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Tubuloclustin analogues with ether moiety: synthesis and evaluation of tubulin clustering and antimitotic activity in cancer cells

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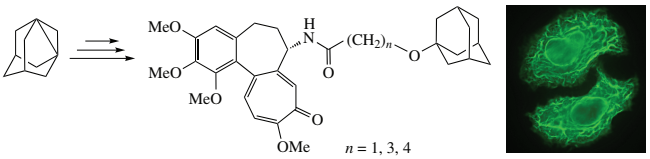
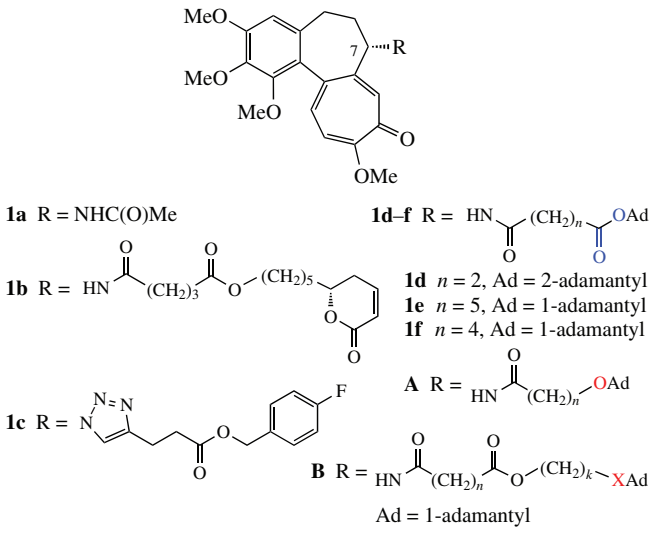
New analogues of anticancer agent tubuloclustin *N*-[7-(adamantan-1-yloxy)-7-heptanoyl]-*N*-deacetylcolchicine with ether moiety in the linker between colchicine and adamantane fragments were synthesized from ω-(adamantan-1-yloxy)-alkan-1-ols. These compounds effectively inhibited growth of human lung carcinoma cell line A549 (IC_{50} = 5–15.5 nM), induced both apoptosis and formation of tubulin clusters. The conjugates lacking ester carbonyl in the linker exhibit improved metabolic stability and are promising for further cytotoxicity studies *in vivo*.



Keywords: adamantane, dehydroadamantane, colchicine, tubuloclustin, metabolic stability, tubulin, colchicine binding site, depolymerization of microtubules, carcinoma A549 cell line.

Antiproliferative activity of colchicine is based on its ability to depolymerize cell microtubules (MT) into α,β-tubulin dimers.[1] Numerous colchicine (**1a**) C^7 derivatives were synthesized in order to improve anticancer activity and reduce systemic toxicity of colchicine[2] (for recent examples, see structures **1b–f**,[3–7] Figure 1). Compound **1d** (tubuloclustin) and its equipotent analogue **1e** are more active than colchicine *in vitro*, have different toxicological profile *in vivo* and induce formation of tubulin clusters in cultured cells.[5,8] Importantly, clustering strength correlates with antiproliferative activity for many



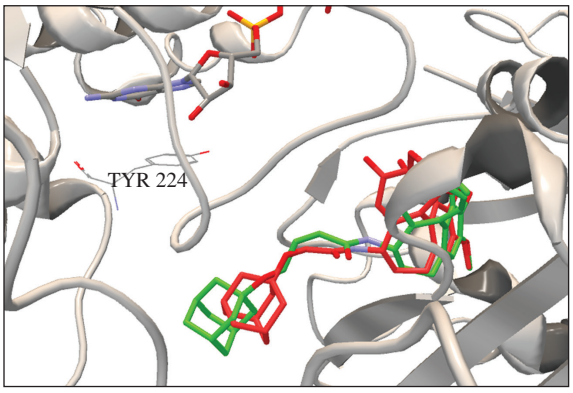


Figure 1 Colchicine (**1a**) and some of its C^7 derivatives, including tubuloclustin (**1d**) and its close analogue **1e**. **A** and **B** – general structures of tubuloclustin analogues studied in the present work. Right panel shows predicted binding modes for conjugates **A** (n = 1 shown in red and n = 3 shown in green) in α,β-tubulin colchicine binding site.

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Table 1 Results of biological activity tests for compounds **4a–c** and **6**.

Compound	Microtubules disassembly[a]		Tubulin clustering[b]					Cell growth inhibition IC_{50}/nM	Cytotoxicity EC_{50}/nM
	50 nM	100 nM	50 nM	100 nM	200 nM	400 or 800 nM	1200 nM		
4a	+	+	++	++	+++	+++	+++	6.4 ± 1	7.8 ± 2
4b	+	+	++	+++	+++	+++	+++	5.0 ± 1	6.7 ± 2
4c	+/–	+	–	–	+/–	+	++	15.5 ± 3	18.4 ± 4
6	+	+	+/–	+	+	++	++	13.4 ± 2	15.8 ± 4
1d	+	+	++	+++	+++	+++	+++	6.1	7.0
1e	+	+	n.d.	n.d.	n.d.	+++	+++	n.d.	11
1a	n.d.	+	–	–	–	–	–	n.d.	30

[a] Effect on microtubules of A549 lung carcinoma cells at marked concentration after 24 h treatment; full (+) or partial (+/–) MT depolymerization is indicated [see Figure 2(*b*),(*c*)]. [b] Relative clustering intensity at marked concentration (at 2400 nM the intensity does not change, data not shown): no effect (–), point-like clusters (+), moderate clusters (++), long clusters (+++) [see Figure 2(*d*)–(*f*)]; n.d. – not determined.

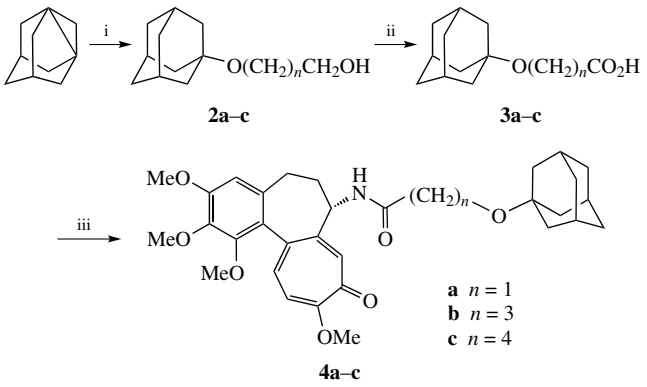
derivatives of **1d** and **1e**.[6,9] We have recently demonstrated that upon shifting the ester group in conjugate **1e**, both clustering ability and activity are retained.[9] This observation led to an idea of tubuloclustin analogues with improved metabolic stability that lack ester carbonyl in the linker (see Figure 1, general formula **A**). The aim of the present work was to synthesize and test conjugates with an ether bond.

Linker length in the compounds of general formula **A** was chosen based on a molecular docking study carried out using 3D model of colchicine binding site in α,β-tubulin (PDB ID : 4O2B),[10] AutoDock Vina 1.1.2 software[11] and USCF Chimera 1.13.1 program.[12] The proposed binding mode of ligands **A** (n = 1–3) was close to that of tubuloclustin (see ref. 5 and refs. herein), but without a hydrogen bond to αTyr224 residue (see Figure 1). Interestingly, this binding mode was not observed for ligands **A** with longer linker chain (n = 4, 5). Based on these data we selected compounds **A** (n = 1, 3 and 4 as a control) for synthesis. To enhance structural diversity of tubuloclustin analogues we also included one compound with both ether and ester groups in the linker – structure **B** (n = 3, k = 2, X = O) with length equal to that of recently studied and very active conjugates **B** (n = 4, k = 1, X = CH_2 and n = 5, k = 0, X = CH_2).[8]

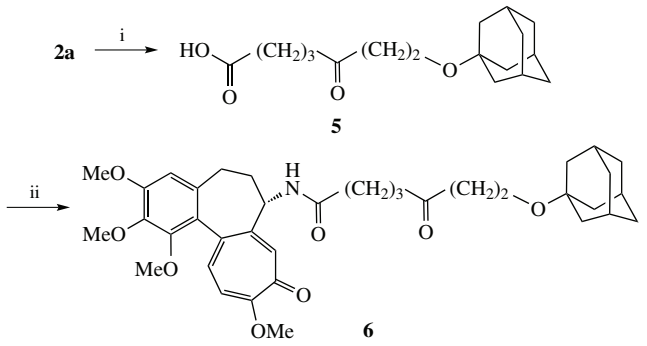
Target compounds were synthesized as outlined in Schemes 1 and 2. Using recently described procedure,[13] 1,3-dehydroadamantane was treated with the corresponding alkanediols to give ω-(adamantan-1-yloxy)alkan-1-ols **2a–c** which were further oxidized by chromium(VI) oxide to afford corresponding acids **3a–c** in fair yields (52–61%).† Amidation of acids **3a–c** with *N*-deacetylcolchicine produced by a described procedure[14] was carried out in the presence of *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ, see Scheme 1). Yields of target conjugates **4a–c** were 28–36%. In ^{1}H NMR spectrum of compounds **4a–c**, the C^7–proton resonance is shifted downfield (4.63–4.68 ppm) compared to that in *N*-deacetylcolchicine (3.71 ppm). ^{1}H and ^{13}C NMR spectra, mass spectra (*m/z* 549 $[M]^+$, 577 $[M]^+$, and 591 $[M]^+$ for **4a**, **4b** and **4c**, respectively) and elemental analysis confirm the formation of target compounds **4a–c**.

Conjugate **6** was prepared from alcohol **2c** which was acylated with glutaric anhydride in the presence of *N*,*N*-dimethylaminopyridine (DMAP) to give the monoester **5** (see Scheme 2). Reaction of the latter with *N*-deacetylcolchicine in the presence of EEDQ led to the target conjugate **6**. Characteristic signals at C^7 atom in compound **6** have chemical shifts of 52.16 and 4.63 ppm in ^{13}C and ^{1}H NMR and spectra, respectively. ^{1}H and ^{13}C NMR spectra and mass spectra (*m/z* 650 $[M]^+$) confirm the formation of target conjugate **6**.

† For synthetic details and characteristics of all novel compounds, see Online Supplementary Materials.



Scheme 1 *Reagents and conditions*: i, alkanediol, Et_2O, 30–40 °C, 2–3 h;[13] ii, Jones reagent, THF, room temperature, 5 min; iii, *N*-deacetylcolchicine, EEDQ, CH_2Cl_2, room temperature, 12 h.



Scheme 2 *Reagents and conditions*: i, glutaric anhydride, 4-DMAP, CH_2Cl_2, room temperature, 24 h; ii, *N*-deacetylcolchicine, EEDQ, CH_2Cl_2, room temperature, 12 h.

Biological activity tests for compounds **4a–c** and **6** were carried out using human epithelial lung carcinoma cell line A549. Microtubule morphology was studied by immunofluorescence microscopy as described previously.[15] Antimitotic activity was evaluated using MTT assay[16] and cell growth inhibition test (direct cell counting by microscopy over 24 h of culturing).[15] The ability of compounds to induce apoptosis was also investigated.[17] Test results are presented in Table 1 and Figure 2(*a*)–(*f*) (for the experimental data on apoptosis, see Online Supplementary Materials).

At 50 nM concentrations, the compounds caused total (**4a**, **4b**, **6**) or partial (**4c**) MT depolymerization in A549 cells after 24 h of treatment. Cluster formation efficacy decreased in the following sequence: **4b** > **4a** > **6** ≫ **4c**. The structure–clustering activity relationship (SAR) in series **4a**, **4b** and **4c** correlates

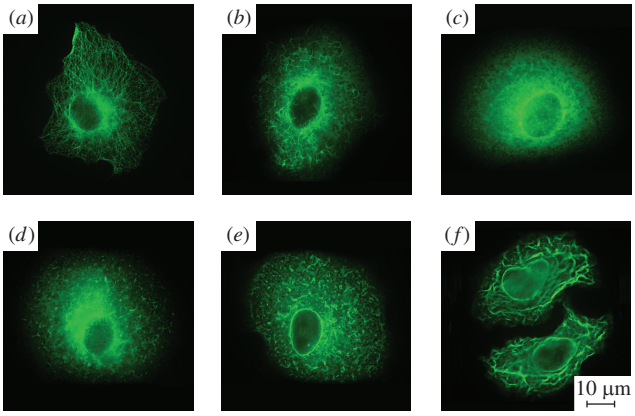


Figure 2 Tubulin in A549 cells treated with (*a*) 0.5% DMSO (intact MT); (*b*) 50 nM of **4c** (partial depolymerization of MT, no clustering); (*c*) 100 nM of **4c** (complete depolymerization of MT, no tubulin clustering); (*d*) 100 nM of **6** (point-like clustering); (*e*) 400 nM of **6** (moderate clustering); (*f*) 1200 nM of **4b** (strong clustering).

with the molecular docking data (see above), which predicted different tubulin binding modes for a pair of structurally close conjugates **4b/4c** (but not for a shorter **4a/4b** pair). Namely, the biotest data for compounds **4a**,**b** indicated that tubuloclustin analogues with ether bond in the linker could induce formation of strong tubulin clusters [see Figure 2(*f*)].

Biological testing revealed that tubulin clustering ability of novel compounds correlates with cytotoxicity, cell growth inhibition (see Table 1) and earlier SAR studies for tubuloclustin derivatives.[6,9] Conjugate **6** activity was close to that of its analogues **B** ($n = 4$, $k = 1$, X = CH_2 and $n = 5$, $k = 0$, X = CH_2),[9] suggesting that clustering ability is negligibly affected by ester bond position. The best IC_{50} and EC_{50} values in low nanomolar concentration range were observed for compounds **4a** and **4b**. The latter was equally active to tubuloclustin in both antiproliferative assays. Each of conjugates **4a–c** and **6** induced nucleus fragmentation in A549 cells which is typical for the cells undergoing apoptosis.[18] The ability to cause apoptosis was close for all new compounds, but there was no exact correlation between apoptotic index (highest for **4c**) and tubulin clustering activity and cytotoxicity (see Online Supplementary Materials).

In conclusion, novel tubuloclustin analogues with ether moiety in the linker between colchicine and adamantane fragments were synthesized and tested in a series of bioassays. The data obtained unambiguously prove that these conjugates retain the ability to induce strong tubulin clustering. Compound **4b** represents tubuloclustin analogue with extremely high antiproliferative activity and improved metabolic stability and therefore it is promising for further *in vivo* studies.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2020.01.035.

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