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Synthesis and antimalarial activities of novel 3,3,6,6-tetraalkyl-1,2,4,5-tetraoxanes

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Abstract—The oxidative system H_2O_2/fluorinated alcohol (TFE, HFIP) was used for direct acid- and $MeReO_3$-catalyzed synthesis of 1,2,4,5-tetraoxanes from cyclic (C6, C7, and C12) and acyclic ketones. The influence of ring size and alkyl chain length were studied and antimalarial activities of synthetic 3,3,6,6-tetraalkyl-1,2,4,5-tetraoxanes were determined. Variations in their antimalarial activities were significant, although they share similar electrochemical properties of the peroxide bond.

1. Introduction

Malaria is still one of the major communicable diseases, estimated to cause or contribute to as many as 3 million deaths per year.[1] Mortality caused by malaria has increased in recent years due mainly to the pathogen having developed resistance to available antimalarial drugs.[2–4] Artemisinin and its semi-synthetic and synthetic endoperoxides that are active against drug-resistant *Plasmodium* have emerged as potent alternative non-alkaloidal drugs. While the mechanism of their antimalarial activity is still unclear, it is known that the peroxide unit in these compounds is essential for their activity.[5–11] Dispiro-1,2,4,5-tetraoxanes (TO), having two endoperoxide bonds, have been found to be potent and inexpensive antimalarial agents.[12–15] The easiest path for their synthesis is acid-catalyzed cyclization of cyclic ketones with hydrogen peroxide, but this is successful only with selected substrates, and leads to mixture of compounds only one of which is the desired tetraoxane.[16–19] Alternative synthetic routes to tetraoxanes include ozonolysis of cycloalkylidene-cycloalkanes,[20] enol ethers,[21,22] and *O*-methyl oximes,[19,23] and the recently reported reaction of *gem*-bishydroperoxides with acetals or ketals catalyzed by boron trifluoride etherate.[24] There are a few reports of the synthesis of 1,2,4,5-tetraoxanes (TO) from acyclic ketones by acid-catalyzed peroxidation. This reaction requires the use of highly concentrated hydrogen peroxide or a tenfold excess of sulfuric and glacial acetic acid.[16,25–27]

Fluorinated alcohols such as hexafluoro-2-propanol, $(CF_3)_2CHOH$ or HFIP, and 2,2,2-trifluoroethanol, TFE, are known activators of hydrogen peroxide in various oxidation reactions by virtue of their high hydrogen bond donor strength, combined with their promotion of template catalysis.[28–30] We have used fluorinated alcohols in the methyltrioxorhenium (MTO)-catalyzed peroxidation of 4-methylcyclohexanone with 30% aqueous H_2O_2 in neutral conditions and shown that the reaction leads to the formation of the *gem*-dihydroperoxide, which, with another carbonyl compound under acid conditions, is further transformed to a non-symmetric TO.[31] We showed that by proper modification of the catalyst, concentration, and temperature 4-substituted cyclohexanones could be selectively transformed into corresponding dispiro-TOs with good yields. The reaction can be controlled in order to avoid the formation of trimeric by-products.[32]

Herein we show that fluorinated alcohols permit synthesis of 1,2,4,5-tetraoxanes directly from cyclic, as well as

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the less reactive acyclic ketones. The simple structure of the products, with a tetraoxane ring and alkyl groups of different length and branching, enables modification of the polarity of TOs and the steric hindrance around the O–O bond without affecting the properties of pharmacophoric peroxide unit. This offers an insight into the correlation between the structure of TOs and their antimalarial activity.

2. Results and discussion

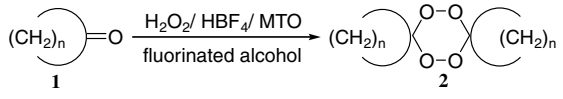
First, the effect of ring size on the formation of TO by acid-catalyzed peroxidation using 30% H_2O_2/ MTO/ fluorinated alcohols was tested. Previous results of such peroxidation of 4-substituted cyclohexanones in fluorinated alcohols at room temperature showed that reactions in HFIP produce lactones as products of Baeyer–Villiger rearrangement, while in TFE, tetraoxanes are formed selectively.[32] We mixed equimolar amounts of the ketone **1**, H_2O_2 (30% aqueous solution), and HBF_4 (54% ethereal solution), and treated the mixture with 0.1 mol% MTO in TFE for one hour at room temperature. Cyclopentanone **1a**, with a ring that is strained compared to that of cyclohexanone, gave under these conditions tetrahydro-2*H*-pyran-2-one, while TO was formed only in a trace amount (Table 1). The less strained cyclohexanone **1b** and cycloheptanone **1c** were successfully transformed into corresponding TOs **2b** and **2c** in TFE without noticeable lactone formation. Further enlargement of the size of the ring resulted in a decrease of conversion and selectivity for TO formation; reaction of the cyclooctanone **1d**, cyclodecanone **1e**, and cyclododecanone **1f** yielded complex reaction mixtures with minimal TO formation. Use of HFIP, a more activating solvent,[32] failed to lead to improvement in the formation of TO from ketones **1d** and **1e**, but in contrast, use of HFIP provided selective conversion of cyclododecanone **1f** to the corresponding tetraoxane **2f** in 55% yield.

The differing behavior of the various cycloalkanones in these peroxidations led us to examine the reactions of acyclic dialkyl ketones and the influence on TO yield of length and branching of the alkyl chain at positions α- and β- to the carbonyl group.

This method (H_2O_2/HBF_4/MTO in TFE) is successful for the transformation of acyclic ketones to TO. Table 2 shows the effect of extension of the alkyl chains on the yield of TO. It can be seen that extension of both the alkyl chains of diethyl ketone **3a** to dipropyl ketone **3b** resulted in a lower TO yield, which remained unchanged by additional chain lengthening leading to dibutyl ketone **3c**. Comparison of results for given family of ethyl ketones (**3a** and **3d**–**3h**) shows that extension of one of the alkyl chains from C_2H_5 to C_7H_{15} has no significant influence on TO yield. The best yield of TO was observed when the ethyl group in **3h** was replaced with a methyl group, in **3i**. Tetraoxanes from unsymmetrical ketones form *cis* and *trans* isomers, however, they could not be separated. Tetraoxane ring inversion is very fast at room temperature leading to broad signals in NMR spectra. When the spectra were recorded at lower temperature (−40 °C), conformation of tetraoxane ring freezes and reveals that a mixture of isomers was formed.

Table 3 shows the influence on TO yield of alkyl chain branching by introduction of a methyl substituent at the α- and β-positions on one or both sides of the carbonyl group. It can be observed that introduction of α-methyl group into diethyl ketone **3a** to give ethyl *iso*-propyl ketone (**5a**) reduces the yield by 50% and a second methyl group at the α′ position (di-*iso*-propyl ketone **5b**) totally inhibits TO formation. Introduction of methyl substituents to the β- and β′-positions (**5c** and **5d**) has a similar effect on the formation of TO. Steric influence therefore has an important role in the formation of TO, although it proved to be possible to trans-

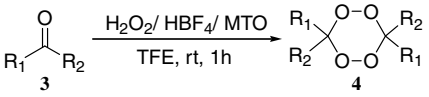
Table 1. The effect of ring size of cycloalkanone on tetraoxane yield



Ketone	*n*	Fluorinated alcohol	TO yield[a] (%]
1a	4	TFE	**2a**: <5[b]
1b	5	TFE	**2b**: 88
1c	6	TFE	**2c**: 45
1d	7	TFE	—[c]
1e	9	TFE	—[c]
1f	11	HFIP	**2f**: 55

[a] React. cond.: solution of equimolar amounts of **1**, H_2O_2, and HBF_4, 0.1 mol% MTO was stirred for 1 h at room temp. Yields refer to isolated products.
[b] Tetrahydro-2*H*-pyran-2-one was the major product as determined by ^{1}H NMR of crude reaction mixture.
[c] Complex reaction mixture with low conversion.

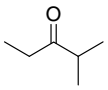
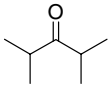
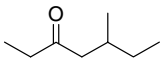
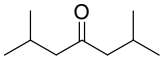
Table 2. Influence on tetraoxane yield of the length of alkyl chains in acyclic ketones



Ketone			TO yield[a] (%)
	R_1	R_2	
3a	Et	Et	**4a**: 57
3b	Pr	Pr	**4b**: 45
3c	Bu	Bu	**4c**: 44
3d	Et	Pr	**4d**: 56
3e	Et	Bu	**4e**: 55
3f	Et	Pent	**4f**: 59
3g	Et	Hex	**4g**: 55
3h	Et	Hept	**4h**: 57
3i	Me	Hept	**4i**: 72

[a] React. cond.: solution of equimolar amounts of **1**, H_2O_2, and HBF_4, 0.1 mol% MTO was stirred for 1 h at room temp. Yields refer to isolated products.

Table 3. The effect of branching of the alkyl chain at the α- and β-positions to carbonyl group on TO yield

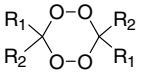
	Ketone	TO yield[a] (%)
5a		**6a**: 26
5b		**6b**: 0
5c		**6c**: 20
5d		**6d**: 0
5e		**6e**: 15

[a] React. cond.: solution of equimolar amounts of **1**, H_2O_2, and HBF_4, 0.1 mol% MTO was stirred for 1 h at room temp. Yields refer to isolated products.

form methyl *tert*-butyl ketone **5e** into the corresponding TO **6e** in 15% yield.

Dispiro-1,2,4,5-tetraoxanes are known antimalarials, but there are no published data on the antimalarial activity of 3,3,6,6-tetraalkyl-1,2,4,5-tetraoxanes. Therefore, we have determined in vitro antimalarial activities of synthesized TOs using FCB1 strain of *Plasmodium falciparum*, which is resistant to chloroquine (IC_{50} = 115 ± 25 nM). The dispiro TO **2c** showed the highest antimalarial activity (Table 4). Extension of the alkyl chain in TO does not influence the electronic properties of the peroxide bond as alkyl chains have similar electron-donating abilities. In spite of this, notable differences in the antimalarial activities of the various compounds were observed. The measured IC_{50} values of tetraalkyl-TOs **4** ranged from 151.4 nM for 3,6-dibutyl-3,6-diethyl-1,2,4,5-tetraoxane **4e** to 387.1 nM for 3,6-diethyl-3,6-dihexyl-1,2,4,5-tetraoxane **4g**, while tetraoxane with methyl substituent **4i** was essentially inactive (IC_{50} > 1 μM).

Table 4. In vitro antimalarial activities against *Plasmodium falciparum* strain FCB1 for synthesized tetraoxanes and their log*P* values

	Tetraoxane 		IC_{50} (nM[a])	log*P*[b]
	R_1	R_2		
2c	$-(CH_2)_6-$		82.9	4.73
2f	$-(CH_2)_{11}-$		Totally insoluble	10.32
4b	*n*-Pr	*n*-Pr	183.4	5.94
4d	Et	*n*-Pr	179.4	4.88
4e	Et	*n*-Bu	151.4	5.94
4f	Et	*n*-Pent	243.7	7.00
4g	Et	*n*-Hex	387.1	8.06
4i	Me	*n*-Hept	>1000	8.06
6a	Et	*i*-Pr	478.1	4.62
6e	Me	*t*-Bu	>1000	4.36
6c	Et	$CH_2CH(CH_3)_2$	135.8	6.74
	Artemether (ref)		3.5	

[a] IC_{50} values were means of three independent experiments.
[b] log*P* values were calculated using program http://www.daylight.com.

The small amount of experimental data on the effect of lipophilicity on the in vivo antimalarial activity suggests that within a given peroxide chemical family, the more lipophilic compounds possess superior antimalarial activity.[15,33,34] In this respect, TOs are interesting prototypes with which to seek correlations between the structure of TO (lipophilicity and steric effects around peroxide bond) and in vitro antimalarial activities. The alkyl chains in tetraalkyl TO are a major part of the structure and their variation has no significant effect on the electronic properties of pharmacophoric peroxide bond because of their similar electron-donating abilities and absence of other functional groups. Accordingly, we correlated the antimalarial activity of selected tetraoxanes with calculated values for their lipophilicity (log*P*). It was found that as the alkyl chains in a given TO family become longer, the calculated lipophilicity, expressed as log*P*, increases and the antimalarial activity decreases, as shown in Figure 1A. A good correlation between log*P* and IC_{50} was observed for related tetraoxanes with linear alkyl chains over a polarity range of more than three orders of magnitude (Fig. 1B), but the TO **4d** with a shorter alkyl substituent was not so correlated and the TOs with a methyl substituent on TO ring (**4i**, **6c**) have IC_{50} values above 1 μM. A further comparison with TOs with branched alkyl chains that influence the sterical hindrance around O–O bond indicates that polarity, or lipophilicity might not be the only significant parameter. Comparison of the antimalarial activity of diethyl dipropyl TO **4d** with that of diethyl di-*iso*-propyl TO (**6a**) reveals that the methyl substituent at the position α to the ring carbon drastically decreases the activity; the tetraoxane **4d** is more than twice as potent than its branched analog **6a**, although they share similar lipophilicity (log*P* 4.88 and 4.62, respectively).

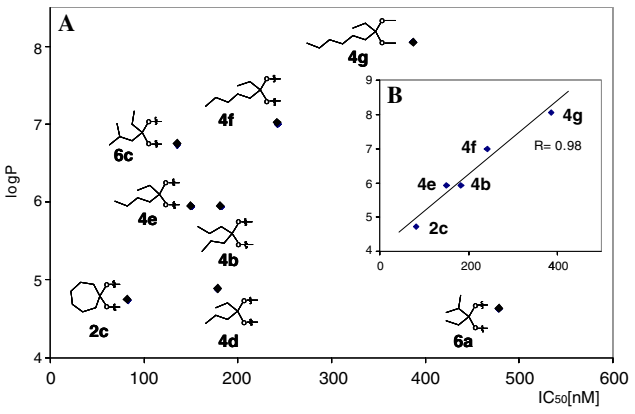


Figure 1. Correlation between antimalarial activity and lipophilicity (log*P*) of selected tetraoxanes.

3. Conclusion

The oxidative system—H_2O_2/fluorinated alcohol (TFE, HFIP)—has proved useful for direct acid- and MTO-catalyzed synthesis of tetraoxanes from cyclic (C_6, C_7, and C_{12}) and acyclic ketones. Cyclopentanone was converted mainly to a lactone, while cyclooctanone and cyclodecanone were less reactive. The reactions of acyclic ketones revealed that the length of the alkyl chain and its branching around carbonyl group has a significant influence on TO yield. As selected substrates have similar substituents in terms of electron-donating properties, differences in their reactivity were attributed to their geometry and lipophilicity. It was observed that chain length does not have a great influence on TO yield as long as one of the two alkyl chains is short. Introduction of α- and β-substituents leads to reduction of TO yield.

The 3,3,6,6-tetraalkyl-1,2,4,5-tetraoxanes have antimalarial activities against a chloroquine-resistant strain of *P. falciparum* similar to that of their dispiro analogs. The variations in antimalarial activities of the TOs studied were significant, although they share similar electronic properties of peroxide bonds. These can probably be attributed to the combination of the influence of polarity (higher lipophilicity, lower activity) and steric hindrance (branching and substitution) of the peroxide bond.

4. Experimental

4.1. General remarks

Ketones and methyltrioxorhenium were obtained from commercial sources and were used as received. 30% H_2O_2 (Perhydrol) was obtained from Merck KGaA. Trifluoroethanol (TFE), 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), and other solvents were distilled before use.

^1H and ^{13}C NMR spectra were obtained with TMS and $CDCl_3$, respectively, as standards on a Varian Unity-300 spectrometer. Chemical shifts are reported in ppm from the internal standard. Melting points were determined on a Büchi 535 apparatus and are uncorrected. Elemental analyses were performed at the Microanalytisches Labor Pascher. IR spectra were recorded on Perkin-Elmer 1310 spectrometer. Standard KBr pellet procedures were used to obtain IR spectra of solids, while a film of neat material was used to obtain IR spectra of liquid products.

Caution. Although we have encountered no difficulties in working with these relatively stable tetraoxanes, routine precautions (shields, fume hoods, and avoidance of transition metal salts) should be observed whenever possible, as organic peroxides are potentially hazardous compounds.

4.2. Reaction procedure for synthesis of tetraoxanes

MTO (0.5 mg, 2 μmol), 30% H_2O_2 (0.227 mL, 2 mmol), and 54% HBF_4 solution in Et_2O (0.275 mL, 2 mmol) were dissolved in TFE (2 mL). Substrate (2 mmol) was added and stirred at room temperature for one hour. CH_2Cl_2 (20 mL) was added and the solution washed twice with 20 mL of H_2O. The organic phase was dried with Na_2SO_4 and the solvent evaporated. Tetraoxanes **2a**–**2c**, **4a**–**4i**, and **6a**–**6e** were purified by column chromatography (SiO_2, CH_2Cl_2/petrol ether 4:1). They were the first products eluted.

For synthesis of **2f** HFIP (2 mL) was used as solvent and **2f** was isolated by filtration of the reaction mixture and washed with CH_2Cl_2.

4.2.1. 7,8,15,16-Tetraoxa-dispiro[5.2.5.2]hexadecane (2b). Compound **2b** was isolated as white solid in 88% yield (201 mg): mp 128.8–129 °C (lit.,[35] 130–131 °C); ^1H NMR: δ 1.37–1.51 (m, 4H), 1.51–1.70 (m, 12H), 2.27 (br s, 4H); ^{13}C NMR: δ 22.05 (br), 25.36, 29.54 (br), 31.80 (br), 108.12.

4.2.2. 8,9,17,18-Tetraoxa-dispiro[6.2.6.2]octadecane (2c). Compound **2c** was isolated as white solid in 45% yield (115 mg): mp 99–100 °C (lit.,[26] 98–100 °C); ^1H NMR: δ 1.45–1.84 (m, 20H), 2.42 (br s, 4H); ^{13}C NMR: δ 22.43, 29.55, 30.21, 31.02, 36.00, 112.43.

4.2.3. 13,14,27,28-Tetraoxa-dispiro[11.2.11.2]octacosane (2f). Compound **2f** was isolated as white solid in 55% yield (220 mg): mp 176–179 °C (dec); ^1H NMR: δ 1.05–1.76 (m, 40H), 2.23 (br s, 4H); ^{13}C NMR: δ 18.00 (br), 19.41 (br), 21.64 (br), 22.23, 25.89 (br), 25.99, 29.24 (br), 112.00; IR (cm^{-1}): 2940, 2840, 1470, 1450, 1440, 1160, 1175, 1080, 1060, 1000, 950, 910. Anal. Calcd for $C_{24}H_{44}O_4$ (414.62): C, 69.52; H, 11.18. Found C,69.67; H, 10.89.

4.2.4. 3,3,6,6-Tetraethyl-[1,2,4,5]tetraoxane (4a). The tetraoxane **4a** was isolated as colorless oil in 57% yield (116 mg): ^1H NMR: δ 0.95 (t, 7.6 Hz, 12H), 1.64 (br s, 4H), 2.23 (br s, 4H); ^{13}C NMR: δ 6.60 (br), 7.93 (br), 23.48 (br), 26.39 (br), 110.89.[25]

4.2.5. 3,3,6,6-Tetrapropyl-[1,2,4,5]tetraoxane (4b). The tetraoxane **4b** was isolated as white solid in 45% yield (116 mg): mp 52–53 °C (lit.,[26] 52–54 °C); ^1H NMR: δ 0.8–1.04 (m, 12H), 1.28–1.5 (m, 8H), 1.55 (m, 4H), 2.13 (br s, 4H); ^{13}C NMR: δ 14.31, 15.71 (br), 17.00 (br), 33.26 (br), 35.99 (br), 110.47.

4.2.6. 3,3,6,6-Tetrabutyl-[1,2,4,5]tetraoxane (4c). The tetraoxane **4c** was isolated as white solid in 44% yield (138 mg): mp 36.3–36.9 °C (lit.,[26] 37–39 °C); ^1H NMR: δ 0.92 (m, 12H), 1.35 (m, 16H), 1.48–1.78 (m, 4H), 2.17 (br s, 4H); ^{13}C δ NMR: 13.87, 22.88, 24.40 (br), 25.66 (br), 30.67 (br), 33.55 (br), 110.63.

4.2.7. 3,6-Diethyl-3,6-dipropyl-[1,2,4,5]tetraoxane (4d). The tetraoxane **4d** was isolated as colorless oil in 56% yield (130 mg): ^1H NMR: δ 0.95 (m, 12H), 1.30–1.53 (m, 4H), 1.61 (br s, 4H), 2.21 (br s, 4H); ^{13}C NMR: δ 6.42 (br), 7.529 (br), 14.32, 15.50 (br), 16.60 (br), 23.97 (br), 26.68 (br), 32.80, 35.18 (br), 110.76; IR (cm^{-1}): 2960, 2860, 1460, 1160, 1140, 1000, 970, 950, 910. Anal. Calcd for $C_{12}H_{24}O_4$ (232.32): C, 62.04; H, 10.41. Found C, 61.85; H, 10.33.

4.2.8. 3,6-Dibutyl-3,6-diethyl-[1,2,4,5]tetraoxane (4e). The tetraoxane **4e** was isolated as colorless oil in 55% yield (142 mg): ^{1}H NMR: δ 0.82–1.06 (m, 12H), 1.22–1.48 (m, 8H), 1.60 (br s, 4H), 2.22 (br s, 4H); ^{13}C NMR: δ 6.62 (br), 7.52 (br), 13.84, 22.87, 23.88 (br), 25.48 (br), 26.95 (br), 30.31 (br), 32.99 (br), 110.76; IR (cm^{-1}): 2960, 2870, 1460, 1160, 1140, 990, 960, 950. Anal. Calcd for $C_{14}H_{28}O_4$ (260.37): C, 64.58; H, 10.84. Found C, 64.57; H, 10.75.

4.2.9. 3,6-Diethyl-3,6-dipentyl-[1,2,4,5]tetraoxane (4f). The tetraoxane **4f** was isolated as colorless oil in 59% yield (171 mg): ^{1}H NMR: δ 0.81–1.03 (m, 12H), 1.18–1.50 (m, 12H), 1.61 (br s, 4H), 2.21 (br s, 4H); ^{13}C NMR: δ 6.55 (br), 7.97 (br), 13.92, 21.57 (br), 22.42, 24.08 (br), 26.78 (br), 30.52 (br), 31.94, 33.13 (br), 110.93; IR (cm^{-1}): 2960, 2860, 1460, 1160, 1140, 1000, 940, 930. Anal. Calcd for $C_{16}H_{32}O_4 \cdot 1/10H_2O$ (290.22): C, 66.17; H, 11.18. Found C, 66.08; H, 11.08.

4.2.10. 3,6-Diethyl-3,6-dihexyl-[1,2,4,5]tetraoxane (4g). The tetraoxane **4g** was isolated as colorless oil in 55% yield (173 mg): ^{1}H NMR: δ 0.81–1.05 (m, 12H), 1.15–1.48 (m, 16H), 1.60 (br s, 4H), 2.20 (br s, 4H); ^{13}C NMR: δ 6.36 (br), 7.96 (br), 14.03, 21.96 (br), 22.54, 23.63 (br), 26.84 (br), 29.45, 31.59 (br), 33.12 (br), 110.78; IR (cm^{-1}): 2950, 2920, 2850, 1460, 1160, 1140, 1000, 960, 940. Anal. Calcd for $C_{18}H_{36}O_4$ (316.48): C, 68.31; H, 11.47. Found C, 68.33; H, 11.44.

4.2.11. 3,6-Diethyl-3,6-diheptyl-[1,2,4,5]tetraoxane (4h). The tetraoxane **4h** was isolated as colorless oil in 57% yield (196 mg): ^{1}H NMR: δ 0.77–1.01 (m, 12H), 1.12–1.47 (m, 20H), 1.60 (br s, 4H), 2.20 (br s, 4H); ^{13}C NMR: δ 6.53 (br), 7.92 (br), 14.06, 21.86 (br), 22.63, 23.81 (br), 26.94 (br), 29.07, 29.75, 30.46 (br), 31.74, 33.05 (br), 110.76; IR (cm^{-1}): 2950, 2920, 2850, 1460, 1150, 1140, 1000, 970, 950. Anal. Calcd for $C_{20}H_{40}O_4$ (344.53): C, 69.72; H, 11.70. Found C, 69.59; H, 11.64.

4.2.12. 3,6-Diheptyl-3,6-dimethyl-[1,2,4,5]tetraoxane (4i). The tetraoxane **4i** was isolated as colorless oil in 72% yield (228 mg): ^{1}H NMR: δ 0.82–0.95 (m, 6H), 1.1–1.5 (m, 23H), 1.60 (br s, 2H), 1.74 (br s, 3H), 2.18 (br s, 2H); ^{13}C NMR: δ 14.04, 19.15 (br), 20.02 (br), 22.22 (br), 22.63, 23.92 (br), 29.07, 29.67, 31.73, 32.46 (br), 36.48 (br), 109.23; IR (cm^{-1}): 2950, 2920, 2850, 1460, 1380, 1200, 1150, 1100, 940. Anal. Calcd for $C_{18}H_{36}O_4$ (316.48): C, 68.31; H, 11.47. Found C, 68.23; H, 11.34.

4.2.13. 3,6-Diethyl-3,6-di*iso*propyl-[1,2,4,5]tetraoxane (6a). The tetraoxane **6a** was isolated as colorless oil in 26% yield (60 mg): ^{1}H NMR: δ 0.73–1.13 (m, 18H), 1.25–2.65 (m, 4H), 3.23 (br s, 2H); ^{13}C NMR: δ 7.37 (br), 16.29, 22.58 (br), 31.70 (br), 111.76; IR (cm^{-1}): 2980, 2940, 2850, 1460, 1160, 1120, 1010, 970, 960, 940, 930. Anal. Calcd for $C_{12}H_{24}O_4$ (232.32): C, 62.04; H, 10.41. Found C, 62.15; H, 10.27.

4.2.14. 3,6-Diethyl-3,6-*bis*-(2-methyl-butyl)-[1,2,4,5]tetraoxane (6c). The tetraoxane **6c** was isolated as colorless oil in 20% yield (59 mg): ^{1}H NMR: δ 0.75–1.05 (m, 18H), 1.05–2.58 (m, 14H); ^{13}C NMR: δ 6.57 (br), 8.31 (br), 11.25, 20.66 (br), 24.48 (br), 26.78 (br), 29.39 (br), 30.62, 36.68 (br), 40.21 (br), 111.65; IR: 2950, 2870, 1460, 1380, 1000, 980, 950, 930 cm^{-1}. Anal. Calcd for $C_{16}H_{32}O_4 \cdot 1/4H_2O$ (292.92): C, 65.27; H, 11.18. Found C, 64.98; H, 10.74.

4.2.15. 3,6-Di-*tert*-butyl-3,6-dimethyl-[1,2,4,5]tetraoxane (6e). The tetraoxane **6e** was isolated as white solid in 15% yield (34 mg): mp 123.8–124 °C (lit.,[36] 122.5–123.5 °C); ^{1}H NMR: δ 1.04 (s, 18H), 1.74 (s, 6H); ^{13}C NMR: δ 15.35, 24.70, 38.89, 111.79.

4.3. In vitro assays

Chloroquine-resistant *P. falciparum* strain FCB1 (Colombia) was maintained in a continuous culture on human erythrocytes as described by Trager and Jensen.[37] In vitro antiplasmodial activity of compounds was determined using a modification of the semi-automated microdilution technique of Desjardins et al.[38] Stock solutions of tested compounds were prepared in DMSO. Drug solutions were serially diluted with the culture medium and added to parasite cultures synchronized at the ring stages (1% parasitemia and 1% final hematocrit) in 96-well plates. Parasite growth was assessed by adding 0.5 μCi of [^{3}H]hypoxanthine (10–30 Ci/mmol, Amersham Biosciences Europe GmbH) to each well. Plates were incubated for 48 h at 37 °C in the appropriate atmosphere. Immediately after incubation, the plates were frozen and thawed to lyse erythrocytes. The contents of each well were collected on filter microplates, washed using a cell harvester, and dried. Scintillation cocktail was added to each filter, and radioactivity incorporated by the parasites was measured in a scintillation counter. The growth inhibition for each drug concentration was determined by comparison of the radioactivity incorporated in the treated culture with that in the control culture (without drug). The drug concentration causing 50% inhibition (IC_{50}) was determined by non-linear regression analysis of log(dose)–response curves. Values are the average of three experiments. DMSO introduced into the cultures never exceeded 0.1% and did not affect parasite growth.

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