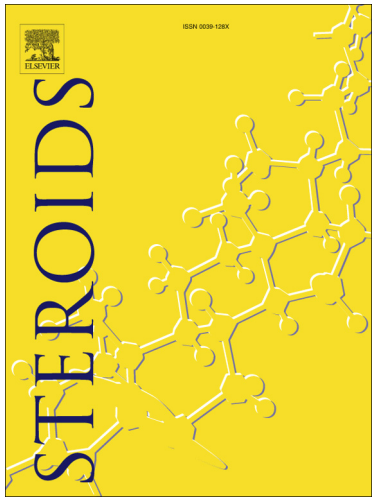

Catalytic cyclometallation in steroid chemistry VI: Targeted synthesis of hybrid molecules based on steroids and tetradeca-5Z,9Z-diene-1,14-dicarboxylic acid and study of their antitumor activity

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Catalytic cyclometallation in steroid chemistry VI[1]: Targeted synthesis of hybrid molecules based on steroids and tetradeca-5Z,9Z-diene-1,14-dicarboxylic acid and study of their antitumor activity

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Abstract: *Hybrid molecules based on a number of steroids (cholesterol, pregnenolone, androsterone) and 1,14-tetradeca-5Z,9Z-dienedicarboxylic acid linked via mono- and diethylene glycol spacers were synthesized for the first time and studied for antitumor activity in vitro. The acid was prepared using catalytic cyclomagnesiation of oxygenated 1,2-dienes with Grignard reagent in the presence of Cp_2TiCl_2 as the key synthetic step. Using flow cytometry, the new molecules were shown for the first time to be efficient apoptosis inducers in the HeLa, Hek293, U937, Jurkat, and K562 cell cultures and to have dose-dependent effect on the S and G2 phases of the cell cycle.*

Keywords: Cross-cyclomagnesiation, Grignard reagent, Steroids, Hybrid molecules, Antitumor activity

1. Introduction

The synthesis of new hybrid molecules for the design of modern efficient pharmacophores and the modeling of existing and well-proven therapeutic agents is a vigorously developing area of medicinal chemistry, as indicated by the avalanche growth of the number of relevant publications in the world literature in the last 10–15 years [1-15].

Steroid molecules attract close attention of researchers, which is largely caused by their high biological activity and participation in the most important cell processes that occur in human and animal bodies. The beginning of steroid application as therapeutic agents dates back to the end of the 1940s. Back in the late 1930s, German chemists demonstrated that the adrenal cortex produces steroid hormones. In 1937, first, deoxycorticosterone and then cortisone and

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hydrocortisone were isolated from the adrenal cortex. The broad range of biological activities of hydrocortisone and cortisone predetermined the possible use of these compounds as therapeutic agents. A typical feature of most natural steroids is the presence of an oxygenated substituent in the C-3 position, which opens up a simple and efficient way for building-up and modification of the molecule.

The broad range of biological activity, the presence of various functional groups arranged around a rigid tetracyclic core, and the clear-cut ability of the steroid molecules to penetrate through the cell membrane and to bind to definite hormonal receptors make this class of compounds a convenient platform for the design of therapeutic agents with unique pharmacological properties.

For the development of new therapeutic drugs meant for the prevention and treatment of a broad range of cardiovascular and autoimmune diseases, cancer, states of shock of any type (post-traumatic, operative, toxic, anaphylactic, burn, and cardiogenic shock) and for transplantation of organs and tissues to suppress the rejection, a lot of hope is now placed on hybrid compounds, in which the pharmacophores of optically active components are linked to steroid residues [6-16].

Recently [17], we have shown that the hybrid molecules synthesized from steroids and tetradeca-5Z,9Z-diene-1,14-dicarboxylic acid, prepared using the catalytic cross-cyclomagnesiation of 1,2-dienes with Grignard reagents in the presence of Cp_2TiCl_2 [18], show high antitumor and cytotoxic activities *in vitro* against a number of tumor cell lines and are efficient inhibitors of human topoisomerase I [19,20].

In view of the above and the practical value and relevance of development of new efficient, low-toxic, and selective antitumor agents, in this communication, we present the results of studies dealing with the synthesis of new hybrid molecules composed of cholesterol, pregnenolone, androsterone and tetradeca-5Z,9Z-diene-1,14-dicarboxylic acid linked via a mono- or diethylene glycol spacer.

This choice of spacers is caused by the fact that ethylene glycols show anchoring behavior for biological receptors and ligands, which reduces non-specific binding of proteins and other biologically active molecules [21,22].

Also, the hybrid molecules we synthesized were studied for cytotoxicity against several tumor cell lines (HeLa, Hek293, U937, Jurkat, and K562) and for the ability to induce apoptosis and the effect on the cell cycle using advanced flow cytometry and fluorescence microscopy methods.

2. Experimental

2.1. Chemistry

Cholesterol, 5-pregnen-β-ol-20-on, 5-androsten-β-ol-17-on, hepta-5,6-dien-1-ol, 4-dimethylaminopyridine (DMAP), *N*-[3-(methylamino)propyl]-*N'*-ethylcarbodiimide hydrochloride (EDC·HCl) were obtained from Sigma-Aldrich and Acros organics. Dichloromethane was and freshly distilled before use. Optical rotations were measured on a Perkin–Elmer 341 polarimeter. Melting points were recorded on Stuart SMP3. IR spectra were recorded on Bruker VERTEX 70V using KBr discs over the range of 400–4000 cm^{-1}. ^{1}H and ^{13}C NMR spectra were obtained using a Bruker Ascend 500 spectrometer in $CDCl_3$ operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C and a Bruker AVANCE 400 spectrometer in $CDCl_3$ operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. Mass spectra of MALDI TOF/TOF positive ions (matrix of sinapic acid) are recorded on a mass spectrometer Bruker Autoflex™ III Smartbeam. Elemental analyses were measured on 1106 Carlo Erba apparatus. Individuality and purity of the synthesized compounds were controlled using TLC on Sorbfil plates; anisic aldehyde in acetic acid was used as a developer. Column chromatography was carried out on Acrus silica gel (0.060–0.200 мм).

*2.1.1. General procedure for the synthesis of mono- and diethylene glycol derivatives of steroids **3a-c**, **4a-c***

Steroids tosylates **2a-c** (1 equiv) prepared from steroids and tosyl chloride was refluxed for 6h with diol (6 equiv), in dry dioxane [23]. The insolubles were removed by filtration. The filtrate was concentrated in vacuo, the residue redissolved in dichoromethane, washed with brine, dried over anhydrous Na_2SO_4 and concentrated on rotary evaporator. The residue was purified on a silica gel chromatography column to get **3a-c, 4a-c**, yield ~70–80%.

*2.1.1.1. 2-[(3β)-Cholest-5-en-3-yloxy]ethanol (**3a**)*

White crystals, 78% yield; m.p. 96–98 °C; $[\alpha]_D^{19.5}$ – 29.0 (*c* 0.72, $CHCl_3$); IR (KBr) ν_{max} 2930, 2868, 1459, 1375, 1365, 1238, 1163, 1100, 1053, 911, 811, 756 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.34 (1H, m, H-6), 3.70 (2H, m, CH_2OH), 3.57 (2H, m, CH_2O), 3.20 (1H, m, H-3), 2.59 (1H, br s, OH), 2.38–0.90 (28H, m), 0.99 (3H, s, H-19), 0.92 (3H, d, *J* = 6.5 Hz, H-21), 0.87 (6H, d, *J* = 6.5 Hz, H-26, H-27), 0.67 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 140.6 (C-5), 121.7 (C-6), 79.4 (C-3), 69.1 (CH_2O), 61.9 (CH_2OH), 56.8 (C-14), 56.2 (C-17), 50.2 (C-9), 42.3 (C-13), 39.8 (C-12), 39.5 (C-24), 38.1 (C-4), 37.2 (C-1), 36.8 (C-10), 36.2 (C-22), 35.8 (C-20),

31.9 (C-7, C-8), 28.4 (C-2), 28.4 (C-16), 27.9 (C-25), 24.3 (C-15), 23.9 (C-23), 22.8 (C-27), 22.6 (C-26), 21.1 (C-11), 19.4 (C-19), 18.7 (C-21), 11.9 (C-18); anal. calcd for $C_{29}H_{50}O_2$: C, 81.87; H, 11.70; found C, 81.79; H, 11.68. MALDI TOF: m/z 453.354 ($[M+Na]^+$, calcd 453.371).

*2.1.1.2. (3β)-3-(2-Hydroxyethoxy)pregn-5-en-20-one (**3b**)*

White crystals, 75% yield; m.p. 96–98 °C; $[\alpha]_D^{18}$ – 5.1 (*c* 1.09, $CHCl_3$); IR (KBr) ν_{max} 2961, 2933, 2871, 1704, 1451, 1400, 1383, 1256, 1111, 1068, 1020, 954, 884, 593 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.36 (1H, m, H-6), 3.73 (2H, m, CH_2OH), 3.59 (2H, m, CH_2O), 3.20 (1H, m, H-3), 2.57–1.00 (20H, m), 2.13 (3H, s, H-21), 1.01 (3H, s, H-19), 0.64 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 209.6 (C-20), 140.7 (C-5), 121.4 (C-6), 79.3 (C-3), 69.0 (CH_2O), 63.7 (C-17), 62.1 (CH_2OH), 56.9 (C-14), 50.0 (C-9), 43.9 (C-13), 39.0 (C-4), 38.8 (C-12), 37.2 (C-1), 36.9 (C-10), 31.8 (C-7, C-8), 31.5 (C-21), 28.4 (C-2), 24.5 (C-15), 22.8 (C-16), 21.1 (C-11), 19.4 (C-19), 13.2 (C-18); anal. calcd for $C_{23}H_{36}O_3$: C, 76.62; H, 10.06; found C, 76.56; H, 10.01. MALDI TOF: m/z 383.255 ($[M+Na]^+$, calcd 383.256), 399.220 ($[M+K]^+$, calcd 399.230).

*2.1.1.3. (3β)-3-(2-Hydroxyethoxy)-17,2'-spiro([1,3]-dioxolane)androst-5-en (**3c**)*

White crystals, 74% yield; m.p. 122–124 °C; $[\alpha]_D^{19}$ – 69.6 (*c* 1.19, $CHCl_3$); IR (KBr) ν_{max} 2948, 2904, 2866, 1457, 1400, 1433, 1382, 1306, 1226, 1169, 1101, 1061, 1040, 961, 882, 869, 760, 579 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.31 (1H, m, H-6), 3.88–3.81 (4H, m, H-4', H-5'), 3.67 (2H, m, CH_2OH), 3.54 (2H, m, CH_2O), 3.16 (1H, m, H-3), 2.67 (1H, br s, OH), 2.36–0.92 (19H, m), 0.97 (3H, s, H-19), 0.82 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 140.6 (C-5), 121.4 (C-6), 119.4 (C-17), 79.3 (C-3), 69.1 (CH_2O), 65.1, 64.5 (C-4', C-5'), 61.9 (CH_2OH), 50.6 (C-14), 49.9 (C-9), 45.7 (C-13), 39.0 (C-4), 37.2 (C-1), 36.8 (C-10), 34.2 (C-16), 32.1 (C-8), 31.2 (C-7), 30.5 (C-12), 28.3 (C-2), 22.7 (C-15), 20.5 (C-11), 19.4 (C-19), 14.2 (C-18); anal. calcd for $C_{23}H_{36}O_4$: C, 73.37; H, 9.64; found C, 73.28; H, 9.62. MALDI TOF: m/z 399.231 ($[M+Na]^+$, calcd 399.251), 415.219 ($[M+K]^+$, calcd 415.225).

*2.1.1.4. 2-{2-[(3β)-Cholest-5-en-3-yloxy]ethoxy}ethanol (**4a**)*

Colorless waxy solid, 72% yield; $[\alpha]_D^{22}$ – 28.0 (*c* 1.28, $CHCl_3$).; IR (KBr) ν_{max} 2929, 2863, 1464, 1379, 1365, 1235, 1160, 1100, 1049, 921, 863, 811, 754 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.36 (1H, H-6), 3.74 (2H, m, CH_2OH), 3.69 (2H, m, CH_2O), 3.66 (2H, m, CH_2O), 3.64 (2H, m, CH_2O), 3.21 (1H, m, H-3), 2.60 (1H, br s, OH), 2.42–0.93 (28H, m), 1.01 (3H, s, H-19), 0.92 (3H, d, *J* = 6.5 Hz, H-21), 0.88 (6H, d, *J* = 7.0 Hz, H-26, H-27), 0.69 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 140.8 (C-5), 121.7 (C-6), 79.6 (C-3), 72.6 (CH_2O), 70.8 (CH_2O), 67.4 (CH_2O), 61.9 (CH_2OH), 56.8 (C-14), 56.2 (C-17), 50.2 (C-9), 42.3 (C-13), 39.8 (C-12),

39.5 (C-24), 38.9 (C-4), 37.2 (C-1), 36.9 (C-10), 36.2 (C-22), 35.8 (C-20), 31.9 (C-7, C-8), 28.3 (C-2), 28.2 (C-16), 28.0 (C-25), 24.3 (C-15), 23.8 (C-23), 22.8 (C-27), 22.6 (C-26), 21.1 (C-11), 19.4 (C-19), 18.7 (C-21), 11.9 (C-18); anal. calcd for $C_{31}H_{54}O_3$: C, 78.43; H, 11.46; found C, 78.34; H, 11.43. MALDI TOF: m/z 497.381 ($[M+Na]^+$, calcd 497.397), 513.364 ($[M+K]^+$, calcd 513.371).

*2.1.1.5. (3β)-3-[2-(2-Hydroxyethoxy)ethoxy]pregn-5-en-20-one (**4b**)*

Colorless waxy solid, 71% yield; $[\alpha]_D^{23}$ – 3.0 (*c* 0.71, $CHCl_3$); IR (KBr) ν_{max} 2933, 2869, 1703, 1509, 1451, 1437, 1383, 1357, 1229, 1132, 1100, 1069, 953, 894, 594 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.28 (1H, m, H-6), 3.67 (2H, m, CH_2OH), 3.60 (2H, m, CH_2O), 3.58 (2H, m, CH_2O), 3.56 (2H, m, CH_2O), 3.15 (1H, m, H-3), 3.05 (1H, br s, OH), 2.50–0.90 (20H, m), 2.08 (3H, s, H-21), 0.94 (3H, s, H-19), 0.57 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 209.4 (C-20), 140.7 (C-5), 121.3 (C-6), 79.4 (C-3), 72.5 (CH_2O), 70.7 (CH_2O), 67.4 (CH_2O), 63.6 (C-17), 61.7 (CH_2OH), 56.8 (C-14), 49.9 (C-9), 43.9 (C-13), 38.9 (C-4), 38.8 (C-12), 37.1 (C-1), 36.8 (C-10), 31.8 (C-8), 31.7 (C-7), 31.5 (C-21), 28.2 (C-2), 24.4 (C-15), 22.8 (C-16), 21.0 (C-11), 19.3 (C-19), 13.2 (C-18); anal. calcd for $C_{25}H_{40}O_3$: C, 74.22; H, 9.97; found C, 74.09; H, 9.95. MALDI TOF: m/z 427.265 ($[M+Na]^+$, calcd 427.282), 443.249 ($[M+K]^+$, calcd 443.256).

*2.1.1.6. (3β)-3-[2-(2-Hydroxyethoxy)ethoxy]androst-5-en-17-one (**4c**)*

Colorless waxy solid, 70% yield; $[\alpha]_D^{19}$ + 4.6 (*c* 0.85, $CHCl_3$); IR (KBr) ν_{max} 2965, 2945, 2931, 2871, 2836, 1747, 1666, 1464, 1451, 1398, 1368, 1245, 1180, 1131, 1085, 1027, 997, 964, 899, 799, 584 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.33 (1H, m, H-6), 3.66 (2H, m, CH_2OH), 3.61 (2H, m, CH_2O), 3.58 (2H, m, CH_2O), 3.56 (2H, m, CH_2O), 3.15 (1H, m, H-3), 3.00 (1H, br s, OH), 2.44–0.92 (19H, m), 0.97 (3H, s, H-19), 0.83 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 220.9 (C-17), 140.9 (C-5), 120.9 (C-6), 79.3 (C-3), 72.5 (CH_2O), 70.7 (CH_2O), 67.4 CH_2O), 61.7 (CH_2OH), 51.7 (C-14), 50.2 (C-9), 47.5 (C-13), 38.9 (C-4), 37.1 (C-1), 36.9 (C-10), 35.8 (C-16), 31.4 (C-8, C-12), 30.8 (C-7), 28.2 (C-2), 21.8 (C-15), 20.3 (C-11), 19.4 (C-19), 13.5 (C-18); anal. calcd for $C_{23}H_{36}O_4$: C, 73.37; H, 9.64; found C, 73.23; H, 9.61. MALDI TOF: m/z 399.248 ($[M+Na]^+$, calcd 399.251), 415.208 ($[M+K]^+$, calcd 415.225).

^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) spectral data for (5Z,9Z)-tetradeca-5.9-dienedioic acid (**8**) are in agreement with the literature data [17].

*2.1.2. General procedure for the synthesis of steroidal derivatives of 5Z,9Z-dienoic acid **9a-c**, **10a-c***

To a solution of mono-or diethylene glycol derivatives of steroids **3a-c** (or **4a-c**) (1.0 mmol) in dichloromethane (10 ml) was added the (5Z,9Z)-tetradeca-5.9-dienedioic acid (**8**) (0.51 g, 2.0 mmol) followed by *N*-[3-(methylamino)propyl]-*N*'-ethylcarbodiimide hydrochloride (0.48 g, 2.5 mmol) and 4-dimethylaminopyridine (18 mg, 0.15 mmol) under argon. The mixture was stirred at room temperature for 18 h until the reaction was complete (TLC monitoring, hexane/ethyl acetate = 1/1). The mixture was diluted with H_2O (10 ml) and the CH_2Cl_2 layer was separated, dried over $MgSO_4$, and concentrated. The crude product was purified by column chromatography (silica gel) using hexane/ethyl acetate = 5/1 as the elution solvent to afford 5Z,9Z-dienoic acids of steroid.

*2.1.2.1. (5Z,9Z)-14-{2-[(3β)-Cholest-5-en-3-yloxy]ethoxy}-14-oxotetradeca-5,9-dienoic acid (**9a**)*

Colorless waxy solid, 0.49 g, 74% yield; $[\alpha]_D^{19}$ – 15.3 (*c* 0.26, $CHCl_3$); IR (KBr) ν_{max} 2932, 2854, 1738, 1708, 1622, 1456, 1431, 1384, 1323, 1238, 1180, 1151, 1116, 1077, 1025, 878, 800 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.41 (2H, m, H-6', H-9'), 5.37 (2H, m, H-5', H-10'), 5.35 (1H, m, H-6), 4.22 (2H, m, CH_2O), 3.69 (2H, m, CH_2O), 3.19 (1H, m, H-3), 2.36 (2H, m, H-2'), 2.35 (2H, m, H-13'), 2.38–0.90 (28H, m), 2.10 (4H, m, H-4', H-11'), 2.08 (4H, m, H-7', H-8'), 1.71 (2H, m, H-3'), 1.70 (2H, m, H-12'), 1.01 (3H, s, H-19), 0.92 (3H, d, *J* = 6.5 Hz, H-21), 0.88 (6H, d, *J* = 6.5 Hz, H-26, H-27), 0.69 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 178.9 (C-1'), 173.9 (C-14'), 140.7 (C-5), 130.4, 130.2 (C-6', C-9'), 129.0, 128.8 (C-5', C-10'), 121.7 (C-6), 79.6 (C-3), 65.9 (CH_2O), 63.9 (CH_2O), 56.8 (C-14), 56.2 (C-17), 50.2 (C-9), 42.3 (C-13), 39.8 (C-12), 39.5 (C-24), 38.9 (C-4), 37.2 (C-1), 36.8 (C-10), 36.2 (C-22), 35.8 (C-20), 33.6 (C-13'), 33.3 (C-2'), 31.9 (C-7, C-8), 28.3 (C-2), 28.2 (C-16), 28.0 (C-25), 27.3 (C-7', C-8'), 26.6 (C-11'), 26.5 (C-4'), 24.9 (C-12'), 24.6 (C-3'), 24.3 (C-15), 23.8 (C-23), 22.8 (C-27), 22.6 (C-26), 21.1 (C-11), 19.4 (C-19), 18.7 (C-21), 11.9 (C-18); anal. calcd for $C_{43}H_{70}O_5$: C, 77.43; H, 10.58; found C, 77.36 H, 10.55. MALDI TOF: m/z 689.415 ($[M+Na]^+$, calcd 689.512), 705.377 ($[M+K]^+$, calcd 705.486).

*2.1.2.2. (5Z,9Z)-14-Oxo-14-(2-{[(3β)-20-oxopregn-5-en-3-yl]oxy}ethoxy)tetradeca-5,9-dienoic acid (**9b**)*

Colorless waxy solid, 0.43 g, 72% yield; $[\alpha]_D^{18}$ – 0.45 (*c* 0.88, $CHCl_3$); IR (KBr) ν_{max} 2938, 2870, 1737, 1709, 1455, 1436, 1384, 1307, 1240, 1219, 1170, 1109, 1042, 960, 800, 753 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.41 (2H, m, H-6', H-9'), 5.35 (3H, m, H-5', H-10', H-6),

4.21 (2H, m, CH_2O), 3.68 (2H, m, CH_2O), 3.19 (1H, m, H-3), 2.55–0.99 (20H, m), 2.37 (2H, m, H-2'), 2.34 (2H, m, H-13'), 2.13 (3H, s, H-21), 2.10 (4H, m, H-4', H-11'), 2.07 (4H, m, H-7', H-8'), 1.71 (2H, m, H-3'), 1.68 (2H, m, H-12'), 1.00 (3H, s, H-19), 0.63 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 209.8 (C-20), 179.6 (C-1'), 173.9 (C-14'), 140.7 (C-5), 130.4, 130.2 (C-6', C-9'), 129.0, 128.8 (C-5', C-10'), 121.4 (C-6), 79.5 (C-3), 65.9 (CH_2O), 63.8 (CH_2O), 63.7 (C-17), 56.9 (C-14), 50.0 (C-9), 44.0 (C-13), 38.9 (C-4), 38.8 (C-12), 37.2 (C-1), 36.8 (C-10), 33.6 (C-13'), 33.3 (C-2'), 31.8 (C-7, C-8), 31.5 (C-21), 28.3 (C-2), 27.3 (C-7', C-8'), 26.6 (C-11'), 26.5 (C-4'), 24.9 (C-12'), 24.6 (C-3'), 24.5 (C-15), 22.8 (C-16), 21.1 (C-11), 19.3 (C-19), 13.2 (C-18); anal. calcd for $C_{37}H_{56}O_6$: C, 74.46; H, 9.46; found C, 74.41; H, 9.44. MALDI TOF: m/z 619.348 ($[M+Na]^+$, calcd 619.397), 635.317 ($[M+K]^+$, calcd 635.371).

*2.1.2.3. (5Z,9Z)-14-Oxo-14-(2-{[(3β)-17,2'-spiro([1,3]-dioxolane)androst-5-en-3-yl]oxy}ethoxy)tetradeca-5,9- dienoic acid (**9c**)*

Colorless waxy solid, 0.45 g, 73% yield; $[\alpha]_D^{19.5}$ – 24.0 (*c* 0.94, $CHCl_3$); IR (KBr) ν_{max} 2938, 2870, 1737, 1709, 1455, 1436, 1384, 1307, 1240, 1219, 1170, 1109, 1042, 960, 837, 800, 753 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.42 (2H, m, H-6', H-9'), 5.36 (3H, m, H-5', H-10', H-6), 4.22 (2H, m, CH_2O), 3.93–3.87 (4H, m, H-4'', H-5''), 3.69 (2H, m, CH_2O), 3.22 (1H, m, H-3), 2.38–0.99 (19H, m), 2.36 (2H, m, H-2'), 2.35 (2H, m, H-13'), 2.11 (4H, m, H-4', H-11'), 2.09 (4H, m, H-7', H-8'), 1.71 (2H, m, H-3'), 1.69 (2H, m, H-12'), 0.99 (3H, s, H-19), 0.88 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 178.3 (C-1'), 173.9 (C-14'), 140.7 (C-5), 130.4, 130.2 (C-6', C-9'), 129.0, 128.9 (C-5', C-10'), 121.5 (C-6), 119.5 (C-17), 79.5 (C-3), 65.9 (CH_2O), 65.2, 64.6 (C-4'', C-5''), 63.9 (CH_2O), 50.6 (C-14), 50.0 (C-9), 45.7 (C-13), 38.9 (C-4), 37.2 (C-1), 36.9 (C-10), 34.2 (C-16), 33.6 (C-13'), 33.2 (C-2'), 32.2 (C-8), 31.3 (C-7), 30.6 (C-12), 28.3 (C-2), 27.3 (C-7', C-8'), 26.6 (C-11'), 26.5 (C-4'), 24.9 (C-12'), 24.6 (C-3'), 22.8 (C-15), 20.5 (C-11), 19.4 (C-19), 14.2 (C-18); anal. calcd for $C_{37}H_{56}O_7$: C, 72.51; H, 9.21; found C, 72.46; H, 9.19. MALDI TOF: m/z 635.327 ($[M+Na]^+$, calcd 635.392).

*2.1.2.4. (5Z,9Z)-14-(2-{2-[(3β)-Cholest-5-en-3-yloxy]ethoxy}ethoxy)-14-oxotetradeca-5,9-dienoic acid (**10a**)*

Colorless waxy solid, 0.49 g, 70% yield; $[\alpha]_D^{22}$ – 8.5 (*c* 1.20, $CHCl_3$); IR (KBr) ν_{max} 2934, 2867, 1737, 1712, 1619, 1458, 1382, 1260, 1138, 1107, 1024, 956, 874, 801 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.41 (2H, m, H-6', H-9'), 5.36 (2H, m, H-5', H-10'), 5.36 (1H, m, H-6), 4.24 (2H, m, CH_2O), 3.72 (2H, m, CH_2O), 3.65 (4H, m, CH_2O), 3.20 (1H, m, H-3), 2.36 (2H, m, H-2'), 2.35 (2H, m, H-13'), 2.37–0.93 (28H, m), 2.10 (4H, m, H-4', H-11'), 2.08 (4H, m, H-7', H-8'), 1.72 (2H, m, H-3'), 1.70 (2H, m, H-12'), 1.01 (3H, s, H-19), 0.92 (3H, d, *J* = 6.4 Hz, H-

21), 0.88 (6H, d, *J* = 6.4 Hz, H-26, H-27), 0.69 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 178.1 (C-1'), 173.8 (C-14'), 140.9 (C-5), 130.4, 130.2 (C-6', C-9'), 128.9 (C-5', C-10'), 121.6 (C-6), 79.6 (C-3), 70.8 (CH_2O), 69.2 (CH_2O), 67.3 (CH_2O), 63.5 (CH_2O), 56.8 (C-14), 56.2 (C-17), 50.2 (C-9), 42.3 (C-13), 39.8 (C-12), 39.5 (C-24), 39.0 (C-4), 37.2 (C-1), 36.9 (C-10), 36.2 (C-22), 35.8 (C-20), 33.6 (C-13'), 33.2 (C-2'), 31.9 (C-7, C-8), 28.3 (C-2), 28.2 (C-16), 28.0 (C-25), 27.3 (C-7', C-8'), 26.6 (C-11'), 26.5 (C-4'), 24.8 (C-12'), 24.6 (C-3'), 24.3 (C-15), 23.8 (C-23), 22.8 (C-27), 22.6 (C-26), 21.1 (C-11), 19.4 (C-19), 18.7 (C-21), 11.9 (C-18); anal. calcd for $C_{45}H_{74}O_6$: C, 76.01; H, 10.49; found C, 75.96 H, 10.46. MALDI TOF: m/z 733.425 ($[M+Na]^+$, calcd 733.538), 749.378 ($[M+K]^+$, calcd 749.512).

*2.1.2.5. (5Z,9Z)-14-Oxo-14-[2-(2-{[(3β)-20-oxopregn-5-en-3-yl]oxy}ethoxy)ethoxy]tetradeca-5,9-dienoic acid (**10b**)*

Colorless waxy solid, 0.44 g, 69% yield; $[\alpha]_D^{26}$ – 0.1 (*c* 0.99, $CHCl_3$); IR (KBr) ν_{max} 2933, 2870, 1736, 1705, 1455, 1417, 1383, 1359, 1242, 1179, 1169, 1139, 1108, 1045, 954, 800, 736 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.40 (2H, m, H-6', H-9'), 5.36 (3H, m, H-5', H-10', H-6), 4.24 (2H, m, CH_2O), 3.72 (2H, m, CH_2O), 3.65 (4H, m, CH_2O), 3.19 (1H, m, H-3), 2.56–0.95 (20H, m), 2.37 (2H, m, H-2'), 2.33 (2H, m, H-13'), 2.13 (3H, s, H-21), 2.09 (4H, m, H-4', H-11'), 2.06 (4H, m, H-7', H-8'), 1.71 (2H, m, H-3'), 1.68 (2H, m, H-12'), 1.00 (3H, s, H-19), 0.63 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 209.8 (C-20), 178.5 (C-1'), 173.8 (C-14'), 140.9 (C-5), 130.2, (C-6', C-9'), 128.9 (C-5', C-10'), 121.3 (C-6), 79.5 (C-3), 70.8 (CH_2O), 69.2 (CH_2O), 67.3 (CH_2O), 63.7 (C-17), 63.4 (CH_2O), 56.9 (C-14), 50.0 (C-9), 44.0 (C-13), 38.9 (C-4), 38.8 (C-12), 37.2 (C-1), 36.9 (C-10), 33.6 (C-13'), 33.3 (C-2'), 31.8 (C-7, C-8), 31.5 (C-21), 28.3 (C-2), 27.3 (C-7', C-8'), 26.6 (C-11'), 26.5 (C-4'), 24.8 (C-12'), 24.6 (C-3'), 24.5 (C-15), 22.8 (C-16), 21.1 (C-11), 19.4 (C-19), 13.2 (C-18); anal. calcd for $C_{39}H_{60}O_7$: C, 73.09; H, 9.44; found C, 72.97; H, 9.43. MALDI TOF: m/z 633.351 ($[M+Na]^+$, calcd 633.424), 679.305 ($[M+K]^+$, calcd 679.398).

*2.1.2.6. (5Z,9Z)-14-oxo-14-[2-{[(3β)-17-oxoandrost-5-en-3-yl]oxy}ethoxy)ethoxy]tetradeca-5,9-dienoic acid (**10c**)*

Colorless waxy solid, 0.42 g, 68% yield; $[\alpha]_D^{23}$ + 1.0 (*c* 1.02, $CHCl_3$); IR (KBr) ν_{max} 2933, 2859, 1737, 1454, 1376, 1245, 1180, 1141, 1105, 1059, 800 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.40 (2H, m, H-6', H-9'), 5.37 (2H, m, H-5', H-10', H-6), 5.36 (1H, m, H-6), 4.23 (2H, m, CH_2O), 3.72 (2H, m, CH_2O), 3.64 (4H, m, CH_2O), 3.19 (1H, m, H-3), 2.38–1.00 (19H, m), 2.37 (2H, m, H-2'), 2.33 (2H, m, H-13'), 2.10 (4H, m, H-4', H-11'), 2.08 (4H, m, H-7', H-8'), 1.70 (2H, m, H-3'), 1.68 (2H, m, H-12'), 1.02 (3H, s, H-19), 0.89 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 221.3 (C-17), 177.9 (C-1'), 173.8 (C-14'), 141.1 (C-5), 130.3, 130.2 (C-6', C-9'),

128.9 (C-5', C-10'), 120.8 (C-6), 79.4 (C-3), 70.8 (CH_2O), 69.3 (CH_2O), 67.3 (CH_2O), 63.4 (CH_2O), 51.8 (C-14), 50.3 (C-9), 47.6 (C-13), 39.0 (C-4), 37.1 (C-1), 36.9 (C-10), 35.8 (C-16), 33.6 (C-13'), 33.2 (C-2'), 31.5 (C-8), 31.4 (C-7), 30.8 (C-12), 28.2 (C-2), 27.3 (C-7', C-8'), 26.6 (C-11'), 26.5 (C-4'), 24.8 (C-12'), 24.6 (C-3'), 21.9 (C-15), 20.3 (C-11), 19.4 (C-19), 13.5 (C-18); anal. calcd for $C_{37}H_{56}O_7$: C, 72.51; H, 9.21; found C, 72.47; H, 9.18. MALDI TOF: m/z 635.382 ($[M+Na]^+$, calcd 635.392), 651.341 ($[M+K]^+$, calcd 651.366).

2.2. Biological assays

2.2.1. Cell culturing

Cells (Jurkat, K562, U937) were purchased from Russian Cell Culture Collection (Institute of Cytology of the Russian Academy of Sciences) and cultured according to standard mammalian tissue culture protocols and sterile technique. Human cancer cell lines HEK293 and HeLa were obtained from the HPA Culture Collections (UK). All cell lines used in the study were tested and shown to be free of mycoplasma and viral contamination.

Hek293, HeLa cell lines and fibroblasts were cultured as monolayers and maintained in Dulbecco's modified Eagle's medium (DMEM, Gibco BRL) supplemented with 10% foetal bovine serum and 1% penicillin-streptomycin solution at 37 °C in a humidified incubator under a 5% CO_2 atmosphere.

Cells were maintained in RPMI 1640 (Jurkat, K562, U937) (Gibco) supplemented with 4 mM glutamine, 10% FBS (Sigma) and 100 units/ml penicillin-streptomycin (Sigma). All types of cells were grown in an atmosphere of 5 % CO_2 at 37 °C. The cells were subcultured at 2-3 days intervals. Adherent cells (HEK293, HeLa, fibroblasts) were suspended using trypsin/EDTA and counted after they have reached 80% confluency. Cells were then seeded in 24 well plates at $5x10^4$ cells per well and incubated overnight. Jurkat, K562, U937 cells were subcultured at 2 day intervals with a seeding density of 1×10^5cells per 24 well plates in RPMI with 10% FBS.

2.2.2. Cytotoxicity assay

Viability (Live/dead) assessment was performed by staining cells with 7-AAD (7-Aminoactinomycin D) (Biolegend). Cells after treatment with compounds **9a-c**, **10a-c** at various concentrations (2, 1, 0.5, 0.25, 0.1 μM) and incubated in an atmosphere of 5 % CO_2 at 37 °C during 24 hours were harvested, washed 1-2 times with phosphate-buffered saline (PBS) and centrifuged at 400g for 5 minutes. Cell pellets were resuspended in 200 uL of flow cytometry staining buffer (PBS without $Ca^{2+}$and Mg^{2+}, 2,5% FBS) and stained with 5 uL of 7-AAD staining solution for 15 minutes at room temperature in the dark. Samples were acquired on

NovoCyteTM 2000 FlowCytometry System (ACEA) equipped with 488 nm argon laser. Detection of 7-AAD emission was collected through a 675/30 nm filter in FL4 channel.

2.2.3. Viability and apoptosis

Apoptosis was determined by flow cytometric analysis of Annexin V and 7-aminoactinomycin D staining. U937 tumor cells after treatment with compounds **9b** or **10b** at concentrations (0.3, 0.2, 0.1 μM) during 24 hours were harvested, washed 1-2 times with phosphate-buffered saline (PBS) and centrifuged at 400g for 5 minutes. Cell pellets were resuspended in 200 uL of flow cytometry staining buffer (PBS without $Ca^{2+}$and Mg^{2+}, 2,5% FBS). Then, 200 μl of Guava Nexin reagent (Millipore, Bedford, MA, USA) was added to 5 × 10^5 cells in 200 μl, and the cells were incubated with the reagent for 20 min at room temperature in the dark. At the end of incubation, the cells were analyzed on NovoCyteTM 2000 FlowCytometry System (ACEA).

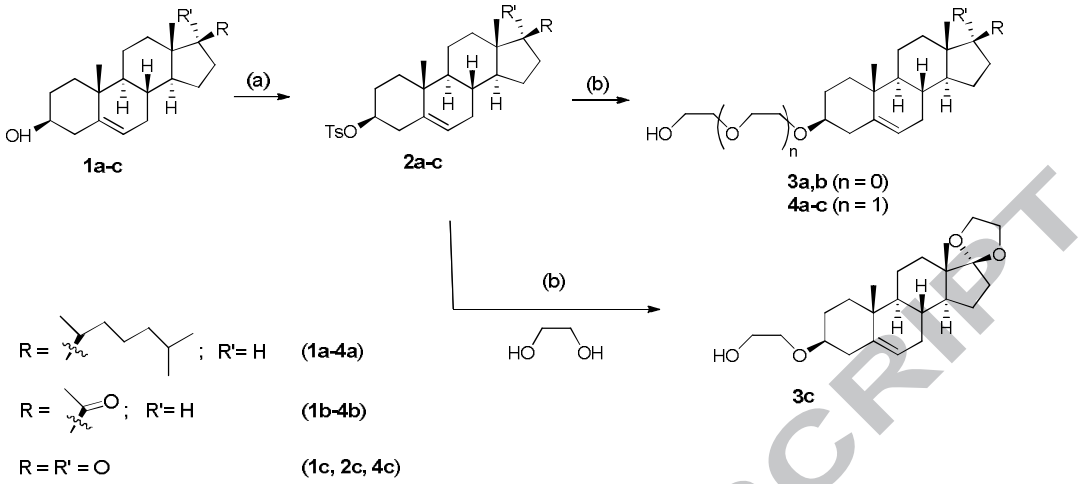
2.2.4. Cell cycle analysis

Cell cycle was analyzed using the method of propidium iodide staining. U937 tumor cells after treatment with compound **9b** at concentrations (0.4, 0.3, 0.2, 0.1 μM) during 24 hours were harvested, washed 1-2 times with phosphate-buffered saline (PBS) and centrifuged at 400g for 5 minutes. Cell pellets were resuspended in 200 uL of flow cytometry staining buffer (PBS without $Ca^{2+}$and Mg^{2+}, 2,5% FBS). Then, cells were plated in 24-well round bottom plates at a density 10×10^5 cells per well, centrifuged at 450× g for 5 minutes, and fixed with ice-cold 70% ethanol for 24 hour at 0 °C. Cells were then washed with PBS and incubated with 250 μl of Guava Cell Cycle Reagent (Millipore) for 30 minutes at room temperature in the dark. Samples were analyzed on NovoCyteTM 2000 FlowCytometry System (ACEA).

3. Results and discussion

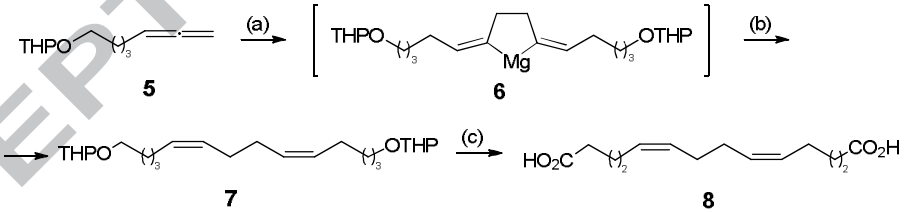
3.1. Chemistry

The investigation program was started with the synthesis of mono- and diethylene glycol derivatives of cholesterol, pregnenolone and androsterone **3a-c**, **4a-c** by refluxing the corresponding steroid tosylates **2a-c** with an excess (6 eq.) of mono- or diethylene glycol in 1,4-dioxane (Scheme 1) [23]. It is noteworthy that under these conditions, androsterone tosylate **2c** reacts with ethylene glycol via both the tosyl group and the oxo group at C-17 to give the monoethylene glycol derivative of androsterone with spiro-connected dioxolane moiety **3c**.



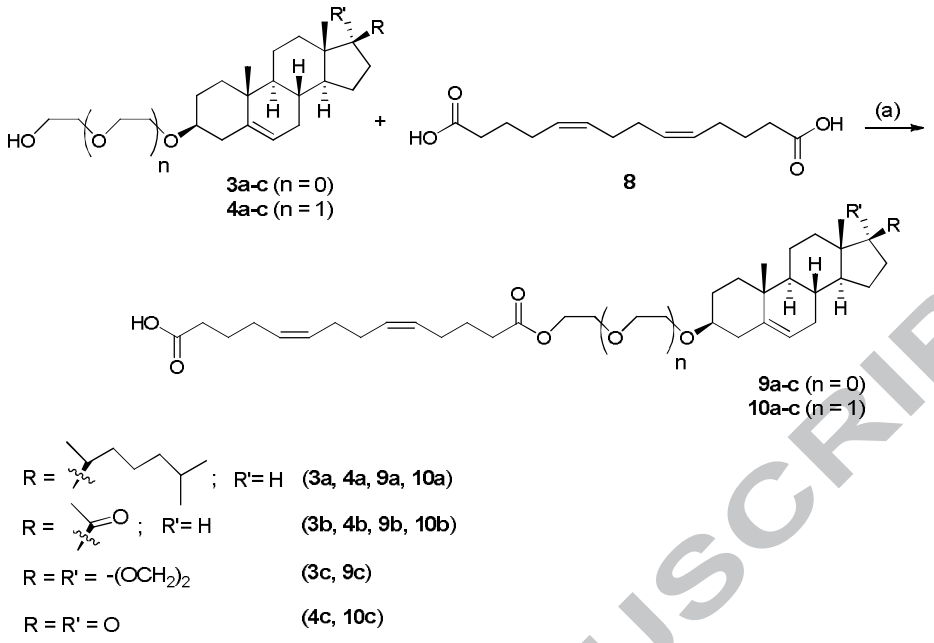
Scheme 1. Synthesis of mono(di)ethylene glycol derivatives of steroids **3a-c**, **4a-c**: (a) TsCl, anhydrous pyridine, 0 °C; (b) ethylene glycol (or diethylene glycol), dioxane, reflux.

The initial tetradeca-5Z,9Z-diene-1,14-dicarboxylic acid **8** was synthesized in two steps using the previously developed [24,25] homo-cyclomagnesiation of the tetrahydropyran ether of 5,6-hepta-5,6-dien-1-ol **5** with EtMgBr in the presence of the Cp_2TiCl_2 catalyst (5 mol. %), which was followed by hydrolysis of magnesacyclopentane **6** and oxidation of thus formed 1,14-bis-tetrahydropyranyl-5Z,9Z-diene-1,14-diol **7** with the Jones reagent. The overall yield was ~60% (Scheme 2).



Scheme 2. Synthesis of (5Z,9Z)-tetradeca-5,9-dienedioic acid **8**. (a) EtMgBr, Mg, Cp_2TiCl_2 (5 mol%), diethyl ether; (b) H_3O^+; (c) H_2CrO_4/H_2SO_4, acetone, CH_2Cl_2.

The subsequent reaction of tetradeca-5Z,9Z-diene-1,14-dicarboxylic acid **8** with mono- (**3a-c**) and diethylene glycol (**4a-c)** steroid derivatives takes place in the presence of N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC·HCl) and catalytic amounts of dimethylaminopyridine (DMAP) and gives the target 5Z,9Z-dienoic acids **9a-c**, **10a-c** (Scheme 3).



Scheme 3. Synthesis of steroid-containing 5Z,9Z-dienoic fatty acids **9a-c**, **10a-c**: (a) EDC·HCl, DMAP, CH_2Cl_2.

The structures of hybrid molecules **9a-c** and **10a-c** were established by ^{1}H and ^{13}C NMR spectroscopy, and 2D heteronuclear correlation experiments (HSQC, HMBC).

3.2. Biological evaluation

All of the synthesized hybrid molecules **9a-c** and **10a-c** were tested for the first time for the antitumor activity *in vitro* using the Jurkat, K562, Hek293, HeLa, and U937 cell lines; the study included determination of CC_{50}, the apoptosis-inducing activity, and the effect on the cell cycle using flow cytometry.

The cytotoxic activities of compounds **9a-c** and **10a-c** *in vitro* against the Jurkat, K562, U937, and HeLa tumor cultures and the cell line obtained from the Hek293 human embryonic kidney cells are summarized in Table 1.

Table 1

Cytotoxic activities of synthesized hybrid steroids **9a-c** and **10a-c** measured on tumor cell cultures (Jurkat, K562, Hek293, HeLa, U937) and on normal fibroblasts (CC_{50}, μM).

Compound	**Jurkat**	**K562**	**Hek293**	**HeLa**	**U937**	**Fibroblasts**
9a	0.18±0.019	0.15±0.011	0.51±0.015	0.79±0.047	0.26±0.048	1.17±0.011
9b	0.06±0.023	0.15±0.012	0.42±0.014	0.15±0.017	0.10±0.011	0.67±0.033
9c	0.13±0.011	0.14±0.012	0.03±0.043	0.68±0.031	0.16±0.034	0.69±0.038
10a	0.20±0.029	0.39±0.043	0.48±0.012	0.75±0.036	0.30±0.042	0.82±0.031
10b	0.14±0.012	0.11±0.017	0.41±0.038	0.53±0.012	0.13±0.028	0.77±0.015
10c	0.33±0.037	0.21±0.024	0.62±0.011	0.74±0.048	0.21±0.024	0.64±0.028
Camp.	1.03±0.015	1.29±0.019	1.41±0.015	1.19±0.049	0.98±0.025	3.64±0.017

The cytotoxic effect of the hybrid molecules determined by flow cytometry against five tumor cell lines and human fibroblasts follows a clear-cut dose-dependent pattern, which is specific to each compound (Table 1 and Fig. 1 and Fig. 2). It can be seen from the Table that the inhibitory concentrations CC_{50} for acids **9b** and **10b**, found by exposure of the test compounds to the above-mentioned cell lines followed by cell staining with the dye 7AAD, generally varies depending on the cell culture, but the lowest values were found for two lines, Jurkat and U937. The Jurkat value was the lowest, being 0.06 μM for compound **9b** and 0.14 μM for compound **10b**. In the case of U937 culture, CC_{50} was 0.10 μM for compound **9b** and 0.13 μM for **10b**. The CC_{50} values for normal fibroblasts were 0.67 μM for **9b** and 0.77 μM for **10b**. Thus, the half maximal inhibitory concentration of **9b** is almost 10 times lower for the Jurkat cells and 5 times lower for the U937 cells than for normal fibroblasts. A similar situation is observed for compound **10b** (Table 1). Simultaneously, it have been shown that mono- (**3a-c**) and diethylene glycol (**4a-c**) steroid derivatives to have a 5-7-fold lower cytotoxic effect on tumor cell lines than the corresponding hybrid molecules.

Determination of the apoptosis induced by compounds **9b** and **10b** using the vital double staining with AnnexinV/7AAD and flow cytometry detection was performed in relation to Jurkat and U937 tumor cells, with the test compound concentrations varying from 0.1 μM to 0.3 μM. Compounds **9b** and **10b** were found to induce the tumor cell death for the given cell lines by apoptotic mechanism (see Fig. 1).

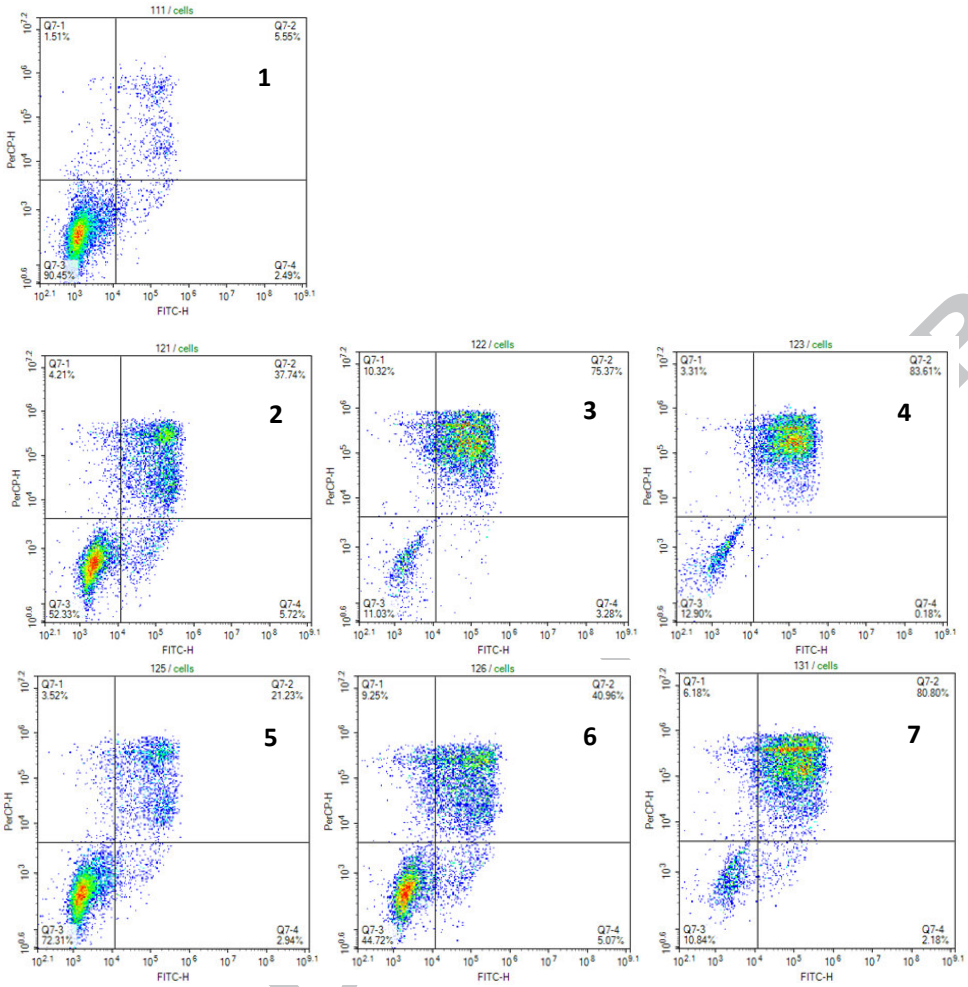


Fig. 1. Tumor cell line U937 treated with compounds **9b** (2-4) and **10b** (5-7) in various concentrations, stained by annexin V/7AAD, and analyzed by flow cytometry. (1) control; (2) **9b** (0.1 μM); (3) **9b** (0.2 μM); (4) **9b** (0.3 μM), (5) **10b** (0.1 μM). (6) **10b** (0.2 μM). (7) **10b** (0.3 μM).

Table 2

Results of measurement of apoptosis in the Jurkat and U937 cell cultures induced by acids **9b** and **10b**.

Compound	Jurkat		U937	
	Early apoptosis (%)	Late apoptosis (%)	Early apoptosis (%)	Late apoptosis (%)
9b (0.1 μM)	4.5	35.8	5.7	37.7
9b (0.2 μM)	2.4	54.8	3.3	75.4
9b (0.3 μM)	1.2	74.2	0.2	83.6
10b (0.1 μM)	2.2	20.1	2.9	21.2
10b (0.2 μM)	4.7	38.9	5.1	40.9
10b (0.3 μM)	2.2	78.9	2.2	80.8
Campt. (1 μM)	5.1	78.9	5.7	83.1

As the concentration of hybrid steroids **9b** and **10b** is increased, the number of cells that have reached early and late apoptosis stages statistically significantly increases. The highest

amount of apoptotic cells occurring at early and late apoptosis stages, which were overally AnV^+7AAD^-, AnV^+7AAD^+, and AnV^-7AAD^+ positive, was found for U937 cells and was 83.6% at 0.3 μM concentration of **9b**. Thus, the obtained results attest to the ability of compounds **9b** and **10b** to exert selective cytotoxic action on tumor cells and induce apoptotic cell death.

Figure 2 shows the results of analysis of kinetic characteristics of the cell cycle for U937 cells determined by DNA flow cytometry after treating the cells with compound **9b** in various concentrations and incubation for 24 h.

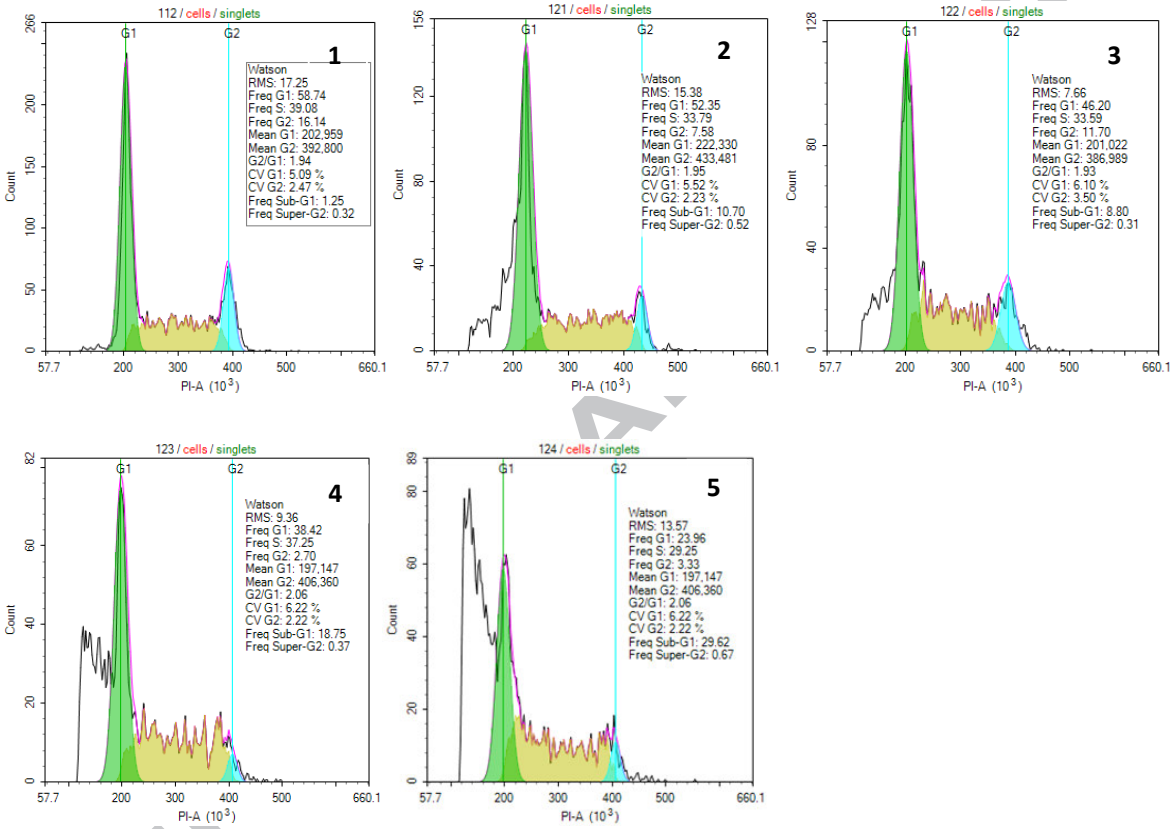


Fig. 2. Phases of the cell cycle for the U937 cells treated with compound **9b**. (1) control; (2) **9b** (0.1 μM); (3) **9b** (0.2 μM); (4) **9b** (0.3 μM); (5) **9b** (0.4 μM). The incubation time of **9b** with the cells is 24 h.

It can be seen from Fig. 2 that hybrid product **9b** affects mainly the S and G2 cell cycle phases, with this effect being dose-dependent. The most pronounced changes in the S and G2 phases arise when the test compound is taken in 0.4 μM concentration (Fig. 2). Also, with increasing concentration of compound **9b**, a subG0 peak appears, indicating the apoptotic death of tumor cells. The most pronounced enrichment of subG0 cell population is induced by 0.4 μM concentration.

4. Conclusion

Synthetic analogues of natural 5Z,9Z-dienoic acids, namely, hybrid molecules based on cholesterol, progesterone, and androsterone and 1,14-tetradeca-5Z,9Z-dienedicarboxylic acid linked by mono- and diethylene glycol spacers were synthesized and studied for the antitumor activity *in vitro*. Using flow cytometry, the new hybrid molecules were shown to have high cytotoxicity *in vitro* against HeLa, Hek293, U937, Jurkat, and K562 tumor cells, to exert phase-specific cytotoxic action on the S and G2 phase cells, and to be efficient apoptosis inducers. These results open up prospects for the development of new highly efficient antitumor drugs.

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References

[1] M. Decker, Hybrid Molecules incorporating natural products: applications in cancer therapy, neurodegenerative disorders and beyond, Curr. Med. Chem. 18 (2011) 1464–1475.

[2] Shaveta, S. Mishra, P. Singh, Hybrid molecules: The privileged scaffolds for various pharmaceuticals, Eur. J. Med. Chem. 124 (2016) 500–536.

[3] S. Vandekerckhove, M. D'hooghe, Quinoline-based antimalarial hybrid compounds, Bioorg. Med. Chem. 23 (2015) 5098–5119.

[4] S. Sandhu, Y. Bansal, O. Silakari, G. Bansal, Coumarin hybrids as novel therapeutic agents, Bioorg. Med. Chem. 22 (2014) 3806–3814.

[5] X. Zhang, X. He, Q. Chen, J. Lu, S. Rapposelli, R. Pi, A review on the hybrids of hydroxycinnamic acid as multi-targetdirected ligands against Alzheimer's disease, Bioorg. Med. Chem. 26 (2015) 543–550.

[6] R. Yehuda, S.R. Brand, J.A. Golier, R.K.Yang, Clinical correlates of DHEA associated with post-traumatic stress disorder, Acta Psychiatr. Scand. 114 (2006) 187–193.

[7] R.S. Jiang, S.H. Wu, K.L. Liang, J.Y. Shiao, C.H. Hsin, M.C. Su, Steroid treatment of posttraumatic anosmia, Eur. Arch. Otorhinolaryngol. 267 (2010) 1563–1567.

[8] H.S. Kim, B.S. Choi, K.C. Kwon, S.O. Lee, H.J. Kwak, C.H. Lee, Synthesis and antimicrobial activity of squalamine analogue, Bioorg. Med. Chem. 8 (2000) 2059–2065.

[9] S. Khabnadideh, C.L. Tan, S.L. Croft, H. Kendrick, V. Yardley, I.H. Gilbert, Squalamine analogues as potential anti-trypanosomal and anti-leishmanial compounds, Bioorg. Med. Chem. Lett. 10 (2000) 1237–1239.

[10] E.W.Sugandhia, C. Slebodnicka, J.O. Falkinham, R.D. Gandour, Synthesis and antimicrobial evaluation of water-soluble, dendritic derivatives of epimeric 5α-cholestan-3-amines and 5α-cholestan-3-yl aminoethanoates, Steroids 2 (2007) 615–626.

[11] C. Loncle, J.M. Brunel, N. Vidal, M. Dherbomez, Y. Letourneux, Synthesis and antifungal activity of cholesterol-hydrazone derivatives, Eur. J. Med. Chem. 39 (2004) 1067–1071.

[12] K. Kikuchi, E.M. Bernard, A. Sadownik, S.L. Regen, D. Armstrong, Antimicrobial activities of squalamine mimics, Antimicrob. Agents Chemother. 41 (1997) 1433–1438.

[13] A. Gupta, B.S. Kumar, A.S. Negi, Current status on development of steroids as anticancer agents, J. Steroid Biochem. Mol. Biol. 137 (2013) 242–270.

[14] J.A. Salvador, J.F. Carvalho, M.A.Neves, S.M. Silvestre, A.J.Leitao, M.M. Silva, M.L..Sá e Melo, Anticancer steroids: linking natural and semi-synthetic compounds, Nat. Prod. Rep. 30 (2013) 324–374.

[15] P. Saha, C. Debnath, G. Bérubé, Steroid-linked nitrogen mustards as potential anticancer therapeutics: a review, J. Steroid Biochem. Mol. Biol. 137 (2013) 271–300.

[16] A.H. Bandaya, B.P. Mira, I.H. Lonea, K.A. Suri, H.M. Sampath Kumar, Studies on novel D-ring substituted steroidal pyrazolines as potential anticancer agents, Steroids 75 (2010) 805–809.

[17] V.A. D'yakonov, L.U. Dzhemileva, R.A. Tuktarova, A.A. Makarov, I.I. Islamov, A.R. Mulyukova, U.M. Dzhemilev, Catalytic cyclometallation in steroid chemistry III: Synthesis of steroidal derivatives of 5Z,9Z-dienoic acid and investigation of its human topoisomerase I inhibitory activity, Steroids 102 (2015) 110–117.

[18] V.A. D'yakonov, A.A. Makarov, L.U. Dzhemileva, E.Kh. Makarova, E.K. Khusnutdinova, U.M. Dzhemilev, The facile synthesis of the 5Z,9Z-dienoic acids and their topoisomerase I inhibitory activity, Chem. Commun. 49 (2013) 8401–8403.

[19] V.A. D'yakonov, L.U. Dzhemileva, R.A. Tuktarova, S.R. Ishmukhametova, M.M. Yunusbaeva, I.R. Ramazanova, U.M. Dzhemilev, Novel hydrid molecules on the basis of steroids and (5Z,9Z)-tetradeca-5,9-dienoic acid: synthesis, anti-cancer studies and human topoisomerase I inhibitory activity, Anticancer Agents Med. Chem. 16 (2017) 1–10.

[20] V.A. D'yakonov, L.U. Dzhemileva, U.M. Dzhemilev, Advances in the Chemistry of Natural and Semisynthetic Topoisomerase I/II Inhibitors, Studies in Natural Prod. Chem. 54 (2017) 21–86.

[21] S. Svedhem, C.A. Hollander, J. Shi, P. Konradsson, B. Liedberg, S.C.T. Svensson, Synthesis of a series of oligo(ethylene glycol)-terminated alkanethiol amides designed to address structure and stability of biosensing interfaces, J. Org. Chem. 66 (2001) 4494–4503.

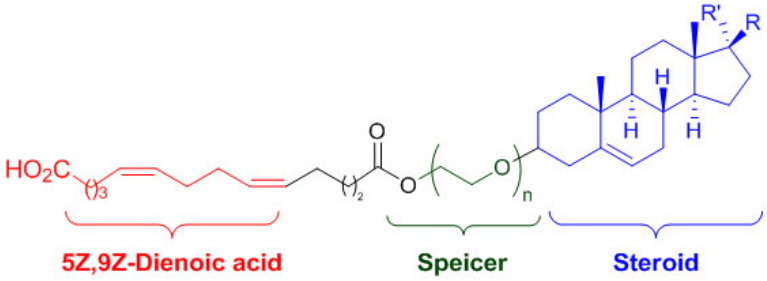
[22] X.Wu, Y. Wu, L. Hai, Y. Bao, Synthesis of cholesterylated carboxylic acids with oligo(polyethylene glycols) units for gene delivery, Lett. Org. Chem. 3 (2006) 911–914.

[23] S.W. Cha, J. Jin, D.C. Kim, W.C. Zin, Combined type liquid crystalline poly(oxy-1,4-phenyleneoxyterephthaloyl)s bearing cholesterol pendants attached through polymethylene spacers, Macromolecules 34 (2001) 5342–5348.

[24] V.A. D'yakonov, A.A. Makarov, E.Kh. Makarova, L.M. Khalilov, U.M. Dzhemilev, Synthesis and transformations of metallacycles 41. Cyclomagnesiation of O-containing 1,2-dienes with Grignard reagents in the presence of Cp_2TiCl_2, Russ. Chem. Bull. 61 (2012) 1943–1949.

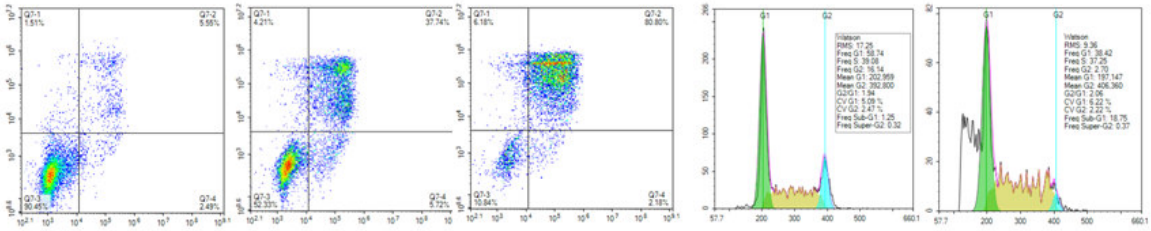
[25] V.A. D'yakonov, A.A. Makarov, E.Kh. Makarova, U.M. Dzhemilev, Novel organomagnesium reagents in synthesis. Catalytic cyclomagnesiation of allenes in the synthesis of N-, O-, and Si-substituted 1Z,5Z-dienes, Tetrahedron 69 (2013) 8516–8526.

Graphical Abstract



Cytotoxicity (CC50, nM)

Jurkat	0.06-0.33
K562	0.11-0.39
Hek293	0.03-0.62
HeLa	0.15-0.79
U937	0.10-0.30
Fibroblasts	0.67-1.17



Induction of apoptosis and cell cycle analysis by flow cytometry

Highlights

- Synthesis of steroid containing 5Z,9Z-dienoic acids have been developed.
- New molecules are efficient apoptosis inducers in cancer cells.
- The structures of all novel compounds were confirmed by NMR measurements.