Design and Synthesis of Novel Artemisinin-Like Ozonides with Antischistosomal Activity

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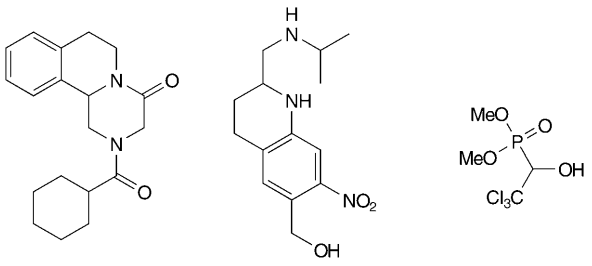
To develop new drugs for prevention and treatment of schistosomiasis, a series of novel artemisinin-like ozonides **10** were synthesized *via* a facile three-step procedure starting with the degraded product of artemisinin (*Scheme*). The *Criegee* ozonolysis reaction of the unsaturated lactone intermediates **14** is the key step which provided the target molecules **10**. The *in vivo* pharmacological results suggested that this type of artemisinin analogues exhibited moderate antischistosomal activity (*Table*).

Introduction. – Schistosomiasis is a chronic and debilitating parasitic disease caused by flatworms belonging to the genus *Schistosoma*, which is widespread throughout tropical and subtropical areas and ranks second behind malaria in terms of socio-economic and public-health importance [1]. It is currently estimated that more than 600 million people in 74 countries live at risk of infection, and 200 million people are suffering from schistosomiasis [2].

There is yet no vaccine available [3], and the current mainstay of schistosomiasis control is chemotherapy with praziquantel (**1**) as the drug of choice [2a][4] (*Fig. 1*). But left with a single efficacious antischistosomal drug is a dangerous situation since oxamniquine (**2**) is difficult to obtain, and metrifonate (**3**) was withdrawn from the market in 1998 (*Fig. 1*). In view of rapid re-infection following treatment and concern about the development of tolerance and/or resistance to praziquantel, there is an urgent need



1 Praziquantel **2** Oxamniquine **3** Metrifonate

Fig. 1. *Three known antischistosomal drugs*

for research and development of novel drugs for the prevention and treatment of schistosomiasis.

Fortunately, artemisinin (**4**) [5] and its derivatives such as dihydroartemisinin (**5**) [6], artemether (**6**) [7], arteether (**7**) [8], and artesunate (**8**) [9], besides the widely acknowledged antimalarial effect, also exhibited good antischistosomal activity (*Fig. 2*). After massive clinic trials, the Chinese Ministry of Public Health approved artemether and artesunate for chemoprophylaxis of oriental schistosomiasis in 1996 as an additional tool for transmission control. Laboratory studies revealed that artemether exhibited the highest activity against juvenile stages of the parasites, while adult worms were significantly less susceptible [7b].

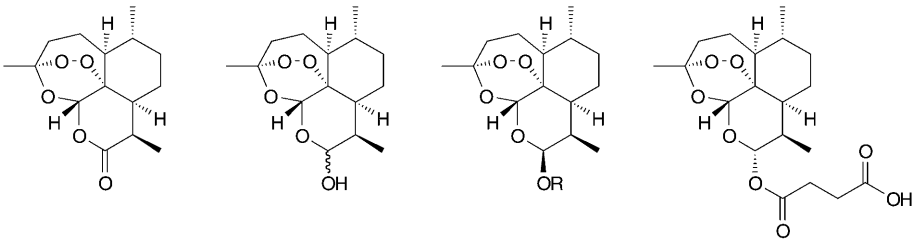


Fig. 2. *Artemisinin and several of its important semi-synthetic derivatives*

Recently, *Vennerstrom* and co-workers reported a large series of novel spiro 1,2,4-trioxolanes (= ozonides) and dispiro 1,2,4-trioxolanes **9** which displayed excellent antimalarial and antischistosomal activities (*Fig. 3*) [10]. Among them, a drug candidate **OZ277** has entered phase-I clinical trials in the UK and would be promising to become a new type of antimalarial and antischistosomal drug [11].

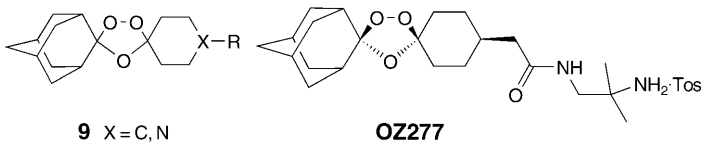


Fig. 3. *Spiro[adamantane-2,3′-[1,2,4]trioxolanes] as antimalarials and schistosomicides*

The pharmacological results suggested that the antischistosomal mechanism of artemisinins might be analogous to what has been proposed for the mechanism of action of artemisinin derivatives on malaria parasites, which involves activation of artemisinins within the parasites by intraparasitic heme-iron, leading to the cleavage of the endoperoxide bridge and generation of C-centered free radicals which attacked the targets, probably some important proteins, to kill the parasites [12]. Therefore, the peroxidic bond in the artemisinins and ozonides would be the key factor contributing to high antiparasitic activity.

Based on the aforementioned knowledge/rationale, we designed and synthesized a series of novel artemisinin-like ozonides and evaluated their *in vivo* antischistosomal activity. The remarkable structure feature of these ozonides resides in the artful replacement of the 1,2,4-trioxane moiety of the parent artemisinins by a 1,2,4-trioxolane moiety.

Results and Discussion. – *Chemistry.* A series of artemisinin-analogous ozonides **10** were synthesized from diketo methyl ester **11** [13], the acid-catalyzed (TFA = CF_3-COOH) degradation product of artemisinin (**4**), *via* a facile three-step procedure (*Scheme*). According to our previous work [14], compound **12** was obtained by intramolecular condensation of **11** in the presence of barium hydroxide, after acidification with dilute hydrochloric acid to pH 4–5. But if the reaction mixture was acidified to pH < 3, then a lactone by-product **13** was formed, which was present as a co-crystal mixture with the target compound, thus hampering the subsequent purification. At $-20°$, treatment of compound **12** with various *Grignard* reagents followed by acidification with dilute sulfuric acid afforded the unsaturated lactone intermediate **14**, commonly accompanied by a corresponding conjugated-diene-acid by-product **15**. Generally, compounds **14** and **15** could be purified by acid–base methods without column chromatography. Finally, the desired artemisinin-like ozonides **10** were prepared by *Criegee* ozonolysis of intermediate **14** at $-78°$. Of course, inevitably **10** was accompanied by small amount of over-ozonolyzed product **16**. The new ozonides **10** could be purified by column chromatography followed by recrystallization from AcOEt/petroleum ether. It was interesting that the structure of ozonide **10a** not only is highly similar to that of the parent artemisinin (**4**), but that **10a** is also its homologue. In general, these new ozonides were stable without any decomposition for several months at room temperature.

When ^{i}BuMgBr was employed to react with compound **12**, another rearrangement product **17** was obtained besides the expected compounds **14e** and **15c**. This result may be correlated to the relatively large steric bulk of the isobutyl substituent, which could lead to an easier elimination of this group as compared to other substituents, but the detailed reaction mechanism of the formation of **17** is not clear yet.

Antischistosomal Activity. The novel artemisinin-like ozonides **10a**–**e** were tested *in vivo* for their antischistosomal activity on *Schistosoma japonicum* with *O*-[(*tert*-butoxy)carbonyl]dihydroartemisinin [15] as the positive control (*Table*). These preliminary results showed that ozonides **10** exhibited moderate antischistosomal activity at a one-off dose of 300 mg/kg (*p.o.*). Among them, ozonide **10a** displayed the highest worm-reduction rate. Furthermore, it seemed that the activity was decreasing with increasing bulk of the substituent R, which suggests that antischistosomal activity decreases when the peroxide bond is sterically inaccessible to heme-iron species in parasites.

In addition, there was no indication of toxicosis after absorption of the above dose of ozonides, as checked at different intervals for one month post medication.

Conclusions. – In summary, a set of novel artemisinin-homologous 1,2,4-trioxolanes were synthesized, and their *in vivo* antischistosomal activity on mice model infected with *Schistosoma japonicum* was evaluated. The ozonides **10a**–**e** exhibited moderate parasiticidal potency.

Scheme. *Synthesis of artemisinin-like ozonides*

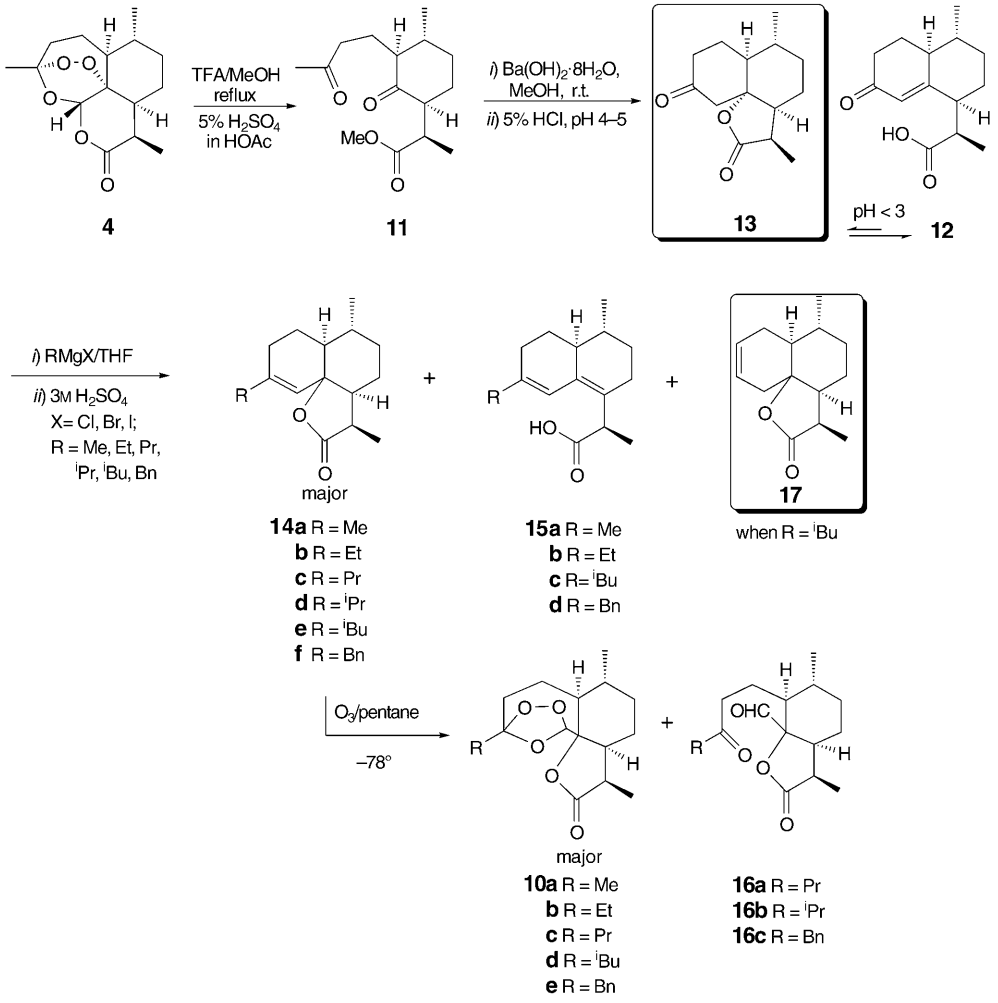


Table. *Antischistosomal Activity of Ozonides* **10** in vivo *against* Schistosoma japonicum *at a Dose of 300 mg/kg*

	R	Animals	Total worms (mean ± s.d.)	Worm-reduction rate [%]
10a	Me	5	12 ± 2	60
10b	Et	5	14 ± 4	52
10c	Pr	5	18 ± 3	40
10d	^{i}Bu	5	20 ± 3	33
10e	Bn	5	20 ± 4	33
Positive control	–	5	10 ± 2	67
Blank control	–	6	30 ± 2	–

These results are expected to be useful in guiding the design of new antischistosomal drugs of high efficacy and low toxicity. We expect that these novel ozonides could well complement existing control strategies and have the potential to become an important component of integrated control approaches to significantly reduce the current burden of schistosomiasis in many parts of the world.

We thank the *Shanghai Institute of Animal Parasitology, Chinese Academy of Agricultural Sciences*, for carrying out the *in vivo* antischistosomal test.

Experimental Part

1. *General.* All commercially available reagents were used without further purification unless otherwise stated. The solvents used were all AR grade and were distilled under positive pressure of dry N_2 where necessary. Yields were of purified compounds and were not optimized. Anal. TLC (reaction monitoring): home-made *$HSGF_{254}$* precoated silica gel plates; visualization by UV or development with I_2. M.p.: open capillary tubes; *Büchi 510* melting-point apparatus; uncorrected. IR Spectra (4000–600 cm^{-1}); *Perkin-Elmer 599B* spectrophotometer; KBr pellets or thin films; in cm^{-1}. ^{1}H-NMR Spectra: $CDCl_3$ solns. at r.t.; *Bruker AMX* spectrophotometer; chemical shifts δ in ppm downfield from $SiMe_4$ as the internal standard, coupling constants *J* in Hz. EI-MS: *Finnigan MAT-95* mass spectrometer; in *m/z* (rel. %). Elemental analyses: *CE-1106* elemental analyzer; all results within $\pm 0.4\%$ of the theoretical values.

2. *Compound* **14a**–**f**, **15a**–**d**, and **17**. To a soln. of **12** (2.36 g, 10.0 mmol) in dry THF (50 ml), the *Grignard* reagent, prepared from alkyl halide (40 mmol) and Mg powder (1.2 g, 50.0 mmol) in Et_2O (80 ml) was added dropwise at $-20°$ under continuous stirring. After the addition, the mixture was stirred for 3 h at r.t. Then the soln. was acidified with dil. sulfuric acid (3M) at $-20°$, quenched with H_2O (15 ml) and extracted with Et_2O. The combined extract was washed with 10% aq. Na_2SO_3 soln., 5% aq. NaOH soln., H_2O, and brine, dried ($MgSO_4$), and evaporated, and the crude product **14** purified by flash chromatography or by recrystallization. The aq. alkaline phase was acidified with 10% aq. HCl soln. to pH 5 and extracted with Et_2O. The org. layer was washed with H_2O and brine, dried ($MgSO_4$), and evaporated and the residue purified by column chromatography or recrystallization to give product **15**.

*(3*R*,3a*S*,6*R*,6a*S*)-3,3a,4,5,6,6a,7,8-Octahydro-3,6,9-trimethyl-2*H*-naphtho[8a,1-*b*]furan-2-one* (**14a**): Yield 75%. Colorless oil or crystals. M.p. 69–71° (from AcOEt/petroleum ether). IR (film): 2931, 2860, 1763 (C=O), 1666 (C=C), 1452, 1379, 1331, 1217, 1163, 1013, 926, 910. ^{1}H-NMR ($CDCl_3$, 400 MHz): 5.63 (*s*, 1 H); 3.14 (*quint.*, *J* = 6.87, 1 H); 2.15–1.97 (*m*, 3 H); 1.92–1.84 (*m*, 1 H); 1.68 (*s*, 3 H); 1.74–1.60 (*m,* 3 H); 1.46–1.36 (*m*, 1 H); 1.24–1.17 (*m*, 2 H); 1.14 (*d*, *J* = 7.14, 3 H); 1.09–0.99 (*m*, 1 H); 0.93 (*d*, *J* = 6.59, 3 H). Anal. calc. for $C_{15}H_{22}O_2$ (234.34): C 76.88, H 9.46; found: C 76.72, H 9.60.

*(α*R*,4*R*,4a*S*)-2,3,4,4a,5,6-Hexahydro-α,4,7-trimethylnaphthalene-1-acetic Acid* (**15a**) [16]. Yield 22%. Colorless crystals. M.p. 135–137°. IR (KBr): 3300 (chel OH), 2970, 2925, 2825, 1705 (C=O), 1610 (C=C), 1460, 1380, 1240, 1080, 950. ^{1}H-NMR ($CDCl_3$, 60 MHz): 12.10 (*s*, 1 H); 6.13 (*s*, 1 H); 3.78 (*q*, *J* = 7.00, 1 H); 1.82 (*s*, 3 H); 1.20 (*d*, *J* = 7.00, 3 H); 1.03 (*d*, *J* = 4.00, 3 H). Anal. calc. for $C_{15}H_{22}O_2$ (234.34): C 76.88, H 9.46; found: C 76.57, H 9.54.

*(3*R*,3a*S*,6*R*,6a*S*)-9-Ethyl-3,3a,4,5,6,6a,7,8-octahydro-3,6-dimethyl-2*H*-naphtho[8a,1-*b*]furan-2-one* (**14b**): Yield 75%. White needles. M.p. 73–75° (from AcOEt/petroleum ether). IR (KBr): 2974, 2941, 2897, 1751 (C=O), 1668 (C=C), 1454, 1377, 1213, 1180, 906, 849. ^{1}H-NMR ($CDCl_3$, 600 MHz): 5.59 (*s*, 1 H); 3.14 (*quint.*, *J* = 6.84, 1 H); 2.14–2.06 (*m*, 2 H); 2.05–1.98 (*m*, 1 H); 1.96 (*q*, *J* = 7.32, 2 H); 1.90–1.84 (*m*, 1 H); 1.67–1.60 (*m*, 3 H); 1.45–1.37 (*m*, 1 H); 1.24–1.14 (*m*, 2 H); 1.12 (*d*, *J* = 7.43, 3 H); 1.06–0.99 (*m*, 1 H); 0.97 (*t*, *J* = 7.57, 3H); 0.91 (*d*, *J* = 6.35, 3 H). Anal. calc. for $C_{16}H_{24}O_2$ (248.37): C 77.38, H 9.74; found: C 77.66 H 9.93.

*(α*R*,4*R*,4a*S*)-7-Ethyl-2,3,4,4a,5,6-hexahydro-α,4-dimethylnaphthalene-1-acetic Acid* (**15b**): Yield 24%. White crystalline particles. M.p. 138–140° (from AcOEt/petroleum ether). Yield: 24%. IR (KBr): 3300 (chel. OH), 2968, 2924, 2825, 1705 (C=O), 1614 (C=C), 1458, 1419, 1380, 1242, 1078, 949, 675. ^{1}H-NMR ($CDCl_3$, 600 MHz): 6.09 (*s*, 1 H); 3.81 (*q*, *J* = 6.84, 1 H); 2.18–1.98 (*m*, 7 H); 2.06 (*q*, *J* = 6.84, 2 H); 1.72–1.66 (*m*, 1 H); 1.60 (*t*, *J* = 10.99, 1 H); 1.29–1.22 (*m*, 1 H); 1.20 (*d*, *J* = 7.32, 3 H); 1.19–1.10 (*m*, 2 H); 1.02 (*t*, *J* = 7.33, 3 H); 0.98 (*d*, *J* = 5.86, 3 H). Anal. calc. for $C_{16}H_{24}O_2$ (248.37): C 77.38 H 9.74; found: C 77.51, H 10.02.

*(3*R*,3a*S*,6*R*,6a*S*)-3,3a,4,5,6,6a,7,8-Octahydro-3,6-dimethyl-9-propyl-2*H*-naphtho[8a,1-*b*]furan-2-one* (**14c**). Yield 52%. Solidified colorless oil. IR (KBr): 2956, 2929, 2872, 1759 (C=O), 1664 (C=C), 1460, 1375, 1219,

1184, 1171, 930, 914, 841, 723. ^{1}H-NMR ($CDCl_3$, 400 MHz): 5.62 (*s*, 1 H); 3.16 (*quint.*, *J* = 6.87, 1 H); 2.16–2.08 (*m*, 2 H); 2.07–1.99 (*m*, 1 H); 1.94 (*t*, *J* = 7.70, 2 H); 1.91–1.85 (*m*, 1 H); 1.76–1.60 (*m*, 3 H); 1.47–1.37 (*m*, 3 H); 1.26–1.18 (*m*, 2 H); 1.15 (*d*, *J* = 7.15, 3 H); 1.10–0.99 (*m*, 1 H); 0.94 (*d*, *J* = 6.60, 3 H); 0.87 (*t*, *J* = 7.36, 3 H). Anal. calc. for $C_{17}H_{26}O_2$ (262.39): C 77.82, H 9.99; found: C 78.09, H 10.28.

*(3*R*,3a*S*,6*R*,6a*S*)-3,3a,4,5,6,6a,7,8-Octahydro-9-isopropyl-3,6-dimethyl-2*H*-naphtho[8a,1-*b*]furan-2-one* (**14d**): Yield 70%. Colorless oil. IR (film): 2956, 2860, 1767 (C=O), 1659 (C=C), 1462, 1379, 1217, 1171, 1010, 912, 882, 843, 721. ^{1}H-NMR ($CDCl_3$, 400 MHz): 5.62 (*s*, 1 H); 3.16 (*quint.*, *J* = 6.78, 1 H); 2.23–1.97 (*m*, 4 H); 1.94–1.86 (*m*, 1 H); 1.76–1.57 (*m*, 3 H); 1.48–1.38 (*m*, 1 H); 1.26–1.17 (*m*, 2 H); 1.15 (*d*, *J* = 7.14, 3 H); 1.10–1.03 (*m*, 1 H); 1.00 (*dd*, *J* = 6.87, 6 H); 0.93 (*d*, *J* = 6.60, 3 H). EI-MS: 262 (38, *M*), 219 (88, $[M - {}^{i}Pr]^+$), 218 (99, $[M - CO_2]^+$), 203 (31, $[M - CO_2]^+$), 189 (84, $[M - CH(Me)CO_2H]^+$), 175 (100, $[M - Me - CH(Me)CO_2]^+$).

*(3*R*,3a*S*,6*R*,6a*S*)-3,3a,4,5,6,6a,7,8-Octahydro-9-isobutyl-3,6-dimethylnaphtho[8a,1-*b*]furan-2-one* (**14e**): Yield 27%. Colorless oil. IR (film): 2953, 2870, 1768 (C=O), 1662 (C=C), 1464, 1381, 1215, 1163, 914, 897. ^{1}H-NMR ($CDCl_3$, 600 MHz): 5.58 (*s*, 1 H); 3.13 (*quint.*, *J* = 6.84, 1 H); 2.12–2.05 (*m*, 2 H); 2.02–1.94 (*m*, 1 H); 1.89–1.82 (*m*, 2 H); 1.80–1.60 (*m*, 5 H); 1.45–1.37 (*m*, 1 H); 1.22–1.15 (*m*, 2 H); 1.13 (*d*, *J* = 6.84, 3 H); 1.07–0.99 (*m*, 1 H); 0.91 (*d*, *J* = 6.83, 3 H); 0.83, 0.81 (*dd*, *J* = 6.35, 6.83, 6 H). EI-MS: 276 (24, M^+), 232 (100, $[M - CO_2]^+$), 219 (44, $[M - {}^{i}Bu]^+$), 203 (31, $[M - CH(Me)COOH]^+$), 189 (83, $[M - Me - CH(Me)COO]^+$).

*(3*R*,3a*S*,6*R*,6a*S*)-3,3a,4,5,6,6a,7,10-Octahydro-3,6-dimethyl-2*H*-naphtho[8a,1-*b*]furan-2-one* (**17**): Yield 35%. White needles. M.p. 78–80° (from AcOEt-petroleum ether). IR (KBr): 3030, 2980, 2941, 2856, 1755 (C=O), 1649 (C=C), 1452, 1380, 1211, 1178, 1047, 928, 914, 883, 713. ^{1}H-NMR ($CDCl_3$, 600 MHz): 5.94–5.89 (*m*, 1 H); 5.87–5.83 (*m*, 1 H); 3.10 (*quint.*, *J* = 6.84, 1 H); 2.24–1.97 (*m*, 1 H); 2.13–2.03 (*m*, 2 H); 1.89–1.83 (*m*, 1 H); 1.73–1.67 (*m*, 3 H); 1.46–1.38 (*m*, 1 H); 1.26–1.14 (*m*, 2 H); 1.12 (*d*, *J* = 7.32, 3 H); 1.07–1.00 (*m*, 1 H); 0.92 (*d*, *J* = 6.84, 3 H). Anal. calc. for $C_{14}H_{20}O_2$ (220.31): C 76.33, H 9.15; found: C 76.38, H 9.35.

*(α*R*,4*R*,4a*S*)-2,3,4,4a,5,6-Hexahydro-7-isobutyl-α,4-dimethylnaphthalene-1-acetic Acid* (**15c**): Yield 5%. Colorless oil. IR (film): 3400 (chel. OH), 2953, 2926, 2860, 1705 (C=O), 1460, 1379, 1223, 1167, 926. ^{1}H-NMR ($CDCl_3$, 600 MHz): 6.06 (*s*, 1 H); 3.80 (*q*, *J* = 7.03, 1 H); 2.13–1.96 (*m*, 4 H); 1.95–1.80 (*m*, 2 H); 1.80–1.40 (*m*, 4 H); 1.22 (*d*, *J* = 7.32, 3 H); 0.98 (*d*, *J* = 5.86, 3 H); 0.83, 0.82 (2*d*, *J* = 6.35, 6 H). EI-MS: 276 (55, M^+), 231 (20, $[M - COOH]^+$), 219 (26, $[M - {}^{i}Bu]^+$), 203 [100, $M - CH(Me)COOH]^+$], 189 (22, $[M - Me - CH(Me)COO]^+$).

*(3a*S*,3*R*,6*R*,6a*S*)-9-Benzyl-3,3a,4,5,6,6a,7,8-octahydro-3,6-dimethyl-2*H*-naphtho[8a,1-*b*]furan-2-one* (**14f**): Yield 80%. Solidified colorless oil. IR (KBr): 3010, 2924, 2849, 1755 (C=O), 1668, 1495 (C=C), 1452, 1377, 1331, 1184, 1161, 910, 897, 735, 702. ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.32–7.25 (*m*, 2 H); 7.24–7.18 (*m*, 1 H); 7.15–7.11 (*m*, 2 H); 5.67 (*s*, 1 H); 3.28 (*s*, 2 H); 3.10 (*quint.*, *J* = 6.88, 1 H); 2.16–2.04 (*m*, 2 H); 2.03–1.92 (*m*, 1 H); 1.90–1.82 (*m*, 1 H); 1.66–1.62 (*m*, 3 H); 1.48–1.38 (*m*, 1 H); 1.26–1.18 (*m*, 2 H); 1.14 (*d*, *J* = 7.22, 3 H); 1.10–0.99 (*m*, 1 H); 0.92 (*d*, *J* = 6.55, 3 H). Anal. calc. for $C_{21}H_{26}O_2$ (310.44): C 81.25, H 8.44; found: C 81.21, H 8.52.

*(α*R*,4*R*,4a*S*)-7-Benzyl-2,3,4,4a,5,6-hexahydro-α,4-dimethylnaphthalene-1-acetic Acid* (**15d**): Yield 10%. Colorless oil. IR (KBr): 3400 (chel. OH), 3026, 2922, 2831, 1705 (C=O), 1603, 1495 (C=C), 1454, 1377, 1240, 1074, 937, 700. ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.31–7.25 (*m*, 2 H); 7.22–7.16 (*m*, 3 H); 6.21 (*s*, 1 H); 3.80 (*q*, *J* = 7.05, 1 H); 3.38 (*s*, 2 H); 2.18–1.98 (*m*, 5 H); 1.74–1.67 (*m*, 1 H); 1.62 (*t*, *J* = 9.82, 1 H); 1.22 (*d*, *J* = 7.05, 3 H); 1.19–1.12 (*m*, 3 H); 0.98 (*d*, *J* = 6.04, 3 H). EI-MS: 310 (60, M^+), 219 (100, $[M - Bn]^+$), 265 (14, $[M - COOH]^+$), 237 (44, $[M - CH(Me)COOH]^+$), 145 (84, $[M - Bn - CH(Me)COOH]^+$), 91 (97, Bn^+).

3. *Ozonides* **10a**–**e** *and* **16**. Through a soln. of **14** (2.0 mmol) in dry pentane (30 ml) was bubbled ozone at −78°, until the soln. turned into light blue. After completion, the soln. was flushed with N_2 for 2 min before being evaporated at r.t. The residue was purified by flash chromatography followed by recrystallization to provide the desired ozonides **10a**–**e** in 10–30% yield and over-ozonolyzed products **16a**–**c**.

*(3*R*,3a*S*,6*R*,6a*S*)-Octahydro-3,6,9-trimethyl-9,12-epoxy-12*H*-furo[3,2-*k*][2,3]benzodioxocin-2(3*H*)-one*) (**10a**): Yield 28%. White needles. M.p. 142–144° (from AcOEt/petroleum ether) ([16]: 135–140°). IR (KBr): 2941, 2860, 1763 (C=O), 1452, 1381, 1227, 1182, 1149, 1088, 1013, 939. ^{1}H-NMR ($CDCl_3$, 300 MHz): 5.35 (*s*, 1 H); 2.85 (*quint.*, *J* = 7.29, 1 H); 2.39–2.16 (*m*, 2 H); 2.09 (*dt*, *J* = 14.62, 3.20, 1 H); 1.82–1.54 (*m*, 5 H); 1.50 (*s*, 3 H); 1.49–1.33 (*m*, 2 H); 1.17 (*d*, *J* = 7.29, 3 H); 1.15–1.08 (*m*, 1 H); 0.98 (*d*, *J* = 6.46, 3 H). Anal. calc. for $C_{15}H_{22}O_5$ (282.34): C 63.81, H 7.85; found: C 64.08, H 7.75.

*(3*R*,3a*S*,6*R*,6a*S*)-9-Ethyloctahydro-3,6-dimethyl-9,12-epoxy-12*H*-furo[3,2-*k*][2,3]benzodioxocin-2(3*H*)-one* (**10b**): Yield 15%. White catkin/flossy crystals. M.p. 143–145° (from AcOEt/petroleum ether). IR (KBr): 2972, 2939, 2860, 1763 (C=O), 1464, 1377, 1188, 1122, 1086, 1024, 957, 679. ^{1}H-NMR ($CDCl_3$, 300 MHz): 5.36 (*s*, 1 H); 2.84 (*quint.*, *J* = 7.22, 1 H); 2.32 (*q*, *J* = 6.65, 1 H); 2.28–2.12 (*m*, 1 H); 2.03 (*dt*, *J* = 14.53, 2.99, 1 H); 1.80 (*q*,

$J = 7.61$, 2 H); 1.74–1.56 (*m*, 5 H); 1.56–1.42 (*m*, 1 H); 1.40–1.28 (*m*, 1 H); 1.16 (*d*, $J = 7.29$, 3 H); 1.14–1.06 (*m*, 1 H); 0.99 (*t*, $J = 7.50$, 3 H); 0.98 (*d*, $J = 6.32$, 3 H). Anal. calc. for $C_{16}H_{24}O_5$ (296.36): C 64.84, H 8.16; found: C 65.08, H 7.95.

*(3*R*,3a*S*,6*R*,6a*S*)-Octahydro-3,6-dimethyl-9-propyl-9,12-epoxy-12*H*-furo[3,2-*k*][2,3]benzodioxocin-2(3*H*)-one* **10c**: Yield 10%. White needles. M.p. 144–146° (from AcOEt/petroleum ether). IR (KBr): 2939, 2860, 1774, 1761 (C=O), 1454, 1380, 1188, 1148, 1122, 989, 957, 920, 677. ^{1}H-NMR ($CDCl_3$, 400 MHz): 5.35 (*s*, 1 H); 2.84 (*quint.*, $J = 7.25$, 1 H); 2.32 (*q*, $J = 6.69$, 1 H); 2.26–2.14 (*m*, 1 H); 2.03 (*dt*, $J = 14.71$, 3.20, 1 H); 1.76–1.70 (*m*, 3 H); 1.70–1.57 (*m*, 3 H); 1.57–1.40 (*m*, 4 H); 1.40–1.30 (*m*, 1 H); 1.17 (*d*, $J = 7.29$, 3 H); 1.14–1.08 (*m*, 1 H); 0.98 (*d*, $J = 6.60$, 3 H); 0.92 (*t*, $J = 7.36$, 3 H). Anal. calc. for $C_{17}H_{26}O_5$ (310.39): C 65.78, H 8.44; found: C 65.77, H 8.40.

*(3*R*,3a*S*,6*R*,7*S*)-Hexahydro-3,6-dimethyl-2-oxo-7-(3-oxohexyl)benzofuran-7a(2*H*)-carboxaldehyde* (**16a**): Yield 12%. Colorless oil. IR (film): 2960, 2875, 2720 (C(O)–H), 1790 (C=O, lactone), 1732 (C=O, aldehyde), 1709 (C=O, ketone), 1456, 1379, 1183, 1032, 953, 926, 875. ^{1}H-NMR ($CDCl_3$, 400 MHz): 9.74 (*s*, 1 H); 2.61–2.48 (*m*, 2 H); 2.46–2.31 (*m*, 1 H); 2.34 (*t*, $J = 7.22$, 2 H); 1.89–1.62 (*m*, 6 H); 1.62–1.52 (*m*, 2 H); 1.48–1.38 (*m*, 1 H); 1.16–1.06 (*m*, 2 H); 1.10 (*d*, $J = 7.15$, 3 H); 0.99 (*d*, $J = 6.47$, 3 H); 0.89 (*d*, $J = 7.36$, 3 H). EI-MS: 294 (1, M^+), 266 (12, $[M - CO]^+$), 265 (36, $[M - CHO]^+$), 251 (12, $[M - Pr]^+$), 237 (100, $[M - CHO - CO]^+$).

*(3*R*,3a*S*,6*R*,7*S*)-Hexahydro-3,6-dimethyl-7-(4-methyl-3-oxopentyl)-2-oxobenzofuran-7a(2*H*)-carboxaldehyde* (**16b**): Yield 21%. Colorless oil. IR (film): 2933, 2860, 2720 (C(O)–H), 1778 (C=O, lactone), 1732 (C=O, aldehyde), 1705 (C=O, ketone), 1466, 1383, 1342, 1182, 1032, 955, 926, 878, 733. ^{1}H-NMR ($CDCl_3$, 400 MHz): 9.75 (*s*, 1 H); 2.63–2.51 (*m*, 2 H); 2.49–2.37 (*m*, 2 H); 1.92–1.64 (*m*, 4 H); 1.64–1.54 (*m*, 2 H); 1.50–1.40 (*m*, 1 H); 1.24–1.19 (*m*, 2 H); 1.11 (*d*, $J = 7.01$, 3 H); 1.07 (*d*, $J = 6.88$, 6 H); 1.00 (*d*, $J = 6.46$, 3 H). EI-MS: 294 (1, M^+), 266 (20, $[M - CO]^+$), 265 (40, $[M - CHO]^+$), 251 (24, $[M - {}^i\text{Pr}]^+$), 237 (100, $(M - CHO - CO]^+$).

*(3*R*,3a*S*,6*R*,6a*S*)-Octahydro-3,6-dimethyl-9-(2-methylpropyl)-9,12-epoxy-12*H*-furo[3,2-*k*][2,3]benzodioxocin-2(3*H*)-one* (**10d**): Yield 30%. White needles. M.p. 150–152° (from AcOEt/petroleum ether). IR (KBr): 2959, 2860, 1770 (C=O), 1468, 1359, 1219, 1188, 1146, 1119, 1036, 995, 957, 922. ^{1}H-NMR ($CDCl_3$, 400 MHz): 5.34 (*s*, 1 H); 2.84 (*quint.*, $J = 7.25$, 1 H); 2.34 (*q*, $J = 6.56$, 1 H); 2.28–2.14 (*m*, 1 H); 2.04 (*dt*, $J = 15.99$, 3.80, 1 H); 1.91–1.80 (*m*, 1 H); 1.76–1.60 (*m*, 5 H); 1.60–1.56 (*m*, 2 H); 1.54–1.46 (*m*, 1 H); 1.44–1.31 (*m*, 1 H); 1.17 (*d*, $J = 7.29$, 3 H); 1.15–1.07 (*m*, 1 H); 0.98 (*d*, $J = 6.46$, 3 H); 0.94 (*d*, $J = 6.60$, 6 H). Anal. calc. for $C_{18}H_{28}O_5$ (324.42): C 66.64, H 8.70; found: C 66.97, H 8.37.

*(3*R*,3a*S*,6*R*,6a*S*)-9-Benzyl-octahydro-3,6-dimethyl-9,12-epoxy-12*H*-furo[3,2-*k*][2,3]benzodioxocin-2(3*H*)-one* (**10e**): Yield 15%. White needles. M.p. 148–150° (from AcOEt/petroleum ether). Yield: 15%. IR (KBr): 2945, 2860, 1772 (C=O), 1498 (C=C), 1456, 1244, 1186, 1146, 1113, 1086, 1003, 957, 754. ^{1}H-NMR ($CDCl_3$, 300 MHz): 7.38–7.20 (*m*, 5 H); 5.35 (*s*, 1 H); 3.06 (*s*, 2 H); 2.81 (*quint.*, $J = 7.08$, 1 H); 2.28 (*q*, $J = 6.74$, 1 H); 2.19–2.03 (*m*, 1 H); 1.95 (br. *d*, $J = 14.58$, 1 H); 1.80–1.58 (*m*, 5 H); 1.47–1.37 (*m*, 1 H); 1.35–1.23 (*m*, 1 H); 1.15 (*d*, $J = 7.15$, 3 H); 1.11–1.02 (*m*, 1 H); 0.95 (*d*, $J = 6.46$, 3 H). Anal. calc. for $C_{21}H_{26}O_5$ (358.43): C 70.37, H 7.31; found: C 70.72, H 7.14.

*(3*R*,3a*S*,6*R*,7*S*)-Hexahydro-3,6-dimethyl-2-oxo-7-(3-oxo-4-phenylbutyl)benzofuran-7a(2*H*)-carboxaldehyde* (**16c**): Yield 12%. Colorless oil. IR (film): 3100, 3030, 2929, 2860, 2720 (C(O)–H), 1778 (C=O, lactone), 1732 (C=O, aldehyde), 1713 (C=O, ketone), 1600, 1497 (C=C, Ar), 1454, 1381, 1182, 1032, 953, 735, 702. ^{1}H-NMR ($CDCl_3$, 400 MHz): 9.66 (*s*, 1 H); 7.35–7.17 (*m*, 5 H); 3.67 (*s*, 2 H); 2.66–2.50 (*m*, 2 H); 2.50–2.30 (*m*, 2 H); 1.90–1.60 (*m*, 6 H); 1.44–1.34 (*m*, 1 H); 1.10 (*d*, $J = 7.14$, 3 H); 1.07–0.95 (*m*, 1 H); 0.91 (*d*, $J = 6.46$, 3 H). EI-MS: 342 (10, M^+), 314 (30, $[M - CO]^+$); 285 (44, $[M - CO - CHO]^+$), 251 ([64, $M - Bn]^+$), 223 (60, $[M - CO - Bn]^+$), 195 (56, $[M - PhCH_2CO - CO]^+$), 179 (88, $[M - Bn - CH(Me)COO]^+$), 91 (100, Bn^+).

4. *Mice and* Schistosoma *Parasites.* Mice (Kunming strain) of both sexes, weighing 18–24 g, were purchased from the *Shanghai Animal Centre*, *Chinese Academy of Sciences*, Shanghai, China. They were maintained on a rodent food and H_2O *ad libitum*. An Anhui isolate of *S. japonicum*, released from laboratory-bred Oncomelania hupensis snails, was provided by *Shanghai Institute of Animal Parasitology, Chinese Academy of Agricultural Sciences*. Each mouse was infected with 50–55 cercariae via the shaved abdominal skin.

5. *Assessment of Drug Effects.* Ozonides **10a**–**e** were suspended in 1% (carboxymethyl)cellulose (CMC) soln. All reagents used were of anal. grade. To five mice as a group, at 30 days post-infection, the ozonides were administered intragastrically at a one-off dose of 300 mg/kg. At 30 days post-medication, mice were killed by exsanguination, and the *Schistosoma* were collected by perfusion with ice-cold HBSS from the mesenteric veins and livers. Worm motor activity, alterations in the tegument, damage to the gut, and parasite survival were assessed by careful examination of the worms under an inverted microscope. Parasite death was defined as no motor activity during 2 min of observation and extensive tegumental alterations, i.e., > 50% of the tegument characterized by vesiculation, collapse, and peeling.

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