Journal of Medicinal Chemistry

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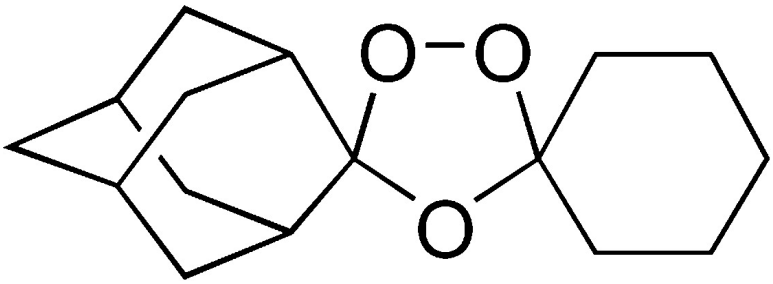
J. Med. Chem., **2005**, 48 (15), 4953-4961• DOI: 10.1021/jm049040u • Publication Date (Web): 25 June 2005



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Spiro and Dispiro-1,2,4-trioxolanes as Antimalarial Peroxides: Charting a Workable Structure−Activity Relationship Using Simple Prototypes

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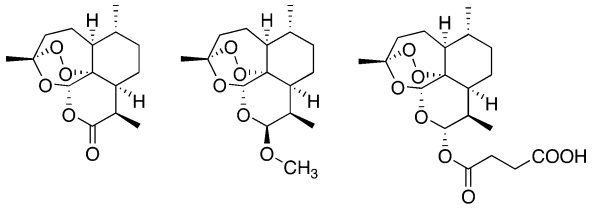
Received November 27, 2004

This paper describes the discovery of synthetic 1,2,4-trioxolane antimalarials and how we established a workable structure−activity relationship in the context of physicochemical, biopharmaceutical, and toxicological profiling. An achiral dispiro-1,2,4-trioxolane (**3**) in which the trioxolane is flanked by a spiroadamantane and spirocyclohexane was rapidly identified as a lead compound. Nonperoxidic 1,3-dioxolane isosteres of **3** were inactive as were trioxolanes without the spiroadamantane. The trioxolanes were substantially less effective in a standard oral suspension formulation compared to a solubilizing formulation and were more active when administered subcutaneously than orally, both of which suggest substantial biopharmaceutical liabilities. Nonetheless, despite their limited oral bioavailability, the more lipophilic trioxolanes generally had better oral activity than their more polar counterparts. In pharmacokinetic experiments, four trioxolanes had high plasma clearance values, suggesting a potential metabolic instability. The toxicological profiles of two trioxolanes were comparable to that of artesunate.

The semisynthetic artemisinins, most notably artemether and artesunate, are important antimalarials because they rapidly reduce parasite burden and have good therapeutic indices.[1] Although these are potent and



rapid-acting antimalarial drugs, they have poor biopharmaceutical properties, and when used alone, they must be administered over a period of 5−7 days, leading to noncompliance and recrudescence.[2] Although many relatively potent synthetic antimalarial peroxides have been prepared, most suffer from low oral activity.[3] It is evident that within a given peroxide chemical family, the more lipophilic members are more active than their more polar counterparts.[3] This poses a challenge to identify peroxide structures with the required "druglike" physicochemical properties[4] to ensure good absorption and bioavailability following oral administration. Although synthetic 1,2-dioxanes, 1,2,4-trioxanes, and 1,2,4,5-tetraoxanes have been relatively well explored,[3] very little is known about the antimalarial properties of 1,2,4-trioxolanes, more commonly known as secondary ozonides, aside from two reports[5] that disclose the in vitro antimalarial activity of several tricyclic di- and trisubstituted trioxolanes, the best of which had an IC_{50} of 2000 ng/mL against *P. falciparum*.

Given these considerations, we were intrigued by the discovery of Griesbaum et al.[6] that tetrasubstituted 1,2,4-trioxolanes (secondary ozonides) could be conveniently obtained by a novel coozonolysis of *O*-alkyl ketone oximes in the presence of carbonyl compounds. From the outset of this work, we considered only tetrasubstituted 1,2,4-trioxolanes as viable targets; since they have no α-H atoms, heterolytic peroxide fragmentation reactions driven by formation of stable carbonyl-containing products are precluded. The process leading to the identification of a dispiro-1,2,4-trioxolane clinical candidate has recently been described.[7] In this paper, we more fully describe the initial discovery of the trioxolanes as a new class of synthetic peroxide antimalarials and how we established a workable structure−activity-relationship (SAR) in the context of physicochemical, biopharmaceutical, and toxicological profiling.

Chemistry

Target trioxolanes **1**−**5** and **8**−**27** were obtained in 15−65% yield using the Griesbaum coozonolysis reac-

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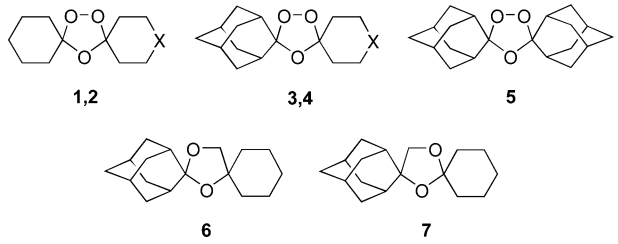
10.1021/jm049040u CCC: $30.25
Published on Web 06/25/2005

Table 1. Activity of Trioxolane Prototypes **1**−**5** and Nonperoxidic Isosteres **6** and **7** against *P. falciparum* in Vitro and *P. berghei* in Vivo



compd	X	IC_{50} (ng/mL) K1[a]	IC_{50} (ng/mL) NF54[a]	activity (%)[b] po/sc	survival[c] (days) po/sc
none				0	5.4
1	CH_2	100 ± 37 (3)	504, 411 (2)	0/0	5.3/5.3
2	C=O	15 ± 2.1 (3)	39, 36 (2)	0/0	5.0/5.0
3	CH_2	0.97 ± 0.68 (5)	1.4 ± 0.9 (4)	>99.99/>99.99	14.3/28
4	C=O	0.48 ± 0.37 (5)	0.73 ± 0.20 (5)	>99.99/>99.99	10.7/30
5		950 (1)	779 (1)	0/0	5.3/5.3
6		>1000 (3)	>1000 (2)	0/0	5.6/5.6
7		>1000 (3)	>1000 (2)	0/0	5.8/5.6
ART[d]		1.6 ± 0.1 (50)	2.8 ± 0.2 (6)	98/>99.99	7.3/14
AM[d]		0.74 ± 0.11 (10)	1.2 ± 0.1 (9)	99.95/>99.99	14.7/14.7

[a] Mean ± SD (*n*) for chloroquine-resistant (K1) and chloroquine-sensitive (NF54) strains of *P. falciparum*. [b] Groups of three *P. berghei*-infected MORO mice were treated 1 day postinfection with trioxolanes (100 mg/kg) dissolved or suspended in 3% ethanol and 7% Tween-80. Antimalarial activity was measured by percent reduction in parasitemia on day 3 postinfection, and survival times were compared to an untreated control group. Individual measurements generally differed by less than 10%. [c] Survival to day 30 postinfection is considered to be a cure. [d] ART = artemisinin. AM = artemether.

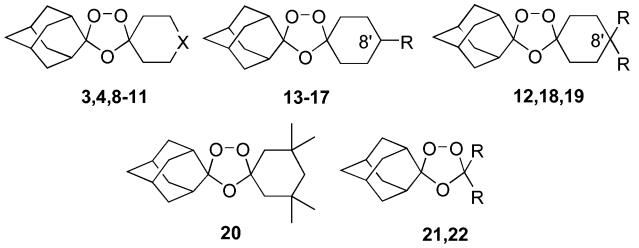
tion.[8] Oxime ether and ketone reaction partners were selected such that the electron-withdrawing groups were present on the latter to increase their dipolarophilicities to the *O*-methyl oxime-derived carbonyl oxides. Except for **8**, the spiroadamantyl trioxolanes were prepared by a common *O*-methyl 2-adamantanone oxime precursor; for the former, because of the poor dipolarophilicity of cyclododecanone,[6] the reaction partners *O*-methyl cyclododecanone oxime and 2-adamantanone were used. Symmetrical oxime ethers such as these preclude the syn−anti isomerism of the resulting carbonyl oxide intermediates and ensure that the stereochemistry of the cycloaddition product trioxolanes is only a function of the starting material ketones. For achiral ketones, the trioxolane products (**1**−**5**, **8**−**12**, **18**, **19**, **21**−**24**) are achiral; for 4-substituted cyclohexanones, two cis and trans achiral diastereomeric trioxolane products (**14**−**17**, **25**) are possible. Trioxolane **17** was assigned a cis configuration based on X-ray crystallographic analysis (Supporting Information). The data revealed a chair cyclohexane with the phenyl and peroxo groups at the equatorial and axial positions, respectively. Assuming that the stereochemistry of the cycloaddition remains unchanged for 4-substituted cyclohexanones,[9] this result lends support to cis assignments for trioxolanes **14**−**16** and **25**. For **14**−**17** and **25**, only the major isomers were separated by flash chromatography or crystallization and individually characterized. For **26** and **27**, oxime ethers of 4-substituted cyclohexanones were employed, and as a consequence, these trioxolanes were isolated as 3:2 and 3:1 mixtures of achiral diastereomers, respectively.[10] As previously described,[9] trioxolane alcohol **13** was obtained as a 1:1 mixture of achiral diastereomers by sodium borohydride reduction of trioxolane ketone **4**. 1,3-Dioxolanes **6** and **7** were obtained by acid-catalyzed reactions of the requisite diols with 2-adamantanone and cyclohexanone, respectively.

Antimalarial Activity

In vitro and in vivo antimalarial activities were measured using the chloroquine-resistant K1 and chloroquine-sensitive NF54 strains of *P. falciparum* and *P. berghei*-infected mice, respectively. In vivo data from the first experiment (Table 1) was determined using 100 mg/kg oral (po) and subcutaneous (sc) doses. First, **1** and **2**, trioxolanes without the sterically bulky spiroadamantane, are 1−2 orders of magnitude less potent than artemisinin in vitro and completely inactive in vivo. Second, **5**, a compound in which the trioxolane is flanked by two spiroadamantanes, is similarly inactive. In light of the iron activation hypothesis for antimalarial peroxides,[11] these data suggest that antimalarial activity falls off when the trioxolane peroxide bond is too exposed (metabolically unstable) or is sterically inaccessible to iron(II) species. A favorable balance of peroxide bond shielding and accessibility is apparently achieved for **3** and **4** in which one side of the trioxolane is sterically hindered but the other allows for an energetically favorable interaction of iron (II) with a relatively sterically unhindered peroxide oxygen atom; these two trioxolanes have activities comparable to those of the artemisinin controls. Third, 1,3-dioxolanes **6** and **7**, nonperoxidic isosteres of **3**, are devoid of antimalarial activity. This clearly demonstrates that the chemical reactivity of the peroxide bond is key to the antimalarial activity **3** and its analogues. From this first set of data it was immediately apparent that 100 mg/kg doses did not adequately differentiate between trioxolanes **3** and **4**, so 10 mg/kg doses were selected for subsequent primary screening (Tables 2 and 3).

When the spirocyclohexane in **3** was replaced with spirododecane in **8**, in vitro potency decreased by an order of magnitude but in vivo activity was little changed (Table 2). The relatively polar achiral heterocyclic analogues of **3** (trioxolane ether **9**, trioxolane carbamate **10**, and trioxolane sulfone **11**) were as potent

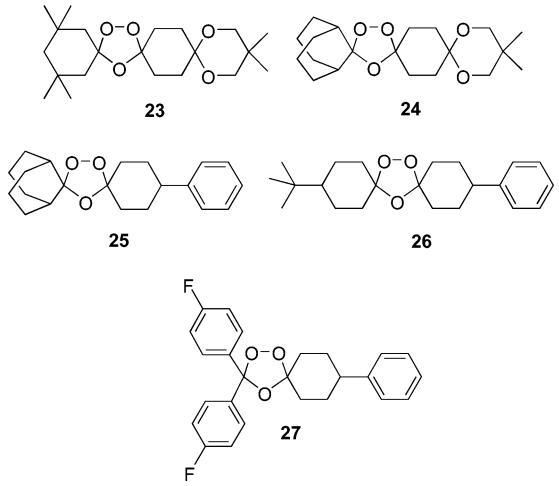
Table 2. Activity of Trioxolanes **3**, **4**, and **8**−**22** against *P. falciparum* in Vitro and *P. berghei* in Vivo



compd	X or R	log *P*/PSA(Å^2)	IC_{50} (ng/mL) K1/NF54[a]	activity (%)[b] po/sc	survival (days) po/sc
none				0	5.4
3	CH_2	6.1[c]/27.7	0.97/1.4	94/>99.99	7.2/11.9
4	C=O	3.8[c]/44.8	0.48/0.73	94/99.7	7.1/8.6
8	$(CH_2)_7$	7.2/27.7	15/20	97/90	7.4/7.0
9	O	2.5/36.9	0.32/0.56	35/99.6	6.0/7.7
10	NCO_2Et	5.3[c]/57.2	0.29/0.57	99.5/99.9	7.7/8.9
11	SO_2	1.8/70.2	1.1/1.2	77/97	7.0/7.3
12	$OCH_2C(CH_3)_2CH_2O$	6.1[c]/46.2	1.5/2.7	99.99/>99.99	11.4/18
13	OH	3.9[c]/47.9	0.25/0.51	79/91	6.7/8.7
14	*n*-propyl	8.1/27.7	1.1/2.1	99.9/99.97	8.4/16.5
15	isopropyl	8.0/27.7	1.4/2.0	99/99.8	7.3/9.0
16	*tert*-butyl	8.3/27.7	63/84	0/0	5.0/5.3
17	phenyl	8.6[c]/27.7	2.2/4.8	99.97/99.92	12.6/17.1
18	methyl	7.5[c]/27.7	2.3/3.5	80/99.97	6.7/11.7
19	phenyl	9.9/27.7	42/60	0/0	5.3/5.7
20		8.6/27.7	3.1/5.0	88/99.9	7.0/9.7
21	phenyl	7.3[c]/27.7	2.3/2.6	68/92	6.0/7.0
22	benzyl	6.2/27.7	3.1/3.0	88/99.7	6.7/8.0
AM[d]		3.3[c]/46.2	0.74/1.2	98/99.78	7.9/9.1
AS[d]		3.5/100.5	1.3/1.6	65/65	6.8/6.8

[a] With the exception of **3**, **4**, artemether (AM), and artesunate (AS), values represent the average of $n = 2$ measurements. [b] Groups of three *P. berghei* infected MORO mice were treated 1 day postinfection with trioxolanes (10 mg/kg) dissolved or suspended in 3% ethanol and 7% Tween-80. Individual measurements generally differed by less than 10%. [c] Experimental log *P* value. [d] AM = artemether. AS = artesunate.

Table 3. Activity of Trioxolanes **23**−**27** against *P. falciparum* in Vitro and *P. berghei* in Vivo



compd	log *P*/PSA (Å^2)	IC_{50} (ng/mL) K1/NF54[a]	activity (%)[b] po/sc	survival (days) po/sc
none			0	5.4
23	8.1/46.2	24/62	0/0	5.7/5.7
24	6.1[c]/46.2	41/49	1/6	5.0/5.3
25	8.1[c]/27.7	>100/>100	0/0	5.0/5.0
26	8.9/27.7	77/62	0/NA	7.3/NA
27	8.4[c]/27.7	44/36	0/0	5.3/5.7

[a] Values represent the average of $n = 2$ measurements. [b] Groups of three *P. berghei* infected MORO mice were treated 1 day postinfection with trioxolanes (10 mg/kg) dissolved or suspended in 3% ethanol and 7% Tween-80. Individual measurements generally differed by less than 10%. [c] Experimental log *P* value.

as the parent, and of these, **10** had superior oral activity in vivo. Trioxolane ketal **12**, a potential prodrug of trioxolane ketone **4**, had especially good oral activity. As expected, **12** undergoes a first-order decay process under acidic conditions to form **4**. No appreciable decomposition of **12** was observed in parallel experiments performed in neutral media (deionized water or isotonic phosphate buffer, pH 7.4) over an 11 h incubation period. The more polar trioxolane alcohol **13**, a reductive metabolite of **4** formed rapidly in the red cell (vide infra), had potent activity in vitro but was much less active than its ketone parent in vivo. For trioxolanes **14**−**16**, in vivo activity declined in proportion to the size of the 8′-alkyl groups, although **15** was no less potent than **14**. Like **14**, the 8′-phenyl analogue **17** had an excellent activity profile, with noteworthy oral activity and survival. In comparison, **18** and **20**, the 8′,8′-dimethyl and 7′,7′,9′,9′-tetramethyl analogues of **3**, were substantially less active. Trioxolane **19**, the 8′,8′-diphenyl analogue of **3**, was without significant antimalarial activity. We suggest that **16** and **19** are inactive for the same reason that **5** is inactive: steric hindrance to electron transfer from iron(II) to the peroxide bond. Diaryl and dibenzyl spiro trioxolanes **21** and **22**, were both quite potent, although only **22** had significant oral activity.

Trioxolanes **23** and **24**, analogues of **12** in which the spiroadamantane was replaced with a tetramethylcyclohexane or spirobicyclo[3.3.1]nonane, were each weakly potent and completely inactive in vivo (Table 3). Trioxolanes **25**−**27**, analogues of **17** in which the spiroadamantane was replaced with a spirobicyclo[3.3.1]nonane, *tert*-butylspirocyclohexyl, and diphenyl, respectively, were similarly inactive. These data confirm that at least one side of the trioxolane heterocycle must be relatively sterically hindered to retain good antimalarial activity

and further demonstrate the unique contribution of the spiroadamantane ring system to the antimalarial activity in these trioxolanes.

From the physicochemical and primary antimalarial screening data in Tables 1−3, we gain a number of other useful insights. First, although trioxolanes with poor potency in vitro also had poor to no in vivo activity, a number of trioxolanes such as **11**, **13**, and **21** with high in vitro potency had poor oral activity in vivo, demonstrating that in vitro data alone are insufficient to form the required SAR to guide compound optimization. Second, more lipophilic trioxolanes generally, but not always (**10**), had better oral activity than their more polar counterparts, an outcome consistent with that seen for other synthetic peroxides.[3] Third, trioxolanes were almost always more active when administered subcutaneously than orally, suggesting substantial biopharmaceutical liabilities (vide infra). Indeed, most of the trioxolanes had log *P* values greater than 5, and even **4**, one of the more polar trioxolanes, had an aqueous solubility in phosphate-buffered saline (pH 7.4) of only 0.05 μg/mL. Fourth, calculated polar surface area (PSA) values of between 16 and 53 Å^2 indicate that the polarity of these trioxolanes will not be a rate-limiting factor for membrane permeability,[12] and it is therefore unlikely to limit oral bioavailability. Fifth, experimental log *P* values determined for 33 (12 in Tables 2 and 3) selected trioxolanes,[13] artemisinin, artemether, and dihydroartemisinin by a rapid and accurate RP-HPLC method[14] suggested that high lipophilicity and the resulting poor aqueous solubility were likely to be limiting factors for oral absorption for many of these trioxolanes.

The physicochemical and antimalarial data described above provided a workable SAR from which to move forward in compound optimization. When a progression criterion of ≥75% activity at a 3 mg/kg oral dose (data not shown) was used, only trioxolanes **12** (98% activity), **14** (88% activity), and **17** (86% activity) would move on to secondary antimalarial screens, metabolism, and pharmacokinetic experiments. However, to establish a broader SAR with this rather small number of trioxolanes in the context of biopharmaceutical and toxicological profiling, we selected **4**, **10**, **12**, and **17**, four trioxolanes with a wider range of physicochemical properties.

In Table 4, the effect of formulation and single vs multiple dose administration for the selected trioxolanes was examined using oral ED_{50}/ED_{90} data. In the first two columns are data from parallel single-dose experiments using two different formulations. The first is 3% ethanol and 7% Tween-80 (T/A), the same formulation used in all of the primary in vivo experiments, and the second is a standard suspending vehicle (SSV) comprising 0.5% w/v carboxymethyl cellulose, 0.5% v/v benzyl alcohol, 0.4% v/v Tween-80, and 0.9% w/v sodium chloride in water. The three trioxolanes tested were substantially less effective in the standard oral suspension formulation SSV compared to the solubilizing formulation T/A. This evidence supports the hypothesis that poor solubility severely restricts oral absorption for these trioxolanes. In contrast, the antimalarial activity for the more polar control antimalarial drugs artemisinin, artemether, and chloroquine seemed to be nearly formulation-independent. Peters et al.[15] similarly observed that the relatively lipophilic synthetic antimalarial peroxides arteflene and fenozan B07, but not artemether, were considerably less active orally using SSV than a solubilizing 10% aqueous DMSO vehicle. In the third column are data from the well-known 4-day suppressive test of Peters.[16] As is evident, the multiple-dose Peters ED_{50}/ED_{90} data nicely parallel the single-dose ED_{50}/ED_{90} data. Onset and recrudescence data (Figure 1) for **4**, **12**, and **17** suggest that, like artemether and artesunate, the trioxolanes are rapidly acting antimalarial agents. Recrudescence (>5% parasitemia) occurred on day 6 for artesunate, on day 7 for artemether and **4**, on day 10 for **12**, and on day 14 for **17**. For **12** and **17**, the two more lipophilic trioxolanes, recrudescence was delayed the longest and was comparable to that of chloroquine and mefloquine.

Table 4. Effect of Formulation and Single vs Multiple Dose Administration Using Oral Effective Dose Experiments

	ED_{50}/ED_{90} ((mg/kg)/day)		
compd	T/A (1 day)[a]	SSV (1 day)[a]	Peters (4 day)[b]
4	3.5/7.0	8.7/12	1.5/6.1
10	1.6/5.5	NA	0.9/2.0
12	1.5/2.8	4.4/6.2	1.8/3.1
17	1.9/3.2	2.9/5.7	1.3/2.6
AS[c]	5.0/13	5.9/21	2.4/13
AM[c]	2.0/4.3	2.7/6.2	3.1/5.0
CQ[c]	3.0/4.2	1.9/4.2	1.7/3.1

[a] $n = 1$ for **10**, **12**, and **17**; mean of $n = 2$ for **4**, artesunate (AS), artemether (AM), and chloroquine (CQ). Individual measurements generally differed by less than 10%. Compounds dissolved or suspended in T/A or SSV are given on day 1 postinfection, and parasitemia is measured on day 3 postinfection. [b] In the 4 day suppressive test (Peters, 1987), starting on the day of infection, compounds dissolved or suspended in water containing Tween-80 (0.2%) or DMSO (10%) are given once daily for 4 consecutive days, and parasitemia is measured on day 4 postinfection. [c] AS = artesunate. AM = artemether. CQ = chloroquine.

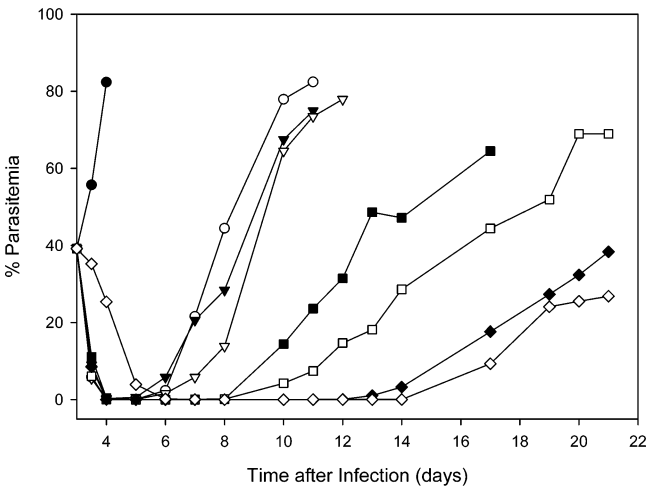


Figure 1. Onset of action and recrudescence after a single oral dose of 100 mg/kg to a group of five mice on day 3 postinfection with *P. berghei*. Parasitemia reduction was monitored initially at 12 h after treatment, and the time of recrudescence was assessed by daily blood smears followed by intermittent assessment for up to 60 days: control (filled circles), artesunate (open circles), artemether (open inverse triangles), chloroquine (filled squares), mefloquine (open diamonds), **4** (filled inverse triangles), **12** (open squares), and **17** (filled diamonds) are shown. Error bars have been omitted from the figure for clarity. The coefficient of variation was generally less than 10%.

Table 5. Pharmacokinetic Parameters after Intravenous and Oral Administration to Rats (Mean ± SD for $n = 3$)

	intravenous administration			oral administration bioavailability (%)
compd	half-life (min)	volume of distribution (L/kg)	plasma clearance ((mL/min)/kg)	
4[a]	117 ± 41	12 ± 6	0 ± 19	nd[c]
10	344 ± 22	23 ± 5	47 ± 11	1.5 ± 0.9
12	155 ± 25	18 ± 8	83 ± 34	0.25, 0.73
17	97 ± 9	5 ± 1	33 ± 9	9.5[d]
DHA[b]	26 ± 2	3 ± 1	72 ± 19	nd[c]
AM[e]	52 ± 6	8 ± 2	114 ± 21	1.4 ± 0.6

[a] Parameters for the reduced metabolite, **13**, following administration of **4**. [b] Dihydroartemisinin (DHA), the lactol of artemisinin and primary metabolite of artesunate. [c] nd = not detected. [d] Only $n = 1$ determination available. [e] AM = artemether

Metabolism and Pharmacokinetics

After intravenous administration, the elimination half-lives of **4** (detected as the reduced metabolite **13**), **10**, **12**, and **17** ranged from 97.3 min for **17** to 343.5 min for **10**; these values were significantly longer than those for the control compounds (26.3 min for dihydroartemisinin and 52.2 min for artemether) (Table 5). Trioxolane ketone **4** was rapidly converted to the reduced metabolite, trioxolane alcohol **13**, which displayed a half-life of approximately 117 min. In the case of **10** and **12**, the long half-lives can most likely be attributed to the high volume of distribution. Although marginally lower than for the comparator compounds, these trioxolanes exhibited high plasma clearance values, suggesting possible high metabolic instability.

Unfortunately, each of the trioxolanes tested displayed limited oral bioavailability in rats, most likely a result of their high lipophilicity and poor aqueous solubility as well as their high plasma clearance. Since the spiroadamantane trioxolane pharmacophore is inherently lipophilic, it is expected that the clearance of these compounds occurs predominantly through hepatic metabolism. Of the compounds tested, **17** had the highest bioavailability at approximately 10% and also had the lowest plasma clearance, supporting the hypothesis that metabolic instability (i.e., high clearance) may have contributed in part to the very low oral bioavailability of **4**, **10**, and **12**.

Toxicology

Screening versions of the Ames test and the in vitro micronucleus test (MNT) did not indicate a genotoxic liability of **13** or **17** (±S9); however, because of its poor solubility, reservations should be applied to the results obtained with **17**. Even though reported clinical neurotoxicity for the semisynthetic artemisinins is very rare,[17] neurotoxicity is a potential concern for antimalarial peroxides of any structural class. Against the NB2a neuroblastoma cell line,[18] trioxolanes **3**, **4**, and **5** had relatively high IC_{50} values of 13, 31, and 44 μM, respectively. In this same screen, dihydroartemisinin ($IC_{50} = 0.22$ μM), the presumed metabolite of all of the semisynthetic artemisinins, showed a high neurotoxic potential.

The toxic liabilities of **10** and **13**, with artesunate as a comparator drug, were investigated in an exploratory tolerance study in male Wistar rats. Trioxolanes **10** and **13** were administered orally in 100 or 300 (mg/kg)/day doses over 5 days in SSV and artesunate was administered orally in 30 and 100 (mg/kg)/day doses over 5 days in SSV. Body weight development was reduced during the treatment period for animals receiving the high dose of **10** or artesunate but was mostly compensated during the recovery period. Clinical laboratory investigations revealed minimal and essentially reversible changes mostly in high-dose-group animals. Liver weights were minimally to slightly increased in animals treated with **10**, **13**, or artesunate. Histopathological examinations indicated slight gastric irritation at the high doses of **10** and artesunate. No evidence of plasma accumulation of the trioxolanes, artesunate, or dihydroartemisinin, the major metabolite of artesunate, was seen. Overall, the toxicological profiles of these two trioxolanes were comparable to that of artesunate.

In summary, we identified spiro- and dispiro-1,2,4-trioxolanes as a new class of synthetic antimalarial peroxides and discovered **3** as a novel antimalarial lead. A trioxolane structure−activity relationship in the context of physicochemical, biopharmaceutical, and toxicological data was established that provides several new avenues for compound optimization as exemplified by **10**, **12**, **14**, and **17**.

Experimental Section

General. Melting points are uncorrected. Using $CDCl_3$ as solvent, ^1H and ^{13}C NMR spectra were recorded on a 300 MHz spectrometer for **1**−**3**, **5**, **8**−**10**, **12**, **14**, **16**, **17**, and **20** and on a 500 MHz spectrometer for the remaining compounds. All chemical shifts are reported in parts per million (ppm) and are relative to internal $(CH_3)_4Si$ (0 ppm) for ^1H and $CDCl_3$ (77.0 ppm) for ^{13}C NMR.

Physicochemical Properties. Calculated values for polar surface area (PSA) and log *P* were obtained using the ACD/Labs Log D suite software, version 7.04 (ACD/Labs, Toronto, Ontario). For calculated log *P* values, the software was trained based on experimentally determined log *P* values for structurally related trioxolanes.

General Procedure for the Preparation of 1,2,4-Trioxolanes. Ozone was produced with an OREC ozone generator (0.6 L/min O_2, 60 V), passed through an empty gas washing bottle that was cooled to −78 °C, and bubbled through a solution of an *O*-methyl ketone oxime[19] and a ketone in pentane or pentane/CH_2Cl_2 at 0 °C. *O*-Methyl oximes of 2-adamantanone, cyclohexanone, 4-phenylcyclohexanone, 4-*tert*-butylcyclohexanone, 3,3,5,5-tetramethylcyclohexanone, and bicyclo[3.3.1]nonan-9-one (1 mmol) were consumed within 3 min while *O*-methyl cyclododecanone oxime (1 mmol) required 6 min to disappear. After completion, the solution was flushed with oxygen for 5 min before being concentrated in vacuo at room temperature to give a residue that was purified by flash chromatography. Although we encountered no difficulties in working with these 1,2,4-trioxolanes (secondary ozonides), routine precautions such as the use of shields, fume hoods, and avoidance of metal salts should be observed whenever possible. Differential scanning calorimetry experiments revealed that these 1,2,4-trioxolanes had good thermal stabilities; decomposition occurred at temperatures greater than 145 °C with enthalpies ranging from 300 to 700 J/g.

7,14,15-Trioxadispiro[5.1.5.2]pentadecane (1). A solution of *O*-methyl cyclohexanone oxime[20] (1.27 g, 10 mmol) and cyclohexanone (1.96 g, 20 mmol) in pentane (100 mL) was treated with ozone according to the general procedure. The crude product was purified by flash chromatography (silica gel, 2% ether in petroleum ether) to afford **1**[6] (1.23 g, 58%) as a colorless oil. ^1H NMR δ 1.20−2.00 (m, 20H); ^{13}C NMR δ 23.80, 24.91, 34.65, 108.84.

3-Oxo-7,14,15-trioxadispiro[5.1.5.2]pentadecane (2). A solution of *O*-methyl cyclohexanone oxime (1.27 g, 10 mmol)

and 1,4-cyclohexanedione (2.24 g, 20 mmol) in pentane (60 mL) and CH_2Cl_2 (40 mL) was treated with ozone according to the general procedure. The crude product was purified by flash chromatography (silica gel, 10% ether in petroleum ether) to afford **2**[6] (0.88 g, 39%) as a colorless solid: mp 52−54 °C (lit.[6] 53 °C); ^{1}H NMR δ 1.30−1.90 (m, 10H), 2.16 (t, J = 7.0 Hz, 4H), 2.53 (t, J = 7.0 Hz, 4H); ^{13}C NMR δ 23.77, 24.81, 32.97, 34.41, 37.78, 106.89, 110.03, 203.07.

Adamantane-2-spiro-3′-1′,2′,4′-trioxaspiro[4.5]decane (3). A solution of *O*-methyl 2-adamantanone oxime (1.79 g, 10 mmol) and cyclohexanone (1.96 g, 20 mmol) in pentane (100 mL) was treated with ozone according to the general procedure. The crude product was purified by flash chromatography (silica gel, 3% ether in petroleum ether) to afford **3**[21] (1.38 g, 52%) as a colorless oil. ^{1}H NMR δ 1.30−2.10 (m, 24H); ^{13}C NMR δ 23.84, 24.97, 26.48, 26.89, 34.73, 34.77, 34.81, 36.40, 36.79, 108.85, 111.15.

Adamantane-2-spiro-3′-1′,2′,4′-trioxolane-5′-spiro-2″-adamantane (5). A solution of *O*-methyl 2-adamantanone oxime[22] (1.80 g, 10 mmol) and 2-adamantanone (3.00 g, 20 mmol) in pentane (200 mL) was treated with ozone according to the general procedure. The crude product was purified by flash chromatography (silica gel, 2% ether in petroleum ether) to afford **5**[21] (1.38 g, 40%) as a colorless solid: mp 150 °C dec (lit.[21] 140−144 °C dec); ^{1}H NMR δ 1.50−2.20 (m, 28H); ^{13}C NMR δ 26.52, 26.97, 34.70, 34.95, 36.58, 36.81, 111.19.

Adamantane-2-spiro-2′-1′,3′-dioxaspiro[4.5]decane (6). *p*-Toluenesulfonic acid monohydrate (0.04 g, 0.21 mmol) was added to a mixture of 1-hydroxymethylcyclohexanol[23] (0.20 g, 1.5 mmol), 2-adamantanone (0.30 g, 2.0 mmol), and CH_2Cl_2 (15 mL). The reaction mixture was stirred at room temperature overnight, washed with saturated aqueous $NaHCO_3$ (15 mL), water (15 mL), and brine (15 mL), dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by flash chromatography (silica gel, 10% EtOAc in hexane) to afford **6** as a colorless oil (0.30 g, 75%). ^{1}H NMR δ 1.22−1.80 (m, 20H), 1.96 (d, J = 12.2 Hz, 2H), 2.03 (d, J = 11.7 Hz, 2H), 3.73 (s, 2H); ^{13}C NMR δ 23.8, 25.5, 26.8, 27.1, 34.88, 34.91, 37.1, 37.3, 38.2, 73.3, 80.2, 111.4. HRMS-FAB for $C_{17}H_{26}O_2$ $[M]^+$.

Adamantane-2-spiro-2′-1′,4′-dioxaspiro[4.5]decane (7). *p*-Toluenesulfonic acid monohydrate (0.04 g, 0.21 mmol) was added to a mixture of 2-hydroxymethyl-2-adamantanol[24] (0.20 g, 1.1 mmol), cyclohexanone (0.22 g, 2.2 mmol), and CH_2Cl_2 (20 mL). The reaction mixture was stirred at room temperature overnight, washed with saturated aqueous $NaHCO_3$ (20 mL), water (20 mL), and brine (20 mL), dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by flash chromatography (silica gel, 10% EtOAc in hexane) to afford **7** as a colorless oil (0.27 g, 96%). ^{1}H NMR δ 1.34−1.41 (m, 2H), 1.54−1.64 (m, 12 H), 1.68−1.71 (m, 2H), 1.74−1.82 (m, 6H), 2.20 (brd, J = 12.2 Hz, 2H), 3.86 (s, 2H); ^{13}C NMR δ 24.1, 25.3, 26.8, 27.0, 33.6, 35.9, 37.2, 37.40, 37.45, 72.2, 84.2, 109.0. HRMS-FAB for $C_{17}H_{26}O_2$ $[M]^+$.

Adamantane-2-spiro-3′-1′,2′,4′-trioxaspiro[4.11]hexadecane (8). A solution of *O*-methyl cyclododecanone oxime[25] (2.11 g, 10 mmol) and 2-adamantanone (3.0 g, 20 mmol) in pentane (90 mL) and CH_2Cl_2 (10 mL) was treated with ozone according to the general procedure. The crude product was purified by flash chromatography (silica gel, 2% ether in petroleum ether) to afford **8** (1.88 g, 54%) as a colorless solid: mp 73−75 °C (ethanol/H_2O 3:1); ^{1}H NMR δ 1.18−1.60 (m, 18H), 1.62−2.10 (m, 18H); ^{13}C NMR δ 20.07, 22.05, 22.37, 25.81, 26.07, 26.49, 26.88, 31.37, 34.76, 34.86, 36.38, 36.79, 111.33, 112.59. Anal. ($C_{22}H_{36}O_3$) C, H.

Adamantane-2-spiro-3′-1′,2′,4′,8′-tetraoxaspiro[4.5]decane (9). A solution of *O*-methyl 2-adamantanone oxime (0.90 g, 5 mmol) and tetrahydro-4*H*-pyran-4-one (1.00 g, 10 mmol) in pentane (100 mL) was treated with ozone according to the general procedure. The crude product was purified by flash chromatography (silica gel, 2−10% ether in petroleum ether) to afford **9** (0.87 g, 65%) as a colorless oil. ^{1}H NMR δ 1.20−2.30 (m, 18H), 3.50−4.10 (m, 4H); ^{13}C NMR δ 26.33, 26.73, 34.60, 34.68, 35.43, 36.30, 36.60, 65.67, 105.91, 111.76. Anal. ($C_{15}H_{22}O_4$) C, H.

Adamantane-2-spiro-3′-8′-ethoxycarbonyl-1′,2′,4′-trioxa-8′-azaspiro[4.5]decane (10). A solution of *O*-methyl 2-adamantanone oxime (0.90 g, 5 mmol) and 1-ethoxycarbonyl-4-piperidone (1.71 g, 10 mmol) in pentane (80 mL) and CH_2Cl_2 (20 mL) was treated with ozone according to the general procedure. The crude product was purified by flash chromatography (silica gel, 10−20% ether in petroleum ether) to afford **10** (0.43 g, 26%) as a colorless solid: mp 44−46 °C (ethanol/H_2O 5:2); ^{1}H NMR δ 1.27 (t, J = 7.0 Hz, 3H), 1.60−2.10 (m, 18H), 3.40−3.75 (m, 4H), 4.14 (q, J = 7.1 Hz, 2H); ^{13}C NMR δ 14.66, 26.40, 26.79, 34.35, 34.71, 34.79, 36.35, 36.68, 41.69, 61.42, 106.88, 112.06, 155.33. Anal. ($C_{18}H_{27}NO_5$) C, H, N.

Adamantane-2-spiro-3′-1′,2′,4′-trioxa-8′-thiaspiro[4.5]-decane 8′,8′-dioxide (11). A solution of *O*-methyl 2-adamantanone oxime (0.90 g, 5 mmol) and 1,1-dioxotetrahydrothiopyran-4-one[26] (0.74 g, 5 mmol) in pentane (25 mL) and CH_2Cl_2 (50 mL) was treated with ozone according to the general procedure. The crude product was purified by flash chromatography (silica gel, 50% ether in hexanes) to afford **11** (0.23 g, 15%) as a colorless solid: mp 128−129 °C (ethanol/H_2O 1:1); ^{1}H NMR δ 1.60−2.05 (m, 14H), 2.38 (t, J = 6.3 Hz, 4H), 3.10−3.30 (m, 4H); ^{13}C NMR δ 26.36, 26.76, 32.31, 34.74, 34.84, 36.31, 36.59, 48.81, 104.97, 113.33. Anal. ($C_{15}H_{22}O_5S$) C, H, S.

Adamantane-2-spiro-3′-11′,11′-dimethyl-1′,2′,4′,9′,13′-pentaoxadispiro[4.2.5.2]pentadecane (12). A solution of *O*-methyl 2-adamantanone oxime (1.80 g, 10 mmol) and 3,3-dimethyl-1,5-dioxaspiro[5.5]undecan-9-one (1.98 g, 10 mmol) in pentane (100 mL) was treated with ozone according to the general procedure. The crude product was purified by flash chromatography (silica gel, 4% ether in petroleum ether) to afford **12** (1.43 g, 39%) as a colorless solid: mp 123−125 °C (ethanol); ^{1}H NMR 0.99 (s, 6H), 1.61−2.14 (m, 22H), 3.51 (s, 4H); ^{13}C NMR δ 22.66, 26.43, 26.84, 29.41, 30.16, 30.46, 34.73, 34.82, 36.30, 36.75, 70.24, 70.19, 96.67, 108.47, 111.51. Anal. ($C_{21}H_{32}O_5$) C, H.

Adamantane-2-spiro-3′-8′-propyl-1′,2′,4′-trioxaspiro-[4.5]decane (14). A solution of *O*-methyl 2-adamantanone oxime (0.90 g, 5 mmol) and 4-propylcyclohexanone (1.40 g, 10 mmol) in pentane (100 mL) was treated with ozone according to the general procedure. The crude product was purified by flash chromatography (silica gel, 2% ether in petroleum ether) to afford **14** (0.89 g, 58%) as a colorless solid: mp 49−51 °C (ethanol/H_2O 2:1); ^{1}H NMR δ 0.88 (t, J = 7.2 Hz, 3H), 1.05−1.45 (m, 7H), 1.50−2.10 (m, 20H); ^{13}C NMR δ 14.31, 20.18, 26.49, 26.89, 30.12, 34.29, 34.78, 35.83, 36.39, 36.82, 38.52, 109.15, 111.07. Anal. ($C_{19}H_{30}O_3$) C, H.

Adamantane-2-spiro-3′-8′-isopropyl-1′,2′,4′-trioxaspiro-[4.5]decane (15). A solution of *O*-methyl 2-adamantanone oxime (0.90 g, 5 mmol) and 4-isopropylcyclohexanone (1.40 g, 10 mmol) in pentane (100 mL) was treated with ozone according to the general procedure. The crude product was purified by flash chromatography (silica gel, 2% ether in petroleum ether) to afford **15** (0.47 g, 31%) as a colorless solid: mp 67−69 °C (ethanol); ^{1}H NMR δ 0.85 (d, J = 6.8 Hz, 6H), 1.02−1.13 (m, 1H), 1.17−1.32 (m, 2H), 1.40−1.52 (m, 1H), 1.60−2.10 (m, 20H); ^{13}C NMR δ 19.82, 26.54, 26.85, 26.94, 32.12, 34.54, 34.81, 34.83, 36.44, 36.87, 42.57, 109.11, 111.10. Anal. ($C_{19}H_{30}O_3$) C, H.

Adamantane-2-spiro-3′-8′-*tert*-butyl-1′,2′,4′-trioxaspiro-[4.5]decane (16). A solution of *O*-methyl 2-adamantanone oxime (1.80 g, 10 mmol) and 4-*tert*-butylcyclohexanone (3.09 g, 20 mmol) in pentane (100 mL) was treated with ozone according to the general procedure. The crude product was purified by flash chromatography (silica gel, 2% ether in petroleum ether) to afford **16** (1.68 g, 52%) as a colorless solid: mp 123−124 °C (ethanol); ^{1}H NMR δ 0.84 (s, 9H), 0.89−1.10 (m, 1H), 1.14−1.35 (m, 2H), 1.55−1.85 (m, 12H), 1.86−2.10 (m, 8H); ^{13}C NMR δ 24.71, 26.49, 26.89, 27.57, 32.27, 34.79, 36.38, 36.82, 46.66, 108.95, 111.12. Anal. ($C_{20}H_{32}O_3$) C, H.

Adamantane-2-spiro-3′-8′-phenyl-1′,2′,4′-trioxaspiro-[4.5]decane (17). A solution of *O*-methyl 2-adamantanone oxime (0.90 g, 5 mmol) and 4-phenylcyclohexanone (1.74 g, 10 mmol) in pentane (80 mL) and CH_2Cl_2 (20 mL) was treated

with ozone according to the general procedure. The crude product was purified by flash chromatography (silica gel, 5% ether in petroleum ether) to afford **17** (0.83 g, 49%) as a colorless solid: mp 103−105 °C (ethanol/H_2O 2:1); ^{1}H NMR δ 1.55−2.20 (m, 22H), 2.45−2.65 (m, 1H), 7.10−7.40 (m, 5H); ^{13}C NMR δ 26.47, 26.87, 31.42, 34.58, 34.72, 34.79, 36.39, 36.79, 42.93, 108.39, 111.37, 126.14, 126.75, 128.37, 146.14. Anal. ($C_{22}H_{28}O_3$) C, H.

Adamantane-2-spiro-3′-8′,8′-dimethyl-1′,2′,4′-trioxaspiro-[4.5]decane (18). A solution of *O*-methyl 2-adamantanone oxime (0.90 g, 5 mmol) and 4,4-dimethylcyclohexanone[27] (1.26 g, 10 mmol) in pentane (100 mL) was treated with ozone according to the general procedure. The crude product was purified by flash chromatography (silica gel, 2% ether in petroleum ether) to afford **18** (0.72 g, 49%) as a colorless solid: mp 125−127 °C (ethanol/H_2O 3:1); ^{1}H NMR δ 0.92 (s, 3H), 0.95 (s, 3H), 1.42 (t, J = 6.4 Hz, 4H), 1.62−2.10 (m, 18H); ^{13}C NMR δ 26.46, 26.72 (br), 26.87, 28.87 (br), 29.41, 30.80, 34.75, 34.83, 36.37, 36.52, 36.79, 109.07, 111.19. Anal. ($C_{18}H_{28}O_3$) C, H.

Adamantane-2-spiro-3′-8′,8′-diphenyl-1′,2′,4′-trioxaspiro-[4.5]decane (19). A solution of *O*-methyl 2-adamantanone oxime (0.90 g, 5 mmol) and 4,4-diphenylcyclohexanone[28] (1.25 g, 5 mmol) in pentane (60 mL) and CH_2Cl_2 (40 mL) was treated with ozone according to the general procedure. The crude product was purified by flash chromatography (silica gel, 5% ether in petroleum ether) to afford **19** (0.48 g, 23%) as a colorless solid: mp 155−157 °C (ethanol); ^{1}H NMR δ 1.40−2.20 (m, 18H), 2.32−2.65 (m, 4H), 7.00−7.42 (m, 10H); ^{13}C NMR δ 26.52, 26.91, 31.51, 34.05, 34.79, 34.87, 36.45, 36.83, 45.47, 108.66, 111.46, 125.79, 125.88, 126.72, 127.17, 128.30, 128.46, 145.94, 147.63. Anal. ($C_{28}H_{32}O_3$) C, H.

Adamantane-2-spiro-3′-7′,9′-tetramethyl-1′,2′,4′-trioxa-spiro[4.5]decane (20). A solution of *O*-methyl 2-adamantanone oxime (0.90 g, 5 mmol) and 3,3,5,5-tetramethylcyclohexanone (1.54 g, 10 mmol) in pentane (100 mL) was treated with ozone according to the general procedure. The crude product was purified by flash chromatography (silica gel, 2% ether in petroleum ether) to afford **20** (0.77 g, 48%) as a colorless solid: mp 71−72 °C (ethanol/H_2O 1:1); ^{1}H NMR δ 1.03 (s, 6H), 1.07 (s, 6H), 1.24 (s, 1H), 1.25 (s, 1H), 1.59 (s, 4H), 1.61−2.10 (m, 14H); ^{13}C NMR δ 26.50, 26.91, 31.47, 31.69, 32.36, 34.77, 34.92, 36.38, 36.83, 45.70, 51.46, 110.26, 110.96. Anal. ($C_{20}H_{32}O_3$) C, H.

Adamantane-2-spiro-3′-5′,5′-diphenyl-1′,2′,4′-trioxo-lane (21). A solution of *O*-methyl 2-adamantanone oxime (0.90 g, 5 mmol) and benzophenone (0.91 g, 5 mmol) in pentane (90 mL) and CH_2Cl_2 (10 mL) was treated with ozone according to the general procedure. The crude product was purified by flash chromatography (silica gel, 2% ether in petroleum ether) to afford **21** (0.55 g, 32%) as a colorless solid: mp 105−107 °C (ethanol/H_2O 2:1); ^{1}H NMR δ 1.60−2.10 (m, 12H), 2.16−2.30 (m, 2H), 7.25−7.42 (m, 6H), 7.45−7.60 (m, 4H); ^{13}C NMR δ 26.56, 26.98, 34.86, 35.07, 36.21, 36.88, 109.68, 113.92, 126.97, 128.05, 128.56, 140.06. Anal. ($C_{23}H_{24}O_3$) C, H.

Adamantane-2-spiro-3′-5′,5′-dibenzyl-1′,2′,4′-trioxo-lane (22). A solution of *O*-methyl 2-adamantanone oxime (0.90 g, 5 mmol) and 1,3-diphenylacetone (1.10 g, 5 mmol) in pentane (60 mL) was treated with ozone according to the general procedure. The crude product was purified by flash chromatography (silica gel, 1% ether in hexanes) to afford **22** (1.10 g, 58%) as a colorless solid: mp 86−88 °C (ethanol/H_2O 1:1); ^{1}H NMR δ 1.40−2.10 (m, 14H), 2.93 (d, J = 4.2 Hz, 2H), 3.04 (d, J = 4.2 Hz, 2H), 7.10−7.40 (m, 10H); ^{13}C NMR δ 26.49, 26.93, 34.81, 34.90, 36.13, 36.80, 41.92, 110.37, 112.48, 126.58, 127.89, 130.89, 135.70. Anal. ($C_{25}H_{28}O_3$) C, H.

2,2,4,4,14,14-Hexamethyl-7,12,16,19,20-pentaoxatrispiro-[5.1.2.5.2.2]icosane (23). A solution of *O*-methyl 3,3,5,5-tetramethylcyclohexanone oxime[13] (0.92 g, 5 mmol) and 3,3-dimethyl-1,5-dioxaspiro[5.5]undecan-9-one (1.98 g, 10 mmol) in pentane (100 mL) was treated with ozone according to the general procedure. The crude product was purified by flash chromatography (silica gel, 4% ether in petroleum ether) to afford **23** (0.70 g, 38%) as a colorless solid: mp 95−97 °C (ethanol); ^{1}H NMR δ 0.97 (s, 6H), 1.03 (s, 6H), 1.04 (s, 6H), 1.20−1.29 (m, 2H), 1.55 (d, J = 3.2 Hz, 2H), 1.63 (d, J = 3.7 Hz, 2H), 1.83 (t, J = 6.4 Hz, 4H), 1.86−2.04 (m, 4H), 3.50 (s, 4H); ^{13}C NMR δ 22.68, 29.47, 30.20, 30.40, 30.91, 32.21, 32.30, 45.59, 51.43, 70.29, 96.70, 107.94, 110.58. Anal. ($C_{21}H_{36}O_5$) C, H.

Bicyclo[3.3.1]nonane-9-spiro-3′-11′,11′-dimethyl-1′,2′,4′,9′,13′-pentaoxadispiro[4.2.5.2]pentadecane (24). A solution of *O*-methyl bicyclo[3.3.1]nonan-9-one oxime[13] (0.84 g, 5 mmol) and 3,3-dimethyl-1,5-dioxaspiro[5.5]undecan-9-one (0.99 g, 5 mmol) in pentane (100 mL) was treated with ozone according to the general procedure. The crude product was purified by flash chromatography (silica gel, 5% ether in petroleum ether) to afford **24** (0.72 g, 41%) as a colorless solid: mp 122−124 °C (ethanol/H_2O 5:1); ^{1}H NMR δ 0.97 (s, 6H), 1.40−1.56 (m, 2H), 1.62−2.16 (m, 20H), 3.49 (s, 4H); ^{13}C NMR δ 20.48, 20.91, 22.71, 29.40, 29.54, 29.73, 30.21, 30.64, 36.34, 70.30, 70.33, 96.76, 108.46, 111.50. Anal. ($C_{20}H_{32}O_5$) C, H.

Bicyclo[3.3.1]nonane-9-spiro-3′-8′-phenyl-1′,2′,4′-trioxa-spiro[4.5]decane (25). A solution of *O*-methyl bicyclo[3.3.1]-nonan-9-one oxime (0.84 g, 5 mmol) and 4-phenylcyclohexanone (0.87 g, 5 mmol) in pentane (80 mL) and CH_2Cl_2 (20 mL) was treated with ozone according to the general procedure. The crude product was purified by flash chromatography (silica gel, 3% ether in petroleum ether) to afford **25** (0.54 g, 33%) as a colorless solid: mp 120−122 °C (ethanol/H_2O 4:1); ^{1}H NMR δ 1.41−1.60 (m, 2H), 1.61−2.20 (m, 20H), 2.49−2.60 (m, 1H), 7.14−7.35 (m, 5H); ^{13}C NMR δ 20.51, 20.93, 29.47, 29.68, 31.49, 34.88, 36.44, 43.05, 108.32, 111.35, 126.16, 126.78, 128.40, 146.22. Anal. ($C_{21}H_{28}O_3$) C, H.

3-*tert*-Butyl-11-phenyl-7,14,15-trioxadispiro[5.1.5.2]pentadecane (26). A solution of *O*-methyl 4-*tert*-butylcyclohexanone oxime[22] (1.83 g, 10 mmol) and 4-phenylcyclohexanone (1.74 g, 10 mmol) in hexanes (100 mL) and CH_2Cl_2 (100 mL) was treated with ozone according to the general procedure. The crude product was purified by flash chromatography (silica gel, 10% EtOAc in hexanes) and further triturated with ethanol to afford **26** (0.60 g, 17%, 3:2 mixture of two diastereomers) as a colorless solid: mp 110−115 °C dec;^{1}H NMR δ 0.86 (s, 5.4H), 0.88 (s, 3.6H), 0.90−1.08 (m, 1H), 1.19−1.41 (m, 2H), 1.51−2.12 (m, 14H), 2.48−2.57 (m, 1H), 7.15−7.32 (m, 5H); ^{13}C NMR δ 24.54, 24.69, 27.56, 27.69, 31.37, 31.42, 32.26, 32.31, 34.55, 34.57, 34.60, 34.65, 42.86, 42.92, 46.63, 47.26, 108.12, 108.61, 108.90, 108.92, 126.13, 126.16, 126.73, 126.76, 128.36, 128.38, 146.04, 146.11. Anal. ($C_{22}H_{32}O_3$) C, H.

3,3-Bis(4-fluorophenyl)-8-phenyl-1,2,4-trioxaspiro[4.5]-decane (27). A solution of *O*-methyl 4-phenylcyclohexanone oxime[13] (1.02 g, 5 mmol) and 4,4′-difluorobenzophenone (1.09 g, 5 mmol) in pentane (90 mL) and CH_2Cl_2 (10 mL) was treated with ozone according to the general procedure. The crude product was purified by flash chromatography (silica gel, 3% ether in petroleum ether) to afford **27** (0.56 g, 27%, 3:1 mixture of two diastereomers) as a colorless solid: mp 87−90 °C (ethanol/H_2O 2.5:1); ^{1}H NMR δ 1.60−2.15 (m, 8H), 2.51−2.70 (m, 1H), 6.99−7.09 (m, 4H), 7.16−7.36 (m, 5H), 7.44−7.53 (m, 4H); ^{13}C NMR δ 31.23, 31.34, 34.20, 34.48, 42.85, 43.01, 108.84, 109.29, 111.20, 111.29, 115.15 (d, J = 21.4 Hz), 115.18 (d, J = 21.4 Hz), 126.29, 126.33, 126.74, 126.83, 128.47, 128.95 (d, J = 8.4 Hz), 129.01 (d, J = 6.1 Hz), 135.33, 135.36, 145.74, 145.79, 163.05 (d, J = 248.0 Hz). Anal. ($C_{25}H_{22}F_2O_3$) C, H.

Antimalarial Screens. In vitro and in vivo antimalarial data were obtained as previously described.[7,29,30]

Neurotoxicity Screen. As described by Fishwick et al.,[18] in vitro neurotoxicity was assessed using NB2a neuroblastoma cells.

Ames and in Vitro Micronucleus Test (MNT). Five *Salmonella typhimurium* tester strains (TA1535, TA97, TA98, TA100, and TA102) were employed in a microsuspension version of the Ames assay.[31] Exponentially growing L 5178Y tk+/− mouse lymphoma cells were used in the in vitro MNT test.[32] Both tests were performed in the absence or in the presence of an exogenous metabolic activation system (S9)

derived from the livers of phenobarbital/5,6-benzoflavone treated male Sprague Dawley rats.

Exploratory Toxicity Study in Rats. All compounds were suspended in a standardized suspending vehicle (SSV) and administered at a constant volume of 5 (mL/kg)/day. Control animals received the vehicle (SSV) at a volume of 5 (mL/kg)/day. Six animals per group were treated for 5 consecutive days and 6 animals per group were kept for an additional 1 week recovery period. Examinations included clinical observations, body weight development, and clinical laboratory investigations (hematology, clinical chemistry, and urine analysis) at the end of the treatment and recovery periods. At the end of the scheduled study period, the rats were sacrificed and necropsied and selected organs were examined histopathologically. Plasma levels were analyzed using validated HPLC/MS assays, and the data were examined for evidence of drug accumulation over the course of the study.

Pharmacokinetic Studies. Pharmacokinetic experiments in rats were carried out as previously described.[7] All animal studies were conducted in accordance with the National Institutes of Health "Guidelines for the Care and Use of Laboratory Animals" and were approved by the institutional animal experimentation ethics committee. On the day prior to dosing, surgical anesthesia was initiated and maintained by inhaled isofluorane. Jugular vein and carotid artery cannulations were performed with polyethylene (PE) tubing (o.d. 0.96 mm, i.d. 0.58 mm) to allow iv dosing and blood sampling, respectively. Animals were fasted overnight, and water was available ad libitum. On the following day, compounds were administered to conscious, free-moving rats ($n = 3$ per group) either by a 5 min infusion of a 1 mL solution in aqueous 0.1 M Captisol into the jugular vein or by oral gavage of a 1 mL suspension in carboxymethylcellulose (0.5% w/v), polysorbate 80 (0.4% v/v), benzyl alcohol (0.5% v/v), and sodium chloride (0.9% w/v). Intravenous doses were 10 mg/kg for **10**, **17**, and AM, 20 mg/kg for **4** and DHA, and 30 mg/kg for **12**, and oral doses were 30 mg/kg for **17**, 50 mg/kg for **10** and AM, and 80 mg/kg for **12**. Blood samples (250 μL) were collected into heparinized tubes periodically over 7 h postdosing via the carotid cannula, and plasma was collected following centrifugation. Plasma samples were stored at −20 °C until analysis within approximately 2 weeks. Samples were analyzed using validated LC/MS methods.

Acknowledgment. This investigation received financial support from the UNDP/WORLD BANK/WHO Special Programme for Research and Training in Tropical Diseases (TDR ID No. 960275) and Medicines for Malaria Venture (MMV). We thank Geoffrey Edwards of the University of Liverpool and Stephan Kirchner and Wolfgang Muster of F. Hoffmann-La Roche Ltd. for the in vitro neurotoxicity and Ames/MNT data, respectively. We also thank W. Peters of the International Institute of Parasitology for determining the effective dose data shown in the third column of Table 4. We acknowledge the Nebraska Center for Mass Spectrometry for the HRMS data.

Supporting Information Available: Elemental analysis and HRMS data for **6**−**12** and **14**−**27**, and X-ray structural data for **17**. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM049040U