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Homologous series of novel adamantane–colchicine conjugates: synthesis and cytotoxic effect on human cancer cells

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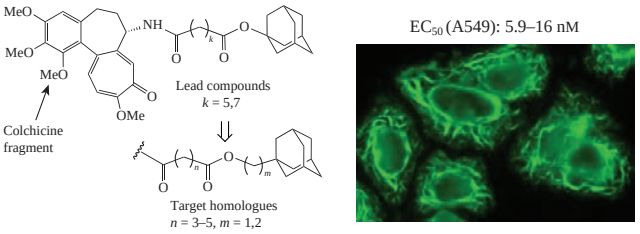
Homologues of *N*-[7-(adamantan-1-yloxy)-7-oxoheptanoyl]-*N*-deacetylcolchicine with sequential shift of the ester group in the chain connecting colchicine and adamantane moieties were synthesized to find an optimal position of this group. All homologues possessed very high cytotoxicity to human lung carcinoma cell line A549 demonstrating a weak dependence of toxic activity on the ester group position. The cytotoxicity (EC_{50} = 5.9 nM) of the most active compound was close to that of clinically used anti-tubulin anticancer drug taxol.



In drug design the homologous series are usually synthesized for the purpose of lipophilicity modulation, for switching from receptor agonists to antagonists or to establish the optimal length of alkyl linker chain (see typical examples in refs. 1–5). In this work we present a quite rare example of homology application to the search for the best position of functional group in the linker connecting two molecular fragments.

Earlier we synthesized conjugates of anticancer agent colchicine with adamantane **1a**,**b** (Figure 1), which were cytotoxic to cancer cells and promoted depolymerization of cellular microtubules followed by tubulin assembly to clusters.[4] The purpose of the present work was to find an optimal position of ester group in the linker connecting colchicine and adamantane moieties in the conjugates. Since the alkyl chain elongation from 5 (in **1a**) to 7 (in **1b**) methylene groups did not significantly change the cytotoxicity[4] we suggested synthesizing a series of homologues of **1a**,**b** by sequential shift of the ester group in their linker chain (see Figure 1).

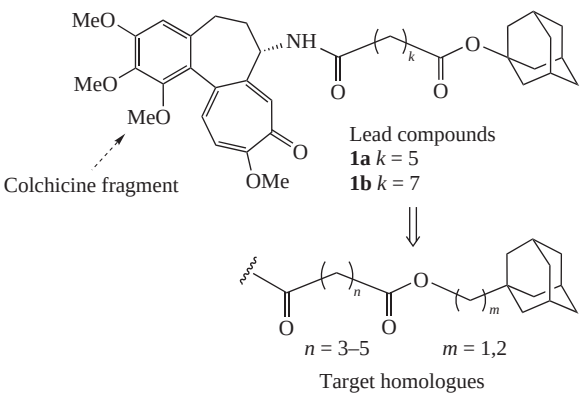
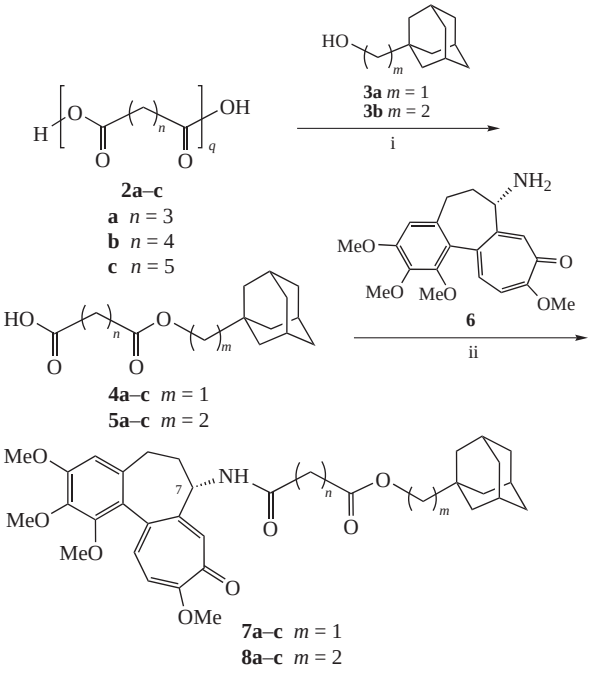


Figure 1 Structural modification in the lead compounds **1a**,**b** comprises a shift of ester group position relative to colchicine and adamantane moieties.

Target compounds were synthesized as outlined in Scheme 1. Reactions of (poly)anhydrides of glutaric, adipic and pimelic acids **2a**–**c** (obtained from the corresponding acids and acetic anhydride) with (adamantan-1-yl)methanol **3a** or 2-(adamantan-1-yl)ethanol **3b** in the presence of 4-dimethylaminopyridine (DMAP) afforded six monoesters of the corresponding dicarboxylic acids with adamantane alcohols (**4a**–**c** and **5a**–**c**) in moderate yields of 49–69%. In ^{1}H NMR spectra of compounds **4a**–**c** and **5a**–**c** the resonances of



Scheme 1 *Reagents and conditions:* i, DMAP, CH_2Cl_2, ~20°C, 24 h; ii, EEDQ, CH_2Cl_2, ~20°C, 12 h.

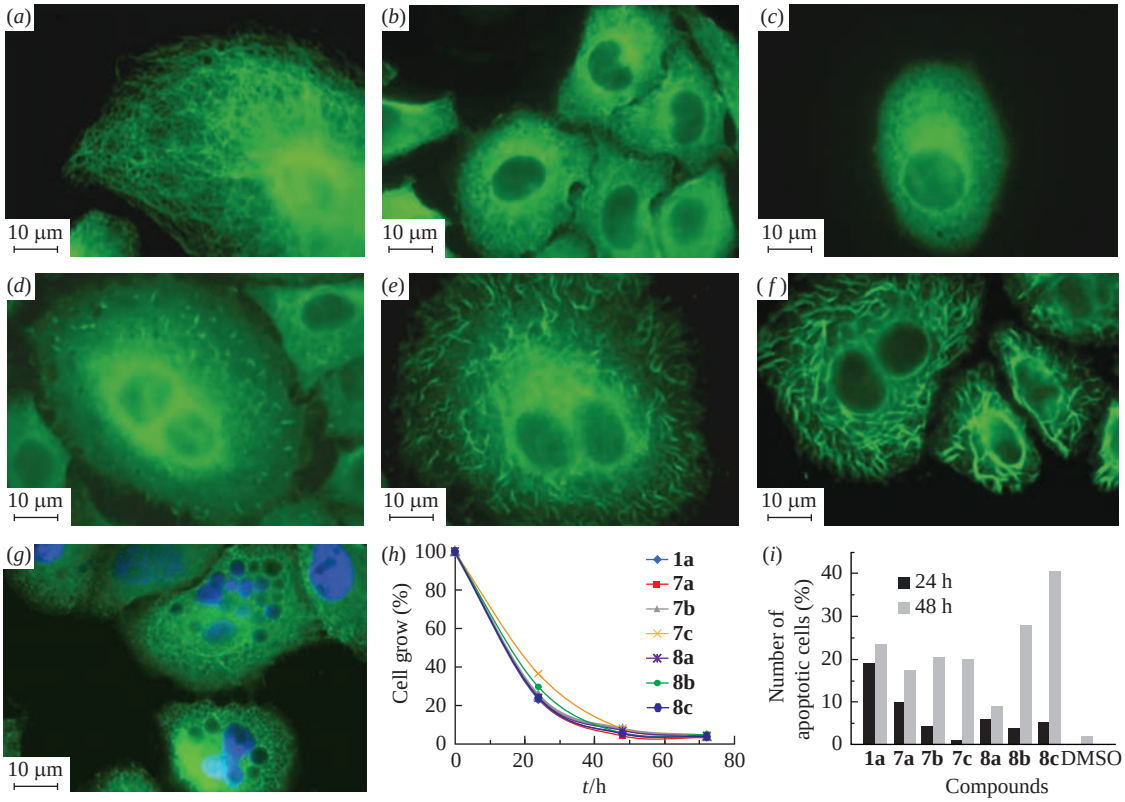


Figure 2 Immunofluorescence microscopy images of the microtubules network in A549 lung carcinoma cells treated with compounds **7a**–**c** and **8a**–**c** or control compounds (DMSO, **1a**): (*a*) intact microtubules (0.5% DMSO, negative control), (*b*) partial depolymerization, (*c*) full depolymerization, (*d*) weak clustering (+), (*e*) moderate clustering (++), (*f*) strong clustering (+++), (*g*) nuclear fragmentation in the cells undergoing apoptosis caused by tested compounds. (*h*) Cell growth inhibition curves. (*i*) Apoptotic index diagram.

AdCH_2 or AdCH_2CH_2 protons are shifted downfield with respect to the corresponding peaks of initial alcohols (3.65, 3.67, 3.64 ppm for **4a**–**c** and 3.17 ppm for **3a**; 4.13, 4.10, 4.12 ppm for **5a**–**c** and 3.71 ppm for **3b**).†

Amidation reaction of monoesters **4a**–**c** and **5a**–**c** with *N*-deacetylcolchicine **6** (synthesized in three steps from colchicine[6]) was carried out in the presence of *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) and led to the target conjugates **7a**–**c** and **8a**–**c** in yields of 36–59%. The formation of amide bond is proved by the downfield shift of C^7-proton resonance in ^{1}H NMR spectra of **7a**–**c** and **8a**–**c** (4.65–4.68 ppm) in comparison with the corresponding peak of starting compound **6** (~3.71 ppm). The data of ^{1}H and ^{13}C NMR spectra, mass spectra and elemental analysis prove the formation of target compounds.

The synthesized compounds **7a**–**c** and **8a**–**c** were tested for cytotoxicity to the human epithelial lung carcinoma cell line A549 in MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay[7] (see procedures in refs. 8–10). The effect on cell growth was also studied using direct cell counting by microscopy over 24, 48 and 72 h of culturing.[11] The ability of compounds to alter the microtubule dynamics and to stimulate apoptosis was investigated using immunofluorescence microscopy as described.[5,12]

The results of biotests (Table 1, Figure 2) revealed the fact that all homologous conjugates **7a**–**c** and **8a**–**c** were highly cytotoxic to A549 cancer cells (in low nanomolar range) and strongly inhibited cell proliferation.

The best EC_{50} values (< 10 nM) were obtained for compounds **7a** and **8a**. Moreover, after cell treatment for 24 h at the concentration 400 nM all the conjugates caused total depolymerization of microtubules [Figure 2(*c*)], and at 800 nM they stimulated the formation of pronounced or moderate tubulin clusters [see Table 2, Figure 2(*e*),(*f*)]. Futhermore, all the compounds at 800 nM strongly inhibited cell growth and an extent of inhibition correlated with the MTT data [Figure 2(*h*)]. The cells treated with each conjugate of the series **7a**–**c** and **8a**–**c** underwent apoptosis [revealed by a number of cells with nucleus fragmentation, see Figure 2(*g*)]. Interestingly, the ability to cause apoptosis did not correlate with MTT data and tubulin clustering activity for each conjugate [Figure 2(*i*)].

The biotesting results clearly demonstrated a weak dependence of cellular activity of the homologues synthesized on the ester

Table 1 Results of biotests for compounds **7a**–**c** and **8a**–**c**.

Compound	Cytotoxicity EC_{50}/nM[a]	Microtubules disassembly[b]		Tubulin clustering[c]	
		100 nM	200 nM	200 nM	800 nM
7a	5.9 ± 0.2	–	–	++	+++
7b	13 ± 1	+/–	+/–	+	+++
7c	11.5 ± 1.5	+	–	+	+++
8a	8.5 ± 1.5	+	–	++	+++
8b	12.5 ± 3	+	+/–	+	++
8c	16 ± 3	+	+/–	+	++
1a	11 ± 1[4]	n.d.[d]	–	+	+++
1b	29 ± 1[4]	n.d.[d]	+/–	n.d.[d]	++
Tubuloclustin	6.0 ± 0.2[4]	n.d.[d]	–	n.d.[d]	+++
Colchicine	30 ± 2	n.d.[d]	–	–	–

[a] The average of 3–6 experiments. [b] Effect on microtubules at the noticed concentration; '+' stands for no effect [Figure 2(*a*)], '+/–' means the partial depolymerization [Figure 2(*b*)]; '–' denotes the full depolymerization [Figure 2(*c*)]; at 400 nM all compounds cause full depolymerization of microtubules. [c] Relative intensity of clustering ability indicated by different number of '+' [see Figure 2(*d*)–(*f*)], '–' means the absence of clustering; at 1200 nM the intensity of clustering ability does not change. [d] Not determined.

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

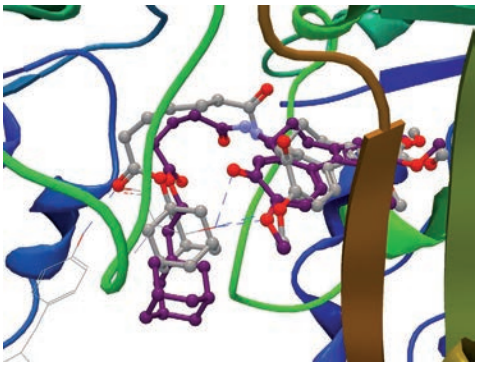


Figure 3 Most advantageous location of the conjugates **1a** (shown in gray) and **8a** (shown in black) in tubulin dimer as predicted by automated docking (AutoDock 4.2; visualized using CLC Drug Discovery Workbench). α-Subunit is presented on the left and β-subunit – on the right (hydrogen bonds are shown in dotted lines, hydrogen atoms are omitted for clarity). On the left αTyr224 residue is shown, its hydroxyl being hydrogen bonded with carbonyl oxygen in **1a**, but not with **8a** (the distance from αTyr224 phenol hydroxyl to both oxygen atoms of ester group in **8a** is about 5 Å).

group position in respect to adamantane and colchicine fragments. Indeed, comparison of the activity data for the conjugates with the total number of methylene groups in the linker equal to five (**7b**, **8a**, **1a**) indicates that both EC_{50} values and intensity of clustering effect are very close. Similar relations are observed for the pairs with six (**7c** and **8b**) and seven (**8c** and **1b**[4]) methylene groups in the linker (see Table 1).

Molecular modeling study demonstrates that oxygen atoms of ester group in **1a** can make a hydrogen bond with phenolic hydroxyl of αTyr224 in tubulin, while in the case of conjugates **8a**–**c** (with ester group noticeably shifted from adamantane) this bond is not formed (Figure 3). Thus, the similar activity for all conjugates **7a**–**c** and **8a**–**c** was not expected and may be explained either by low contribution of this bond to the interaction with protein or by possible deviation of adamantane positions in **1a** and **8a**–**c** from the predicted by modeling (due to high conformational flexibility of the linker chains in these compounds).

Interestingly, the homologue with the shortest linker in the series, namely **7a**, was the most active in all tests. It was more cytotoxic to A549 cells than colchicine or lead molecule **1a** and was the only one compound in both series **7a**–**c** and **8a**–**c** that caused total depolymerization of microtubules at the concentration as low as 100 nM (see Table 1).

Compound **7a** was equally cytotoxic to the earlier synthesized tubuloclustin,[4] but was more potent than the latter (data not shown) and conjugate **1a** [see Figure 2(*i*)] in the cancer cell growth inhibition. Antiproliferative activity of conjugate **7a** was comparable to that of clinically used anti-tubulin anticancer drug taxol. Thus, this compound represents an interesting candidate for testing *in vivo* and stimulates further studies of structure–activity relationships among analogues of compound **1a** with short ($n \leqslant 4$) or structurally different linkers. This work is now in progress and will be published in a due course.

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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.2018.05.027.

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