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Design, synthesis, and bioactivity of putative tubulin ligands with adamantane core

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ABSTRACT

Several adamantane-based taxol mimetics were synthesized and found to be cytotoxic at micromolar concentrations and to cause tubulin aggregation. The extent of the aggregation is maximal for *N*-benzoyl-(2*R*,3*S*)-phenylisoseryloxyadamantane (**5**) and is very sensitive to the structural modifications. A hybrid compound (**15**), combining adamantane-based taxol mimetic with colchicine was synthesized and found to possess both microtubule depolymerizing and microtubule bundling activities in A549 human lung carcinoma cells.

Taxol (paclitaxel, **1a**, Fig. 1), isolated from the bark extracts of *Taxus brevifolia*, and its synthetic analogue taxotere (docetaxel, **1b**) possess high antitumor activity due to their ability to cause spontaneous polymerization of the intracellular protein tubulin into stable microtubules and to stabilize preformed microtubules, thereby preventing cell division.[1,2] The most important contribution to tubulin binding is provided by the C^{13} side chain taxol and taxotere (i.e., *N*-benzoyl- or *N*-*tert*-butoxycarbonyl-(2*R*,3*S*)-phenylisoseryl), while C^2 (–OBz), C^4 (–OAc) substituents, and the oxetan fragment also play a role in this binding.[1,2]

The intricate molecular structure of taxane compounds and the necessity to obtain them semi-synthetically from natural sources make the development of taxol and taxotere analogues with a simpler structure important. During the last ten years there appeared several publications about such attempts.[3–9]

In 2002, based on the hypothesis, that the main function of the taxane skeleton is to provide proper orientation of the substituents important for tubulin binding,[10] we suggested a general theoretical model of a 'simplified' taxol analogue with a bicyclo[3.3.1]nonane core (structurally similar to the AB rings of the parent molecule).[11] Later, we synthesized a number of such compounds, including structure **2**, and demonstrated that they possess weak cytotoxicity and can cause slight tubulin aggregation, but not polymerization to microtubules.[12] Because one of the reasons for this result might be improper substituents positions in structure **2** and similar compounds due to the relative conformational freedom of the bicyclo[3.3.1]nonane framework, we suggested to synthesize their analogues with a rigid adamantane core. Here, we present the results of molecular modeling and synthesis of structure **3** and the data of biological tests of compound **3** and two other adamantane-based taxol mimetics **4** and **5** (Fig. 1).

The model of tubulin structure (kindly granted to us by Prof. J. Snyder, USA)[13] was used for the molecular docking of compound **3** to the taxol binding site. The obtained binding mode of ester **3** is presented in Figure 2.[14,15] According to the modeling results, in case of an identical location of taxol and compound **3** side chains in the protein, the oxetan oxygen of **3** can be hydrogen bonded to the Thr 276 amino group. This interaction was not observed for the compound **2**,[12] but it corresponds exactly to the bond formed by the oxetan oxygen in taxol.[13] Moreover, the carbonyl oxygen of structure **3** ester linker can be hydrogen bonded with Arg284 (Fig. 2).

For the synthesis of compound **3** double esterification was performed for the TMS-protected 1-hydroxyadamantane-5-carboxylic acid **6**, first by 3-hydroxyoxetan (obtained in five steps from glycerin) with the formation of ester **7**,[16] and then by an oxazolidine-protected amino acid (Scheme 1).[17] The subsequent opening of the oxazolidine ring in the product **8** led to the ester **3**.[18]

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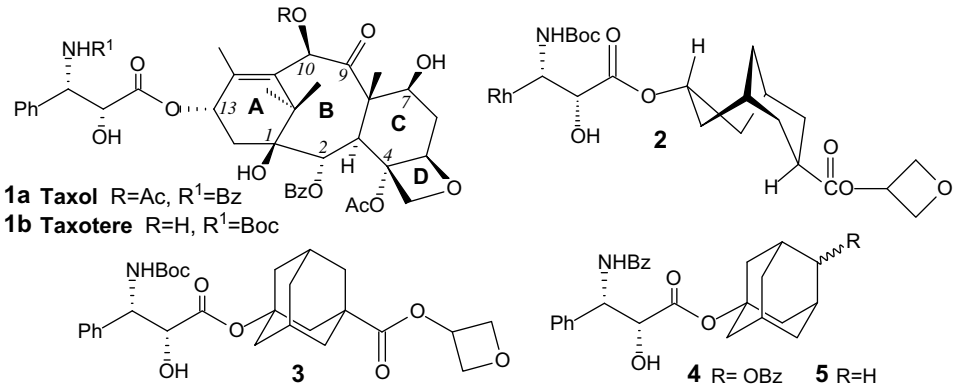


Figure 1. Structures of taxoids and their 'simplified' analogues.

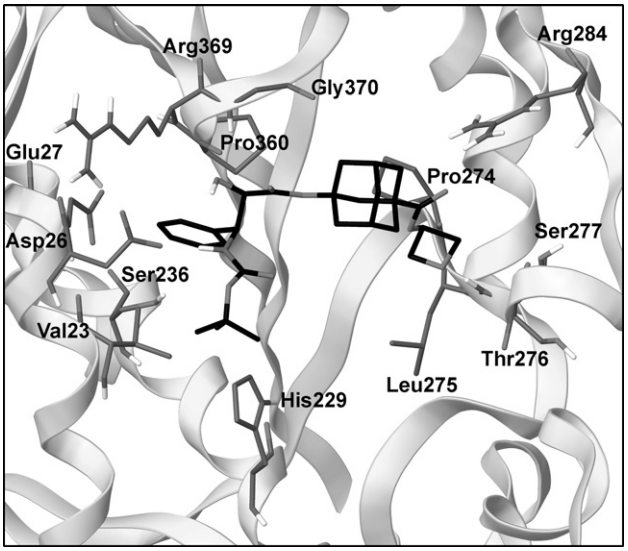
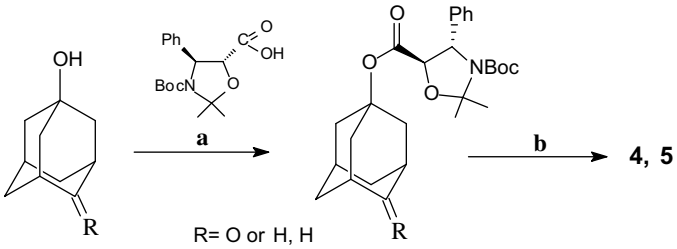


Figure 2. Structure **3** inside the taxol binding site of β-tubulin (hydrogen atoms are not indicated).

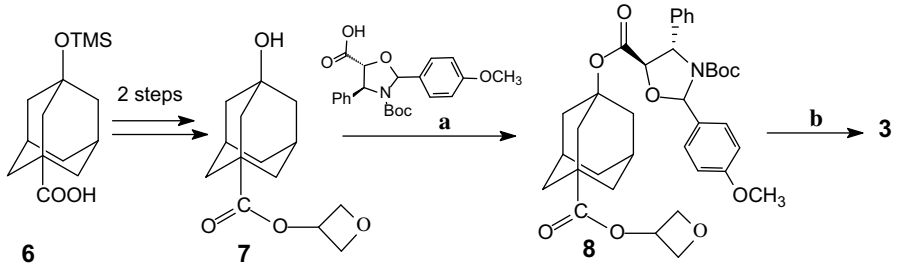
Compounds **4** and **5** were synthesized by esterification of kemantane (1-hydroxy-adamantane-4-one)[19] and adamantane-1-ol[20] correspondingly using slightly different oxazolidine amino acid protections[21] (general Scheme 2). It should be noted that this type of protection is unsuitable for the synthesis of compound **3** due to the destruction of the oxetan ring during amino acid regeneration by formic acid.

The synthesized adamantane-based taxol mimetics were evaluated for their *in vitro* cytotoxicity against the A549 human lung carcinoma cell line. The results are presented in Table 1. These data indicate that compounds **3**–**5** exhibit cytotoxicity at the micromolar level, that is, the same as the bicyclo[3.3.1]nonane esters (e.g., **2**)[12] and other simplified taxol analogues (e.g., indolizidinones[7] and macrocycles[9]).



Scheme 2. Reagents and conditions: (a) DCC, DMAP, CH_2Cl_2, 25 °C, 12 h, 99% from kemantane, 82% from adamantanol; (b) for **4**: 1—$NaBH_4$, MeOH, Et_2O, 0 °C, 99%; 2—BzCl, Et_3N, DMAP, CH_2Cl_2, 25 °C, 12 h, 74%; 3—HCO_2H, 25 °C, 2 h, then BzCl, $NaHCO_3$, EtOAc, H_2O, 25 °C, 15 min, 83%. For **5**: HCO_2H, 25 °C, 2 h, then BzCl, $NaHCO_3$, EtOAc, H_2O, 25 °C, 15 min, 44%.

To determine if the 'adamantane taxoids' **3**–**5** are able to cause tubulin polymerization, the samples of tubulin purified from bovine brain[23] were incubated with compounds **3**–**5** or with taxol as a positive control and studied by means of video-enhanced contrast light microscopy (AVEC-DIC microscopy).[24] This study showed that all tested taxol mimetics were not able to promote microtubule assembly of isolated tubulin (no microtubule bundles were observed). However, sedimentation assays[25] revealed that these compounds induced tubulin aggregation (Fig. 3A). Remarkably, ester **5** showed the highest ability to promote tubulin sedimentation—57%, whereas for all other compounds (including **2** and similar structures)[12] it varied between 25% and 33% (Fig. 3B).



Scheme 1. Reagents and conditions: (a) DCC, DMAP, CH_2Cl_2, 25 °C, 12 h, 49%; (b) TsOH, MeOH, 25 °C, 2 h, 89%.

Table 1
In vitro cytotoxicities against the A549 cell line

Compound	IC_{50} (μM)
Taxol	0.002
2	3.8 (Ref. 12)
3	2.5
4*	9.5
5	5.6
15	0.0006

* Ester **4** was tested as a mixture of *trans*- and *cis*-isomers (in the ratio 2:1).[22]

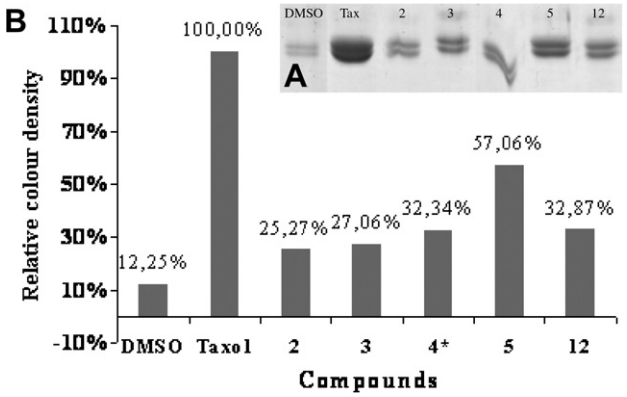


Figure 3. Sedimentation analysis of the tubulin after incubation in the presence of compounds **2**–**5** and **12**. (A) SDS–PAGE of tubulin pellets. (B) Estimation of the relative amount of tubulin in the sediment (100% corresponds to the amount of tubulin pelleted after incubation with taxol; DMSO was used as a negative control).

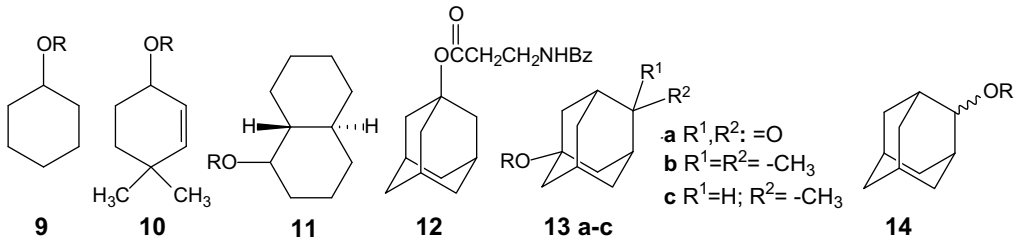
Although we were unable to observe aggregates of tubulin by AVEC-DIC microscopy in all tested adamantane ester samples (**3**–**5**), electron microscopy of these samples stained with uranyl acetate showed the presence of amorphous aggregates (data not shown). Thus, the results of the sedimentation assay and electron microscopy indicate that compounds **3**–**5** induce tubulin aggregation, and this ability is remarkably high for ester **5**. It is interesting that both unsubstituted adamantane core and taxol side chain seem to play a part in this ability, because specially synthesized esters **9**–**12** as well as **13a**–**c** demonstrate a relative amount of tubulin in the sedimentation assay of less than 35% (e.g., **12** in Fig. 3). At the same time, the taxol side chain shift to the 2-position of adamantane (compound **14**) maintains the high level of tubulin aggregation.

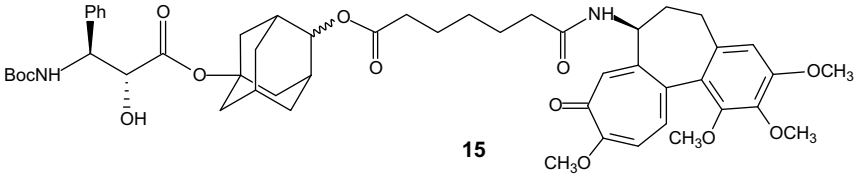
The interesting ability of structure **5** enabled us to chose taxol/taxotere side chain substituted adamantane as a structural fragment in a hybrid compound **15** analogous to recently obtained hybrid taxol-colchicine ligand colchitaxel.[26] (It is known that taxol and colchicine interact with tubulin at different binding sites and the latter inhibit the protein polymerization and microtubule formation.)[27] We hypothesized that binding of a 'colchicine part' of compound **15** with colchicine binding site might induce the 'adamantane-based taxoid mimic part' to interact effectively with the taxol binding site of tubulin.

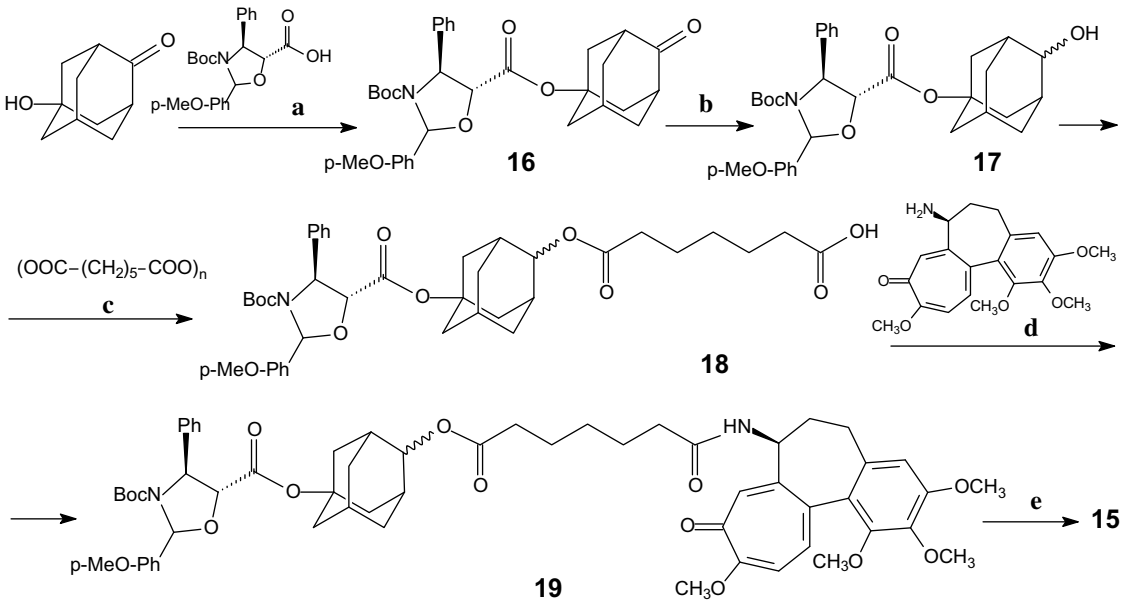
The synthesis of the structure **15** is presented in Scheme 3. The esterification of kemantane by an oxazolidine-type-protected *N*-*tert*-butoxycarbonylphenylisoserine led to the ester **16**, and subsequent reduction of its keto-group by $NaBH_4$ gave the corresponding alcohol **17** (*trans*-/*cis*-isomer ratio 2:1). The further esterification of **17** by the polyanhydride of pimelinic acid led to isomeric esters **18**. Finally, deacetylcolchicine (obtained in three steps from colchicine)[27] was attached to the carboxylic group of compound **18**, and the following deprotection of amino acid in the product **19** led to compound **15** (*trans*-/*cis*-isomers ratio 2:1).

Compound **15** was found to possess very high cytotoxicity against human lung carcinoma cell line A549 (Table 1). Though it is caused mostly by interaction with colchicine binding site, it is interesting to mention that immuno-fluorescent microscopy of microtubules in the A549 cells[28] revealed the existence of a small amount of microtubule bundles, characteristic for the taxol action among microtubules and their shortened aggregates in cells treated with 5 μM of **15**. This observation needs, however, additional investigation, because visually similar structures might be a result of compound **15** interacting with a vinca-alkaloid binding site on tubulin. These investigations are currently in progress.

In summary, the synthesized adamantane derivatives, though being cytotoxic, turned out to be unable to promote microtubule assembly of purified tubulin in vitro like most of the known simplified taxol analogues (e.g., Refs. 4,6,7,9,12). This indicates that the 'simplification' in the presented structures is too drastic in comparison with the parent molecule. Nevertheless, the *N*-benzoyl-(2*R*,3*S*)-phenylisoseryloxyadamantane (**5**) demonstrated the ability to cause significant aggregation of the protein, whereas the hybrid compound **15** revealed high and rather unusual cytotoxicity profile and may be considered as a structural clue for the further studies.







Scheme 3. Reagents and conditions: (a) DCC, DMAP, CH_2Cl_2, rt, 12 h, 46%; (b) $NaBH_4$, Et_2O, rt, 10 h, 96%; (c) DMAP, CH_2Cl_2, rt, 24 h, 46 %; (d) EEDQ, CH_2Cl_2, rt, 24 h, 76%; (e) TsOH, MeOH, rt, 2 h, 63%.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.07.116.

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28. In this assay A549 cells were treated with compound **15**, fixed with polyformaldehyde and stained with antibodies (primary mouse anti-α-tubulin secondary fluorescent labelled rabbit anti mouse-ALEXA 488).