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RESEARCH ARTICLE

A new phenolic glycoside from *Spiraea prunifolia* var. *simpliciflora* twigs

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Abstract The phytochemical investigation of the methanol extract from the twigs of *Spiraea prunifolia* var. *simpliciflora* (Rosaceae) using column chromatography led to the isolation of a new phenol glycoside, 1-*O*-(*E*)-caffeoyl-2-*O*-*p*-(*E*)-coumaroyl-*β*-D-glucopyranose (**1**), together with 16 known phenolic compounds (**2**–**17**). The structure of this new compound was elucidated by analysis of spectroscopic data including 1D, 2D nuclear magnetic resonance and HR-FAB-MS data. The isolated compounds were tested for cytotoxicity against four human tumor cell lines in vitro using the sulforhodamine B bioassay.

Keywords *Spiraea prunifolia* var. *simpliciflora* · Phenolic glycoside · Cytotoxicity

Introduction

Spiraea prunifolia var. *simpliciflora* (Nakai) Nakai (Rosaceae) is distributed throughout China and Korea. The twigs and roots of this source have been used to treat colds, sore throat, phlegm, neuralgia and malaria in Chinese folk medicine remedies (Lee 2003). Previous phytochemical investigations on *Spiraea* species reported the isolation of terpenoids (Oh et al. 2013; Park et al. 2009), alkaloids (Lui et al. 2007, 2009), flavonoids (Uzma et al. 2012) and phenolic compounds (Yoshida et al. 2010), and the methanol extract of this species have been reported to exhibit anti-inflammatory (Jun et al. 2007; Oh et al. 2003) and antioxidant activities (Park et al. 2013). As part of our ongoing research of Korean medicinal plants, we have studied the methanol extract of twigs of *S. prunifolia* var. *simpliciflora*, and isolated a new phenolic glycoside and 16 known phenolic constituents (Fig. 1). The structure of the new compound, 1-*O*-(*E*)-caffeoyl-2-*O*-*p*-(*E*)-coumaroyl-*β*-D-glucopyranose (**1**) was elucidated by spectroscopic methods, including 1D, 2D nuclear magnetic resonance (NMR) and HR-FAB-MS. All isolated compounds (**1**–**17**) were evaluated for their cytotoxic activities against four human cancer cell lines.

Materials and methods

General experimental procedure

Thin layer chromatography (TLC) was performed using Merck precoated silica gel F_{254} plates and RP-18 F_{254s} plates. Spots were detected on TLC under ultraviolet (UV) light or by heating after spraying with 10 % H_2SO_4 in C_2H_5OH (v/v). Silica gel 60 (70–230 and 230–400 mesh, Merck, Germany) and RP-C_{18} silica gel (230–400 mesh, Merck, Germany) was used for open column chromatography. Sep-Pak® (Vac 12 cm^3, Waters, MA, USA) was also used for column chromatography. Optical rotations were measured on a Jasco P-1020 polarimeter (Jasco, Easton, MD, USA). NMR spectra, including ^1H–^1H correlation spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC) and hetero nuclear multiple bond correlation (HMBC) were recorded on an AvanceIII

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NMR spectrometer operating at 700 MHz (^{1}H) and 175 MHz (^{13}C) (Bruker, Germany), with chemical shifts given in ppm (δ). HR-FAB-MS data were obtained on a JEOL JMS700 mass spectrometer (JEOL, Japan). Preparative HPLC was performed using a Gilson 306 pump (Gilson, Germany) with a Shodex refractive index detector (Shodex, New York, NY, USA) and Apollo Silica 5μ column (250 × 22 mm, Alltech, Nicholasville, KY, USA) or Econosil® RP-18 10μ column (250 × 22 mm, Alltech, Nicholasville, KY, USA). Silica gel 60 (230–400 mesh, Merck, Germany) was used for column chromatography.

Plant materials

Spiraea prunifolia var. *simpliciflora* (Rosaceae) (7.0 kg) was collected from Goesan-gun in Chungchoengbuk-do, Korea in March 2013. The plants were authenticated by one of the authors (K.R. Lee). A voucher specimen (SKKU-NPL-1301) of the plant was deposited at the School of Pharmacy, Sungkyunkwan University, Suwon, Korea.

Extraction and isolation

The twigs of *S. prunifolia* var. *simpliciflora* (7.0 kg) were extracted at 85 °C with 80 % MeOH (3 × 5 L every 3 days) and evaporated under reduced pressure to give a residue (380 g), which was dissolved in water (800 mL) and solvent-partitioned to give *n*-hexane (28 g), $CHCl_3$ (29 g), EtOAc (12 g) and *n*-BuOH fractions (47 g). The $CHCl_3$ soluble fraction (19 g) was separated over a silica gel column with $CHCl_3$:MeOH (30:1–1:1 gradient) as the eluent to yield 10 fractions (C1–10). Fraction C3 (616 mg) was subjected to RP-C_{18} silica gel column chromatography (50 % MeOH) as eluent to give seven subfractions (C31–37). Subfraction C32 (60 mg) was purified with a silica semi prep. HPLC (*n*-hexane:EtOAc:MeOH = 5:1:1) to yield compounds **5** (15 mg, R_t = 15.1 min), **6** (7 mg, R_t = 17.2 min), **9** (14 mg, R_t = 23.8 min), and **10** (8 mg, R_t = 27.1 min). Fraction C4 (1.3 g) was subjected to RP-C_{18} silica gel column chromatography (50–100 % MeOH gradient) as eluent to give six subfractions (C41–46). Subfraction C41 (316 mg) was separated over silica gel column chromatography with a solvent system of $CHCl_3$:MeOH (20:1) as the eluent to give four subfractions (C411–414). Subfraction C414 (123 mg) was purified with an RP-C_{18} semi prep. HPLC (25 % ACN) to yield compounds **8** (12 mg, R_t = 14.8 min), **12** (4 mg, R_t = 15.0 min), **15** (6 mg, R_t = 19.7 min), and **16** (10 mg, R_t = 23.2 min). Fraction C5 (3.5 g) was subjected to RP-C_{18} silica gel column chromatography (50–100 % MeOH gradient) to give six subfractions (C51–56). Subfraction C51 (985 mg) was separated over silica gel column chromatography with a solvent system of *n*-hexane:EtOAc:MeOH (3:1:1) as the eluent to give eight subfractions (C511–518). Subfraction C512 (106 mg) was purified with an RP-C_{18} semi prep. HPLC (35 % MeOH) to yield compound **7** (15 mg, R_t = 19.4 min). The EtOAc soluble fraction (10 g) was separated over a silica gel column with $CHCl_3$:MeOH:H_2O (4:1:0.1) as the eluent to yield seven fractions (E1–7). Fraction E3 (750 mg) was subjected to RP-C_{18} silica gel column chromatography (40 % MeOH) to give nine subfractions (E31–39). Subfraction E35 (228 mg) was separated over a silica gel column chromatography with a solvent system of *n*-hexane:EtOAc:MeOH (4:1:1) as the eluent to give four subfractions (E351–354). Subfraction E353 (50 mg) was purified with an RP-C_{18} semi prep. HPLC (20 % ACN) to yield compound **11** (12 mg, R_t = 24.2 min). Subfraction E4 (1.4 g) was subjected to RP-C_{18} silica gel column chromatography (50 % MeOH) to give seven subfractions (E41–47). Subfraction E44 (118 mg) was purified with an RP-C_{18} semi prep. HPLC (40 % MeOH) to yield compounds **3** (7 mg, R_t = 19.2 min), **4** (11 mg, R_t = 21.5 min), **13** (4 mg, R_t = 22.4 min), **14** (8 mg, R_t = 25.3 min), and **17** (6 mg, R_t = 28.3 min). Subfraction E45 (1.0 mg) was separated over a silica gel column chromatography with a solvent system of $CHCl_3$:MeOH:H_2O (4:1:0.1) as the eluent to give four subfractions (E451–454). Subfraction E451 was purified with an RP-C_{18} semi-prep. HPLC (30 % ACN) to yield compounds **1** (4 mg, R_t = 15.1 min) and **2** (6 mg, R_t = 16.2 min).

1-*O*-(*E*)-caffeoyl-2-*O*-*p*-(*E*)-coumaroyl-β-D-glucopyranose (1)

Yellow gum, $[\alpha]_D^{25}$ −14.0° (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 203, 227, 314 nm; IR (KBr) ν_{max} 3385, 1703, 1631, 1604, 1515, 1262, 1170, 1075, 832 cm^{-1}; HR-FAB-MS *m/z* 487.1236 $[M-H]^-$ (calc. $C_{24}H_{23}O_{11}$, 487.1235); ^{1}H- (CD_3OD, 700 MHz) and ^{13}C-NMR (CD_3OD, 175 MHz), see Table 1.

2-*O*-(*E*)-caffeoyl-1-*O*-*p*-(*E*)-coumaroyl-β-D-glucopyranose (2)

Yellow gum, $[\alpha]_D^{25}$ −0.8° (c 0.25, MeOH); FAB-MS *m/z* 487.12 $[M-H]^-$; ^{1}H- (CD_3OD, 700 MHz) and ^{13}C-NMR (CD_3OD, 175 MHz), see Table 1.

1,2-Di-*O*-(*E*)-caffeoyl-β-D-glucopyranose (3)

Yellow gum, $[\alpha]_D^{25}$ −15.3° (c 0.7, MeOH); FAB-MS *m/z* 503.10 $[M-H]^-$; ^{1}H-NMR (CD_3OD, 700 MHz): δ 3.49 (1H, m, H-5″), 3.53 (1H, t, J = 9.0 Hz, H-4″), 3.72 (1H, t, J = 9.0 Hz, H-3″), 3.74 (1H, dd, J = 5.0, 12.0 Hz, H-6″b), 3.94 (1H, dd, J = 2.0, 12.0 Hz, H-6″a), 5.06 (1H, dd,

Table 1 ^{1}H- and ^{13}C-NMR spectral data of compounds **1** and **2**

Positions	Compound **1**		Positions	Compound **2**	
	δ_H	δ_C		δ_H	δ_C
1		127.2	1		126.8
2	7.45 d (8.5)	131.4	2	7.42 d (9.0)	131.4
3	6.80 d (2.0)	116.9	3	6.78 d (9.0)	116.8
4		150.2	4		161.5
5	6.80 d (2.0)	116.9	5	6.78 d (9.0)	116.8
6	7.45 d (8.5)	131.4	6	7.43 d (9.0)	131.4
7	7.67 d (16.0)	147.5	7	7.65 d (16.0)	147.7
8	6.36 d (16.0)	113.9	8	6.25 d (16.0)	114.6
9		167.3	9		167.2
1′		127.6	1′		127.5
2′	7.04 d (2.0)	115.4	2′	7.01 d (2.0)	115.2
3′		147.0	3′		146.6
4′		161.5	4′		149.5
5′	6.79 d (2.0)	116.6	5′	6.74 d (8.0)	116.4
6′	6.95 dd (2.0, 8.0)	123.5	6′	6.91 dd (2.0, 8.0)	123.1
7′	7.61 d (16.0)	147.0	7′	7.58 d (16.0)	148.5
8′	6.21 d (16.0)	114.9	8′	6.27 d (7.5)	115.2
9′		168.5	9′		168.3
1″	5.81 d (8.5)	93.8	1″	5.82 d (8.0)	93.8
2″	5.08 dd (8.5, 9.5)	74.0	2″	3.73 dd (8.0, 9.0)	74.1
3″	3.76 m[a]	75.4	3″	5.09 dd (8.0, 9.0)	75.9
4″	3.54 m[a]	71.0	4″	3.56 t (9.0)	71.1
5″	3.54 m[a]	78.9	5″	3.54 m	78.9
6″-a	3.76 m[a]	62.0	6″-a	3.76 dd (5.0, 12.0)	62.1
6″-b	3.93 m		6″-b	3.90 dd (2.0, 12.0)	

[1] H- and ^{13}C-NMR run at 700 and 175 MHz (CD_3OD), respectively. Proton coupling constants (*J*) in Hz are given in parentheses

[a] Overlapped signals

$J = 8.0, 9.0$ Hz, H-2″), 5.79 (1H, d, $J = 8.0$ Hz, H-1″), 6.18 (1H, d, $J = 8.0$ Hz, H-8), 6.27 (1H, d, $J = 8.0$ Hz, H-8′), 6.75 (1H, d, $J = 8.0$ Hz, H-5), 6.76 (1H, d, $J = 8.0$ Hz, H-5′), 6.91 (1H, dd, $J = 2.0$, 8.0 Hz, H-6), 6.93 (1H, dd, $J = 2.0$, 8.0 Hz, H-6′), 7.04 (1H, d, $J = 2.0$ Hz, H-1), 7.57 (1H, d, $J = 16.0$ Hz, H-9′), 7.58 (1H, d, $J = 16.0$ Hz, H-9); ^{13}C-NMR (CD_3OD, 175 MHz): δ 62.2 (C-6″), 71.1 (C-4″), 74.2 (C-2″), 75.9 (C-3″), 78.9 (C-5″), 93.9 (C-1″), 113.7 (C-8), 114.6 (C-8′), 115.2 (C-2), 115.3 (C-2′), 116.5 (C-5,5′), 123.1 (C-6), 123.4 (C-6′), 127.2 (C-1′) 127.4 (C-1), 147.7 (C-7), 148.9 (C-7′), 149.6 (C-4′), 149.7 (C-3,3′), 149.9 (C-4′), 167.2 (C-9), 168.3 (C-9′).

1-Caffeoyl-6-tuliposide A (4)

Yellow gum, $[\alpha]_D^{25}$ +4.5° (c 0.1, MeOH); FAB-MS *m/z* 439.1 $[M-H]^-$; ^{1}H-NMR (CD_3OD, 700 MHz): δ 2.53 (2H, t, $J = 6.5$ Hz, H-3′), 3.41 (1H, t, $J = 9.0$ Hz, H-4″), 3.45 (1H, dd, $J = 9.0$, 7.5 Hz, H-2″), 3.48 (1H, t, $J = 9.0$ Hz, H-3″), 3.66 (2H, t, $J = 6.5$ Hz, H-4′), 3.67 (1H, m, H-5″), 4.33 (1H, dd, $J = 5.5$, 12.0 Hz, H-6″a), 4.48 (1H, dd, $J = 2.0$, 12.0 Hz, H-6″b), 5.56 (1H, d, $J = 7.5$ Hz, H-1″), 5.69 (1H, d, $J = 1.5$ Hz, H-5′), 6.24 (1H, d, $J = 1.5$ Hz, H-5′), 6.30 (1H, d, $J = 16.0$ Hz, H-8), 6.78 (1H, d, $J = 8.5$ Hz, H-5), 6.97 (1H, dd, $J = 2.0$, 8.5 Hz, H-6), 7.06 (1H, d, $J = 2.0$ Hz, H-2), 7.66 (1H, d, $J = 16.0$ Hz, H-7); ^{13}C-NMR (CD_3OD, 175 MHz): δ 34.9 (C-3′), 60.2 (C-4′), 63.1 (C-6″), 69.9 (C-4″), 72.5 (C-2″), 74.7 (C-5″), 76.4 (C-3″), 94.3 (C-1″), 112.8 (C-2), 115.1 (C-8), 121.9 (C-9), 126.1 (C-4), 126.7 (C-5′), 113.8 (C-5), 137.1 (C-2′), 145.4 (C-6), 147.0 (C-3), 148.5 (C-7), 166.2 (C-1), 166.7 (C-1′).

(−)-Nortrachelogenin (5)

Yellow gum, $[\alpha]_D^{25}$ −29.8° (c 0.3, MeOH); EI-MS *m/z* 374 $[M]^+$; ^{1}H-NMR ($CDCl_3$, 700 MHz): δ 2.43 (1H, m, H-8′), 2.46 (1H, dd, $J = 4.5$, 13.5 Hz, H-7′b), 2.78 (1H, dd,

J = 4.5, 13.5 Hz, H-7′a), 2.84 (1H, d, J = 13.5 Hz, H-7b), 3.11 (1H, d, J = 13.0 Hz, H-7a), 3.77 (3H, s, 3′-OCH_3), 3.80 (3H, s, 3-OCH_3), 3.97 (2H, d, J = 7.5 Hz, H-9′), 6.56 (1H, dd, J = 2.0, 8.0 Hz, H-6), 6.58 (1H, dd, J = 2.0, 8.0 Hz, H-6′), 6.66 (1H, d, J = 2.0 Hz, H-2′), 6.68 (1H, d, J = 2.0 Hz, H-2), 6.69 (1H, d, J = 2.0 Hz, H-5′), 6.77 (1H, dd, J = 2.0, 8.0 Hz, H-5); ^{13}C-NMR ($CDCl_3$, 175 MHz): δ 31.7 (C-7′), 42.2 (C-7), 43.9 (C-8′), 55.9 (3-OCH_3), 56.0 (3′-OCH_3), 70.1 (C-9′), 76.4 (C-8), 111.4 (C-2′), 112.6 (C-2), 114.3 (C-5), 114.5 (C-5′), 121.5 (C-6′), 123.2 (C-6), 126.0 (C-1), 130.2 (C-1′), 144.4 (C-4′), 145.1 (C-4), 146.6 (C-3), 146.6 (C-3′), 178.3 (C-9).

Lariciresinol (6)

Yellow gum, $[\alpha]_D^{25}$ +17.1° (c 0.4, MeOH); FAB-MS m/z 359 $[M-H]^-$; ^{1}H-NMR (CD_3OD, 700 MHz): δ 2.44 (1H, dd, J = 2.0, 8.0 Hz, H-6), 2.73 (1H, dd, J = 2.0, 8.0 Hz, H-6′), 2.92 (2H, d, J = 7.5 Hz, H-9′), 3.76 (3H, s, 3′-OCH_3), 3.80 (3H, s, 3-OCH_3), 4.06 (1H, d, J = 2.0 Hz, H-2′), 4.81 (1H, d, J = 2.0 Hz, H-2), 5.49 (1H, d, J = 2.0 Hz, H-5′), 6.85 (1H, m, H-5); ^{13}C-NMR (CD_3OD, 175 MHz): δ 33.3 (C-7′), 42.5 (C-7), 52.6 (C-8′), 55.9 (3,3′-OCH_3), 61.1 (C-9), 72.9 (C-9′), 82.8 (C-8), 109.1 (C-2′), 111.2 (C-2), 114.4 (C-5), 118.0 (C-5′), 121.2 (C-6′), 132.3 (C-6), 135.6 (C-1), 144.0 (C-1′), 146.6 (C-4′), 148.5 (C-4), 149.2 (C-3).

(−)-Olivil (7)

Yellow gum, $[\alpha]_D^{25}$ −60.8° (c 0.3, MeOH); FAB-MS m/z 375 $[M-H]^-$; ^{1}H-NMR (CD_3OD, 700 MHz): δ 2.29 (1H, ddd, J = 5.5, 6.0, 7.0 Hz, H-8), 2.98 (1H, d, J = 14.0 Hz, H-7′a), 2.91 (1H, d, J = 14.0 Hz, H-7′b), 3.59 (1H, d, J = 9.0 Hz, H-9′a), 3.72 (1H, dd, J = 6.0, 11.5 Hz, H-9a), 3.80 (1H, d, J = 9.0 Hz, H-9′a), 3.83 (1H, dd, J = 6.0, 11.5 Hz, H-9b), 3.84 (6H, s, 3,3′-OCH_3), 4.71 (1H, d, J = 7.0 Hz, H-7), 6.73 (1H, d, J = 8.0 Hz, H-5′), 6.75 (1H, d, J = 8.0 Hz, H-5), 6.77 (1H, dd, J = 2.0, 8.0 Hz, H-6), 6.93 (1H, d, J = 2.0, 8.0 Hz, H-6′), 7,17 (1H, d, J = 2.0 Hz, H-2); ^{13}C-NMR (CD_3OD, 175 MHz): δ 40.6 (C-7′), 48.0 (C-8), 56.6 (3,3′-OCH_3), 60.8 (C-9), 78.0 (C-9′), 82.6 (C-8′), 85.7 (C-7), 112.0 (C-2′), 115.7 (C-5), 116.0 (C-5′), 116.0 (C-2), 121.0 (C-6′), 124.1 (C-6), 130.6 (C-1), 135.5 (C-1′), 146.4 (C-4′), 147.4 (C-4), 148.7 (C-3), 149.1 (C-3′).

(−)-Berchemol (8)

Yellow gum, $[\alpha]_D^{25}$ −8.3° (c 0.1, MeOH); FAB-MS m/z 375 $[M-H]^-$; ^{1}H-NMR (CD_3OD, 700 MHz): δ 2.44 (1H, d, J = 12.5, 13.0 Hz, H-7′a), 2.61 (1H, m, H-8′), 3.07 (1H, d, J = 4.0, 13.0 Hz, H-7′b), 3.64 (1H, d, J = 11.5 Hz, H-9a), 3.72 (1H, dd, J = 5.0, 8.5 Hz, H-9′a), 3.88 (3H, s, 3-OCH_3), 3.89 (1H, d, J = 11.5 Hz, H-9b), 3.90 (3H, s, 3′-OCH_3), 4.17 (1H, dd, J = 6.5, 8.5 Hz, H-9′b), 4.85 (1H, s, H-7), 6.70 (1H, d, J = 2.0 Hz, H-2′), 6.79 (1H, dd, J = 2.0, 8.0 Hz, H-6), 6.85 (1H, d, J = 8.0 Hz, H-5′), 6.90 (1H, d, J = 2.0 Hz, H-2), 6.92 (1H, d, J = 8.0 Hz, H-5), 6.69 (1H, dd, J = 2.0, 8.0 Hz, H-6′); ^{13}C-NMR (CD_3OD, 175 MHz): δ 40.7 (C-7′), 48.0 (C-8), 56.6 (3′,3″-OCH_3), 60.8 (C-9), 78.0 (C-9′), 82.6 (C-8′), 85.7 (C-7), 112.0 (C-2′), 115.7 (C-2), 115.8 (C-5), 115.9 (C-5′), 120.9 (C-6′), 124.0 (C-6), 130.5 (C-1′), 135.5 (C-1′), 146.3 (C-4′), 147.3 (C-4), 148.7 (C-3), 149.2 (C-3′).

(+)-1-Hydroxypinoresinol (9)

Yellow gum, $[\alpha]_D^{25}$ +11.5° (c 0.1, MeOH); FAB-MS m/z 373 $[M-H]^-$; ^{1}H-NMR (CD_3OD, 700 MHz): δ 3.03 (1H, m, H-5), 3.75 (1H, dd, J = 6.0, 9.0 Hz, H-4b), 3.83 (1H, d, J = 9.5 Hz, H-8b), 3.84 (6H, s, 3′,3″-OCH_3), 4.02 (1H, d, J = 9.5 Hz, H-8a), 4.45 (1H, dd, J = 9.0, 9.0 Hz, H-4a), 4.66 (1H, s, H-2), 4.83 (1H, d, J = 5.0 Hz, H-6), 6.77 (1H, d, J = 8.0 Hz, H-5″), 6.78 (1H, d, J = 8.0 Hz, H-5′), 6.86 (1H, dd, J = 2.0, 8.0 Hz, H-6″), 7.03 (1H, d, J = 2.0 Hz, H-2′); ^{13}C-NMR (CD_3OD, 175 MHz): δ 33.4 (C-7), 42.4 (C-8), 52.6 (C-8′), 55.9 (3,3′-OCH_3), 60.9 (C-9′), 72.9 (C-9), 82.8 (C-7′), 108.2 (C-2′), 111.6 (C-2), 114.1 (C-5′), 114.4 (C-5), 118.7 (C-6′), 121.2 (C-6), 131.6 (C-1′), 134.8 (C-1), 144.0 (C-4), 145.0 (C-4′), 146.5 (C-3), 146.6 (C-3′).

(+)-Fraxiresinol (10)

Yellow gum, $[\alpha]_D^{25}$ +22.1° (c 0.1, MeOH); EI-MS m/z 403 $[M]^+$; ^{1}H-NMR (CD_3OD, 700 MHz): δ 3.06 (1H, ddd, J = 5.5, 6.5, 8.5 Hz, H-5), 3.79 (1H, dd, J = 6.5, 9.0 Hz, H-4b), 3.88 (6H, s, 3′,5′-OCH_3), 3.89 (3H, s, 3″-OCH_3), 3.90 (1H, d, J = 9.0 Hz, H-8b), 4.09 (1H, d, J = 9.0 Hz, H-8a), 4.49 (1H, dd, J = 8.5, 9.0 Hz, H-4a), 4.70 (1H, s, H-2), 4.87 (1H, d, J = 6.0 Hz, H-6), 6.75 (2H, s, H-2′,6′), 6.79 (1H, d, J = 8.0 Hz, H-5″), 6.89 (1H, dd, J = 2.0, 8.0 Hz, H-6″), 7.07 (1H, d, J = 2.0 Hz, H-2″); ^{13}C-NMR (CD_3OD, 175 MHz): δ 33.3 (C-7), 42.4 (C-8), 52.6 (C-8′), 55.9 (3,3′-OCH_3), 60.9 (C-9′), 72.9 (C-9), 82.8 (C-7′), 108.2 (C-2′), 111.6 (C-2), 114.4 (C-5), 114.1 (C-5′), 118.7 (C-6′), 121.21 (C-6), 134.8 (C-1), 131.6 (C-1′), 145.0 (C-4′), 144.0 (C-4), 146.5 (C-3), 146.6 (C-3′).

(+)-1-Hydroxypinoresinol 1-*O*-*β*-D-glucopyranoside (11)

Yellow gum, $[\alpha]_D^{25}$ −9.6° (c 0.05, MeOH); FAB-MS m/z 535 $[M-H]^-$; ^{1}H-NMR (CD_3OD, 700 MHz): δ 2.87 (1H, m, H-5″), 3.03 (1H, dt, J = 2.5, 5.0 Hz, H-2″), 3.14 (2H, m, H-3″,4″), 3.40 (1H, m, H-8), 3.51 (1H, dd, J = 6.0,

12.0 Hz, H-6″a), 3.68 (dd, J = 2.0, 12.0 Hz, H-6″b), 3.80 (1H, dd, J = 6.0, 9.0 Hz, H-9b), 3.86 (3H, s, 3′-OCH_3), 3.87 (3H, s, 3-OCH_3), 3.96 (1H, d, J = 10.0 Hz, H-9′b), 4.37 (1H, d, J = 7.5 Hz, H-1″), 4.49 (1H, dd, J = 7.0, 9.0 Hz, H-9a), 4.52 (1H, d, J = 10.0 Hz, H-9′a), 4.71 (1H, s, H-7), 4.82 (1H, s, H-7′), 6.75 (1H, d, J = 8.0 Hz, H-5′), 6.83 (1H, d, J = 8.0 Hz, H-5), 6.89 (1H, dd, J = 2.0, 8.0 Hz, H-6′), 6.91 (1H, dd, J = 2.0, 8.0 Hz, H-6), 7.06 (1H, d, J = 2.0 Hz, H-2), 7.12 (1H, d, J = 2.0 Hz, H-2′); ^{13}C-NMR (CD_3OD, 175 MHz): δ 55.3 (3-OCH_3), 55.4 (3′-OCH_3), 59.0 (C-8), 61.3 (C-6″), 70.9 (C-4″), 72.1 (C-9), 73.6 (C-9′), 76.8 (C-2″), 77.1 (C-5″), 78.2 (C-3″), 85.6 (C-7), 88.8 (C-7′), 98.0 (C-8′), 98.8 (C-1″), 109.5 (C-2), 112.9 (C-2′), 114.0 (C-5′), 115.1 (C-5), 118.7 (C-6), 121.2 (C-6′), 127.4 (C-1′), 131.9 (C-1), 146.1 (C-4′), 146.3 (C-4), 147.2 (C-3′), 148.1 (C-3).

(−)-Secoisolariciresinol (12)

Yellow gum, $[\alpha]_D^{25}$ −21.7° (c 0.1, MeOH); FAB-MS m/z 361 $[M-H]^-$; ^{1}H-NMR (CD_3OD, 700 MHz): δ 1.92 (2H, m, H-8,8′), 2.57 (2H, m, H-7b,7′b), 2.68 (2H, dd, J = 8.0, 14.0 Hz, H-7a,7′a), 3.60 (4H, m, H-9,9′), 3.75 (6H, s, 3, 3′-OCH_3), 6.56 (2H, dd, J = 2.0, 8.0 Hz, H-6,6′), 6.61 (2H, d, J = 2.0 Hz, H-2,2′), 6.67 (2H, d, J = 8.0 Hz, H-5,5′); ^{13}C-NMR (CD_3OD, 175 MHz): δ 36.2 (C-7,7′), 44.2 (C-8,8′), 56.1 (3,3′-OCH_3), 62.3 (C-9,9′), 114.2 (C-2,2′), 116.1 (C-5,5′), 122.8 (C-6,6′), 134.0 (C-1,1′), 145.6 (C-4,4′), 148.9 (C-3,3′).

(+)-9-*O*-*β*-D-glucopyranosyl lyoniresinol (13)

Yellow gum, $[\alpha]_D^{25}$ +36.2° (c 0.1, MeOH); FAB-MS m/z 581 $[M-H]^-$; ^{1}H-NMR (CD_3OD, 700 MHz): δ 1.80 (2H, t, J = 3.0, 10.0 Hz, H-8′), 2.18 (2H, m, H-8), 2.84 (2H, m, H-1), 3.21 (1H, dd, J = 8.0, 9.0 Hz, H-2″), 3.39 (1H, dd, J = 3.0, 12.0 Hz, H-9a), 3.66 (1H, dd, J = 6.0, 12.0 Hz, H-9′a), 3.68 (1H, t, J = 5.0, 10.0 Hz, H-9a), 3.73 (1H, t, J = 3.0, 12.0 Hz, H-9b), 3.77 (3H, s, 3-OCH_3), 3.80 (3H, s, 3′-OCH_3), 3.86 (1H, brd, J = 8.0 Hz, H-7′), 3.87 (1H, dd, J = 2.0, 12.0 Hz, H-6″b), 4.00 (1H, dd, J = 4.0, 10.0 Hz, H-9′b), 4.29 (1H, d, J = 8.0 Hz, H-1″), 6.19 (1H, s, H-5), 6.61 (1H, dd, J = 2.0, 8.0 Hz, H-6′), 6.65 (1H, s, H-2), 6.68 (1H, d, J = 2.0 Hz, H-2′), 6.74 (1H, d, J = 8.0 Hz, H-5′); ^{13}C-NMR (CD_3OD, 175 MHz): δ 33.9 (C-7), 39.6 (C-8), 47.9 (C-7′), 46.0 (C-8′), 56.5 (3,3′-OCH_3), 62.8 (C-6″), 65.3 (C-9), 69.6 (C-9′), 71.7 (C-4″), 75.2 (C-2″), 77.9 (C-5″), 78.2 (C-3″), 105.2 (C-1″), 112.6 (C-2), 114.1 (C-2′), 116.1 (C-5′), 117.5 (C-5), 123.3 (C-6′), 129.2 (C-1), 134.3 (C-6), 138.7 (C-1′), 146.1 (C-4′), 145.3 (C-4), 147.1 (C-3), 149.0 (C-3′).

(+)-9-*O*-*β*-D-glucopyranosyl isolariciresinol (14)

Yellow gum, $[\alpha]_D^{25}$ +3.9° (c 0.1, MeOH); FAB-MS m/z 521 $[M-H]^-$; ^{1}H-NMR (CD_3OD, 700 MHz): δ 1.80 (2H, t, J = 3.0, 10.0 Hz, H-8′), 2.18 (2H, m, H-8), 2.84 (2H, m, H-1), 3.21 (1H, dd, J = 8.0, 9.0 Hz, H-2″), 3.39 (1H, dd, J = 3.0, 12.0 Hz, H-9′a), 3.66 (1H, dd, J = 6.0, 12.0 Hz, H-9a), 3.68 (1H, t, J = 5.0, 10.0 Hz, H-9a), 3.73 (1H, t, J = 3.0, 12.0 Hz, H-9′b), 3.77 (3H, s, 3-OCH_3), 3.80 (3H, s, 3′-OCH_3), 3.86 (1H, brd, J = 8.0 Hz, H-7′), 3.87 (1H, dd, J = 2.0, 12.0 Hz, H-6″b), 4.00 (1H, dd, J = 4.0, 10.0 Hz, H-9b), 4.29 (1H, d, J = 8.0 Hz, H-1″), 6.19 (1H, s, H-5), 6.61 (1H, dd, J = 2.0, 8.0 Hz, H-6′), 6.65 (1H, s, H-2), 6.68 (1H, d, J = 2.0 Hz, H-2′), 6.74 (1H, d, J = 8.0 Hz, H-5′); ^{13}C-NMR (CD_3OD, 175 MHz): δ 33.9 (C-7), 37.5 (C-8), 48.0 (C-7′), 48.3 (C-8′), 56.5 (3,3′-OCH_3), 61.8 (C-9′), 62.9 (C-1″), 71.8 (C-4″), 73.9 (C-9), 75.3 (C-2″), 78.1 (C-5″), 78.2 (C-3″), 104.7 (C-1″), 112.6 (C-2), 114.1 (C-2′), 116.1 (C-5′), 117.5 (C-5), 123.3 (C-6′), 129.2 (C-1), 134.3 (C-6), 138.7 (C-1′), 146.0 (C-4′), 145.4 (C-4), 147.3 (C-3), 149.1 (C-3′).

7*R*,8*S*-dihydrodehydrodiconiferyl alcohol (15)

Yellow gum, $[\alpha]_D^{25}$ −19.0° (c 0.1, MeOH); FAB-MS m/z 383 $[M+Na]^+$; ^{1}H-NMR (CD_3OD, 700 MHz): δ 2.78 (1H, dd, J = 4.5, 13.5 Hz, H-7′a), 2.84 (1H, d, J = 13.5 Hz, H-7b), 3.77 (3H, s, 3′-OCH_3), 3.11 (1H, d, J = 13.0 Hz, H-7a), 3.80 (3H, s, 3-OCH_3), 3.97 (2H, d, J = 7.5 Hz, H-9′), 6.56 (1H, dd, J = 2.0, 8.0 Hz, H-6), 6.58 (1H, dd, J = 2.0, 8.0 Hz, H-6′), 6.66 (1H, d, J = 2.0 Hz, H-2′), 6.68 (1H, d, J = 2.0 Hz, H-2), 6.69 (1H, d, J = 2.0 Hz, H-5′), 6.77 (1H, dd, J = 2.0, 8.0 Hz, H-5); ^{13}C-NMR (CD_3OD, 175 MHz) δ: 32.7 (C-7), 35.9 (C-8), 55.1 (C-8), 55.6 (3-OCH_3), 56.8 (3′-OCH_3), 61.5 (C-9), 64.4 (C-9′), 89.1 (C-7′), 110.6 (C-2), 114.2 (C-2), 116.5 (C-5′), 117.6 (C-6), 119.8 (C-6′), 130.0 (C-1), 134.9 (C-1′), 137.0 (C-5), 145.3 (C-3), 147.6 (C-3′,4), 149.2 (C-4′).

7*R*,8*S*-5-methoxydihydrodehydroconiferyl alcohol (16)

Yellow gum, $[\alpha]_D^{25}$ −11.4° (c 0.1, MeOH); FAB-MS m/z 389 $[M-H]^-$; ^{1}H-NMR (CD_3OD, 700 MHz): δ 2.43 (1H, m, H-8′), 2.46 (1H, dd, J = 4.5, 13.5 Hz, H-7′b), 2.78 (1H, dd, J = 4.5, 13.5 Hz, H-7′a), 2.84 (1H, d, J = 13.5 Hz, H-7b), 3.11 (1H, d, J = 13.0 Hz, H-7a), 3.77 (3H, s, 3′-OCH_3), 3.80 (3H, s, 3-OCH_3), 3.97 (2H, d, J = 7.5 Hz, H-9′), 6.56 (1H, dd, J = 2.0, 8.0 Hz, H-6), 6.58 (1H, dd, J = 2.0, 8.0 Hz, H-6′), 6.66 (1H, d, J = 2.0 Hz, H-2′), 6.68 (1H, d, J = 2.0 Hz, H-2), 6.69 (1H, d, J = 2.0 Hz, H-5′), 6.77 (1H, dd, J = 2.0, 8.0 Hz, H-5); ^{13}C-NMR

(CD_3OD, 175 MHz): δ 32.0 (C-7′), 34.6 (C-8′), 53.8 (C-8), 56.0 (3-OCH_3), 56.3 (3′-OCH_3), 62.2 (C-9′), 63.7 (C-9), 88.1 (C-7), 103.1 (C-2,6), 112.4 (C-2′), 115.9 (C-6′), 127.7 (C-5′), 132.3 (C-1), 134.6 (C-4), 135.5 (C-1′), 144.2 (C-3′), 146.5 (C-4′), 147.1 (C-3, 5).

Dihydrodehydrodiconiferyl alcohol 4-*O*-*β*-D-glucopyranoside (17)

Yellow gum, $[\alpha]_D^{25}$ −8.2° (c 0.3, MeOH); FAB-MS *m/z* 545 $[M+Na]^+$; ^{1}H-NMR (CD_3OD, 700 MHz): δ 1.81 (2H, m, H-8′), 2.62 (2H, t, $J = 6.5$ Hz, H-7′), 3.56 (2H, t, $J = 6.5$ Hz, H-9′), 3.83 (3H, s, 3-OCH_3), 3.86 (3H, s, 3-OCH_3), 5.56 (1H, d, $J = 6.0$ Hz, H-7), 6.72 (2H, d, $J = 2.0$ Hz, H-2′,6′), 6.93 (1H, dd, $J = 2.0$, 8.5 Hz, H-6), 7.03 (1H, d, $J = 2.0$ Hz, H-2), 7.14 (1H, d, $J = 8.5$ Hz, H-5); ^{13}C-NMR (CD_3OD, 175 MHz): δ 32.9 (C-7′), 35.8 (C-8′), 55.6 (C-8), 56.7 (3-OCH_3), 56.8 (3′-OCH_3), 62.2 (C-9′), 62.5 (C-6″), 65.1 (C-9), 71.3 (C-4″), 74.4 (C-1″), 77.8 (C-5″), 78.2 (C-3″), 88.5 (C-7), 102.8 (C-1″), 112.2 (C-2), 114.3 (C-2′), 118.0 (C-6′), 118.2 (C-5), 119.4 (C-6), 129.6 (C-1′), 137.1 (C-5′), 138.4 (C-1), 145.2 (C-3′), 147.5 (C-4), 147.6 (C-4′), 150.9 (C-3).

Acid hydrolysis of compound 1

Compound **1** (1.5 mg) was heated in an ampoule with 1 mL of 2 N HCl (aq.) at 90 °C for 3 h. After cooling, the reaction mixture was extracted with $CHCl_3$. The $CHCl_3$ solvent was evaporated *in vacuo*, and identified as coumaroyl acid and caffeoyl acid by co-TLC [$CHCl_3$:MeOH (10:1, R_f 0.45 of coumaroyl acid, R_f 0.6 of caffeoyl acid)] with an authentic sample (Sigma, St. Louis, MO, USA). The H_2O layer yielded D-glucose which was identified with an authentic sample (Sigma, St. Louis, MO, USA) using silica gel co-TLC with a solvent system of $CHCl_3$:-MeOH:H_2O at a 2:1:0.1 ratio with an R_f value of 0.3 (Cho et al. 2014; Lee et al. 2014).

Test for cytotoxicity in vitro

Sulforhodamine B (SRB) bioassays were used to screen the compounds for cytotoxicity (Skehan et al. 1990). Cytotoxicity assays for each compound were performed in vitro against four cultured human tumor cell lines (National Cancer Institute, Bethesda, MD, USA) at the Korean Research Institute of Chemical Technology. The cell lines used were A549 (non-small cell lung adenocarcinoma), SK-OV-3 (ovarian cancer cells), A498 (kidney carcinoma cells), and HCT15 (colon cancer cells). Etoposide was used as a positive control.

Results and discussion

Compound **1** was obtained as a yellow gum. From the HR-FAB-MS *m/z* 487.1236 $[M-H]^-$ (calc. $C_{24}H_{23}O_{11}^-$, 487.1235) and ^{1}H- and ^{13}C-NMR spectral data, the molecular formula was deduced to be $C_{24}H_{24}O_{11}$. The ^{1}H-NMR spectrum showed the presence of seven aromatic protons signals at δ_H 7.45 (2H, d, $J = 8.0$ Hz), 7.04 (1H, d, $J = 1.5$ Hz), 6.96 (1H, dd, $J = 2.0$, 8.0 Hz), 6.80 (2H, d, $J = 8.0$ Hz) and 6.79 (1H, d, $J = 8.0$ Hz), four olefinic proton signals δ_H 7.68 (1H, d, $J = 16.0$ Hz), 7.62 (1H, d, $J = 16.0$ Hz), 6.37 (1H, d, $J = 16.0$ Hz) and 6.20 (1H, d, $J = 16.0$ Hz). The ^{13}C-NMR spectrum exhibited 18 carbon signals of each aglycone composed of the signals at δ_C 168.5 (C-9), 150.2 (C-4), 149.0 (C-7), 147.0 (C-3), 127.6 (C-1), 123.5 (C-6), 116.6 (C-5), 115.4 (C-2), and 113.9 (C-8) for the caffeoyl moiety, as well as other signals at δ_C 167.3 (C-9′), 161.5 (C-4′), 147.5 (C-7′), 131.4 (C-2′,6′), 127.2 (C-1′), 117.0 (C-3′,5′) and 114.9 (C-8′) for coumaroyl moiety. These spectral data suggested that compound **1** was a phenolic glycoside (Jiang et al. 2001). Overall NMR spectral data were similar to those of 2-*O*-(*E*)-caffeoyl-1-*O*-*p*-(*E*)-coumaroyl-*β*-D-glucopyranose (**2**) (Jiang et al. 2001), except for slight shift of H-8 (δ_H 6.36) and H-8′ (δ_H 6.21), and aromatic protons at δ_H 6.80 (H-3,5) and 6.79 (H-5′). The D-glucose position was established by a HMBC experiment, in which correlations were observed between the H-1″ (δ_H 5.82) of D-glucopyranose and C-9′ (δ_C 168.46) of caffeoyl moiety, and between H-2″ (δ_H 5.10) of D-glucopyranose and C-9 (δ_C 167.33) of coumaroyl moiety. The coupling constant ($J = 8.5$ Hz) of the anomeric proton of D-glucose suggested that it was the *β* form. An analysis of the ^{1}H–^{1}H COSY, HMQC and HMBC correlations led to the establishment of the structure for **1** (Fig. 2).

Acid hydrolysis of **1** yielded the caffeic acid, coumaric acid and D-glucopyranose. The caffeic acid and coumaric acid were identified by co-TLC confirmation with authentic samples [$CHCl_3$:MeOH (10:1, R_f 0.45 of coumaric acid, R_f 0.6 of caffeoyl acid)]. D-Glucopyranose was identified through co-TLC [$CHCl_3$:MeOH:H_2O (2:1:0.1, R_f 0.3 D-glucopyranoside)] and specific optical rotation $\{[\alpha]_D^{25} + 62.2$ (c = 0.05, H_2O)$\}$. Thus, structure of **1** was identified as 1-*O*-(*E*)-caffeoyl-2-*O*-*p*-(*E*)-coumaroyl-*β*-D-glucopyranose.

The structures of the known compounds were identified to be 2-*O*-(*E*)-caffeoyl-1-*O*-*p*-(*E*)-coumaroyl-*β*-D-glucopyranose (**2**) (Jiang et al. 2001), 1,2-di-*O*-(*E*)-caffeoyl-*β*-D-glucopyranose (**3**) (Jiang et al. 2001), 1-caffeoyl-6-tuliposide A (**4**) (Park et al. 2013), (−)-nortrachelogenin (**5**) (Woo et al. 2011), lariciresinol (**6**) (Fonseca et al. 1978), (−)-olivil (**7**) (Hans et al. 1983), (−)-berchemol (**8**) (Sakurai et al. 1989), (+)-1-hydroxypinoresinol (**9**), (+)-fraxiresinol (**10**) (Ando

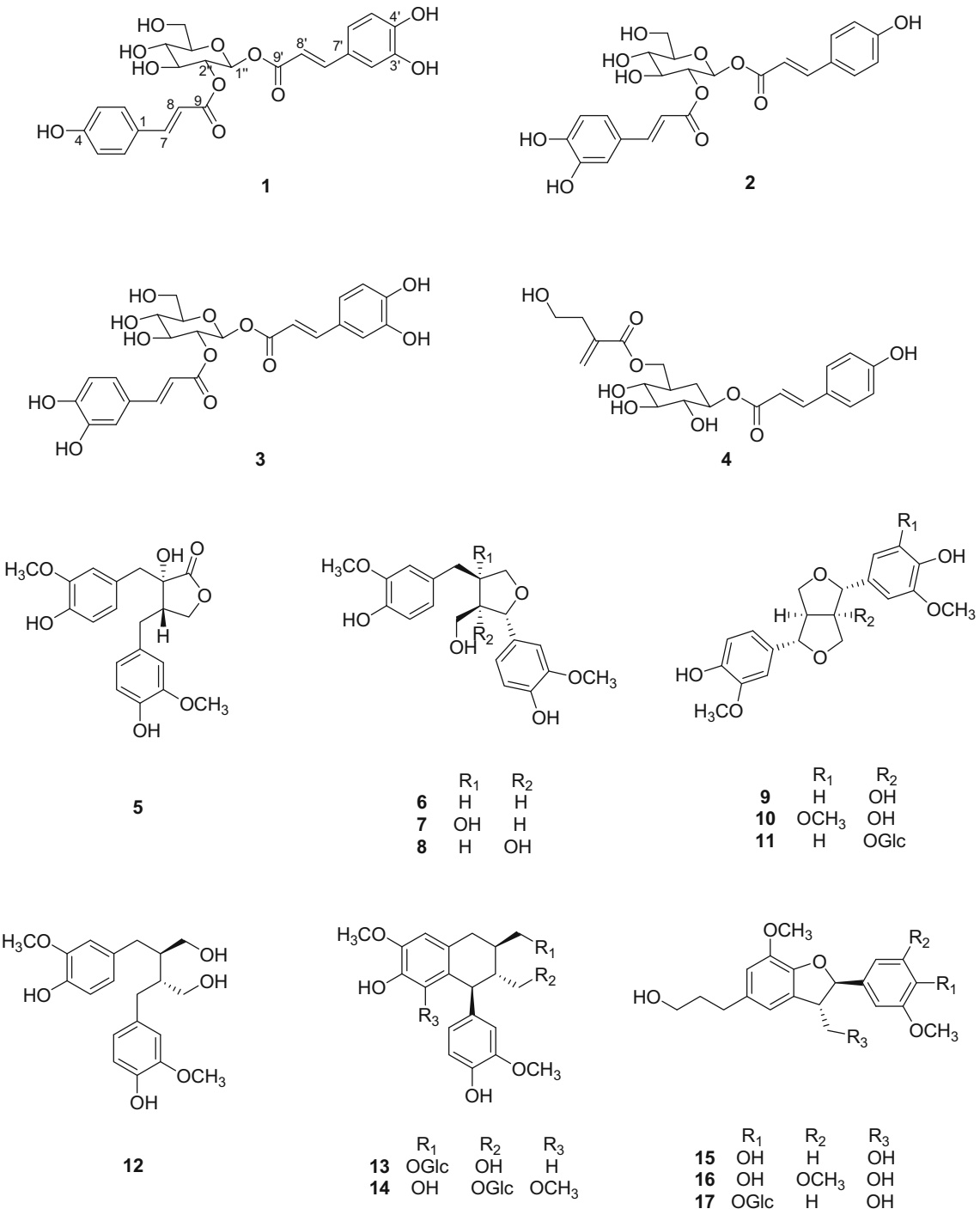

Fig. 1 Structures of compounds **1**–**17** from the twigs of *S. prunifolia* var. *simpliciflora*

et al. 2007), (+)-1-hydroxypinoresinol 1-*O*-*β*-D-glucopyranoside (**11**) (Yang et al. 2007), (−)-secoisolariciresinol (**12**) (Park et al. 2009), (+)-9-*O*-*β*-D-glucopyranosyl lyoniresinol (**13**) (Tanaka et al. 2004), (+)-9-*O*-*β*-D-glucopyranosyl isolariciresinol (**14**) (Otsuka et al. 2000), 7*R*,8*S*-dihydrodehydrodiconiferyl alcohol (**15**) (Fang et al. 1992), 7*R*,8*S*-5-methoxydihydrodehydroconiferyl alcohol (**16**) (Wang et al. 2010), and dihydrodehydrodiconiferyl alcohol 4-*O*-*β*-D-glucopyranoside (**17**) (Matsuda et al. 1996) by comparing the spectroscopic data with those in the literature.

The isolated compounds **1**–**17** were tested for cytotoxicity against four human tumor cells in vitro using the

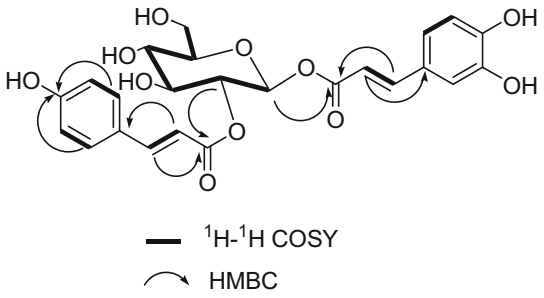

Fig. 2 Key 2D NMR (^{1}H–^{1}H COSY and HMBC) correlations of compound **1**

Table 2 Cytotoxic activities of compounds isolated from *S. prunifolia* var. *simpliciflora*

Compounds	IC_{50} (μM)[a]			
	A549	SK-OV-3	A498	HCT15
4	8.92	8.14	4.05	>10.0
5	>10.0	>10.0	9.27	>10.0
10	>10.0	>10.0	9.14	>10.0
17	>10.0	9.56	6.27	6.44
Etoposide[b]	1.65	1.73	0.72	1.80

[a] IC_{50} value of compounds against cancer cell lines, defined as the concentration (μM) that cause 50 % inhibition of cell growth in vitro

[b] Etoposide as positive control

SRB assay. Of them the compounds **4**, **5**, **10**, and **17** exhibited significant cytotoxicities against A549, SK-OV-3, A498, and HCT15 cell lines with the IC_{50} values (compound **4**: 8.92, 8.14, 4.05, and >10.0 μM, compound **5**: >10.0, >10.0, 9.27, and >10.0 μM, compound **10**: >10.0, >10.0, 9.14, and >10.0 μM, and compound **17**: >10.0, 9.56, 6.27, and 6.44 μM, respectively) and the other compounds showed little cytotoxicity (IC_{50} >10 μM). IC_{50} values for the cytotoxicity of the control compound, etoposide, against A549, SK-OV-3, A498, and HCT15 were 1.65, 1.73, 0.72, and 1.80 μM, respectively (see Table 2). Interestingly, although the structures of **1**–**3** and **4** are quite similar except of the presence of a 4-hydroxy-2-methylene-butyrate instead of a phenylpropanoid, they differed substantially with respect to their cytotoxic effects. The obtained data suggest that the presence of the 4-hydroxy-2-methylene-butyrate at C-6″ in glucose unit is important for the cytotoxic activity on A549, SK-OV-3, and A498 cell lines though more phenolic glycosides need to be tested to confirm this hypothesis.

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