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Design, synthesis and bioactivity of simplified taxol analogues on the basis of bicyclo[3.3.1]nonane derivatives

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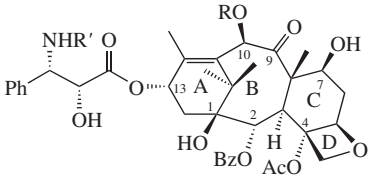
Four specially designed bicyclo[3.3.1]nonane-based structures were synthesised and found to be cytotoxic at micromolar concentrations and to cause slight non-specific tubulin aggregation.

Taxol (paclitaxel), isolated from the bark extracts of *Taxus brevifolia*, and its synthetic analogue taxotere (docetaxel) are current clinically used antitumor drugs (Figure 1). The action of these compounds is based on their ability to cause spontaneous polymerisation of intracellular protein tubulin to stable microtubules and to stabilise preformed microtubules thereby preventing cell division.[1,2]



Taxol: R = Ac, R′ = Bz
Taxotere: R = H, R′ = Boc

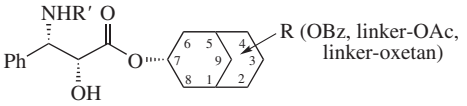


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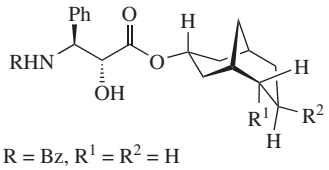
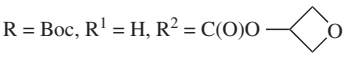
Figure 1 Taxane structures and a model of their 'simplified' analogues.

The intricate molecular structure of taxane compounds leads to the necessity to obtain them semi-synthetically from natural sources. Therefore, the development of taxol and taxotere analogues with a simpler structure is an important task. During the last ten years, such attempts have been reported.[3–8]

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,[9] we suggested a general theoretical model of a 'simplified' taxol analogue with bicyclo[3.3.1]nonane core (**1**, Figure 1).[10] This moiety has a definite structural similarity to the AB rings of the parent molecule. Suggesting model **1**, we were guided by the fact that taxol and taxotere C(13) side chain [*i.e.*, *N*-benzoyl- or *N*-*tert*-butoxycarbonyl-(2*R*,3*S*)-phenylisoseryl] makes the most important contribution to binding with tubulin, whereas C(2) (OBz), C(4) (OAc) substituents and the oxetan fragment also play a role in this binding.[1,2] Here, we present the results of molecular modeling, synthesis and biological tests of mono- and disubstituted bicyclo[3.3.1]nonanes **2**–**5**, corresponding to model compound **1**.



2 R = Bz, $R^1 = R^2 = H$
3 R = Bz, R^1 = OBz, R^2 = H
4 R = Bz, R^1 = H, $R^2 = CH_2OC(O)Me$



5 R = Boc, R^1 = H, R^2 = C(O)O

Molecular modeling.† The proper bridgehead core positions for the introduction of the substituents in model **1** were determined by the overlay of bicyclo[3.3.1]nonane with taxol in conformations with a minimal energy (chair–chair and T-conformation,[12] respectively) (Figure 2). Taking into account that the introduction of the taxol-like amino acid side chain at the 7-position of bicyclo[3.3.1]nonane brings changes to the conformation of the corresponding cyclohexane ring from chair to boat, the position of this chain in compounds **2**–**5** might differ from the one in the parent molecule. Therefore, we performed a molecular docking of two compounds (**3** and **5**) using the taxol binding site model (kindly granted to us by Professor J. Snyder[12]).

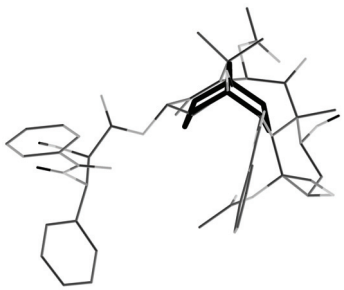


Figure 2 Overlay of bicyclo[3.3.1]nonane with the taxol molecule.

† The molecular docking of compounds **3** and **5** was performed automatically with the Glide program of the MacroModel package.[11] The binding site was defined as a box with a side of 29 Å with a center in the centroid of taxol atoms. For all other parameters their default values were used. The full flexibility of the ligand was allowed.

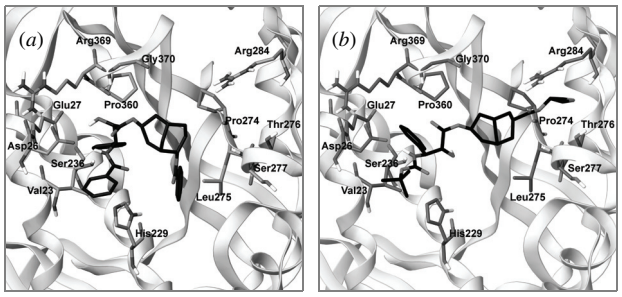


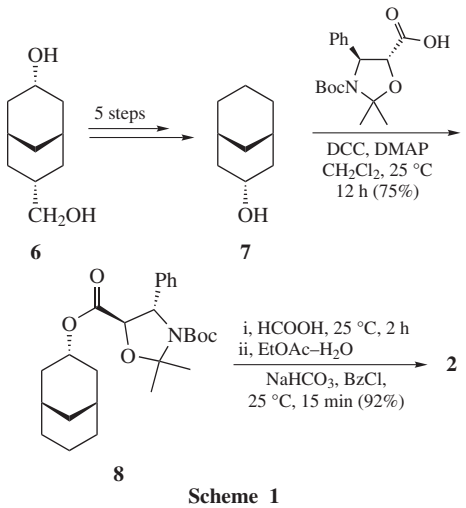
Figure 3 Structures of (*a*) **3** and (*b*) **5** inside the taxol binding site of β-tubulin (hydrogen atoms are not indicated).

This study revealed that in the case of the identical location of taxol and compound **3** *N*-benzoyl-(2*R*,3*S*)-phenylisoseryl in the protein, the aromatic ring of *endo*-bezoyloxy group in the 7-position of **3** is able to provide an important interaction with His229 [Figure 3(*a*)].

The carbonyl oxygen of ester **5** can be hydrogen bonded to the Thr 276 amino group (this bond is formed by oxetan oxygen in taxol[2,12]). The oxetan oxygen of compound **5** can form a hydrogen bond with the Thr276 hydroxyl and the Arg284 side chain [Figure 3(*b*)].

In general, the molecular docking data confirm the expected supposition that taxol binding site, which is able to hold the tetracyclic parent molecule, is too large to hold the bridged core. Thus, many unpredictable bicyclo[3.3.1]nonane positions in the binding site are possible.

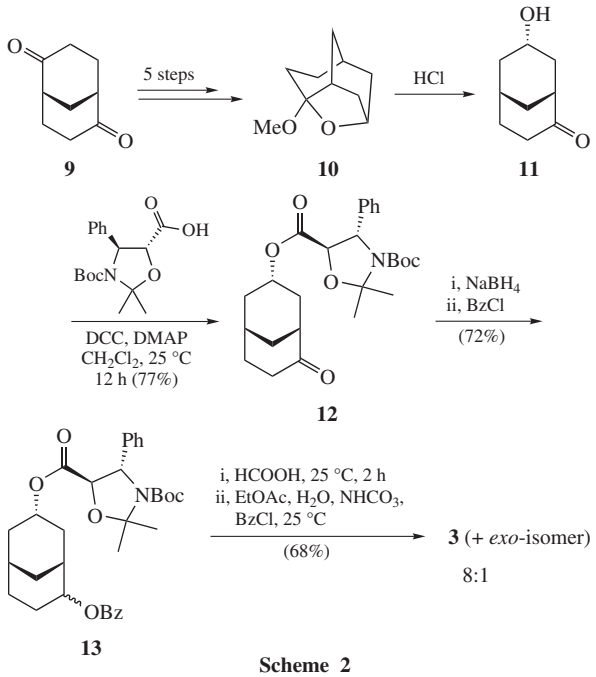
Synthesis.‡ Compounds **2**–**5** were synthesised by the esterification of corresponding alcohols by oxazolidine protected *N*-benzoylphenylisoserine with the subsequent opening of the oxazolidine ring.[13] Thus, ester **2** was synthesised from bicyclo[3.3.1]nonan-3-ol **7** (obtained by a five-step procedure from diol **6**[14]) (Scheme 1). The *endo*:*exo* isomer ratio in bicyclo[3.3.1]nonan-3-ol **7** was 9:1 but after the esterification only the wanted *endo*-isomers of esters **8** and **2** were isolated.



Scheme 1

For the synthesis of ester **3**, *endo*-bicyclo[3.3.1]nonan-2-one **11** was obtained from bicyclo[3.3.1]nonanedione **9** as described in ref. 15 (Scheme 2). Note that key tricyclic compound **10** (its hydrolysis in acidic media leads to desired ketol **11**) can also be synthesised from 4-hydroxyadamantan-2-one.[16] The esterification of ketol **11** by protected amino acid gave product **12**, and the subsequent selective reduction of carbonyl group in **12** followed by benzoylation of the obtained hydroxyl led to the mixture of two isomeric benzoyloxy derivatives **13** with a ratio

‡ For the synthetic details and the characteristics of all the compounds see Online Supplementary Materials.



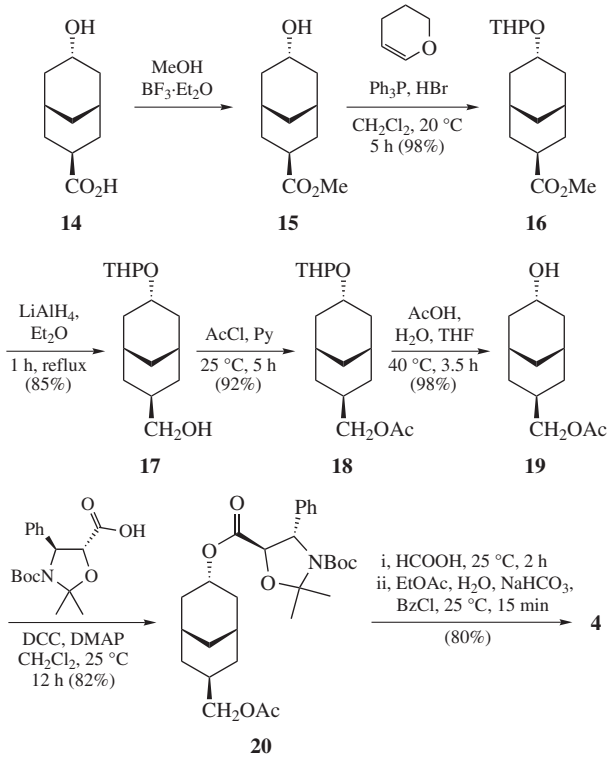
Scheme 2

of 8:1 (*endo*:*exo*[17]). The same ratio was observed in final product **3**:*exo*-isomer (8:1).

Esters **4** and **5** were synthesised from 7-*endo*-hydroxybicyclo[3.3.1]nonane carboxylic acid **14**, which was obtained by alkaline ring opening of 2-oxahomoadamantan-2-one. The resulting acid serves as a template with a proper configuration of substituents (*endo*-*exo*).[18,19] The synthesis of compound **4** was carried out according to Scheme 3.

To obtain compound **5**, double esterification was performed for TMS protected 7-*endo*-hydroxybicyclo[3.3.1]nonane carboxylic acid **14**, first by 3-hydroxyoxetane (obtained in five steps from glycerol)[20] and then by a protected amino acid (Scheme 4).

Note that the amino acid protection used for the synthesis of compounds **2**–**4** (Schemes 1–3) is unsuccessful in this case,



Scheme 3

Table 1 Results of biological tests.

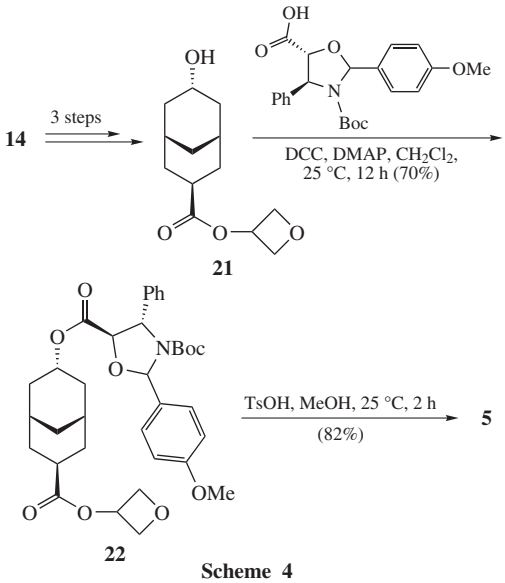
Compound	IC_{50}/μM	Tubulin sedimentation related to taxol (%)
Taxol	0.002	100
2	not determined	<40
3[a]	2.25	38
4	2.25	37
5	3.8	25
DMSO	—	12

[a]Compound **3** was tested in a mixture with its *exo*-isomer due to the small amount of the latter and the broad concentration intervals in biotests.

because the oxetane ring undergoes destruction during amino acid regeneration from the corresponding ester. Therefore, we used a slightly different amino acid protection[21] to obtain compound **5** (Scheme 4).

Biotests.[§] The results of biological activity of synthesised taxol analogues are presented in Table 1. These data indicate that, according to an estimation of IC_{50} for the A549 human lung carcinoma cell line, compounds **3**–**5** exhibit cytotoxicity at a micromolar level. In spite of a noticeable (taking into account the extent of structural simplification) cytotoxicity, all compounds were found to be unable to promote microtubule assembly of purified tubulin *in vitro*. Nevertheless, a sedimentation assay revealed that compounds **2**–**5** induce slight non-specific tubulin aggregation.

Thus, the synthesised compounds display the same kind of activity as the majority of the known simplified taxol analogues, *e.g.*, indolizidinones[5] and macrocycles.[7] The inability of compounds **2**–**5** to promote tubulin polymerization might be caused by different reasons, such as: (i) insufficiency of the two substituents in the bridgehead core, or (ii) their wrong orientation in the protein due to too bulky binding site, or (iii) the lack of the general rigidity of bicyclic framework. The results of this work indicate that the 'simplification' in the presented compounds is too drastic in comparison with the parent molecules and requires more thorough structural 'tuning' of the fragments to make it bioisosteric to a taxane skeleton.



Scheme 4

[§] For biological tests of esters **2**–**5** initial solutions of these compounds and taxol in DMSO were prepared. The tests were carried out in a concentration range of 1–60 μM. A taxol solution was used as a positive control and pure DMSO as negative control. The ability of tested compounds to stimulate *in vitro* assembly of microtubules was determined using tubulin (1–2 mg cm^{-3}) isolated from bovine brain.[22] The formation of microtubules was monitored by light video-enhanced contract microscopy (AVEC DIC microscopy)[23] and sedimentation analysis.[24] The ability of compounds **3**–**5** to inhibit cell growth (IC_{50}) was studied using phase contrast microscopy of human lung carcinoma cell line A549.

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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.2008.07.003.

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