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Steroidal Tetraoxanes - New Class of Antitumor Compounds

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Abstract: Cholic acid derived steroidal tetraoxanes possessing methyl ester, carboxylic acid, primary and secondary amide termini were synthesised and their *in vitro* antitumor activity against Fem-X and HeLa cell cultures was determined. IC_{50} range between 3.1 and 166 μM. Five out of seven tested compounds exhibited cell rounding and / or apoptic activity. Their liquid secondary ionization (LSI) and electronspray ionization (ESI) spectra confirmed the tetraoxane structure.

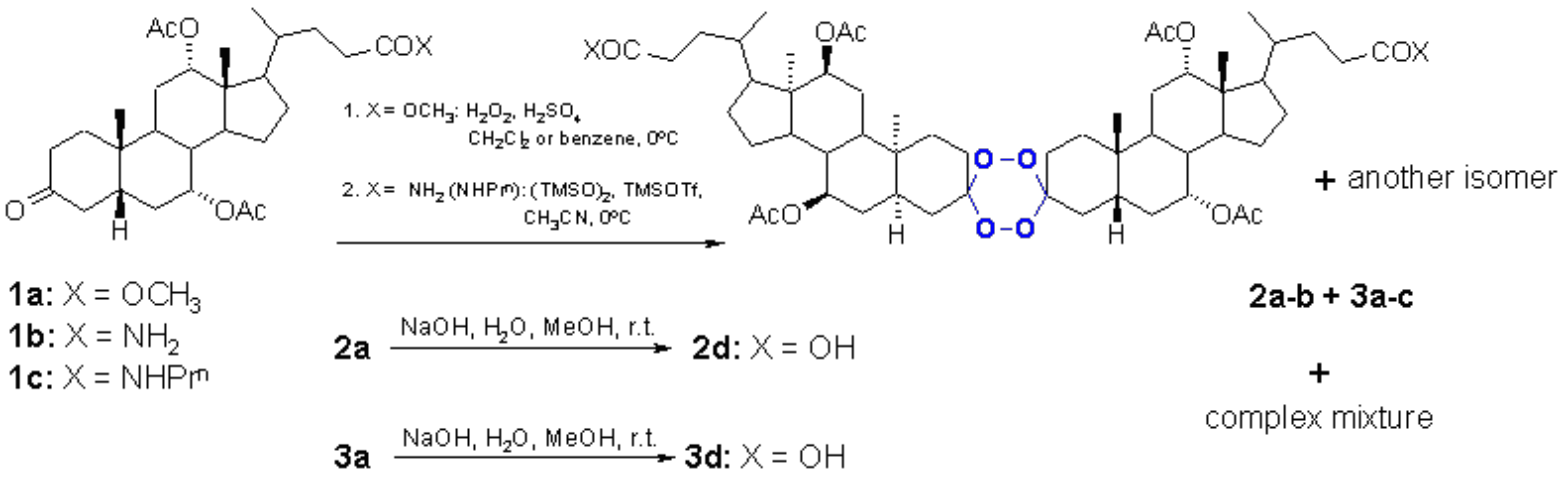
Keywords: Steroids, cholic acid, tetraoxanes, LSIMS, ESI, antitumor activity.

Introduction

1,2,4,5-Tetraoxanes are a class of compounds usually synthesised for ring-enlargement purposes [1], and more recently, for their established antimalarial activity [2]. Activity of 1,2,4,5-tetraoxanes against *Plasmodium falciparum* D6 and W2 clones is very similar to that of trioxanes such as naturally occurring artemisinin, its semi-synthetic derivatives [3] and related compounds [4]. Cholestane based steroidal tetraoxanes were prepared [5] in order to explore the influence of bulky substituent(s) on 1,2,4,5-tetraoxane ring on their activity, as well as the possible influence of natural carrier. So far no report on tetraoxane antitumor activity has been published.

Here, we present the results of our further studies on steroidal tetraoxanes, the synthesis, (preliminary) structure determination and *in vitro* antitumor activity of some of cholic acid derived tetraoxanes [6].

Scheme 1



Results and Discussion

Tetraoxanes **2a** (15%, 250-251 ^{o}C) and **3a** (13%, mp 167-171 ^{o}C) were synthesised from parent ketone **1a** (X = OCH_3) using procedure given in [5], Scheme 1. The acids **2d** (56%, mp 206-210 ^{o}C) and **3d** (47 %, mp 189-192 ^{o}C) were obtained by alkali hydrolysis of corresponding esters. Tetraoxanes **2b** (26%, mp 211-217 ^{o}C), **3b** (24%, mp 196-199 ^{o}C) and **3c** (20%, mp 171-174 ^{o}C) were prepared using $(TMSO)_2$ / TMSOTf method of Jefford et al. [7]. In each case (except hydrolysis reaction) the complex mixture of products was obtained, from which the given tetraoxanes were obtained by chromatography on SiO_2 column. Two isomeric methyl esters (**2a** and **3a**, hence two acids **2d** and **3d**) were isolated, as well as a pair of primary amides (**2b** and **3b**). Propyl amide **1c** afforded only tetraoxane **3c**. The stereochemistry of steroid substituents at 1,2,4,5-tetraoxane ring is not determined as yet (lowest energy conformations of the pair of stereoisomers is shown in Figure 1 (X = OCH_3)). The ^{1}H and ^{13}C NMR spectra (vide infra) of each pair are almost identical (at the level of 200 MHz spectrometer), but clear distinction between tetraoxanes within each pair can be made based on the appearance of corresponding acetate methyl groups (compare ^{1}H NMR spectra, Figure 2). In one series, the acetate methyls appear as broad singlet (at ca. 2.10 ppm), in the another, as broad singlet (at ca. 2.12 ppm) followed by another smaller broad singlet at 2.07 ppm. Tetraoxane **3c** belongs to the second group. Liquid secondary ionization and electrospray ionization mass spectra of the above compounds resulted in molecular ion peaks in all of the cases. These experiments along with the LSIMS high resolution probe accurate mass measurements confirmed that we are dealing with tetraoxanes and not with hexaoxanes [8], Table 1.

The effect of tetraoxanes **2a**-**3d** on Fem-x and HeLa cell survival, 72 h after the agent's action is given in Table 2. Investigated compounds expressed the dose dependent antiproliferative action toward investigated cell lines. In order to compare the extent of the antiproliferative action between the members of this group, IC_{50} were determined under exactly the same conditions. They were similar for Fem-X and HeLa cells with exemption for **3c**. This compound inhibited selectively more melanoma then HeLa cell growth. Morphological examination of target cells on inverted microscope showed that cytotoxic action of **3a**, **2b**, **3b**, **2d** and **3d** resulted in target cell rounding and / or cell fragmentation to apoptic bodies, typical for action of known cytostatic cis-diamminedichloroplatinum(II), an apoptosis inducing agent. Tetraoxane **3c** inhibited HeLa cell growth without the signs of direct cell cytotoxicity up to 66 μM.

Figure 1. Computer generated structures of two possible diastereomeric tetraoxanes (X = OCH_3)

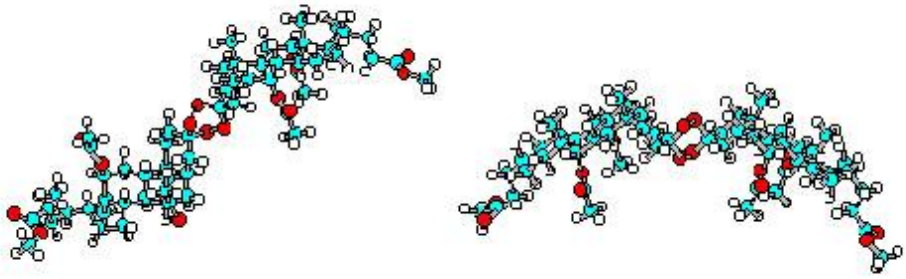
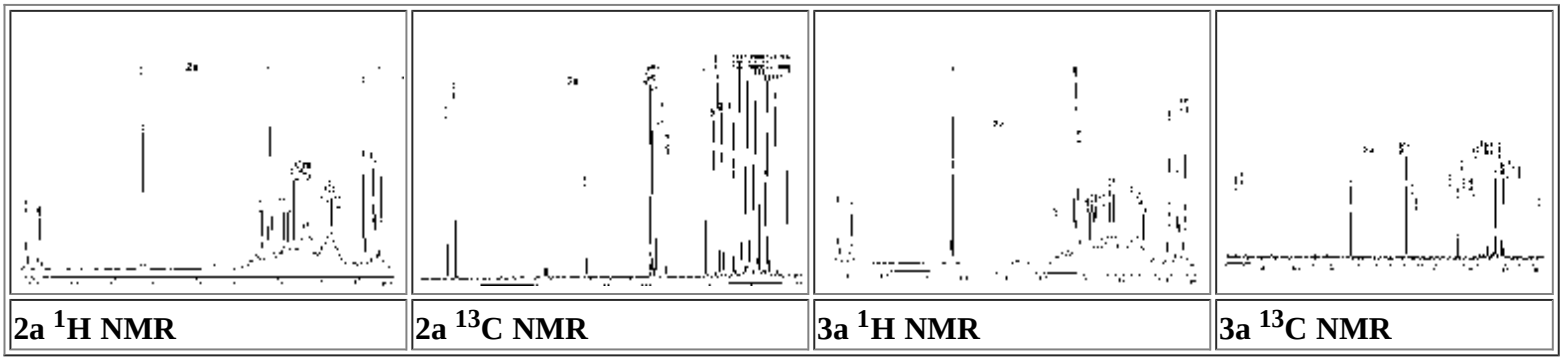


Figure 2. ^{1}H and ^{13}C NMR spectra of **2a** and **3a**, respectively.



2a ^{1}H NMR	**2a ^{13}C NMR**	**3a ^{1}H NMR**	**3a ^{13}C NMR**

Table 1. MS data for tetraoxanes **2a-3d**

Compound	**[M+H]+** **[M+Na]$^{+}$**	**ESI** Spectra were recorded on a Autospec instrument in positive ion mode using CH_3CN : H_2O (1:1) with 1 % AcOH as carrying solvent solution. The stock solutions of the samples were further diluted using the same buffer. The source temperature was 75 ^{o}C, and the cone voltage was set to 40 V.
2a	1041.7 (10) **1063.8 (8)**	981.6 (4), 921.7 (14), 597.0 (13), 579.0 (20), 537.4 (61), 477.3 (50), 417.3 (18), 238.0 (60), 197.0 (100), 178.2 (54), 149.0 (35)
3a	1041.7(100) **1063.8 (10)**	981.7 (15), 921.7 (23), 861.7 (4), 537.4 (70), 477.4 (82), 385.3 (18), 238.1 (16), 197.0 (26), 178.2 (23), 149.0 (14)
		LSIMS LSI mass spectra were recorded on a VG-ZAB-T instrument using ceasium ion gun. Accelerating voltage was set to 8 kV ising MNBA as matrix. Probe accurate mass measurements were performed in the presence of PEG internal callibrant at 5000 resolution.
2d	**1035.6 (43)**	1012.6 (14), 993.6 (23), 969.6 (19), 951.6 (34), 891.5 (100), 849.5 (29), 794.0 (26), 780.0 (45), 766.1 (27), 749.1 (29), 735.0 (66%), 719.0 (34), 691.0 (36), 674.0 (47), 658.0 (42), 643.0 (53), 631.0 (58)
3d	1013.5(21) **1035.6 (100)**	1011.4 (17), 993.5 (27), 951.4 (41), 909.4 (11), 893.4 (51), 849.5 (12), 833.4 (17), 779.9 (14), 734.9 (17), 699.5 (15), 673.9 (13), 644.9 (16), 628.9 (15)
2b	1011.6 (100)	967.7 (17), 909.6 (16), 891.6 (29), 851.6 (15), 793.6 (17), 766.4 (20), 735.5 (20), 677.5 (24), 647.5 (15), 613.2 (95), 577.4 (14), 561.4 (20)
3b	1011.6 (80)	967.6 (6), 919.3 (10), 851.6 (6), 766.2 (14), 735.5 (10), 677.5 (11), 613.2 (100), 581.2 (9), 566.2 (11)

3c	1095.8 (100)	1035.8 (5), 967.7 (2), 909.6 (2), 851.7 (2), 793.6 (3), 766.2 (4), 677.5 (3), 613.2 (16)

Table 2. IC_{50} (μM) for the antiproliferative action of tetraoxanes **2a**-**3d** on Fem-X and HeLa cells, determined 72 h after continuous agents' action, by MTT test

	IC_{50} (μM) 72h	
Compound	**Fem-X**	**HeLa**
2a	>29	>29
3a	19.5	20.5
2b	**3.1**	**3.7**
3b	6.2	6.0
3c	94	166
2d	4.2	5.2
3d	7.6	9.2

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