Synthesis and Antimalarial Activity of Cyclic Peroxides, 1,2,4,5,7-Pentoxocanes and 1,2,4,5-Tetroxanes

Hye-Sook Kim,[†] Yasuharu Shibata,[†] Yusuke Wataya,*[,†] Kaoru Tsuchiya,[‡] Araki Masuyama,[‡] and Masatomo Nojima[‡]

Faculty of Pharmaceutical Sciences, Okayama University, Okayama 700-8530, Japan, and Department of Materials Chemistry, Faculty of Engineering, Osaka University, Suita, Osaka 565-0871, Japan

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A variety of 1,2,4,5,7-pentoxocane and 1,2,4,5-tetroxane derivatives were prepared as potential peroxide antimalarial agents. In both series of cyclic peroxides, the steric and electronic effects of the substituents attached to the peroxide ring exert a remarkable influence on the antimalarial activity. For some cyclic peroxides, which were found to be highly effective in vitro, the study in vivo has been also conducted.

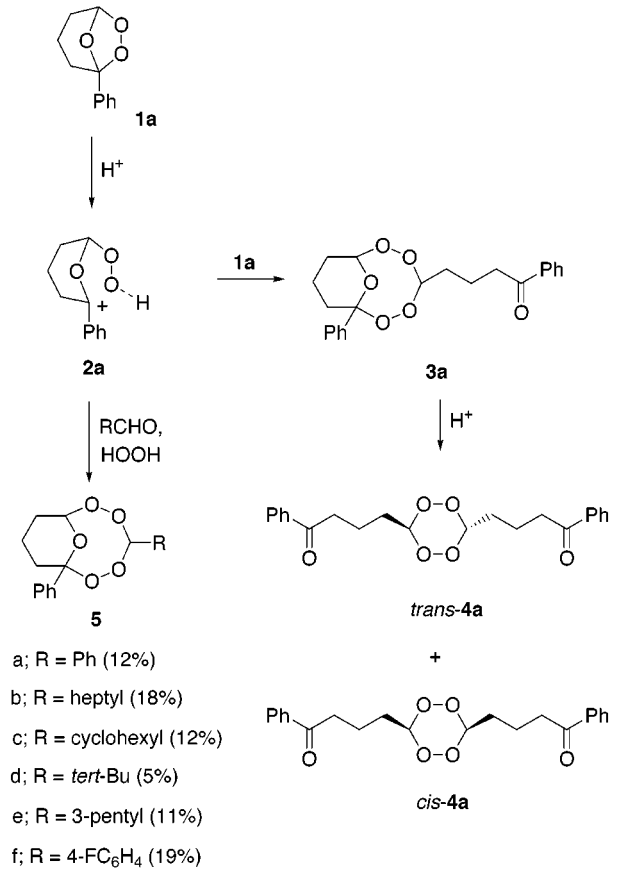
Introduction

As a result of an apparent association between the peroxide functional group and antimalarial activity, a substantial effort has been devoted to developing new peroxide antimalarials.[1] Of these, we have been particularly interested in the fact that dispiro-1,2,4,5-tetroxanes, e.g., 3,12-dimethyl-7,8,15,16-tetraoxadispiro-[5.2.5.2]hexadecane, easily prepared by the acid-catalyzed condensation of cyclohexanones and hydrogen peroxide, exhibit remarkable antimalarial activities in vitro and in vivo.[2] To understand the detailed structure−activity relationships, therefore, we have prepared a number of 3,6-disubstituted 1,2,4,5-tetroxanes and the related 1,2,4,5,7-pentoxocanes and have found that these cyclic peroxides show significant antimalarial activities in vitro, the activity being a marked function of the substituent steric and electronic effects of these cyclic peroxides. Some of the cyclic peroxides have been found to be active in vivo, too.

Results and Discussion

Synthesis of 1,2,4,5,7-Pentoxocanes. About 2 decades ago, we found that acidolysis of 1-phenylcyclopentene ozonide (**1a**) gives the novel 1,2,4,5,7-pentoxocane derivative **3a**, together with two stereoisomeric tetroxanes, *trans*- and *cis*-**4a**.[3] Also, the acidolysis of a mixture of **1a**, benzaldehyde, and hydrogen peroxide in acetic acid led to the formation of the phenyl-substituted 1,2,4,5,7-pentoxocane **5a** (Scheme 1).[4] To see the possibility of 1,2,4,5,7-pentoxocanes as a potential antimalarial drug, we prepared in this study a series of new pentoxocane derivatives **5b**−**f**. Although the yields were poor, pure compounds could be easily isolated by recrystallization and/or column chromatography on silica gel (Scheme 1). Also, pentoxocanes **3b**,**c** were prepared by the acid-catalyzed dimerization of aryl-substituted cyclopentene ozonides **1b**,**c**. By the acid-catalyzed cyclocondensation of (4-fluorophenyl)cyclopentene ozonide (**1c**) and aldehydes in the presence of H_2O_2, the corresponding pentoxocanes **6a**−**c** were obtained (Scheme 2).

Scheme 1



Synthesis of 1,2,4,5-Tetroxanes. By the acid-catalyzed dimerization of ozonides **1b**,**c**, *trans*-3,6-bis-(aryloxypropyl)-1,2,4,5-tetroxanes **4b**,**c** were obtained together with **3b**,**c** (Schemes 1 and 2). As an alternative and more general method for the synthesis of 1,2,4,5-tetroxanes, Jefford and co-workers[5] reported that trimethylsilyl trifluoromethanesulfonate (TMSOTf)-catalyzed condensation of an aldehyde and dioxybis[trimethylsilane] provides the symmetrically substituted 1,2,4,5-tetroxane in high yield (Scheme 3). We repeated this reaction to prepare a series of 3,6-dialkyl-substituted 1,2,4,5-tetroxanes **8a**−**d**.

* Corresponding author: Yusuke Wataya. Phone: +81-86-251-7976. Fax: +81-86-251-7974. E-mail: wataya@cc.okayama-u.ac.jp.
[†] Okayama University.
[‡] Osaka University.

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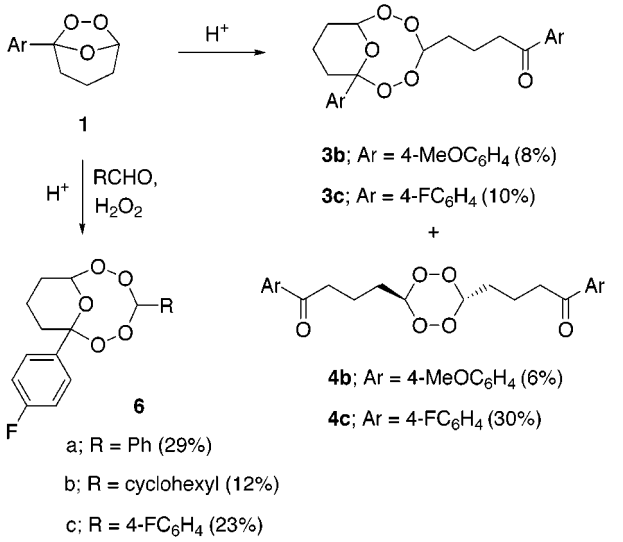
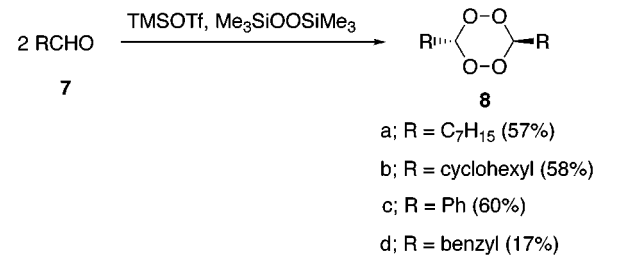
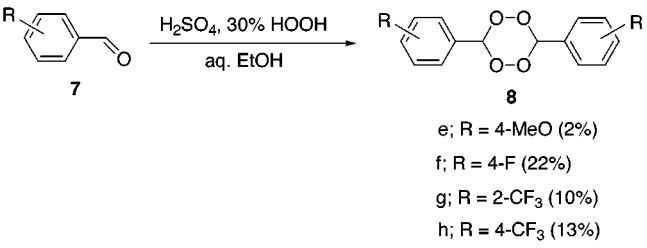
Scheme 2



Scheme 3



Scheme 4



In the case of the 3,6-diaryl-substituted tetroxanes, the more conventional method was found to be effective, i.e., H_2SO_4-catalyzed cyclocondensation of aldehydes and hydrogen peroxide provided the expected tetroxanes **8e**−**h** (Scheme 4).[2] In the case of 3,6-bis(4-methoxylphenyl)-1,2,4,5-tetroxane (**8e**), the yield was very poor. Our trial to prepare the same compound **8e** by the method of Jefford[5] also failed. The reason is obscure.

Antimalarial Activities of 1,2,4,5,7-Pentoxocanes and 1,2,4,5-Tetroxanes in Vitro. With a series of 1,2,4,5,7-pentoxocanes in hand, we tested the antimalarial activities against *P. falciparum* and cytotoxicities against FM3A cells (Table 1).[6,7] The results are summarized as follows. (a) EC_{50} values of pentoxocanes **3a**−**c** and **5a**,**c**,**e** against *P. falciparum* are in the ranges of (1.4×10^{-6})−(2.1×10^{-7}) M, and moreover, the selectivities are notable. (b) The nature of the alkyl substituent at C-4 shows a remarkable influence on the activity, the cyclohexyl- and 3-pentyl-substituted ones, **5c**,**e**, being the most attractive. (c) When **3a**−**c** are compared, it is noticed that substitution by the electron-withdrawing fluorine increases the activity. Also, the selectivity of **3c** was remarkable (>141). In this respect,

Table 1. In Vitro Antimalarial Activities of Pentoxocanes against *P. Falciparum* and Cytotoxicities against FM3A Cells[a]

	EC_{50} values (M)		
pentoxocane	*P. falciparum*[b]	FM3A[c]	selectivity[d]
3a, Ar = Ph	1.3×10^{-6}	NT[e]	>77
3b, Ar = 4-$MeOC_6H_4$	9.3×10^{-7}	1.8×10^{-5}	19
3c, Ar = 4-FC_6H_4	7.1×10^{-7}	NT[e]	>141
5a, R = Ph	1.4×10^{-6}	5.0×10^{-5}	36
5b, R = heptyl	1.5×10^{-6}	1.2×10^{-5}	8
5c, R = cyclohexyl	2.1×10^{-7}	1.0×10^{-5}	48
5d, R = *tert*-butyl	1.7×10^{-5}	2.6×10^{-5}	2
5e, R = 3-pentyl	5.1×10^{-7}	3.3×10^{-5}	65
5f, R = 4-FC_6H_4	2.8×10^{-6}	1.7×10^{-5}	6
6a, R = Ph	2.9×10^{-6}	8.1×10^{-6}	4
6b, R = cyclohexyl	1.5×10^{-7}	2.5×10^{-6}	17
6c, R = 4-FC_6H_4	1.9×10^{-6}	9.0×10^{-6}	5
artemisinin	7.8×10^{-9}	1.0×10^{-5}	1280

[a] In vitro antimalarial activities and cytotoxicities are described in the Experimental Section. [b] Chloroquine-sensitive (FCR-3 strain). [c] Mouse mammary tumor FM3A cells in culture as a control for mammalian cell cytotoxicity. [d] Selectivity = (mean of EC_{50} value for FM3A cells)/(mean of EC_{50} value for *P. falciparum*). [e] NT, not toxic at a 1×10^{-4} M concentration.

Table 2. In Vitro Antimalarial Activities of Tetroxanes against *P. Falciparum* and Cytotoxicities against FM3A Cells[a]

	EC_{50} values (M)		
tetroxane	*P. falciparum*[b]	FM3A[c]	selectivity[d]
trans-**4a**, Ar = Ph	4.0×10^{-7}	NT[e]	>250
cis-**4a**, Ar = Ph	1.2×10^{-5}	NT[e]	>8
4b, Ar = 4-$MeOC_6H_5$	2.0×10^{-5}	NT[e]	>5
4c, Ar = 4-FC_6H_4	5.1×10^{-7}	NT[e]	>169
8a, R = heptyl	1.5×10^{-6}	NT[e]	>67
8b, R = cyclohexyl	2.0×10^{-7}	1.1×10^{-5}	55
8c, R = phenyl	1.1×10^{-6}	NT[e]	>91
8d, R = benzyl	1.4×10^{-5}	NT[e]	>7
8e, R = 4-$MeOC_6H_4$	7.9×10^{-7}	NT[e]	>127
8f, R = 4-FC_6H_4	5.0×10^{-7}	NT[e]	>200
8g, R = 2-$CF_3C_6H_4$	6.0×10^{-7}	3.0×10^{-6}	5
8h, R = 4-$CF_3C_6H_4$	NT[e]	NT[e]	

[a] In vitro antimalarial activities and cytotoxicities are described in the Experimental Section. [b] Chloroquine-sensitive (FCR-3 strain). [c] Mouse mammary tumor FM3A cells in culture as a control for mammalian cell cytotoxicity. [d] Selectivity = (mean of EC_{50} value for FM3A cells)/(mean of EC_{50} value for *P. falciparum*). [e] NT, not toxic at a 1×10^{-4} M concentration.

Porter and co-workers[8] have found a similar fluorine atom effect on the antimalarial activity for 1,4-diaryl-substituted 3,4-dioxabicyclo[2.2.2]octanes. In the case of fluorophenyl-substituted pentoxocanes, **5f** and **6c**, however, the activity was moderate and, moreover, the selectivity was poor. In summary, 1,2,4,5,7-pentoxocanes are a new class of antimalarial peroxides; considering the ease of the preparation, the modification of the structure would lead to the discovery of the promising antimalarial drugs.

A similar substituent electronic effect was observed for 1,2,4,5-tetroxanes **4a**−**c**, too: i.e., the activity follows the sequence: *trans*-**4a** (Ar = Ph) = **4c** (Ar = 4-FC_6H_4) ≫ **4b** (Ar = 4-$MeOC_6H_4$) (Table 2). It was surprising to note that in contrast to *trans*-**4a**, the stereoisomeric *cis*-**4a** was inactive, suggesting that the difference in structure of the tetroxanes plays an important role. For a series of 3,6-diaryl- or 3,6-dialkyl-substituted tetroxanes **8a**−**h**, the diaryl-substituted tetroxanes **8c**,**e**,**f** were found to show remarkable activities against *P. falciparum*. In marked contrast, 3,6-bis(trifluoromethylphenyl)-1,2,4,5-tetroxanes **8g**,**h** were ineffective. Also, the dialkyl-substituted 1,2,4,5-tetroxanes **8a**,**b**,**d** showed

Table 3. Antimalarial Activities of 1,2,4,5,7-Pentoxocanes and 1,2,4,5-Tetroxanes in Vivo[a]

compd	growth inhibition (%) of *P. berghei* in mice[b]	
	20 mg/kg/day	50 mg/kg/day
Tetroxane		
trans-**4a**, Ar = Ph	30	40
4c, Ar = 4-FC_6H_4	0	30
8b, R = cyclohexyl	50	96
8c, R = phenyl	6	50
8f, R = 4-FC_6H_4	50	90
Pentoxocane		
3c, Ar = 4-FC_6H_4	0	0
5c, R = cyclohexyl	0	15
5e, R = 3-pentyl	15	18
artemisinin	93	100

[a] In vivo antimalarial activity is described in the Experimental Section. [b] Growth inhibition (%) = [(mean % parasitemia in controls − mean % parasitemia in treated)/mean % parasitemia in controls] × 100.

moderate to poor activities against *P. falciparum*, the activity following the sequence **8b** (R = cyclohexyl) > **8a** (R = heptyl) > **8d** (R = benzyl) (Table 2).

By the intensive study on the origin of the notable antimalarial activity of artemisinin and some related synthetic organic endoperoxides, Posner and co-workers[9] have proposed that the first step of the action involves homolytic cleavage of the peroxide bridge by single electron transfer from an Fe(II) species to produce an oxy radical; subsequent intramolecular 1,5-hydride shift leads to the formation of the carbon radical which, as an active intermediate, would kill malaria parasites. For an active antimalarial drug, arteflene, too, intervention of a radical intermediate, derived from the O−O and the subsequent C−C bond scission, has been suggested.[10] The structure−activity relationship observed for 1,2,4,5,7-pentoxocanes and 1,2,4,5-tetroxanes, however, may suggest that a different factor would be also important. If the activity were controlled by the stability of the carbon radical, the *tert*-butyl-substituted pentoxocane **5d** and dibenzyl-substituted tetroxane **8d** might have been most active. This is clearly inconsistent with the experimental observations (Tables 1 and 2). Together with the remarkable difference in activity between *trans*- and *cis*-**4a**, we prefer to consider that the substituent-dependent ease in access of an Fe(II) species such as heme to the peroxide bridge would play an important role, particularly when complexation precedes activation.[11] Perhaps in accordance with this, Avery[12] has demonstrated that large substituents at C-3 in artemisinin homologues suppress activity, irrespective of their electronic qualities.

Antimalarial Activities of 1,2,4,5,7-Pentoxocanes and 1,2,4,5-Tetroxanes in Vivo. In vivo antimalarial activity for 1,2,4,5,7-pentoxocanes and 1,2,4,5-tetroxanes against *P. berghei* NK 65 strain was determined.[13] Results shown in Table 3 indicate that 1,2,4,5-tetroxanes had potent antimalarial activities compared with those of 1,2,4,5,7-pentoxocanes in vivo. The activity of 1,2,4,5-tetroxanes follows the sequence: **8b** (R = cyclohexyl) = **8f** (R = 4-FC_6H_4) > *trans*-**4a** (Ar = Ph) > **8c** (R = phenyl) > **4c** (Ar = 4-FC_6H_4). Especially, tetroxanes **8b**,**f** seem to be potent antimalarial agents against *P. berghei* (ED_{50}: approximately 20 mg/kg). Regretfully, **8b**,**f** have, however, weak antimalarial activity relative to artemisinin (ED_{50}: 5.4 mg/kg; data will be published elsewhere). Although growth of the parasites was suppressed during administration with **8b**,**f** (each of 50 mg/kg/day), malaria parasites were still observed in blood stream after the 4-day suppressive test. Thus, all of the mice treated with **8b**,**f** died due to *P. berghei* infection. The similar trend was observed for artemisinin. Our experiment disclosed that artemisinin at a dose of 50 mg/kg/day also failed to resolve infection in mice.

Particularly interesting is the remarkably low toxicity of these two agents (**8b**,**f**): no signs, including weight loss and diarrhea, were observed at a total dose of 200 mg/kg (ip). 1,2,4,5,7-Pentoxocanes were either inactive or less active than were 1,2,4,5-tetroxanes. It is also interesting to note that the fluorine-substituted pentoxocane **3c** and tetroxane **4c** were ineffective in vivo, although they showed notable characteristics in vitro.[8]

In summary, we have prepared a number of new 1,2,4,5-tetroxanes and 1,2,4,5,7-pentoxocanes to test antimalarial activity. The study in vitro demonstrates that these types of cyclic peroxides are active, the extent being a marked function of the steric and electronic effects of the substituents attached to the peroxide ring. By the study in vivo, 3,6-dicyclohexyl- and 3,6-bis(4-fluorophenyl)-1,2,4,5-tetroxanes have been found to be promising as new antimalarial agents. These results may help in the design of better chemotherapeutic 1,2,4,5-tetroxanes in the worldwide fight against malaria.

Experimental Section

General Procedure. ^1H (270 MHz) and ^{13}C (67.5 MHz) NMR spectra were obtained in $CDCl_3$ with $SiMe_4$ as standard. Ozonides **1a**−**c**,[3] pentoxocanes **3a**[3] and **5a**,[4] and tetroxanes *trans*- and *cis*-**4a**[3] and **8c**[5] were prepared by the reported method.

(4-Methoxyphenyl)cyclopentene ozonide (1b): mp 64−65 °C (from hexane/ether); ^1H NMR δ 1.8−2.3 (m, 6 H), 3.82 (s, 3 H), 5.98 (s, 1 H), 6.92 (d, J = 8.9 Hz, 2 H), 7.48 (d, J = 8.9 Hz, 2 H); ^{13}C NMR δ 16.08, 29.09, 32.61, 55.27, 103.66, 107.92, 113.71, 127.55, 129.85, 130.35, 160.48, 163.48. Anal. ($C_{12}H_{14}O_4$) C, H.

(4-Fluorophenyl)cyclopentene ozonide (1c): an oil; ^1H NMR δ 1.8−2.4 (m, 6 H), 5.99 (s, 1 H), 7.07 (t, J = 8.8 Hz, 2 H), 7.53 (dd, J = 8.8 and 5.3 Hz, 2 H); ^{13}C NMR δ 16.06, 29.00, 32.97, 103.79, 107.60, 115.37 (d, J = 20.8 Hz), 128.12, 131.82, 163.38 (d, J = 249 Hz). Anal. ($C_{11}H_{11}FO_3$) C, H.

Caution: Since organic peroxides are potentially hazardous compounds, they must be handled with due care; avoid exposure to strong heat or light, mechanical shock, oxidizable organic materials, and transition-metal ions. No particular difficulties were experienced in handling any of the new peroxides synthesized in this work using the reaction scales and procedures described below together with the safeguard mentioned above.

Synthesis of 1,2,4,5,7-Pentoxocanes by the Acid-Catalyzed Cyclocondensation of Arylcyclopentene Ozonides 1a−c, Aldehydes, and Hydrogen Peroxide. The reaction of 1-phenylcyclopentene ozonide (**1a**) with octanal is representative. An equimolar mixture of **1a** (600 mg, 3.13 mmol) and hydrogen peroxide (30%; 355 mg, 3.13 mmol) in the presence of chlorosulfonic acid (36 mg, 0.31 mmol) in acetic acid−CH_2Cl_2 (35 mL, 5:2, v/v) was stirred at room temperature for 30 min. Then, octanal (400 mg, 3.13 mmol) was added, and the mixture was stirred for a further 90 min. Workup of the reaction mixture was carried out by pouring it into water, extracting the latter with ether, and washing the organic extracts with portions of aqueous $NaHCO_3$ and saturated brine. After evaporation of the solvent, the neutral products

were isolated by column chromatography on silica gel, using benzene−hexane (3:2) as an eluent. The first fraction contained pentoxocane **5b** (184 mg, 18%).

1,4-Diphenyl-2,3,5,6,11-pentaoxabicyclo[5.3.1]undecane (5a):[3] mp 134−135 °C (from methanol); ^{1}H NMR δ 1.4−2.4 (m, 6 H), 5.73 (s, 1 H), 6.62 (s, 1 H), 7.1−7.7 (m, 10 H). Anal. ($C_{18}H_{18}O_5$) C, H.

4-Heptyl-1-phenyl-2,3,5,6,11-pentaoxabicyclo[5.3.1]undecane (5b): mp 64−65 °C (from methanol); ^{1}H NMR δ 0.85 (t, J = 6.9 Hz, 3 H), 1.2−2.1 (m, 18 H), 5.64 (s, 1 H), 5.68 (t, J = 5.9 Hz, 1 H), 7.3−7.6 (m, 5 H); ^{13}C NMR δ 13.95, 22.48, 24.89, 28.29, 28.81, 28.92, 29.00, 31.52, 33.89, 43.78, 98.24, 102.86, 109.17, 125.28, 125.84, 127.92, 127.98, 142.08. Anal. ($C_{19}H_{28}O_5$) C, H.

4-Cyclohexyl-1-phenyl-2,3,5,6,11-pentaoxabicyclo[5.3.1]undecane (5c): mp 121−122 °C (from methanol); ^{1}H NMR δ 1.2−2.1 (m, 17 H), 5.44 (d, J = 6.8 Hz, 1 H), 5.62 (s, 1 H), 7.3−7.6 (m, 5 H); ^{13}C NMR δ 14.72, 24.76, 25.41, 25.52, 25.86, 27.80, 28.56, 33.87, 38.08, 98.24, 102.84, 111.50, 125.39, 125.89, 127.98, 128.32, 142.16. Anal. ($C_{18}H_{24}O_5$) C, H.

4-*tert*-Butyl-1-phenyl-2,3,5,6,11-pentaoxabicyclo[5.3.1]undecane (5d): mp 141−142 °C (from methanol); ^{1}H NMR δ 0.95 (s, 9 H), 1.5−2.1 (m, 6 H), 5.35 (s, 1 H), 5.61 (s, 1 H), 7.3−7.6 (m, 5 H); ^{13}C NMR δ 14.74, 24.80, 25.84, 33.55, 34.92, 98.17, 102.70, 113.26, 125.43, 125.91, 127.89, 128.36, 128.85, 142.16. Anal. ($C_{16}H_{22}O_5$) C, H.

4-(3-Pentyl)-1-phenyl-2,3,5,6,11-pentaoxabicyclo[5.3.1]undecane (5e): mp 68−69 °C (from methanol); ^{1}H NMR δ 0.86 (t, J = 6.9 Hz, 6 H), 1.2−2.2 (m, 11 H), 5.60 (br s, 2 H), 7.2−7.4 (m, 5 H); ^{13}C NMR δ 11.23, 11.28, 14.81, 21.62, 22.07, 24.83, 33.76, 42.01, 98.33, 102.84, 110.31, 125.42, 127.98, 129.88, 142.28. Anal. ($C_{17}H_{24}O_5$) C, H.

4-(4-Fluorophenyl)-1-phenyl-2,3,5,6,11-pentaoxabicyclo[5.3.1]undecane (5f): mp 113−114 °C (from methanol); ^{1}H NMR δ 1.5−2.3 (m, 6 H), 5.73 (s, 1 H), 6.61 (s, 1 H), 6.96 (t, J = 8.9 Hz, 2 H), 7.3−7.4 (m, 5 H), 7.58 (d, J = 7.9 Hz, 2 H); ^{13}C NMR δ 14.81, 24.80, 33.89, 98.69, 103.36, 107.83, 115.31 (d, J = 21.9 Hz), 125.35, 127.15, 128.12, 129.23, 129.36, 142.03, 163.53 (d, J = 249.1 Hz). Anal. ($C_{18}H_{17}FO_5$) C, H.

1-(4-Fluorophenyl)-4-phenyl-2,3,5,6,11-pentaoxabicyclo[5.3.1]undecane (6a): mp 122−123 °C (from methanol); ^{1}H NMR δ 1.5−2.2 (m, 6 H), 5.70 (s, 1 H), 6.62 (s, 1 H), 7.02 (t, J = 8.8 Hz, 2 H), 7.3−7.8 (m, 7 H); ^{13}C NMR δ 14.81, 24.78, 33.93, 98.65, 102.98, 108.53, 114.91 (d, J = 22.0 Hz), 127.22, 127.27, 128.38, 128.46, 130.04, 131.07, 138.02, 162.52 (d, J = 245 Hz). Anal. ($C_{18}H_{17}FO_5$) C, H.

4-Cyclohexyl-1-(4-fluorophenyl)-2,3,5,6,11-pentaoxabicyclo[5.3.1]undecane (6b): mp 131−132 °C (from methanol); ^{1}H NMR δ 1.2−2.1 (m, 17 H), 5.42 (d, J = 6.6 Hz, 1 H), 5.60 (s, 1 H), 7.03 (t, J = 8.6 Hz, 2 H), 7.52 (dd, J = 8.3 and 5.3 Hz, 2 H); ^{13}C NMR δ 14.75, 24.78, 25.57, 25.93, 28.05, 28.59, 33.96, 38.17, 98.31, 102.60, 111.57, 114.70, 115.02, 127.33, 127.44, 138.17, 162.51 (d, J = 247 Hz). Anal. ($C_{18}H_{23}FO_5$) C, H.

1,4-Bis(4-fluorophenyl)-2,3,5,6,11-pentaoxabicyclo[5.3.1]undecane (6c): mp 112−113 °C (from methanol); ^{1}H NMR δ 1.5−2.2 (m, 6 H), 5.72 (s, 1 H), 6.60 (s, 1 H), 7.0−7.1 (m, 4 H), 7.3−7.4 (m, 2 H), 7.5−7.6 (m, 2 H); ^{13}C NMR δ 14.81, 24.76, 33.94, 98.72, 103.07, 107.83, 114.96 (d, J = 20.7 Hz), 115.54 (d, J = 22.0 Hz), 127.24, 127.37, 129.22, 129.34, 137.93, 137.98, 162.57 (d, J = 246 Hz), 163.61 (d, J = 249 Hz). Anal. ($C_{18}H_{16}F_2O_5$) C, H.

Acid-Catalyzed Dimerization of Arylcyclopentene Ozonides. Reaction of (4-fluorophenyl)cyclopentene ozonide (**1c**) is representative. A mixture of ozonide **1c** (210 mg, 1.00 mmol) and $ClSO_3H$ (0.3 equiv) in CH_2Cl_2 (10 mL) was stirred at room temperature for 30 min. After workup as described above, the mixture of the crude products was triturated with ether to give the tetroxane **4c** (41 mg, 10%). After evaporation of the ether, the residue was column chromatographed on silica gel. Elution with ether−hexane (1:9) gave the pentoxocane **3c** (125 mg, 30%).

1-(4-Methoxyphenyl)-4-[1-(4-methoxyphenyl)-2,3,5,6,11-pentaoxabicyclo[5.3.1]undec-4-yl]-1-butanone (3b): mp 132−133 °C (from methanol); ^{1}H NMR δ 1.5−2.1 (m, 10 H), 2.89 (t, J = 6.9 Hz, 2 H), 3.80 (s, 3 H), 3.86 (s, 3 H), 5.62 (s, 1 H), 5.74 (t, J = 5.6 Hz, 1 H), 6.8−7.0 (m, 4 H), 7.46 (d, J = 8.6 Hz, 2 H), 7.87 (d, J = 8.90 Hz, 2 H); ^{13}C NMR δ 14.79, 19.57, 24.76, 28.01, 34.07, 37.52, 55.24, 55.44, 98.44, 103.02, 108.88, 113.39, 113.64, 122.28, 126.68, 130.28, 134.57, 159.28, 163.36, 198.16. Anal. ($C_{24}H_{24}O_8$) C, H.

***trans*-4,4′-(1,2,4,5-Tetroxane-3,6-diyl)bis[1-(4-methoxyphenyl)-1-butanone] (4b)**: mp 149−150 °C (from methanol); ^{1}H NMR δ 1.5−2.0 (m, 8 H), 2.96 (t, J = 7.3 Hz, 4 H), 3.87 (s, 6 H), 5.94 (t, J = 5.3 Hz, 2 H), 6.92 (d, J = 8.9 Hz, 4 H), 7.91 (d, J = 8.9 Hz, 4 H); ^{13}C NMR δ 18.26, 29.11, 37.32, 55.49, 108.59, 113.75, 129.85, 130.28, 163.48, 212.59. Anal. ($C_{24}H_{28}O_8$) C, H.

1-(4-Fluorophenyl)-4-[1-(4-fluorophenyl)-2,3,5,6,11-pentaoxabicyclo[5.3.1]undec-4-yl]-1-butanone (3c): mp 126−127 °C (from methanol); ^{1}H NMR δ 1.5−2.1 (m, 10 H), 2.92 (t, J = 7.6 Hz, 2 H), 5.62 (s, 1 H), 5.74 (t, J = 5.9 Hz, 1 H), 7.02 (t, J = 8.7 Hz, 2 H), 7.11 (t, J = 8.6 Hz, 2 H), 7.51 (dd, J = 8.9 and 5.6 Hz, 2 H), 7.91 (dd, J = 8.9 and 5.3 Hz, 2 H); ^{13}C NMR δ 14.74, 19.21, 24.71, 27.91, 34.00, 37.75, 98.45, 102.82, 108.84, 114.93 (d, J = 22 Hz), 115.63 (d, J = 22 Hz), 127.28, 130.65, 133.26, 138.04, 161.68 (d, J = 246.1 Hz), 162.52 (d, J = 246.1 Hz), 197.75. Anal. ($C_{22}H_{22}F_2O_6$) C, H.

***trans*-4,4′-(1,2,4,5-Tetroxane-3,6-diyl)bis[1-(4-fluorophenyl)-1-butanone] (4c)**: mp 155−156 °C (from ether); ^{1}H NMR δ 1.5−2.0 (m, 8 H), 2.99 (t, J = 7.3 Hz, 4 H), 5.95 (t, J = 5.6 Hz, 2 H), 7.13 (t, J = 8.9 Hz, 4 H), 7.95 (dd, J = 8.9 and 5.3 Hz, 4 H); ^{13}C NMR δ 17.99, 29.00, 37.57, 108.53, 115.72 (d, J = 21.9 Hz), 130.53, 130.67, 133.13, 165.76 (d, J = 253 Hz), 197.37. Anal. ($C_{22}H_{22}F_2O_6$) C, H.

Synthesis of 1,2,4,5-Tetroxanes by TMSOTf-Catalyzed Cyclocondensation of Aldehydes and Dioxybis[trimethylsilane]. The synthesis of tetroxane **8b** is representative. To an ice-cold solution of TMSOTf (666 mg, 3.00 mmol) in CH_3CN (15 mL) was added dioxybis[trimethylsilane][14] (540 mg, 3.00 mmol) by a syringe over 4 min under argon. Then, a solution of cyclohexanecaboxaldehyde (336 mg, 3.00 mmol) in CH_3CN (20 mL) was added by a syringe during 30 min at 0 °C. After stirring for more than 90 min at the same temperature, the mixture was poured into ether (70 mL). Then, the organic layer was washed with ice-cold $NaHCO_3$ and saturated brine and dried over anhydrous $MgSO_4$. After evaporation of the solvent under vacuum, the residue was separated by column chromatography on silica gel. Elution with ether−hexane (1:50) gave the tetroxane **8b** (223 mg, 58%).

3,6-Diheptyl-1,2,4,5-tetroxane (8a): mp 57−58 °C (from methanol); ^{1}H NMR δ 0.88 (t, J = 6.3 Hz, 6 H), 1.2−1.6 (m, 24 H), 5.87 (t, J = 5.6 Hz, 2 H); ^{13}C NMR δ 14.05, 22.61, 23.49, 24.84, 29.15, 29.69, 31.69, 109.40. Anal. ($C_{16}H_{32}O_4$) C, H.

3,6-Dicyclohexyl-1,2,4,5-tetroxane (8b): mp 68−69 °C (from methanol); ^{1}H NMR δ 1.5−2.0 (m, 22 H), 5.66 (t, J = 6.3 Hz, 2 H); ^{13}C NMR δ 25.34, 25.88, 26.81, 39.03, 110.96. Anal. ($C_{14}H_{24}O_4$) C, H.

3,6-Dibenzyl-1,2,4,5-tetroxane (8d): mp 112−113 °C (from methanol); ^{1}H NMR δ 2.85 (d, J = 5.3 Hz, 4 H), 6.08 (t, J = 5.6 Hz, 2 H), 7.0−7.4 (m, 10 H); ^{13}C NMR δ 36.24, 108.57, 127.20, 128.57, 129.45, 133.10. Anal. ($C_{16}H_{16}O_4$) C, H.

Synthesis of 1,2,4,5-Tetroxanes by the H_2SO_4-Catalyzed Cyclocondensation of Arylaldehydes with Hydrogen Peroxide. Reaction of 4-fluorobenzaldehyde is representative. Aqueous H_2SO_4 (36 wt %; 50 mL) was added by slow dropwise addition to a solution of aqueous ethanol (1:1 v/v, 50 mL) at 0 °C. Then, a solution of 4-fluorobenzaldehyde (5.00 g, 40 mmol) and 30% H_2O_2 (2.5 mL, 22.06 mmol) in ethanol (10 mL) was added in one portion, and the mixture was stirred for 1 day. After workup as described above, tetroxane **8f** was isolated by recrystallization (1.22 g, 22%).

3,6-Bis(4-methoxyphenyl)-1,2,4,5-tetroxane (8e): mp 214−215 °C (from methanol); ^{1}H NMR δ 3.83 (s, 6 H), 6.84 (s, 2 H), 6.94 (d, J = 8.9 Hz, 4 H), 7.45 (d, J = 8.9 Hz, 4 H). Anal. ($C_{16}H_{16}O_6$) C, H.

3,6-Bis(4-fluorophenyl)-1,2,4,5-tetroxane (8f): mp 222−223 °C (from methanol); ^{1}H NMR δ 6.89 (s, 2 H), 7.14 (t, J =

8.7 Hz, 4 H), 7.52 (dd, J = 8.9 and 5.3 Hz, 4 H). Anal. ($C_{14}H_{10}F_2O_4$) C, H.

3,6-Bis(2-trifluoromethylphenyl)-1,2,4,5-tetroxane (8g): mp 172−173 °C (from methanol); ^{1}H NMR δ 7.32 (s, 2 H), 7.6−7.9 (m, 8 H); ^{13}C NMR δ 104.53, 123.54 (q, J = 274 Hz), 126.18, 126.25, 129.38, 129.70, 131.57, 132.40. Anal. ($C_{16}H_{10}F_6O_4$) C, H.

3,6-Bis(4-trifluoromethylphenyl)-1,2,4,5-tetroxane (8h): mp 206−207 °C (from methanol); ^{1}H NMR δ 7.00 (s, 2 H), 7.65 (d, J = 8.6 Hz, 4 H), 7.73 (d, J = 8.6 Hz, 4 H). Anal. ($C_{16}H_{10}F_6O_4$) C, H.

Antimalarial Activities of 1,2,4,5,7-Pentoxocanes and 1,2,4,5-Tetroxanes in Vitro. Malaria parasites: *Plasmodium falciparum* (ATCC 30932, FCR-3 strain) was used in our study. *P. falciparum* was cultivated by a modification of the method of Trager and Jensen[15] using a 5% hematocrit of type A human red blood cells suspended in RPMI 1640 medium (Gibco, NY) supplemented with heat-inactivated 10% type A human serum. The plates were placed in a $CO_2-O_2-N_2$ incubator (5% CO_2, 5% O_2, and 90% N_2 atmosphere) at 37 °C, and the medium was changed daily until 5% parasitemia (which means that 5 parasite-infected erythrocytes in every 100 erythrocytes were existing).

Mammalian Cells: Mouse mammary tumor FM3A cells (wild-type, subclone F28-7)[16] were supplied by the Japanese Cancer Research Resources Bank (JCRB). FM3A cells were maintained in suspension culture at 37 °C in a 5% CO_2 atmosphere in plastic bottles containing ES medium (Nissui Pharmaceuticals, Tokyo, Japan) supplemented with 2% heat-inactivated fetal bovine serum (Gibco, NY).

In vitro antimalarial activity of 1,2,4,5-tetroxanes and 1,2,4,5,7-pentoxocanes: The following procedures were used for assay of antimalarial activity.[6,7] Asynchronously cultivated *P. falciparum* were used. Various concentrations of compounds in dimethyl sulfoxide were prepared. Five microliters of each solution was added to individual wells of a multidish, 24 wells. Erythrocytes with 0.3% parasitemia were added to each well containing 995 μL of culture medium to give a final hematocrit level of 3%. The plates were incubated at 37 °C for 72 h in a $CO_2-O_2-N_2$ incubator (5% CO_2, 5% O_2, and 90% N_2 atmosphere). To evaluate the antimalarial activity of test compound, we prepared thin blood films from each culture and stained them with Giemsa (E. Merck, Germany). Total 1×10^4 erythrocytes/1 thin blood film were examined under microscopy. All of the test compounds were assayed in duplicate at each concentration. Drug-free control cultures were run simultaneously. All data points represent the mean of three experiments. Parasitemia in control reached between 4% and 5% at 72 h. The EC_{50} value refers to the concentration of the compound necessary to inhibit the increase in parasite density at 72 h by 50% of control.

Toxicity against mammalian cell line: FM3A cells grew with a doubling time of about 12 h. Prior to exposure to drugs, cell density was adjusted to 5×10^4 cells/mL. A cell suspension of 995 μL was dispensed to the test plate, and compound at various concentrations suspended in dimethyl sulfoxide (5 μL) was added to individual wells of a multidish, 24 wells. The plates were incubated at 37 °C in a 5% CO_2 atmosphere for 48 h. All of the test compounds were assayed in duplicate at each concentration. Cell numbers were measured using a microcell counter CC-130 (Toa Medical Electric Co., Japan). All data points represent the mean of three experiments. The EC_{50} value refers to the concentration of the compound necessary to inhibit the increase in cell density at 48 h by 50% of control. Selectivity refers to the mean of EC_{50} value for FM3A cells per the mean of EC_{50} value for *P. falciparum*.

Antimalarial Activities of 1,2,4,5,7-Pentoxocanes and 1,2,4,5-Tetroxanes in Vivo. In vivo antimalarial activities of 1,2,4,5,7-pentoxocanes and 1,2,4,5-tetroxanes were determined in mice infected with *P. berghei* (NK 65 strain).[13] Five-week-old ICR male mice obtained in sterile containers from Charles River Breeding Laboratories, Inc. (Yokohama, Japan) weighing 22−25 g were used. They were housed under a natural day−night cycle at 25 °C. The mice were randomly assigned to treated groups and housed in cages each containing five individuals. Parasites were collected by cardiac puncture in a heparinized syringe from a donor mouse harboring about 15% parasitemia. The blood was diluted with 0.9% NaCl solution to a final concentrations of 1×10^6 infected erythrocytes/0.2 mL of infecting suspension. Test compounds were prepared at doses of 20 and 50 mg/kg in dimethyl sulfoxide. Five animals were treated with each dose. Initially, each mouse was inoculated intravenously in the tail vein with 1×10^6 parasitized erythrocytes (infecting suspension in 0.2 mL of 0.9% NaCl solution). The compounds were administrated once a day starting on day 0 and continued on day 1, day 2, and day 3. The first administration of test compound intraperitoneally started 2 h after parasite inoculation. Parasitemia levels were determined on the day following the last treatment (on day 4). To evaluate the antimalarial activity of the compounds, we prepared tail blood smears and stained them with Giemsa (E. Merck, Germany). Total 1×10^4 erythrocytes/1 thin blood film were examined under microscopy. On day 4, parasitemia of control mice were between 18% and 22%. The suppression of parasitemia for the dose of 1,2,4,5,7-pentoxocanes and 1,2,4,5-tetroxanes were caluated by the formula: [(average % parasitemia in controls (sham-treated) − average % parasitemia, in treated mice)/average % parasitemia in controls (sham-treated)] × 100. Five infected, dimethyl sulfoxide-dosed mice were used as a control. Treatment was considered curative when no parasites were detected after 60 days. The data shown are the mean values from five mice in one test. The care and treatment of mice were in accordance with the guidelines (No. 141, 1987) issued by the Science and International Affairs Bureau of the Japanese Ministry of Education, Science, Culture and Sports.

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