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Novel C2–C3′ N-peptide linked macrocyclic taxoids. Part 1: Synthesis and biological activities of docetaxel analogues with a peptide side chain at C3′

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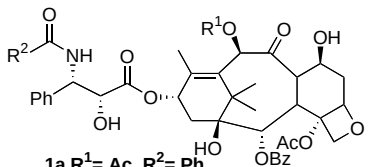
Abstract—A series of novel docetaxel analogues possessing a peptide side chain at the C3′-N position was synthesized. These compounds were designed to mimic a region of the α-tubulin loop that is equivalent to the paclitaxel binding pocket in β-tubulin. Eight new peptidic taxoids were obtained and evaluated as inhibitors of microtubule disassembly, as well as for their cytotoxicity.

Taxol® (paclitaxel)[1] **1a** and its semi-synthetic analogue Taxotere® (docetaxel)[2] **1b** are two complex diterpenoids useful as chemotherapeutic drugs in the treatment of breast, ovarian, and nonsmall-cell lung cancers,[3] and are also active against prostate cancer.[4] Their therapeutic effect is due, at least in part, to their interaction with microtubules. In vitro, paclitaxel and docetaxel promote the assembly of tubulin into microtubules[5] and inhibit the disassembly of microtubules.[6] Microtubules are cytoskeletal elements essential in all eukaryotic cells, with functions extending from cellular transport to cell motility and mitosis. They are made of repeating αβ-tubulin heterodimers that bind head to tail into protofilaments. In 1998, the structure of tubulin was determined by electron crystallography on zinc-induced sheets of tubulin stabilized with paclitaxel **1a**.[7]



1a R^1= Ac, R^2= Ph
1b R^1= H, R^2= O*t*Bu

The 3.7 Å resolution allows localization of the paclitaxel **1a** binding site on the β-subunit and also to visualize the region of α-tubulin that is equivalent to the paclitaxel binding pocket.[8,9] Depending on their secondary structure, α- and β-tubulins could be superimposed with only few differences. One of these is that the paclitaxel site on β-tubulin is occupied in α-tubulin by a series of eight amino acids, which form part of a loop connecting strands B9 and B10 (Fig. 1).[8,9]

We performed molecular modeling to study the superimposition of the α- and β-tubulin structures in this region, and we observed conformational similarities between the octapeptide Val362-Val-Pro-Gly-Gly-Asp-Leu-Ala369 and the structure of paclitaxel when it is bound to β-tubulin (Fig. 2). Thus, six amino acids, Val363-Pro-Gly-Gly-Asp-Leu368, of the α-tubulin octapeptide showed a good insertion between positions 3′ and 2 of paclitaxel **1a**, while the other amino acids mimic a part of the paclitaxel skeleton. Moreover, this superposition could be related to that of the conformationally restricted macrocyclic taxoids synthesized in our group to mimic the 'nonpolar' and 'T-shaped' conformation of paclitaxel and docetaxel.[10–12]

As shown earlier, bioactive taxoids bind to the β-tubulin subunit in a specific binding conformation, and several studies (X-rays, NMR, and molecular modeling) have been performed to elucidate the nature of the bioactive conformation as 'nonpolar',[13,14] 'polar'[15] or

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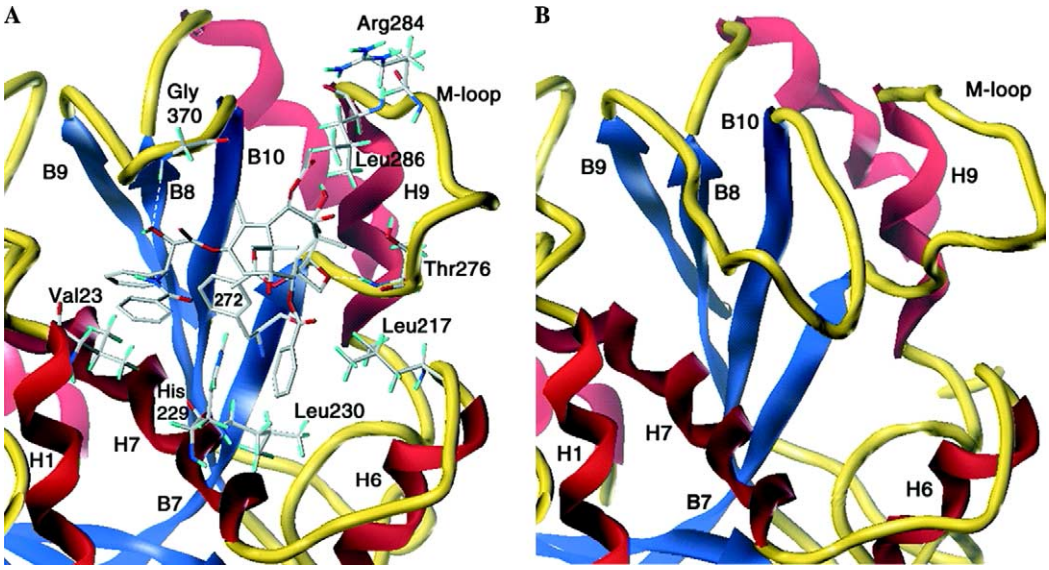


Figure 1. The paclitaxel binding site in β-tubulin (A) and the B9–B10 loop in α-tubulin (B).[9]

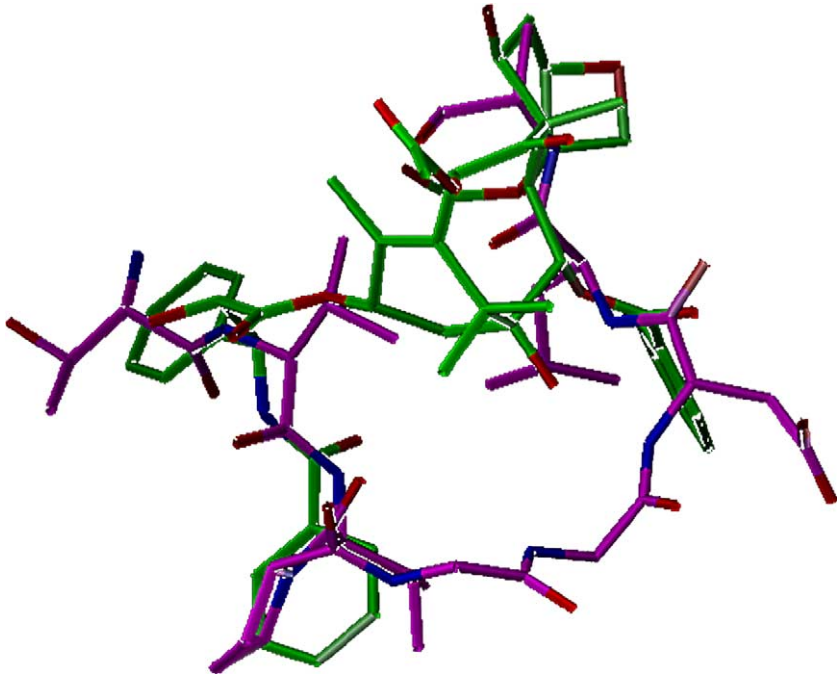


Figure 2. Overlapping of paclitaxel (green) and octapeptide A362–A369 (magenta) after manual superimposition of α- and β-tubulin secondary structures, followed by an optimization of the position of the octapeptide (Sybyl software).

'T-shaped'[9,16] forms. Our previous studies were based on the synthesis of macrocyclic docetaxel analogues with bridges linking the C3′ nitrogen to the C2 oxygen using variable chain sizes to describe the 'active' conformation.[10–12] The results obtained from this work seem to confirm that the 'T-shaped' form is preferred for a good interaction with the β-tubulin binding pocket.[12] With the goal of synthesizing novel macrocyclic docetaxel analogues, we designed new derivatives by insertion of specific amino acids of the α-tubulin loop between the 3′-NH and the 2-C positions of docetaxel **1b** (Fig. 3). These could constitute another approach to lock the

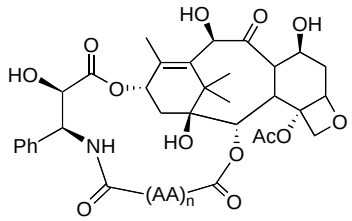


Figure 3. Macrocyclic peptidic taxoids (AA, amino acids of the α-tubulin region equivalent to the paclitaxel binding site).

docetaxel conformation and improve the taxoid β-tubulin interaction in the same way that the octapeptide interacts within α-tubulin.

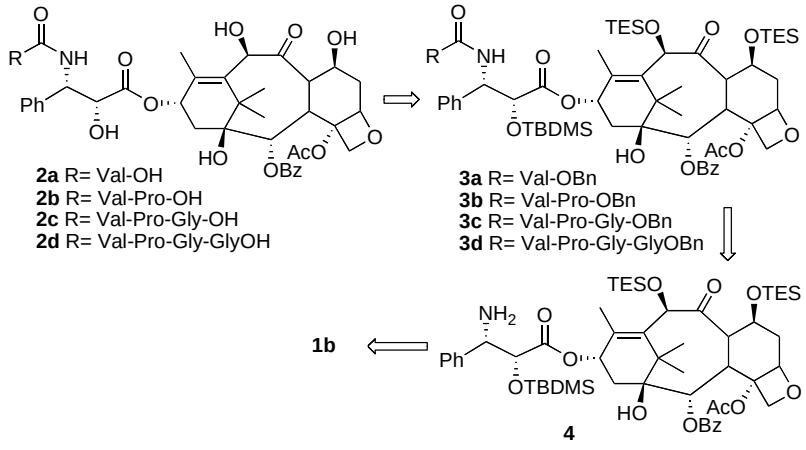
In this paper, we describe, as a preliminary result, the synthesis of novel taxoids **2a**–**2d** bearing at the 3′N position from one to four amino acids (Val-Pro-Gly-Gly) which are part of the α-tubulin loop that is equivalent to the β-tubulin paclitaxel binding site. The variable length of the peptide allows us to correlate step by step the biological activities to the number of added amino acids and to verify if the addition of amino acids possessing a free or a protected amino group at the C3′ position is not deleterious to the antitubulin activity. To confirm the specificity of the interaction between tubulin and the peptide analogues, we also synthesized three other compounds in which the amino acids at C3′ (Val-Phe-Met-OMe, Gly-Ser(OBn)-Tyr(OBn)OBn, and Gly-Ser-TyrOH) are different from those constituting the octapeptide residue. To our knowledge, no taxoids bearing a peptide side chain directly linked to the C3′ position have been previously described. However, linking of amino acids to taxoids at positions 2′, 7, and 10 has been reported with the goal of promoting water solubility or of obtaining prodrugs of antitumor taxoids.[17–21]

Our retrosynthetic scheme of C3′ taxoid analogues linked to amino acids **2a**–**2d** is shown in Scheme 1. Compounds **2a**–**2d** could be obtained from compounds **3a**–**3d**, respectively, after deprotection of the C7, C10, and C2′ hydroxyl groups and hydrogenolysis of the benzyl ester protecting the acid group of the amino acids, which have been added step by step to compound **4**. The latter can be readily synthesized from docetaxel **1b** through double protection at C7 and C10, and removal of the Boc group.[10]

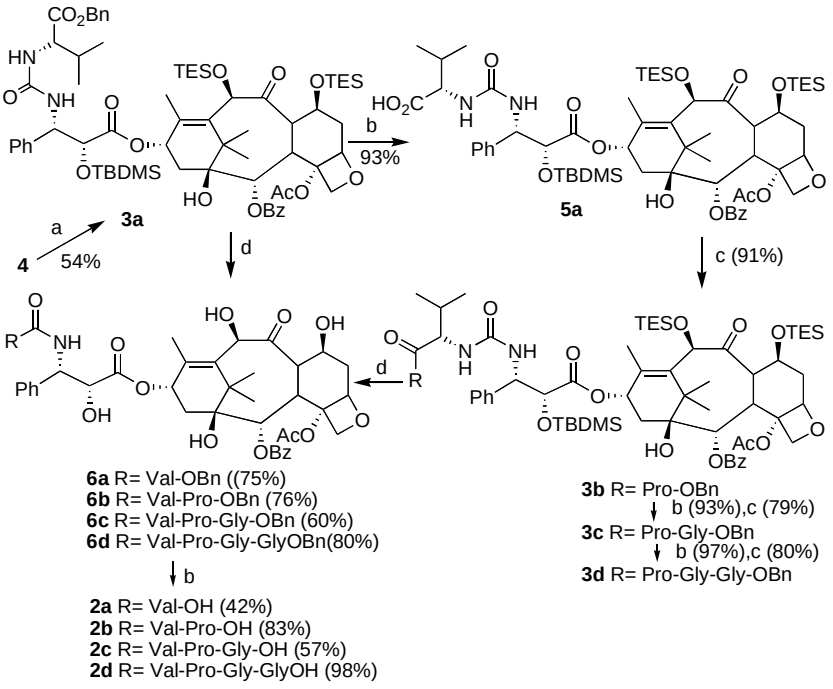
To mimic the desired α-tubulin loop, it was necessary to have the N-terminal part of the peptide linked to the 3′-position and the C-terminal part to the 2-position. Thus, the connection of **4** to the first amino acid (valine) was realized by introduction of a urea function using carbonyldiimidazole (CDI) (Scheme 2).

Optimization of the conditions for the addition of valine benzyl ester gave compound **3a** in 54% yield. Hydrogenolysis of **3a** led to the deprotected amino acid taxoid **5a** in 93% yield. The second amino acid (proline) was then added by creation of a peptide bond with the free acid function of valine using 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide hydrochloride[22] (EDCI) as the coupling reagent and 1-hydroxybenzotriazole[23] (HOBt) to limit undesirable side reactions.[24] Moreover, we used a low dielectric constant solvent, such as CH_2Cl_2, to minimize possible racemization via formation of an oxazolone intermediate.[25] Thus, proline benzyl ester was added to valine to give compound **3b** (91%). To increase the peptide chain length further, the benzyl ester was removed by hydrogenolysis, and the other amino acids were introduced using the same pathway (Scheme 2). All these compounds were deprotected at C7, C10, and C2′ by HF/pyridine with good yields, affording compounds **6a**–**6d**, while the terminal amino acids were all deprotected by hydrogenolysis, leading to compounds **2a**–**2d** in 57–98% yields. In the same way, we also prepared analogues **8** and **11** bearing the tripeptides Val-Phe-Met-OMe and Gly-Ser(OBn)-Tyr(OBn)-OBn, respectively (Scheme 3). Compound **8** was obtained by addition of the dipeptide Phe-Met-OMe[26] to **5a**. Preparation of compound **11** was initiated from the glycine derivative **9** resulting from the attachment of glycine to **4** by a urea function, as described earlier for the formation of **3a** (Scheme 2). Coupling of **9** with the dipeptide Ser(OBn)-Tyr(OBn)-OBn led to **11** after deprotection of the hydroxyl groups (Scheme 3). Finally, the peptide part of taxoid **11** was deprotected by hydrogenolysis to give **12**.

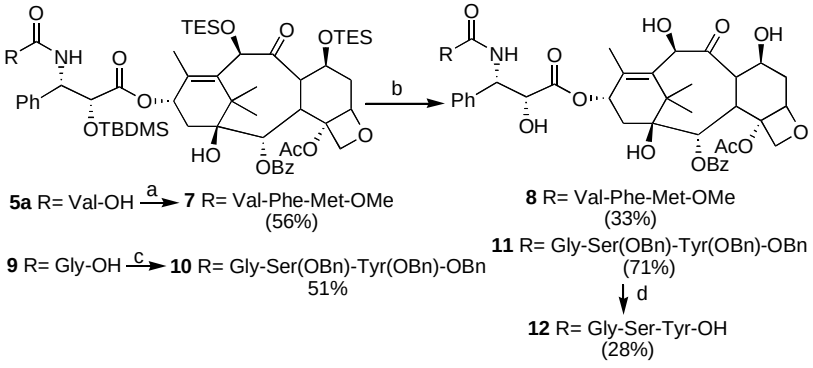
The new compounds **2a**–**2d**, **6a**–**6d**, and **8**, **9**, and **12** as well as docetaxel **1b**, were evaluated for their inhibition of cold-induced microtubule disassembly[27] and for their cytotoxicity against the KB cancer cell line[28] (Table 1). In the series **2a**–**2d** and **6a**–**6d**, most of the compounds exhibited very good interaction with microtubules, showing IC_{50} values similar to that of docetaxel. Except for analogue **2b**, which is three to four times less active than docetaxel, there were no crucial differences in the antitubulin activity of



Scheme 1. Retrosynthetic pathway of docetaxel analogues containing amino acids at 3′-NH.



Scheme 2. Reagents and conditions: (a) Val-OBn (10 equiv), CDI (20 equiv), NMM (33 equiv), CH_2Cl_2/CH_3CN (1/1), 60 °C; (b) Pd/C 10%, H_2, MeOH/AcOH (9/1), rt; (c) AA-OBn (5 equiv), EDCI (5 equiv), HOBt (5 equiv), CH_2Cl_2, rt; (d) HF/pyridine 70% (45 equiv), CH_3CN, 0 °C and rt.



Scheme 3. Reagents and conditions: (a) Phe-Met-OMe (5 equiv), ECDI (5 equiv), HOBt (5 equiv), CH_2Cl_2, rt; (b) HF/pyridine 70% (45 equiv), CH_3CN, 0 °C and rt; (c) Ser(OBn)-Tyr(OBn)-OBn (5 equiv), ECDI (5 equiv), HOBt (5 equiv), CH_2Cl_2, rt; (d) Pd/C 10%, H_2, MeOH, rt.

protected and unprotected analogues, showing that hydrophobicity of the side chain is not necessary for the interaction with tubulin. We also noted a slight systematic increase in antitubulin activity, following the step-by-step addition of amino acids. With regard to cytotoxicity, compounds **2a**–**2d** bearing a deprotected amino acid side chain are less cytotoxic than their corresponding protected analogues **6a**–**6d**. This is probably due to the presence of the free acidic function in compounds **2a**–**2d** that hampers cell penetration. Among these compounds, **6a** has a cytotoxicity comparable to that of docetaxel, and it is noteworthy that the cytotoxic effects of the compounds decrease with the length of the amino acid side chain. Compounds **8** and **12** also interact with microtubules but to a lesser extent than the previous series, and, curiously, compound **11**, which was found inactive on microtubules, showed significant cytotoxicity toward KB cells.

Table 1. Microtubule disassembly inhibition and cytotoxicity of docetaxel **1b** and compounds **2a**–**2d**, **6a**–**6d**, and **8**, **11**, and **12**

Compounds	Microtubules disassembly IC_{50} (μM)[a]	Cytotoxicity against KB IC_{50} (μM)[b]
Docetaxel **1b**	0.5	0.001
2a	0.8	0.67
6a	0.8	0.004
2b	1.7	4.50
6b	0.5	0.05
2c	0.6	>10
6c	0.4	0.10
2d	0.5	>10
6d	0.3	4.00
8	2.6	1.10
11	Inactive	0.09
12	1.0	1.05

[a] IC_{50} is the concentration that inhibits 50% of the rate of microtubule disassembly.

[b] IC_{50} measures the drug concentration required for the inhibition of 50% cell proliferation after 72 h incubation.

In summary, we have synthesized 11 new taxoids with a peptide side chain at C3′, and we observed that the substitution of the Boc group of docetaxel by these amino acids does not induce any loss of activity on microtubule disassembly but leads, except for compound **6a**, to a decrease in cytotoxicity, especially when the groups are hydrophilic. These preliminary results have encouraged us to continue our work on the synthesis of macrocyclic peptide analogues of docetaxel mimicking the amino acid region of α-tubulin that is similar to that of the taxol binding site. This study will be presented as the second part of this research program in a future publication.

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