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# Synthesis and biological evaluation of taxinine analogues as orally active multidrug resistance reversal agents in cancer

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**Abstract**—Three novel taxinine analogues were prepared and tested for their activity as multidrug resistance (MDR) reversal agents in comparison with verapamil. In vitro testing demonstrated that compounds **8**–**10** possess MDR-reversal activity in the KB/V cell line. Half-hour after treatment with 5, 10, and 20 μmol/L compound **9**, the intracellular rhodamine123 concentration increased 2.3, 2.9, and 3.2-fold, respectively, higher than 1.88-fold of 10 μmol/L verapamil in KB/V cell line. In vivo studies with VCR-resistant KB/V tumor xenografts showed that compound **9** in combination with VCR significantly inhibited tumor growth. Treatment with VCR or **9** alone did not result in growth inhibition. These results reveal that three taxinine analogues are good modifiers of MDR in tumor cells.


Multidrug resistance (MDR) in tumor cells is a significant obstacle to the success of chemotherapy in many cancers. MDR is a phenomenon whereby tumor cells that have been exposed to one cytotoxic agent develop cross-resistance to a range of structurally and functionally unrelated compounds. The drug resistance that develops in cancer cells often results from elevated expression of particular proteins, such as cell-membrane transporters, which can result in an increased efflux of the cytotoxic drugs from the cancer cells, thus lowering their intracellular concentrations. The classic resistance to the cytotoxic drugs has most often been linked to the overexpression of P-glycoprotein (P-gp), a 170-kd ATP-dependent membrane transporter that acts as a drug efflux pump, resulting in increased efflux of chemotherapeutic agents from cancer cells.[1] P-gp transports a broad range of substrates. To inhibit P-gp as a method to reverse MDR in cancer patients has been studied extensively, some compounds have been developed to reverse MDR in vitro and in vivo.[2] Some nontaxol-type taxoids, including taxinine (**1**) isolated from Japanese *yew Taxus cuspidate*, have been reported as reversal agents.[3] Sinenxan A (SIA) (**2**) is readily available as a biosynthetic taxane in good yield.[4] It has similar skeleton with taxinine (**1**). The development of a procedure using SIA (**2**) as starting material for the preparation of bioactive taxinine analogues would be of significance (Fig. 1).

In this paper we describe the preparation of three taxinine analogues synthesized from SIA (**2**), the effects on cellular accumulation of rhodamine123 (Rho.123) in MDR tumor cells, and the reversal activity in tumor xenografts.

The procedure for the synthesis of compounds **8**–**10** from SIA (**2**) is outlined in Scheme 1. The acetyl at C-14 was removed by KOH/MeOH/THF at 0 °C to give compound **3** in approximately 30% yield, accompanied

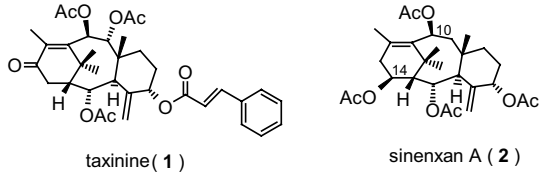


**Figure 1.** The structures of taxinine (**1**) and sinenxan A (**2**).

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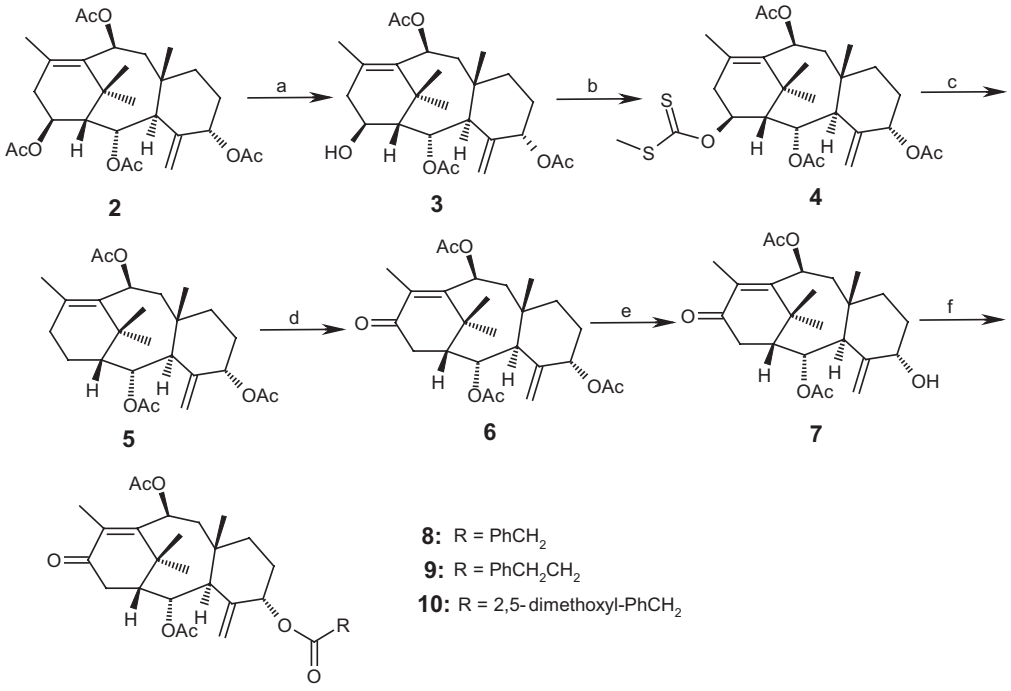


**Scheme 1.** Reagents and reaction conditions: (a) 10 mol/L KOH, MeOH, THF, 0 °C, 20 min, 30–40%; (b) NaH, $CS_2$, dry THF, $N_2$, reflux, 15 h; then MeI, 40 °C, 3 h, 85% (two steps); (c) $H_3PO_2$, AIBN, dioxane, cyclohexene, reflux, 5 h, 82%; (d) 70% *t*-BuOOH, $CrO_3$, $CH_2Cl_2$, rt, 5 h, 73%; (e) *t*-BuOK, dry THF, $N_2$, −78 °C, 2 h, 90%; (f) RCOOH, DCC, DMAP, dry toluene, rt, 6 h, **8** (85%), **9** (88%), **10** (83%).

by 10-OH-SIA and recovered SIA (**2**). The treatment of compound **3** with $CS_2$/NaH/MeI in THF gave xanthated compound **4** in 85% yield. By radical deoxygenation and allylic oxidation,[5] 13-keto derivative (**6**) could be obtained. The 5-*O*-acetate of **6** could be regio-selectively cleaved by *t*-BuOK in THF to give **7** in high yield.[6] The target compounds **8**, **9**, **10** were obtained by esterification with the corresponding acid. The structures of the compounds **8**, **9**, **10** were confirmed by $^1$H NMR, $^{13}$C NMR, and FABMS.[7]

The taxinine analogues prepared in this study were tested for their reversal activity in epidermoid KB human cancer cell line and its MDR subline KB/V cell. Compound **9** was then further evaluated for their activity to increase accumulation of intracellular Rho.123 and in vivo sensitizing activity with human epidermoid (KB, KB/V) cancer xenografts. Chemosensitivity in vitro was measured by means of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) colorimetric assay performed in 96-well plates.[8] The concentration that caused 50% inhibition of cancer cell growth against various cancer cell lines were expressed as $IC_{50}$ values. The results are presented in Table 1. The reversal activity of target compounds were expressed reversal folds ($IC_{50}$ value for vincristine (VCR) of KB/V cell with the reversing agents divided by $IC_{50}$ value for VCR of KB/V cell without the reversing agents). Compounds **8**–**10** showed potent MDR reversal activity in vitro in a dose-dependent way. In order to obtain more meaningful relative activities, verapamil was tested at the same time. Overexpression of P-gp was the main cause of MDR in KB/V cell line.[9] Furthermore, we measured the effect of compound **9** on P-gp function by Rho.123 dye-efflux assay. Compound **9** resulted in significantly increased intracellular levels of Rho.123 in a dose-dependent way (Fig. 2).

**Table 1.** Effects of MDR-reversing agents on the cytotoxicity of VCR against MDR KB/V cell line

| Compd | $IC_{50}$ (nM) | Fold reversal[a] |
|---|---|---|
| VCR | 1230.48 | — |
| VCR + compd **8** (5 μM) | 218.02 | 5.64 |
| VCR + compd **8** (10 μM) | 7.30 | 168.56 |
| VCR + compd **9** (5 μM) | 19.43 | 63.32 |
| VCR + compd **9** (10 μM) | 2.80 | 439.46 |
| VCR + compd **10** (5 μM) | 325.42 | 3.78 |
| VCR + compd **10** (10 μM) | 8.93 | 137.79 |
| VCR + taxinine (10 μM) | 124.18 | 9.91 |
| VCR + verapamil (10 μM) | 18.14 | 67.81 |

Cell survival was determined by MTT assay.

[a] The reversal activity of target compounds were expressed reversal folds ($IC_{50}$ value for VCR of KB/V cell with the reversing agents divided by $IC_{50}$ value for VCR of KB/V cell without the reversing agents).

The sensitizing activity of compound **9** was investigated in nude mice. Experiments were carried out in the human tumor xenografts KB and MDR subline KB/V (Fig. 3A and B). As shown in Figure 3A, VCR administered at a dose of 0.5 mg/kg ip resulted in significant tumor growth inhibition ($p < 0.01$, day 24). On the other hand, as shown in Figure 3B, VCR administered at a dose of 0.5 mg/kg ip potently produced any growth inhibition in VCR-resistant KB/V tumor xenografts. The same was true when orally treated with compound **9** (330 mg/kg) alone. When treated concomitantly with

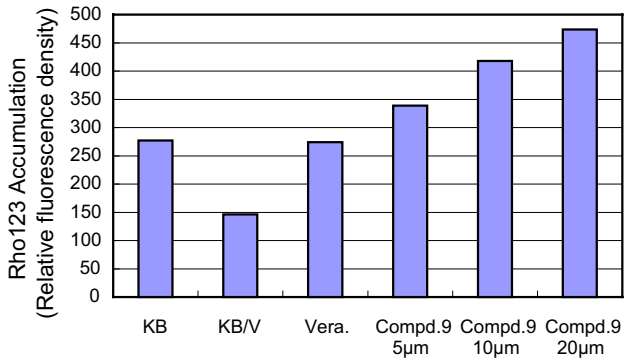


**Figure 2.** Accumulation of Rho.123 in KB and KB/V cells.

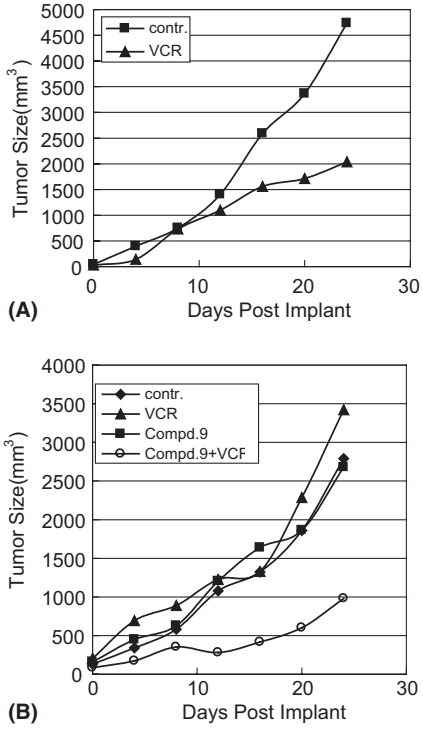


**Figure 3.** (A) Inhibition effect of VCR on VCR-sensitive KB tumor in athymic mice. There were six mice per group. VCR, 0.5 mg/kg (▲) was administered ip as single agent on days 0, 4, 8, 12, 16, and 20. Control animals (■) were not treated. (B) Inhibition effect of VCR combined compound **9** on VCR-resistant KB/V tumor in athymic mice. VCR, 0.5 mg/kg (▲) ip, compound **9**, 330 mg/kg (■) orally was administered as single agent on days 0, 4, 8, 12, 16, and 20. Combined therapy (○) included VCR, 0.5 mg/kg (▲) ip, compound **9**, 330 mg/kg orally on days 0, 4, 8, 12, 16, and 20. Control animals (◆) were not treated.

compound **9** (330 mg/kg po), and VCR (0.5 mg/kg ip) resulted in significant growth inhibition ($p < 0.01$, day 24). The tumor growth inhibition observed with the combination in VCR-resistant KB/V tumor xenografts was just like that of VCR in VCR-sensitive KB tumor xenografts ($p > 0.05$).

In conclusion, three taxinine analogues (**8**–**10**) at 10 μM markedly reversed the resistance to VCR in KB/V cells (overexpress P-gp), while taxinine (**1**) showed weak effect. Compound **9** increased the Rho.123 accumulation in multidrug-resistant KB/V cells and had stronger activity than that of verapamil. In addition, we found that compound **9** given orally enhanced the chemotherapeutic effect of VCR in KB/V-bearing mice. These taxinine analogues (**8**–**10**) may be useful for the treatment of tumors when they are used in combination with anticancer agents, since they increase the sensitivity to anticancer agents. These taxinine analogues (**8**–**10**) may interact directly with P-gp and inhibit the active efflux of antitumor agents, thus overcome MDR in vitro and in vivo.

## Acknowledgements

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7. *Compound* **8**: mp 51–52 °C, $^1$H NMR (500 MHz, $CDCl_3$, $\delta$ ppm): 7.35–7.24 (m, 5H, Ph), 6.08 (dd, 1H, $J = 12$, 5.5 Hz, H-10), 5.48 (dd, 1H, $J = 2$, 6.5 Hz, H-2), 5.27 (s, 1H, H-20), 5.24 (t, 1H, $J = 2$ Hz, H-5), 4.83 (s, 1H, H-20), 3.55 (q, 2H, $J = 17$ Hz, 2′-$CH_2$), 3.10 (d, 1H, $J = 6.5$ Hz, H-3), 2.84 (dd, 1H, $J = 7$, 19.7 Hz, H-14), 2.50 (dd, 1H, $J = 11.7$, 15 Hz, H-9), 2.37 (d, 1H, $J = 19.5$ Hz, H-14), 2.24 (s, 3H, 10-OAc-$CH_3$), 2.62 (dd, 1H, $J = 2$, 7 Hz, H-1), 2.10 (s, 3H, 2-OAc-$CH_3$), 2.06 (s, 3H, 18-$CH_3$), 1.95 (m, 1H, H-7), 1.80 (m, 3H, 2×6-H, H-9), 1.70 (s, 3H, 16-$CH_3$), 1.29 (m, 1H, H-7), 1.15 (s, 3H, 17-$CH_3$), 0.89 (s, 3H, 19-$CH_3$); FABMS: 537.5; HR-FABMS (Gly/*m*-NBA): found 537.2755, calcd 537.2852, formula: $C_{32}H_{41}O_7$.
*Compound* **9**: mp 117 °C, $^1$H NMR (500 M Hz, $CDCl_3$, $\delta$ ppm): 7.33–7.21 (5H, Ph), 6.06 (dd, 1H, $J = 12.3$, 6 Hz, H-10), 5.48 (dd, 1H, $J = 1.5$, 6.7 Hz, H-2), 5.28 (s, 1H, H-20), 5.24 (br s, 1H, H-5), 4.84 (s, 1H, H-20), 3.17 (d, 1H, $J = 6$ Hz, H-3), 2.96 (t, 2H, $J = 8$ Hz, 2′-$CH_2$), 2.81 (dd, 1H, $J = 6.5$, 20 Hz, H-14), 2.63 (m, 1H, 3′-$CH_2$), 2.53 (m, 1H, 3′-$CH_2$), 2.50 (dd, 1H, $J = 12$, 15 Hz, H-9), 2.32 (d, 1H, $J = 20$ Hz, H-14), 2.18 (s, 3H, 10-OAc-$CH_3$), 2.16 (dd, 1H, $J = 1.5$, 6.5 Hz, H-1), 2.12 (s, 3H, 2-OAc-$CH_3$), 2.09 (s, 3H, 18-$CH_3$), 1.91 (m, 1H, H-7), 1.78 (m, 3H, 2×H-6, H-9), 1.71 (s, 3H, 16-$CH_3$), 1.28 (m, 1H, H-7), 1.15 (s, 3H, 17-$CH_3$), 0.92 (s, 3H, 19-$CH_3$); FABMS: 551.3 (M + 1); HR-FABMS (Gly/*m*-NBA): found 550.2946, calcd 550.2931, formula: $C_{33}H_{42}O_7 + H^+$.
*Compound* **10**: mp 69–71 °C, $^1$H NMR (500 M Hz, $CDCl_3$, $\delta$ ppm): 6.82–6.75 (m, 3H, Ph), 6.09 (dd, 1H, $J = 5.5$, 12 Hz, H-10), 5.48 (dd, 1H, $J = 1.5$, 6.5 Hz, H-2), 5.27 (s, 1H, H-20), 5.25 (br s, 1H, H-5), 4.82 (s, 1H, H-20), 3.77 (s, 3H, 5′-$OCH_3$), 3.76 (s, 3H, 2′-$OCH_3$), 3.59 (d, 1H, $J = 17$ Hz, H-2′), 3.41 (d, 1H, $J = 17$ Hz, H-2′), 3.23 (d, 1H, $J = 6.5$ Hz,

H-3), 2.81 (dd, 1H, $J=7$, 20 Hz, H-14), 2.50 (dd, 1H, $J=12.3$, 14.7 Hz, H-9), 2.39 (d, 1H, $J=20$ Hz, H-14), 2.24 (s, 3H, 10-OAc-$CH_3$), 2.16 (d, 1H, $J=5.5$ Hz, H-1), 2.09 (s, 3H, 2-OAc-$CH_3$), 2.07 (s, 3H, 18-$CH_3$), 2.17–1.96 (m, 1H, H-7), 1.83 (m, 2H, 2×H-6), 1.77 (dd, 1H, $J=14.7$, 5.7 Hz, H-9), 1.70 (s, 3H, 16-$CH_3$), 1.28 (m, H-7), 1.14 (s, 3H, 17-$CH_3$), 0.91 (s, 3H, 19-$CH_3$); FABMS: 596.3; HR-FABMS (Gly/*m*-NBA), found 596.2970, calcd 596.2985, formula: $C_{34}H_{44}O_9$.

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