Synthesis of steroid analogs of tubuloclustin, their cytotoxicity and effect on microtubules of A549 carcinoma cells

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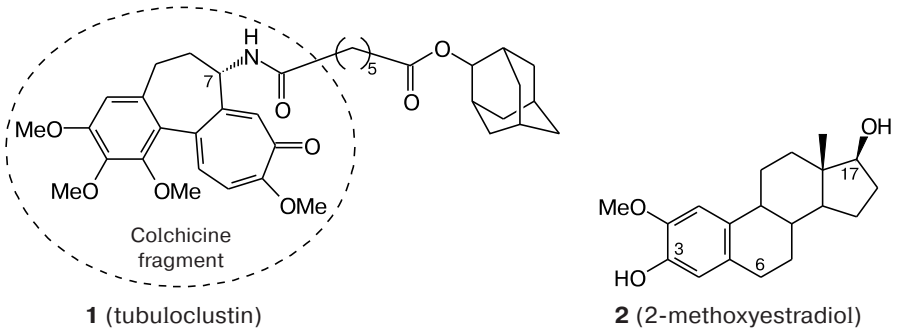
Synthesis of analogs of tubuloclustin (*N*-(7-adamant-2-yloxy-7-oxoheptanoyl)-*N*-deacetylcolchicine (**1**)) with the colchicine fragment replaced with 2-methoxyestradiol scaffold attached *via* phenolic hydroxy group was described. Esters **3a—c** exhibit moderate cytotoxicity (EC_{50} = 5—6 μmol L^{-1}) and exert a weak effect on the microtubule network in A549 human lung carcinoma cells similar to the clustering effect of tubuloclustin and its derivatives. Conjugates **6a—c** and **7a—c** with the phenolic ester bond are low stable and compounds **7a—c** are inactive to the microtubules of A549 cells, while compounds **6a—c** cause an unusual effect of curling of the microtubules.

Key words: 2-methoxyestradiol, adamantane, colchicine, tubuloclustin, tubulin, microtubule network, cytotoxicity, A549 lung carcinoma cells.

Tubuloclustin (**1**), an adamantane conjugate of anticancer drug colchicine, exhibits high antiproliferative activity both *in vitro* (IC_{50} ≈ 6 μmol L^{-1})[1] and *in vivo*.[2] It not only inhibits tubulin polymerization into microtubules as typical for colchicine[3] but also causes unusual tubulin clustering (the intensity of tubulin-clustering ability in a series of analogs of tubuloclustin **1** correlates with their cytotoxicity against tumor cells).[1] Data on the efficiency of tubuloclustin *in vivo* prompted synthesis of its analogs by replacing the colchicine fragment with other ligands of the tubulin colchicine domain.[2,4—6] Among these ligands, 2-methoxyestradiol (**2**) (Fig. 1) that exhibits low general toxicity[7,8] is of special interest. The attempt to link the adamantane-substituted chain to the C(6) position of 2-methoxyestradiol **2** (the C(6) atom of 2-methoxyestradiol was the closest to the C(7) atom of colchicine when both molecules were simultaneously docked at the colchicine-binding domain of tubulin[9]) led to unstable and inactive compounds.[2] Inactive conjugates (EC_{50} > 50 μmol L^{-1}) were also obtained by linking the



1 (tubuloclustin) **2** (2-methoxyestradiol)

Fig. 1. Schematic representation of the replacement of the colchicine moiety in the structure of tubuloclustin (**1**) with 2-methoxyestradiol **2** (in the present work, we linked the 2-methoxyestradiol moiety to the C(3)OH group).

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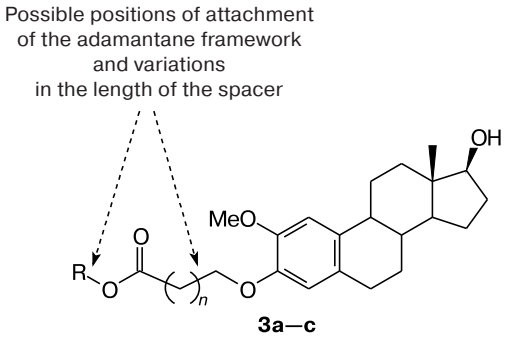
adamantane-containing spacer to the C(17) hydroxy group of 2-methoxyestradiol.[5]

The aim of the present work was the synthesis of tubuloclustin-like conjugates of 2-methoxyestradiol *via* modification of the phenolic hydroxy group at C(3) of steroid (see Fig. 1) and evaluation of biological activity of the synthesized compounds. Our goal was to synthesize stable conjugates bearing a flexible linker, namely, esters of the general formula **3**.



3: *n* = 5, R = adamant-2-yl (**a**), adamant-1-yl (**b**);
n = 6, R = adamant-2-yl (**c**)

Since modification of the C(3) hydroxy group of the starting steroid can result in a decrease in cytotoxicity,[10] we docked compounds **3** with the alkyl spacers of different length (*n* = 5—8) and with differently positioned adamantane framework (at either the bridgehead position or the C(2) atom) into the 3D model of colchicine-binding site of tubulin (PDB ID: 1SA0) using CLC Drug Discovery Workbench software. Molecular modeling showed that the steroid moieties of the suggested conjugates **3** and 2-methoxyestradiol **2** are positioned differently (Fig. 2) but the position of the adamantane framework of **3** is close to that

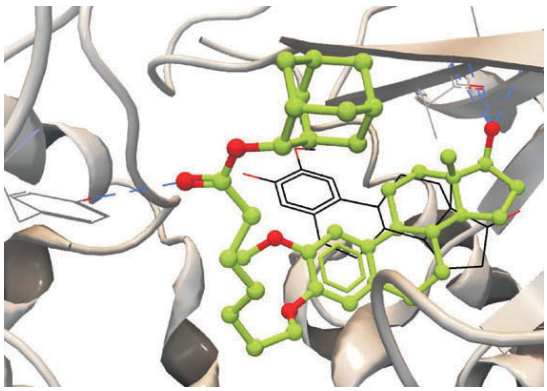
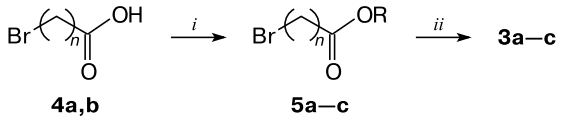


Fig. 2. Arrangement of molecule **3a** in the α,β-tubulin dimer (β-subunit is shown on the right; H-bond between the carbonyl oxygen atom of compound **3a** and the hydroxy group of αTyr224 residue is shown by the dashed line (on the left); arrangement of 2-methoxyestradiol **2** is shown by thin lines for a comparison). *Note.* Figure 2 is available in full color on the web page of the journal (https://link.springer.com/journal/11172).

of tubuloclustin and the carbonyl oxygens of compounds **3a—c** form the H-bond with phenolic hydroxy group of the αTyr224 residue as typical for tubuloclustin.[1] These results stimulated us to synthesize and study compounds **3a—c**.

Synthesis of compounds **3a—c** was accomplished in two steps from 7-bromoheptanoic (**4a**) and 8-bromooctanoic acids (**4b**) (Scheme 1). In turn, acids **4a**,**b** were obtained by the reaction of the corresponding cyclic lactones[11] with HBr in AcOH as described by Hrabálek and co-workers.[12]

Scheme 1



Reagents, conditions, and yields: *i*. ROH, DCC, DMAP, CH_2Cl_2, 25 °C; *ii*. 2-methoxyestradiol (**2**), K_2CO_3, acetone, 85 °C; **3a** (55% from **5a**), **3b** (51% from **5b**), **3c** (27% from **5c**).

4: *n* = 6 (**a**), 7 (**b**)
5: *n* = 6, R = adamant-2-yl (**a**), adamant-1-yl (**b**),
n = 7, adamant-2-yl (**c**)

The first step involves esterification of acids **4a**,**b** with either adamant-1-ol or adamant-2-ol (ROH) in the presence of DCC and DMAP to give brominated monoesters **5a—c**.[4] In the second step, compounds **5a—c** were reacted with 2-methoxyestradiol **2** in the presence of K_2CO_3 *via* the modified procedure[13] to afford the target conjugates **3a—c**. ^{1}H NMR spectra of compounds **3a—c** lack the signal of the phenolic hydroxy group and show the triplet signals of the side chain CH_2O protons at δ 3.97 (**3a**) and 3.98 (**3b**,**c**). MALDI-TOF mass spectrometry data (*m*/*z* 564 $[M]^+$ for **3a**,**b** and 578 $[M]^+$ for **3c**) and elemental analysis confirm the structure and compositions of compounds **3a—c**.

The standard MTT assay[14] on A549 carcinoma cells revealed moderate cytotoxicity of compound **3a—c** (in submicromolar concentration range), which is independent on both the length of the spacer and the position of its linking to the adamantane framework (Table 1). A decrease in cytotoxicity of conjugates **3a—c** as compared with tubuloclustin **1** is apparently due to the difference in the position of the steroid moieties of compounds **3a—c** and 2-methoxyestradiol **2** in the colchicine-binding site of tubulin found by molecular modeling (see Fig. 2) (despite the fact that the ligand—receptor (tubulin) scoring functions for compounds **3a—c** and 2-methoxyestradiol **2** have the same values).

Immunofluorescence microscopy (see Table 1 and Fig. 3) showed that compounds **3a—c** exerted the same weak effect on microtubule network similar to the effects

Table 1. Effects of compounds **1**, **2**, **3a—c**, **6a—c**, and **7a—c** on A549 cells

Compound	Cytotoxicity[a] IC_{50}/μmol L^{-1}	Effect on microtubules[b]
3a	5±1	Low clustering effect
3b	6±1	Low clustering effect
3c	5.2±0.8	Low clustering effect
6a	—[c]	Microtubule curling
6b	—[c]	Microtubule curling
6c	—[c]	Microtubule curling
7a	—[c]	No effect
7b	—[c]	No effect
7c	—[c]	No effect
2	0.20±0.05[15]	Depolymerization
1	0.006[1]	Depolymerization and clustering

[a] The average of three experiments.
[b] In a dose of 100 μmo l L^{-1}.
[c] Not determined.

of tubuloclustin and its derivatives[1] (see Figs 3, *c* and 3, *d*) only when applied at extremely high concentrations (100 μmol L^{-1}). This also agrees with the molecular docking results (see above) predicting for compounds **1** and **3a—c** similar orientation of the adamantane moieties playing an essential role in the appearance of this effect.[1]

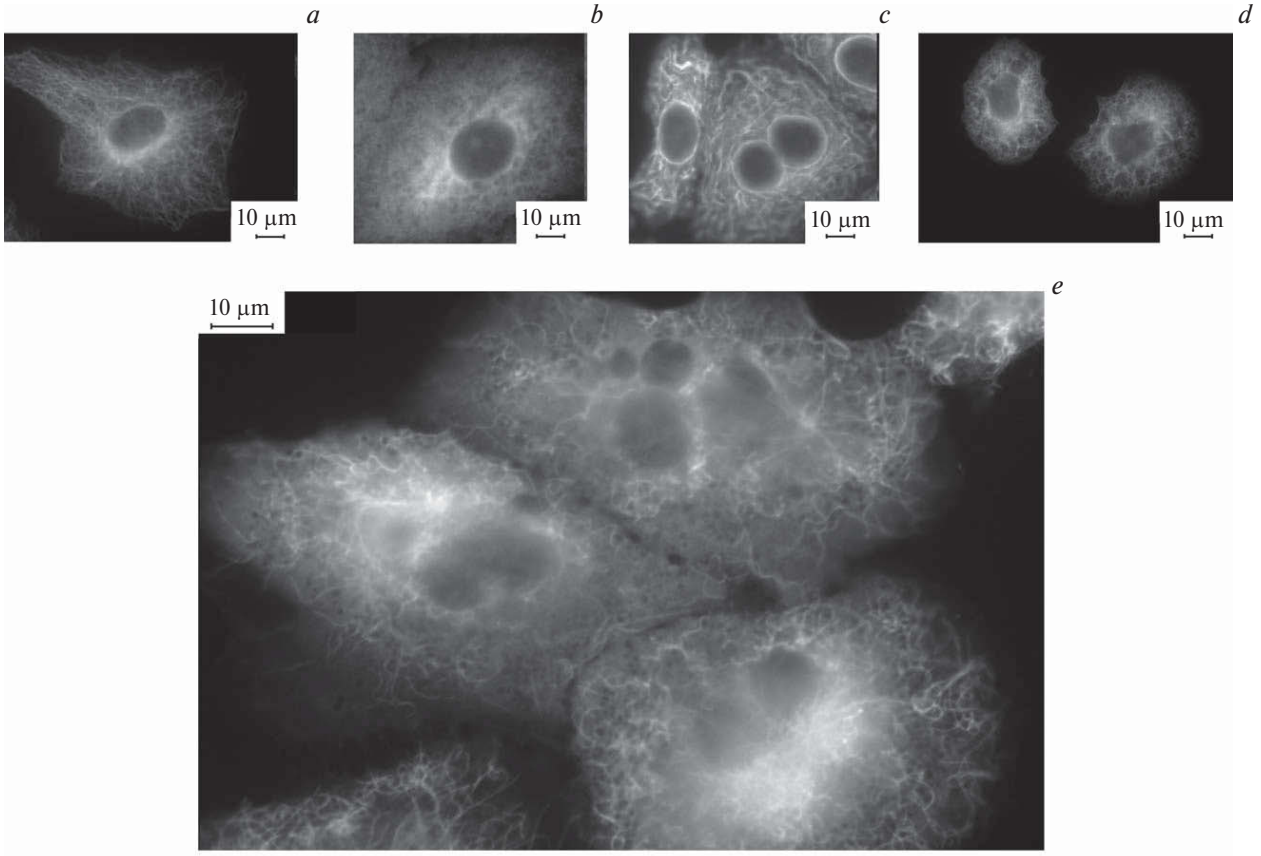
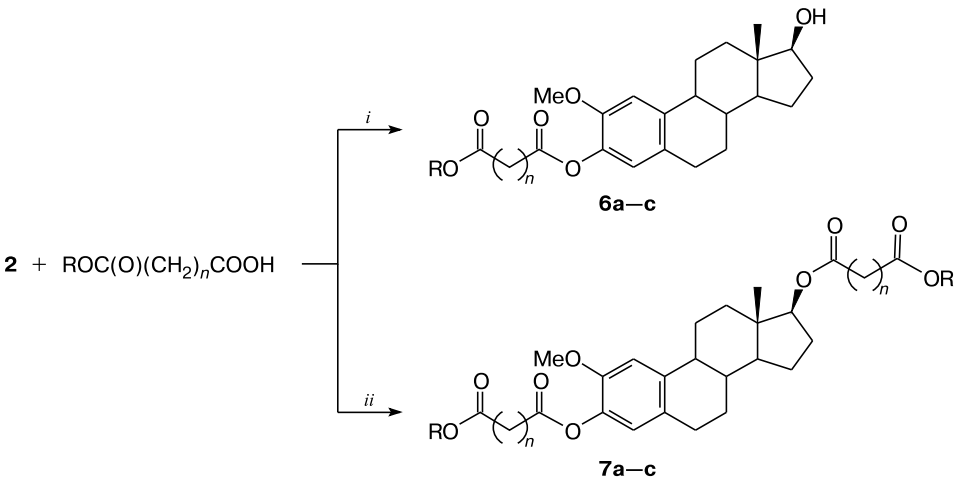


Fig. 3. Immunofluorescence microscopy images of the A549 cells treated as follows: 0.5% aqueous DMSO for 48 h (negative control; intact microtubule network) (*a*); 2-methoxyestradiol **2** at a dose of 100 μmol L^{-1} (positive control; depolymerization of microtubules) (*b*); tubuloclustin **1** at a dose of 1 μmol L^{-1} (positive control: strong clustering effect) (*c*), compound **3a** at a dose of 100 μmol L^{-1} (weak clustering effect) (*d*), compound **6c** at a dose of 100 μmol L^{-1} (curling of microtubules; nuclear fragmentation typical for the apoptotic cells is also visible) (*e*).

Scheme 2



R =

Compound	*n*	Isolated yield (%)
6a	5	16
6b	6	21
6c	7	31
7a	5	16
7b	6	21
7c	7	22

Reagents and conditions: *i* and *ii*. DCC, DMAP, CH_2Cl_2, ~20 °C (for *i*, a solution of $ROC(O)(CH_2)_nCOOH$ (n = 5—7) and DCC was added dropwise to a solution of 2-methoxyestradiol **2** and DMAP; for *ii*, simultaneous mixing of all components).

According to the published data,[10] highly cytotoxic C(3)-modified derivatives of 2-methoxyestradiol generally contain unsaturated moiety at the atom two bonds distant from the C(3) atom. Therefore, we attempted synthesis of such conjugates by linking the adamantane-containing spacer at the steroid core *via* the ester bond.

Esterification of 2-methoxyestradiol **2** with monoadamantyl esters of pimelic, suberic, and azelaic acids[4] at a ratio of **2** : mono-ester = 1 : 1 gives compounds **6a**—**c** (Scheme 2). However, products **6a**—**c** were significantly contaminated by the starting compound **2** and dicarboxylic acid mono-esters; the latter resulted from hydrolysis of the target products upon silica gel chromatography (content of impurities was about 30%, see Experimental). It should be noted that the change in the reaction protocol (see Scheme 2 and Experimental) gives conjugates **7a**—**c** modified at both hydroxy groups of the starting steroid. Compounds **7a**—**c** are also unstable and contaminated by hydrolysis products (content of impurities of about 15%) as well.

Since compounds **6a**—**c** and **7a**—**c** are unstable, we performed no studies of their cytotoxicity and the immunofluorescence microscopy measurements were performed using very high concentrations of the test compounds (100 μmol L^{-1}) to ensure a sufficient number of unhydrolyzed molecules in the cells. Conjugates **7a**—**c** exert no effect on microtubules; while compounds **6a**—**c** cause unusual microtubule curling (see Table 1 and Fig. 3, *e*). No similar microtubule curling was observed in the presence of neither tubuloclustin (**1**) and compounds **3a**—**c** nor the products of hydrolysis of the latter, *i.e.*, 2-methoxyestradiol **2** and compounds **5a**—**c**. It is noteworthy that according to molecular modeling (1) the adamantane framework of conjugates **6a**—**c** is closer to the protein β-subunit than that of both tubuloclustin **1** and compounds **3a**—**c** and (2) conjugates **6a**—**c** form no H-bond with the phenolic hydroxy group of the αTyr224 residue. These are the apparent reasons for the observed differences in the modes of action of the studied compounds on the microtubule network.

In general, the replacement of the colchicine unit in the structure of tubuloclustin with the 2-methoxyestradiol moiety *via* linking to the phenolic hydroxy group of the steroid results in the compounds exhibiting moderate cytotoxicity (in a micromolar concentration range) against cancer cells and compounds interesting due to their effects on the microtubule network of the A459 cells.

Experimental

Automated docking into the 3D model of tubulin—*N*-deacetyl-*N*-(2-mercaptoacetyl)colchicine complex (predetermined radius of 16 Å) was performed using CLC Drug Discovery Workbench (Version 1.5): Evaluation license (2016). The reaction course and purity of the synthesized compounds were monitored by TLC on Silufol-UV254 pates. For column chromatography, Acros Organics silica gel (40—60 μm) was used. ^1H and ^{13}C NMR spectra were run on an Agilent 400-MR instrument (working frequencies of 400 (^1H) and 100 MHz (^{13}C)) in $CDCl_3$ at 28 °C. The chemical shifts are given in the δ scale relative to the solvent signal ($CDCl_3$; δ_H 7.26, δ_C 77.36). Elemental analysis was

performed with a Vario micro cube CHN analyzer. IR spectra were recorded with a ThermoNicolet IR-200 FT-IR spectrometer in the KBr pellets. Matrix assisted laser desorption/ionization time-of-flight mass spectrometry was performed with an Autoflex II instrument operating in the reflecting mode (a nitrogen laser operated at 337 nm, accelerating voltage of 20 kV). Acetone was dried by distillation over P_2O_5.

Synthesis of compounds 3a—c (general procedure). To a solution of 2-methoxyestradiol **2** (0.040 g, 0.132 mmol) in anhydrous acetone (50 mL), calcined K_2CO_3 (0.248 g, 1.797 mmol) was added. After 20 min, a solution of ω-bromocarboxylic acid adamantyl ester[4] (0.100 g, 0.292 mmol) in acetone (1 mL) was added. The mixture was heated at 80—85 °C for 5 h under argon, concentrated *in vacuo*, and the residue was subjected to silica gel column chromatography (if not stated otherwise, gradient elution with ethyl acetate—petroleum ether (b.p. 40—70 °C), 1 : 9→1 : 8).

Adamant-2-yl 7-[(17β)-17-hydroxy-2-methoxyestra-1,3,5(10)-trien-3-yloxy]heptanoate (3a) was synthesized according to the general procedure from compound **2**, adamant-2-yl 7-bromoheptanoate[4] (**5a**), and K_2CO_3. Yield 0.041 g (55%), colorless heavy oil. ^{1}H NMR ($CDCl_3$), δ: 0.80 (s, 3 H, C(18)Me); 1.17—1.58 (m, 14 H); 1.65—1.91 (m, 14 H); 1.95—2.06 (m, 5 H); 2.09—2.24 (m, 2 H); 2.29—2.33 (m, 1 H); 2.34 (t, 2 H, CH_2CO_2, J = 7.5 Hz); 2.74—2.87 (m, 2 H, H(6)); 3.75 (dd, 1 H, H(17α), J = 8.3 Hz, J = 8.6 Hz); 3.85 (s, 3 H, OMe); 3.98 (t, 2 H, CH_2O, J = 6.7 Hz); 4.94 (m, 1 H, $H(2)_{Ad}$); 6.60 (s, 1 H, H(4)); 6.84 (s, 1 H, H(1)). ^{13}C NMR ($CDCl_3$), δ: 11.0 (C(18)), 23.1, 25.1, 25.7, 26.5, 26.9, 27.2, 27.3, 28.9, 29.0, 29.2, 30.6, 31.7 (Ad), 31.8 (Ad), 34.8, 36.3 (Ad), 36.7, 37.3, 38.8, 43.2, 44.2, 50.0, 56.2 (OMe), 68.8 (CH_2O), 76.7 ($C(2)_{Ad}$), 81.8 (C(17)), 109.5 (C(1)), 113.6 (C(4)), 128.7 (C(5)), 132.2 (C(10)), 146.5 (C(2)), 147.3 (C(3)), 173.2 (C=O). MS (MALDI-TOF), *m/z*: 564 $[M]^+$, 587 $[M + Na]^+$, 603 $[M + K]^+$. Found (%): C, 76.52; H, 9.18. $C_{36}H_{52}O_5$. Calculated (%): C, 76.56; H, 9.28.

Adamant-1-yl 7-[(17β)-17-hydroxy-2-methoxyestra-1,3,5(10)-trien-3-yloxy]heptanoate (3b) was synthesized according to the general procedure from compound **2**, adamant-1-yl 7-bromoheptanoate[4] (**5b**), and K_2CO_3. Column was eluted first with benzene and then with ethyl acetate—benzene (1 : 10). Yield 0.038 g (51%), colorless heavy oil. ^{1}H NMR ($CDCl_3$), δ: 0.80 (s, 3 H, C(18)Me); 1.17—1.54 (m, 12 H); 1.57—1.74 (m, 9 H); 1.80—1.91 (m, 3 H); 1.97 (dt, 1 H, J = 12.5 Hz, J = 3.0 Hz); 2.11—2.19 (m, 11 H), 2.22 (t, 2 H, CH_2CO_2, J = 7.4 Hz); 2.29—2.33 (m, 1 H); 2.73—2.86 (m, 2 H, H(6)); 3.74 (dd, 1 H, H(17α), J = 8.4 Hz, J = 8.6 Hz); 3.84 (s, 3 H, OMe); 3.97 (t, 2 H, CH_2O, J = 6.7 Hz); 6.59 (s, 1 H, H(4)); 6.83 (s, 1 H, H(1)). ^{13}C NMR ($CDCl_3$), δ: 11.1 (C(18)), 23.1, 25.1, 25.7, 26.5, 27.4, 28.8, 29.1, 29.2, 30.7, 30.8 ($C(3,5,7)_{Ad}$), 35.6, 36.2 ($C(4,6,10)_{Ad}$), 36.8, 38.8, 41.4 ($C(2,8,9)_{Ad}$), 43.3, 44.2, 50.0, 56.3 (OCH_3), 68.9 (CH_2O), 80.1 ($C(1)_{Ad}$), 81.9 (C(17)), 109.7 (C(1)), 113.7 (C(4)), 128.8 (C(5)), 132.3 (C(10)), 146.6 (C(2)), 147.3 (C(3)), 172.9 (C=O). MS (MALDI-TOF), *m/z*: 564 $[M]^+$, 587 $[M + Na]^+$, 603 $[M + K]^+$. Found (%): C, 76.58; H, 9.27. $C_{36}H_{52}O_5$. Calculated (%): C, 76.56; H, 9.28.

Adamant-2-yl 8-[(17β)-17-hydroxy-2-methoxyestra-1,3,5(10)-trien-3-yloxy]octanoate (3c) was synthesized according to the general procedure from compound **2** (0.045 g, 0.149 mmol), adamant-2-yl 8-bromooctanoate[4] (**5c**) (0.109 g, 0.305 mmol), and K_2CO_3 (0.286 g, 2.072 mmol). Yield 0.023 g (27%), colorless heavy oil. ^{1}H NMR ($CDCl_3$), δ: 0.80 (s, 3 H, C(18)Me); 1.17—1.58 (m, 16 H); 1.65—1.90 (m, 14 H); 1.95—2.06 (m, 5 H); 2.09—2.24 (m, 2 H); 2.29—2.33 (m, 1 H); 2.35 (t, 2 H, CH_2CO_2, J = 7.4 Hz); 2.74—2.87 (m, 2 H, H(6)); 3.75 (dd, 1 H, H(17α), J = 8.2 Hz, J = 8.6 Hz); 3.85 (s, 3 H, OCH_3); 3.98 (t, 2 H, CH_2O, J = 6.7 Hz); 4.94 (m, 1 H, $H(2)_{Ad}$); 6.60 (s, 1 H, H(4)); 6.84 (s, 1 H, H(1)). ^{13}C NMR ($CDCl_3$), δ: 11.1 (C(18)), 23.1, 25.1, 25.9, 26.5, 27.0, 27.2, 27.4, 29.0, 29.0, 29.2, 29.3, 30.6, 31.8 (Ad), 31.9 (Ad), 34.9, 36.3 (Ad), 36.8, 37.4, 38.8, 43.3, 44.2, 50.0, 56.3 (OCH_3), 69.0 (CH_2O), 76.7 ($C(2)_{Ad}$), 81.9 (C(17)), 109.7 (C(1)), 113.7 (C(4)), 128.8 (C(5)), 132.3 (C(10)), 146.6 (C(2)), 147.4 (C(3)), 173.2 (C=O). MS (MALDI-TOF), *m/z*: 578 $[M]^+$, 601 $[M + Na]^+$, 617 $[M + K]^+$. Found (%): C, 76.80; H, 9.38. $C_{37}H_{54}O_5$. Calculated (%): C, 76.78; H, 9.40.

Reaction of 2-methoxyestradiol 2 with carboxylic acid monoadamantyl esters (general procedure). Method *A*. To a solution of 2-methoxyestradiol **2** (0.050 g, 0.165 mmol) and catalytic amounts of DMAP (0.01—0.02 g), a solution of carboxylic acid and DCC (0.040 g, 0.194 mmol) in CH_2Cl_2 (10 mL) was added dropwise over a period of 30 min and the mixture was then stirred at room temperature for additional 24 h. The volatiles were removed *in vacuo*, the residue was dissolved in ethyl acetate (10—20 mL) and kept at 0—4 °C for 2—3 h. Precipitated crystalline or amorphous *N*,*N*′-dicyclohexylurea was filtered off, washed with chilled ethyl acetate (2×10 mL) and the filtrate was successively washed with brine (10 mL) and water (10 mL), dried over Na_2SO_4, and concentrated *in vacuo*. The residue was chromatographed three times (gradient elution with ethyl acetate—petroleum ether (b.p. 40—70 °C), 1 : 11→1 : 9).

Adamant-2-yl (17β)-17-hydroxy-2-methoxyestra-1,3,5(10)-trien-3-ylheptanedioate (6a) was synthesized according to method *A* from pimelic acid mono-2-adamantyl ester (0.058 g, 0.197 mmol). Yield 0.015 g (16%), colorless oil with chemical purity of ~70%. ^{1}H NMR ($CDCl_3$), δ: 0.79 (s, 3 H, C(18)Me); 1.17—1.64 (m, 16 H); 1.68—1.90 (m, 10 H); 1.95—2.03 (m, 5 H); 2.09—2.18 (m, 1 H); 2.20—2.34 (m, 2 H); 2.37 (t, 2 H, $C\underline{H}_2CO_2Ad$, J = 7.4 Hz); 2.58 (t, 2 H, $C\underline{H}_2CO_2Ar$, J = 7.4 Hz); 2.75—2.86 (m, 2 H, H(6)); 3.74 (dd, 1 H, H(17α), J = 8.2 Hz, J = 8.6 Hz); 3.79 (s, 3 H, OMe); 4.94 (m, 1 H, $H(2)_{Ad}$)); 6.72 (s, 1 H, H(4)); 6.89 (s, 1 H, H(1)). MS, *m/z*: 601 $[M + Na]^+$, 617 $[M + K]^+$.

Adamant-2-yl (17β)-17-hydroxy-2-methoxyestra-1,3,5(10)-trien-3-yloctanedioate (6b) was synthesized according to method *A* from suberic acid mono-2-adamantyl ester (0.051 g, 0.194 mmol). Yield 0.021 g (21%), colorless oil with chemical purity of ~70%. ^{1}H NMR ($CDCl_3$), δ: 0.79 (s, 3 H, C(18)Me); 1.16—1.62 (m, 18 H); 1.67—1.89 (m, 10 H); 1.95—2.03 (m, 5 H); 2.07—2.18 (m, 1 H); 2.20—2.32 (m, 2 H); 2.35 (t, 2 H, CH_2CO_2Ad, J = 7.5 Hz); 2.56 (t, 2 H, CH_2CO_2Ar, J = 7.5 Hz); 2.75—2.84 (m, 2 H, H(6)); 3.74 (dd, 1 H, H(17α), J = 8.5 Hz, J = 8.6 Hz); 3.79 (s, 3 H, OMe); 4.93 (m, 1 H, $H(2)_{Ad}$); 6.72 (c, 1 H, H(4)); 6.89 (c, 1 H, H(1)). MS, *m/z*: 615 $[M + Na]^+$, 631 $[M + K]^+$.

Adamant-2-yl (17β)-17-hydroxy-2-methoxyestra-1,3,5(10)-trien-3-ylnonanedioate (6c) was synthesized according to method *A* from azelaic acid mono-2-adamantyl ester (0.053 g, 0.165 mmol). Yield 0.031 g (16%), colorless oil with chemical purity of ~70%. ^{1}H NMR ($CDCl_3$), δ: 0.79 (s, 3 H, C(18)Me); 1.15—1.61 (m, 20 H); 1.65—1.90 (m, 10 H); 1.93—2.04 (m, 5 H); 2.10—2.19 (m, 1 H); 2.21—2.32 (m, 2 H); 2.35 (t, 2 H, CH_2CO_2Ad, J = 7.5 Hz); 2.56 (t, 2 H, CH_2CO_2Ar, J = 7.5 Hz); 2.75—2.86 (m, 2 H, H(6)); 3.74 (dd, 1 H, H(17α), J = 8.5 Hz, J = 8.6 Hz); 3.80 (s, 3 H, OMe); 4.93 (m, 1 H, $H(2)_{Ad}$); 6.73

(s, 1 H, H(4)); 6.90 (s, 1 H, H(1)). MS, *m/z*: 629 $[M + Na]^+$, 645 $[M + K]^+$.

Method *B*. To a solution of carboxylic acid monoester in CH_2Cl_2 (10 mL), 2-methoxyestradiol **2** (0.050 g, 0.165 mmol), DCC (0.040 g, 0.194 mmol), and catalytic amount of DMAP (0.01—0.02 g) were added at room temperature. The residue was chromatographed twice (gradient elution with ethyl acetate—petroleum ether (b.p. 40—70 °C), 1 : 25→1 : 20).

Adamant-2-yl (17β)-17-[7-(2-adamantyloxy)-7-oxoheptanoyloxy]-2-methoxyestra-1,3,5(10)-trien-3-ylheptanedioate (7a) was synthesized according to method ***B*** from pimelic acid mono-2-adamantyl ester (0.055 g, 0.187). Yield 0.023 g (16%), colorless heavy oil with chemical purity of ~85%. ^{1}H NMR ($CDCl_3$), δ: 0.84 (s, 3 H, C(18)Me); 1.26—1.59 (m, 18 H); 1.65—1.90 (m, 24 H); 2.00—2.04 (m, 8 H); 2.19—2.32 (m, 3 H); 2.34 (t, 2 H, CH_2CO_2, J = 7.4 Hz); 2.36 (t, 2 H, CH_2CO_2, J = 7.4 Hz); 2.38 (t, 2 H, CH_2CO_2, J = 7.4 Hz); 2.59 (t, 2 H, CH_2CO_2Ar, J = 7.4 Hz); 2.77—2.84 (m, 2 H, H(6)); 3.80 (s, 3 H, OMe); 4.71 (dd, 1 H, H(17α), J = 7.8 Hz, J = 9.0 Hz); 4.94 (m, 2 H, $H(2)_{Ad}$); 6.73 (s, 1 H, H(4)); 6.89 (s, 1 H, H(1)). MS, *m/z*: 878 $[M + Na]^+$, 894 $[M + K]^+$.

Adamant-2-yl (17β)-17-[8-(2-adamantyloxy)-8-oxooctanoyloxy]-2-methoxyestra-1,3,5(10)-trien-3-yloctanedioate (7b) was synthesized according to method ***B*** from suberic acid mono-2-adamantyl ester (0.058 g, 0.188 mmol). Yield 0.031 g (21%), colorless heavy oil with chemical purity of ~85%. ^{1}H NMR ($CDCl_3$), δ: 0.85 (s, 3 H, C(18)Me); 1.26—1.58 (m, 22 H); 1.63—1.90 (m, 24 H); 2.00—2.04 (m, 8 H); 2.18—2.30 (m, 3 H); 2.33 (t, 2 H, CH_2CO_2, J = 7.6 Hz); 2.35 (t, 2 H, CH_2CO_2, J = 7.6 Hz); 2.36 (t, 2 H, CH_2CO_2, J = 7.4 Hz); 2.59 (t, 2 H, CH_2CO_2Ar, J = 7.4 Hz); 2.78—2.86 (m, 2 H, H(6)); 3.80 (s, 3 H, OMe); 4.72 (dd, 1 H, H(17α), J = 8.0 Hz, J = 9.0 Hz); 4.94 (m, 2 H, $H(2)_{Ad}$); 6.73 (s, 1 H, H(4)); 6.89 (s, 1 H, H(1)). MS, *m/z*: 906 $[M + Na]^+$, 922 $[M + K]^+$.

Adamant-2-yl (17β)-17-[9-(2-adamantyloxy)-9-oxononanoyloxy]-2-methoxyestra-1,3,5(10)-trien-3-ylnonanedioate (7c) was synthesized according to method ***B*** from azelaic acid mono-2-adamantyl ester (0.061 g, 0.187 mmol). Yield 0.033 g (22%), colorless heavy oil with chemical purity of ~85%. ^{1}H NMR ($CDCl_3$), δ: 0.84 (s, 3 H, C(18)Me); 1.26—1.58 (m, 26 H); 1.61—1.90 (m, 24 H); 1.99—2.04 (m, 8 H); 2.18—2.30 (m, 3 H); 2.32 (t, 2 H, CH_2CO_2, J = 6.9 Hz); 2.33 (t, 2 H, CH_2CO_2, J = 7.5 Hz); 2.36 (t, 2 H, CH_2CO_2, J = 7.6 Hz); 2.56 (t, 2 H, CH_2CO_2Ar, J = 7.4 Hz); 2.77—2.82 (m, 2 H, H(6)); 3.79 (s, 3 H, OMe); 4.71 (dd, 1 H, H(17α), J = 8.0 Hz, J = 9.0 Hz); 4.93 (m, 2 H, $H(2)_{Ad}$); 6.72 (s, 1 H, H(4)); 6.88 (s, 1 H, H(1)). MS, *m/z*: 934 $[M + Na]^+$, 950 $[M + K]^+$.

Cytotoxicity of the synthesized compounds was evaluated against the lung alveolar epithelial carcinoma cell line A549 (CCL-185) using MTT viability assay following the earlier described procedure.[15] **Effect of the compounds on the microtubule network** was analyzed by immunofluorescence microscopy as earlier described.[1]

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