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Synthesis and antimalarial activity of 3′-trifluoromethylated 1,2,4-trioxolanes and 1,2,4,5-tetraoxane based on deoxycholic acid

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ABSTRACT

A series of new steroidal peroxides – 3′-trifluoromethylated 1,2,4-trioxolanes and 1,2,4,5-tetraoxanes based on deoxycholic acid were prepared via the reactions of the Griesbaum coozonolysis and peroxycondensation, respectively. 1,2,4-Trioxolanes were synthesized by the interaction of methyl *O*-methyl-3-oximino-12α-acetoxy-deoxycholate with $CF_3C(O)CH_3$ or $CF_3C(O)Ph$ and O_3 as the mixtures of four possible stereoisomers at ratios of 1:2:2:1 and in yields of 50% and 38%, respectively. The major diastereomer of methyl 12α-acetoxy-5β-cholan-24-oate-3-spiro-5′-(3′-methyl-3′-trifluoromethyl-1′,2′,4′-trioxolane) was isolated via crystallization of a mixture of stereoisomers from hexane and its (3*S*,3′*R*)-configuration was determined using X-ray crystallographic analysis. Peroxycondensation of methyl 3-bishydroperoxy-12α-acetoxy-deoxycholate with $CF_3C(O)CH_3$ or acetone led to 1,2,4,5-tetraoxanes in yields of 44% and 37%, respectively. Antimalarial activity of these new steroidal peroxides was evaluated *in vitro* against the chloroquine-sensitive (CQS) T96 and chloroquine-resistant (CQR) K1 strains of *Plasmodium falciparum*. Deoxycholic acid 3′-trifluoromethylated 1,2,4,5-tetraoxane demonstrated a good IC_{50} value against CQR-strain (IC_{50} (K1) = 7.6 nM) of *P. falciparum*. Tetraoxane with the acetone subunit demonstrated the best results among all tested peroxides with an IC_{50} value of 3 nM against the CQ-resistant K1 strain. In general, 1,2,4-trioxolanes of deoxycholic acid are less active than 1,2,4,5-tetraoxanes.

1. Introduction

The interest in chemical and pharmacological properties of natural metabolites whose molecules contain a peroxide structural fragment [1–3] has sharply increased after the discovery of a unique antimalarial activity of artemisinin [4]. Since then, the development of drugs for treating malaria based on peroxide compounds has proceeded along three main avenues: synthetic transformations of artemisinin [5], development of new methods [6] and synthesis of peroxide compounds of different structural types [7–9], study on physicochemical properties and mechanisms of the antimalarial action of 1,2,4-trioxolanes and 1,2,4,5-tetraoxanes [10–14]. Advances have been achieved along these lines. As a result, notable compounds based on 1,2,4-trioxolane and 1,2,4,5-tetraoxane pharmacophores have been investigated with enhanced antimalarial properties as compared to artemisinin [15] (Fig. 1). In 2012, ozonide OZ277, also known as arterolane maleate, was approved for marketing in India as a combination product with piperaquine phosphate (Synriam) to treat malaria [16]. Several synthetic peroxides are under development, and regarding OZ439 [10] and RKA182 [17] the most accurate results have to be achieved to provide a new form of antimalarial therapies. Recently, O'Neill et al. has reported the synthesis of a series of antimalarial aryloxy 1,2,4,5-tetraoxanes, among which the compound E209 displays nanomolar efficacy against multiple strains of *P. falciparum* and *P. vivax* and overcomes PfK13-C580Y dependent artemisinin resistance [18,19].

A promising avenue is the search for new platforms to introduce cyclic peroxide groups. Steroids and their derivatives possessing different biological properties [20] still represent a remarkable scaffold in antimalarial drug design. Opsenica and Šolaja have synthesized a new class of steroid derivatives (1,2,4,5-tetraoxanes) based on cholic acids (Fig. 1) [21]. An investigation into the antimalarial, antitubercular and antitumor properties showed that the use of cholic acid as the 1,2,4,5-

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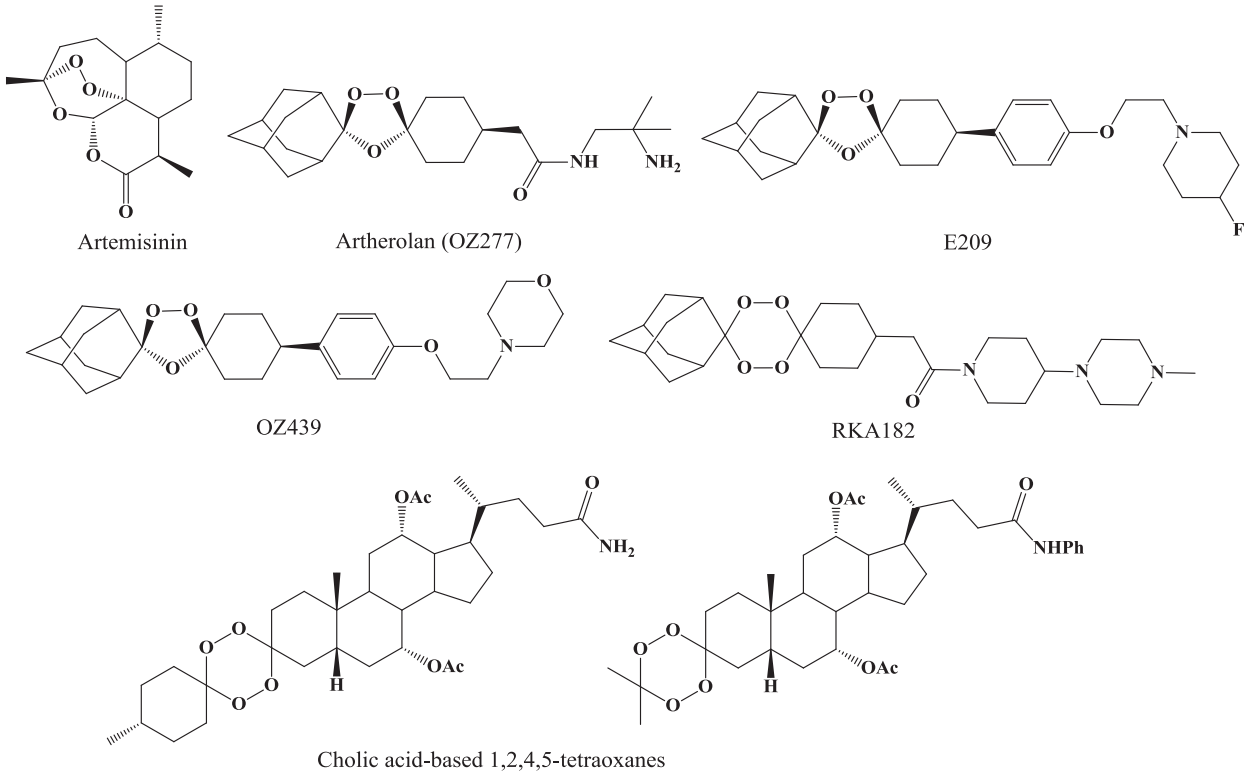

Fig. 1. The structures of artemisinin, arterolan (OZ277), 1,2,4-trioxolane OZ439, 1,2,4,5-tetraoxanes RKA182 and E209, and cholic acid-based 1,2,4,5-tetraoxanes.

tetraoxane carrier had improved the pharmacological profile of the diperoxides produced.

There are some previous publications devoted to the comparison of pharmacological properties of structurally analogous adamantane-based 1,2,4-trioxolanes, 1,2,4,5-tetraoxanes and 1,2,4-trioxanes [22,23]. They showed that ozonide and diperoxide isosteres possessed relatively similar *in vitro* and *in vivo* profiles. However, no research of that kind has been undertaken on cholic acid-based 1,2,4,5-tetraoxanes. This is most probably due to the fact that the introduction of 1,2,4-trioxolane fragments into the structure of complex polycyclic compounds still remains a non-trivial task because of high affinity of reaction intermediates to rearrangements [24–31]. Recently, we have reported the first synthesis of lithocholic acid-based ozonides via the Griesbaum coozonolysis [32] that opens the possibility of applying this method in principle to natural steroids or triterpenoids. Thus, the aim of this work is to synthesize deoxycholic acid-based 1,2,4-trioxolanes via the Griesbaum coozonolysis of steroidal *O*-methylated oxime and carbonyl compounds with CF_3-moiety, with subsequent investigations into antimalarial properties as compared to homologous 1,2,4,5-tetraoxanes.

2. Experimental

Melting points were determined on a Boetius apparatus. Specific rotations were determined on a Perkin-Elmer 241 MC polarimeter at a given temperature. ^{1}H and ^{13}C NMR-spectra were recorded using a Bruker AM-300 spectrometer (at 300 and 75.5 MHz, respectively) in deuterated chloroform as the solvent and using TMS as the internal standard. Chemical shifts are expressed in ppm (δ) values and coupling constants (J) in Hz. The elemental analysis was carried out using a Euro EA-3000 CHNS analyzer, with acetanilide as the basic standard. Individuality and purity of the synthesized compounds were controlled using thin-layer chromatography (TLC). TLC was performed on precoated silica-gel "Sorbfil" plates using 10% H_2SO_4solution with subsequent heating to 100 °C to indicate peroxides, eluents $CHCl_3$ – EtOAc (40:1). Deoxycholic acid, $CF_3C(O)Ph$, $CF_3C(O)CH_3$ and CH_3ONH_2·HCl were commercial products (Aldrich). Ozone was generated with the aid of an Ozon-4K ozonizer. The presence of peroxide ozonolysis products was checked by the potassium iodide test. The XSA was performed on a Bruker SMART APEX II diffractometer. Methyl 3-oxo-12α-acetoxy-5β-cholan-24-oate **1** and methyl 3,3-dihydroperoxy-12α-acetoxy-5β-cholan-24-oate **6** were synthesized according to [33] and [34], respectively.

2.1. General procedure for synthesis of compound 2

CH_3ONH_2·HCl (0.17 g, 2 mmol) was added to the solution of compound **1** (0.45 g, 1 mmol) in a mixture of pyridine and dry methanol (30 mL, 1:1, v/v). The reaction mixture was refluxed for 8 h with a back condenser, cooled to room temperature and quenched with 5% HCl (150 mL). The precipitate was filtered off, washed with water and air-dried.

2.1.1. Methyl O-methyl-3-oxyimino-12α-acetoxy-5β-cholan-24-oate (2)

White amorphous residue; Yield 0.43 g (90%) as a mixture of *syn*- and *anti*-isomers; $[\alpha]_D^{20}$ + 94° (*c* 0.075, $CHCl_3$); R_f 0.26; IR ($CHCl_3$) υ_{max}: 2937, 2870, 1729, 1446, 1377, 1245, 1193, 1167, 1053, 1028, 875, 754 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.73, 0.92 (2s, 6H, $2CH_3$), 0.79 (d, 3H, J = 6.09 Hz, H-21), 0.99–2.50 (m, 24H, CH, CH_2), 2.04 (s, 3H, OAc), 2.99 (dd, 2H, ^1J = 14.7 Hz, ^2J = 15.7 Hz, H-2), 3.64 (s, 3H, OCH_3), 3.79 and 3.80 (s, 3H, −$NOCH_3$, 1:1), 5.08 (wide s, 1H, H-12); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 12.4 (C-18), 17.5 (C-21), 20.3 (C-26), 21.3 (C-19), 22.7 and 22.8 (C-2, 3:2), 23.4 (C-15), 25.5 and 25.6 (C-7, 2:3), 25.8 (C-11), 26.4 and 26.7 (C-6, 1:1), 27.3 (C-16), 30.8 (C-22), 30.9 (C-23), 32.1 (C-4), 34.3 and 34.5 (C-1, 3:2), 34.7 and 34.8 (C-9, 3:2), 35.4 and 35.8 (C-10, 3:2), 36.7 (C-20), 42.4 (C-8), 43.8 (C-5), 45.0 (C-13), 47.6 (C-17), 49.4 (C-14), 51.5 (C-27), 61.0 (C-28, $NOCH_3$), 75.8 (C-12), 160.5 and 160.7 (C-3, 3:2), 170.4 (C-25, OAc), 174.6 (C-24). Anal. Calcd. for $C_{28}H_{45}NO_5$: C, 70.70; H, 9.54; N, 2.94. Found: C, 71.16; H, 9.36; N, 3.01.