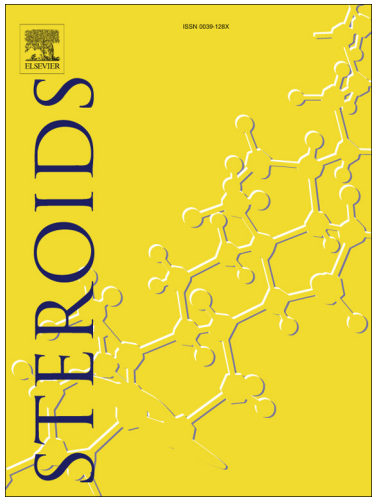

Synthesis and *in vitro* anticancer activity of 23(23')*E*-benzylidenespirostanols derived from steroid sapogenins

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Graphical Abstract

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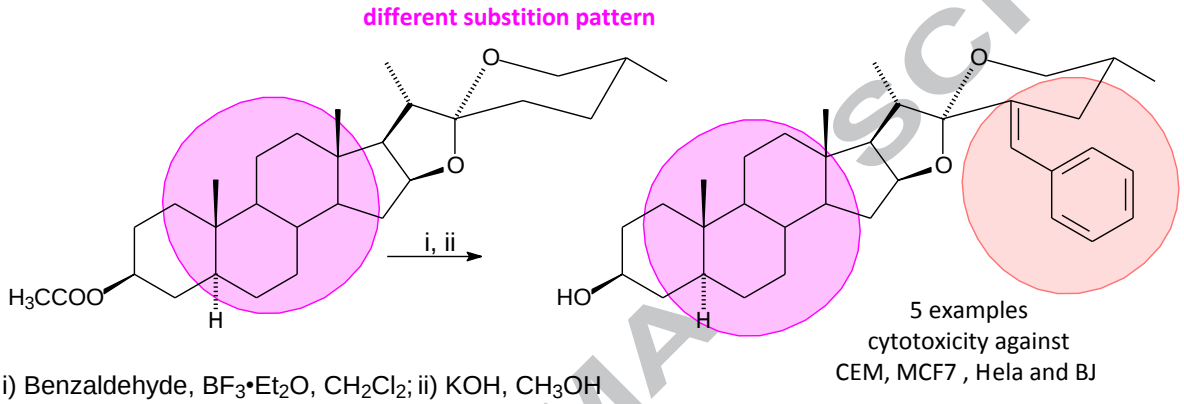

i) Benzaldehyde, BF_3•Et_2O, CH_2Cl_2; ii) KOH, CH_3OH

Synthesis and *in vitro* anticancer activity of 23(23´)*E*-benzylidenespirostanols derived from steroid sapogenins

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Abstract: Benzylidenespirostanols were prepared by two-step synthesis including BF_3•Et_2O-catalyzed aldol condensation of several acetylated steroid sapogenins with benzaldehyde followed by saponification. The obtained compounds showed moderate cytotoxicity against three cancer cell lines (T-lymphoblastic leukemia cell line CEM, breast carcinoma cell line MCF7 and cervical carcinoma cell line HeLa) and normal human fibroblasts (BJ). The most active of the five tested substances was **3c** (lowest IC_{50} for MCF7 cells 19.9 ± 0.1 µM) without any selectivity towards human cancer and normal cells, respectively.

Keywords: Aldol condensation; Benzylidenespirostanols; Cytotoxicity; Cancer cell lines, Fibroblasts

Introduction

Cytotoxic steroids have been the focus of intensive research for more than 30 years. In the main, a large number of naturally occurring steroids is biologically tested to assess their antiproliferative and/or cytotoxic activity against different cancer cell lines *in vitro*. The already large number of naturally occurring cytotoxic steroids is thus continuously increasing. The diversity of these compounds is vast and includes polyhydroxy sterols [1], saponins [2], monomeric spiroketals like steroid sapogenins (SS) [3] and hippuristanols [4] as well as the dimmeric spiroketals, cephalostatins and ritterazines [5] amongst many others.

The comprehensive research directed to identifying potential antitumor agents includes the synthesis of non-natural cytotoxic steroids by introduction of structural modifications into

bioactive natural compounds and *de novo* design and synthesis of new steroid candidate molecules. Both approaches have produced a plethora of synthetic cytotoxic steroids [6].

Previous studies reported that side chain modified steroid sapogenins (SS) showed cytotoxic activity against a wide spectrum of cancer cell lines [7]. Sandoval *et. al*. found that while diosgenin exhibited cytotoxic activity against cervical carcinoma cell line HeLa (IC_{50} 36.17 μM) and CaSki (IC_{50} 31.35 μM), the oxime of 23-acetyldiosgenin showed an increased activity against HeLa (IC_{50} 10.9 μM) and CaSki (IC_{50} 10.51 μM) [7a]. On the other hand Santillan *et. al*. described a set of transformations on the SS side chain that resulted in the synthesis of a family of 23-ethylidene-26-hydroxy-22-oxo-cholestanes that exhibited cytotoxic activity against CEM cells (IC_{50} 5.9 – 40.9 μM) [7b].

Due to the vast structural diversity of cytotoxic steroids, finding accurate SAR is a complicated, if not impossible, task. Nevertheless, the gathered evidence of anticancer activities allows the association of the cytotoxicity with the presence of certain substructures. Fuchs *et. al.* suggested that oxocarbenium ions, formed as a result of the cleavage of spiroketal moieties, may act as alkylating agents of nucleophilic biomolecules and that this underlies the cytotoxicity observed in cephalostatins and ritterazines[8]. Although this hypothesis is yet not confirmed, the large number of cytotoxic steroid spiroketals, as well as the inverse relationship between the cytotoxicity and the enthalpy of oxocarbenium ion formation [8b] suggest that this putative explanation may be, at least, well oriented.

23*E*-Benzylidene spirostanes, a new family of steroid spiroketals derived from the BF_3•Et_2O catalyzed aldol condensation of SS and benzaldehyde, that we recently described

[9a] exhibit a reactivity profile that differ from that of the parent SS and leads to different mode of cleavage of the spiroketal moiety [9b,c]. Bearing in mind that the native steroid sapogenins demonstrated cytotoxicity and that the presence of the 23*E*-benzylidene moiety in the spiroketal side chain produces drastic changes in the reactivity of this fragment, we decided to explore the cytotoxicity of some members of this new derivatives of SS. In this paper, we describe the synthesis and cytotoxic activity against human cancer cells and normal fibroblasts of 23*E*-benzylidenespirostanols derived from various steroid sapogenins.

Experimental

Reactions were monitored by TLC on ALUGRAM® SIL G/UV254 plates from MACHEREY-NAGEL. Chromatographic plates were sprayed with a 1% solution of vanillin in 50% $HClO_4$ and heated until color developed. Melting points were measured on a Melt-Temp II instrument . Mass spectra were obtained from a Thermo-Electron spectrometer model DFS (Double Focus Sector). NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 solutions on a Varian INOVA 400 spectrometer using the solvent signals as reference. NMR signal assignments were carried out with the aid of a combination of 1D and 2D NMR techniques that included ^{1}H, ^{13}C, COSY, Nuclear Overhauser Effect Spectroscopy (NOESY), Heteronuclear Single Quantum Correlation (HSQC) and Heteronuclear Multiple Bond Correlation (HMBC). Compounds **2a** to **2d** were obtained as previously described [9a].

[23(23´)E,25R]-23(23´)-benzylidenspirost-7-en-3β-ol acetate **(2e)**. Freshly distilled BF_3•Et_2O (40.56 ml) was added to solution of the steroid sapogenin 2e (3.10 g, 6.8 mmol)

and benzaldehyde (1.43 g, 13.5 mmol) in dry CH_2Cl_2 (200 ml) and the mixture was stirred under argon at room temperature for 4 h. The reaction mixture was washed with water (10 x 75 ml), dried (anh. Na_2SO_4) and evaporated to produce a syrupy residue. The obtained oil was leached with hot methanol and the solid precipitate was washed with methanol to obtain the desired product. Yield: 1.73 g, 47 %. Mp 177.9 – 179.7 °C*(from acetone)* ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 7.33 (dd, *J*=8.6, 6.7 Hz, 2H, H-meta), 7.25 – 7.19 (m, 3H, H-ortho H-para), 6.57 (d, *J*=1.9 Hz, 1H, H-23´), 5.15 (dd, *J*=4.7, 2.3 Hz, 1H, H-7), 4.70 (tt, *J*=11.3, 4.6 Hz, 1H, H-3α), 4.52 (m, 1H, H-16α), 3.65 – 3.51 (m, 2H, H-26 ax. and H-26 eq.), 2.79 (ddd, *J*=13.4, 4.2, 1.6 Hz, 1H, H-24 eq.), 2.58 (p, *J*=7.0 Hz, 1H, H-20β), 2.10 (m, 1H, H-24 ax.), 1.12 (d, *J*=7.0 Hz, 3H, H-21), 0.84 (s, 3H, H-19), 0.80 (d, *J*=6.6 Hz, 3H, H-27), 0.72 (s, 3H, H-18). ^{13}C NMR($CDCl_3$, 100.53 MHz) δ ppm: δ 36.8 C-1; 27.4 C-2; 73.3 C-3, 33.8 C-4, 40.1 C-5, 29.4 C-6, 117.9 C-7, 138.4 C-8, 48.9 C-9, 34.3 C-10, 21.4 C-11, 39.6 C-12, 41.6 C-13, 54.8 C-14, 31.0 C-15, 80.2 C-16, 61.5 C-17, 16.4 C-18, 13.0 C-19, 37.7 C-20, 14.8 C-21, 110.6 C-22; 137.1 C-23, 32.9 C-24, 33.1 C-25, 66.1 C-26, 17.2 C-27, 170.7 C=O acetyl, 21.4 CH_3 acetyl, 137.1 *ipso*, 129.1 *ortho*, 128.1 *meta*, 126.5 *para*, 122.9 C-23´. ESIMS *m/z*: 545 M^++1 (2.5), 454 (26.8), 440 (13.6), 99(13.9), 85 (100.0), 69 (15.0), 55 (10.8).

General procedure for the saponification of benzylidene spirostanes: KOH (600 mg) was added to a solution of the benzylidene spirostane **2a-e** (200 mg) in methanol (10 mL) and dioxane (5 mL) and the mixture was stirred until the starting material was consumed (TLC). The volatile solvent was evaporated under reduced pressure and ethyl acetate (50 mL) was added. The organic phase was washed with H_2O (6x25 mL) and brine (2x25 mL), dried (anhydrous Na_2SO_4) and evaporated. The produced solid was purified in a

chromatographic column packed with silica gel (10 g) employing hexane/ethyl acetate (6/1 for **3a**, **3b** and **3d**; 4/1 for **3d**; 3/2 for **3c**) as eluent, to afford the desired compound.

[23(23´)*E*,25*R*]-23(23´)-Benzylidene-5α-spirostan-3β-ol (**3a)** Yield 183.9 mg (99%). Mp 200.8 – 201.8 °C *(from ethyl acetate)*. ^{1}H NMR($CDCl_3$, 400 MHz) δ ppm: 7.33 (dd, *J*=8.2, 7.0 Hz, 2H, H-meta), 7.26 – 7.20 (m, 3H, H-ortho and H-para), 6.57 (d, *J*=2.2 Hz, 1H, H-23´), 4.43 (dt, *J*=9.0, 7.1 Hz, 1H, H-16α), 3.66 – 3.47 (m, 3H, H-3α, H-26 ax. and H-26 eq.), 2.78 (ddd, *J*=13.3, 4.4, 1.5 Hz, 1H, H-24 eq.), 2.60 (q, *J*=6.9 Hz, 1H, H-20β), 2.10 (ddd, *J*=13.5, 11.7, 1.9 Hz, 1H, H-24 ax.), 2.00 (ddd, *J*=12.2, 7.5, 5.2 Hz, 1H, H-15β), 1.89 (dd, *J*=9.0, 7.1 Hz, 1H, H-17α), 1.10 (d, *J*=6.9 Hz, 3H, H-21), 0.84 (s, 3H, H-18), 0.83 (s, 3H, H-19), 0.80 (d, *J*=6.6 Hz, 3H, H-27), 0.67 (ddd, *J*=12.2, 10.3, 4.1 Hz, 1H, H-9α). ^{13}C NMR($CDCl_3$, 100.52 MHz) δ ppm: δ 36.9 C-1; 31.5 C-2; 71.3 C-3, 38.2 C-4, 44.8 C-5, 28.6 C-6, 32.3 C-7, 35.0 C-8, 54.4 C-9, 35.6 C-10, 21.1 C-11, 40.3 C-12, 40.8 C-13, 56.3 C-14, 31.6 C-15, 80.5 C-16, 61.6 C-17, 16.6 C-18, 12.4 C-19, 37.3 C-20, 14.8 C-21, 110.6 C-22; 137.1 C-23, 32.8 C-24, 33.1 C-25, 66.1 C-26, 17.2 C-27, 137.1 ipso, 129.1 ortho, 128.1 meta, 126.6 para, 122.9 C-23´. ESIMS *m/z*: 505 M^++1 (9.3), 454 (42.8), 440 (22.3), 99 (14.3), 98 (11.5), 85 (100), 69 (15.9), 55 (12.1).

[23(23´)*E*,25*S*]-23(23´)-Benzylidene-5β-spirostan-3β-ol (**3b)** Yield 153.8 mg (83%). Mp 129.6 – 130.8 °C *(from CH_2Cl_2/acetone)* ^{1}H NMR($CDCl_3$, 400 MHz) δ ppm: 7.36 – 7.29 (m, 2H, H-meta), 7.23 (d, *J*=7.5 Hz, 3H, H-ortho and H-para), 6.72 (s, 1H, H-23´), 4.47 (dt, *J*=8.9, 7.1 Hz, 1H, H-16α), 4.15 – 4.09 (m, 1H, H-3α), 4.05 (dd, *J*=11.0, 4.2 Hz, 1H, H-26 ax.), 3.32 (dt, *J*=11.2, 1.7 Hz, 1H, H-26 eq.), 2.72 (ddd, *J*=13.7, 5.8, 1.7 Hz, 1H, H-24 eq.),

2.49 (p, *J*=6.9 Hz, 1H, H-20β), 2.29 (dd, *J*=13.7, 5.2 Hz, 1H, H-24 ax.), 1.11 (d, *J*=6.9 Hz, 3H, H-21), 0.99 (s, 3H, H-19), 0.99 (d, *J*=6.9 Hz, 3H, H-27), 0.85 (s, 3H, H-18). ^{13}C NMR($CDCl_3$, 100.52 MHz) δ ppm: δ 29.9 C-1; 27.8 C-2; 67.1 C-3, 33.5 C-4, 36.5 C-5, 26.5 C-6, 26.5 C-7, 35.2 C-8, 39.8 C-9, 35.3 C-10, 20.9 C-11, 40.4 C-12, 40.9 C-13, 56.4 C-14, 31.6 C-15, 81.0 C-16, 61.8 C-17, 16.7 C-18, 23.9 C-19, 39.7 C-20, 14.7 C-21, 111.5 C-22; 136.7 C-23*, 30.8 C-24, 30.3 C-25, 65.1 C-26, 18.3 C-27, 137.2 ipso*, 129.0 ortho, 128.0 meta, 126.5 para, 125.0 C-23´. ESIMS *m/z*: 505 M^++1 (7.9), 454 (36.7), 440 (18.8), 99 (14.5), 98 (10.1), 85 (100), 69 (15.7), 55 (12.4). * interchangeable.

[23(23´)*E*,25*R*]-23(23´)-Benzylidene-3β-hydroxy-5α-spirostan-12-one (**3c)** Yield 135.4 mg (73%). Mp 126.2 - 127.1 °C *(from* CH_2Cl_2/MeOH). ^{1}H NMR($CDCl_3$, 400 MHz) δ ppm: 7.33 (t, *J*=7.5 Hz, 2H, H-meta), 7.26 – 7.15 (m, 3H, H-ortho and H-para), 6.55 (d, *J*=1.9 Hz, 1H, H-23´), 4.38 (dt, *J*=8.9, 6.8 Hz, 1H, H-16α), 3.69 – 3.51 (m, 1H, H-3α), 2.79 (dd, *J*=13.4, 3.7 Hz, 1H, H-24 eq.), 2.67 (dd, *J*=9.1, 7.0 Hz, 1H, H-17α), 2.51 – 2.37 (m, 1H, H-20β), 2.25 (dd, *J*=14.4, 5.0 Hz, 1H, H-11eq.), 2.12 – 2.05 (m, 1H, H-24 ax.), 1.20 (d, *J*=6.9 Hz, 3H, H-21), 1.11 (s, 3H, H-18), 0.91 (s, 3H, H-19), 0.80 (d, *J*=6.6 Hz, 3H, H-27).). ^{13}C NMR($CDCl_3$, 100.52 MHz) δ ppm: δ 36.5 C-1; 31.2 C-2; 70.9 C-3, 37.8 C-4, 44.6 C-5, 28.3 C-6, 31.0 C-7, 34.2 C-8, 55.5 C-9, 36.1 C-10, 37.8 C-11, 213.4 C-12, 55.3 C-13, 55.7 C-14, 31.6 C-15, 78.8 C-16, 52.9 C-17, 16.2 C-18, 12.0 C-19, 37.8 C-20, 13.6 C-21, 110.6 C-22; 137.0 C-23, 32.9 C-24, 33.0 C-25, 66.1 C-26, 17.2 C-27, 137.0 ipso, 129.0 ortho, 128.0 meta, 126.6 para, 123.0 C-23´. ESIMS *m/z*: 541 M^+Na (<1), 454 (36.1), 440 (18.3), 99 (14.0), 85 (100.0), 69 (14.8), 55 (11.2).

[23(23´)*E*,25*R*]-23(23´)-Benzylidene-5α-spirost-5-en-3β-ol (**3d)** Yield 171.8 mg (91%). Mp 148.3 – 150.6 °C *(from CH_2Cl_2/MeOH).* ^{1}H NMR($CDCl_3$, 400 MHz) δ ppm: 7.36 – 7.30 (m, 2H, H-meta), 7.25 – 7.20 (m, 3H, H-otro and H-para), 6.58 (s, 1H, H-23´), 5.35 (m, 1H, H-6), 4.46 (dt, *J*=9.0, 7.0 Hz, 1H, H-16β), 3.68 – 3.44 (m, 3H, H-3, H-26 ax and H-26 eq.), 2.78 (dd, *J*=13.3, 3.5 Hz, 1H, H-24 eq.), 2.60 (p, *J*=7.0 Hz, 1H, H-20β), 2.10 (ddd, *J*=13.5, 11.8, 2.0 Hz, 1H, H-24 ax.), 1.11 (d, *J*=6.9 Hz, 3H, H-21), 1.03 (s, 3H, H-19), 0.86 (s, 3H, H-18), 0.80 (d, *J*=6.5 Hz, 3H, H-27). ^{13}C NMR($CDCl_3$, 100.52 MHz) δ ppm: δ 37.2 C-1; 31.6 C-2; 71.7 C-3, 42.3 C-4, 140.8 C-5, 121.4 C-6, 32.1 C-7, 31.4 C-8, 50.1 C-9, 36.7 C-10, 20.9 C-11, 40.0 C-12, 40.5 C-13, 56.5 C-14, 31.7 C-15, 80.4 C-16, 61.5 C-17, 16.5 C-18, 19.4 C-19, 37.3 C-20, 14.9 C-21, 110.6 C-22; 137.1 C-23, 32.9 C-24, 33.1 C-25, 66.1 C-26, 17.2 C-27, 137.1 ipso, 129.1 ortho, 128.1 meta, 126.5 para, 122.9 C-23´. ESIMS *m/z*: 541 M^+K (<1), 454 (39.8), 440 (25.9), 99 (15.1), 98 (17.0), 97 (13.7), 95 (10.6), 85 (100.0), 69 (15.7), 55 (12.1).

[23(23´)E,25R]-23(23´)-Benzylidene-5α-spirost-7-en-3β-ol (**3e)** Yield 124.4 mg (67%). Mp 156.1 - 158.3 *(from CH_2Cl_2/hexane)*.^{1}H NMR($CDCl_3$, 400 MHz) δ ppm: 7.33 (t, *J*=7.6 Hz, 2H, H-meta), 7.25 – 7.20 (m, 3H, H-ortho and H-para), 6.57 (s, 1H, H-23´), 5.16 (m, 1H, H-7), 4.52 (m, 1H, H-16β), 3.68 – 3.43 (m, 2H, H-26 ax. and H-26 eq.), 2.79 (m, 1H, H-24 eq.), 2.58 (q, *J*=7.0 Hz, 1H, H-20β), 1.12 (d, *J*=6.8 Hz, 3H. H-21), 0.82 (s, 3H, H-19), 0.80 (d, *J*=6.9 Hz, 3H, H-27), 0.72 (s, 3H, H-18). ^{13}C NMR($CDCl_3$, 100.52 MHz) δ ppm: δ 37.1 C-1; 31.4 C-2; 71.0 C-3, 37.9 C-4, 40.3 C-5, 29.6 C-6, 118.0 C-7, 138.5 C-8, 49.1 C-9, 34.3 C-10, 21.5 C-11, 39.6 C-12, 41.6 C-13, 54.9 C-14, 31.0 C-15, 80.2 C-16, 61.5 C-17, 16.4 C-18, 13.1 C-19, 37.8 C-20, 14.8 C-21, 110.6 C-22; 137.1 C-23*, 32.9 C-24, 33.1 C-

25, 66.1 C-26, 17.2 C-27, 137.0 ipso*, 129.1 ortho, 128.1 meta, 126.5 para, 122.9 C-23´. ESIMS *m/z*: 541 M^+K (<1), 454 (46.7), 440 (25.8), 99 (14.0), 85 (100), 69 (15.5), 55 (11.3). * interchangeable.

Biological tests

Cell culture

Stock solutions (10 mmol/L) of the tested compounds were prepared by dissolving an appropriate quantity of each substance in dimethylsulfoxide (DMSO). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), L-glutamine, penicillin and streptomycin were purchased from Sigma Aldrich (MO, USA). Calcein AM was obtained from Molecular Probes (ThermoFisher, MA, USA). The screening cell lines (T-lymphoblastic leukemia cell line CEM, breast carcinoma cell line MCF7, cervical carcinoma cell line HeLa and human fibroblasts BJ) were obtained from the American Type Culture Collection (Manassas, VA, USA). CEM cell line was cultured in RPMI 1640 medium with 20 % fetal bovine serum and all other cells were cultured in DMEM medium (Sigma Aldrich, MO, USA), supplemented with 10% fetal bovine serum, L-glutamine (2 mmol/L), penicillin and streptomycin (1 %). The cell lines were maintained under standard cell culture conditions at 37 ºC and 5% CO_2 in a humid environment. Cells were subcultured twice or three times a week using the standard trypsinization procedure.

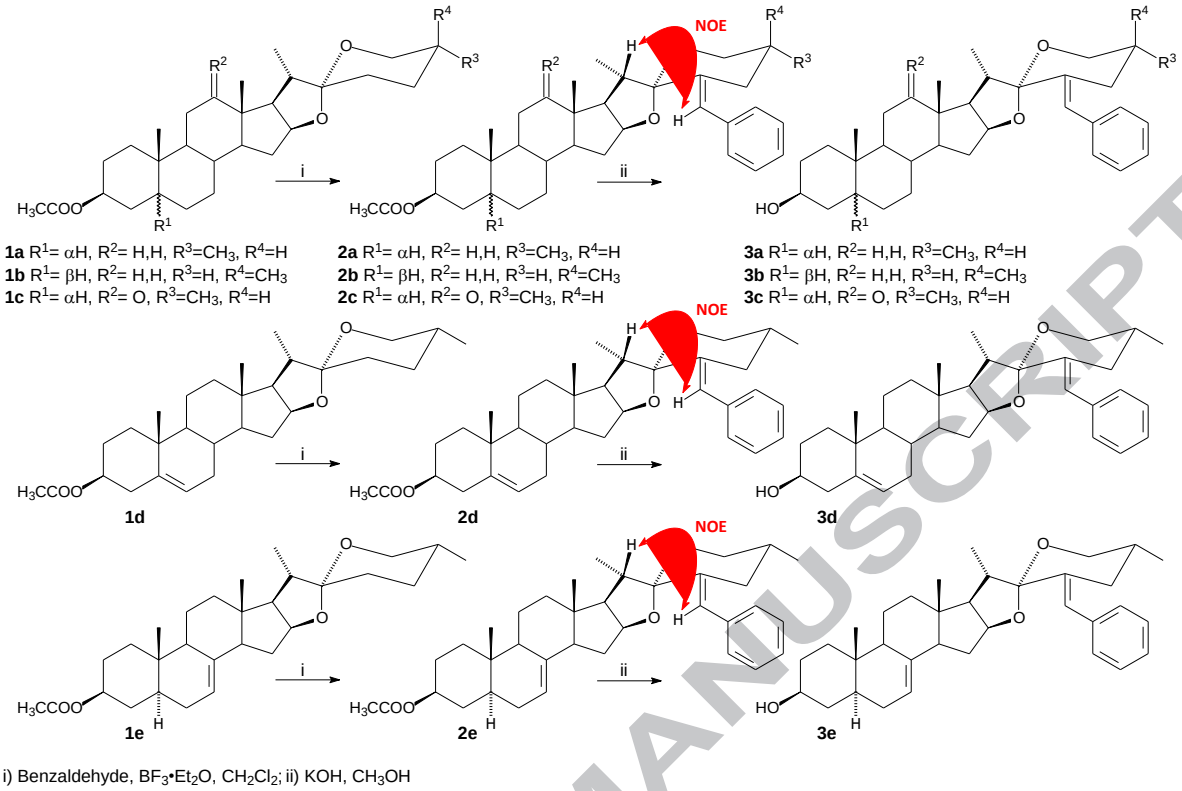
Calcein AM assay

Suspensions of tested cell lines (ca. 1.0×10^5 cells/mL) were placed in 96-well microtiter plates and after 24 h of stabilization (time zero) the tested compounds were added (in three 20 µL aliquots) in serially diluted concentrations in dimethylsulfoxide (DMSO). Control

cultures were treated with DMSO alone, and the final concentration of DMSO in the incubation mixtures never exceeded 0.6%. The test compounds were typically evaluated at six 3-fold dilutions and the highest final concentration was generally 50 µM. After 72 h incubation, Calcein AM solution (100 µL, Molecular Probes, ThermoFisher, MA, USA) was added, and incubation was continued for another hour. The fluorescence of viable cells was then quantified using a Fluoroskan Ascent instrument (Labsystems, Finland). The percentage of surviving cells in each well was calculated by dividing the intensity of the fluorescence signals from the exposed wells by the intensity of signals from control wells and multiplying by 100. These ratios were then used to construct dose-response curves from which IC_{50} values, the concentrations of the respective compounds that were lethal to 50% of the tumor cells, were calculated. All the experiments were repeated three times in triplicates.

Results and discussion

In a previous communication we described that treatment of the native steroid sapogenins with BF_3•Et_2O and benzaldehyde in CH_2Cl_2 afforded good yields of the acetylated benzylidenespirostanols **2a-d** [9a]. When this methodology was applied to Δ^7-tigogenin acetate **(1e)**, a new acetylated 23*E*-benzylidenespirostane **2e** was obtained in a moderate yield. As previously reported for **2a-d** [9a], the observation of the H-20↔H-23' NOE effect allows the easy verification of the *E* configuration of the benzylidene in compound **2e**. Saponification of **2a-e** with KOH in methanol led to the desired 23*E*-benzylidenespirostanols **3a-e** in good to excellent yields. (Scheme 1).



Scheme 1. Synthesis of benzylidene spirostanols **3a-e**.

Antiproliferative activities *in vitro*

The *in vitro* antiproliferative activities of the studied compounds were tested in cancer cell lines of various histopathological origins, including T-lymphoblastic leukaemia CEM, breast adenocarcinoma MCF7, and cervical carcinoma HeLa lines. The results were compared with the cytotoxicity towards normal human BJ fibroblasts. The cells of all of these lines were exposed to six serial 3-fold dilutions of each drug for 72 h, proportions of surviving cells were then estimated and IC_{50} values (50% inhibitory concentrations) calculated. The results from Calcein AM assays are presented in Table 1. All tested compounds showed moderate cytotoxic activity towards all cell lines including normal human fibroblasts. The most active of the five substances was **3c** (lowest IC_{50} for MCF7 cells 19.9 ± 0.1 μM). None of the benzylidenespirostanols was selective for any cell line.

Table 1 IC_{50} (μM) values obtained from Calcein AM assays using three cancer cell lines (CEM, MCF7 and HeLa) and normal human fibroblasts (BJ) treated with tested substances for 72 h.

	IC_{50} (μM)			
Compound	**CEM**	**MCF7**	**HeLa**	**BJ**
3a	26.8 ± 2.4	33.0 ± 4.2	36.9 ± 3.1	41.9 ± 1.4
3b	31.5 ± 5.1	44.7 ± 1.6	42.6 ± 2.5	42.9 ± 0.1
3c	23.2 ± 0.4	19.9 ± 0.1	35.7 ± 3.5	45.3 ± 2.3
3d	28.0 ± 1.3	40.3 ± 3.3	41.0 ± 3.5	44.1 ± 1.2
3e	28.9 ± 2.8	36.6 ± 4.7	39.2 ± 7.1	41.6 ± 1.5

Although these results are preliminary and the differences are small, a tendency can be seen. Naturally occurring steroids bearing an oxygenated substituent in ring C like cephalostatine-1, ritterazine-F [5b], porrigenin C [3e] and hippuristanols [4e] are among the most cytotoxic steroids and compared with the partner bearing a non-oxygenated ring C, they showed increased cytotoxicity. This suggests that in the natural products the presence of an oxygenated substituent in ring C can lead to enhanced cytotoxicity. Interestingly the most active of the studied compounds was **3c** that bears a carbonyl group at position C-12 on ring C. Additionally, the obtained results suggest that compounds bearing a *trans*-fused AB ring system with a saturated B ring **(3a** and **3c**) exhibit higher activity than those with a *cis*-fused AB core (**3b**) or an unsaturated B ring (**3d** and **3e**).

Conclusions

We found that benzylidenespirostanols readily obtained from steroid sapogenins by condensation of spirostanes and benzaldehyde followed by saponification, exhibit moderate cytotoxicity towards three different human cancer cell lines and normal human fibroblasts. Further experiments to explore the effects of the modification of the of benzylidene moiety on cytotoxicity are in progress.

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Highlights

- Condensation of spirostanes and benzaldehyde led to benzylidenspirostanes
- Saponification of benzylidenspirostanes led to benzylidenspirostanols
- New benzylidenspirostanols showed cytotoxicity against three cancer cell lines
- The most active compound bears a trans AB core and a carbonyl group at C-12