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Tubulin targeted antimitotic agents based on adamantane lead compound: synthesis, SAR and molecular modeling

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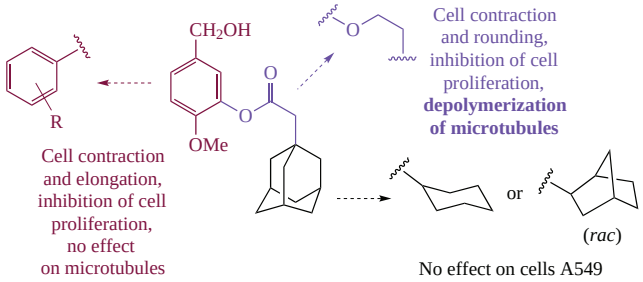
5-Hydroxymethyl-2-methoxyphenyl adamantane-1-acetate inhibits cell proliferation and stimulates depolymerization of microtubules of cancer cells to free tubulin. Its analogues were synthesized *via* the Steglich or Mitsunobu reactions to determine the role of structural subunits of the molecule in tubulin binding. Based on the structure–activity relationship studies, metabolically stable 1-[2-(5-hydroxymethyl-2-methoxyphenyl)ethyl]adamantane was invented, which exhibits a dual-target profile and retains *in vitro* activity observed for the lead compound.



Keywords: adamantane, tubulin, colchicine binding site, lung carcinoma A549, dual-target profile, structure–activity relationship.

Adamantane moiety is occasionally used in drug design for enhancement of lipophilicity or bulkiness of the lead molecule.[1,2] Such modification can improve both pharmacokinetic properties and toxicological profile of the compound, which is especially important for anticancer drugs. Anticancer agents comprising adamantane moiety belong mostly to DNA cross-linking agents, inhibitors of protein tyrosine kinases and ligands of retinoic acid receptors.[1] Recently, we designed a novel tubulin targeted agent, *viz*. 5-hydroxymethyl-2-methoxyphenyl adamantane-1-acetate **1** (Figure 1) and proved its ability to stimulate depolymerization of microtubules (MTs) of cancer cells to free tubulin.[3] The structure of compound **1** is not typical of the tubulin ligands.[4–7] Therefore, in the present work we synthesized and tested a series of its analogues with the purpose to identify the role of adamantane and other subunits for tubulin binding.

Lead compound **1** comprises three substructures, namely, adamantane, the substituted aromatic ring and the linker, which were sequentially modified in the work (see Figure 1). Adamantane residue was replaced by other lipophilic groups such as cyclohexyl or *rac-exo*-norbornyl ones. The corresponding mono-esters **2a**,**b** were synthesized by the Steglich esterification which also afforded bis-esters **3a**,**b** (Scheme 1, for the synthetic details and characteristics of novel compounds see Online Supplementary Materials).

To examine the role of benzylic hydroxyl in the parent molecule **1**, we obtained aryl esters **4a–d** with at least one methoxy group (typical for the vast majority of ligands of colchicine binding site in tubulin[5,7–9]). The Steglich esterification of some phenols provided the target compounds **4a–d** in good yields (Scheme 2).

Two ethers **6** and **7** were synthesized to examine the role of carbonyl moiety in the linker of the lead-molecule **1** and compound **4a**. The Mitsunobu reaction between phenols and 2-(1-adamantyl)ethanol led to ethers **5** and **7**. The following

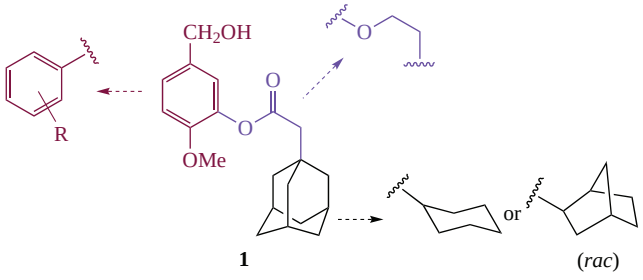
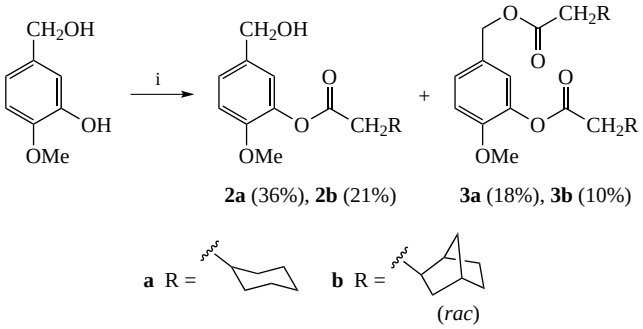
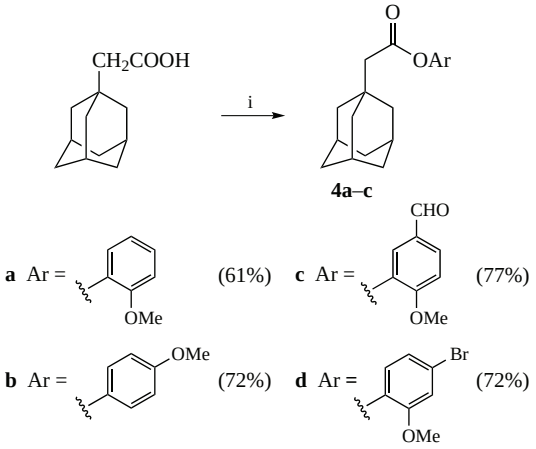


Figure 1 Structure of the lead compound **1** and its modifications carried out in the work.



Scheme 1 *Reagents and conditions*: i, RCH_2COOH, DCC, DMAP, CH_2Cl_2, room temperature, 48 h.



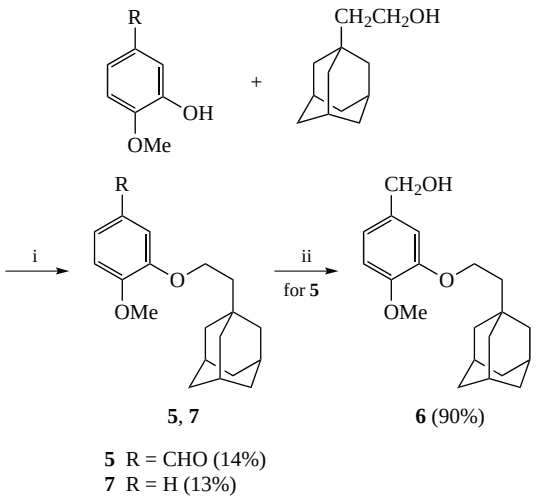
Scheme 2 *Reagents and conditions*: i, ArOH, DCC, DMAP, CH_2Cl_2, room temperature, 48 h.

reduction of formyl group in compound **5** gave the target benzylic alcohol derivative **6** (Scheme 3).

All synthesized compounds were evaluated *in vitro* for their ability to alter cell proliferation and microtubule dynamics in the human epithelial lung carcinoma cells A549 using immunofluorescence microscopy as described.[10] The results are presented in Table 1 and Figure 2. Both cyclohexane (**2a**) and norbornane (**2b**) containing esters were inactive even at high concentration of 100 μM, demonstrating that the adamantane residue of molecule **1** is a key structural unit determining the efficiency. Interestingly, one of the bis-esters, namely **3a**, exhibited cytostatic activity, which was not connected with the action on MTs (see Table 1).

Esters **4a**–**d** at 100 μM exhibited either potent (**4a**–**c**) or weak (**4d**) cytostatic activity and caused the changes in cell morphology [cell contraction and elongation, see Figure 2(*b*)], however none of the compounds **4a**–**d** caused any effect on MTs (see Table 1). The results indicate the importance of benzylic hydroxyl in the lead molecule. Moreover, the loss of potency by formyl analogue of the lead (**4c**) allows one to propose that hydroxyl group in molecule **1** interacts with tubulin as hydrogen bond donor.

This proposition is confirmed by molecular modeling data, which shows the possibility of hydrogen bond formation between hydroxyl in compound **1** and carbonyl oxygen atom of amino acid residue Val315(β) [Figure 3(*a*)]. Interestingly, the similar hydrogen bond is formed by benzylic hydroxyl of cyclohexane analogue of the lead (structure **2a**), while the position of



Scheme 3 *Reagents and conditions*: i, DIAD, PPh_3, THF, room temperature, 48 h; ii, $NaBH_4$, MeOH, room temperature, 2 h.

Table 1 Biotesting results for new compounds (cell line: human lung carcinoma A549).

Compound	Action on the cell proliferation, cell morphology and MTs (100 μM, 24 h)[a]
1	Cell contraction and rounding, inhibition of cell proliferation, depolymerization of MTs
2a	No effect
2b	No effect
3a	Cell contraction and elongation, inhibition of cell proliferation, no effect on MTs
3b	No effect
4a	Cell contraction and elongation, inhibition of cell proliferation, no effect on MTs
4b	Cell contraction and elongation, inhibition of cell proliferation, no effect on MTs
4c	Cell contraction and elongation, inhibition of cell proliferation, no effect on MTs
4d	Some cell contraction and elongation, very week inhibition of cell proliferation, no effect on MTs
6	Cell contraction and rounding, inhibition of cell proliferation, depolymerization of MTs
7	Cell contraction and elongation, some cell rounding, inhibition of cell proliferation, no effect on MTs

[a] Results of three independent experiments.

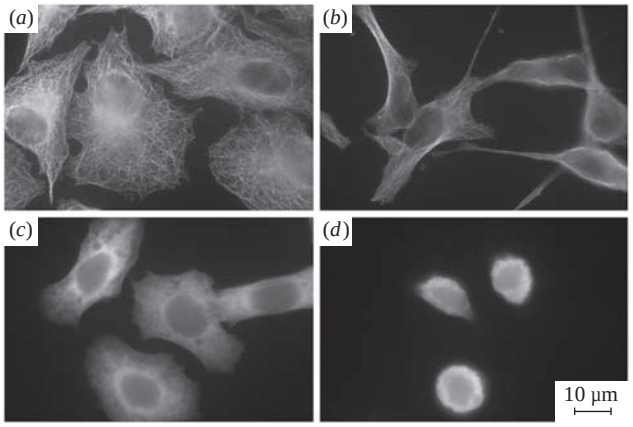


Figure 2 Immunofluorescence microscopy of MTs in human lung carcinoma cells A549 treated with: (*a*) 0.5% DMSO (intact MTs); (*b*) 100 μM **4c** (contracted elongated cells, intact MTs), similar effect was observed for **3a**, **4a**, **4b**; (*c*) 100 μM **6** (totally depolymerized MTs); (*d*) 100 μM **6** (contracted rounded cells).

cyclohexane ring differs essentially from that of adamantane in the parent molecule [see Figure 3(*a*)]. This difference can explain the loss of potency by compound **2a**.

At 100 μM concentration, ether **6** exhibited cytostatic action and altered MTs dynamics by causing complete depolymerization

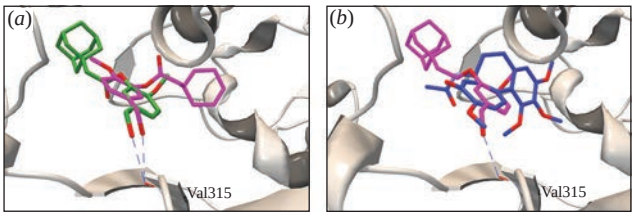


Figure 3 Location of the compounds in colchicine binding site of α,β-tubulin dimer as predicted by molecular docking (PDB ID:4O2B, ref. 11, and AutoDock Vina 1.1.2, refs. 12, 13; for the description of the docking procedure, see Online Supplementary Materials): (*a*) **1** (in green) and **2a** (in magenta); (*b*) **6** (in magenta), the position of colchicine is shown in blue for comparison (α-subunit is presented at left and β-subunit – at right, hydrogen bonds with Val315(β) are indicated by dashed lines, hydrogen atoms are omitted for clarity).

of MTs in 24 h [see Table 1 and Figure 2(*c*),(*d*)]. So, carbonyl oxygen atom in the linker chain of parent ester **1** plays insignificant role in binding with tubulin. This finding is also in agreement with molecular modeling data, namely, the location of compound **6** in a complex with tubulin can be similar to that of the lead molecule **1** [see Figure 3(*b*)]. In this complex the benzylic hydroxyl of **6** is hydrogen bonded to Val315(β). And the carbonyl oxygen of **1** does not participate in binding with the protein [see Figure 3(*a*)]. The important role of benzylic hydroxyl in ether **6** (analogously to ester **1**) was proved by inability of ether **7** to alter MTs dynamics (see Table 1).

Additional study of ability of ether **6** to inhibit the cell growth using microscopy for direct cell counting over 24 and 48 h of culturing demonstrated that at 100 μM concentration compound **6** completely inhibited proliferation of cancer cells. Cytotoxicity value (EC_{50}) of comtpound **6** evaluated in a standard calorimetric MTT assay (the specific procedure see in the ref. 14) is in micromolar concentration range (9.8 μM) and is in a close range to that of the parent molecule **1** (EC_{50} = 4.3 μM). It is noteworthy that ether **6** is more stable metabolically than lead ester **1**.

An interesting observation was revealed during additional biotesting of compounds **1** and **6**. Both compounds essentially inhibited cell proliferation at the concentration of 10 μM at which MTs remained intact in the cells (no effect on MTs was detected at this concentration). Therefore, the antimitotic activity of adamantane-based compounds **1** and **6** is caused by their interaction not only with tubulin, but also with at least one another molecular target in cancer cells. This second target could probably be identical to that of compounds **4a–c** (which inhibit cell proliferation without effect on MTs) and could belong, in particular, to a family of protein tyrosine kinases or retinoic acid receptors.[1]

In conclusion, the structure–activity relationship studies for ten analogues of 5-hydroxymethyl-2-methoxyphenyl adamantane-1-acetate **1** revealed several compounds with cytostatic activity and led to the invention of 1-[2-(5-hydroxymethyl-2-methoxyphenyl)ethyl]adamantane **6** that retained ability to stimulate depolymerization of MTs in cancer cells but was more stable metabolically. Both antimitotic agents **1** and **6** comprise adamantane moiety as a basic part of the molecule, which is unusual for tubulin targeted compounds (see *e.g.* refs. 15–17 for comparison). A multitarget profile exhibited by the adamantane-based tubulin ligands makes them interesting for further 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.2020.07.005.

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