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Synthesis and antimalarial activity of 6-cycloalkylvinyl substituted 1,2,4-trioxanes☆

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Abstract—6-Cycloalkylvinyl substituted 1,2,4-trioxanes 6–15 have been prepared and tested against multi-drug resistant *Plasmodium yoelii* in mice. The most active trioxane 11 provides 80% protection to the treated mice. Further derivatization of 11 leads to decrease in antimalarial activity.

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1. Introduction

Malaria is a major parasitic disease of the tropics, affecting around 300-500 million people of which more than one million die each year.¹ The emergence of malarial parasites resistant to contemporary drugs has added a new dimension to malaria problem. Currently, artemisinin 1 and its derivatives are the drugs of choice to treat multi-drug resistant malaria.² Peroxide group, present in the form of 1,2,4-trioxane, is essential for the antimalarial activity of artemisinin-based compounds. In recent years synthesis of a large number of structurally simple trioxanes have been reported.³ Earlier we have reported a new photooxygenation route for the synthesis of 1,2,4trioxanes. Preparation of β -hydroxyhydroperoxide by photooxygenation of allylic alcohols is the key step of this method. Several 6-arylvinyl substituted trioxanes prepared by this method have shown significant in vivo activity against chloroquine-sensitive P. berghei in mice⁴ by i.p. route but are weakly active against multi-drug resistant Plasmodium yoelii by i.m. route. Replacement of aryl group by alkyl and arylalkyl groups leads to improvement in activity.^{5,6} In the present study we have prepared cyclopropyl and cyclohexyl substituted allylic alcohols 4a-c and investigated their potential for the

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synthesis of a new series of 1,2,4-trioxanes by photooxygenation route. The twin objective of this study is to study the behavior of these alcohols, particularly the cyclopropyl substituted alcohols **4a** and **4c**, under photooxygenation condition and the effect of cycloalkyl groups on antimalarial activity. We have also prepared several derivatives of the most active trioxane (**11**) of the series.



2. Chemistry

3-Cyclopropyl and 3-cyclohexyl substituted butenoates 3a and 3b were prepared as inseparable mixtures of *cis* and *trans* isomers. Reduction of 3a and 3b with LiAlH₄ furnished allylic alcohols 4a and 4b in 89% and 85% yields, respectively. Compounds 4a and 4b are mixture of *cis* and *trans* isomers in the ratio of 1:3. Photooxygenation of 4a furnished β -hydroxyhydroperoxide 5a in 50% yield. A similar photooxygenation of 4b furnished

Keywords: Artemisinin; Antimalarial 1,2,4-trioxane; Photooxygenation; *Plasmodium yoelii*.

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β-hydroxyhydroperoxide **5b** in 35% yield. Interestingly both these reactions were highly regiospecific. Acid catalyzed condensation of **5a** with benzaldehyde, acetone, cyclopentanone, cyclohexanone, cycloheptanone, and 2-adamantanone furnished trioxanes **6**, **7**, **8**, **9**, **10**, and **11** in 31%, 67%, 61%, 75%, 37%, and 75% yields, respectively. β-Hydroxyhydroperoxide **5b** on condensation with acetone, cyclopentanone, cyclohexanone, and 2adamantanone gave trioxanes **12**, **13**, **14**, and **15** in 57%, 78%, 80%, and 83% yields, respectively (Scheme 1). Trioxanes **6–15** gave satisfactory ¹H NMR, ¹³C NMR, and MS data and all these compounds except trioxanes **7**, **8**, **9**, **12**, and **13**, which are volatile oils, gave satisfactory elemental analysis.

Trioxane 11 the most active trioxane of the series (see activity section) on reaction with 3-chloroperbenzoic acid (mCPBA) furnished epoxide 16 as mixture of diastereomers, which on hydrolysis furnished diol 17 also as a mixture of diastereomers. Reaction of diol 17 with succinic anhydride in the presence of Et₃N furnished hemisuccinate 18 as mixture of diastereomers. The diols 17 were also separated by column chromatography to pure diastereomers 17a and 17b (stereochemistry not assigned). Reaction of diol 17a with succinic anhydride in the presence of Et₃N furnished hemisuccinate derivative 18a. Similar treatment of diol 17b gave hemisuccinate derivative 18b. Acid catalyzed condensation of 17 with cyclopentanone furnished spiro derivative 19, which was separated into diastereomers 19a (higher $R_{\rm f}$) and **19b** (lower R_f) by column chromatography. Spiro derivatives 20 and 21 were prepared similarly and separated into diastereomers 20a,b and 21a,b. All these trioxanes 16–21 were fully characterized by ¹H NMR and MS spectra. These compounds also gave correct elemental analysis. Compounds 18a and 18b on treatment with



Scheme 1. Reagents and conditions: (a) triethylphosphonoacetate, NaH, DME, reflux, 4–30h; (b) LiAlH₄, Et₂O, 0°C, 2h; (c) h ν , O₂, methylene blue, MeCN, 0°C, 6h; (d) aldehyde/ketone, PTSA, CH₂Cl₂, rt, 2h.

Fable 1.	In vivo	antimala	rial activ	ity of tric	oxanes 6–1	5, 17–21	and 24	1
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Compound	Dose	%Suppression	No of mice	
	(mg/kg/day)	on day 4 ^a	survived on day 28	
6	96	70.0	0/5	
	48	57.0	0/5	
7	96	89.0	0/5	
	48	83.0	0/5	
8	96	82.0	0/5	
	48	68.0	0/5	
9	96	90.0	0/5	
	48	79.0	0/5	
10	96	84.0	0/5	
	48	61.0	0/5	
11	96	91.0	4/5	
	48	65.0	0/5	
12	96	23.0	0/5	
13	96	96.0	0/5	
14	96	28.0	0/5	
15	96	36.0	0/5	
17	96	72.0	0/5	
	48	53.0	0/5	
18	96	56.0	0/5	
	48	22.0	0/5	
19a	96	18.0	0/5	
19b	96	53.0	0/5	
20a	96	16.0	0/5	
20b	96	19.0	0/5	
21a	96	Nil	0/5	
21b	96	53.0	0/5	
24	96	97.0	0/5	
Artemisinin	48	100.0	5/5	
	24	100.0	4/5	
Vehicle control			0/15	

^a Percent suppression = $[(C-T)/C] \times 100$; where C = parasitemia in control group, and T = parasitemia in treated group.

diazomethane furnished methyl ester derivatives **25a** and **25b**, respectively, which were characterized by ¹H NMR, ¹³C NMR, and MS spectra.

Dicyclopropyl substituted allylic alcohol **4c** prepared from dicyclopropyl ketone in two steps on photooxygenation furnished β -hydroxyhydroperoxide **5c** in 28% yield. Condensation of **5c** with acetone and cyclohexanone furnished trioxanes **22** and **23** in very poor yields and therefore were not tested for antimalarial activity. 6-Phenylvinyl substituted trioxane **24** was prepared according to the published procedure.⁴

3. Antimalarial activity

Trioxanes 6–15, 17–21, and 24 were tested against multidrug resistant *P. yoelii* in swiss mice at 96 and 48 mg/kg by intramuscular route.⁷ The results are summarized in Table 1.

4. Results and discussion

As can be seen from Table 1, trioxane **11** is the most active compound of the series. It shows more than 90% clearance of parasitemia on day 4 at 96 mg/kg and 80% of treated mice survive beyond day 28. It is more active than the corresponding phenyl vinyl substituted trioxane **24**. All other cyclopropylvinyl substituted trioxanes (7–10) except trioxane **6** show more than 80% suppression of parasitemia on day 4 but none of the treated mice survive up to day 28. Cyclohexylvinyl substituted trioxanes **12–15**, on the other hand, are poorly active at 96 mg/kg. Trioxane **13** though shows 96% suppression on day 4 at 96 mg/kg but none of the treated mice survive up to day 28. Derivatization of the most active trioxane **11** leads to decrease in activity as all the derivatives (**17–21**) show less than 80% suppression of parasitemia on day 4 and none of the treated mice survive up to day 28.



5. Conclusion

We have investigated the potential of cyclopropyl, cyclohexyl, and dicyclopropyl substituted allylic alcohols 4a-c for the preparation of 1,2,4-trioxanes by photooxygenation route. Both 4a and 4b undergo regiospecific photooxygenation to furnish β-hydroxyhydroperoxides in reasonable yields and both these hydroxyhydroperoxides undergo facile acid catalyzed condensation with various ketones to provide 1,2,4trioxanes. On the other hand while photooxygenation of dicyclopropyl substituted allylic alcohol 4c provides β -hydroxyhydroperoxide **5c** in acceptable yield, the subsequent condensation of this hydroperoxide furnished trioxanes in extremely poor yields. Trioxane 11, the best compound of the series has shown 80% protection against multi-drug resistant P. voelii in mice and is more active than the corresponding cyclohexylvinyl and phenylvinyl substituted trioxanes 15 and 24 and its own derivatives 16-21.

6. Experimental

Melting points were taken in open capillaries on COMP-LAB melting point apparatus and are uncorrected. Infrared spectra (cm^{-1}) were recorded on Perkin–Elmer RXI FT-IR spectrophotometer. ¹H NMR and ¹³C NMR were recorded on Bruker Supercon Magnet DPX-200/DRX-300 MHz using CDCl₃ as solvent. Tetramethylsilane (0.00 ppm) was used as an internal standard in ¹H NMR and CDCl₃ (δ 77.0 ppm) was used in ¹³C NMR. Fast atom bombardment mass spectra (FABMS) were obtained on JEOL SX-102 spectrometer using glycerol or *m*-nitro benzyl alcohol as matrix. Electron ionization mass spectra (EIMS) were obtained on JEOL-JMS-600H spectrometer. Elemental analysis was performed on Elementar Vario EL III analyzer. The progress of the reaction was monitored by silica gel thin layer chromatography with detecting agents: iodine vapors and/or spraying with an aq solution of vanillin in 10% sulfuric acid followed by heating at 150°C. Chromatographic purification was performed over silica gel (60-120 mesh) obtained from Qualigens (India). All chemicals and reagents were obtained from Aldrich (USA), Lancaster (UK) or Spectrochem Pvt. Ltd (India) and were used without further purification. Esters **3a**,**b**, and **3c** were prepared by known procedures.^{8,9}

6.1. 3-Cyclopropyl-but-2-en-1-ol (4a)

To an ice cooled slurry of lithium aluminum hydride (4.92g, 129.47 mmol) in dry ether (100 mL), in a threenecked round bottomed flask, was added ethyl 3-cyclopropyl-2-butenoate **3a** (10.00 g, 64.94 mmol) in dry ether (50mL) under nitrogen atmosphere and stirred for 2h. Excess lithium aluminum hydride was quenched first by adding ordinary ether (25mL) followed by water (10mL) and then 10% aq NaOH (5mL). Ether layer was decanted, dried over anhyd Na₂SO₄, and concentrated to give the crude product, which on column chromatography over silica gel using EtOAc-hexane (1:9) as eluant furnished **4a** (6.50 g, 89% yield) as oil: FT-IR (neat, cm^{-1}) 1658.9, 3349.3; ¹H NMR (200 MHz, CDCl₃): δ 0.47–0.65 (m, 4H), 1.20 (br s, 1H, OH), 1.33–1.43 (m, 1H), 1.46 and 1.56 (2×s, 3H), 4.15 and 4.28 $(2 \times d, 2H, J = 7.0 \text{ Hz} \text{ each}), 5.46$ (t, 1H, J = 7.0 Hz; FABMS (*m*/*z*) 113 (M+H)⁺.

Compounds **4b** and **4c** were also prepared by the procedure described for **4a**.

6.2. 3-Cyclohexyl-but-2-en-1-ol (4b)

Yield 85%, oil: FT-IR (neat, cm⁻¹) 1610.5, 3390.6; ¹H NMR (200 MHz, CDCl₃): δ 1.06–1.37 (m, 5H), 1.64 and 1.65 (2×s, 3H), 1.66–1.85 (m, 6H), 4.16 (d, 2H, J = 6.8 Hz), 5.39 (2×t, 1H, J = 6.8 Hz each); FABMS (*m*/*z*) 155 (M+H)⁺.

6.3. 3,3-Dicyclopropyl-prop-2-en-1-ol (4c)

Yield 84%, oil: FT-IR (neat, cm⁻¹) 1649.0, 3344.7; ¹H NMR (200 MHz, CDCl₃): δ 0.36–0.39 (m, 2H), 0.54–0.57 (m, 2H), 0.65–0.76 (m, 4H), 0.92–0.96 (m, 1H),

1.62–1.69 (m, 2H), 4.30 (d, 2H, J = 6.8 Hz), 5.32 (t, 1H, J = 6.8 Hz); FABMS (*m*/*z*) 139 (M+H)⁺.

6.4. 3-Cyclopropyl-2-hydroperoxy-but-3-en-1-ol (5a)

A solution of allylic alcohol **4a** (1.00 g, 8.92 mmol) and methylene blue (5 mg) in acetonitrile (75 mL) was irradiated with a 500 W tungsten-halogen lamp maintained at 0 °C, while oxygen was bubbled slowly into the reaction mixture for 6h. Solvent was evaporated under vacuum at rt to furnish crude product, which on column chromatography over deactivated silica gel (12% v/w of water) using EtOAc-hexane (2:8) as eluant furnished **5a** (0.64 g, 50% yield) as oil: FT-IR (neat, cm⁻¹) 1644.5, 3350.0; ¹H NMR (200 MHz, CDCl₃) 0.45–0.54 (m, 2H), 0.68–0.75 (m, 2H), 1.18–1.41 (m, 1H), 2.31 (br s, 1H, OH), 3.81 (d, 2H, J = 5.6Hz), 4.61 (t, 1H, J = 5.6Hz), 4.87 and 5.00 (2×s, 2H), 9.02 (br s, 1H, OOH); FABMS (*m*/*z*) 145 (M+H)⁺.

6.5. 3-Cyclohexyl 2-hydroperoxy-but-3-en-1-ol (5b)

A solution of allylic alcohol **4b** (1.00 g, 6.49 mmol) and methylene blue (5 mg) in acetonitrile (75 mL) was irradiated with a 500 W tungsten-halogen lamp maintained at 0°C, while oxygen was bubbled slowly into the reaction mixture for 6h. Solvent was evaporated under vacuum at rt to furnish crude product, which on column chromatography over deactivated silica gel (12% v/w of water) using EtOAc-hexane (2:8) as eluant furnished pure **5b** (0.42 g, 35% yield) as a white solid: mp 84– 86°C; FT-IR (KBr, cm⁻¹) 1645.0, 3394.3; ¹H NMR (200 MHz, CDCl₃): δ 1.01–1.23 (m, 5H), 1.60–1.84 (m, 6H), 2.20 (br s, 1H, OH), 3.60–3.66 (m, 2H), 4.48 (dd, 1H, J = 6.6, 4.2 Hz), 4.99 and 5.03 (2 × s, 2H), 8.43 (br s, 1H, OOH); FABMS (*m*/*z*) 187 (M+H)⁺.

6.6. 3-Cyclopropyl-3-cyclopropylidene-2-hydroperoxypropan-1-ol (5c)

A solution of allylic alcohol **4c** (1.00 g, 7.24 mmol) and methylene blue (5 mg) in acetonitrile (75 mL) was irradiated with a 500 W tungsten–halogen lamp maintained at 0 °C, while oxygen was bubbled slowly into the reaction mixture for 6h. Solvent was evaporated under vacuum at rt to furnish crude product, which on column chromatography over deactivated silica gel (12% v/w of water) using EtOAc–hexane (2:8) as eluant furnished **5c** (0.35 g, 28% yield) as oil: FT-IR (neat, cm⁻¹) 1407.5, 3381.1; ¹H NMR (300 MHz, CDCl₃): δ 0.40–0.74 (m, 4H), 0.99–1.15 (m, 4H), 1.47–1.52 (m, 1H), 2.88 (br s, 1H, OH), 3.81 (dd, 1H, J = 12.0, 3.3 Hz), 4.04 (dd, 1H, J = 12.0, 8.4 Hz), 4.74 (dd, 1H, J = 8.4, 3.3 Hz), 9.02 (br s, 1H, OOH); FABMS (m/z) 171 (M+H)⁺.

6.7. 6-(1-Cyclopropyl-vinyl)-3-phenyl-[1,2,4]trioxane (6)

To a solution of hydroperoxide 5a (0.64g, 4.44 mmol) in dichloromethane (15mL) was added benzaldehyde (1.20g, 11.3 mmol) and *p*-toluenesulfonic acid monohydrate (0.05g, 0.26 mmol). Reaction mixture was stirred at rt for 2h and poured into saturated aq. NaHCO₃

solution (50 mL). Organic layer was separated, aqueous layer was extracted with dichloromethane (3×25 mL), dried over anhyd Na₂SO₄ and evaporated under vacuum at rt to furnish the crude trioxane, which on column chromatography over silica gel using EtOAc–hexane (0.5:99.5) as eluant furnished **6** (0.32 g, 31% yield) as oil: FT-IR (neat, cm⁻¹) 1451.7, 1641.8; ¹H NMR (200 MHz, CDCl₃): δ 0.45–0.53 (m, 2H), 0.68–0.77 (m, 2H), 1.29–1.41 (m, 1H), 3.99 (dd, 1H, J = 11.5, 10.5 Hz), 4.25 (dd, 1H, J = 11.5, 2.5 Hz), 4.92 and 4.99 ($2 \times s$, 2H), 4.98 (dd, 1H, J = 10.5, 2.5 Hz), 6.16 (s, 1H), 7.45 (m, 5H); ¹³C NMR (50 MHz, CDCl₃): δ 6.50, 6.86, 14.28, 70.04, 82.25, 104.42, 111.99, 127.40, 128.82, 130.34, 134.65, 145.06; FABMS (*m*/*z*) 233 (M+H)⁺. Anal. Calcd for C₁₄H₁₆O₃: C 72.38; H 6.94. Found: C 72.20; H 7.44.

Compounds 7–15 and 22–23 were also prepared by the procedure described for 6.

6.8. 6-(1-Cyclopropyl-vinyl)-3,3-dimethyl-[1,2,4]trioxane (7)

Yield 67%, oil: FT-IR (neat, cm⁻¹) 1642.4; ¹H NMR (200 MHz, CDCl₃): δ 0.45–0.47 (m, 2H), 0.67–0.72 (m, 2H), 1.26–1.33 (m, 1H), 1.38 (s, 3H), 1.65 (s, 3H), 3.80 (dd, 1H, *J* = 11.8, 2.6Hz), 4.00 (dd, 1H, *J* = 11.8, 10.1 Hz), 4.74 (dd, 1H, *J* = 10.1, 2.6Hz), 4.87 and 4.94 (2×s, 2H); ¹³C NMR (50 MHz, CDCl₃): δ 6.39, 6.75, 14.10, 20.43, 25.84, 63.67, 81.83, 102.66, 111.33, 145.66; FABMS (*m*/*z*) 185 (M+H)⁺.

6.9. 8-(1-Cyclopropyl-vinyl)-6,7,10-trioxa-spiro[4,5]decane (8)

Yield 61%, oil: FT-IR (neat, cm⁻¹) 1642.4; ¹H NMR (200 MHz, CDCl₃): δ 0.42–0.47 (m, 2H), 0.65–0.74 (m, 2H), 1.28–1.39 (m, 1H), 1.68–1.96 (m, 7H), 2.43–2.55 (m, 1H), 3.83–3.90 (m, 2H), 4.80 (dd, 1H, *J* = 7.8, 5.3 Hz), 4.86 and 4.92 (2 × s, 2H); ¹³C NMR (50 MHz, CDCl₃): δ 6.41, 6.78, 14.13, 23.70, 25.10, 33.02, 37.44, 65.34, 81.89, 111.36, 114.81, 145.56; FABMS (*m/z*) 211 (M+H)⁺.

6.10. 3-(1-Cyclopropyl-vinyl)-1,2,5-trioxa-spiro[5,5]undecane (9)

Yield 75%, oil: FT-IR (neat, cm⁻¹) 1640.6; ¹H NMR (200 MHz, CDCl₃): δ 0.42–0.49 (m, 2H), 0.65–0.74 (m, 2H), 1.26–1.37 (m, 1H), 1.43–1.61 (m, 8H), 1.92–2.05 (m, 1H), 2.14–2.24 (m, 1H), 3.77 (dd, 1H, J = 11.7, 3.1 Hz), 4.02 (dd, 1H, J = 11.7, 10.2 Hz), 4.74 (dd, 1H, J = 10.2, 3.1 Hz), 4.87 and 4.93 (2 × s, 2H); ¹³C NMR (50 MHz, CDCl₃): δ 6.40, 6.75, 14.15, 22.66, 22.71, 25.95, 29.32, 35.07, 62.92, 81.89, 102.78, 111.27, 145.78; FABMS (m/z) 225 (M+H)⁺.

6.11. 3-(1-Cyclopropyl-vinyl)-1,2,5-trioxa-spiro[5,6]dode-cane (10)

Yield 37%, oil: FT-IR (neat, cm⁻¹) 1642.5; ¹H NMR (200 MHz, CDCl₃): δ 0.42–0.49 (m, 2H), 0.64–0.73 (m, 2H), 1.28–1.39 (m, 1H), 1.47–1.77 (m, 10H), 2.14–2.27

z), 5.01 and 5.04 (2>

(m, 2H), 3.76 (dd, 1H, J = 11.8, 3.2Hz), 3.96 (dd, 1H, J = 11.8, 10.3Hz), 4.73 (dd, 1H, J = 10.3, 3.2Hz), 4.86 and 4.92 (2×s, 2H); ¹³C NMR (50MHz, CDCl₃): δ 6.35, 6.75, 14.07, 22.48, 22.61, 29.73, 30.16, 31.35, 38.67, 63.10, 81.71, 107.27, 111.06, 145.81; FABMS (*m*/*z*) 239 (M+H)⁺. Anal. Calcd for C₁₄H₂₂O₃·0.3H₂O: C 68.98; H 9.34. Found: C 68.63; H 9.36.

6.12. Trioxane 11

Yield 75%, white solid: mp 82–84°C; FT-IR (KBr, cm⁻¹) 1641.9; ¹H NMR (200 MHz, CDCl₃): δ 0.42–0.49 (m, 2H), 0.67–0.74 (m, 2H), 1.26–1.40 (m, 1H), 1.61–2.09 (m, 13H), 2.93 (br s, 1H), 3.77 (dd, 1H, J = 11.7, 3.1 Hz), 3.99 (dd, 1H, J = 11.7, 10.4 Hz), 4.75 (dd, 1H, J = 10.4, 3.1 Hz), 4.87 and 4.93 (2×s, 2H); ¹³C NMR (50 MHz, CDCl₃): δ 6.43, 6.79, 14.25, 27.58, 29.71, 33.39, 33.66, 33.86, 33.95, 36.65, 37.63, 62.45, 81.74, 104.87, 111.31, 145.86; FABMS (*m*/*z*) 277 (M+H)⁺. Anal. Calcd for C₁₇H₂₄O₃·0.1H₂O: C 73.39; H 8.77. Found: C 73.38; H 9.10.

6.13. 6-(1-Cyclohexyl-vinyl)-3,3-dimethyl-[1,2,4]trioxane (12)

Yield 57%, oil: FT-IR (neat, cm⁻¹) 1639.4; ¹H NMR (200 MHz, CDCl₃): δ 1.10–1.31 (m, 5H), 1.37 (s, 3H), 1.45–1.93 (m, 6H), 1.65 (s, 3H), 3.70 (dd, 1H, *J* = 11.9, 2.9 Hz), 3.91 (dd, 1H, *J* = 11.9, 10.3 Hz), 4.69 (dd, 1H, *J* = 10.3, 2.9 Hz), 5.03 (s, 2H); FABMS (*m*/*z*) 227 (M+H)⁺.

6.14. 8-(1-Cyclohexyl-vinyl)-6,7,10-trioxa-spiro[4,5]decane (13)

Yield 78%, oil: FT-IR (neat, cm⁻¹) 1641.6; ¹H NMR (200 MHz, CDCl₃): δ 1.10–1.31 (m, 5H), 1.60–1.92 (m, 13H), 2.45–2.52 (m, 1H), 3.77–3.81 (m, 2H), 4.76 (dd, 1H, J = 8.5, 4.5 Hz), 5.00 and 5.03 (2×s, 2H); ¹³C NMR (50 MHz, CDCl₃): δ 23.65, 25.10, 26.47, 26.90, 32.65, 33.01, 37.44, 43.28, 65.72, 80.79, 112.89, 114.64, 149.75; FABMS (*m*/*z*) 253 (M+H)⁺.

6.15. 3-(1-Cyclohexyl-vinyl)-1,2,5-trioxa-spiro[5,5]undecane (14)

Yield 80%, oil: FT-IR (neat, cm⁻¹) 1640.6; ¹H NMR (200 MHz, CDCl₃): δ 1.10–1.31 (m, 5H), 1.46–2.01 (m, 15H), 2.15–2.29 (m, 1H), 3.67 (dd, 1H, *J* = 11.8, 2.96 Hz), 3.94 (dd, 1H, *J* = 11.8, 10.4 Hz), 4.70 (dd, 1H, *J* = 10.4, 2.96 Hz), 5.02 and 5.04 (2×s, 2H); ¹³C NMR (50 MHz, CDCl₃): δ 22.67, 22.71, 25.95, 26.47, 26.89, 29.34, 32.61, 35.17, 43.39, 63.36, 80.76, 102.65, 112.87, 149.98; FABMS (*m*/*z*) 267 (M+H)⁺. Anal. Calcd for C₁₆H₂₆O₃·0.8H₂O: C 68.43; H 9.90. Found: C 68.77; H 10.20.

6.16. Trioxane 15

Yield 83%, oil: FT-IR (neat, cm⁻¹) 1640.0; ¹H NMR (200 MHz, CDCl₃): δ 1.11–1.32 (m, 5H), 1.55–2.09 (m, 19H), 2.94 (br s, 1H), 3.67 (dd, 1H, J = 11.8, 2.92 Hz), 3.91 (dd, 1H, J = 11.8, 10.4 Hz), 4.72 (dd, 1H,

J = 10.4, 2.92 Hz), 5.01 and 5.04 (2 × s, 2H); ¹³C NMR (50 MHz, CDCl₃): δ 26.49, 26.89, 27.59, 29.72, 32.59, 33.39, 33.65, 33.86, 33.97, 36.76, 37.63, 43.54, 62.92, 80.59, 104.74, 112.90, 150.05; FABMS (*m*/*z*) 319 (M+H)⁺. Anal. Calcd for C₂₀H₃₀O₃·H₂O: C 71.39; H 9.58. Found: C 71.70; H 9.29.

6.17. 6-(Cyclopropyl-cyclopropylidene-methyl)-3,3dimethyl-[1,2,4]trioxane (22)

Yield 8%, oil: FT-IR (neat, cm⁻¹) 1452.0; ¹H NMR (200 MHz, CDCl₃): δ 0.52–0.70 (m, 5H), 1.09 (s, 4H), 1.38 (s, 3H), 1.67 (s, 3H), 3.70 (dd, 1H, *J* = 11.8, 2.8 Hz), 4.30 (dd, 1H, *J* = 11.8, 10.9 Hz), 4.84 (dd, 1H, *J* = 10.9, 2.8 Hz); ¹³C NMR (50 MHz, CDCl₃): δ 1.67, 2.1, 5.7, 6.1, 13.7, 20.4, 25.9, 62.4, 82.0, 102.4, 122.2, 124.5; FABMS (*m*/*z*) 211 (M+H)⁺.

6.18. 3-(Cyclopropyl-cyclopropylidene-methyl)-1,2,5-trioxa-spiro[5.5]undecane (23)

Yield 8%, oil: FT-IR (neat, cm⁻¹) 1448.9; ¹H NMR (200 MHz, CDCl₃): δ 0.53–0.69 (m, 5H), 0.96–1.08 (m, 4H), 1.48–1.59 (m, 8H), 1.93–2.06 (m, 1H), 2.19–2.29 (m, 1H), 3.67 (dd, 1H, *J* = 11.7, 2.9 Hz), 4.33 (dd, 1H, *J* = 11.7, 10.8 Hz), 4.85 (dd, 1H, *J* = 10.8, 2.9 Hz); ¹³C NMR (50 MHz, CDCl₃): δ 1.72, 2.15, 5.73, 6.22, 13.81, 22.70, 22.77, 26.00, 29.34, 35.26, 61.70, 82.15, 102.67, 122.27, 124.68; FABMS (*m*/*z*) 251 (M+H)⁺.

6.19. Trioxane 16

To a magnetically stirred mixture of trioxane 11 (2.00 g, 7.24 mmol) and NaHCO₃ (2.20 g, 26.19 mmol) in dichloromethane (100 mL) was added mCPBA (2.18 g, 26.03 mmol) portion wise over 30 min and stirred at rt for 24h. Reaction mixture was diluted with water $(50 \,\mathrm{mL})$ and extracted with dichloromethane $(3 \times 75 \text{ mL})$. Combined organic layer was washed first with 10% ag NaOH solution (50mL), then with water $(2 \times 25 \text{ mL})$ and dried over anhyd Na₂SO₄. Solvent was removed under vacuum at rt to furnish the crude product, which on column chromatography over silica gel using EtOAc-hexane (0.5:99.5) as eluant furnished 16 (1.40g, 66% yield) as mixtures of diastereomers as viscous oil: ¹H NMR (200 MHz, CDCl₃): δ 0.16–0.55 (m, 4H), 1.16–1.42 (m, 1H), 1.55–2.03 (m, 13H), 2.53 (d, 1H, J = 5.0 Hz), 2.71, and 2.91 (2×d, 1H, J = 5.0 Hz each), 2.59 and 2.86 (2 × br m, 1H), 3.75– 4.53 (m, 3H); ¹³C NMR (50 MHz, CDCl₃): δ 0.11, 0.27, 2.29, 2.76, 10.65, 12.28, 27.51, 30.04, 31.21, 33.39, 33.63, 33.79, 34.90, 36.14, 37.55, 49.33, 50.34, 57.08, 57.36, 58.22, 58.91, 76.82, 77.45, 78.09, 80.03, 81.86, 105.22; FABMS (*m*/*z*) 293 (M+H)⁺. Anal. Calcd for C₁₇H₂₄O₄·0.3H₂O: C 68.56, H 8.33. Found: C 68.48, H 8.56.

6.20. Trioxane 17

To an ice cooled magnetically stirred solution of **16** (1.0 g, 3.42 mmol) in 1,4-dioxane (15 mL) was added 25% aq HClO₄ (7.2 mL) and stirred at 0 °C for 2h. Reaction mixture was diluted with water (50 mL) and

extracted with ether $(3 \times 50 \text{ mL})$. Combined organic layer was washed with satd NaHCO₃ solution (25 mL), water (2 × 25 mL) and dried over anhyd Na₂SO₄. Solvent was removed under vacuum at rt to furnish the crude product, which on column chromatography over silica gel using acetone–dichloromethane (2:98) as eluant furnished **17a** (350 mg, 33.01% yield) eluting first and **17b** (230 mg, 21.69% yield) eluting next.

Compound **17a**: mp 129–130 °C; FT-IR (KBr, cm⁻¹) 3407.6; ¹H NMR (200 MHz, CDCl₃): δ 0.34–0.56 (m, 4H), 0.72–0.85 (m, 1H), 1.64–2.05 (m, 14H), 2.67 (br s, 1H), 3.51 (d, 1H, *J* = 11.6 Hz), 3.94 (d, 1H, *J* = 11.6 Hz), 3.94–4.00 (m, 1H), 4.19 (dd, 1H, *J* = 11.7, 8.3 Hz), 4.34 (dd, 1H, *J* = 8.3, 3.1 Hz); EI-MS (*m*/*z*) 310 (M)⁺. Anal. Calcd for C₁₇H₂₆O₅·0.2H₂O: C 65.02; H 8.47. Found: C 65.16; H 8.60.

Compound **17b**: mp 123–124 °C; FT-IR (KBr, cm⁻¹) 3415.2; ¹H NMR (200 MHz, CDCl₃): δ 0.35–0.52 (m, 4H), 0.71–0.88 (m, 1H), 1.64–2.07 (m, 14H), 2.31 (br s, 1H), 2.48 (s, 1H), 2.73 (br s, 1H), 3.48 (d, 1H, J = 11.5 Hz), 3.82 (d, 1H, J = 11.5 Hz), 3.90 (dd, 1H, J = 11.6, 3.0Hz), 4.23 (dd, 1H, J = 11.6, 9.4Hz), 4.37 (dd, 1H, J = 9.4, 3.0Hz); FABMS (m/z) 311 (M+H)⁺. Anal. Calcd for C₁₇H₂₆O₅·0.2H₂O: C 65.02; H 8.47. Found: C 64.91; H 8.54.

6.21. Trioxane 18a

To a solution of 17a (200 mg, 0.65 mmol) in dichloromethane (5mL), were added succinic anhydride (120 mg, 1.2 mmol), triethylamine (0.5 mL), and DMAP (5mg) and stirred for 1h at rt. Reaction mixture was poured into 10% HCl (20mL). Organic layer was separated, aqueous layer extracted with dichloromethane $(2 \times 25 \text{ mL})$ and combined organic layer dried over anhyd Na₂SO₄. Solvent was removed under vacuum at rt to furnish the crude product, which on column chromatography over silica gel using MeOH-CHCl₃ (2:98) as eluant furnished 18a (250 mg, 94.69% yield) as oil: FT-IR (neat, cm⁻¹) 1726.1, 3477.8; ¹H NMR (200 MHz, CDCl₃): δ 0.36–0.55 (m, 4H), 0.79–0.86 (m, 1H), 1.62– 2.04 (m, 14H), 2.68 (s, 4H), 3.94 (dd, 1H, J = 11.7, 3.4 Hz), 4.16 (dd, 1H, J = 11.7, 9.0 Hz), 4.23 (s, 2H), 4.36 (dd, 1H, J = 9.0, 3.4 Hz); FABMS (*m*/*z*) 411 $(M+H)^+$. Anal. Calcd for $C_{21}H_{30}O_8 \cdot 0.7H_2O$: C 59.61; H 7.48. Found: C 59.40; H 7.57.

6.22. Trioxane 18b

To a solution of **17b** (160 mg, 0.52 mmol) in dichloromethane (5 mL), were added succinic anhydride (100 mg, 1.0 mmol), triethylamine (0.5 mL), and DMAP (5 mg) and stirred for 1 h at rt. Reaction mixture was poured into 10% HCl (20 mL). Organic layer was separated, aqueous layer extracted with dichloromethane (2 × 25 mL) and combined organic layer dried over anhyd Na₂SO₄. Solvent was removed under vacuum at rt to furnish the crude product, which on column chromatography over silica gel using MeOH–CHCl₃ (2:98) as eluant furnished **18b** (150 mg, 71.42% yield) as white solid. Mp 82–84 °C; FT-IR (KBr, cm⁻¹) 1724.0, 3471.4; ¹H NMR (200 MHz, CDCl₃) 0.33–0.54 (m, 4H), 0.77–0.81 (m, 1H), 1.65–2.07 (m, 13H), 2.69 (s, 4H), 2.80 (br s, 1H), 3.82 (dd, 1H, J = 11.6, 2.6 Hz), 4.14– 4.30 (m, 3H), 4.44 (dd, 1H, J = 10.2, 2.6 Hz); FABMS (*m*/*z*) 411 (M+H)⁺. Anal. Calcd for C₂₁H₃₀O₈·0.7H₂O: C 59.61; H 7.48. Found: C 59.76; H 7.40.

6.23. Trioxanes 19a and 19b

To a magnetically stirred solution of diol 17 (0.60 g, 1.93 mmol, a mixture of 17a and 17b) and cyclopentanone (1.10 g, 13.09 mmol) in dichloromethane (15 mL) was added concd HCl (five drops) and stirred at rt for 3h. Reaction mixture was poured into saturated aq NaHCO₃ solution (25 mL) and organic layer was separated. Aqueous layer was extracted with dichloromethane (2×25 mL). Combined organic layer was dried over anhyd Na₂SO₄. Solvent was removed under vacuum at rt to furnish the crude product, which on column chromatography over silica gel using EtOAc-hexane (0.5:99.5) as eluant furnished 19a (0.15 g, 21% yield) eluting first and 19b (0.17 g, 23% yield) eluting next.

Compound **19a**: viscous oil, ¹H NMR (200 MHz, CDCl₃): δ 0.32–0.51 (m, 4H), 0.87–0.97 (m, 1H), 1.56–2.1 (m, 21H), 2.89 (br s, 1H), 3.68 (d, 1H, J = 8.6Hz), 3.75 (dd, 1H, J = 11.2, 2.4Hz), 4.11 (d, 1H, J = 8.6Hz), 4.26 (dd, 1H, J = 11.2. 10.8Hz), 4.41 (dd, 1H, J = 10.8, 2.4Hz); FABMS (*m*/*z*) 377 (M+H)⁺. Anal. Calcd for C₂₂H₃₂O₅·0.5H₂O: C 68.54; H 8.63. Found: C 68.19; H 8.80.

Compound **19b:** viscous oil, ¹H NMR (200 MHz, CDCl₃): δ 0.39–0.46 (m, 4H), 0.85–0.98 (m, 1H), 1.55–2.08 (m, 21H), 2.85 (br s, 1H), 3.67 (d, 1H, J = 8.6 Hz), 3.84 (dd, 1H, J = 11.7, 3.0 Hz), 4.09 (dd, 1H, J = 11.7, 10.4 Hz), 4.22 (d, 1H, J = 8.6 Hz), 4.36 (dd, 1H, J = 10.4, 3.0 Hz); FABMS (m/z) 377 (M+H)⁺. Anal. Calcd for C₂₂H₃₂O₅·0.5H₂O: C 68.54; H 8.63. Found: C 68.24; H 8.93.

Trioxanes 20a,b and 21a,b were prepared by the same procedure as described for 19a,b.

6.24. Trioxanes 20a and 20b

Compound **20a**: yield 19%, white solid: mp 110–112°C; ¹H NMR (200 MHz, CDCl₃): δ 0.32–0.55 (m, 4H), 0.87– 0.97 (m, 1H), 1.35–2.10 (m, 23H), 2.90 (br s, 1H), 3.70 (d, 1H, J = 8.7Hz), 3.77 (dd, 1H, J = 11.4, 2.4Hz), 4.20 (d, 1H, J = 8.7Hz), 4.29 (dd, 1H, J = 11.4, 10.8Hz), 4.45 (dd, 1H, J = 10.8, 2.4Hz); ¹³C NMR (50 MHz, CDCl₃): δ –0.30, 2.25, 12.05, 24.08, 24.32, 25.43, 27.56, 29.82, 33.42, 33.69, 33.84, 33.93, 35.92, 36.72, 37.39, 37.62, 58.72, 72.44, 80.22, 83.27, 105.16, 110.56; FABMS (*m*/*z*) 391 (M+H)⁺. Anal. Calcd for C₂₃H₃₄O₅·0.4H₂O: C 69.45; H 8.82. Found: C 69.59; H 9.12.

Compound **20b**: yield 20%, white solid: mp 94–96 °C; ¹H NMR (200 MHz, CDCl₃): δ 0.40–0.49 (m, 4H), 0.85–0.93 (m, 1H), 1.36–2.00 (m, 23H), 2.87 (br s, 1H), 3.72 (d, 1H, J = 8.6Hz), 3.89 (dd, 1H, J = 11.6, 3.0Hz),

4.12 (dd, 1H, J = 11.6, 10.4 Hz), 4.24 (d, 1H, J = 8.6 Hz), 4.35 (dd, 1H, J = 10.4, 3.0 Hz); ¹³C NMR (50 MHz, CDCl₃): δ 0.49, 1.48, 16.09, 24.29, 25.53, 27.57, 30.02, 33.48, 33.92, 35.59, 36.26, 36.49, 37.64, 59.18, 69.92, 80.89, 82.81, 105.00, 110.93; FABMS (*m*/*z*) 391 (M+H)⁺. Anal. Calcd for C₂₃H₃₄O₅·0.5H₂O: C 69.14; H 8.83. Found: C 68.81; H 8.98.

6.25. Trioxanes 21a and 21b

Compound **21a**: yield 25%, white solid: mp 156–157 °C; ¹H NMR (200 MHz, CDCl₃): δ 0.26–0.38 (m, 2H), 0.43– 0.58 (m, 2H), 0.87–0.98 (m, 1H), 1.55–2.10 (m, 27H), 2.90 (br s, 1H), 3.70 (d, 1H, J = 8.5 Hz), 3.77 (dd, 1H, J = 10.9, 2.0 Hz), 4.21 (d, 1H, J = 8.5 Hz), 4.32 (dd, 1H, J = 10.9 Hz each), 4.44 (dd, 1H, J = 10.9, 2.0 Hz); FABMS (*m*/*z*) 443 (M+H)⁺. Anal. Calcd for C₂₇H₃₈O₅·0.7H₂O: C 71.23, H 8.72. Found: C 71.36, H 8.76.

Compound **21b**: yield 24%, white solid: mp 159–160 °C; ¹H NMR (200 MHz, CDCl₃): δ 0.37–0.52 (m, 4H), 0.85– 0.91 (m, 1H), 1.55–2.00 (m, 27H), 2.88 (br s, 1H), 3.74 (d, 1H, *J* = 8.5 Hz), 3.91 (dd, 1H, *J* = 11.5, 2.8 Hz), 4.13 (dd, 1H, *J* = 11.5, 10.2 Hz), 4.25 (d, 1H, *J* = 8.5 Hz), 4.36 (dd, 1H, *J* = 10.2, 2.8 Hz); FABMS (*m*/z) 443 (M+H)⁺. Anal. Calcd for C₂₇H₃₈O₅: C 73.27, H 8.65. Found: C 73.03, H 8.83.

6.26. Trioxane 25a

To an ice-cooled solution of 18a (150mg, 0.36mmol) in ether (10mL) was added ice-cooled solution of CH₂N₂ in ether (15mL), prepared by the reaction of N-nitroso N-methyl urea (180 mg, 1.74 mmol) with 40% aq KOH (15mL), and stirred for 30min. Solvent was evaporated to furnish the crude product, which on column chromatography over silica gel using EtOAc-hexane (15:85) as eluant furnished 25a (0.15g, 96.77% yield) as solid: mp 128–130 °C; FT-IR (KBr, cm⁻¹) 1739.2, 3492.3; ¹H NMR (200 MHz, CDCl₃): δ 0.36–0.53 (m, 4H), 0.77– 0.87 (m, 1H), 1.63–1.99 (m, 14H), 2.32 (s, 1H, OH), 2.66 (s, 4H), 3.69 (s, 3H), 3.94 (dd, 1H, J=11.6, 3.1 Hz), 4.12-4.29 (m, 3H), 4.36 (dd, 1H, J = 8.9,3.1 Hz); ¹³C NMR (50 MHz, CDCl₃): δ -0.83, 0.0, 13.99, 27.08, 28.89, 29.17, 30.33, 33.00, 33.22, 33.36, 33.43, 35.02, 37.11, 51.89, 57.56, 67.99, 71.66, 81.88, 104.89, 172.13, 172.73; EIMS (m/z) 424 $(M)^+$. Anal. Calcd for C₂₂H₃₂O₈: C 62.25; H 7.60. Found: C 62.86; H 8.15.

6.27. Trioxane 25b

To an ice-cooled solution of **18b** (80 mg, 0.19 mmol) in ether (10 mL) was added ice-cooled solution of CH_2N_2 in ether (15 mL), prepared by the reaction of *N*-nitroso *N*-methyl urea (100 mg, 0.97 mmol) with 40% aq KOH (10 mL), and stirred for 30 min. Solvent was evaporated to furnish the crude product, which on column chromatography over silica gel using EtOAc–hexane (15:85) as eluant furnished **25b** (0.08 g, 97.56% yield) as oil: FT-IR (neat, cm⁻¹) 1737.9, 3490.3; ¹H NMR (200 MHz, CDCl₃): δ 0.39–0.58 (m, 4H), 0.78–0.88 (m, 1H), 1.65– 2.03 (m, 13H), 2.62 (s, 1H, OH), 2.66 (s, 4H), 2.81 (br s, 1H), 3.70 (s, 3H), 3.82 (dd, 1H, J = 11.6, 2.5Hz), 4.14–4.31 (m, 3H), 4.44 (dd, 1H, J = 10.0, 2.5Hz); ¹³C NMR (50 MHz, CDCl₃): δ –0.51, 0.38, 12.68, 27.51, 29.40, 29.67, 30.07, 33.38, 33.63, 33.80, 33.89, 36.27, 37.56, 52.41, 58.30, 68.71, 71.68, 82.03, 105.29, 173.09, 173.36; EIMS (*m*/*z*) 424(M)⁺. Anal. Calcd for C₂₂H₃₂O₈: C 62.25; H 7.60. Found: C 61.88; H 7.92.

7. In vivo antimalarial efficacy test

The in vivo efficacy of compounds was evaluated against *P. yoelii* (MDR) in Swiss mice model. The colony bred Swiss mice $(25 \pm 1 \text{ g})$ were inoculated with 1×10^6 parasitized RBC on day 0 and treatment was administered to a group of five mice at each dose, from day 0 to day 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 neutralized groundnut oil and administered intramuscularly for each dose. Parasitemia level were recorded from thin blood smears between day 4 and 28.¹⁰ Mice treated with artemisinin served as positive controls.

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