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Geraniol-Derived 1,2,4-Trioxanes with Potent In-Vivo Antimalarial Activity☆

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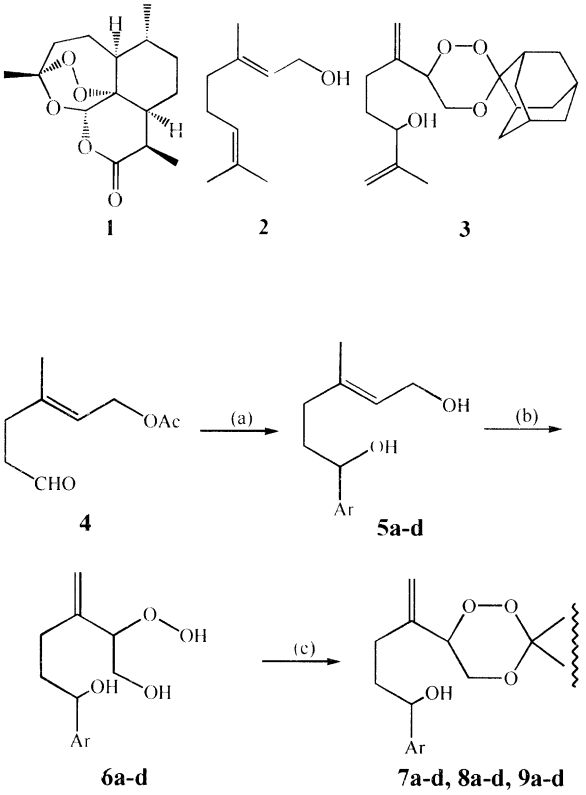
Abstract—Geraniol, an abundantly available naturally occurring allylic alcohol, has been used as a starting material to prepare a series of 6-[α-(3′-aryl-3′-hydroxypropyl)vinyl]-1,2,4-trioxanes. Some of these novel trioxanes have shown very promising antimalarial activity against multi-drug resistant *Plasmodium yoelii* in mice by both intramuscular (im) and oral routes.

Artemisinin **1**, the antimalarial principle of Chinese traditional drug *Artemisia annua* and its derivatives are highly potent antimalarials, active against multi-drug resistant malaria.[1] The fact that these compounds owe their antimalarial activity to peroxide group, present in the form of a 1,2,4-trioxane in their molecular structures, has led to the current interest in synthetic 1,2,4-trioxanes.[2]

In continuation with our studies in this area,[2g,3] we have recently reported the synthesis and antimalarial activity of a series of geraniol-based 1,2,4-trioxanes.[4] Trioxane **3**, the best compound in this series showed only moderate activity against multi-drug resistant *Plasmodium yoelii* in mice. The methodology for preparation of these trioxanes also suffered from two serious limitations; (i) photooxygenation of geraniol **2**, the key step in the synthesis, gave a complex mixture of hydroperoxides resulting in very poor yield of the desired compounds, (ii) only limited structural variations were possible. In the present study, we have used aldehyde acetate **4**, easily accessible from geranyl acetate in two steps,[5] to prepare a series of new hydroxy-functionalized 1,2,4-trioxanes. Several of these trioxanes have shown promising antimalarial activity against multi-drug resistant *P. yoelii* in mice by both oral and intramuscular (im) routes.



Scheme 1. Reaction conditions: (a) (i) ArMgBr, dry Et_2O, 0 °C to rt, 3 h; (ii) H_2O, 0 °C; (b) hv, O_2, methylene blue, MeCN, 0 °C, 4–6 h; (c) ketone, TsOH, CH_2Cl_2, rt, 1 h.

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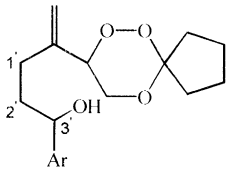
Table 1. In vivo antimalarial activity of trioxanes against *P. yoelii* in Swiss mice

Compd	Dose (mg/kg/day)	Route	% Suppression on day 4[a]	Mice alive on day 28	Mean survival time[b] (MST)±SE
3	96		100.0	2/5	19.57±2.14
	48	im	100.0	0/5	14.80±2.23
	24		67.7	0/5	12.20±2.24
7a	96		100.0	0/5	14.80±1.06
	48	im	97.0	0/5	12.60±1.12
	96	oral	30.0	0/5	08.20±0.53
7b	96		100.0	2/5	14.57±0.83
	48	im	98.4	0/5	14.75±0.75
	96	oral	29.6	0/5	07.40±0.51
7c	96		100.0	1/5	14.75±1.48
	48	im	93.4	0/5	15.75±2.37
	96	oral	81.3	0/5	10.40±0.93
7d	96		100.0	2/5	17.33±1.75
	48	im	100.0	0/5	16.75±1.23
	24		85.2	0/5	11.80±1.53
	96		100.0	0/5	16.00±1.22
	48	oral	94.3	0/5	12.00±1.14
8a	96		94.5	0/5	14.40±1.21
	48	im	90.5	0/5	12.60±1.21
	96	oral	65.0	0/5	11.60±1.12
8b	96		100.0	5/5	>28
	48	im	97.1	0/5	16.00±1.93
	96	oral	88.8	0/5	10.20±0.53
8c	96		100.0	4/5	16.00±0.00
	48	im	93.4	0/5	14.20±1.33
	96	oral	68.4	0/5	13.00±1.67
8d	96		96.4	4/5	14.00±0.00
	48	im	81.0	0/5	10.60±0.68
	96		100.0	0/5	13.80±0.53
	48	oral	77.5	0/5	11.20±1.36
9a	96		100.0	5/5	>28
	48	im	98.1	2/5	15.57±0.33
	96		100.0	2/5	16.57±1.21
	48	oral	62.4	0/5	09.00±0.71
9b	96		100.0	5/5	>28
	48	im	78.9	0/5	15.00±0.95
	96		100.0	5/5	>28
	48	oral	90.8	0/5	10.20±0.85
9c	96	im	72.13	2/5	15.33±2.31
	96		100.0	3/5	17.00±3.00
	48	oral	100.0	2/5	15.57±1.75
	24		87.1	0/5	10.40±1.24
9d	96	im	79.0	3/5	14.50±0.50
	96		100.0	2/5	17.33±1.45
	48	oral	100.0	2/5	17.57±2.02
	24		90.4	0/5	08.80±0.53
Artemisinin	48		100.0	5/5	>28
	24	im	100.0	4/5	16.00±0.00
Chloroquine	96		100.0	4/5	20.00±0.00
	48	oral	100.0	2/5	17.60±1.33
Vehicle control	—	—	—	0/15	07.00±0.14

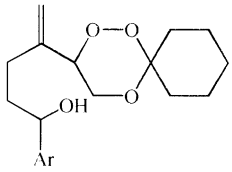
[a]Percent suppression = [(C−T)/C]×100; where C = parasitaemia in control group, and T = parasitaemia in treated group.
[b]MST calculated for the mice which died during 28-day observation period.

Chemistry

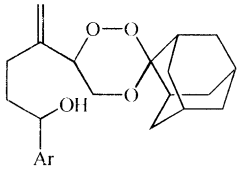
Geranyl acetate was converted to aldehyde acetate **4** using a known procedure.[5] Reaction of **4** with excess of Grignard reagents prepared from bromobenzene, 4-bromochlorobenzene, 1-bromonaphthalene and 4-bromobiphenyl furnished allylic alcohols **5a–d** in 60-75% yields. Methylene blue sensitized photooxygenation of allylic alcohols **5a–d** in MeCN furnished β-hydroxyhydroperoxides **6a–d** in 30–45% yield, as inseparable mixture of diastereomers. Acid catalyzed condensation of β-hydroxyhydroperoxides **6a–d** with cyclopentanone, cyclohexanone, and 2-adamantanone furnished hydroxy-functionalized 1,2,4-trioxanes **7a–d**, **8a–d**, **9a–d** in 50–74% yields (Scheme-1), again as inseparable mixture of diastereomers. In most of the cases these diastereomers were indistinguishable even by ^{1}H NMR, and only ^{13}C NMR could differentiate them (Scheme 1).[6]



7a-d



8a-d



9a-d

a; Ar = Ph
b; Ar = 4-ClC$_6$H$_4$
c; Ar = 1-naphthyl
d; Ar = 4-PhC$_6$H$_4$

Antimalarial Activity

Trioxanes **7a–d**, **8a–d**, and **9a–d** were initially screened for their antimalarial activity against multi-drug resistant *P. yoelii* in Swiss mice[7] at a highest dose of 96 mg/kg[8] by both intramuscular (im) and oral routes. The trioxanes showing activity at 96 mg/kg by either routes were further evaluated at 48 and 24 mg/kg. The results are shown in Table 1.

Results and discussion

Trioxane **9b** is the best compound in the series. It shows 100% clearance of parasitaemia on day 4 at 96 mg/kg by both im and oral routes and all the animals survive beyond day 28. Trioxane **9a** is the next best compound in the series. At 96 mg/kg given im, this compound shows complete clearance of parasitaemia on day 4 and all the animals survive beyond day 28. Even at 48 mg/kg, this compound shows almost complete clearance of parasitaemia on day 4 and 40% of the animals survive beyond day 28. This trioxane also shows complete clearance of parasitaemia on day 4 at 96 mg/kg by oral route and 40% of the animals survive beyond day 28. Both these trioxanes are derived from 2-adamantanone. The other two trioxanes derived from 2-adamantanone (**9c** and **9d**) also show significant activities. **9c** provides 60% protection at 96 mg/kg and 40% protection at 48 mg/kg when given orally. Similarly trioxane **9d** provides 40% protection when given im at 96 and 48 mg/kg. Both these trioxanes show complete clearance of parasitaemia on day 4 when given orally at 96 and 48 mg/kg. Trioxanes **8b** and **8c** derived from cyclohexanone also show complete clearance of parasitaemia on day 4 at 96 mg/kg given im and provide 100 and 80% protection, respectively. Trioxane **8d** though shows only 96% clearance of parasitaemia on day 4 but provides 80% protection when given im. Trioxanes **7b**, **7c**, and **7d** derived from cyclopentanone also show complete clearance of parasitaemia on day 4 at 96 mg/kg given by im route and provide 40, 20 and 40% protection, respectively. Trioxanes **7c** and **7d** also show significant suppression of parasitaemia on day 4 when given orally but none of the treated tice survives beyond day 28. Similarly, trioxanes **7a** and **8a** show significant suppression of parasitaemia on day 4 at 96 and 48 mg/kg given im, but none of the treated animals survive, beyond day 28. Trioxane **3**, the best compound of the earlier series, shows 100% clearance of parasitaemia at 96 and 48 mg/kg on day 4 when given im but provides only 40% protection at 96 mg/kg (Table 1).

A high order of activity shown by trioxanes having adamantane moiety (**9a–d**) is in agreement with our earlier observations.[3] In this series, the introduction of very hydrophobic aryl groups such as naphthyl and biphenyl (**9c** and **9d**) leads to decrease in the activity. For trioxanes with adamantane moiety a phenyl/chlorophenyl in the side chain provides the desired level of hydrophobicity to these molecules. Introduction of naphthyl and biphenyl groups in the side chain of the trioxanes derived from cyclohexanone (**8c** and **8d**) has favorable effect on the activity.

Conclusion

Using geraniol derived trioxane **3** as lead, we have prepared a new series of hydroxy-functionalized 1,2,4-trioxanes, several of which have shown very promising activity against multi-drug resistant *P. yoelii* in mice both by oral and im routes.

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6. Selected data: Trioxane **7b** (mixture of diastereomers): FT-IR (neat, cm^{-1}) 1597.4, 1646.8, 3435.1; ^1H NMR (200 MHz, $CDCl_3$) δ 1.70–1.92 (m, 9H), 2.06–2.16 (m, 2H), 2.38–2.44 (m, 1H), 3.78–3.82 (m, 2H), 4.64–4.76 (m, 2H), 5.05 (s, 2H), 7.20–7.40 (m, 4H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 23.33 (t), 24.70 (t), 29.84 (t), 32.73 (t), 36.97 (t), 37.27 (t), 64.46 (t), 73.04 (d), 73.07 (d), 81.14 (d), 81.22 (d), 114.47 (s), 114.50 (t), 127.23 (2×d), 128.67 (2×d), 133.31 (s), 142.97 (s), 143.10 (s); FABMS (*m*/*z*) 339 and 341 ($M^+ + 1$). Anal. calcd C 63.81%, H 6.84%; found C 63.58%, H 7.02%. Trioxane **8c** (mixture of diastereomers): FT-IR (neat, cm^{-1}) 1597.5, 1645.4, 3443.8; ^1H NMR (200 MHz, $CDCl_3$) δ 1.42–1.57 (m, 8H), 1.84–2.31 (m, 6H), 3.68 (dd, 1H, $J = 11.8, 3.0$), 3.86–4.01 (m, 1H), 4.69 (dd, 1H, $J = 10.2, 3.0$), 5.04 and 5.06 (2×s, 2H), 5.45 (m, 1H), 7.45–8.08 (m, 7H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 22.67 (t), 22.71 (t), 25.94 (t), 29.41 (t), 30.67 (t), 30.72 (t), 35.00 (t), 36.77 (t), 36.82 (t), 62.53 (t), 62.57 (t) 70.80 (d), 70.95 (d), 81.68 (d), 81.75 (d), 102.84 (s), 114.64 (t) 114.87 (t), 123.20 (d), 123.31 (d), 123.49 (d), 125.81 (d), 125.98 (d), 126.48 (d), 126.52 (d), 128.44 (d), 128.48 (d), 129.31 (d), 130.77 (s), 134.28 (s), 140.49 (s)140.58 (s), 143.94 (s), 144.01 (s); FABMS (*m*/*z*) 369 ($M^+ + 1$). Anal. calcd C 74.97%, H 7.66%; found C 75.20%, H 7.49%. Trioxane **9d** (mixture of diastereomers): FT-IR (neat, cm^{-1}) 1600.8, 1646.5, 3407.3; ^1H NMR (200 MHz, $CDCl_3$) δ 1.50–2.18 (m, 17H), 2.88 (bs, 1H), 3.72 (dd, 1H, $J = 11.8$, 3.0 Hz), 3.94 (dd, 1H, $J = 11.8$, 10.5 Hz), 4.70–4.80 (m, 2H), 5.07 (s, 2H), 7.33–7.60 (m, 9H); FABMS (*m*/*z*) 447 ($M^+ + 1$). Anal. calcd: C 78.00%, H 7.67%; found C 77.69%, H 8.01%.

7. The in vivo efficacy of compounds was evaluated against *P. yoelii* (MDR) in Swiss mice model. The colony bred Swiss mice (25 ± 1 g) were inoculated with 1×10^6 parasitised RBC on day zero and treatment was administered to a group of five mice at each dose, from day 0 to 3, in two divided doses daily. The drug dilutions were prepared so as to contain the required amount of the drug (1.2 mg for a dose of 96 mg/kg and 0.6 mg for a dose of 48 mg/kg) in 0.1 mL and administered either intramuscularly or orally for each dose. Parasitaemia level were recorded from thin blood smears between days 4 and 28.[9] Mice treated with artemisinin and chloroquine served as positive controls.

8. Since artemisinin is active at a dose of 48 mg/kg, we have chosen 96 mg/kg as the highest dose in the primary screening of trioxanes.

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