4-1999

Synthesis and Antimalarial Activity of 11 Dispiro-1,2,4,5-tetraoxane Analogues of WR 148999. 7,8,15,16-Tetraoxadispiro[5.2.5.2]hexadecanes Substituted at the 1 and 10 Positions with Unsaturated and Polar Functional Groups

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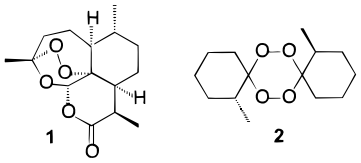
Received December 11, 1998

Eleven novel dispiro-1,2,4,5-tetraoxanes **3** bearing unsaturated and polar functional groups were designed to enhance the oral antimalarial activity of the prototype tetraoxane **2** (WR 148999). With the exception of **3g** and **3h**, tetraoxanes **3** were available via the peroxidation of corresponding cyclohexanone derivatives in H_2SO_4/CH_3CN. Tetraoxanes **3g** and **3h** were prepared by hydrolysis of ester tetraoxanes **3e** and **3i**, respectively. Five of the 11 tetraoxanes were inactive, but six tetraoxanes had IC_{50} values of 6−26 nM against the K1 and NF54 strains of *Plasmodium falciparum* compared to corresponding IC_{50} values of 28 and 39 nM for **2**, and 10 and 12 nM for artemisinin (**1**). Ester tetraoxane **3e** was the most active in vitro, some 2-fold more potent than **1**. However, none of the six tetraoxanes active in vitro were as effective as either **1** or **2** in vivo; at single doses of 100 mg/kg most possessed little to no vivo activity in mice infected with *Plasmodium berghei*. Unsaturated tetraoxane **3a** was uniquely more active when administered per os (po) than subcutan (sc). For this series of tetraoxanes, the discrepancy between vitro and vivo activities underscores the limitations of conclusions drawn solely from in vitro antimalarial data and illustrates a practical benefit of complementary single-dose in vivo antimalarial screens.

The discovery of artemisinin (qinghaosu, **1**), a naturally occurring endoperoxide sesquiterpene lactone, stimulated a substantial effort to elucidate its structure−activity relationship (SAR) and initiated an ongoing quest to identify structurally simple and synthetically accessible antimalarial peroxides.[1] Among synthetic antimalarial peroxides, dispiro-1,2,4,5-tetraoxanes such as **2** (WR 148999) are notable in that they differ considerably in structure from **1**, are readily prepared in one step from substituted cyclohexanones, and possess antimalarial activity comparable to **1**.[2,3]



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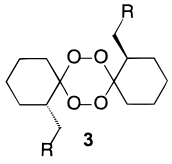
The poor to modest oral bioavailability[4] of artemisinin and its semisynthetic derivatives has been attributed to a high first-pass drug metabolism. Whether the poor oral antimalarial activity of tetraoxane **2** is due to poor absorption or rapid inactivating metabolism is unknown. However, the excellent ip activity of **2**[3] suggests that this tetraoxane has some resistance to inactivating first-pass hepatic drug metabolism as a major exit mechanism for drugs from the peritoneal cavity is by way of the portal circulation.[5] It follows that the low oral activity of **2** may be due either to inactivating gut metabolism or to an inadequate dissolution rate which is a function of its intrinsic solubility, stability to stomach acid, and formulation.

Design

To address the hypothesis that tetraoxane oral bioavailability is a function of oral absorption, we envisioned the synthesis of more water soluble tetraoxanes bearing polar functional groups.[6] These analogues might be more easily absorbed than their alkyl-substituted counterparts, an important consideration if lack of absorption is a significant factor in the low oral activity of tetraoxane **2**. Using tetraoxane **2** as a prototype, we designed target dispiro-1,2,4,5-tetraoxanes (7,8,15,16-tetraoxadispiro[5.2.5.2]hexadecanes) **3** bearing unsaturated and functional groups at the 1 and 10 positions. As exemplified by **2**, dispiro-1,2,4,5-tetraoxanes derived from 2-alkyl cyclohexanones are always formed as single centrosymmetric stereoisomers.[2,7] We anticipated that this predictable stereochemical outcome would hold true for tetraoxanes **3** as well.



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10.1021/jm980698f CCC: $18.00
Published on Web 03/25/1999

Table 1. Antimalarial Activity against *P. falciparum* in Vitro

		IC_{50}, nM[a]	
cmpd	R	K1	NF54
3a	$CH{=}CH_2$	19	23
3b	$C{\equiv}CH$	13	13
3c	C_6H_5	>200	>200
3d	CH_2COOEt	14	16
3e	COOEt	6.2	6.5
3f	CH_2COOH	>200	>200
3g	COOH	>200	>200
3h	OH	>200	>200
3i	$OCOC_6H_5$	>200	>200
3j	OMe	15	16
3k	$OCH_2C_6H_5$	18	26
2	H	28	39
1		10	12

[a] Average of $n = 2$.

Tetraoxanes **3** were designed as derivatives of **2** by replacing one methyl hydrogen atom of each of the two methyl groups in **2** with unsaturated (**3a**−**3c**), carboxylic (**3d**−**3g, 3i**), hydroxylic (**3h**) and ether (**3j**, **3k**) substituents (Table 1). Five of the target tetraoxanes could also conceivably function as prodrugs in vivo. For example, **3j** and **3k** could both be converted to **3h** via metabolic O-dealkylation reactions. In addition, **3d**, **3e**, and **3i** could be converted to **3f**, **3g**, and **3h**, respectively, via plasma esterase enzymes.

Chemistry

Nine tetraoxanes were prepared according to the peroxidation method developed by McCullough et al.[8] in which cyclohexanones are treated with hydrogen peroxide in H_2SO_4/CH_3CN. The unoptimized yields ranged from 4 to 12%. Tetraoxanes **3g** and **3h** were not formed using this method and were instead indirectly prepared by the hydrolysis of tetraoxane esters **3e** and **3i** in yields of 72 and 60%, respectively. The failure to form **3g** and **3h** via acid-catalyzed peroxidation is possibly associated with formation of persistent perhydrates, in agreement with reported data[9] in which 2-chloro- and 2-bromocyclohexanone afforded isolable perhydrates instead of tetraoxanes due to the formation of an intramolecular hydrogen bond between the hydroperoxide hydrogen and the halogen substituent.

Tetraoxanes **3a**−**3k** are white crystalline solids stable at room temperature for months if not years. In ^1H NMR spectra of these tetraoxanes, as is also the case for tetraoxane **2**,[2] a characteristic peak for the two methine protons at the 1 and 10 positions falls between 2.7 and 3.4 ppm. In the corresponding ^{13}C NMR spectra, a diagnostic signal is present at 108−110 ppm, corresponding to the spiro quaternary carbon atoms.[2,7]

Antimalarial Activity

The in vitro antimalarial data (Table 1) reveal that six of the tetraoxanes were at least as potent as the parent tetraoxane **2**. Of these, **3b**, **3d**, and **3j** were nearly as potent as artemisinin, and **3e** was notably more potent than artemisinin. From these data, we can conclude that the presence of alkene (**3a**), alkyne (**3b**), and ether (**3j**, **3k**) moieties maintain or enhance antimalarial potency whereas carboxylic acid (**3f**, **3g**) and alcohol (**3h**) moieties decrease antimalarial potency. The situation for esters is less clear; ethyl esters **3d** and **3e** had good activity, whereas **3i**, the dibenzoate ester of **3h**, was inactive. We also note that the six active tetraoxanes, like artemisinin, were more potent against the chloroquine-resistant K1 strain than against the drug-sensitive NF54 strain of *Plasmodium falciparum*, although in each case, the potency difference was small.

Table 2. Antimalarial Activity against *P. berghei* in Vivo

	activity (%)[b]		survival (days)[c]	
cmpd[a]	sc	po	sc	po
3a	15	93		7
3b	9	0		
3d	0	0		
3e	0	0		
3j	100	18	24.7	
3k	0	0		
2	100	99.7	27.7	7.7
1	100	98	14	7.3

[a] Compounds in a solution or a suspension containing 3% ethanol and 7% Tween 80 were administered on day 1 post-infection at a single dose of 100 mg/kg. [b] Reduction of parasitemia on day 3 post-infection. [c] On average, mice in a control group survive 5.2 days post-infection.

The six active tetraoxanes, along with **1** and **2** as controls, were selected for in vivo antimalarial evaluation in mice infected with the ANKA strain of *Plasmodium berghei*. Consistent with existing multiple-dose Thompson test data,[2,3] the vivo antimalarial efficacies for **1** and **2** differed only slightly. From the vivo data (Table 2), it is clear that none of these functionalized tetraoxanes possessed useful oral antimalarial activity and all were less effective than either **1** or **2**. We attribute the complete lack of vivo activity for ester tetraoxanes **3d** and **3e** to in vivo chemical or enzymatic hydrolysis to the inactive tetraoxane acids **3f** and **3g**, respectively. Although methyl ether tetraoxane **3j** had good vivo activity sc, it was almost completely inactive po due probably to first-pass O-dealkylative metabolism to the inactive tetraoxane alcohol **3h**. Unsaturated tetraoxane **3a** was uniquely more active po than sc, due possibly to formation of some active metabolite.

Discussion

In this work, we identified six functionalized tetraoxanes with in vitro potency equal or superior to prototype tetraoxane **2** and one diester tetraoxane (**3e**) with in vitro potency superior to **1**. It is conceivable that some of the tetraoxanes were well absorbed orally but suffered from a lack of metabolic stability. Nevertheless, the poor vivo antimalarial activity of these tetraoxanes provides no justification for additional experiments (i.e., blood level determinations) to assess their oral bioavailability. For this series of tetraoxanes, the discrepancy between vitro and vivo activities underscores the limitations of conclusions drawn solely from in vitro antimalarial data and illustrates how the single-dose in vivo antimalarial screen used in this study provided a necessary and more rigorous test of antimalarial potential.

As these functionalized tetraoxanes were based on tetraoxane **2** as a structural template, they manifest the advantage of both defined and predictable stereochemistry but also the liability of their centrosymmetric symmetry in which each tetraoxane (ketone diperoxide) necessarily bears two functional groups present singly in the starting material cyclohexanones. Existing tetraoxane synthetic methods[10] do not allow for the synthesis of nonsymmetrical tetraoxanes. Even with this

limitation, incorporation of unsaturated and polar functional groups at other positions in dispiro-1,2,4,5-tetraoxanes is possible, and this work provides a guide to functional group selection in the search for more orally active tetraoxane antimalarials.

Experimental Section

Melting points are uncorrected. Unless noted otherwise, ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra were recorded on a Varian XL-300 spectrometer using $CDCl_3$ as a solvent. All chemical shifts are reported in parts per million (ppm) and are relative to internal $(CH_3)_4Si$ for ^{1}H and $CDCl_3$ (77.0 ppm) for ^{13}C NMR. Microanalyses were performed by M-H-W-laboratories, Phoenix, AZ. 2-Allyl- and 2-carboethoxymethylcyclohexanone were purcased from Aldrich Chemical Co. 2-Benzyl-, 2-propargyl-, 2-carboethoxyethyl-, and 2-benzyloxymethylcyclohexanone were prepared by enamine alkylation reactions described by Stork et al.[11] 2-Hydroxymethylcyclohexanone was prepared from cyclohexanone and paraformaldehyde.[12] 2-Benzoyloxymethylcyclohexanone was synthesized by acylation of 2-hydroxymethylcyclohexanone with benzoyl chloride in pyridine/dichloromethane. 2-Carboxyethyl- and 2-carboxymethylcyclohexanone were prepared by hydrolysis of the corresponding commercially available ethyl esters in KOH/MeOH followed by acidification using concentrated HCl.

General Procedure[8] for the Preparation of Tetraoxanes 3a−3f and 3i−3k. A cold (−20 °C) solution of a parent cyclohexanone (10 mmol) in acetonitrile (2 mL) was added dropwise to a stirred, cold (−30 °C) solution of 50% hydrogen peroxide (0.60 mL, 11 mmol) and concentrated sulfuric acid (1 mL) in acetonitrile (4 mL). After being stirred for an additional 1 h at −30 to −20 °C, the solution was kept at −20 °C for 2 days. If the required product precipitated or crystallized out of the reaction solvent (**3a**, **3c**, **3e**, **3f**, **3i**, **3k**), a simple filtration and recrystallization sufficed for purification. If the product tetraoxane did not precipitate (**3b**, **3d**, **3j**), extraction with dichloromethane and subsequent silica gel flash column chromatography eluting with petroleum ether/ether (9:1) were applied to isolate the product. In each case, product tetraoxanes were isolated as white crystalline solids.

1,10-Bis(2-propenyl)-7,8,15,16-tetraoxadispiro[5.2.5.2]-hexadecane (3a): yield 5%; mp 101−102 °C (CH_3CN); ^{1}H NMR 1.05−1.45 (m, 4H), 1.46−1.82 (m, 12H), 1.85−2.15 (m, 2H), 2.40−2.70 (m, 2H), 2.80−3.10 (m, 2H), 4.90−5.17 (m, 4H), 5.55−5.90 (m, 2H); ^{13}C NMR 22.31, 24.54, 27.01, 29.91, 32.06, 43.27, 109.28, 116.46, 136.84. Anal. ($C_{18}H_{28}O_4$) C, H.

1,10-Bis(2-propynyl)-7,8,15,16-tetraoxadispiro[5.2.5.2]-hexadecane (3b): yield 10%; mp 167−168 °C dec (CH_3CN); ^{1}H NMR 1.15−2.40 (m, 20H), 2.50−2.79 (m, 2H), 2.80−3.04 (bs, 2H); ^{13}C NMR 17.54, 22.18, 24.59, 27.19, 29.83, 42.97, 69.44, 82.51, 108.67. Anal. ($C_{18}H_{24}O_4$) C, H.

1,10-Dibenzyl-7,8,15,16-tetraoxadispiro[5.2.5.2]hexadecane (3c): yield 13%; mp 182−184 °C dec (CH_3CN); ^{1}H NMR 1.10−1.42 (m, 4H), 1.48−1.78 (m, 10H), 1.82−1.98 (m, 2H), 2.34−2.53 (m, 2H), 3.03−3.19 (m, 2H), 3.20−3.37 (m, 2H), 7.09−7.37 (m, 10H); ^{13}C NMR 22.36, 24.61, 26.66, 30.08, 33.71, 45.65, 109.31, 125.99, 128.29, 129.21, 140.24. Anal. ($C_{26}H_{32}O_4$) C, H.

1,10-Bis(carboethoxyethyl)-7,8,15,16-tetraoxadispiro-[5.2.5.2]hexadecane (3d): yield 9%; mp 87 °C; ^{1}H NMR 1.26 (t, J = 7.2 Hz, 6H), 1.20−1.85 (m, 18H), 1.90−2.50 (m, 6H), 2.80−3.15 (bs, 2H), 4.13 (q, J = 7.1 Hz, 4H); ^{13}C NMR 14.17, 23.08, 24.39, 27.31, 29.64, 29.72, 32.59, 42.91, 60.24, 109.55, 173.40. Anal. ($C_{22}H_{36}O_8$) C, H.

1,10-Bis(carboethoxymethyl)-7,8,15,16-tetraoxadispiro-[5.2.5.2]hexadecane (3e): yield 4%; mp 110 °C (pentane); ^{1}H NMR 1.26 (t, J = 7.1 Hz, 6H), 1.20−1.85 (m, 14H), 2.05−2.40 (m, 4H), 2.70−2.89 (m, 2H), 2.90−3.12 (bs, 2H), 4.13 (q, J = 7.1 Hz, 4H); ^{13}C NMR 14.18, 22.17, 24.70, 28.36, 29.89, 33.34, 40.33, 60.50, 108.80, 172.38. Anal. ($C_{20}H_{32}O_8$) C, H.

1,10-Bis(carboxyethyl)-7,8,15,16-tetraoxadispiro[5.2.5.2]-hexadecane (3f): yield 12%; mp 178 °C (CH_3OH:H_2O, 1:1); ^{1}H NMR (DMSO-d_6) 1.05−1.80 (m, 18H), 1.81−2.05 (bs, 2H), 2.06−2.40 (m, 4H), 2.70−2.95 (bs, 2H); ^{13}C NMR (DMSO-d_6) 22.19, 23.00, 24.25, 27.16, 29.64, 32.06, 42.58, 109.51, 174.89. Anal. ($C_{18}H_{28}O_8$) C, H.

1,10-Bis(benzoyloxymethyl)-7,8,15,16-tetraoxadispiro-[5.2.5.2]hexadecane (3i): yield 10%; mp 195 °C dec (ether:pentane, 1:1); ^{1}H NMR 1.15−2.01 (m, 14H), 2.04−2.32 (bs, 2H), 2.90−3.25 (bs, 2H), 4.10−4.49 (bs, 2H), 4.50−4.80 (bs, 2H), 7.30−7.75 (m, 6H), 7.90−8.30 (m, 4H); ^{13}C NMR 22.03, 24.55, 25.72, 30.04, 43.30, 62.90, 109.13, 128.32, 129.56, 130.10, 132.92, 166.42. Anal. ($C_{28}H_{32}O_8$) C, H.

1,10-Bis(methoxymethyl)-7,8,15,16-tetraoxadispiro-[5.2.5.2]hexadecane (3j): yield 8%; mp 117−118 °C; ^{1}H NMR 1.10−2.05 (m, 16H), 2.75−3.00 (bs, 2H), 3.32 (s, 6H), 3.15−3.40 (m, 2H), 3.55−3.80 (m, 2H); ^{13}C NMR 22.17, 24.38, 25.84, 29.90, 43.91, 58.84, 70.36, 109.14. Anal. ($C_{16}H_{28}O_6$) C, H.

1,10-Bis(benzyloxymethyl)-7,8,15,16-tetraoxadispiro-[5.2.5.2]hexadecane (3k): yield 10%; mp 131 °C (pentane); ^{1}H NMR 1.20−1.82 (m, 12H), 1.85−2.10 (bs, 4H), 2.70−3.00 (bs, 2H), 3.20−3.55 (bs, 2H), 3.60−3.95 (bs, 2H), 4.30−4.70 (m, 4H), 7.15−7.50 (m, 10H); ^{13}C NMR 22.17, 24.39, 26.02, 29.93, 44.06, 68.22, 73.14, 109.18, 127.52, 127.54, 128.32, 138.35. Anal. ($C_{28}H_{36}O_6$) C, H.

Procedure for Preparation of 3g and 3h. A mixture of diester tetraoxane **3e** or **3i** (1 mmol), dichloromethane (1 mL), MeOH (10 mL), and KOH (6 mmol, 0.35 g) dissolved in water (1 mL) was heated to 70 °C for 1 h. Diacid tetraoxane **3g** was obtained by adding concentrated HCl (1 mL) to the cooled reaction mixture followed by filtration and recrystallization from DMSO. Tetraoxane diol **3h** was obtained by adding water (20 mL) to the cooled reaction mixture followed by filtration and recrystallization from MeOH. Tetraoxanes **3g** and **3h** were isolated as white crystalline solids.

1,10-Bis(carboxymethyl)-7,8,15,16-tetraoxadispiro-[5.2.5.2]hexadecane (3g): yield 72%; mp 198−199 °C dec; ^{1}H NMR (DMSO-d_6) 1.10−1.80 (m, 14H), 1.90−2.25 (m, 4H), 2.00−2.20 (m, 2H), 2.75−3.00 (bs, 2H); ^{13}C NMR (DMSO-d_6, 30 °C) 21.72, 23.89, 27.96, 29.07, 32.79, 39.68, 108.44, 172.88. Anal. ($C_{16}H_{24}O_8$) C, H.

1,10-Bis(hydroxymethyl)-7,8,15,16-tetraoxadispiro-[5.2.5.2]hexadecane (3h): yield 60%; mp 182 °C dec; ^{1}H NMR (DMSO-d_6) 1.05−2.10 (m, 16H), 2.56−2.89 (bs, 2H), 3.05−3.32 (m, 2H), 3.60−3.89 (m, 2H), 4.54 (t, J = 5.3 Hz, 2H); ^{13}C NMR (DMSO-d_6) 21.93, 23.62, 25.13, 29.19, 45.85, 58.34, 108.99. Anal. $C_{14}H_{24}O_6$: C, H.

Antimalarial Screens. In vitro antimalarial activity against the drug-sensitive NF54 and chloroquine-resistant K1 strains of *P. falciparum* was determined by a modified tritiated hypoxanthine uptake method of Desjardins et al.[13] as previously described in detail.[14] Data were expressed as IC_{50} values, estimated as described by Huber and Koella.[15] In vivo antimalarial activity was assessed using Moro SPF mice infected with the ANKA strain of *P. berghei* as described by Ridley et al.[14] Groups of three mice were treated on day 1 post-infection with tetraoxanes dissolved or suspended in 3% ethanol and 7% Tween 80. Tetraoxanes were administered as single 100 mg/kg doses sc and po. Antimalarial activity was measured by percent reduction in parasitemia on day 3 post-infection and by survival times compared to an untreated control group.

Acknowledgment. We gratefully acknowledge the expert technical assistance of Ms. A. Luginbühl with the vitro and vivo antimalarial assays. This investigation received financial support from the UNDP/WORLD BANK/WHO Special Program for Research and Training in Tropical Diseases (TDR ID No. 960275) and DHHS/NIH/NIAID (R15 AI39670-01).

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JM980698F