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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and in vitro and in vivo antimalarial activity of novel 4-anilinoquinoline Mannich base derivatives

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Abstract Development of resistance has severely limited the choice of available antimalarial drugs, which clearly highlights the urgent need of novel chemotherapeutic agents for the treatment of malaria. The purpose of this study was to develop new potential antimalarial agents with 4-anilinoquinoline ring. A series of novel 4-anilinoquinoline Mannich base drug molecules have been synthesized. The synthesis involves the preparation of Mannich base and these bases subsequently coupled with 4,7-dichloroquinoline to get targeted drug molecules (Burckhalter *et al.* in J Am Chem Soc 70:1363–1373, 1948). Their structures were confirmed by IR, NMR, and mass spectral data. The synthesized molecules were evaluated for in vitro and in vivo antimalarial activity against the chloroquine sensitive 3D7 (West Africa) and RKL-2 strain of *Plasmodium falciparum* and rodent malaria parasite *Plasmodium yoelii* (strain N-67) in Swiss mice model, respectively (Charmot *et al.* in Prev Med 15:889, 1986). Except one molecule (containing diphenylamine), all the tested molecules showed activity while one of them (containing morpholine) showed promising in vitro and significant in vivo antimalarial activity under given test conditions.

Keywords 4-Anilinoquinoline · Mannich base · Antimalarial · In vitro · In vivo · *P. falciparum*

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Introduction

Chloroquine (CQ) resistance in *Plasmodium falciparum* malaria has become a major health concern of the developing world. Such resistance has incited a reexamination of the pharmacology of other antimalarial agents that may be effective against resistant strains (Watkins *et al.*, 1984; Olliaro *et al.*, 1996). Amodiaquine (AQ) (**2**), a Mannich base derivative, was come into the market in the 1950s, has been shown to be a superior alternative for CQ (Nevill *et al.*, 1994; Panali *et al.*, 1994; Muller *et al.*, 1996; White, 1996). In 1980s, the use of AQ declined due to agranulocytosis and hepatitis (Douer *et al.*, 1985; Neftel *et al.*, 1986). However, further investigations have shown that AQ is no more toxic than CQ to treat uncomplicated *P. falciparum* malaria with therapeutic dose. The data of clinical trials reflects that adverse drug reactions to AQ are likely to occur only during prophylaxis (White, 1996).

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 (Doberstyn, 1985; Smrkovski *et al.*, 1985; Charmot *et al.*, 1986; LeHesran *et al.*, 1997). 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 mechansim and yet retain activity. This advantage can then be used in the redesign of new Mannich base antimalarials. Amopyroquine (**3**), a structural analog 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 CQ-resistant strains of *P. falciparum* isolates (Thompson *et al*., 1958; Pussard *et al*., 1988). 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**, Fig. 1).

Considering the above said facts, we have designed and synthesized a novel series 4-anilinoquinoline of Mannich base derivatives (Table 4) that could modulate the antimalarial activity.

Materials and methods

Chemistry

All the compounds (BM-1 to BM-7) were synthesized using synthetic grade chemicals of Rankem and Merck without further purification and obtained from commercial suppliers. Analytical thin-layer chromatography (TLC) was performed on aluminum sheets pre-coated with silica gel obtained from Merck. Visualization was accomplished by UV light (254 nm). Infrared (IR) spectra were recorded on Perkin Elmer—Spectrum RX-I and Shimadzu 8201PC spectrophotometers using KBr pellets. The ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Avance—II 400 NMR spectrometer at 400 and 100 MHz, respectively. Chemical shift values are given in δ (ppm) scale using TMS as an internal standard. Significant ^{1}H NMR data are written in order: number of protons, multiplicity (b, broad; s, singlet; d, doublet; t, triplet; m, multiple), coupling constants in hertz, assignment. The mass spectra were recorded on MS 500 IT with 410 Prostar Binary LC, Varian Inc, USA Mass spectrometer. The *m*/*z* values of the more intense peaks were mentioned.

4-(7-Chloro-quinolin-4-ylamino)-2-morpholin-4-ylmethyl-phenol (BM-1)

Step I. 4-Hydroxyacetanilide (0.05 mol) was subjected to a Mannich reaction with a morpholine (0.1 mol) and aqueous formaldehyde (0.1 mol) in ethanol. After reflux (48 h) period, the solvent was removed under reduced pressure, and the residue dissolved in dichloromethane (50 ml). The organic solution was extracted with dilute hydrochloric acid (0.1 M, 2 × 75 ml). This solution was basified (pH 9–10) and extracted with dichloromethane (3 × 75 ml). The combined extracts were washed with water (1 × 100 ml) and dried (anhydrous sodium sulfate) and then solvent was evaporated under reduced pressure to give the product and the crude product was re-crystallized with suitable solvent.

Step II. A solution of Step I product (*N*-(3-substituted-aminomethyl-4-hydroxy-phenyl)-acetamide) (0.005 mol) in hydrochloric acid (20%, 15 ml) was heated under reflux for 1 h. This solution was concentrated under reduced pressure and then co-evaporated with ethanol. The residue was dissolved in ethanol (20 ml), 4,7-dichloroquinoline (0.005 mol) was added, and then the solution heated under reflux for 2 h. The solution was concentrated under reduced pressure to give a viscous residue which was poured into ice-cold ammonium hydroxide (5%, 100 ml). The sticky solid which separated was dissolved in dichloromethane (50 ml) and separated from the basic solution. The organic solution was washed with water (75 ml), dried (anhydrous sodium sulfate), and evaporated to dryness under reduced pressure to give the crude product (pale brown solid, 64.86%), and the product was re-crystallized. M.p. = 171–173°C;^{1}H NMR (400 MHz, $CDCl_3$): δ 8.44 (d, 1H, $J = 4.80$ Hz, quinoline-H), δ 7.98 (d, 1H, $J = 1.60$ Hz, quinoline-H), δ 7.91 (d, 1H, $J = 8.40$ Hz, quinoline-H), δ 7.42 (dd, 1H, $J = 8.4$, 1.6 Hz, quinoline-H), δ 7.10 (d, 1H, $J = 1.20$ Hz, quinoline-H), δ 6.93 (d, 1H, $J = 12.80$ Hz, Ar–H), δ 6.88 (d, 1H, $J = 8.80$ Hz, Ar–H), δ 6.64 (s, 1H, Ar–OH/–NH), δ 6.66 (dd, 1H,

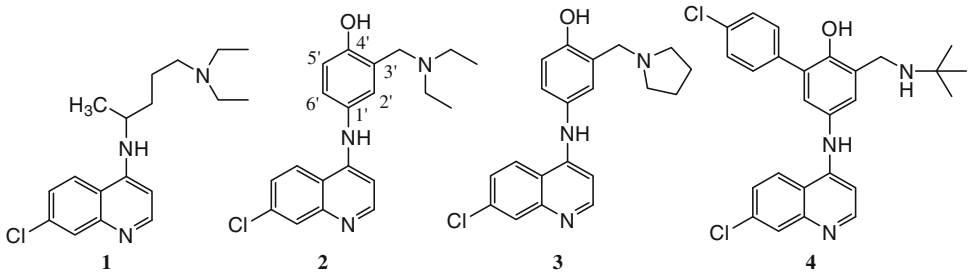


Fig. 1 Chloroquine (**1**), Amodiaquine (**2**), Amopyroquine (**3**), and Tebuquine (**4**)

$J = 5.20, 5.20$ Hz, Ar–H), δ 3.73 (t, 4H, morpholinyl-H), δ 2.54 (s, 2H, CH_2), δ 1.58 (t, 4H, morpholinyl-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 155.63 (C-1), 151.58 (C-2′), 149.59 (C-4′), 149.42 (C-9′), 135.13 (C-4), 129.95 (C-7′), 128.46 (C-8′), 125.81 (C-6′), 125.62 (C-3), 125.40 (C-5), 123.53 (C-2), 121.39 (C-5′), 117.48 (C-6), 117.16 (C-10′), 101.20 (C-3′), 66.64, 59.00, 53.18 (C-7); IR (in KBr disk): 3422, 3201, 1614, 1573, 1454, 1374, 1247, 1116, 1073, 859, 813 cm^{-1}; MS (*m*/*z*): 369.9 (m+).

2-Butylaminomethyl-4-(7-chloro-quinolin-4-ylamino)-phenol (BM-2)

This compound was prepared in a similar manner to BM-1 to give the product as a golden yellow solid residue (73%); m.p. = 59–62°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.69 (d, 1H, $J = 4.80$ Hz, quinoline-H), δ 8.14 (d, 1H, $J = 8.80$ Hz, quinoline-H), δ 7.99 (d, 1H, $J = 3.20$ Hz, quinoline-H), δ 7.44 (dd, 1H, $J = 8.80, 6.00$ Hz, quinoline-H), δ 7.06 (d, 1H, $J = 7.60$ Hz, quinoline-H), δ 6.93 (d, 1H, $J = 15.20$ Hz, Ar–H), δ 6.85 (d, 1H, $J = 8.00$ Hz, Ar–H), δ 6.75 (s, 1H, Ar–OH/–NH), δ 6.63 (dd, 1H, $J = 5.20, 4.40$ Hz, Ar–H), δ 3.37 (s, 2H, CH_2), δ 1.25(s, 1H, amine), δ 3.91 (m, 2H, *n*-butyl-H), δ 2.70 (m, 2H, *n*-butyl-H), δ 1.56 (t, 2H, *n*-butyl-H), δ 1.35 (t, 3H, *n*-butyl-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.48 (C-1), 151.24 (C-2′), 149.79 (C-4′), 149.58 (C-9′), 135.18 (C-4), 129.93 (C-7′), 128.28 (C-8′), 125.61 (C-6′), 125.54 (C-3), 125.30 (C-5), 123.49 (C-2), 121.82 (C-5′), 117.44 (C-6), 117.24 (C-10′), 101.10 (C-3′), 52.13 (C-7), 48.38, 31.44, 20.46, 13.70; IR (in KBr disk): 3423, 3070, 1614, 1575, 1460, 1375, 1256, 1157, 1119, 874, 815, 768 cm^{-1}; MS (*m*/*z*): 356.0 (m+).

4-(7-Chloro-quinolin-4-ylamino)-2-[(diphenylamino)-methyl]-phenol (BM-3)

This compound was prepared in a similar manner to BM-1 to give the product as a light gray solid residue (66.67%); m.p. = 44–46°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.70 (d, 1H, $J = 5.20$ Hz, quinoline-H), δ 8.11 (d, 1H, $J = 1.6$ Hz, quinoline-H), δ 8.00 (d, 1H, $J = 1.6$ Hz, quinoline-H), δ 7.44 (dd, 1H, $J = 4.80, 2.00$ Hz, quinoline-H), δ 7.06 (d, 1H, $J = 8.00$ Hz, quinoline-H), δ δ 6.97 (d, 1H, $J = 2.00$ Hz, Ar–H), δ 6.90 (d, 1H, $J = 7.20$ Hz, Ar–H), δ 6.87 (s, 1H, Ar–OH/–NH), δ 6.68 (dd, 1H, $J = 10.80, 5.20$ Hz, Ar–H), δ 3.85 (s, 2H, CH_2); δ 7.58 (d, 1H, $J = 8.80$ Hz, phenyl-H), 7.01 (d, 1H, $J = 2.80$ Hz, phenyl-H), δ 6.94 (d, 1H, $J = 10.00$ Hz, phenyl-H), ^{13}C NMR (100 MHz, $CDCl_3$): δ 152.50 (C-1), 151.23 (C-2′), 149.66 (C-4′), 149.40 (C-9′), 142.65, 135.65 (C-4), 129.60 (C-7′), 129.25, 128.61 (C-8′), 125.56 (C-6′), 124.97 (C-3), 124.45 (C-5), 123.52 (C-2), 121.42 (C-5′), 120.45, 119.86, 117.81 (C-6), 117.16 (C-10′), 101.13 (C-3′), 40.45 (C-7); IR (in KBr disk): 3405, 3060, 1608, 1560, 1440, 1375, 1310, 1260, 1119, 875, 816 cm^{-1}; MS (*m*/*z*): 452.1 (m+).

4-(7-Chloro-quinolin-4-ylamino)-2-dibutylaminomethyl-phenol (BM-4)

This compound was prepared in a similar manner to BM-1 to give the product as a light yellow solid residue (65.53%); m.p. = 126–128°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.46 (d, 1H, $J = 5.36$ Hz, quinoline-H), δ 7.99 (d, 1H, $J = 2.08$ Hz, quinoline-H), δ 7.86 (d, 1H, $J = 8.92$ Hz, quinoline-H), δ 7.40 (dd, 1H, $J = 2.08, 2.04$ Hz, quinoline-H), δ 7.10 (d, 1H, $J = 2.52$ Hz, quinoline-H), δ 6.92 (d, 1H, $J = 2.4$ Hz, Ar–H), δ 6.86 (d, 1H, $J = 8.48$ Hz, Ar–H), δ 6.75 (s, 1H, Ar–OH/–NH), δ 6.63 (dd, 1H, $J = 5.44, 5.36$ Hz, Ar–H), δ 3.76 (s, 2H, CH_2), δ 2.53 (t, 2H, di-*n*-butyl-H), δ 1.54 (m, 2H, di-*n*-butyl-H), δ 1.32 (m, 2H, di-*n*-butyl-H), δ 0.919 (t, 3H, di-*n*-butyl-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.65 (C-1), 151.83 (C-2′), 149.56 (C-4′), 149.44 (C-9′), 135.28 (C-4), 129.88 (C-7′), 128.74 (C-8′), 125.81 (C-6′), 125.65 (C-3), 125.39 (C-5), 123.56 (C-2), 121.22 (C-5′), 117.47 (C-6), 117.16 (C-10′), 101.37 (C-3′), 58.03, 53.29 (C-7), 28.43, 20.65, 14.04; IR (in KBr disk): 3420, 3205, 2957, 2872, 1614, 1566, 1454, 1371, 1265, 1122, 876, 818, 749 cm^{-1}; MS (*m*/*z*): 412.1 (m+).

4-(7-Chloro-quinolin-4-ylamino)-2-[(diisopropylamino)-methyl]-phenol (BM-5)

This compound was prepared in a similar manner to BM-1 to give the product as a dark brown solid residue (58.33%); m.p. = 131–132°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.37 (d, 1H, $J = 5.20$ Hz, quinoline-H), δ 8.07 (d, 1H, $J = 8.88$ Hz, quinoline-H), δ 7.89 (d, 1H, $J = 1.12$ Hz, quinoline-H), δ 7.34 (dd, 1H, $J = 1.92, 1.92$ Hz, quinoline-H), δ 7.29 (d, 1H, $J = 8.16$ Hz, quinoline-H), δ 6.97 (d, 1H, $J = 7.68$ Hz, Ar–H), δ 6.72 (d, 1H, $J = 8.40$ Hz, Ar–H), δ 6.85 (s, 1H, Ar–OH/–NH), δ 6.58 (dd, 1H, $J = 5.28, 5.20$ Hz, Ar–H), δ 3.76 (s, 2H, CH_2), δ 3.10 (m, 2H, diisopropyl-H), δ 1.05 (d, 12H, diisopropyl-H);^{13}C NMR (100 MHz, $CDCl_3$): δ 157.00 (C-1), 151.84 (C-2′), 149.59 (C-4′), 149.46 (C-9′), 135.13 (C-4), 129.86 (C-7′), 128.68 (C-8′), 125.64 (C-6′), 125.25 (C-3), 125.09 (C-5), 123.60 (C-2), 121.41 (C-5′), 117.51 (C-6), 117.07 (C-10′), 101.30 (C-3′), 49.56, 48.14 (C-7), 19.77; IR (in KBr disk): 3425, 3061, 2970, 2897, 2876, 1612, 1568, 1454, 1367, 1261, 1123, 874, 812 cm^{-1}; MS (*m*/*z*): 384.0 (m+).

4-(7-Chloro-quinolin-4-ylamino)-2-(isopropylamino-methyl)-phenol (BM-6)

This compound was prepared in a similar manner to BM-1 to give the product as a dark brown solid residue (46.78%);

m.p. = 56–57°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.43 (d, 1H, J = 1.00 Hz, quinoline-H), δ 7.99 (d, 1H, J = 2.00 Hz, quinoline-H), δ 7.88 (d, 1H, J = 9.00 Hz, quinoline-H), δ 7.36 (dd, 1H, J = 2.12, 2.12 Hz, quinoline-H), δ 7.07 (d, 1H, J = 2.60 Hz, quinoline-H), δ 6.92 (d, 1H, J = 2.52 Hz, Ar–H), δ 6.87 (d, 1H, J = 4.52 Hz, Ar–H), δ 6.85 (s, 1H, Ar–OH/–NH), δ 6.57 (dd, 1H, J = 4.44, 4.40 Hz, Ar–H), δ 3.97 (s, 2H, CH_2), δ 1.25 (s, 1H, amine), δ 2.91 (m, 1H, isopropyl-H), δ 1.16 (d, 6H, J = 3.52 Hz, isopropyl-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.74 (C-1), 151.79 (C-2′), 149.65 (C-4′), 149.39 (C-9′), 135.16 (C-4), 130.02 (C-7′), 128.59 (C-8′), 125.66 (C-6′), 125.13 (C-3), 124.95 (C-5), 123.61 (C-2), 121.45 (C-5′), 119.93 (C-6), 117.49 (C-10′), 101.29 (C-3′), 49.95, 48.38 (C-7), 22.38; IR (in KBr disk): 3423, 3030, 2968, 2889, 1612, 1574, 1443, 1373, 1254, 1159, 1123, 874, 814 cm^{-1} MS (*m*/*z*): 342.0 (m+).

4-(7-Chloro-quinolin-4-ylamino)-2-*n*-propylamino-methyl-phenol (BM-7)

This compound was prepared in a similar manner to BM-1 to give the product as a brown solid residue (55.56%); m.p. = 58–59°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.43 (d, 1H, J = 5.36 Hz, quinoline-H), δ 7.99 (d, 1H, J = 2.04 Hz, quinoline-H), δ 7.88 (d, 1H, J = 2.80 Hz, quinoline-H), δ 7.42 (dd, 1H, J = 2.12, 2.08 Hz, quinoline-H), δ 7.08 (d, 1H, J = 2.64 Hz, quinoline-H), δ 6.93 (d, 1H, J = 2.60 Hz, Ar–H), δ 6.87 (d, 1H, J = 1.72 Hz, Ar–H), δ 6.90 (s, 1H, Ar–OH/–NH), δ 6.65 (dd, 1H, J = 5.40, 5.28 Hz, Ar–H), δ 3.98 (s, 2H, CH_2), δ 1.25 (s, 1H, amine), δ 2.66 (t, 2H, *n*-propyl-H), δ 1.58 (m, 2H, *n*-propyl-H), δ 0.96 (t, 3H, *n*-propyl-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.70 (C-1), 151.75 (C-2′), 149.66 (C-4′), 149.36 (C-9′), 135.21 (C-4), 130.01 (C-7′), 128.56 (C-8′), 126.45 (C-6′), 125.70(C-3), 125.30 (C-5), 123.62 (C-2), 121.47 (C-5′), 119.94 (C-6), 117.49 (C-10′), 101.31 (C-3′), 52.50, 50.62 (C-7), 22.69, 11.65; IR (in KBr disk): 3424, 3205, 2957, 2874, 1606, 1568, 1445, 1371, 1258, 1121, 878, 816, 740 cm^{-1}; MS (*m*/*z*): 342.0 (m+).

Pharmacology

In vitro antimalarial activity

The in vitro antimalarial assay of synthesized compounds was carried out against two different CQ sensitive strains of *P. falciparum*; first three compounds (BM-1 to BM-3) were evaluated against 3D7 (West Africa) strain in the CDRI, Lucknow, while rest of the compounds (BM-4 to BM-7) were evaluated against MRC-RKL-2 strain in RMRC, Dibrugarh.

Methodology

Giemsa stained slide method (Rieckmann *et al*., 1978), the cultures of *P. falciparum* strain were maintained in medium RPMI 1640 supplemented with 25 mM HEPES, 1% D-glucose, 0.23% sodium bicarbonate, and 10% heat inactivated human serum. The asynchronous parasites of *P. falciparum* were synchronized after 5% D-sorbitol treatment to obtain only the ring stage parasitized cells. For carrying out the assay, an initial ring stage parasitemia of 0.8–1.5% at 3% hematocrit in a total volume of 200 µl of medium RPMI-1640 was uniformally maintained. A stock solution of 5 mg/ml of each of the test samples was prepared in DMSO, and subsequent dilutions were prepared with culture medium. The diluted samples in 20 µl volume were added to the test wells so as to obtain final concentrations (at fivefold dilutions) ranging between 0.4 and 100 µg/ml in duplicate wells containing parasitized cell preparation. The culture plates were incubated at 37°C in a candle jar. After 36–40 h incubation, the blood smears from each well were prepared and stained with Giemsa stain.

For BM-1, BM-2, and BM-3 compounds the slides were microscopically observed to record maturation of ring stage parasites into trophozoites and schizonts in the presence of different concentrations of the test agents. The test concentration which inhibited the complete maturation into schizonts was recorded as the minimum inhibitory concentrations (MICs) (Table 1). While for the BM-4, BM-5, BM-6, and BM-7 compounds, the slides were microscopically observed to record percentage of dead rings and schizonts in the presence of different concentrations of the test agents. The test concentration which showed the complete dead rings and schizonts was recorded (Table 2). Chloroquine was used as the standard reference drug.

In vivo antimalarial activity

The in vivo antimalarial activity of the compound BM-1 was carried out in the CDRI, Lucknow. The aqueous suspensions of the test sample were prepared after making a paste with few drops of Tween 80 and a few drops of water. The volume was adjusted so as to obtain the required dose in 1.0 ml volume. The treatment to a group of 5 *Plasmodium yoelii* infected mice was administered via oral route, once daily for four consecutive days from days 0 to 3. One group of five mice was administered the aqueous vehicle used for preparing suspension and served as untreated control.

The thin blood smears were prepared from each animal on day 4 i.e., 24 h after the last treatment dose. The degree of infection was microscopically recorded in terms of number of *P. yoelii* infected cells per 100 RBC (i.e., percent parasitemia). The mean value determined for a group of five mice was used to calculate the percent suppression

Table 1 Antimalarial activity (in vitro) of synthesized compounds against chloroquine sensitive *P. falciparum* (3D7, West Africa)

Compound	Concentrations evaluated (μg/ml)	Number of parasites/100 infected RBCs			Percent schizont maturation inhibition
		Rings	Trophozoites	Schizonts	
BM-1	100	100	0	0	100
	50	100	0	0	100
	10	100	0	0	100
	2.0	100	0	0	100
	1.0	100	0	0	100
	0.5	100	0	0	100
	0.25	100	0	0	100
	0.125	100	0	0	100
	0.063	***100***	***0***	***0***	***100***
	0.031	68	8	24	73.33
BM-2	100	0	100	0	100
	50	0	100	0	100
	10	***0***	***100***	***0***	***100***
	2.0	55	23	16	82.22
	1.0	40	30	26	71.11
BM-3	100	0	45	55	38.88
	50	0	15	85	0.05
Chloroquine	0.50	100	0	0	100
	0.25	***100***	***0***	***0***	***100***
	0.125	95	3	2	97.1
	0.0625	70	32	8	88.4
Control-1	–	0	10	90	–

Bold-italic values indicate minimum concentration at which hundred percent schizont maturation inhibition

Table 2 Antimalarial activity (in vitro) of synthesized compounds against chloroquine sensitive *P. falciparum* (RKL-2)

Compound	Dosase (μg/ml)	% Dead rings + schizonts[a]
BM-4	100	5.0
BM-5	100	3.0
BM-6	100	6.5
BM-7	100	6.5
Chloroquine (standard)	0.4	54.0

[a] Mean of two replicates. Counted against 100 asexual parasites per replicate

of parasitemia with respect to the untreated control group. The animals which did not show any parasites on day 4 were subsequently monitored twice weekly till day 28 post-infection. The animals which did not develop any infection till day 28 were recorded as cured (Table 3).

Result and discussion

Chemistry

In this study, a series of novel 4-anilinoquinoline Mannich base derivatives were prepared (Fig. 2) by using 4-hydroxyacetanilide as starting materials (Burckhalter *et al*., 1948). In this series, the 3′-diethylamino function of AQ is replaced by a 3′-primary or secondary amino function. The synthesis involves the preparation of Mannich base of the 4-hydroxyacetanilide followed by hydrolysis of the amide function of the Mannich base. The hydrolysis product (Mannich substituted 4-aminophenol) is subsequently coupled with 4,7-dichloroquinoline to provide target compounds (Table 4). The new compounds were characterized by the use of TLC and melting point techniques. Moreover, the structures of the novel compounds were determined using IR, 1H NMR, 13C NMR, and mass spectroscopy.

Pharmacology

Initially, all the compounds (BM-1 to BM-7) were tested for in vitro antimalarial activity against the CQ sensitive 3D7 (West Africa) and RKL-2 strain of *P. falciparum*. All the compounds showed activity but only two compounds (BM-1 and BM-2) showed activity with MIC 0.063 and 10 μg/ml, respectively, as compared to Chloriquine (MIC = 0.25 μg/ml). The antimalarial screening result reflects that the compound (BM-1) with six member heterocyclic ring (Morpholine) at the side chain showed

Table 3 In vivo antimalarial profile of BM-1 against *P. yoelii* N67 in Swiss mice

Dose mg/kg	No. of mice	Percent suppression on day 4	No. of mice showing nil parasitemia on day						Remarks
			4	7	10	14	21	28	
500	5	100	5/5	5/5	5/5	4/5	2/5	2/5	Curative response in 2/5 mice
Control	5	–	0/5						

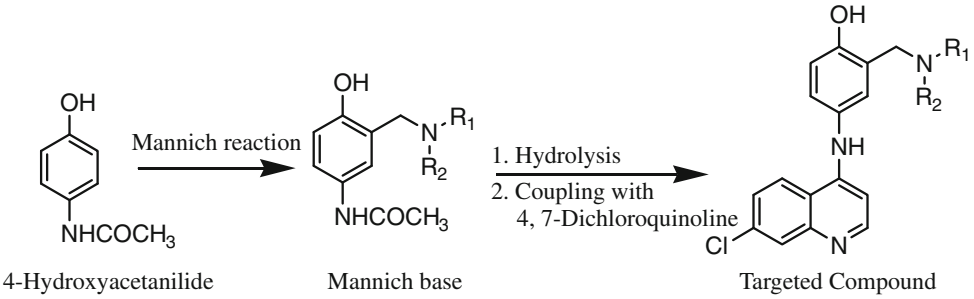
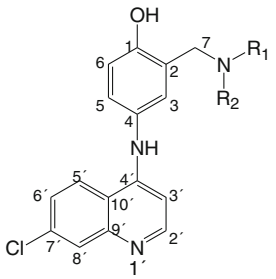


Fig. 2 Reaction scheme for the synthesis of novel 4-anilinoquinoline Mannich base compounds. R_1R_2NH is primary or secondary amines

Table 4 List of the designed compounds



Compounds	R_1	R_2
BM-1	$–(CH_2)_2O(CH_2)_2–$	
BM-2	H	*n*-Butyl
BM-3	Phenyl	Phenyl
BM-4	*n*-Butyl	*n*-Butyl
BM-5	Isopropyl	Isopropyl
BM-6	H	Isopropyl
BM-7	H	*n*-Propyl

promising activity while the compound (BM-2) with aliphatic side chain (*n*-butylamine) showed moderate activity.

Subsequently, compound BM-1 was investigated for antimalarial activity in the in vivo assay against CQ resistant *P. yoelii* at an oral dose of 500 mg/kg/day × 4 days. This compound displayed complete suppression of parasitemia on day 4. Fortunately out of the group, two animals which survived till day 28 were found to be completely cured.

Conclusion

New 4-anilinoquinoline Mannich base derivatives were prepared for evaluation of their antimalarial activity. It was found that morpholine substituted 4-anilinoquinoline Mannich base derivative (BM-1) exhibited strong suppressive activity against CQ resistant *P. yoelii* infection in swice mice.

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