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# SYNTHESIS, ANTIPROLIFERATIVE ACTIVITY, AND EFFECT ON CARCINOMA A549 CELL MICROTUBULES OF NEW TUBULOCLUSTIN ANALOGS

**N. A. Zefirov,[1, 2] Yu. A. Evteeva,[1] A. R. Fatkulin,[1] S. Schulz,[3] S. A. Kuznetsov,[3] and O. N. Zefirova[1, 2,*]**

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Combretastatin analogs of the antitumor agent tubuloclustin {*N*-[7-(adamant-2-yloxy)-7-oxoheptanoyl]-*N*-deacetylcolchicine} were prepared via esterification of combretastatin by monoesters of pimelic or adipic acid with adamantan-2-ol or (adamantan-1-yl)methanol. These conjugates were stable and cytotoxic to human lung carcinoma A549 cells ($EC_{50} \approx 50 – 70$ nM) and caused depolymerization of microtubules and slight clustering of tubulin. Tubuloclustin analogs with shortened linkers were prepared via amidation by *N*-deacetylcolchicine of monoesters of adipic or succinic acids with adamantan-1-ol or (adamantan-1-yl)methanol. The conjugate *N*-[6-(adamantyl)-6-oxohexanoyl]-*N*-deacetylcolchicine was more active ($EC_{50} \approx 4$ nM) than tubuloclustin and promoted strong tubulin clusterization. All compounds induced apoptosis of A549 cells. Tests *in vivo* of *N*-[6-(adamantyl)-6-oxoheaxnoyl]-*N*-deacetylcolchicine on carcinoma A549 experimental models were concluded to be promising.

**Keywords:** carcinoma A549, tubuloclustin, colchicine, combretastatin, adamantane.

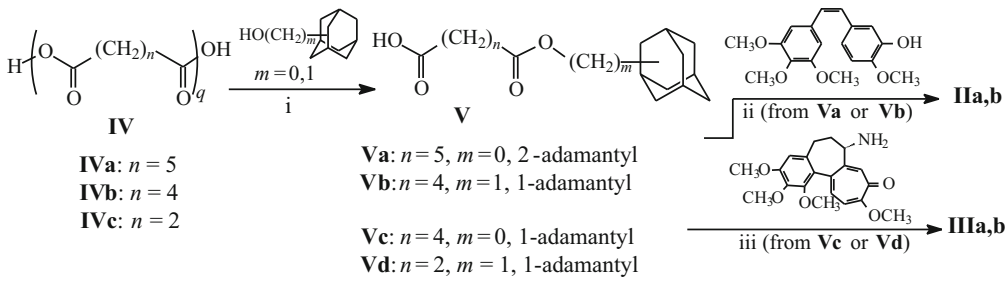
The antitumor activity of ligands for the colchicine domain of the dimeric protein α,β-tubulin is due to their ability to inhibit its polymerization to microtubules, which play an important role in the cell life cycle [1, 2]. Although many such compounds, both natural and synthetic, have now been prepared and studied, only one example is used in clinical practice because of high general toxicity [3] or low efficacy *in vitro* and/or *in vivo* [1, 4]. The pairing of molecular fragments has been used extensively in the last decade to design more active and less toxic ligands of the tubulin colchicine domain [5, 6], including combining colchicine (reacting mainly with the protein β-subunit) with groups capable of binding to the tubulin α-subunit (e.g., **Ia-e** in Fig. 1) [7-10].

This approach was used by us to prepare tubuloclustin (**Id**) and its bridgehead analog **Ie**, which caused tubulin clusterization *in vitro* that was uncharacteristic of colchicine [10] and exhibited high antiproliferative activity against tumor cells *in vitro* [11] and *in vivo* [12].

Further attempts to increase the activity and decrease the general toxicity of tubuloclustin had limited success [12 – 16]. Therefore, the goals of the present work were to prepare two pairs of new analogs, to determine their antiproliferative activity and effect on tumor cell microtubule networks, and to explain the results using computer molecular modeling.

Compounds **IIa-b** and **IIIa-b** were proposed for synthesis based on previously obtained structure—activity data for tubuloclustin analogs [11, 13, 15] (Fig. 2). Although all studied versions of replacing colchicine in **Id** and **Ie** by fragments of other ligands of the tubulin colchicine domain with lower general toxicity than colchicine (2-methoxyestradiol, combretastatin A-4, etc.) produced compounds with low activity [12, 14 – 16], we made yet another attempt in this direction. Pair **IIa**,**b** were analogs of starting **Id** and **Ie** in which a combretastatin bonded to a phenol hydroxyl through an ester bond (this type of linker had not been studied by us earlier) replaced the colchicine fragment. The overall linker chain length in **IIb** did not change compared with lead com-

[1] M. V. Lomonosov Moscow State University, Moscow, 119991 Russia.
[2] Institute of Physiologically Active Compounds, Russian Academy of Sciences, Chernogolovka, Moscow Oblast, 142432 Russia.
[3] Institute of Biological Sciences, University of Rostock, Rostock, Germany.
[*] e-mail: olgaz@org.chem.msu.ru




**Scheme 1.** Reagents and conditions: i) 4-DMAP, $CH_2Cl_2$, RT, 24 h [**Va** from **IVa** and adamantan-2-ol, 71%; **Vb** from **IVb** and (adamantan-1-yl)methanol, 60%; **Vc** from **IVb** and adamantan-1-ol, 49%; **Vd** from **IVc** and (adamantan-1-yl)methanol, 50%]; ii) combretastatin A-4, **Va** or **Vb**, DCC, $-DMAP, DMF, RT (**IIa** from **Va**, 67%; **IIb** from **Vb**, 59%); iii) *N*-deacetylcolchicine, **Vc** or **Vd**, EEDQ, $CH_2Cl_2$, RT, 12 h (**IIIa** from **Vc**, 56%; **IIIb** from **Vd**, 25%).

pound **Ie**. However, this ester bond was shifted from the adamantane fragment to provide greater conformational flexibility to the terminal conjugate fragment.

The structures of conjugates **IIIa**,**b** were proposed based on published results [13] that showed the antiproliferative activity could be increased somewhat by using the modified oxoheptanoyl chain of **Ie** and that analogs with linkers containing four and less methylenes should be further studied.

Target conjugates **IIa**,**b** and **IIIa**,**b** were synthesized in steps. First, the reaction of polyanhydrides of pimelic or adipic acids or succinic anhydride (**IVa-c**, prepared by reacting the corresponding dibasic acids with acetic anhydride) with adamantan-2-ol, (adamantan-1-yl)methanol, or adamantan-1-ol in the presence of a catalytic amount of 4-dimethylaminopyridine (4-DMAP) produced monoesters **Va-d** in 49-71% yields (Scheme 1). $^{13}C$ NMR spectra of previously unreported **Vc** and **Vd** (see Experimental section) showed a characteristic resonance for ester C atoms (173.55 ppm for **Vc** and 172.18 ppm for **Vd**). The PMR spectrum of **Vd** exhibited also a characteristic shift of the Ad–C$\underline{H}_2$–O– protons (3.70 ppm) to weak field as compared with those in the starting framework alcohol (3.17 ppm).

Target pair **IIa**,**b** was synthesized by esterification of monoesters of carboxylic acids **Va**,**b** with combretastatin A-4 in the presence of *N*,*N*-dicyclohexylcarbodiimide (DCC) and 4-DMAP. Formation of the esters was confirmed by two resonances for ester C atoms in $^{13}C$ NMR spectra of conjugates **IIa**,**b** at 171.42 and 172.97 ppm for **IIa** and 172.24 and 173.63 ppm for **IIIa**. MALDI-TOF mass spectra exhibited peaks for a molecular ion with *m*/*z* 592 [$M^+$]. It is noteworthy that compounds **IIa**,**b** were demonstrated to be stable in $CDCl_3$ solution for 3 d according to NMR spectroscopy although combretastatin A-4 esters are rather stable [17].

*N*-Deacetylcolchicine was synthesized in three steps as before [18, 19] to produce the target pair of colchicine conjugates **IIIa**,**b**. Amidation of monoesters **Vc** and **Vd** by

**TABLE 1.** Biological Test Results for Human Lung Carcinoma A549 Cell Line

| Compound | Cytotoxicity* $EC_{50}$, nM | Effect on microtubule network** |
|---|---|---|
| **IIa** | 70 ± 6 | 10 μM: 24 h, microtubule depolymerization; 72 h, point clusterization in several cells |
| **IIb** | 53 ± 5 | 10 μM: 24 h, microtubule depolymerization, point clusterization in several cells |
| **IIIa** | 4.2 ± 0.5 | 100 nM: 24 h, complete microtubule depolymerization; 800 nM: 24 – 72 h, strong clusterization |
| **IIIb** | 13 ± 2 | 1 μM: 24 h, complete microtubule depolymerization; 72 h, moderate clusterization |
| **Id** (tubuloclustin) | 6 [11] | 1 μM: 24 h, depolymerization and strong clusterization |
| **II** [R= $-CH_2-(CH_2)_5C(O)O-^2Ad$] | 1040 [15] | 10 μM: 24 h, microtubule depolymerization; 72 h, point clusterization in several cells [15] |
| **Ie** | 11 [11] | 1 μM: 24 h, depolymerization and strong clusterization |
| CA-4 | 10 ± 2 | 1 μM: 24 – 72 h, microtubule depolymerization and no clusterization |
| Colchicine | 31 ± 5 | 1 μM: 24 – 72 h, microtubule depolymerization and no clusterization |

* Average of three independent experiments;
** effect on microtubular network at the given concentrations and incubation periods.

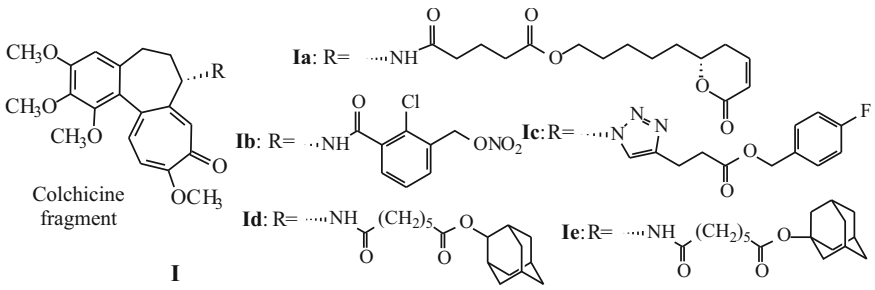



**Fig. 1.** Reported conjugates of colchicine and groups interacting with tubulin α-subunit; **Id** is tubuloclustin.

*N*-deacetylcolchicine in the presence of *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) afforded conjugates **IIIa**,**b** in 56 and 25% yields. PMR spectra of **IIIa**,**b** showed colchicine $C_7$ resonances shifted to weaker field (4.65 ppm) relative to the resonance of the same proton in the spectrum of *N*-deacetylcolchicine (~3.71 ppm). This confirmed that the amide bond formed. The carboxyl C atom resonance of starting acids **Va**,**b** (178.42 – 179.52 ppm) disappeared in $^{13}C$ NMR spectra of **IIIa**,**b**. Mass spectrometry and elemental analysis confirmed the structures of all previously unreported compounds.

Antiproliferative activity (cytotoxicity) of target combretastatin conjugates **IIa**,**b** and colchicine conjugates **IIIa**,**b** against human lung carcinoma A549 cells was determined using the standard colorimetric assay with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazole hydrobromide (MTT [20]) and direct counting of A549 cells under a microscope 24 and 48 h after treatment with the tested compounds. The ability of the synthesized compounds to affect A549 cell microtubular networks and stimulate apoptosis was studied using immunofluorescent spectroscopy (Table 1, Fig. 3, a-f).

The MTT assay showed that combretastatin conjugates **IIa**,**b** possessed high cytotoxicity against carcinoma A549 cells with $EC_{50}$ values of 50-70 nM, i.e., approximately equal to $EC_{50}$ of colchicine and slightly greater than $EC_{50}$ of combretastatin A-4 (Table 1). Direct cell counting showed that cell proliferation was completely inhibited 24 h after treatment with **IIa**,**b** (10 μM). Both conjugates also induced apoptosis of tumor cells [% cells with fragmented nuclei characteristic of apoptosis (Fig. 3*e* ) after treatment with **IIa** (10 μM): 24 h, 18%; 48, 42; 72, 48; **IIb**: 24 h, 42%; 48, 47; 72, 60].

Immunofluorescent microscopy gave interesting results (Fig. 3, a-d). Both **IIa** and **IIb** depolymerized completely A549 cell microtubules and caused small point clusters of tubulin to form in several of them (Fig. 3*c* ). This was characteristic of several tubuloclustin analogs and for less cytotoxic conjugate **II** [R = $–CH_2–(CH_2)_5C(O)O–^2Ad$] with a similar structure that was prepared by us earlier [16] (Table 1). First, these data indicated that hydrolysis of the ester bond at the phenol hydroxyl of **IIa, b** by nonspecific cellular esterases, if it occurred at all, was insignificant. This meant that a large number of unhydrolyzed molecules remained in the cells (because the hydrolysis products did not possess clustering effects). Also, the results confirmed the hypothesis proposed by us previously that tubuloclustin analogs with various ligands of the tubulin colchicine domain could exhibit clustering effects [15].

Computer docking of **IIa, b** and **II** [R = $–CH_2–(CH_2)_5C(O)O–^2Ad$] into three-dimensional models of complexes of the tubulin dimer with various ligands of the colchicine domain (PDB ID: ISAO, 4O2B) using CLC Drug Discovery Workbench (Version 1.5) revealed a version for positioning three of these molecules in the 4O2B model (from several with minima in estimating functions) that explained the obtained test results (Fig. 4).

Figure 4 shows that the linkers to adamantane in **IIa, b** had similar positions that were different from those of **II** [R = $–CH_2–(CH_2)_5C(O)O–^2Ad$]. However, the adamantane framework in all three molecules occupied lipophilic regions

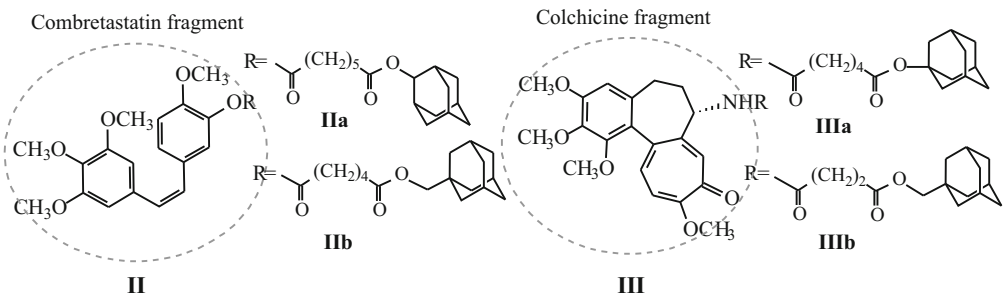



**Fig. 2.** Structures of new analogs **Ia** and **Ib** with the colchicine fragment replaced by combretastatin and a modified linker.

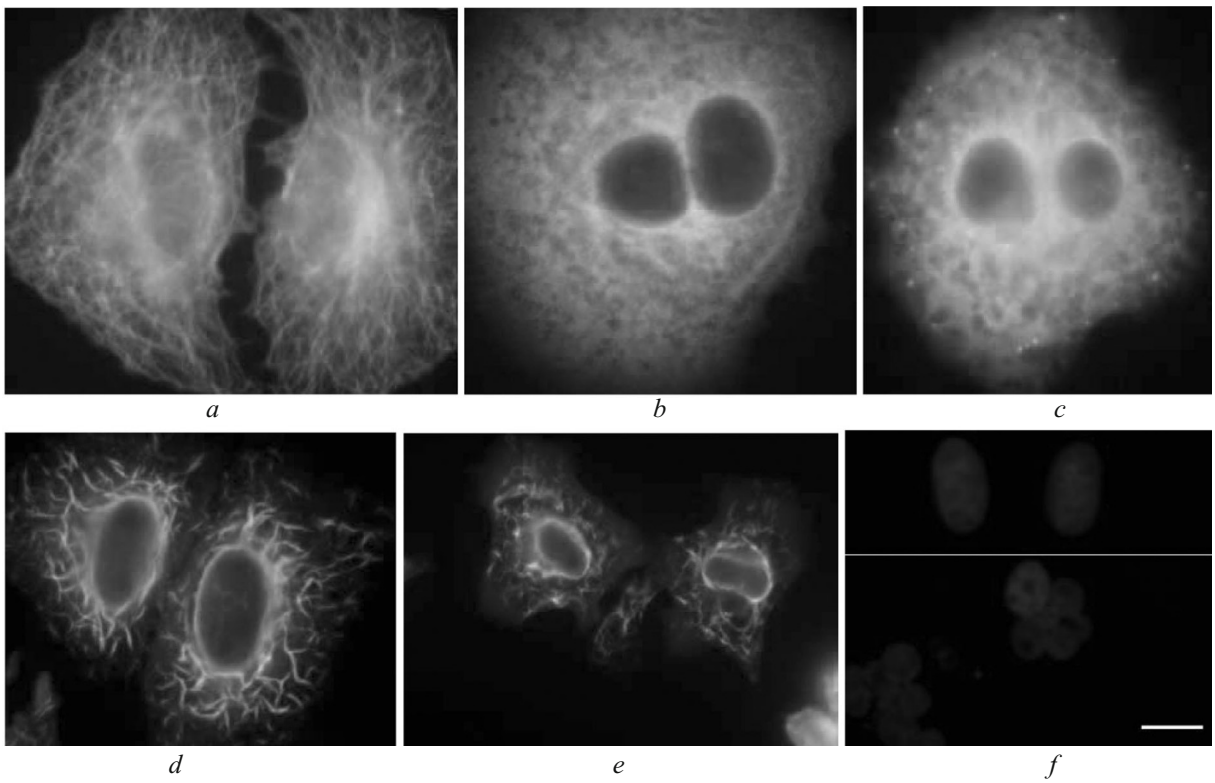



**Fig. 3.** Tubulin accumulation in carcinoma A549 cells treated with tested or control compounds: whole microtubules (negative control, 0.5% DMSO) (a); diffuse distribution of tubulin after depolymerization of microtubules (CA-4, positive control) (b); weak (point) tubulin clusterization (c); strong tubulin clusterization analogous to the effect of tubuloclustin (d); moderate tubulin clusterization (e); normal unfragmented nuclei (top), nuclear fragmentation characteristic of apoptotic cells (bottom). Scale, 10 μm.

of the protein α-subunit that were close to amino-acid residue αTyr224. This was characteristic of compounds capable of causing the clustering effect characteristic of tubuloclustin and its analogs [10, 15]. The locations in the protein of the combretastatin fragments of the examined molecules were close to that for free combretastatin for conjugates **IIa, b** and distinctly different from it for **II** [R = $–CH_2–(CH_2)_5C(O)O–^2$Ad]. Apparently, limited free rotation around the phenol ester bond in **IIa, b** was responsible for the difference because the carbonyl O atom of this group was not involved in H-bonding with protein amino-acid residues according to the modeling data.

According to MTT assays of colchicine conjugates with shortened linker chains (**IIIa, b**), both were highly cytotoxic against tumor cells with an $EC_{50}$ value for **IIIa** that was an order of magnitude greater than that of colchicine. Moreover, this compound was also more cytotoxic than tubuloclustin **Id** and its bridgehead analog **Ie** (Table 1). It caused complete tubulin depolymerization already at a concentration of 100 nM (Table 1) and gave a strong clustering effect (formation of point clusters began at much lower concentrations) at 800 nM (Fig. 3*d*). Both compounds at a concentration of 1 μM inhibited completely cell growth and induced apoptosis (determined from fragmented nuclei, Fig. 3*e*).

In general, the results demonstrated that tubuloclustin analogs with the colchicine fragment replaced by a less toxic tubulin colchicine site ligand could remain highly cytotoxic against tumor cells and stimulated further research in this area. The cytotoxicity of **IIIa** against human lung carcinoma A549 cells was comparable to that of one of the most effective tubulin-directed clinically used antitumor agents, taxol. Therefore, **IIIa** was interesting for further *in vivo* testing.

## EXPERIMENTAL CHEMICAL PART

The course of reactions and purity of compounds were monitored by TLC on Silufol-UV254 plates. Column chromatography used Acros silica gel (40 – 60 μm). PMR and $^{13}$C NMR spectra were recorded at 28°C on a Bruker Avance 400 spectrometer (400 and 100 MHz, respectively). Chemical shifts were given on the δ scale (ppm) vs. residual solvent resonances. Spin—spin coupling constants were given in Hz. Elemental analyses were performed on a Vario micro cube CHN analyzer. MALDI-TOF mass spectra were recorded on a Bruker Autoflex II instrument. Colchicine (Sigma-Aldrich, 95% by HPLC) and tubuloclustin synthesized by the literature method [11] (purity >95% by PMR spectroscopy and elemental analysis) were used in the work.

**7-(2-Adamantyloxy)-7-oxoheptanoic acid (Va)** was prepared in 71% yield by the literature method [11].

**6-(Adamant-1-ylmethoxy)-6-oxohexanoic acid (Vb)** was prepared in 60% yield by the literature method [13].

**6-(Adamant-1-yl)-6-oxohexanoic acid (Vc).** Adamantan-1-ol (0.217 g, 1.31 mmol) in $CH_2Cl_2$ (5 mL) was treated with adipic acid polyanhydride (0.500 g, 3.9 mmol) and a catalytic amount of DMAP (0.01 g). The reaction mixture was stirred at room temperature for 48 h. The solvent was evaporated in a rotary evaporator. The solid was chromatographed [eluent EtOAc–petroleum ether (40 – 70°C), 1:51:3 gradient] to afford **Vc** (0.180 g, 49%) as a colorless oil. PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 1.51 – 1.52 (6H, m), 1.63 – 1.74 (10H, m), 1.98 (3H, m), 2.34 – 2.40 (4H, m), 10.70 (1H, br.s, COOH). $^{13}C$ NMR spectrum ($CDCl_3$, δ, ppm): 24.06, 24.32, 27.96, 33.08, 33.61, 33.88, 36.89, 39.21, 173.55 [C(O)O], 179.52 (COOH). $C_{16}H_{24}O_4$.

**4-(Adamant-1-ylmethoxy)-4-oxobutanoic acid (Vd).** Adamantan-1-ylmethanol (0.341 g, 2.05 mmol) in THF (5 mL) was treated with succinic anhydride (0.612 g, 6.12 mmol) and a catalytic amount of DMAP (0.01 g). The reaction mixture was refluxed for 24 h. The solvent was evaporated. The residue was chromatographed [eluent EtOAc–petroleum ether (40 – 70°C) 1:71:4 gradient] to afford **Vd** (0.270 g, 50%) as a colorless oil. $^{13}C$ NMR spectrum ($CDCl_3$, δ, ppm): 27.96, 28.92, 29.03, 33.13, 36.87, 39.13, 74.39 ($AdCH_2$), 172.18 (COO), 178.42 (COOH). $C_{15}H_{22}O_4$.

**1-Adamantan-2-yl-7-{2-methoxy-5-[(*Z*)-3,4,5-trimethoxystyryl]phenyl}heptanedioate (IIa).** Compound **Va** (0.020 g, 0.068 mmol) in $CH_2Cl_2$ (10 mL) was treated with combretastatin A-4 (0.20 g, 0.063 mmol), DCC (0.028 g, 0.135 mmol), and a catalytic amount of DMAP (0.01 g). The mixture was stirred at room temperature for 12 h, treated with HOAc (5 – 10 μL), and evaporated after 15 min. The precipitate was dissolved in EtOAc (10 – 20 mL) and stored at 0 – 4°C for 2 – 3 h. The resulting precipitate of *N*,*N*′-dicyclohexylurea was filtered off and rinsed with cold EtOAc (2 × 10 mL). The filtrate was washed with saturated NaCl solution (10 mL) and $H_2O$ (10 mL), dried over $Na_2SO_4$, and evaporated. The residue was chromatographed [eluent EtOAc–petroleum ether (40 – 70°C), 1:8 – 1:5] to afford **IIa** (0.025 g, 67%) as a colorless oil. PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 1.46 (2H, m); 1.58 (3H, m); 1.67 – 1.78 (3H, m); 1.84 (6H, m); 1.99 (3H, m); 2.36 (2H, m, J = 6.9 Hz); 2.43 (1H, m, J = 6.9 Hz); 2.55 (2H, t, J = 7.5 Hz); 3.68 (2H, s, $AdCH_2$); 3.71 (6H, s, $OCH_3$); 3.80 (3H, s, $OCH_3$); 3.84 (3H, s, $OCH_3$); 4.93 (1H, m); 6.46 (2H, d, CH=CH, J = 1.5 Hz); 6.51 (2H, s, Ar); 6.85 (1H, d, Ar, J = 8.6 Hz); 7.00 (2H, d, Ar, J = 2.1 Hz); 7.12 (1H, dd, Ar, J = 2.1, 8.4 Hz). $^{13}C$ NMR spectrum ($CDCl_3$, δ, ppm): 24.61, 24.79, 28.50, 31.77, 32.72, 33.71, 34.65, 36.30, 37.35, 55.89, 56.07, 60.86, 76.77, 103.35, 105.79, 111.91, 120.28, 123.12, 127.58, 128.57, 129.44, 130.04, 132.42, 139.44, 150.20, 152.92, 171.42 (C=O), 172.97 (C=O). Mass spectrum (MALDI-TOF) *m*/*z*: 592 $[M^+K]^+$.

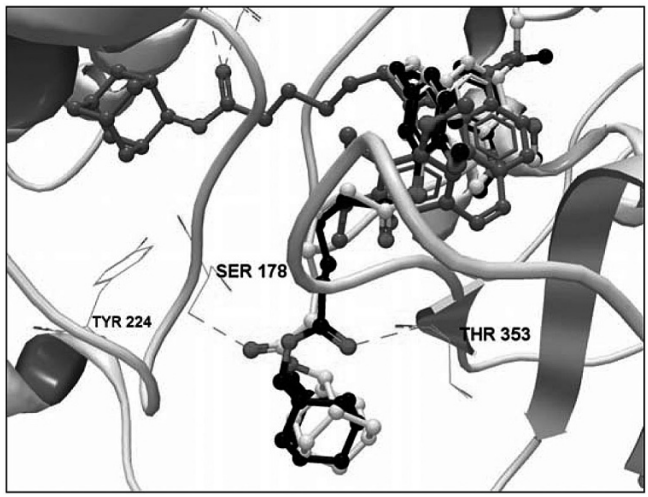



**Fig. 4.** Positioning of structures of **IIa** (light-gray), **IIb** (black), and **II** [R = $–CH_2–(CH_2)_5C(O)O–^2$Ad] (gray) in α, β-tubulin dimer (β-subunit shown on the right; α-subunit, on the left; H-bonds are shown as dotted lines; most H atoms were omitted for clarity).

**Adamant-1-ylmethoxy-6-{2-methoxy-5-[(*Z*)-3,4,5-trimethoxystyryl]phenyl}hexanedioate (IIb)** was synthesized analogously to **IIa** from **Vb** (0.021 g, 0.071 mmol), combretastatin A-4 (0.020 g, 0.063 mmol), and DCC (0.028 g, 0.135 mmol) to afford **IIb** (0.022 g, 59%) as a yellow oil. PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 1.53 (6H, m); 1.60 – 1.78 (9H, m); 1.98 (3H, m); 2.34 – 2.40 (2H, m, J = 6.9 Hz); 2.57 (2H, t, J = 6.9 Hz); 3.68 (2H, s, $AdCH_2$); 3.71 (6H, s, $OCH_3$); 3.80 (3H, s, $OCH_3$); 3.84 (3H, s, $OCH_3$); 6.45 (2H, d, CH=CH, J = 1.3 Hz); 6.50 (2H, s, Ar); 6.85 (1H, d, Ar, J = 8.5 Hz); 7.00 (2H, d, Ar, J = 2.1 Hz); 7.12 (1H, dd, Ar, J = 2.1, 8.5 Hz). $^{13}C$ NMR spectrum ($CDCl_3$, δ, ppm): 24.30, 24.42, 27.99, 33.11, 33.52, 33.89, 36.91, 39.23, 55.83, 55.87, 60.84, 73.95, 105.77, 111.88, 123.07, 127.60, 128.54, 129.44, 130.01, 132.41, 137.08, 139.39, 150.16, 152.91, 171.17 (C=O), 173.49 (C=O). Mass spectrum (MALDI-TOF) *m*/*z*: 592 $[M^+K]^+$.

***N*-[6-(Adamantyl)-6-oxohexanoyl]-*N*-deacetylcolchicine (IIIa).** Compound **Vc** (0.050 g, 0.15 mmol) in $CH_2Cl_2$ (10 mL) was stirred, treated with EEDQ (0.037 g, 0.15 mmol) and *N*-deacetylcolchicine (0.48 g, 0.13 mmol), stirred at room temperature for 24 h, and evaporated. The residue was chromatographed [eluent EtOAc—petroleum ether (40 – 70°C), 1:5; then $CH_2Cl_2$—MeOH, 30:1] to afford **IIIa** (0.045 g, 56%) as a yellow oil. PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 1.51 – 1.69 (m, 8H), 1.84 – 1.90 (m, 2H), 2.07 (m, 6H), 2.14 (m, 3H, $H^6$colch), 2.18 – 2.29 (m, 5H), 2.37 – 2.42 (m, 1H), 2.51 (dd, 1H, J 13.5, 6.2 Hz, $H^5$colch), 3.65 (s, 3H, $OCH_3$), 3.90 (s, 3H, $OCH_3$), 3.94 (s, 3H, $OCH_3$), 3.99 (s, 3H, $OCH_3$), 4.65 (m, 1H, $H^7$colch), 6.53 (s, 1H, $H^4$colch), 6.83 (d, 1H, J 11.0 Hz, $H^{11}$colch), 6.91 (br.s., 1H, NH), 7.30 (d, 1H, J 11.0 Hz, $H^{12}$colch), 7.43 (s, 1H, $H^8$colch). $^{13}C$ NMR spectrum ($CDCl_3$, δ, ppm): 24.58, 24.98,

25.83, 28.67, 29.93, 30.26, 33.36, 34.26, 34.28, 34.69, 35.96, 36.98, 51.64 ($OCH_3$), 52.15 ($C^7$colch), 56.12 ($OCH_3$), 56.44 ($OCH_3$), 61.40 ($OCH_3$), 61.62 ($OCH_3$), 66.91, 107.38, 113.02, 125.56, 130.64, 134.22, 135.82, 138.70, 141.68, 146.41, 152.19, 153.57, 164.02, 172.51 (C=O), 173.02 (C=O), 177.47 ($C^9$colch=O). $C_{36}H_{45}NO_8$.

***N*-[4-(Adamant-1-ylmethoxy)-4-oxobutanoyl]-*N*-deacetylcolchicine (IIIb)** was synthesized analogously to **IIIa** from **Vd** (0.041 g, 0.15 mmol), EEDQ (0.037 g, 0.15 mmol), and *N*-deacetylcolchicine (0.050 g, 0.14 mmol) to afford **IIIb** (0.021 g, 25%) as a yellow oil. PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 1.51 – 1.69 (m, 5H), 1.84 – 1.90 (m, 2H), 2.07 (m, 6H), 2.14 (m, 3H, $H^6$colch), 1.83 (m, 1H), 1.93 – 1.98 (m, 3H), 2.25 (m, 1H), 2.42 (m, 1H), 2.69 – 2.70 (m, 2H), 3.64 (s, 3H, $OCH_3$), 3.91 (s, 3H, $OCH_3$), 3.94 (s, 3H, $OCH_3$), 4.00 (s, 3H, $OCH_3$), 4.65 (ddd, 1H, J 12.9, 6.7, 5.9 Hz, $H^7$colch), 6.49 (br.s., 1H, NH), 6.53 (s, 1H, $H^4$colch), 6.86 (d, 1H, J 11.0 Hz, $H^{11}$colch), 7.33 (d, 1H, J 11.0 Hz, $H^{12}$colch), 7.50 (s, 1H, $H^8$colch). $^{13}C$ NMR spectrum ($CDCl_3$, δ, ppm): 20.67, 27.97, 29.91, 33.08, 33.48, 35.15, 36.90, 37.04, 39.21, 52.28 ($C^7$colch), 56.08 ($OCH_3$), 56.51 ($OCH_3$), 61.34 ($OCH_3$), 61.59 ($OCH_3$), 74.00 ($Ad\underline{C}H_2$), 107.37, 112.74, 125.67, 130.65, 134.26, 135.42, 136.65, 141.64, 151.25, 153.52, 153.45, 163.93, 172.24 (C=O), 173.63 (C=O), 179.15 ($C^9$colch=O). $C_{35}H_{43}NO_8$.

## EXPERIMENTAL BIOLOGICAL PART

**The MTT assay for cytotoxicity** was conducted on human lung carcinoma A549 cell line (CCL-185) using the published method [13]. Each compound was tested in triplicate. Results were processed statistically (nonlinear regression, Excel: XLSTAT software). $EC_{50}$ values were determined from sigmoidal curves (values with $p < 0.05$ were considered statistically significant).

**Cell growth and tests with immunofluorescent-labeled microtubules** were studied using the same cell line and published methods [16]. Differences among effects on the microtubule network of control and test compounds were determined visually using a Nikon Diaphot 300 microscope (Nikon GmbH, Dusseldorf, Germany) equipped with a SenSys camera (Photometrics, Munich, Germany).

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