Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 3587–3589

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Isolation and Antimalarial Activity of Peroxydisulfate Oxidation Products of Primaquine

Virendra K. Dua,[a,*] Sukesh N. Sinha,[a] Sukla Biswas,[b] N. Valecha,[b] S. K. Puri[c] and V. P. Sharma[b]

[a]*Malaria Research Centre, Field Station, Sector III, BHEL, Ranipur, Hardwar-249 403, India*
[b]*Malaria Research Centre, 22 Sham Nath Marg, Delhi-110 054, India*
[c]*Central Drug Research Institute, Lucknow, India*

Received 5 June 2002; accepted 12 September 2002

Abstract—Five compounds formed by peroxydisulfate oxidation of primaquine were isolated using chromatographic methods and evaluated for antimalarial activity in vitro. One compound 6-methoxy-5,8 bis(4′-amino-1′-methylbutylamino)quinoline [**P_1**] was found to have good gametocytocidal activity against *Plasmodium yoelli* infected mice at 10 mg kg^{-1} dose in vivo.

Primaquine, an 8-aminoquinoline, is the clinical drug of choice for the radical cure of relapsing malaria.[1] However, its usefulness has been restricted by toxic side effects, especially with patients deficient in glucose-6-phosphate dehydrogenase.[2] Therefore, there is always a recognized need for investigations of new, less toxic tissue schizontocide. Several analogues of primaquine have been synthesized, using chemical[3–6] and oxidative[7] methods, and tested for their antimalarial activity but none was found better than primaquine. We have isolated five compounds formed by the peroxydisulfate oxidation of primaquine using chromatographic methods and tested for antimalarial activity. In vitro gametocytocidal studies showed that two compounds have more gametocytocidal activity than primaquine while in vivo results indicated only one compound with gametocytocidal activity against *P. yoelli* infected mice. The results are reported here.

Chemistry

Primaquine, on oxidation with peroxydisulfate ion in neutral medium gave pale yellow to orange, violet and than yellow colour within one h after initiation of reaction. Five compounds were isolated in greater than 90% purity using Bio-Gel P-2 column chromatography and HPLC from the reaction mixture.[8,9] The structures of all compounds were determined using IR, MS and ^{1}H NMR studies and are given in Figure 1.

Biological Evaluation[10]

Antimalarial activity: in vitro

Five compounds isolated from the oxidation of primaquine were tested for their in vitro schizontocidal and gametocytocidal activity at different concentrations. Results are given in Table 1. Compounds **P_1** and **P_2** showed higher gametocytocidal activity than primaquine, while the compounds **P_3**, **P_4** and **P_5** had lower activity than primaquine or no gametocytocidal effects. The IC_{50} and IC_{90} of compound P_1 were 0.026 and 0.055 μg/well respectively while of compound **P_2** were 0.036 and 0.062 μg/well, respectively. The schizontocidal activity of all five compounds were many fold lower than chloroquine. However, the schizontocytocidal activity of compounds **P_1** and **P_2** were more than primaquine.

Effort were made to find structure-gemetocytocidal activity correlation of these compounds with reference to primaquine. Results indicates that a substitution at 5-position of primaquine with 4-amino 1-methylbutylamine had increased 4-fold gametocytocidal activity of primaquine. Similarly a substitution at 5 position with another molecule of primaquine also increased 4-fold

*Corresponding author. E-mail: virendradua@hotmail.com

0960-894X/02/$ - see front matter
PII: S0960-894X(02)00802-8

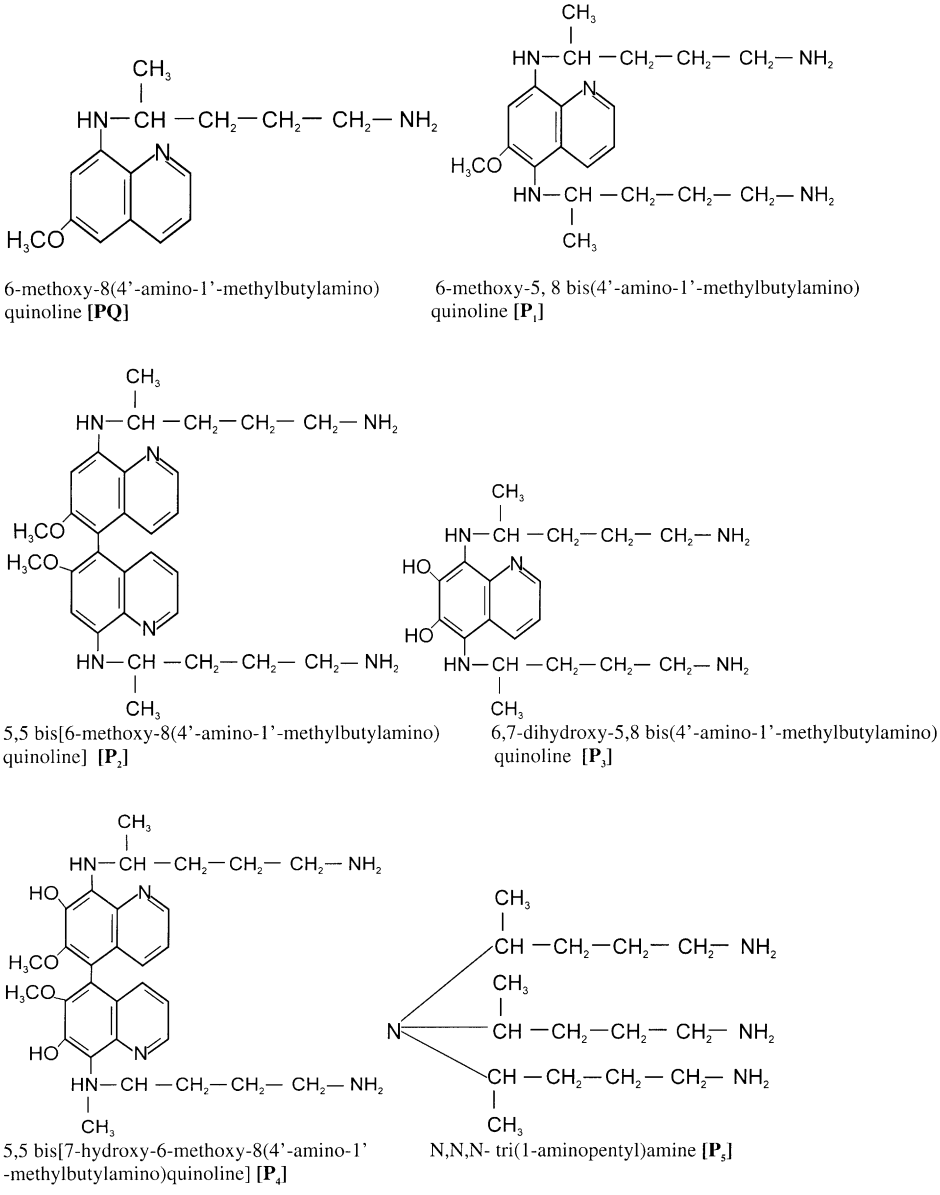

Figure 1.

Table 1. In vitro antimalarial activity of primaquine (PQ) and 5 oxidation products on *P. falciparum*

Activity		Compd concentration (μg/well)						
		CQ	**PQ**	**P_1**	**P_2**	**P_3**	**P_4**	**P_5**
Gamatocidal	IC_{50}		0.115	0.026	0.036	>150	>150	0.115
	IC_{90}	—	0.38	0.055	0.062	>150	>150	0.38
Schizontocidal	IC_{50}	0.011	nd	0.046	0.031	>50	0.49	0.155
	IC_{90}	0.015	nd	0.235	0.19	>50	1.75	0.245

gametocytocidal action. It is to note that *N*-acetyl primaquine and dimmer of *N*-acetyl primaquine were found inactive by in vitro studies,[11,12] which implied that terminal amino group on the side chain of primaquine may be necessary for antimalarial activity. An addition of –OH group to **P_1** and **P_2** decreased antimalarial activity substantially. Similar observation was also recorded earlier for hydroxyprimaquine derivatives.[13]

In vivo

The compounds **P_1** and **P_2** were tested for in vivo gametocytocidal activity against *P. yoelli* infected mice. Results are given in Table 2 which revealed that compound **P_1** showed good gametocytocidal activity in mice and there was no infectivity in mice after treatment with **P_1** at the dose of 10 mg kg^{-1}. This was confirmed by feeding *An. stephensi* mosquitoes on *P. yoelli* infected mice before and after the treatment. Results showed that there was complete lose of infectivity in mosquitoes after treatment with compound **P_1** while the infectivity was confirmed in mosquitoes fed on animals before treatment. Primaquine was taken as control compound. **P_2** did not possessed any gametocytocidal effect against *P. yoelli* infected mice (Table 2).

Table 2. In vivo gametocytocidal activity of primaquine and compounds $\mathbf{P_1}$ and $\mathbf{P_2}$ on *P. yoelli* infected mice

Compd	Mice No.	Mosquito feeding time (h)	Oocyst	Mosquito +ve/dissected
Primaquine	1	−1	280.7±124.5	18/20
		+6	—	0/20
		+24	—	0/20
$\mathbf{P_1}$	1	−1	315±130	15/15
		+6	—	0/20
		+24	—	0/20
	2	−1	240±82.6	16/20
		+6	—	0/20
		+24	—	0/20
	3	−1	185±60	8/11
		+6	—	0/10
$\mathbf{P_2}$	1	−1	>300	18/20
		+6	>300	16/20
		+24	>300	10/12
	2	−1	260.3±96.2	13/15
		+6	221.3±131.69	12/15
		+24	68.33	5/15

In conclusion 6-methoxy-5, 8 bis(4′-amino-1′-methylbutylamino) quinoline is found to be a novel antimalarial compound with good gametocytocidal activity.

References and Notes

1. Carson, P. E. In *Antimalarial Drugs II*; Peters, W.; Richards, W. H. G., Ed; Springer-Verlag: New York, 1984; pp 83–121.
2. Beutler, E.; Dern, R. J.; Alving, A. S. *J. Lab. Clin. Med.* **1954**, *44*, 177.
3. Milhous, W. K.; Robinson, B. L.; Breuckner, R. P.; Schuster, B. G.; Peters, W. *Am. J. Trop. Med. Hyg.* **1992**, *47* (Suppl.), 89.
4. Jain, R.; Gupta, R. C.; Anand, N. *Ind. J. Chem.* **1994**, *33*, 792.
5. Lamontagne, M. P.; Blumbergs, P.; Strube, R. E. *J. Med. Chem.* **1982**, *25*, 1094.
6. Lamontagne, M. P.; Markovac, A.; Khan, M. S. *J. Med. Chem.* **1982**, *25*, 964.
7. Brossi, A.; Gessner, W.; Hufford, C. D.; Baker, J. K.; Homo, F.; Millet, P.; Landau, I. *FEBS Lett.* **1987**, *223*, 77.
8. Dua, V. K.; Sinha, S.; Sharma, V. P. *J. Chromatog.* **1998**, *708*, 316.

9. **Isolation of oxidation products:** an aqueous solution of 2.5 mg mL^{-1} primaquine (2 mL) and 2.4 mg mL^{-1} K_2 S_2 O_8 (2 mL) were mixed together. After 40 min, of initiation, the oxidation products were fractionated on a Bio-Gel P-2 (<45 mesh) column of 2.0 × 100 cm using water for elution. Each fraction was tested for its purity using HPLC. The HPLC apparatus consisted of a Waters 510 pump, a Rheodyne 7125 injector, a variable wave length UV 486 detector operated at 254 nm and an integrator. The mobile phase acetonitrile–methanol–(1 M) perchloric acid–water (30:7:1:95 v/v) was pumped at a flow rate of 1.0 mL/min through μBondapak C_{18} reverse phase column (300 × 3.9 mm; particle size, 5). Retention time of compounds $\mathbf{P_1}$, $\mathbf{P_2}$, $\mathbf{P_3}$, $\mathbf{P_4}$, $\mathbf{P_5}$ and primaqine were 9.0, 4.6, 3.0, 5.4, 4.0 and 10.0 min respectively. Small scale preparative chromatography was carried out with a combination of Bio-Gel column chromatography and reverse phase HPLC.

10. **Biological methods: in vitro:** Antimalarial efficacy was checked in gametocyte producing *P. falciparum* culture lines (FDL-HD). Culture was maintained in in vitro O +ve RBC at 10% haematocrit in AB +ve serum in RPMI 1640 medium supplemented with d-glucose and l-glutamine. The culture was treated with selected concentrations of different compounds and allow to grow at 37 °C in candle jar for 72 h. After incubation, blood smears were prepared and stained with Giemsa stain. Percentage inhibition for gametocytes and asexual stages were calculated by comparing growth in control sets. Primaquine and chloroquine were taken as standard references. Gametocytocidal and schizontocidal concentrations were expressed as 50% (IC_{50}) and 90% (IC_{90}) inhibitory concentrations. **In vivo:** Primaquine and the test compounds $\mathbf{P_1}$ and $\mathbf{P_2}$ were administered in mice in the dose of 10 mg kg^{-1} (Intraperitoneal) and mosquito feeding was repeated at −1, 6 and 24 h. after injection. The fed mosquitoes were kept at 24±2 °C and 70±5% relative humidity in the insectory. Mosquito dissection were done on the 4th day and average oocyst were calculated to find infectivity. Activity is assessed by comparing the number of oocytes on the midgets of mosquitoes fed on drug treated mice with those of the insects fed on untreated animals. A group of infected mice without being administered any drug was kept an untreated control.

11. Clark, A. M.; Hufford, C. D.; McChesney, J. D. *Antimicrob. Agents Chemother.* **1981**, *19*, 337.
12. Clark, A. M.; Hufford, C. D.; Puri, R. K.; McChesney, J. D. *Appl. Environ. Microbiol.* **1984**, *47*, 540.
13. Bhasin, V. K.; Trager, W. In *Primaquine; Pharmacokinetics, Metabolism, Toxicity and Activity;* Wernsdorfer, W. H. Trigg, P.I., Ed.; John Wiley & Sons, 1984; pp 145–153.