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Synthesis and SAR requirements of adamantane–colchicine conjugates with both microtubule depolymerizing and tubulin clustering activities

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ABSTRACT

A series of analogues of conjugate **1**, combining an adamantane-based paclitaxel (taxol) mimetic with colchicine was synthesized and tested for cytotoxicity in a cell-based assay with the human lung carcinoma cell line A549. The most active compounds (**10** EC_{50} 2 ± 1.0 nM, **23** EC_{50} 6 ± 1.4 nM, **26** EC_{50} 5 ± 1.8 nM, **28** EC_{50} 11 ± 1.7 nM, **30** EC_{50} 4.8 ± 0.5 nM) were found to interfere with the microtubule dynamics in an interesting manner. Treatment of the cells with these compounds promoted disassembly of microtubules followed by the formation of stable tubulin clusters. Structure–activity relationships for the analogues of **23** revealed the sensitivity of both cytotoxicity and tubulin clustering ability to the linker length. The presence of adamantane (or another bulky hydrophobic and non-aromatic moiety) in **23** was found to play an important role in the formation of tubulin clusters. Structural requirements for optimal activity have been partially explained by molecular modeling.

1. Introduction

Natural compounds such as paclitaxel (taxol), colchicine and vinca-alkaloids (vincristine and vinblastine) possess high antitumour activity due to their interaction with the intracellular protein tubulin. Although these compounds have the same target protein, their binding sites and their biological activities differ considerably. Thus, paclitaxel causes spontaneous tubulin polymerization into stable microtubules and stabilize preformed microtubules whereas colchicine and vinca-alkaloids inhibit tubulin polymerization and microtubule formation.[1,2]

Among the different approaches to the design of new antitubulin agents, studies of various hybrid ligands recently attracted much attention.[3] The rationale behind the combination of two or more tubulin ligands in a single molecule is that the hybrid ligand may have higher activity and/or better pharmacological profile than the equivalent combination of individual compounds. Besides, this approach leads sometimes to a discovery of molecules with unusual types of activity.

Earlier we synthesized a hybrid ligand combining an adamantane-based paclitaxel mimetic with colchicine (**1**, *trans*/*cis* 2:1, Fig. 1),[4] which possessed very high cytotoxicity against A549 human lung carcinoma cells. Moreover the preliminary test indicated that hybrid **1** had an unusual cytotoxicity profile, namely both microtubule destabilizing activity and ability to promote the formation of stable tubulin structures resembling the action of paclitaxel with microtubule bundling or vinblastine-like activity with tubulin paracrystal formation.

In the present work we carried out additional biological tests to verify the effect of **1** on tubulin and studied the structure–activity relationships for a number of analogues of the lead compound. Several aspects were suggested for the investigation: the linker length, the possibility of amino acid side chain removal, the position of the linker attachment and the role of adamantane moiety. Molecular modeling was performed for a better understanding SAR results.

2. Results and discussion

2.1. Biological assays for the lead compound

The hybrid **1** was first tested using in vitro tubulin polymerization assay.[5] Purified bovine brain tubulin was polymerized in the presence of 10% DMSO and supplemented with 10 μM of **1** or 10 μM colchicine, as positive control. After 1 h of incubation the resulting preparations were analyzed by Allen Video-Enhanced

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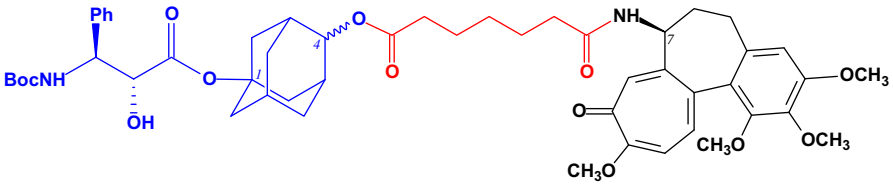
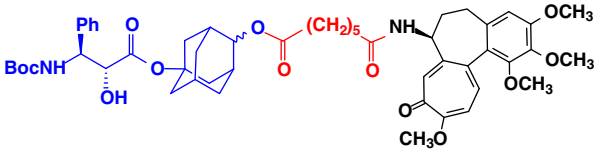
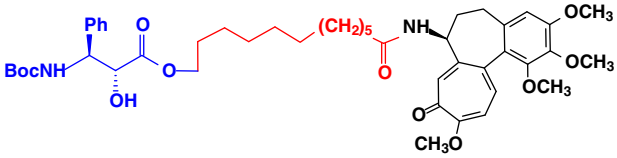
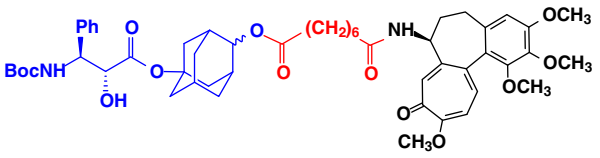
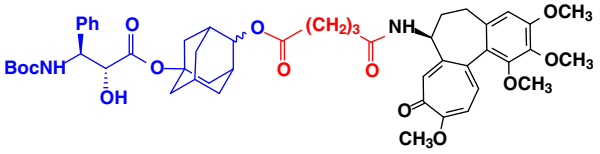


Figure 1. Adamantane-based paclitaxel mimetic—colchicine hybrid.

Table 1
Cytotoxicity and effect of the hybrid compounds on A549 cellular microtubules

N	Compound	Cytotoxicity[a] EC_{50} ± SD (nM)	Effect on cellular microtubules[b] at 1 μM
1		73± ± 2.9	Depolymerization and tubulin clustering ++
4		41 ± 9.8	Depolymerization and tubulin clustering +
10		2 ± 1.0	Depolymerization and tubulin clustering +++
11		220 ± 12	ND
Colchicine		27 ± 1.5	Depolymerization only
Paclitaxel (Taxol)		4.6 ± 0.7	Microtubule bundling

[a] The average of three to six experiments.
[b] The number of '+' symbolizes the relative strength of the effect.

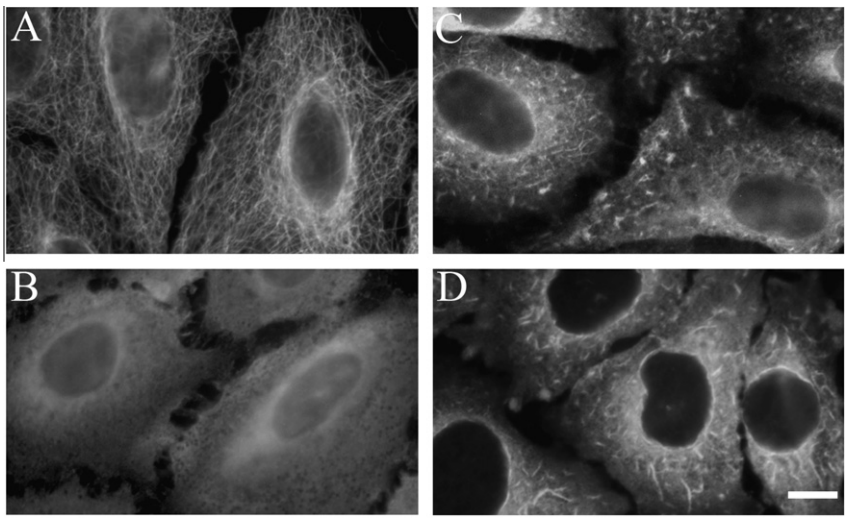


Figure 2. Effect of conjugate **1** on the microtubule network in A549 lung carcinoma cells analyzed by indirect immunofluorescence microscopy. Cells were treated: A—with 0.6% DMSO (negative control); B—with 1 μM of colchicine (positive control); C and D—with 1 μM and 5 μM of compound 1 respectively. Destabilizing effect of 1 on the microtubule network and tubulin clustering effect are clearly seen in C and D. Bar 20 μm.

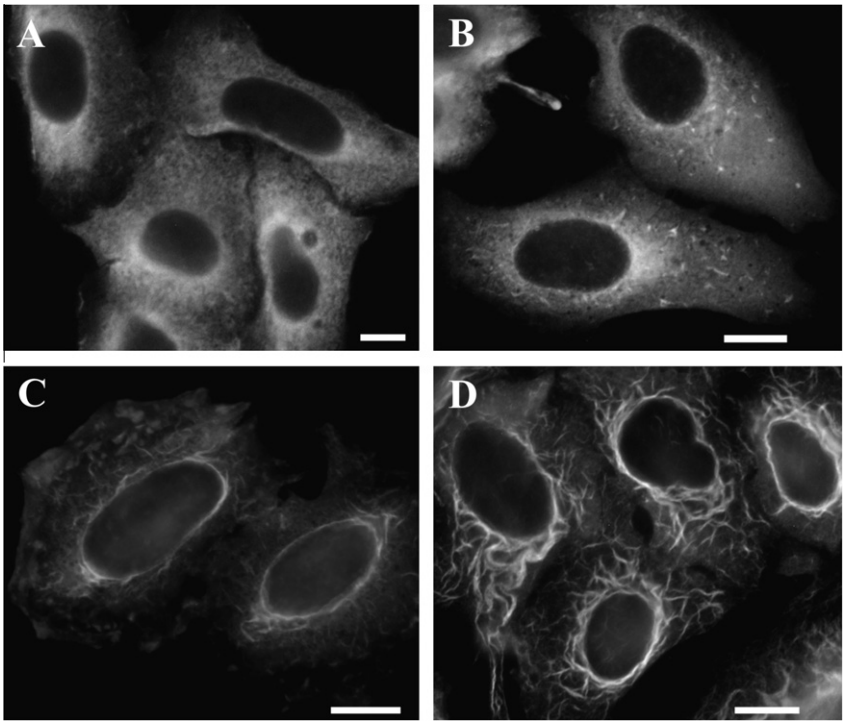


Figure 3. Typical patterns of tubulin distribution in treated cells used for evaluation of the effect of tested compounds on microtubules as indicated in Tables 1 and 2. A—'depolymerization only'; B—tubulin clustering '+'; C—tubulin clustering '++'; D—tubulin clustering '+++'. Bar 20 μm.

Differential Interference Contrast light microscopy.[6] According to the images obtained hybrid **1** completely inhibited the microtubule assembly in vitro. This effect was more pronounced than that of colchicine, in the presence of which few small aggregates and short microtubules have been detected (see Supplementary data).

Compound **1** was previously found to inhibit strongly the cell growth of human lung carcinoma cell line A549 in a cell proliferation test.[4] In this study cytotoxic properties of **1** were further evaluated by A549 cell-based viability assay with the standard MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay protocol.[7] The EC_{50} value was found to be close to that of colchicine (see Table 1)[†].

The effect of hybrid **1** on microtubule dynamics in A549 cells was also investigated and compared to that of colchicine. Cells were incubated with 1 μM and 5 μM of **1** for 8 h, fixed in 4% paraformaldehyde and stained with primary mouse monoclonal antibodies against α-tubulin followed by incubation with ALEXA-Fluor488 labelled goat anti-mouse IgG as the secondary antibody.[8] Images of the samples were analyzed by fluorescence microscopy and are shown on Figure 2 A–D.

At a concentration of 1 μM hybrid **1** had a clearly visible destabilizing effect on the microtubule network: the samples displayed only very few small microtubule fragments. Although the effect of the tested compound was slightly weaker than that of colchicine, contrary to the latter, hybrid **1** in addition caused a formation of tubulin clusters which were detected among short microtubules and diffusely labeled tubulin (Fig. 2C). Notably, at a concentration of 5 μM no intact microtubules were observed and the clustering effect of **1** was more prominent (Fig. 2D).

These experiments proved the ability of hybrid **1** to cause tubulin clustering in addition to the colchicine-like activity. We named the former ability 'tubulin clustering' because the morphology of these clusters is different from paclitaxel-induced microtubule bundles and vinblastine-induced tubulin paracrystals (see also Fig. 3). Interestingly, the 'clustering effect' cannot be caused by the simultaneous cell treatment with 5 μM paclitaxel and 5 μM colchicine for eight hours, thus being a specific property of the hybrid molecule.

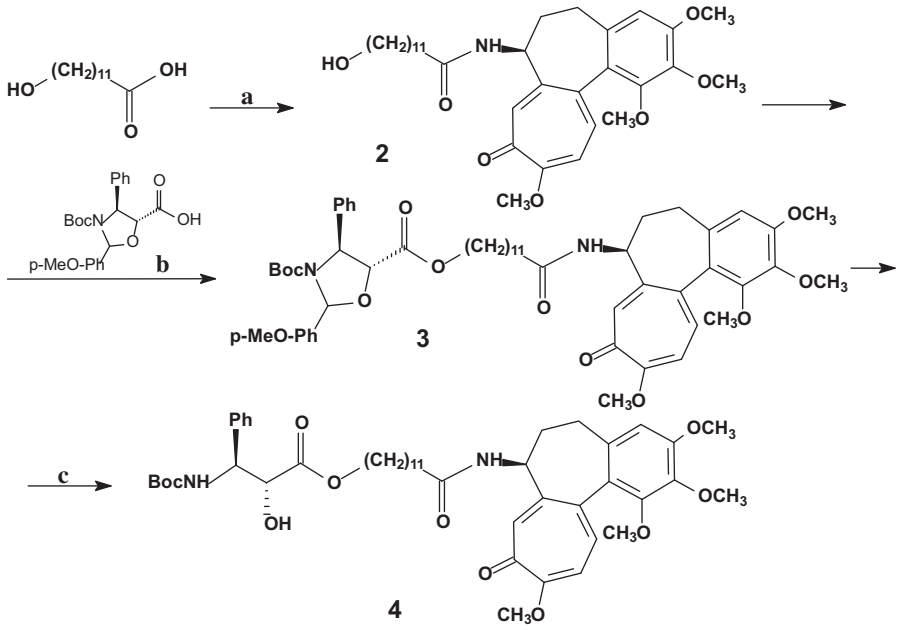
2.2. Chemistry

To determine the structural requirements for the tubulin clustering effect and cytotoxicity a structure–activity investigation was performed for analogues of **1**. The derivative of hybrid **1** without adamantane moiety (structure **4**) was synthesized from 12-hydroxydodecanoic acid and *N*-deacetylcolchicine (prepared in three steps from colchicine[9]) as shown in Scheme 1. Esterification of the obtained amide **2** by an oxazolidine-type protected N-*tert*-butoxycarbonylphenylisoserine[10] led to the conjugate **3** and deprotection of the latter afforded the purpose compound **4**.

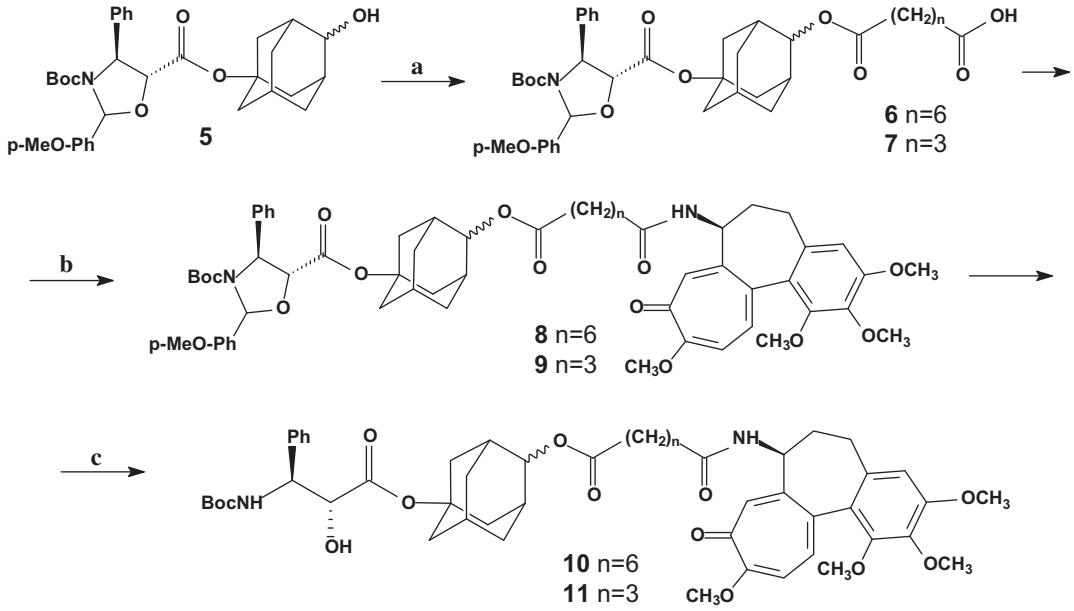
The synthesis of two hybrids **10**, **11** with different linker lengths was analogous to that of **1** (Scheme 2). The esterification of 1-hydroxy-adamantantane-4-one by protected amino acid and subsequent reduction of the resulting keto-ester gave a mixture of isomeric alcohols **5**.[4] Further esterification of **5** by either suberic acid polyanhydride[11] or glutaric acid anhydride led to isomeric esters **6** and **7** respectively. Finally, *N*-deacetylcolchicine was attached to the carboxylic group of compounds **6** and **7**. The following deprotection of amino acid in the products **8** and **9** afforded hybrids **10** (*trans/cis* 7:3) and **11** (*trans/cis* 1:1).

All derivatives of **1** without amino acid side chain (**23**–**29**) and their analogues with hydrophobic substituents other than

[†] It should be mentioned that this value was higher than the IC_{50} value for compound **1** determined earlier by a different method (i.e. the proliferation assay, where the cells were counted directly with hemocytometer cell count calculator).[4] Actually it is not clear, why the two methods give so different absolute values, while the relative values obtained for the different compounds are in a good agreement. However in the present investigation MTT experiments were carried out under identical conditions for all the tested compounds. The differences in the effects were also confirmed by immunofluorescence microscopy.



Scheme 1. *Reagents and conditions:* (a) EEDQ, CH_2Cl_2, rt, 60 h; (b) DCC, DMAP, CH_2Cl_2, rt, 12 h; (c) pTSA, MeOH, rt, 2.5 h.



Scheme 2. *Reagents and conditions:* (a) $(OOC–(CH_2)_n–CO)_m$, DMAP, CH_2Cl_2, rt, 24-48 h; (b) *N*-deacetylcolchicine, EEDQ, CH_2Cl_2, rt, 24 h; (c) pTSA, MeOH, rt, 2.5 h.

adamantane (**30–33**) were prepared from the corresponding alcohols as shown in Scheme 3.

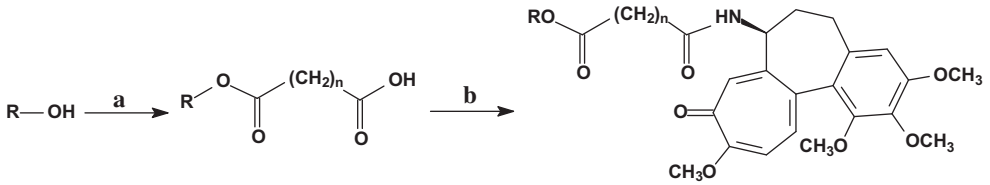
2.3. Biotests and structure–activity relationships

The inhibitory effect of the synthesized compounds on proliferation of human lung carcinoma A549 cells was evaluated using MTT test.[7] Effect on microtubule dynamics was investigated as described in section 2.1 (the typical patterns of tubulin clusters in cells treated with different compounds are shown in Fig. 3). The results of both tests are presented in Tables 1 and 2.

As evidenced from Table 1 an analogue of hybrid **1** without adamantane moiety and with the similar distance between colchicine and the amino acid (hybrid **4**) exhibited only slightly higher cytotoxicity than the parent compound and a strongly less pronounced clustering effect. Thus the adamantane fragment was maintained in the next two series of compounds.

The data for **1**, **10** and **11** clearly indicate that the elongation of the spacer length improves cytotoxity, the hybrid **10** with a six-methylene linker being one order of magnitude more potent than colchicine and as potent as paclitaxel (see Table 1).

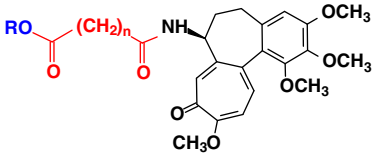
To check the role of amino acid substituent in the parent structure we synthesized compound **23**, which is an analogue of **1** without *N*-tert-butoxycarbonyl-(*2R,3S*)-phenylisoseryl moiety. This amino acid represents a C^{13} side chain of paclitaxel derivative taxoter and is known to provide the most important contribution to the paclitaxel-binding site interaction.[1,2] As seen in Table 2 conjugate **23** exhibits very high cytotoxicity and its clustering activity is

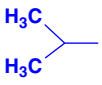


12 R=2-Ad, n=5
13 R=2-Ad, n=4
14 R=2-Ad, n=3
15 R=2-Ad, n=6
16 R=2-Ad, n=7
17 R=1-Ad, n=5
18 R=1-Ad, n=7
19 R= cyclohexyl, n=5
20 R=Me, n=5
21 R=i-Pr, n=5
22 R=Ph, n=5
23 R=2-Ad, n=5
24 R=2-Ad, n=4
25 R=2-Ad, n=3
26 R=2-Ad, n=6
27 R=2-Ad, n=7
28 R=1-Ad, n=5
29 R=1-Ad, n=7
30 R= cyclohexyl, n=5
31 R=Me, n=5
32 R=i-Pr, n=5
33 R=Ph, n=5

Scheme 3. *Reagents and conditions:* (a) (OOC–$(CH_2)_n$–CO)$_m$, DMAP, CH_2Cl_2, rt, 24–36 h; (b) *N*-deacetylcolchicine, EEDQ, CH_2Cl_2, rt, 12–24 h, (c) TFA, CH_2Cl_2, rt, 24 h.

Table 2
Cytotoxicity and effect of the colchicine conjugates on A549 cellular microtubules



Compound			Cytotoxicity[a] EC_{50} ± SD (nM)	Effect on cellular microtubules[b] at 1 μM
N	R	*n*		
23		5	6 ± 1.4	Depolymerization and tubulin clustering +++
24		4	830 ± 140	Depolymerization and tubulin clustering ++
25		3	200 ± 62	depolymerization only
26		6	5 ± 1.8	Depolymerization and tubulin clustering +++
27		7	3800 ± 630	Depolymerization and tubulin clustering +
28		5	11 ± 1.7	Depolymerization and tubulin clustering +++
29		7	29 ± 1.2	ND
30		5	4.8 ± 0.5	Depolymerization and tubulin clustering +++
31	H_3C—	5	32 ± 2.1	Depolymerization only
32		5	30 ± 2.7	Depolymerization and tubulin clustering +
33		5	5700 ± 820	Weak depolymerization only
Colchicine			27 ± 1.5	Depolymerization only
Paclitaxel (Taxol)			4.6 ± 0.7	Microtubule bundling

[a] The average of three to six experiments.
[b] The number of '+' symbolizes the relative strength of the effect.

improved in comparison with **1**. This result is remarkable and signifies that the clustering effect does not depend upon the presence of taxoter amino acid side chain and, consequently, is hardly connected to an interaction with the paclitaxel binding site of tubulin. Thus, these data are in accordance with the results of our study on the morphology of the clusters (see Section 2.1).

As though the structure of conjugate **23** is much simpler than that of **1**, we have chosen **23** as a new lead compound for subsequent structure–activity investigations.

A SAR study in a series of analogues of **23** with different linker length **23**–**27** (Table 2) shows a maximum of cytotoxicity for ligands with five- and six-methylene linkers (**23**, **26**). Further elongation of the spacer (**27**) or its shortening (**24**, **25**) leads to a considerable decrease of activity. Interestingly, all compounds in the series are in fact rather simple colchicine derivatives with a modified side chain at C^7 (it is generally accepted that substitution at this position is highly tolerated (see e.g.[12])). Nevertheless a strong dependence of both cytotoxicity and clustering effect on

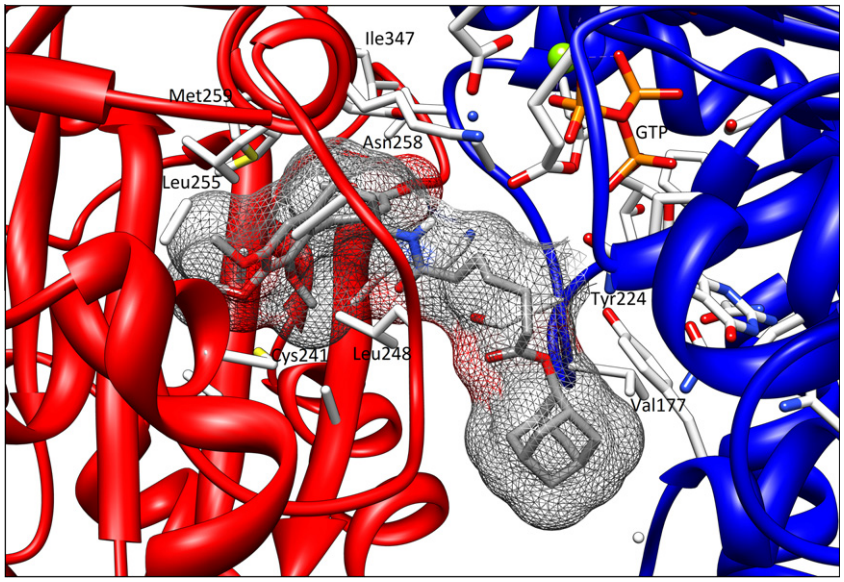


Figure 4. Location of compound **23** at the interface of α/β tubulin subunits (β-subunit is colored in red, α-subunit in blue, hydrogen atoms are not shown for clarity, molecular surface mesh is indicated).

the length of this substituent was observed. Thus, three-methylene ligand **25** maintains only the microtubule depolymerizing activity while its four-methylene analogue **24** possesses also a noticeable clustering effect.

The structural modification of the lead **23** next studied was a change of the position of linker attachment to adamantane. Conjugate **28** with the linker attached to a bridgehead atom possessed high cytotoxicity and clustering effect close to that of **23**, thus suggesting that the movement of the linker was tolerated. Interestingly, the two-methylene elongation of the spacer length in **28**—structure **29**—only slightly reduced the cytotoxicity, while in the pair **23**/**27** it was detrimental for the activity.

The final structure–activity relationship was established for a series of conjugates similar to **23** but with lipophilic moieties distinct from adamantane. Replacement of a bridgehead core for cyclohexane led to compound **30** with the same high cytotoxicity and close tubulin clustering effect. Unexpectedly weak activity was observed for ligand **33** with a phenyl substituent, moreover its clustering ability was lost. Cytotoxicity of methyl (**31**) and isopropyl (**32**) derivatives was almost equal to that of colchicine and for the former only the colchicine-like depolymerizing effect was observed. These results indicate, that the presence of a bulky hydrophobic and non-aromatic moiety in the studied conjugates plays an important role in the tubulin-clustering effect.

Since all the most cytotoxic compounds synthesized (**10**, **23**, **26**, **28**, **30**) possess the strongest tubulin clustering ability (see Tables 1 and 2), we suppose that this property might be an important factor in the increment of mitostatic activity. This proposition is also confirmed by comparison of the activities in the triad of structurally close compounds colchicine—**31**–**23**. A noticeably higher cytotoxicity is observed for the compound **23** with high clustering effect, while compounds with depolymerizing ability only have equal lower cytotoxicity.

It should be mentioned, that the effect of the lead compound **23** on microtubule dynamics was also studied using cancer cell line, different from A549, namely HeLa human cervical carcinoma cells. In these cells we also observed the intensive formation of tubulin clusters, which proves the fact that this ability of **23** is not only limited to A549 cancer cells.

2.4. Molecular modeling

To gain more knowledge about the mechanism of action of the synthesized compounds we conducted a molecular modeling study using a three-dimensional model of the structure of the tubulin dimer–colchicine–vinblastine complex (PDB ID: 1Z2B). Conjugate **23** was chosen for the modeling among the synthesized compounds with highest cytotoxicity and maximal clustering effect (**10**, **23**, **26**, **28**, **30**). As though the clustering ability of the conjugates does not depend upon the presence of taxoter side chain (see Section 2.3), molecular docking was performed to the colchicine binding site located at β-subunit at the interface of α/β tubulin subunits. The study was carried out with the help of the AutoDock Vina program, which takes ligands flexibility into account.[13] Ligand-tubulin complexes with the best values of the corresponding scoring function calculated by AutoDock Vina were chosen. The obtained binding mode of **23** is presented in Figure 4.

According to the modeling results, while colchicine fragment of **23** binds to the corresponding site of β-subunit (formed by the residues of Cys241, Leu248, Leu255, Asn 258, Met 259 etc.), the adamantane moiety of the conjugate is located in a binding site formed by hydrophobic residues Tyr224 and Val177 in the α-subunit. Thus, compound **23** connects two binding sites located on two opposite surfaces of α and β tubulin subunits[‡].

Each of oxygen atoms of the linker ester group of **23** may form a hydrogen bond with the hydroxyl of Tyr224. This hydrogen bonding seems to be important for providing the location of the adamantane moiety at the interface of two subunits and can be the reason for the observed dependence of the activity on spacer length.

A similar molecular modeling study was carried out for compound **31**, which possesses no tubulin clustering ability. The result

[‡] It should be mentioned that if the docking study is carried out using the model of β-subunit only, then in the best binding mode of **23** its adamantane moiety is located in an additional binding site formed by hydrophobic residues Ile347 and Pro348 along with hydrophobic parts of Thr314 and Asn 349. Both carbonyl oxygens of the linker in **23** may form hydrogen bonds with Asn 258 and Met 259 residues and the importance of these bonds might be the reason for the observed dependence of activity on spacer length. However this docking study does not explain the difference in binding of compounds with and without tubulin clustering ability as it does the docking to both α- and β-subunits of the tubulin dimer (see below).

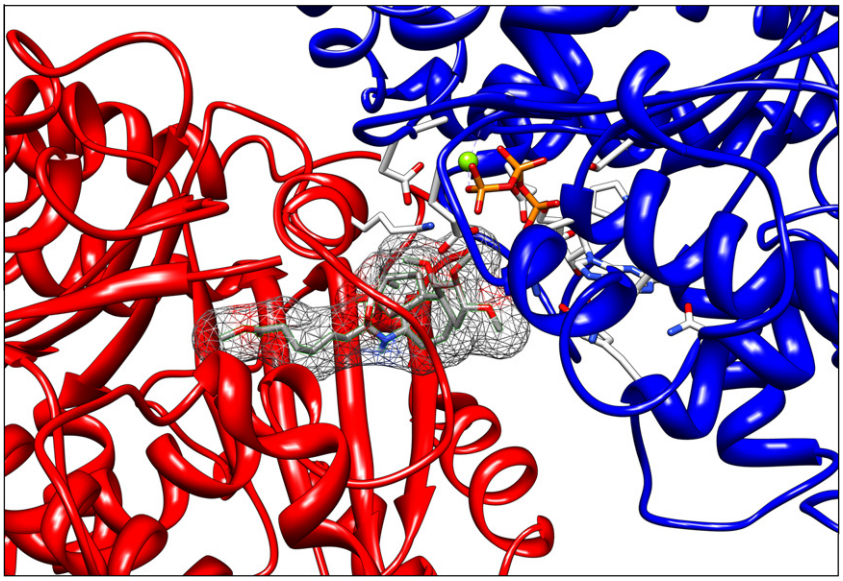


Figure 5. The best-binding mode of compound **31** with tubulin (β-subunit is colored in red, α-subunit in blue, hydrogen atoms are not shown for clarity, molecular surface mesh is indicated). The direction of the linker of **31** towards the inner space of the β-subunit is clearly seen.

indicates that its best binding mode is different from that of **23** (Fig. 5). The linker and methyl group of **31** are not exposed to the interface of tubulin subunits but are directed towards the inner space of the β-subunit protein globe (Fig. 5). The analogues binding mode (i.e. the direction of the side chain to the internal area of β-subunit) was observed for conjugate **33** with phenyl substituent (data not shown).

Since compounds **31** and **33** do not exhibit the tubulin-clustering effect it is logical to propose that this property might be connected to the ability of the adamantane moiety of **23** to interact with the hydrophobic site on the α-subunit, providing a kind of 'crosslinking' of the tubulin dimer subunits. Binding of the conjugate **23** to other tubulin areas is also possible. Further experiments are ongoing to understand the details of the mechanisms by which the compounds effect microtubule dynamics and induce tubulin clustering in cultured cells and to evaluate an anti-tumor effect and an overall toxicity of the most active conjugates in vivo.

3. Conclusion

In summary, a hybrid of adamantane-based paclitaxel mimetic with colchicine (**1**) was proved to interfere with the microtubule dynamics in an unusual manner, i.e. to promote disassembly of microtubules followed by the formation of stable tubulin clusters. This ability was found to be sensitive to the length and structure of the linker. More pronounced tubulin clustering effect was observed for the derivative of **1** without amino acid side chain—conjugate **23**. Structure—activity relationships for the analogues of **23** indicate that both cytotoxicity and clustering ability are very sensitive to the linker length and much less sensitive to the shift of the position of linker attachment to adamantane. Replacement of the bridgehead core in **23** for other lipophilic groups revealed, that the presence of a bulky hydrophobic and non-aromatic moiety in the studied conjugates plays an important role in bringing about the tubulin-clustering effect. These structure—activity data were partially explained by molecular modeling studies.

The most cytotoxic compounds synthesized in this work (**10**, **23**, **26**, **28**, **30**) possess activity in nanomolar concentrations and are several times more cytotoxic than colchicine.

4. Experimental section

4.1. Chemistry

Reaction control was carried out by thin-layer chromatography on 'Silufol' plates. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ at 400 and 100 MHz correspondingly and are referenced to residual chloroform (δ 7.26 ppm ^{1}H; δ 77.0 ppm ^{13}C). Chemical shifts of the second isomer are given in square brackets. Attached proton test (APT) technique was used for signal assignment in some ^{13}C NMR spectra. Electron impact mass spectra were obtained with a typical voltage of 70 eV. GC-EIMS spectra were recorded on 'JMS-D300' mass spectrometer with 'HP-5890' chromatograph (150 °C, 70 eV). Elemental analysis of the synthesized compounds was performed on CNH analyser 'Carlo-Erba' ER-20. Infrared spectra (IR) were registered on 'Thermo Nicolet IR200' apparatus in KBr plates and reported in cm^{-1}. Melting points were measured in a block with sealed capillaries and are uncorrected.

Compounds were purified by flash and column chromatography, which were performed on silica gel Acros (40–60 μm). Some of the oily compounds obtained can be solidified into glassy solids on standing in refrigerator.

4.2. Synthesis and characteristics of the final compounds (synthetic protocols and analytical data for compounds 2, 3, 6–9, 12–21 are provided within Supplementary data)

4.2.1. Opening of the oxazolidine-type protected N-*tert*-butoxycarbonylphenylisoserine (general procedure A)

The solution of a conjugate with oxazolidine-type protected N-Boc-phenylisoserine and *p*-toluenesulfonic acid (pTSA) in methanol was stirred 2.5 h at room temperature. After neutralization by 5% solution of $NaHCO_3$ methanol was evaporated and the residue was extracted by CH_2Cl_2. The organic layer was dried over Na_2SO_4 and subjected to column chromatography (methanol/CH_2Cl_2 1:50).

4.2.1.1. (2*R*,3*S*)-*N*-(*tert*-Butoxycarbonyl)phenylisoserine 12-oxo-12-(*N*-deacetylcolchicin-7-*N*-yl)dodecyl ester (4). It was prepared from **3** (0.077 g, 0.082 mmol) and pTSA (0.012 g,

0.115 mmol) according to procedure A. The product **4** was isolated as white semisolid foam, 0.039 g, yield 59%). $C_{46}H_{62}N_2O_{11}$ requires: C, 67.46; H, 7.63; N, 3.42. Found: C, 67.22; H, 7.68; N, 3.40. ^{1}H NMR: 1.21 (9H, s, tBu), 1.13–1.38 (14H, m), 1.54 (2H, m), 1.65 (2H, m), 1.81 (1H, m, J = 6.7, 6.0 Hz, H^{6colch}), 2.17–2.36 (3H, m), 2.34 (1H, m, J = 13.2, 6.7, Hz, H^{5colch}), 2.48 (1H, dd, J = 13.2, 6.0 Hz, H^{5colch}), 3.63 (3H, s, OMe), 3.81 (1H, br s, OH), 3.88 (3H, s, OMe), 3.92 (3H, s, OMe), 3.97 (3H, s, OMe), 4.18 (2H, t, J = 6.7 Hz, $CH_2O(O)C$), 4.43 (1H, m, $H^{2isoserinyl}$), 4.65(1H, m, J = 6.6, 6.0 Hz, H^{7colch}), 5.18 (1H, m, $H^{3isoserinyl}$), 5.40–5.51 (1H, br s, NH), 6.50 (1H, s, H^{4colch}), 6.83 (1H, d, J = 10.8 Hz, $H^{11colch}$), 7.04 (1H, m, H^{4Ph}), 7.27 (1H, d, J = 10.8 Hz, $H^{12colch}$), 7.24–7.36 (5H, m), 7.46 (1H, s, H^{8colch}); ^{13}C NMR: 25.4, 25.67, 28.31, 28.42, 29.14, 29.25, 29.35 ($C(Me)_3$), 29.46, 29.98, 36.29, 36.84, 52.11 (C^{7colch}), 56.07 (OMe), 56.14 (OMe), 56.41 (OMe), 61.44 (OMe), 61.66 ($C^{3isoserinyl}$), 66.55 (OMe), 73.64, 79.77 ($C(Me)_3$), 107.37, 112.6, 125.69, 126.75, 127.64, 128.56, 130.71, 134.25, 135.34, 136.63, 139.36, 141.69, 151.27, 151.91, 153.51, 155.1, 164.02, 171.39 (C═O), 172.92 (C═O), 179.46 (C═O); IR: 3438, 3303(NN), 3060, 2929–2854 (C–H), 1741 (C═O), 1716, 1678, 1655, 161 4, 1589, 1560, 1535, 1489, 1460, 1433, 1400, 1365, 1350, 1323, 1282, 1252, 1194, 1171, 1140, 1095, 1049, 1018, 987, 924, 904, 843, 800, 777, 702, 671, 592, 484.

4.2.1.2. 1-{[(2*R*,3*S*)-*N*-(*tert*-Butoxycarbonyl)phenylisoserinyl]oxy}-4-adamantyl 8-oxo-8-(*N*-deacetylcolchicin-7-*N*-yl)octanoate (10).

It was prepared from **8** (0.043 g, 0.04 mmol) and pTSA (0.009 g, 0.047 mmol) according to procedure A. Column chromatography of the product did not lead to the separation of individual isomers and compound **10** was isolated as mixture of *trans*- and *cis*-stereoisomers (ca. 7:3) (0.027 g, yield 70%, white solid). Mp = 32–34 °C. $C_{52}H_{66}N_2O_{13}$ requires: C, 67.37; H, 7.18; N, 3.02. Found: C, 67.40; H, 7.16; N, 3.03. MS (MALDI) m/z found for $(M+Na)^+$ 950.1. ^{1}H NMR: 1.43 (9H, s, *t*Bu), 1.60–2.43 (28H, m), 2.52–2.58 (1H, m), 3.16 (1H, br s, OH), 3.67 [3.63] (3H, s,OMe), 3.92 [3.90] (3H, s, OMe), 3.97 [3.96] (3H, s, OMe), 4.02 [3.99] (3H, s, OMe), 4.39 (1H, br, $H^{2isoserinyl}$), 4.67 (1H, m, H^{7colch}), 4.82 (0.3H, t, $J_{4eq\text{-}3/5eq}$ = 3.1 Hz, H^{4adam}), 4.95 (0.7H, t, $J_{4ax\text{-}3/5eq}$ = 4.0 Hz, H^{4adam}), 5.26 (1H, m, $H^{3isoserinyl}$), 5.41 (0.7H, d, J = 8.5 Hz, NH), 5.52 (0.3H, d, J = 8.5 Hz, NH), 6.09–6.12 (1H, br s, NH), 6.55 [6.53] (1H, s, H^{4colch}), 6.81–6.85 (1H, m, $H^{11colch}$), 7.28–7.39 (7H, m, arom.).

4.2.1.3. 1-{[(2*R*,3*S*)-*N*-(*tert*-Butoxycarbonyl)phenylisoserinyl]oxy}-4-adamantyl 5-oxo-5-[*N*-deacetylcolchicino]pentanoate (11).

It was prepared from **9** (0.015 g, 0.08 mmol) and pTSA (0.067 g, 0.0668 mmol) according to procedure A. Column chromatography of the product did not lead to the separation of individual isomers and compound **11** was isolated as mixture of *trans*- and *cis*-stereoisomers (ca. 1:1) (0.032 g, yield 48%, white semisolid foam). $C_{49}H_{60}N_2O_{13}$ requires: C, 66.50; H, 6.83; N 3.17. Found: C, 66.52; H, 6.78; N, 3.15.^{1}H NMR: 1.42 (9H, s, tBu), 1.69–2.55 (23, m), 3.24 (1H, s, OH), 3.67 [3.64] (3H, s, OMe), 3.91 [3.87] (3H, s, OMe), 3.95 [3.93] (3H, s, OMe), 4.00 (3H, s, OMe), 4.40 (1H, br s, $H^{2isoserinyl}$), 4.62 (1H, m, H^{7colch}), 4.92 [4.81] (1H, two br s 1:1, H^{4adam}, 5.24 (1H, d, J = 8.84 Hz, $H^{3isoserinyl}$), 5.43 [5.38] (1H, d, J = 9.35 Hz, NH), 6.55 [6.45] (1H, s), 6.80–6.86 (1H, two d, J = 11.2 Hz, $H^{11colch}$), 7.20–7.48 (7H, m, arom.).^{13}C NMR: 20.82 [20.70], 28.33 ($C(Me)_3$), 29.70 [26.56], 30.26, 33.63, 34.65, 34.77, 35.12, 39.06, 39.32, 40.73, 52.11 (C^{7colch}), 56.05 (CHNHBoc), 56.11(OMe), 56.37 (OMe), 61.40 (OMe), 61.57 (OMe), 74.16 [73.65] ($C^{2isoserinyl}$), 75.21 [75.00] (C^{4adam}), 79.71 ($C(Me)_3$), 82.04 [81.86] (C^{1adam}), 107.32. 112.43, 125.69 [125.62], 126.73 [126.49], 127.55 [127.36], 128.50 [128.40], 130.85, 134.29, 134.85, 135.29, 136.35, 141.55, 151.18, 151.36, 153.48, 154.97, 164.00, 171.85(C═O), 172.34(C═O), 172.42(C═O), 179.51(C═O).

4.2.2. Preparation of amides from dicarboxylic acids monoesters (general procedure B)

The solution of dicarboxylic acid mono-ester in CH_2Cl_2, 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) and *N*-deacetylcolchicine were stirred together at room temperature for 12–60 h. The mixture was concentrated and the residue was purified by column chromatography.

4.2.2.1. *N*-(7-Adamant-2-yloxy-7-oxoheptanoyl)-*N*-deacetylcolchicine (23).

It was prepared by method B from **12** (0.094 g, 0.319 mmol), EEDQ (0.085 g, 0.344 mmol) and *N*-deacetylcolchicine (0.095 g, 0.266 mmol). Chromatographic purification (ethyl acetate/petroleum ether 2:3, then CH_2Cl_2/methanol 20:1) gave **23** as yellowish oil (0.092 g, yield 54%). $C_{37}H_{47}NO_8$ requires: C, 70.12; H, 7.47; N, 2.21; Found: C, 69.98; H, 7.52; N, 2.21; MS (MALDI) m/z found for $(M+Na)^+$ 656.8. ^{1}H NMR: 1.24–1.34 (2H, m), 1.48–1.55 (6H, m), 1.68–1.95 (13H, m), 2.11–2.38 (6H, m), 2.47 (1H, dd, J = 11.4, 7.0 Hz, H^{5colch}), 3.62 (3H, s, OMe), 3.86 (3H, s, OMe), 3.90 (c, 3H, MeO), 3.97 (c, 3H, MeO), 4.63 (1H, m, J = 6.3, 5.8 Hz, H^{7colch}), 4.85 (1H, m, H^{2adam}), 6.50 (1H, s, H^{4colch}), 6.84 (1H, d, J = 10.7 Hz, $H^{11colch}$), 7.30 (1H, d, J = 10.7 Hz, $H^{12colch}$), 7.53 (1H, s, H^{8colch}), 7.66 (1H, br s, NH); ^{13}C NMR: 24.81, 25.00, 27.17, 31.73, 31.82, 34.61, 35.86, 36.30, 36.79, 37.18, 37.35, 52.19 (C^{7colch}), 56.09 (OMe), 56.41 (OMe), 61.38 (OMe), 61.62 (OMe), 76.73 (C^{2adam}), 107.35, 112.96, 125.77, 130.70, 134.29, 135.63, 136.97, 141.60, 151.13, 151.50, 152.3, 164.04, 171.11 (C═O), 172.70 (C═O), 179.13 (C═O); IR: 3286 (NH), 2925–2854 (C–H), 1726 (C═O), 1675, 1657, 1616, 1589, 1562, 1487, 1460, 1431, 1400, 1350, 1323, 1282, 1252, 1194, 1174, 1140, 1095, 1045, 1018, 985, 964, 923, 904, 841, 484.

4.2.2.2. *N*-(6-Adamant-2-yloxy-6-oxohexanoyl)-*N*-deacetylcolchicine (24).

It was prepared by method B from **13** (0.03 g, 0.170 mmol), EEDQ (0.03 g, 0.120 mmol) and *N*-deacetylcolchicine (0.034 g, 0.095 mmol). Chromatographic purification (ethyl acetate/petroleum ether 1:2, then CH_2Cl_2/methanol 20:1) gave **24** as yellowish oil (0.054 g, yield 92). $C_{36}H_{45}NO_8$ requires: C 69.77, H 7.32, N 2.26. Found: C, 69.80; H, 7.30; N, 2.19. ^{1}H NMR: 1.51–1.57 (2H, m), 1.63–1.88 (13H, m), 1.95–1.98 (4H, m), 2.22–2.45 (6H, m), 2.52 (1H, dd, J = 13.2, 6.1 Hz, H^{5colch}), 3.65 (3H, s, OMe), 3.90 (3H, s, OMe), 3.94 (3H, s, OMe), 3.99 (3H, s, OMe), 4.65 (1H, m, J = 6.6, 6.0 Hz, H^{7colch}), 4.90 (1H, m, H^{2adam}), 6.53 (1H, s, H^{4colch}), 6.81 (1H, br s, NH), 6.83 (1H, d, J = 10.8 Hz, $H^{11colch}$), 7.32 (1H, d, J = 10.8 Hz, $H^{12colch}$), 7.44 (1H, s, H^{8colch}); ^{13}C NMR: 24.59, 24.82, 26.97, 27.19, 29.94, 31.75, 31.82, 34.4, 35.7, 36.3, 36.81, 37.36, 52.15 (C^{7colch}), 56.09 (OMe), 56.36 (OMe), 61.39 (OMe), 61.59 (OMe), 76.87 (C^{2adam}), 107.33, 112.5, 125.66, 130.73, 134.21, 135.24, 136.53, 141.64, 151.22, 151.77, 153.46, 164.0, 172.27 (C═O), 172.94 (C═O), 179.48 (C═O).

4.2.2.3. *N*-(5-Adamant-2-yloxy-5-oxopentanoyl)-*N*-deacetylcolchicine (25).

It was prepared by method B from **14** (0.027 g, 0.101 mmol), EEDQ (0.027 g, 0.109 mmol) and *N*-deacetylcolchicine (0.030 g, 0.084 mmol). Chromatographic purification (ethyl acetate/petroleum ether 1:2, then CH_2Cl_2/methanol 20:1) gave **25** as yellowish oil (0.033 g, yield 64%). $C_{35}H_{43}NO_8$ requires: C, 69.40; H, 7.16; N, 2.31. Found: C, 69.51; H, 7.11; N, 2.46. ^{1}H NMR: 1.51–1.54 (2H, m), 1.72-1.76 (4H, m), 1.81–1.98 (9H, m), 2.25–2.37 (5H, m), 2.41 (1H, m, J = 13.2, 6.7, Hz, H^{5colch}), 2.53 (1H, dd, J = 13.2, 6.3 Hz, H^{5colch}), 3.67 (3H, s, OMe), 3.91 (3H, s, OMe), 3.95 (3H, s, OMe), 4.00 (3H, s, OMe), 4.65 (1H, m, J = 6.6, 6.0 Hz, H^{7colch}), 4.90 (1H, m, H^{2adam}), 6.52 (1H, s, H^{4colch}), 6.85 (1H, d, J = 10.8 Hz, $H^{11colch}$), 7.32 (1H, d, J = 10.8 Hz, $H^{12colch}$), 7.33 (1H, br s, NH), 7.48 (1H, s, H^{8colch}); ^{13}C NMR: 20.86, 26.97, 27.16, 29.93, 31.74, 31.83, 34.04, 35.16, 36.31, 36.87, 37.34, 52.11

(C^{7colch}), 56.1 (OMe), 56.36 (OMe), 61.39 (OMe), 61.58 (OMe), 77.06 (C^{2adam}), 107.33, 112.42, 125.65, 130.73, 134.19, 135.24, 136.42, 141.65, 151.22, 151.53, 153.47, 164.01, 171.86 (C=O), 172.58 (C=O), 179.45 (C=O).

4.2.2.4. *N*-(8-Adamant-2-yloxy-8-oxooctanoyl)-*N*-deacetylcolchicine (26). It was prepared by method B from **15** (0.060 g, 0.195 mmol), EEDQ (0.052 g, 0.213 mmol) and *N*-deacetylcolchicine (0.063 g, 0.177 mmol). Chromatographic purification (ethyl acetate/ petroleum ether 1:2, then CH_2Cl_2/methanol 15:1) gave **26** as yellowish oil (0.036 g, yield 32%). $C_{38}H_{49}NO_8$ requires: C, 70.46; H, 7.62; N, 2.16. Found: C, 70.35; H, 7.72; N, 2.04.^{1}H NMR: 1.33 (4H, m), 1.55–1.66 (6H, m), 1.74-1.84 (9H, m), 1.99–2.02 (4H, m), 2.21–2.45 (6H, m), 2.55 (1H, dd, *J* = 12.8, 6.6 Hz, H^{5colch}), 3.67 (3H, s, OMe), 3.92 (3H, s, OMe), 3.96 (3H, s, OMe), 4.02 (3H, s, OMe), 4.68 (1H, m, *J* = 6.8, 5.8 Hz, H^{7colch}), 4.93 (1H, m, H^{2adam}), 6.22 (1H, br s, NH), 6.55 (1H, s, H^{4colch}), 6.85 (1H, d, *J* = 10.4 Hz, $H^{11colch}$), 7.34 (1H, d, *J* = 10.4 Hz, $H^{12colch}$), 7.45 (1H, s, H^{8colch}); ^{13}C NMR: 24.94; 25.22; 26.96; 27.17; 28.77; 28.92; 29.92; 31.73; 31.83; 34.74; 35.93; 36.29; 36.64; 37.35; 52.20 (C^{7colch}); 56.08 (OMe); 56.36 (OMe); 61.34 (OMe); 61.57 (OMe); 76.66 (C^{1adam}); 107.33; 112.68; 125.62; 130.64; 134.26; 135.33; 136.74; 141.60; 151.18; 152.30; 153.45; 163.97; 172.86 (C=O); 173.14 (C=O); 179.45 (C=O).

4.2.2.5. *N*-(9-Adamant-2-yloxy-9-oxononanoyl)-*N*-deacetylcolchicine (27). It was prepared by method B from **16** (0.120 g, 0.372 mmol), EEDQ (0.096 g, 0.388 mmol) and *N*-deacetylcolchicine (0.076 g, 0.212 mmol). Chromatographic purification (ethyl acetate/petroleum ether 1:5, then CH_2Cl_2/methanol 20:1) gave **27** as yellowish oil (0.165 g, yield 67%). $C_{39}H_{51}NO_8$ requires: C, 70.78; H, 7.77; N, 2.12. Found: C, 70.69; H, 7.78; N, 2.10. ^{1}H NMR: 1.25 (6H, m), 1.52–1.58 (6H, m), 1.71–1.76 (4H, m), 1.81–1.89 (5H, m), 1.96–2.0 (4H, m), 2.18–2.31 (5H, m), 2.37 (1H, m, *J* = 13.2, 6.6 Hz, H^{5colch}), 2.49 (1H, dd, *J* = 13.2, 6.1 Hz, H^{5colch}), 3.65 (3H, s, OMe), 3.90 (3H, s, OMe), 3.94 (3H, s, OMe), 3.99 (3H, s, OMe), 4.65 (1H, m, *J* = 6.3, 6.0 Hz, H^{7colch}), 4.89 (1H, m, H^{2adam}), 6.53 (1H, s, H^{4colch}), 6.85 (1H, d, *J* = 10.8 Hz, $H^{11colch}$), 7.32 (1H, d, *J* = 10.8 Hz, $H^{12colch}$), 7.34 (1H, br s, NH), 7.48 (1H, s, H^{8colch}).^{13}C NMR: 25.09, 25.29, 26.98, 27.20, 28.90, 29.12, 29.93, 31.76, 31.85, 34.8, 36.11, 36.31, 36.75, 37.37, 52.14 (C^{7colch}), 56.09 (OMe), 56.36 (OMe), 61.37 (OMe), 61.6 (OMe), 76.66 (C^{2adam}), 107.32, 112.54, 125.66, 130.64, 134.2, 135.25, 136.59, 141.63, 151.21, 151.98, 153.45, 163.99, 172.83 (C=O), 173.25 (C=O), 179.47 (C=O).

4.2.2.6. *N*-(7-Adamant-1-yloxy-7-oxoheptanoyl)-*N*-deacetylcolchicine (28). It was prepared by method B from **17** (0.045 g, 0.153 mmol), EEDQ (0.047 g, 0.190 mmol) and *N*-deacetylcolchicine (0.068 g, 0.190 mmol). Chromatographic purification (ethyl acetate/petroleum ether 2:3, then CH_2Cl_2/methanol 20:1) gave **28** as yellowish oil (0.090 g, yield 93%). $C_{37}H_{47}NO_8$ requires: C, 70.12; H, 7.47; N, 2.21. Found: C, 70.21; H, 7.48; N, 2.18. ^{1}H NMR: 1.38 (2H, m), 1.49–1.61 (10H, m), 1.86 (1H, m, *J* = 11.8, 6.6, 6.4 Hz, H^{6colch}), 2.02–2.29 (14H, m) 2.37 (1H, m, *J* = 12.9, 6.6, Hz, H^{5colch}), 2.49 (1H, dd, *J* = 12.9, 6.1 Hz, H^{5colch}), 3.64 (3H, s, OMe), 3.88 (3H, s, OMe), 3.92 (3H, s, OMe), 3.96 (3H, s, OMe), 4.63 (1H, m, *J* = 6.4, 5.8 Hz, H^{7colch}), 6.51 (1H, s, H^{4colch}), 6.83 (1H, d, *J* = 10.8 Hz, $H^{11colch}$), 7.30 (1H, d, *J* = 10.8 Hz, $H^{12colch}$), 7.38 (1H, br s, NH), 7.47 (1H, s, H^{8colch}); ^{13}C NMR: 24.73, 25.06, 28.69, 29.93, 30.75, 35.44, 35.88, 36.18, 36.70, 41.31, 52.17 (C^{7colch}), 56.09 (OMe), 56.34 (OMe), 61.35 (OMe), 61.58 (OMe), 80.01 (C^{1adam}), 107.32, 112.47, 125.68, 130.68, 134.21, 135.2, 136.55, 141.63, 151.21, 151.93, 153.43, 163.99, 172.64 (C=O), 172.85 (C=O), 179.43 (C=O).

4.2.2.7. *N*-(9-Adamant-1-yloxy-9-oxononanoyl)-*N*-deacetylcolchicine (29). It was prepared by method B from **18** (0.060 g, 0.186 mmol), EEDQ (0.048 g, 0.194 mmol) and *N*-deacetylcolchicine (0.038 g, 0.106 mmol). Chromatographic purification (ethyl acetate/petroleum ether 2:3, then CH_2Cl_2/methanol 20:1) gave **29** as yellowish oil (0.037 g, yield 53%). $C_{39}H_{51}NO_8$ requires: C, 70.78; H, 7.77; N, 2.12. Found: C, 70.85; H, 7.80; N, 2.12.^{1}H NMR: 1.23 (6H, m), 1.45–1.69 (10H, m), 1.84 (1H, m, *J* = 11.8, 6.4 Hz, H^{6colch}), 2.03–2.32 (14H, m), 2.37 (1H, m, *J* = 13.0, 6.4, Hz, H^{5colch}), 2.49 (1H, dd, *J* = 13.0, 6.1 Hz, H^{5colch}), 3.65 (3H, s, OMe), 3.89 (3H, s, OMe), 3.93 (3H, s, OMe), 3.99 (3H, s, OMe), 4.60–4.69 (1H, m, *J* = 6.8, 6.4 Hz, H^{7colch}), 6.52 (1H, s, H^{4colch}), 6.84 (1H, d, *J* = 10.8 Hz, $H^{11colch}$), 7.31 (1H, d, *J* = 10.8 Hz, $H^{12colch}$), 7.29–7.34 (1H, br s, NH), 7.48 (1H, s, H^{8colch}); ^{13}C NMR: 25.05, 25.31, 28.83, 28.94, 29.13, 29.94, 30.77, 35.66, 36.13, 36.2, 36.75, 41.33, 52.15 (C^{7colch}), 56.09 (OMe), 56.38 (OMe), 61.38 (OMe), 61.62 (OMe), 79.97 (C^{1adam}), 107.32, 112.5, 125.67, 130.64, 134.23, 135.24, 136.56, 141.63, 151.23, 151.96, 153.45, 163.99, 172.84 (C=O), 173.03 (C=O), 179.46 (C=O).

4.2.2.8. *N*-(7-Cyclohexyloxy-7-oxoheptanoyl)-*N*-deacetylcolchicine (30). It was prepared by method B from **19** (0.040 g, 0.165 mmol), EEDQ (0.044 g, 0.178 mmol) and *N*-deacetylcolchicine (0.030 g, 0.084 mmol). Chromatographic purification (ethyl acetate/petroleum ether 1:2, then CH_2Cl_2/methanol 20:1) gave **30** as yellowish oil (0.043 mmol, yield 51%). $C_{33}H_{43}NO_8$ requires: C, 68.14; H, 7.45; N, 2.41. Found: C, 67.99; H, 7.38; N, 2.55. ^{1}H NMR: 1.22–1.43 (7H, m), 1.50–1.53 (5H, m), 1.67–1.74 (2H, m), 1.76–1.89 (3H, m), 2.19–2.31 (5H, m), 2.40 (1H, m, *J* = 13.2, 6.8, Hz, H^{5colch}), 2.52 (1H, dd, *J* = 13.2, 6.1 Hz, H^{5colch}), 3.66 (3H, s, OMe), 3.91 (3H, s, OMe), 3.94 (3H, s, OMe), 4.00 (3H, s, OMe), 4.65 (1H, m, *J* = 6.6, 6.1 Hz, H^{7colch}), 4.72 (1H, m, $H^{1cyclohex}$), 6.53 (1H, s, H^{4colch}), 6.83 (1H, d, *J* = 10.8 Hz, $H^{11colch}$), 6.89 (1H, br s, NH), 7.32 (1H, d, *J* = 10.8 Hz, $H^{12colch}$), 7.44 (1H, s, H^{8colch}). ^{13}C NMR: 23.75, 24.83, 25.14, 25.37, 28.77, 31.34, 31.65, 32.57, 34.64, 36.62, 51.47 (C^{7colch}), 55.96 (OMe), 56.87 (OMe), 60.84 (OMe), 61.39 (OMe), 72.36 ($C^{1cyclohex}$), 109.22, 112.61, 128.86, 132.94, 135.11, 136.12, 137.25, 140.67, 152.04, 152.39, 153.48, 163.55, 173.13 (C=O), 175.07 (C=O), 179.13 (C=O).

4.2.2.9. *N*-(7-Methoxy-7-oxoheptanoyl)-*N*-deacetylcolchicine (31). It was prepared by general method B from **20** (0.050 g, 0.287 mmol), EEDQ (0.077 g, 0.311 mmol) and *N*-deacetylcolchicine (0.085 g, 0.239 mmol). Chromatographic purification (ethyl acetate/petroleum ether 1:2, then CH_2Cl_2/methanol 10:1) gave **31** as yellowish oil (0.05 g, yield 41%). $C_{52}H_{35}NO_8$ requires: C, 65.48; H, 6.87; N, 2.73. Found: C, 65.45; H, 7.83; N, 2.74. ^{1}H NMR: 1.30 (2H, m, *J* = 1.9 Hz), 1.53–1.62 (4H, m, *J* = 7.6, 1.9 Hz), 1.85 (1H, m, *J* = 11.8, 6.7 Hz, H^{6colch}), 2.21–2.27 (5H, m), 2.39 (1H, m, *J* = 13.2, 7.0 Hz, H^{5colch}), 2.47–2.55 (1H, m, *J* = 13.2, 6.7 Hz, H^{5colch}), 3.63 (3H, s, OMe), 3.65 (3H, s, OMe), 3.89 (3H, s, OMe), 3.93 (3H, s, OMe), 3.99 (3H, s, OMe), 4.59–4.68 (1H, m, *J* = 6.5, 5.8 Hz, H^{7colch}), 6.52 (1H, s, H^{4colch}), 6.84 (1H, d, *J* = 10.7 Hz,, $H^{11colch}$), 7.16 (1H, br s, NH), 7.31 (1H, d, *J* = 10.7 Hz, $H^{12colch}$), 7.46 (1H, s, H^{8colch}); ^{13}C NMR: 24.51, 24.98, 28.70, 29.92, 33.78, 35.96, 36.89, 51.45 (OMe), 52.11 (C^{7colch}), 56.11 (OMe), 56.35 (OMe), 61.38 (OMe), 61.59 (OMe), 107.37, 112.48, 125.66, 130.67, 134.16, 135.25, 136.48, 141.70, 151.24, 151.61, 153.13, 153.48, 164.02, 172.47 (C=O), 179.44 (C=O).

4.2.2.10. *N*-(7-Isopropyloxy-7-oxoheptanoyl)-*N*-deacetylcolchicine (32). It was prepared by general method B from **21** (0.017 g, 0.084 mmol), EEDQ (0.033 g, 0.134 mmol) and *N*-deacetylcolchicine (0.02 g, 0.056 mmol). Chromatographic purification (ethyl acetate/petroleum ether 1:2, then CH_2Cl_2/methanol 10:1)

gave **32** as yellowish oil (0.032 g, yield 70%). $C_{30}H_{39}NO_8$ requires: C, 66.52; H, 7.26; N, 2.59. Found: C, 66.47; H, 7.13; N, 2.57. MS (EI) *m/z*, (%): 542 ($M+H^+$, 3), 540 ($M-H^+$, 11), 312 (40), 43 (100). ^{1}H NMR: 1.19 (3H, d, *J* = 6.3 Hz, $CHMe_2$), 1.20 (3H, d, *J* = 6.3 Hz, $CHMe_2$), 1.30 (2H, m, *J* = 1.9 Hz), 1.53–1.62 (4H, m, *J* = 7.6, 1.9 Hz), 1.85 (1H, m, *J* = 11.8, 6.8 Hz, H^{6colch}), 2.21–2.27 (5H, m), 2.38 (1H, m, *J* = 13.1, 7.0 Hz, H^{5colch}), 2.47–2.54 (1H, m, *J* = 13.1, 6.5 Hz, H^{5colch}), 3.66 (3H, s, OMe), 3.90 (3H, s, OMe), 3.94 (3H, s, OMe), 4.00 (3H, s, OMe), 4.62–4.68 (1H, m, *J* = 6.8, 5.3 Hz, H^{7colch}), 4.97 (1H, *J* = 6.3 Hz, $CHMe_2$), 6.53 (1H, s, H^{4colch}), 6.84 (1H, d, *J* = 10.8 Hz, $H^{11colch}$), 6.99 (1H, m, *J* = 5.3 Hz, NH), 7.31 (1H, d, *J* = 10.8 Hz, $H^{12colch}$), 7.47 (1H, s, H^{8colch}); ^{13}C NMR: 21.83 $CHMe_2$, 24.62, 25.02, 28.71, 29.92, 34.41, 39.95, 36.85, 52.11 (C^{7colch}), 56.11 (OMe), 56.35 (OMe), 61.38 (OMe), 61.59 (OMe), 67.38 ($CHMe_2$), 107.35, 112.48, 125.67, 130.69, 134.18, 135.24, 136.49, 141.69, 151.24, 151.67, 153.47, 164.01, 172.52, 173.23 (C═O), 179.47 (C═O).

4.2.2.11. *N*-(7-Phenyloxy-7-oxoheptanoyl)-*N*-deacetylcolchicine (33). It was prepared by method B from **22** (0.025 g, 0.106 mmol), EEDQ (0.030 g, 0.121 mmol) and *N*-deacetylcolchicine (0.034 g, 0.095 mmol). Chromatographic purification (ethyl acetate/petroleum ether 1:2, then CH_2Cl_2/methanol 20:1) gave **33** as yellowish oil (0.04 g, yield 73%). $C_{33}H_{37}NO_8$ requires: C, 68.85; H, 6.48; N, 2.43. Found: C, 68.88; H, 6.50; N, 2.39. ^{1}H NMR: 1.42 (2H, m), 1.70–1.84 (5H, m), 2.26 (1H, m, H^{6colch}), 2.40 (1H, m, *J* = 13.2, 6.8, Hz, H^{5colch}), 2.49–2.60 (5H, m, H^{β} + H^{5colch}), 3.67 (3H, s, OMe), 3.91 (3H, s, OMe), 3.94 (3H, s, OMe), 3.99 (3H, s, OMe), 4.65 (1H, m, *J* = 6.6, 6.0 Hz, H^{7colch}), 6.54 (1H, s, H^{4colch}), 6.86 (1H, d, *J* = 10.8 Hz, $H^{11colch}$), 6.96 (1H, br s, NH), 7.07 (2H, m, *J* = 8.0 Hz, H^{2Ph}), 7.21 (1H, t, *J* = 7.7 Hz, H^{4Ph}), 7.34 (1H, d, *J* = 10.8 Hz, $H^{12colch}$), 7.36 (2H, m, *J* = 8.0, 7.7 Hz, H^{3Ph}) 7.54 (1H, s, H^{8colch}); ^{13}C NMR: 24.52, 25.04, 28.69, 29.97, 34.12, 35.93, 36.91, 52.15 (C^{7colch}), 56.15 (OMe), 56.39 (OMe), 61.42 (OMe), 61.64 (OMe), 107.44, 112.72, 121.63, 125.7, 125.8, 129.38, 130.8, 134.24, 135.39, 136.67, 141.74, 150.76, 151.27, 151.85, 153.54, 164.06, 172.6 (C═O), 177.3 (C═O), 179.55 (C═O).

4.3. Biology

4.3.1. Cell culture

A549 human lung epithelial carcinoma cells (CCL-185™) were cultured with Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum and 1% antibiotic penicillin/streptomycin at 37 °C under a humidified 5% CO_2 atmosphere.

4.3.2. MTT cytotoxicity assay

Cells were seeded in 96-well plates at a density of 3×10^3 cells per well. Stock solutions of test compounds were prepared in dimethylsulfoxide (DMSO). Cells were treated for 24 h with selected compounds at 1–12000 nM or with colchicine at 5–100 nM or taxol at 1–15 nM as positive controls (8 wells for each concentration). DMSO (0.4%) served as a negative control. The number of surviving cells was determined by the colorimetric MTT assay.[7] MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyl-2*H*-tetrazoliumbromid, Roth GmbH, Karlsruhe, Germany) was prepared at 5 mg/mL in phosphate buffered saline (PBS) and filtered through a 0.22 μm filter. 20 μL of sterile MTT solution (final concentration 0.5 mg/mL) was added to each well 2 h before the end of compound exposure. Then the supernatant was removed, and 100 μL of DMSO containing 10% SDS and 0.6% acetic acid was added to each well. Resulting formazan crystals were solubilised by thorough mixing on a plate shaker. Optical density was measured at 590 nm with 690 nm reference filter using a EL808 Ultra Microplate Reader (BioTek Instruments, Winooski, USA). Experiments for all compounds were repeated at least three times and EC_{50} values were determined by sigmoidal curve fitting using Excel-based software.

4.3.3. Immunofluorescence staining of cellular microtubules

For microtubule staining A549 cells were cultured in 12-well plates on small glass coverslips (11 mm diameter) at a density of 2×10^4 cells per coverslip. Cells were incubated with selected compounds or colchicine and taxol as positive controls at concentrations of 1 and 5 μM at 37 °C and 5% CO_2 for 8 h. 0.5% DMSO served as a negative control. The whole process of cell fixation and staining was described previously.[8] Fixed cells were labelled with mouse monoclonal antibody against α-tubulin at a dilution of 1:400 (Sigma, St. Louis. USA), followed by incubation of Alexa Fluor488 labelled goat anti-mouse IgG at a dilution of 1:200 (Invitrogen, Germany). Images of all samples were acquired with a Nikon Diaphot 300 inverted microscope (Nikon GmbH, Düsseldorf, Germany) equipped with a cooled charge-couple device camera system (SenSys; Photometrics, Munich, Germany).

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Supplementary data

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References and notes

1. Kingston, D. G. I. *Chem. Commun.* **2001**, 867.
2. Zefirova, O. N.; Nurieva, E. V.; Ryzhov, A. N.; Zyk, N. V.; Zefirov, N. S. *Russ. J. Org. Chem.* **2005**, *41*, 329.
3. Breen, E. S.; Walsh, J. J. *Curr. Med. Chem.* **2010**, *17*, 609.
4. Zefirova, O. N.; Nurieva, E. V.; Lemcke, H.; Ivanov, A. A.; Shishov, D. V.; Weiss, D. G.; Kuznetsov, S. A.; Zefirov, N. S. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5091.
5. Kuznetsov, S. A.; Rodionov, V. I.; Bershadsky, A. D.; Gelfland, V. I.; Rosenblat, V. A. *Cell Biol. Int. Rep.* **1980**, *4*, 1017.
6. Weiss, D. G. In *Cell Biology: A Laboratory Handbook*; Celis, J. E., Ed.; Academic Press: San Diego, 2006; Vol. 3, pp 57–65.
7. Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55.
8. Al-Haddad, A.; Shonn, M. A.; Redlich, B.; Blocker, A.; Burkhardt, J. K.; Yu, H.; Hammer, J. A., 3rd; Weiss, D. G.; Steffen, W.; Griffiths, G.; Kuznetsov, S. A. *Mol. Biol. Cell* **2001**, *12*, 2742.
9. Lebeau, L.; Ducray, P.; Mioskowski, C. *Synth. Commun.* **1997**, *27*, 293.
10. (a) Bourzat, J. D.; Commerçon, A. *Tetrahedron Lett.* **1993**, *34*, 6049; (b) Didier, E.; Fouque, E.; Taillepied, I.; Commerçon, A. *Tetrahedron Lett.* **1994**, *35*, 2349.
11. Hill, J. W.; Carothers, W. H. *JACS* **1933**, *55*, 5023.
12. Zefirova, O. N.; Diikov, A. G.; Zyk, N. V.; Zefirov, N. S. *Russ. Chem. Bull., Int. Ed.* **2007**, *56*, 680.
13. Trott, O.; Olson, A. J. *J. Comput. Chem.* **2010**, *31*, 455.