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Weak base dispiro-1,2,4-trioxolanes: Potent antimalarial ozonides

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Abstract—Thirty weak base 1,2,4-dispiro trioxolanes (secondary ozonides) were synthesized. Amino amide trioxolanes had the best combination of antimalarial and biopharmaceutical properties. Guanidine, aminoxy, and amino acid trioxolanes had poor antimalarial activity. Lipophilic trioxolanes were less stable metabolically than their more polar counterparts.

The discovery of artemisinin (ART)[1] and its semisynthetic derivatives dihydroartemisinin (DHA), artemether (AM) and artesunate (AS) (Fig. 1) triggered[2] the search for superior semisynthetic artemisinins[3] and synthetic peroxide antimalarials.[4]

The first attempts to improve synthetic peroxide[5] and semisynthetic artemisinin[6] antimalarial specificity and biopharmaceutical properties by incorporating weak base functional groups and heterocycles were largely unsuccessful. Since that time, however, continued work[7–12] in this area has produced some encouraging results as illustrated by synthetic peroxides **1** (OZ209)[13] and **2** (trioxaquine),[14] and semisynthetic artemisinin **3** (artemisone)[15] (Fig. 2). In this paper, we describe the synthesis[16] and antimalarial properties of thirty 1,2,4-dispiro trioxolanes (secondary ozonides) containing azole heterocycles and aliphatic and aromatic amine functional groups. Metabolism and pharmacokinetic

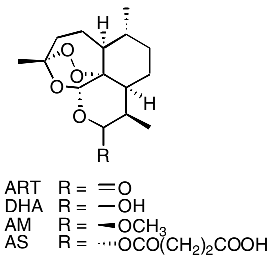
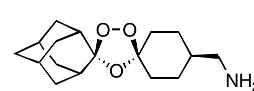


Figure 1. Artemisinin and its semisynthetic derivatives.



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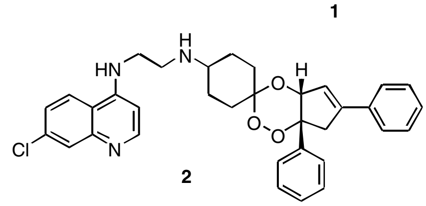

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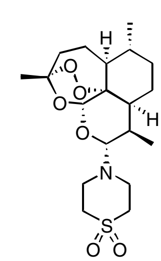

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Figure 2. Weak base antimalarial peroxides.

Keywords: 1,2,4-Trioxolanes; Secondary ozonides; Antimalarial; Peroxide; Artemisinin.
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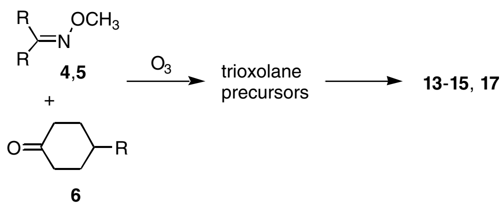


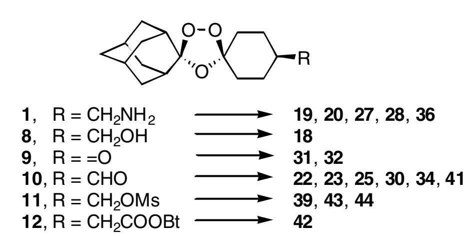
Scheme 1. Trioxolane synthesis by Griesbaum coozonolysis and post-ozonolysis transformations.

data are presented for selected trioxolanes. Our aim was to identify a potent weak base analog of **1** with high oral activity, good biopharmaceutical properties, and low toxicity.

Trioxolanes **13**, **14**, **15**, and **17** were obtained by post-ozonolysis transformations of their precursor trioxolane esters and phthalimides; the latter were obtained by Griesbaum coozonolysis[17,18] reactions between the *O*-methyl oximes of 2-adamantanone (**4**) or 4-*tert*-butyl cyclohexanone (**5**) (for **13**) and the appropriate 4-substituted cyclohexanones **6** (Scheme 1). Symmetrical oxime ethers such as **4** preclude the *syn-anti* isomerism of the resulting carbonyl oxide intermediates, and ensure that the stereochemistry of the cycloaddition is only a function of the starting material ketones. For 4-substituted cyclohexanones, the major trioxolane isomers are uniformly *cis* with the substituent and peroxo groups at the equatorial and axial positions, respectively.[19,20] With the exception of **31**, each of the trioxolanes reported herein is single *cis* isomer. Indeed, X-ray crystallographic analysis[21] of **7**,[19] the phthalimide precursor of **14**, reveals that it has a *cis* configuration (Fig. 3).

Alcohol **17** (96%) was obtained by lithium borohydride/lithium triethylborohydride[22] reduction of its precursor methyl ester (*cis*). Conversion of **17** to its mesylate, followed by azide formation and triphenyl phosphine reductions afforded amine **15** (52% overall). Azole **45** (61%) was formed by treatment of the mesylate derivative of **17** with imidazole/NaH. Amines **13** (65%) and **14** (69%) were obtained by hydrazinolysis of their precursor phthalimides. Unlike amine **15**, amines **1** and **14** were unstable as hydrochloride salts, but were quite stable as mesylate or tosylate salts.

Alkylation of **1** with 2-bromoacetamide and 2-chloropyrimidine in the presence of K_2CO_3 afforded **28** (44%) and **36** (20%) (Scheme 2). Carbamate **19** (63%) and guanidine **20** (56%) were obtained by treatment of **1** with ethyl chloroformate/Et_3N and 1*H*-pyrazole-1-carboxamidine hydrochloride/Et_3N. Amine **27** (55%) was obtained by reductive amination of formaldehyde with amine **1**. Aminoxy trioxolane **18** (69% overall) was obtained by Mitsunobu reaction of alcohol **8** with *N*-hydroxyphthalimide followed by hydrazine deprotection. Reductive amination reactions were used to obtain amines **32** (74%) and **31** (45%) (1:1 mixture of *cis* and *trans* achiral diastereomers) from ketone **9** and amines **22** (36%), **23** (39%),**25** (46%), **30** (61%), and **34** (49%) from aldehyde **10** (Scheme 2). Imidazole **41** (70%) was obtained from **10** by treatment with 40% aq glyoxal followed by 7 N methanolic ammonia. Reaction of mesylate **11** with the anions of pyrazole and methyl 4-imidazole carboxylate afforded **39** (81%) and the isomeric imidazole ester precursors of **43** (20%) and **44** (47%). Successive treatment of these esters with 15% KOH, BOC anhydride, and 7 N methanolic ammonia afforded imidazole amides **43** (11% overall) and **44** (27% overall). Tetrazole **42** (69% overall) was obtained by successive treatment of active ester **12** with 3-aminopropionitrile, trimethylsilyl azide/TPP/DIAD, sodium bicarbonate, and 1 M HCl according to the method of Johansson et al.[23] Trioxolanes **1**, **7–12**, **16**, **21**, **24**, **26**, **29**, **33**, **35**, **37**, **38**, and **40** were obtained as previously described.[19,20]



Scheme 2. Trioxolane synthesis via post-ozonolysis transformations.

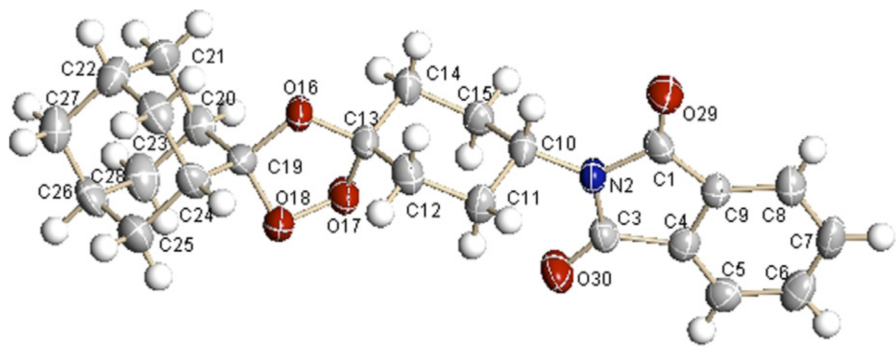


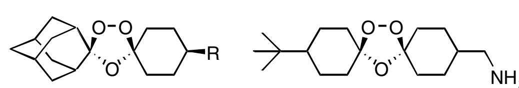
Figure 3. Ellipsoid plot of **7** showing the atom numbering used for the X-ray crystallographic report. The phthalimide and epoxide substituents are in equatorial positions on the cyclohexane ring, and the peroxide substituent is in the axial position. Displacement ellipsoids are shown at the 50% probability level.

In vitro and in vivo antimalarial activities[13] were measured using the chloroquine-resistant K1 and chloroquine-sensitive NF54 strains of *Plasmodium falciparum*, and *Plasmodium berghei*-infected mice, respectively. In vivo data were determined using single 10 mg/kg oral doses of the trioxolanes administered on day 1 post-infection in a non-solubilizing, standard suspension vehicle (SSV) formulation 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 complete lack of activity for **13** demonstrates the essential contribution of the spiroadamantane ring system to the antimalarial properties of **1** and its analogs. For the homologous series of primary amines **14**, **1**, and **15**, in vitro potencies and metabolic stabilities were essentially invariant, but in vivo activity was the highest for **1** (Table 1). The primary alcohol isosteres **16**, **8**, and **17** were similarly potent in vitro, but were not as effective in vivo. The weaker in vivo activities of the alcohols may be due in part to their decreased metabolic stabilities (predicted hepatic ER > 0.5), presumably a function of their greater lipophilicities. The poor antimalarial profile of **18**, the aminoxy isostere of **15**, shows that a basic amino group is required for optimal antimalarial activity. On the other hand, the data for **20** show that substituting a more basic guanidine for the primary amine in **1** diminishes antimalarial potency by an order of magnitude. Ethyl carbamate **19**, a potential prodrug of **1**, had an antimalarial profile equal to that of **1**, but was considerably less metabolically stable due, presumably, to hydrolysis of the carbamate functional group.

The data for secondary and tertiary aliphatic amines are depicted in Table 2. With the exception of amino acid **23**, all of the secondary amines were quite potent (IC_{50s} <1 ng/mL). Of these, only cyclopropyl amine **21** and diamine **26** had activities <99.5%. Data for aminoester **22**, aminoacid **23**, and aminoamide **24** show that **22** was rapidly metabolized (predicted hepatic ER > 0.99), probably by conversion to the metabolically stable **23**, and that **24** provides an optimal combination of functional groups (amine, amide). Aminoamide **25**, the homolog of **24**, also had a very good antimalarial profile and had a metabolic stability similar to that of **24**. Although tertiary amines **27** and **28** had good potency in vitro, they were not very active in vivo. When the tertiary amine was the proximal N atom of a piperazine heterocycle (**29**, **30**), in vivo activity improved substantially.

The data for secondary aromatic amines are depicted in Table 3. All of these had good potencies in vitro (IC_{50s} <5 ng/mL), and with the exception of **36**, had in vivo activities ⩾ 99.5%. Anilides and sulfanilamides **31-34** were considerably more lipophilic ($\log D_{pH}$ 7.4 >5) than their aliphatic amine counterparts shown in Table 2. Not surprisingly, **31** and **32** were rapidly metabolized as was **34** (predicted ER > 0.7). Interestingly, pyridine

Table 1. Lipophilicity, metabolic stability, and activity of primary amino trioxolanes and their alcohol and aminoxy isosteres, and carbamate and guanidine derivatives against *P. falciparum* in vitro and *P. berghei* in vivo



1, 10, 14-20   **13**

Compound	R	Log P/$D_{pH\ 7.4}$[a]	IC_{50}[b] (ng/ml) K1/NF54	Activity (%)[c]	ER[d]
None	—	—	—	0	—
13[e]	—	3.3D	>100/>100	0	ND
14[f]	NH_2	2.7D	0.81/0.31	98	<0.3
1[e]	CH_2NH_2	2.6D	0.39/0.42	99.98	0.24
15[g]	$(CH_2)_2NH_2$	2.9D	0.15/0.48	99.08	<0.3
16[h]	OH	3.9	0.25/0.51	93	0.62
8[h]	CH_2OH	5.1	0.83/0.20	99.15	0.51
17	$(CH_2)_2OH$	5.6	0.30/0.75	89	0.65
18[e]	CH_2ONH_2	5.6D	28/20	47	ND
19	$CH_2NHCO_2CH_2CH_3$	6.2	0.40/0.56	99.94	0.57
20[g]	$CH_2C{=}NH(NH_2)$	1.2D	6.2/7.6	9	ND
AM[h]	—	3.3	0.74/1.2	99.36	0.89
AS[h]	—	3.5	1.3/1.6	67	0.43[i]

[a] Calculated as previously described,[13,20] $\log D_{pH\ 7.4}$ denotes the octanol/buffer partition coefficient at pH 7.4 which is relevant for the ionizable analogs.

[b] Mean from (n = 2–3). Individual measurements differed by less than 50%.

[c] Groups of three *P. berghei*-infected MORO mice were treated orally one day post-infection with trioxolanes dissolved or suspended in SSV. Antimalarial activity was measured by percent reduction in parasitemia on day three post-infection. Individual measurements differed by less than 10%.

[d] Predicted hepatic extraction ratios (ER) using human liver microsomes.[30]

[e] Mesylate salt.

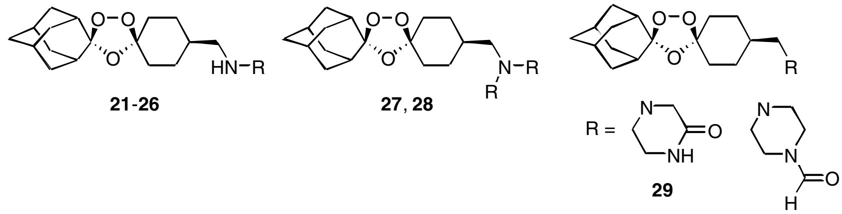
[f] Tosylate salt.

[g] Hydrochloride salt.

[h] Data from Dong et al.[25]

[i] Value for DHA, the primary metabolite of AS.

Table 2. Lipophilicity, metabolic stability, and activity of secondary and tertiary amino trioxolanes against *P. falciparum* in vitro and *P. berghei* in vivo

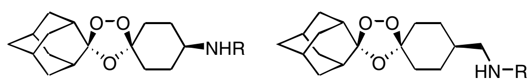


21-26 **27, 28** **29** **30**

Compound	R	$\log D_{pH\ 7.4}$	IC_{50} (ng/ml) K1/NF54	Activity (%)	ER
21[a]	Cyclopropyl	2.8	0.56/0.45	59	0.33
22[a]	CH_2COOEt	5.7	0.42/0.45	99.64	>0.99
23[a]	CH_2COOH	2.5	11/17	99.80	0.25
24	CH_2CONH_2	3.6	0.30/0.59	99.67	0.40
25[a]	$(CH_2)_2CONH_2$	2.4	0.41/0.91	99.92	0.36
26[b]	$CH_2C(CH_3)_2NH_2$	3.4	0.49/0.83	95	ND
27[a]	CH_3	3.8	0.35/0.75	74	0.34
28	CH_2CONH_2	3.8	1.7/2.0	86	ND
29	—	4.0	1.3/1.8	99.81	0.50
30	—	4.6	1.6/2.4	99.01	ND

[a] Mesylate salt.
[b] Dimesylate salt.

Table 3. Lipophilicity, metabolic stability, and activity of aromatic amino trioxolanes against *P. falciparum* in vitro and *P. berghei* in vivo



31, 32 **33-37**

Compound	R	$\log D_{pH\ 7.4}$	IC_{50} (ng/ml) K1/NF54	Activity (%)	ER
31	$4\text{-}CONH_2C_6H_5$	6.2	1.1/1.0	99.98	0.74
32	$4\text{-}SO_2NH_2C_6H_5$	5.8	2.7/3.0	99.98	0.73
33	$4\text{-}CONH_2C_6H_5$	6.3	0.94/1.6	99.73	ND
34	$4\text{-}SO_2NH_2C_6H_5$	5.9	2.0/3.0	99.95	0.84
35	3-Pyridyl	5.4	0.25/0.34	99.58	ND
36	2-Pyrimidinyl	5.3	0.65/1.2	80	ND
37[a]	2-Thiazolyl	6.4	2.3/2.2	99.49	ND

[a] Mesylate salt.

35 and pyrimidine **36** were similarly potent in vitro, but the less basic **36** was much less active than **35** in vivo.

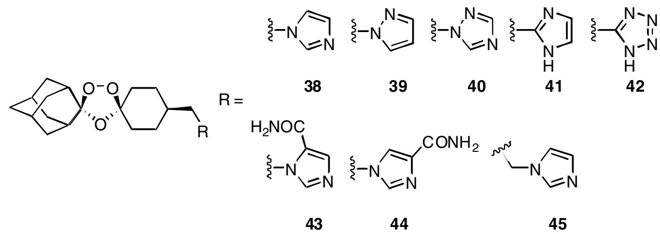
The data for the azoles are depicted in Table 4. With the exception of acidic tetrazole **42**, all had IC_{50s} < 2 ng/mL. Compared to imidazole **38**, the less basic pyrazole (**39**) and triazole (**40**) isosteres were equally potent in vitro, but only **40** was as active in vivo, and it was unexpectedly less stable metabolically. Compared to *N*-alkyl imidizole **38**, the more polar 2-substituted imidazole **41** was more potent in vitro, but, it was much less active in vivo. Imidazoles **43** and **44** show that increasing the polarity of **38** by carboxamide substitution maintains antimalarial efficacy, but where measured (**44**), decreases metabolic stability. Imidazole **45** shows that extending the link between the cyclohexane and imidazole heterocycle increases lipophilicity but does not enhance antimalarial efficacy.

To assess whether some of the new weak base trioxolanes could cure *P. berghei*-infected mice, we administered a 3×10 mg/kg divided dose on days +1, +2, and +3 post-infection. In this experiment, **1** was completely curative, semisynthetic artemisinins AM and AS provided no cures,[13] and **24**, **31**, **32**, and **38** cured 1/5, 2/5, 4/5, and 0/5 of the infected mice, respectively.

Selected trioxolanes were administered intravenously (IV) and orally (PO) to rats[24] and pharmacokinetic data for **1**, **8**, **24**, **38**, DHA, and AM are shown in Table 5. The data indicated that alcohol **8** was rapidly cleared after IV dosing by conversion to its less active[25] carboxylic acid metabolite. After PO dosing, plasma concentrations of **8** were not detected indicating very low oral bioavailability. Weak base trioxolanes **1**, **24**, and **38** each had a considerably longer half-life compared to **8** and oral bioavailabilities ranging from 30% to 60%.

Preliminary toxicological investigations (5-day toxicity studies in male rats with daily oral administration) indicated toxicological profiles of **1**, **24**, and **38** similar to that of artesunate, including gastric irritation, hepatocellular

Table 4. Lipophilicity, metabolic stability, and activity of azole trioxolanes against *P. falciparum* in vitro and *P. berghei* in vivo



Compound	$\mathrm{Log}\,D_{\mathrm{pH}\ 7.4}$	IC_{50} (ng/ml) K1/NF54	Activity (%)	ER
38[a]	5.4	1.3/1.1	99.78	0.23
39	6.0	1.0/1.2	78	ND
40[b]	4.9	0.90/1.7	99.88	0.41
41	4.9	0.29/0.42	71	ND
42	3.1	46/55	40	ND
43	5.0	0.58/0.97	99.77	ND
44	5.0	0.68/1.3	99.76	0.89
45	5.9	0.44/0.40	99.82	ND

[a] Hydrochloride salt.
[b] Mesylate salt.

Table 5. Pharmacokinetic parameters[a] after intravenous and oral administration to rats

Compound	Intravenous administration			Oral administration bioavailability (%)
	Half-life (min)	Vol of distribution (L/kg)	Plasma clearance (mL/min/kg)	
1	150	39	177	31
8	27	5.3	136	nd[b]
24	94	13	102	58
38	47	4.1	60.1	36
DHA[c]	26	3.0	72.0	Not dosed PO
AM[c]	52	8.0	114	1.4

[a] Values represent the average of 2–3 determinations.
[b] nd, plasma concentrations were not detected following PO administration.
[c] Data from Dong et al.[20]

hypertrophy, renal tubular changes, and atrophy of lymphatic tissues. No signs of neurotoxicity were seen. The overall toxicity of **24** and **38** was significantly lower than that of **1**. Findings tended to be reversible at the end of a 1-week recovery period. Preliminary genotoxicity tests (Ames microsuspension[26] and MNT in vitro[27] assays) were not indicative of a relevant genotoxic or clastogenic/aneugenic potential. hERG patch-clamp assays revealed IC_{50} values of 1.8 and 2.7 μM[28] for **1** and **24**, similar to those of 2.5 and 2.6 μM[29] for chloroquine and mefloquine.

In summary, compared to primary amino trioxolanes **14**, **1**, and **15**, alcohol (**16**, **8**, **17**) and aminoxy (**18**) isosteres, and guanidine (**20**) and amino acid (**23**) analogs had inferior antimalarial and biopharmaceutial profiles. As exemplified by imidazole **38**, the good antimalarial profiles of several weak base azoles show that trioxolanes do not require an aliphatic amino functional group for high antimalarial activity. The reduced potency of **23** and **42** is consistent with our previous observation[25] that trioxolane carboxylic acids have weak antimalarial activities. Although none of these new weak base trioxolanes had antimalarial profiles superior to that of **1**, amino amides **24**, **25**, **29**, and **31**–**34** were nearly as effective; however each of these was less stable metabolically than **1**. Indeed, lipophilic trioxolanes tended to be less stable metabolically than their more polar counterparts. Importantly, **1**, **24**, and **38** each displayed an improved half-life and oral bioavailability relative to DHA and AM, and the latter (**24**, **38**) were less toxic than **1**. Future studies will determine the potential of weak base trioxolanes as antimalarial drug development candidates.

Acknowledgments

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21. Crystallographic data (excluding structural factors) for structure **7** in this paper have been deposited with the Cambridge Crystallographic Data Centre (CCDC) as supplementary publication number CCDC 298211. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK [fax: +44 (0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].
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24. Compounds were dosed to male Sprague Dawley rats following an overnight fast. IV doses were administered via a cannula previously inserted into the jugular (cannulation on the day prior to dosing) and oral dosing was via gavage. Doses included **8** 4 mg/kg IV in 40% v/v propylene glycol (PG), 10% v/v ethanol (EtOH), water and 10 mg/kg PO as a suspension in 0.5% w/v aqueous hydroxypropylmethyl cellulose (HPMC); **38** 10 mg/kg IV in 10% v/v PG, 10% v/v EtOH, water and 50 mg/kg PO as a suspension in 0.5% w/v aqueous HPMC; **24** 25 mg/kg IV in aqueous citrate buffer, pH 3, and 50 mg/kg PO as a suspension in 0.5% w/v aqueous HPMC; **1** 25 mg/kg IV in aqueous citrate buffer, pH 3, and 50 mg/kg PO as a suspension in 0.5% w/v aqueous HPMC; DHA 17 mg/kg IV in 0.1 M Captisol®. Sequential blood samples were collected through a cannula inserted in the carotid artery on the day prior to dosing. Blood samples were centrifuged immediately after sampling and plasma separated and stored at −80 °C prior to analysis by LC/MS. Concentrations were quantified by comparison to a calibration curve prepared in plasma and analyzed along with the study samples.
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