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Antimalarial *t*-Butylperoxyamines

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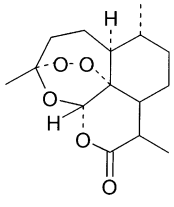
Abstract—Twelve *t*-butylperoxyamines (**6**–**17**) were synthesized as targeted antimalarials and evaluated for antimalarial activity in vivo against *Plasmodium berghei* in mice and in vitro against both chloroquine sensitive and chloroquine resistant strains of *Plasmodium falciparum*. Compound **8** was found to have highest potency with activity at 80 and 160 mg/kg dose in vivo and compound **11** exhibited highest efficacy in vitro.

Naturally occurring peroxides have been found to exhibit potent antimalarial activity; artemisinin[1–5] (**1**), dihydroascaridole (**2**) and an endoperoxide[6] **3** are a few examples. However, simple peroxides such as hydrogen peroxide[7] (**4**) and *t*-butyl hydroperoxide[8,9] (**5**) also show activity, but are less potent than **1** and cause hemolysis of uninfected erythrocytes at parasiticidal concentrations. The efficacy of compounds **1**–**5** depends on the observation that malaria infected red cells are selectively damaged by oxidants.



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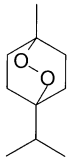

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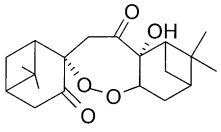

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It has been proved beyond doubt from structure–activity studies that the endoperoxide moiety in artemisinin (**1**) and its analogues is absolutely essential for antimalarial activity,[10] which is mediated by activated oxygen (superoxide, H_2O_2 and/or hydroxy radicals) or carbon-free radicals. It suggests an oxidative mode of action.[11–14] The increase in the potency of **1** with an increase in oxygen tensions ranging from 3 to 30% and decrease in the potency of **1** when co-administered with reducing agents support this oxidative mode of action.[15]

The amines containing *t*-butoxy-peroxides are reported to have antimalarial activity in vitro against *Plasmodium falciparum*. Vennerstorm[16] reported a series of such peroxides with potential antimalarial activity approximately one order of magnitude more potent than *t*-butyl hydroperoxide (**5**) in vitro against the chloroquine sensitive (D-6) and chloroquine resistant (W-2) clones of *P. falciparum*. But all of these amine peroxides were inactive in vivo against *Plasmodium berghei*.

Recently, we have reported the antimalarial activity of monoterpenic fragment analogues of aplasmomycin[17,18] and of 3-hydroxy-3-aryl-2-methylene-propionic acid derivatives.[19] In continuation of our search for new antimalarials, we report herein the synthesis of peroxyamines and their antimalarial activity in vitro against *P. falciparum* and in vivo against *P. berghei* in mice.

Chemistry[20]

The compounds **6**–**13** were obtained from the corresponding secondary amines by the treatment with formaldehyde (33% aqueous) and *t*-butyl hydroperoxide (70% aqueous) in methanol at 4 °C. Whereas the compounds **14**–**17** were prepared by treatment of the aldehydes, namely citronellal, furfural, thiophene-2-carboxaldehyde with primary amines, namely benzylamine and ethanolamine to give corresponding imines. The imines were then reduced using sodium borohydride to give corresponding secondary amines. The secondary amines thus obtained were then converted to amine peroxides by the above-mentioned procedure (Scheme 1). A

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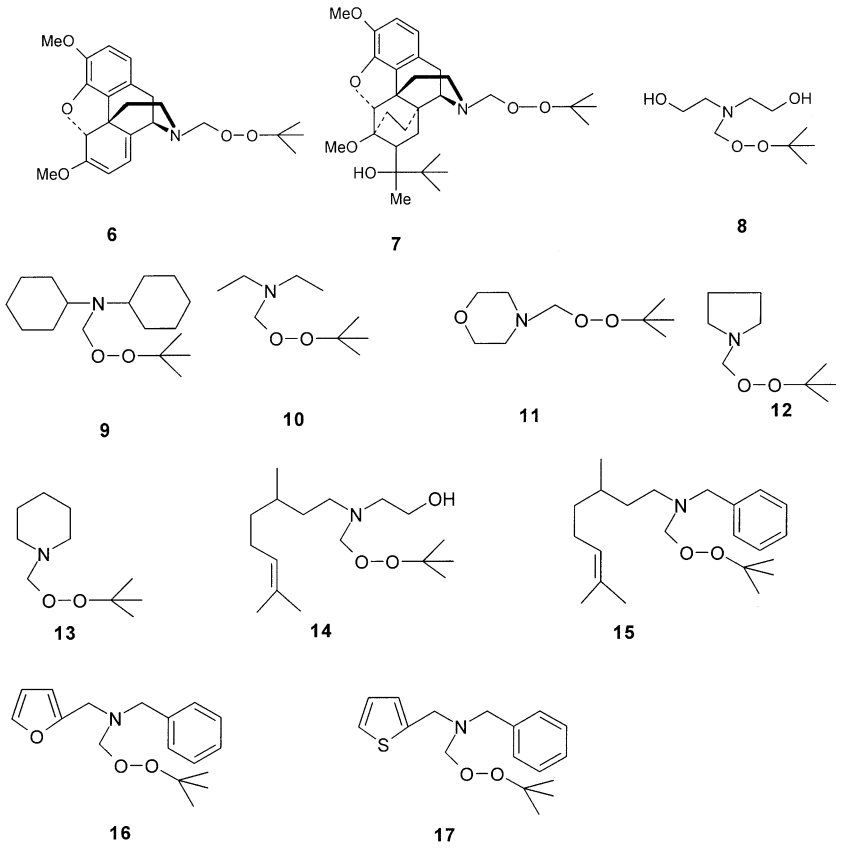


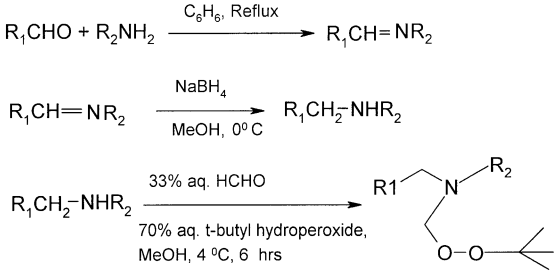
singlet at δ 1.24–1.26 (9H) and a singlet at δ 4.5–4.7 (2H) in the ^{1}H NMR spectra confirmed the incorporation of the *t*-butyl and the methylene groups, respectively, in the molecules.

Biological Evaluation[21]

Antimalarial activity

In vitro. The compounds **6**–**17** were evaluated against both chloroquine sensitive (FJB D9) and chloroquine resistant (FSH 14) strains of *P. falciparum* at different doses starting from 25 μmol/well onwards with 2-fold serial dilution. Doses were kept constant for all compounds to have a comparative profile. Results obtained from the in vitro schizont maturation inhibition and parasite growth inhibition in the case of both chloroquine sensitive (FJB D9) and chloroquine resistant (FSH 14) strains of *P. falciparum* are summarised in Tables 1 and 2, respectively. Compound **11** was found to exhibit maximum efficacy in vitro.

In vivo. The compounds **6**–**17** were evaluated for antimalarial activity in vivo against virulent strains of *P. berghei* in mice using Rane's schizontocidal method described by Osdene et al.[22] The dose range selected was 20, 40, 80, 160 mg/kg and a minimum of five mice per dose were used. Artemisinin (20 and 40 mg/kg) was kept as standard drug in trial for comparison. On comparison of activities (Table 3) compound **8** was found to be



Scheme 1.

Table 1. In vitro antimalarial activity of compounds against chloroquine sensitive *P. falciparum* strain FJB D9[a]

Compounds (μmol/well)	IC_{50}		IC_{90}	
	SMI	PGI	SMI	PGI
6	0.135	1.8	0.47	6.4
7	1.6	1.45	4.6	4.0
8	22	>25	>25	>25
9	1.5	5.0	21	25
10	>25	>25	>25	>25
11	0.058	0.15	2.6	3.0
12	>25	>25	>25	>25
13	3.6	5.5	22.5	>25
14	3.2	7.8	>25	>25
15	1.6	4.4	9.0	10.0
16	1.45	2.1	2.8	5.5
17	0.9	2.7	6.0	4.8
Artemisinin	0.01	0.015	0.028	0.03

[a]SMI, schizont maturation inhibition; PGI, total parasite growth inhibition.

Table 2. In vitro antimalarial activity of compounds against chloroquine resistant *P. falciparum* strain FSH 14[a]

Compounds (μmol/well)	IC_{50}		IC_{90}	
	SMI	PGI	SMI	PGI
6	0.5	0.45	0.92	5.0
7	2.65	1.95	5.6	5.0
8	10.0	>25	>25	>25
9	2.1	5.0	>25	>25
10	>25	>25	>25	>25
11	0.064	0.16	2.9	2.15
12	>25	>25	>25	>25
13	2.9	5.0	24	>25
14	1.4	3.8	>25	>25
15	1.6	2.5	10	12
16	1.35	2.25	4.5	5.4
17	0.5	2.2	11.5	12.0
Artemisinin	0.01	0.01	0.028	0.029

[a]SMI, Schizont maturation inhibition; PGI, total parasite growth inhibition.

Table 3. In vivo antimalarial activity of compounds against *P. berghei* in mice

Compounds	Untreated control (days)	Mean survival time (days) Doses (mg/kg)				Remarks
		20	40	80	160	
6	6.2	5.2	6.1	6.8	7.2	Inactive
7	6.2	7.0	8.4	10.0	12.6	Active at 160 mg/kg
8	6.2	10.2	11.6	12.8	14.0	Active at 80 and 160 mg/kg
9	6.5	7.6	8.6	9.80	13.4	Active at 160 mg/kg
10	6.5	7.2	8.4	9.40	12.2	Inactive
11	6.5	6.8	7.6	8.80	11.4	Inactive
12	6.4	5.4	7.0	9.80	11.8	Active at 160 mg/kg
13	5.8	5.2	6.6	8.20	10.2	Inactive
14	6.8	6.2	7.3	11.2	15.0	Active at 160 mg/kg
15	6.0	5.2	6.8	10.8	12.2	Active at 160 mg/kg
16	6.8	6.4	7.2	10.8	12.0	Inactive
17	6.8	5.8	6.6	10.2	14.0	Active at 160 mg/kg
Artemisinin	6.3	10.8	12.8			Active from 40 mg/kg

most potent with activity at 80 and 160 mg/kg dose. The compounds **7**, **9**, **12**, **14**, **15** and **17** showed activity at 160 mg/kg dose level.

In conclusion, *t*-butylperoxyamines were found to be novel antimalarials among which the compound **8** has the highest potency in vivo against *P. berghei*. Compound **11** was found to exhibit maximum efficacy in vitro against *P. falciparum*. The present study assumes greater significance because of the facile synthesis of these compounds and their antimalarial activity against *P. berghei* in mice.

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20. **Experimental: general procedures**

Conversion of secondary amine to *t*-butylperoxyamines

To a solution of secondary amine (10 mmol) in methanol (5 mL) at 0 °C was added 70% aqueous *t*-butyl hydroperoxide (1.08 g, 12 mmol) followed by 37% aqueous formaldehyde solution (0.36 g, 12 mmol). The resulting mixture was stirred for 4 h at 4 °C, the mixture was extracted with solvent ether, and the organic layer was dried using anhydrous K_2CO_3. Removal of solvent in vacuo provided the peroxide as a clear oil. The compound was further purified by column chromatography using neutral alumina with ethyl acetate and petroleum ether as eluants.

Synthesis of imines. A mixture of amine (10 mmol) and aldehyde (10 mmol) in 20 mL of dry benzene was refluxed for 8 h using Dean–Stark apparatus until all the water that had formed was removed. Removal of solvent in vacuo gave the imine, which was directly taken to the next step without purification.

Reduction of imine using $NaBH_4$. To a solution of imine (0.01 mol) in methanol, an alkaline solution of $NaBH_4$ (2 g) in NaOH solution (2 mL) and water (20 mL) was added dropwise at such a rate that the temperature doesn't rise above 50 °C and the mixture was stirred for 1 h. The solvent was then distilled off, diluted with water and the amine was extracted with $CHCl_3$. The organic layer was dried over anhydrous Na_2SO_4. Removal of the solvent in vacuo and purification using alumina gave the required amine.

21. **Biological activity in vitro**. Two *P. falciparum* strains were obtained from well adapted in vitro culture lines. One chloroquine sensitive, FJB D9 (Jabalpur, Central India) and one chloroquine resistant, FSH 14 (Shankagarh, North India) isolate collected from patients in 1990 and 1993, respectively, were adapted and maintained in vitro by candle-jar technique.[12] Parasites were cultured in O+ erythrocyted in RPMI 1640 media enriched with 10% AB+ serum and supplemented with 25 mM HEPES buffer and 25 mM $NaHCO_3$. Parasite culture was synchronised at ring forms using density gradient method.[13] Assay was done at 10% haematocrit containing 1% ring stage parasite in 96-well flat-bottom tissue culture plate. Compounds were dosed in wells in duplicate at concentrations of 25, 10, 5, 2.5, 1, 0.5, 0.25, 0.1, 0.05, 0.025 and 0.01 μmol per well. Control culture was done in complete media without any synthesised compounds. To determine the activity of various compounds, assay was done in two sets. The first set of bioassay was conducted to determine the effect of schizont maturation after 24 h and the second set of assay was conducted to determine the effect of total growth of the parasites after 72 h. The volume of culture media per well for both sets of assay was 200 μL. Growth of the parasites from duplicate wells of each concentration was monitored microscopically in JSB strained[14] smears by counting number of schizonts per 200 asexual parasites and total number of parasites per 5000 RBCs. Percentage schizont maturation inhibition and total growth inhibition were calculated by the formula; $(1-Nt/Nc)\times 100$ where Nt and Nc represent the number of schizont in the test and control well, respectively. Inhibitory concentrations at 50 and 90% were calculated.

Biological activity in vivo (Rane's test). The compounds were evaluated for their activity against virulent strains of *P. berghei yoelli* (NK 65) using Rane's schizontocidal method described by Osdane et al.[22] Four-week-old mice weighing 20–25 g each received intraperitoneal inoculum of 1×10^{-6} parasitized *P. berghei* red cells. The test solutions of synthesised compounds in distilled water were prepared by homogenisation with two drops of 1% Tween-80 and injected once subcutaneously 72 h post infection. A control group of infected mice that was not administered any drug was kept as untreated control. The dose range selected was 20, 40, 80 and 160 mg/kg and a minimum of five mice per dose were used. Artemisinin (20 and 40 mg/kg), cycloguanil hydrochloride (25 mg/kg) and DDS (20 mg/kg) were kept as standard drugs in trial for comparison. Deaths occurring within 24 h of treatment classified as death due to toxicity. All mice receiving synthetic compounds showed survival time of 12–18 days. Testing was evaluated by calculating mean survival time (MST) of the treated and controlled group of mice.

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