. M. *Anal. Sci.* **1989**, *5*, 641.
(18) Rode, B. M.; Schwendinger, M. G.; Kokpol, S. U.; Hannongbua, S. V.; Polman, S. *Monatsh. Chem.* **1989**, *120*, 913.
(19) Nguyen-Cong, V.; Rode, B. M. *J. Chem. Inf. Comput. Sci.* **1996**, *36*, 114.
(20) de Dios, A. C.; Pearson, J. G.; Oldfield, E. *Science* **1993**, *260*, 1491.
(21) de Dios, A. C.; Oldfield, E. *J. Am. Chem. Soc.* **1994**, *116*, 5307.
(22) de Dios, A. C.; Pearson, J. G.; Oldfield, E. *J. Am. Chem. Soc.* **1993**, *115*, 9768.
(23) Spiesecke, H.; Schneider, W. G. *J. Chem. Phys.* **1961**, *35*, 722.
(24) Spiesecke, H.; Schneider, W. G. *J. Chem. Phys.* **1961**, *35*, 731.
(25) Lauterbur, P. C. *J. Chem. Phys.* **1965**, *43*, 360.
(26) Tokuhiro, T.; Wilson, N. K.; Fraenkel, G. *J. Am. Chem. Soc.* **1968**, *90*, 3622.
(27) Tokuhiro, T.; Fraenkel, G. *J. Am. Chem. Soc.* **1969**, *91*, 5005.
(28) Karplus, M.; Pople J. A. *J. Chem. Phys.* **1963**, *38*, 2803.
(29) Alger, T. D.; Grant, D. M.; Paul, E. G. *J. Am. Chem. Soc.* **1966**, *88*, 5397.
(30) Strub, H.; Beeler, A. J.; Grant, D. M.; Michl, J.; Cutts, P. W.; Zilm, K. W. *J. Am. Chem. Soc.* **1983**, *105*, 3333.
(31) Casabianca, L. B.; Faller, C. M.; de Dios, A. C. *J. Phys. Chem. A* **2006**, *110*, 234.
(32) Becke, A. D. *J. Chem. Phys*. **1993**, *98*, 5648.
(33) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785.
(34) Hehre, W. J.; Ditchfield, R.; Pople, J. A. *J. Chem. Phys.* **1972**, *56*, 2257.
(35) Glukhovstev, M. N.; Pross, A.; McGrath, M. P.; Radom, L. *J. Chem. Phys.* **1995**, *103*, 1878−1885. Basis sets were obtained from the Extensible Computational Chemistry Environment Basis Set Database, Version 02/25/04, as developed and distributed by the Molecular Science Computing Facility, Environmental and Molecular Sciences Laboratory which is part of the Pacific Northwest Laboratory, P.O. Box 999, Richland, WA 99352, U.S.A., and funded by the U.S. Department of Energy. The Pacific Northwest Laboratory is a multiprogram laboratory operated by Battelle Memorial Institute for the U.S. Department of Energy under contract DE-AC06-76RLO 1830. Contact Karen Schuchardt for further information.
(36) Ditchfield, R. *Mol. Phys.* **1974**, *27*, 789.
(37) Hehre, W. J.; Radom, L.; Schleyer, P.; Pople, J. A. *Ab Initio Molecular Orbital Theory*; John Wiley and Sons: New York, 1986.
(38) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A., Jr.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Baboul, A. G.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98, Revision A.7;* Gaussian, Inc.: Pittsburgh, PA, 1998.
(39) de Dios, A. C.; Tycko, R.; Ursos, L. M. B.; Roepe, P. D. *J. Phys. Chem. A* **2003**, *107*, 5821.
(40) Dascombe, M. J.; Drew, M. G. B.; Marris, H.; Wilairat, P.; Auparakkitanon, S.; Moule, W. A.; Alizadeh-Shekalgourabi, S.; Evans, P. G.; Lloyd, M.; Dyas, A. M.; Carr, P.; Ismail, F. M. D. *J. Med. Chem.* **2005**, *48*, 5423−5436.
(41) Moreau, S.; Perly, B.; Biguet, J. *Biochimie* **1982**, *64*, 1015.
(42) Leed, A.; DuBay, K.; Ursos, L. M. B.; Sears, D. N.; de Dios, A. C.; Roepe, P. D. *Biochemistry* **2002**, *41*, 10245.
(43) de Dios, A. C.; Casabianca, L. B.; Kosar, A. D.; Roepe, P. D. *Inorg. Chem.* **2004**, *43*, 8078.