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Aporphine Alkaloids and their Reversal Activity of Multidrug Resistance (MDR) from the Stems and Rhizomes of *Sinomenium acutum*

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Chromatographic separation of the MeOH extract from the stems and rhizomes of *Sinomemium acutum* led to the isolation of nine alkaloids and a lignan. Their structures were determined to be dauriporphine (**1**), bianfugecine (**2**), dauriporphinoline (**3**), menisporphine (**4**), (-)-syringaresinol (**5**), N-feruloyltyramine (**6**), acutumine (**7**), dauricumine (**8**), sinomenine (**9**), and magnoflorine (**10**) by spectroscopic means. These compounds were examined for their P-gp mediated MDR reversal activity in human cancer cells. Compound **1** showed the most potent P-gp MDR inhibition activity with an ED_{50} value 0.03 μg/mL and 0.00010 μg/mL in the MES-SA/DX5 and HCT15 cells, respectively.

Key words: *Sinomenium acutum*, Menispermaceae, MDR, Aporphine alkaloid, Lignan

INTRODUCTION

The stems and rhizomes of *Sinomemium acutum* (Menispermaceae) were widely used in traditional Chinese medicine as an antirheumatic and analgesic agent for treating rheumatic arthritis, articular swelling and pain (Yan *et al.*, 1999). Alkaloids and lignans have been isolated from *S. acutum* (Wang *et al.*, 2002: Otsuka *et al.*, 1993).

As part of an ongoing study into biological active compounds in Korean medicinal plants, *S. acutum* was examined. By repeated column chromatographic separation of the MeOH extract of the stems and rhizomes of *S. acutum,* nine alkaloids and a lignan were isolated. Their structures were determined to be dauriporphine (**1**), bianfugecine (**2**), dauriporphinoline (**3**), menisporphine (**4**), (-)-syringaresinol (**5**), *N*-feruloyltyramine (**6**), acutumine (**7**), dauricumine (**8**), sinomenine (**9**), and magnoflorine (**10**) by spectroscopic means. The compounds **2**, **4**, **5**, and **8** were first reported from this source. The compounds were tested for their cytotoxicity against five tumor cell lines *in vitro* by SRB method. Of them, the marginal or non cytotoxic compounds were examined for their MDR reversal activity. This paper reports the characterization and the MDR reversal activities of these compounds isolated from *S. acutum.*

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MATERIALS AND METHODS

General experimental procedures

The melting points were determined on a Gallenkamp melting point apparatus and were uncorrected. The optical rotations were determined using a Jasco P-1020 polarimeter. The IR spectra were recorded as KBr discs on a Bruker Vector 22 FT-IR spectrometer. The UV spectra were obtained using a Shimadzu UV-1601 UV/Visuble (Japan) and PDA detector (Waters Co.). The NMR spectra were recorded on Varian VXR-500 and JNM-LA400. The EI-MS data were obtained using a JMS700 spectrometer (Jeol Co.). The LC-ESI-MS/MS data were acquired using a Quattro micro (Waters Co.). The prep-HPLC was performed with Prep Nova-Pak HR C18 (6 μm, 19×300 mm) column using a PDA detector (Waters Co., model 2996) and a RI detector (Waters Co., model 2414). Silica gel 60 (0.063-0.200 mm, Merck Co.) was used for column chromatography. Kiesel gel 60F254 precoated plates (Merck Co.) and RP-18 F254s precoated plates (Merck

Co.) were used for TLC. Sephadex LH-20 (Pharmacia Co.) was used as the packing material used in molecular sieve column chromatography. DIAION HP-20 (Mitsubishi Co.) was used as the reversed-phase adsorption resin.

Plant material

The Stem and rhizome of *S. acutum* was purchased in May, 2002, Seoul, Korea, and a voucher specimen (SKKU-2002-06) was deposited in the college of Pharmacy at Sungkyunkwan University.

Extraction and isolation

The dried, chopped stem and rhizome (2.5 kg) were extracted three times with MeOH (5 L × 3) at room temperature. The resulting extracts (200 g) were suspended in distilled water (3 L), followed by fractionated with n-hexane (44 g), chloroform (16 g), and n-butanol (35 g). Each fraction was examined for its anti-MDR activity against the SK-OV-3 (P-gp non-expressing MDR cell), HCT15 and MES-SA/DX5 (P-gp expressing MDR cell) cell lines. The n-hexene and chloroform fractions showed moderate anti-MDR activity (Table I).

The n-hexane layer (40 g) was chromatographed through a silica gel column using the hexane : ethylacetate (10:1 → 2:1) gradient solvent system as eluents to give nine sub-fractions (H-1~H-9). Subfraction H-7 was purified on a Sephadex LH-20 column (CH_2Cl_2:MeOH=1:1) and RP prep. HPLC (80% MeOH) to afford compounds **1** (64 mg) and **2** (2 mg). Subfraction H-9 was purified on a silica gel column (hexane:ethylacetate=10:1) and a Sephadex LH-20 column (CH_2Cl_2:MeOH=1:1) to afford compound **3** (14 mg).

The chloroform layer (14 g) was subjected to silica gel column chromatography using the hexane : ethylacetate : MeOH (10:10:1) solvent system as eluents to give eight sub-fractions (C-1~C-8). Subfraction C-2 (300 mg)was purified on a Sephadex LH-20 column (CH_2Cl_2:MeOH =1:1) and RP prep. HPLC (80% MeOH) to afford compound **4** (10 mg). Subfraction C-5 (1.5 g) was purified on a Sephadex LH-20 column (CH_2Cl_2:MeOH=1:1) and RP LPLC (80% MeOH) to afford compounds **5** (81 mg) and **6** (493 mg). Subfraction C-7 (800 mg) was purified in a Sephadex LH-20 column (CH_2Cl_2:MeOH=1:1) and RP LPLC (60% MeOH) to afford compounds **7** (25 mg) and **8** (9 mg).

Table I. MDR reversal activity of fractions obtained from *S. acutum* (net growth as % of control)

Compounds	ED_{50} (μg/mL)		
	SK-OV-3	HCT15	MES-SA/DX5
Paclitaxel	0.0004	0.1130	0.817
P + MeOH Fr.	0.0003	0.0190	0.374
P + Hexane Fr.	0.0007	0.0090	0.286
P + $CHCl_3$ Fr.	0.0003	0.0030	0.347
P + BuOH Fr.	0.0007	0.0260	0.307
P + verapamil	0.0007	0.0003	0.022

The n-butanol layer (34 g) was loaded onto a DIAION HP-20 resin column, and eluted with 100% distilled water and 30% MeOH. The 30% MeOH layer (18 g) was chromatographed through a silica gel column using the ethylacetate : MeOH : water (10:4:2) solvent system to give seven sub-fractions (B30-1~B30-7). Subfraction B30-5 (5.1 g) was purified with RP prep. HPLC (30% MeOH, 0.1% TFA) to afford compound **9** (190 mg). Subfraction B30-7 (2.8 g) was purified with RP prep. HPLC (30% MeOH, 0.1% TFA) to give compound **10** (972 mg).

Dauriporphine (1)

Yellow needle (CH_2Cl_2/MeOH), mp : 160°C; UV λ_{max}^{MeOH} nm : 211, 262; IR ν_{max}^{KBr} cm^{-1} : 1646, 1603, 1457, 1352, 1023; EI-MS *m/z* (rel. int.) : 351 (M^+, 100), 336, 322, 306, 336, 293; ^{1}H-NMR (400 MHz, $CDCl_3$) : δ 3.97 (3H, s, $-OCH_3$), 4.03 (3H, s, $-OCH_3$), 4.15 (3H, s, $-OCH_3$), 4.25 (3H, s, $-OCH_3$), 7.32 (1H, dd, *J* = 2.7, 8.8 Hz, H-10), 7.86 (1H, d, *J* = 2.7 Hz, H-8), 7.96 (1H, d, *J* = 5.6 Hz, H-3), 8.67 (1H, d, *J* = 5.6 Hz, H-2), 8.85 (1H, d, *J* = 8.8 Hz, H-11); ^{13}C-NMR (100 MHz, $CDCl_3$) : δ 55.71 (br, $-OCH_3$), 61.82 ($-OCH_3$), 62.87 ($-OCH_3$), 109.53, 114.71, 116.39, 120.02, 121.63 (very intense peak), 126.80, 128.40, 135.08, 152.99, 181.18 (C=O).

Bianfugecine (2)

Yellow powder, UV λ_{max}^{MeOH} nm : 206, 256; EI-MS *m/z* (rel. int.) : 291 (M+, 100), 276 (M^+- CH3), 261, 248, 220, 177, 146; ^{1}H-NMR (400 MHz, $CDCl_3$) : δ 4.02 (3H, s, $-OCH_3$), 4.08 (3H, s, $-OCH_3$), 7.41 (1H, dd, *J* = 2.8, 8.7 Hz), 7.45 (1H, d, *J* = 2.5 Hz), 7.68 (1H, d, *J* = 5.9 Hz), 7.88 (1H, d, *J* = 2.8 Hz), 8.31 (1H, d, *J* = 2.5 Hz), 8.66 (1H, d, *J* = 5.9 Hz), 9.01 (1H, br s).

Dauriporphinoline (3)

Yellow needle (CH_2Cl_2/MeOH), mp : 190~192°C; UV λ_{max}^{MeOH} nm : 215, 233, 256; IR ν_{max}^{KBr} cm^{-1} : 3500~3300, 1655 (conj. C=O), 1611, 1487, 1223, 1014; EI-MS *m/z* (rel. int.) : 337 (M^+, 100), 322, 294, 251, 208; ^{1}H-NMR (500 MHz, $CDCl_3$) : δ 4.04 (3H, s, $-OCH_3$), 4.13 (3H, s, $-OCH_3$), 4.38 (3H, s, $-OCH_3$), 7.50 (1H, dd, *J* = 2.9, 8.8 Hz), 7.99 (1H, d, *J* = 2.9 Hz), 8.06 (1H, br s), 8.83 (1H, d, *J* = 5.4 Hz), 9.13 (1H, br s).

Menisporphine (4)

Yellow powder, UV λ_{max}^{MeOH} nm : 212, 270; IR ν_{max}^{KBr} cm^{-1} : 1660 (conj. C=O), 1603, 1478, 1283, 1013; EI-MS *m/z* (rel. int.) : 321 (M^+, 100), 306 (M^+ - CH3), 292; ^{1}H-NMR (500 MHz, $CDCl_3$) : δ 4.01 (3H, s, $-OCH_3$), 4.13 (3H, s,

-OCH_3), 4.18 (3H, s, -OCH_3), 7.41 (1H, dd, *J* = 2.5, 9.0 Hz), 7.45 (1H, s), 7.68 (1H, d, *J* = 5.9 Hz), 7.90 (1H, d, *J* = 2.5 Hz), 8.69 (1H, d, *J* = 5.9 Hz), 9.09 (1H, br s).

(-)-Syringaresinol (5)

Colorless needle, mp : 170°C; [α]: -47.0° (*c* = 0.1, $CHCl_3$); UV λ_{max}^{MeOH} nm : 209, 237, 272; IR ν_{max}^{KBr} cm^{-1} : 2943, 2870, 1611, 1519, 1457, 1111; EI-MS *m/z* (rel. int.) : 418 (M^+, 100), 235, 205, 193, 181, 167; ^{1}H-NMR (500 MHz, CDCl3) : δ 3.10 (2H, m, H-8, 8'), 3.91 (12H, s, -OCH_3), 3.90-3.95 (2H, m), 4.3 (2H, m), 4.75 (2H, d, *J* = 4.3 Hz, H-7, 7'), 5.49 (s, -OH), 6.60 (4H, s, H-2, 2', 6, 6'); ^{13}C-NMR (125 MHz, $CDCl_3$) : δ 54.38 (C-9, 9'), 56.40 (-OCH3), 71.81 (C-8, 8'), 86.07 (C-7, 7'), 102.77 (2, 2', 6, 6'), 132.14 (1, 1'), 134.37 (4, 4'), 147.18 (3, 3', 5, 5').

***N*-Feruloyltyramine (6)**

Colorless crystal, mp :92~93°C; UV λ_{max}^{MeOH} nm : 221, 294, 320; IR ν_{max}^{KBr} cm^{-1} : 3363, 1656, 1587, 1454, 1250, 1032; EI-MS *m/z* (rel. int.) : 313 (M^+), 193, 192, 177 (100), 145, 120; ^{1}H-NMR (500 MHz, $CDCl_3$+CD_3OD) : δ 2.69 (2H, t, *J* = 6.8 Hz), 3.45 (2H, t, *J* = 6.8 Hz), 3.76 (phenolic OH), 3.80 (3H, s, -OCH_3), 6.19 (1H, d, *J* = 15.6 Hz), 6.68 (2H, m), 6.75 (1H, m), 6.85-6.98 (4H, m), 7.39 (1H, d, *J* = 15.6 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$+CD_3OD) : δ 34.34 (C-β), 41.00 (C-α), 55.56 (OCH_3), 110.09 (C-3), 114.95 (C-3", C-5"), 115.16 (C-5'), 117.10 (C-2'), 121.98 (C-6'), 126.75 (C-3'), 129.45 (C-2", C-6"), 129.59 (C-1"), 141.18 (C-2), 147.26 (C-4'), 147.86 (C-1'), 155.13 (C-4"), 167.23 (C-1).

Acutumine (7)

Colorless powder, mp : 228~230°C (decomp.); UV λ_{max}^{MeOH} nm : 250; IR ν_{max}^{KBr} cm^{-1} : 3429, 2969, 2808, 1689, 1604, 1452, 1356, 1213, 1098; EI-MS *m/z* (rel. int.) : 399, 397 (M+), 362 (M^+-Cl), 360, 334, 209 (100), 166, 150; ^{1}H-NMR (500 MHz, pyridine-d_5) : δ 1.64 (1H, m, H-14a), 2.41 (3H, N-Me), 2.46 (1H, m, H-15a), 2.54 (1H, d, *J* = 15.7 Hz, H-5a), 2.68 (3H, m, H-9a, H-14b, H-15b), 3.04 (1H, d, *J* = 15.7 Hz, H-5b), 3.14 (1H, t, *J* = 12.4 Hz, H-9a), 3.73 (3H, s, O-Me), 3.80 (3H, s, O-Me), 4.04 (3H, s, O-Me), 5.03 (1H, s, H-1), 5.20 (1H, dd, *J* = 6.7, 12.4 Hz, H-10), 5.60 (1H, s, H-3); ^{13}C-NMR (125 MHz, pyridine-d_5) : δ 36.34 (C-16), 38.47 (C-14), 41.43 (C-9), 47.17 (C-5), 51.73 (C-15), 53.25 (C-12), 57.80 (C-10), 58.84 (C-17), 60.17 (C-18), 60.46 (C-19), 68.34 (C-11), 70.68 (C-1), 73.06 (C-13), 105.56 (C-3), 139.03 (C-7), 159.60 (C-8), 188.98 (C-2), 192.84 (C-6), 201.37 (C-4).

Dauricumine (8)

Colorless powder, UV λ_{max}^{MeOH} nm : 249; IR ν_{max}^{KBr} cm^{-1} : 3462, 2943, 2784, 1698, 1652, 1606, 1457, 1234, 1073; EI-MS *m/z* (rel. int.) : 399, 397 (M+), 362 (M+-Cl), 360, 334, 209 (100), 166, 150; ^{1}H-NMR (400 MHz, CD_3OD) : δ 1.97 (1H, ddd, *J* = 2.9, 5.4, 13.3 Hz, H-14a), 2.18 (1H, m, H-14b), 2.27 (1H, dd, *J* = 7.1, 13.3 Hz, H-9a), 2.35 (3H, s, -NCH_3), 2.39 (1H, ddd, *J* = 5.4, 9.9, 10.1 Hz, H-15a), 2.45 (1H, d, *J* = 17.1 Hz, H-5a), 2.53 (1H, dd, *J* = 11.9, 13.3 Hz, H-9b), 2.68 (1H, ddd, *J* = 2.9, 7.0, 10.1 Hz, H-15b), 2.77 (1H, d, *J* = 17.1 Hz, H-5b), 3.64 (3H, s, -OCH3), 3.92 (3H, s, -OCH_3), 4.13 (3H, s, -OCH_3), 4.47 (1H, s, H-1), 4.51 (1H, dd, *J* = 7.1, 11.9 Hz, H-10), 5.41 (1H, s, H-3); ^{13}C-NMR (100 MHz, CD3OD) : δ 36.52 (C-16), 41.56 (C-14), 41.57 (C-9), 49.85 (C-5), 52.97 (C-15), 53.63 (C-12), 59.88 (C-17), 60.83 (C-18), 61.38 (C-19), 61.42 (C-10), 69.67 (C-11), 74.80 (C-13), 75.82 (C-1), 106.59 (C-3), 139.60 (C-7), 163.31 (C-8), 191.25 (C-2), 196.62 (C-6), 205.38 (C-4).

Sinomenine (9)

Colorless crystal, mp : 160°C; UV λ_{max}^{MeOH} nm : 206, 231, 264; IR ν_{max}^{KBr} cm^{-1} : 3443, 2910, 1738, 1693, 1629, 1483, 1278, 1054; EI-MS *m/z* (rel. int.) : 329 (M+), 314 (100), 301, 286, 242, 192, 178; ^{1}H-NMR (400 MHz, CDCl3) : δ 1.90-2.01 (2H, m), 2.15 (1H, m), 2.45 (1H, d, *J* = 15.6 Hz, H-5a), 2.49 (3H, s, -NCH_3), 2.65 (1H, d like s), 2.79 (1H, m), 2.99 (1H, d like s), 3.16 (1H, br s), 3.28 (1H, br s), 3.47 (3H, s, -OCH_3), 3.79 (3H, s, -OCH_3), 4.33 (1H, d, *J* = 15.6 Hz, H-5b), 5.41 (1H, d, *J* = 1.9 Hz, H-8), 6.52 (1H, d, *J* = 8.3 Hz, H-2), 6.62 (1H, d, *J* = 8.3 Hz, H-1); ^{13}C-NMR (100 MHz, $CDCl_3$) : δ 24.32 (C-10), 35.35 (C-15), 40.10 (C-13), 42.38 (N-Me), 44.99 (C-14), 47.34 (C-16), 48.75 (C-5), 54.82 (O-Me), 56.05 (C3-O-Me), 57.01 (C-9), 109.15 (C-2), 114.03 (C-8), 118.33 (C-1), 122.04 (C-12), 129.47 (C-11), 144.70 (C-4), 145.15 (C-3), 152.45(C-7), 193.57 (C-6).

Magnoflorine (10)

Colorless crystal, mp : 207~210°C (decomp.); UV λ_{max}^{MeOH} nm : 223, 269, 302; IR ν_{max}^{KBr} cm^{-1} : 3420, 2971, 2844, 1679, 1459, 1202, 1126; LC-ESI-MS/MS m/z (rel. int.) : 342 (M^+, 78), 297 (100), 265 (57); ^{1}H-NMR (500 MHz, $CDCl_3$+CD_3OD) : δ 2.65 (1H, t, *J* = 12.7 Hz), 2.90 (1H, m), 2.92 (3H, s, -N^+CH_3), 3.11 (1H, dd, *J* = 2.9, 12.7 Hz), 3.24 (1H, m), 3.31 (1H, m), 3.32 (3H, s, -N^+CH_3), (1H, dd, *J* = 4.9, 13.2 Hz), 3.81 (3H, s, -OCH_3), 3.82 (3H, s, -OCH_3), 4.13 (1H, d, *J* = 13.2 Hz), 6.64 (1H, s), 6.77 (1H, d, *J* = 8.3 Hz), 6.82 (1H, d, *J*=8.3 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$+ CD_3OD) : δ 23.47 (C-4), 30.50 (C-7), 42.70 (N^+-Me), 53.45 (N^+-Me), 55.78 (O-Me), 55.87 (O-Me), 61.06 (C-5), 69.67 (C-6a), 109.58 (C-3), 110.75 (C-9), 118.86 (C-1b), 119.47 (C-1a), 119.70 (C-11a#), 119.75 (C-3a#), 119.88 (C-8), 124.30 (C-7a), 141.89 (C-11*), 142.00 (C-1*), 148.68 (C-10), 149.77 (C-2). #,* Signals may be reversed

Cytotoxicity test *in vitro*

A sulforhodamin B Bioassay (SRB) was used for the

cytotoxicity test. The activity ofthe compounds was tested at several concentrations against the following five cultured human tumor cells *in vitro* (Skehan *et al.*, 1990). A549 (non small cell lung adenomcarcinoma), SK-OV-3 (ovarian), SK-MEL-2 (skin melanoma), XF498 (CNS) and HCT15 (colon).

MDR reversal activity

The human ovarian cancer cell line, SK-OV-3, the human colorectal cancer cell line, HCT15 cell, the human uterine sarcoma cell line, MES-SA, and the human MDR uterine sarcoma cell line, MES-SA/DX5 were provided by the National Cancer Institute (NCI). The SK-OV-3 and MES-SA cells were P-gp non-expressing and non-multidrug resistant cancer cells. The HCT15 and MES-SA/DX5 cells showed a high level of P-gp expression. The cells were cultured in RPMI1640 medium supplied with 5% FBS, as previously reported (Choi *et al.*, 1996). The cells were incubated with serial dilutions of paclitaxel for 72 h in the presence or absence of each isolated compound (10 μM) or verapamil (10 μM). The procedure for calculating the survival fractions was identical to that used in the cytotoxicity assay. In this assay, the wells containing each of the isolated compounds or verapamil without paclitaxel were used as the controls.

RESULTS AND DISCUSSION

Compound **1** (dauriporphine, Kunitomo *et al.*, 1985), compound **5** ((-)-syringaresinol, Vermes *et al.*, 1991), compound **6** (*N*-feruloyl-tyramine, Fukuda *et al.*, 1983), compound **7** (acutumine, Sugimoto *et al.*, 2001), compound **9** (sinomenine, Yoshihiro *et al.*, 1975), compound **10** (magniflorine, Marsaioli *et al.*, 1979; Saxena *et al.*, 1979; Al-Howiriny *et al.*, 2001) were characterized by comparing their physical and spectroscopic data with those reported in the literatures.

Compound **2** was obtained as a yellow powder. The UV and ^{1}H-NMR spectra of compound **2** were similar to those of compound **1**. The molecular ion peak of compound **2** in EI-MS spectrum was at *m/z* 291 as the base peak. The ^{1}H-NMR spectrum showed two methoxyl groups at δ 4.02 and 4.08, H-2 and H-3 signals of isoquinoline A ring at δ 8.66 and 7.68 (d, *J* = 5.9 Hz), AMX spin system of

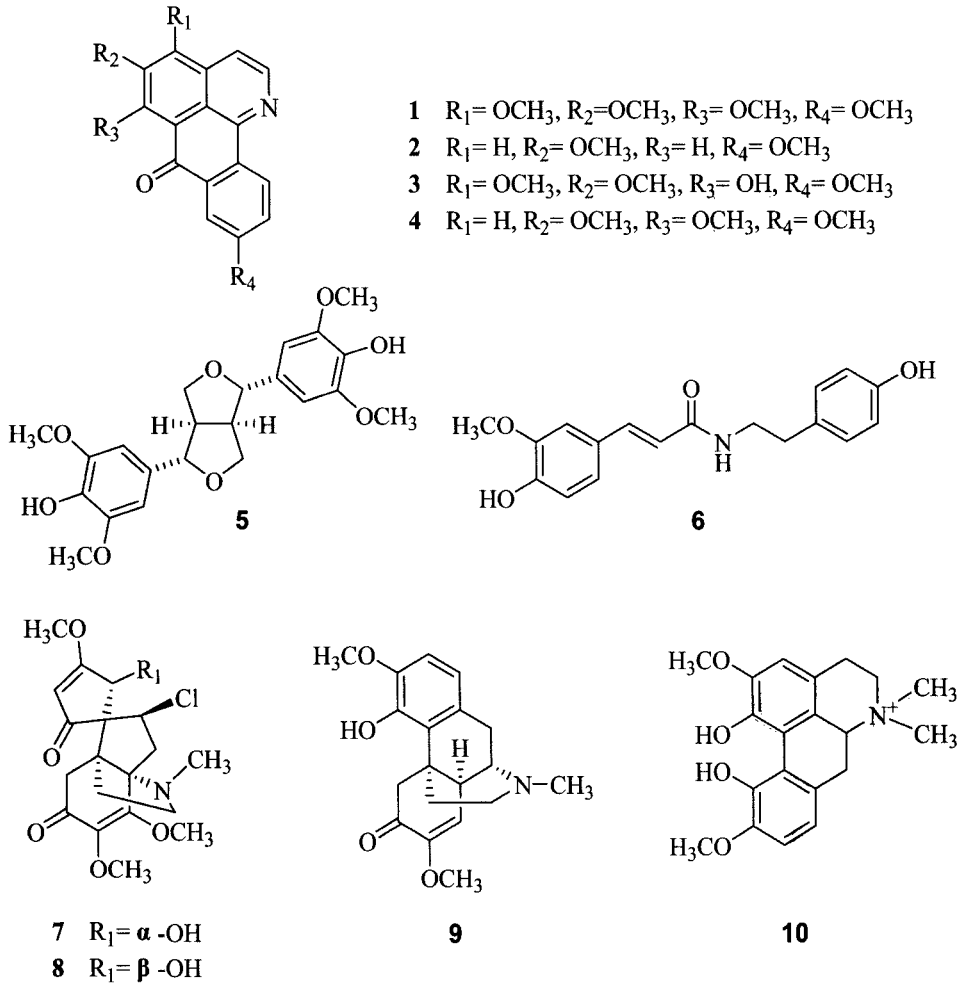

Fig. 1. Structures of compounds (**1~10**) isolated from *S. acutum*

benzene ring at δ 7.88 (1H, d, *J* = 2.8 Hz, H-8), 7.32 (1H, dd, *J* = 2.8, 8.7 Hz, H-10) and 9.01 (1H, br s, H-11), and AX spin system of isoquinoline B ring at δ 7.45 and 8.31 (d, *J* = 2.5 Hz). On the basis of the above spectral data, compound **2** was supposed to be 5,9-dimethoxy oxoisoaporphine derivative (Sugimoto *et al.*, 1999; Kunitomo *et al.*, 1983). Thus, the structure of compound **2** was determined to be bianfugecine. The NMR data and physical data of compound **2** were in good agreement with those reported in the literature (Hou *et al.*, 1985).

Compound **3** was obtained as yellow needles. The UV, and ^{1}H-NMR spectra of compound **3** were almost same with those of compound **1**. The ^{1}H-NMR spectrum showed three methoxyl groups at δ 4.04, 4.13, and 4.38, while compound **1** showed four methoxyl signals at δ 3.97, 4.03, 4.15, and 4.25 in the ^{1}H-NMR spectrum. On the basis of above consideration and literatures survey, the structure of compound **3** was determined to be 4,5,9-trimethoxy 6-hydroxy oxoisoaporphine derivative, dauriporphinoline (Sugimoto *et al.*, 1999; Kunitomo *et al.*, 1983). The NMR and physical data of compound **3** were in good agreement with those reported in the literature (Shouxun *et al.*, 1989).

Compound **4** was obtained as yellow powder. The IR spectrum showed the presence of a conjugated C=O group at 1660 cm^{-1}. The molecular ion peak of compound **4** in EI-MS spectrum was at *m/z* 321 as the base peak. The ^{1}H-NMR spectrum showed three methoxyl groups at δ 4.01, 4.13, and 4.18, H-2 and H-3 signals of isoquinoline A ring at δ 8.69 and 7.68 (d, *J* = 5.9 Hz), AMX spin system of benzene ring at δ 7.90 (d, *J* = 2.5 Hz), 7.41 (dd, *J* = 2.5, 9.0 Hz) and 9.09 (1H, br s). and isoquinoline B ring proton at δ 7.45 (s). On the basis of spectral data, the structure of compound **4** was determined to be 5,6,9-trimethoxy oxoisoaporphine derivative, menisporphine (Sugimoto *et al.*, 1999; Kunitomo *et al.*, 1983). The NMR data and physical data of compound **4** were in good agreement with those reported in the literature (Kunitomo *et al.*, 1982).

Compound **8** was obtained as colorless powder. The UV spectrum of compound **8** was similar to that of compound **7**. The IR spectrum showed the presence of an OH group at 3462 cm^{-1} and two conjugated C=O group at 1698 and 1652 cm^{-1}. The presence of a chlorine atom in the molecule was suggested by its EI-MS spectrum, in which appeared the molecular ion peak at *m/z* 397 and characteristic isotope peak at m/z 399 with a relative intensity of 3 : 1. The EI-MS [M$^+$], EI-MS fragment pattern and ^{1}H-, ^{13}C-NMR spectral data of compound **8** were almost same with compound **7** (acutumine), but retention time of compound **7** and compound **8** were different (*R*t = 3.87 min. in compound **7**; *R*t = 5.72 min. in compound **8**) in the HPLC chromatography profile (column=RP18, 5 um, Ø 4.6×150 mm; eluent=gradient 20% MeOH→90% MeOH, 15 min.). These data suggested that compound **7** and compound **8** were diastereomer. Therefore, the structure of compound **8** was determined to be dauricumine. The NMR data and physical data of compound **8** were in good agreement with those reported in the literature (Sugimoto *et al.*, 2001).

Compounds **1**, **5~10** were noncytotoxic to the human cancer cell lines, A549, SK-OV-3, SK-MEL-2, XF498 and HCT15 (Table II). These compounds were tested for their MDR reversal activities in the P-gp non-expressing (non-MDR) cell line, MES-SA, and in the P-gp expressing MDR cell lines, MES-DA/DX5 and HCT15 cells. Compounds **1** and **5** inhibited P-gp MDR. In particular, the MDR reversal activity of compound **1** was comparable to verapamil, having an ED_{50} value 0.03 μg/μL and 0.00010 μg/μL in the MES-SA/DX5 and HCT15 cells, respectively (Table III).

Table II. Cytotoxic activity of compounds (**1~10**) isolated from *S. acutum*

Compounds	ED_{50} (μg/mL)				
	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
1	103.42	17.93	68.71	44.15	76.29
2	5.87	60.47	5.91	5.85	9.68
3	90.25	8.33	23.18	9.04	9.28
4	5.57	2.86	4.07	3.73	9.72
5	>100.0	82.29	>100.0	>100.0	>100.0
6	95.11	71.84	105.62	100.61	>100.0
7	>100.0	>100.0	>100.0	>100.0	>100.0
8	>100.0	>100.0	>100.0	>100.0	>100.0
9	>100.0	98.64	>100.0	>100.0	>100.0
10	>100.0	99.74	>100.0	>100.0	>100.0
Doxorubicin	0.009	0.092	0.009	0.017	0.107

Table III. MDR reversal activity of compounds (**1**, **5~10**) isolated from *S. acutum*

Compounds	ED_{50} (μg/mL)		
	MES-SA	MES-SA/DX5	HCT15
Paclitaxel (P)	0.00004	0.99	0.00091
P + **1**	0.00004	0.03	0.00010
P + **5**	0.00007	0.44	0.00074
P + **6**	0.00010	0.82	0.00076
P + **7**	0.00004	0.87	0.00104
P + **8**	0.00007	1.19	0.00076
P + **9**	0.00008	1.23	0.00079
P + **10**	0.00011	0.85	0.00096
P + verapamil	0.00005	0.02	0.00009

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