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Stable Tricyclic Antitubercular Ozonides Derived from Artemisinin

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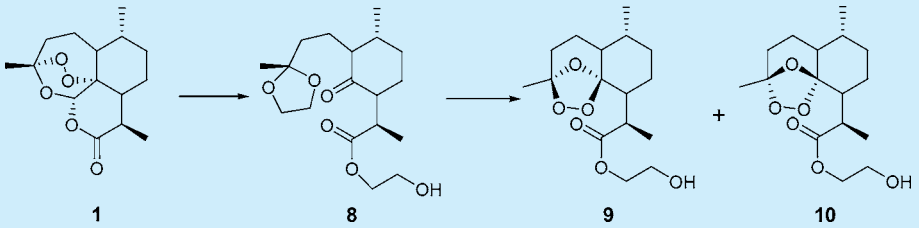
Supporting Information



ABSTRACT: New, highly stable tricyclic antitubercular ozonides **9** and **10** derived from artemisinin are reported in 39 and 9% yields, respectively. The ozonide groups of **9** and **10** were found to be stable under strong basic and acidic conditions. The absolute configuration of ozonides **9** was confirmed by X-ray crystallography. Ozonide **10** shows promising antitubercular activity against *M. tuberculosis* $H_{37}Ra$ and *M. tuberculosis* $H_{37}Rv$ with MIC values of 0.39 and 3.12 μg/mL, respectively.

Artemisinin **1**[1] is a sesqueterpene lactone endoperoxide and has a unique tetracyclic framework having the pharmacophoric 1,2,4-trioxane ring responsible for antimalarial activity.[2] In addition to providing a series of antimalarial drugs, such as dihydroartemisinin **2**, artemether **3**, arteether **4**, and artesunic acid **5** (Figure 1), to combat multidrug-resistant malaria, the discovery has also revealed that the malarial parasite can be selectively killed by providing an additional oxidative stress in the form of a peroxide molecule.[3]

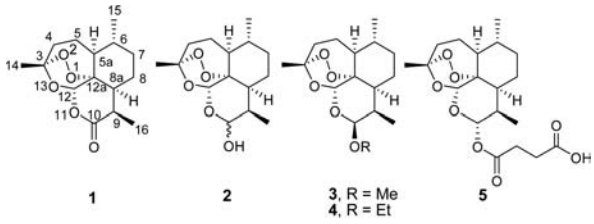


Figure 1. Artemisinin **1** and its derivatives **2**−**5**.

Apart from antimalarial activity, several other biological activities, such as anti-infective,[4] antifungal,[5] anticancer,[6] antiproliferative,[5] antiinflamatory,[5] and antiarrhythmic[7] activities, have also been reported in artemisinin derivatives.

Organic peroxides, such as 1,2-dioxolanes, endoperoxides, and 1,2,4,5-tetraoxanes, possess antitubercular activity.[8] Recently, the first example of mycobactin-artemisinin conjugate **6** has been shown to possess dual potency against tuberculosis and malaria.[9]

In addition, mixed tetraoxanes **7a** and **7b** not only shown promising antimalarial activity against *P. falciparum* in vitro but also exhibit significant antitubercular activity (Figure 2).[10] Thus, it can be speculated that these activities in artemisinin are due to the presence of the peroxide linkage.

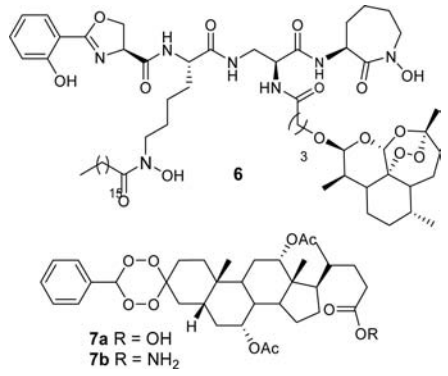


Figure 2. Structures of peroxides having antimalarial and antitubercular activity.

For the last two decades, 1,2,4-trioxolane (ozonide) has been identified as an important scaffold for drug discovery research because ozonides are endowed with a wide range of biological activities.[11] Several naturally occurring and synthetic ozonides are known to possess antimalarial,[11a−d] antibacterial,[11e] antischitosomal,[11f] and *F. hepatica*[11g] activities. In general, ozonides are prepared via ozonolysis of olefins, but few

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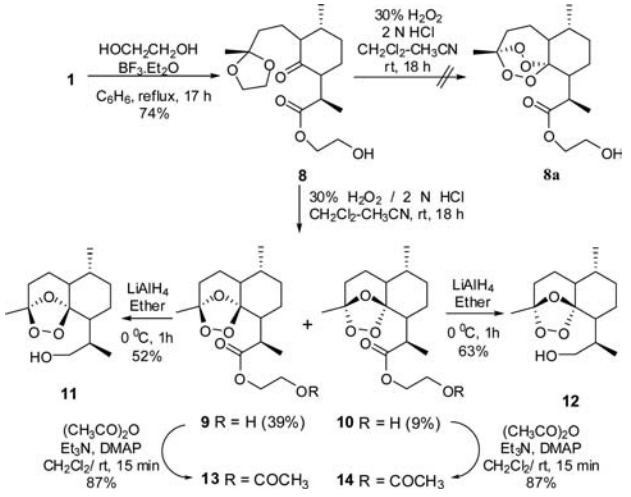
instances are reported in the literature to prepare ozonides via H_2O_2 treatment on diketo compounds.[12]

Earlier, we had emphasized the importance of tetracyclic framework of artemisinin, which clearly indicates its intrinsic antimalarial activity.[13] To better understand the importance of each group and the stereochemistry of each chiral center and to further study the groups responsible for antimalarial activity in artemisinin, we planned to replace the 1,2,4-trioxane moiety of artemisinin with an ozonide moiety and then study its effect on antimalarial activity. Herein, we report new ozonide chemistry in artemisinin, albeit with high cost. For the first time, we report the synthesis of tricyclic antitubercular ozonides. We also report the synthesis of 1,2,4-trioxolane alcohols.

To test these ideas, we treated compound **8**, easily accessible in a single step and in good yield from artemisinin,[14] with 30% H_2O_2/2 N HCl using a mixture of DCM and CH_3CN as solvents to furnish a diastereomeric mixture of ozonides **9** and **10**.[15] Interestingly, tetraoxane **8a** was not detected (Scheme 1).[15,16] After column chromatography, the structures of these

Scheme 1. Synthesis of Diastereomeric Ozonides 9 and 10



ozonides were characterized by ^{1}H NMR, ^{13}C NMR, MASS, and elemental analysis. Although the synthesis of ozonides structurally related to artemisinin has been reported previously,[11f] this is the first example of conversion of artemisinin to its tricyclic ozonide analogues.

The high stability of ozonides **9** and **10** is confirmed by their formation under 2 N HCl conditions. Moreover, these ozonides can withstand temperatures up to 65 °C.

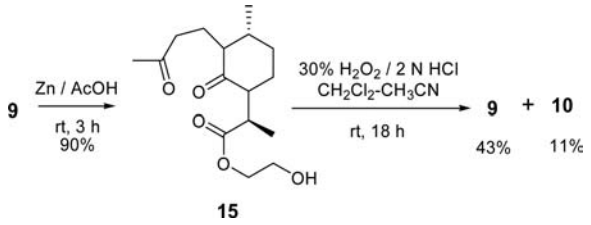
Reduction of ozonides **9** and **10** with $LiAlH_4$ furnished ozonide alcohols **11** and **12**, respectively, which also confirms high stability of these tricyclic ozonides toward strong basic conditions (Scheme 1).

Ozonide **9** on reaction with Zn/AcOH furnished diketoester **15**, which on treatment with H_2O_2/HCl furnished the same mixture of diastereomeric ozonides **9** and **10**, thus confirming that the two ozonides differ in stereochemistry only at carbons linked with the peroxy group (Scheme 2).

Ozonides **9** and **10** on reaction with Ac_2O/Et_3N furnished corresponding acetates **13** and **14**, respectively, of which only compound **13** furnished crystals that were sufficient to be analyzed by X-ray crystallography.

The structure of ozonide **13** was confirmed by single crystal X-ray diffraction analysis.[17] The ORTEP view of the molecule

Scheme 2. Stereochemical Assignment of Diastereomeric Ozonides 9 and 10



(at 30% probability) with atomic numbering is depicted in Figure 3. The molecule consists of a fused tricyclic ring system

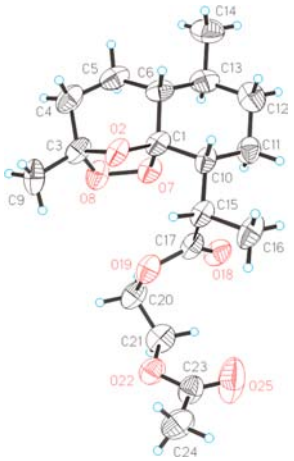


Figure 3. ORTEP diagram (at 30% probability) of ozonide **13**.

(two six-membered and one five-membered) having a peroxide bridge. Both the six-membered rings exist in chair conformation whereas the ozonide ring adopts an envelope conformation.

A probable mechanism for the formation of ozonides **9** and **10** from ketoester **8** via α-hydroxyhydroperoxide intermediate **16** is illustrated in Figure 4.[18]

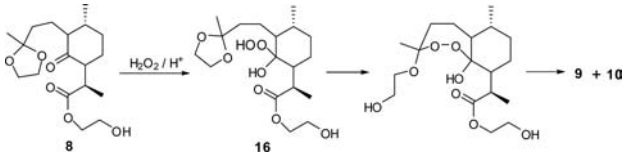


Figure 4. Possible mechanism for the formation of ozonides **9** and **10**.

A preliminary in vivo screening of ozonides **9** and **10** and their derivatives **11**−**14** against multidrug-resistant *P. yoelii nigeriensis* at a dose of 96 mg/kg × 4 days in Swiss mice was assessed.[19] These ozonides were found to be less active than **1** and **2** as antimalarials. This further confirms the importance of 1,2,4-trioxanes as pharmacophores for antimalarial activity and also emphasizes the necessity of the tetracyclic ring system present in artemisinin (Table 1).

Artemisinin possesses antitubercular activity;[9] thus, ozonides **9** and **10** were also evaluated for antitubercular activity against avirulent strain *M. Tuberculosis* H_{37}Ra (using MABA method)[21] and against virulent strain *M. Tuberculosis* H_{37}Rv (using agar Proportion Assay)[22] at concentrations ranging from 0.39 to 12.5 μg/mL (Table 1). Of the two stereoisomers (**9** and **10**), stereoisomer **10** showed antitubercular activity against *M.*

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Table 1. Antimalarial and Antitubercular Activity of Ozonides 9 and 10

compound	in vivo antimalarial activity against *P. yoelii nigeriensis* (cured/treated)[20]	MABA MIC (μg/mL) against *M. tuberculosis* $H_{37}Ra$	agar microdilution MIC (μg/mL) against *M. tuberculosis* $H_{37}Rv$
9	0/5	12.5	inactive[a]
10	0/5	0.39	3.12
1	5/5		
2	5/5		
Rifampicin		0.1	0.20
Ethambutol		nd[b]	2.0

[a]Inactive = >12.5. [b]Not done (nd).

Tuberculosis $H_{37}Ra$ and *M. Tuberculosis* $H_{37}Rv$ with MIC values of 0.39 and 3.12 μg/mL, respectively (Table 1).

In conclusion, our study uncovered tricyclic antitubercular ozonides **9** and **10**, which were prepared from the antimalarial agent artemisinin. These ozonides were found to be chemically stable under strong acidic and basic conditions. Most importantly, these ozonides show good antitubercular activity comparable to that of the clinically available antitubercular drugs Rifampicin and Ethambutol in vitro. This finding opens a new area in the chemotherapy of tuberculosis. The difference in biological activity displayed by the two stereoisomers and understanding the exact mechanism of action, importance of stereochemistry of peroxide linkage, and their proper structure–activity relationship will be reported elsewhere in due course.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b02296.

Experimental details and characterization data, purity/characterization table, ^{1}H NMR and ^{13}C NMR spectra of compounds **9**–**15** (PDF)

CIF file for compound **13** (CIF)

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Notes

The authors declare no competing financial interest.

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(16) The possibility of the formation of tetraoxane **8a** cannot be ruled out.
(17) Crystal data for **13**: $C_{18}H_{28}O_7$, monoclinic, M = 356.4, $P2_1$, a = 9.874(1), b = 10.305(2), c = 18.765(3) Å, β = 99.09(1)°, V = 1885.4(5) Å^3, T = 293(2) K, Z = 4, D_c= 1.256 gcm^{-3}, μ = 0.096 mm^{-1}, λ (Mo K_α) = 0.71073 Å, $F_{(000)}$ = 768, reddish block, crystal size 0.250 × 0.125 × 0.050 mm, 4528 reflections measured (R_{int} = 0.0844), 3874 unique, $R1$= 0.0731 for 2050 $Fo > 4\sigma(Fo)$, and 0.1535 for all 3874 data, S = 1.047 for all data, 1 restraints and 460 parameters. The determination of unit cell and intensity data collection (2θ = 50.02°) was performed using a Bruker P4 diffractometer at 293(2) K. Structure solutions were resolved by direct methods and refinements were resolved by the full-matrix-least-squares methods on F^2. Programs: XSCANS (Siemens Analytical X-ray Instruments Inc., Madison, Wisconsin, USA, 1996) was used for data collection and data processing, and SHELXTL-NT (Bruker AXS Inc., Madison, Wisconsin, USA, 1997) was used for the determination of structure, refinements and molecular graphics. Crystallographic data (excluding structure factors) for the structures in this manuscript have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 658109. Copies of the data can be obtained, without any charge, upon application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: + 44 1223 336033 or email: deposit@ccdc.cam.ac.uk).
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