Reduction of the Double Bond of 6-Arylvinyl-1,2,4-trioxanes Leads to a Remarkable Increase in Their Antimalarial Activity against Multidrug-Resistant *Plasmodium yoelii nigeriensis* in a Swiss Mice Model

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**ABSTRACT:** Novel 6-arylethyl-1,2,4-trioxanes *6a*–*i* and *7a*–*i* are easily accessible in one step from the diimide reduction of 6-arylvinyl-1,2,4-trioxanes *5a*–*i*. All of these new trioxanes were assessed for their oral antimalarial activity against multidrug-resistant *Plasmodium yoelii nigeriensis* in a Swiss mice model. Most of the saturated trioxanes *6c, 6f, 6g, 6h,* and *6i*, the active compounds of the series, provided 100% protection to the malaria-infected mice at a dose of 24 mg/kg × 4 days. Further, trioxane *6i*, the most active compound of the series, also showed 100% protection even at a dose of 12 mg/kg × 4 days and 20% protection at a dose of 6 mg/kg × 4 days. In this model, β-arteether provided 100% protection at a dose of 48 mg/kg × 4 days and only 20% protection at a dose of 24 mg/kg × 4 days via the oral route, which was found to exhibit 4-fold antimalarial activity compared with the currently used drug β-arteether.

**INTRODUCTION**

Since ancient times, humankind has had to struggle against the persistent onslaught of pathogenic microorganisms and is still suffering. Malaria, a vector-borne disease caused by *Plasmodium* sp., is still one of the world’s most deadly diseases that threaten nearly 40% of the world’s population, putting 3.2 billion people at risk in 107 countries, and infects approximately 300−500 million people annually worldwide, mainly in tropical and subtropical areas.1 It is estimated that there are between 1 and 3 million deaths every year due to malaria. In Africa alone, more than 1 million people die because of malaria and most of them are children under 5 years of age.2 The economic toll of malaria is tremendous, as it has been estimated that the African continent has suffered almost $100 billion loss in GDP over the last 35 years due to malaria alone.3 Malaria ranks third among the major infectious diseases in causing deaths after pneumococcal acute respiratory infections and tuberculosis and accounts for approximately 2.6% of the total disease burden of the world.4,5

Indeed, the emergence of malaria as a worldwide epidemic can largely be attributed to the indiscriminate use of conventional drugs, due to which there has been a rapid development of resistant varieties of the malaria parasite. In that regard, the discovery of artemisinin 1, a sesquiterpene lactone endoperoxide, isolated from the Chinese traditional medicinal herb *Artemisia annua*, has proven to be a milestone in malaria chemotherapy.6−13 It was found to be active against both chloroquine-sensitive and chloroquine-resistant strains of malaria, and its semisynthetic derivatives artether 2, arteether 3, and artesunic acid 4 (Figure 1) have shown tremendous potential and are presently the drugs of choice for...
the treatment of multidrug-resistant malaria caused by Plasmodium falciparum.14−19 Several analogues of artemisinin have been synthesized so far that have shown potential antimalarial activity.20−24 The very fact that it is the endoperoxide linkage of artemisinin and its semisynthetic analogues in the form of a 1,2,4-trioxane ring system, which is responsible for its antimalarial activity, has led to the development of several synthetic trioxanes and various other related peroxides that have shown potent antimalarial activity both in vivo and in vitro.25−37

As part of an endeavor to develop synthetic substitutes for artemisinin and its derivatives, we had earlier reported a photooxygenation route for the preparation of 6-arylvinyl-1,2,4-trioxanes.38,39 Preparation of β-hydroxyhydroperoxides by the photooxygenation of allylic alcohols and their acid-catalyzed condensation with ketones are the key steps of this method (Scheme 1). Several 1,2,4-trioxanes prepared by this route have shown promising activity against multidrug-resistant Plasmodium yoelii nigeriensis in Swiss mice.40−50

Scheme 1. Preparation of 6-Arylvinyl-1,2,4-trioxanes

![Scheme 1](https://acsomega.org/journal/acsodf/content/6/13/30791/ACSOmega2105041.html)

A unique feature of these 6-arylvinyl-substituted 1,2,4-trioxanes is that they undergo a highly facile fragmentation under basic conditions by the extraction of an acidic C-6 proton to furnish α,β-unsaturated keto alcohols, which react very efficiently with various amines and thiols to afford Michael adducts (Scheme 2).51

Based on these results, we had earlier suggested that this facile formation of α,β-unsaturated keto systems under mild basic conditions and their equally facile reaction with amines and thiols might have relevance to their mechanism of action as antimalarials.51 This suggestion naturally brings the role of the double bond as the key group for the activity of this group of 1,2,4-trioxanes and calls for the preparation and antimalarial assessment of corresponding saturated analogues as proof for their mechanism of action, as it is expected that they should be less active. Toward this end, we prepared several saturated analogues (6-arylethyl-1,2,4-trioxanes) and assessed them for their antimalarial efficacy. A graphical representation of the evolution of our work on trioxanes resulting in the current series of molecules is shown in Figure 2. In this article, we report details of this study.

### CHEMISTRY

Diimide reduction52−56 of the double bond of 6-arylvinyl-1,2,4-trioxanes 5a−i using hydrazinium carboxylate (N$_2$H$_3$COON$_2$H$_5$) and 30% H$_2$O$_2$ as reported by us earlier57 furnished 6-arylethyl-1,2,4-trioxanes 6a−i (less polar or higher R$_2$) and 7a−i (more polar or lower R$_2$) as a mixture of diastereomers in very good yields. (Scheme 3).

Thus, the reaction of trioxanes 5a−i with N$_2$H$_3$COON$_2$H$_5$ and 30% H$_2$O$_2$ in a 1:1 mixture of THF/EtOH at rt furnished saturated trioxanes 6a−i (less polar) and 7a−i (more polar) as a mixture of diastereomers in a ratio of 2:3 in 35−97% yield. The two diastereomers were separated by repeated flash chromatography and characterized separately.

**Antimalarial Activity.** Parent 6-arylvinyl-1,2,4-trioxanes 5a−i and their saturated derivatives 6a−i (less polar) and 7a−i (more polar) were assessed for their antimalarial activity against multidrug-resistant P. yoelii nigeriensis in mice by the oral route using Peter’s procedure.58−60 The activity data of trioxanes 5a−i against P. yoelii nigeriensis have already been reported earlier, but it has been included in the present study just for the sake of comparison.43,45 In this model, β-arteether 3 provides 100% protection to the mice infected with multidrug-resistant P. yoelii nigeriensis at a dose of 48 mg/kg × 4 days via the oral route as all of the treated mice survived beyond day 28. At a dose of 24 mg/kg × 4 days, β-arteether provides only 20% protection to the treated mice. Among the 6-arylvinyl-1,2,4-trioxanes 5a−i that were initially tested at 96 mg/kg × 4 days orally, double the effective dose of β-arteether, only compounds 5h and 5i provided 100% protection and compound 5g provided 80% protections at this dose. Compound 5i also provided 100% protection at a dose of 48 mg/kg × 4 days but provided only 80% protection at a dose of 24 mg/kg × 4 days via the oral route. However, compounds 5g and 5h provided only 60% protection at a dose of 48 mg/kg × 4 days. Among the saturated trioxanes 6a−i and 7a−i, compounds 6a−i (less polar) and 7i (more polar) were found effective at a dose of 48 mg/kg × 4 days—the effective dose of β-arteether. Compounds 6c, 6f, 6g, 6h, and 6i were found 100% curative even at a dose of 24 mg/kg × 4 days, double the effective dose of β-arteether. Compound 6i was
found effective even at a dose of 12 mg/kg × 4 days. Compounds 7a–g were found less effective at their respective doses at which they were screened. The results are summarized in Table 1.

RESULTS AND DISCUSSION

As seen in Table 1, although several 6-aryylvinyl trioxanes 5a–i prepared by the process as shown in Scheme 1 have shown promising antimalarial activity, a large number of such trioxanes were less effective than β-arteether. 6-Arylethyl-1,2,4-trioxanes showed very surprising results, as, contrary to our expectations, these trioxanes were found far more active than their parent counterparts. The interesting feature about their activity was that the less polar isomer (higher $R_f$) was far more active in comparison to the more polar isomer (lower $R_f$), which showed only moderate activity.

Among these 6-arylethyl 1,2,4-trioxanes compounds, 6i was found to be the most active compound of the series, as it provided 100% clearance of parasitemia when administered at a dose of 48 mg/kg × 4 days, 24 mg/kg × 4 days, and 12 mg/kg × 4 days via the oral route. Compound 6i also provided 20% protection at a dose of 6 mg/kg × 4 days. Its corresponding more polar isomer 7i also provided 100% protection at a dose of 48 mg/kg × 4 days but showed no protection at a dose of 24 mg/kg × 4 days. Compounds 6c and 6g–i were the next most active compounds of the series, as they provided 100% protection to the treated mice at a dose of 24 mg/kg × 4 days. Compounds 6a,b and 6d,e showed 100% clearance of parasitemia at a dose of 48 mg/kg × 4 days. Compound 6a also provided 20% protection to the treated mice at a dose of 24 mg/kg × 4 days.

The corresponding more polar isomers 7a–g showed only partial to 100% suppression of parasitemia until day four, as compounds 7f and 7h provided 100% suppression, but none of the mice survived at a dose of 48 mg/kg × 4 days. Although compounds 7c and 7g provided 100% and 99% suppression of parasitemia, respectively, the rest of the compounds 7a,b, and 7d,e provided only partial suppression at a dose of 96 mg/kg × 4 days until day four, as none of the mice survived beyond day 28 in all of these cases.

A careful analysis of Table 1 reveals that there is a direct correlation between in vivo oral antimalarial activity and log $p$ values in this new series of 6-arylethyl-1,2,4-trioxanes, and the compounds having log $p$ values in the range of 6.74–5.56 are the most active. Recent reports on antimalarial activity have shown that compounds having high log $p$ values are highly active by the oral route.49 Compound 6i, the most active compound of the series, having the highest log $p$ value of 6.74 provided 100% protection at a dose of 12 mg/kg × 4 days, while the next best compounds 6g and 6h having a log $p$ value of 6.00, compound 6f having a log $p$ value of 5.84, and compound 6c having a log $p$ value of 5.56 provided 100% protection at a dose of 24 mg/kg × 4 days.

Comounds that have relatively small log $p$ values, for example, compound 6b having a log $p$ value of 5.49, compound 6d having a log $p$ value of 5.16, compound 6a having a log $p$ value of 5.01, and compound 6e having a log $p$ value of 4.88 exhibited 100% clearance of parasitemia only at a dose of 48 mg/kg × 4 days and none of the compounds provided 100% protection at a dose of 24 mg/kg × 4 days.

Analysis of Table 1 also shows that the saturated derivatives have log $p$ values higher than that of their parent unsaturated counterparts; for example, compound 5a has a log $p$ value of...
Table 1. Comparative Oral Antimalarial Activity of 6-Arylvinyl-1,2,4-trioxanes 5a–i versus 6-Arylethyl-1,2,4-trioxanes 6a–i and 7a–i against Multidrug-Resistant *P. yoelii nigeriensis* in Swiss Mice

| Compound | Log P | Dose mg/kg | % Suppression on day 4<sup>a</sup> | Mice alive on day 28 |
|----------|-------|------------|----------------------------------|---------------------|
| 5a       | 4.65  | 96         | 96                               | 0/5                 |
| 6a       | 5.01  | 96         | 48                               | 5/5                 |
|          |       | 48         | 100                              | 5/5                 |
|          |       | 24         | 100                              | 5/5                 |
|          |       | 12         | 100                              | 1/5                 |
| 7a       | 5.01  | 96         | 40                               | 0/5                 |
| 5b       | 5.14  | 96         | 95                               | 0/5                 |
| 6b       | 5.49  | 48         | 100                              | 5/5                 |
|          |       | 24         | 96                               | 0/5                 |
| 7b       | 5.49  | 96         | 32                               | 0/5                 |
| 5c       | 5.21  | 96         | 100                              | 0/5                 |
| 6c       | 5.56  | 48         | 100                              | 5/5                 |
|          |       | 24         | 100                              | 5/5                 |
|          |       | 12         | 100                              | 2/5                 |
| 7c       | 5.56  | 96         | 100                              | 0/5                 |
| 5d       | 4.81  | 96         | 99                               | 0/5                 |
| 6d       | 5.16  | 48         | 100                              | 5/5                 |
|          |       | 24         | 100                              | 5/5                 |
|          |       | 12         | 100                              | 0/5                 |
| 7d       | 5.16  | 96         | 33                               | 0/5                 |
| 5e       | 4.53  | 96         | 99                               | 0/5                 |
| 6e       | 4.88  | 48         | 100                              | 5/5                 |
|          |       | 24         | 100                              | 0/5                 |
|          |       | 12         | 82                               | 0/5                 |
| 7e       | 4.88  | 96         | 11                               | 0/5                 |
| 5f       | 5.48  | 96         | 99                               | 0/5                 |
| 6f       | 5.84  | 48         | 100                              | 5/5                 |
|          |       | 24         | 100                              | 5/5                 |
|          |       | 12         | 100                              | 0/5                 |

<sup>a</sup> Data from ACS Omega, http://pubs.acs.org/journal/acsodf

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4.65, while its saturated derivatives 6a and 7a have a log $p$ value of 5.01. A similar pattern is observed in the rest of the compounds as well. This could be one of the reasons for their increased activity in comparison to their parent trioxanes. The difference in activity of the two diastereomers is still uncertain and could be due to certain unknown stereochemical factors, as there are numerous reports in the literature where one isomer has been found active and the other less active or sometimes even toxic.61

These observations also indicated that the mechanism of action for antimalarial activity, certainly not in the case of 6-arylvinyl-1,2,4-trioxanes but at least in the case of 6-arylethyl 1,2,4-trioxanes, is not by the process as shown in Scheme 2.

### CONCLUSIONS

In conclusion, in our efforts to assess the role of the double bond of 6-arylvinyl-1,2,4-trioxanes toward antimalarial activity, we have prepared a new series of saturated 1,2,4-trioxanes using the chemistry of the double bond and studied their structure—activity relationship. Several of these trioxanes (6a–i) have shown a better activity profile than the parent trioxanes 5a–i. The trioxane 6i, the most active compound of the series, has four times better oral antimalarial activity than that of the clinically used drug, β-artether. Hopefully, the outcome of our finding, the antimalarial activity of current trioxanes by oral route, helps scientists in finding the better drug candidate in fighting against malaria.

### EXPERIMENTAL SECTION

**General.** All glass apparatus were oven-dried prior to use. Melting points were determined in open capillaries on a comlab melting-point apparatus and are uncorrected. Infrared spectra were recorded on a PerkinElmer Fourier transform infrared (FT-IR) RXI spectrophotometer. $^1$H NMR and $^{13}$C NMR spectra were recorded using a Bruker Supercon Magnet DRX-300 spectrometer (operating at 300 MHz for $^1$H and 75
MHz for $^{13}$C using CDCl$_3$ as the solvent. Tetramethylsilane ($\delta$ 0.00 ppm) served as an internal standard in $^1$H NMR and CDCl$_3$ ($\delta$ 77.23 ppm) in $^{13}$C NMR. Chemical shifts are reported in parts per million. Splitting patterns are described as singlet (s), doublet (d), triplet (t), and multiplet (m). In NMR, the numbering of atoms is presented according to the usual numbering in artesiminin, as indicated in the text. Fast atom bombardment mass spectra (FAB-MS) were obtained on a JEOL SX-102/DA-6000 mass spectrometer using argon/xenon (6 kV, 10 mA) as the FAB gas. Glycerol or $m$-nitrobenzyl alcohol was used as the matrix. Electrospray mass spectrometry (ES-MS) was performed on a Micromass Quattro II triple quadrupole mass spectrometer. High-resolution electron impact mass spectra (HR-EIMS) were obtained on a JEOL MS route 600H instrument. Elemental analyses were performed on a Vario EL-III CHNS analyzer (Germany) and values were within $\pm 0.4\%$ of the calculated values except where noted. Column chromatography was performed over Merck silica gel (particle size: 60–120 mesh) procured from Qualigens (India) and flash silica gel (particle size: 230–400 mesh). All chemicals and reagents were obtained from Aldrich (Milwaukee, WI), Lancaster (England), or Spectrochem (India) and were used without further purification. $log_{10}$ values of the compounds were calculated using ChemDraw Ultra 15.1 software. The purity of final tested compounds was typically determined to be $>95\%$ by elemental analysis.

Procedure for the Preparation of a Hydrazinocarbazate Solution. In an ice-cooled hydrazine hydrate ($N_2H_5H_2O$, 103 g, 2.06 mol), a slow stream of CO$_2$ gas was bubbled until the weight of the reaction mixture became constant (150 g, which corresponds to a 2:1 adduct of $N_2H_4H_2O$ and CO$_2$). One gram of this highly viscous material (density 1.45) was dissolved in 100 mL of water for the measurement of pH, which was found to be 7.51, while the pH value of 1\% aqueous solution of $N_2H_4H_2O$ was found to be 9.79.

General Procedure for Diimide Reduction of 1,2,4-Trioxanes Using Hydrazinocarbazate ($N_2H_5COONH_4$H$_2$O) and 30\% H$_2$O$_2$. Reduction of 1,2,4-trioxane 5a as a representative: to a stirred and ice-cooled solution of trioxane 5a (3.00 g, 9.62 mmol) and hydrazinocarbazate (9.55 mL, 10 equiv) in a 1:1 mixture of EtOH/tetrahydrofuran (THF) (150 mL) was added 30\% H$_2$O$_2$ (32.69 mL, 30 equiv) dropwise over 30 min, and the reaction mixture was allowed to stir at rt for 9 days. The reaction mixture was concentrated under vacuum, diluted with water (20 mL), and extracted with ether (2 $\times$ 150 mL). The combined organic extract was washed successively with 10\% HCl (30 mL), water (30 mL), and with saturated aqueous NaHCO$_3$ (30 mL), dried over anhydrous Na$_2$SO$_4$, concentrated under vacuum, and the crude product was purified by column chromatography over silica gel to furnish saturated trioxanes 6a and 7a (2.92 g, 97\% yield) as a mixture of diastereomers in approximately 2:3 ratio, which on flash chromatography using the eluent EtOAc/hexane (1:9) furnished the pure isomers 6a (less polar, oil) and 7a (more polar, white solid, mp 84–85 °C).

$^{15}$, $^{35}$, $^{55}$, $^{6}$S, $^{7}$S, $^{6}$-6'-(R)-(1-Phenylethyl)spiro-[adamantane-2,3'-[1,2,4]trioxane] (6a). Yield (1.17 g, 39\%) as an oil; FT-IR (neat cm$^{-1}$) 763, 1025, 1117, 1223, 1602, 2914; $^{1}$H NMR (300 MHz, CDCl$_3$) $\delta$ 1.27 (d, 3H, $J = 7.3$ Hz), 1.55–2.08 (m, 13H), 2.35 (s, 3H), 2.78 (s, 1H), 2.84 (quin, 1H, $J = 7.3$ Hz), 2.76 (dd, 1H, $J = 11.6$ and 3.4 Hz), 3.83 (dd, 1H, $J = 11.6$ and 6.9 Hz), 4.47 (dd, 1H, $J = 6.9$, 7.7, and 3.4 Hz) 7.13 (s, 4H, Ar); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 17.62 (CH$_3$), 21.23 (CH$_3$), 27.38 (CH), 27.42 (CH$_2$), 29.80 (CH), 33.27 (CH$_3$), 33.40 (CH$_2$), 33.62 (CH$_2$), 33.74 (CH$_3$), 36.17 (CH), 37.48 (CH$_2$), 40.41 (CH), 60.95 (CH$_3$), 82.69 (CH), 104.59 (CH), 127.71 (2 CH), 129.40 (2 CH), 136.42 (C), 139.47 (C); FAB-MS (m/z) 329 [M + H$^+$]; EI-HRMS calcd for C$_{21}$H$_{28}$O$_3$ [M$^+$]: 328.2039. Found: 328.2039; Anal. Calcd for C$_{21}$H$_{28}$O$_3$: %C 76.79, %H 8.59. Found: %C 76.79, %H 8.58; purity 99.86%.

$^{15}$, $^{35}$, $^{55}$, $^{6}$S, $^{7}$S, $^{6}$-6'-(R)-(1-4-Chlorophenylethyl)spiro-[adamantane-2,3'-[1,2,4]trioxane] (6c). Yield (1.14 g, 38\%) as a white solid, mp 74–76 °C; FT-IR (Kbr cm$^{-1}$) 767, 1041, 1216, 1636, 2926; $^{1}$H NMR (300 MHz, CDCl$_3$) $\delta$ 1.27 (d, 3H, $J = 7.3$ Hz), 1.55–2.08 (m, 13H), 2.35 (s, 3H), 2.78 (s, 1H), 2.84 (quin, 1H, $J = 7.3$ Hz), 2.76 (dd, 1H, $J = 11.6$ and 3.4 Hz), 3.83 (dd, 1H, $J = 11.6$ and 6.9 Hz), 4.47 (dd, 1H, $J = 6.9$, 7.7, and 3.4 Hz) 7.13 (s, 4H, Ar); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 17.62 (CH$_3$), 21.23 (CH$_3$), 27.38 (CH), 27.42 (CH$_2$), 29.80 (CH), 33.27 (CH$_3$), 33.40 (CH$_2$), 33.62 (CH$_2$), 33.74 (CH$_3$), 36.17 (CH), 37.48 (CH$_2$), 40.41 (CH), 60.95 (CH$_3$), 82.69 (CH), 104.59 (CH), 127.71 (2 CH), 129.40 (2 CH), 136.42 (C), 139.47 (C); FAB-MS (m/z) 329 [M + H$^+$]; EI-HRMS calcd for C$_{21}$H$_{28}$O$_3$ [M$^+$]: 328.2039. Found: 328.2039; Anal. Calcd for C$_{21}$H$_{28}$O$_3$: %C 76.79, %H 8.59. Found: %C 76.79, %H 8.58; purity 99.86%.
as a white solid, mp 92–94 °C; FT-IR (KBr cm⁻¹) 768, 1091, 1112, 1127, 1655, 2917; 1³H NMR (300 MHz, CDCl₃) δ 6.16 (d, 3H, J = 6.9 Hz), 1.59–2.03 (m, 13H), 2.76 (s, 1H), 2.81 (quin, 1H, J = 11.8 & 2.5 Hz), 3.36 (dd, 1H, J = 11.8 & 2.5 Hz), 3.59 (dd, 1H, J = 11.7 & 9.4 Hz), 4.28 (dt, 1H, J = 9.1 & 2.3 Hz), 7.13 (d, 2H, J = 8.4 Hz, Ar), 7.29 (2H, J = 8.4 Hz, Ar); ¹³C NMR (75 MHz, CDCl₃) δ 18.49 (CH₃), 27.31 (2 × CH), 30.19 (CH), 33.20 (CH₂), 34.42 (CH₂), 33.60 (2 × CH₂), 35.52 (CH), 37.36 (CH₂), 40.28 (CH), 60.90 (CH₂), 82.96 (CH), 104.62 (C), 129.08 (2 × CH), 129.14 (2 × CH), 132.95 (C), 140.56 (C); FAB-MS (m/z) 349 [M + H⁺]; EI-IRMS calc'd for C₂₀H₂₅ClO₃ [M⁺]: 348.1492. Found: 348.1429; Anal. Calc'd for C₂₀H₂₅ClO₃: % C 68.86, % H 7.22. Found: % C 68.99, % H 7.34; purity 99.67%.

(15,35,55,6'S,7'S,5′-6′-(S)-1-(4-Chlorophenyl)ethyl)spiro-[adamantane-2,3′-[1,2,4]trioxane] (7c). Yield: (1.72 g, 57%) as a white solid, mp 114–115 °C; FT-IR (KBr cm⁻¹) 772, 1089, 1113, 1220, 1636, 2918; ¹³H NMR (300 MHz, CDCl₃) δ 1.26 (d, 3H, J = 7.2 Hz), 1.55–2.06 (m, 13H), 2.72 (s, 1H), 2.85 (quin, 1H, J = 7.2 Hz), 3.78–3.80 (m, 2H), 4.41 (brdd, 1H), 7.16 (d, 2H, J = 8.3 Hz, Ar), 7.29 (d, 2H, J = 8.3 Hz, Ar); ¹³C NMR (75 MHz, CDCl₃) δ 17.68 (CH₂), 27.31 (CH), 27.35 (CH), 29.90 (CH), 33.23 (CH₃), 33.35 (CH₂), 33.56 (CH₂), 33.68 (CH), 35.94 (CH), 37.40 (CH₂), 40.18 (CH), 60.87 (CH₂), 82.45 (CH), 104.72 (C), 128.81 (2 × CH), 129.22 (2 × CH), 132.66 (C), 141.06 (C); FAB-MS (m/z) 349 [M + H⁺]; EI-IRMS calc'd for C₁₉H₂₀ClO₃ [M⁺]: 348.1492. Found: 348.1438; Anal. Calc'd for C₁₉H₂₀ClO₃: % C 68.86, % H 7.22. Found: % C 68.98, % H 7.34; purity 99.68%.

(15,35,55,6'S,7'S,5′-6′-(S)-1-(4-Methoxyphenyl)ethyl)spiro-[adamantane-2,3′-[1,2,4]trioxane] (7e). Yield: (1.70 g, 57%) as a white solid, mp 60–62 °C; FT-IR (KBr cm⁻¹) 757, 1033, 1113, 1615, 2928; ¹³H NMR (300 MHz, CDCl₃) δ 1.24 (d, 3H, J = 7.2 Hz), 1.52–2.04 (m, 13H), 2.73 (s, 1H), 2.80 (quin, 1H, J = 7.2 Hz), 3.71 (dd, 1H, J = 11.6, 3.4 Hz), 3.78 (3H, J = 3.4 Hz), 3.88 (d, 2H, J = 8.7 Hz, Ar), 7.12 (d, 2H, J = 8.7 Hz, Ar); ¹³C NMR (75 MHz, CDCl₃) δ 16.78 (CH₂), 27.34 (CH), 27.37 (CH), 29.73 (CH), 33.23 (CH₃), 33.37 (CH₂), 33.59 (CH₂), 33.71 (CH), 36.15 (CH), 37.44 (CH₂), 39.96 (CH), 55.43 (CH₂), 60.91 (CH₂), 82.72 (CH), 104.57 (C), 114.10 (2 × CH), 128.78 (2 × CH), 134.51 (C), 158.55 (C); FAB-MS (m/z) 345 [M + H⁺]; EI-IRMS calc'd for C₂₀H₂₅O₂Br [M⁺]: 344.1988. Found: 344.1988; Anal. Calc'd for C₂₀H₂₅O₂Br: % C 73.23, % H 8.19. Found: % C 73.30, % H 8.40; purity 99.96%.
as an oil; FT-IR (neat cm−1: 30797, 1122, 1655, 2917; 1H NMR (300 MHz, CDCl3) δ 1.38 (d, 3H, J = 7.2 Hz), 1.54–2.07 (m, 13H), 2.77 (s, 1H), 3.05 (quin, 1H, J = 11.9 Hz, J = 7.2 Hz), 3.80 (dd, 1H, J = 11.7 & 3.6 Hz), 3.87 (dd, 1H, J = 11.7 & 9.5 Hz), 4.60 (dd, 1H, J = 9.5 & 7.6, 3.6 Hz) (m, 2H, Ar), 7.75–7.79 (m, 1H, Ar), 7.88 (d, 1H, J = 1.5 Hz, Ar), 8.01 (d, 1H, J = 6.5 Hz, Ar), 13C NMR (75 MHz, CDCl3) δ 18.98 (CH3), 27.30 (CH), 27.35 (CH), 30.29 (CH), 33.21 (CH2), 33.46 (CH), 33.59 (CH3), 33.62 (CH2), 33.62 (CH2), 34.39 (CH), 37.38 (CH), 60.92 (CH2), 83.91 (CH2), 104.61 (CH), 122.96 (CH), 124.46 (CH), 125.78 (2 × CH), 126.39 (CH), 127.52 (CH), 129.27 (CH), 131.75 (CH), 134.20 (C), 138.66 (C); ESI-MS (m/z) 365 [M + H]+; EI-HRMS calculated for C27H30O3 [M]+: 364.2195; Found: 364.2194; Anal. Calcd for C27H30O3: %C 79.09, %H 7.74. Found: %C 79.29, %H 7.81; purity 99.25%.

(15,35,55,6′S,7′S)-6′-((S)-1-(Naphthalen-2-yl)ethyl)spiro[adamantane-2,3′-[1,2,4]trioxane] (7g). Yield (1.62 g, 54%) as a white solid, mp 136–138 °C; FT-IR (KBr cm−1) 739, 1086, 1113, 1596, 2912; 1H NMR (300 MHz, CDCl3) δ 1.34 (d, 3H, J = 7.3 Hz), 1.59–2.03 (m, 13H), 2.77 (s, 1H), 2.95 (quin, 1H, J = 7.3 Hz), 3.79 (dd, 1H, J = 11.7 & 3.6 Hz), 3.85 (dd, 1H, J = 11.7 & 3.6 Hz) (m, 4H, Ar), 7.53–7.55 (m, 1H, Ar), 7.57–7.78 (m, 2H, Ar); 13C NMR (75 MHz, CDCl3) δ 17.89 (CH3), 27.38 (2 × CH), 29.82 (CH), 32.28 (CH2), 33.41 (CH3), 33.63 (CH3), 33.75 (CH3), 36.19 (CH), 37.12 (CH), 37.47 (CH), 41.02 (CH), 61.03 (CH), 82.76 (CH), 104.67 (CH), 119.94 (CH), 120.04 (CH), 124.51 (CH), 125.20 (CH), 126.60 (CH), 126.68 (CH), 126.91 (CH), 140.72 (C), 141.24 (C), 141.84 (C), 143.49 (C), 143.79 (C); ESI-MS (m/z) 403 [M + H]+; EI-HRMS calculated for C27H30O3 [M]+: 402.2195; Found: 402.2194; Anal. Calcd for C27H30O3: %C 80.56, %H 7.51. Found: %C 80.76, %H 7.90; purity 99.33%.

(15,35,55,6′S,7′S)-6′-((S)-1-(9H-Fluoren-2-yl)ethyl)spiro[adamantane-2,3′-[1,2,4]trioxane] (7i). Yield (1.62 g, 54%) as a white solid, mp 136–138 °C; FT-IR (KBr cm−1) 739, 1086, 1113, 1596, 2912; 1H NMR (300 MHz, CDCl3) δ 1.34 (d, 3H, J = 7.3 Hz), 1.59–2.03 (m, 13H), 2.77 (s, 1H), 2.95 (quin, 1H, J = 7.3 Hz), 3.79 (dd, 1H, J = 11.7 & 3.6 Hz), 3.85 (dd, 1H, J = 11.7 & 3.6 Hz) (m, 4H, Ar), 7.53–7.55 (m, 1H, Ar), 7.57–7.78 (m, 2H, Ar); 13C NMR (75 MHz, CDCl3) δ 17.89 (CH3), 27.38 (2 × CH), 29.82 (CH), 32.28 (CH2), 33.41 (CH3), 33.63 (CH3), 33.75 (CH3), 36.19 (CH), 37.12 (CH), 37.47 (CH), 41.02 (CH), 61.03 (CH), 82.76 (CH), 104.67 (CH), 119.94 (CH), 120.04 (CH), 124.51 (CH), 125.20 (CH), 126.60 (CH), 126.68 (CH), 126.91 (CH), 140.72 (C), 141.24 (C), 141.84 (C), 143.49 (C), 143.79 (C); ESI-MS (m/z) 403 [M + H]+; EI-HRMS calculated for C27H30O3 [M]+: 402.2195; Found: 402.2194; Anal. Calcd for C27H30O3: %C 80.56, %H 7.51. Found: %C 80.76, %H 7.90; purity 99.33%.

ASSOCIATED CONTENT
Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.1c05041.

1H NMR and 13C NMR spectra of compounds 6a–i and 7a–i (PDF)

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Notes
The authors declare no competing financial interest.

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References
(1) WHO. World Malaria Report. 2019, https://www.who.int/publications/i/item/world-malaria-report-2019 (accessed March 1, 2020).
(2) W.H.O. Drug Information Bulletin 1999, 13, 9.
(3) Go, M.-L. Novel antiplasmodial agents. Med. Res. Rev. 2003, 23, 456–487.
(4) Wiesner, J.; Ortman, R.; Jomaa, H.; Schlitzer, M. New antimalarial drugs. Angew. Chem., Int. Ed. 2003, 42, 5274–5293.
(5) Schlitzer, M. Malaria chemotherapy part I: History of antimalarial drug development, currently used therapeutics and drugs in clinical development. Chem. Med. Chem. 2007, 2, 944–986.
(6) Klayman, D. L. Qinghaosu (artemisinin): an antimalarial drug from China. Science 1985, 228, 1049–1055.
(7) Luo, X. D.; Shen, C. C. The chemistry, pharmacology, and clinical applications of qinghaosu (artemisinin) and its derivatives. Med. Res. Rev. 1987, 7, 29–92.
(8) Cumming, J. N.; Ploypradith, P.; Posner, G. H. Antimalarial activity of artesinin (qinghaosu) and related trioxanes. Adv. Pharmacol. 1997, 37, 253–297.
(9) Bhattacharya, A. K.; Sharma, R. P. Recent developments on the chemistry and biological activity of artemisinin and related antimalarials. Heterocycles 1999, 51, 1681–1745.
(10) Borstnik, K.; Paik, I.; Shapiro, T. A.; Posner, G. H. Antimalarial chemotherapeutic peroxides: artemisinin, yinghaosu A and related compounds. Int. J. Parasitol. 2002, 32, 1661–1667.
(11) Ploypradith, P. Development of artemisinin and its structurally simplified trioxane derivatives as antimalarial drugs. Acta Trop. 2004, 89, 329–342.
(12) O’Neill, P. M.; Posner, G. H. A Medicinal chemistry perspective on artemisinin and related endoperoxides. J. Med. Chem. 2004, 47, 2945–2964.
(13) Kumari, A.; Karnatak, M.; Singh, D.; Shankar, R.; Jat, J. L.; Sharma, S.; Yadav, D.; Shrivastava, R.; Verma, V. P. Current scenario of artemisinin and its analogues for antimalarial activity. Eur. J. Med. Chem. 2019, 163, 804–829.
(14) Lin, A. J.; Klayman, D. L.; Milhous, W. K. Antimalarial activity of new water-soluble dihydroartemisinin derivatives. J. Med. Chem. 1987, 30, 2147–2150.
(15) Jung, M.; Li, X.; Bustos, D. A.; Eshohy, H. N.; McChesney, J. D.; Milhous, W. K. Synthesis and antimalarial activity of (+)-deoxyartemisinin. J. Med. Chem. 1990, 33, 1516–1518.
(16) Jung, M.; Lee, K.; Kendrick, H.; Robinson, B. L.; Croft, S. L. Synthesis, stability, and antimalarial activity of new hydrologically stable and water-soluble (+)-deoxyartemellic acid. J. Med. Chem. 2002, 45, 4940–4944.
(17) O’Neill, P. M.; Bishop, L. P.; Storr, R. C.; Hawley, S. R.; Maggs, J. L.; Ward, S. A.; Park, B. K. Mechanism-based design of parasite-targeted artemisinin derivatives: Synthesis and antimalarial activity of benzylamino and alkylamino ether analogues of artemisinin. J. Med. Chem. 1996, 39, 4511–4514.
(18) O’Neill, P. M.; Searle, N. L.; Kan, K. W.; Storr, R. C.; Maggs, J. L.; Ward, S. A.; Raynes, K.; Kevin, B. Novel, potent, semisynthetic antimalarial canula analogues of the first-generation 1,2,4-trioxane artesmether. J. Med. Chem. 1999, 42, 5487–5493.
(19) Avery, M. A.; Alvim-Gaston, M.; Vroman, J. A.; Wu, B.; Ager, A.; Peters, W.; Robinson, B. L.; Charman, W. Structure-activity relationships of the antimalarial agent artemisinin. 7. direct modification of (+)-artemisinin and in vivo antimalarial screening of new, potential preclinical antimalarial candidates. J. Med. Chem. 2002, 45, 4321–4335.
(20) Haynes, R. K.; Ho, W.-Y.; Chan, H.-W.; Fugmann, B.; Stetter, J.; Croft, S. L.; Vivas, L.; Peters, W.; Robinson, B. L. Highly Antimalaria-Active Artemisinin Derivatives: Biological Activity Does Not Correlate with Chemical Reactivity. Angew. Chem., Int. Ed. 2004, 43, 1381–1385.
(21) Singh, C.; Chaudhary, S.; Puri, S. K. New Orally Active Artemisinin Derivatives with High Efficacy against Multidrug-Resistant Malaria in Mice. J. Med. Chem. 2006, 49, 7227–7233.
(22) Posner, G. H.; Paik, I.-H.; Chang, W.; Borstnik, K.; Sinishtaj, S.; Rosenthal, A. S.; Shapiro, T. A. Malaria-Infected Mice Are Cured by a Single Dose of Novel Antimalarial Derivatives. J. Med. Chem. 2007, 50, 2516–2519.
(23) Asthana, O. P.; Srivastava, J. S.; Valecha, N. Current status of the artemisinin derivatives in the treatment of malaria with focus on artether. J. Parasit. Dis. 1997, 211, 1–12.
(24) Jambou, R.; Legrand, E.; Niang, M.; Khim, N.; Lim, P.; Volney, B.; Therese Ekala, M.; Bouchier, C.; Estere, P.; Fandeur, T.; Mercereau-Puijalon, O. Resistance of Plasmodium falciparum field isolates to in-vitro artesether and point mutations of the SERCA-type PiATPase 6. Lancet 2005, 366, 1960–1963.
(25) Payne, G. B.; Smith, C. W. Tungstic acid catalyzed hydroxylation of cyclohexene in nonaqueous media. J. Org. Chem. 1957, 22, 1696–1698.
(26) Kepler, J. A.; Philip, A.; Lee, Y. W.; Matthew C. Morey, M. C.; F. Iry Carroll, F. I. 1,2,4-Trioxanes as potential antimalarial agents. J. Med. Chem. 1988, 31, 713–716.
(27) Peters, W.; Robinson, B. L.; Rossier, J. C.; Jefford, C. W. The chemotherapy of rodent malaria. XLVIII. The activities of some synthetic 1,2,4-trioxanes against chloroquine-sensitive and chloroquine-resistant parasites. Part 1: Studies leading to the development of novel cis-fused cyclopenteno derivatives. Ann. Trop. Med. Parasitol. 1993, 87, 1–7.
(28) Peters, W.; Robinson, B. L.; Rossier, J. C.; Jefford, C. W. et al. The chemotherapy of rodent malaria. XLI. The activities of some synthetic 1,2,4-trioxanes against chloroquine-sensitive and chloroquine-resistant parasites. Part 2: Structure-activity studies on cis-fused cyclopenteno-1,2,4-trioxanes (fenozans) against drug-sensitive and drug-resistant lines of Plasmodium berghei and P. yoelii. NS in vivo. Ann. Trop. Med. Parasitol. 1993, 87, 9–16.
(29) Peters, W.; Robinson, B. L.; Tovey, G.; Rossier, J. C.; Jefford, C. W. The chemotherapy of rodent malaria. L. The activities of some synthetic 1,2,4-trioxanes against chloroquine-sensitive and chloroquine-resistant parasites. Part 3: Observations on ‘Fenozan-50F’, a difluorinated 3,3-spirocyclopentane 1,2,4-trioxane. Ann. Trop. Med. Parasitol. 1993, 87, 111–123.
(30) Posner, G. H.; Maxwell, J. P.; O’Dowd, H.; Krasavin, M.; Xie, S.; Shapiro, T. A. Antimalarial sulfide, sulfone, and sulfonamide trioxanes. Bioorg. Med. Chem. 2000, 8, 1361–1370.
(31) Posner, G. H.; Jeon, H. B.; Parker, M. H.; Krasavin, M.; Paik, I.-H.; Shapiro, T. A. Antimalarial simplified 3-aryltrioxanes: Synthesis and preclinical efficacy/toxicity testing in rodents. J. Med. Chem. 2001, 44, 3054–3058.
(32) Posner, G. H.; Jeon, H. B.; Ploypradith, P.; Paik, I.-H.; Borstnik, K.; Xie, S.; Shapiro, T. A. Orally active, water-soluble antimalarial 3-
aryltrioxanes: short synthesis and preclinical efficacy testing in rodents. J. Med. Chem. 2002, 45, 3824−3828.

(33) Griesbeck, A. G.; El-Idreesy, T. T.; Fiege, M.; Brun, R. Synthesis of antimalarial 1,2,4-trioxanes via photooxygenation of a chiral allylic alcohol. Org. Lett. 2002, 4, 4193−4195.

(34) O'Neill, P. M.; Mukhtar, A.; Ward, S. A.; Bickley, J. F.; Davies, J.; Bachi, M. D.; Stocks, P. A. Application of thiol-olein cooxygenation methodology to a new synthesis of the 1,2,4-trioxane pharmacopeia. Org. Lett. 2004, 6, 3035−3038.

(35) Vennerstrom, J. L; Arbe-Barens, S.; Brun, R.; Charman, S. A.; Chiu, F. C. K.; Chollet, J.; Dong, Y.; Dorn, A.; Hunziker, D.; Matile, H.; McIntosh, K.; Padmanilayam, M.; Tomas, J. S.; Scheurer, C.; Scorneaux, B.; Tang, Y.; Urwyler, H.; Witten, S.; Charman, W. N. Identification of an antimalarial synthetic trioxadone drug development candidate. Nature 2004, 430, 900−904.

(36) Dong, Y.; Chollet, J.; Matile, H.; Charman, S. A.; Chiu, F. C. K.; Charman, W. N.; Scorneaux, B.; Urwyler, H.; Tomas, J. S.; Scheurer, C.; Snyder, C.; Dorn, A.; Wang, X.; Karle, J. M.; Tang, Y.; Urwyler, H.; Burn, B.; Vennerstrom, J. L. Spiro and Dispiro-1,2,4-trioxanes as Antimalarial Peroxides: Charting a Workable Structure-Activity Relationship Using Simple Prototypes. J. Med. Chem. 2005, 48, 4953−4961.

(37) Griesbeck, A. G.; El-Idreesy, T. T.; Hönicke, L.-O.; Lex, J.; Brun, R. Novel spironellated 1,2,4-trioxanes with high in vitro antimalarial activities. Bioorg. Med. Chem. Lett. 2005, 15, 595−597.

(38) Singh, C. Preparation of β-hydroxyhydroperoxides by photooxygenation of allylic alcohols and their elaboration into 1,2,4-trioxanes. Tetrahedron Lett. 1990, 31, 6901−6902.

(39) Singh, C.; Gupta, N.; Puri, S. K. Photooxygenation of 3-aryl-2-cyclohexenols: Synthesis of a new series of 1,2,4-trioxadiones. Tetrahedron Lett. 2005, 46, 205−207.

(40) Singh, C.; Misra, D.; Saxena, G.; Chandra, S. Synthesis of in vivo potent antimalarial 1,2,4-trioxanes. Bioorg. Med. Chem. Lett. 1992, 2, 497−500.

(41) Singh, C.; Misra, D.; Saxena, G.; Chandra, S. In vivo potent antimalarial 1,2,4-trioxanes: Synthesis and activity of 8-(α-arylvinyl)-6,7,10-trioxaspiro[4,5]decanes and 3-(α-arylvinyl)-1,2,5-trioxaspiro[5,5]undecanes against Plasmodium berghei mice. Bioorg. Med. Chem. Lett. 1995, 5, 1913−1916.

(42) Singh, C.; Gupta, N.; Puri, S. K. Geraniol-derived 1,2,4-trioxanes with potent in vivo antimalarial activity. Bioorg. Med. Chem. Lett. 2003, 13, 3447−3450.

(43) Singh, C.; Gupta, N.; Puri, S. K. Synthesis of new 6-alkylvinyl/arylalkylvinyl substituted 1,2,4-trioxanes active against multi-drug resistant Plasmodium berghei in mice. Bioorg. Med. Chem. 2004, 12, 5533−5562.

(44) Shukla, M.; Hassam, M.; Yadav, D. K.; Sharma, S.; Singh, C.; Shivratvasta, R.; Puri, S. K.; Verma, V. P. Synthesis of novel 1,2,4-trioxanes and antimalarial evaluation against multidrug-resistant Plasmodium yoelii nigeriensis. Bioorg. Med. Chem. Lett. 2021, 49, No. 128305.

(45) Singh, C.; Kanchan, R.; Sharma, U.; Puri, S. K. New adamantane-based spiro 1,2,4-trioxanes orally effective against rodent and simian malaria. J. Med. Chem. 2007, 50, 521−527.

(46) Karnatak, M.; Hassam, M.; Vanangamudi, M.; Sharma, S.; Yadav, D. K.; Singh, C.; Puri, S. K.; Rawat, V.; Verma, V. P. Novel naphthyl based 1,2,4-trioxanes: Synthesis and in vivo efficacy in the Plasmodium yoelii nigeriensis in Swiss mice. Bioorg. Med. Chem. Lett. 2021, 51, No. 128372.

(47) Tripathi, R.; Mishra, D.; Rizvi, A.; Singh, C. Evaluation of some adamantane-based synthetic trioxanes against Plasmodium knowlesi in Rhesus monkeys. Life Sci. 2007, 81, 1544−1548.

(48) Singh, C.; Verma, V. P.; Naikade, N. K.; Singh, A. S.; Hassam, M.; Puri, S. K. Novel bis- and tris-1,2,4-trioxanes: Synthesis and antimalarial activity against multidrug-resistant Plasmodium yoelii in Swiss mice. J. Med. Chem. 2008, 51, 7581−7592.

(49) Singh, C.; Hassam, M.; Naikade, N. K.; Verma, V. P.; Singh, A. S.; Puri, S. K. Synthesis and Antimalarial Assessment of a New Series of Orally Active Amino-Functionalized Spiro 1,2,4-Trioxanes. J. Med. Chem. 2010, 53, 7587−7598.