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Water-soluble constituents from the bark of *Elaeagnus pungens* and their cytotoxic activities

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ORIGINAL ARTICLE

Water-soluble constituents from the bark of *Elaeagnus pungens* and their cytotoxic activities

Yan-Bing Wu[a], Yong Gu[b] and Ming-An Ouyang[b]*

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Three new water-soluble compounds, pungens A–C, along with four known compounds including two phenol glycosides, one *secoiso*-flavanol and one phenol ether, have been isolated from the bark of *Elaeagnus pungens.* Among them, pungen C (**7**) (200 μg/ml) was tested in SGC-7901 and BEL-7404 tumor cell lines, and showed moderate cytotoxic activity. The structures of the new compounds were elucidated on the basis of spectroscopic data and chemical evidence.

Keywords: *Elaeagnus pungens*; phenols and phenol glycosides; isopropyl alcohol glycoside; cytotoxicity; SGC-7901 cell line; BEL-7404 cell line

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1. Introduction

Elaeagnus pungens Thunb is a shrub and belongs to the Elaeagnaceae family, which is primarily distributed in South Chinese provinces such as Fujian, Guangdong, Guangxi, and Yunnan. The fruits, roots, and leaves of *E. pungens* have long been used as a herbal remedy for the treatment of variety of diseases, such as cough, asthma, hemoptysis, carbuncle, tumor, and other ailments in the Chinese traditional medicine [1].

Earlier papers have reported the isolation of tannin, triterpene, alkaloid, phenol, and flavonoid glycoside [2,3]. From the leaves of *E. pungens*, Zhao *et al.* [4,5] reported the isolation and identification of 4-hydroxybenzoic acid, 3,3′-dimethoxyquercetin, caffeic acid methyl ester, methyl-3,4-dihydroxybenzoate, spingic acid, 4-methoxybenzoic acid, 3-methyl-kaempferol, kaempferol-3-*O*-β-D-glucoside, and daucosterol, and screening of their cytotoxic activities. As part of our continuing study, this paper deals with the isolation and structural elucidation of three new compounds, pungens A (**1**), B (**4**), and C (**7**), along with four known compounds, rhyncoside C (**2**), rhyncoside B (**3**) [6], hovetrichoside B (**5**) [7], and compound (**6**) (see Figure 1), and their cytotoxicity activities against SGC-7901 and BEL-7404 cell lines. It was found that compound **7** had moderate activity against both cells at the measured concentration.

2. Results and discussion

Pungen A (**1**) was obtained as a colorless powder and had a molecular formula of $C_{19}H_{28}O_{13}$, based on the negative HR-FAB-MS spectrum. The IR spectrum showed the presence of a hydroxyl group ($3350\,cm^{-1}$) and an aromatic ring ($1601\,cm^{-1}$). The ^{13}C NMR spectral data of **1** gave 19 carbon signals, 8 of which were assigned to the aglycone part, while

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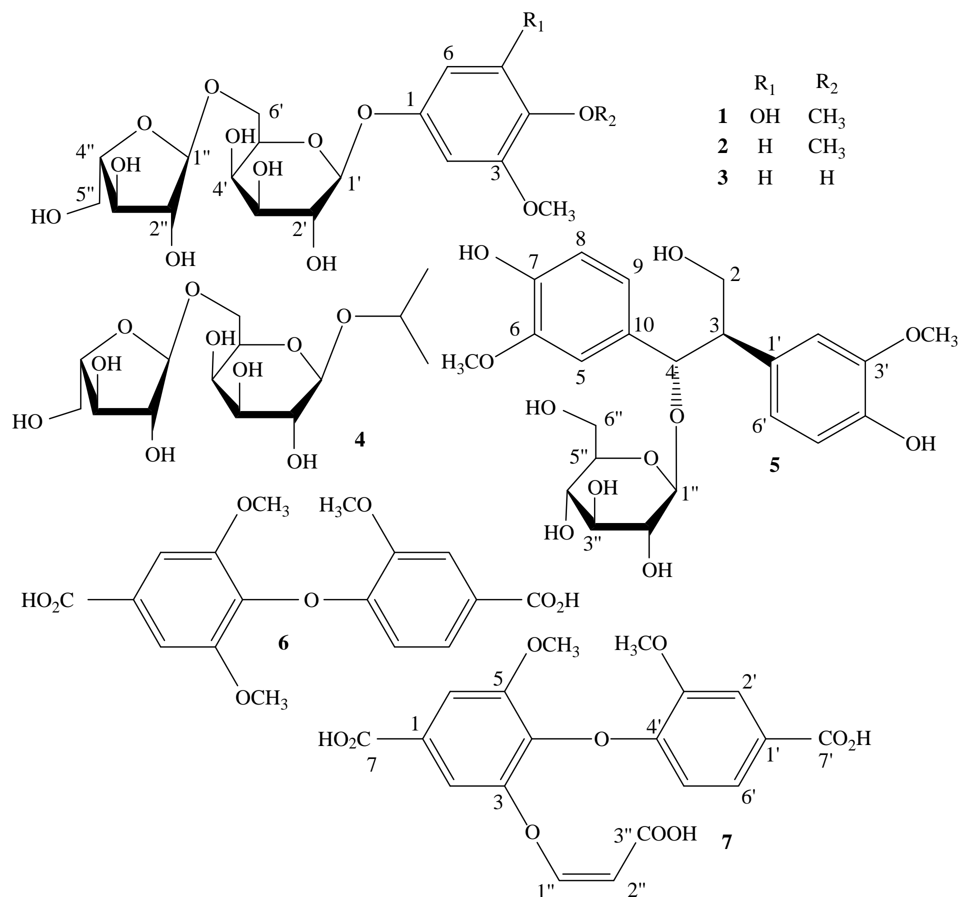

Figure 1. Structures of compounds **1**–**7**.

11 carbons came from the carbohydrate moiety. The two methoxy signals at δ_C 56.1 and δ_H 3.75 (s, 3H) and δ_C 60.7 and δ_H 3.82 (s, 3H) and two methine signals at δ_C 99.3 and δ_H 7.02 (d, $J = 2.5$ Hz, 1H) and δ_C 94.4 and δ_H 6.63 (d, $J = 2.5$ Hz, 1H) are observed in the ^{13}C and ^{1}H NMR spectra of **1**. Combining the proton signals in the ^{1}H NMR spectrum, the aglycone of **1** was indicated as a tetra-substituted aromatic ring. According to the HMBC and NOESY spectra of **1**, the key correlations were observed between protons and carbons or between protons and protons: H_{OMe}-4 (δ 3.82)/C-4 (δ 133.3) and H_{OMe}-3 (δ 3.75)/C-3 (δ 152.6); H_{OMe}-4 (δ 3.82)/H_{OMe}-3 (δ 3.75) and H-2 (δ 6.63)/H_{OMe}-3 (δ 3.75). The correlations revealed two methoxy groups at positions C-3 and C-4, respectively. On the other hand, the carbon signals C-3, 5 and the carbon–proton signals C-2, 6 (see Table 1) were neither symmetrical nor overlapping; the evidence indicated that the hydroxyl group at position C-5 was bare or not methylated. Thus, the structure of the genin of **1** was established to be 1,5-dihydroxy-3,4-dimethoxy-benzene.

The ^{1}H and ^{13}C NMR spectra of **1** also exhibited two anomeric signals at δ_C 103.4 and δ_H 5.47 (d, $J = 7.4$ Hz), δ_C 110.1 and δ_H 5.64 (br s), respectively. Acid hydrolysis of **1** gave two monosaccharides, arabinose and galactose (1:1), which were analyzed by GC as their derivatives. The absolute configurations of the sugars

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Table 1. ^{13}C and ^{1}H NMR spectral data of compounds **1**–**4** in pyridine-d_6.

No.	**1**	C	H	**2**			**3**			**4**		
1	C	155.7		C	153.5		C	152.4		CH	71.2	4.21, m
2	CH	94.4	6.63, d, 2.5	CH	103.8	7.01, d, 2.5	CH	104.3	7.09, overlap	CH_3	24.0	1.28, d, 5.9
3	C	152.6		C	150.7		C	149.2		CH_3	22.2	1.17, d, 5.9
4	C	133.3		C	145.4		C	143.6				
5	C	154.5		CH	113.6	6.88, d, 8.8	CH	116.5	7.17, d, 8.6			
6	CH	99.3	7.02, d, 2.5	CH	108.8	7.07, dd, 8.8, 2.5	CH	110.2	7.08, overlap			
	3-OCH_3	56.1	3.75, s	3-OCH_3	56.6	3.70, s	3-OCH_3	56.2	3.81, s			
	4-OCH_3	60.7	3.82, s	4-OCH_3	55.9	3.64, s						
	Gal			Gal			Gal			Gal		
1′	CH	103.4	5.47, d, 7.4	CH	103.7	5.45, d, 7.3	CH	104.0	5.37, d, 7.3	CH	102.8	4.83, d, 7.8
2′	CH	75.0	4.26, overlap	CH	75.0	4.29, overlap	CH	75.1	4.18, t, 9.0	CH	75.2	3.93, t, 8.8
3′	CH	78.4	4.27, overlap	CH	78.4	4.30, overlap	CH	78.3	4.21, t, 9.0	CH	78.4	4.79, overlap
4′	CH	72.2	4.12, overlap	CH	72.2	4.13, t, 7.8	CH	72.2	4.13, t, 8.3	CH	74.4	4.04, t, 9.3
5′	CH	76.8	4.13, overlap	CH	76.8	4.19, overlap	CH	76.8	4.08, m	CH	76.4	4.00, m
6′	CH_2	68.5	4.74, br d, 8.8	CH_2	68.6	4.78, overlap	CH_2	68.6	4.73, overlap	CH_2	68.4	4.67, dd, 10.8, 2.5
			4.11, overlap			4.20, overlap			3.98, dd, 11.2, 5.3			4.17, overlap
	Ara			Ara			Ara			Ara		
1″	CH	110.1	5.64, br s	CH	110.1	5.65, br s	CH	110.1	5.61, br s	CH	109.8	5.66, br s
2″	CH	82.3	4.89, br s	CH	82.3	4.87, br s	CH	82.3	4.79, br s	CH	82.1	4.86, br s
3″	CH	78.0	4.81, dd, 5.4, 2.0	CH	78.0	4.81, overlap	CH	77.9	4.72, overlap	CH	78.0	4.16, overlap
4″	CH	84.2	4.94, dd, 11.6, 5.4	CH	84.2	4.94, dd, 11.4, 5.6	CH	84.4	4.85, dd, 11.5, 5.4	CH	84.3	4.96, dd
5″	CH_2	62.4	4.53, dd, 11.6, 5.3	CH_2	62.4	4.53, dd, 11.4, 5.3	CH_2	62.5	4.55, dd, 11.5, 5.2	CH_2	62.4	4.54, dd, 11.6, 5.3
			4.45, dd, 11.6, 6.3			4.44, dd, 11.4, 6.0			4.37, dd, 11.5, 6.2			4.44, dd, 11.6, 6.2

were shown to be L-arabinose and D-galactose. The 2D NMR techniques HMBC and ROESY were used to determine the nature of the monosaccharides and the sequence of the oligosaccharide chain of **1**. The anomeric configurations and ring sizes of each sugar were obtained following analysis on the H-1 vicinal coupling constants ($^3J_{HH}$ and $^1J_{CH}$) and their chemical shifts, and comparing their ^{13}C NMR spectral data with those of methyl glycosides. According to the coupling constants of H-1 (7.4, < 1.0 Hz), the anomeric hydroxyls of galactose and arabinose were determined as β- and α-configurations, respectively. From the assigned carbons and protons of the arabinose moiety, the sugar should indicate a furan ring of the arabinose [8]. Based on the evidence, the two sugars and their anomeric configurations in **1** were determined to be β-D-galactopyranose and α-L-arabinofuranose. The sequence of the oligosaccharide chain was deduced from HMBC and ROESY spectra. The C-1 of the galactosyl group was attached to the 1-OH of the aglycone according to the correlation between H-1 (δ 5.47) of the galactosyl group and C-1 (δ 155.7) of the aglycone. The C-1 of the arabinosyl group was linked at 6-OH of the galactosyl group according to the correlation between H-1 (δ 5.64) and C-6 (δ 68.5) in the HMBC spectrum (Figure 2). Based on the above findings, the structure of **1** was determined to be 1-*O*-α-L-arabinofuranosyl-(1 → 6)-β-D-galactopyranosyl-5-hydroxy-3,4-dimethoxy-benzene, named pungen A.

Pungen B (**4**) was a colorless powder and had a molecular formula of $C_{14}H_{26}O_{10}$, based on the negative HR-FAB-MS spectrum. The IR spectrum gave only the absorption bands of the hydroxyl group and sp^3 C—H signals. The NMR spectra exhibited the same sugars and oligosaccharide sequence as in compound **1**. Compound **4** had a simple aglycone with three carbon signals including two methyls [δ_C 24.0 and δ_H 1.28 (d, $J = 5.9$ Hz), δ_C 22.2 and δ_H 1.17 (d, $J = 5.9$ Hz)] and a methine [δ_C 71.2 and δ_H 4.21 (m)]. The two methyl signals were not overlapping, and were shielded by sugar configurations. Therefore, **4** was established as 1-*O*-α-L-arabinofuranosyl-(1 → 6)-β-D-galactopyranosyl-isopropyl alcohol, and named pungen B.

Pungen C (**7**) was a white powder and had a molecular formula $C_{19}H_{16}O_{10}$, as determined by negative FAB-MS and its ^{13}C NMR spectrum. The IR spectrum gave an aromatic ring (1601 cm^{-1}), a conjugated acid (1689 cm^{-1}), and a double bond (1630 cm^{-1}). The ^{13}C NMR spectrum showed 19 signals, of which 12 signals were assigned to two aromatic rings and other signals included a double bond, two methoxyl groups, and three carbonyl carbon signals (see Table 2). The ^{1}H NMR spectrum exhibited an ABX spin system in the aromatic ring at δ_H 8.16 (1H, dd, $J = 8.2$, 1.7 Hz), 8.07 (1H, d, $J = 1.7$ Hz), 7.29 (1H, d, $J = 8.2$ Hz), tetra-substituted aromatic ring at δ_H 7.89

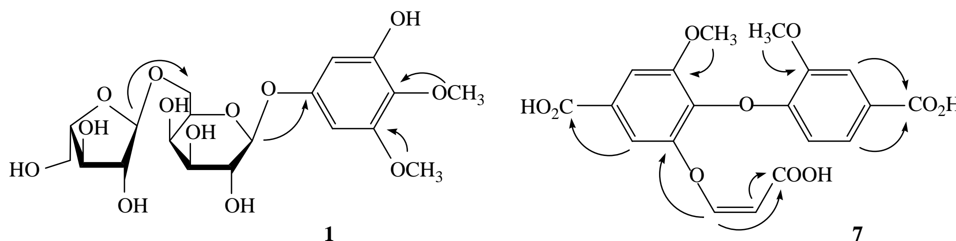

Figure 2. Key HMBC correlations of compounds **1** and **7**.

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Table 2. ^{13}C and ^{1}H NMR spectral data of compounds **6** and **7** in pyridine-d_6.

No.		**6**		**7**	
1	C	122.2		123.3	
2	CH	108.5	7.89, s	108.4	7.89, s
3	C	148.8		153.3	
4	C	142.4		142.4	
5	C	148.8		148.7	
6	CH	108.5	7.89, s	108.4	7.89, s
7	C	169.3		169.2	
3,5-OCH_3		56.4	3.81, s	56.3	3.80, s
1′	C	123.5		123.6	
2′	CH	113.9	8.07, d, 1.7	113.8	8.07, d, 1.7
3′	C	152.9		152.8	
4′	C	148.4		148.3	
5′	CH	116.3	7.30, d, 8.0	116.2	7.29, d, 8.2
6′	CH	125.0	8.16, dd, 8.0, 1.7	124.9	8.16, dd, 8.2, 1.7
7′	C	169.3		169.1	
3′-OCH_3		55.9	3.75, s	55.8	3.73, s
1″	CH			142.1	7.50, d, 9.8
2″	CH			101.2	5.80, d, 9.8
3″	C			165.6	

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(2H, s), *cis*-olefinic protons at δ_H 7.50 (1H, d, $J = 9.8$ Hz) and 5.80 (1H, d, $J = 9.8$ Hz), and two methoxyl groups at δ_H 3.80 (3H, s) and 3.73 (3H, s). Comparing the spectral data of compounds **6** and **7**, the signal of C-3 (δ_C 153.3) of **7** shifted toward downfield by 4.5 ppm, and this evidence should be caused by a deshielding effect. The HMBC correlations between protons and carbons in the HMBC spectrum, H-1″ (δ 7.50)/C-3 (δ 153.3), C-3″ (δ 165.6), were observed. Based on this, the acrylic acid moiety was linked to the C-3 position of the aromatic ring. Due to the influences of the aromatic ring and the double bond, the carbonyl signal of acrylic acid was at δ 165.6. Two benzene rings were connected by one oxygen atom due to the almost identical spectral data to compound **6** and the molecular formula of compound **7**. Thus, pungen C was elucidated as 4-*O*-3′-methoxy-4′-hydroxy benzoic acid-3-*O*-acrolactic acid-5-methoxy-gallic acid.

The cytotoxic activities of compounds **1**–**7** against SGC-7901 and BEL-7404 cell lines were tested using MTT assay approach. Compound **7** (200 μg/ml) displayed 90.26% inhibition against SGC-7901 cell line and 87.54% against BEL-7404 cell line. But compound **6** showed moderate activity (see Table 3). The reason is that compound **7** possessed acrylic acid at position C-3. In addition, compounds **1**–**5** showed weak cytotoxicities from 5.01 to 40.80% against SGC-7901 cell line and from 8.51 to 29.61% against BEL-7404 cell line. Except compound **7**, all other compounds were unworthy of in-depth research.

3. Experimental

3.1 General experimental procedures

Optical rotations were taken on a JASCO-20C digital polarimeter (JASCO, Essex, UK) and the IR spectrum was recorded with a Perkin-Elmer 1750 FT-IR spectrometer (Cambridge Scientific Products, Cambridge, MA, USA). ^{1}H and ^{13}C NMR spectra were obtained with a Bruker DRX-500 spectrometer (Billerica, MA, USA) with TMS as an internal standard and the solvent is C_6D_6N. FAB-MS were taken on a VG Autospec 3000 system spectrometer. Column chromatography (CC) was carried out on silica gel G (100–200 mesh,

Table 3. Cytotoxic activities of compounds **1**–**7**.

Compound no.	Inhibition rate[a] (%)	Inhibition rate[b] (%)
Fr. 1[c]	57.00 ± 0.07	51.21 ± 0.11
Fr. 2[d]	–	–
Fr. 3	75.02 ± 0.11	67.22 ± 0.09
1	40.80 ± 0.19	29.61 ± 0.03
2	19.36 ± 0.17	21.65 ± 0.04
3	27.00 ± 0.14	16.26 ± 0.03
4	5.01 ± 0.18	8.51 ± 0.07
5	14.44 ± 0.13	29.20 ± 0.06
6	49.05 ± 0.07	31.09 ± 0.06
7	90.26 ± 0.02	87.54 ± 0.10
CDDP[e]	84.24 ± 0.18	89.14 ± 0.68

Note: The concentration of all compounds is 200 μg/ml.
[a] Inhibition rate of compounds against SGC-7901 cells.
[b] Inhibition rate of compounds against BEL-7404 cells.
[c] Fractions 1–3 were crude extract, liposoluble extract, and water-soluble extract, respectively.
[d] No inhibitory activity.
[e] Cis-diaminedichloroplatinum (CDDP, cisplatin) diluted in 20 μg/ml was used as the positive control.

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200–300 mesh; Qingdao Marine Chemical Co., Qingdao, China), silica gel H (10–40 μm; Qingdao Marine Chemical Co.), and Sephadex LH-20 (25–100 μm; Amersham Pharmacia Biotech AB, Uppsala, Sweden). The following solvent systems were used: $CHCl_3$–MeOH (100:5 to 100:20) for silica gel column and MeOH–H_2O (0–100%) for the Sephadex LH-20 column. Thin-layer chromatography was conducted on silica gel plates GF_{254} (Qingdao Marine Chemical Co.). Spots on chromatograms were detected by spraying with 10% H_2SO_4–EtOH.

3.2 Plant material

The plant bark (*E. pungens*) was collected from the suburb of Quanzhou, Fujian, China, in September 2004. The specimen was identified by Prof. Ke-Cuo He of College of Plant Protection, Fujian Agriculture and Forestry University. A voucher specimen (No. 219145) has been deposited in the Department of Botany, Fujian, Agriculture and Forestry University.

3.3 Extraction and isolation

The dry bark of *E. pungens* (6 kg) was extracted with methanol for 5 days. The extract was concentrated to dryness under reduced pressure, and the residue (700 g) was dissolved and suspended in water (3 liters), and then the water layer was evaporated *in vacuo* to give a residue of 102 g. The residue was subjected to dry CC on silica gel (1.8 kg), eluted with $CHCl_3$–MeOH (10:1) to get 15 fractions. Fraction 2 (CH_2Cl_2–EtOAc–MeOH, 10:10:1) gave compounds **6** (21 mg) and **7** (32 mg), and fraction 4 (CH_3Cl–MeOH, 10:1.2) was extensively chromatographed over columns of silica gel and Sephadex LH-20 to afford **5** (24 mg). The H_2O–MeOH (3:2) and H_2O–MeOH (2:3) eluates were subjected to reverse-phased silica gel column and Sephadex LH-20 to give compounds **1** (26 mg), **4** (54 mg), **3** (39 mg), and **2** (23 mg).

3.3.1 Compound 1

A colorless powder, $[\alpha]_D^{27}$ −22.1 ($c = 0.11$, MeOH); UV (MeOH) λ_{max} ($\log \varepsilon$): 228 (3.97), 278 (3.23) nm; IR ν_{max}: 3350, 2917, 1601, 1594, 1415 cm^{-1}; ^{13}C and ^{1}H NMR spectral data: see Table 1; FAB-MS *m/z*: 463 $[M-H]^-$, 448 $[M-H-CH_3]^-$, 331 $[M-H-132]^-$, 169 $[M-H-132-162]^-$; HR-FAB-MS

m/z: 463.1452 $[M-H]^-$ (calcd for $C_{19}H_{27}O_{13}$, 463.1452).

3.3.2 Compound **4**

A colorless powder, $[\alpha]_D^{27}$ −56.1 ($c = 0.11$, MeOH); IR ν_{max}: 3284, 2941, 1446, 1294, 1108 cm^{-1}; ^{13}C and ^1H NMR spectral data: see Table 1; FAB-MS *m/z*: 353 $[M-H]^-$, 221 $[M-H-132]^-$; HR-FAB-MS *m/z*: 353.1445 $[M-H]^-$ (calcd for $C_{14}H_{25}O_{10}$, 353.1448).

3.3.3 Compound **6**

A white powder, $[\alpha]_D^{27}$ +22.9 ($c = 0.24$, MeOH); IR ν_{max}: 2527, 1683, 1605, 1260, 1047 cm^{-1}; ^{13}C and ^1H NMR spectral data: see Table 2. FAB-MS *m/z*: 347 $[M-H]^-$, 332 $[M-H-15]^-$; HR-FAB-MS *m*/z: 347.0764 $[M-H]^-$ (calcd for $C_{17}H_{15}O_8$, 347.0767).

3.3.4 Compound **7**

A white powder, $[\alpha]_D^{27}$ +10.1 ($c = 0.10$, MeOH); UV (MeOH) λ_{max} ($\log \varepsilon$): 208 (4.45), 234 (4.11), 268 (3.30), 284 (3.3.34) nm; IR ν_{max}: 2540, 1689, 1630, 1601, 1262, 1042 cm^{-1}; ^{13}C and ^1H NMR spectral data: see Table 2; FAB-MS *m/z*: 403 $[M-H]^-$, 388 $[M-H-15]^-$, 317, 165, 151; HR-FAB-MS *m/z*: 403.0664 $[M-H]^-$ (calcd for $C_{19}H_{15}O_{10}$, 403.0665).

3.3.5 Acid hydrolysis

A solution of each compound (5 mg) was heated under reflux at 100°C in 2 M aqueous CF_3COOH (5 ml) on a water bath for 3 h. The reaction mixture was then diluted with H_2O (15 ml) and extracted with CH_2Cl_2 (3× 5 ml). The combined CH_2Cl_2 extracts were washed with H_2O and then evaporated to dryness *in vacuo*. After evaporation to dryness of the aqueous layer with MeOH until neutral, the sugars were analyzed by comparison with an authentic sample (solvent system $CHCl_3$–MeOH–H_2O (7:3:1) lower-layer 9 ml + 1 ml of HOAc) on silica gel HPTLC as development and aniline-*O*-phthalic acid as detection, comparing with the authentic samples: glucose (R_f 0.26), galactose (R_f 0.19), and arabinose (R_f 0.42). The extract of sugars was derivatized with thiazolidine, as the reported method [9]. Monosaccharides were detected by GC and conditions: column, Supelco SPB-1 0.25 mm × 27 m; 230°C, column temperature; N_2, carrier gas; t_R, L-arabinose (7.0 min), D-arabinose (6.8 min), L-glucose (13.3 min), D-glucose (13.8 min), L-galactose (14.2 min), D-galactose (14.6 min); and D-galactose and L-arabinose were detected in **1**–**4** and D-glucose was detected in **5**.

3.4 Cytotoxic activities

3.4.1 Cell lines and culture

Human gastric cancer cells SGC-7901 and human hepatoma cells BEL-7404 were provided by Fujian Medical University. These cells were maintained in a RPMI-1640 medium supplemented with 10% fetal bovine serum and 10% penicillin–streptomycin solution in an atmosphere of 5% CO_2 at 37°C.

Compounds were dissolved in DMSO and diluted to the required concentrations before use. Cells grown in media containing an equivalent amount of DMSO without compound treatment served as a negative control.

3.4.2 MTT assay

Cancer cells were plated onto 96-well culture dishes at a density of 1×10^3/well in 180 μl medium. After plating for 24 h, cells were treated with compounds ranging from 12.5 to 200 μg/ml (using DMSO as the vehicles at a maximum concentration of 0.1%). Cells were incubated with various concentrations of the agents for 72 h, then 20 μl of 2 mg/ml MTT was added,

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and the absorbance at 570 nm was determined by a microtiter plate reader [10]. CDDP at a concentration of 20 μg/ml was used as the positive control. Experiments were conducted in triplicate.

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References

[1] Z.W. Xie, *Quanguo Zhongcaoyao Huibian* (A Collection of Chinese Herbal Medicine) (People's Health Publishing House, Beijing, 1992), pp. 584–585.

[2] H. Huang, X. Zhao, and B. Jiang, *Chin. Tradit. Herb Drugs* **37**, 307 (2006).

[3] K. Tagahara, J. Koyama, and T. Sugita, *Shoyakugaku Zasshi* **35**, 340 (1981).

[4] X. Zhao, R.L. Zhu, H. Jiang, and H. Huang, *China J. Chin. Mater. Med.* **31**, 472 (2006).

[5] X. Zhao, H. Huang, and R.L. Zhu, *Chin. Tradit. Pat. Med.* **28**, 403 (2006).

[6] S.Y. Bao, Y. Ding, Z.W. Deng, P. Proksch, and W.H. Lin, *Chem. Pharm. Bull.* **55**, 1175 (2007).

[7] K. Yoshikawa, N. Mimura, and S. Arihara, *J. Nat. Prod.* **61**, 1137 (1998).

[8] D.Q. Yu and J.S. Yang, *Analysis of NMR Spectrum* (Chemical Industry Publishing House, Beijing, 1999), Vol. 7, p. 902.

[9] T. Miyase, H. Saitoh, K. Shiokawa, and A. Ueno, *Chem. Pharm. Bull.* **43**, 466 (1995).

[10] P.R. Twentyman, N.E. Fox, and J.K.H. Rees, *Br. J. Haematol.* **71**, 19 (1989).

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