

# Structure–activity relationships for ferriprotoporphyrin IX association and $\beta$ -hematin inhibition by 4-aminoquinolines using experimental and ab initio methods



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## ABSTRACT

In order to probe structure–activity relationships of association with ferriprotoporphyrin IX ( $\log K$ ) and inhibition of  $\beta$ -hematin formation, a series of 4-aminoquinolines with varying substituents at the 7-position (X) have been synthesized. These have been further elaborated by introduction of two different R groups on the 4-amino nitrogen atom in the form of methyl (R = Me) and ethylamine (R = EtNH<sub>2</sub>) side chains. Data for a previously investigated series containing an *N,N*-diethyl-ethylamine side chain were also compared with the findings of this study. Experimentally,  $\log K$  values for the simple 4-aminoquinoline series (R = H) were found to correlate with the hydrophobicity constant ( $\pi$ ) of the group X. The  $\log K$  values for the series with R = Me and EtNH<sub>2</sub> were found to correlate with those of the series with R = H. The  $\log$  of the 50%  $\beta$ -hematin inhibitory activity ( $\log \text{BHIA}_{50}$ ) was found to correlate with  $\log K$  and either *meta* ( $\sigma_m$ ) or *para* ( $\sigma_p$ ) Hammett constants for the series with R = Me and EtNH<sub>2</sub>, but not the simple series with R = H. To further improve predictability, correlations with ab initio electrostatic parameters, namely Mulliken and CHelpG charges were investigated. The best correlations were found with CHelpG charges which indicated that  $\log K$  values can be predicted from the charges on atom H-8 and the group X in the quinolinium species computed in vacuum, while  $\log \text{BHIA}_{50}$  values can be predicted from the CHelpG charges on C-7, C-8 and N-1 for the neutral species in vacuum. These correlations indicate that association and inhibition of  $\beta$ -hematin formation are separately determined. They also suggest that electron withdrawing groups at the 7-position, but not necessarily hydrophobic groups are required for hemozoin inhibition. The upshot is that the correlations imply that considerably more hydrophilic hemozoin inhibitors are feasible.

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## 1. Introduction

Quinoline antimalarials such as quinine and chloroquine have had a long and successful history.<sup>1</sup> Subsequent emergence of chloroquine resistance has led to the use of other quinoline derivative antimalarials such as mefloquine and amodiaquine. Indeed, the development and clinical deployment of new quinoline antimalarials continues to this day. For example, piperazine, a bis-4-aminoquinoline effective against chloroquine-resistant strains that consists of two 4-aminoquinoline moieties attached by a linker has only recently been introduced into clinical use.<sup>2</sup> Ferroquine, a 4-amino-7-chloroquinoline with a ferrocenyl side-chain is currently in phase IIB clinical trials.<sup>3</sup> In addition, research on new quinoline antimalarials retains considerable interest, such as that on so-called ‘reversed chloroquinones’ and related compounds that

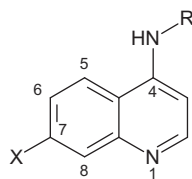
are both antimalarially active and inhibit the chloroquine resistance mechanism of the parasite.<sup>4–10</sup>

The class of 4-aminoquinoline antimalarials is believed to act by inhibiting the incorporation of ferriheme (Fe(III)PPIX), a toxic by-product of hemoglobin digestion, into hemozoin. This is a less toxic insoluble microcrystalline form of Fe(III)PPIX which is produced in the acidic digestive vacuole of the malaria parasite. Indeed, recently chloroquine has been shown to increase free Fe(III)PPIX and decrease hemozoin in the *Plasmodium falciparum* cell in a dose-dependent manner.<sup>11</sup> The detailed mechanism of inhibition has been a matter of debate, with one school of thought proposing that it involves complex formation in solution,<sup>12–14</sup> while another postulates inhibition of crystal growth via interaction with the crystal surface.<sup>15</sup> It is known that quinolines form complexes with Fe(III)PPIX in solution,<sup>12,16</sup> while it is also the case that the inhibitory effects of these compounds can be described by a surface interaction model.<sup>15,17,18</sup>

Despite the good efficacy of 4-aminoquinolines in treating malaria, a number of adverse drug effects are known for this class of

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R = H (**9**) and X = Me (**a**), CF<sub>3</sub> (**b**), Cl (**c**), CONH<sub>2</sub> (**d**), CN (**e**), H (**f**), NH<sub>2</sub> (**g**), NO<sub>2</sub> (**h**), OMe (**i**)

R = Me (**10**) and X = Me (**a**), CF<sub>3</sub> (**b**), Cl (**c**), CONH<sub>2</sub> (**d**), CN (**e**), H (**f**), NH<sub>2</sub> (**g**), NO<sub>2</sub> (**h**), OMe (**i**)

R = EtNH<sub>2</sub> (**11**) and X = Me (**a**), CF<sub>3</sub> (**b**), Cl (**c**), CN (**e**), H (**f**), NO<sub>2</sub> (**h**), OMe (**i**)

R = EtN(Et)<sub>2</sub> (**13**) and X = Me (**a**), CF<sub>3</sub> (**b**), Cl (**c**), H (**f**), NH<sub>2</sub> (**g**), NO<sub>2</sub> (**h**), OMe (**i**), F (**j**), Br (**k**), I (**l**)

Scheme 1.

compound. One of the most important drug liabilities is cardiotoxicity.<sup>19</sup> The human *ether-a-go-go related gene* (hERG) encodes a K<sup>+</sup> channel that plays a key role in regulating cardiac action potential.<sup>20</sup> Inhibition of this channel can lead to arrhythmias and in extreme cases heart failure. Cytochrome P450 enzymes (CYPs) are known to be inhibited by many drug candidates and also represent a potential liability for 4-aminoquinolines.<sup>21</sup> Indeed, Gleeson has shown that weak base compounds, of which the 4-aminoquinolines are an example, commonly exhibit adverse properties with respect to hERG toxicity and CYP inhibition. However, the likelihood of such liabilities is reduced in compounds that have decreased lipophilicity (clogP <4) and relatively low molecular weight (<400).<sup>22</sup> Thus, suitably modified quinolines may exhibit improved drug toxicity profiles.

In order to design 4-aminoquinolines with improved toxicity profiles, it is a prerequisite to have an understanding of structure–activity relationships at the target level. A large number of 4-aminoquinolines with activity against the parasite are known, but these data are often difficult to interpret because of the complexity of the biological system, which includes factors such as accumulation in the digestive vacuole, access to the parasite and lipophilicity. A number of studies have been undertaken investigating effects of quinoline structure on Fe(III)PPIX association and inhibition of synthetic hemozoin (β-hematin) formation.<sup>23–29</sup> Some of these have been qualitative, while others have provided some quantitative information. In some cases however, it has been difficult to account for the strong activity of 4-amino-7-chloroquinolines relative to other 4-aminoquinolines.<sup>24,28</sup>

Here we have conducted a structure–activity relationship study to establish the minimum structural criteria for Fe(III)PPIX association and β-hematin inhibition in this class of compound. In this endeavor we have started with a series of simple 4-aminoquinolines and built up the lateral side chain to determine the effects on both association constant with Fe(III)PPIX (logK) and 50% β-hematin inhibitory activity (BHIA<sub>50</sub>). This involved preparation of a series of 4-aminoquinolines in which the group at position 7 of the quinoline ring (X) and the lateral side-chain on the 4-amino group (R) were varied (Scheme 1), as well as comparisons with a previous series (R = EtN(Et)<sub>2</sub> in Scheme 1) prepared in our laboratory.<sup>24</sup> The values of logK and logBHIA<sub>50</sub> were then correlated with empirical properties, namely hydrophobicity and Hammett constants and correlations between logBHIA<sub>50</sub> and measured logK values were also explored. Furthermore, correlations with atomic charges, computed using density functional theory (DFT), were also investigated.

## 2. Results

Previous studies on 4-aminoquinolines have shown that association constants with Fe(III)PPIX and ability to inhibit β-hematin forma-

Table 1  
LogK with Fe(III)PPIX and (BHIA<sub>50</sub>) for 4-aminoquinolines

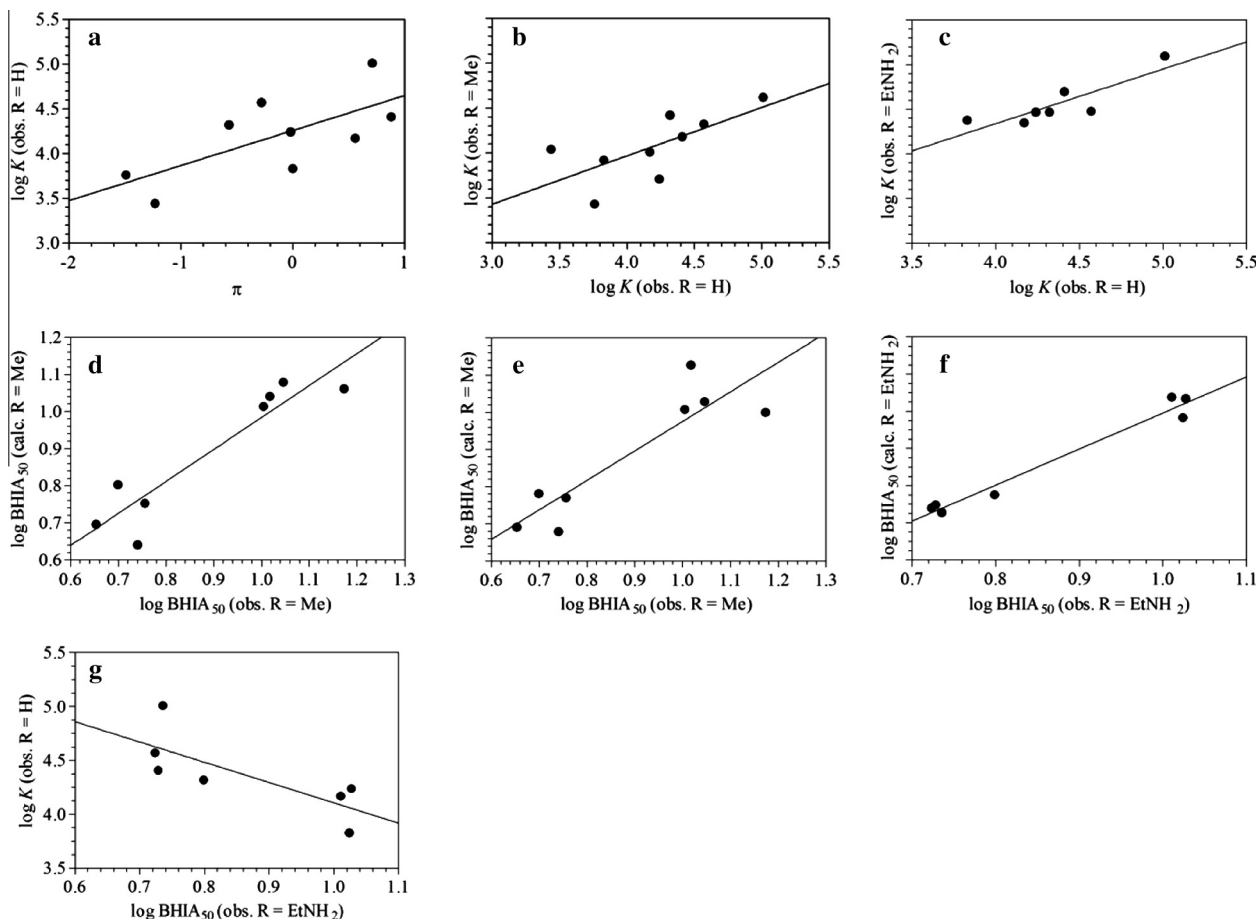
R	X	Compound	LogK	BHIA <sub>50</sub> <sup>a</sup>
H	CH <sub>3</sub>	<b>9a</b>	4.17 ± 0.03 <sup>b</sup>	10.8 ± 0.6 <sup>b</sup>
H	CF <sub>3</sub>	<b>9b</b>	4.41 ± 0.01 <sup>b</sup>	5.6 ± 0.7 <sup>b</sup>
H	Cl	<b>9c</b>	5.01 ± 0.06 <sup>b</sup>	5.8 ± 0.9 <sup>b</sup>
H	CONH <sub>2</sub>	<b>9d</b>	3.76 ± 0.12 <sup>b</sup>	10.3 ± 0.4 <sup>b</sup>
H	CN	<b>9e</b>	4.32 ± 0.28 <sup>b</sup>	6.1 ± 0.9 <sup>b</sup>
H	H	<b>9f</b>	3.83 ± 0.02 <sup>b</sup>	10.0 ± 0.3 <sup>b</sup>
H	NH <sub>2</sub>	<b>9g</b>	3.44 ± 0.20 <sup>b</sup>	>20 <sup>b</sup>
H	NO <sub>2</sub>	<b>9h</b>	4.57 ± 0.11 <sup>b</sup>	5.7 ± 0.6 <sup>b</sup>
H	OCH <sub>3</sub>	<b>9i</b>	4.24 ± 0.02 <sup>b</sup>	15.6 ± 0.5 <sup>b</sup>
CH <sub>3</sub>	CH <sub>3</sub>	<b>10a</b>	4.01 ± 0.01 <sup>b</sup>	11.1 ± 0.9 <sup>b</sup>
CH <sub>3</sub>	CF <sub>3</sub>	<b>10b</b>	4.18 ± 0.02 <sup>b</sup>	5.0 ± 0.4 <sup>b</sup>
CH <sub>3</sub>	Cl	<b>10c</b>	4.62 ± 0.03 <sup>b</sup>	5.7 ± 0.5 <sup>b</sup>
CH <sub>3</sub>	CONH <sub>2</sub>	<b>10d</b>	3.43 ± 0.07 <sup>b</sup>	10.1 ± 0.5 <sup>b</sup>
CH <sub>3</sub>	CN	<b>10e</b>	4.42 ± 0.03 <sup>b</sup>	4.5 ± 0.3 <sup>b</sup>
CH <sub>3</sub>	H	<b>10f</b>	3.92 ± 0.01 <sup>b</sup>	14.9 ± 0.3 <sup>b</sup>
CH <sub>3</sub>	NH <sub>2</sub>	<b>10g</b>	4.04 ± 0.01 <sup>b</sup>	>20 <sup>b</sup>
CH <sub>3</sub>	NO <sub>2</sub>	<b>10h</b>	4.32 ± 0.08 <sup>b</sup>	5.5 ± 0.3 <sup>b</sup>
CH <sub>3</sub>	OCH <sub>3</sub>	<b>10i</b>	3.71 ± 0.03 <sup>b</sup>	10.4 ± 0.3 <sup>b</sup>
CH <sub>2</sub> CH <sub>2</sub> NH <sub>2</sub>	CH <sub>3</sub>	<b>11a</b>	4.35 ± 0.01 <sup>b</sup>	10.3 ± 0.3 <sup>b</sup>
CH <sub>2</sub> CH <sub>2</sub> NH <sub>2</sub>	CF <sub>3</sub>	<b>11b</b>	4.70 ± 0.01 <sup>b</sup>	5.4 ± 0.2 <sup>b</sup>
CH <sub>2</sub> CH <sub>2</sub> NH <sub>2</sub>	Cl	<b>11c</b>	5.10 ± 0.01 <sup>b</sup>	5.4 ± 0.5 <sup>b</sup>
CH <sub>2</sub> CH <sub>2</sub> NH <sub>2</sub>	CN	<b>11e</b>	4.47 ± 0.03 <sup>b</sup>	6.3 ± 0.8 <sup>b</sup>
CH <sub>2</sub> CH <sub>2</sub> NH <sub>2</sub>	H	<b>11f</b>	4.38 ± 0.03 <sup>b</sup>	10.6 ± 0.4 <sup>b</sup>
CH <sub>2</sub> CH <sub>2</sub> NH <sub>2</sub>	NO <sub>2</sub>	<b>11h</b>	4.48 ± 0.07 <sup>b</sup>	5.3 ± 0.4 <sup>b</sup>
CH <sub>2</sub> CH <sub>2</sub> NH <sub>2</sub>	OCH <sub>3</sub>	<b>11i</b>	4.47 ± 0.01 <sup>b</sup>	10.6 ± 0.3 <sup>b</sup>
CH <sub>2</sub> CH <sub>2</sub> N(CH <sub>2</sub> CH <sub>3</sub> ) <sub>2</sub>	CH <sub>3</sub>	<b>13a</b>	4.86 ± 0.01 <sup>c</sup>	17 ± 4 <sup>c</sup>
CH <sub>2</sub> CH <sub>2</sub> N(CH <sub>2</sub> CH <sub>3</sub> ) <sub>2</sub>	CF <sub>3</sub>	<b>13b</b>	4.67 ± 0.02 <sup>c</sup>	8 ± 2 <sup>c</sup>
CH <sub>2</sub> CH <sub>2</sub> N(CH <sub>2</sub> CH <sub>3</sub> ) <sub>2</sub>	Cl	<b>13c</b>	5.81 ± 0.01 <sup>c</sup>	2.2 ± 0.2 <sup>c</sup>
CH <sub>2</sub> CH <sub>2</sub> N(CH <sub>2</sub> CH <sub>3</sub> ) <sub>2</sub>	F	<b>13j</b>	4.60 ± 0.02 <sup>c</sup>	9.7 ± 0.8 <sup>c</sup>
CH <sub>2</sub> CH <sub>2</sub> N(CH <sub>2</sub> CH <sub>3</sub> ) <sub>2</sub>	Br	<b>13k</b>	4.99 ± 0.01 <sup>c</sup>	4.4 ± 0.7 <sup>c</sup>
CH <sub>2</sub> CH <sub>2</sub> N(CH <sub>2</sub> CH <sub>3</sub> ) <sub>2</sub>	I	<b>13l</b>	5.02 ± 0.02 <sup>c</sup>	2.8 ± 0.2 <sup>c</sup>
CH <sub>2</sub> CH <sub>2</sub> N(CH <sub>2</sub> CH <sub>3</sub> ) <sub>2</sub>	H	<b>13f</b>	4.75 ± 0.03 <sup>c</sup>	>20 <sup>c</sup>
CH <sub>2</sub> CH <sub>2</sub> N(CH <sub>2</sub> CH <sub>3</sub> ) <sub>2</sub>	NH <sub>2</sub>	<b>13g</b>	4.35 ± 0.02 <sup>c</sup>	>20 <sup>c</sup>
CH <sub>2</sub> CH <sub>2</sub> N(CH <sub>2</sub> CH <sub>3</sub> ) <sub>2</sub>	NO <sub>2</sub>	<b>13h</b>	4.65 ± 0.02 <sup>c</sup>	3.1 ± 0.6 <sup>c</sup>
CH <sub>2</sub> CH <sub>2</sub> N(CH <sub>2</sub> CH <sub>3</sub> ) <sub>2</sub>	OCH <sub>3</sub>	<b>13i</b>	4.83 ± 0.01 <sup>c</sup>	>20 <sup>c</sup>

<sup>a</sup> Equivalents relative to Fe(III)PPIX.

<sup>b</sup> This study.

<sup>c</sup> From Kaschula et al.<sup>24</sup>

tion depend on both lipophilic and electronic properties of the group at position 7.<sup>23–25</sup> For this reason, a series of 4-aminoquinolines with various substituents at position 7 (X) were chosen (Scheme 1). This was done with the aid of a Craig plot of Hammett constant (σ<sub>p</sub>) versus lipophilicity constant (π) so as to minimize correlation between these two properties.<sup>30</sup> They were prepared according to well established procedures. Briefly, five 4-chloro-7-X-quinolines were synthesized either by reaction of *meta*-substituted anilines with diethyl ethoxymethylenemalonate in a five step process with moderate yields or by reaction of the aniline with Meldrum's acid (2,2-dimethyl-1,3-dioxane-4,6-dione) and triethyl orthoformate in a two-step process. Three additional 4-chloro-7-X-quinolines were commercially available (X = Cl,



**Figure 1.** Experimentally observed correlations of  $\log K$  and  $\log \text{BHIA}_{50}$ . (a) Single linear correlation of  $\log K$  versus  $\pi$  for the series where  $R = \text{H}$ . Values of  $\pi$  were obtained from Hansch and Leo.<sup>32</sup>  $\log K = 0.4(2)\pi + 4.3(1)$ ,  $r^2 = 0.48$ ,  $P = 0.039$ . (b) Single linear correlation of  $\log K$  of the series where  $R = \text{Me}$  versus  $\log K$  of the series where  $R = \text{H}$ .  $\log K (R = \text{Me}) = 0.5(2) \log K (R = \text{H}) + 1.8(9)$ ,  $r^2 = 0.48$ ,  $P = 0.038$ . (c) Single linear correlation of  $\log K$  of the series where  $R = \text{EtNH}_2$  versus  $\log K$  of the series where  $R = \text{H}$ .  $\log K (R = \text{EtNH}_2) = 0.6(2) \log K (R = \text{H}) + 1.9(7)$ ,  $r^2 = 0.73$ ,  $P = 0.014$ . (d) Multiple linear correlation of  $\log \text{BHIA}_{50}$  with  $\log K$  and  $\sigma_m$  for the series  $R = \text{Me}$  showing the calculated versus observed  $\log \text{BHIA}_{50}$  values. Calculated values given by  $\log \text{BHIA}_{50} = -0.2(1) \log K - 0.5(1)\sigma_m + 1.8(4)$ ,  $r^2 = 0.86$ ,  $F = 15.5$  ( $>F^{0.99} = 13.27$ ). (e) Multiple linear correlation of  $\log \text{BHIA}_{50}$  with  $\log K$  and  $\sigma_p$  for the series  $R = \text{Me}$  showing the calculated versus observed  $\log \text{BHIA}_{50}$  values. Calculated values given by  $\log \text{BHIA}_{50} = -0.2(1) \log K - 0.3(1)\sigma_p + 1.9(5)$ ,  $r^2 = 0.79$ ,  $F = 9.66$  ( $>F^{0.95} = 5.79$ ). (f) Multiple linear correlation of  $\log \text{BHIA}_{50}$  with  $\log K$  and  $\sigma_p$  for the series  $R = \text{EtNH}_2$  showing the calculated versus observed  $\log \text{BHIA}_{50}$  values. Calculated values given by  $\log \text{BHIA}_{50} = -0.37(5) \log K - 0.28(3)\sigma_p + 2.1(2)$ ,  $r^2 = 0.97$ ,  $F = 69.19$  ( $>F^{0.99} = 13.27$ ). (g) Single linear correlation of  $\log K$  for the series  $R = \text{H}$  with  $\log \text{BHIA}_{50}$  of the series  $R = \text{EtNH}_2$ .  $\log K = -1.9(7) \log \text{BHIA}_{50} + 6.0(6)$ ,  $r^2 = 0.58$ ,  $P = 0.045$ .

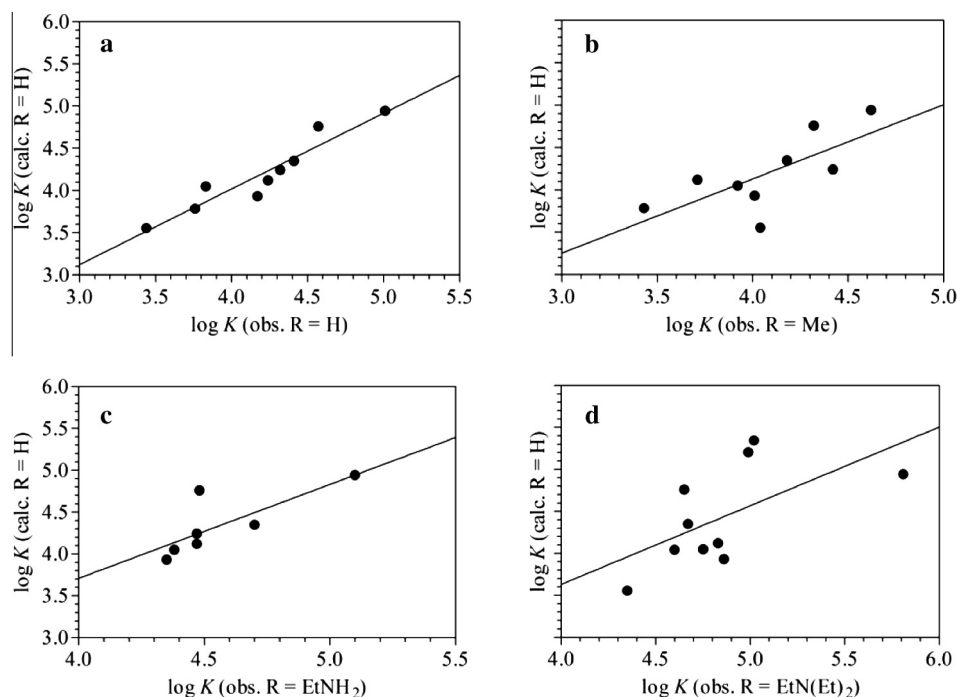
H and  $\text{CF}_3$ ). Further modifications to these 7-substituted quinolines were made by varying the lateral side chain at position 4 (Scheme 1). This was accomplished by displacement of the 4-chloro group in an aromatic substitution reaction with either ammonia or two primary amines, namely methylamine or ethanediamine. Finally, compounds with  $X = \text{NH}_2$  were prepared from the corresponding 7-nitroquinoline by reduction with  $\text{SnCl}_2$ . Full synthetic details for all of the above reactions are supplied as [Supplementary data](#).

For each of the compounds synthesized, the  $\log K$  with  $\text{Fe(III)PPIX}$  was measured in 40% (v/v) aqueous DMSO. UV–visible spectroscopic changes were consistent with those reported for compounds which  $\pi$ -stack in solution.<sup>31</sup> In addition, all compounds were tested for inhibition of  $\beta$ -formation mediated by acetate. The conditions used for measurement of these quantities were chosen to facilitate comparison with previously collected data for a related series of compounds with a *N,N*-diethylaminoethyl side chain ( $R = \text{EtN}(\text{Et})_2$  in Scheme 1).<sup>24</sup> Values of  $\log K$  and the 50%  $\beta$ -hematin inhibitory activity ( $\text{BHIA}_{50}$ ) are presented in Table 1.

In a previous study of 4-aminoquinolines with  $R = \text{EtN}(\text{Et})_2$ , it was found that  $\log K$  is correlated with the substituent hydrophobicity constant ( $\pi$ ) of the  $X$  substituent.<sup>24</sup> A similar correlation is observed for the series prepared here with  $R = \text{H}$  (Fig. 1a). The same trend is also seen with  $\text{clog} P$  (not shown). These correlations sug-

gest that the association of the 4-aminoquinoline with  $\text{Fe(III)PPIX}$  becomes stronger as the substituent  $X$  becomes more lipophilic. No correlation between  $\log K$  and substituent constants was found for the series with  $R = \text{Me}$  or  $\text{EtNH}_2$ . However, a statistically significant correlation ( $P < 0.05$ ) was found between the  $\log K$  values for these two series and those for the series with  $R = \text{H}$  (Fig. 1b and c). On the other hand, no correlation of  $\log K$  for the series with  $R = \text{EtN}(\text{Et})_2$  with  $\log K$  for the series with  $R = \text{H}$  was found.

In the earlier study, using multiple linear correlation analysis (MLCA), a correlation was also found between  $\log \text{BHIA}_{50}$  and both  $\log K$  and  $\sigma_m$ .<sup>24</sup> In the present work, a similar correlation was found in the series with  $R = \text{Me}$  (Fig. 1d). In addition,  $\log \text{BHIA}_{50}$  also correlated with both  $\log K$  and  $\sigma_p$  for both the series with  $R = \text{Me}$  and  $\text{EtNH}_2$  (Fig. 1e and f). A further interesting observation was that there is a simple linear correlation between  $\log \text{BHIA}_{50}$  for the series with  $R = \text{EtNH}_2$  and the  $\log K$  values for the corresponding compounds in the  $R = \text{H}$  series (Fig. 1g). A similar relationship was found between the  $R = \text{Me}$  and  $R = \text{H}$  series, but in this case the correlation between  $\log \text{BHIA}_{50}$  and  $\log K$  falls just short of statistical significance at the 95% confidence level. Thus, inhibition of  $\beta$ -hematin formation appears to be favored by stronger association with  $\text{Fe(III)PPIX}$  and by more electron withdrawing  $X$  substituents. Somewhat surprisingly, no such correlation between  $\log \text{BHIA}_{50}$



**Figure 2.** Correlation of observed  $\log K$  values with calculated  $\log K$  values obtained for the quinolinium species with  $R = H$  using CHelpG charges on atom/group X and atom H-8, calculated in vacuum. (a)  $\log K$  (obs.) for the series where  $R = H$  versus  $\log K$  (calc.) for the same series obtained by multiple linear correlation. Calculated values given by  $\log K = -3.6(8)X - 21(3)H - 8 + 7.6(5)$ ,  $r^2 = 0.90$ ,  $F = 25.95$  ( $>F^{0.99} = 10.93$ ). (b) Single linear correlation of  $\log K$  (obs.) for the series where  $R = Me$  versus calculated  $\log K$  values for the series where  $R = H$  as determined in (a), with  $r^2 = 0.52$  and  $P = 0.029$ . (c) Single linear correlation of  $\log K$  (obs.) for the series where  $R = EtNH_2$  versus calculated  $\log K$  values for the series where  $R = H$  as determined in (a), with  $r^2 = 0.61$  and  $P = 0.039$ . (d) Single linear correlation of  $\log K$  (obs.) for the series where  $R = EtN(Et)_2$  versus calculated  $\log K$  values for the series where  $R = H$  as determined in (a), with  $r^2 = 0.37$  and  $P = 0.061$ .

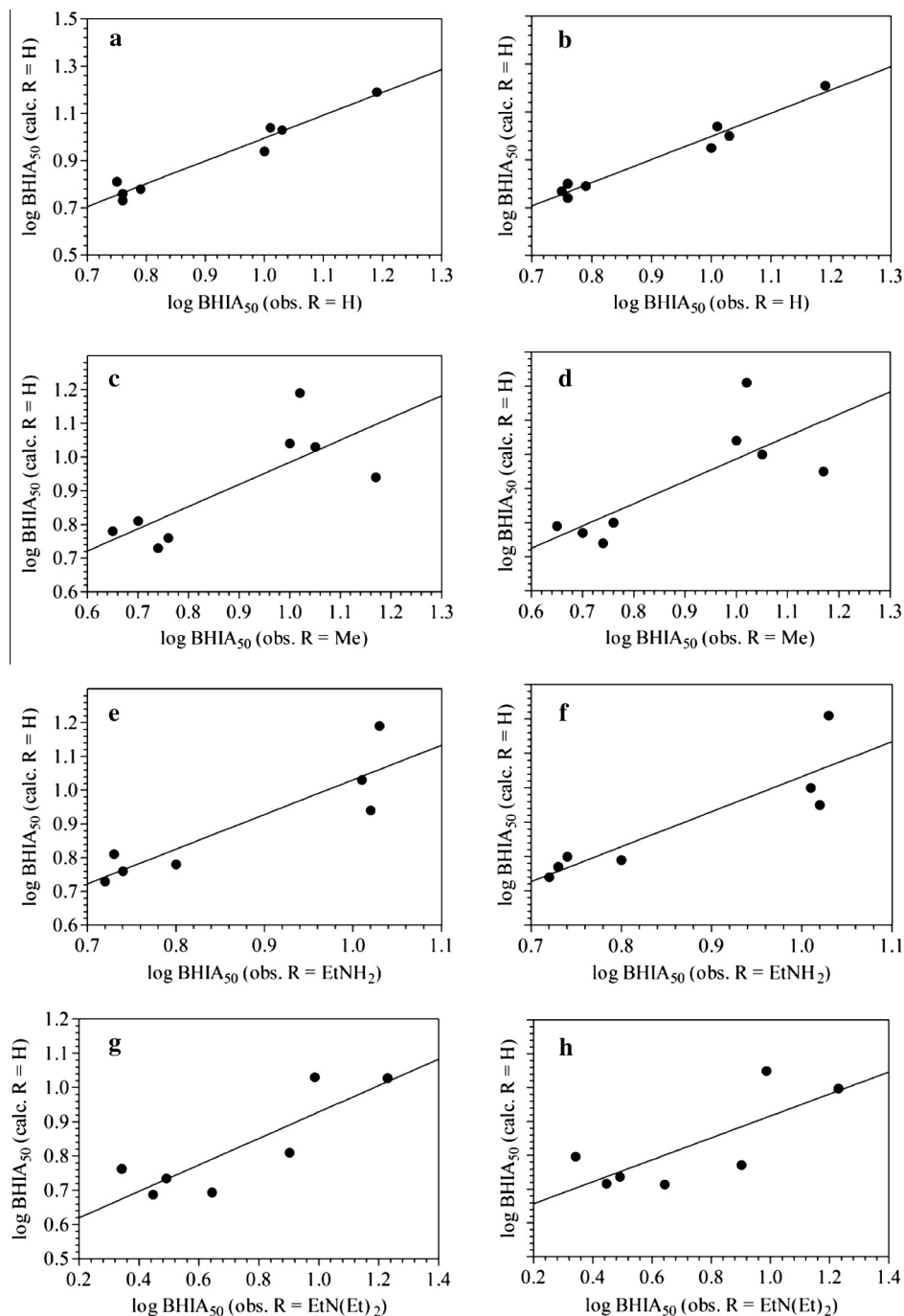
and  $\log K$  was found for the series with  $R = H$  itself. However, correlations were found between  $\log BHIA_{50}$  for the series with  $R = H$  and those with  $R = Me$  and  $EtNH_2$ . As in the case of  $\log K$ , no such correlation was found for the series with  $R = EtN(Et)_2$ .

Given the role found for electronic properties (Hammett constants) in the inhibition of  $\beta$ -hematin formation described above, it was decided to investigate whether there were any correlations between atomic charges on the quinoline moiety calculated using quantum mechanical methods and  $\log K$ ,  $\log BHIA_{50}$ , or both. For this purpose, Mulliken charges were calculated for all atoms in the entire series with  $R = H$  as well as three compounds not prepared in this study with  $R = H$  and  $X = F$ ,  $Br$  and  $I$ . These were calculated for both the neutral and protonated positively charged (quinolinium) forms in both vacuum and aqueous medium using an implicit water model (SMD).<sup>33</sup> In the case of  $\log K$ , eight statistically significant ( $P < 0.05$ ) correlations with single atomic charges were found. Using MLCA, two additional correlations were obtained. The best correlation was between  $\log K$  and the charges on C-6 & C-8a calculated for the neutral form in water (see Supplementary data Fig. S1). Association is predicted to be favored by greater negative charge on C-8a and reduced negative charge on C-6. However, values of  $\log K$  predicted in this way do not correlate with experimental values for any of the other series.

In the case of  $\log BHIA_{50}$  many correlations with Mulliken atomic charges were found. However, an additional criterion could be used in the selection of more reliable predictors, namely the predicted activity of the compound with  $X = NH_2$ . Although no experimental values could be obtained for derivatives with  $X = NH_2$  because their  $\beta$ -hematin inhibitory activities were found to be too weak to measure using the assay, these compounds could be used as test molecules. Only correlations which predicted the corresponding  $X = NH_2$  compound in the series to have a  $\log BHIA_{50}$

higher than the maximum value tested ( $>1.3$ ) were considered to be realistic. This additional constraint reduced the many correlations found by MLCA to just three. These correlations were found for the quinolinium species in both vacuum and water and involved atomic charges N-1 & H-8, N-1 & H-6 and N-1 & H-1 (see Supplementary data Fig. S2). The strongest correlation was between  $\log BHIA_{50}$  and N-1 & H-8, with increased activity favored by a less negative charge on N-1 and a more positive charge on H-8. The predicted  $\log BHIA_{50}$  for this series were also then found to correlate significantly ( $P < 0.05$ ) with the experimental  $\log BHIA_{50}$  values for the series with  $R = Me$ ,  $EtNH_2$  and  $EtN(Et)_2$ .

The electrostatic surface potential of a molecule is likely to be a major determinant of non-covalent interactions with other molecules given the prominent role of electrostatic forces in such interactions. The CHelpG charge scheme provides a model of electrostatic surface potential using localized atomic point charges. The possibility was therefore considered that these might provide better correlations than the Mulliken charges. CHelpG charges were therefore calculated for the same molecules as the Mulliken charges. Once again, these were calculated for both the neutral and quinolinium forms in both vacuum and water. In the case of the series with  $R = H$ , there were three significant linear correlations with  $\log K$  involving single atomic charges. Using MLCA with pairs of atomic charges eight further correlations of  $\log K$  were found at or above the 95% confidence level. The strongest correlation was found using the MLCA approach and exhibited considerably greater statistical significance than the best correlation obtained with Mulliken charges ( $F = 26.0$  vs  $6.3$ ). This was for the quinolinium species in vacuum and involved atoms X & H-8 (Fig. 2a). The association with Fe(II)PPiX is favored by a more negative potential on X and a less positive potential on H-8, with the latter having a greater influence. By contrast to the results obtained using Mulliken charges, the  $\log K$  values calculated from the



**Figure 3.** Correlation of observed  $\log\text{BHIA}_{50}$  values versus calculated  $\log\text{BHIA}_{50}$  values obtained for the neutral species with  $R = \text{H}$  using CHelpG charges on atoms N-1 and C-8 (left panels) and atoms N-1 and C-7 (right panels), calculated in vacuum. (a)  $\log\text{BHIA}_{50}$  (obs.) for the series where  $R = \text{H}$  versus  $\log\text{BHIA}_{50}$  (calcd) for the same series obtained by multiple linear correlation. Calculated values given by  $\log\text{BHIA}_{50} = -12(2)\text{N}-1 - 0.6(2)\text{C}-7(1)$ ,  $r^2 = 0.96$ ,  $F = 56.98$  ( $>F^{0.99} = 13.27$ ). (b)  $\log\text{BHIA}_{50}$  (obs.) for the series where  $R = \text{H}$  versus  $\log\text{BHIA}_{50}$  (calcd) for the same series obtained by multiple linear correlation. Calculated values given by  $\log\text{BHIA}_{50} = -13(1)\text{N}-1 + 0.26(8)\text{C}-7 - 8(1)$ ,  $r^2 = 0.97$ ,  $F = 71.35$  ( $>F^{0.99} = 13.27$ ). (c) Single linear correlation of  $\log\text{BHIA}_{50}$  (obs.) for the series where  $R = \text{Me}$  versus values of  $\log\text{BHIA}_{50}$  calculated for the series where  $R = \text{H}$  as described in (a), with  $r^2 = 0.60$  and  $P = 0.024$ . (d) Single linear correlation of  $\log\text{BHIA}_{50}$  (obs.) for the series where  $R = \text{Me}$  versus values of  $\log\text{BHIA}_{50}$  calculated for the series where  $R = \text{H}$  as described in (b), with  $r^2 = 0.60$  and  $P = 0.025$ . (e) Single linear correlation of  $\log\text{BHIA}_{50}$  (obs.) for the series where  $R = \text{EtNH}_2$  versus values of  $\log\text{BHIA}_{50}$  calculated for the series where  $R = \text{H}$  as described in (a), with  $r^2 = 0.80$  and  $P = 0.007$ . (f) Single linear correlation of  $\log\text{BHIA}_{50}$  (obs.) for the series where  $R = \text{EtNH}_2$  versus values of  $\log\text{BHIA}_{50}$  calculated for the series where  $R = \text{H}$  as described in (b), with  $r^2 = 0.79$  and  $P = 0.008$ . (g) Single linear correlation of  $\log\text{BHIA}_{50}$  (obs.) for the series where  $R = \text{EtN}(\text{Et})_2$  versus values of  $\log\text{BHIA}_{50}$  calculated for the series where  $R = \text{H}$  as described in (a), with  $r^2 = 0.73$  and  $P = 0.015$ . (h) Single linear correlation of  $\log\text{BHIA}_{50}$  (obs.) for the series where  $R = \text{EtN}(\text{Et})_2$  versus values of  $\log\text{BHIA}_{50}$  calculated for the series where  $R = \text{H}$  as described in (b), with  $r^2 = 0.58$  and  $P = 0.046$ .

relationship above using CHelpG charges produced a statistically significant linear correlation with the experimental  $\log K$  values for the series with  $R = \text{Me}$  and  $\text{EtNH}_2$  ( $P < 0.05$ ). In the case of the series with  $R = \text{EtN}(\text{Et})_2$ , the correlation is not statistically significant, albeit only by a small margin (Fig. 2b–d).

As with the Mulliken charges, in the case of  $\log\text{BHIA}_{50}$  only those correlations that successfully predicted the inactivity of compounds with  $X = \text{NH}_2$  were deemed worthy of consideration. For the series with  $R = \text{H}$ , nine correlations, four simple linear correlations and five multiple linear correlations involving pairs of



**Table 2**

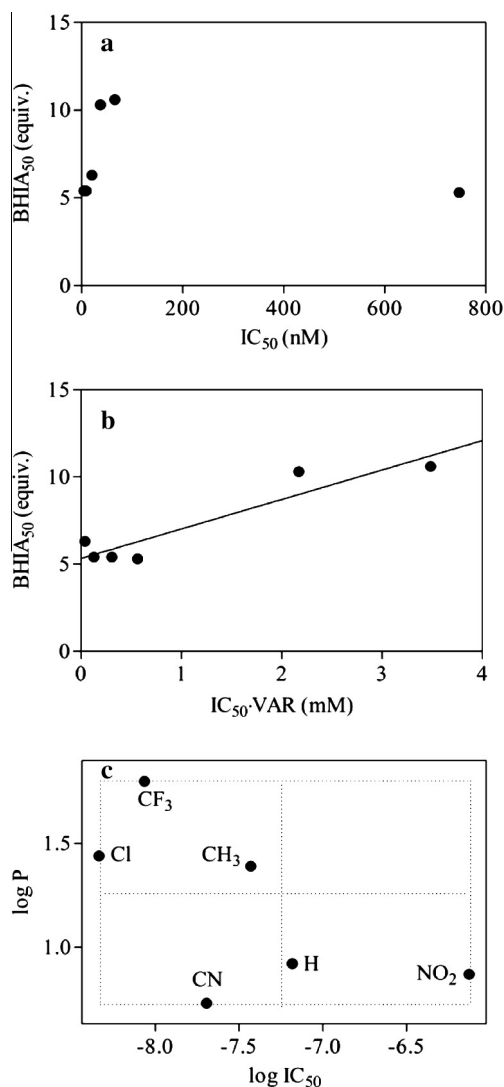
In vitro activity against the NF54 strain of *P. falciparum*, predicted vacuolar accumulation ratio (VAR) and calculated log*P* values for the compound series with R = EtNH<sub>2</sub>

X	Compound	p <i>K</i> <sub>a1</sub> <sup>a</sup>	p <i>K</i> <sub>a2</sub> <sup>a</sup>	VAR <sup>b</sup>	Log <i>P</i> <sup>c</sup>	IC <sub>50</sub> (nM)
CH <sub>3</sub>	<b>11a</b>	8.51	9.65	58,545	1.39	37
CF <sub>3</sub>	<b>11b</b>	7.52	9.62	35,894	1.80	8.6
Cl	<b>11c</b>	7.30	9.62	27,978	1.44	4.6
CN	<b>11e</b>	5.85	9.62	1962	0.73	20
H	<b>11f</b>	8.13	9.63	53,181	0.92	66
NO <sub>2</sub>	<b>11h</b>	5.31	9.62	753	0.87	747

<sup>a</sup> Calculated using MarvinSketch.<sup>34</sup>

<sup>b</sup> VAR =  $\frac{1+10^{pH_v-pH_e}}{1+10^{pH_v-pH_e}+10^{pK_{a1}+pH_v-2pH_e}} + \frac{10^{pK_{a2}+pH_v-2pH_e}}{1+10^{pH_v-pH_e}+10^{pK_{a2}+pH_v-2pH_e}}$  where pH<sub>v</sub> is the vacuolar pH taken as 5 and pH<sub>e</sub> is the external pH taken as 7.4.

<sup>c</sup> Calculated using MarvinSketch with the VG method.



**Figure 4.** In vitro antimalarial activity of compounds **11a–h** compared to  $\beta$ -hematin inhibitory activity and log*P*. (a) No correlation was observed between BHIA<sub>50</sub> and IC<sub>50</sub> in the chloroquine-sensitive NF54 strain. (b) When the IC<sub>50</sub> was corrected for predicted vacuolar accumulation through pH trapping there was a strong correlation with BHIA<sub>50</sub>. BHIA<sub>50</sub> = 1.7(3) IC<sub>50</sub> VAR (mM) + 5.3(5), *r*<sup>2</sup> = 0.87, *P* = 0.0064. (c) A plot of log*P* versus log IC<sub>50</sub> shows that this series of compounds fall into three categories. The 7-Cl, 7-CF<sub>3</sub> and 7-CH<sub>3</sub> derivatives displayed good activity (low log IC<sub>50</sub>), but high lipophilicity (top left quadrant), while the 7-H and 7-NO<sub>2</sub> derivatives showed weak activity, but low lipophilicity (bottom right quadrant). Only the 7-CN derivative displayed both strong activity and low lipophilicity (bottom left quadrant).

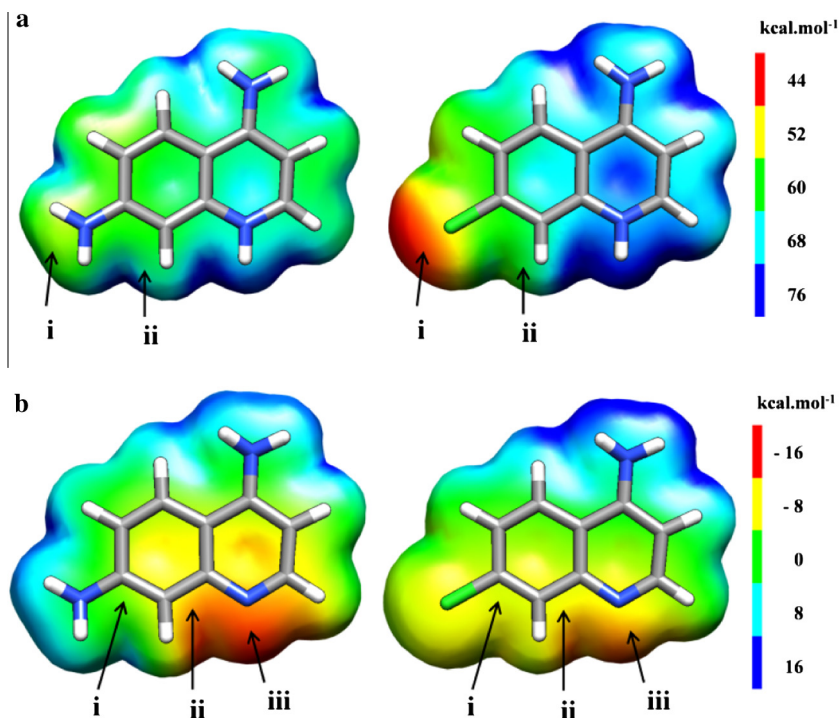
charges were found. As observed in the case of log*K*, the strongest correlations obtained with CHelpG charges had considerably greater statistical significance than those obtained using Mulliken charges. In particular, two correlations found by MLCA were significant at better than the 99% confidence level in one case and 99.9% in the other. These involved charges calculated for the neutral species in vacuum on atoms N-1 & C-8 and N-1 & C-7, respectively (Fig. 3a and b). As seen with the log*K* values, the predicted logBHIA<sub>50</sub> values for this series were also then found to correlate significantly (*P* < 0.05) with the experimental logBHIA<sub>50</sub> values for the series with R = Me and EtNH<sub>2</sub>. In addition, because CHelpG charges could be computed for further compounds not synthesized, a similar correlation was now uncovered with series with R = EtN(Et)<sub>2</sub> (Fig. 3c–h). Thus,  $\beta$ -hematin inhibitory activity was found to improve with decreasing negative potential on N-1, decreasing negative potential on C-8 and decreasing positive potential on C-7.

A notable observation is that as the lateral side chain becomes more lipophilic, the sensitivity of logBHIA<sub>50</sub> to the identity of the X substituent decreases. This is observed as a decrease in slope in Figure 3a–h as log*P* increases (see Supplementary data Fig. S3).

In order to determine whether these observations are likely to be useful in designing new antimalarials, some of the compounds were tested for in vitro antimalarial activity against a chloroquine-sensitive strain of *Plasmodium falciparum*, NF54. Previous studies on closely related compounds have shown that a weak base N atom is required in the side chain for strong biological activity.<sup>24</sup> This group causes the compound to pH trap in the acidic environment of the digestive vacuole of the parasite. For this reason, only the series with R = EtNH<sub>2</sub> was investigated. The p*K*<sub>a</sub> values of the protonated terminal amino group and quinolinium N atom were estimated using the program MarvinSketch.<sup>34</sup> These values, together with calculated log*P* values are reported in Table 2. Of particular note is the 7-CN compound. While it was not as active as the 7-Cl and 7-CF<sub>3</sub> compounds, it nonetheless had a low IC<sub>50</sub> of 20 nM. In keeping with previous studies,<sup>8,24</sup> these compounds showed no direct correlation between IC<sub>50</sub> for inhibition of parasite growth and BHIA<sub>50</sub> (Fig. 4a). However, when the vacuolar accumulation ratio was taken into account, a strong linear correlation was observed (Fig. 4b). Comparison of lipophilicity and biological activity among this entire series of compounds shows that the 7-CN group provides the best combination of strong activity and low lipophilicity (Fig. 4c).

### 3. Discussion

This study was designed to probe relationships between the structures of the simplest Fe(III)PPIX-associating and  $\beta$ -hematin-inhibiting 4-aminoquinolines and their derivatives with the aim of determining the roles of the group at the 7-position and the lateral side chain in these interactions. This investigation also clarified, where possible, the relationship between Fe(III)PPIX binding and inhibition of  $\beta$ -hematin formation. To these ends, the simple undecorated 4-aminoquinoline derivatives (R = H), were used as the starting point. The side chain on the 4-amino group was then gradually built up to observe its effect, starting with a –CH<sub>3</sub> group and then proceeding to an EtNH<sub>2</sub> group. The association constants with Fe(III)PPIX and the inhibition of  $\beta$ -hematin formation values were found to be correlated, except in the series with R = H. The parameter  $\pi$  was found to correlate with log*K* in the case of the series with R = H, similar to that found previously by Kaschula et al. for the series with R = EtN(Et)<sub>2</sub>.<sup>24</sup> The parameter  $\sigma_p$  in combination with log*K* correlated with logBHIA<sub>50</sub> of the series with R = Me and EtNH<sub>2</sub>, while  $\sigma_m$  and log*K* correlated in the case where R = Me, similar to that found by Kaschula and co-workers in the case of the series with R = EtN(Et)<sub>2</sub>. The R = H series was also used



**Figure 5.** Electrostatic surface potentials of species with R = H and X = NH<sub>2</sub> (left panel) and Cl (right panel). Negative and positive potentials represented by red and blue shading, respectively. (a) Quinolinium species calculated in vacuum illustrating atoms/groups that predict association constants with Fe(III)PPIX. Association was found to be favored by increased negative potential at X (i) and decreased positive potential at H-8 (ii). (b) Neutral species calculated in vacuum illustrating atoms predictive of  $\beta$ -hematin inhibition. Inhibition was found to be favored by decreased positive potential on C-7 (i) and decreased negative potential on both C-8 (ii) and N-1 (iii).

to confirm that the quinoline ring is the primary component for the inhibition of  $\beta$ -hematin formation as previously suggested.<sup>23</sup> These relationships all suggest that both an increase in association constant and the presence of an electron withdrawing group at the 7-position enhances inhibition of  $\beta$ -hematin formation in this class of molecule.

Calculated atomic charges, whether Mulliken or CHelpG charges obtained from quantum mechanical calculations were better predictors of logK than tabulated substituent constants. Specifically, the CHelpG charges were found to be the more predictive of the two and further discussion is limited to these. Charges on atom (or group) X together with H-8 for the quinolinium species in vacuum were found to be the major predictors of logK. Not only did they correlate strongly with logK for the series with R = H, but the predicted values for this series also cross-correlated with the experimental logK values for the series with R = Me and EtNH<sub>2</sub>. More specifically, increased negative potential on X and decreased positive potential on H-8 apparently favors association with Fe(III)PPIX. This is graphically illustrated by the electrostatic surface potential (ESP) shown in Figure 5a.

Similarly atomic charges, particularly CHelpG charges, were good predictors of the trend in logBHIA<sub>50</sub>. Here, the best predictors were the CHelpG charges on N-1 and C-7, with N-1 and C-8 being almost as good. In contrast to the logK prediction, the neutral form (also in vacuum) was found to produce the strongest correlation. Decreased negative potential on N-1 in combination with either a less negative potential on C-8 or a less positive potential on C-7 was found to favor  $\beta$ -hematin inhibition (Fig. 5b). The predicted activities for the series with R = H again cross-correlated with the experimental values for the series with R = Me and EtNH<sub>2</sub> and was even found to predict activities in the series reported by Kaschula et al. with R = EtN(Et)<sub>2</sub>.<sup>24</sup> An interesting observation is that the sensitivity of logBHIA<sub>50</sub> to the changes in ESP brought about

by altering the X-substituent decreases as the lateral side chain becomes more lipophilic.

Several notable features emerge from this investigation. Atomic charges that predict logK are for the most part not the same as those that predict logBHIA<sub>50</sub> and furthermore the correlations involve different species, namely quinolinium and neutral, respectively. Indeed, in the case of the series with R = H there is no experimental correlation between logK and logBHIA<sub>50</sub>, suggesting that inhibition of  $\beta$ -hematin formation is not directly related to association between Fe(III)PPIX and the 4-aminoquinoline compound in solution. This appears to support the hypothesis that inhibition of  $\beta$ -hematin formation primarily occurs via interaction of the inhibitor with the growing crystal surface, rather than via complex formation in solution. It must be noted however, that there is some correlation between logK and logBHIA<sub>50</sub> as the lateral side chain is lengthened. Thus, a role for solution association cannot necessarily be ruled out for all series of 4-aminoquinoline compounds. Finally, the correlation equations permit the prediction of both logK and logBHIA<sub>50</sub> for these series of 4-aminoquinolines. Interestingly, they do not suggest that the lipophilicity of the group X plays a major role in  $\beta$ -hematin inhibition. This is an important finding, since as noted in the introduction there is evidence that less lipophilic and lower molecular weight weak base compounds possess fewer undesirable drug liabilities, such as hERG toxicity. In this regard, it is worth remarking on the observed activity of the analogs with X = CN, which are relatively hydrophilic compared to those with X = Cl, but retain rather strong  $\beta$ -hematin inhibitory activity. This indicates that 4-aminoquinolines with improved toxicity properties, but which maintain strong hemozoin-inhibiting characteristics are feasible.

Preliminary investigation of biological activities of compounds in the series with R = EtNH<sub>2</sub> indicate that these findings are indeed likely to be pertinent to the design of new 4-aminoquinoline

antimalarials. While there was no direct correlation between activity and strength of inhibition of  $\beta$ -hematin formation, the correlation was strong if vacuolar accumulation through pH trapping was taken into account. This is consistent with previous studies on similar families of compounds.<sup>8,24</sup> However, the strong activity of the 7-CN derivative, a compound with a particularly low lipophilicity is noteworthy. This contrasts with the 7-NO<sub>2</sub> derivative which exhibited poor activity despite being a strong  $\beta$ -hematin inhibitor. The weak activity of the latter compound can be ascribed to its particularly strong electron withdrawing effect. This lowers the pK<sub>a</sub> of the quinolinium NH<sup>+</sup> group to such an extent that accumulation in the parasite digestive vacuole is too low for it to exert strong biological activity. On the other hand, the 7-CH<sub>3</sub> and 7-H derivatives appear anomalously active given their weak inhibition of  $\beta$ -hematin formation. In this case, the electron releasing characteristics of these groups ensure higher pK<sub>a</sub> values and hence greater vacuolar accumulation that compensates for weak  $\beta$ -hematin inhibition. The 7-CN derivative presents the best compromise of properties if low lipophilicity is desired and is worth pursuing further.

#### 4. Conclusion

Compounds with electron withdrawing substituents, namely NO<sub>2</sub>, CN, Cl and CF<sub>3</sub>, were found to be strong inhibitors of  $\beta$ -hematin formation. Additionally, all of the hydrophilic and electron withdrawing compounds with X = CN were found to show strong inhibition of  $\beta$ -haematin formation. Analogs with electron releasing groups at the 7-position were found to be poor inhibitors or non-inhibitors of  $\beta$ -haematin formation.

Derivatives containing electron withdrawing groups at the 7-position were also found to bind strongly to Fe(III)PPIX, while those with electron releasing groups were weaker binders. Lipophilicity was found to be an important factor in Fe(III)PPIX binding.

The lateral side chain was found to play a positive role in Fe(III)PPIX binding. An increase in the length of the chain resulted in an increase in logK. The extension of the lateral side-chain was not found to have a significant impact on  $\beta$ -haematin inhibition, but was found to modulate the influence of the substituent at the 7-position. These findings could prove useful in the design of new 4-aminoquinoline  $\beta$ -hematin inhibitors with potential antimalarial activity, especially in view of the observed good activity of the 7-CN derivative of the series with R = EtNH<sub>2</sub>.

#### 5. Experimental

##### 5.1. Synthesis

Chemicals and solvents were purchased from Sigma–Aldrich Chemical Company or Fluka. Column chromatography was carried out using Silica gel 60 from Sigma–Aldrich. All products were characterized by <sup>1</sup>H and <sup>13</sup>C NMR and were recorded on a Bruker Avance (400 MHz) or Varian EM (300 MHz) spectrometer. Compound identities were further confirmed by high resolution electrospray measurements performed on a Bruker Daltonis MicroTOF mass spectrometer. The purity of final compounds was confirmed by high performance liquid chromatography (HPLC). This was performed on a Spectra Systems HPLC equipped with a C18 5  $\mu$ m, 4.6 mm  $\times$  150 mm, 100A analytical column (Waters, xBridge). Some compounds were purified using preparative HPLC on the same system with a preparative column (Prep C18, 5  $\mu$ m, 19 mm  $\times$  250 mm, xBridge). Compounds were dissolved in DMSO and water and injected through a 50  $\mu$ L loop. The solvent system used consisted of two mobile phases. Mobile phase A: NH<sub>4</sub>CO<sub>3</sub>/H<sub>2</sub>O, pH 10.4; mobile phase B: NH<sub>4</sub>CO<sub>3</sub>/MeOH, pH 10.4. Flow rates of 1.2 mL/min for analytical and 20 mL/min

for preparative methods were used with 20 min runs. The chromatographic purities of all the compounds were  $\geq$ 98%.

Briefly, the compounds investigated were prepared via a series of 4-chloroquinolines with differing substituents at position 7 on the quinoline ring. All of these are known compounds and are available commercially. Full synthetic details of those prepared in this study are provided as [Supplementary data](#). These were prepared in two different ways (see [Supplementary Schemes S1 and S2](#)).<sup>24,35–37</sup> In most cases, the starting materials were 3-X-anilines (X = CH<sub>3</sub>, CN, NO<sub>2</sub> or OCH<sub>3</sub>) and diethyl ethoxymethylenemalonate which were heated in equimolar ratio at 110 °C for 45–60 min to yield a diethyl 2-((3-X-phenylamino)methylene)malonate. This was then cyclized to a corresponding ethyl-4-hydroxy-7-X-quinoline-3-carboxylate by refluxing in diphenyl ether (265 °C) for 30–60 min. The resulting ester was hydrolyzed by reflux in 2 M aqueous NaOH for 30–60 min yielding the corresponding acid. This was decarboxylated by refluxing in diphenyl ether for 45–60 min, except for 4-hydroxy-7-nitroquinoline-3-carboxylic acid which was first converted to a silver salt before decarboxylation.<sup>38</sup> The alternative approach involved reacting triethyl orthoformate with a slight excess of 2,2-dimethyl-1,3-dioxane-4,6-dione (Meldrum's acid) to form an intermediate (ethoxymethylene Meldrum's acid) to which 3-X-aniline (X = CONH<sub>2</sub>) was then added after 1 h at 90 °C (1 equiv relative to Meldrum's acid) in situ. The resulting mixture was refluxed for 2 h to yield 3-((2,2-dimethyl-4,6-dioxo-1,3-dioxan-5-ylidene)methylamino)benzamide which was isolated by precipitation from ice cold ethanol. The product was then directly cyclized to 4-hydroxyquinoline-7-carboxamide by refluxing in diphenyl ether for 5 min.<sup>36,37</sup> This approach was also used as a second method for preparing the derivatives with X = NO<sub>2</sub> or OCH<sub>3</sub>. In a final step, each of the 4-hydroxy-7-X-quinolines was refluxed in excess phosphorus oxychloride (POCl<sub>3</sub>) for 20–30 min to yield the corresponding 4-chloro-7-X-quinoline which was then isolated by precipitation after neutralization with ice cold 2 M NaOH. Three additional 4-chloro-7-X-quinolines (X = CF<sub>3</sub>, Cl or H) were sourced commercially (Sigma–Aldrich).

The series of 4-amino-7-X-quinolines (X = CH<sub>3</sub>, Cl, CONH<sub>2</sub>, CN, H, NO<sub>2</sub> or OCH<sub>3</sub>) were prepared by reacting the corresponding 4-chloro-7-X-quinoline with excess aqueous NH<sub>3</sub> in a sealed tube at 70 °C for 4 h ([Supplementary Scheme S3](#)). Products were recovered by cooling and filtration and were then washed with 5% NH<sub>3</sub> and isolated by preparative HPLC. For the compound with X = CF<sub>3</sub>, the 4-chloroquinoline was reacted with excess NaN<sub>3</sub> at 90 °C in 8:3 DMF/H<sub>2</sub>O for 2 h. The resulting 4-azidoquinoline was extracted into CHCl<sub>3</sub> with NH<sub>4</sub>Cl and isolated by evaporation. This product was reduced to the 4-aminoquinoline with a small excess of PPh<sub>3</sub> over 2 h in 1:1 THF/H<sub>2</sub>O at room temperature ([Supplementary Scheme S4](#)).<sup>39,40</sup> The product was extracted with aqueous HCl and then into CHCl<sub>3</sub> with 1 M NaOH and isolated by evaporation. Finally, 4,7-diaminoquinoline was prepared from 4-amino-7-nitroquinoline by reduction with excess stannous chloride (SnCl<sub>2</sub>) over 2 h in HCl and glacial acetic acid at 60 °C ([Supplementary Scheme S5](#)).<sup>41</sup> The product was obtained after base extraction into CHCl<sub>3</sub> with NaOH after stirring, filtering and washing with acetone.

The series of N-methyl-(7-X-quinoline-4-yl)ethane-1,2-diamines (X = CH<sub>3</sub>, CF<sub>3</sub>, Cl, CONH<sub>2</sub>, CN, H, NO<sub>2</sub> or OCH<sub>3</sub>) were prepared by reaction of the corresponding 4-chloro-7-X-quinoline with excess methylamine in a sealed tube at 90 °C for 6 h ([Supplementary Scheme S3](#)). The products were purified by silica gel column chromatography. The compound with X = NH<sub>2</sub> was obtained by reduction of the nitro derivative with SnCl<sub>2</sub> as described for 4,7-diaminoquinoline above ([Supplementary Scheme S5](#)).

Lastly, the series of N<sup>1</sup>-(7-X-quinolin-4-yl)ethane-1,2-diamines (X = CH<sub>3</sub>, CF<sub>3</sub>, Cl, CN, H, NO<sub>2</sub> or OCH<sub>3</sub>) was prepared by reaction of the 4-chloro-7-X-quinoline with 5 equiv of ethylene diamine in a sealed tube at 110 °C for 4–6 h ([Supplementary Scheme S3](#)).



The crude products were obtained after base extraction into  $\text{CHCl}_3$  and were purified by preparative HPLC.

Full synthetic details for previously reported compounds are provided as [Supplementary data](#), while details for novel compounds are provided below.

#### 5.1.1. 4-Aminoquinolin-7-carboxamide (9d)

Compound **7d** was heated in a sealed tube with excess aqueous ammonia at 70 °C for 4 h. The mixture was cooled to room temperature and the solid filtered. Solid was then washed with 5%  $\text{NH}_3$  (aq) and dried. Following silica gel chromatography and preparative HPLC where purity was above 98% the product was obtained as a white solid (50 mg, 40%). mp 132–134 °C. NMR— $\delta_{\text{H}}$  (400 MHz; DMSO) 5.12 (2H, br s,  $-\text{NH}_2$ ), 6.66 (1H, d,  $J$  5.1 Hz, H-3), 7.80 (1H, d,  $J$  8.8 Hz, H-6), 8.34 (1H, d,  $J$  8.8 Hz, H-5), 8.42 (1H, s, H-8), 8.56 (1H, d,  $J$  5.1 Hz, H-2).  $\delta_{\text{C}}$  (100 MHz; DMSO) 116.8 (C-3), 121.8 (C-4a), 124.9 (C-5), 125.3 (C-6), 126.8 (C-8), 127.7 (C-7), 143.1 (C-8a), 153.2 (C-2), 153.6 (C-4), 156.3 ( $-\text{CONH}_2$ ). HPLC  $t_{\text{R}}$  = 9.51 min, purity  $\geq$  98%. HRMS (ESI)  $m/z$  187.1980 ( $\text{C}_{10}\text{H}_9\text{N}_3\text{O}$ ,  $\text{M}^+$  requires 187.1969).

#### 5.1.2. N,7-Dimethylquinolin-4-amine (10a)

Compound **7a** and excess methylamine (33% in MeOH) were heated in a sealed tube at 90 °C for 6 h. The mixture was allowed to cool to room temperature and concentrated under reduced pressure. The product was then purified by silica gel chromatography using MeOH:EtOAc:Et<sub>3</sub>N (1:98:1) and obtained as a white solid (80 mg, 65%), mp 133–135 °C. NMR— $\delta_{\text{H}}$  (400 MHz; DMSO) 2.51 (3H, s, Ar- $\text{CH}_3$ ), 3.07 (3H, d,  $J$  4.6 Hz,  $\text{NHCH}_3$ ), 6.66 (1H, d,  $J$  7.0 Hz, H-3), 7.49 (1H, dd,  $J$  1.5, 8.7 Hz, H-6), 7.75 (1H, br s, H-8), 8.43 (1H, d,  $J$  6.9 Hz, H-2), 8.45 (1H, d,  $J$  8.7 Hz, H-5), 9.34 (1H, br s,  $\text{NHCH}_3$ ).  $\delta_{\text{C}}$  (100 MHz; DMSO) 20.9 (Ar- $\text{CH}_3$ ), 29.1 ( $-\text{NHCH}_3$ ), 97.2 (C-3), 116.4 (C-4a), 121.4 (C-5), 125.7 (C-6), 127.1 (C-8), 138.4 (C-7), 147.3 (C-8a), 149.8 (C-3), 151.1 (C-4). HPLC  $t_{\text{R}}$  = 10.60 min, purity  $\geq$  98%. HRMS (ESI)  $m/z$  173.1062 ( $\text{C}_{11}\text{H}_{12}\text{N}_2$ ,  $[\text{M}+\text{H}]^+$  requires 173.1079).

#### 5.1.3. N-Methyl-7-(trifluoromethyl)quinolin-4-amine (10b)

Compound **7b** and excess methylamine (33% in MeOH) were heated in a sealed tube at 90 °C for 6 h. The mixture was allowed to cool to room temperature and concentrated under reduced pressure. The product was then purified by silica gel chromatography using MeOH:EtOAc:Et<sub>3</sub>N (1:98:1) and obtained as a white solid (70 mg, 60%), mp 227–229 °C. NMR— $\delta_{\text{H}}$  (400 MHz; DMSO) 2.87 (3H, d,  $J$  4.7 Hz,  $\text{NHCH}_3$ ), 6.47 (1H, d,  $J$  5.4 Hz, H-3), 7.51 (1H, d,  $J$  4.3 Hz,  $\text{NHCH}_3$ ), 7.64 (1H, dd,  $J$  1.9, 8.8 Hz, H-6), 8.04 (1H, br s, H-8), 8.34 (1H, d,  $J$  8.8 Hz, H-5), 8.48 (1H, d,  $J$  5.3 Hz, H-2).  $\delta_{\text{C}}$  (100 MHz; DMSO) 29.8 ( $\text{NHCH}_3$ ), 100.0 (C-3), 119.4 (C-6), 121.3 (C-5), 124.1 (C-4a), 126.8 (Ar- $\text{CF}_3$ ), 126.8 (C-8), 129.4 (C-7), 147.9 (C-8a), 151.3 (C-2), 152.8 (C-4). HPLC  $t_{\text{R}}$  = 9.49 min, purity  $\geq$  98%. HRMS (ESI)  $m/z$  227.0782 ( $\text{C}_{11}\text{H}_9\text{F}_3\text{N}_2$ ,  $[\text{M}+\text{H}]^+$  requires 227.0796).

#### 5.1.4. 4-(Methylamino)quinolin-7-carboxamide (10d)

Compound **7d** and excess methylamine (33% in MeOH) were heated in a sealed tube at 90 °C for 6 h. The mixture was allowed to cool to room temperature and concentrated under reduced pressure. The product was then purified by silica gel chromatography using MeOH:EtOAc:Et<sub>3</sub>N (1:98:1) and obtained as a white solid (60 mg, 50%), mp 165–166 °C. NMR— $\delta_{\text{H}}$  (400 MHz; DMSO) 2.82 (1H, d,  $J$  4.6 Hz,  $\text{NHCH}_3$ ), 5.88 (1H, d,  $J$  3.9 Hz,  $\text{NHCH}_3$ ), 6.68 (1H, d,  $J$  5.5 Hz, H-3), 7.69 (1H, d,  $J$  8.4 Hz, H-5), 7.91 (1H, d,  $J$  0.8 Hz, H-8), 8.06 (1H, dd,  $J$  1.3, 8.5 Hz, H-6), 8.52 (1H, d,  $J$  5.4 Hz, H-2).  $\delta_{\text{C}}$  (100 MHz; DMSO) 31.1 ( $\text{NHCH}_3$ ), 102.7 (C-3), 110.4 (C-4a), 123.0 (C-5), 128.0 (C-6), 135.0 (C-8), 147.3 (C-7), 149.6 (C-8a), 149.8 (C-2), 151.9 (C-4). HPLC  $t_{\text{R}}$  = 9.58 min, purity  $\geq$  98%. HRMS (ESI)  $m/z$  201.0700 ( $\text{C}_{11}\text{H}_{11}\text{N}_3\text{O}$ ,  $\text{M}^+$  requires 201.0902).

#### 5.1.5. 4-(Methylamino)quinolin-7-carbonitrile (10e)

Compound **7e** and excess methylamine (33% in MeOH) were heated in a sealed tube at 90 °C for 6 h. The mixture was allowed to cool to room temperature and concentrated under reduced pressure. The product was then purified by silica gel chromatography using MeOH:EtOAc:Et<sub>3</sub>N (1:98:1) and obtained as a white solid (90 mg, 65%), mp 252–254 °C. NMR— $\delta_{\text{H}}$  (400 MHz; DMSO) 2.87 (3H, d,  $J$  4.7 Hz,  $\text{NHCH}_3$ ), 6.47 (1H, d,  $J$  5.4 Hz, H-3), 7.51 (1H, d,  $J$  4.4 Hz,  $\text{NHCH}_3$ ), 7.68 (1H, dd,  $J$  1.7, 8.7 Hz, H-6), 8.22 (1H, d,  $J$  1.7 Hz, H-8), 8.28 (1H, d,  $J$  8.7 Hz, H-5), 8.48 (1H, d,  $J$  5.4 Hz, H-2).  $\delta_{\text{C}}$  (100 MHz; DMSO) 29.8 ( $\text{NHCH}_3$ ), 100.3 (C-7), 111.9 (Ar-CN), 119.2 (C-3), 121.8 (C-5), 124.0 (C-4a), 125.0 (C-6), 135.0 (C-8), 147.7 (C-8a), 151.2 (C-2), 153.0 (C-4). HPLC  $t_{\text{R}}$  = 9.51 min, purity  $\geq$  98%. HRMS (ESI)  $m/z$  184.0874 ( $\text{C}_{11}\text{H}_9\text{N}_3$ ,  $[\text{M}+\text{H}]^+$  requires 184.0796).

#### 5.1.6. N-Methyl-7-nitroquinolin-4-amine (10h)

Compound **7h** and excess methylamine (33% in MeOH) were heated in a sealed tube at 90 °C for 6 h. The mixture was allowed to cool to room temperature and concentrated under reduced pressure. The product was then purified by silica gel chromatography using MeOH:EtOAc:Et<sub>3</sub>N (1:98:1) and obtained as a yellow solid (40 mg, 55%), mp  $>$ 330 °C. NMR— $\delta_{\text{H}}$  (400 MHz; DMSO) 2.94 (3H, s,  $\text{NHCH}_3$ ), 6.55 (1H, d,  $J$  5.3 Hz, H-3), 8.10 (1H, dd,  $J$  2.0, 9.2 Hz, H-6), 8.48 (1H, d,  $J$  9.2 Hz, H-5), 8.54 (1H, d,  $J$  1.8 Hz, H-8), 8.57 (1H, d,  $J$  5.3 Hz, H-2).  $\delta_{\text{C}}$  (100 MHz; DMSO) 31.1 ( $\text{CH}_3$ ), 102.7 (C-3), 110.5 (C-8), 123.0 (C-6), 128.0 (C-5), 135.0 (C-4a), 147.3 (C-8a), 149.6 (C-3), 149.8 (C-7), 151.9 (C-4). HPLC  $t_{\text{R}}$  = 5.30 min, purity  $\geq$  98%. HRMS (ESI)  $m/z$  204.0792 ( $\text{C}_{10}\text{H}_9\text{N}_3\text{O}_2$ ,  $[\text{M}+\text{H}]^+$  requires 204.0773).

#### 5.1.7. 7-Methoxy-N-methylquinolin-4-amine (10i)

Compound **7i** and excess methylamine (33% in MeOH) were heated in a sealed tube at 90 °C for 6 h. The mixture was allowed to cool to room temperature and concentrated under reduced pressure. The product was then purified by silica gel chromatography using MeOH:EtOAc:Et<sub>3</sub>N (1:98:1) and obtained as a white solid (100 mg, 60%), mp 75–78 °C. NMR— $\delta_{\text{H}}$  (400 MHz; DMSO) 2.94 (3H, br s,  $-\text{NHCH}_3$ ), 3.85 (3H, s, Ar- $\text{OCH}_3$ ), 6.44 (1H, d,  $J$  6.4 Hz, H-3), 7.13 (1H, dd,  $J$  2.6, 9.2 Hz, H-6), 7.25 (1H, d,  $J$  2.6 Hz, H-8), 8.22 (1H, d,  $J$  9.3 Hz, H-5), 8.26 (1H, s, Ar- $\text{NHCH}_3$ ), 8.33 (1H, d,  $J$  6.4 Hz, H-2).  $\delta_{\text{C}}$  (100 MHz; DMSO) 30.0 ( $-\text{NHCH}_3$ ), 56.1 (Ar- $\text{OCH}_3$ ), 103.7 (C-8), 112.3 (C-3), 117.1 (C-4a), 124.5 (C-6), 144.3 (C-5), 146.0 (C-8a), 154.3 (C-2), 161.8 (C-4), 164.8 (C-7). HPLC  $t_{\text{R}}$  = 8.91 min, purity  $\geq$  98%. HRMS (ESI)  $m/z$  189.1026 ( $\text{C}_{11}\text{H}_{12}\text{N}_2\text{O}$ ,  $[\text{M}+\text{H}]^+$  requires 189.1028).

#### 5.1.8. N<sup>4</sup>-Methylquinolin-4,7-diamine (10g)

Stannous chloride ( $\text{SnCl}_2$ ) (0.87 g, 4.57 mmol) dissolved in concentrated HCl (3 mL) was added slowly to a stirred solution of **10h** (0.12 g, 0.57 mmol) in glacial acetic acid (20 mL). The mixture was stirred at 60 °C for 2 h. After the mixture was cooled, acetone (50 mL) was added and it was stirred vigorously. Precipitate was collected by filtration, washed with acetone, and suspended in water (250 mL). The suspension was made basic (pH 12) with sodium hydroxide and the product was extracted with chloroform (5  $\times$  50 mL). The crude product was purified by silica gel chromatography with chloroform–methanol (10:1) containing 0.5% triethylamine. Solvent was evaporated to obtain **10g** (84 mg, 70%) as a yellow solid mp 283–285 °C. NMR— $\delta_{\text{H}}$  (400 MHz; DMSO) 2.91 (3H, d,  $J$  4.7 Hz,  $-\text{NHCH}_3$ ), 6.53 (1H, d,  $J$  5.4 Hz, H-3), 7.45 (1H, bs,  $-\text{NHCH}_3$ ), 8.08 (1H, dd,  $J$  2.3, 9.2 Hz, H-6), 8.36 (1H, d,  $J$  9.2 Hz, H-5), 8.52 (1H, d,  $J$  2.3 Hz, H-8), 8.54 (1H, d,  $J$  5.3 Hz, H-2).  $\delta_{\text{C}}$  (100 MHz; DMSO) 29.8 ( $-\text{NHCH}_3$ ), 100.7 (C-3), 117.1 (C-8), 123.1 (C-4a), 124.5 (C-5), 124.9 (C-6), 148.0 (C-7), 150.3 (C-8a), 151.5

(C-2), 153.6 (C-4). HPLC  $t_R$  = 7.86 min, purity  $\geq$ 98%. HRMS (ESI)  $m/z$  174.1031 ( $C_{10}H_{11}N_3$ ,  $[M+H]^+$  requires 174.1029).

#### 5.1.9. *N*<sup>1</sup>-(7-Methylquinolin-4-yl)ethane-1,2-diamine (11a)

Compound **7a** (1 equiv) and excess (5 equiv) ethylene diamine were heated in a sealed tube at 120 °C for 4–6 h. The reaction mixture was cooled to room temperature and a mixture of 1 M NaOH and  $CHCl_3$  (1:4) was added to the mixture. The organic layer was washed with brine and dried with  $Na_2SO_4$ . Solvent was removed and resulting solid purified by column chromatography and further purified by HPLC where purity was found to be below 98%. The product was obtained as a white solid (80 mg, 50%). NMR— $\delta_H$  (400 MHz; DMSO) 2.44 (3H, s, Ar- $CH_3$ ), 2.83 (3H, t,  $J$  6.3 Hz,  $CH_2$ ), 3.24 (2H, t,  $J$  6.2 Hz,  $CH_2$ ), 6.38 (1H, d,  $J$  5.3 Hz, H-3), 6.87 (1H, br s, Ar-NH-), 7.22 (1H, dd,  $J$  1.3, 8.5 Hz, H-6), 7.55 (1H, br s, H-8), 8.08 (1H, d,  $J$  8.5 Hz, H-5), 8.32 (1H, d,  $J$  5.3 Hz, H-2).  $\delta_C$  (100 MHz; DMSO) 21.6 (Ar- $CH_3$ ), 38.7 (C-11), 41.8 (C-10), 98.5 (C-3), 122.5 (C-4a), 124.5 (C-5), 129.0 (C-6), 129.5 (C-8), 139.4 (C-7), 145.8 (C-8a), 148.7 (C-2), 150.2 (C-4). HPLC  $t_R$  = 11.79 min, purity  $\geq$ 98%. HRMS (ESI)  $m/z$  202.1350 ( $C_{12}H_{15}N_3$ ,  $[M+H]^+$  requires 202.1344).

#### 5.1.10. 4-(2-Aminoethylamino)quinoline-7-carbonitrile (11e)

Compound **7e** (1 equiv) and excess (5 equiv) ethylene diamine were heated in a sealed tube at 120 °C for 4–6 h. The reaction mixture was cooled to room temperature and a mixture of 1 M NaOH and  $CHCl_3$  (1:4) was added to the mixture. The organic layer was washed with brine and dried with  $Na_2SO_4$ . Solvent was removed and resulting solid purified by column chromatography and further purified by HPLC where purity was found to be below 98%. The product was obtained as a white solid (60 mg, 50%), mp 161–163 °C. NMR— $\delta_H$  (400 MHz; DMSO) 2.81 (3H, t,  $J$  6.4 Hz,  $CH_2$ ), 3.26 (3H, t,  $J$  5.3 Hz,  $CH_2$ ), 6.57 (1H, d,  $J$  5.5 Hz, H-3), 7.35 (1H, br s, Ar-NH), 7.68 (1H, dd,  $J$  1.7, 8.7 Hz, H-6), 8.22 (1H, d,  $J$  1.7 Hz, H-8), 8.39 (1H, d,  $J$  8.7 Hz, H-5), 8.46 (1H, d,  $J$  5.4 Hz, H-2).  $\delta_C$  (100 MHz; DMSO) 46.3 (C-10), 100.6 (C-7), 111.8 (Ar-CN), 119.2 (C-3), 121.9 (C-5), 124.4 (C-4a), 124.9 (C-6), 135.1 (C-8), 147.9 (C-8a), 150.6 (C-2), 152.9 (C-4). HPLC  $t_R$  = 12.92 min, purity  $\geq$ 98%. HRMS (ESI)  $m/z$  213.1156 ( $C_{12}H_{12}N_4$ ,  $[M+H]^+$  requires 213.1140).

#### 5.1.11. *N*<sup>1</sup>-(7-Nitroquinolin-4-yl)ethane-1,2-diamine (11h)

Compound **7h** (1 equiv) and excess (5 equiv) ethylene diamine were heated in a sealed tube at 120 °C for 4–6 h. The reaction mixture was cooled to room temperature and a mixture of 1 M NaOH and  $CHCl_3$  (1:4) was added to the mixture. The organic layer was washed with brine and dried with  $Na_2SO_4$ . Solvent was removed and resulting solid purified by column chromatography and further purified by HPLC where purity was found to be below 98%. The product was obtained as a yellow solid (90 mg, 55%), mp 170–172 °C. NMR— $\delta_H$  (400 MHz; DMSO) 2.82 (2H, t,  $J$  6.1 Hz,  $CH_2$ ), 3.22 (2H, t,  $J$  6.1 Hz,  $CH_2$ ), 6.79 (1H, d,  $J$  5.5 Hz, H-3), 7.74 (1H, d,  $J$  8.4 Hz, H-5), 7.91 (1H, d,  $J$  1.2 Hz, H-8), 8.09 (1H, dd,  $J$  1.3, 8.5 Hz, H-6), 8.54 (1H, d,  $J$  5.5 Hz, H-2).  $\delta_C$  (100 MHz; DMSO) 45.9 (C-10), 103.2 (C-3), 110.6 (C-8), 122.5 (C-6), 128.1 (C-5), 134.6 (C-4a), 147.4 (C-8a), 148.8 (C-2), 149.8 (C-7), 151.8 (C-4). HPLC  $t_R$  = 10.38 min, purity  $\geq$ 98%. HRMS (ESI)  $m/z$  256.097 ( $C_{11}H_{12}N_4O_2$ ,  $[M+H+Na]^+$  requires 256.0998).

### 5.2. Biophysical measurements and in vitro *P. falciparum* assay

Association constants of the various 4-aminoquinolines with Fe(III)PPIX were determined in 40% aqueous (v/v) DMSO, pH 7.5 by spectrophotometric titration using methods previously described and without modification.<sup>13</sup> The  $\beta$ -hematin inhibitory activity was also determined by methods previously reported,

again without modification.<sup>42</sup> The assay uses 4.5 M acetate to mediate  $\beta$ -hematin formation and 5% aqueous pyridine reacts with unconverted Fe(III)PPIX to form a low-spin bis-pyridyl complex which can be measured colorimetrically.

Compounds **11a–h** were tested in vitro against the chloroquine-sensitive NF54 strain of *P. falciparum* using the [<sup>3</sup>H]-hypoxanthine incorporation assay, basically as described elsewhere.<sup>43</sup>

### 5.3. Computational methods

DFT calculations on neutral and cationic (quinolinium) structures of 7-substituted 4-aminoquinolines ( $R = H$ ) were performed using the GAUSSIAN09 software package.<sup>44</sup> Geometry optimizations of all structures were completed in vacuum using the B3LYP functional and 6-31G(d,p) basis set, B3LYP/6-31G(d,p), except in the case of the iodine analog where the iodine atom was described by the 6-311G(d) basis set (retrieved from the EMSL Basis Set Exchange portal)<sup>45</sup> which makes use of a d-type polarization function.<sup>46</sup> Frequencies of final geometry-optimized structures were generated to ensure a stationary point was obtained. In order to test the reliability of the chosen functional and basis set, the structure of CQ was computed and compared to experimental crystal structure data.<sup>47</sup> The average bond length and angle deviations of the quinoline ring were 0.01 Å and 0.5°, respectively, indicating this method can produce computed structures in good agreement with experimental data. CHelpG charges were calculated using the B3LYP functional in vacuum or under the implicit water conditions of the SMD solvent model,<sup>33</sup> and were constrained to reproduce the dipole moment. Atoms were described using the larger 6-311+G(2df,p) basis set, B3LYP/6-311+G(2df,p), or in the case of the iodine atom in the  $X = I$  molecule, the 6-311G(d) basis set (described above for geometry optimization). For bromine and iodine atoms, atomic radii used to determine CHelpG charges were set to the van der Waals radii of 1.85 and 1.98 Å, respectively.<sup>48</sup> CHelpG as well as Mulliken atomic charges (calculated in the same manner as for CHelpG charges) for all 7-substituted 4-aminoquinolines ( $R = H$ ) in both neutral and quinolinium forms, are listed in Supplementary data Tables S2–S5. Electrostatic surface potentials were generated using the USCF Chimera software package<sup>49</sup> by mapping the electrostatic potential (obtained using the same functional and basis sets as described for CHelpG atomic charges) onto a density isosurface of 0.002 e au<sup>-3</sup>.

### 5.4. Correlation analyses

Single linear correlation analysis (SLCA) and multiple linear correlation analysis (MLCA) were performed using the computed CHelpG and Mulliken atomic charges for the neutral and quinolinium species in both vacuum and in the implicit SMD water model. In order to facilitate correlations, the atomic charges of any X substituent consisting of two or more atoms were summed to give an overall atomic charge of the X group. For SLCA, a correlation between atomic charge and logK was deemed significant if the P-statistic was less than 0.05. While the same criterion were required for statistical significance between atomic charge and logBHIA<sub>50</sub>, an additional condition was also imposed, namely that the relationship should predict that the logBHIA<sub>50</sub> value for the  $X = NH_2$  molecule be greater than the experimental assay limit (1.30). For MLCA, correlations between atomic charge and logK were deemed significant if they produced a  $F$  value greater than  $F^{crit}$  at the 95% confidence level and both correlation coefficients produced a  $t$  value greater than  $t^{crit}$  at the 95% confidence level. The same requirements were enforced for correlations between atomic charges and logBHIA<sub>50</sub>, but as in the case of SLCA, the additional criterion of predicting that the logBHIA<sub>50</sub> of the  $X = NH_2$  molecule be greater than 1.30 was also imposed.

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.bmc.2013.04.040>.

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