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Synthesis and Evaluation of Artificial Taxoids with Antitumor and Multi-Drug Resistance Reversing Activities

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Abstract

Artificial taxoids were synthesized and subjected to evaluation of their ability of multi-drug resistance reversing and antitumor activities. While the taxoid **4** could not increase cellular accumulation of vincristine in multi-drug resistant tumor cells, the C4-hydroxy analog **15** showed significant effect. However, these compounds showed weak activities on growth inhibition of cancer cells.

Keywords: Antineoplastics; Antitumor compds; Terpenes and terpenoids

Taxol® (**1**)[3] [1] is one of the most promising agent in cancer chemotherapy due to its strong antitumor activity and intriguing mechanism of action. It is now currently used for treatment of advanced ovarian and breast cancer, and further clinical trials for other cancers undergoing. However taxol, likewise other anticancer agents, have proved to be inactive for multi-drug resistance (MDR) tumors [2]. It is now clear that the MDR is caused by overexpressed membrane transporter named p-glycoprotein which decreases cellular accumulation of anticancer agent by acting as efflux pump [3,4]. In the past decade, much effort have been put on modifying taxol to gain improved activity as well as bioavailability and to decrease side effects [5,6]. Recently, there have been much interest in new taxoids with inhibiting activity for p-glycoprotein mediated drug efflux, because such compounds may be effective against MDR cancer. Ojima et al. reported some semisynthetic taxol analogs derived from 10-deacetylbaccatin III [7-10], and Kobayashi et al. reported naturally occurring taxoids named taxuspines with such activity [11-13]. During the course of synthetic studies on taxol, we have obtained in our hands some interesting intermediates having taxol-like, but different

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3. Taxol is the registered trademark for molecule with the generic name paclitaxel.

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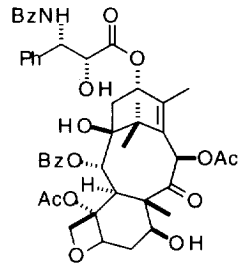
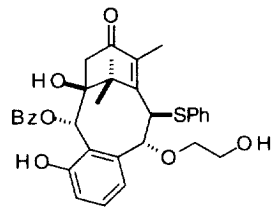
skeletons [14-16]. Under these circumstances, we explored the possibility to generate completely artificial analogs of taxol possessing both of MDR reversing and antitumor activities by using such compounds, although these two activities were entirely different.[1] In the preceding communication, we described that the C-aromatic taxoid **2** showed significant MDR reversing activity as potent as verapamil [17]. The present communication describes our attempts to design, synthesize, and evaluate the artificial taxoids with MDR reversing and antitumor activities.



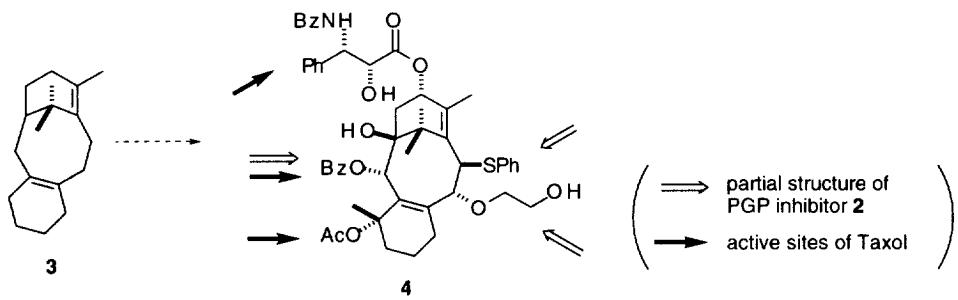
Taxol® (1)



2

Design

First of all, we decided to use **3**, which consists of almost all the carbon framework of taxol except for the oxetane ring and C3-C8 double bond, as a template structure in terms of synthetic convenience and structural interest (Figure 1)[2] [18,19]. The design of a target molecule **4** was based on the information for MDR reversing activity obtained from the previous study [17], in combination with the widely known structure-activity relationship (SAR) of taxol for antitumor activity [5,6]. SAR studies of taxol analogs have suggested that the *N*-acylphenylisoserine side chain at C13, the benzoyl group at C2, and the acetoxy group at C4 were essential for antitumor activity. These parts should be preserved and introduced to the template **3**. The acetoxyoxetane moiety in taxol was substituted by C4α-acetoxy-β-methyl functionalities as a mimic in the point of reducing structural complexity. On the other hand, the structure around C9 and C10 have been known to be less important for antitumor activity. Therefore, this part could be replaced with the structure of the C-aromatic taxoid **2**



3 **4**

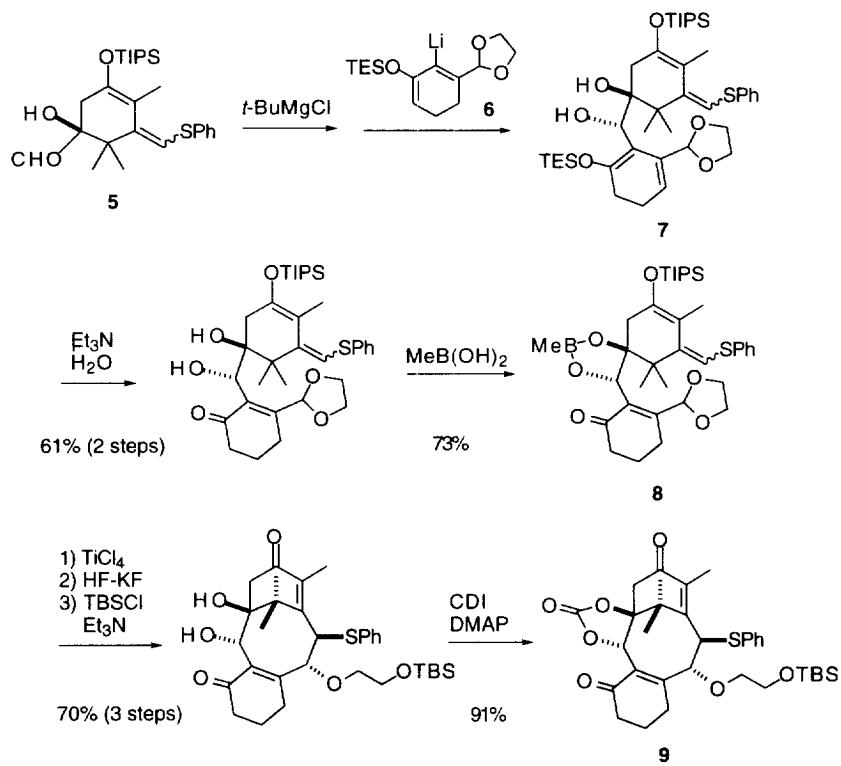
1. In general, a desirable MDR reversing agent is non-toxic to the cells. However, if a compound having these two different activities is materialized, it might be an anti-MDR-cancer agent by itself.
2. In this study, C-Aromatic taxoid like compound **2** was not used as a core structure because of the following reason. Two research groups have already reported the lack of activity or weak activity of *C-Aromatic* artificial taxoids. The diminished potency could be due to the lack of the C4-acetoxyoxetane moiety, which is believed to be essential for Taxol-like activity in these compounds. See, references 18, 19.

in order to acquire MDR reversing activity. In addition, it seemed quite favorable that the C2 benzoyl group was crucial for both activities.

Synthesis.

We planned that a target compound **4** could be prepared from a key intermediate **9** containing the carbon framework of **3**. Preparation of **9** was carried out based on the previous paper [14-17] as shown in scheme 1. Coupling reaction of the A-ring hydroxyaldehyde **5** [20] with the C-ring aryl lithium **6** was achieved under chelation control to yield a 1,2-adduct **7** as a single diastereomer. The adduct **7** was then transformed to cyclization precursor **8** via selective hydrolysis of the C-ring dienol silyl ether and protection of the vicinal diol moiety as a methylboronate. Lewis acid mediated B-ring cyclization of **8** followed by adjustment of protective groups, afforded the intermediate **9**.

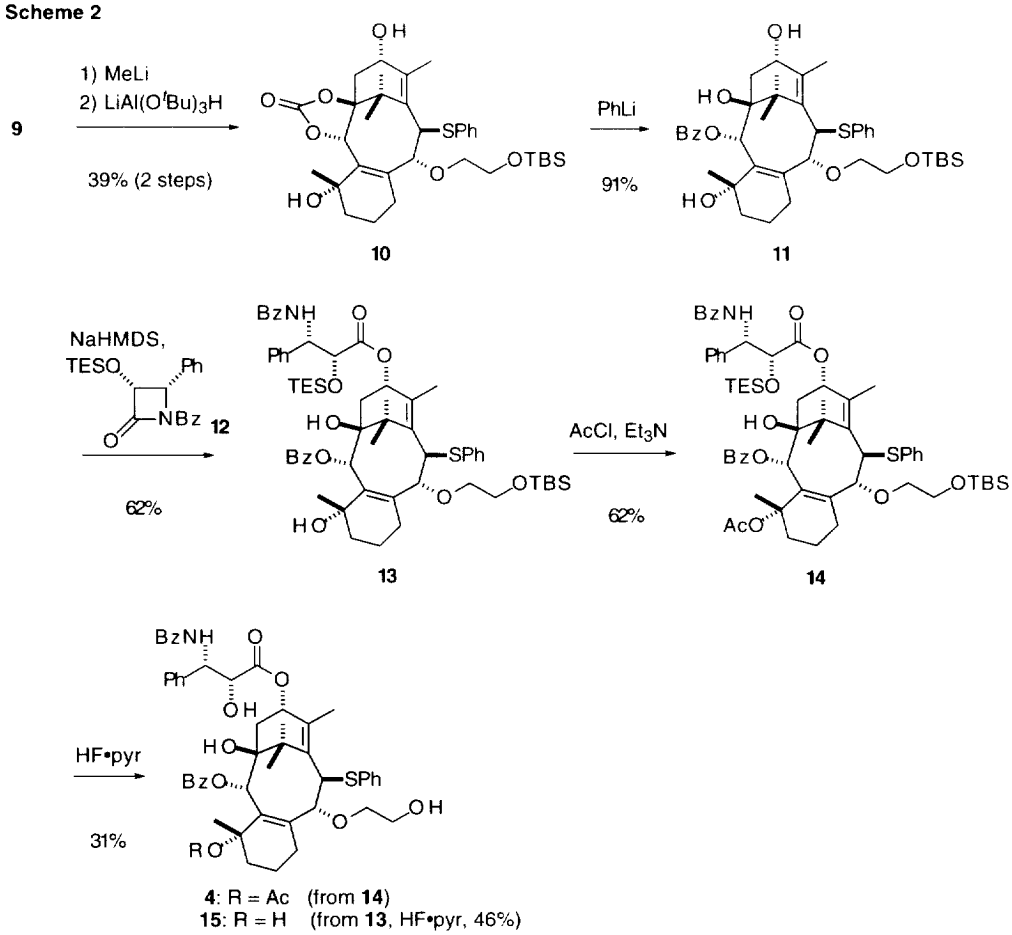
Scheme 1



The synthesis of the target molecule **4** from the intermediate **9** was shown in scheme 2. The reaction of **9** with methyl lithium took place regioselectively at the C4 site, giving a C4α-hydroxy-C4β-methyl derivative. Then the remaining C13 carbonyl group was reduced to an α-oriented OH to produce the allyl alcohol **10**. Conversion of the carbonate moiety of **10** to a benzoate **11** with phenyl lithium followed by an attachment of the C13 side chain according

to the Ojima's procedure [21] using a β-lactam **12** gave **13** in good yield. Finally, acetylation of the C4-OH and successive removal of the two silyl groups afforded the target molecule **4**. In a similar manner, a C4-hydroxyl analog **15** was prepared by desilylation of **13**, which was used for evaluation of C4 structure-activity relationship.

Scheme 2



Biological Evaluation.

MDR reversing activity of the artificial taxoids **4** and **15** was evaluated as an increasing effect on vincristine (VCR) cellular accumulation in ovarian MDR cancer cells (2780AD). It is known that p-glycoprotein is expressed in the cells, and vincristine uptake is decreased compared to normal cells. By using these cells, intracellular uptake of [^{3}H]-vincristine was measured under the presence of the test compounds at 1.0 and 10 μg/mL concentrations. Results were shown in Table 1 as % of the control value that was measured without the test compound.[1] Verapamil was used as a positive control. Relative activities of the tested

1. SDs were less than 5% of each mean values.

compounds against verapamil at 1.0 and 10 μg/mL concentrations were expressed in parentheses. The taxoid **4** was proved to have no effect on VCR accumulation in the cell. In contrast, the compound **15** showed significant activity, although its value was slightly lower than verapamil.

Table 1.
Enhancing Effect of Taxoids on VCR accumulation in 2780AD cells.

compound	VCR accumulation (% of control) 1.0 μg / mL	10 μg / mL
4	80[a] (57)[b]	102[a] (33)[b]
15	151 (108)	217 (70)

a) The relative amounts of VCR accumulation in 2780AD in the presence of taxoids to the control value without taxoids. b) VCR accumulation was compared to that obtained with verapamil and expressed as percentage of the value with verapamil.

Antitumor activity of the synthesized taxoids was evaluated as inhibition of tumor cell growth by using a panel screening based on the method of National Cancer Institute. The screening was accomplished using a sulforhodamine-B assay in 38 human tumor cell lines and mouse P338 leukemia cells. The taxoid **4** exhibited modest activities in 7 cells[1] at the range of GI_{50} = 5 - 10 μM.[2] In contrast, **15** proved to have less activity and showed GI_{50} = 9.5 μM only in BSY-1 cell. Although it seemed desirable to evaluate their antitumor activity using drug-resistant cancer cell lines expressing MDR phenotype, further biological assays were not examined because of the low anti-proliferating activity of **4** and **15** as compared with that of Taxol [22].

The taxoid **4** had no MDR reversing activity, however, the C4-deacetyl analog **15** showed modest activity as described above. It should be pointed out here that Taxol, which possesses C4-acetoxy group, has been proved to have no such activity [11]. It thus appears to be interesting that the activity is modified by a slight difference of the C4 functionality.

These taxoids showed antitumor activity, however, the potency was far lower than Taxol [22], and thus the concept of dual inhibitor was not materialized unfortunately. The reason for their low antitumor activity is unclear at present, but may be speculated to conformational change induced by the structural difference of the C-ring from Taxol.

While many attempts for modification of natural taxoids have been reported, artificial analogs derived from a synthesized core skeleton have been rarely known. From this context, the activities of **4** and **15** clearly suggests the possibility of the artificial taxoids as new leads of analog studies. Further SAR studies of these new taxoids are now in progress.

1. The 7 cells were BSY-1, HCT-116, NCI-H522, DMS-114, OVCAR-3, MKN-28, and MKN-74.
2. The concentration of the tested compound causing a 50% growth inhibition. Taxol showed potent activity at sub nanomolar level in the same assay system. See, ref. 22.

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