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Modulation of Multidrug Resistance in Tumor Cells by Taxinine Derivatives

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Abstract: Among a series of taxinine (**1**) and its designed derivatives (**2** ~ **33**), two taxoids (**29** and **33**) increased cellular accumulation of vincristine in multidrug-resistant tumor cells more potently than verapamil, while the activities of eight taxoids (**11**, **14** ~ **16**, **22**, and **30** ~ **32**) were comparable with that of verapamil. These results reveal that some taxinine derivatives are good modifiers of multidrug resistance in tumor cells.

Keywords: biologically active compounds; glycopeptides/glycoproteins; molecular design; taxoids

When tumor cells acquire resistance against a certain chemotherapeutic drug, they often show cross resistance to a variety of antitumor drugs. The mechanism of multidrug resistance (MDR) has been well studied, and it has been found that P-glycoprotein, an efflux pump for hydrophobic antitumor drugs, plays a key role in MDR. Some compounds such as verapamil and cyclosporin A have been reported to reverse MDR *in vitro* and *in vivo*. The reversal agents competitively inhibit the binding of antitumor drugs to P-glycoprotein in MDR tumor cells, and increase the intracellular accumulation of antitumor drugs and overcome MDR.[1~4] We previously reported that among a number of new and known taxoids isolated from the Japanese yew *Taxus cuspidata*,[5,6] some non-taxol-type taxoids having neither an oxetane ring at C-4 and C-5 nor an *N*-acylphenylisoserine group at C-13 increased cellular accumulation of vincristine (VCR) in multidrug-resistant tumor cells as potent as verapamil, and efficiently inhibited [^{3}H]-azidopine photolabeling of P-glycoprotein.[5,6] More recently, we also found that taxuspine C (**1**) enhanced the chemotherapeutic effect of VCR in P388/VCR-bearing mice.[7] These results indicate that the taxoids showing such potent activity possess in common a cinnamoyl or a 3-*N*,*N*-dimethylamino-3-phenylpropanoyl group at C-5. In this paper we describe the preparation of various derivatives (**2** ~ **33**) of taxinine (**1**), and the effects of them to cellular accumulation of VCR in MDR tumor cells.

Derivatization of Taxinine

Taxinine (**1**), a major taxoid isolated from the Japanese yew *Taxus cuspidata*,[5] was chemically modified to yield compounds **2** ~ **33** as follows.

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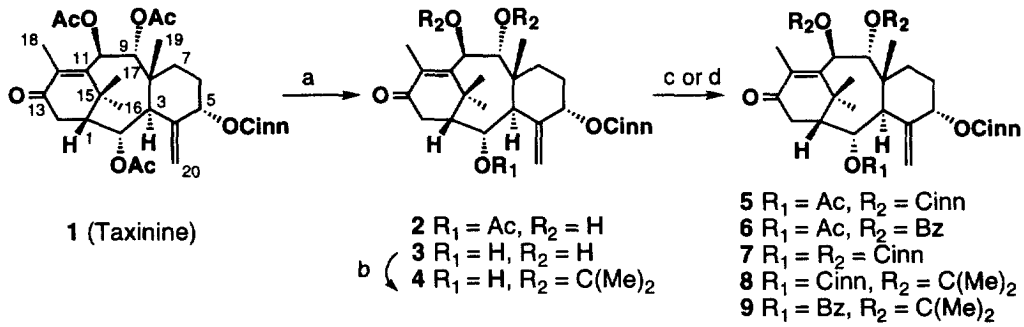
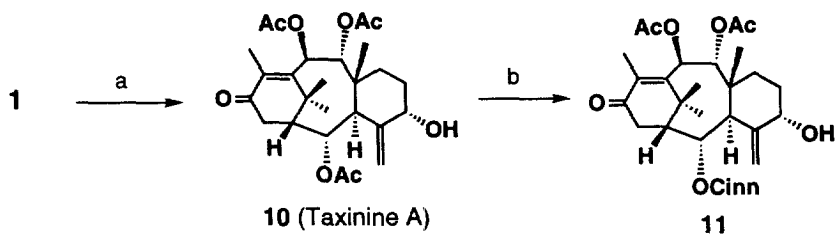
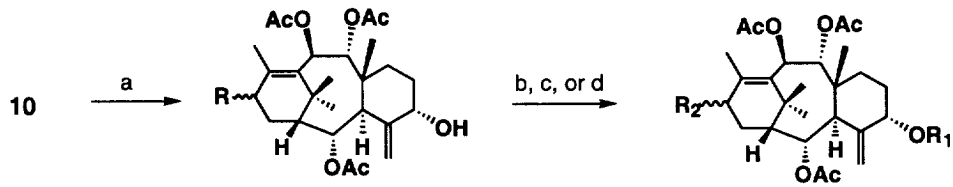


Scheme 1. a) K_2CO_3, MeOH, dioxane, rt [**2** (91%) for 80 min; **2** (70%) and **3** (30%) for 6 h]; b) $(CH_3)_2C(OMe)_2$, PPTS, CH_2Cl_2, rt, 8 h, 60%; c) Cinnamic acid, DCC, CH_2Cl_2, rt [**2** to **5** (28%) for 1 h; **3** to **7** (24%) for 6 h; **4** to **8** (60%) for 12 h]; d) BzCl, pyr., rt [**2** to **6** (56%) for 12 h; **3** to **9** (96%) for 13 h]

Methanolysis of taxinine (**1**) with K_2CO_3 in MeOH at room temperature for 80 min gave 9,10-di-*O*-deacetyltaxinine (**2**, 91%), while the reaction continued for 6h afforded a mixture of **2** (70%) and 2,9,10-tri-*O*-deacetyltaxinine (**3**, 30%), which were separated by a silica gel column (hexane/EtOAc, 2:1).[8,9] 9,10-Acetonide (**4**) was derived from **3** with 2,2-dimethoxypropane in CH_2Cl_2 containing pyridinium *p*-toluenesulfonate (PPTS). Esterification of compounds **2**, **3**, and **4** with cinnamic acid and 1,3-dicyclohexylcarbodiimide (DCC) afforded the 9,10-di-*O*-cinnamate (**5**, 28%), 2,9,10-tri-*O*-cinnamate (**7**, 24%), and 2-*O*-cinnamate (**8**, 60%), respectively (Scheme 1). On the other hand, treatment of **2** and **4** with benzoyl chloride in pyridine gave the 9,10-di-*O*-benzoate (**6**, 56%) and 2-*O*-benzoate (**9**, 96%), respectively (Scheme 1). Hydrolysis of taxinine (**1**) with hydroxylamine sulfate in EtOH/THF/H_2O (1:1:1) containing triethylamine afforded taxinine A (**10**, 80%).[10] Protection of the 5-OH group in **10** with triethylsilyl chloride (TESCl) and imidazole followed by treatment with DIBAL-H in anhydrous THF afforded 2-*O*-deacetyl-5-*O*-TES-taxinine A (31%, 2 steps), which was esterified with cinnamic acid and DCC followed by treatment with tetrabutylammonium fluoride (TBAF) to yield compound **11** (40%, 2 steps) (Scheme 2).



Scheme 2. a) $NH_2OH{\bullet}H_2SO_4$, Et_3N, THF/EtOH/H_2O (1:1:1), reflux, 8 h, 80%; b) (i) TESCl, imidazole, CH_2Cl_2, rt, 4 h, 100%; (ii) DIBAL-H, THF, rt, 6 h, 31%; (iii) Cinnamic acid, DCC, CH_2Cl_2, rt, 16 h, 62%; (iv) TBAF, THF, rt, 3 h, 64%

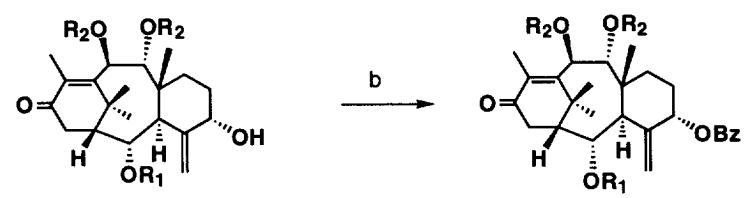


12 R = α-OH
13 R = β-OH

14 R_1 = H, R_2 = α-*O*-Cinn
15 R_1 = H, R_2 = α-*O*-Bz
16 R_1 = Bz, R_2 = α-OH
17 R_1 = H, R_2 = β-*O*-Cinn
18 R_1 = Cinn, R_2 = β-*O*-Cinn
19 R_1 = Bz, R_2 = β-*O*-Bz

Scheme 3. a) $NaBH_4$, THF/MeOH (1:1), rt, 15 min, **12** (50%) and **13** (50%); b) Cinnamic acid, DCC, CH_2Cl_2, rt [**12** to **14** (52%) for 24 h; **13** to **17** (52%) for 12 h]; c) Cinnamoyl chloride, DMAP, pyr., 90°C, 24 h [**13** to **18** (50%)]; d) BzCl, pyr., rt [**12** to **15** (59%) and **16** (40%) for 4 h; **13** to **19** (63%) for 12 h]

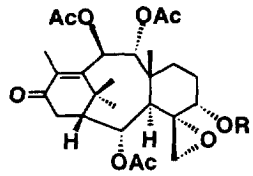
$NaBH_4$ reduction of **10** in THF/MeOH (1:1) gave a 1:1 mixture of the 13-α-OH (**12**, 50%) and 13-β-OH (**13**, 50%) derivatives, which were separated by a silica gel column (hexane/EtOAc, 2:1). Esterification of **12** and **13** with cinnamic acid and DCC afforded the 13α-*O*-cinnamate (**14**, 52%) and 13β-*O*-cinnamate (**17**, 52%), respectively. Treatment of **12** with benzoyl chloride in pyridine gave the 13α-*O*-benzoate (**15**, 59%) and 5-*O*-benzoate (**16**, 40%), and the benzoylation of **13** under the same condition yielded the 5,13-β-di-*O*-benzoate (**19**, 63%). Treatment of **12** with cinnamoyl chloride in pyridine containing 4-dimethylaminopyridine (DMAP) afforded the 5,13-β-di-*O*-cinnamate (**18**, 50%) (Scheme 3). Methanolysis of taxinine A (**10**) with K_2CO_3 in MeOH/dioxane (1:5) yielded 9,10-di-*O*-deacetyltaxinine A (**20**, 74%). 5,9,10-Tri-*O*-benzoate (**23**) and 2,5-di-*O*-benzoate (**24**) were obtained by benzoylation of **20** and taxuspine G (**21**)[11], respectively (Scheme 4). Treatment of taxinine A (**10**) with benzyloxymethyl chloride (BOMCl) led to compound **33** (78%). The structures of **2** ~ **9**, **11** ~ **21**, **23**, **24**, and **33** were confirmed by the ^1H



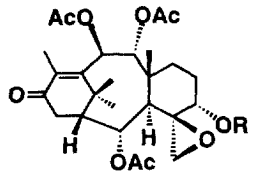
a
10 R_1 = R_2 = Ac
20 R_1 = Ac, R_2 = H
21 R_1 = H, R_2 = Ac

22 R_1 = R_2 = Ac
23 R_1 = Ac, R_2 = Bz
24 R_1 = Bz, R_2 = Ac

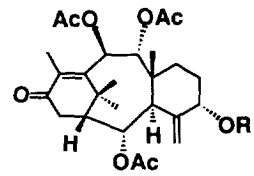
Scheme 4. a) K_2CO_3, MeOH/dioxane (1:5), rt, 4 h, 74%; b) BzCl, pyr., rt [**20** to **23** (15%) for 6 h; **21** to **24** (67%) for 24 h]



25 R = H
27 R = Bz
29 R = Cinn
30 R = TES



26 R = H
28 R = Bz
31 R = TES



32 R = TES
33 R = BOM

Figure 1

and ^{13}C NMR and MS spectral data and comparison with those of known compounds,[8,9] while compounds **22** and **25** ~ **32** were synthesized from **10** as previously reported (Figure 1).[12]

Increased Cellular Accumulation of Vincristine in Multidrug-Resistant Cells by Taxoids (1 ~ 33)

The cellular accumulation of vincristine (VCR) is reduced in multidrug-resistant (MDR) tumor cells as compared with the parental cells. MDR-reversing agents such as verapamil increase the reduced accumulation of antitumor agents in MDR cells and overcome multidrug resistance. The effect of taxoids (**1** ~ **33**) on the cellular accumulation of VCR in multidrug-resistant human ovarian cancer 2780AD cells was examined and the results were shown in Table 1. Verapamil at 1 and 10 μg/mL increased the VCR accumulation in a dose dependent manner. The increased cellular accumulation of VCR by taxinine (**1**) and taxinine A (**10**) corresponded to be 80 and 60% of that by verapamil, respectively.[6] In this activity it was found that taxoids **29** and **33** were 1.4 and 1.5 times more potent than verapamil, respectively. Compounds **11**, **14** ~ **16**, **22**, and **30** ~ **32** increased the VCR accumulation as potent as verapamil, while the activities of taxoids **2**, **3**, **12**, **17**, **19**, **24**, **25**, **27**, and **28** were comparable to those of taxinine (**1**) or taxinine A (**10**). Compounds **5** ~ **9**, **13**, **18**, **21**, **23**, and **26** showed weak activity. The activities of 9,10-di-*O*-deacetyl derivatives (**2** and **3**) of taxinine (**1**) were almost equal to that of **1**, while the 9,10-di-*O*-cinnamoyl (**5** and **7**), 9,10-di-*O*-benzoyl (**6**), and 9,10-*O*-acetonide (**8** and **9**) derivatives showed less activity. The 2-*O*-cinnamoyl (**11**), 5-*O*-benzoyl (**22**), 13-*O*-cinnamoyl (**14** and **17**), 13-*O*-benzoyl (**15**), 5-*O*-TES (**32**), and 5-*O*-BOM (**33**) derivatives of taxinine A (**10**) were more active than taxinine A (**10**), while the 5,13-di-*O*-cinnamoyl (**18**), 5,13-di-*O*-benzoyl (**19**), and 5,9,10-tri-*O*-benzoyl (**23**) derivatives showed less activity. Among compounds **12**, **13**, **14**, and **17**, the taxoids (**12** and **14**) having an α-oriented functional group at C-13 were more active than those (**13** and **17**) possessing the corresponding β-oriented functional group at C-13. The activity of **22** having a ketone group at C-13 was similar to that of **16** with a hydroxy group at C-13. Among compounds (**25** ~ **31**) with an epoxide at C-4 and C-20, the taxoids (**29** ~ **30**) having a cinnamoyl or TES group at C-5 were as active as verapamil, while those (**25** ~ **28**) with a hydroxy or benzoyloxy group at C-5 showed less activity. The activities of the α-epoxides (**27** and **30**) were almost equal to those of the corresponding β-epoxides (**28** and **31**), respectively, while the α-epoxide (**25**) with a hydroxy group at C-5 was more active than the coressponding β-epoxide (**26**).

Table 1. Effects of Taxoids (1 ~ 33) on the Accumulation of Vincristine (VCR) in Multidrug-Resistant Cells.

Compound	VCR accumulation (% of control)[a] with a taxoid concentration of		Compound	VCR accumulation (% of control)[a] with a taxoid concentration of	
	1 μg/mL	10 μg/mL		1 μg/mL	10 μg/mL
1 (Taxinine)	195	571	**18**	157	234
2	274	619	**19**	197	391
3	225	518	**21**	139	271
5	181	273	**22**	184	645
6	141	210	**23**	132	324
7	186	230	**24**	146	470
8	198	234	**25**	180	364
9	126	210	**26**	144	238
10 (Taxinine A)	153	461	**27**	140	402
11	207	727	**28**	200	391
12	164	422	**29**	358	1027
13	149	290	**30**	233	752
14	249	699	**31**	227	748
15	185	643	**32**	213	669
16	151	649	**33**	401	1110
17	213	551	**Verapamil**	254	739

a) The amounts of VCR accumulated in multidrug-resistant human ovarian cancer 2780AD cells were determined in the presence of 1 and 10 μg/mL of taxoids. The values represent means of triplicate determinations, and are expressed as the relative amounts of VCR accumulated in the cells as compared with the control experiment.

In summary some taxinine derivatives (**11**, **14**, **15**, **16**, **22**, **29**, **32**, and **33**) containing a cinnamoyloxy, a benzoyloxy, a TES, or a BOM group at C-2, C-5, or C-13 effectively increased the cellular accumulation of VCR in MDR tumor cells, while another taxoids (**5**, **6**, **7**, **8**, **9**, and **23**) having cinnamoyloxy, benzoyloxy, or acetonide groups at both C-9 and C-10 showed remarkable reduction of the activity. Since the 6/8/6-membered ring system of these taxinine derivatives take commonly "cage" -like backbone structures such as taxinine[13], the presence of the bulky group at C-2, C-5, or C-13 oriented to inside of the "cage" structure may be important for its effective binding to P-glycoprotein, whereas the existence of the bulky groups at C-9 and C-10 directed to outside of the "cage" structure may result in its less binding. It is noted that among these taxinine derivatives (**2** ~ **32**) the two taxoids **29** and **33** were 1.4 and 1.5 times more potent than verapamil, respectively. Compounds **29** and **33** showed weak or no cytotoxicity against murine lymphoma L1210 cells with IC_{50} values of 3.0 and 8.0 μg/mL, respectively, and human epidermoid carcinoma KB cells with IC_{50} values of >10 and 9.0 μg/mL, respectively, *in vitro*, while the other taxoids (**1** ~ **28** and **30** ~ **32**) also exhibited weak or no cytotoxicity. Thus, this study showed that some taxinine derivatives are good modifiers of multidrug resistance in tumor cells.

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