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Synthesis and Antimalarial Activity of New Isotebuquine Analogues

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Amodiaquine (AQ) and tebuquine are 4-aminoquinoline antimalarials with Mannich base side chain and are highly effective against chloroquine (CQ)-resistant strains of Plasmodium falciparum. Clinical use of AQ has been severely restricted due to hepatotoxicity and agranulocytosis side effects associated with its long term use. Lysosomal accumulation and bioactivation to generate reactive quinoneimine metabolite are implicated to be the cause of the observed AQ toxicities. To avoid the quinoneimine formation and thus the toxicity, a series of isotebuquine analogues and their $N^\alpha$-oxides with hydroxy group meta to the amino rather than in para position of the aniline moiety were prepared. The new Mannich bases are highly active against both CQ-sensitive (D6) and -resistant (W2 and TM91C235) clones of P. falciparum with IC$_{50}$ in the range of 0.3−120 ng/mL. New compounds are 1000-fold less toxic (IC$_{50}$ = 0.7−6 μg/mL) to mouse macrophage cell line than to parasite cell lines. Mono-Mannich bases are more active than bis-Mannich bases. Mono-Mannich base 1a (IC$_{50}$ = 0.3 ng/mL) is 20-fold more active than the corresponding trifluoromethyl analogue 1b. No appreciable difference in either toxicity or efficacy were observed between the new Mannich bases (m-hydroxyaniline derivatives) 1a or 2a and the corresponding p-hydroxyaniline derivatives.

Introduction

Malaria is one of the most widespread diseases in many regions of the world, exposing millions of people to risk of infection and death. The 4-aminoquinolines such as chloroquine (CQ) are the most important and widely used class of drugs for treatment of malaria. Chloroquine has been the drug of choice for malaria treatment since the early 1950s, providing effective and safe treatment for millions of Plasmodium falciparum infected patients a year. Development of drug resistance to chloroquine in malaria has become a major health concern in malaria endemic areas, which has prompted a search for alternative antimalarials against the CQ-resistant strains.1,2 As a result, several new classes of antimalarial drugs were developed; none has reached the same status of recognition as the drug of choice in malaria therapy as CQ.

Amodiaquine (AQ), a new Mannich base 4-aminoquinoline, is effective against chloroquine-resistant strains of P. falciparum.3 However, the clinical use of AQ has been severely restricted because of the hepatotoxicity and agranulocytosis side effects associated with its long term use.4,5 Recent reports indicate that AQ was metabolized by cytochrome P-450 oxidation to form reactive quinoneimine metabolite with subsequent conjugate addition of glutathione or cysteinyl function of the enzymes.6,7 Lysosomal accumulation and bioactivation of reactive quinoneimine metabolite are implicated to be the cause of the observed AQ in vivo toxicity.8,9 To avoid the quinoneimine metabolite formation, a series of isoquine and related analogues were reported in the literature.10−12 Isoquine (IQ) is an analogue of AQ, in which the 4'-hydroxy group on the aniline ring of AQ is interchanged with a 3'-Mannich base side chain. IQ was demonstrated to possess higher antimalarial activity against Plasmodium yoelii than AQ. In contrast to AQ, IQ was excreted primarily as glucuronide, instead of glutathione conjugate.10

Another promising compound from the class of the 4-aminoquinolines is tebuquine (TQ), which is a biaryl analogue of AQ with a p-chlorophenyl moiety substituted at the 5-position of the 4-hydroxyaniline side chain of AQ. TQ is significantly more active than AQ and CQ in both in vitro and in vivo tests.13−15 Similar to AQ, TQ forms active quinoneimine metabolite (Scheme 1) and consequently develops the same toxic side effects as AQ in prolonged use.

In this study, we described the synthesis of a series of isotebuquine analogues with a hydroxy group in meta rather than para position to the amino group of the aniline moiety. Unable to produce the toxic quinoneimine metabolite, new isotebuquine analogues may exhibit similar or better activity than tebuquine with no or low liver toxicity. In vitro antimalarial activities of the new compounds were assessed in CQ-sensitive (D6) and CQ-resistant (W2) cell lines of P. falciparum, and their in vitro toxicities were measured in a murine monocyte-like macrophage line J774. Furthermore, in vivo antimalarial efficacy values against Plasmodium berghei of the new compounds are presented.

Chemistry

The initial step of this work was the synthesis of bis- and mono-(tert-butylaminomethyl)-5-(7-chloroquinolin-4-ylamino)-

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biphenyl-3-ols, the so-called isotebuquine analogues. The general approach to prepare the new Mannich bases 1a, 1b-5a, 1b is illustrated in Scheme 2. The intermediate 1-bromo-3,5-dinitrobenzene (6) was prepared by addition of the N-bromosuccinimide in small portions to the commercially available starting material 1,3-dinitrobenzene in sulfuric acid at 80–90 °C. 16 3-Bromo-5-nitroanisole (7) was obtained by treatment of 6 with sodium methoxide in absolute methanol. 17 The coupling of bromoanisole 7 with the 4-chlorophenylboronic acid was achieved using a modified Suzuki method to afford substituted biaryl compound 8a in a good yield. 18 Two types of catalysts were used in the Suzuki coupling reaction to prepare compounds 8a and 8b. Method A utilized tetrakis-(triphenylphosphine) palladium(0), 18 while method B employed Pd(OAc)2 as a catalyst (Scheme 3). 19 The compound 8a was prepared by method A, while trifluoromethyl analogue 8b was synthesized by method B. Both coupling methods A and B were efficient to prepare the biaryl intermediates when 3,5-dinitro-bromobenzene was used. However, when the 1-iodo-3,5-dinitrobenzene

**Scheme 1.** Biotransformation of Tebuquine by P-450 Oxidation and Further Conjugation with Cysteinyi Function of Enzymes

**Scheme 2.**

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was used as a starting material in the Suzuki coupling reaction, it gave a poorer yield than when 1-bromo-3,5-dinitrobenzene was used, due to incomplete reaction and tedious purification procedure.

The reduction of the nitro-group by catalytic hydrogenation of 8 using palladium on activated carbon afforded the amino-biphenyl derivatives 9 in a quantitative yield.20 The intermediate amines obtained were used for the next reaction without further purification. The boron tribromide-catalyzed demethylation of 9a in dichloromethane solution gave a desired aminophenol 10a in high yield (70%). The trifluoromethyl-containing analogue 10b was obtained using an alternative reagent, boron tribromide-catalyzed demethylation in dichloromethane solution.

The intermediates 11a and 11b for the synthesis of isotebuquine analogues were obtained by treatment of 4,7-dichloroquinoline with corresponding biarylaminoethanols 11a,b. Introduction of the Mannich base side chain, tert-butylaminomethyl, into the key intermediate 11 was a challenging task due to low selectivity of the reaction. When 11a or 11b was subjected to Mannich reaction in the presence of tert-butylamine and 37% formaldehyde, a mixture consisting of five products was obtained. The mixture was separated by the use of a silica gel column followed by fractional crystallization. The product ratio of this reaction was highly dependent on the ratio of the reagents used. Formation of bis-Mannich base 3 was favored when the reaction was conducted using a stoichiometric amount of reagents, 11, tert-butylamine, and formaldehyde. Doubling the ratio of the reagents, tert-butylamine and formaldehyde, led to the formation of both mono-Mannich base 1 as well as bis-Mannich base 3. Further addition of reagents to the same reaction mixture gave other analogues, 2 (mono-) and 4 (bis-), in small yield (7–10%). The yield of compound 5 was insignificant, isolation of which was possible only in large-scale synthesis. The products were first separated by column chromatography, followed by repeated recrystallization from chloroform and diethyl ether mixture. Purification of products 1 and 4 could not be accomplished by chromatography because of their similarity in Rf values on TLC under various solvent systems. However, separation of the products was achieved by fractional crystallization from methanol.

The structures of all products were characterized by elemental analysis, mass spectrometry, and 1H and 13C NMR spectroscopy.

As an extension of this work, two Nα-oxide isotebuquine analogues (13 and 14) were prepared to improve solubility and therefore enhance the bioavailability of this type of antimalarial.

Synthetic pathway for Nα-oxides was based on the method developed for the synthesis of isotebuquine derivatives (Scheme 3). Thus, p-chloro-biarylaminophenol 10a was treated with 4,7-dichloroquinoline 1-oxide to provide corresponding 4-chloro-5-(7-chloro-1-oxoquinolin-4-ylamino)-biphenyl-3-ol (12) as outlined in Scheme 4. Contrary to the synthesis of Mannich base 1–5, which was rather straightforward, the final step in the synthesis of the target Nα-oxide 13 and 14 was a challenging task. In an attempt to apply the same conditions for the Mannich reaction that has been utilized for the synthesis of regular isotebuquine analogues 1–5, which was rather straightforward, the final step in the synthesis of the target Nα-oxide 13 and 14 was a challenging task. In an attempt to apply the same conditions for the Mannich reaction that has been utilized for the synthesis of regular isotebuquine analogues 1–5 (3 equiv of tert-butylamine, 4 equiv of formaldehyde, DMF, room temperature, 3 days), no reaction was observed. Heating at 100 °C gave a complicated mixture of high lipophilic byproducts. When the Mannich reaction was carried out in diluted DMF solution (15 mg/mL) at 65 °C for 7 days, two new Nα-oxide derivatives, mono- (13) and bis-Mannich base (14), were obtained in low yield. On scale-up synthesis, two deoxygenated byproducts, 1a and 3a, were isolated.

### 1H NMR Studies

Due to poor solubility of some of the final products in CDCl₃, most of the NMR spectra were taken in CD₂OD. When compounds are soluble in both chloroform and methanol, the spectrum in both solvents, CDCl₃ and CD₂OD, are reported. The structure assignments of 1–5 were based on comparison of their chemical shifts with the key intermediate 11. The assignment of all protons in compound 11 is straightforward (Table 1). The 1H NMR spectrum of 11b shows three triplets for protons Hh, Hi, and Hj of the aminophenol moiety at 6.91, 6.96, and 7.15 ppm, respectively, with small coupling constant J = 1.6 Hz. Two sets of doublets at 7.10 and 8.33 ppm were attributed to Hb and He of the quinoline ring, and the doublets at 7.75 and 7.82 ppm were assigned to Hf, respectively. The proton chemical shifts of intermediate 11 are informative for the structure determination of its Mannich
Table 1. H NMR Chemical Shifts (δ) for Aromatic Protons of Isofetbuquine Derivatives (11 and 1–5b) and Their N-Oxides (12–14) in CD3OD Solution

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<tr>
<th>Comp</th>
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<th>Hβ</th>
<th>Hε</th>
<th>Hδ</th>
<th>Hγ</th>
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a MB = Mannich base side chain.

Experimental Section

Melting points were determined in open capillary tubes on a Mettler FP62 melting point apparatus (Mettler Toledo, USA) and are uncorrected. 1H NMR and 13C NMR spectra were recorded using a Bruker Avance-300 spectrometer (Bruker Instrument, Inc., Wilmington, DE) at a frequency of 300.13 MHz. Chemical shifts are given in ppm (δ) relative to tetramethylsilane (TMS) as internal standard. Analytical thin-layer chromatography (TLC) was performed using HPLC-HLF normal phase 150 micrometer silica gel plates or silica gel GF/UF254, 500 micrometer plates (Analtech, Newark, DE). Visualization of the developed chromatograms was performed using UV absorbance or iodine stain. Flash chromatography was conducted with silica gel 60 Å (200–400 mesh) from Sigma-Aldrich Co. Solvents and reagents obtained from commercial sources were used without purification, unless noted. Reactions were carried out under an inert atmosphere of nitrogen. Elemental analysis was performed by Atlantic Microlab, Inc. (Norcross, GA). Where analyses are indicated by symbols of the elements, the analytical results obtained were within ±0.4% of the theoretical values.

1-Bromo-3-methoxy-5-nitrobenzene (7). To the solution of compound 6 (12 g, 48.6 mmol) in dry methanol (120 mL) was added sodium methoxide (3.24 g, 60 mmol). The mixture was boiled for 2 h and allowed to cool to room temperature. The reaction was quenched by addition of 1 N solution of HCl, and the mixture was extracted with dichloromethane (2 × 250 mL). Organic layers were combined, washed with brine, dried over sulfuric acid, filtered, and evaporated to dryness in vacuum. The crude product was purified by flash column chromatography on silica gel, eluting with a gradient of 2% ethyl acetate in hexane followed by 10–12% ethyl acetate in hexane to yield 9.25 g (82%) of product 8a as a yellow solid, mp 86.9 °C (lit., 87.5 °C). 1H NMR (CDCl3): δ 7.99 (t, J = 1.3 Hz, 1H), 7.70 (t, J = 2.1 Hz, 1H), 7.40 (t, J = 2.2 Hz, 1H), 3.92 (s, 3H). 13C NMR: 160.62, 149.50, 123.92, 123.01, 118.98, 107.78, and 56.20.

4-Chloro-5-methoxy-3-nitro-biphenyl (8a). Method A. A solution of compound 7 (6.93 g, 30 mmol) and sodium carbonate (30 mL of 2 M solution) were suspended in a mixture of toluene and ethanol (120 mL: 4:1). To the bilayer mixture were sequentially added 4-chlorophenylboronic acid (5.16 g, 33 mmol) and tetrakis(triphenylphosphine) palladium(0) with stirring. The reaction mixture was refluxed for 2 h under nitrogen atmosphere. On cooling to room temperature, the solution was poured onto ice water. Solid precipitate formed was collected, dried, and purified by flash column chromatography on silica gel eluting with a gradient of 1% ethyl acetate in hexane followed by 10% ethyl acetate in hexane to give 6.33 g (80%) of product 8a as a pale yellow solid, mp 131.7 °C. 1H NMR (CDCl3): δ 8.05 (t, J = 1.7 Hz, 1H), 7.74 (t, J = 2.1 Hz, 1H), 7.56 (d, J = 8.6 Hz, 2H), 7.47 (d, J = 8.6 Hz, 2H), 7.41 (t, J = 1.7 Hz, 1H), 3.97 (s, 3H). 13C NMR: 160.53, 149.73, 142.44, 137.17, 134.89, 129.33, 128.41, 119.72, 114.41, 106.85, 56.04. MS (m/z): 264.11 (MH+).

5-Methoxy-3-nitro-4-(trifluoromethyl)-biphenyl (8b). Method B. To the solution of compound 6 (2.7 g, 11.64 mmol) and 4-(trifluoromethyl)phenylboronic acid (2.43 g, 12.80 mmol) in a mixture of acetone and water (57 mL, 27:30) were added potassium carbonate (4.0 g, 29.0 mmol) and palladium(II) acetate (100 mg). The deep black mixture was refluxed for 2 h and allowed to cool to room temperature. The mixture was extracted with ethyl acetate (2 × 150 mL), and the organic layer was passed through a layer of Celite. The solution was dried by Na2SO4 and evaporated in vacuum to dryness. The residue was purified by flash column chromatography on silica gel eluting with a gradient of 2% ethyl acetate in hexane followed by 10% ethyl acetate in hexane to give 3.2 g (92%) of the desired product as a pale yellow solid, mp 54.6 °C. 1H NMR (CDCl3): δ 8.08 (t, J = 1.6 Hz, 1H), 7.78 (t, J = 2.1 Hz, 1H), 7.76 (d, J = 3.0 Hz, 4H), 7.45 (t, J = 1.6 Hz, 1H), 3.98 (s, 3H). 13C NMR: 160.61, 149.73, 142.17, 130.92, 130.48, 129.05, 129.68, 129.23, 127.56, 126.17, 126.12, 126.07, 126.02, 125.79, 122.19, 120.05, 118.7, 114.65, 107.40, 56.08. MS (m/z): 298.14 (MH+).

4-Chloro-5-methoxy-biphenyl-3-yamine (9a). A solution of 8a (6.0 g, 22.75 mmol) with a catalytic amount of 10% palladium on activated carbon (0.6 g, 10% of the weight) was hydrogenated in tetrahydrofuran under 40 psi pressure at room temperature for 2 h. The black mixture was passed through a thin layer of Celite, and the yellow solution was evaporated in vacuum to give 5.10 g (96%) of crude product as a gum, which was pure enough to be used for next step synthesis without further purification. A portion of the mixture was purified to give pure product as a gum. 1H NMR (CDCl3): δ 7.49 (d, J = 8.6 Hz, 2H), 7.39 (d, J = 8.6 Hz, 2H), 6.51 (t, J = 1.7 Hz, 1H), 6.49 (t, J = 1.7 Hz, 1H), 6.27 (t, J = 2.1 h).
ethanol, and recrystallized from chloroform to give bis-substituted Mannich base 3 as a pale yellow solid in 40% yield. The dimethylformamide filtrates were combined and diluted with cool water (100 mL). The precipitate obtained was collected, washed with water, and dried. The crude product was purified by silica gel column chromatography eluting with mixture of CHCl₃/EtOAc: MeOH (10:1:1) to give compounds 2 and 5 which were recrystallized from CHCl₃/ether. Fractions, which contained both compounds 1 and 4, were collected and evaporated in vacuum to dryness. The products were separated by fractional crystallization from methanol to give first pure 1, and then compound 4, which was recrystallized from ether.

6-(tert-Butylamino)methyl)-4′-chboro-5-(7-chloroquinolin-4-ylamino)-biphenyl-3-ol (1a). Yield: 18%, mp > 220 °C dec. H NMR (CD₂OD): see Table 1. 13C NMR: 163.80, 161.93, 152.00, 151.22, 150.74, 147.77, 138.93, 135.45, 130.64, 129.28, 126.94, 125.50, 124.93, 123.85, 123.21, 121.38, 119.35, 117.63, 114.25, 113.51, 101.36, 52.80, 39.89, 26.29. MS (m/z): 551.1 (M⁺). Anal. (C₁₂H₁₃N₂OCl₂·H₂O) C, H, N.

6-(tert-Butylamino)methyl)-4′-chboro-5-(7-chloroquinolin-4-ylamino)-biphenyl-3-ol (1b). Yield: 10%, mp > 220 °C dec. H NMR (CD₂OD): see Table 1. 13C NMR: 163.80, 161.93, 152.00, 151.22, 150.74, 147.77, 138.93, 135.45, 130.64, 129.28, 126.94, 125.50, 124.93, 123.85, 123.21, 121.38, 119.35, 117.63, 114.25, 113.51, 101.36, 52.80, 39.89, 26.29. MS (m/z): 551.1 (M⁺). Anal. (C₁₂H₁₃N₂OCl₂·H₂O) C, H, N.

6-Bis-(tert-butylamino)methyl)-4′-chboro-5-(7-chloroquinolin-4-ylamino)-biphenyl-3-ol (3a). Yield: 40%, mp > 220 °C dec. H NMR (CD₂OD): see Table 1. 13C NMR: 160.04, 151.12, 150.43, 148.71, 141.11, 139.38, 137.98, 135.34, 133.04, 130.38, 128.05, 126.49, 125.40, 123.15, 120.15, 118.54, 117.66, 114.98, 101.06, 52.21, 51.48, 41.18, 38.13, 26.76, 26.22. MS (m/z): 551.1 (M⁺). Anal. (C₁₂H₁₃N₂OCl₂·H₂O) C, H, N.

6-Bis-(tert-butylamino)methyl)-4′-chboro-5-(7-chloroquinolin-4-ylamino)-biphenyl-3-ol (3b). Yield: 43%, mp 196 °C. H NMR (CD₂OD): see Table 1. 13C NMR: 162.32, 151.13, 150.29, 148.73, 144.94, 140.55, 137.99, 135.36, 129.59, 129.31, 128.80, 126.50, 125.24, 124.83, 124.79, 124.75, 124.70, 123.13, 121.15, 119.01, 117.68, 114.92, 101.01, 51.64, 50.29, 41.16, 37.99, 27.07, 26.45. MS (m/z): 293.1 (M⁺ + 2H). Anal. (C₁₂H₁₀N₂OCl₂) C, H, N.

General Procedure for Synthesis of Mannich Base. A mixture of tert-butylamine (23 mmol) and 37% formaline (28 mmol) in N,N-dimethylformamide (10 mL) was added to a solution of compound 11 (7 mmol) in N,N-dimethylformamide (30 mL). The reaction mixture was stirred at room temperature for 3 days. The resulting precipitate was collected, washed with water followed by
Chulay et al. Three P. falciparum malaria parasite clones, from CDC Indochina III (W-2), CDC Sierra Leone I (D-6), and Southeast Asia Isolates (TM91C235), were utilized in susceptibility testing. They were derived by direct visualization and micromanipulation from patient isolates. The W-2 clone is susceptible to mefloquine (MQ) but resistant to chloroquine (CQ), sulfadoxine, pyrimethamine, and quinine, whereas the D-6 clone is naturally resistant to MQ but susceptible to CQ, sulfadoxine, pyrimethamine, and quinine. The TM91C235 is a multi-drug resistance P. falciparum isolate from Southeast Asia. Test compounds were initially dissolved in DMSO and diluted 400-fold in RPMI 1640 culture medium supplemented with 25 mM Hepes, 32 mM NaHCO3, and 10% Albumax I (Gibco BRL, Grand Island, NY). These solutions were subsequently serially diluted 2-fold with a Biomek 1000 (Beckman, Fullerton, CA) over 11 different concentrations. The parasites were exposed to serial dilutions of each compound for 48 h and incubated at 37 °C with 5% CO2/95% air at 37 °C. Cell viability was calculated. The results are shown in Table 2.

(ii) In Vitro Toxicity Assay. Selected compounds were tested for toxicity in vitro against a subclone (G8) of the murine monocyte/macrophage line J774. The cell line was obtained from Dr. Jose Alunda, Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad Complutense, Madrid, Spain. Murine cells for toxicity in vitro against a subclone (G8) of the murine monocyte/macrophage line J774. The cell line was obtained from Dr. Jose Alunda, Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad Complutense, Madrid, Spain. Murine cells were maintained in 75 cm2 tissue culture flasks in Dulbecco’s modified Eagle medium (GIBCO) supplemented with 10% fetal calf serum, 2 mM l-glutamine 50 μg/mL gentamicin under humidified 5% CO2/95% air at 37 °C.

Toxicity tests were performed in 96-well tissue culture plates using an aqueous tetrazolium/formazan system as described. Cells were plated at a density of 1 x 104 cells/well in 100 μL of culture medium. After 24 h under culture conditions, 10 μL of drug (experimental) or solvent (control) diluted to the appropriate concentration in culture medium was added to each well. After the mixture was incubated for 72 h, 20 μL of a solution containing 3-(4,5-dimethylthiazol-2-yl)-2,5-(3-carboxymethoxy-phenyl)-2-(4-sulfo-phenyl)-2H-tetrazolium, inner salt (MTS), (Technical Bulletin #TB245, Promega Corporation) was added, and the plates were cultured for 1 to 2 h at 37 °C. A Spectra MAX Plus microtiter plate reader ( Molecular devices) was used to measure the optical density (OD) at a wavelength of 490 nm. Solvent control values were subtracted from experimental values.

### Table 2. Growth Inhibition of Isoquine Analogues against P. falciparum and Macrophage Line J774 Cell Lines

<table>
<thead>
<tr>
<th>Compd</th>
<th>Macrophase inhibition (IC50, μg/mL)</th>
<th>P. falciparum (IC50, μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D6</td>
<td>W2</td>
</tr>
<tr>
<td>1a</td>
<td>6.1 ± 0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>2a</td>
<td>18.6 ± 1.2</td>
<td>7.1</td>
</tr>
<tr>
<td>1b</td>
<td>1.6 ± 0.3</td>
<td>1.4</td>
</tr>
<tr>
<td>2b</td>
<td>1.7 ± 0.3</td>
<td>1.8</td>
</tr>
<tr>
<td>3a</td>
<td>3.6 ± 0.1</td>
<td>2.2</td>
</tr>
<tr>
<td>3b</td>
<td>0.75 ± 0.2</td>
<td>1.9</td>
</tr>
<tr>
<td>4a</td>
<td>2.8 ± 0.3</td>
<td>2.8</td>
</tr>
<tr>
<td>4b</td>
<td>ND</td>
<td>1.8</td>
</tr>
<tr>
<td>5a</td>
<td>ND</td>
<td>1.2</td>
</tr>
<tr>
<td>1a</td>
<td>ND</td>
<td>8.8</td>
</tr>
<tr>
<td>1b</td>
<td>ND</td>
<td>15</td>
</tr>
<tr>
<td>12</td>
<td>ND</td>
<td>397</td>
</tr>
<tr>
<td>13</td>
<td>ND</td>
<td>4.3</td>
</tr>
<tr>
<td>14</td>
<td>ND</td>
<td>4.1</td>
</tr>
</tbody>
</table>

### Biological Studies. (i) In Vitro Antimalarial Studies

The in vitro assays were conducted by using a modification of the semiautomated microdilution technique of Desjardins et al. and...
and are more active than those with two Mannich base functions highly active against W2 clone in the test, with IC\textsubscript{50} of 8.8 and compounds with mono Mannich base side chain (1a appeared no cross resistance with CQ, except 1b).

In Vivo Antimalarial Activity against \textit{P. berghei} in Mice.  The three most active compounds—1a, 2a, and 3a—were tested in mice infected with \textit{P. berghei} by oral administration (Thompson test). None showed significant activity on 3-day treatment with a daily dose up to 192 mg/kg. Likewise, the N-oxide analogues, 13 and 14, possess only marginal activity with a minimum active dose of 450 mg/kg. The marginal efficacy results in Thompson test may be a result of poor oral bioavailability of these compounds.

In Vitro Toxicity. Macrophage line J774 was used to assess the toxicity of the new Mannich base analogues synthesized in this study (Table 2). The results indicated that the concentration required to inhibit the normal cell line growth is 1000-fold higher than that for the inhibition of parasite growth, especially the chloroquine-sensitive cell line D-6, with therapeutic index of ≥1000 and is comparable to that of the positive control drug, tebuquine and tebuquine N\textsuperscript{\textit{\textalpha}}-oxide.

Conclusion

Methods for synthesis of isotebuquine analogues and their N\textsuperscript{\textalpha}-oxides have been developed. New derivatives exhibit promising in vitro antimalarial activity against both chloroquine-sensitive (D6) and chloroquine-resistant (W2) clones of \textit{P. falciparum} cell growth at a concentration comparable to that of tebuquine. However, the new isotebuquine analogues showed only marginal antimalarial activity in the Thompson test against \textit{P. berghei} by oral administration. The poor solubility in organic solvents and water may be partially responsible for the poor oral activity observed.

Acknowledgment

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Supporting Information Available: Elemental analysis results. This material is available free of charge via the Internet at http://pubs.acs.org.

References

Supporting Information

Synthesis and Antimalarial Activity of New Isotebuquine Analogs

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Division of Experimental Therapeutics, Walter Reed Army Institute of Research, 503 Robert Grant Avenue, Silver Spring, Maryland 20910

Elemental Analysis Data

<table>
<thead>
<tr>
<th>Compd</th>
<th>Formula</th>
<th>Calcd</th>
<th>Found</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>C_{26}H_{25}N_{3}OCl_{2} \cdot 0.2 \text{ H}_2\text{O}</td>
<td>C, 66.44; H, 5.45; N, 8.94</td>
<td>C, 66.56; H, 5.40; N, 9.01</td>
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<tr>
<td>1b</td>
<td>C_{27}H_{25}N_{3}OClF_{3}</td>
<td>C, 64.86; H, 5.04; N, 8.40</td>
<td>C, 64.75; H, 5.03; N, 8.35</td>
</tr>
<tr>
<td>2a</td>
<td>C_{28}H_{25}N_{3}OCl_{2}</td>
<td>C, 66.95; H, 5.40; N, 9.01</td>
<td>C, 66.62; H, 5.89; N, 8.42</td>
</tr>
<tr>
<td>2b</td>
<td>C_{27}H_{25}N_{3}OClF_{3}</td>
<td>C, 64.86; H, 5.04; N, 8.40</td>
<td>C, 64.30 H, 5.05; N, 8.20</td>
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<tr>
<td>3a</td>
<td>C_{31}H_{36}N_{4}OCl_{2} \cdot 1.9 \text{ H}_2\text{O}</td>
<td>C, 63.56; H, 6.85; N, 9.56</td>
<td>C, 63.66; H, 6.54; N, 9.46</td>
</tr>
<tr>
<td>3b</td>
<td>C_{32}H_{36}N_{4}OClF_{3}</td>
<td>C, 65.69; H, 6.20; N, 9.58</td>
<td>C, 65.77; H, 6.15; N, 9.47</td>
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<tr>
<td>4a</td>
<td>C_{31}H_{36}N_{4}OCl_{2} \cdot 0.75 \text{ H}_2\text{O}</td>
<td>C, 65.89; H, 6.69; N, 9.92</td>
<td>C, 65.82; H, 6.39; N, 9.75</td>
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<tr>
<td>4b</td>
<td>C_{32}H_{36}N_{4}OClF_{3}</td>
<td>C, 65.69; H, 6.20; N, 9.58</td>
<td>C, 65.37; H, 6.11; N, 9.47</td>
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<td>5b</td>
<td>C_{27}H_{25}N_{3}OClF_{3} \cdot 0.25 \text{ H}_2\text{O}</td>
<td>C, 64.29; H, 5.09; N, 8.33</td>
<td>C, 64.21; H, 4.92; N, 8.24</td>
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<td>11a</td>
<td>C_{21}H_{14}N_{2}OCl_{2} \cdot 0.4 \text{ H}_2\text{O}</td>
<td>C, 64.93; H, 3.84; N, 7.21</td>
<td>C, 64.94; H, 3.49; N, 7.13</td>
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<tr>
<td>11b</td>
<td>C_{22}H_{14}N_{2}OClF_{3} \cdot 0.9 \text{ H}_2\text{O}</td>
<td>C, 61.31; H, 3.69; N, 6.50</td>
<td>C, 61.31; H, 3.49; N, 6.46</td>
</tr>
<tr>
<td>12</td>
<td>C_{21}H_{14}N_{2}OCl_{2} \cdot 0.3 \text{ H}_2\text{O}</td>
<td>C, 62.64; H, 3.65; N, 6.96</td>
<td>C, 62.71; H, 3.58; N, 7.04</td>
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<tr>
<td>13</td>
<td>C_{26}H_{25}N_{3}OCl_{2} \cdot \text{ H}_2\text{O}</td>
<td>C, 62.40; H, 5.44; N, 8.40</td>
<td>C, 62.40; H, 5.40; N, 8.24</td>
</tr>
<tr>
<td>14</td>
<td>C_{31}H_{36}N_{4}OCl_{2} \cdot 0.3 \text{ H}_2\text{O}</td>
<td>C, 65.28; H, 6.55; N, 9.64</td>
<td>C, 65.03; H, 6.51; N, 9.52</td>
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</tbody>
</table>