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ORIGINAL ARTICLE

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The polar neutral and basic taxoids isolated from needles and twigs of *Taxus cuspidata* and their biological activity

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Abstract Twelve basic taxoids and 22 neutral taxoids were isolated from basic and polar neutral fractions of the extracts of needles and twigs of *Taxus cuspidata*. Among them, taxine NA-13, 3,11-cyclotaxinine NN-1, taxinine NN-6, 11(15→1)*abeo*-taxinine NN-1, taxine NA-8, and taxine NA-4 were isolated first from natural sources by us. The cytotoxic activity of isolated compounds was evaluated against three human cell lines: normal human fibroblast cells (WI-38), malignant tumor cells induced from WI-38 (VA-13), and human liver tumor cells (HepG2). 7-Epitaxol, 7-epicephalomannine, taxinine NN-6, taxine NA-2, taxuspine H, and taxagifine were active toward VA-13 cells and 7-epitaxol, 7-epicephalomannine, taxinine NN-1, 9,10-deacetyltaxinine, and taxagifine were active toward HepG2 cells. The multidrug-resistant (MDR) cancer reversal activity of isolated compounds was evaluated on the basis of the amount of vincristine (VCR) accumulated in MDR human ovarian cancer 2780 AD. Taxine NA-8, taxine NA-2, 5-cinnamoyl-10-acetyltaxicin II, and taxinine NN-1 indicated stronger MDR cancer reversal activity than verapamil. The result of primary screening based on 39 human cancer cell lines suggests that taxinine NN-1 belongs to a new mechanistic class and is a new anticancer agents. 7-Epicephalomannine was found to be an effective anticancer agent with tubulin as its molecular target, which is the same as paclitaxel.

Key words *Taxus cuspidata* · Taxoid · Cytotoxic activity · MDR-cancer reversal activity · Taxinine NN-1

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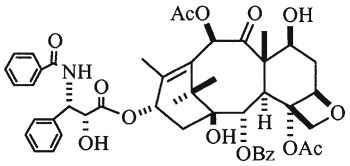
Part of this work was presented at the 74th Annual Meeting of the Chemical Society of Japan, Tanabe, March 1998, and the 41st Symposium on the Chemistry of Terpenes, Essential Oils, and Aromatics, Gifu, December 1998. The results of this work were also presented as patent documents at PCT Int: Appl WO 2001007040 A1, February 2001; Appl WO 2000-Jp 5036, 27 July 2000. Priority: JP 1999-214273, 28 July 1999; JP 1999-224652, 6 August 1999; JP 2000-76404, 14 March 2000

Introduction

Since the discovery of the clinical application of paclitaxel (taxol) (Fig. 1) toward refractory ovarian, breast, and other carcinomas, much attention has been paid to the isolation of paclitaxel, its semisynthetic intermediates, and new bioactive taxoids from various species of yew trees.[1,2] For this purpose, the harvest of enough yew trees from natural sources is impossible, because their growth is very slow, their numbers in nature are limited, their cost is very high, and they are often protected in national forest. The needles and twigs of yew tree are its only reproducible and constantly available plant material. The needles and twigs of Japanese yew, *Taxus cuspidata*, contain an impressive array of taxoids.[3–9] However, the content of the antitumor paclitaxel in the needles and twigs of this species is generally low. Taxane derivatives occurring in consistently large amounts are taxinine (**19**)[10–12] as a neutral taxoid, and taxine NA-1 (2′-hydroxytaxine II) (**8**)[6] and taxine II (**13**)[13] as basic taxoids. As a part of ongoing study of the new type of anticancer agents and multidrug-resistant (MDR) cancer reversal agents belonging to taxoids, we have conducted a further

Fig. 1. Structure of paclitaxel (taxol)



investigation on polar neutral taxoids and basic taxoids isolated from the extracts of needles and twigs of *T. cuspidata* as a source of useful bioactive taxoids.

Experimental

General

The instrumentation and chromatography were the same as described previously.[6–8]

Plant material

The needles and twigs of *Taxus cuspidata* were collected from trees of 3-m height in Aobayama, Sendai, Japan, on November 15, 1997.

Extraction and isolation

Fresh needles and twigs of *T. cuspidata* (4.77 kg) were extracted successively with hexane, ethyl acetate (EtOAc), and methanol (MeOH). The MeOH-soluble portion was extracted with chloroform ($CHCl_3$). The $CHCl_3$ extract gave a polar neutral taxoid fraction (107.62 g) after washing with acid and base to eliminate basic and acidic compounds. The EtOAc extract was subject to acid and base extractions. The extract with acid gave a crude fraction of basic taxoids (9.26 g) after neutralization and successive extraction with $CHCl_3$ (Fig. 2).

The crude fraction of basic taxoids (9.26 g) was separated by column chromatography (CC) on alumina (920 g) into 17 fractions with gradient elution with EtOAc–hexane–MeOH [F1(3:7:0); F2,3 (5:5:0); F4,5 (7:3:0); F6,7 (1:0:0); F8 (9:0:1); F9 (8:0:1); F10 (7:0:1); F11 (5:0:5); F12–15 (MeOH); and F16,17 (acetone)]. The following separations were performed by high performance liquid chromatography [HPLC; reversed-phase column (ODS) and gradient elution with MeOH–0.05 M NH_4OAc buffer (pH 4.8)–MeCN].[6] Fractions F1–4 (449 mg) gave taxinine (**19**, 48.8 mg), taxuspine C (**25**, 3.2 mg), and taxinine NN-2 (**34**, 6.7 mg). Separation of residue of F1–4 (382 mg), F5–7 (622 mg), F8 (2.20 g), and F9 (2.33 g) gave taxine II (**13**, 890 mg). Separation of the combined lower polarity fraction of F1–9 gave taxine NA-8 (**9**, 16.6 mg), 2′-deacetylaustrospicatine (**10**, 28.4 mg), 7,2′-didesacetoxyaustrospicatine (**14**, 37.0 mg), 2′-desacetoxyaustrospicatine (**15**, 55.7 mg), taxine NA-3 (**16**, 47.6 mg), taxinine NN-1 (**23**, 7.00 mg). Separation of the combined higher polarity fraction of F1–7 gave new compound 3,11-cyclotaxinine NN-1 (**2**, 7.7 mg), and taxine NA-2 (**17**, 38.5 mg), taxuspine H (**18**, 25.3 mg), taxagifine (**26**, 5.5 mg), taxuspine F (**27**, 2.8 mg), taxinine A (**30**, 15.8 mg), decinnamoyltaxinine J (**31**, 2.0 mg), and 12(3→20)*abeo*-taxinine NN-2 (**33**, 1.5 mg). Separation of F8, F9, and F10–17 gave 11(15→1)*abeo*-taxinine NN-1 (**7**, 10.9 mg) and taxine NA-1 (**8**, 1.82 g). Further separation of the combined residue of F8, F9, and F10–17 gave taxinine NN-6 (**5**, 7.8 mg), taxine NA-11 (**11**, 13.6 mg), taxine NA-4 (**12**, 43.0 mg), taxine NA-2 (**17**, 222 mg), taxuspine H (**18**, 39.0 mg), taxagifine (**26**, 3.20 mg), and taxinine A (**30**, 30.8 mg) (Fig. 3).

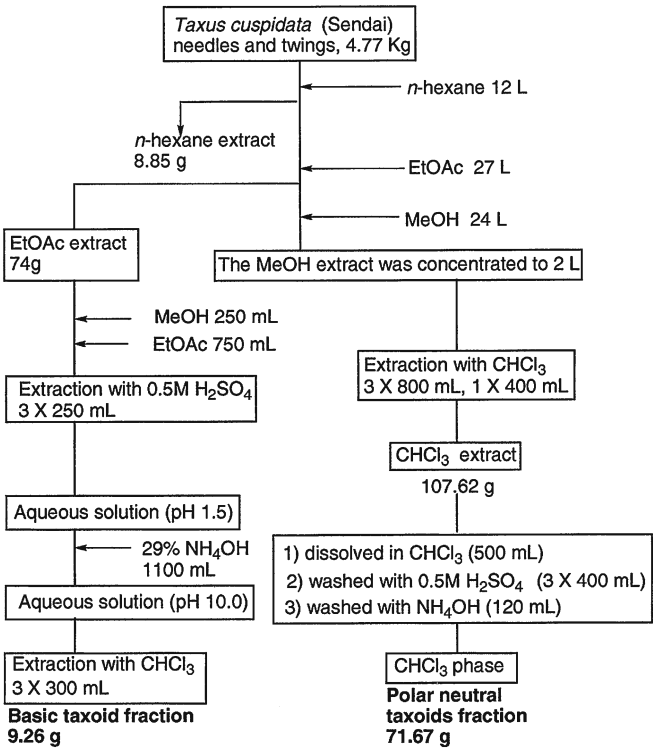


Fig. 2. Extraction procedures of the needles and twigs of *Taxus cuspidata*

The crude polar neutral taxoid fraction (71.67 g) was separated by CC on silica gel (1400 g) into fractions F1–15. Fractions F6–10 (4.77 g) were further separated into 10 fractions (f1–10) by CC on silica gel (250 g, hexane–EtOAc–MeOH gradient). Fractions f5–7 (2.66 g) were separated into 4 fractions (F1′–4′) by flash CC on silica gel (150 g, hexane–EtOAc–MeOH gradient). Fraction F4′ (1.38 g) was further separated into 9 fractions (F4′-1–F 4′-9) by flash CC on silica gel (150 g, hexane–EtOAc–MeOH gradient). Separation of F4′–6 (36.7 mg) gave 5-cinnamoyloxy-10-acetyltaxicin II (**20**, 9.9 mg), taxagifine (**26**, 2.5 mg), taxuspine F (**27**, 7.5 mg) by separation with normal phase HPLC (silica gel column, hexane–EtOAc gradient). F4′–7 (342 mg) afforded 7-epitaxol (**3**, 38.7 mg), 7-epicephalomannine (**4**, 14.1 mg), 2,9,10-deaceyltaxinine (**21**, 3.6 mg), taxagifine (**26**, 17.4 mg), taxuspine F (**27**, 24.3 mg), and 2-deacetyl-5α-decinnamoyltaxinine J (**28** 3.8 mg), and F4′–8 (356 mg) afforded 7-epitaxol (**3**, 6.4 mg), taxuspine F (**27**, 1.9 mg), and taxezopidine F (**29**, 3.1 mg) by combination of

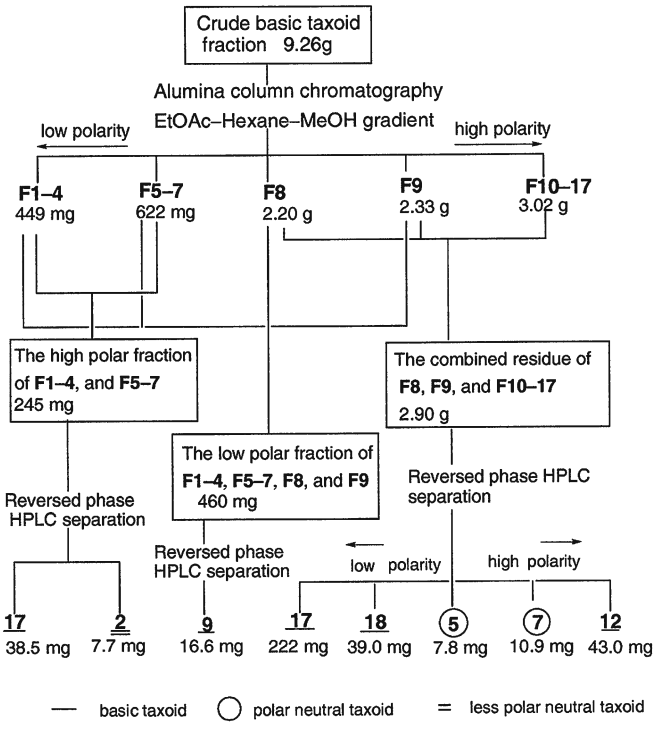


Fig. 3. Separation procedures of crude basic fraction

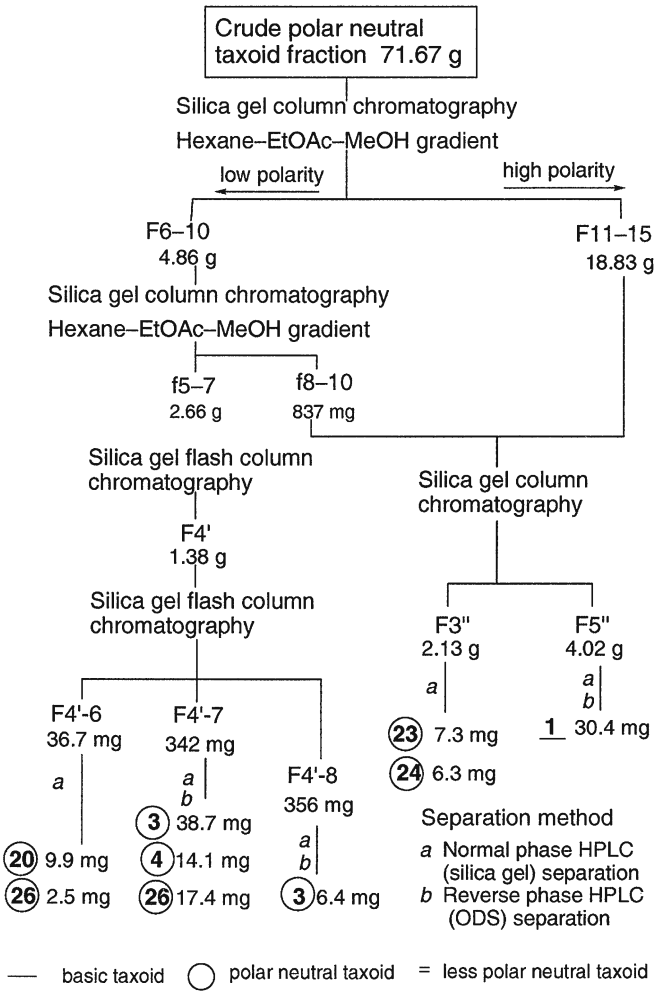


Fig. 4. Separation procedures of crude polar neutral fraction

normal phase HPLC (silica gel column, hexane–EtOAc gradient)[7,8] and reversed-phase HPLC [ODS column, H_2O–MeOH–acetonitrile (CH_3CN) gradient elution].[6] Repeated separation of F4′–9 (413.7 mg) afforded 9α,10β,13α-triacetoxy-5α-cinnamoyloxytaxa-4(20),11-diene (**22**,2.7 mg) by reversed-phase HPLC (ODS column, H_2O–MeOH–CH_3CN gradient elution).[6] The combined fractions (19.67 g) of f8–10 and F11–15 were separated into 9 fractions (F1″–9″) by CC on silica gel (1 kg, hexane–EtOAc–MeOH gradient). F3″ (2.13 g) gave taxinine (**19**, 39.7 mg), taxinine NN-1 (**23**, 7.3 mg), 9,10-deacetyltaxinine (**24**, 6.3 mg), and taxinine A (**30**, 110 mg), and F4″ (1.44 g) afforded taxinine A (**30**, 31.5 mg) by separation with HPLC (silica gel column, hexane–EtOAc gradient). F5″ (4.02 g) gave taxine NA-13 (**1**, 30.4 mg), taxayuntin (**6**, 30.9 mg), taxinine (**19**, 53.1 mg), taxuspine F (**27**, 5.8 mg), taxinine A (**30**, 46.7 mg), and decinnamoyltaxagifine (**32**, 30.4 mg) by separation with the combination of normal phase HPLC (silica gel column, hexane–EtOAc, gradient)[7,8] and reversed-phase HPLC (ODS column, H_2O–MeOH–CH_3CN gradient) (Fig. 4).[6]

Identification of isolated compounds

Taxine NA-13 (**1**) and 3,11-cyclotaxinine NN-1 (**2**) were the first isolated compounds from natural sources by us and their physical constants and nuclear magnetic resonance (NMR) and high resolution (HR) electron impact ionization mass spectrometry (EI-MS) spectroscopic data are given below. The detailed discussion of structure determination of **1** and **2** will be reported elsewhere separately. Taxinine NN-6 (**5**),[14] 11(15→1)*abeo*-taxinine NN-1 (**7**),[15] taxine NA-8 (**9**),[15] and taxine NA-4 (**12**)[15] are formerly reported by us as patents, which are their only references. Their structures were established by NMR and high resolution EI-MS spectroscopic analyses as shown in Fig. 5.

Taxine NA-13. **1** was obtained as colorless granular crystals; mp 96°–100°C; $[\alpha]^{20}_D$ +34.2° (*c* 0.15, $CHCl_3$). ^{1}H NMR ($CHCl_3$): δ 7.42–7.41 (2H, m, 3″-H, 5″-H), 7.36–7.29 (3H, m, 2″-H, 4″-H, 6″-H), 5.80 (1H, br s, 9-H), 5.80 (1H, br s, 10-H), 5.46 (1H, dd, J = 6.6, 2.0 Hz, 2-H), 5.40 (1H, br s, 20-Ha), 5.31 (1H, br s, 5-H), 4.87 (1H, d, J = 2.9 Hz, 4′-Hα), 4.85 (1H, br s, 20-Hb), 4.28 (1H, d, J = 2.7 Hz, 4′-Hβ), 4.22 (1H, d, J = 7.6 Hz, 2′-H), 3.48 (1H, d, J = 7.6 Hz, 3′-H), 2.95 (1H, br d, J = 6.6 Hz, 3-H), 2.71 (1H, dd, J = 19. 8, 7.1 Hz, 14-Hβ), 2.25 (3H, s, NMe), 2.19 (1H, d, J = 19.8 Hz, 14-Hα), 2.12 (1H, dd, J = 6.8, 1.7 Hz, 1-H), 2.05 (3H, s, acetyl methyl), 2.04 (3H, s, acetyl methyl), 2.02 (3H, s, acetyl methyl), 1.71 (1H, m, 6-Hβ), 1.65 (3H, s, 17-H_3), 1.64 (1H, m, 7-Hα), 1.64 (1H, m, 6-Hα), 1.54 (3H, s, 18-H_3), 1.37 (1H, m, 7-Hβ), 1.04 (3H, s, 16-H_3), 0.85 (3H, s, 19-H_3). ^{13}C NMR ($CHCl_3$): δ 198.8 (13-C), 171.2 (1′-C), 169.8 (9-O acetyl carbonyl), 169.7 (10-O acetyl carbonyl), 169.4 (2-O acetyl carbonyl), 149.0 (11-C), 141.1 (4-C), 138.0 (12-C), 137.6 (1″-C), 128.6 (2″-C,

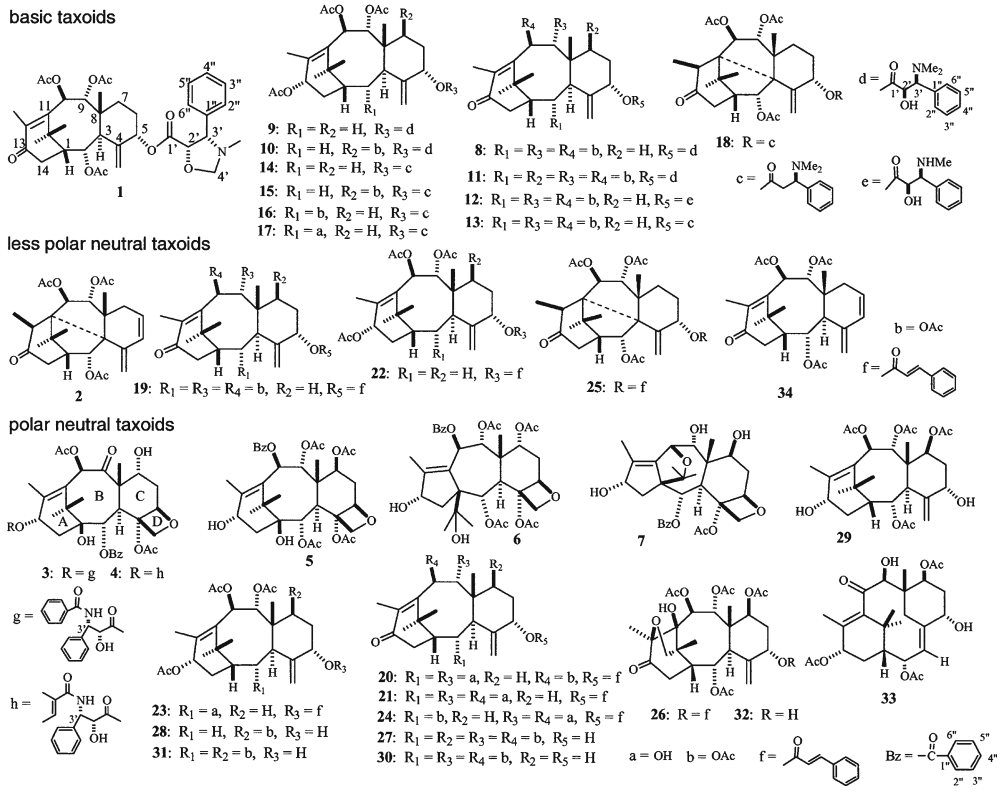


Fig. 5. Compounds from *Taxus cuspidata*

6″-C), 128.4 (3″-C, 5″-C), 128.0 (4″-C), 118.6 (20-C), 89.3 (4′-C), 82.3 (2′-C), 78.8 (5-C), 75.7 (9-C), 73.5 (3′-C), 73.1 (10-C), 69.5 (2-C), 48.4 (1-C), 44.4 (8-C), 43.1 (3-C), 37.3 (15-C), 37.2 (16-C), 36.5 (N-Me), 35.8 (14-C), 28.3 (6-C), 27.3 (7-C), 25.1 (17-C), 24.4, 20.8, 20.7 (acetyl methyl), 17.5 (19-C), 13.2 (18-C). Infrared (IR) ($CHCl_3$): ν_{max} cm^{-1}1744, 1674. EI-MS *m/z* (rel. int.): 666 $[M+H]^+$ (100); HR EI-MS *m/z* 666.3279$[M+H]^+$ (calcd for $C_{37}H_{47}O_{10}N$, $[M+H]^+$, 666.3279).

3,11-Cyclotaxinine NN-1. **2** was obtained as white amorphous powder; mp 45°–47°C; $[\alpha]^{20}_D$–108.1° (*c* 0.060, $CHCl_3$). ^{1}H NMR ($CHCl_3$): δ 6.26 (1H, dd, *J* = 9.1, 2.0 Hz, 5-H), 6.14 (1H, d, *J* = 5.1 Hz, 2-H), 6.10 (1H, ddd, *J* = 9.1, 6.6, 2.7 Hz, 6-H), 5.56 (1H, s, 20-Ha), 5.54 (1H, d, *J* = 9.0 Hz, 9-H), 5.23 (1H, d, *J* = 9.0 Hz, 10-H), 5.10 (1H, s, 20-Hb), 2.68 (1H, d, *J* = 20.3 Hz, 14-Hα), 2.68 (1H, d, *J* = 7.3 Hz, 12-H), 2.49 (1H, dd, *J* = 20.4, 7.3 Hz, 14-Hβ), 2.20 (1H, dd, *J* = 14.7, 6.57 Hz, 7-Hα), 2.17 (1H, m, 1-H), 2.10 (3H, s), 2.06 (3H, s, acetyl methyl), 2.00 (3H, s, acetyl methyl), 1.66 (1H, ddd, *J* = 14.7, 2.7, 2.0 Hz, 7-Hβ), 1.63 (3H, s, 16-H_3), 1.37 (3H, s, 19-H_3), 1.19 (3H, d, *J* = 7.3 Hz, 18-H_3), 1.19 (3H, s, 17-H_3). ^{13}C NMR ($CHCl_3$): δ 214.1 (13-C), 170.9 (10-O acetyl carbonyl), 169.8 (9-O acetyl carbonyl), 169.6 (2-O acetyl carbonyl), 143.2 (4-C), 134.4 (5-C), 131.5 (6-C), 117.0 (20-C), 83.3 (9-C), 80.3 (10-C), 76.3 (2-C), 66.9 (3-C), 57.8 (11-C), 52.2 (12-C), 49.3 (8-C), 48.1 (1-C), 42.1 (15-C), 38.7 (14-C), 34.5 (7-C), 28.1 (16-C), 26.9 (19-C), 26.7 (17-C), 21.4, 21.1, 21.0 (acetyl methyl), 15.8 (18-C). IR ($CHCl_3$): ν_{max} cm^{-1}1746, 1706. EI-MS *m/z* (rel. int.): 458 $[M]^+$ (68), 314 (7), 298 (5), 268 (9), 253 (8), 239 (6), 58 (100); HR EI-MS *m/z* 458.2306 (calcd for $C_{26}H_{34}O_7$, 458.2305).

Taxinine NN-6. **5** was obtained as white amorphous powder; mp 241°–242°C; $[\alpha]^{20}_D$–41.1° (*c* 0.560, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 7.86 (2H, br d, *J* = 7.8 Hz, 2″-H, 6″-H), 7.56 (1H, br t, *J* = 7.5 Hz, 4″-H), 7.43 (2H, br dd, *J* = 7.8, 7.5 Hz, 3″-H, 5″-H), 6.55 (1H, d, *J* = 10.7 Hz, 10-H), 6.16 (1H, d, *J* = 10.7 Hz, 9-H), 6.12 (1H, d, *J* = 7.8 Hz, 2-H), 5.55 (1H, dd, *J* = 8.3, 8.3 Hz, 7-H), 4.95 (1H, br d, *J* = 8.0 Hz, 5-H), 4.53 (1H, d, *J* = 7.7 Hz, 20-Ha), 4.47 (1H, br d, *J* = 7.1 Hz, 13-H), 4.41 (1H, br d, *J* = 7.7 Hz, 20-Hb), 3.16 (1H, br d, *J* = 7.8 Hz, 3-H), 2.55 (1H, ddd, *J* = 15.6, 8.0, 8.0 Hz, 6-Hα), 2.26 (1H, dd, *J* = 15.1, 7.57 Hz, 14-Hα), 2.17, 2.08 (each 3H, s, acetyl methyl), 2.04 (3H, s, 18-H_3), 2.03 (3H, s, acetyl methyl), 1.91 (1H, br dd, *J* = 15.6, 8.3 Hz, 6-Hβ), 1.75 (3H, s, acetyl methyl), 1.69 (3H, s, 19-H_3), 1.59 (1H, dd, *J* = 15.3, 7.23 Hz, 14-Hβ), 1.17, 1.06 (each 3H, s, 16-H_3, 17-H_3). ^{13}C NMR ($CDCl_3$): δ 171.2 (4-O acetyl carbonyl), 169.8 (3C, 2-, 7-, 9-O acetyl carbonyl), 164.0 (10-O benzoyl carbonyl), 151.4 (11-C), 134.0 (12-C), 133.4 (4″-C), 129.5 (2″-C and 6″-C), 129.0 (1″-C), 128.8 (3″-C, 5″-C), 85.0 (5-C), 80.0 (4-C), 77.2 (13-C), 76.5 (9-C), 75.5 (1-C), 75.0 (20-C), 70.3 (7-C), 69.2 (10-C), 68.0 (2-C), 67.7 (15-C), 44.0 (8-C), 43.7 (3-C), 39.6 (14-C), 37.8 (6-C), 27.5 (17-C), 25.2 (C-16), 22.4, 21.6, 21.4, 20.7 (acetyl methyl), 12.6 (19-C), 11.9 (18-C). IR ($CHCl_3$) ν_{max} cm^{-1} 3576, 1740, 1606. EI-MS *m/z* (rel. int.): 654 $[M-H_2O]^+$ (<1), 636 (10), 536 (7), 506 (2), 105 (100).

11(15→1)abeo-Taxinine NN-14. **7** was obtained as white amorphous powder; mp 147°–149°C; $[\alpha]^{20}_D$–9.6° (*c* 0.230, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 8.02 (2H, br d, *J* = 8.0 Hz, 2″-H, 6″-H), 7.61 (1H, br t, *J* = 7.5 Hz, 4″-H), 7.43 (2H, br dd, *J* = 7.8, 7.8 Hz, 3″-H, 5″-H), 6.07 (1H, d, *J* = 7.3 Hz, 2-H), 4.89 (1H, br d, *J* = 8.2 Hz, 5-H), 4.53 (1H, d, *J* = 10.0 Hz,

10-H), 4.46 (1H, d, J = 7.8 Hz, 20-Ha), 4.34 (1H, d, J = 10.0 Hz, 9-H), 4.23 (1H, dd, J = 7.0, 7.2 Hz, 13-H), 4.21 (1H, t, J = 8.5 Hz, 7-H), 4.09 (1H, d, J = 7.8 Hz, 20-Hb), 3.02 (1H, d, J = 7.3 Hz, 3-H), 2.58 (1H, ddd, J = 15.5, 8.5, 8.1 Hz, 6-Hα), 2.26 (1H, dd, J = 14.8, 7.2 Hz, 14-Hα), 2.21, (3H, s, acetyl methyl), 1.95 (3H, s, 18-H), 1.87 (3H, s, 19-H), 1.86 (1H, br dd, J = 15.5, 8.1 Hz, 6-Hβ), 1.78 (1H, dd, J = 14.8, 7.0 Hz, 14-Hβ), 1.12, 1.06 (each 3H, s, 17-H_3, 16-H_3). ^{13}C NMR ($CDCl_3$): δ 171.0 (4-O acetyl carbonyl), 166.0 (2-O benzoyl carbonyl), 146.9(C-12), 137.4 (C-11), 133.5 (4″-C), 129.9 (1″-C), 129.6 (2″-C, 6″-C), 128.6 (3″-C, 5″-C), 85.0 (C-5), 80.7 (C-9), 80.5 (C-4), 77.7 (C-13), 76.5 (C-1), 74.8 (C-20), 72.4 (C-7), 68.8(C-10), 68.8 (C-2), 67.6 (C-15), 45.1(C-3), 42.6 (C-8), 39.8 (C-14), 37.4 (C-6), 27.8 (C-16), 25.1 (C-17), 22.5 (acetyl methyl), 12.1 (C-19), 11.3 (C-18). IR ($CHCl_3$) ν_{max} cm^{-1} 3432, 1712, 1678. EI-MS m/z (rel. int.): 528 [M]$^+$ (2), 494 (3), 355 (100).

Taxine NA-8. **9** was obtained as white amorphous powder; mp 186°–188°C; $[\alpha]^{20}_D$ +80.2° (c 0.500, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 7.42–7.30 (2H, m, 3″-H, 5″-H), 7.26–7.23 (3H, m, 2″-H, 4″-H, 6″-H), 6.04 (1H, d, J = 10.8 Hz, 10-H), 5.82 (1H, d, J = 10.8 Hz, 9-H), 5.81 (1H, m, 13-H), 5.30 (1H, br d, J = 2.5 Hz, 5-H), 5.23 (1H, br d, J = 1.5 Hz, 20-Ha), 4.85 (1H, br d, J = 1.5 Hz, 20-Hb), 4.53 (1H, d, J = 9.0 Hz, 2′-H), 3.72 (1H, d, J = 9.0 Hz, 3′-H), 2.75 (1H, br d, J = 5.5 Hz, 3-H), 2.68 (1H, ddd, J = 15.0, 10.5, 5.5 Hz, 14-Hβ), 2.24 (6H, s, NMe_2), 2.19 (3H, s, 18-H_3), 2.14, 2.05, 2.03 (each 3H, s, acetyl methyl), 1.81 (1H, m, 1-H), 1.69 (1H, br dd, J = 16.0, 5.5 Hz, 2-Hβ), 1.65 (1H, br dd, J = 16.0, 5.5 Hz, 2-Hα), 1.59 (3H, s, 17-H_3), 1.45 (1H, m, 7-Hβ), 1.34 (1H, m, 6-Hα), 1.33 (1H, m, 7-Hα), 1.09 (3H, s, 16-H_3), 0.99 (1H, dd, J = 15.0, 7.0 Hz, 14-Hα), 0.63 (3H, s, 19-H_3), 0.44 (1H, ddd, J = 11.0, 5.0, 2.0 Hz, 6-Hβ). ^{13}C NMR ($CDCl_3$): δ 172.6 (1′-C), 171.1 (13-O acetyl carbonyl), 170.3 (9-O acetyl carbonyl), 170.1 (10-O acetyl carbonyl), 147.7 (4-C), 136.9 (12-C), 134.7 (11-C), 132.6 (1″-C), 130.0 (2″-C, 6″-C), 128.5 (4″-C), 128.1 (3″-H, 5″-C), 115.2 (20-C), 77.2 (9-C), 76.1 (5-C), 72.4 (10-C), 72.1 (3′-C), 70.3 (13-C), 69.9 (2′-C), 42.7 (8-C), 41.2 (NMe_2), 40.2 (1-C), 39.2 (15-C), 38.0 (3-C), 31.9 (14-C), 31.0 (16-C), 28.0 (2-C), 27.3 (7-C), 27.1 (17-C), 27.0 (6-C), 21.1, 20.9, 20.8 (acetyl methyl), 17.7 (19-C), 15.2 (18-C). IR ($CHCl_3$) ν_{max} cm^{-1} 3328, 1740. EI-MS m/z (rel. int.): 653 [M]$^+$ (23), 651 (32), 635 (7), 594 (100), 535 (7), 518 (10); HR EI-MS m/z 653.3558 (calcd for $C_{37}H_{51}O_9N$, 653.3564).

Taxine NA-4. **12** was obtained as white granular crystals; mp 59°–62°C; $[\alpha]^{20}_D$ + 12.3° (c 0.065, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 7.42–7.30 (2H, m, 3″-H, 5″-H), 7.26–7.23 (3H, m, 2″-H, 4″-H, 6″-H), 5.97 (1H, d, J = 10.3 Hz, 10-H), 5.81 (1H, d, J = 10.3 Hz, 9-H), 5.47 (1H, dd, J = 6.4, 2.2 Hz, 2-H), 5.34 (1H, s, 20-Ha), 4.86 (1H, br s, 20-Hb), 4.86 (1H, br s, 5-H), 4.10 (1H, d, J = 7.6 Hz, 3′-H), 3.72 (1H, d, J = 7.6 Hz, 2′-H), 2.93 (1H, br d, J = 6.1 Hz, 3-H), 2.76 (1H, dd, J = 20.3, 6.8 Hz, 14-Hβ), 2.29 (3H, s, HNMe), 2.24 (1H, d, J = 20.3 Hz, 14-Hα), 2.23 (3H, s, 18-H_3), 2.16 (1H, dd, J = 6.8, 1.95 Hz, 1-H), 2.05, 2.05, 2.04 (each 3H, s, acetyl methyl), 1.72 (3H, s, 17-H_3), 1.60 (1H, m, 7-Hβ), 1.44 (1H, m, 7-Hα), 1.44 (1H, ddd, J = 11.0, 5.0, 2.0 Hz, 6-Hβ), 1.13 (3H, s, 16-H_3), 1.05 (1H, m, 6-Hα), 0.83 (3H, s, 19-H_3). ^{13}C NMR ($CDCl_3$): δ 200.2 (13-C), 172.2 (1′-C), 169.8 (10-O acetyl carbonyl), 169.8 (9-O acetyl carbonyl), 169.4 (2-O acetyl carbonyl), 152.1 (11-C), 141.1 (4-C), 138.8 (12-C), 132.7 (1″-C), 128.4 (2″-C, 6″-C), 128.1 (3″-C, 5″-C), 127.8 (4″-C), 118.1 (20-C), 78.7 (5-C), 75.7 (3′-C), 75.7 (9-C), 73.4 (10-C), 69.3 (2-C), 67.8 (2′-C), 48.6(1-C), 44.6 (8-C), 42.7 (3-C), 37.7 (15-C), 37.6 (16-C), 35.8 (14-C), 34.2 (NHMe), 28.0 (6-C), 27.4 (7-C), 25.1 (17-C), 21.4, 20.9, 20.7 (acetyl methyl), 17.4 (19-C), 14.3 (18-C). IR ($CHCl_3$) ν_{max} cm^{-1} 3520, 1746, 1666. EI-MS m/z (rel. int.): 653 [M]$^+$ (2), 594 (3), 196 (100).

The structures of the known compounds isolated here were confirmed by comparison of their spectral data and physical data with those reported previously for 7-epitaxol (**3**),[16–18] 7-epicephalomannine (**4**),[19,20] taxayuntin (**6**),[21,22] taxine NA-1 (**8**),[6,14,15] 2′-deacetylaustrospicatine (**10**),[15,23] taxine NA-11 (**11**),[15,24] taxine II (**13**),[13,15] 7,2′-didesacetoxyaustrospicatine (**14**),[25] 2′-desacetoxyaustrospicatine (**15**),[23] taxine NA-3 (**16**),[15,26] taxine NA-2 (**17**),[15,26,27] taxuspine H (**18**),[5] taxinine (**19**),[10–12] 5-cinnamoyl-10-acetyltaxicin II (**20**),[28] 2,9,10-deaceyltaxinine (**21**),[29,30] 9α,10β,13α-triacetoxy-5α-cinnamoyloxytaxa-4(20),11-diene (**22**),[31] taxinine NN-1 (**23**),[15,32] 9,10-deacetyltaxinine (**24**),[30] taxuspine C (**25**),[3,33,34] taxagifine (**26**),[35–37] taxuspine F (**27**),[5] 2-deacetyl-5α-decinnamoyltaxinine J (**28**),[38] taxezopidine F (**29**),[32,39] taxinine A (**30**),[40,41] decinnamoyltaxinine J (**31**),[42] decinnamoyltaxagifine (**32**),[36,43] 12(3→20)*abeo*-taxinine NN-2 (**33**),[15,44] and taxinine NN-2 (**34**).[45] The structures of these compounds are shown in Fig. 5.

Classification of isolated compounds

The taxoids isolated here (Fig. 5) possess from four to seven oxygen functional groups at 1, 2, 4, 5, 7, 9, 10, 13, and 20 positions of the taxane skeleton or the rearranged taxane skeleton. In them, the compound possessing modified 3-phenylisoseryloxy- or 3-amino-3-phenylpropanoyloxy group at 5α-position was designated as basic taxoid. The compound for which all functional groups are acyloxy or 13-carbonyl group was designated as less polar taxoid. The compound possessing more than one free hydroxyl group in their oxygen functional groups was designated as polar taxoid. Because paclitaxel (taxol) has an oxetane D ring as a reaction site of the anticancer agent, the taxoid with an oxetane D ring was designated as paclitaxel analog. Thus, the following classification of the taxoides isolated here is possible. Compounds **1** and **8–18** belong to the basic taxoids; **c**ompounds **2**, **19**, **22**, **25**, and **34** belong to the less polar neutral taxoids; compounds **3–7**, **20**, **21**, **23**, **24**, and **26–33** belong to the polar neutral taxoids; and compounds **3–7** belong to the paclitaxel analogs. Because the nitrogen bond at the 3′-position of modified 3-phenylisoseryloxy side chain at the 13-position of paclitaxel and its analogs **3** and **4** are amide bonds and not basic and compounds **3–7** have more than two free hydroxyl groups, they also belong to the polar neutral taxoids.

Cell growth inhibitory activity of compounds to WI-38 fibroblast cell, VA-13 malignant tumor cell, and HepG2 human liver tumor cell in vitro

Cells. WI-38 is the normal human fibroblast derived from female human lung. VA-13 is malignant tumor cells induced from WI-38 by infection of SV-40 virus. HepG2 is human liver tumor cells. These cell lines are available from the Institute of Physical and Chemical Research (RIKEN), Tsukuba, Ibaraki, Japan. WI-38 and VA-13 cells were maintained in Eagle's MEM medium (Nissui Pharmaceutical, Tokyo, Japan) and RITC 80-7 medium (Asahi Technoglass, Chiba, Japan), respectively, both supplemented with 10% (v/v) fetal bovine serum (FBS) (Filtron, Australia) with 80 μg/ml of kanamycin. HepG2 cells were maintained in D-MEM medium (Invitrogen) supplemented with 10% (v/v) FBS (Filtron) with 80 μg/ml of kanamycin.

Procedures. Medium (100 μl) containing ca. 5000 cells (WI-38, VA-13, HepG2) was incubated at 37°C in a humidified atmosphere of 5% CO_2 for 24 h in 96-well micro plate. Then test samples dissolved in dimethyl sulfoxide (DMSO) were added to the medium and incubation was continued for a further 48 h in the same conditions. Coloration substrate, WST-8 [2-(2-methyl-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium monosodium salt] was added to the medium. The resulting formazan concentration was determined by measuring the absorption at 450 nm. Cell viability (%) was calculated as [(experimental absorbance – background absorbance)/(control absorbance – background absorbance)] × 100. Cell viability at different concentration of compounds was plotted and 50% inhibition of growth was calculated as IC_{50}.

Cellular accumulation of [^{3}H]-vincristine (VCR)

The multidrug-resistant 2780AD cells were maintained in PPMI 1640 medium (Nissui, Tokyo, Japan) supplemented with 5% heat-inactivated fetal bovine serum and kanamycin (100 μg/ml). 2780AD cells (1×10^6 cells per well) were seeded in a 24-well plate and cultured for 18 h before the assay. The cells were treated with 1×10^5 disintegrations per minute (dpm) of [^{3}H]-VCR (222 Gbq/mmol; Amersham Pharmacia Biotech, Tokyo, Japan) in the presence or absence of verapamil or taxoids. Immediately after incubation for 2 h at 37°C, the cells were washed five times with ice-cold phosphate-buffered saline containing 0.1 mg/ml of nonradioactive vincristine (VCR) and lysed with 500 μl of 0.2 M NaOH. After incubation for 45 min at 56°C, lysates were neutralized with 2 M acetic acid, and the radioactivity was counted in ACS II (Amersham Pharmacia Biotech).

Analyses of the anticancer activity of compounds based on the 39 human cancer cell lines panel (HCC panel)

Experimental details of anticancer activity of compounds based on the HCC panel and the data analysis were described previously.[46]

Results and discussion

Cytotoxic activities of compounds toward human cancer and normal cells

The side reaction of anticancer agents toward normal cells in the human body is a serious problem in cancer chemotherapy. An effective anticancer agent with no side reaction is desirable. We employed the combination of human lung fibroblast cells (WI-38) and malignant lung tumor cells (VA-13) induced from WI-38 as a model of in vitro assay in order to find effective cytotoxic compounds against human lung tumor cells with no cytotoxicity toward their parental normal cells.

There is no effective anticancer agent for liver cancer, because the liver is where toxic substances including anticancer agents are detoxified. The reaction mechanisms for liver cells to transfer a toxic substance from inside to outside the cells in the course of detoxification are analogous with those of MDR cancer cells. Thus, an effective anticancer agent for liver cancer is also expected to be a new type of anticancer agent toward MDR cancer.

Therefore, we decided to examine the cell growth inhibitory activity (IC_{50}) of compounds **1**, **3–6**, **8–13**, **15–21**, **23–30**, and **32** against the three cell lines, WI-38, VA-13, and HepG2 cells (Table 1). 7-Epitaxol (**3)** showed the smallest

Table 1. Cell growth inhibitory activities of taxanes **1**, **3–6**, **8–13**, **15–21**, **23–30**, and **32** against WI-38, VA-13, and HepG2 cells

Compound	IC_{50} (μg/ml)		
	WI-38	VA-13	HepG2
1	95.7	72.1	88.1
3	89.2	0.0063	8.97
4	11.1	0.024	0.49
5	>100	5.2	45.2
6	68.7	49.1	72.3
8	9.8	63.3	64.2
9	>100	71.5	51.9
10	79.6	>100	85.6
11	83.4	>100	>100
12	25.9	>100	62.0
13	21.8	88.8	65.2
15	70.3	96.4	62.5
16	>100	>100	>100
17	8.33	0.99	17.7
18	68.0	6.1	25.9
19	45.1	60.9	74.0
20	49.4	59.2	25.7
21	28.1	65.1	19.7
23	31.9	63.7	6.1
24	7.7	43.1	7.8
25	91.9	>100	39.9
26	9.5	6.1	8.3
27	>100	61.5	74.4
28	80.7	53.2	53.8
29	>100	>100	89.5
30	65.8	72.8	>100
32	68.7	49.1	72.3
Paclitaxel	0.034	0.0043	6.9
Adriamycin	0.38	0.22	0.69

IC_{50} is the concentration that causes 50% growth inhibition; values given are means of duplicate determinations. Paclitaxel and adriamycin are positive controls

IC_{50} values toward VA-13 (IC_{50} = 0.0063 μg/ml) in paclitaxel analogs (**3–5**) and a rearranged paclitaxel analog **6** possessing an oxetane D ring. Interestingly, compound **3** showed very weak cytotoxic activity toward parental normal cells, WI-38 (89.2 μg/ml). Although the cytotoxic activities of **3** toward VA-13 and HepG2 were slightly weaker than those of paclitaxel, the cytotoxic activity of **3** toward WI-38 was 2600 times weaker than that of pacritaxel. These results suggest that **3** possesses desirable properties as an anticancer agent. Displacement of the N-acyl group at 3′-C of the 3-phenylisoseryloxy side chain of **3** from the benzoyl group to the 2-butene-2-carbonyl group of **4** induced the decrease of activity toward VA-13 (IC_{50} = 0.024 μg/ml) but induced an increase of activity toward HepG2 (IC_{50} = 0.49 μg/ml). Elimination of the 3-phenylisoseryl group of **3** and **4** induced a decrease of activity of taxinine NN-6 (**5**) toward VA-13 (IC_{50} = 5.20 μg/ml) and HepG2 (IC_{50} = 45.2 μg/ml). Cytotoxic activity of **6** against VA-13 (IC_{50} = 49.1 μg/ml) was decreased remarkably by the skeletal rearrangement of the taxane skeleton of **5** to the 11(15→1)*abeo*-taxane skeletone of taxayuntin (**6**). Taxine NA-1 (**8**) and its analogs **9–12** with the 5α-(3-phenylisoseryloxy) side chain showed very weak or no cytotoxic activity toward WI-38, VA-13, and HepG2. Taxine II (**13**) and its analogs **15** and **16** with the 5α-[3′-(dimethylamino)-3′-phenylpropanoyloxy] side chain also showed very weak or no cytotoxic activity toward WI-38, VA-13, and HepG2. Interestingly, taxine NA-2 (**17**), the 13α-acetoxy-2α-hydroxy derivative of **13**, showed significant activity toward VA-13 (IC_{50} = 0.99 μg/ml) and weak activity toward HepG2 (IC_{50} = 17.7 μg/ml). Taxuspine H (**18**) with 3,11-cyclotaxane derivative of **13**, showed moderate activity toward VA-13 (IC_{50} = 6.10 μg/ml). Taxinine NN-1 (**23**) and 9,10-deacetyltaxinine (**24**) showed moderate cytotoxic activity toward HepG2 (IC_{50} = 6.10 μg/ml for **23**; IC_{50} = 7.80 μg/ml for **24**) in taxinine (**19**) and its analogs, **20–24** possessing cinnamoyloxy group at 5α positon. Taxagifine (**26**), the 7β-acetoxy-12,16-epoxy derivative of taxinine, showed moderate cytotoxic activity toward WI-38, VA-13, and HepG2 cells (IC_{50} = 9.5, 6.1, and 8.3 μg/ml, respectively). Other taxane derivatives **27–30** and **32** with no side chain on the 5α-oxygen atom showed very weak or no cytotoxic activity toward WI-38, VA-13, and HepG2.

MDR-cancer reversal activity of compounds

In cancer chemotherapy, the 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 drugs.[47–49] P-gp is a transporter for a wide range of reagents utilizing

Table 2. Effects of compounds **7**, **8**, **9, 12**, **16**, and **23** on the accumulation of vincristine (VCR) in multidrug-resistant cells 2780AD

Compound	VCR accumulation[a]					Evaluation
	Concentration (μg/ml)	Average[b] (dpm/well)	Against control[c] (%)	Activity[d]	Against verapamil[e] (%)	Maximum verapamil concentration
7	0.1	490	94	±	88	N
	1	475	91	±	62	
	10	477	91	±	34	
8	0.1	557	107	±	100	N
	1	627	120	+	82	
	10	873	167	+	62	
9 (1)	0.1	481	92	±	86	R
	1	668	128	+	87	114%
	10	1612	308	++	114	10 μg/ml
12	0.1	441	84	–	79	N
	1	1324	253	+	82	
	10	1775	339	++	88	
16	0.1	443	85	–	79	N
	1	778	149	+	101	
	10	1336	255	+	94	
23 (1)	0.1	642	123	+	115	R
	1	1324	253	+	172	172%
	10	1775	339	++	126	1 μg/ml
Control	0	523	100			
	0.1	558	107	±	100	
Verapamil	1	767	147	+	100	
	10	1414	270	+	100	

dpm, Disintegrations per minute; N, no activity; R, positive: the activity is stronger than that of verapamil (verapamil% > 100%) and reexamined to check the reproducibility of the assay result

[a]The amount of VCR accumulated in multidrug-resistant human ovarian cancer 2780AD cells was determined with the control in the presence of 0.1, 1, and 10 μg/ml of taxoid

[b]Values represent means of triplicate determination

[c]Relative amount of VCR accumulated in the cell compared with the control experiment

[d]Indices expressed on a seven-step scale by the range of the relative amount of VCR accumulation as compared with the control experiment: ++, 301%–500%; +, 111%–300%; ±, 91%–110%; –, <90%

[e]Relative amount of VCR accumulation in the cell as compared with that of verapamil

energy by hydrolysis of ATP. 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 that showed MDR reversal activity have been reported.[4,50–56] We have previously reported the isolation of MDR reversal agents from *Taxus cuspidata*,[7,8,15] and their production by callus culture.[46,57–59] In this article, we report the results of our investigation of MDR reversal activity of compounds **7–10**, **12**, **16**, **17**, **20**, **22**, **23**, **26**, and **33**.

The effects of compounds **7–9**, **12**, **16**, and **23**; **10**, **26**, and **33**; and **17**, **20**, and **22** on the cellular accumulation of vincristine (VCR) in human ovarian MDR cancer 2780AD cells were examined and the preliminary results are summarized in Tables 2 and 3, respectively. Compound **7** had no effect (<100% of control) and compounds **8**, **9**, **12**, **16**, and **23** were effective compounds (>100% of control). Of these, compounds **9** and **23** showed stronger activity than that of verapamil (verapamil maximum = 114% and 172% at 10 and 1 μg/ml, respectively). Compounds **9** and **23** were selected for reexamination to check reproducibility of the assay results (Table 2).

Compounds **26** and **33** had no effect (≤100% of control) and compound **10** showed weak activity (230% of control at 10 μg/ml). Because compound **10** showed weaker activity than that of the positive control (<100% of verapamil activity), the activity of compound **10** was judged to be negative. All of the compounds **17**, **20**, and **22** showed stronger activities than that of verapamil (106%, 122%, and 121% of verapamil activity at 10, 1, and 1 μg/ml, respectively) and were selected for reexamination to check reproducibility of the assay results (Table 3).

The results of reexamination of the effects of compounds **9**, **17**, **20**, **22**, and **23** on the accumulation of VCR in MDR 2780AD are summarized in Table 4. Of these, compounds **9**, **17**, **20**, and **23** again showed stronger activity than that of verapamil (200%, 105%, 145%, and 323% of verapamil activity at 10, 10, 1, and 1 μg/ml, respectively) and were evaluated to be positive (P). The results suggest that the active compounds contains one aromatic ring such as N,N-dimethyl-3-phenylisoseryloxy-, 3-(dimethylamino)-3-phenylpropanoyloxy-, and cinnamoyloxy-groups at C-5 and free hydroxyl group at C-2 of the taxane skeleton or the 2′ position of side chain.

Table 3. Effects of compounds **10**, **17**, **20**, **22, 26**, and **33** on the accumulation of VCR in multidrug-resistant cells 2780AD

Compound	VCR accumulation[a]					Evaluation
	Concentration (μg/ml)	Average[b] (dpm/well)	Against control[c] (%)	Activity[d]	Against verapamil[e] (%)	Maximum verapamil concentration
10	0.1	369	87	–	76	N
	1	384	90	±	56	
	10	976	230	+	73	
26	0.1	413	97	±	84	N
	1	406	96	±	60	
	10	451	106	±	34	
33	0.1	388	91	±	79	N
	1	403	95	±	59	
	10	411	97	±	31	
Control	0	523	100			
Verapamil	0.1	558	107	±	100	
	1	767	147	+	100	
	10	1414	270	+	100	
17 (1)	0.1	255	89	–	87	R
	1	341	119	+	86	106%
	10	1019	356	++	106	10 μg/ml
20 (1)	0.1	252	88	–	86	R
	1	480	168	+	122	122%
	10	1088	380	++	113	1 μg/ml
22 (1)	0.1	323	113	+	111	R
	1	477	167	+	121	121%
	10	698	244	+	73	1 μg/ml
Control	0	286	100			
	0.1	291	102	±	100	
Verapamil	1	395	138	+	100	
	10	959	335	++	100	

[a]The amount of VCR accumulated in multidrug-resistant human ovarian cancer 2780AD cells was determined with the control in the presence of 0.1, 1, and 10 μg/ml of taxoid
[b]Values represent means of triplicate determination
[c]Relative amount of VCR accumulated in the cell compared with the control experiment
[d]Indices expressed on a seven-step scale by the range of the relative amount of VCR accumulation as compared with the control experiment
[e]Relative amount of VCR accumulation in the cell as compared with that of verapamil

Table 4. Reexamination of effects of compounds **9**, **17**, **20**, **22**, and **23** on the accumulation of VCR in multidrug-resistant cells 2780AD

Compound	VCR accumulation[a]					Evaluation
	Concentration (μg/ml)	Average[b] (dpm/well)	Against control[c] (%)	Activity[d]	Against verapamil[e] (%)	Maximum verapamil concentration
9 (2)	0.1	259	114	+	97	P
	1	726	318	++	153	200%
	10	2421	1062	++++	200	10 μg/mL
23 (2)	0.1	339	149	+	127	P
	1	1527	670	+++	323	323%
	10	2143	940	+++	177	1 μg/ml
Control	0	228	100			
Verapamil	0.1	226	117	+	100	
	1	473	207	+	100	
	10	1211	531	+++	100	
17 (2)	0.1	254	98	±	102	P
	1	365	140	+	93	105%
	10	921	354	++	105	10 μg/ml
20 (2)	0.1	265	102	±	106	P
	1	563	217	+	145	145%
	10	1053	405	++	120	1 μg/ml
22 (2)	0.1	255	98	±	99	N
	1	410	158	+	100	100%
	10	659	253	+	75	1 μg/ml
Control	0	260	100			
Verapamil	0.1	249	96	±	100	
	1	391	150	+	100	
	10	880	338	++	100	

P, Positive
[a]The amount of VCR accumulated in multidrug-resistant human ovarian cancer 2780AD cells was determined with the control in the presence of 0.1, 1, and 10 μg/ml of taxoid
[b]Values represent means of triplicate determination
[c]Relative amount of VCR accumulated in the cell compared with the control experiment
[d]Indices expressed on a seven-step scale by the range of the relative amount of VCR accumulation as compared with the control experiment: ++++, >1000%–2000%; +++, 501%–1000%
[e]Relative amount of VCR accumulation in the cell as compared with that of verapamil

Table 5. Summary of evaluation of compound **4** based on the 39 humancancer cell lines

Parameters of effective concentrations				COMPARE analysis			
	GI_{50}	TGI	LC_{50}	Rank	Compound	*r*	Molecular targets/drug type
MG-MID	–7.15	–6.07	–6	1	Paclitaxel	0.9	Tubulin
				2	Paclitaxel	0.861	Tubulin
				3	Paclitaxel	0.859	Tubulin

GI_{50}, The 50% growth inhibition parameter; TGI, the total growth inhibition parameter; LC_{50}, the lethal concentration parameter; *r*, Pearson correlation coefficient. The correlation coefficient of the mean graph of compounds was compared with those of known anticancer agents by the COMPARE analysis by the computer program. $r < 0.5$: COMPARE negative means the compound is a new anticancer agent with a new reaction mechanism. $0.5 < r < 0.75$: COMPARE marginal means that the compound is a borderline compound. $0.75 < r$: COMPARE positive means the compound possesses a similar reaction mechanism with a known anticancer agent; MG-MID, Mean-graph midpoint. Mean-graph midpoint (MG-MID) is an average value of $\log GI_{50}$, logTGI, and $\log LC_{50}$ of compounds toward 39 human cancer cells expressed by M concentration.
The mean graph of the tested compound was compared with those of 200 standard compounds using COMPARE analysis. Drugs were ordered according to the correlation coefficient

Evaluation of compounds based on the 39 human cancer cell lines panel

The cytotoxic activities of 7-epicephalomannine (**4**), 11(15→1)*abeo*-taxinine NN-1 (**7**), taxine NA-8 (**9**), and taxinine NN-1 (**23**) were examined on the 39 human cancer cell lines panel (HCC panel).[60,61] The results for compounds **4**, **7**, **9**, and **23** are shown in Tables 5, 6, 7, and 8, respectively. The effective concentration of **4** is low enough and differential growth inhibition is clearly recognized. Because the COMPARE result of **4** is positive ($r > 0.85$), it belongs to a known mechanistic class. The fingerprint is quite similar to that of paclitaxel. The analysis result suggests that the molecular target is tubulin (Table 5).

The cell growth inhibition effect expressed by mean-graph midpoint (MG-MID) of GI_{50} (–4.28) and differential growth inhibition of compound **7** are weak. Because the COMPARE result of **7** is positive ($r > 0.67$), it belongs to a known mechanistic class. The fingerprint is similar to that of paclitaxel. The analysis result suggests that the molecular target is tubulin. Thus, compound **7** is not an effective anticancer agent (Table 6).

Table 6. Summary of evaluation of compound **7** based on the 39 human cancer cell lines

Parameters of effective concentrations				COMPARE analysis			
	GI_{50}	TGI	LC_{50}	Rank	Compound	*r*	Molecular targets/drug type
MG-MID	–4.28	–4.03	–4	1	Paclitaxel	0.743	Tubulin
				2	Paclitaxel	0.677	Tubulin
				3	Paclitaxel	0.673	Tubulin

The mean graph of the tested compound was compared with those of 200 standard compounds using COMPARE analysis. Drugs were ordered according to the correlation coefficient

Table 7. Summary of evaluation of compound **9** based on the 39 human cancer cell lines

Parameters of effective concentrations				COMPARE analysis			
	GI_{50}	TGI	LC_{50}	Rank	Compound	*r*	Molecular targets/drug type
MG-MID	–4.64	–4.1	–4	1	Interferon-α	0.403	Tubulin
				2	UCN-01	0.382	Protein kinase C
				3	Navelbine	0.353	Tubulin

Table 8. Summary of evaluation of compound **23** based on the 39 human cancer cell lines[a]

Parameters of effective concentrations				COMPARE analysis			
	GI_{50}	TGI	LC_{50}	Rank	Compound	*r*	Molecular targets/drug type
MG-MID	–5.33	–4.23	–4.03	1	Interferon-α	0.488	Protein kinase C
				2	UCN-01	0.411	Antimetabolite
				3	Navelbine	0.404	Tubulin

The cell growth inhibition effect (MG-MID of GI_{50} = –4.64) and differential growth inhibition of compound **9** are weak. The COMPARE result of **9** is negative ($r < 0.5$), thus it belongs to a new mechanistic class. The results suggest that compound **9** is not an effective anticancer agent (Table 7).

The effective concentration of **23** is low enough (MG-MID of $GI_{50} < -5$). Although the value was weak, differential growth inhibition is recognized (Delta = 0.43, Range = 1.3). Because the COMPARE result of **23** is negative ($r < 0.5$), it possibly belongs to a new mechanistic class and may be a new anticancer agent (Table 8).

Conclusions

Compound **23** was identified as the most interesting compound in the results of bioassay. This is explained in terms of the following points. (1) Compound **23** showed the same level of cytotoxic activity toward HepG2 as paclitaxel, but the cytotoxic activity of **23** toward human normal cell, WI-38, was 900 times less than that of paclitaxel. This is a desirable result in the screening of anticancer reagents. (2) Compound **23** was the most effective compound in tested compounds on the accumulation of vincristine in MDR 2780AD cells. With the activity of **23** being three times higher than that of verapamil and the cytotoxic activity of **23** toward human normal cell, WI-38, being weak, compound **23** is expected to be a new MDR reversal agent. (3) Evaluation of the anticancer activity of compound **23** based on the HCC panel suggests **23** as a new mechanistic class and a new anticancer agent.

Compounds **3**, **4**, **5**, **17**, **18**, and **26** showed effective cytotoxic activity toward V-13 cells. Of these, compounds **3**, **4**, **5**, **17**, and **18** are desirable antitumor agents, because their ratios of IC_{50} toward WI-38/IC_{50} toward V-13 were 1460, 462, >20, 8.3, and 11, respectively, and larger than 1.

Compounds **4** and **23** showed stronger cytotoxic activities toward HepG2 than paclitaxel. The analyses of the HCC panel revealed that compound **4** was an effective anticancer agent and the molecular target of **4** is tubulin as we expected from its structural analogy with paclitaxel.

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