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Synthesis of new arylaminoquinoxalines and their antimalarial activity in mice

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Abstract

2-Arylaminoquinoxalines were prepared by the condensation of 2-chloroquinoxaline with the appropriate Mannich bases in the presence of HCl. To synthesize the Mannich bases, 4-acetamidophenol was reacted with formaldehyde and dialkylamine to yield 3-[(dialkylamino) methyl]-4-hydroxyacetanilide, followed by hydrolysis. Antimalarial activities of the new arylaminoquinoxalines were evaluated against the rodent malaria parasite *Plasmodium yoelii* at a dose of 75 mg kg^{-1}. Three compounds synthesized (2-[3-[(diethylamino) methyl]-4-hydroxy-anilino]-quinoxaline dihydrochloride (**2b**), 2-[3-[(pyrrolidinyl) methyl]-4-hydroxyanilino]-quinoxaline dihydrochloride (**2f**), and 2-[3-[(piperidinyl) methyl]-4-hydroxyanilino]-quinoxaline dihydrochloride (**2g**)) showed moderate antimalarial activity.

Introduction

Malaria remains a major health threat in the world as it has for the last century, and a major cause of death, particularly among children and women, primarily in Africa (Su et al 1999). Chloroquine was the most effective and widely used drug in malaria therapy because of its rapid onset of action, good tolerability and low cost (Hoffman 1996). However, emergence of strains of *Plasmodium falciparum* resistant to chloroquine and many other drugs, including primaquine, pyrimethamine and mefloquine, in succession has stimulated efforts to identify new antimalarial agents. 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. Recent efforts in modifying presently available drugs has led to the discovery of potent molecules that are active against resistant strains of *P. falciparum* (Barlin et al 1993; Ridley et al 1996; De et al 1998; Chibale et al 2000). Amodiaquine (**1**, Figure 1), a Mannich base derivative, has been shown to be a superior alternative to chloroquine in areas of high chloroquine resistance (Churchill et al 1985). Subsequent research into the synthesis of Mannich base compounds containing side chains afforded many diverse Mannich base derivatives (Raynes et al 1999). Tebuquine, a hybrid compound combining structural elements of amodiaquine and some 2-(dialkyl-amino)-o-cresol derivatives have shown more potent activity than amodiaquine (Werbel et al 1986).

From time to time reports of antimalarial activity with quinoxaline derivatives have appeared in the literature (Haworth & Robinson 1948; Growther et al 1949).

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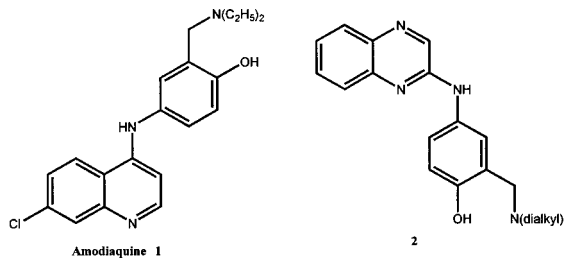

Figure 1 The structures of amodiaquine **1** and the new arylaminoquinoxalines **2**.

In light of these observations, we have attempted to combine a quinoxaline system with a series of Mannich base derivatives to develop new arylaminoquinoxalines (**2**, Figure 1) for antimalarial evaluation.

Materials and Methods

Chemical procedures

All melting points were determined on a Buchi model 530 melting point apparatus and were uncorrected. Infrared spectra were recorded on a JASCO IR report-100 infrared spectrophotometer and were given in cm^{-1}. ^1H NMR spectra were recorded using a Bruker AC 300F NMR spectrometer. Chemical shifts were reported in parts per million (δ) relative to tetramethylsilane as internal standard. All the HCl salts were characterized by elemental analyses. Elemental analyses are indicated by the symbols of the elements and were within ± 0.4 of the theoretical values. Hydrochloride salts were obtained by the dropwise addition of a saturated, anhydrous solution of ethereal HCl into a cold solution of the free base in anhydrous ether until further addition of ethereal HCl did not produce any more precipitate. Reactions were performed under a nitrogen atmosphere.

2-Hydroxyquinoxaline (3)

A mixture of n-butylgyloxylate (12.75 g, 0.10 mol) and o-phenylenediamine (10.8 g, 0.10 mol) in ethanol (100 mL) was heated at reflux for 2 h. The reaction mixture was cooled to give 2-hydroxyquinoxaline. The product was purified by dissolving in 10% NaOH solution (125 mL), treated with charcoal and then precipitated by addition of dilute HCl to give the title compound. Yield 11.4 g, 78%, mp 268–269°C (Atkenson et al (1956): 267–269°C).

2-Chloroquinoxaline (4)

Phosphorous oxychloride (75 mL) was added slowly dropwise to 2-hydroxyquinoxaline (**3**) (14.6 g, 0.10 mol) at 0°C. A few drops of DMF were added to the reaction mixture and heated to reflux for 45 min. Excess of phosphorous oxychloride was removed under reduced pressure and the residue was then poured into crushed ice and extracted with Et_2O (5×50 mL), washed with H_2O, dried ($MgSO_4$) and evaporated to give 2-chloroquinoxaline and was recrystallized from n-pentane to give a pure compound. Yield 14.5 g, 88%; mp 46–48°C (Gowenlock et al (1945): 46°C)

General procedure for the preparation of 2-[3-[(dialkylamino) methyl]-4-hydroxyanilino]-quinoxaline dihydrochloride (2a–2j)

A mixture of 4-acetamidophenol (3.8 g, 25 mmol), 37% formaldehyde (2.0 mL) and dialkylamine (25 mmol) suspended in alcohol (50 mL) was heated on a steam bath for 3 h. The volatile materials were removed under reduced pressure, 20% HCl (20 mL) was added and heated at refluxing temperature for 3 h. The reaction mixture was cooled. The pH was adjusted to alkaline with ammonia solution, extracted with CH_2Cl_2 (4×30 mL), washed with H_2O, dried ($MgSO_4$) and evaporated to give the free amine. The mixture of the above amine, 2-chloroquinoxaline (25 mmol) was dissolved in ethanol (20 mL), pH was adjusted to pH 4 with hydrochloric acid and was heated to reflux for 20 h. The reaction mixture was cooled and pH was adjusted to alkaline with NH_4OH and extracted with CH_2Cl_2 (4×50 mL), dried ($MgSO_4$), evaporated under pressure and purified by column chromatography (CH_2Cl_2/CH_3OH, 20:1) to yield freebase and the freebase was converted to their respective hydrochloride salts.

2-[3-[(Dimethylamino) methyl]-4-hydroxyanilino]-quinoxaline dihydrochloride (2a)

Yield 49%; mp 86–88°C. IR (cm^{-1}): 3522 (OH), 3216 (NH), 1507, 1375 (aromatic). ^1H NMR (DMSO-d_6, freebase): δ 2.4 (s, 6H, $2 \times CH_3$), 3.7 (s, 2H, CH_2), 6.9–8.4 (m, 8H, ArH), 11.6 (bs, 1H, OH). Anal. ($C_{17}H_{18}N_4O$. 2HCl) C, H, N.

2-[3-[(Diethylamino) methyl]-4-hydroxyanilino]-quinoxaline dihydrochloride (2b)

Yield 58%; mp 249–251°C. IR (cm^{-1}): 3390 (OH), 3198 (NH), 1546, 1418 (aromatic). ^1H NMR (DMSO-d_6, freebase): δ 1.1 (t, 6H, $2 \times CH_3$), 2.4 (m, 4H, $2 \times CH_2$),

4.1 (s, 2H, CH_2), 6.7–8.3 (m, 8H, ArH), 11.8 (bs, 1H, OH). Anal. ($C_{19}H_{22}N_4O$. 2HCl.0.25H_2O) C, H, N.

2-[3-[(Dipropylamino) methyl]-4-hydroxyanilino]-quinoxaline dihydrochloride (2c)

Yield 73%; mp 271–273°C. IR (cm^{-1}): 3342 (OH), 3163 (NH), 1561, 1459 (aromatic). ^{1}H NMR (DMSO-d_6, freebase): δ 0.98 (t, 6H, $2 \times CH_3$), 1.4 (m, 4H, $2 \times CH_2$), 2.6 (m, 4H, $2 \times CH_2$), 3.6 (s, 2H, CH_2), 6.7–8.2 (m, 8H, ArH), 12.0 (bs, 1H, OH). Anal. ($C_{21}H_{26}N_4O$. 2HCl) C, H, N.

2-[3-[(Diisopropylamino) methyl]-4-hydroxyanilino]-quinoxaline dihydrochloride (2d)

Yield 64%; mp 209–211°C. IR (cm^{-1}): 3436 (OH), 3184 (NH), 1459, 1376 (aromatic). ^{1}H NMR (DMSO-d_6, freebase): δ 1.1 (m, 12H, $4 \times CH_3$), 1.37 (s, 1H, CH), 2.61 (q, 2H, CH_2), 3.97 (s, 2H, CH_2), 6.62–9.42 (m, 8H, ArH), 11.98 (bs, 1H, OH). Anal. ($C_{21}H_{26}N_4O$. 2HCl) C, H, N.

2-[3-[(Dibutylamino) methyl]-4-hydroxyanilino]-quinoxaline dihydrochloride (2e)

Yield 66%; mp 280–282°C. IR (cm^{-1}): 3361 (OH), 3114 (NH), 1512, 1459 (aromatic). ^{1}H NMR (DMSO-d_6, freebase): δ 0.9 (t, 6H, $2 \times CH_3$), 1.89 (m, 8H, $4 \times CH_2$), 2.89 (m, 4H, $2 \times CH_2$), 3.93 (s, 2H, CH_2), 6.47–8.49 (m, 8H, ArH), 12.2 (bs, 1H, OH). Anal. ($C_{23}H_{30}N_4O$. 2HCl) C, H, N.

2-[3-[(Pyrrolidinyl) methyl]-4-hydroxyanilino]-quinoxaline dihydrochloride (2f)

Yield 54%; mp 276–278°C. IR (cm^{-1}): 3384 (OH), 3207 (NH), 1558, 1543 (aromatic). ^{1}H NMR (DMSO-d_6, freebase): δ 1.74 (m, 4H, $2 \times CH_2$), 2.49 (m, 4H, $2 \times CH_2$), 3.78 (s, 2H, CH_2), 6.4–8.6 (m, 8H, ArH), 11.84 (bs, 1H, OH). Anal. ($C_{19}H_{20}N_4O$. 2HCl.0.5H_2O) C, H, N.

2-[3-[(Piperidinyl) methyl]-4-hydroxyanilino]-quinoxaline dihydrochloride (2g)

Yield 49%; mp 261–263°C. IR (cm^{-1}): 3405 (OH), 3190 (NH), 1524, 1477 (aromatic). ^{1}H NMR (DMSO-d_6, freebase): δ 1.68 (m, 6H, $3 \times CH_2$), 2.52 (m, 4H, $2 \times CH_2$), 3.73 (s, 2H, CH_2), 6.5–8.1 (m, 8H, ArH), 11.92 (bs, 1H, OH). Anal. ($C_{20}H_{22}N_4O$. 2HCl.0.25H_2O) C, H, N.

2-[3-[(N-2-Methyl-piperdinyl) methyl]-4-hydroxyanilino]-quinoxaline dihydrochloride (2h)

Yield 43%; mp 178–180°C. IR (cm^{-1}): 3428 (OH), 3188 (NH), 1561, 1493 (aromatic). ^{1}H NMR (DMSO-d_6, freebase): δ 0.97 (s, 3H, CH_3), 1.1–1.7 (m, 6H, $3 \times CH_2$), 2.42 (t, 2H, CH_2), 2.71 (m,1H, CH), 3.92 (s, 2H, CH_2), 6.9–8.3 (m, 8H, ArH), 11.82 (bs, 1H, OH). Anal. ($C_{21}H_{24}N_4O$. 2HCl) C, H, N.

2-[3-[(N-Methylpiperazin-1-yl) methyl]-4-hydroxyanilino]-quinoxaline dihydrochloride (2i)

Yield 41%; mp 174–176°C. IR (cm^{-1}): 3386 (OH), 3212 (NH), 1558, 1458 (aromatic). ^{1}H NMR (DMSO-d_6, freebase): δ 1.12 (s, 3H, CH_3), 2.6–2.95 (m, 8H, $4 \times CH_2$), 3.69 (s, 2H, CH_2), 6.7–8.1 (m, 8H, ArH), 11.87 (bs, 1H, OH). Anal. ($C_{20}H_{23}N_5O$. 2HCl) C, H, N.

2-[3-[(Morpholinyl) methyl]-4-hydroxyanilino]-quinoxaline dihydrochloride (2j)

Yield 48%; mp 279–281°C. IR (cm^{-1}): 3386 (OH), 3212 (NH), 1558, 1458 (aromatic). ^{1}H NMR (DMSO-d_6, freebase): δ 2.34–2.46 (m 4H, $2 \times CH_2$), 3.31 (m, 4H, $2 \times CH_2$), 4.20 (s, 2H, CH_2), 6.1–8.5 (m, 8H, ArH), 11.98 (bs, 1H, OH). Anal. ($C_{19}H_{20}N_4O_2$. 2HCl) C, H, N.

Antimalarial activity

The institutional ethics committee of the Birla Institute of Technology and Science, Pilani, India, approved the animal experiments.

The in-vivo studies were carried out following the method described by Puri & Dutta (1989) for anti-malarial evaluation of new arylamine derivatives. *Plasmodium yoelii nigerensis* was obtained from the National Malaria Research Center, New Delhi, India. Swiss albino mice (27 ± 2 g) were allowed free access to water and food. For the evaluation of blood schizontocidal activity against rodent malaria, the mice, in groups of five, were inoculated with 1×10^6 parasites. Inoculum for infection was prepared from the previously infected donor mouse with rising parasitaemia (20%). Blood was drawn from the heart of an infected mouse under anaesthesia in a sterile, heparinized disposable

Table 1 Antimalarial activity of 2-arylaminoquinoxalines against blood-induced *Plasmodium yoelli* in mice.

Compound	NR	Mean day four parasitaemia (mean ± s.e.m.)	Mean survival time (mean ± s.e.m.)
2a	Dimethylamino	20.19 ± 3.6	5.50 ± 1.6
2b	Diethylamino	10.23 ± 0.96*	10.54 ± 1.92*
2d	Diisopropylamino	26.64 ± 2.48	5.94 ± 0.88
2e	Dibutylamino	24.48 ± 4.22	6.12 ± 2.20
2f	Pyrrolidinyl	9.42 ± 4.02*	11.20 ± 0.48*
2g	Piperidinyl	4.96 ± 1.08*	13.24 ± 2.24*
2h	N-(2-methyl) piperidinyl	21.79 ± 2.12	6.40 ± 1.48
2i	N-methylpiperazinyl	20.08 ± 3.16	7.12 ± 2.18
2j	Morpholinyl	16.38 ± 1.12	8.96 ± 2.96
Chloroquine		−ve	> 15
Control		23.12 ± 3.2	6.18 ± 0.46

*$P < 0.05$ compared with the control group.

syringe. The test compounds, 2-arylaminoquinoxalines at 75 mg kg^{-1}, were administered orally. The compounds were triturated with 1% w/v tragacanth solution for uniform mixing and finally reconstituted with water. The drug treatment was administered for four days from day 0 to day 3. Blood smears were prepared on day 4, fixed in methanol and stained with giemsa stain. The slides were observed under oil immersion lens (1000 ×) to obtain the percentage of parasitaemia. A group of five infected mice served as negative control, while a similar test with mice treated with chloroquine 20 mg kg^{-1} for four days served as a positive control. The percentage parasitaemia on day 4 and extension of mean survival time of treated mice was interpreted as evidence of antimalarial activity. Table 1 shows the antimalarial activity of the new 2-arylaminoquinoxaline derivatives.

Statistical analysis

The data were expressed as mean ± s.e.m. Statistical tests were performed by one-way analysis of variance followed by Dunnett's test for multiple comparisons of test groups with control group. The significance level was $P < 0.05$.

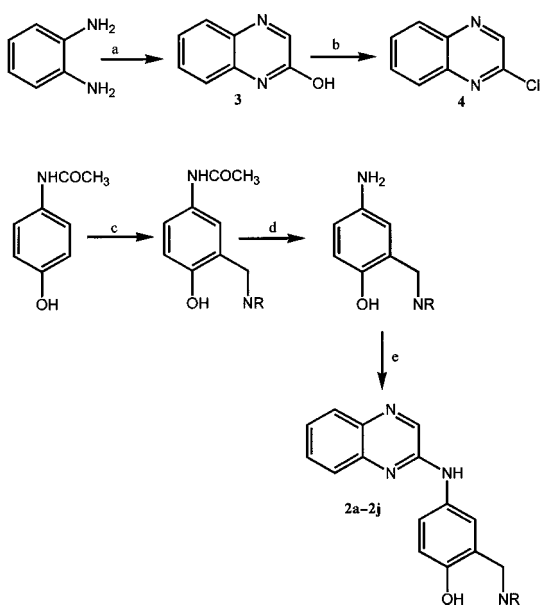

Figure 2 Synthesis of 2-arylaminoquinoxalines (**2a–2j**). Reagents are : a, n-butylgyloxalate, ethanol, reflux; b, $POCl_3$, reflux; c, HCHO, dialkylamine (Mannich Reaction), ethanol, reflux; d, 6 M HCl, reflux; e, 2-chloroquinoxaline (**4**), pH 4, ethanol.

Results and Discussion

The arylaminoquinoxalines were prepared by the condensation of 2-chloroquinoxaline with Mannich arylamines (Figure 2). 2-Chloroquinoxaline (**4**) was synthesized according to the method of Gowenlock et al (1945) by chlorinating 2-hydroxyquinoxaline (**3**) with $POCl_3$ in high yield. 2-Hydroxyquinoxaline was prepared according to the method of Atkenson et al (1956) by reacting o-phenylenediamine and n-butylgyloxylate. The procedure of Burckhalter et al (1948) was used for the synthesis of 4-hydroxy-3-[(dialkylamino) methyl] ani-

lines. Reactions of equimolar quantities of 4-hydroxy acetanilide, formaldehyde and requisite amine were refluxed in ethanol to yield 4-hydroxy-3-[(dialkylamino) methyl] acetanilide. Acetanilide derivatives were converted to free amines using 20% hydrochloric acid. After hydrolysis, the pH of the reaction mixture was adjusted to pH 4 and refluxed with 2-chloroquinoxaline for 20 h in ethanol to bring about the condensation of the in-situ free amine (Banks 1944).

All the compounds were tested for their antimalarial activity in mice infected with *P. yoelii*. The antimalarial activity of the 2-arylaminoquinoxalines is shown in Table 1. From the ten compounds tested, only compounds **2b**, **2f** and **2g** showed moderate antimalarial activity at 75 mg kg^{-1}. The mean survival time of the mice treated with chloroquine was more than 15 days, where as the mean survival time of control mice was 6.18 days. Arylaminoquinoxalines containing diethylamine, pyrrolidine and piperidine moieties were found to have better antimalarial activity than the other amines. However, none of the tested compounds had significant antimalarial activity when compared with chloroquine.

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