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Synthesis and biological evaluation of novel taxoids designed for targeted delivery to tumors

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Abstract—The use of drug–antibody conjugates affords a method for the targeted delivery of anticancer drugs specifically to cancer cells. Monoclonal antibodies alone usually do not possess high therapeutic efficacy, however, they are capable of targeting tumor markers selectively. We have prepared taxoids with significantly higher cytotoxicity than paclitaxel and docetaxel. These taxoids now meet the high potency required for use in a targeted-delivery approach using monoclonal antibodies. The synthesis and biological evaluation of these taxoids are reported.

The advent of monoclonal antibody (mAb) technology has led to the development of a large number of antibodies directed against tumor-associated antigens.[1] Despite their tumor specificity, most antibodies are found to be only weakly cytotoxic themselves and therefore are not therapeutically useful. However, their high specificity and long circulation half life (several weeks for a humanized antibody) render them excellent vehicles for the targeted delivery of cytotoxic drugs to the tumor in the form of an antibody–drug conjugate.

The amount of mAb localized at the tumor site in humans has been reported to be only a fraction of the total injected dose. As a result of this limitation, the drug component of the conjugate is required to possess a very high potency ($IC_{50} < 1 \times 10^{-10}$ M) in order to achieve a therapeutic dose at the tumor site.[2] We have previously developed antibody conjugates of highly potent drugs such as the maytansinoids and CC-1065 analogs.[3] These conjugates were shown to display high, antigen specific cytotoxicity in vitro, and remarkable antitumor activity in human tumor models in mice.

The natural product paclitaxel (**1**), and its semisynthetic analog, docetaxel (**2**), are members of the taxane diterpenoids family,[4] and two of the most active anticancer agents in clinical use today, being commonly used against ovarian and breast cancers. Despite their widespread use these taxanes possess various undesirable qualities, such as low tumor selectivity, the development of multidrug resistance (MDR), and poor solubility in aqueous solutions. In light of these limitations, the targeted delivery of taxanes in the form of antibody–taxane conjugate offers a means of greatly enhancing the tumor selectivity, and hence, the therapeutic efficacy of taxanes. Initial efforts on the tumor-specific delivery of taxoids have been previously described.[5] However, the taxoids utilized in these studies were found to be only moderately potent, and thus not ideal for use in the targeted delivery approach.

In an effort to identify new highly potent taxanes that are suitable for use in targeted delivery, our ongoing studies on the structure–activity relationships (SAR) of taxoids revealed that C-2 modified taxoids, such as **3**, were 30–100 fold more potent than paclitaxel in various assays.[6] In order to further investigate the SAR of these C-2 modified taxoids, we were interested in evaluating the effects of modification at the C-3′ and C-3′*N* positions. Herein, we report the design, synthesis, and biological evaluation of these novel taxoids.

The synthesis of taxanes **8**–**29** began by constructing the required baccatin core **7**. Thus, starting with the commercially available natural product 10-deacetylbaccatin

Keywords: Taxol; Taxotere; Paclitaxel; Docetaxel; Taxane; Cancer; Monoclonal antibody.
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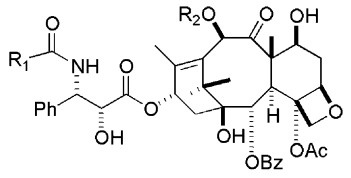
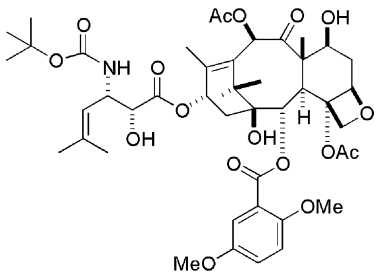


R_1=Ph, R_2=Ac, Taxol® (Paclitaxel); **1**
R_1=^{t}BuO, R_2=H, Taxotere® (Docetaxel); **2**



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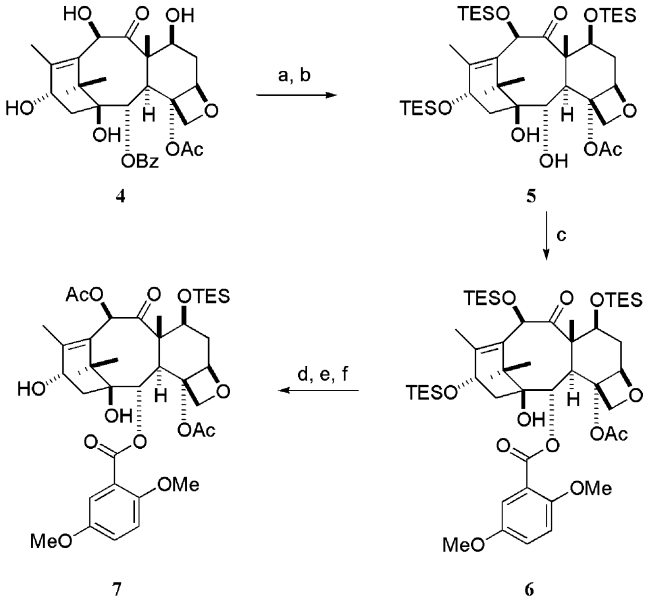
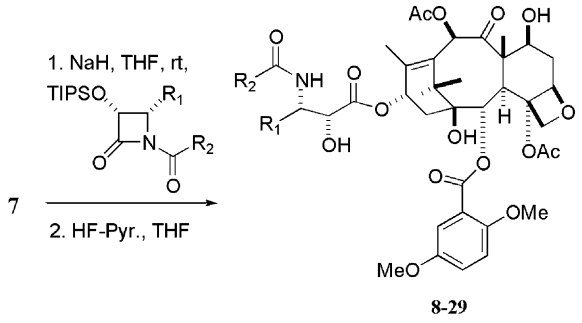
III (**4**), exhaustive protection of the C-7, C-10, and the C-13 hydroxyl groups as their triethylsilyl ethers, followed by de-esterification of the C-2 benzoate gave the protected C-1,2 diol **5** in good yields. While it has been shown in the literature that esterification at the C-2 position of baccatin can easily be achieved using a variety of coupling agents such as EDC,[7a] DIC,[7b] and DCC,[7c] we found this was not the case with a more bulky acid, such as 2,5-dimethoxybenzoic acid. Utilizing either DIC or EDC resulted in the formation of virtually no product even with mild heating for several days. More forcing conditions, using a large excess of reagents and higher temperatures resulted in the formation of significant amounts of the undesired oxetane ring-opened product, making purification extremely difficult. After detailed studies, we have found that the coupling could be achieved by using DCC (10 equiv) and 4-PP (0.1 equiv) in toluene at 60 °C for 8–9 days. These conditions gave a 95% conversion of starting material and 85–90% isolated yields of the desired product with no apparent side products.

Treatment of baccatin **6** with HF/pyridine to remove the silyl protecting groups, followed by selective acylation at C-10, employing Holton's method,[8] and reprotection of the C-7 hydroxyl as its triethylsilyl ether provided the desired baccatin derivative **7** in six steps (Scheme 1).



Scheme 1. Synthesis of the baccatin derivative **7**. Reagents and conditions: (a) chlorotriethylsilane, imidazole, DMF, rt; (b) Red-Al, THF, rt; (c) 2,5-dimethoxybenzoic acid, DCC, 4-PP, $PhCH_3$, 60 °C; (d) HF–pyridine, THF, 0 °C–rt; (e) Ac_2O, $CeCl_3$, THF, rt; (f) chlorotriethylsilane, imidazole, DMF, rt.

In order to thoroughly investigate the SAR with modifications at the C-3′ and C-3′*N* positions we prepared a variety of different β-lactams using known methods.[9] The couplings of these β-lactams with baccatin **7** were carried out using NaH, followed by removal of the silyl protecting groups with HF–pyridine to give the desired taxoids (**8**–**29**) (Scheme 2).



Scheme 2. Synthesis of taxoids **8**–**29**.

The biological evaluation of these novel taxoids modified at the C-2, C-3′, and C-3′*N* positions is summarized in Table 1.[10] In contrast to the parent taxanes paclitaxel and docetaxel, which possess an unsubstituted benzoyl group at the C-2 position all the compounds in this SAR study had a dimethoxybenzoyl substituent at the C-2 position. Introduction of this substituent along with the modifications at the C-3′ and C-3′*N* positions resulted in taxoids that were up to 68-fold more potent than paclitaxel. This level of increase in cytotoxicity was not observed when the C-2 benzoyl remained unchanged and the C-3′ and C-3′*N* were modified simultaneously,[11] suggesting that the dimethoxybenzoyl group at the C-2 position is responsible for this high potency. To further investigate this phenomenon we prepared taxoids **28** and **29**. As expected it is clear that the introduction of the dimethoxybenzoyl group at the C-2 posi-

Table 1. Cytotoxicity data (IC_{50},[a] nM) of taxoids

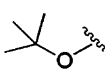
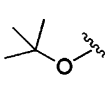
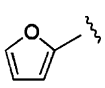
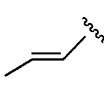
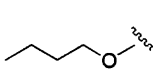
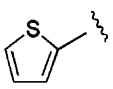
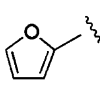
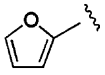
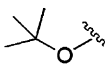
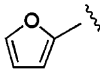
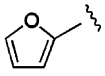
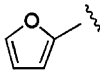
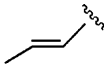
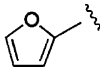
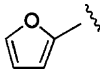
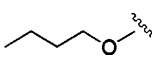
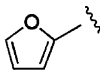
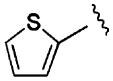
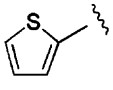
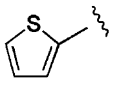
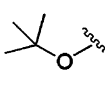
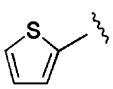
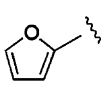
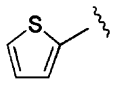
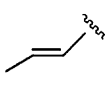
Taxoid[b]	R_1	R_2	A549[c]	MCF7[d]
Paclitaxel, **1**			1.7	0.8
Docetaxel, **2**			1.0	1.0
3			0.040	0.043
8			0.025	0.020
9			0.060	0.038
10			0.030	0.012
11			0.041	0.041
12			0.042	0.032
13			0.042	0.032
14			0.037	0.041
15			0.031	0.042
16			0.090	0.120
17			0.162	0.131
18			0.221	0.210
19			0.036	0.031
20			0.100	0.061
21			0.047	0.050
22			0.033	0.031
23			0.055	0.065

Table 1 (*continued*)

Taxoid[b]	R_1	R_2	A549[c]	MCF7[d]
24			0.180	0.161
25			>3.0	>3.0
26			0.048	0.048
27			0.036	0.036
28			0.130	0.032
29			0.052	0.063

[a] The concentration of taxoid that kills 50% of the growth of cancer cell line population after 72 h of exposure.
[b] Taxoids 1 and 2 do not bear a dimethoxybenzoyl group at C-2.
[c] Non-small-cell lung carcinoma.
[d] Breast cancer.

tion greatly contributes to the cytotoxicity of taxoids possessing the paclitaxel and docetaxel side chains.

Introduction of the isobuteneyl substituent at the C-3′ position, resulted in highly potent taxoids, regardless of the substituent at the C-3′*N* position. Thus taxoid **3** and **8**–**13**, bearing seven different substituents at C-3′*N* were all highly cytotoxic, with IC_{50} values in the 0.01–0.06 nM range. Taxoids bearing a 2-furyl or 2-thiazolyl substituent at the C-3′ position were also potent when the C-3′*N* position had a *t*-BOC, isobuteneyl or *n*-BOC group. An important and interesting result of this SAR study is the interactive effects between the substituents.

For example, the 2-furyl and the crotonyl groups at the C-3′*N* position showed lower cytotoxicity with the 2-furyl group at the C-3′ position (analogs **16** and **17**), while the same groups showed higher cytotoxicity than with the isobuteneyl group at the C-3′ position (analogs **9** and **10**). Introduction of the benzoyl group at the C-3′*N* position (analogs **18** and **25**) gave lower potency, suggesting that the benzoyl group may not be the best choice for this position.

This SAR study has revealed that the C-3′ and C-3′*N* positions of these taxoids can tolerate a variety of substituents, while retaining high potency. This finding opens the door to the incorporation of appropriate functional groups at these positions to enable linkage to monoclonal antibodies. Efforts to prepare such 'linkable' and highly cytotoxic taxoids for conjugation with a mAb are currently underway and will be reported in the near future.

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10. The in vitro cytotoxicities of these new taxoids were evaluated using a clonogenic assay. Cells were plated in six-well tissue culture plates and exposed for 72 h to the taxoid. The medium was then replaced and the cells were incubated until colonies had formed (5–10 days). Cells were then fixed and stained with crystal violet, and the surviving fractions were determined. The clonogenic (plating efficiency) assay is a direct and accurate method to determine the survival of cells following their exposure to a cytotoxic drug (Sellers, J. R.; Cook, S.; Goldmacher, V. S. *J. Immunol. Methods*, **1994**, *172*, 255–264). We found indirect methods, such as MTT (Mosmann, T. *J. Immunol. Methods*, **1983**, *65*, 55–63) and SRB (Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J., Vistica, D., Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.*, **1990**, *82*, 1107–1112) less accurate and reproducible when used for evaluation of highly cytotoxic compounds. The IC_{50} values varied dramatically depending on the conditions, such as the cell number, and the length of cell culturing following their exposure to a drug.
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