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Synthesis of C-Ring Aromatic Taxoids and Evaluation of Their Multi-Drug Resistance Reversing Activity

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Abstract

The C-aromatic taxoids were synthesized to develop effective inhibitors against drug efflux mediated by p-glycoproteins. Among those tested using multi-drug resistant tumor cells (2780AD), the benzoate **11** exhibited significant activity as potent as verapamil, a well-established MDR reversing agent.

Keywords: Antineoplastics; Antitumor compds; Terpenes and terpenoids

In the present cancer chemotherapy, drug resistance of cancer cells to anticancer agents is one of the major drawbacks. Among the different mechanisms involved, multi-drug resistance (MDR) mediated by the transport protein, named p-glycoprotein [1,2], is the major problem. P-glycoprotein is overexpressed on the MDR cell membrane and functions as an ATP-dependent drug efflux pump. The inhibition of p-glycoprotein may thus result in intracellular accumulation of anticancer agents and is expected to be effective in overcoming MDR. To date, a number of compounds possessing this activity have been reported [3]. Recently, Kobayashi et al. reported that taxuspines, naturally occurring taxoids isolated from Japanese yew tree, showed enhancing effect of cellular accumulation of vincristine in MDR tumor cells [4,5], and Ojima et al. reported highly effective taxane based MDR reversing agents [6,7]. Interestingly, also described was that Taxol[3] [8,9], a major congener of taxane diterpenoids and the most promising anticancer agent, showed no such activity [4]. This prompted us to explore possible separation of antitumor and MDR reversing activities.

During the course of the synthetic studies on Taxol, we prepared several compounds with various kinds of taxane-like skeletons [10,11]. Among such compounds, C-aromatic taxoids [10-13] are structurally simple analogs of natural taxanes, which may exert interesting MDR reversing activity. In this communication, we report the synthesis of C-aromatic taxoids **5** and evaluation of its derivatives **6-12** as MDR reversing agents.

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

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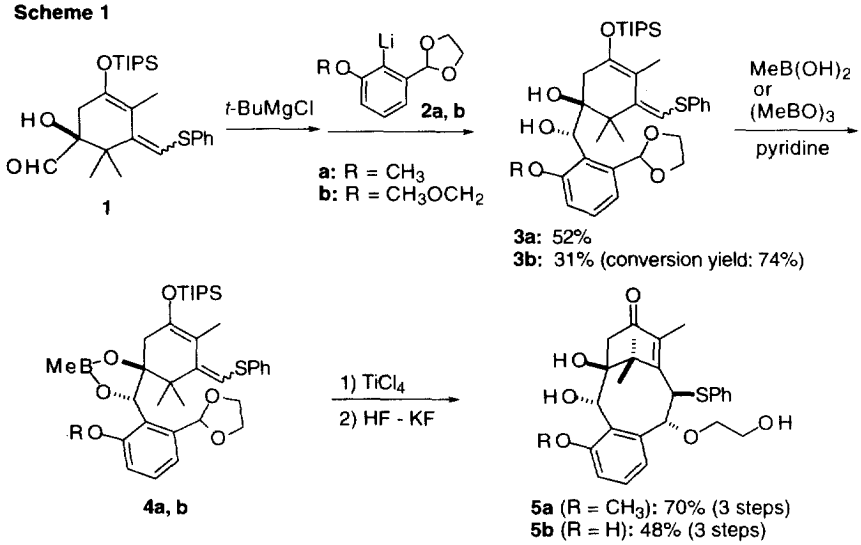
Chemistry

C-aromatic taxoids **5**, key intermediates of this series, were synthesized as shown in Scheme 1 in a similar manner to the previous report [11]. An optically active hydroxy-aldehyde **1** (A-ring fragment) [14] was treated with an aryl lithium **2** (C-ring fragment) under chelation control to afford an adduct **3** as a single diastereomer in both **a**- and **b**-series. The vicinal diol moiety in **3** was protected as a methyl boronate to yield **4**, which was a key precursor to the B-ring cyclization. Lewis acid mediated aldol-type cyclization reaction of **4** followed by removal of the boronate with hydrogen fluoride afforded **5** as a sole product in good yield.

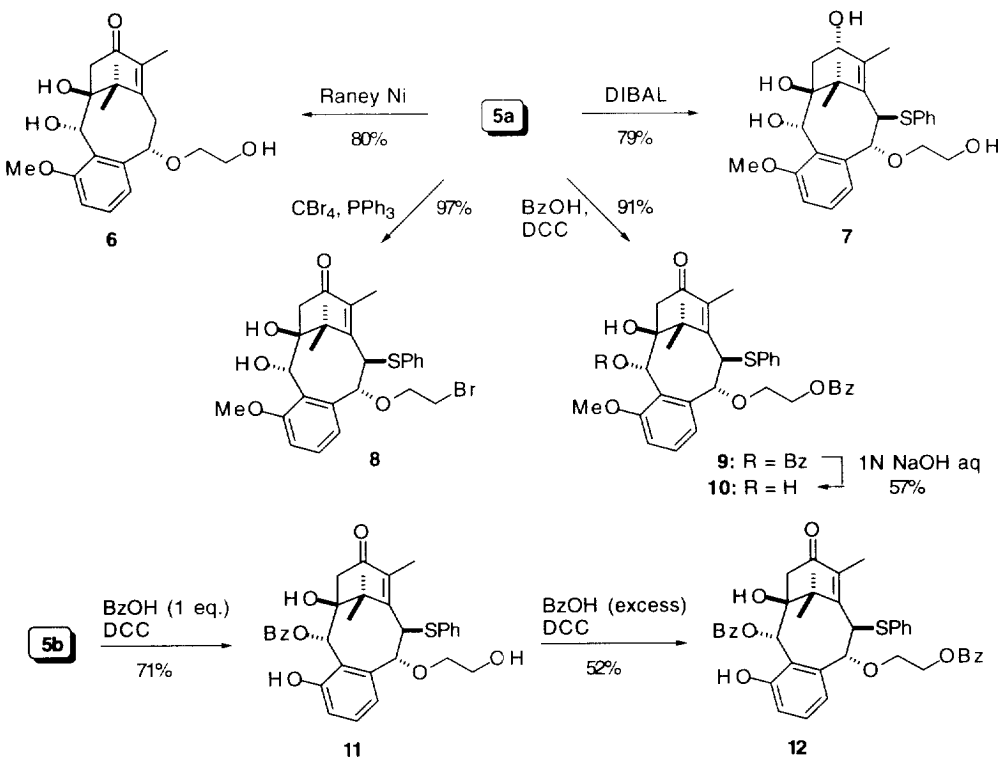
Scheme 1



The modification of these intermediates **5a** and **5b** were designed to address two points. First, it was anticipated that hydrophobicity of a molecule was important for p-glycoprotein affinity [3], because aromatic functional groups were incorporated in most of the active compounds known to date. Thus, a benzoyl group was chosen and incorporated to the hydroxyl groups of **5a** and **5b**. Secondly, functional group modification seemed necessary in order to identify the structural parts essential to the biological activity.[1] Transformation of **5a** and **5b** were therefore carried out as shown in Scheme 2. The phenylthio group of **5a** was removed with Raney-Ni to afford **6**. Stereoselective reduction of the C13 carbonyl group with DIBAL gave an allyl alcohol **7**. Bromination of the hydroxylethyl group at C9 afforded a bromide **8**. In the esterification of **5a**, no selectivity was observed and a dibenzoate **9** was obtained. Mild hydrolysis of **9** yielded a monobenzoate **10**. In contrast, esterification of **5b** with equimolar amounts of benzoic acid and DCC afforded a C2 benzoate **11**. A dibenzoate **12** was obtained by treatment of **11** with excess amount of the same reagents for a longer reaction period. All the intermediates and products were subjected to the biological assay.

1. In this point of view, it seemed desirable to remove the hydroxyethyl group at C9. However, this transformation unfortunately caused unexpected 8-membered B-ring opening via a retro-aldol type reaction.

Scheme 2



Biological Evaluation.

MDR reversing activity of the synthesized taxoids was evaluated as enhancing effect of vincristine accumulation in ovarian MDR cancer cells (2780AD). It is well-known that p-glycoprotein is expressed in the cells and vincristine uptake is decreased compared to the parent cells. The intracellular uptake of [^{3}H]-vincristine was measured in the absence and presence of the test compounds at 1.0 and 10 μg/mL concentrations. The results were described in Table 1 and the activity was expressed as % of the incorporated radioactivity to the control value measured without the test compounds.[1] Verapamil was used as a positive control. Relative activities of the tested compounds against verapamil at 1 and 10 μg/mL concentrations were expressed in parentheses.

The intermediate **5a** exhibited a weak activity compared to verapamil. Most of the functional group transformation were not effective unfortunately: removal of the phenylthio group, reduction of the C13 carbonyl group, and bromination of the hydroxyl group failed to improve activity significantly (**6**, **7**, **8**). However, distinct enhancement of the activity was observed in the benzoate derivatives. Thus, the monobenzoates **10** and **11**, especially the C2-benzoate **11**, exhibited the same potency as verapamil. Additional incorporation of a benzoyl group was again ineffective in enhancing activity as observed for **9** and **12**.

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

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

compound	VCR accumulation (% of control) 1.0 μg / mL	10 μg / mL
5a	112[a] (76)[b]	171[a] (48)[b]
6	111 (63)	124 (41)
7	109 (62)	144 (47)
8	93 (53)	166 (55)
9	106 (62)	211 (66)
10	140 (80)	256 (84)
11	238 (154)	569 (108)
12	106 (76)	142 (46)

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.

These results indicate that an aromatic functional group on the B-ring may play an important role in the interaction of the taxoids with p-glycoprotein. However, it is not clear why the benzoyl groups at different site are both effective as observed for **10** and **11**. Work is currently underway to obtain more informations of structure-activity relationship, and to improve MDR reversing activity of these artificial taxoids. Having the potent activity of the compound **11** as strong as verapamil clearly shows the possibility of the C-aromatic taxoid as a lead of MDR reversing agent.

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