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Structure–activity relationships of some taxoids as multidrug resistance modulator

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Abstract—1,7-Deoxy-4-deacetylbaccatin III (**12**) and its five analogues **6**–**9**, **13**, and their oxetane ring opened derivatives **14**, **16**, and **17**, which were synthesized from taxinine, showed significant activity as MDR reversal agent by the assay of the calcein accumulation toward MDR human ovarian cancer 2780AD cells. The most effective compound **12** in this assay is actually efficient for the recovery of cytotoxic activity of paclitaxel (taxol®), adriamycin (ADM), and vincristine (VCR) toward MDR 2780AD cells at the same level toward parental 2780 cells. This activity of **12** is very interesting because baccatin III (**4**) has no such MDR reversal activity but has cytotoxic activity. The essential functional groups inducing such a difference in biological activity between **4** and **12** are 4α-acetoxyl for **4** and 4α-hydroxyl for **12**. In seven compounds possessing MDR reversal activity, compound **12** is the most desirable compound for anti-MDR cancer reversal agent, because it has the highest accumulation ability of anticancer agent in MDR cancer cells and weak cytotoxic activity. Compounds **8** and **13** showed significant cytotoxic activity toward HepG2 and VA-13, respectively, as well as MDR reversal activity. They are expected to become lead compounds for new types of anticancer agent or anti-MDR cancer agent.

In cancer chemotherapy, occurrence of multidrug resistance (MDR) in cancer cells caused by repeated administration of anticancer agents is a serious problem. One mechanism of MDR is overexpression of the P-glycoprotein (P-gp), which is the efflux pump of anticancer agent.[1,2] P-gp is a transporter for a wide range of reagents utilizing energy by hydrolysis of ATP. P-gp is expressed on the small intestine, capillary of brain, kidney, and liver. Its physiological role is considered as a defense mechanism against the toxic materials in the cell. When P-gp is expressed on the cell membrane of a cancer cell, it transports various kinds of anticancer agents from inside of the cell to the outside. Many taxane derivatives, which showed MDR reversal activity, have been reported.[3–11] We have previously reported the isolation of MDR reversal agents from *Taxus caspidata*,[12] their production by callus culture,[13] and the synthesis of taxinine NN-1, the most efficient MDR reversal agents in natural taxane.[12a,14] (Fig. 1).

Structure–activity relationship (SAR) studies of the functional groups of taxanes revealed that substitutions of the OH group at the C-7 position to hydrophobic side groups are effective for MDR reversal activity.[15]

Many taxane derivatives for SAR studies were synthesized from 10-deacetyl-baccatin III or 14β-hydroxybaccatin III with an acetoxy group at the C-4 position.[7,8,10,11] It has been reported that deacetylation at the C-4 position results in the loss of cytotoxicity in pacritaxel.[16]

Keywords: Taxoid; Taxinine; 1,7-Deoxy-4-deacetylbaccatin III; MDR; Cytotoxicity.
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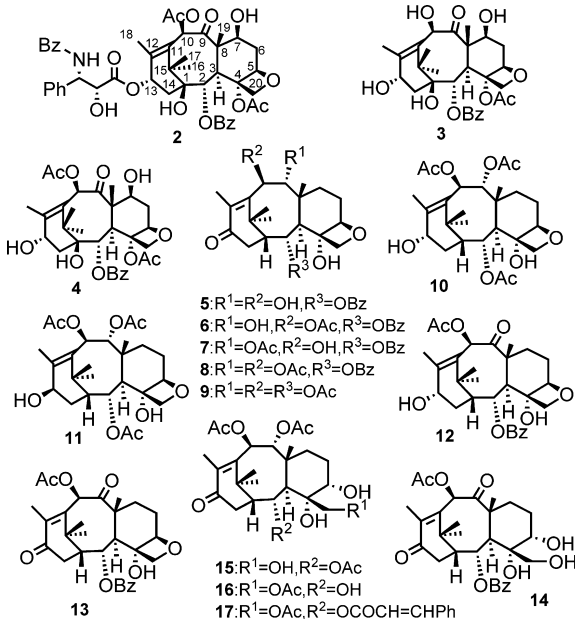


Figure 1.

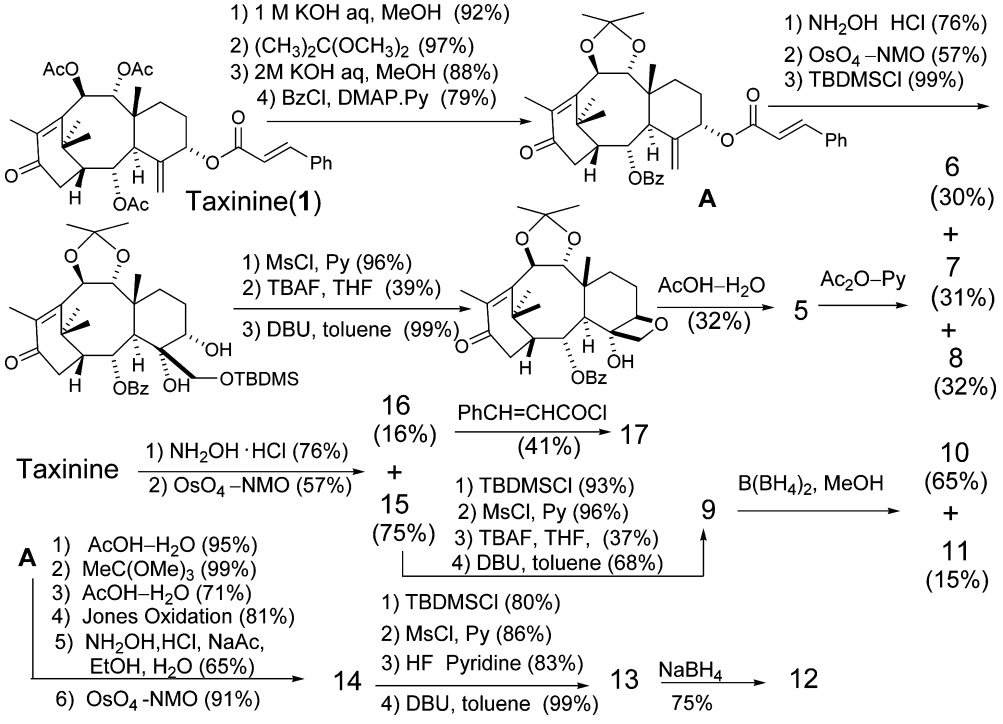
From the viewpoint of MDR reversal agent, it is desirable that the compound has no cytotoxicity and has high MDR reversal activity at the time when it is used with an anticancer reagent toward MDR cancer cells.

We synthesized some taxane derivatives with a 5β,20-epoxy ring (4,5-oxetane ring) and with hydroxyl group at the C-4 position from taxinine (**1**) by the method shown in Scheme 1. The effects of 15 kinds of taxane derivatives, **3**–**17,** on the cellular accumulation of calcein in MDR human ovarian cancer 2780AD cells were examined (Table 1).

Baccatin III (**4**) and 10-deacetyl baccatin III (**3**) showed no activity toward calcein accumulation in MDR 2780AD cells. Among the compounds possessing an oxetane ring, compounds **6**–**8** with a benzoyloxy group at C-2 and an acetoxy group at C-9 and/or C-10 showed significant effect on the calcein accumulation and the strength of their activities is almost the same. However the corresponding compound **5** with hydroxyl groups at both C-9 and C-10 showed weaker activity than those of **6**–**8**. These results suggested that compound **5** possessing hydroxyl groups at both C-9 and C-10 positions decreased the activity of the cellular accumulation of calcein in MDR 2780AD cells.

Compound **9** with a 13-carbonyl, 5β,20-epoxy ring (4,5-oxetane ring), and three acetoxyl groups at C-2, C-9, and C-10 showed significant activity toward calcein accumulation in MDR 2780AD cells. On the other hand, the corresponding 13α- and 13β-hydroxyl derivatives, **10** and **11**, the reduction products of the carbonyl group at C-13 of **9**, lost the activity.

The structure change of **6** to **13** is the hydroxyl group at C-9 of **6** to a carbonyl group of **13**. Since the activity of **6** and **13** is almost the same, the effect of carbonyl and hydroxyl groups at C-9 showed the same efficiency. Compound **12** with structure change of the 13-carbonyl group of **13** to a 13α-hydroxyl group showed further stronger activity. These results showed that the 13α-hydroxyl group is more efficient than the 13-carbonyl group in this case. The structure–activity relationship of compounds **9**–**13** probably indicated that the exis-



Scheme 1.

Table 1. Effects of compounds on the accumulation of calcein in 2780AD cells, and cytotoxicities of compounds against WI-38, VA-13, and HepG2 cells

Compound	Calcein accumulation[a,b] (% control)			Cytotoxicity IC_{50}[c] (μg/ml)		
	0.25μg/ml	2.5μg/ml	25μg/ml	WI-38	VA-13	HepG2
Verapamil	99	103	140	—	—	—
2	—	—	—	0.034	0.0043	6.90
3	95	90	93	—	—	—
4	93	84	81	16.1	0.84	9.91
5	105	110	106	>100	>100	67.7
6	99	102	140	42.3	>100	77.7
7	115	118	134	0.67	67.0	43.2
8	97	119	135	0.82	51.9	7.32
9	115	120	123	77.0	>100	>100
10	94	95	95	8.39	81.5	>100
11	82	95	96	76.2	>100	>100
12	96	129	163	54.2	77.0	69.9
13	101	120	137	7.38	7.93	30.1
14	88	114	112	57.4	59.6	33.4
15	79	94	88	85.3	9.73	>100
16	88	97	126	>100	8.47	>100
17	88	116	145	68.1	82.3	2.14

[a] The amount of calcein accumulated in multidrug-resistant human ovarian cancer 2780AD cells was determined compared with the control in the presence of 0.25, 2.5, and 25 μg/ml of test compounds and verapamil (positive control).
[b] The values are the relative amount of calcein accumulated in the cell compared with the control experiment. The values represent the mean of triplicate determination.
[c] IC_{50} represents the mean of duplicate determination.

tence of one carbonyl group at C-13 or C-9 is desirable for the expression of the activity.

Compound **12** showed the strongest activity among the compounds tested but **4** had no activity for calcein accumulation in MDR 2780AD cells. The structure changes from **4** to **12** occur at the C-1, C-4, and C-7 positions. Two hydroxyl groups at C-1 and C-7 of **4** were lost in **12** and the acetoxyl group at C-4 of **4** was displaced by a hydroxyl group in **12**. These changes of functional groups of **4** to **12** enhanced remarkably the accumulation of calcein in MDR 2780AD cells. Compound **14** possessing 5α-,20-dihydroxyl groups instead of a 5β,20-epoxy ring (4,5-oxetane ring) in **13** showed weaker activity than that of **13**. Analogously, compound **15** possessing 5α-,20-dihydroxyl groups instead of 5β,20-epoxy ring (4,5-oxetane ring) in **9** showed no activity of accumulation of calcein in 2780 AD cells, although **9** had significant activity. These results indicated that the 5β,20-epoxy ring (4,5-oxetane ring) is an important functional group and 5α-,20-dihydroxyl group has no effect for the expression of activity of the taxane derivatives above mentioned. On the contrary, compound **16** possessing 5α-hydroxy-20-acetoxy groups instead of the 5α,20-dihydroxyl groups of **15** showed significant activity. Compound **17**, 2-cinnamoyloxy derivative of **16,** showed more efficient activity than that of **16**. This enhancement of the activity was induced by the change of the functional group at C-2 from hydroxyl group to a cinnamoyloxyl group.

The above-mentioned compounds, **5**–**17**, are all oxygenated at C-2, C-4, C-5, C-9, C-10, C-13, and C-20 positions and synthesized for this research from taxinine, which is the major component of the Japanese yew tree, *T. caspidata*.

In 15 taxane derivatives tested in this research, 10 compounds showed moderate to strong activity on calcein accumulation in MDR 2780AD cells. These results seemed to indicate that the taxane skeleton has special meaning toward the MDR reversal activity although the strength of activity depends on the combination of the functional groups on the taxane skeleton.

We tested the effect of the compound **12** on the cytotoxicity of taxol, adriamycin (ADM), and vincristine (VCR) toward MDR 2780AD cells and its parental cells (A2780) comparing with verapamil, which is a well-known MDR reversal agent. The IC_{50} values of taxol for A2780 and MDR 2780AD cells were 0.7 and 535 nM, respectively (Table 2). When compound **12** was added at a final concentration of 0.2, 2.0, and 10 μM, the IC_{50} values of taxol for MDR 2780AD cells were shifted to 375, 10, and 1 nM, respectively. The enhancing effect of **12** for taxol was comparable to that of verapamil. Compound **12** is also effective for ADM and VCR. The IC_{50} values of ADM for A2780 and MDR 2780AD cells were 13 and 909 nM, respectively. The IC_{50} values for MDR 2780AD cells were shifted to 556, 107, and 17 nM in the presence of **12** at a final concentration of 0.2, 2.0, and 10 μM, respectively. The IC_{50} values of VCR for A2780 and 2780AD cells were 0.9 and 895 nM, respectively. The IC_{50} values for MDR 2780AD cells were shifted to 336, 30, and 7 nM in the presence of **12** at a final concentration of 0.2, 2.0, and 10 μM, respectively. The enhancing effects of **12** for taxol, ADM, and VCR were the same levels as that of verapamil toward MDR 2780AD cells. On the other hand, compound **12** showed no enhancing effect for taxol, ADM, and VCR toward the parental 2780 cells. Thus, compound **12** can modulate the multidrug resistance of cancer cells as well as verapamil in vitro.

Table 2. Effect of compound **12** on the cytotoxicity of anticancer agents toward A2780 and MDR 2780AD cells[a]

Cell lines	MDR modulator (μM)		IC_{50} (nM) of anticancer agents		
			Taxol	ADM	VCR
2780	No modulator		0.7	13	0.9
	Verapamil	0.2	0.7	11	1
		2	0.8	9	0.8
		10	0.9	14	1.1
	12	0.2	0.7	8	1
		2	0.9	10	0.8
		10	0.8	7	0.8
2780AD	No modulator		535	909	895
	Verapamil	0.2	197	615	563
		2	11	109	29
		10	0.9	59	7
	12	0.2	375	556	336
		2	10	107	30
		10	1	17	7

The values represent the mean of triplicate determination.

[a] Enhancing effects of verapamil and compound **12** on the cytotoxicity of Taxol, adriamycin (ADM), and vincristin (VCR) toward A2780 cells and MDR A2780 (2780AD) cells were determined in the presence of 0.2, 2.0, and 10 μM of each compound.

Cell growth inhibitory activity (IC_{50}) of compounds **4**–**17** to three different cell lines was examined (Table 1). The three cell lines employed in this experiment are human lung fibroblast cells (WI-38), malignant lung tumor cells (VA-13) induced from WI-38, and human liver cancer Hepatoma G2 cells (HepG2). Compound **8** showed the smallest IC_{50} values toward VA-13 and HepG2 in compounds **5**–**8**. The results suggested that displacement of the acetoxyl group at C-9 and/or C-10 of **8** with a hydroxyl group induced the decrease of activity in **5**, **6**, and **7**. Cytotoxic activity of **9** against WI-38, VA-13, and HepG2 decreased remarkably on displacement of the 2α-benzoyloyl group of **8** with an acetoxyl group. Compound **13** showed moderate and weak activities to VA-13 and HepG2, respectively. Compound **14** with 5α-,20-dihydroxyl groups instead of 5α,20-oxetane ring in **13** showed weaker activity to VA-13 than that of **13**. Compounds **15** and **16** with a 5α-,20-dihydroxyl and 5α-hydroxy-20-acetoxy groups, respectively, showed significant activity toward VA-13. On the contrary, compound **17**, 2-cinnamoyloxy derivative of **16**, showed significant activity not to VA-13 but HepG2. These results suggested that some modifications of the functional groups on the taxane skeleton induced a new cytotoxicity to a different cell line.

Compounds **6**, **9**, and **12** showed MDR reversal activities but have no cytotoxicity. These compounds are expected to be lead compounds of MDR cancer reversal agents. On the other hand, cytotoxic activity of compounds **8** and**17** against HepG2 is the same level or more than that of taxol (**2**) or baccatin III (**4**). Since compounds **8** and **17** also showed significant MDR reversal activity, they are expected as lead compounds of new type anticancer agents. Compounds **13**, **15**, and **16** showed significant cytotoxic activity toward VA-13. Among them, **13** and **16** showed significant MDR reversal activity and are expected to be a new type of anticancer agents.

Compound **13** with a carbonyl group at C-13 showed both MDR reversing and cytotoxic activities in vitro. On the other hand, the corresponding 13α-hydroxy derivative **12** showed efficient MDR reversing activity but its cytotoxic activity decreased drastically. Introduction of an isoserine moiety to the 13α-hydroxyl group of taxane derivatives increases cytotoxic activity remarkably as shown in the change from baccatin III (**4**) to taxol (**2**) in Table 1. Compound **12** is also expected to be a lead compounds of anti-MDR cancer agents or anticancer agents after introduction of the isoserine moiety on the13α-hydroxyl group of **12**.

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