

Expedited Articles

New 4-Aminoquinoline Mannich Base Antimalarials. 1. Effect of an Alkyl Substituent in the 5'-Position of the 4'-Hydroxyanilino Side Chain

Kaylene J. Raynes, Paul A. Stocks, Paul M. O'Neill,[‡] B. Kevin Park, and Stephen A. Ward*

Departments of Pharmacology and Therapeutics and of Chemistry, The University of Liverpool, P.O. Box 147, Liverpool L69 3GE, U.K.

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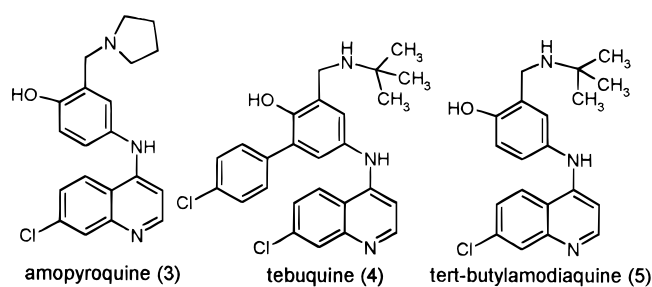
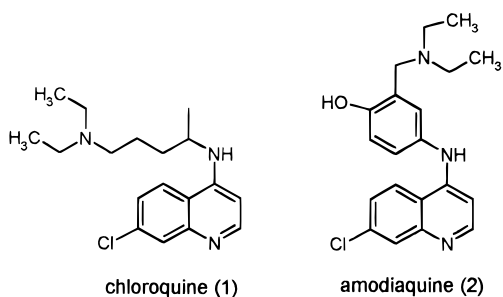
A new series of 4-aminoquinoline Mannich base derivatives have been synthesized, in which the 3'-diethylamino function of amodiaquine (AQ) is replaced by a 3'-*tert*-butylamino group and an aliphatic hydrocarbon entity is incorporated into the 5'-position of the 4'-hydroxyanilino side chain. Seven alkyl Mannich base derivatives were screened and found to be active against both chloroquine-sensitive and -resistant strains of *Plasmodium falciparum* in vitro. The propyl and isopropyl alkyl derivatives were found to be the most active; consequently these derivatives were tested against a nonsensitive strain of *Plasmodium berghi* in vivo and found to be 3-fold more active than AQ, irrespective of the route of administration (oral or intraperitoneal).

Introduction

Events of the last 40 years have seen the evolution of multidrug-resistant strains of the malaria parasite (*Plasmodia*).¹ The efficacy of chloroquine (**1**, CQ), a 4-aminoquinoline derivative, has rapidly decreased such that the drug is now virtually useless in many malaria-

cytosis and hepatitis when the drug was used in prophylaxis.^{6,7} However detailed investigations have shown that AQ is no more toxic than CQ when used therapeutically to treat uncomplicated *Plasmodium falciparum* malaria.^{2–4,8} In fact, results collated from 40 different clinical trials held in the late 1980s suggest that adverse drug reactions to AQ are likely to occur only during prophylaxis.⁸

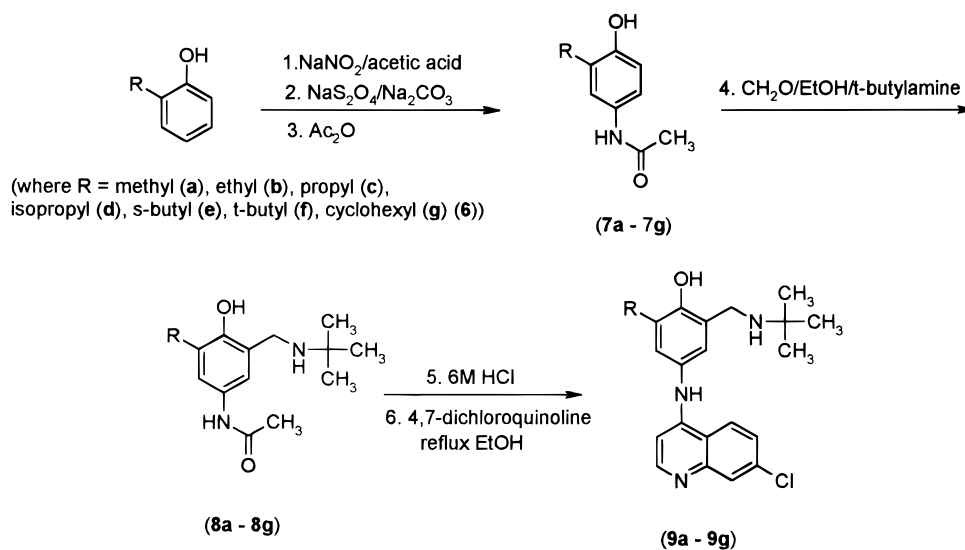
Despite some clinical evidence of AQ sensitivity, laboratory analysis has revealed cross-resistance between CQ and AQ which has transformed into clinical resistance in some settings.^{9–12} The emergence of multidrug-resistant strains of *Plasmodia* has created a near-desperate situation, where the need for new inexpensive antimalarials to circumvent the parasite's resistance mechanism has become vital. Unfortunately the design and subsequent synthesis of new antimalarials are hindered by the fact that the mechanism of resistance is not fully understood. Therefore it is imperative to establish the structural features of the Mannich base antimalarials which enable a compound to evade the drug-resistance mechanism and yet retain activity. This advantage can then be used in the redesign of new Mannich base antimalarials. Amopyroquine (**3**), a structural analogue to AQ where the diethylamino side chain is replaced with a pyrrolidine group, was shown to be more active than both CQ and AQ against 11 CQ-resistant strains of *P. falciparum* isolates.^{1,3,14} Subsequent research into the synthesis of Mannich base compounds containing side chains which were less susceptible to metabolism afforded many diverse Mannich base derivatives, one of which was tebuquine (**4**). Tebuquine (**4**), a substituted biphenyl-aminoquinoline, exhibited greater antimalarial activity than AQ in vivo^{15,16} and in vitro.^{17,18} Further investigations, however, revealed chronic toxicity in experimental animals, including the presence of foamy macrophages (L. M. Werbal, personal communication).



infected areas, such as Southeast Asia. Amodiaquine (**2**, AQ), a Mannich base derivative which was introduced into the field in the late 1950s, has been shown to be a superior alternative to CQ in areas of high CQ resistance.^{2–5} Unfortunately in the mid-1980s, the use of AQ declined abruptly following initial reports of agranulo-

* Address correspondence to S. A. Ward. Tel: 0151-794-8219. Fax: 0151-794-8217. E-mail: SAWARD@liv.ac.uk.

[‡] Department of Chemistry.

Scheme 1. Synthesis of Compounds **8a–8g**

Recent studies have suggested that the ability of antimalarials to overcome CQ resistance *in vitro* is dependent on the degree of drug lipophilicity.¹⁹ These authors have shown that an increase in drug lipophilicity correlates with a decrease in cross-resistance to CQ.¹⁹ Indeed, the greater efficacy of tebuquine (**4**) against CQ-resistant parasites may be due to the presence of a *p*-chlorophenyl substituent in the 5'-position of the side chain, thereby increasing lipid solubility.¹⁷

In light of these observations, we have synthesized a new series of Mannich base derivatives where the diethylamino function of AQ has been replaced by a *N*-*tert*-butylamino group to prevent the side chain being metabolized to metabolites which display cross-resistance.^{18,19} In addition, to evaluate the importance of lipophilicity with respect to antimalarial activity, a series of alkyl substituents were placed in the 5'-position of the 4'-hydroxyanilino side chain. The ability of each compound within the series (**9a–9g**) to arrest the growth of CQ-sensitive and -resistant strains of *Plasmodium* *in vitro* and *in vivo* has been measured in anticipation that these derivatives may circumvent the mechanism of drug resistance.

Chemistry

The alkyl-substituted Mannich base compounds were prepared using a modified procedure of Kesten et al. which is depicted in Scheme 1.¹⁶ All the 2-alkylphenols were commercially available, with the exception of the *o*-cyclohexylphenol. Friedel–Craft alkylation of phenol with cyclohexyl chloride, using ferric chloride as the catalyst, gave a mixture of the *o*- and *p*-cyclophenol isomers²⁰ which could be readily separated by column chromatography. Alkylation of phenol with cyclohexyl chloride proved to be the most difficult step in the preparation of compound **9g**. The favored isomer during the alkylation of phenol using ferric chloride as the catalyst was the *p*-cyclohexylphenol isomer. Higher temperatures only enhanced the yield of the para isomer (above 190 °C), while lower temperatures (below 120 °C) decreased the overall yield of both isomers. Best results were observed using a temperature range between 160 and 180 °C, yielding 13% and 35% of the ortho and para

isomers, respectively. These results disagree with a previous literature report for the synthesis of *o*-cyclohexylphenol.²⁰ The reasons for the discrepancies are unknown.

The synthetic route of 7-chloro-4-[3'-alkyl-4'-hydroxy-5'-[(*tert*-butylamino)methyl]phenyl]aminoquinolines **9a–9g** employs multiple steps from the substituted 2-alkylphenol. In general, the 2-alkylphenols were diazotized, and without isolation, the diazo group was converted to an amino function using sodium dithionite. However, the 2-alkylanilines readily decomposed when exposed to air; consequently, without purification, the amino substituent was acetylated to give 3-alkyl-4-hydroxyacetanilides **7a–7g**. Compounds **7a–7g** were purified and allowed to react with excess *N*-*tert*-butylamine and aqueous formaldehyde in ethanol to give 5-alkyl-4-hydroxy-3-[(*tert*-butylamino)methyl]acetanilides **8a–8g**. Acid hydrolysis of the acetanilides **8a–8g** followed by treatment with the commercially available precursor 4,7-dichloroquinoline in ethanol gave the target compounds **9a–9g** in an overall yield of approximately 50%.

Antimalarial Activity

The capacity of AQ (**2**), *N*-*tert*-butylamodiaquine (TBAQ, **5**), and the 5'-alkyl-substituted Mannich side chain derivatives **9a–9g** to arrest the growth of CQ-sensitive (19 nM for CQ) and CQ-resistant (182 nM for CQ) strains of *P. falciparum* was determined (Table 1). As reported previously, both AQ (**2**) and TBAQ (**5**) arrested parasite growth in the two different strains with different efficiencies.¹⁹ The marked difference in the potency of AQ against CQ-sensitive (HB3) and CQ-resistance (K1) strains is illustrated by the calculated resistance factor of 5.4 (Table 1). All 5'-alkyl derivatives examined in this study displayed antimalarial activity, although some members were more effective than others. A general correlation between the size and shape of the 5'-alkyl substituent of the 4'-hydroxyanilino side chain and the efficacy of these drugs against both strains of the parasite was observed. The introduction of large nonplanar substituents, such as a *N*-*tert*-butyl (**9f**) or cyclohexyl (**9g**) group, substantially decreased antimalarial activity, while alkyl groups which contained a 3-carbon backbone with no greater than one

Table 1. IC₅₀ Values for Inhibition of Growth of *P. falciparum* in Vitro by Alkyl Mannich Base Derivatives

| compd | R | HB3 (nM) | K1 (nM) | resistance factor IC ₅₀ (K1)/ IC ₅₀ (HB ₃) |
|-----------|------------|------------------------------|------------------------------|--|
| CQ | | 19 ± 3 (2) | 182 ± 17 (2) | 9.57 |
| 2 | | 2.47 ± 0.50 (4) ^a | 13.2 ± 2.5 (3) | 5.35 |
| 5 | | 1.47 ± 0.40 (3) | 3.43 ± 0.53 (3) | 2.33 |
| 9a | Me | 2.99 ± 1.25 (3) | 11.2 ± 2.1 (4) | 3.76 |
| 9b | Et | 2.97 ± 1.50 (4) | 7.90 ± 1.82 (3) | 2.65 |
| 9c | propyl | 0.98 ± 0.2 (4) ^c | 1.83 ± 0.42 (3) ^b | 1.86 |
| 9d | isopropyl | 1.24 ± 0.32 (4) ^c | 1.87 ± 0.56 (3) ^b | 1.50 |
| 9e | sec-butyl | 1.97 ± 0.31 (3) | 5.77 ± 0.23 (4) | 2.92 |
| 9f | tert-butyl | 8.35 ± 2.30 (3) | 21.2 ± 4.2 (3) | 2.54 |
| 9g | cyclohexyl | 6.80 ± 1.64 (3) | 14.5 ± 1.2 (3) | 2.13 |

^a Number of experiments. ^b Not significant ($p > 0.05$). ^c Significantly different ($p > 0.05$).

Table 2. ED₅₀ Values for Inhibition of Growth of *P. falciparum* in Vivo by Alkyl Mannich Base Derivatives

| compd | oral (mg/kg) | intraperitoneal (mg/kg) |
|---------------------------|--------------|-------------------------|
| 2 (AQ) | 1.92 ± 0.42 | 3.30 ± 1.70 |
| 9c (R = propyl) | 0.56 ± 0.03 | 0.80 ± 0.01 |
| 9d (R = isopropyl) | 0.38 ± 0.06 | 0.78 ± 0.10 |

branch, as seen with compounds **9c–9e**, gave optimum antimalarial activity.

In addition, the two best derivatives in vitro (**9c** and **9d**) were assessed for their ability to arrest the growth of NS strain of *P. berghei* compared to AQ in vivo (Table 2). Two different routes of drug administration were used (oral and intraperitoneal) to determine whether antimalarial activity was influenced by variable drug absorption. There was a significant difference ($p > 0.05$) between the two different routes of administration (oral and intraperitoneal) for all three drugs investigated (AQ, **9c**, and **9d**). The drugs were almost 2-fold more active when administered orally compared to intraperitoneal route of administration, probably as a result of the acidic environment of the gut facilitating solubilization.

Discussion

All of the 7-alkyl-substituted Mannich base derivatives prepared (**9a–9g**) inhibited growth of both the CQ-sensitive (HB3) and the CQ-resistant (K1) parasites by varying degrees in vitro (Table 1). It was found that the replacement of the diethylamino function of AQ with a *N*-*tert*-butylamine group of the 4'-hydroxyanilino side chain (TBAQ, **5**) led to a substantial increase in antimalarial activity against both strains. There was a 1.5-fold increase activity of TBAQ against the CQ-sensitive strain and almost a 4-fold increase in activity against the CQ-resistant strain. This result is in agreement with previous studies.^{17,18} Earlier studies have suggested that the enhanced potency of TBAQ may be due to inherent differences in the level of accumulation between AQ and TBAQ. The cellular accumulation ratio (CAR) for TBAQ was found to be 5–9 times greater in CQ-sensitive isolates compared to AQ and 2–3-fold greater in CQ-resistant strains.¹⁷

A similar decrease in the level of cross-resistance was seen between AQ and TBAQ was observed between AQ and the new alkyl derivatives **9a–9g**. Our data (Table 1) suggest that the shape/length of the 5'-alkyl substituent had a marked effect on drug activity. Activity

increased as the length of the alkyl group increased with optimum activity observed with the propyl and isopropyl derivatives. Replacement of the linear alkyl group containing only one branch with a more bulky, nonplanar group such as a *N*-*tert*-butyl or cyclohexyl substituent markedly reduced the efficacy of these derivatives against both strains compared to AQ or TBAQ. All the 5'-alkyl Mannich base derivatives displayed less cross-resistance than AQ, with resistant factors between 1.5 and 3.7 compared to 5.3 for AQ. In fact, the 5'-propyl- and 5'-isopropyl-substituted derivatives (**9c** and **9d**) were 2-fold more active than TBAQ and 7-fold more active than AQ against CQ-resistant (K1) isolate. The reason for the greater activity for these two derivatives is not known. However, given the structural similarities between TBAQ and the new derivatives (**9a–9g**), it is probable that the 5'-alkyl derivatives and TBAQ accumulate to similar levels in the CQ-resistant parasite, resulting in an increased activity.

The mechanism of accumulation of the 4-aminoquinolines in the malarial parasite remains quite controversial.^{17,21–24} Nevertheless most hypotheses are based on a central theme, that accumulation is dependent on the ability of drug to bind to an intraparasitic receptor, whether that be free heme^{21,22} and/or an active exchanger.^{23,24} Whatever the accumulation mechanism, it is apparent from the antimalarial activity data presented here that antimalarial activity of the new Mannich base derivatives (**9a–9g**) is dependent on more than one factor, the most likely factors being drug lipophilicity and the type of substituent in the 5'-position of the 4'-hydroxyanilino side chain.

Our results indicate that these Mannich base derivatives (**9a–9g**) exhibit greater activity in vivo compared to AQ when administered orally. It is possible that the increased efficacy of these drugs is at least in part due to their increased bioavailability. The metabolism of AQ has been widely investigated.^{25–27} Although it has a high absorption from the gut, due to a large first-pass effect, AQ has a low bioavailability.²⁶ It has been shown that AQ rapidly undergoes deethylation to the bis-deethylated metabolite and is ultimately excreted into the urine.^{25,28,29} In addition, further studies have shown that AQ is excreted into rat bile exclusively as the 5'-glutathionyl thioether²⁷ and subsequently into the intestines to be excreted into the feces or undergoes enterohepatic recirculation.³⁰ However both of these two processes of metabolic clearance have been avoided by the introduction of a 5'-alkyl substituent and the *N*-*tert*-butylamino group.

Experimental Section

All the *o*-alkylphenols, with the exception of *o*-cyclohexylphenol, were obtained from Aldrich Chemical Co., Gillingham, Dorset, England. Merck Kiesegel 60 F254 precoated silica plates for TLC were obtained from BDH, Poole, Dorset, U.K. Column chromatography was carried out on Merck 938S silica gel.

Proton NMR spectra were recorded using a Perkin-Elmer R34 (220 MHz) NMR spectrometer. The NMR solvent was deuterated chloroform unless otherwise stated in text, and tetramethylsilane was used as an internal reference. Full details are given for derivatives **9a–9g**. Mass spectra were recorded at 70 eV using a VG7070E mass spectrometer. The samples were introduced using a direct insertion probe. In the text the molecular ion (M^+) is given followed by peaks

corresponding to major fragment losses. Melting points were performed using a Gallenkamp melting point apparatus and are reported uncorrected.

***o*-Cyclohexylphenol (6).** The condensation of phenol with cyclohexyl chloride was performed using a modified procedure of Abdurasuleva et al.²⁰ A mixture of phenol (0.5 mol), cyclohexyl chloride (0.1 mol), and ferric chloride (5.5 mmol) was heated (170 °C) with continuous stirring for 2 h. After the mixture cooled to room temperature, sodium hydroxide was added (20%, 20 mL) and the mixture stirred. To facilitate the precipitation of the sodium salt of the product, diethyl ether was added (50 mL). The salt was collected by filtration and dissolved in diluted hydrochloric acid (1 M, 50 mL), and the solution was extracted with dichloromethane to give a mixture of *p*- and *o*-cyclohexylphenols (7.5 g, 42%). The two isomers were separated using a silica column and dichloromethane as the mobile phase (*o*-cyclohexylphenol, $R_f = 0.48$; *p*-cyclohexylphenol, $R_f = 0.80$).

3-Alkyl-4-hydroxyacetanilides 7a–7g. All the acetanilide derivatives were prepared from the method of Kesten et al.¹⁶ Yields and melting point data were all comparable to the literature values.

5'-Methyl-4'-hydroxyl-3'-[(*tert*-butylamino)methyl]acetanilide (8a). 3-Methyl-4-hydroxyacetanilide (7a) (5 mmol) was subjected to a Mannich reaction with *N*-*tert*-butylamine (10 mmol) and aqueous formaldehyde (10 mmol) in ethanol. After refluxing for 48 h the solvent was removed under reduced pressure and the residue dissolved in dichloromethane (10 mL). The organic solution was extracted with dilute hydrochloric acid (0.1 M, 2 × 20 mL). This solution was basified (pH 9–10) and extracted with dichloromethane (3 × 20 mL). The combined extracts were washed with water (1 × 20 mL) and dried (MgSO₄), and the was solvent evaporated under reduced pressure to give the product as a crude oil. Recrystallization was achieved using toluene/light petroleum ether (40–60 °C) (20/80 v/v) to afford a light-brown solid, sufficiently pure for the next reaction (65%, mp 245–247 °C): ¹H NMR δ 1.1 (s, 12H, *tert*-butyl, -CH₃), 1.9 (s, 3H, NH-C(O)-CH₃), 2.2 (3H, -CH₃), 3.7 (s, 2H, Ar-CH₂-NH-C(O)-CH₃), 6.8 (s, 1H, Ar), 7.0 (s, 1H, Ar), 8.1 (s, 1H, NH-C(O)-).

The synthesis of **8b–8g** was completed by a similar procedure as that of **8a**, where **7a** was replaced by the appropriate acetanilide **7b–7g**.

7-Chloro-4-[5'-methyl-4'-hydroxyl-3'-[(*tert*-butylamino)methyl]phenyl]aminoquinoline (9a). A solution of **8a** (5.0 g, 23 mmol) in hydrochloric acid (25 mL of 6 N HCl) was heated under reflux for 1 h. This solution was concentrated by reduced pressure and then coevaporated with ethanol. The residue was dissolved in ethanol (30 mL), 4,7-dichloroquinoline (4.5 g, 23 mmol) was added, and the solution heated under reflux for 2 h. The solution was concentrated by reduced pressure to give a viscous residue which was poured into ice-cold ammonium hydroxide (5%, 200 mL). The sticky solid which separated was dissolved in dichloromethane (100 mL) and separated from the basic solution. The organic solution was washed with water (100 mL), dried (MgSO₄), and evaporated to dryness under reduced pressure to give the crude product. This solid was recrystallized twice from aqueous ethanol to give an analytical product (48%, mp 209–210 °C): ¹H NMR δ 1.1 (s, 12H, *tert*-butyl, -CH₃), 2.2 (3H, -CH₃), 3.9 (s, 2H, Ar-CH₂-NH-C(O)-CH₃), 6.5 (br s, 1H, -NH-), 6.6 (d, $J = 5$ Hz, H-3), 6.7 (s, 1H, Ar), 7.0 (s, 1H, Ar), 7.4 (dd, $J = 9, 2$ Hz, 1H, H-6), 7.8 (dd, $J = 9, 2$ Hz, 1H, H-5), 7.9 (d, $J = 2$ Hz, 1H, H-8), 8.4 (d, $J = 5$ Hz, 1H, H-2); MS m/z 369.5 (M + 1). Anal. (C₂₁H₂₄N₃OCl) C, H, N.

7-Chloro-4-[5'-ethyl-4'-hydroxyl-3'-[(*tert*-butylamino)methyl]phenyl]aminoquinoline (9b): yellow solid (51%); mp 195–196 °C (from aqueous EtOH); ¹H NMR δ 1.1 (s, 12H, *tert*-butyl, -CH₂CH₃), 2.6 (q, $J = 7$ Hz, -CH₂CH₃), 3.7 (s, 2H, Ar-CH₂-NH-), 6.5 (br s, 1H, -NH-), 6.6 (d, $J = 5$ Hz, H-3), 6.8 (s, 1H, Ar), 6.9 (s, 1H, Ar), 7.4 (dd, $J = 9, 2$ Hz, 1H, H-6), 7.8 (dd, $J = 9, 2$ Hz, 1H, H-5), 7.9 (d, $J = 2$ Hz, 1H, H-8), 8.4 (d, $J = 5$ Hz, 1H, H-2); MS m/z 383.5 (M + 1). Anal. (C₂₂H₂₆N₃OCl) C, H, N.

7-Chloro-4-[5'-propyl-4'-hydroxyl-3'-[(*tert*-butylamino)methyl]phenyl]aminoquinoline (9c): off-white solid (54%); mp 168–169 °C (from aqueous EtOH); ¹H NMR δ 0.9 (t, $J = 7$ Hz, 3H, -CH₂CH₃) 1.1 (s, 9H, *tert*-butyl), 1.6 (m, 2H, -CH₂-CH₂-CH₃), 2.6 (q, $J = 7$ Hz, -CH₂-CH₂-CH₃), 3.7 (s, 2H, Ar-CH₂-NH-), 6.5 (br s, 1H, -NH-), 6.6 (d, $J = 5$ Hz, H-3), 6.8 (s, 1H, Ar), 6.9 (s, 1H, Ar), 7.4 (dd, $J = 9, 2$ Hz, 1H, H-6), 7.8 (dd, $J = 9, 2$ Hz, 1H, H-5), 7.9 (d, $J = 2$ Hz, 1H, H-8), 8.4 (d, $J = 5$ Hz, 1H, H-2); MS m/z 398.5 (M + 1). Anal. (C₂₃H₂₈N₃OCl) C, H, N.

7-Chloro-4-[5'-isopropyl-4'-hydroxyl-3'-[(*tert*-butylamino)methyl]phenyl]aminoquinoline (9d): yellow solid (52%); mp 188.5–190.0 °C (from aqueous EtOH); ¹H NMR δ 1.1 (m, 15H, *tert*-butyl, 2 -CH₃), 1.6 (m, 2H, -CH₂-CH₃), 3.7 (s, 2H, Ar-CH₂-NH-), 6.5 (br s, 1H, -NH-), 6.6 (d, $J = 5$ Hz, H-3), 6.7 (s, 1H, Ar), 7.0 (s, 1H, Ar), 7.4 (dd, $J = 9, 2$ Hz, 1H, H-6), 7.8 (dd, $J = 9, 2$ Hz, 1H, H-5), 7.9 (d, $J = 2$ Hz, 1H, H-8), 8.4 (d, $J = 5$ Hz, 1H, H-2); MS m/z 398.5 (M + 1). Anal. (C₂₃H₂₈N₃OCl) C, H, N.

7-Chloro-4-[5'-(*sec*-butyl)-4'-hydroxyl-3'-[(*tert*-butylamino)methyl]phenyl]aminoquinoline (9e): fawny solid (48%); mp 217 °C (from aqueous EtOH); ¹H NMR δ 0.9 (t, $J = 7$ Hz, 3H, -CH₂CH₃), 1.1 (m, 12H, *tert*-butyl, -CH₃), 1.6 (m, 2H, -CH₂-CH₃), 3.0 (m, -CH(CH₃)-CH₂-CH₃), 3.7 (s, 2H, Ar-CH₂-NH-), 6.5 (br s, 1H, -NH-), 6.6 (d, $J = 5$ Hz, H-3), 6.7 (s, 1H, Ar), 7.0 (s, 1H, Ar), 7.4 (dd, $J = 9, 2$ Hz, 1H, H-6), 7.8 (dd, $J = 9, 2$ Hz, 1H, H-5), 7.9 (d, $J = 2$ Hz, 1H, H-8), 8.4 (d, $J = 5$ Hz, 1H, H-2); MS m/z 412.5 (M + 1). Anal. (C₂₄H₃₀N₃OCl) C, H, N.

7-Chloro-4-[5'-(*tert*-butyl)-4'-hydroxyl-3'-[(*tert*-butylamino)methyl]phenyl]aminoquinoline (9f): fawny solid (38%); mp 225–226 °C (from aqueous EtOH); ¹H NMR δ 1.1 (s, 9H, *tert*-butyl), 1.3 (s, 9H, *tert*-butyl), 3.7 (s, 3H, Ar-CH₂-NH-), 6.5 (br s, 1H, -NH-), 6.6 (d, $J = 5$ Hz, H-3), 6.7 (s, 1H, Ar), 7.0 (s, 1H, Ar), 7.4 (dd, $J = 9, 2$ Hz, 1H, H-6), 7.8 (dd, $J = 9, 2$ Hz, 1H, H-5), 7.9 (d, $J = 2$ Hz, 1H, H-8), 8.4 (d, $J = 5$ Hz, 1H, H-2); MS m/z 412.5 (M + 1). Anal. (C₂₄H₃₀N₃OCl) C, H, N.

7-Chloro-4-[5'-(cyclohexyl)-4'-hydroxyl-3'-[(*tert*-butylamino)methyl]phenyl]aminoquinoline (9g): fawny solid (39%); mp 189–190 °C (from aqueous EtOH); ¹H NMR δ 1.1 (s, 9H, *tert*-butyl), 1.3–1.8 (m, 10H, cyclohexyl), 3.0 (m, 1H, -CH of cyclohexyl), 3.7 (s, 3H, Ar-CH₂-NH-), 6.5 (br s, 1H, -NH-), 6.6 (d, $J = 5$ Hz, H-3), 6.7 (s, 1H, Ar), 7.0 (s, 1H, Ar), 7.4 (dd, $J = 9, 2$ Hz, 1H, H-6), 7.8 (dd, $J = 9, 2$ Hz, 1H, H-5), 7.9 (d, $J = 2$ Hz, 1H, H-8), 8.4 (d, $J = 5$ Hz, 1H, H-2); MS m/z 438.5 (M + 1). Anal. (C₂₆H₃₂N₃OCl) C, H, N.

Antimalarial Activity. Two strains of *P. falciparum* from Thailand were used in this study: (a) the uncloned K1 strain which is known to be CQ-resistant and (b) the HB3 strain which is sensitive to all antimalarials. Parasites were maintained in continuous culture using the method of Trager and Jensen.³¹ Cultures were grown in culture flasks containing human erythrocytes (2–5%) with parasitemia in the range of 1–10% suspended in RPMI 1640 medium, supplemented with 25 mM HEPES and 32 mM NaHCO₃, and 10% human serum (complete medium). Cultures were gassed with a mixture of 3% O₂, 4% CO₂, and 93% N₂.

(a) In Vitro Testing. Antimalarial activity was assessed using an adaption of the 48-h sensitivity assay of Desjardins et al.³² using [³H]hypoxanthine incorporation as an assessment of parasite growth. Stock drug solutions were prepared in 100% dimethyl sulfoxide (DMSO) and diluted to the appropriate concentration using complete medium. Assays were performed in sterile 96-well microtiter plates; each plate contained 200 μL of parasite culture (2% parasitemia, 0.5% hematocrit) with or without 10-μL drug dilutions. Each drug was tested in triplicate and parasite growth compared to control wells (which constituted 100% parasite growth). After 24 h incubation at 37 °C, 0.5 μCi of hypoxanthine was added to each well. Cultures were incubated for a further 24 h before they were harvested onto filtermats, dried for 1 h at 55 °C, and counted using a Wallac 1450 Microbeta Trilux liquid scintillation and

luminescence counter. IC₅₀ values were calculated by interpolation of the probit transformation of the log dose–response curve.

(b) In Vivo Testing. Male Swiss albino mice weighing 18–22 g were inoculated intraperitoneally with 10⁷ parasitized erythrocytes with *P. berghei* NS strain. Animals were then dosed daily via two routes (intraperitoneal and oral) for 4 consecutive days beginning on the day of infection. Compounds were dissolved or suspended in the vehicle solution consisting of methanol, phosphate-buffered saline, and DMSO (2:5:3). The parasitemia was determined on the day following the last treatment and the ED₅₀ (50% suppression of parasites when compared to vehicle-only-treated controls) determined from a plot of log dose against parasitemia.

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