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Bioorganic & Medicinal Chemistry Letters 15 (2005) 73–76
Bioorganic & Medicinal Chemistry Letters

Design, synthesis and evaluation of 5-substituted amino-2,4-diamino-8-chloropyrimido-[4,5-*b*]quinolines as novel antimalarials

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Received 7 August 2004; accepted 12 October 2004
Available online 2 November 2004

Abstract—Novel 5-substituted amino-2,4-diamino-8-chloropyrimido-[4,5-*b*]quinolines were designed based on a pharmacophore developed for potent antimalarial activity using Chem-X and MOE softwares. The designed molecules were synthesized by following a novel route and were evaluated by Rane's test for blood schizonticidal activity in mice infected by *Plasmodium berghei*. Based on the Mean Survival Time (MST) data, of the nine compounds evaluated, three had curative potential when compared with chloroquine.

Malaria is a serious health problem and as per the recent report of WHO, there are 300 million new cases of malaria every year and about a million children die each year of the disease.[1] Moreover, drug resistance is a serious problem in malaria and it can be attributed to the use of single drug (monotherapy) for treatment and to the adaptation of the malarial parasite by developing alternate pathways for survival. Hence, the present strategy for new drug development is directed towards identifying the essential enzyme systems in the parasite and developing molecules to inhibit them. With this in view, the inactivation of potent drugs like chloroquine by malarial parasites has been studied in depth.[2]

The present work is aimed towards developing novel molecules with improved potential for treating malaria and with decreased probability for developing drug resistance. It is proposed to achieve this by generating a common pharmacophore from the structures of potent antimalarials belonging to different classes, by designing novel molecules, synthesizing and evaluating them.

A three-point pharmacophore was developed by using structures of potent antimalarials like chloroquine (blood schizonticidal activity), pyrimethamine (tissue schizonticide), quinacrine (gametocidal activity against *P. vivax* and *P. malariae*), sulfadoxine (long duration of action against blood schizonts) and pyronaridine (inhibits *P. falciparum* topoisomerase II).[3] The study was carried out using Chem-X[4] and Molecular Operating Environment (MOE)[5] softwares on Pentium IV 1.6 GHz computer. The conformers of each molecule were generated by using dynamic simulations at the temperature of 310 °C and the sampling time was taken as 5×10^{-4} s. The lowest energy conformations were identified for each molecule and were then used for arriving at a three-point pharmacophore. The basic nucleus, 2,4,5-triamino-8-chloropyrimido-[4,5-*b*]quinoline, was built using the features of the pharmacophore.

Synthesis of the basic nucleus is outlined in Scheme 1. Reaction of guanidine nitrate **1** with malononitrile **2** in the presence of sodium alkoxide in dry ethanol or methanol yielded 2,4,6-triaminopyrimidine **3** (mp 249 °C). This was condensed with 2,4-dichlorobenzoic acid **4**, in the presence of activated copper bronze powder at 180–190 °C to yield N-(2,4-diamino-6-pyrimidino)-4-chloroanthranilic acid **5** (mp 181 °C) which on cyclization using concentrated sulfuric acid yielded 5-oxo-(10*H*)-2,4-diamino-8-chloropyrimido-[4,5-*b*]quinoline **6** (mp 160 °C). This was reacted with phosphorous oxychloride to get 2,4-diamino-5,8-dichloropyrimido-[4,5-*b*]quinoline **7** (mp 151 °C) which on stirring with liquor ammonia at room temperature gave 2,4,5-triamino-8-chloropyrimido-[4,5-*b*]quinoline **8** (mp 200 °C, dec.).

Keywords: Malaria; Pyrimido-[4,5-*b*]quinolines; Antimalarials.
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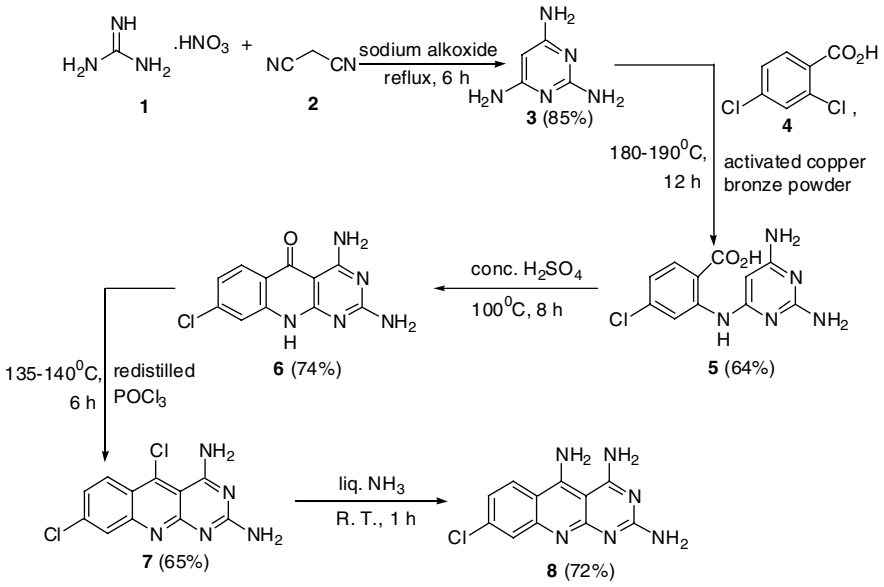
doi:10.1016/j.bmcl.2004.10.037



Scheme 1. Synthesis of 2,4,5-triamino-8-chloropyrimido-[4,5-*b*]quinoline **8**.

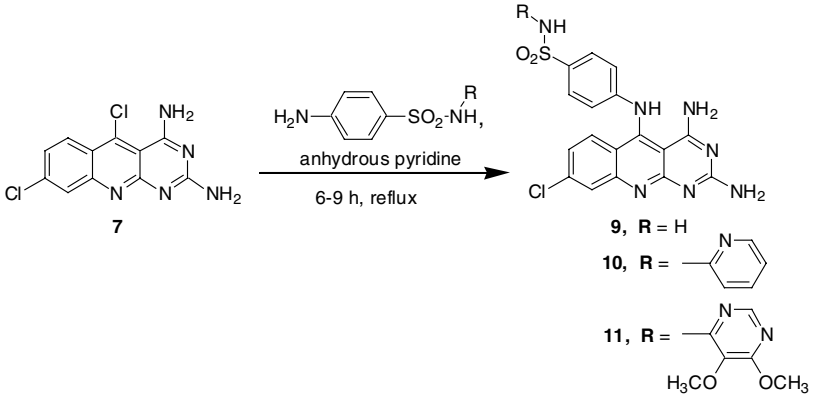
Compound **8** was found to be active against *P. berghei* infected mice in vivo at 160 mg/kg.

In order to achieve enhanced potency, compound **7** was condensed with substituted aminobenzenesulfonamides in the presence of dry pyridine to get compounds **9**–**11** (Scheme 2). Similarly, on condensing compound **7** with 3-substituted and 3,5-disubstituted 4-hydroxyanilines gave compounds **12** and **14**, and, **13** and **15**, respectively (Scheme 3). Finally reaction of compound **7** with methyl- and ethyl-amines and then with formaldehyde and *t*-butyl hydroperoxide gave compounds **16** and **17**, respectively (Scheme 4). All the compounds were purified by silica gel column chromatography. The physical constants and spectral characteristics of the final compounds are mentioned in Table 1.

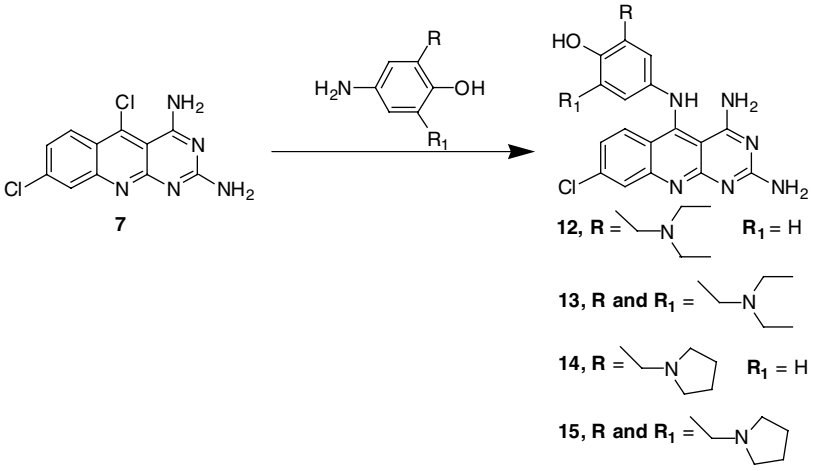
During the synthesis of compound **5**, activated copper bronze gave improved yields and reduced reaction time when compared to the use of copper powder. Cyclization of compound **5** with conc. H_2SO_4 gave good quality product compared to reaction using polyphosphoric acid. Attempts at cyclization and halogenation of compound **5** using phosphorous oxychloride in a single step led to a drastic reduction in yield.

The final compounds **8**–**17** were evaluated for antimalarial activity in vivo against virulent strains of *Plasmodium berghei* in mice using Rane's blood schizonticidal method described by Osdene et al.[6] Four-week old mice weighing 18–20 g each received intra-peritoneal inoculum of 1×10^6 parasitized red blood cells. A group of five infected mice were kept as the control group. The test solutions of the synthesized compounds and chloroquine were prepared in water with the addition of one drop of 1% Tween 80 and were injected to mice subcutaneously 72 h post-infection. The dose range selected was 20, 40, 80 and 160 mg/kg for the test compounds and 20 mg/kg for chloroquine as the standard group and five mice per dose were used. Mean Survival Time (MST) was calculated for control, the standard and test groups and based on this, compounds were classified as active, curative and inactive (Table 2).

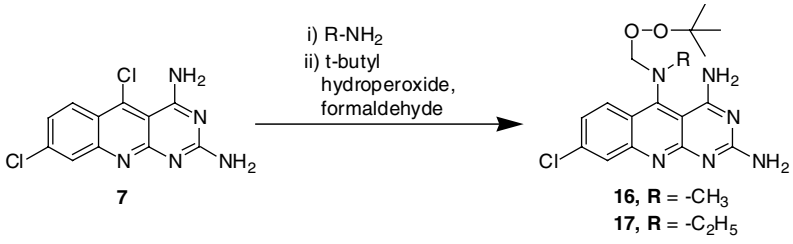
From the results it may be observed that among the aminobenzenesulfonamides, the compound with bulky



Scheme 2. Synthesis of compounds **9**–**11**.

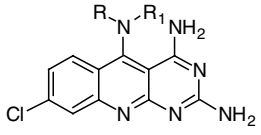


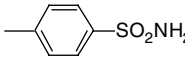
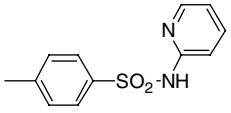
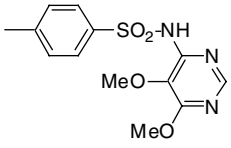
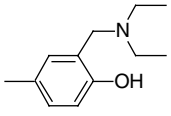
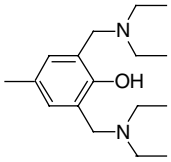
Scheme 3. Synthesis of compounds **12**–**15**.



Scheme 4. Synthesis of compounds **16** and **17**.

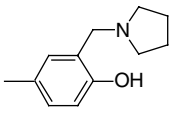
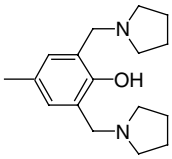
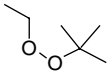
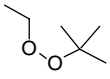
Table 1. Physical constants and spectral data of the final compounds



Compd	R	R_1	Melting point (°C)	Yield (%)	^{1}H NMR (δ in ppm)
9		H	159	60	δ 2.5 (s, 1H, –NH), δ 7.5–7.9 (m, 7H, Ar-H)
10		H	166	48	δ 2.5 (s, 1H, –NH), 3.1 (s, 4H, –NH_2), 7.5–7.9 (m, 11H)
11		H	178	72	δ 2.5 (s, 1H, –NH), 3.6 (s, 3H, –OCH_3), 3.9 (s, 3H, –OCH_3), 6.5 (s, 2H, Ar-H), 6.6 (s, 2H, Ar-H), 7.5–8.4 (m, 4H, Ar-H)
12		H	165	65	δ 1.00 (t, 6H, –CH_2–CH_3), 2.3 (q, 4H, CH_2–CH_3), 2.5 (s, 1H, –NH), 3.6 (s, 4H, –CH_2–), 4.9 (br s, 1H, O–H), 7.5–7.9 (m, 6H, Ar-H)
13		H	191	70	δ 0.9 (t, 12H, –CH_2–CH_3), 2.2 (q, 8H, CH_2–CH_3), 2.5 (s, 1H, –NH), 3.4 (s, 4H, –CH_2–), 5.0 (br s, 1H, O–H), 7.5–7.9 (m, 5H, Ar-H)

(*continued on next page*)

Table 1 (*continued*)

Compd	R	R_1	Melting point (°C)	Yield (%)	^{1}H NMR (δ in ppm)
14[a]		H	235	57	δ 1.5 (s, 4H, –CH_2– of pyrrolidine), 2.2 (s, 4H, –CH_2–N), 2.4 (s, 1H, –NH), 3.6 (s, 2H, –CH_2–), 5.2 (br s, 1H, O–H), 7.4–7.9 (m, 6H, Ar-H)
15		H	182	66	δ 1.52 (s, 8H, –CH_2– of pyrrolidine), 2.0 (s, 8H, –CH_2–N), 2.4 (s, 1H, –NH), 3.4 (s, 4H, –CH_2–), 5.0 (br s, 1H, O–H), 7.4–7.9 (m, 5H, Ar-H)
16	–CH_3		210	72	δ 1.2 (s, 9H, C–(CH_3)$_3$), 2.2 (s, 3H, N–CH_3), 5.3 (s, 2H, N–CH_2–O), 7.4, 7.6, 8.0 (s, 3H, Ar-H)
17	–C_2H_5		232	77	δ 1.0 (q, 3H, N–CH_2–CH_3), 1.4 (s, 9H, C–(CH_3)$_3$), 3.2 (t, 2H, N–CH_2–CH_3), 5.3 (s, 2H, N–CH_2–O), 7.4, 7.6, 8.0 (s, 3H, Ar-H)

[a] Isolated as hydrochloride salt.

Table 2. In vivo antimalarial activity of compounds against *P. berghei* in mice

Compd	Untreated control (days)	Mean survival time (days) at doses (mg/kg)				Remarks
		20	40	80	160	
8	10	15	16	18	20	Active (160 mg/kg)
9	11	12	13	15	14	Inactive
10	10	11	14	15	13	Inactive
11	10	>60	>60	>60	>60	Curative (all doses)
12	11	12	13	15	14	Inactive
13	13	>60	>60	>60	>60	Curative (all doses)
14	12	15	20	25	26	Active (80, 160 mg/kg)
15	11	>60	>60	>60	>60	Curative (all doses)
16	14	28	30	20	14	Active (20, 40 mg/kg)
17	10	13	12	14	15	Inactive
Chloroquine	10	15[a]	—	—	—	Curative (20 mg/kg)

[a] Only three mice died from this group.

substitution (compound **11**) was curative while among the substituted aminophenols the disubstituted compounds (**13** and **15**) were curative. Hence it may be concluded that the nature and size of the substituents at the 5-amino function in the pyrimidoquinoline class of compounds significantly influences the antimalarial activity.

Acknowledgements

The authors thank University Grants Commission (UGC), New Delhi for the financial support and Dr. (Mrs.) V. R. Kamath of Haffkine Institute, Parel, Mumbai 400012 for antimalarial evaluation.

References and notes

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