New Nervogenic Acid Derivatives from *Liparis nervosa*

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Key words
- Orchidaceae
- *Liparis nervosa*
- benzamide acid derivatives
- nervogenic acid derivatives
- cytotoxicity

Abstract

Ten new nervogenic acid derivatives (**1**–**4, 6**–**11**) and one known compound (**5**) have been isolated from *Liparis nervosa*. Their structures were determined using extensive spectroscopic analysis, including 1D and 2D NMR experiments. Compounds **3, 4, 9, 10**, and **11** were evaluated for their cytotoxicity against A549, H460, Hela, MCF-7, Caco2, and HepG2 human cancer cell lines.

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Introduction

Liparis nervosa (Thunb.) Lindl, a member of the Orchidaceae family, is an herbaceous plant that is widely distributed in China. This plant usually grows in broad-leaved forests, particularly on moist slopes at altitudes from 850 to 1000 m. *Liparis nervosa* historically has been used in folk medicine as an antipyretic, detoxicating, and hemostatic agent [1]. The chemical components of this plant have been investigated by Kunisuke Nishkawa, leading to the identification of a new pyrrolizidine alkaloid, nervosine [2, 3]. However, the nervogenic acid derivatives and their bioactivities have not been studied in detail yet, which prompted us to undertake a systematic study of this plant. Here we report on the isolation and structural elucidation of new nervogenic acid derivatives (**1**–**4, 6**–**11**) (**Fig. 1**) by means of 1D and 2D NMR experiments. In addition, one known compound was isolated and identified as methyl 3,5-bis (3-methyl-2-butenyl)-4-O-(β-D-glucopyranosyl) benzoate (**5**) by comparison of its spectroscopic data with those reported in the literature [4]. To the best of our knowledge, the cytotoxic activity of the nervogenic acid derivatives has not been studied yet, and we have here investigated part of the isolated compounds for their cytotoxicity *in vitro* against several human cancer cell lines.

Materials and Methods

General experimental procedures

Optical rotations were measured on a Perkin-Elmer 341 polarimeter. 1D and 2D NMR spectra were recorded on a Bruker AV 400 or a Varian Unity INOVA 400/54 NMR spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C) with TMS as an internal standard. IR spectra were obtained with a Thermo Fisher Nicolet 6700 spectrometer, KBr pellets in cm^{-1}. UV spectra were determined with a Shimadzu UV-2450 spectrophotometer. HR-ESI-MS were measured using a Q-TOF micro mass spectrometer (Waters). GC analyses were performed using a Hewlett Packard GC6890 instrument on an Agilent HP-5 column (0.25 mm, 30 m, i.d., 0.25 µm). Silica gel (Qingdao Haiyang Chemical Co., Ltd., 200–300 mesh), Sephadex LH-20 (Pharmacia Co.), RP-18 silica gel (Merck, 40–60 µm), and D-101 macroporous resin (Rohm & Haas) were used for column chromatography (CC). Semipreparative HPLC was carried out on a Waters Symmetry Prep™ C-18 column (7 µm, ∅ 19.0 × 300 mm) with a Waters 600 controller and Waters 2487 detector. TLC plates were precoated with silica gel GF_{254} (Qingdao Haiyang Chemical Co., Ltd.) and visualized under a UV lamp at 254 nm or by spraying 5% vanillin-H_2SO_4 (*w*/*v*) or by iodine.

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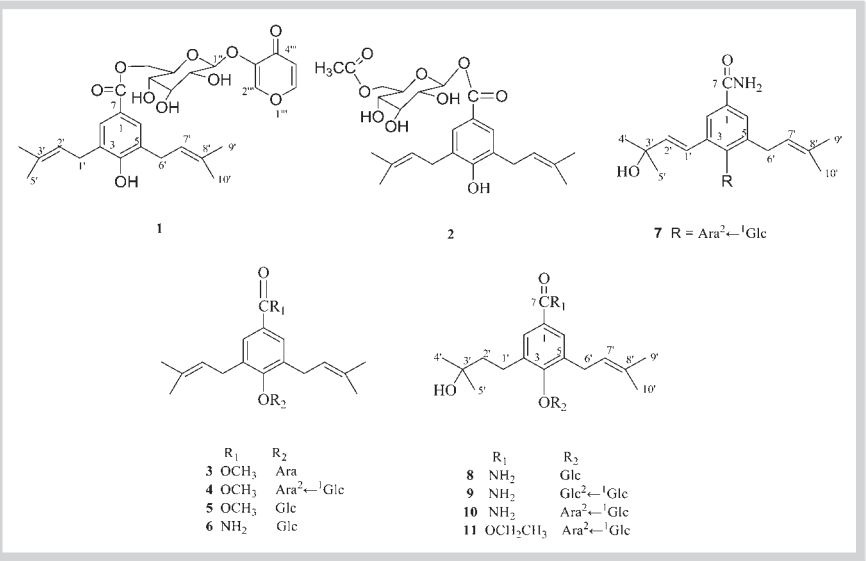

Fig. 1 Structures of compounds **1**–**11** isolated from *Liparis nervosa*.

Plant material

The whole plant of *L. nervosa* was collected in Chongqing, China in July 2007. The plant was identified by Professor Liangke Song in the School of Life Science and Engineering, Southwest Jiaotong University, Sichuan Province, China, where a voucher specimen was deposited (No. Z36150701).

Extraction and isolation

The shade dried *L. nervosa* (5.0 kg) was powdered and extracted with 95% EtOH (3 × 20 L) at room temperature for 3 days. After the solvent was removed by evaporation, the ethanol extract (300.0 g) was recovered. The extract was then suspended in water (1 L) at 50 °C and adjusted to pH 2.8 with HCl. The aqueous solution was then extracted with $CHCl_3$ (500 mL × 3) and EtOAc (500 mL × 3) to obtain the $CHCl_3$ (82.0 g) and EtOAc extracts (25.0 g), respectively. The pH of the aqueous layer was adjusted to 9.4 with aqueous ammonia solution and then extracted with EtOAc (500 mL × 3) and *n*-butanol (500 mL × 3) to obtain the crude alkaline EtOAc extract (10.0 g) and the *n*-butanol extract (50.0 g), respectively.

The *n*-butanol extract (50.0 g) was subjected to a column (⌀ 9.5 × 60 cm) using D101 resin and eluting with H_2O, 30%, 60%, and 95% EtOH sequentially, to afford four fractions (I, II, III, and IV).

Fraction II (6.5 g) was separated by RP-18 silica gel column CC (⌀ 4.0 × 45 cm) using MeOH and H_2O as the mobile phase with a gradient from 2% to 100% to afford fractions F_{II-1}-F_{II-6} based on TLC analysis. F_{II-2} (400 mg) was subjected to another silica gel column (⌀ 1.5 × 30 cm) and eluted with $CHCl_3$: MeOH : EtOAc (12 : 1 : 1) to yield compound **10** (42 mg). F_{II-3} (1.5 g) was subjected to a silica gel column (⌀ 3.5 × 45 cm) and eluted with $CHCl_3$: MeOH : EtOAc (25 : 1 : 1) to yield six fractions (F_{II-3-1}–F_{II-3-6}). F_{II-3-2} was subjected to a Sephadex LH-20 column (⌀ 1.5 × 60 cm) and eluted with MeOH to yield compound **9** (43 mg). F_{II-3-3} was first purified by column chromatography using a Sephadex LH-20 column (⌀ 1.5 × 60 cm) and eluted with MeOH. It was further purified by a preparative HPLC, eluted with MeOH : H_2O (58 : 42) at a flow rate of 8 mL/min to afford compound **8** (6 mg).

Fraction III (9.0 g) was subjected to a silica gel column (⌀ 5.0 × 50 cm) and eluted with $CHCl_3$: EtOAc : MeOH (10 : 10 : 1) to yield six fractions (F_{III-1}–F_{III-6}), among which F_{III-3} (400 mg) was further purified by an RP-18 silica gel column (⌀ 2.0 × 45 cm) with MeOH : H_2O (45 : 55) as the mobile phase to yield compounds **6** (7 mg), **7** (10 mg), and **11** (16 mg), respectively.

Meanwhile, the EtOAc extract (25.0 g) was subjected to RP-18 silica gel column (⌀ 7.0 × 60 cm) and eluted with MeOH : H_2O (0 : 1–1 : 0) to yield five fractions (F_{1-1}-F_{1-5}). F_{1-3} (2.0 g) was purified using a Sephadex LH-20 column (⌀ 1.5 × 60 cm, MeOH) first and then by an HPLC system using MeOH : H_2O (62 : 38) to afford compounds **1** (6 mg) and **2** (4 mg). F_{1-5} (1.7 g) was subjected to a silica gel column (⌀ 3.5 × 45 cm) and eluted with EtOAc : MeOH : H_2O (120 : 1 : 1) to yield compounds **3** (25 mg), **4** (22 mg), and **5** (25 mg).

Isolated compounds

{6-O-[(4-oxo-4H-pyran-3-yloxy)-O-β-D-glucopyranosyl]}-4-hydroxy-3,5-bis(3-methyl-2-butenyl) benzoate (**1**): Amorphous solid, purity: 96% (HPLC); $[\alpha]_D^{20}$ − 14.4 (*c* 0.305, MeOH); UV (MeOH) λ_{max} (log ε), 223 (3.27), 261 (3.46) nm; IR (KBr) ν_{max} 3401, 2970, 2925, 1711, 1642, 1607, 1439, 1384, 1310, 1284, 1241, 1194, 1072, 875, 842, 769 cm^{-1}; ^1H and ^{13}C NMR data, see **Tables 1** and **3**. HR-ESI-MS at *m/z* 553.2064 $[M + Na]^+$ (calcd. for $C_{28}H_{34}O_{10}Na$, 553.2050).

{1-O-[6-acetyl-O-β-D-glucopyranosyl]}-4-hydroxy-3,5-bis(3-methyl-2-butenyl) benzoate (**2**): Amorphous solid, purity: 95% (HPLC); $[\alpha]_D^{20}$ − 6.2 (*c* 0.225, MeOH); UV (MeOH) λ_{max} (log ε) 226 (2.72), 267 (2.44) nm; IR (KBr) ν_{max} 3411, 2972, 2926, 1724, 1607, 1438, 1375, 1308, 1282, 1247, 1186, 1077, 875, 769 cm^{-1}; ^1H and ^{13}C NMR data, see **Tables 1** and **3**. HR-ESI-MS at *m/z* 477.2132 $[M - H]^-$ (calcd. for $C_{25}H_{33}O_9$, 477.2125).

Methyl 3,5-bis(3-methyl-2-butenyl)-4-O-(α-L-arabinopyranosyl) benzoate (**3**): Amorphous solid, purity: 96% (HPLC); $[\alpha]_D^{20}$ + 26.4 (*c* 0.700, MeOH); UV (MeOH) λ_{max} (log ε) 238 (2.64) nm; IR (KBr) ν_{max} 3400, 2973, 2927, 1715, 1649, 1604, 1435, 1384, 1313, 1265, 1238, 1082, 1018, 951, 912, 772 cm^{-1}; ^1H and ^{13}C NMR data, see **Tables 1** and **3**. HR-ESI-MS at *m/z* 443.2038 $[M + Na]^+$ (calcd. for $C_{23}H_{32}O_7Na$, 443.2046).

Methyl 3,5-bis(3-methyl-2-butenyl)-4-O-[β-D-glucopyranosyl(1 → 2)-α-L-arabinopyranosyl] benzoate (**4**): White amorphous powder, purity: 97% (HPLC); $[\alpha]_D^{20}$ − 6.0 (*c* 0.300, MeOH); UV (MeOH) λ_{max} (log ε) 243 (3.44) nm; IR (KBr) ν_{max} 3377, 2926, 2875, 1722,

Table 1 ^{1}H NMR data of compounds **1**–**6**.

H	1[a, b]	2[a, b]	3[a, c]	4[a, d]	5[c]	6[b]
2	7.64 s	7.73 s	7.67 s	7.68 s	7.64 s	7.67 s
6	7.64 s	7.73 s	7.67 s	7.68 s	7.64 s	7.67 s
1′	3.34 d (7.2)	3.34 d (7.2)	3.45 d (7.2)	3.55 d (7.6)	3.46 d (7.2)	3.32 d (7.1)
2′	5.31 m	5.32 m	5.21 t (7.2)	5.29 t (7.6)	5.19 t (7.2)	5.30 t (7.1)
4′	1.72 s	1.73 s	1.67 s	1.74 s	1.63 s	1.75 s
5′	1.76 s	1.76 s	1.71 s	1.77 s	1.68 s	1.76 s
6′	3.34 d (7.2)	3.34 d (7.2)	3.45 d (7.2)	3.55 d (7.6)	3.46 d (7.2)	3.32 d (7.1)
7′	5.31 m	5.32 m	5.21 t (7.2)	5.29 t (7.6)	5.19 t (7.2)	5.30 t (7.1)
9′	1.72 s	1.73 s	1.67 s	1.74 s	1.63 s	1.75 s
10′	1.76 s	1.76 s	1.71 s	1.77 s	1.68 s	1.76 s
	Glc	Glc	Ara	Ara	Glc	Glc
1″	4.79 d (7.2)	5.65 d (8.0)	4.54 d (7.6)	4.75 d (7.2)	4.66 d (7.6)	4.72 d (7.6)
2″	3.49 m	3.48 m	4.02 br.t (8.8)	4.15 dd (7.2, 8.8)	3.62–3.79 m	3.53 m
3″	3.49 m	3.48 m	3.69 m	3.81 m	3.62–3.79 m	3.43 m
4″	3.47 m	3.45 m	3.93 m	3.91 m	3.62–3.79 m	3.43 m
5″	3.74 m	3.64 m	a 3.34 br.d (12.4) b 3.93 br.d (12.4)	a 3.41 ov b 3.93 ov	3.15 m	3.15 m
6″	a 4.42 dd (6.4, 12.0) b 4.63 dd (2.0, 12.0)	a 4.22 dd (5.6, 12.0) b 4.37 dd (2.0, 12.0)	–	–	a 3.49 dd (6.0,12.0) b 3.70 ov	a 3.65 dd (5.6, 12.0) b 3.79 dd (2.0, 12.0)
				Glc		
1′″	–	–	–	4.72 d (8.0)	–	–
2′″	8.17 d (0.6)	–	–	3.37 m	–	–
3′″	–	–	–	3.43 m	–	–
4′″	–	–	–	3.88 m	–	–
5′″	6.46 d (5.6)	–	–	3.35 m	–	–
6′″	7.97 dd (5.6, 0.6)	–	–	a 3.69 dd (5.2,12.0) b 3.80 ov	–	–
-$OCOCH_3$	–	2.05 s	–	–	–	–
-OCH_3	–	–	3.86 s	3.88 s	3.86 s	–

[a] Data are based on DEPT, HSQC, and HMBC experiments; ^{1}H NMR (400 MHz, δ, J in Hz in parentheses).[b] Data in CD_3OD; [c] data in $CDCl_3$; [d] data in $CDCl_3$+ CD_3OD; ov means overlapped

1604, 1436, 1383, 1319, 1284, 1224, 1082, 1039, 1017, 956, 896, 772 cm^{-1}; ^{1}H and ^{13}C NMR data, see **Tables 1** and **3**. HR-ESI-MS at *m/z* 605.2574 [M + Na]$^{+}$ (calcd. for $C_{29}H_{43}O_{12}Na$, 605.2574).

Methyl 3,5-bis(3-methyl-2-butenyl)-4-O-(β-D-glucopyranosyl) benzoate (**5**): White amorphous powder, purity: 90% (HPLC); $[\alpha]_D^{20}$ − 6.0 (*c* 0.450, MeOH); UV (MeOH) λ_{max} (log ε) 240 (3.46) nm; IR (KBr) ν_{max} 3390, 2923, 2853, 1722, 1601, 1456, 1435, 1377, 1323, 1284, 1237, 1082, 1196, 1180, 1068, 1010, 903, 773 cm^{-1}; ^{1}H and ^{13}C NMR data, see **Tables 1** and **3**. HR-ESI-MS at *m/z* 473.2140 [M + Na]$^{+}$ (calcd. for $C_{28}H_{34}O_8Na$, 473.2151).

3,5-bis(3-Methyl-2-butenyl)-4-O-(β-D-glucopyranosyl) benzamide (**6**): Amorphous solid, purity: 96% (HPLC); $[\alpha]_D^{20}$ − 5.3 (*c* 0.150, MeOH); UV (MeOH) λ_{max} (log ε) 240 (3.52) nm; IR (KBr) ν_{max} 3419, 2923, 2953, 1659, 1648, 1456, 1398, 1384, 1261, 1195, 1162, 1077, 1034, 776 cm^{-1}; ^{1}H and ^{13}C NMR data, see **Tables 1** and **3**. HR-ESI-MS at *m/z* 436.2324 [M + H]$^{+}$ (calcd. for $C_{23}H_{34}NO_7$, 436.2335).

3-[(1E)-(3-Hydroxy-3-methyl-1-butenyl)-4-O-[β-D-glucopyranosyl-(1 → 2)-α-L-arabinopyranosyl]-5-(3-methyl-2-butenyl) benzamide (**7**): Amorphous solid, purity: 97% (HPLC); $[\alpha]_D^{20}$ + 4.4 (*c* 0.350, MeOH); UV (MeOH) λ_{max} (log ε) 236 (2.65) nm; IR (KBr) ν_{max} 3364, 2969, 2925, 1660, 1613, 1579, 1423, 1384, 1265, 1200, 1070, 1018, 902, 775 cm^{-1}; ^{1}H and ^{13}C NMR data, see **Tables 2** and **3**. HR-ESI-MS at *m/z* 606.2529 [M + Na]$^{+}$ (calcd. for $C_{28}H_{41}NO_{12}Na$, 606.2526).

3-(3-Hydroxy-3-methylbutyl)-4-O-(β-D-glucopyranosyl)-5-(3-methyl-2-butenyl) benzamide (**8**): White amorphous powder, purity: 96% (HPLC); $[\alpha]_D^{20}$ + 9.4 (*c* 0.900, MeOH); UV (MeOH) λ_{max} (log ε) 239 (3.82) nm; IR (KBr) ν_{max} 3363, 2968, 2924, 1660, 1611, 1604, 1579, 1423, 1384, 1265, 1199, 1162, 1075, 1041, 925, 899, 774 cm^{-1}; ^{1}H and ^{13}C NMR data, see **Tables 2** and **3**. HR-ESI-MS at *m/z* 476.2262 [M + Na]$^{+}$ (calcd. for $C_{23}H_{35}NO_8Na$, 476.2260).

3-(3-Hydroxy-3-methylbutyl)-4-O-[β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl]-5-(3-methyl-2-butenyl) benzamide (**9**): White amorphous powder, purity: 97% (HPLC); $[\alpha]_D^{20}$ − 20.8 (*c* 0.125, MeOH); UV (MeOH) λ_{max} (log ε) 241 (4.22) nm; IR (KBr) ν_{max} 3364, 2970, 2925, 1660, 1610, 1579, 1423, 1384, 1261, 1200, 1159, 1078, 1035, 950, 909, 778 cm^{-1}; ^{1}H and ^{13}C NMR data, see **Tables 2** and **3**. HR-ESI-MS at *m/z* 638.2766 [M + Na]$^{+}$ (calcd. for $C_{29}H_{45}NO_{13}Na$, 638.2789).

3-(3-Hydroxy-3-methylbutyl)-4-O-[β-D-glucopyranosyl-(1 → 2)-α-L-arabinopyranosyl]-5-(3-methyl-2-butenyl) benzamide (**10**): Amorphous solid, purity: 95% (HPLC); $[\alpha]_D^{20}$ + 23.0 (*c* 0.300, MeOH); UV (MeOH) λ_{max} (log ε) 238 (3.31) nm; IR (KBr) ν_{max} 3392, 2971, 2929, 1716, 1602, 1459, 1383, 1371, 1311, 1272, 1190, 1078, 1031, 948, 912, 772 cm^{-1}; ^{1}H and ^{13}C NMR data, see **Tables 2** and **3**. HR-ESI-MS at *m/z* 608.2668 [M + Na]$^{+}$ (calcd. for $C_{28}H_{43}NO_{12}Na$, 608.2683).

1-(Ethoxy)-3-[(3-hydroxy-3-methylbutyl)-4-O-[β-D-glucopyranosyl-(1 → 2)-α-L-Arabinopyranosyl]]-5-(3-methyl-2-butenyl) benzoate (**11**): White amorphous solid, purity: 94% (HPLC); $[\alpha]_D^{20}$ − 22.7 (*c* 0.600, MeOH); UV (MeOH) λ_{max} (log ε) 236 (3.02) nm; IR (KBr) ν_{max} 3415, 3338, 2970, 2971, 2877, 1638, 1610, 1579, 1420, 1384, 1297, 1259, 1238, 1194, 1100, 1070, 1017, 929, 894,

Table 2 ^{1}H NMR data of compounds **7**–**11**.

H	7[a, b]	8[a, b]	9[a, c]	10[a, c]	11[a, b]
2	7.88 d (2.0)	7.58 d (2.3)	7.60 br.s	7.58 br.s	7.72 d (2.0)
6	7.57 d (2.0)	7.54 d (2.3)	7.51 br.s	7.50 br.s	7.65 d (2.0)
1′	7.27 d (16.2)	3.13 m	2.86 m	2.82 m	2.90 m
2′	6.42 d (16.2)	1.80 m	1.79 br.t (8.4)	1.77 br.t (7.6)	1.79 m
4′	1.42 s	1.27 s	1.30 s	1.29 s	1.28 s
5′	1.42 s	1.27 s	1.30 s	1.29 s	1.28 s
6′	3.55 d (7.2)	3.59 d (7.2)	3.55 ov	3.52 ov	3.54 d (7.2)
7′	5.32 t (7.2)	5.30 t (7.2)	5.29 br.s	5.28 br.s	5.30 t (7.2)
9′	1.74 s	1.75 s	1.71 s	1.70 s	1.74 s
10′	1.74 s	1.75 s	1.71 s	1.70 s	1.76 s
	Ara	Glc	Glc-I	Ara	Ara
1″	4.74 d (7.2)	4.72 d (7.6)	4.95 d (7.2)	4.88 ov	4.70 d (7.6)
2″	4.14 dd (7.2, 8.6)	3.53 m	4.01 br.t (8.0)	4.24 br.t (8.0)	4.16 dd (6.8, 8.0)
3″	3.84 m	3.43 m	3.80 m	3.99 m	3.87 m
4″	3.86 m	3.43 m	3.56 m	3.99 m	3.88 m
5″	a 3.39 ov b 3.88 dd (2.8, 8.4)	3.14 m	3.26 m	a 3.46 ov b 3.88 ov	a 3.40 ov b 3.88 ov
6″	–	a 3.67 dd (5.6,12.0) b 3.76 dd (2.0,12.0)	a 3.73 ov b 3.88 br.d (12.0)	–	–
	Glc	–	Glc-II	Glc	Glc
1‴	4.76 d (7.8)	–	5.00 d (8.0)	4.90 ov	4.80 d (6.4)
2‴	3.35 m	–	3.44 m	3.39 m	3.28 m
3‴	3.40 m	–	3.76 m	3.46 m	3.40 m
4‴	3.35 m	–	3.42 m	3.52 m	3.37 m
5‴	3.30 m	–	3.58 m	3.46 m	3.31 m
6‴	a 3.67 dd (5.0, 11.3) b 3.77 dd (2.2, 11.3)	–	a 3.77 ov b 3.81 brd (8.8)	a 3.75 ov b 3.85 ov	a 3.70 dd (5.7, 12.0) b 3.82 dd (2.0, 12.0)
-O$\underline{CH_2}CH_3$	–	–	–	–	4.32 q (7.2)
-OCH$_2\underline{CH_3}$	–	–	–	–	1.37 t (7.2)

[a] Data are based on DEPT, HSQC, and HMBC experiments; ^{1}H NMR (400 MHz, δ, J in Hz in parentheses). [b] Data in CD_3OD; [c] data in D_2O; ov means overlapped

775 cm^{-1}; ^{1}H and ^{13}C NMR data, see ◉ **Tables 2** and **3**. HR-ESI-MS at *m/z* 637.2824 $[M + Na]^+$ (calcd. for $C_{30}H_{46}O_{13}Na$, 637.2836).

Acid hydrolysis

Compound **1** (2 mg) was heated in 1 M HCl-dioxane (1 : 1, 4 mL) at 80 °C for 4 h. After cooling, the solution was diluted with H_2O (3 mL), neutralized with 1 M NaOH and then extracted with $CHCl_3$ (3 × 3 mL). The aqueous layer was concentrated under a stream of nitrogen. The residue was then dissolved in anhydrous pyridine (0.1 mL), followed by the addition of 0.1 M L-cysteine methyl ester hydrochloride (0.2 mL). The resulting solution was stirred at 60 °C for 1 h, followed by the addition of trimethysilylation reagent HMDS-TMCS-pyridine (hexamethyldisilazane-trimethylchlorosilane-pyridine, 3 : 1 : 9). It was then stirred at 60 °C for additional 30 min. The thiazolidine derivatives were analyzed by GC for sugar identification. D-glucose and L-arabinose were confirmed by comparison with the retention time of the authentic standards at 11.81 min and 9.04 min, respectively. The same analysis was carried out on compounds **2**–**11** [5].

HPLC analysis

HPLC analyses were carried out at 40 °C on a Waters Symmetry-Shield™ RP-18 column (5 µm, 4.6 × 250 mm, detection at UV 254 nm) with water and methanol as the mobile phases with a linear gradient from 5 to 95% of methanol and 40 min eluting time. The flow rate was kept constant at 1 mL/min.

Cell culture and cytotoxicity assay

A549 (non-small cell lung cancer, NSCLC), H460 (human lung adenocarcinoma), Hela (human cervical cancer), MCF-7 (human breast cancer), Caco2 (human colon cancer), HepG2 (human hepatoblastoma) cell lines were obtained from ATCC. The cells were maintained in a growth medium containing RPMI 1640 or Dulbecco's MEM with glutamate supplemented with 10% fetal bovine serum (FBS) and antibiotics. All cells were cultured at 37 °C with 5% CO_2 (*v*/*v*). All cell culture media supplements for the cell culture were purchased from Invitrogen. Treatments with xenobiotics were carried out in growth medium minus serum. Cells treated with DMSO (0.1% *v*/*v*) were used as negative controls, while adriamycin (Sigma–Aldrich) was used as the positive control. Cells were seeded in a 96-well plate at 1 × 10^4 cells per well. After 24 h, cells were treated with compounds **3, 4, 9, 10**, and **11** at different concentrations, respectively, in a medium minus FBS for 24 h and then compared to the untreated cells. Cell viability was determined with an MTS Cell Proliferating Assay Kit (Promega) [6, 7].

Supporting information

The 1D, 2D NMR, UV, and HR-ESI-MS spectra of compounds **1**–**11** as well as the data of cell viability of compounds **3, 4, 9, 10**, and **11** on different cell lines are available as Supporting Information.

Table 3 ^{13}C NMR data of compounds **1**–**11**.

C	1[a, b]	2[a, b]	3[a, c]	4[a, d]	5[c]	6[b]	7[a, b]	8[a, b]	9[a, e]	10[a, e]	11[a, b]
1	122.0 s	121.5 s	126.7 s	125.5 s	126.9 s	131.1 s	131.1 s	131.1 s	132.2 s	132.1 s	127.7 s
2	130.1 d	130.5 d	129.3 d	128.3 d	129.3 d	128.3 d	125.0 d	128.2 d	129.8 d	130.0 d	130.0 d
3	129.5 s	129.6 s	135.6 s	135.2 s	135.5 s	137.1 s	132.2 s	138.2 s	139.8 s	139.6 s	138.3 s
4	158.7 s	159.0 s	155.9 s	155.9 s	155.6 s	156.7 s	156.1 s	156.8 s	157.0 s	157.5 s	158.2 s
5	129.5 s	129.6 s	135.6 s	135.2 s	135.5 s	137.1 s	138.2 s	137.1 s	138.7 s	138.5 s	137.2 s
6	130.1 d	130.5 d	129.3 d	128.3 d	129.3 d	128.3 d	129.0 d	128.4 d	130.0 d	130.0 d	129.9 d
7	168.3 s	167.1 s	167.0 s	166.9 s	166.9 s	172.6 s	172.2 s	172.4 s	174.6 s	174.6 s	168.1 s
1′	29.4 t	29.4 t	28.6 t	27.7 t	29.4 t	29.8 t	123.2 d	26.0 t	26.9 t	27.3 t	26.2 t
2′	123.0 d	123.0 d	122.4 d	121.8 d	122.4 d	124.2 d	140.9 d	45.4 t	45.8 t	46.0 t	45.4 t
3′	134.2 s	134.1 s	133.4 s	132.3 s	133.7 s	133.7 s	72.1 s	71.6 s	74.1 s	74.1 s	71.4 s
4′	17.9 q	17.9 q	18.0 q	16.7 q	17.9 q	18.2 q	30.1 q	29.7 q	30.1 q	30.4 q	29.5 q
5′	26.0 q	25.9 q	25.7 q	24.5 q	25.7 q	25.9 q	30.0 q	28.9 q	30.7 q	30.4 q	29.2 q
6′	29.4 t	29.4 t	28.6 t	27.7 t	29.4 t	29.8 t	29.9 t	29.7 t	31.1 t	31.1 t	29.7 t
7′	123.0 d	123.0 d	122.4 d	121.8 d	122.4 d	124.2 d	123.8 d	124.2 d	124.8 d	124.5 d	123.9 d
8′	134.2 s	134.1 s	133.4 s	132.3 s	133.7 s	133.7 s	133.7 s	133.7 s	136.9 s	137.1 s	134.1 s
9′	17.9 q	17.9 q	18.0 q	16.7 q	17.9 q	18.2 q	18.2 q	18.2 q	20.0 q	19.9 q	18.2 q
10′	26.0 q	25.9 q	25.7 q	24.5 q	25.7 q	25.9 q	26.0 q	25.9 q	27.5 q	27.5 q	26.0 q
	Glc	Glc	Ara	Ara	Glc	Glc	Ara	Glc	Glc-I	Ara	Ara
1″	103.2 d	95.8 d	104.9 d	102.8 d	104.0 d	105.9 d	105.8 d	105.9 d	105.0 d	105.5 d	105.6 d
2″	74.7 d	74.0 d	72.2 d	78.8 d	74.1 d	75.7 d	81.3 d	75.8 d	81.8 d	80.6 d	81.1 d
3″	77.0 d	78.0 d	73.4 d	72.0 d	76.3 d	78.0 d	74.1 d	78.0 d	79.1 d	74.5 d	73.7 d
4″	71.5 d	71.3 d	68.5 d	67.2 d	69.3 d	71.7 d	69.5 d	71.4 d	71.7 d	70.0 d	69.7 d
5″	75.9 d	76.1 d	66.6 t	65.2 t	75.5 d	78.2 d	67.2 t	78.2 d	78.6 d	67.7 t	66.7 t
6″	64.5 t	64.5 t	–	–	61.2 t	62.8 t	–	62.4 t	63.3 t	–	–
				Glc			Glc		Glc-II	Glc	Glc
1‴	–	–	–	103.3 d	–	–	104.8 d	–	104.6 d	105.2 d	104.6 d
2‴	146.2 d	–	–	73.7 d	–	–	76.0 d	–	76.4 d	76.3 d	75.7 d
3‴	148.0 s	–	–	75.9 d	–	–	77.9 d	–	78.5 d	78.3 d	78.0 d
4‴	175.9 s	–	–	69.5 d	–	–	71.4 d	–	72.2 d	72.0 d	71.6 d
5‴	117.0 d	–	–	75.8 d	–	–	78.2 d	–	78.4 d	78.4 d	78.1 d
6‴	157.8 d	–	–	60.1 t	–	–	62.6 t	–	62.3 t	63.1 t	62.6 t
-O<u>C</u>OCH$_3$	–	172.8 s	–	–	–	–	–	–	–	–	–
-OCO<u>CH</u>$_3$	–	20.6 q	–	–	–	–	–	–	–	–	–
-OCH$_3$	–	–	52.0 q	51.0 q	52.0 q	–	–	–	–	–	–
-O<u>CH</u>$_2$CH$_3$	–	–	–	–	–	–	–	–	–	–	62.0 t
-OCH$_2$<u>CH</u>$_3$	–	–	–	–	–	–	–	–	–	–	14.6 q

[a] Data are based on DEPT, HSQC, and HMBC experiments; ^{13}C NMR (100 MHz, δ). [b] Data in CD_3OD: [c] data in $CDCl_3$; [d] data in $CDCl_3$+ CD_3OD; [e] data in D_2O

Results and Discussion

Compound **1** was obtained as an amorphous solid. Its molecular formula was determined as $C_{28}H_{34}O_{10}$ by HR-ESI-MS at *m/z* 553.2064 $[M + Na]^+$ (calcd. for $C_{28}H_{34}O_{10}Na$, 553.2050). The IR spectrum indicated the presence of hydroxyl groups (3401 cm^{-1}), a carbonyl group (1711 cm^{-1}), and an aromatic ring (1607 and 769 cm^{-1}). The ^1H NMR spectrum (**Table 1**) displayed a singlet at δ 7.64 (2H, s) integrating for two protons, supporting the presence of an aromatic ring. In addition, signals due to two prenyl groups were present, namely, δ 5.31 (2H, m), δ 3.34 (4H, d, J = 7.2 Hz), and two singlets at δ 1.72 (6H, s) and δ 1.76 (6H, s) [8]. Further information was obtained from 2D NMR experiments. HMBC (**Fig. 2**) was observed from H-2 and H-6 (δ 7.64) with the carbon resonance at δ_C 168.3 (C-7), indicating that the carboxyl group was attached to C-1. More HMBC correlations were observed from the same proton signal to the resonances at δ_C 158.7 (C-4) and 129.5 (C-3, C-5), supporting the presence of a benzoic acid derivative. The HMBC correlations observed from H-1′ and H-6′ (δ 3.34) with C-2, C-3, C-4, C-5, C-6, C-2′, C-3′, C-7′, C-8′ and H-2, and H-6 (δ 7.64) with C-1′ revealed the linkage of prenyl units to C-3 and C-5. These data led us to establish the aglycone moiety as 4-hydroxy-3,5-bis(3-methyl-2-butenyl)-benzoic acid, previously described as a nervogenic acid unit [9, 10].

The ^1H NMR spectrum showed three unsaturated protons for the ABX pattern [δ 8.17 (1H, d, J = 0.6 Hz, H-2‴), δ 6.46 (1H, d, J = 5.6 Hz, H-5‴), and δ 7.97(1H, dd, J = 5.6, 0.6 Hz, H-6‴)]. Furthermore, the ^{13}C-NMR and DEPT spectra of **1** revealed the signals of five unsaturated carbons (175.9, 157.8, 148.0, 146.2, and 117.0), consistent with those in a pyromeconic acid unit [11].

Next, the proton resonances of the sugar units were observed. The sugar residue was identified as β-D-glucose by gas chromatography of the hydrolyzed product and by the coupling constant of its anomeric proton δ 4.79 (1H, d, J = 7.2 Hz). In the HMBC spectrum, the long-range correlation between Glc H-1″ [δ 4.79 (d, J = 7.2 Hz)] and C-3‴ (δ 148.0) of the pyromeconic acid unit indicated that the sugar moiety was located at C-3‴.

Furthermore, long-range correlations between Glc H-6″ [δ 4.42 (dd, J = 6.4, 12.0 Hz), 4.63 (dd, J = 2.0, 12.0 Hz)] and C-7 of the nervogenic acid unit were observed.

Based on the above data and analysis, the structure of compound **1** was determined to be {6-O-[(4-oxo-4H-pyran-3-yloxy)-O-β-D-glucopyranosyl]}-4-hydroxy-3,5-bis(3-methyl-2-butenyl) benzoate.

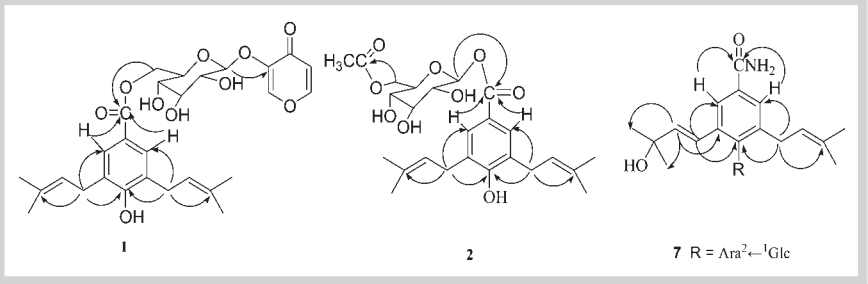

Fig. 2 Key HMBC correlations of compounds **1, 2,** and **7**.

The molecular formula of compound **2** was determined as $C_{25}H_{34}O_9$ by HR-ESI-MS at *m/z* 477.2132 [M – H]$^-$ (calcd. for $C_{25}H_{33}O_9$, 477.2125). From the NMR spectra of **2** (**Tables 1** and **3**), the presence of a nervogenic acid unit as a part of the structure was evident. Furthermore, one *β*-D-glucopyranoside unit and one acetyl unit were confirmed by NMR spectra. Complete structure assignments were obtained from exhaustive analysis of the HMQC, HMBC, and ^{1}H-^{1}H COSY data. The HMBC experiment showed long-range correlations between the anomeric proton signal of Glc at *δ* 5.65 (1H, d, *J* = 8.0 Hz) with C-7, indicating that the Glc unit was linked as an ester linkage (**Fig. 2**). Moreover, the acetyl unit was located at C-6″ of the Glc by the correlations between Glc H-6″ [*δ* 4.22 (dd, *J* = 5.6, 12.0 Hz), 4.37 (dd, *J* = 2.0, 12.0 Hz)] and the carboxyl carbon of the acetyl unit (*δ* 172.8). Thus, structure **2** was determined to be {1-O-[6-acetyl-O-*β*-D-glucopyranosyl]}-4-hydroxy-3,5-bis(3-methyl-2-butenyl) benzoate.

Compound **3** was isolated as an amorphous solid. Its molecular formula was deduced as $C_{23}H_{32}O_7$ by HR-ESI-MS at *m/z* 443.2038 [M + Na]$^+$ (calcd. for $C_{23}H_{32}O_7Na$, 443.2046). The ^{1}H and ^{13}C NMR signals (**Tables 1** and **3**) were assigned by a combination of DEPT, HMQC, and HMBC spectra which included signals due to one nervogenic acid unit, one α-arabinopyranosyl unit, and one methoxy (*δ* 3.86, 52.0) unit. The HMBC experiment showed the long-range correlations from the methoxy unit (*δ* 3.86, 52.0) to C-7, and the Ara H-1″ [*δ* 4.54 (1H, d, *J* = 7.6 Hz)] to C-4 (*δ* 155.9). Thus, structure **3** was determined to be methyl 3,5-bis(3-methyl-2-butenyl)-4-O-(α-L-arabinopyranosyl) benzoate.

Compound **4** was obtained as a white amorphous powder. Its molecular formula was determined as $C_{29}H_{42}NO_{12}$ by HR-ESI-MS at *m/z* 605.2574 [M + Na]$^+$ (calcd. for $C_{29}H_{42}NO_{12}Na$, 605.2574). In the comparison of NMR data of **4** with those of **3**, the main difference was the presence of one *β*-D-glucopyranoside unit. The sugar residues were identified as α-L-arabinopyranosyl and *β*-D-glucopyranoside by gas chromatography of the hydrolyzed product and by the coupling constant of their anomeric protons, Ara H-1″ [*δ* 4.75 (1H, d, *J* = 7.2 Hz)] and Glc H-1‴ [*δ* 4.72 (1H, d, *J* = 8.0 Hz)]. In the HMBC experiment, long-range correlations were observed from Ara H-1″ with C-4, Glc H-1‴ with Ara C-2″, and the methoxy unit (*δ* 3.88, 51.0) to C-7. These data supported the structure of **4** as methyl 3,5-bis(3-methyl-2-butenyl)-4-O-[*β*-D-glucopyranosyl-(1 → 2)-α-L-arabinopyranosyl] benzoate.

Compound **5** was also obtained as an amorphous solid. Its molecular formula was established as $C_{24}H_{34}O_8$ by analysis of HR-ESI-MS at *m/z* 473.2140 [M + Na]$^+$ (calcd. for $C_{24}H_{34}O_8Na$, 473.2151). Comparison of the NMR data of compounds **5** and **3** (**Tables 1** and **3**) indicated that **5** possessed the same aglycone as **3** but differed in the sugar part. The sugar residue was identified as *β*-D-glucopyranoside by gas chromatography of the hydrolyzed product and by the coupling constant of its anomeric protons, Glc H-1″ [*δ* 4.66 (1H, d, *J* = 7.6 Hz)]. By analysis of the HMQC, HMBC, and COSY data, compound **5** was determined to be methyl 3,5-bis(3-methyl-2-butenyl)-4-O-(*β*-D-glucopyranosyl) benzoate [4].

The molecular formula of **6** was determined as $C_{23}H_{33}NO_7$ by HR-ESI-MS at *m/z* 436.2324 [M + H]$^+$ (calcd. for $C_{23}H_{34}NO_7$, 436.2335). The NMR spectroscopic data of **6** were comparable to those of **5**, meaning **6** was also a nervogenic acid derivative with one *β*-D-glucopyranoside unit. Comparison of NMR data to nervogenic acid indicated that the -COOH group at C-7 was replaced by a $CONH_2$ group. Therefore, the structure of **6** was determined to be 3,5-bis(3-methyl-2-butenyl)-4-O-(*β*-D-glucopyranosyl) benzamide.

HR-ESI-MS of compound **7** gave a molecular ion at *m/z* 606.2529 [M + Na]$^+$ (calcd. for $C_{28}H_{41}NO_{12}Na$, 606.2526), corresponding to the molecular formula $C_{28}H_{41}NO_{12}$. The NMR and HR-ESI-MS data of **7** implied that compound **7** should be a benzamide derivative. Comparison of the NMR data of **6** with those of **7** indicated that the modification of one isoprene chain was the most notable difference, revealing the presence of a chain with a *trans* double bond, two methyl groups, and a tertiary hydroxyl group. The HMBC experiment (**Fig. 2**) showed as the most relevant connectivities on this side chain those of the two vinylic protons at *δ* 6.42 (H-2′, d, *J* = 16.2 Hz) and 7.27 (H-1′, d, *J* = 16.2 Hz) with *δ* 132.2 (C-3) and a correlation of the signals at *δ* 1.42 (Me-4′, Me-5′) with *δ* 72.1(C-3′) [8]. The sugar residues were identified as *β*-D-glucopyranoside and α-L- arabinopyranosyl by gas chromatography of the hydrolyzed product and by the coupling constant of their anomeric protons, Ara H-1″ [*δ* 4.74 (1H, d, *J* = 7.2 Hz)] and Glc H-1‴ [*δ* 4.76 (1H, d, *J* = 7.8 Hz)]. Long-range correlations in HMBC experiment were observed between Ara H-1″ with C-4 (*δ* 156.1) and Glc H-1‴ with Ara C-2″ (*δ* 81.3). By analysis of the HMQC, HMBC, and COSY data, the structure of compound **7** was elucidated to be 3-[(1*E*)-3-hydroxy-3-methyl-1-butenyl-4-O-[*β*-D-glucopyranosyl-(1 → 2)-α-L-arabinopyranosyl]]-5-(3-methyl-2-butenyl) benzamide.

The molecular formula of compound **8** was determined as $C_{23}H_{35}NO_8$ by HR-ESI-MS at *m/z* 476.2262 [M + Na]$^+$ (calcd. for $C_{23}H_{35}NO_8Na$, 476.2260). The HR-ESI-MS and ^{13}C NMR (**Table 3**) spectra revealed that compound **8** was also a benzamide derivative. The major differences between compounds **8** and **6** were the absence of the double bond and the presence of the quaternary hydroxy group at one of the prenyl side chains. ^{1}H-NMR spectrum of **8** (**Table 2**) indicated the presence of two aromatic protons at *δ* 7.58 (1H, d, *J* = 2.3 Hz) and 7.54 (1H, d, *J* = 2.3 Hz), characteristic of a 1,3,4,5-tetrasubstituted aromatic ring. Moreover, one 3-hydroxy-3-methylbutyl moiety was confirmed by the presence of signals in the ^{1}H NMR spectrum at *δ* 1.27 (6H, s), 1.80 (2H, m), 3.13 (2H, m) and signals in the ^{13}C NMR spectrum at

δ 26.0 (C-1′), 45.4 (C-2′), 71.6 (C-3′), 29.7 (C-4′), and 28.9 (C-5′) [12].

The linkage position of the isoprene side chain to the aromatic ring was determined by the HMBC experiment, in which correlations between the signals of H-2′/C-3 and H-1′/C-2, C-3, C-4, C-2′, C-3′ were observed. Moreover, the long-range correlation in the HMBC experiment was observed from Glc H-1″ [δ 4.72 (d, *J* = 7.6 Hz)] with C-4 (δ 156.8). Therefore, **8** was elucidated as 3-[(3-hydroxy-3-methylbutyl)-4-O-(β-D-glucopyranosyl)]-5-(3-methyl-2-butenyl) benzamide.

The molecular formula of compound **9** was determined as $C_{29}H_{45}NO_{13}$ (HR-ESI-MS). The ^1H (**Table 2**) and ^{13}C NMR data (**Table 3**) of **9** showed close structural similarity to the aglycone moiety of compound **8**, indicating that they had the same aglycone moiety. Complete assignment of the glycosidic protons and carbons along with an acid hydrolysis experiment indicated the presence of two β-D-glucopyranosyl units. Their sequencing patterns were obtained from the HMBC spectrum. Long-range correlations in the HMBC experiment were observed from Glc-I H-1″ [δ 4.95 (d, *J* = 7.2 Hz)] with C-4 (δ 157.0) and Glc-II H-1‴ [δ 5.00 (d, *J* = 8.0 Hz)] with Glc-I C-2″ (δ 81.8). Thus, structure **9** was assigned to be 3-(3-hydroxy-3-methylbutyl)-4-O-[β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl]-5-(3-methyl-2-butenyl) benzamide.

The molecular formula of **10** was determined as $C_{28}H_{43}NO_{12}$ by HR-ESI-MS at *m/z* 608.2668 $[M + Na]^+$ ($C_{28}H_{43}NO_{12}Na$, 608.2683). Comparison of the NMR data of compounds **10** and **9** (**Tables 2** and **3**) indicated that **10** possessed the same aglycone moiety as **9**. After hydrolysis, sugars were identified as D-glucose and L-arabinose, in a ratio of 1 : 1, based on the GC analysis of their chiral derivatives. Their sequencing was identified as the same as in compound **4** by the HMBC experiment. On the basis of these spectral data, the structure of **10** was established as 3-(3-hydroxy-3-methylbutyl)-4-O-[β-D-glucopyranosyl-(1 → 2)-α-L-arabinopyranosyl]-5-(3-methyl-2-butenyl) benzamide.

The molecular formula of compound **11** was determined as $C_{30}H_{46}O_{13}$ by HR-ESI-MS at *m/z* 637.2824 $[M + Na]^+$ (calcd. for $C_{30}H_{46}O_{13}Na$, 637.2836). It was found to have a similar structure to compound **10** by comparison of their NMR data (**Tables 2** and **3**). The main difference observed was that the $-NH_2$ group at C-7 was replaced by a carboxyethyl group [δ_H 1.37 (3H, t, *J* = 7.2 Hz), 4.32 (2H, q, *J* = 7.2 Hz), and δ_C 14.6 and 62.0]. The position of the carboxyethyl group was unambiguously defined by the HMBC experiment. Structure **11** was therefore determined to be 1-(ethoxy)-3-[(3-hydroxy-3-methylbutyl)-4-O-[β-D-glucopyranosyl-(1 → 2)-α-L-arabinosyl]-5-(3-methyl-2-butenyl) benzoate.

In order to evaluate the biological activitiy of these newly identified compounds isolated from the whole plant of *L. nervosa* for future applications, compounds **3, 4, 9, 10**, and **11** were tested individually for their *in vitro* cytotoxicities against A549, H460, HeLa, MCF-7, Caco2, and HepG2 human cell lines by the MTS method, as described previously in the literature [6, 7]. None of these compounds showed significant inhibitory activity against the tumor cells used (IC_{50} > 10 μM, n = 3).

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Conflict of Interest

All the authors have no conflict of interest to declare.

References

1 *Hua YX, Liu SF, Yang ZQ.* The Chinese herbal, Vol. 12. Shanghai: Shanghai Science and Technology Publishing House; 1999: 997
2 *Nishikawa K, Hirata Y.* Chemotaxonomical alkaloid studies. I. Structure of nervosine. Tetrahedron Lett 1967; 27: 2591–2596
3 *Nishikawa K, Miyamura M, Hirata Y.* Chemotaxonomical alkaloid studies structures of *Liparis* alkaloids. Tetrahedron 1969; 25: 2723–2741
4 *Abe F, Yamauchi T.* Glycosyl nervogenic acid esters of carbohydrates from *Anodendron affine* (Anodendron. VI). Chem Pharm Bull 1985; 33: 2712–2720
5 *Tang L, Jiang Y, Chang HT, Zhao MB, Tu PF, Cui JR, Wang RQ.* Triterpene saponins from the leaves of *Ilex kudingcha*. J Nat Prod 2005; 68: 1169–1174
6 *Chou TC, Motzer RJ, Tong Y, Bosl GJ.* Computerized quantitation of synergism and antagonism of taxol, topetecan, and cisplatin against human teratocarcinoma cell growth: a rational approach to clinical protocol design. J Natl Cancer Inst 1994; 86: 1517–1524
7 *Buttke TM, McCubrey JA, Owen TC.* Use of an aqueous soluble tetrazolium/formazan assay to measure viability and proliferation of lymphokine-dependent cell lines. J Immunol Methods 1993; 157: 233–240
8 *Flores N, Jiménez IA, Giménez A, Ruiz G, Gutiérrez D, Bourdy G, Bazzocchi IL.* Benzoic acid derivatives from *Piper* species and their antiparasitic activity. J Nat Prod 2008; 71: 1538–1543
9 *Šlapetová T, Šmejkal K, Innocenti G, Dall'Acqua S, Heilmann J, Babula P, Hamzová E, Žemličk M.* Glycosylated nervogenic acid derivatives from *Liparis condylobulbon* (Reichb.f.) leaves. Carbohydr Res 2009; 344: 1770–1774
10 *Orjala J, Erdelmeier CAJ, Wright AD, Rali T, Sticher O.* Five new prenylated p-hydroxybenzoic acid derivatives with antimicrobial and molluscicidal activity from *Piper aduncum* leaves. Planta Med 1993; 59: 546–551
11 *Hashidoko Y.* Pyromeconic acid and its glucosidic derivatives from leaves of *Erigeron annuus*, and the siderophile activity of pyromeconic acid. Biosci Biotechnol Biochem 1995; 59: 886–890
12 *Kuo WL, Huang YL, Shen CC, Shieh BJ, Chen CC.* Prenylated benzoic acids and phenanthrenes from *Liparis nakaharai*. J Chin Chem Soc 2007; 54: 1359–1362