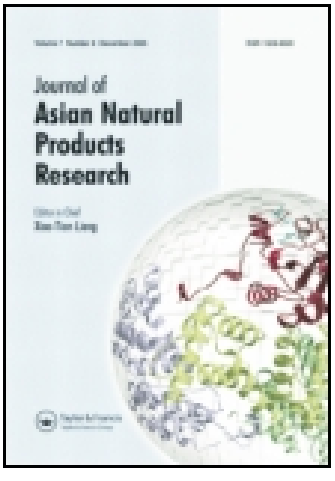

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Three new lignan glycosides from the fruits of Forsythia suspense

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Three new lignan glycosides from the fruits of *Forsythia suspense*

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Two new lignan glycosides forsythiayanoside A (**1**) and forsythiayanoside B (**2**), one new stereoisomer of lignan glycoside (+)-8-hydroxyepipinoresinol-4-*O*-β-D-glucopyranoside (**3**), together with seven known compounds (**4**–**10**), were isolated from the fruits of *Forsythia suspense* (Thunb.) Vahl. Compounds **4** and **10** were isolated from this species for the first time. The structures of **1**–**3** were elucidated on the basis of chemical and spectral analyses, including 1D and 2D NMR data and HR-ESI-MS. The absolute configurations were determined by the circular dichroism method. All isolates were tested for their cytotoxicities against five human cancer cell lines (A549, Colo-205, Hep-3B, HL60, and KB). In particular, compound **3** showed significant cytotoxicity with IC_{50} values of 9.48, 7.75, 0.59, 4.06, and 38.38 μM, respectively.

Keywords: *Forsythia suspense*; lignan glycosides; NMR; CD; cytotoxic activity

1. Introduction

Forsythia suspense (Thunb.) Vahl, a member of the family Oleaceae, is widely distributed in China including Henan, Shanxi, and Shandong Provinces. Its fruits are one of the most important original plants of Traditional Chinese Medicine which have been used for antibacterial, antiviral, anti-inflammation, diuretic, and antidotal purposes in oriental medicine [1]. The literature survey revealed that many kinds of compounds had been isolated from this plant, such as caffeoyl glycosides, phenylethanoid glycosides, lignans, terpenoids, alkaloids, and cyclohexylethanes [2,3]. In this paper, we describe the isolation and the structural elucidation of those new lignan glycosides, along with seven known compounds obtained from the 50% EtOH extract of *F. suspense*. Their structures (Figure 1) were established by extensive spectroscopic data analysis and comparison with those of literature values. Meanwhile, all compounds were evaluated for their cytotoxicities against A549, Colo-205, Hep-3B, HL60, and KB cancer cell lines. Compound **3** showed significant cytotoxicity with IC_{50} values of 9.48, 7.75, 0.59, 4.06, and 38.38 μM, respectively, as compared with positive controls (5-fluorouracil: 25.72, 39.30, 4.80, 11.52, and 44.35 μM, respectively; paclitaxel: 0.43, 7.80, 2.09, 0.01, and 1.00 μM, respectively).

2. Results and discussion

Compound **1** was obtained as a colorless solid (MeOH), with the molecular formula $C_{27}H_{34}O_{12}$ as determined by the HR-ESI-MS at *m/z* 573.1942 $[M + Na]^+$, indicating 11 degrees of unsaturation. The ^1H NMR spectrum of compound **1** (Table 1) displayed six aromatic proton signals at δ_H

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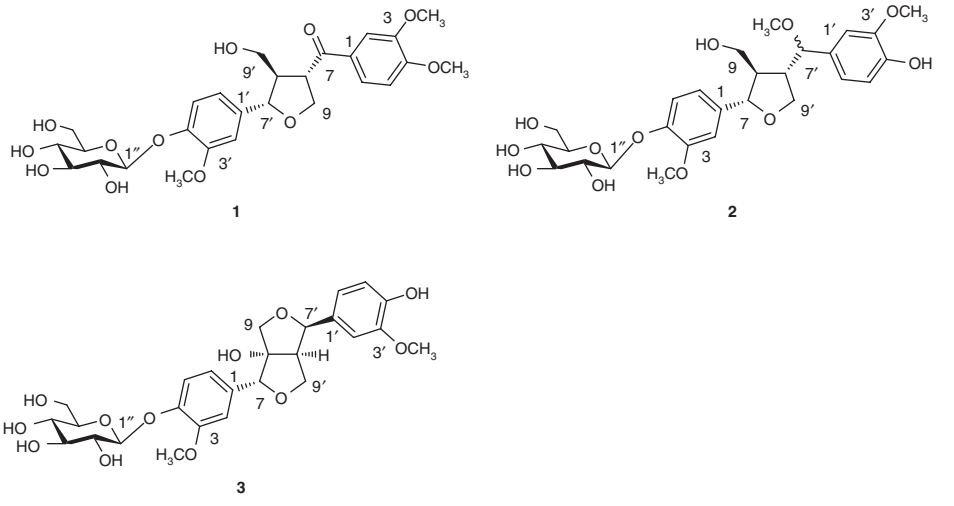

Figure 1. The structures of compounds **1**, **2**, and **3**.

7.47 (1H, d, $J = 1.7$ Hz, H-2), 7.04 (1H, d, $J = 8.6$ Hz, H-5), 7.64 (1H, dd, $J = 8.6$, 1.7 Hz, H-6), 6.94 (1H, d, $J = 1.5$ Hz, H-2′), 7.01 (1H, d, $J = 8.5$ Hz, H-5′), and 6.83 (1H, dd, $J = 8.5$, 1.5 Hz, H-6′), which were assigned to two 1,3,4-trisubstituted benzene rings. In addition, the ^{1}H NMR spectrum also revealed two oxymethylene protons at δ_H 3.97 (1H, dd, $J = 8.4$, 5.2 Hz, H-9β), 4.09 (1H, t, $J = 8.4$ Hz, H-9α), and 3.44–3.50 (2H, m, H-9′), three methine protons at δ_H 4.14–4.17 (1H, m, H-8), 4.56 (1H, d, $J = 8.0$ Hz, H-7′), and 2.49–2.52 (1H, m, H-8′), three methoxyl groups at δ_H 3.72 (3H, s), 3.78 (3H, s), and 3.80 (3H, s), and a glucopyranosyl anomeric proton at δ_H 4.85 (1H, d, $J = 7.2$ Hz, H-1″). The ^{13}C NMR spectrum of **1** (Table 1) showed 27 carbon signals, apart from a glucose unit (δ_C 100.5, 73.7, 77.4, 70.2, 77.3, and 61.1) and three methoxy carbons at δ_C 56.0, 56.1, and 56.2, the remaining 18 carbons could be assigned to a tetrahydrofuranoid lignan

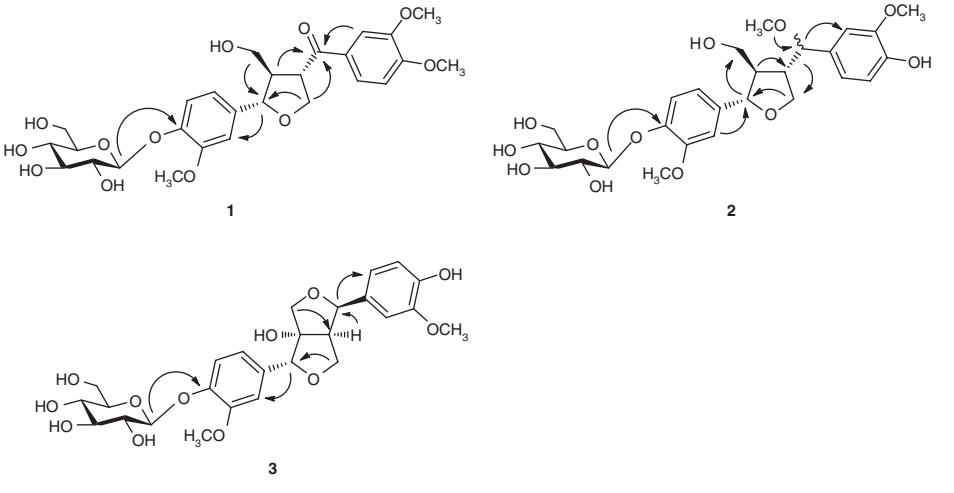

Figure 2. Key HMBC (→) correlations of **1**, **2**, and **3**.

Table 1. ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) spectral data of compounds **1** and **2** in DMSO-d_6.

	1		**2**	
No.	δ_H	δ_C	δ_H	δ_C
1		129.7		136.5
2	7.47 (d, 1.7)	111.1	6.92 (d, 1.6)	111.1
3		149.1		149.25
4		153.7		146.19
5	7.04 (d, 8.6)	111.4	7.04 (d, 8.4)	115.6
6	7.64 (dd, 8.6, 1.7)	123.6	6.82 (dd, 8.4, 1.6)	118.7
7		198.3	4.56 (d, 7.1)	83.0
8	4.14–4.17 (m)	49.3	1.62–1.65 (m)	52.7
9α	4.09 (t, 8.4)	70.5	2.93–2.95 (m)	60.9
9β	3.97 (dd, 8.4, 5.2)		3.06–3.08 (m)	
1′		135.9		131.2
2′	6.94 (d, 1.5)	111.3	6.75 (d, 1.7)	111.5
3′		149.2		148.0
4′		146.4		146.6
5′	7.01 (d, 8.5)	115.5	6.72 (d, 8.0)	115.6
6′	6.83 (dd, 8.5, 1.5)	119.1	6.62 (dd, 8.0, 1.7)	120.8
7′	4.56 (d, 8.0)	83.2	3.91 (d, 9.2)	85.7
8′	2.49-2.52 (m)	53.6	2.39–2.43 (m)	48.5
9′α	3.46–3.48 (m)	60.3	3.82 (dd, 8.8, 7.3)	70.8
9′β			4.05 (dd, 8.8, 4.4)	
1″	4.85 (d, 7.2)	100.5	4.88 (d, 7.3)	100.6
2″	3.21 (m)	73.7	3.25 (m)	73.7
3″	3.25 (m)	77.4	3.26 (m)	77.4
4″	3.12 (m)	70.2	3.16 (m)	70.1
5″	3.22 (m)	77.3	3.29 (m)	77.5
6″	3.62 (m)	61.1	3.46 (m)	61.2
	3.41 (m)		3.67 (m)	
$-OCH_3$	3.72	56.1	3.77	56.2
$-OCH_3$	3.78	56.0	3.73	56.0
$-OCH_3$	3.80	56.2	3.06	56.2

Note: Coupling constants (J) in Hz are given in parentheses; chemical shift values are expressed in ppm.

skeleton [4]. The fact that the carbonyl group is connected to a part of the phenyl group was indicated by a carbonyl carbon signal at δ_C 198.3 and the conjugated property absorption peak shown in the IR spectrum at 1665 cm^{-1}. All of the above spectroscopic data were similar to those of vladinol D [5] with the exception of a methoxy and a glucose unit. Careful analysis of the correlations in the HSQC and HMBC spectra led to the establishment of the planar structure of compound **1** (Figure 1). The hydroxymethyl was linked to C-8′, which was deduced from the long-rang correlations in the HMBC spectrum (Figure 2) from the protons at δ_H 3.46–3.48 (2H, m) to C-8 (δ_C 49.3), C-8′ (δ_C 53.6), and C-7′ (δ_C 83.2). The correlations from H-7′ at δ_H 4.56 (1H, d, J = 8.0 Hz) to C-2′ (δ_C 111.3), C-6′ (δ_C 119.1), and C-1′ (δ_C 135.9) suggested that C-7′ of tetrahydrofuran was connected with C-1′ of the benzene ring. The correlations from H-9 at δ_H 3.97 (1H, dd, J = 8.4, 5.2 Hz, H-9β), 4.09 (1H, t, J = 8.4 Hz, H-9α) to C-7 (δ_C 198.3) indicated that the carbonyl carbon linked to C-8 of the tetrahydrofuran ring.

The relative configuration of **1** was determined by the analysis of ^{1}H NMR data and ROSEY correlations (Figure 3). The chemical shift of H-7′ at δ_H 4.56 (1H, d, J = 8.0 Hz) suggested that H-7′ and

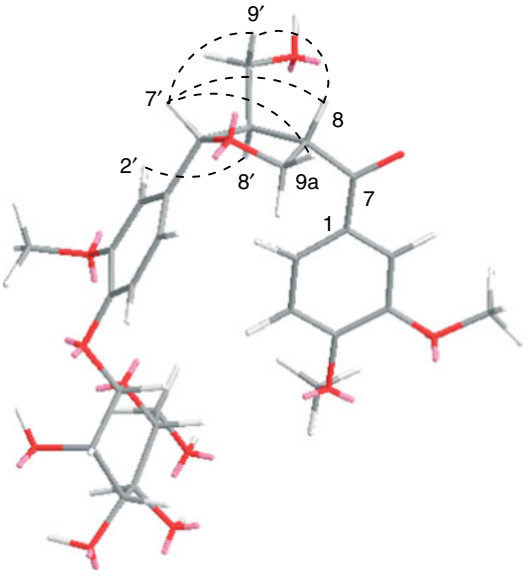

Figure 3. Key ROSEY correlations for **1**.

H-8′ were *trans*-oriented (for a *cis*-orientation of substituents at C-7′ and C-8′, the signal of H-7′ was at around 5.5 ppm) [6,7], and this was supported by a ROSEY correlation between H-2′ and H-8′. Based on ROSEY correlations of H-8/H-7′, H-8/H-9′, and H-7′/H-9′, H-8 and H-7′ were placed on the same side of the tetrahydrofuran ring (*trans*-orientation of H-8 and H-8′). The absolute configuration of **1** was established by the analysis of its circular dichroism (CD) spectrum. A negative Cotton effect at 321 nm and a positive Cotton effect at 294 nm were shown in the CD spectrum of **1**, indicating the (8*S*,7′*S*,8′*R*)-configuration [4,8] in **1**. The anomeric proton at δ_H 4.85 (1H, d, $J = 7.2$ Hz, H-1″) indicated the β-configuration of glucose unit. The component sugar was determined to be D-glucopyranose based on comparison of the acid hydrolyzate of **1** with authentic D-glucopyranose by thin layer chromatography (TLC) and gas chromatography (GC) analyses [9]. In the HMBC spectrum (Figure 2), the anomeric proton at δ_H 4.85 showed a long-range correlation with the aromatic carbon signal at δ_C 146.4 which was assigned to C-4′. Thus, the structure of **1** was determined as (8*S*,7′*S*,8′*R*)-4′,9′-dihydroxy-3,4,3′-trimethoxy-7′,9-epoxylignan-7-oxo-4′-*O*-β-D-glucopyranoside, named forsythiayanoside A.

Compound **2** was obtained as a sticky oil (MeOH) and possessed a molecular formula $C_{27}H_{36}O_{12}$, as revealed from its HR-ESI-MS analysis (*m/z* 575.2098 $[M + Na]^+$), indicating 10 degrees of unsaturation. The ^{1}H NMR spectrum (Table 1) showed proton signals of two ABX systems of aromatic protons [δ_H 6.92 (1H, d, $J = 1.6$ Hz, H-2), 7.04 (1H, d, $J = 8.4$ Hz, H-5), 6.82 (1H, dd, $J = 8.4$, 1.6 Hz, H-6), 6.75 (1H, d, $J = 1.7$ Hz, H-2′), 6.72 (1H, d, $J = 8.0$ Hz, H-5′), and 6.62 (1H, dd, $J = 8.0$, 1.7 Hz, H-6′)], two aromatic CH_3O groups [δ_H 3.73 (3H, s) and 3.77 (3H, s)], indicating the presence of two trisubstituted benzene rings. Six oxygenated aliphatic protons [δ_H 4.56 (1H, d, $J = 7.1$ Hz, H-7), 2.93–2.95 (1H, m, H-9α), 3.06–3.08 (1H, m, H-9β), 3.91 (1H, d, $J = 9.2$ Hz, H-7′), 3.82 (1H, dd, $J = 8.8$, 7.3 Hz, H-9′α), and 4.05 (1H, dd, $J = 8.8$, 4.4 Hz, H-9′β)], together with two

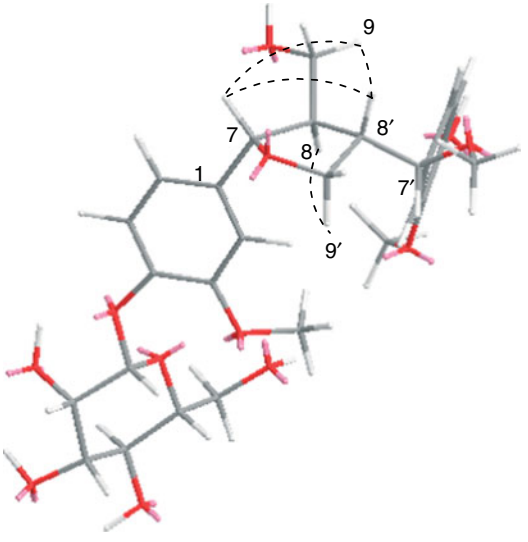

Figure 4. Key NOESY correlations for **2**.

aliphatic protons [δ_H 1.62–1.65 (1H, m, H-8) and 2.39–2.43 (1H, m, H-8′)] were evidences for the presence of a tetrahydrofuranoid lignan skeleton which were similar with compound **1**. In the ^{13}C NMR spectrum (Table 1), apart from the 12 aromatic, a glucose unit, and three methoxyl signals, there were six sp^3 C-atoms, including two oxymethines [δ_C 83.0 and 85.7] and two oxymethylenes [δ_C 60.9 and 70.8]. The remaining CH_3O group was located at C-7′, based on the HMBC correlation (Figure 2) between CH_3O group protons at δ_H 3.06 (3H, s) and C-7′ (δ_C 85.7). The correlations from H-7 at δ_H 4.56 (1H, d, $J = 7.1$ Hz) to C-2 (δ_C 111.1), C-6 (δ_C 118.7), and C-1 (δ_C 136.5) suggested that C-7 of tetrahydrofuran was connected with C-1 of the benzene ring. The correlations from H-9′ at δ_H 3.82 (1H, dd, $J = 8.8$, 7.3 Hz, H-9′α), 4.05 (1H, dd, $J = 8.8$, 4.4 Hz, H-9′β) to C-7′ (δ_C 85.7), from H-7′ at δ_H 3.91 (1H, d, $J = 9.2$ Hz) to C-8′ (δ_C 48.5), C-9′ (δ_C 70.8), C-2′ (δ_C 115.5), and C-6′ (δ_C 120.8) indicated that C-7′ linked to C-2′ of the benzene and C-8′ of the tetrahydrofuran ring.

The relative configuration in the tetrahydrofuran ring was determined by the H-7 chemical shift and NOESY experiment. According to a signal of H-7 at δ_H 4.56, the orientation of H-7/H-8 of compound **2** was determined to be *trans* [10,11]. All *trans*-orientation of H-7/H-8/H-8′ was determined by the cross-peak of H-7/H-9, H-7/H-8′, and H-9/H-8′ in the NOESY spectrum (Figure 4). The CD spectrum of **2** showed two positive peaks at 240 and 283 nm similar to those of analogous compound tinosposide B [12], indicating that C-7, C-8, and C-8′ in **2** have *S*, *R*, and *S*-configurations, respectively. However, there were no definitive conclusions on the relationships between the absolute configuration of C-7′ and CD in this series of tetrahydrofuranoid lignans [11,13]. Acid hydrolysis of **2** afforded D-glucose by TLC and GC analyses [9]. The β-configuration anomeric proton of glucose at δ_H 4.88 (1H, d, $J = 7.3$ Hz) showed HMBC correlations with the aromatic C-4 at δ_C 146.1. Accordingly, compound **2** was established as (7*S*,8*R*,8′*S*)-4,9,4′-trihydroxy-3,3′,7′-trimethoxy-7,9′-epoxylignan-4-*O*-β-D-glucopyranoside, named forsythiayanoside B.

Compound **3** was obtained as a yellow amorphous powder. Its molecular formula

Table 2. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectral data of compound **3** in DMSO-d_6.

	3	
No.	δ_H	δ_C
1		131.4
2	7.00 (d, 1.8)	112.9
3		147.8
4		146.4
5	6.75 (d, 8.2)	115.6
6	6.89 (dd, 8.2, 1.8)	120.3
7	4.29 (s)	88.8
8		90.6
9	3.50 (d, 9.0)	75.9
	4.16 (d, 9.0)	
1′		129.9
2′	6.86 (d, 1.0)	110.2
3′		148.7
4′		145.8
5′	7.03 (d, 8.6)	115.0
6′	6.73 (dd, 8.6, 1.0)	118.3
7′	5.08 (d, 5.2)	81.1
8′	2.01–3.04 (m)	57.6
9′	3.03–3.05 (m)	67.9
	3.82–3.85 (m)	
1″	4.87 (d, 7.3)	100.7
2″	3.17 (m)	77.4
3″	3.26 (m)	73.7
4″	3.17 (m)	70.2
5″	3.28 (m)	77.3
6″	3.47 (m)	61.1
	3.68 (m)	
$-OCH_3$	3.75	56.0
$-OCH_3$	3.76	56.0

Note: Coupling constants (*J*) in Hz are given in parentheses; chemical shift values are expressed in ppm.

was established as $C_{26}H_{32}O_{12}$ by HR-ESI-MS analysis (*m/z* 559.1791 $[M + Na]^+$). The ^{1}H and ^{13}C NMR signals (Table 2) were assigned by the DEPT, HSQC, and HMBC analyses, indicating the presence of one glucosyl and two guaiacyl groups. Two characteristic downfield CH signals at δ_H 5.08 (1H, d, $J = 5.2$ Hz) and 4.29 (1H, s) and six characteristic aliphatic carbon signals at δ_C 81.1 (CH), 57.6 (CH), 67.9 (CH_2), 88.8 (CH), 90.6 (CH), and 75.9 (CH_2) suggested that compound **3** was a 8-hydroxyfuranoid lignan. In the HMBC spectrum (Figure 2), the cross-peaks of H-7 with C-9, C-8′, C-9′, and C-1 and H-7′ with C-9′, C-8, C-9, and C-1′ supported 7.O.9′ and 7′.O.9 oxygen bridges in the ditetrahydrofuran ring moiety. The relative and absolute configurations of the aglycone of compound **3** were determined by comparison of its NMR data and CD spectrum with those of known analogous compounds [14,15]. The ^{13}C NMR spectrum was important in establishing the orientation of the aryl groups. For an equatorial guaiacyl group, C-1′ appeared in downfield at around δ_C 131.3 in DMSO-d_6, while for an axial guaiacyl group, C-1′ appeared in upfield at around δ_C 129.6 [3,14]. Thus, considering the solvent effect on the aryl group, the value of C-1′ at δ_C 129.9 in compound **3** accounted for the appearance of axial guaiacyl group. These spectroscopic data suggested that **3** and known compound (+)-1-hydroxypinoresinol-4′-*O*-β-D-glucopyranoside [14] are epimers at C-7′. Thus, the relative configuration could be proposed as 7,8-*cis*-8,8′-*cis*-7′,8′-*trans*. The CD spectrum of compound **3** was identical with that of (+)-1-hydroxy-6-epipinoresinol which has the absolute configuration 7*R*, 8*S*, 7′*R*, and 8′*R* [14–16]. Therefore, the aglycon of compound **3** was (+)-1-hydroxy-6-epipinoresinol. Acid hydrolysis of **3** afforded D-glucose by TLC and GC analyses [9]. The β-configuration anomeric proton of glucose at δ_H 4.87 (1H, d, $J = 7.3$ Hz) showed HMBC correlation with the aromatic C-atom C-4 (δ_C 146.4). Accordingly, compound **3** was established as (+)-8-hydroxyepipinoresinol-4-*O*-β-D-glucopyranoside.

The known compounds were readily identified as glochidioboside (**4**) [17], (+)-pinoresinol-4-*O*-β-D-glucopyranoside (**5**) [18], (+)-epipinoresinol-4-*O*-β-D-glucopyranoside (**6**) [18], (+)-epipinoresinol-4′-*O*-β-D-glucopyranoside (**7**) [18], (+)-phyllrin (**8**) [18], (−)-matairesinoside (**9**) [18], and (+)-isolariciresino-9-*O*-β-D-glucopyranoside (**10**) [19] by comparing

NMR spectral data with those reported in the literature.

All isolated compounds were evaluated for their cytotoxic activities against lung cancer (A549), colon cancer (Colo-205), hepatoma (Hep-3B), leucocythemia (HL-60), and cervical cancer (KB) cell lines by the MTT method [20], using 5-fluorouracil and paclitaxel as positive controls. Compound **3** showed cytotoxicity against the five human cancer cell lines with IC_{50} values of 9.48, 7.75, 0.59, 4.06, and 38.38 μM, respectively, and was more cytotoxic than 5-fluorouracil (25.72, 39.30, 4.80, 11.52, and 44.35 μM, respectively). Meanwhile, compound **3** displayed similar cytotoxicity to paclitaxel ($IC_{50} = 7.80$ μM) against Colo-205 cells and more cytotoxic than paclitaxel ($IC_{50} = 2.09$ μM) against Hep-3B cells. Other isolates (**1**, **2**, **4**–**10**) are inactive.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on a JASCOP-1020 Polarimeter (Jasco Co., Tokyo, Japan). UV spectra were carried out on a Shimadzu UV-1700 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). IR spectra were obtained on a Shimadzu ftir-8400s spectrophotometer (Shimadzu Corporation). NMR spectra were recorded on Bruker ARX-400 and ARX-600 instruments (Bruker Co., Billerica, MA, USA). HR-ESI-TOF-MS experiments were carried out on a Micro TOF spectrometer (Bruker Co., Karlsruhe, Germany). CD spectra were measured by Bio-Logic (MOS 450) spectrophotometer. High-performance liquid chromatography (HPLC) preparation was carried out on a Hitachi preparative HPLC system (Hitachi Ltd, Tokyo, Japan) equipped with Refractive Index Detector (L-2490) and prep-ODS (10 mm × 250 mm). GC was done on an Agilent 7890A Gas Chromatograph (Agilent Technologies, Inc., Santa Clara, CA, USA) equipped with HP-5 capillary column (30 m × 320 mm × 0.25 μm). Sephadex LH-20 (20–100 μm, Pharmacia Fine Chemical Co. Ltd, Piscataway, NJ, USA), silica gel (200–300 mesh, Qingdao Marine Chemistry Ltd, Qingdao, China), macroporous resin (D101, Cangzhou Bon Adsorber Technology Co., Ltd, Cangzhou, China), and Cosmosil octadecyl silane (ODS; 40–80 μm, Nacalai Tosoh, Inc., Uetikon, Switzerland) were used for column chromatography (CC). TLC was conducted on silica gel GF254 (Qingdao Marine Chemistry Ltd).

3.2 Plant material

The fruits (8.3 kg) of *F. suspense* were collected from Henan Province of China in June 2009, and authenticated by Prof. Jin-Cai Lu, Department of Pharmacognosy, Shenyang Pharmaceutical University. The voucher specimen (No. 20091011) is kept in the Nature Products Laboratory of Shenyang Pharmaceutical University, Shenyang, China.

3.3 Extraction and isolation

The fruits of *F. suspense* (8.3 kg) were crushed to pieces and refluxed with 50% EtOH for three times. The extract was evaporated under reduced pressure to afford a residue (350 g). The residue was suspended in H_2O and then successively extracted with $CHCl_3$, EtOAc, and *n*-butanol. The *n*-butanol extract was evaporated *in vacuo* to give a residue (100 g), which was chromatographed over D101 macroporous resin and a gradient of aqueous EtOH (water, 30%, 60%, and 95%, v/v) to yield four major fractions (A–D). Fraction C (55.0 g) was subjected to silica gel CC (12 × 60 cm) with a gradient mixture of CH_2Cl_2–MeOH (100:0–50:50) to afford five fractions (1–5). Fraction 2 (22.4 g) was further purified over an ODS column chromatography (4 × 45 cm) using MeOH and H_2O as the mobile phases with a gradient from

20% to 60% to afford fractions $F_{2\text{-}1}$–$F_{2\text{-}8}$ based on HPLC analysis. $F_{2\text{-}2}$ (6.1 g) was subjected to another silica gel CC (2 × 30 cm) and eluted with CH_2Cl_2:MeOH: water (8:2:0.25) to afford fractions $F_{2\text{-}2\text{-}1}$–$F_{2\text{-}2\text{-}5}$ based on TLC analysis. $F_{2\text{-}2\text{-}2}$ (500 mg) was subjected to preparative HPLC eluted with CH_3OH–H_2O (15:85) at 3 ml/min (t_R 15 and 17 min) to yield compounds **4** (15 mg) and **9** (36 mg). $F_{2\text{-}2\text{-}3}$ (200 mg) was subjected to semi-preparative HPLC eluted with CH_3CN–H_2O (10:90) at 3.5 ml/min (t_R 15 min) to yield **2** (10 mg), 3.5 ml/min (t_R 20 min) to yield **3** (9 mg), and 3.5 ml/min (t_R 23 min) to yield **7** (15 mg). $F_{2\text{-}3}$ (2.0 g) was subjected to semi-preparative HPLC eluted with CH_3CN–H_2O (20:80) at 3.5 ml/min (t_R 20 min) to yield **1** (20 mg). Fraction 3 (18.0 g) was further purified over an ODS column chromatography (4 × 45 cm) using MeOH and H_2O as the mobile phase with a gradient from 10% to 60% to afford fractions $F_{3\text{-}1}$–$F_{3\text{-}4}$ based on HPLC analysis. $F_{3\text{-}2}$ (10.0 g) was subjected to semi-preparative HPLC eluted with CH_3CN–H_2O (25:75) at 3.5 ml/min (t_R 16 min) to yield **5** (30 mg), 3.5 ml/min (t_R 23 min) to yield **6** (20 mg), and 3.5 ml/min (t_R 40 min) to yield **10** (10 mg). Fraction 4 (2.0 g) was subjected to semi-preparative HPLC eluted with CH_3CN–H_2O (25:75) at 3.5 ml/min (t_R 18 min) to yield **8** (100 mg).

3.3.1 *Forsythiayanoside A (**1**)*

Colorless solid (MeOH); $[\alpha]_D^{25}$ −12.4 ($c = 0.10$, MeOH). IR (KBr) ν_{max} (cm^{-1}): 3405, 2919, 2850, 1665, 1594, 1514, 1384, 1263, and 1046; UV (MeOH) λ_{max}: 206.6, 227.6, 275.6, and 302.2 nm; CD (MeOH): $\Delta\varepsilon_{235.2\,nm}$ −10.17, $\Delta\varepsilon_{276.0\,nm}$ −5.83, $\Delta\varepsilon_{294.0\,nm}$ +3.48, $\Delta\varepsilon_{321.0\,nm}$ −1.25; for ^1H and ^{13}C NMR spectral data (DMSO-d_6), see Table 1; HR-ESI-MS: m/z 573.1942 $[M + Na]^+$ (calcd for $C_{27}H_{34}O_{12}Na$, 573.1942).

3.3.2 *Forsythiayanoside B (**2**)*

Sticky oil (MeOH); $[\alpha]_D^{25}$ −10.3 ($c = 0.05$, MeOH). IR (KBr) ν_{max} (cm^{-1}): 3440, 2918, 2850, 1631, 1466, 1384, 1128, and 1075; UV (MeOH) λ_{max}: 207.0, 227.4, and 278.6 nm; CD (MeOH): $\Delta\varepsilon_{205.1\,nm}$ −4.80, $\Delta\varepsilon_{240.0\,nm}$ +4.69, $\Delta\varepsilon_{283.4\,nm}$ +8.73; for ^1H and ^{13}C NMR spectral data (DMSO-d_6), see Table 1; HR-ESI-MS: m/z 575.2098 $[M + Na]^+$ (calcd for $C_{27}H_{36}O_{12}Na$, 575.2099).

3.3.3 *(+)-8-Hydroxyepipinoresinol-4-O-β-D-glucopyranoside (**3**)*

Yellow amorphous powder (MeOH); $[\alpha]_D^{25}$ −8.1 ($c = 0.05$, MeOH). IR (KBr) ν_{max} (cm^{-1}): 3441, 2919, 2850, 1632, 1384, and 1129; UV (MeOH) λ_{max}: 207.2, 227.0, and 278.2 nm; CD (MeOH): $\Delta\varepsilon_{218.4\,nm}$ −3.72, $\Delta\varepsilon_{236.0\,nm}$ +4.23, $\Delta\varepsilon_{278.2\,nm}$ −7.21; for ^1H and ^{13}C NMR spectral data (DMSO-d_6), see Table 2; HR-ESI-MS: m/z 559.1791 $[M + Na]^+$ (calcd for $C_{26}H_{32}O_{12}Na$, 559.1786).

3.4 *Acid hydrolysis of compounds 1–3*

Each compound (3.0 mg) was hydrolyzed with 2 M HCl (5.0 ml), heated for 4 h at 95°C and extracted with $CHCl_3$ (3 × 5.0 ml). Then, the aqueous layer was concentrated *in vacuo* to appropriate volume, and the solution was examined by TLC (EtOAc–BuOH–H_2O–HOAc, 4:4:1:1), and compared with the authentic sample, glucose was detected. Each remaining aqueous layer was concentrated to dryness to give a residue, which was dissolved in pyridine (1.0 ml), and then L-cysteine methyl ester hydrochloride (2.0 mg) was added to the solution. The mixture was heated at 60°C for 2 h, and 0.5 ml of *N*-tremethylsilyimidazole was added, followed by heating at 60°C for 2 h. The reaction product was subjected to GC analysis on Agilent 7890A (HP-5, 30 m × 320 mm, 0.25 μm) with flame ionization detector detection. Column temperature:

120–280°C with the rate of 8°C/min, and the carrier gas was N_2 (1.4 ml/min); injection temperature: 250°C; injection volume: 1 μl. The absolute configuration of the monosaccharides was confirmed to be D-Glu by comparison of the retention time of its Me_3Si ethers with standard sample [t_R (D-glucose) = 25.873 min].

3.5 Cytotoxicity assay

Human malignant leucocythemia cell lines HL-60, hepatoma cell lines Hep-3B, colon cancer cell lines Colo-205, cervical cancer lines KB, and lung cancer cell lines A549 were obtained from the National Center for Medical Culture Collection (Shanghai, China). They were routinely cultured in Roswell Park Memorial Institute (RPMI) 1640 or Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum and maintained at 37°C in a humidified incubator with 5% CO_2. The *in vitro* cell viability effects of compounds were determined by MTT assay [20]. The cells (1×10^5 cells/ml) were seeded into 96-well culture plates. After overnight incubation, the cells were treated with various concentrations of agents for 72 h. Then, 10 μl of MTT solution (2.5 mg/ml in phosphate-buffered saline) was added to each well, and the plates were incubated for an additional 4 h at 37°C. After centrifugation (200*g*, 10 min), the medium with MTT was aspirated, followed by the addition of 100 μl of DMSO. The optical density of each well was measured at 492 nm with a Biotek Synergy TM HT Reader.

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