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Bioorganic & Medicinal Chemistry Letters 16 (2006) 5542–5545
Bioorganic & Medicinal Chemistry Letters

Antimalarial activity of *N*-alkyl amine, carboxamide, sulfonamide, and urea derivatives of a dispiro-1,2,4-trioxolane piperidine

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Received 8 June 2006; revised 5 August 2006; accepted 8 August 2006
Available online 22 August 2006

Abstract—With an aim to identify a dispiro-1,2,4-trioxolane with high oral activity and good physicochemical properties, 27 derivatives of an achiral piperidine trioxolane were synthesized; most were potent antimalarial peroxides with IC_{50}s ranging from 0.20 to 7.0 ng/mL. The oral efficacies of two of these were superior to artesunate and comparable to artemether. The attractive chemical simplicity of these compounds is balanced only by an apparent metabolic susceptibility.

The semisynthetic artemisinins artemether (AM) and artesunate (AS) (Fig. 1) are potent antimalarial drugs, but they have poor biopharmaceutical properties.[1,2] Many potent synthetic antimalarial peroxides have been prepared, but it has been difficult[3] to identify peroxide structures with the physicochemical and biopharmaceutical properties[4–6] to ensure good absorption and bioavailability following oral administration. We recently described the discovery of dispiro-1,2,4-trioxolane (secondary ozonide) antimalarial prototype **1** (Fig. 1).[7] Although **1** has impressive antimalarial activity comparable to the semisynthetic artemisinins, it is very lipophilic (log *P* 6.1, Table 1).

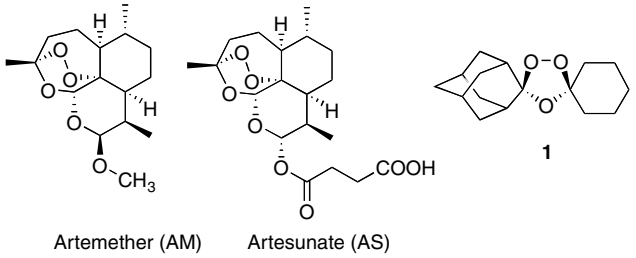

Figure 1.

However, its relatively simple achiral structure is an attractive scaffold. We reasoned that one strategy to retain the achirality and substantially decrease the lipophilicity of **1** was to investigate its piperidine isostere **4** (log *D* 0.25) (Scheme 1). In this paper, we describe the synthesis[8] and antimalarial properties of 27 structurally diverse *N*-alkyl amine (**5**), amide (**6**), sulfonamide (**7**), and urea (**8**) derivatives of **4**[9] (Scheme 1). The latter three functional groups are attractive due to their chemical stability, polarity, and ease of synthesis. Our aim was to identify a piperidine trioxolane with high oral activity and good physicochemical properties.

Amides **6a**, **6g** sulfonamides **7a**, **7b**, and **7d**, and the carbamate, ester, and α-chloroacetamide precursors of **4**, **6b**, **6e**, and **6f** were obtained from coozonolysis[10,11] of adamantanone *O*-methyl oxime (**2**) with the appropriate

Keywords: 1,2,4-Trioxolane; Secondary ozonide; Antimalarial; Artemisinin.
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doi:10.1016/j.bmcl.2006.08.046

Table 1. Activity of *N*-alkyl piperidines **5** against *Plasmodium falciparum* in vitro and *Plasmodium berghei* in vivo

Compound	R	log *P*/PSA(Å^2)[a]	IC_{50}[b] (ng/ml) K1/NF54	Activity[c] (%) po/sc	Survival[d] (days) po/sc
None	—	—	—	0	6–7
1	—	6.1[f]/25.6	0.97/1.4	94/>99.99	7.2/11.9
4[e]	—	0.25[g]/44.2	0.15/0.34	59/84	6.3/7.7
5a	$CH_2CH_2SO_2CH_2CH_3$	2.3[g]/67.7	1.0/2.9	81/92	7.0/8.0
5b	$CH_2C_6H_5$	3.5[g]/28.9	2.5/2.6	40/99.95	6.0/10.0
5c	4-Picolyl	2.7[g]/45.9	0.40/0.67	62/99.02	6.3/11.0
AM	—	3.3[f]/62.2	0.74/1.2	98/99.78	7.9/9.1
AS	—	3.5/111.5	1.3/1.6	65/65	6.8/6.8

[a] 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.

[b] Mean from $n = 2$–3.

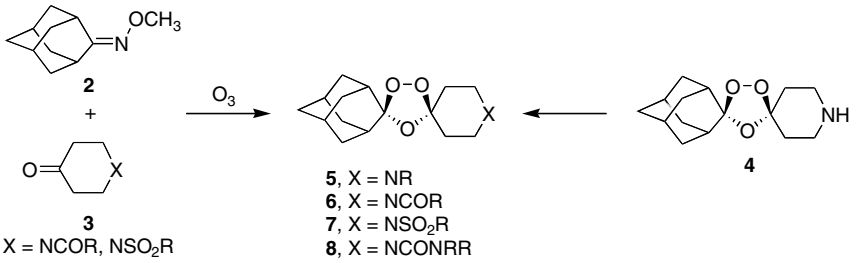
[c] Groups of three *P. berghei*-infected MORO mice were treated one day post-infection with trioxolanes dissolved or suspended in 3% ethanol and 7% Tween 80. Antimalarial activity was measured by percent reduction in parasitemia on day three post-infection and survival times compared to an untreated control group.

[d] Survival to day 30 post-infection is considered to be a cure.

[e] Hydrochloride salt.

[f] Measured Elog *P* value.

[g] $\log D_{\text{pH 7.4}}$.



Scheme 1. Trioxolane piperidine synthesis.

piperidinone derivatives (**3**)[12] in 10–50% yields (Scheme 1). Alcohol **6b** (68%) and acid **6e** (43%) were obtained by aq KOH hydrolysis of their acetate and ethyl ester precursors. Amide **6f** (35%) was obtained by a displacement reaction between its precursor α-chloroacetamide and imidazole. Piperidine **4** was conveniently obtained in 34% yield from its corresponding BOC derivative. The remaining trioxolanes were obtained directly from **4**, a key intermediate that allowed access to target compounds, some of which were impossible to obtain directly[13] by the coozonolysis method (Scheme 1).

Amine sulfone **5a** (72%) was obtained by alkylation of **4** with ethyl vinyl sulfone/Et_3N. Amines **5b** (75%) and **5c** (71%) were obtained by reductive amination (triacetoxyborohydride) reactions of **4** with benzaldehyde and 4-pyridinecarboxaldehyde, respectively. Amides **6c** (96%), **6d** (39%), **6h** (69%), and **6i** (69%), and sulfonamides **7c** (76%) and **7e** (65%) were obtained by reaction of **4** with the requisite acid chlorides in the presence of Et_3N (3 equiv). Urea **8a** (98%) was obtained by treatment of **4** with KOCN in a HOAc/pyridine buffer. Ureas **8b** (51%), **8c** (96%), **8d** (70%), and **8e** (83%) were obtained by treatment of **4** with the appropriate isocyanate in the presence of Et_3N. Ureas **8f** (77%), **8g** (74%), **8h** (42%), **8i** (82%), and **8j** (27%) were obtained by treatment of **4** with the appropriate carbamoyl chloride in the presence of Et_3N.

In vitro and in vivo antimalarial activities (Tables 1–4) were measured using the chloroquine-resistant K1 and chloroquine-sensitive NF54 strains of *Plasmodium falciparum*- and *Plasmodium berghei*-infected mice. In vivo data were determined using single 10 mg/kg oral (po) and subcutaneous (sc) doses administered on day 1 post-infection. Trioxolane **4** was some 4- to 9-fold more potent than artemether, artesunate, and **1** in vitro, and was as active as artesunate, but was less active than artemether and **1** in vivo (Table 1). That **4** is more active sc than po indicates that its oral bioavailability may be low, although sc and po activity differentials per se do not predict oral bioavailability. Sulfone, phenyl, and pyridine *N*-alkyl piperidines **5a**–**5c** had good log *D* values and were quite potent. Of these, only **5a** had better oral activity than **4**, although **5b** and **5c** had high subcutaneous activities and survival times.

With the exception of carboxy amides **6e** and **6i**, amides **6** (Table 2) had IC_{50s} between 0.30 and 1.4 ng/ml. The weakly potent **6e** and **6i** also had low in vivo activities. However, the other amides were as or more active than **4** in vivo. The relatively lipophilic neopentyl amide **6d** had better oral activity than its more polar counterparts **6a**–**6c**, although α-hydroxyacetamide **6b** had better oral activity than the less polar acetamide **6a**. Amide **6h**, a pyridine isostere of benzamide **6g**, was much less active than its more lipophilic prototype.

Table 2. Activity of piperidine carboxamides **6** against *Plasmodium falciparum* in vitro and *Plasmodium berghei* in vivo

Compound	R	log *P*/PSA(Å^2)	IC_{50} (ng/ml) K1/NF54	Activity (%) po/sc	Survival (days) po/sc
6a	CH_3	3.8[a]/79.4	0.82/0.93	81/85	6.7/7.3
6b	CH_2OH	2.4[a]/76.1	0.30/0.60	92/99.8	7.0/8.0
6c	CH_2OCH_3	3.1/65.3	0.95/0.20	92/89	7.3/7.3
6d	$CH_2C(CH_3)_3$	5.0/58.5	0.92/0.35	98/99.94	7.7/8.3
6e	CH_2CH_2COOH	2.3[a]/93.2	7.1/7.2	39/52	6.0/6.3
6f	CH_2Im[b]	2.9[c]/68.6	0.64/0.17	86/87	7.3/7.3
6g	Phenyl	5.6[a]/47.5	0.35/0.58	93/99.9	7.0/8.7
6h	4-Pyridyl	1.8/65.5	1.4/0.34	61/80	6.7/7.0
6i	2-Carboxyphenyl	1.2[c]/93.1	22/13	38/40	6.3/6.3

[a] Measured E log *P* value.
[b] Imidazole.
[c] $\log D_{pH\ 7.4}$.

Table 3. Activity of piperidine sulfonamides **7** against *Plasmodium falciparum* in vitro and *Plasmodium berghei* in vivo

Compound	R	log *P*/PSA(Å^2)	IC_{50} (ng/ml) K1/NF54	Activity (%) po/sc	Survival (days) po/sc
7a	CH_3	1.4/67.6	0.59/0.64	93/99.97	6.7/10.3
7b	CH_2CH_3	1.9/66.8	0.38/0.81	85/99.94	7.0/9.3
7c	$CH_2C_6H_5$	4.2/60.5	1.7/4.1	21/90	5.7/8.0
7d	C_6H_5	5.5[a]/66.4	1.1/1.2	71/>99.99	6.0/10.3
7e	4-$CH_3CONHC_6H_5$	5.0/97.9	1.4/0.50	56/99.98	6.3/12.0

[a] Measured E log *P* value.

Table 4. Activity of piperidine ureas **8** against *Plasmodium falciparum* in vitro and *Plasmodium berghei* in vivo

Compound	R, R	log *P*/PSA(Å^2)	IC_{50} (ng/ml) K1/NF54	Activity (%) po/sc	Survival (days) po/sc
8a	H, H	1.1/82.9	0.69/1.1	99.48/99.44	8.3/8.7
8b	H, $C(CH_3)_3$	2.7/52.8	0.98/1.8	48/99.85	6.7/9.7
8c	H, C_6H_5	3.6/59.4	1.6/0.45	92/99.97	7.0/19.7
8d	H, 4-$CH_3C_6H_5$	4.1/59.1	2.3/2.3	0/99.98	6.0/14.3
8e	H, 4-ClC_6H_5	4.5/58.1	2.5/4.4	38/99.98	6.0/13.7
8f	CH_3, CH_3	2.8/48.4	0.70/1.1	94/72	7.0/7.0
8g	$CH(CH_3)_2$, $CH(CH_3)_2$	4.6/43.2	2.0/6.6	87/99.95	8.3/20.3
8h	$(CH_2)_4$	3.3/47.6	3.9/6.7	87/99.72	7.3/9.7
8i	$CH_2CH_2OCH_2CH_2$	2.3/60.8	1.5/3.0	93/99.87	8.7/9.0
8j	$CH_2CH_2N(CH_3)CH_2CH_2$	2.6[a]/51.0	0.20/0.22	90/97	7.3/8.3

[a] $\log D_{pH\ 7.4}$.

Sulfonamides **7** (Table 3) were also quite potent with IC_{50}s between 0.38 and 4.1 ng/ml. The polar (log *P* 1.4) methanesulfonamide **7a** was as potent in vitro and more active in vivo than its less polar (log *P* 3.8) isosteric acetamide **6a**. Extending the alkyl chain (**7b**) or phenyl substitution (**7c**–**7e**) reduced oral activity, although like methanesulfonamide **7a**, arylsulfonamide **7d** and **7e** had high subcutaneous activities and survival times.

Ureas **8** had IC_{50}s that ranged from 0.20 to 6.7 ng/ml (Table 4). The polar (log *P* 1.1) monosubstituted urea **8a** was as potent, and was significantly more active orally than its less polar acetamide **6a** and sulfonamide **7a** isosteres. There was a substantial loss of oral activity when one of the urea hydrogen atoms of **8a** was replaced with alkyl (**8b**) or phenyl (**8c**–**8e**) groups, although the latter had notably high activities and survival times when they were dosed subcutaneously. The complete lack of oral activity for **8d**, the *p*-methyl analog of **8c**, suggested that it was either not absorbed or was rapidly converted to an inactive metabolite. Oral activity also decreased when both of the urea hydrogen atoms of **8a** were replaced with alkyl (**8f**, **8g**), cyclopentyl (**8h**) or heterocycle (**8i**, **8j**) groups, although weak base *N*-methylpiperidine **8j** was the most potent urea tested.

From the physicochemical and antimalarial data in Tables 1–4, it is immediately evident that in vitro data alone are of little use for compound optimization since they do not predict in vivo activity; however the in vitro data do reveal that acidic derivatives (**6e**, **6i**) are significantly less potent than their neutral and weak base counterparts, an SAR trend consistent with data for other 1,2,4-trioxolanes.[14] Second, these trioxolanes were generally more active when administered subcutaneously than orally. This suggests substantial biopharmaceutical liabilities that are, however, unlikely to be a function of inadequate membrane permeability given the calculated polar surface area (PSA) values of 29–98 Å[15] for these compounds. Third, whereas only the more lipophilic amides (**6d**, **6g**) had oral activities equal to or better than prototype **1**, the highly polar sulfonamide **7a** and urea **8a** had the best oral activities of their respective trioxolane subclasses.

To assess potential mechanisms for the generally poor oral antimalarial activities of these trioxolanes, we examined the metabolic stability of several of these. Using human hepatic microsomes,[16] the predicted hepatic extraction ratios (ER) for **4**, **6c**, **8f**, the ethyl carbamate of **4**,[7], and dihydroartemisinin (the major active metabolite of artesunate) were 0.34, 0.71, 0.75, 0.73, and 0.43, respectively; in each case, ER values were higher (data not shown) when mouse or rat hepatic microsomes were used. These ER values suggest that hepatic metabolism is probably a major clearance mechanism for these compounds. The rapid clearance of these piperidine trioxolanes may arise in part from a more easily reduced peroxide bond due to the inductive effect of the piperidine nitrogen atom. The relative peroxide bond stabilities were tested by examining rates of degradation in the presence of excess $FeSO_4$ using standardized conditions.[17] The resulting pseudo-first order reaction rate constant for piperidine **4** was 4.0 h^{-1}, indicating that peroxide bond cleavage occurred at a rate 10-fold higher than for **1** (0.41 h^{-1}), and significantly faster than for artemisinin (0.05 h^{-1}).

In summary, these piperidine trioxolanes are potent antimalarial peroxides. The oral efficacies of **6d** and **8a** are superior to that of artesunate and comparable to those of **1** and artemether. The attractive chemical simplicity of these compounds is balanced only by an apparent metabolic susceptibility. Future studies will determine the potential of piperidine trioxolanes as antimalarial drug development candidates.

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).

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