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Synthesis and structure–activity relationships of taxuyunnanine C derivatives as multidrug resistance modulator in MDR cancer cells

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Abstract—A series of new generation taxoids bearing a bulky group on different positions such as C-2, C-5, C-7, C-9, C-10 or C-14 were obtained by chemical modifications and biotransformation of taxuyunnanine C (**1**) and its analogs, **4**, **5**, and **10**. Compounds **3**, **5**, **6**, **8**, and **9a** showed significant activity toward calcein accumulation in MDR 2780AD cells. The most effective compound **9a** with a cinnamoyloxy group at C-14 and a hydroxyl group at C-10 was actually efficient for the cellular accumulation of the anticancer agent, vincristine, in MDR 2780AD cells. The enhancing effects of **6** and **9a** for taxol, adriamycin, and vincristine were at the same levels as those of verapamil toward MDR 2780AD cells. Thus, compounds **6** and **9a** can modulate the multidrug resistance of cancer cells. The cytotoxicity (IC_{50}) of the compounds was examined against human normal cell line, WI-38, and cancer model cell lines, VA-13 and HepG2. Since compounds **6** and **8** had no cytotoxicity, they were expected to be lead compounds of MDR cancer reversal agents. On the contrary, compounds **3**, **5**, and **9a** showed cell growth inhibitory activity toward VA-13 and/or HepG2 as well as accumulation activity of calcein and/or vincristine in MDR 2780AD and they were expected to be lead compounds of new-type anticancer agents.

In cancer chemotherapy, occurrence of multidrug resistance (MDR) of cancer cells caused by repeated administration of anticancer agents is a serious problem. One of the mechanisms of MDR is overexpression of P-glycoprotein (P-gp),[1,2] P-gp is originally a transporter for a wide range of reagents and its physiological role is a defense mechanism against the toxic materials in cells. P-gp utilizes energy induced by hydrolysis of ATP for their transport from inside to the outside of cells.

Many taxane derivatives with MDR reversal activity have been reported.[3–11] Effect of the substituents of baccatin III on the MDR reversal activity was widely investigated. However, since baccatin III has no functional group at C-14, only a few reports appeared about the effect of the substituent at C-14 on the MDR reversal activity. Ojima et al. reported structure–activity relationships of 14β-hydroxybaccatin III derivatives with 1,14-carbonate moiety. In this case, hydrophobic groups at C-7 are effective in MDR reversal activity.[12]

Taxuyunnanine C (**1**) and its 14-acyloxy analogs (**2**–**5**) are the major metabolites from callus cultures of *Taxus* species in high yields. We reported their activity toward the accumulation of vincristine in MDR 2780AD cells

Keywords: Taxoid; Taxuyunnanine C; MDR; Cytotoxicity.
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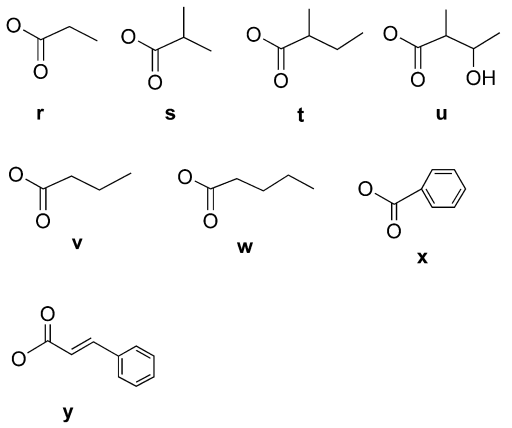
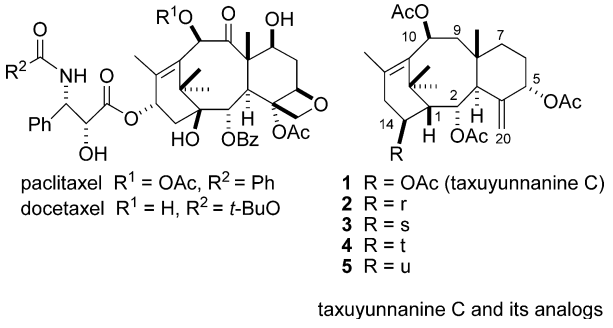


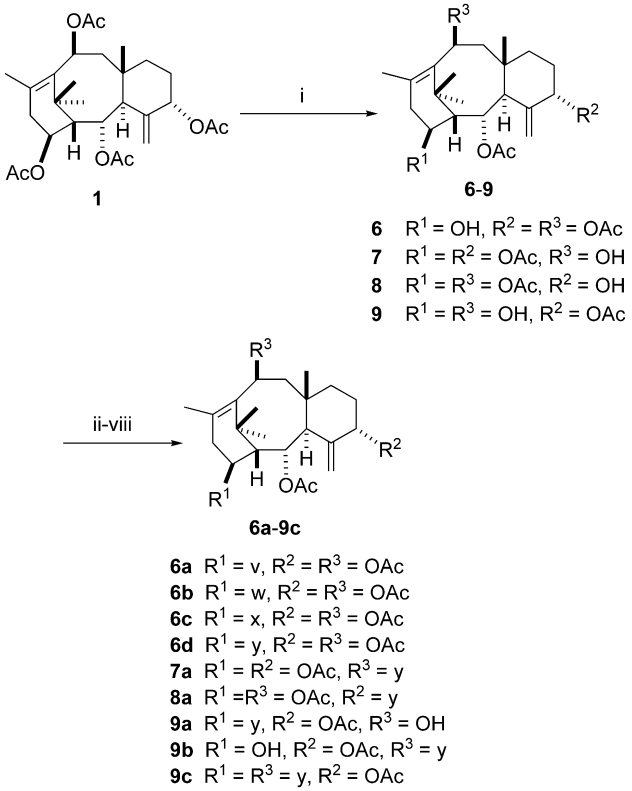
Figure 1. Acyloxy groups *r–y* in the structures of taxuyunnanine C derivatives.

and deduced that a hydrophobic less-hindered alkyl side chain of the C-14 acyloxy group of these compounds played some role to increase the activity.[13]

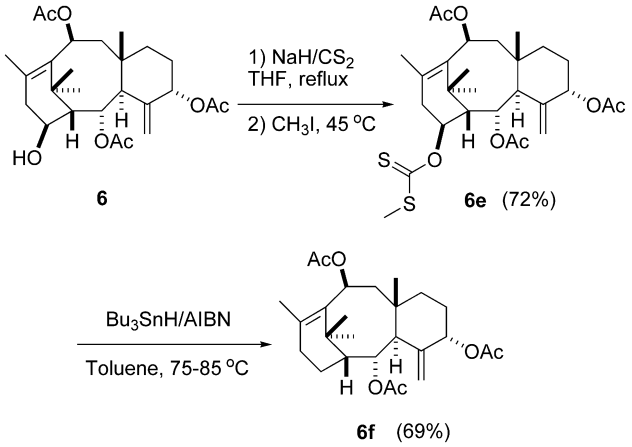


As a part of our study of structure–activity relationships (SAR) of taxoids as MDR reversal agent, we further investigated effect of the substitution of the functional groups at C-14 position on MDR reversal activity. For this purpose, we synthesized a series of new 14-oxygenated taxoids derived from taxuyunnanine C (**1**) and its analogs which are produced by callus cultures of *Taxus cuspidata*.[13–15] (Fig. 1).

We obtained 14-deacetyltaxuyunnanine C (**6**), 10-deacetyltaxuyunnanine C (**7**), 5-deacetyltaxuyunnanine C (**8**), and 10,14-dideacetyltaxuyunnanine C (**9**) by hydrolysis of **1**. 14-Butanoyloxy- (**6a**), 14-pentanoyloxy- (**6b**), 14-benzoyloxy- (**6c**), and 14-cinnamoyloxy- (**6d**) derivatives of **6** were obtained by the acylation of **6** by the corresponding acylchloride. Cinnamoylation of 10-deacetyltaxuyunnanine C (**7**) and 5-deacetyltaxuyunnanine C (**8**) gave **7a** and **8a** bearing a cinnamoyloxy group at C-10 or C-5, respectively. Cinnamoylation of 10,14-dideacetyltaxuyunnanine C (**9**) gave 10- and 14-monocinnamoyloxy analogs, **9b** and **9a**, and 10,14-dicinnamoyloxy analog **9c**, respectively (Scheme 1). 14-Deacetoxytaxuyunnanine C (**6f**) was obtained by Chugaev reaction of **6** (Scheme 2). 2-Benzoyloxy analog, **10a**, was prepared from 2-deacetyltaxuyunnanine C (**10**), which was isolated from the callus culture of *T. cuspidata* (Scheme 3) as a minor product.[16]

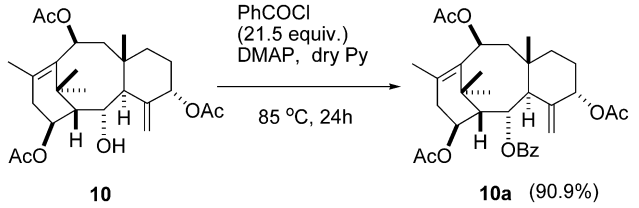


Scheme 1. Reagents and conditions: (i) **1**, 1 M K_2CO_3 (5 equiv), 1:3 THF/MeOH, 45 °C, 7 h (23.9% for **6**, 4.3% for **7**, 0.5% for **8**, 20.7% for **9**); (ii) **6**, $CH_3(CH_2)_2COCl$ (13 equiv), DMAP, dry Py, 80 °C, 12.5 h (87.6% for **6a**); (iii) **6**, $CH_3(CH_2)_3COCl$ (13 equiv), DMAP, dry Py, 80 °C, 13.5 h (88.0% for **6b**); (iv) **6**, PhCOCl (10 equiv), DMAP, dry Py, 80 °C, 15 h (92.7% for **6c**); (v) **6**, PhCH=CHCOCl (8 equiv), DMAP, dry Py, 85 °C, 16 h (92.8% for **6d**); (vi) **7**, PhCH=CHCOCl (8 equiv), DMAP, dry Py, 85 °C, 13 h (91.5% for **7a**); (vii) **8**, PhCH=CHCOCl (20 equiv), DMAP, dry Py, 85 °C, 19h (80% for **8a**); (viii) **9**, PhCH=CHCOCl (2.2 equiv), DMAP, dry Py, 85–90 °C, 23 h (8.4% for **9a**, 29.1% for **9b**, 5.9% for **9c**).



Scheme 2.

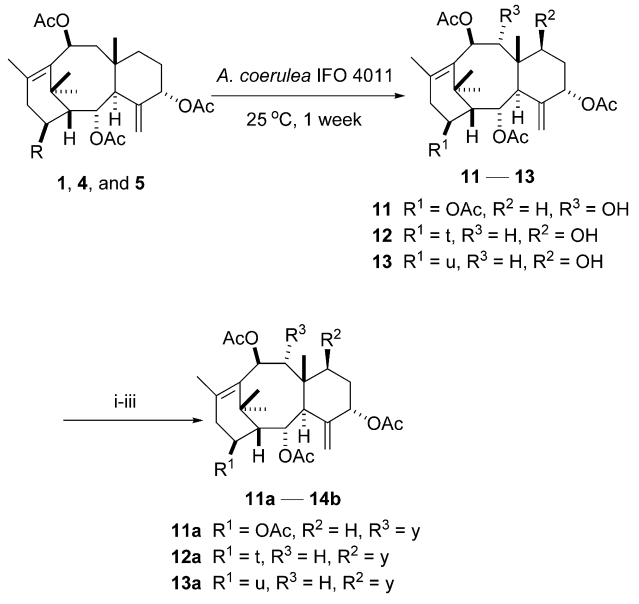
For the preparation of 7- or 9-oxygenated derivatives of **1**, **4**, and **5**, we used a biotransformation. Thus, 14-acyloxytaxoids, **11**–**13**, bearing a hydroxyl group at the C-7 or C-9 position were obtained by treatment of **1**, **4**, and **5** with *Absidia coerulea* IFO 4011. Compounds **11**–**13**



Scheme 3.

were further converted to the corresponding cinnamoyl esters, **11a**, **12a**, and **13a** (Scheme 4).

The effects of 14-oxygenated taxoids mentioned above on the cellular accumulation of calcein in MDR human ovarian cancer 2780AD cells were examined (Table 1). The calcein derived from calcein AM by endogenous esterase was used as an easily operated functional fluorescent probe for this drug efflux protein. Taxuyunnanine C (**1**), its monodeacetyl analogs, **6**–**8** and **10**, and its 9-hydroxylated analog (**11**) showed weak to significant activity toward calcein accumulation in MDR 2780AD cells. In them, compounds **6** and **8**, in which the acetoxyl groups at C-14 or C-5 of **1** were displaced by a hydroxyl group, respectively, showed stronger activity than that of **1**. On the other hand, compounds **7**, **10**, and **11**, in which the acetoxy groups at C-10 or C-2, or one hydrogen at C-9, of **1** were displaced by a hydroxyl group, respectively, showed weaker activity than **1**. Taxoid **6f** in which the acetoxy group at C-14 of **1** was displaced by hydrogen, showed no activity. Compounds **12** and **13** with a hydroxyl group at the C-7 position showed lower activity than those of their parental compounds, **4** and **5**. These results of bioassay suggested that the substitution of the acetoxyl group at C-14 or C-5 of **1** with a more polar substituent such as a hydroxyl group is desirable to increase the activity toward calcein accumulation in MDR 2780AD cells. On the other hand, the substitution of the acetoxyl group at C-2, C-10 or one hydrogen at C-9 of **1** or at C-7 of **4** and **5** with a more polar substituent such as a hydroxyl group is an undesirable change to increase the activity.



Scheme 4. Reagents and conditions: (i) **11**, PhCH=CHCOCl (10 equiv), DMAP, dry Py, 85–90 °C, 9 h (84.6% for **11a**); (ii) **12**, PhCH=CHCOCl (10 equiv), DMAP, dry Py, 85–90 °C, 10 h (88.8% for **12a**); (iii) **13**, PhCH=CHCOCl (6 equiv), DMAP, dry Py, 85–90 °C, 5.5 h (13.9% for **13a**).

Then, the activities of **1**, its seven kinds of C-14 acyloxy analogs (**2**–**5** and **6a**–**d**), 14-deacetyltaxuyunnanine C (**6**), and 14-deacetoxytaxuyunnanine C (**6f**) were compared. The activity order of the compounds was **5** > **6** = **3** > **6c** ⩾ **1** = **6d** ⩾ **2** = **4** ⩾ **6a** ⩾ **6b** ≫ **6f**. These results suggested that the compounds with hydrophilic substituents at C-14 such as **5** and **6**, and the compounds with bulky acyloxy substituents at C-14 such as **3** and **6c** are desirable to increase the activity. On the contrary, the compounds possessing acyloxy groups with less-hindered hydrophobic propyl and butyl side chains such as **6a** and **6b** showed lower activity than the parental compound **1**. Compound **6f**, in which the acetoxy group at C-14 of **1** was displaced by hydrogen, lost the activity completely.

We examined whether functional group at C-14 influenced the effect of structural change at the different position of taxane skeleton on the calcein accumulating activity. When the functional group at C-14 is acetoxyl group such as **7** and **7a**, the activity of **7** with hydroxyl group at C-10 was less than that of **7a** with cinnamoyloxy group at C-10. On the other hand, when functional group at C-14 is cinnamoyl group such as **9a** and **9c**, the activity of **9a** with hydroxyl group at C-10 was higher than that of **9c** with cinnamoyloxy group at C-10. When the functional groups at C-14 are 2-methylbutanoyloxy group such as **12** and **12a**, and 2-methyl-3-hydroxybutanoyloxy group such as **13** and **13a**, the activity of **12** and **13** with hydroxyl group at C-7 and **12a** with cinnamoyloxy group at C-7 was at same level. On the other hand, the activity of **13a** with cinnamoyloxy group at C-7 was higher than that of **13** with hydroxyl group at C-7. Thus, the influences of the functional groups at C-14 on the calcein accumulating activity seemed to be complicated.

Since taxinine derivatives, taxinine NN-1 and taxinine NN-11 bearing a cinnamoyloxy group, exhibited strong MDR reversal activity, we expected the special effect of an aromatic acyloxy group such as a cinnamoyloxy group or a benzoyloxy group on taxuyunnanine C derivatives in calcein accumulation in MDR 2780AD cells. We examined the activity of ten kinds of cinnamoyloxy analogs of **1**. The order of activity was **9a** > **13a** > **7a** = **6d** = **10a** = **11a** ⩾**8a** > **9b** > **12a** ≫ **9c**. In them, compound **9a** with a cinnamoyloxy group at C-14 and a hydroxyl group at C-10 showed the strongest activity. On the contrary, **9b**, regioisomer of **9a**, with a cinnamoyloxy group at C-10 and a hydroxyl group at C-14 showed very weak activity. Compound **13a** with a cinnamoyloxy group at C-7 and a polar acyloxy group, 2-methyl-3-hydroxybutanoyloxy group at C-14 showed significant activity. Interestingly, **12a** with a cinnamoyloxy group at C-7 and less polar 2-methylbutanoyloxy group at C-14 lost activity. Other analogs of

Table 1. Effects of compounds on the accumulation of calcein in multidrug resistant cells 2780AD and cell growth inhibitory activities of compounds against WI-38, VA-13, and HepG2 cells

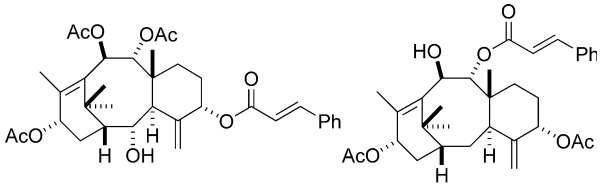
Compound	Calcein accumulation (% of control)[a,b]			Cell growth inhibitory activity (IC_{50} μM)[c]		
	0.25 μg/ml	2.5 μg/ml	25 μg/ml	WI-38	VA-13	HepG2
Paclitaxel				0.04	0.005	8.1
Adriamycin				0.66	0.38	1.2
1	130	141	127	130	53.1	18.5
2	104	98	119	>193	93.7	86.4
3	133	139	148	>188	90.9	28.0
4	100	86	119	>183	61.1	41.7
5	81	94	168	79.4	9.29	18.5
6	104	86	148	133	80.0	216
6a	97	98	114	73.4	17.2	18.5
6b	94	88	111	>183	56.7	16.6
6c	90	107	133	>176	>176	144
6d	123	126	125	154	>169	>169
6f	89	105	91	8.93	12.2	10.3
7	103	98	111	105	12.6	108
7a	92	123	126	>169	>169	106
8	118	118	141	149	105	183
8a	102	95	121	99.0	125	9.95
9a	107	132	190	13.5	37.4	15.7
9b	109	113	113	71.7	11.5	15.3
9c	83	80	80	>147	>147	>147
10	99	99	111	130.4	104	122
10a	98	96	125	125	175	51.9
11	102	101	110	129	>192	128
11a	102	103	125	151	>154	>154
12	94	98	110	81.6	79.1	75.2
12a	96	98	102	>144	>144	>144
13	87	104	107	140	>173	153
13a	97	102	130	62.4	49.9	80.4

[a] The amount of calcein accumulated in multidrug-resistant human ovarian cancer 2780AD cells was determined with the control in the presence of 0.25, 2.5 and 25 μg/ml of test compounds.

[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.

1, **6d**, **7a**, **8a**, and **11a** which possess one cinnamoyloxy group at C-14, C-10, C-5, and C-9, and compound **10a** with one benzoyloxy group at C-2 showed almost the same activity as that of **1**. Compound **9c** with two cinnamoyloxy groups at C-10 and C-14 showed no activity. In conclusion, compound **9a** with a cinnamoyloxy group at C-14 and a hydroxyl group at C-10 has the strongest activity in 26 test samples of taxuyunnanine C analogs toward calcein accumulation in MDR 2780AD cells.



taxinine NN-1 taxinine NN-11

Then, we tested the effects of several taxoids, **6**, **7**, **10**, **11**, **6d**, **7a**, **9a**–**c**, on the accumulation of a widely used anticancer agent, vincristine (VCR), in MDR 2780AD cells comparing with the effects of verapamil as a positive control (Table 2). Compound **9a** possessing a hydroxyl group at the C-10 position and a cinnamoyloxy group at the C-14 position showed the highest activity of cellular accumulation of vincristine in MDR 2780AD cells among the taxoids subjected to the test. Compound **9b**, regioisomer of **9a**, with a cinnamoyloxy group at C-10 and a hydroxyl group at C-14 showed weaker activity than **9a**. Taxoid **9c** bearing cinnamoyloxy groups at both C-10 and C-14 positions showed very weak activity. The activity of taxoid **7a** with a cinnamoyloxy group at the C-10 position was higher than that of taxioid **7** with a hydroxyl group at the corresponding positions. The results were similar to the calcein assay though there were some little differences.

The effects of compounds **6** and **9a** on the cytotoxicity of taxol, adriamycin (ADM), and vincristine (VCR) toward MDR 2780AD cells and its parental cells (A2780) were tested comparing with verapamil, which is a well-known MDR reversal agent (Table 3).

The IC_{50} values of taxol for A2780 and MDR 2780AD cells were 2.8 and 183 nM, respectively. When compound **6** 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 21, 26, and 5.3 nM, respectively. At this time, cytotoxicity of compound **6** against 2780AD cells was equal to that of verapamil, showing more than 70% of cell viability at the concentration of

Table 2. Effects of compounds on the accumulation of vincristine (VCR) in multidrug-resistant cells 2780AD[a]

	Concd (μg/mL)	Average[b] (dpm/well)	% of control[c]	Activities[d]	Verapamil[e] (%)
6	0.1	360	102	±	94
	1	517	147	+	79
	10	853	242	+	53
6d	0.1	336	95	±	87
	1	494	140	+	75
	10	985	280	+	62
7	0.1	324	92	±	84
	1	420	119	+	64
	10	805	229	+	51
7a	0.1	404	132	+	130
	1	641	209	+	118
	10	1385	451	++	81
9a	0.1	425	121	+	111
	1	959	272	+	146
	10	1855	527	+++	116
9b	0.1	402	114	+	105
	1	669	190	+	102
	10	1749	497	++	110
9c	0.1	320	91	±	83
	1	325	92	±	49
	10	429	122	+	27
10	0.1	306	101	±	89
	1	436	144	+	84
	10	786	259	+	74
11	0.1	303	86	—	79
	1	379	108	±	58
	10	592	168	+	37

[a]The amount of VCR accumulated in MDR2780AD cells was determined with the control in the presence of 0.1, 1, and 10 μg/ml of taxoids.
[b] The values represents triplicated determinations.
[c] The values are the relative amount of VCR accumulated in the cell compared with the control experiment.
[d] The indices are expressed on a scale of seven by the range of the relative amount of VCR accumulation as compared with the control experiment(%): +++, 501–1000%; ++, 301–500%; +, 111–300%; ±, 91–100%; —, <90%.
[e] The values are expressed as the relative amount of vincristine(VCR) accumulation in the cell as compared with that of verapamil.

Table 3. Effect of the compounds on the cytotoxicity of anticancer agents toward A2780 and MDR 2780AD cells

Cell lines	MDR modulator (μM)		Viability of the cell (%)[a]	IC_{50} (nM) of anticancer agents[b]		
				Taxol	ADM	VCR
A2780	No modulator	0	100	2.8	5.7	3.1
	Verapamil	0.2	93	2.7	5.8	3.4
		2	82	2.9	6.0	2.0
		10	53	3.5	7.7	0.5
	6	0.2	97	2.6	7.0	2.6
		2	95	2.3	6.0	1.6
		10	20	6.6	7.8	4.8
	9a	0.2	89	2.6	7.0	2.6
		2	74	2.3	6.0	1.6
		10	27	6.6	7.8	4.8
2780AD	No modulator	0	100	183	298	311
	Verapamil	0.2	84	36	269	41
		2	90	5.5	201	25
		10	71	4.9	164	3.9
	6	0.2	101	21	215	55
		2	96	26	256	26
		10	77	5.3	181	11
	9a	0.2	105	22	284	51
		2	86	4.2	138	39
		10	38	3.9	73	23

[a] Cyhotoxicity of the compounds was evaluated in the absence of the anticancer agents.
[b] Enhancing effects of the compounds 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 mM of each compound. The values represent means of triplicate determination.

10 μM, while compound **6** showed significant cytotoxicity against A2780 cells showing 20% of cell viability at the same concentration. From these results, compound **6** enhanced the sensitivity to taxol in MDR 2780AD cells. The IC_{50} of taxol for 2780AD cells in the presence of 10 μM of compound **6** was equal to that for parental A2780 cells (Table 4).

Compound **6** is also effective for ADM and VCR. The IC_{50} values of ADM for A2780 and MDR 2780AD cells were 5.7 nM and 298 nM, respectively. The IC_{50} values for MDR 2780AD cells were shifted to 215, 256, and 181 nM in the presence of **6** 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 3.1 nM and 311 nM, respectively. The IC_{50} values for MDR 2780AD cells were shifted to 55, 26, and 11 nM in the presence of **6** at a final concentration of 0.2, 2.0, and 10 μM, respectively. The enhancing effects of **6** for taxol, ADM, and VCR were at the same levels as that of verapamil toward MDR 2780AD cells. Thus, compound **6** can modulate the multidrug resistance of cancer cells as well as verapamil in vitro.

Cytotoxicity of compound **9a** against 2780AD cells in the absence of anticancer agents was more significant than that of compound **6**, showing 38% of cell viability at the concentration of 10 μM, while the viability of the cell was 27% in the case of parental A2780 cells at the same concentration of compound **9a**. IC_{50} of compound**9a** for MDR 2780AD and parental A2780 was calculated as 8.0 and 6.1 μM, respectively. Thus, compound **9a** showed MDR reversal activity against 2780AD cells.

When compound **9a** 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 22, 4.2, and 3.9 nM, respectively. These results showed that compound **9a** enhanced the sensitivity to taxol in MDR 2780AD cells.

Compound **9a** is also effective for ADM and VCR. The IC_{50} values of ADM for A2780 and MDR 2780AD cells were 5.7 nM and 298 nM, respectively. The IC_{50} values for MDR 2780AD cells were shifted to 284, 138, and 73 nM in the presence of **9a** 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 3.1 nM and 311 nM, respectively. The IC_{50} values for MDR 2780AD cells were shifted to 51, 39, and 23 nM in the presence of **9a** at a final concentration of 0.2, 2.0, and 10 μM, respectively. Thus, compound **9a** can modulate the multidrug resistance of cancer cells as well as verapamil in vitro.

Cell growth inhibitory activity (IC_{50}) of taxoids 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 (HepG2) cells.

Taxuyunnanine C (**1**) and its analogs **6**–**8**, **10**, **11** showed weak activity in many cases. Only taxuyunnanine C (**1**) and its C-10 deacetyl taxuyunnanine C (**7**) showed significant activity against HepG2 and VA-13, respectively. On the contrary, 14-deacetoxytaxuyunnanine C (**6f**) showed significant activities to all three cell lines. This result contrasted with the result of calcein assay and suggested that the modification of the C-14 position of the taxane skeleton significantly affected the biological activities.

The cytotoxic activity on HepG2 of taxuyunnanine C analogs, **2**–**4**, **6a**, and **6b**, bearing different kinds of acyloxy groups at C-14 increased according to the increase of hydrophobicity of their acyloxy group at C-14. In them, **6a** also showed significant cytotoxic activity on VA-13 in addition to the activity on HepG2. However, these compounds showed very weak or no cytotoxic activity against normal cell line, WI-38. This is a desirable result in the screening of anticancer agents.

The cytotoxicity of **7a** was examined, in the results of in vitro primary screening of **7a** based on the 39 human cancer cell lines[17,18] shown in Table 4. Although the effective concentration of **7a** is rather high, differential growth inhibition is recognized. Since the result of COMPARE of **7a** is marginal ($0.5 < r < 0.75$), it possibly belongs to a unique mechanistic class and is a new member of anticancer agents. On the other hand, cell growth inhibition effect (MG-MID of $GI_{50} = -4.41$) and differential growth inhibition of compound **7a** are weak. The result suggests that compound **7a** is not effective as an anticancer agent.

Compounds **6**, **6c**, and **8** showed the activity toward calcein accumulation in MDR 2780AD cells 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 **6f** and **8a** against HepG2 is at the same level of paclitaxel. Since compound **8a** showed moderate activity toward calcein accumulation in MDR 2780AD cells, it is expected as lead compound of new-type anticancer agents. Compounds **5**, **6a**, **7**, and **9b** showed significant cytotoxic activity toward VA-13.

Table 4. Summary of evaluation of compound **7a** based on the 39 human cancer cell lines

	GI_{50}	TGI	LC_{50}
Parameters of effective concentrations			
MG-MID	−4.41	−4.04	−4.00

Rank	Compound	r[a]	Molecular targets/drug type
Results of the COMPARE analysis			
1	ICRF-154	0.581	Topoisomerase
2	ICRF-193	0.580	Topoisomerase
3	TNP-470	0.552	

The mean graph of compound **7a** was compared with those of 200 standard compounds using the COMPARE analysis. Drugs were ordered according to the correlation coefficient. Drugs with correlation coefficients higher than 0.5 ($P < 0.001$) were included.

[a] Pearson's correlation coefficient.

Compound **5** showed significant cytotoxic activity toward VA-13 and HepG2 along with the strong activity toward calcein accumulation in MDR 2780AD cells. Compound **3** showed significant cytotoxic activity toward HepG2 along with the strong activity toward calcein accumulation in MDR 2780AD cells. Compound **9a** showed significant cytotoxic activity toward WI-13 and HepG2 along with the strongest activity in tested samples toward calcein accumulation in MDR 2780AD cells. They are expected to be lead compounds of anti-MDR cancer agents or anticancer agents.

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