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ARTICLE

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Quinoline Antimalarials Containing a Dibemethin Group Are Active against Chloroquinone-Resistant *Plasmodium falciparum* and Inhibit Chloroquine Transport via the *P. falciparum* Chloroquine-Resistance Transporter (PfCRT)

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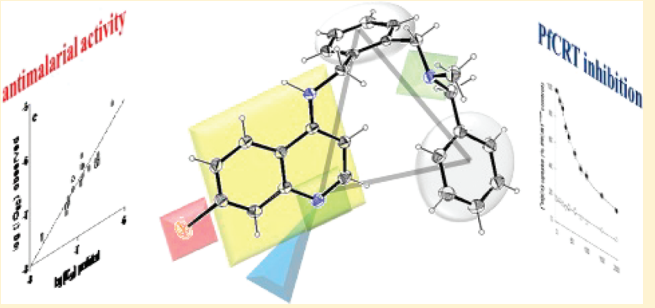
Supporting Information

ABSTRACT: A series of 4-amino-7-chloroquinolines with dibenzylmethylamine (dibemethin) side chains were shown to inhibit synthetic hemozoin formation. These compounds were equally active against cultures of chloroquine-sensitive (D10) and chloroquine-resistant (K1) *Plasmodium falciparum*. The most active compound had an IC_{50} value comparable to that of chloroquine, and its potency was undiminished when tested in three additional chloroquine-resistant strains. The three most active compounds exhibited little or no cytotoxicity in a mammalian cell line. When tested in vivo against mouse malaria via oral administration, two of the dibemethin derivatives reduced parasitemia by over 99%, with mice treated at 100 mg/kg surviving the full length of the experiment. Three of the compounds were also shown to inhibit chloroquine transport via the parasite's chloroquine-resistance transporter (PfCRT) in a *Xenopus* oocyte expression system. This constitutes the first example of a dual-function antimalarial for which the ability to inhibit both hemozoin formation and PfCRT has been demonstrated directly.



INTRODUCTION

Chloroquine (CQ)-resistance has been a major setback to malaria control worldwide[1] and is primarily due to mutations in the *P. falciparum* CQ-resistance transporter (PfCRT). PfCRT is located in the membrane of the parasite's digestive vacuole, the organelle in which CQ exerts its antimalarial effects by interfering with the formation of hemozoin.[2] Expression of PfCRT at the surface of *Xenopus laevis* oocytes has allowed the function of the protein to be studied directly, leading to the demonstration that the resistance-conferring form of PfCRT ($PfCRT^{CQR}$) has the ability to transport CQ out of the digestive vacuole, whereas the CQ-sensitive form ($PfCRT^{CQS}$) does not.[3]

Although parasites have been reported to recover CQ-sensitivity after an extended period in the absence of CQ pressure,[4] it has been shown that this arises from expansion of a CQ-sensitive subpopulation of parasites rather than back mutation of PfCRT.[5] Thus, drug rotation is not a viable option as CQ pressure is likely to result in rapid re-expansion of CQ-resistant strains.[6] Compounds that are able to partially reverse CQ-resistance (known as chemosensitizing agents or CQ-resistance-reversers) have been identified from a wide range of drug families and include the calcium channel blocker verapamil, the antidepressant imipramine (**1**) (Chart 1), and the antihistamine azatadine, as well as antipsychotics such as chlorpromazine.[7] Using the oocyte expression system, it was shown that CQ transport via $PfCRT^{CQR}$ is inhibited by the resistance-reversers verapamil and primaquine, thus providing a mechanistic explanation for the ability of these compounds to reverse CQ-resistance.[3] With the exception of chlorpheniramine,[8,9] however, these compounds have not been tested clinically because most require unacceptably high concentrations to exert significant CQ-resistance reversing activity. This leaves the development of new drugs as the only viable alternative. One approach has been to modify a quinoline so as to avoid cross-resistance. Examples of such re-engineered 4-amino-7-chloroquinolines include ferroquine and isoquine, the first of which is currently in development. These do not exhibit cross-resistance with CQ.[10,11]

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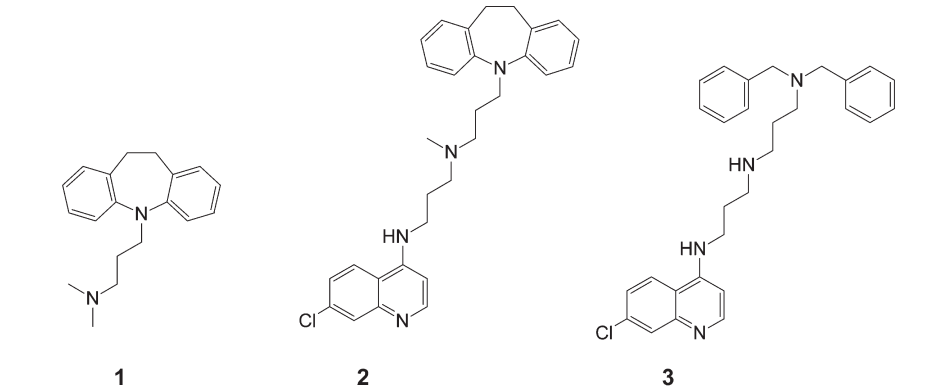
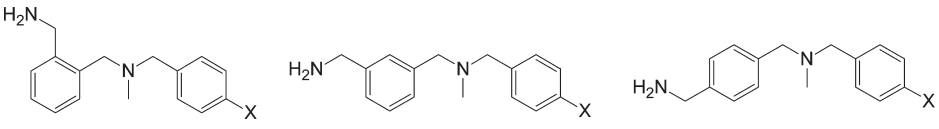
Chart 1. Imipramine (1), a Reversed-CQ (2) Containing a Side-Chain Related to 1, and (3) a Dibemethin Type Side Chain[a]



1 **2** **3**



	X		X		X
4a	H	**5a**	H	**6a**	H
7a	Cl	**8a**	Cl	**9a**	Cl
10a	OCH_3	**11a**	OCH_3	**12a**	OCH_3
13a	$N(CH_3)_2$	**14a**	$N(CH_3)_2$	**15a**	$N(CH_3)_2$

[a]Dibemethin resistance-reversing agents were used in this work as side chains for attachment through the primary amine to 4,7-dichloroquinoline (**4a** − **15a**).

A second strategy has been to generate hybrid compounds that combine the antiplasmodial activity with CQ-resistance-reversing ability.[12,13] Burgess et al. have described such a reversed-CQ compound that is active against both CQ-sensitive and CQ-resistant parasites.[12] It couples the hemozoin-inhibiting 4-amino-7-chloroquinoline pharmacophore of CQ to a resistance-reversing agent, such as **1**, via a linker (**2**) (Chart 1). Similarly, Kelly et al. have shown that an acridone derivative designed to combine both heme-binding and resistance-reversing abilities exhibits additive activity in combination with CQ against a CQ-sensitive strain of *P. falciparum* but synergistic activity against a CQ-resistant strain.[14] Although the reversed-CQs and acridones have been designed to inhibit PfCRT, this activity has not been demonstrated directly.

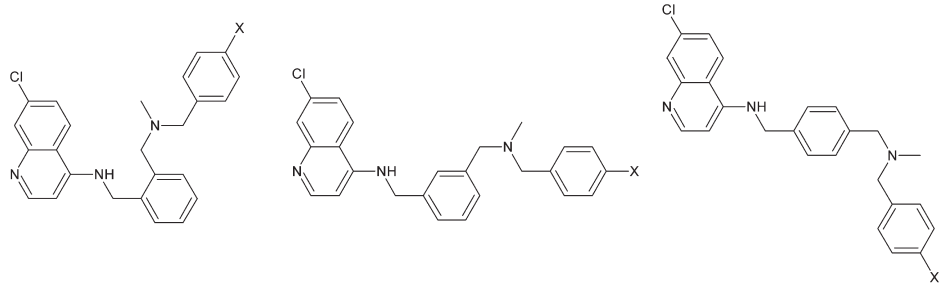
A recent patent[13] has claimed a wide range of reversed-CQs that incorporate the resistance-reversing pharmacophore features identified by Bhattacharjee et al.[15] This pharmacophore consists of two suitably arranged aromatic rings, a three or four carbon chain linked between the rings, and a terminal amino group. The claim includes a dibemethin compound (**3**) with this arrangement (Chart 1). In a recent study,[16] we have shown that resistance-reversing agents need not possess this precise structural motif since a series of aminomethyldibemethins (**4a**−**15a**) also show strong resistance-reversing activity (Chart 1). These compounds were also shown to interact directly with $PfCRT^{CQR}$ to inhibit CQ transport in the *Xenopus* oocyte system.[16]

Here, we demonstrate that linking these molecules to a 7-chloroquinoline nucleus results in compounds that possess antimalarial activity against both CQ-sensitive and CQ-resistant parasites as well as the ability to inhibit $PfCRT^{CQR}$. Structure−activity relationships were determined for the series, and in vivo activity in a mouse model of malaria was assayed. The findings reported herein further validate the viability of the reversed-CQ strategy. The compounds reported here are the subject of a recent patent.[17]

CHEMISTRY

The target molecules (**4**−**15**) (Chart 2) were synthesized in a single step by reaction of the appropriate dibemethin (**4a**−**15a**, described previously by Zishiri et al.)[16] with excess commercially available 4,7-dichloroquinoline in anhydrous *N*-methyl-2-pyrrolidone and in the presence of potassium carbonate and triethylamine as bases. The reaction was conducted in sealed cycloaddition tubes under N_2 at 90−130 °C over periods ranging from 16−48 h. The products were then extracted from an alkaline aqueous solution into ethyl acetate and purified by column chromatography to afford modest yields (between 18 and 37%). All products were characterized by infrared, ^1H and ^{13}C NMR, and mass spectrometry. Well-behaved solids were further characterized by melting point determination and elemental combustion analysis, while oils and hygroscopic solids were subjected to high-resolution mass spectrometry to confirm identity and HPLC to confirm purity. The prototype of this series of compounds (**4**) was crystallized and the structure determined by single crystal X-ray diffraction analysis. The structure is shown in Figure 1, and crystallographic data are presented in Table 1 (see Supporting Information for further details).

Chart 2. Compounds Synthesized in This Study



	X		X		X
4	H	**5**	H	**6**	H
7	Cl	**8**	Cl	**9**	Cl
10	OCH_3	**11**	OCH_3	**12**	OCH_3
13	$N(CH_3)_2$	**14**	$N(CH_3)_2$	**15**	$N(CH_3)_2$

Determination of pK_a Values and β-Hematin Inhibition Activity. The pK_a values of compounds **4**−**15** were determined by pH titration and are reported in Table 2. As expected, all of the compounds exhibit two pK_a values. The lower one, corresponding to the quinoline heteroaromatic N atom was found to lie within a narrow range from 7.33 (**15**) to 7.63 (**5**). The higher pK_a, corresponding to the basic tertiary amino group in the dibemethin side chain ranged from 9.6 (**7**) to 9.9 (**6**).

The IC_{50} values for β-hematin (synthetic hemozoin) inhibition are also presented in Table 2 and were determined using a pyridine based 96-well plate method that we have previously reported in full.[18] The method relies on the fact that a 5% solution (v/v) of aqueous pyridine dissolves hematin but not β-hematin at pH 7.5. The extent of inhibition is then characterized by measuring the intensity of the monomeric pyridine−hematin complex at 405 nm. The IC_{50} values for β-hematin inhibition varied significantly, with the most potent inhibitor (**6**) producing an IC_{50} of 0.32 equivalents and the least active (**12**) a value of 1.44 equivalents.

BIOLOGICAL TESTING

Compounds **4**−**15** were tested in human red blood cells infected with either CQ-sensitive (D10) or CQ-resistant (K1) parasites. The in vitro antimalarial activities of the compounds were measured up to a concentration of 100 ng/mL in the D10-infected cells and to 10 μg/mL in the K1-infected cells. With these cutoff concentrations, three compounds (**7**, **9**, and **14**) were inactive against the D10 strain, and one (**9**) was inactive in the K1 strain. The IC_{50} values for the dibemethin derivatives ranged from 27 nM (**6**) to 178 nM (**13**) in the D10 strain and from 24 nM (**6**) to 1178 nM (**7**) in the K1 strain (Table 2). By contrast, the IC_{50} values determined for CQ against these two strains were 23 nM and 144 nM, respectively. Thus, the most active dibemethin compound has an IC_{50} in CQ-resistant K1 parasites, which is comparable to that of CQ in the CQ-sensitive D10 strain. Compounds **5** and **15** also exhibited potent in vitro antimalarial activities against the D10 and K1 strains, with IC_{50} values between 37 and 48 nM. The resistance index (RI = IC_{50} in the K1 divided by the IC_{50} in the D10 strain) determined for each compound ranged from 0.6 (**8**) to 1.4 (**4**). This variation is small and indicates that the antimalarial activities of these compounds are comparable between the CQ-sensitive D10 and CQ-resistant K1 parasites.

The antimalarial properties of the most active of these compounds (**6**) was tested against a further three strains of CQ-resistant parasites Dd2, W2, and RSA11. As shown in Table 3, no significant cross-resistance was observed. The three most potent in vitro antimalarials (**5**, **6**, and **15**) were also tested for mammalian cytotoxicity in Chinese hamster ovarian (CHO) cells (Table 4). The lowest selectivity index (SI = IC_{50} CHO/IC_{50} D10) was exhibited by **5**, but this exceeded 1000. No cytotoxicity was observed in **6** and **15** up to the maximum concentration tested (100 μg/mL; SI exceeds 9000 and 4700, respectively).

The potent in vitro antimalarial properties of **4**, **5**, and **6**, together with the lack of toxicity of dibemethin derivatives in mammalian cells and the availability of these materials as well behaved solids, encouraged the in vivo testing of this initial subseries of compounds in the *P. berghei* mouse model of malaria (Table 5). The compounds were administered orally at 100 mg/kg and 30 mg/kg. Compound **4** was administered in four treatments (on days 0, 1, 2, and 3 postinfection), whereas the activities of compounds **5** and **6** were investigated using abbreviated regimes owing to the reduced availability of material; compound **5** was administered three times (on days 0, 1, and 2), and compound **6** was given as a single treatment on day 0. All three compounds showed significant activity, reducing parasitemia by over 99% after three or four doses were administered (**4** and **5**) or by over 97% after one dose was delivered (**6**). Compounds **4** and **5** caused all of the mice treated at 100 mg/kg to survive the full 30 days of the experiment, and the mice exhibited no evidence of parasitemia on day 30. Further in vivo studies are being conducted.

To determine whether members of this series of compounds interact with $PfCRT^{CQR}$, we tested compounds **4**−**6** for the ability to inhibit the flux of CQ via this protein. These experiments made use of the recently described system for expressing PfCRT in *Xenopus* oocytes[3] with which direct measurements of CQ transport via $PfCRT^{CQR}$ and its inhibition by potential resistance-reversers or reversed-CQ compounds can be made. The direction of CQ transport in the PfCRT expression system is from the mildly acidic extracellular medium (pH 6.0) into the oocyte cytosol (pH 7.2),[3] which corresponds to the efflux of CQ from the acidic digestive vacuole (pH ~5) into the parasite cytosol

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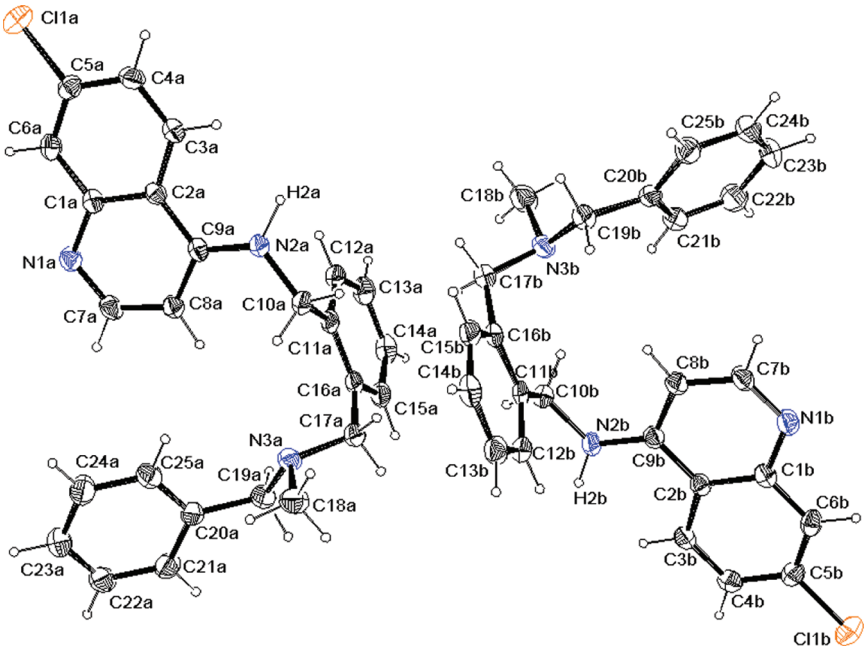

Figure 1. ORTEP drawing of the molecular structure of compound **4** with an ellipsoidal model at 50% probability level, showing the atomic numbering scheme. The molecule exhibits a folded conformation in which the terminal dibemethin phenyl ring wraps back around toward the quinoline ring. The position of the quinoline N relative to the two aromatic rings of the dibemethin side chain resembles the arrangement of the N atom relative to the two aromatic rings in the resistance-reversing analogues of **1**.[15] Pairs of molecules interact via π-stacking of the dibemethin phenyl rings closest to the quinoline group. An interesting feature of the packing (see Supporting Information) is large solvent accessible voids in which no electron density peaks could be located. We suspect that these voids contain highly disordered solvent molecules (hexane and/or ethyl acetate).

Table 1. Crystal Structure Data for Compound 4

empirical formula	$C_{25}H_{24}ClN_3$
formula weight	401.92
crystal system, space group	monoclinic, $P2_1/c$
a (Å)	13.1065(2)
b (Å)	15.7570(3)
c (Å)	21.7792(5)
α (deg)	90
β (deg)	90.5650(10)
γ (deg)	90
V (Å^3)	4497.60(15)
Z	8
λ (Mo−Kα) (Å)	0.71073
F (000)	1696
crystal size (mm)	0.16 × 0.11 × 0.09
range scanned θ (deg)	3.02−25.37
range of indices	h: ± 15
	k: ± 18
	l: ± 26
no. reflections collected	60275
no. unique reflections	8209
S	1.104
R_1	0.0476
wR_2	0.1061
$\Delta\rho$ excursions/e (Å^{-3})	−0.379, 0.329

Table 2. Properties of Compounds 4−15[a]

	code	R	pK_{a1}	pK_{a2}	BHIA$_{50}$[a,b]	IC$_{50}$ D10 (nM)[c]	IC$_{50}$ K1 (nM)[c]	RI[f]
4	VZ1	0	7.57	9.85	1.1(2)	140(2)	122(24)	0.9
5	VZ2	0	7.63	9.77	0.69(5)	41(5)[b]	43(2)[b]	1.0
6	VZ3	0	7.56	9.90	0.32(4)	22(3)[b]	26(3)[b]	1.2
7	2VZ1	−0.15	7.44	9.60	1.4(2)	ND[d]	1128(117)	
8	2VZ2	−0.15	7.55	9.89	0.66(5)	138(7)	85(15)	0.6
9	2VZ3	−0.15	7.44	9.74	0.34(4)	ND[d]	ND[e]	
10	3VZ1	−0.51	7.47	9.85	0.46(4)	175	134(13)	0.8
11	3VZ2	−0.51	7.38	9.67	0.52(7)	91	120(12)	1.3
12	3VZ3	−0.51	7.44	9.70	1.44(9)	130(14)	88(10)	0.7
13	4VZ1	−0.92	7.40	9.71	0.48(2)	178	149(3)	0.8
14	4VZ2	−0.92	7.36	9.67	0.5(3)	ND[d]	281(29)	
15	4VZ3	−0.92	7.33	9.66	0.4(2)	48(2)	37(3)	0.9
CQ			8.4	10.8	1.9(3)[18]	23(4)[b]	144(19)[b]	6.3

[a] Resonance constant (R)(see Chart 2) of each compound is listed along with measured acid dissociation constant (pK_a) values, BHIA50 value, and in vitro antimalarial activities (IC$_{50}$ values) against the D10 and K1 strains of *P. falciparum*, and RI. [b] Equivalents relative to hematin with β-hematin formation mediated by 4.5 M acetate, pH 4.5. [c] Mean (SEM, n = 3). [d] Errors are for duplicate determination except where stated otherwise. [e] Not determined, IC$_{50}$ > 100 ng/mL. [f] Not determined, IC$_{50}$ > 10 μg/mL. [g] RI = IC$_{50}$ (K1)/IC$_{50}$ (D10).

(pH 7.3).[19] The concentration dependence of inhibition of the CQ transport was determined for each of these compounds, and the results are presented in Figure 2 and Table 6. All three compounds blocked the PfCRT^{CQR}-mediated transport of CQ.

The ability of the most active dibemethin derivative (**6**) to enhance the activity of CQ in drug-resistant parasites was also

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investigated. Isobolograms were constructed for drug-resistant (Dd2) and sensitive (D10) strains over a concentration range that was comparable to the in vitro antimalarial IC_{50} value for compound **6**. As shown in Figure 3, compound **6** and CQ were additive in CQ-sensitive parasites but exhibited synergism in the CQ-resistant strain (see Supporting Information for further details).

DISCUSSION

Dependence of pK_a on the Identity of the Terminal Group on the Side Chain. Although the pK_a values for compounds **4**−**15** vary over only a small range of about 0.3 log units, a statistically significant correlation with the physical characteristics of the functional group attached to the terminal phenyl ring of the side chain was observed (Figure 4). Unexpectedly, both pK_{a1} and pK_{a2} increase as the functional group becomes less resonance-releasing (i.e., as the parameter R becomes more positive). One would expect that an electron-releasing group would strengthen the N−H^+ bonds, making deprotonation more difficult and raising rather than decreasing the pK_a. Furthermore, the quinoline N atom is separated from the functional group on the terminal phenyl ring by 15 bonds, and the dibemethin N atom is separated from this group by six bonds. This means that the influence of the terminal functional group on pK_a must be a through-space interaction. The crystal structure of **4** illustrates that these molecules can adopt a folded structure in which the quinoline comes into relatively close contact with the terminal phenyl ring of the side chain. It is possible that electron-withdrawing groups favor intramolecular interactions between the aromatic rings, promoting protonation because of the resulting cation−π interactions. However, without a detailed structural investigation of these intramolecular interactions, this remains speculative, and the observed trend in pK_a values is difficult to rationalize. Given the narrow range of values, we have not attempted to explore this further.

Using eq 1, the predicted accumulation ratio of **4**−**15** in the digestive vacuole of the parasite (VAR) can be calculated.[20]

$$\mathrm{VAR} = \frac{[Q]_v}{[Q]_e} = \frac{1 + 10^{pK_{a1} - pH_v} + 10^{pK_{a1} + pK_{a2} - 2pH_v}}{1 + 10^{pK_{a1} - pH_e} + 10^{pK_{a1} + pK_{a2} - 2pH_e}} \quad (1)$$

Here, $[Q]_v$ is the concentration of the compound in the digestive vacuole of the parasite, $[Q]_e$ is its concentration in the extracellular medium, pH_v is the digestive vacuole pH (taken as 5, the midpoint between two recent estimates of 4.8 and 5.2),[19,21] and pH_e is the pH of the external medium (7.4). Values are given in Table 7. An accumulation-normalized IC_{50} for antimalarial activity can be calculated by multiplying the observed IC_{50} by the VAR value for each compound. Since 4-aminoquinolines are believed to act by inhibiting hemozoin formation in the digestive vacuole, these numbers may be more relevant than the observed IC_{50}. They are, therefore, reported in Table 7.

Correlations among Dibemethin Structure, Biological Activity, and β-Hematin Inhibition. In the case of compounds **4**−**9**, the β-hematin inhibition activity increases markedly in the order of ortho- to meta- to para- derivatives. However, this pattern is reversed in the series **10**−**12**, and no significant differences are seen between compounds **13**−**15**. Thus, the abilities of these compounds to inhibit hemozoin formation appears to depend on both the site of attachment of the aminoquinoline to the dibemethin side chain and the identity of the group on the terminal phenyl ring of the dibemethin, and in a manner that is too complex to determine from the limited number of derivatives used in this study.

Table 3. In Vitro Antimalarial Activities of Compound 6 and CQ against the CQ-sensitive D10 Strain and Three CQ-Resistant Strains of *P. falciparum*[a]

	IC_{50} Dd2 (nM)	RI	IC_{50} W2 (nM)	RI	IC_{50} RSA11(nM)	RI
CQ	150 ± 5[b]	6.5	125 ± 33	5.4	170 ± 17	7.4
6	26 ± 1	1.2	23 ± 2	1.0	38 ± 4	1.7

[a] Mean ± SEM, n = 4. [b] Mean ± SEM, n = 3.

Table 4. In Vitro Cytotoxicity of Selected Compounds in CHO Cells[a]

	IC_{50} D10 (μM)	IC_{50} K1 (μM)	RI	IC_{50} CHO (μM)	SI[b]
5	0.055 ± 0.004	0.065 ± 0.013	1.2	70[c]	1273
6	0.027 ± 0.002	0.025 ± 0.002	0.9	>249	>9222
15	0.047 ± 0.001	0.036 ± 0.002	0.8	>225	>4787
emetine				0.08 ± 0.02	

[a] mean ± SEM, n = 3. [b] IC_{50} (CHO)/IC_{50} (D10). [c] Close to the maximum concentration tested, estimated by interpolation of data points.

Table 5. In Vivo Antimalarial Activity of Selected Compounds in *P. berghei*-Infected Mice[a]

		% parasitized red blood cells[a]						mouse survival/days			
	dose (mg/kg)				av	% of control	% activity				av
4	4 × 100	0.10	0.08	0.10	0.09[b]	0.12	99.9	30[e]	30[e]	30[e]	30.0[e]
	4 × 30	0.11	0.71	0.07	0.30[b]	0.38	99.6	30[e]	16	16	20.7
5	3 × 100	0.09	0.10	0.08	0.09[b]	0.11	99.9	30[e]	30[e]	30[e]	30.0[e]
	3 × 30	0.08	0.10	0.12	0.10[b]	0.13	99.9	16	16	18	16.7
6	1 × 100	0.21	0.10	0.06	0.12[b]	0.16	99.8	10	10	10	10.0
	1 × 30	1.72	1.89	2.88	2.16[c]	2.76	97	7	6	7	6.7
control					78[c,d]						4[f]

[a] The compounds were administered orally. [b] At day 4, each column represents one of three mice used in the experiment. [c] Parasites could not be detected by microscopy. [d] Parasites observed by microscopy. [e] Average of 66.56, 80.24, 70.68, 89.02, and 85.86%. [f] Survived for the full length of the study with no detectable parasitemia at day 30. [g] The parasitemia of all five control mice was measured at day 4, and they were then sacrificed. Control mice in this model die between day 6 and day 7.

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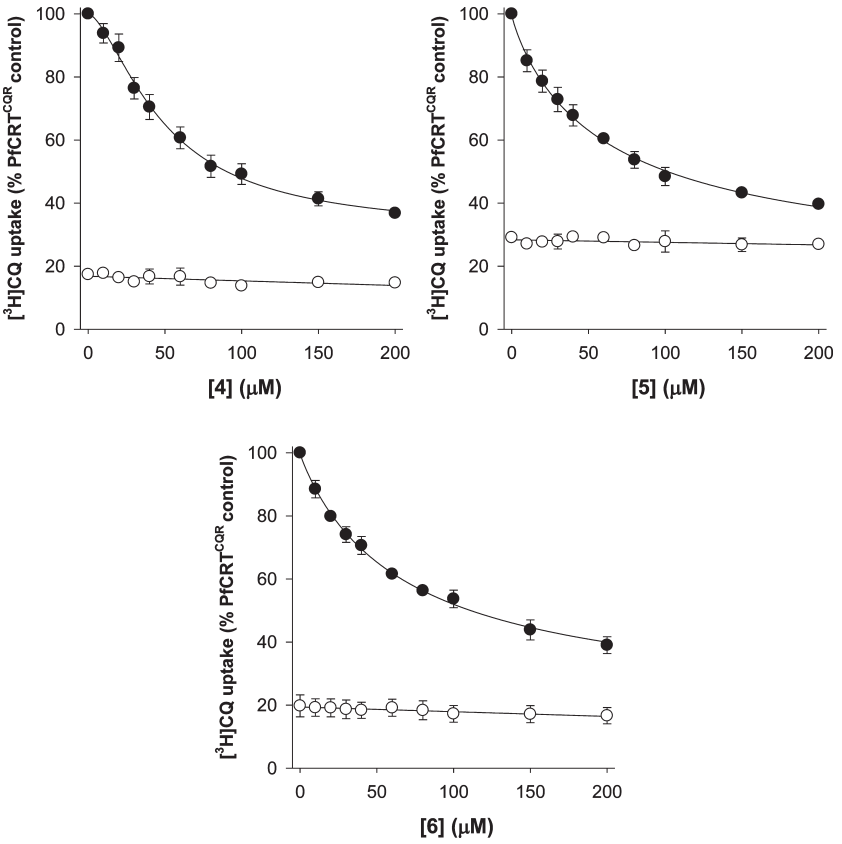

Figure 2. Concentration-dependent effects of **4**, **5**, and **6** on the uptake of [^{3}H]CQ into oocytes expressing Dd2 PfCRT^{CQR} (●) or D10 PfCRT^{CQS} (○). IC_{50} values derived from these data are shown in Table 3. In all panels, uptake is shown as the mean ± SEM from 4−5 separate experiments, within which measurements were made from 10 oocytes per treatment.

Table 6. IC_{50} Values for the Inhibition of PfCRT^{CQR}-Mediated CQ Transport by 4, 5, and 6[a]

compd	IC_{50} (μM)
4	58 ± 6 (n = 4)
5	36 ± 4 (n = 4)
6	69 ± 5 (n = 3)

[a] PfCRT^{CQR}-mediated CQ transport was calculated by subtracting the uptake measured in oocytes expressing PfCRT^{CQS} from that in oocytes expressing PfCRT^{CQR}. The data are shown in Figure 2. IC_{50} values were derived by least-squares fit of the equation $Y = Y_{min} + [(Y_{max} - Y_{min})/(1 + ([inhibitor]/IC_{50})^C]$ where Y is PfCRT^{CQR}-mediated CQ transport, Y_{min} and Y_{max} are the minimum and maximum values of Y, and C is a constant. All values are the mean ± SEM from 4−5 separate experiments, within which measurements were made from 10 oocytes per treatment.

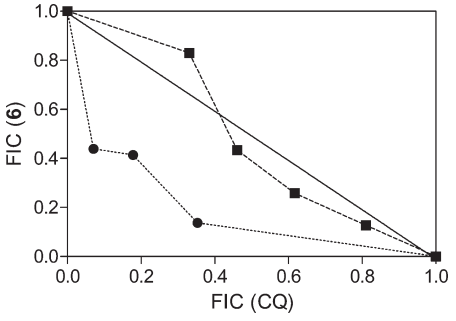

Figure 3. Isobolograms for compound **6** and CQ in the CQ-sensitive D10 (square symbols) and CQ-resistant Dd2 (circular symbols) strains of *P. falciparum*. The straight line represents an additive relationship. Curves for combinations falling below this line are synergistic, whereas those located above the line are antagonistic.

IC_{50} values were not determined for three of the compounds against the D10 strain of parasite, whereas values were obtained for 11 of the 12 compounds against the K1 strain. Hence, we used the K1 IC_{50} data for analyses of structure−activity relationships. Plots of log IC_{50} for in vitro antimalarial activity versus log IC_{50} for inhibition of β-hematin formation (log $BHIA_{50}$) indicate that there was a general decrease in biological activity as the β-hematin inhibition activity decreases (Figure 5a). However, the trend was not statistically significant unless compound **12** was omitted. The correlation was only marginally improved if the antimalarial activity was normalized for accumulation in the digestive vacuole (Figure 5b) and was again only statistically significant if compound **12** was omitted. While this may suggest that either the $BHIA_{50}$ or the IC_{50} value for **12** is an outlier, it is more likely an indication that factors additional to vacuolar accumulation and hemozoin inhibition play a role in biological activity.

In a previous study of short-chain analogues of CQ, vacuolar accumulation and β-hematin inhibition alone correlated with activity.[2] In this study, the structure of the side chain was not kept constant, necessitating the inclusion of a structural descriptor (the position number two, three, and four for ortho-, meta-, and

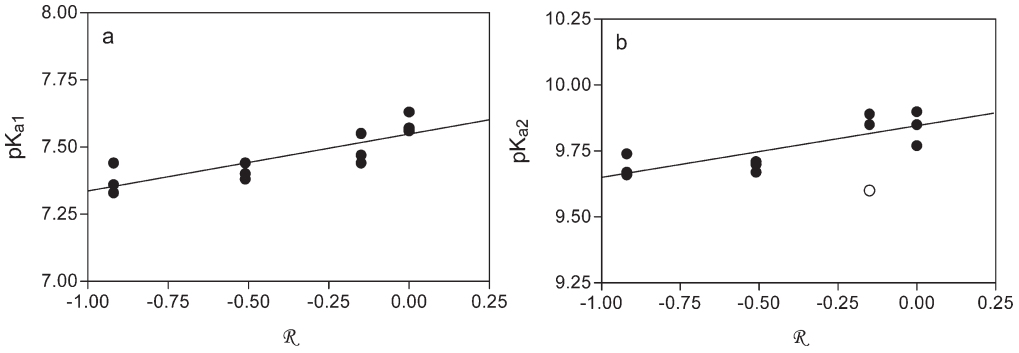

Figure 4. Correlations between pK_a values and resonance constants (R) of the group attached to the terminal phenyl ring (see Table 2). (a) Statistically significant correlation for the quinoline N, pK_{a1} (r^2 = 0.69, P = 0.0008). (b) Correlation for the dibemethin tertiary amino group, pK_{a2}. In this case, the correlation is weaker but is statistically significant if all of the data points are included (r^2 = 0.35, P = 0.042). Omission of a single data point (○) for compound **7** improves the fit considerably (r^2 = 0.65, P = 0.0028).

Table 7. Calculated Vacuolar Accumulation Ratios (VARs) and Accumulation-Normalized IC_{50} Values (VAR·IC_{50}) in the K1 Strain of *P. falciparum*

	VAR[a]	VAR·IC_{50} (mM)
4	15029	2.82
5	15843	1.03
6	14892	0.37
7	13173	14.85
8	14753	1.25
10	13620	1.83
11	12318	1.44
12	13181	1.16
13	12608	1.93
14	12031	3.38
15	11601	0.43

[a] According to eq 1, assuming a vacuolar pH of 5.0.

para- positions, respectively) in the correlation analyses. The log IC_{50} is significantly correlated with position in the order ortho- > meta- > para- (Figure 5c). This correlation improved slightly if the accumulation-normalized IC_{50} values were used (r^2 = 0.55 and P = 0.0087 versus r^2 = 0.52 and P = 0.012 without normalization), and it improved further when the analysis was expanded to include the log $BHIA_{50}$ values (Figure 5d). However, the strongest correlation was achieved when vacuolar accumulation was considered as a third variable in multiple correlation analysis with raw log IC_{50} values (Figure 5e).

The factors affecting in vitro antimalarial activities of this series of compounds are in agreement with our previous study on short chain CQ analogues[2] and with recent studies of reversed-CQ compounds,[22,23] with the additional observation of an independent influence of side-chain structure. The origin of this effect is unknown. It could indicate enhanced uptake of the para-substituted compounds and/or a decrease in the digestive vacuole concentration of ortho-compounds (perhaps as a result of preferential binding to constituents of the cell or culture medium). An alternative explanation is that the ortho-, meta-, or para-substitution pattern of the dibemethin derivatives affects their interaction with $PfCRT^{CQR}$. CQ resistance-reversers are known to exhibit greater intrinsic antiplasmodial activity in parasite strains possessing resistant haplotypes of PfCRT than in those containing the wild-type protein,[24] and this is thought to be because of the ability of the resistance-reverser to inhibit an essential physiological function fulfilled by $PfCRT^{CQR}$.

Cross-Resistance, Cytotoxicity, and Oral Activity. There was no indication of significant cross-resistance of the most active compound in this series (**6**) with CQ in four different strains of the CQ-resistant parasite (K1, Dd2, W2, and RSA11). The lack of cross-resistance with CQ could be the result of the resistance-reversing moiety binding to, and inhibiting, $PfCRT^{CQR}$. Alternatively, the modified quinoline may bind to $PfCRT^{CQR}$ but then fail to undergo translocation because of interference by the altered side chain.

The three most active compounds (**5**, **6**, and **15**) show little or no cytotoxicity in a mammalian cell line (CHO cells), whereas compounds **4**, **5**, and **6** all exhibit oral antimalarial activity in the *P. berghei* mouse model. Although none of the three compounds completely eliminated parasites from the mice after four days, four doses of compound **4** and three doses of **5** (both at 100 mg/kg) appear to have cured the mice since all six mice survived the 30-day duration of the experiment, and no parasites were detected at the end of the experiment. Lower concentrations (30 mg/kg) or a single treatment with **6** either at 100 or 30 mg/kg were much less effective. Lower doses of **4** and **5** reduced the parasite load at day 4 to an extent similar to that of high doses, but mouse survival was reduced from 30 days to between 16−30 days. In this experiment, compound **4** was comparable to CQ, which resulted in an average mouse survival of 29.8 days when administered at 100 mg/kg and 20 days when delivered at 30 mg/kg. These studies demonstrate that the parent compounds are orally active despite not being optimized for oral delivery. Preparation of salts of these compounds and improved formulation would probably increase in vivo activity considerably. Further pharmacological and whole organism toxicity studies will be required to investigate the potential of these compounds for development as antimalarials.

Inhibition of $PfCRT^{CQR}$. Compounds **4**, **5**, and **6** are the first examples of dual action antimalarials, which have been directly demonstrated to inhibit CQ transport via $PfCRT^{CQR}$. It should be noted that the micromolar concentrations of the compounds used here to inhibit $PfCRT^{CQR}$ are physiologically relevant; the dibemethin derivatives are expected to exceed these concentrations within the acidic environment of the parasite's digestive vacuole as a result of weak-base trapping (Table 7). At present, it is not clear whether these and other CQ resistance-reversers inhibit

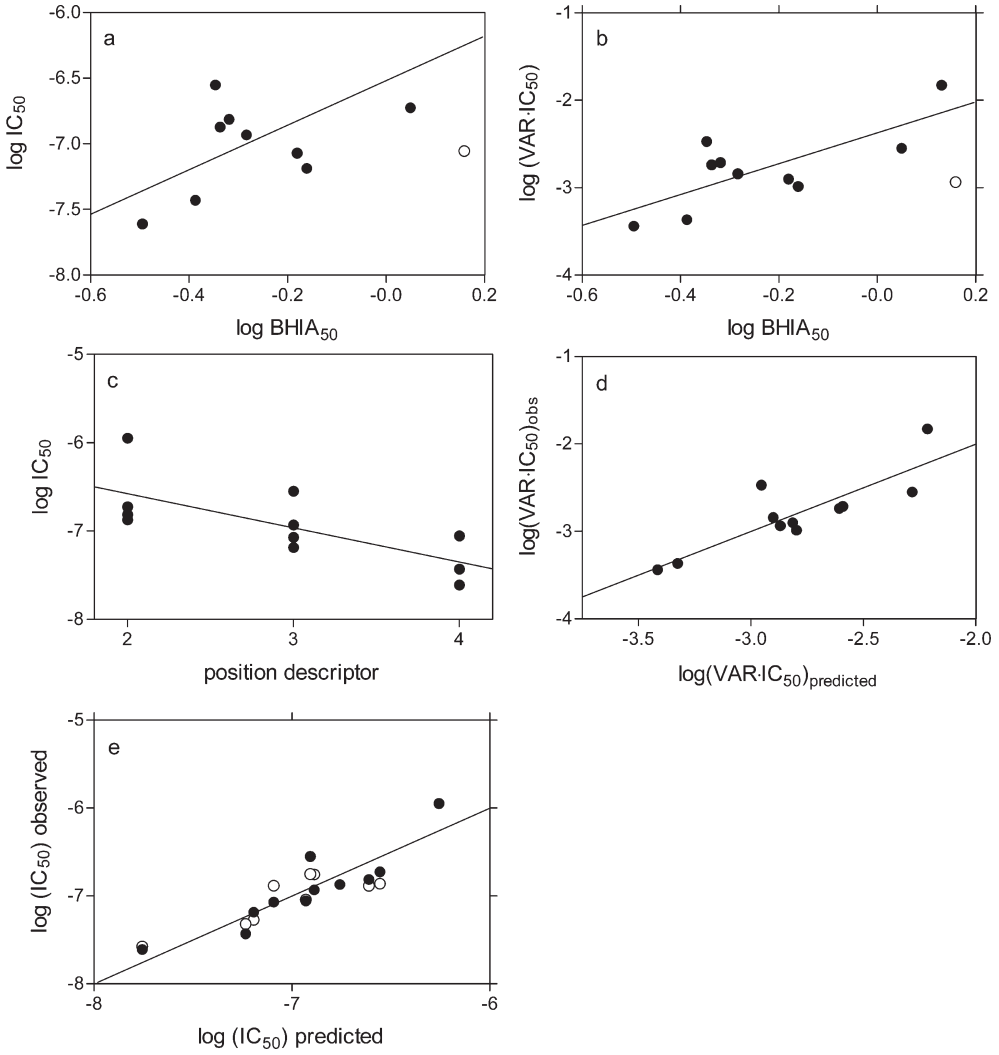

Figure 5. Correlations of biological activity (IC_{50}) with vacuolar accumulation ratio (VAR), IC_{50} for $BHIA_{50}$, and a molecular structure descriptor *pos* (ortho-, meta- or para- indicated by 2, 3, or 4, respectively, and referring to compounds **4**, **7**, **10**, and **13**; **5**, **8**, **11**, and **14**; and **6**, **9**, **12**, and **15**, respectively). (a) Linear correlation between log IC_{50} and log $BHIA_{50}$ is not statistically significant ($r^2 = 0.30$, $P = 0.083$) unless the point for compound **12** is omitted (open circle), which considerably improves the correlation ($r^2 = 0.50$, $P = 0.021$). (b) Linear correlation between the vacuolar accumulation-normalized log IC_{50} (log VAR·IC_{50}) and log $BHIA_{50}$ is also not statistically significant ($r^2 = 0.33$, $P = 0.064$) unless the point for compound **12** is omitted (open circle), which again considerably improves the correlation ($r^2 = 0.51$, $P = 0.012$). (c) Statistically significant linear correlation between log IC_{50} and a structural descriptor for the ortho-, meta-, and para- substituent pattern on the side-chain was observed ($r^2 = 0.52$, $P = 0.012$). (d) Multiple linear correlation between the log of the vacuolar accumulation-normalized IC_{50} (log VAR·IC_{50}) and both log $BHIA_{50}$ and the structural descriptor (*pos*) conform to the equation log VAR·$IC_{50} = 0.84 \times \log BHIA_{50} - 0.34 \times pos - 1.65$. $F = 10.3 > F_{crit} = 8.65$ at the 99% confidence level. (e) Multiple linear correlation of log IC_{50} with VAR, log $BHIA_{50}$, and *pos*. Here, the data conform to the equation $\log IC_{50} = 0.95 \times \log BHIA_{50} - 0.35 \times pos - 3.87 \times \log VAR + 10.24$. $F = 9.70 > 8.45$ at the 95% confidence level. Open circles represent the available IC_{50} values for the D10 strain, which were not used in the correlation.

CQ transport by competing with CQ for translocation via $PfCRT^{CQR}$ or whether the resistance-reversers bind but fail to complete the translocation step. Given the lack of CQ cross-resistance displayed by this series of compounds, if the former applies, the efficiency of transport of compounds **4**, **5**, and **6** via $PfCRT^{CQR}$ must be far lower than that of CQ. Regardless of the mode of inhibition, the fact that these dibemethin derivatives possess the ability to block $PfCRT^{CQR}$ supports the hypothesis that reversed-CQ compounds could combat CQ resistance by inhibiting mutant PfCRT. The observed ability of compound **6** to enhance CQ activity in a CQ-resistant strain, and at physiologically relevant concentrations, further supports this idea.

CONCLUSIONS

The series of novel dibemethin-coupled 4-aminoquinolines described here are structurally simple and achiral. They can be synthesized in a short four-step sequence from relatively cheap starting materials. The final step, which couples the dibemethin side chain to 4-amino-7-chloroquinoline, exhibited relatively low yields and would need to be optimized if these compounds are to be developed further. Several of these compounds (**5**, **6**, and **15**) displayed potent in vitro antimalarial activities against both CQ-sensitive (D10) and CQ-resistant (K1) strains of parasite, with IC_{50} values below 100 nM. None of the tested compounds showed a significant cross-resistance with CQ. The activity of the

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most potent compound (**6**) in four CQ-resistant strains was comparable to that of CQ in the CQ-sensitive strain. The three most active compounds (**5**, **6**, and **15**) exhibited little or no evidence of cytotoxicity, and the three prototype compounds (**4**, **5**, and **6**) all possessed significant oral in vivo activity against mouse malaria.

The activity of this class of compound results from their ability to accumulate in the parasite digestive vacuole and inhibit hemozoin formation, a finding which is consistent with previous structure−activity studies of other CQ analogues. However, a hitherto unknown structural influence of the side chain on activity was uncovered and warrants future investigation. These compounds represent the first examples of reversed-CQ antimalarials that have been directly demonstrated to inhibit both hemozoin formation and the CQ-resistance mechanism. Given this dual-functionality, and their likely increased cost of synthesis, it is conceivable that reversed-CQ compounds like these could be administered in combination with CQ. Such a combination would be expected to have an additive effect in CQS parasites, and a synergistic effect in CQR parasites, providing a cost-effective treatment for drug-resistant malaria.

EXPERIMENTAL SECTION

Chemistry. Solvents, acids, and common salts were obtained from Sarchem, Krugersdorp, South Africa, and 4,7-dichloroquinoline was obtained from Sigma-Aldrich, Vorna Valley, South Africa. The synthesis of the dibemethin side chains has been described elsewhere.[16] Precoated silica gel plates as well as silica and alumina for column chromatography were obtained from Merck, South Africa. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury spectrometer at 300 MHz and a Varian Unity spectrometer at 400 MHz. All spectra were recorded in *d*3-chloroform or *d*4-methanol. Infrared spectra were recorded on a Perkin-Elmer Paragon 1000 FT-IR spectrophotometer in the range 3600−800 cm^{-1}. Mass spectra were recorded on a VG Micromass 16F spectrometer operating at 70 eV with an accelerating voltage of 4 kV and a variable temperature source. Accurate mass determinations were performed on a Kratos Limited MS9/50 spectrometer. All mass spectra were obtained using electron-impact techniques. All compounds for which elemental analyses could not be obtained as well as compounds **5** and **6** were subjected to analytical HPLC to confirm purity using a Spectra Series HPLC with Phenomenex-Luna, 3 m C18 column, and a run time of 13 min (see Supporting Information). Purity exceeded 95% except where otherwise stated. Extensive attempts were made to purify **7**; however, neither crystallization nor chromatography, including preparative thin layer chromatography, could improve the purity of this compound above 86%.

Details for the crystal structure determination can be found in the Supporting Information. CCDC 835831 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

***N*-[{2-(*N*-Benzyl-*N*-methylaminomethyl)phenyl}methyl]-7-chloro-4-quinolinamine (4).** To a stirred solution of **4a** (0.42 g, 1.73 mmol) in anhydrous *N*-methyl-2-pyrrolidone (2 mL) under N_2 were added triethylamine (1.21 mL, 8.67 mmol), K_2CO_3 (0.48 g, 3.47 mmol), and 4,7-dichloroquinoline (1.72 g, 8.67 mmol), and the mixture was heated at 90 °C for 48 h. After the mixture was allowed to cool to room temperature, it was poured into saturated brine (20 mL) and extracted with ethyl acetate (3 × 50 mL). The combined organic extracts were further washed 5 times with saturated brine to ensure the removal of any traces of pyrrolidone before being dried over anhydrous Na_2SO_4 and filtered. Following solvent removal, the resulting crude product was dried under reduced pressure and purified by silica-gel chromatography using mixtures of ethyl acetate/hexane (30:70) to (80:20) as eluent to give **4** as a white crystalline solid (0.26 g, 37%): mp (EtOAc/hexane) 101−103 °C; IR (DMSO) ν_{max} 3583, 3384−3256, 2595, 2140, 2050, 1975, 1659, 1580; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.53 (1H, d, J = 5.1 Hz, H-1), 7.89 (1H, d, J = 2.0 Hz, H-8), 7.49−7.19 (10H, m, Ar H and H-5), 6.85 (1H, dd, J = 2.0, 9.0 Hz, H-6), 6.52 (1H, d, J = 5.1 Hz, H-2), 4.43 (2H, d, J = 5.1 Hz, H-10), 3.61 (2H, s, H-17), 3.58 (2H, s, H-19), 2.14 (3H, s, H-18), 1.27 (1H, s, NH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 152.0 (C-1), 150.2 (Ar_{qu}), 149.3 (Ar_{qu}), 137.4 (Ar_{qu}), 137.2 (Ar_{qu}), 137.1 (Ar_{qu}), 134.5 (Ar_{qu}), 132.0 (C-15), 130.5 (Ar_{C-H}), 129.9 (C-12), 129.9 (Ar_{C-H}),128.4 (C-21/25), 128.4 (C-22/24), 127.9 (C-8), 127.6 (Ar_{C-H}), 124.7 (C-6), 122.2 (C-5), 117.8 (Ar_{qu}), 99.0 (C-2), 61.9 (C-17), 61.0 (C-19), 46.6 (C-10), 41.9 (C-18); Anal. ($C_{25}H_{24}N_3Cl$) C, H, N.

***N*-[{3-(*N*-Benzyl-*N*-methylaminomethyl)phenyl}methyl]-7-chloro-4-quinolinamine (5).** To a stirred solution of **5a** (0.50 g, 2.08 mmol) in anhydrous *N*-methyl-2-pyrrolidone (5 mL) under N_2 were added triethylamine (1.45 mL, 10.4 mmol), K_2CO_3 (0.57 g, 4.16 mmol), and 4,7-dichloroquinoline (2.06 g, 10.4 mmol). The mixture was heated under pressure in a cyclo-addition tube at 130 °C overnight. After the mixture was allowed to cool to room temperature, it was poured into saturated brine (20 mL) and extracted with ethyl acetate (3 × 50 mL). The organic layer was further washed with saturated brine (5 × 50 mL) to ensure the removal of any traces of pyrrolidone. The organic layer was dried (Na_2SO_4) and concentrated in vacuo to afford a crude product, which was purified by column chromatography using mixtures of ethyl acetate/hexane (50:50) to (90:10) as eluent to give **5** as a white solid (0.19 g, 23%): mp (EtOAc/hexane) 103−104 °C; IR (DMSO) ν_{max} 3582, 3395−3279, 2596, 2143, 2060, 1972, 1660, 1579; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.51 (1H, d, J = 5.1 Hz, H-1), 7.98 (1H, d, J = 2.6 Hz, H-8), 7.69 (1H, d, J = 9.0 Hz, H-5), 7.37 (1H, dd, J = 2.6, 9.0 Hz, H-6), 7.34−7.20 (9H, m, ArH), 6.45 (1H, d, J = 5.1 Hz, H-2), 5.43 (1H, br t, J = 5.1 Hz, NH), 4.51 (2H, d, J = 5.1 Hz, H-10), 3.53 (2H, s, H-17), 3.51 (2H, s, H-19), 2.19 (3H, s, N−CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 152.1 (C-1), 149.5 (Ar_{qu}), 149.2 (Ar_{qu}), 140.4 (Ar_{qu}), 139.1 (Ar_{qu}), 137.2 (Ar_{qu}), 134.9 (Ar_{qu}), 128.9 (Ar_{C-H}), 128.8 (Ar_{C-H}), 128.8 (C-21/25), 128.5 (Ar_{C-H}), 128.2 (C-22/24), 127.9 (C-8), 126.9 (Ar_{C-H}), 126.1 (Ar_{C-H}), 125.4 (C-6), 120.9 (C-5), 117.2 (Ar_{qu}), 99.7 (C-2), 61.9 (C-17), 61.6 (C-19), 47.6 (C-10), 42.3 (N−CH_3). Anal. ($C_{25}H_{24}N_3Cl$) C, H. N: calcd, 10.45%; found, 9.87%.

***N*-[{4-(*N*-Benzyl-*N*-methylaminomethyl)phenyl}methyl]-7-chloro-4-quinolinamine (6).** To a stirred solution of **6a** (0.35 g, 1.46 mmol) in anhydrous *N*-methyl-2-pyrrolidone (3.5 mL) under N_2 were added triethylamine (1.02 mL, 7.25 mmol), K_2CO_3 (0.57 g, 4.16 mmol), and 4,7-dichloroquinoline (2.06 g, 10.4 mmol). The mixture was heated under pressure in a cyclo-addition tube at 90 °C for 48 h. After the mixture was allowed to cool to room temperature, it was poured into saturated brine (20 mL) and extracted with ethyl acetate (3 × 50 mL). The organic layer was further washed with saturated brine (5 × 50 mL) to ensure the removal of any traces of pyrrolidone. The organic layer was dried (Na_2SO_4) and concentrated in vacuo, and the resulting crude product was purified by column chromatography using mixtures of ethyl acetate/hexane (50:50) to (90:10) as eluent to give **6** as a colorless solid (0.19 g, 32%): mp (EtOAc/hexane) 120−122 °C; IR (DMSO) ν_{max} 3603, 3394−3225, 2598, 2349, 2140, 2056, 1969, 1903, 1657; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.53 (1H, d, J = 5.1 Hz, H-1), 7.98 (1H, d, J = 1.9 Hz, H-8), 7.68 (1H, d, J = 8.3 Hz, H-5), 7.41−7.22 (9H, m, ArH), 7.28 (1H, dd, J = 1.9, 8.3 Hz, H-6), 6.46 (1H, d, J = 5.1 Hz, H-2), 5.29 (1H, br s, NH), 4.49 (2H, d, J = 5.1 Hz, H-10), 3.53 (2H, s, H-17), 3.51 (2H, s, H-19), 2.19 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 152.1 (C-1), 149.5 (Ar_{qu}), 149.2 (Ar_{qu}), 139.5 (Ar_{qu}), 139.3 (Ar_{qu}), 135.8 (Ar_{qu}), 134.9 (Ar_{qu}), 129.5 (C-21/25), 129.0 (C-23), 128.8 (C-22/24), 128.2 (C-13/15), 127.6 (C-12/16), 127.0 (C-8), 125.5 (C-6), 120.9 (C-5), 117.1 (Ar_{qu}), 99.6 (C-2), 61.9 (C-17), 61.4 (C-19), 47.4 (C-10), 42.3 (C-18). Anal. ($C_{25}H_{24}N_3Cl$) C, H. N: calcd, 10.45%; found, 10.00%.

***N*-[{2-(*N*-*p*-Chlorobenzyl-*N*-methylaminomethyl)phenyl}-methyl]-7-chloro-4-quinolinamine (7).** To a stirred solution of **7a** (0.20 g, 0.73 mmol) in anhydrous *N*-methyl-2-pyrrolidone (2 mL) under N_2 were added triethylamine (0.58 mL, 4.17 mmol), K_2CO_3 (0.23 g, 1.46 mmol), and 4,7-dichloroquinoline (0.82 g, 4.14 mmol). The mixture was heated under pressure in a cyclo-addition tube at 120 °C overnight. After the mixture was allowed to cool to room temperature, it was poured into saturated brine (20 mL) and extracted with ethyl acetate (3 × 50 mL). The organic layer was further washed with saturated brine (5 × 50 mL) to ensure the removal of any traces of pyrrolidone. The organic layer was dried (Na_2SO_4) and concentrated in vacuo to afford a crude product, which was purified by column chromatography using mixtures of ethyl acetate/hexane (50:50) to (80:10) as eluent to give **7** as a yellow oil (98 mg, 31%): IR (DMSO) ν_{max} (cm^{-1}) 3595, 3378−3231, 2142, 2067, 1975, 1657; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.53 (1H, d, J = 5.4 Hz, H-1), 7.91 (1H, d, J = 2.4 Hz, H-8), 7.41−7.12 (8H, m, ArH), 7.26 (1H, d, J = 9.0 Hz, H-5), 6.98 (1H, dd, J = 2.4, 9.0 Hz, H-6), 6.52 (1H, d, J = 5.4 Hz, H-2), 5.29 (1H, s, NH), 4.44 (2H, d, J = 3.0 Hz, H-10), 3.60 (2H, s, H-17), 3.54 (2H, s, H-19), 2.16 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 151.9 (C-1), 150.1 (Ar_{qu}), 149.1 (Ar_{qu}), 137.1 (Ar_{qu}), 136.9 (Ar_{qu}), 135.9 (Ar_{qu}), 134.8 (Ar_{qu}), 133.5 (Ar_{qu}), 132.0 (Ar_{C-H}), 131.1 (C-21/25), 130.5 (Ar_{C-H}), 128.6 (C-22/24), 128.5 (Ar_{C-H}), 128.4 (Ar_{C-H}), 128.0 (C-8), 124.9 (C-6), 121.8 (C-5), 118.0 (Ar_{qu}), 99.1 (C-2), 61.4 (C-19), 60.8 (C-17), 46.5 (C-10), 42.0 (C-18). HRMS (ESI): found, 436.1344; $C_{25}H_{23}N_3Cl_2$ $(M + H)^+$ requires 436.1342. HPLC, 86.1%.

***N*-[{3-(*N*-*p*-Chlorobenzyl-*N*-methylaminomethyl)phenyl}-methyl]-7-chloro-4-quinolinamine (8).** To a stirred solution of **8a** (0.45 g, 1.64 mmol) in anhydrous *N*-methyl-2-pyrrolidone (4 mL) under N_2 were added triethylamine (1.16 mL, 8.32 mmol), K_2CO_3 (0.46 g, 3.33 mmol), and 4,7-dichloroquinoline (1.65 g, 8.32 mmol). The mixture was heated under pressure in a cyclo-addition tube at 120 °C overnight. After the mixture was allowed to cool to room temperature, it was poured into saturated brine (20 mL) and extracted with ethyl acetate (3 × 50 mL). The organic layer was further washed with saturated brine (5 × 50 mL) to ensure the removal of any traces of pyrrolidone. The organic layer was dried (Na_2SO_4) and concentrated in vacuo to afford a crude product, which was purified by column chromatography using mixtures of ethyl acetate/hexane (50:50) to (90:10) as eluent to give **8** as a white solid (0.19 g, 27%): mp (DCM/hexane) 103−104 °C; IR (DMSO) ν_{max} (cm^{-1}) 3620, 3372−3193, 2594, 2345, 2150, 2054, 1971, 1911, 1676, 1626; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.48 (1H, d, J = 5.3 Hz, H-1), 7.97 (1H, d, J = 2.4 Hz, H-8), 7.16 (1H, d, J = 8.7 Hz, H-5), 7.37−7.23 (9H, m, H-6 and ArH), 6.42 (1H, d, J = 5.3 Hz, H-2), 5.62 (1H, br s, NH), 4.51 (2H, d, J = 4.8 Hz, H-10), 3.50 (2H, s, H-17), 3.45 (2H, s, H-19), 2.15 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 151.5 (C-1), 149.8 (Ar_{qu}), 149.8 (Ar_{qu}), 140.1 (Ar_{qu}), 137.6 (Ar_{qu}), 137.2 (Ar_{qu}), 135.1 (Ar_{qu}), 132.6 (Ar_{qu}), 130.0 (C-21/25), 128.9 (C-8), 128.4 (Ar_{C-H}), 128.4 (Ar_{C-H}), 128.3 (C-22/24), 127.8 (Ar_{C-H}), 126.2 (Ar_{C-H}), 125.5 (C-6), 121.2 (C-5), 117.1 (Ar_{qu}), 99.6 (C-2), 61.6 (C-17), 61.0 (C-19), 47.5 (C-10), 42.2 (C-18). Found, 436.1335; $C_{25}H_{23}N_3Cl_2$ $(M + H)^+$ requires 436.1342.

***N*-[{4-(*N*-*p*-Chlorobenzyl-*N*-methylaminomethyl)phenyl}-methyl]-7-chloro-4-quinolinamine (9).** To a stirred solution of **9a** (0.57 g, 2.08 mmol) in anhydrous *N*-methyl-2-pyrrolidone (6 mL)) under N_2 were added triethylamine (1.45 mL, 10.4 mmol), K_2CO_3 (0.57 g, 4.16 mmol), and 4,7-dichloroquinoline (2.06 g, 10.40 mmol). The mixture was heated under pressure in a cyclo-addition tube at 120 °C overnight. After the mixture was allowed to cool to room temperature, it was poured into saturated brine (20 mL) and extracted with ethyl acetate (3 × 50 mL). The organic layer was further washed with saturated brine (5 × 50 mL) to ensure the removal of any traces of pyrrolidone. The organic layer was dried (Na_2SO_4) and concentrated in vacuo, and the resulting crude product was purified by column chromatography using mixtures of ethyl acetate/hexane (50:50) to (90:10) as eluent to give **9** as a colorless crystalline solid (0.19 g, 21%): mp (DCM/hexane) 101−104 °C; IR (DMSO) ν_{max} (cm^{-1}) 3607, 3362−3195, 2344, 2146, 2059, 1947, 1905, 1677, 1627; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.47 (1H, d, J = 5.4 Hz, H-1), 7.95 (1H, d, J = 1.8 Hz, H-8), 7.37−7.25 (1H, d, J = 9.0 Hz, H-5), 7.32 (9H, m, H-6 and ArH), 6.40 (1H, d, J = 5.4 Hz, H-2), 5.73 (1H, br s, NH), 4.48 (2H, d, J = 3.9 Hz, H-10), 3.50 (2H, s, H-17), 3.47 (2H, s, H-19), 2.17 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 151.6 (C-1), 149.8 (Ar_{qu}), 149.8 (Ar_{qu}), 139.0 (Ar_{qu}), 137.8 (Ar_{qu}), 135.8 (Ar_{qu}), 135.0 (Ar_{qu}), 130.1 (C-21/25), 129.4 (C-22/24), 128.4 (Ar_{qu}), 128.3 (C-13/15), 127.5 (C-8), 127.5 (C-12/16), 125.4 (C-6), 121.2 (C-5), 117.1 (Ar_{qu}), 99.5 (C-2), 61.4 (C-17), 61.0 (C-19), 47.3 (C-10), 42.2 (C-18). Found, 436.1335; $C_{25}H_{23}N_3Cl_2$ $(M + H)^+$ requires 436.1342.

7-Chloro-*N*-[{2-(*N*-*p*-methoxybenzyl-*N*-methylamino-methyl)phenyl}methyl]-4-quinolinamine (10). To a stirred solution of **10a** (0.35 g, 1.30 mmol) in anhydrous *N*-methyl-2-pyrrolidone (3.50 mL) under N_2 were added triethylamine (0.91 mL, 6.50 mmol), K_2CO_3 (0.54 g, 3.90 mmol), and 4,7-dichloroquinoline (2.06 g, 10.4 mmol). The mixture was heated under pressure in a cyclo-addition tube at 120 °C overnight. After the mixture was allowed to cool to room temperature, it was poured into saturated brine (20 mL) and extracted with ethyl acetate (3 × 50 mL). The organic layer was further washed with saturated brine (5 × 50 mL) to ensure the removal of any traces of pyrrolidone. The organic layer was dried (Na_2SO_4) and concentrated in vacuo, and the resulting crude product was purified by column chromatography using mixtures of ethyl acetate/hexane (50:50) to (100:0) as eluent to give **10** as a dark-yellow oil (0.12 g, 21%): IR (DMSO) ν_{max} (cm^{-1}) 3618, 3376-3177, 2633, 2595, 2548, 2149, 2059, 1972, 1903, 1681; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.53 (1H, d, J = 5.4 Hz, H-1), 7.91 (1H, d, J = 2.3 Hz, H-8), 7.62 (1H, s, NH), 7.34 − 7.31 (4H, m, ArH), 7.27 (1H, d, J = 9.0 Hz, H-5), 7.09 (2H, d, J = 8.9 Hz, H-21/25), 6.92 (1H, dd, J = 2.3, 9.0 Hz, H-6), 6.75 (2H, d, J = 8.9 Hz, H-22/24), 6.53 (1H, d, J = 5.4 Hz, H-2), 4.42 (2H, s, H-10), 3.77 (3H, s, OMe), 3.61 (2H, s, H-17), 3.53 (2H, s, H-19), 2.16 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 159.1 (Ar_{qu}), 151.7 (C-1), 150.3 (Ar_{qu}), 149.0 (Ar_{qu}), 137.2 (Ar_{qu}), 137.1 (Ar_{C-H}), 134.6 (Ar_{qu}), 132.0 (Ar_{C-H}), 131.0 (C-21/25), 130.5 (Ar_{qu}), 129.3 (Ar_{qu}), 128.3 (Ar_{C-H}), 128.1 (Ar_{C-H}), 127.9 (C-8), 124.7 (C-6), 122.3 (C-5), 117.8 (Ar_{qu}), 113.8 (C-22/24), 99.0 (C-2), 61.5 (C-17), 60.9 (C-19), 55.2 (OMe), 46.5 (C-10), 41.7 (C-18). HRMS (ESI): found, 432.1860; $C_{26}H_{26}N_3O$ Cl $(M + H)^+$ requires 432.1877.

7-Chloro-*N*-[{3-(*N*-*p*-methoxybenzyl-*N*-methylaminome-thyl)phenyl}methyl]-4-quinolinamine (11). To a stirred solution of **11a** (0.40 g, 1.48 mmol) in anhydrous *N*-methyl-2-pyrrolidone (4.00 mL) under N_2 were added triethylamine (1.04 mL, 7.40 mmol), K_2CO_3 (0.61 g, 4.44 mmol), and 4,7-dichloroquinoline (1.47 g, 7.40 mmol). The mixture was heated under pressure in a cyclo-addition tube at 120 °C overnight. After the mixture was allowed to cool to room temperature, it was poured into saturated brine (20 mL) and extracted with ethyl acetate (3 × 50 mL). The organic layer was further washed with saturated brine (5 × 50 mL) to ensure the removal of any traces of pyrrolidone. The organic layer was dried (Na_2SO_4) and concentrated in vacuo, and the resulting crude product was purified by column chromatography using mixtures of ethyl acetate/hexane (50:50) to (90:10) as eluent to give **11** as a light-brown oil (0.13 g, 20%): IR (DMSO) ν_{max} (cm^{-1}) 3600, 3363−3169, 2599, 2350, 2148, 2059, 1973, 1907, 1679, 1618; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.51 (1H, d, J = 5.4 Hz, H-1), 7.98 (1H, d, J = 1.8 Hz, H-8), 7.70 (1H, d, J = 9.0 Hz, H-5), 7.40−7.24 (4H, m, ArH), 7.36 (1H, dd, J = 2.1, 9.0 Hz, H-6), 7.21 (2H, d, J = 8.7 Hz, H-21/25), 6.80 (2H, d, J = 8.7 Hz, H-22/24), 6.45 (1H, d, J = 5.4 Hz, H-2), 5.44 (1H, br s, NH), 4.51 (2H, d, J = 5.4 Hz, H-10), 3.78 (3H, s, OMe), 3.50 (2H, s, H-17), 3.45 (2H, s, H-19), 2.17 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 158.6 (Ar_{qu}), 152.0 (C-1), 149.6 (Ar_{qu}),

149.1 (Ar_{qu}), 140.4 (Ar_{qu}), 137.2 (Ar_{qu}), 134.9 (Ar_{qu}), 131.0 (Ar_{qu}), 130.0 (C-22/24), 128.8 (C-8), 128.7 (Ar_{C-H}), 128.5 (Ar_{C-H}), 128.0 (Ar_{C-H}), 126.1 (Ar_{C-H}), 125.4 (C-6), 121.1 (C-5), 117.2 (Ar_{qu}), 113.6 (C-21/25), 99.6 (C-2), 61.4 (C-17), 61.2 (C-19), 55.2 (OMe), 47.5 (C-10), 42.1 (C-18). HRMS (ESI): found 432.1823; $C_{26}H_{26}N_3OCl$ $(M + H)^+$ requires 432.1877.

7-Chloro-*N*-[{4-(*N*-*p*-Methoxybenzyl-*N*-methylaminomethyl)phenyl}methyl]-4-quinolinamine (12). To a stirred solution of **12a** (0.50 g, 1.85 mmol) in anhydrous *N*-methyl-2-pyrrolidone (5.00 mL) under N_2 were added triethylamine (1.30 mL, 9.25 mmol), K_2CO_3 (0.77 g, 5.55 mmol), and 4,7-dichloroquinoline (1.84 g, 9.25 mmol). The mixture was heated under pressure in a cyclo-addition tube at 120 °C overnight. After the mixture was allowed to cool to room temperature, it was poured into saturated brine (20 mL) and extracted with ethyl acetate (3 × 50 mL). The organic layer was further washed with saturated brine (5 × 50 mL) to ensure the removal of any traces of pyrrolidone. The organic layer was dried (Na_2SO_4) and concentrated in vacuo, and the resulting crude product was purified by column chromatography using mixtures of ethyl acetate/hexane (50:50) to (100:0) as eluent to give **12** as a dark-yellow oil (0.19 g, 24%): IR (DMSO) ν_{max} (cm^{-1}) 3589, 3365−3255, 2148, 2059, 1977, 1910, 1661; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.46 (1H, d, J = 5.7 Hz, H-1), 7.94 (1H, d, J = 2.1 Hz, H-8), 7.69 (1H, d, J = 9.0 Hz, H-5), 7.38−7.25 (7H, m, H-6 and ArH), 6.86 (2H, d, J = 8.7 Hz, H-22/24), 6.40 (1H, d, J = 5.7 Hz, H-2), 5.57 (1H, br s, NH), 4.49 (2H, d, J = 4.8 Hz, H-10), 3.76 (3H, s, OMe), 3.47 (2H, s, H-17), 3.44 (2H, s, H-19), 2.15 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 158.7 (Ar_{qu}), 151.5 (C-1), 149.9 (Ar_{qu}), 149.9 or 148.4 (Ar_{qu}), 139.4 (Ar_{qu}), 135.7 (Ar_{qu}), 135.2 (Ar_{qu}), 131.1 (Ar_{qu}), 130.1 (C-22/24), 129.6 (C-13/15), 128.3 (C-8), 127.6 (C-12/16), 125.6 (C-6), 121.2 (C-5), 117.1 (Ar_{qu}), 113.7 (C-21/25), 99.5 (C-2), 61.2 (C-17), 61.2 (C-19), 55.3 (OMe), 47.4 (C-10), 42.1 (C-18). HRMS (ESI): found, 432.1835; $C_{26}H_{26}N_3OCl$ $(M + H)^+$ requires 432.1877.

7-Chloro-*N*-[{2-(*N*-*p*-dimethylaminobenzyl-*N*-methylaminomethyl)phenyl}methyl]-4-quinolinamine (13). To a stirred solution of **13a** (0.40 g, 1.41 mmol) in anhydrous *N*-methyl-2-pyrrolidone (4.00 mL) under N_2 were added triethylamine (1.00 mL, 7.19 mmol), K_2CO_3 (0.58 g, 4.23 mmol), and 4,7-dichloroquinoline (1.39 g, 7.04 mmol). The mixture was heated under pressure in a cyclo-addition tube at 120 °C overnight. After the mixture was allowed to cool to room temperature, it was poured into saturated brine (20 mL) and extracted with ethyl acetate (3 × 50 mL). The organic layer was further washed with saturated brine (5 × 50 mL) to ensure the removal of any traces of pyrrolidone. The organic layer was dried (Na_2SO_4) and concentrated in vacuo, and the resulting crude product was purified by column chromatography using mixtures of hexane/ethyl acetate/methanol (50:50:0) to (0:90:10) as eluent to give **13** as a dark-yellow oil (112 mg, 18%): IR (DMSO) ν_{max} (cm^{-1}) 3633, 3360−3104, 2706, 2641, 2598, 2342, 2147, 2056, 1970, 1901; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.54 (1H, d, J = 5.4 Hz, H-1), 7.89 (1H, d, J = 2.1 Hz, H-8), 7.79 (1H, br s, NH), 7.40−7.10 (4H, m, ArH), 7.04 (2H, d, J = 8.6 Hz, H-21/25), 6.89 (1H, d, J = 2.4 Hz, H-5), 6.86 (1H, d, J = 2.1 Hz, H-6), 6.57 (2H, d, J = 8.6 Hz, H-22/24), 6.54 (1H, d, J = 5.4 Hz, H-2), 4.41 (2H, s, H-10), 3.61 (2H, s, H-17), 3.50 (2H, s, H-19), 2.92 (6H, s, NMe_2), 2.14 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 152.0 (C-1), 150.3 (Ar_{qu}), 150.0 (Ar_{qu}), 149.2 (Ar_{qu}), 137.4 (Ar_{qu}), 137.4 (Ar_{qu}), 134.4 (Ar_{qu}), 132.0 (Ar_{C-H}), 130.8 (C-21/25), 130.5 (Ar_{C-H}), 128.2 (C-8), 128.0 (Ar_{C-H}), 127.8 (Ar_{C-H}), 124.8 (Ar_{qu}), 124.7 (C-6), 122.6 (C-5), 117.9 (Ar_{qu}), 112.3 (C-22/24), 98.8 (C-2), 61.6 (C-19), 61.0 (C-17), 46.5 (C-10), 41.5 (C-18), 40.4 (NMe_2). HRMS (ESI): found, 445.2172; $C_{27}H_{29}N_4Cl$ $(M + H)^+$ requires 445.2159. HPLC, 93.6%.

7-Chloro-*N*-[{3-(*N*-*p*-dimethylaminobenzyl-*N*-methylaminomethyl)phenyl}methyl]-4-quinolinamine (14). To a stirred solution of **14a** (0.40 g, 1.41 mmol) in anhydrous *N*-methyl-2-pyrrolidone (4.00 mL) under N_2 were added triethylamine (1.00 mL, 7.19 mmol), K_2CO_3 (0.58 g, 4.23 mmol), and 4,7-dichloroquinoline (1.39 g, 7.04 mmol). The mixture was heated under pressure in a cyclo-addition tube at 120 °C overnight. After the mixture was allowed to cool to room temperature, it was poured into saturated brine (20 mL) and extracted with ethyl acetate (3 × 50 mL). The organic layer was further washed with saturated brine (5 × 50 mL) to ensure the removal of any traces of pyrrolidone. The organic layer was dried (Na_2SO_4) and concentrated in vacuo, and the resulting crude product was purified by column chromatography using mixtures of hexane/ethyl acetate/methanol (50:50:0) to (0:90:10) as eluent to give **14** as a dark-yellow oil (0.14 g, 22%): IR (DMSO) ν_{max} (cm^{-1}) 3363−3169, 2599, 2350, 2148, 2059, 1973, 1907, 1679, 1618; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.47 (1H, d, J = 5.2 Hz, H-1), 7.95 (1H, d, J = 2.0 Hz, H-8), 7.70 (1H, d, J = 8.8 Hz, H-5), 7.38−7.21 (4H, m, ArH), 7.31 (1H, dd, J = 2.0, 8.8 Hz, H-6), 7.13 (2H, d, J = 8.6 Hz, H-21/25), 6.61 (2H, d, J = 8.6 Hz, H-22/24), 6.41 (1H, d, J = 5.2 Hz, H-2), 5.58 (1H, br s, NH), 4.47 (2H, d, J = 4.8 Hz, H-10), 3.47 (2H, s, H-17), 3.40 (2H, s, H-19), 2.88 (6H, s, NMe_2), 2.15 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 152.4 (Ar_{qu}), 152.0 (C-1), 149.9 (Ar_{qu}), 149.6 (Ar_{qu}), 140.7 (Ar_{qu}), 137.2 (Ar_{qu}), 135.0 (Ar_{qu}), 130.1 (Ar_{qu}), 129.8 (C-21/25), 128.8 (C-8), 128.8 (Ar_{C-H}), 128.6 (Ar_{C-H}), 128.1 (Ar_{C-H}), 126.1 (Ar_{C-H}), 125.5 (C-6), 121.0 (C-5), 117.2 (Ar_{qu}), 112.5 (C-22/24), 99.7 (C-2), 61.3 (C-17), 61.3 (C-19), 47.7 (C-10), 42.2 (C-19), 40.7 (NMe_2). HRMS (ESI): found, 445.2138; $C_{27}H_{29}N_4Cl$ $(M + H)^+$ requires 445.2159.

7-Chloro-*N*-[{4-(*N*-*p*-dimethylaminobenzyl-*N*-methylaminomethyl)phenyl}methyl]-4-quinolinamine (15). To a stirred solution of **15a** (0.40 g, 1.41 mmol) in anhydrous *N*-methyl-2-pyrrolidone (4.00 mL) under N_2 were added triethylamine (1.00 mL, 7.19 mmol), K_2CO_3 (0.58 g, 4.23 mmol), and 4,7-dichloroquinoline (1.39 g, 7.04 mmol). The mixture was heated under pressure in a cyclo-addition tube at 120 °C overnight. After the mixture was allowed to cool to room temperature, it was poured into saturated brine (20 mL) and extracted with ethyl acetate (3 × 50 mL). The organic layer was further washed with saturated brine (5 × 50 mL) to ensure the removal of any traces of pyrrolidone. The organic layer was dried (Na_2SO_4) and concentrated in vacuo, and the resulting crude product was purified by column chromatography using mixtures of hexane/ethyl acetate/methanol (50:50:0) to (0:90:10) as eluent to give **15** as a dark-yellow oil (0.16 g, 26%): IR (DMSO) ν_{max} (cm^{-1}) 3616, 3378−3212, 2596, 2150, 2062, 1977, 1904, 1674, 1635; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.43 (1H, d, J = 5.4 Hz, H-1), 7.92 (1H, d, J = 2.0 Hz, H-8), 7.70 (1H, d, J = 9.2 Hz, H-5), 7.34−7.28 (4H, m, ArH), 7.26 (1H, dd, J = 2.4, 6.4 Hz, H-6), 7.19 (2H, d, J = 8.4 Hz, H-21/25), 6.68 (2H, d, J = 8.4 Hz, H-22/24), 6.37 (1H, d, J = 5.4 Hz, H-2), 5.82 (1H, br s, NH), 4.43 (2H, d, J = 4.8 Hz, H-10), 3.46 (2H, s, H-17), 3.42 (2H, s, H-19), 2.89 (6H, s, NMe_2), 2.15 (3H, s, $N-CH_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 151.7 (C-1), 149.7 (Ar_{qu}), 149.7 (Ar_{qu}), 148.8 (Ar_{qu}), 139.2 (Ar_{qu}), 135.6 (Ar_{qu}), 134.8 (Ar_{qu}), 129.7 (C-13/15), 129.3 (C-21/25), 128.3 (C-8), 127.3 (C-12/16), 126.7 (C-20), 125.2 (C-6), 121.3 (C-5), 117.1 (Ar_{qu}), 112.4 (C-22/24), 99.5 (C-2), 61.2 (C-17 or 19), 61.0 (C-19 or 17), 47.1 (C-10), 42.0 ($N-CH_3$), 40.6 (NMe_2). HRMS (ESI): found, 445.2171; $C_{27}H_{29}N_4Cl$ $(M + H)^+$ requires 445.2159.

Biological Testing. *In Vitro Antimalarial Testing.* Continuous in vitro cultures of asexual erythrocyte stages of *P. falciparum* were maintained using a modified method of Trager and Jensen.[25] A quantitative assessment of antimalarial activity in vitro was determined via the parasite lactate dehydrogenase assay using a modified method described by Makler.[26]

The samples were prepared to a 2 mg/mL stock solution in 100% DMSO or 100% methanol and sonicated to enhance solubility. Samples were tested as a suspension if not completely dissolved. Stock solutions were stored at −20 °C. Further dilutions were prepared on the day of the experiment. CQ was used as the reference drug in all experiments. A full dose−response was performed for all compounds to determine the

concentration inhibiting 50% of parasite growth (IC_{50} value). Test samples were tested at a starting concentration of 100 ng/mL or 10 μg/mL, which was then serially diluted 2-fold in complete medium to give 10 concentrations. The same dilution technique was used for all samples. CQ was tested at a starting concentration of 1.15 μg/mL. The highest concentration of solvent to which the parasites were exposed had no measurable effect on the parasite viability (data not shown). The IC_{50} values were obtained using a nonlinear dose−response curve fitting analysis via Graph Pad Prism v.3.0 software.[27]

For the construction of the isobolograms, mixtures consisting of 0, 20, 40, 60, 80, and 100% by mass CQ were prepared with compound **6**. The IC_{50} values were determined in the D10 and Dd2 strains by the same method used for pure substances described above. Values were converted from units of ng/mL to nanomolar based on the masses and corresponding molecular masses of each component in the mixture. Fractional IC_{50} values (FICs) were then calculated for CQ and **6** for each mixture (data are presented in Supporting Information) and used to plot the isobolograms.

Cytotoxicity Testing. The MTT assay was used as a colorimetric assay for cellular growth and survival and compares well with other available assays.[28] The tetrazolium salt MTT was used to measure all growth and chemosensitivity. Compound **4** was tested in triplicate on three separate occasions.

The compound was dissolved in 10% DMSO. The initial concentrations of stock solutions were 2 mg/mL. The compound was tested as a suspension and stored at −20 °C until use. The highest concentration of solvent to which the cells were exposed had no measurable effect on the cell viability (data not shown). Emetine was used as the reference drug in all experiments. The initial concentration of emetine was 100 μg/mL, which was serially diluted in complete medium with 10-fold dilutions to give 6 concentrations, the lowest being 0.001 μg/mL. The same dilution technique was applied to the test sample with an initial concentration of 100 μg/mL to give 5 concentrations, with the lowest concentration being 0.01 μg/mL.

The 50% inhibitory concentration (IC_{50}) values for these samples were obtained from dose−response curves, using a nonlinear dose−response curve fitting analysis via GraphPad Prism v.3.0 software.[27]

In Vivo Antimalarial Testing. Compounds **4**, **5**, and **6** were sent to the Parasite Chemotherapy Unit of the Swiss Tropical Institute for testing on *P. berghei* infected female NMRI mice using a modified Peters four-day test.[29] Heparinized blood was taken from a donor mouse with approximately 30% parasitemia and diluted in physiological saline to 108 parasitized erythrocytes per milliliter. Of this suspension, 0.2 mL was injected intravenously (i.v.) into experimental groups of 3 mice and a control group of 5 mice. Four hours postinfection the experimental groups were treated with a single oral dose; the experimental groups were treated with a further single daily dose of **4** and **5** at 24 and 48 h postinfection and with a dose of **4** at 72 h postinfection (owing to problems handling **5** and **6** resulting from the hygroscopic nature of these compounds, insufficient material was available for a fourth treatment with **5** and for any additional tests with **6**). One microliter of tail blood was taken 24 h after the last drug treatment, and the parasitemia was determined with a FACScan. The difference between the mean value of the control group and those of the experimental groups was calculated and expressed as a percent relative to the control group (=activity). The survival of the animals was monitored up to 30 days. Mice surviving for 30 days were checked for parasitemia. A compound was considered curative if the animal survived to day 30 postinfection with no detectable parasites. The results were expressed as (1) the reduction of parasitemia on day 4 in % as compared to the untreated control group and (2) mean survival time.

Measurements of CQ Transport in X. laevis Oocytes Expressing PfCRT. Expression of mutant and wild-type forms of PfCRT (from the strains Dd2 and D10, respectively) at the plasma membrane of *X. laevis* oocytes was performed as described elsewhere.[3] Briefly, oocytes were injected with cRNA encoding PfCRT (30 ng per oocyte), and the uptake of [^{3}H]CQ (0.15 μM; 15 Ci/mmol) was measured 4−6 days postinjection using a method described previously.[30] The influx measurements were made over 1−2 h at 27.5 °C and in medium that contained 96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, 10 mM MES, 10 mM Tris-base (pH 6.0), and 15 μM unlabeled CQ. Statistical comparisons were made with the Student's *t*-test for paired or unpaired samples or with ANOVA in conjunction with Tukey's multiple comparisons test.

Physico-Chemical Measurements. β-Hematin inhibition was measured using a method described in detail elsewhere without modification.[18]

■ ASSOCIATED CONTENT

Supporting Information. Crystal structure data including a packing diagram, tables of bond lengths, angles, and torsion angles for compound **4**; details of data used to construct isobolograms; HPLC traces and details. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ DISCLOSURE

Any opinion, findings and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the funding agencies involved.

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■ ABBREVIATIONS USED:

$BHIA_{50}$, 50% β-hematin inhibitory activity; CHO, Chinese hamster ovarian; CQ, chloroquine; dibemethin, *N,N*-dibenzylmethylamine; PfCRT, *Plasmodium falciparum* chloroquine-resistance transporter; $PfCRT^{CQR}$, chloroquine-resistant form of PfCRT; $PfCRT^{CQS}$, chloroquine-sensitive form of PfCRT; RI, resistance index; SI, selectivity index; VAR, vacuolar accumulation ratio

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