Adamantane acid esters with alkoxyaryl alcohols: synthesis, antiproliferative activity, and influence on microtubule network of tumor cells*

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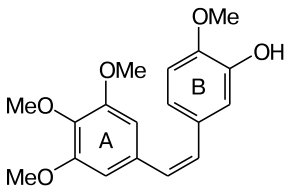
Adamantaneacetic and adamantanecarboxylic acid esters containing 3-hydroxy-4-methoxybenzyl, 3,4,5-trimethoxybenzyl, or 5-(hydroxymethyl)-2-methoxyphenyl groups were synthesized as unusual analogs of natural antitumor and anti-tubulin agents combretastatin A-4 and 2-methoxyestradiol. The compounds were found to possess noticeable cytotoxicity to epithelial human carcinoma cell line A549 (EC_{50} = 4.3—81 μmol L^{-1}). An ability to cause complete depolymerization of microtubule network of A549 cells was demonstrated for 5-(hydroxymethyl)-2-methoxyphenyl adamantan-1-ylacetate (**6a**) at a concentration of 100 μmol L^{-1}. Ester **6a** belongs to a new structural type, which is unusual for the ligands of the tubulin colchicine domain, and is an interesting lead compound for further structural optimization.

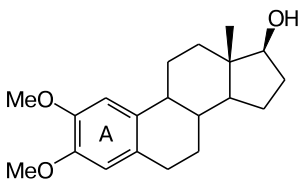
Key words: adamantane, combretastatin A-4, 2-methoxyestradiol, colchicine domain of tubulin, depolymerization of microtubules, antiproliferative activity.

When developing the design of structures for pharmaceutical agents, sometimes cage and bridging groups are used to replace aromatic fragments[1—4] or polycyclic skeletons of parent molecules.[1,2,5—8] The replacement of a planar aromatic ring with the nonplanar cage core is usually directed at maximization of hydrophobic contacts in the target protein cavity,[9,10] while the replacement of a polycyclic system, as a rule, is aimed at simplification of the original molecule structure[2,7] or improvement of its pharmacokinetic properties.[4]

In the present work, we consider a possibility of using a cage group (adamantane) for the development of unusual analogs of known natural antitumor agents combretastatin A-4 (CA-4) and 2-methoxyestradiol (2-ME). The anticancer activity of these compounds is largely due to their ability to interact with the colchicine domain of cell protein tubulin and inhibit its polymerization to microtubules.[11—13]



CA-4



2-ME

To date, neither combretastatin A-4, nor 2-methoxyestradiol, nor their numerous derivatives are used in anticancer therapy, and only a few of them are at different stages of clinical trials.[14] This requires the use of nonstandard approaches to the development of structural analogs of the molecules under consideration, for example, through the modification of their skeletons or the introduction of unusual substituents. It is however important

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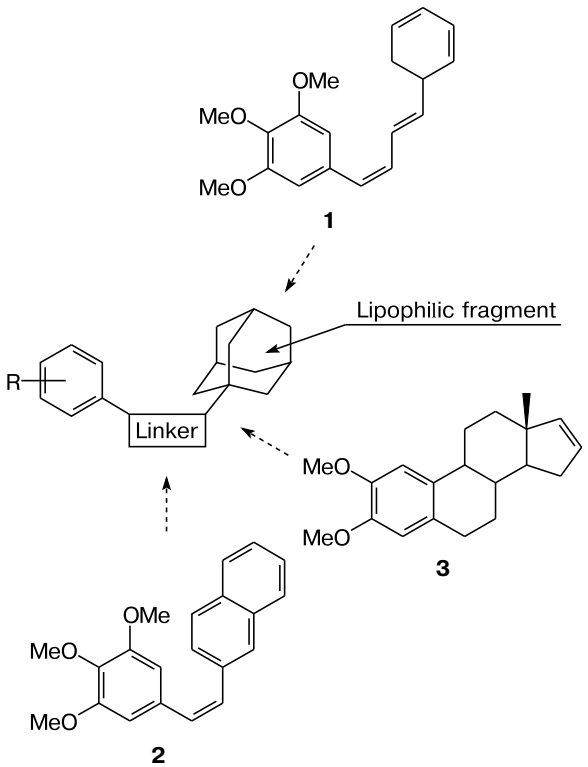
that in the course of such modifications these compounds retain their ability to cause depolymerization of the microtubule network of tumor cells.

In the process of construction of new structural analogs of CA-4 and 2-ME, we relied on the known data on high antitubulin activity and cytotoxicity of CA-4 vinylog, namely, compound **1** (see Ref. 15), as well as on that of CA-4 derivatives, in which ring B of the parent molecule is replaced with the unsubstituted naphthalene core (**2**) or with benzothiophene attached through the atom C(2) of thiophene[16] (Scheme 1). Both cytotoxicity and antitubulin activity are also retained by 2-methoxyestradiol derivatives without hydroxyl group at atom C(17) (**3**).[13] Comparison of these facts leads to the conclusion that for the efficient binding to the colchicine domain of tubulin, it is sufficient to have in the structure a specifically substituted aromatic moiety in combination with a bulky lipophilic fragment (although, according to the classical pharmacophore model of the ligand of the colchicine domain, rings A in CA-4 and in 2-ME have different locations in the protein[17]). The presented data led to the idea to develop new structures through the combination of alkoxyaryl groups with a lipophilic adamantane cage, replacing the aromatic fragment in the structures **1** and **2** or the polycyclic skeleton in the structure **3**. Note that this structural type is not characteristic of tubulin ligands and have not been studied previously for this purpose.

Scheme 1



To test whether in principle such substances can inhibit the polymerization of tubulin, we have suggested a series of six readily available esters **4a**,**b**—**6a**,**b** for synthesis and biotesting (Scheme 2). In these compounds, the alkoxyaryl groups are attached to adamantane through a linker with the length equal to or close to that in molecule **1** (which has better antitubulin activity than CA-4).[15] It was assumed that the greater than in the original compounds **1** and **2** flexibility of the chain linking the aryl ring to the adamantane core will provide the latter with the opportunity to adopt the optimal position in the protein.

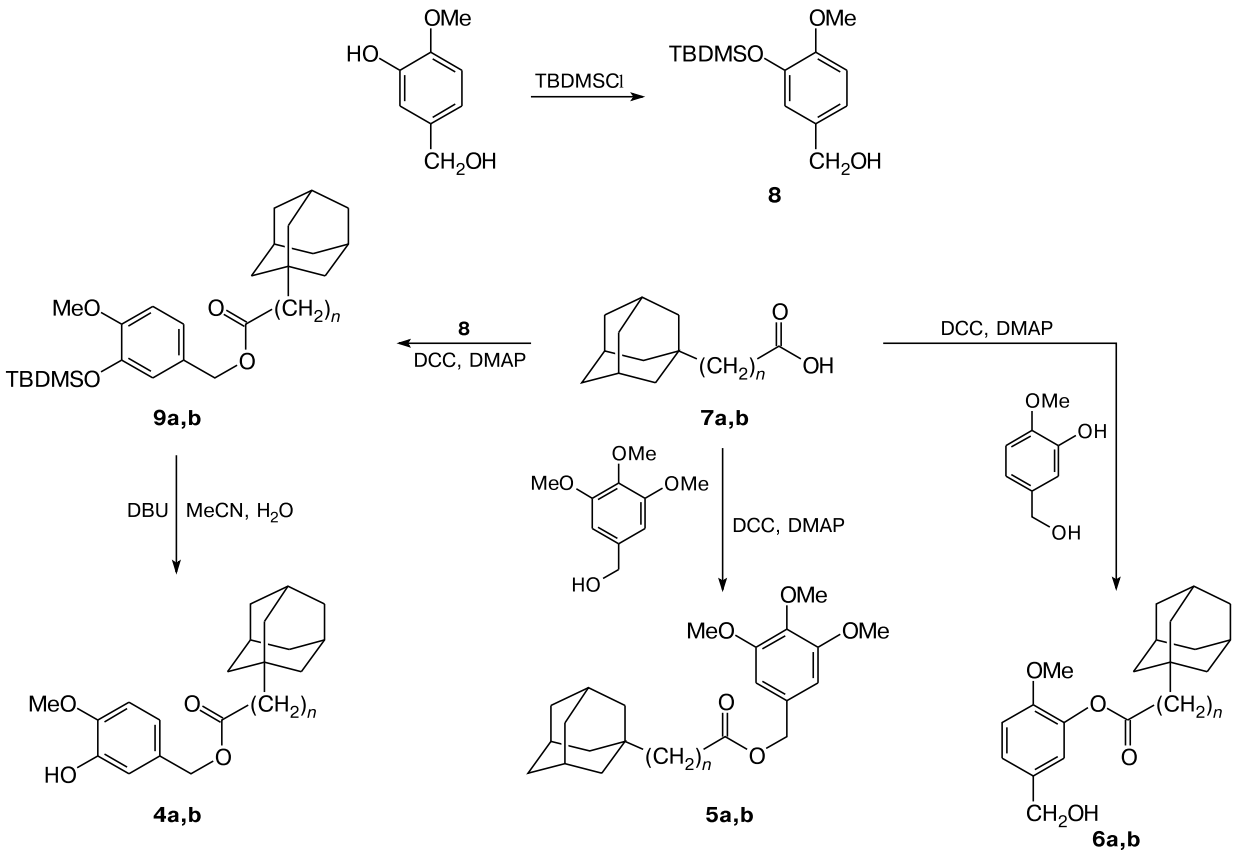
As aromatic fragments, we have chosen methoxyphenyl (**4a**,**b**) and trimethoxyphenyl groups (**5a**,**b**) present in the combretastatin and/or 2-methoxyestradiol molecule, as well as 5-(hydroxymethyl)-2-methoxyphenyl group (**6a**,**b**). In compounds **6a**,**b**, the adamantane framework is attached through the linker to the phenol hydroxyl of 5-(hydroxymethyl)-2-methoxyphenol (in contrast to esters **4a**,**b** with the attachment of the alcohol group to atom C(5)).

The synthesis of all the suggested compounds was carried out similarly (see Scheme 2), namely, by esterification of adamantaneacetic (**7a**) and adamantanecarboxylic (**7b**) acids with protected alcohol **8** (see Ref. 18), or with (3,4,5-trimethoxyphenyl)methanol, or 5-(hydroxymethyl)-2-methoxyphenol in the presence of *N*,*N*′-dicyclohexylcarbodiimide (DCC) and 4-*N*,*N*-dimethylaminopyridine (DMAP).

In the ^{1}H NMR spectra of the intermediate esters **9a** and **9b**, the signals for the protons of the methylene group are downfield shifted (δ 5.00 in **9a** and δ 5.01 in **9b**) as compared to that for alcohol **8** (δ 4.57). The protecting group in compounds **9a**,**b** was removed according to the standard procedure in the presence of 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU). The composition and the structure of the target compounds **4a**,**b**, **5a**,**b**, and **6a**,**b** were confirmed by NMR and IR spectroscopy, mass spectrometry, and elemental analysis (see Experimental section).

The biotesting of the series of synthesized compounds on human lung carcinoma cells A549 in a standard test with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazole hydrobromide (MTT)[19] showed (Table 1) that all the compounds possess a noticeable cytotoxicity, although less than that of CA-4 and 2-ME. Compounds **4b**, **6a**, and **6b** were the most active, their EC_{50} values lie in the submicromolar range of concentrations and are close to that for 2-methoxyestradiol. In addition, we studied the influence of some compounds (**4a**, **4b**, **6a**) on the growth of A549 cells by direct counting under a microscope 24, 48, and 72 h after treatment with a test compound at a concentration of 15 or 100 μmol L^{-1}. In the first case, after 48 h of treatment the amount of cells almost did not decrease (data not shown). However, at a concentration of 100 μmol L^{-1} compounds **4a**, **4b**, and **6a** completely inhibited not only the proliferation of the cells, but also

Scheme 2



n = 1 (**a**), 0 (**b**)

Compound	Yield (%)	Compound	Yield (%)	Compound	Yield (%)	Compound	Yield (%)
4a	88	**5a**	40	**6a**	51	**9a**	96
4b	90	**5b**	35	**6b**	42	**9b**	77

the number of adherent cells, which gradually decreased from the time of treatment until the end of the experiment to near zero (Fig. 1).

The results of studying the effect of all the substances obtained on the morphology of A549 cells and the dynamics of their microtubules (carried out by immuno-

Table 1. Biotesting results for compounds **4a**,**b**, **5a**,**b**, and **6a**,**b** on carcinoma cells A549

Compound	Cytotoxicity* EC_{50}/μmol L^{-1} (c = 100 μmol L^{-1})	Effect on cells	Effect on microtubules (c = 100 μmol L^{-1})
4a	17.8±0.5	Rounding, loss of adhesion	No effect
4b	4.9±0.5	Rounding, loss of adhesion	No effect
5a	22±4	Rounding, loss of adhesion	No effect
5b	81±2	Rounding, loss of adhesion	No effect
6a	4.3±0.2	Rounding, loss of adhesion, vesiculation	Depolymerization
6b	6.6±0.1	Rounding, loss of adhesion	No effect
5-(Hydroxymethyl)-2-methoxyphenol	N.d.	No effect	No effect
$AdCH_2COOH$ и $AdCH_2COOCH_3$	N.d.	No effect	No effect
CA-4	0.01±0.1	No effect	Depolymerization
2-ME	0.8±0.1	Rounding, loss of adhesion	Depolymerization

* The results of 3—4 independent experiments. N.d. means not determined.

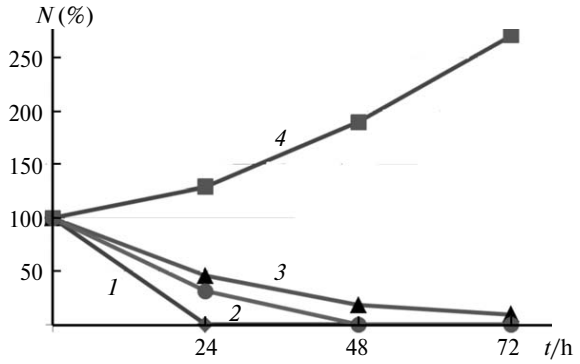


Fig. 1. Influence of compounds **4a** (*1*), **4b** (*2*), and **6a** (*3*) (at a concentration of 100 μmol L^{-1}) on the cell growth in culture (counted using phase contrast microscopy; the control was 0.5% DMSO, *N* is the number of cells, *t* is the duration of experiment).

fluorescence microscopy, see Table 1 and Fig. 2) indicate that at high concentrations (100 μmol L^{-1}) all of them strongly alter the morphology of the cells, causing rounding and loss of adhesion, as well as a reduction in the surface (see Fig. 2, *c*—*e*), but esters **4a**,**b**, **5a**,**b** and **6b** have no effect on the microtubule network. Compound **6a**, however, exhibits a pronounced ability to cause complete depolymerization of microtubules within 72 h (Fig. 2, *f*). Note that at this concentration, compound **6a** causes a noticeable fragmentation of A549 cell nuclei, which is characteristic of apoptosis.

The interesting result obtained for compound **6a** stimulated additional experiments. Although the hydrolysis of the ester bond in molecule **6a** with nonspecific cell esterases is hindered by the presence of a bulky adamantane substituent, we studied the effect of the products of this

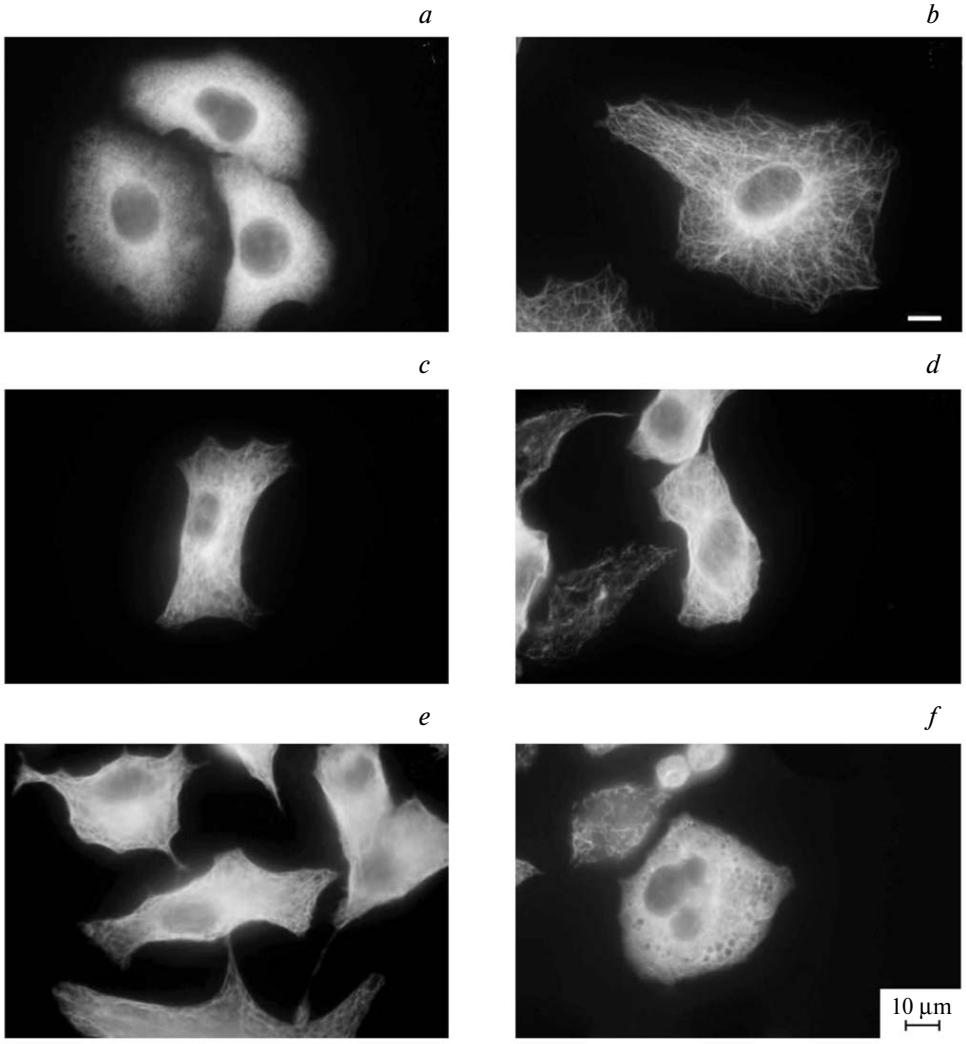


Fig. 2. Immunofluorescence microscopy data for A549 cells treated with CA-4 at a concentration of 1 μmol L^{-1} (*a*, positive control, depolymerization of microtubules); with 0.5% DMSO (*b*, negative control; microtubule network is normal); compounds **4a** or **4b** at a concentration of 100 μmol L^{-1} (*c*) and compounds **5a** or **5b** at a concentration of 100 μmol L^{-1} (*d*, reduction of the cell surface and decrease of the cell density); with compound **6b** at a concentration of 100 μmol L^{-1} (*e*, reduction of the cell surface and, as a consequence, the formation of the cell membrane "protrusions"); with compound **6a** at a concentration of 100 μmol L^{-1} (*f*, complete depolymerization of microtubules; cell vesiculation). The scale is 10 μm.

possible hydrolysis on the microtubule network of A549 cells. However, at a concentration of 100 μmol L^{-1} neither 5-(hydroxymethyl)-2-methoxyphenol, nor adamantaneacetic acid, nor its methyl ester with better permeability through the cell membrane had any effect on microtubules (see Table 1). Thus, all the results obtained confirm that compound **6a** has a reliable cytotoxic effect on A549 carcinoma cells, while its antiproliferative properties are at least partially associated with the influence on the microtubular network.

The automatic computer docking of ligand **6a** to the three-dimensional model of the tubulin colchicine domain (PDB ID: 1SA0) shows that in one of the most favorable arrangement of this structure in protein, the aryl fragment of compound **6a** is located in the region close to that occupied by ring B (CA-4) and ring A (2-ME) (Fig. 3).

In this case, the hydroxy group in the structure **6a** forms two hydrogen bonds with the carbonyl groups of the main chain of the amino acid moieties βVal315 and βAsn350. The adamantane cage is located above the plane of the trimethoxyphenyl group of CA-4 and the steroid skeleton of 2-ME, but it falls into the hydrophobic region formed by the side chains of the residues βVal318, βIle378, and βLeu255. Note that the arrangement of molecule **6a** in the protein shown in Fig. 3 has been chosen from several variants with the minimum values of scoring-function (according to the automatic docking data) as the closest to that for the original CA-4 and 2ME molecules.

In conclusion, we emphasize that only separate examples of adamantane-containing tubulin ligands are described in the literature, in which the framework plays the role of a substituent attached (frequently through a linker) to the molecules of combretastatin A-4, colchicine or podophyllotoxin.[20—23] Therefore, the found in this study ability to cause depolymerization of the microtubule network for the compound with the adamantane moiety as a basic structural unit is important and interesting in terms of expanding the structural diversity of such substances. 5-(Hydroxymethyl)-2-methoxyphenyl adamantan-1-ylacetate (**6a**) itself is promising as a new original lead compound for further optimization.

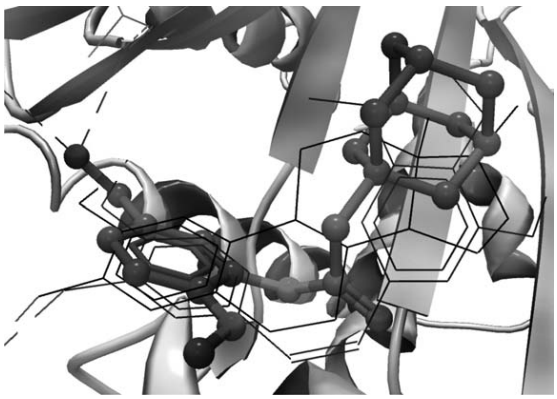


Fig. 3. One of the most energetically favorable arrangement of structure **6a** in the tubulin dimer (β subunit is on the left) based on the results of automatic docking (the CLC Drug Discovery Workbench program). Hydrogen bonds are shown by dashed lines (hydrogen atoms are omitted). For comparison, thin lines show molecules of combretastatin A-4 and 2-methoxyestradiol.

Experimental

Automatic docking in a three-dimensional model of tubulin complex with *N*-deacetyl-*N*-(2-mercaptoacetyl)colchicine (PDB ID: 1SA0) was carried out using the CLC Drug Discovery Workbench program (Version 1.5): Evaluation license (2014). The preset radius value was 16 Å, the number of iterations was 500. The ligand—tubulin complexes with the best values of scoring functions calculated according to this program were selected.

All the solvents for extraction and chromatography were purified and dried according to the standard procedures. Adamantaneacetic and adamantanecarboxylic acids, 3,4,5-trimethoxyphenylbenzaldehyde, 3-hydroxy-4-methoxybenzaldehyde were purchased from Sigma-Aldrich. The starting alcohols, 5-(hydroxymethyl)-2-methoxyphenol and (3,4,5-trimethoxyphenyl)methanol, were synthesized according to the standard procedure by the reduction of the corresponding aldehydes with sodium borohydride, [3-(3-*tert*-butyldimethylsilyloxy)-4-methoxyphenyl]methanol (**8**) was synthesized from 5-(hydroxymethyl)-2-methoxyphenol according to the procedure described earlier.[18] Reaction progress and purity of compounds were monitored by thin-layer chromatography on Silufol-UV254 plates. Chromatographic separation was carried out on columns with Acros silica gel (40—60 μm). ^{1}H and ^{13}C NMR spectra were recorded on a on Bruker Avance 400 spectrometer (400 and 100 MHz, respectively) at 28 °C. Chemical shifts are given relative to the residual signal of the solvent ($CDCl_3$: δ 7.26 (^{1}H NMR); 77.0 (^{13}C NMR)). Elemental analysis was carried out on a Vario Micro Cube CHN-analyzer. IR spectra were recorded on an IR-200 ThermoNicolet spectrophotometer in KBr pellets. MALDI-TOF mass spectra were recorded on a VISION-2000 instrument.

Esterification (general procedure). A corresponding alcohol, *N*,*N*′-dicyclohexylcarbodiimide (DCC), and a catalytic amount of 4-*N*,*N*-dimethylaminopyridine (DMAP) (2—4 mg) were added to a solution of a carboxylic acid in CH_2Cl_2 (10 mL). The reaction mixture was stirred for 24 h at room temperature, the solvent was evaporated *in vacuo*. Then, EtOAc (20 mL) was added and the mixture was allowed to stand for 2—3 h at 4 °C. The crystals of *N*,*N*′-dicyclohexylurea were filtered off and washed with cold EtOAc (2×10 mL), the solvent was evaporated *in vacuo*. The residue was subjected to chromatography (eluent: ethyl acetate—light petroleum ether, 40—70 °C, gradient 1 : 9—1 : 6).

3-[*tert*-Butyl(dimethyl)silyloxy]-4-methoxybenzyl adamantan-1-yl acetate (9a) was obtained according to the general esterification procedure from alcohol **8** (0.240 g, 0.90 mmol), adamantaneacetic acid (0.2 g, 1.03 mmol), and DCC (0.22 g, 1.07 mmol). The yield of compound **9a** was 0.384 g (96%), a colorless oily liquid. ^{1}H NMR ($CDCl_3$), δ: 0.17 (s, 6 H, $Si(Me)_2$); 1.01 (s, 9 H, $Si(Bu^t)$); 1.61—1.64 (m, 9 H); 1.68—1.71

(m, 3 H); 1.96 (m, 3 H); 2.11 (s, 2 H, AdC$\underline{H}_2$); 3.81 (s, 3 H, OMe); 5.00 (s, 2 H, ArC$\underline{H}_2$); 6.82 (d, 1 H, C(5)H, J = 8.2 Hz); 6.88 (d, 1 H, C(2)H, J = 1.9 Hz); 6.92 (dd, 1 H, C(6)H, J = 8.2 Hz, J = 1.9 Hz). ^{13}C NMR ($CDCl_3$), δ: −4.64 (Si(Me)$_2$), 18.45 (Si$\underline{C}$(Me)$_3$), 25.72 (SiC(Me)$_3$), 28.61, 32.87 (C(1-Ad)), 36.72, 42.41, 49.00 (Ad$\underline{C}H_2$), 55.51 (OMe), 65.65 (Ar$\underline{C}H_2$), 111.81 (C(5)), 121.38 (C(2)), 122.06 (C(6)), 128.81 (C(1)), 144.95 (C(3)), 150.95 (C(4)), 171.69 (C=O). IR (KBr, ν/cm^{-1}): 1513, 1731 (C=O); 2902, 2850. MS, *m/z*: 467 [M + Na]$^+$.

(3-Hydroxy-4-methoxybenzyl) adamantan-1-ylacetate (4a). 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) (0.120 g, 0.79 mmol) was added to a solution of ester **9a** (0.350 g, 0.79 mmol) in a mixture of MeCN—H_2O (99 : 1, 10 mL). The mixture was stirred for 2 h at room temperature, diluted with water (20 mL), and extracted with CH_2Cl_2 (3×20 mL), the organic layers were combined, dried with Na_2SO_4, the solvent was evaporated *in vacuo*. The residue was subjected to chromatography (eluent: light petroleum ether (40—70 °C), then ethyl acetate—light petroleum ether (40—70 °C) 1 : 8—1 : 6). The yield of compound **4a** was 0.229 g (88%), a colorless oily liquid. ^{1}H NMR ($CDCl_3$), δ: 1.61—1.65 (m, 9 H); 1.69—1.72 (m, 3 H); 1.96 (m, 3 H); 2.11 (s, 2 H, AdC$\underline{H}_2$); 3.89 (s, 3 H, OMe); 5.00 (s, 2 H, ArC$\underline{H}_2$); 5.72 (s, 1 H, OH); 6.83 (d, 1 H, C(5)H, J = 8.2 Hz); 6.87 (dd, 1 H, C(6)H, J = 8.2 Hz, J = 2.0 Hz); 6.96 (d, 1 H, C(2)H, J = 2.0 Hz). ^{13}C NMR ($CDCl_3$), δ: 28.56, 32.80 (C(1-Ad)), 36.67, 42.33, 48.86 (Ad$\underline{C}H_2$), 55.92 (OMe), 65.62 (Ar$\underline{C}H_2$), 110.42 (C(5)), 114.75 (C(2)), 120.26 (C(6)), 129.39 (C(1)), 145.58 (C(3)), 146.46 (C(4)), 171.65 (C=O). IR (KBr, ν/cm^{-1}): 1513, 1729 (C=O); 2902. Found (%): C, 72.69; H, 7.95. $C_{20}H_{26}O_4$. Calculated (%): C, 72.70; H, 7.93.

3-[*tert*-Butyl(dimethyl)silyloxy]-4-methoxybenzyl adamantane-1-carboxylate (9b) was obtained according to the general esterification procedure from alcohol **8** (0.240 g, 0.90 mmol), adamantanecarboxylic acid (0.180 g, 1 mmol), and DCC (0.206 g, 1 mmol). The yield of compound **9b** was 0.300 g (77%), a colorless oily liquid. ^{1}H NMR ($CDCl_3$), δ: 0.17 (s, 6 H, Si(Me)$_2$); 1.02 (s, 9 H, Si$\underline{Bu}^t$); 1.68—1.76 (m, 6 H); 1.92 (m, 6 H); 2.02 (m, 3 H); 3.81 (s, 3 H, OMe); 5.01 (s, 2 H, ArC$\underline{H}_2$); 6.82 (d, 1 H, C(5)H, J = 8.2 Hz); 6.86 (d, 1 H, C(2)H, J = 2.1 Hz); 6.89 (dd, 1 H, C(6)H, J = 8.1 Hz, J = 2.1 Hz). ^{13}C NMR ($CDCl_3$), δ: −4.66 (Si(Me)$_2$), 18.43 (Si$\underline{C}$(Me)$_3$), 25.69 (SiC(Me)$_3$), 27.92, 36.48, 38.81, 40.70 (C(1-Ad)), 55.48 (OMe), 65.43 (Ar$\underline{C}H_2$), 111.79 (C(5)), 120.69 (C(2)), 121.35 (C(6)), 129.12 (C(1)), 144.92 (C(3)), 150.76 (C(4)), 177.43 (C=O). IR (KBr, ν/cm^{-1}): 1513, 1725 (C=O); 2854, 2906, 2929. MS, *m/z*: 453 [M + Na]$^+$, 469 [M + K]$^+$.

(3-Hydroxy-4-methoxybenzyl) adamantane-1-carboxylate (4b) was obtained similarly to compound **4a** from ester **9b** (0.250 g, 0.581 mmol) and DBU (0.250 g, 0.581 mmol). The yield of compound **4b** was 0.165 g (90%), white crystals, m.p. 107—109 °C. ^{1}H NMR ($CDCl_3$), δ: 1.68—1.76 (m, 6 H); 1.92—1.93 (m, 6 H); 2.02 (m, 3 H); 3.90 (s, 3 H, OMe); 5.01 (s, 2 H, ArC$\underline{H}_2$); 5.66 (s, 1 H, OH); 6.82—6.86 (m, 2 H, C(5)H, C(6)H); 6.93 (d, 1 H, C(2)H, J = 1.2 Hz). ^{13}C NMR ($CDCl_3$), δ: 27.96, 36.51, 38.83, 40.75 (C(1-Ad)), 55.97 (OMe), 65.58 (ArC$\underline{H}_2$), 110.45 (C(5)), 114.20 (C(2)), 119.75 (C(6)), 129.83 (C(1)), 145.61 (C(3)), 146.35 (C(4)), 177.52 (C=O). IR (KBr, ν/cm^{-1}): 1590, 1708 (C=O); 2921. Found (%): C, 72.15; H, 7.67. $C_{19}H_{24}O_4$. Calculated (%): C, 72.13; H, 7.65.

3,4,5-Trimethoxybenzyl adamantan-1-ylacetate (5a) was obtained according to the general esterification procedure from acid **7a** (0.503 g, 2.59 mmol), (3,4,5-trimethoxyphenyl)-methanol (0.426 g, 2.15 mmol), and DCC (0.532 g, 2.58 mmol). The yield was 0.328 g (40%), white crystals, m.p. 35—37 °C. ^{1}H NMR ($CDCl_3$), δ: 1.47—1.50 (m, 9 H); 1.55—1.58 (m, 3 H); 1.82 (m, 3 H); 1.99 (s, 2 H, AdC$\underline{H}_2$); 3.70 (s, 3 H, OMe); 3.72 (s, 6 H, 2 OMe); 4.91 (s, 2 H, ArC$\underline{H}_2$); 6.48 (s, 2 H, Ar). ^{13}C NMR ($CDCl_3$), δ: 28.04, 32.28 (C(1-Ad)), 36.12, 41.80, 48.22 (Ad$\underline{C}H_2$), 55.39 (2OMe), 60.04 (OMe), 65.28 (Ar$\underline{C}H_2$), 104.85 (C(2), C(6)), 131.37 (C(4)), 137.23 (C(1)), 152.67 (C(3), C(5)), 170.69 (C=O). IR (KBr, ν/cm^{-1}): 1592, 1731 (C=O); 2902. MS, *m/z*: 374 [M]$^+$, 397 [M + Na]$^+$, 413 [M + K]$^+$. Found (%): C, 70.53 H, 8.03. $C_{22}H_{30}O_5$. Calculated (%): C, 70.56; H, 8.07.

3,4,5-Trimethoxybenzyl adamantane-1-carboxylate (5b) was obtained according to the general esterification procedure from acid **7b** (0.420 g, 2.33 mmol), (3,4,5-trimethoxyphenyl)-methanol (0.416 g, 2.09 mmol), and DCC (0.480 g, 2.33 mmol). The yield was 0.267 g (35%), white crystals, m.p. 74—76 °C. ^{1}H NMR ($CDCl_3$), δ: 1.67—1.75 (m, 6 H); 1.92—1.93 (m, 6 H); 2.02 (m, 3 H); 3.84 (s, 3 H, OMe); 3.86 (s, 6 H, 2 OMe); 5.03 (s, 2 H, ArC$\underline{H}_2$); 6.55 (s, 2 H, Ar). ^{13}C NMR ($CDCl_3$), δ: 27.78, 36.32, 38.70, 39.12 (C(1-Ad)), 55.93 (2 OMe), 60.66 (OMe), 65.64 (Ar$\underline{C}H_2$), 104.55 (C(2), C(6)), 132.13 (C(4)), 137.45 (C(1)), 153.10 (C(3), C(5)), 177.21 (C=O). MS, *m/z*: 360 [M]$^+$, 383 [M + Na]$^+$, 399 [M + K]$^+$. Found (%): C, 70.04; H, 7.78. $C_{21}H_{28}O_5$. Calculated (%): C, 69.98; H, 7.83.

5-(Hydroxymethyl)-2-methoxyphenyl adamantan-1-ylacetate (6a) was obtained according to the general esterification procedure from acid **7a** (0.450 g, 2.32 mmol), 5-(hydroxymethyl)-2-methoxyphenol (0.324 g, 2.10 mmol), and DCC (0.481 g, 2.33 mmol). The yield of compound **6a** was 0.353 g (51%), a waxy solid compound. ^{1}H NMR ($CDCl_3$), δ: 1.68—1.78 (m, 13 H, H(Ad) + OH); 2.03 (m, 3 H); 2.34 (s, 2 H, AdC$\underline{H}_2$); 3.83 (s, 3 H, OMe); 4.62 (s, 2 H, C$\underline{H}_2$OH); 6.95 (d, 1 H, C(3)H, J = 8.3 Hz); 7.05 (d, 1 H, C(6)H, J = 2.0 Hz); 7.19 (dd, 1 H, C(4)H, J = 2.0 Hz, J = 8.3 Hz). ^{13}C NMR ($CDCl_3$), δ: 28.65, 33.07 (C(1-Ad)), 36.76, 42.25, 48.54 (Ad$\underline{C}H_2$), 55.83 (OMe), 64.62 ($\underline{C}H_2$OH), 112.30 (C(3)), 121.95 (C(6)), 125.39 (C(4)), 133.57 (C(5)), 139.73 (C(1)), 150.62 (C(2)), 169.70 (C=O). IR (KBr, ν/cm^{-1}): 1592, 1731 (C=O); 2902. MS, *m/z*: 353 [M + Na]$^+$, 369 [M + K]$^+$. Found (%): C, 72.74; H, 7.89. $C_{20}H_{26}O_4$. Calculated (%): C, 72.70; H, 7.93.

5-(Hydroxymethyl)-2-methoxyphenyl adamantane-1-carboxylate (6b) was obtained according to the general esterification procedure from acid **7b** (0.420 g, 2.33 mmol), 5-(hydroxymethyl)-2-methoxyphenol (0.324 g, 2.10 mmol), and DCC (0.481 g, 2.33 mmol). The yield of compound **6b** was 0.284 g (42%), white crystals, m.p. 105—107 °C. ^{1}H NMR ($CDCl_3$), δ: 1.78 (m, 6 H); 2.08 (m, 9 H); 2.40 (br.s, 1 H, OH); 3.78 (s, 3 H, OMe); 4.53 (s, 2 H, C$\underline{H}_2$OH); 6.90 (d, 1 H, C(3)H, J = 8.4 Hz); 6.99 (d, 1 H, C(6)H, J = 2.0 Hz); 7.13 (dd, 1 H, C(4)H, J = 2.0 Hz, J = 8.4 Hz). ^{13}C NMR ($CDCl_3$), δ: 27.87, 36.40, 38.73, 40.95 (C(1-Ad)), 55.96 (OMe), 64.25 ($\underline{C}H_2$OH), 112.31 (C(3)), 121.66 (C(6)), 125.04 (C(4)), 133.73 (C(5)), 140.00 (C(1)), 150.46 (C(2)), 175.80 (C=O). IR (KBr, ν/cm^{-1}): 1511, 1733 (C=O); 2903. MS, *m/z*: 339 [M + Na]$^+$, 355 [M + K]$^+$. Found (%): C, 72.10; H, 7.63. $C_{19}H_{24}O_4$. Calculated (%): C, 72.13; H, 7.65.

MTT test for cytotoxicity was carried out on epithelial human carcinoma cells (line A-549, CCL-185) according to the procedures described in the works.[24,25]

Study of cell growth. The A549 cells were plated into 96-well plates (density about 100 cells per well). The cells were treated

during 72 h with a solution of compound **6a** in DMSO at concentrations 15 or 100 μmol L^{-1} or 0.5% DMSO, which was used as a negative control. The cells labelled with Hoechst dye (0.8 mmol L^{-1} in phosphate-buffered solution, PBS) were counted under a microscope directly on the cell counter.

Test with immunofluorescently labelled microtubules. For labelling microtubules, the cell A549 were cultured on small coverslips 11 mm in diameter placed into plates with 12 wells (density about 100 cells per coverslip). The cells were incubated during 8 h with test compounds or colchicine as a positive control at concentrations of 50 and 100 μmol L^{-1} at 37 °C and 5% CO_2. A negative control was 0.5% DMSO. Fixed cells were stained with monoclonal antibodies of mice to α-tubulin (Sigma, St. Louis. USA) in dilution 1 : 400, followed by incubation with fluorescently labelled AlexaFlour488 goat secondary antibodies against mouse immunoglobulins (IgG) (Molecular Probes, Eugene, USA) at dilution 1 : 200. Fixed cells were analyzed using a Nikon Diaphot 300 microscope (Nikon GmbH, Dusseldorf, Germany) equipped with a SenSys camera (Photometrics, Munich, Germany).

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