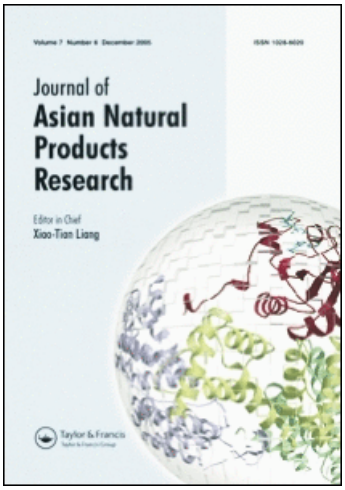

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New flavonol glycosides and new xanthone from *Polygala japonica*

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Three new flavonol glycosides and a new xanthone were isolated from *Polygala japonica* HOUTT. with eight known compounds. Their structures were identified as 1,7-dihydroxy-3,4-dimethoxy-xanthone (**1**), kaempferol-7,4′-dimethyl ether (**2**), physcion (**3**), guazijinxanthone (**4**), rhamnetin (**5**), polygalin A (**6**), 3,5,7-trihydroxy-4′-methoxy-flavone-3-*O*-β-D-galactopyranoside (**7**), 3,5,3′-trihydoxy-7,4′-dimethoxy-flavone-3-*O*-β-D-galactopyranoside (**8**), 3,5,3′,4′-tetrahydroxy-7-methoxy-flavone-3-*O*-β-D-galactopyranoside (**9**), 3,5,3′,4′-tetrahydroxy-7-methoxy-flavone-3-*O*-β-D-glucopyranoside (**10**), polygalin B (**11**), polygalin C (**12**). Among them, compound **4** is a new xanthone, and **6**, **11** and **12** are new flavonol glycosides. Compounds **1**, **4**, **7** and **8** were tested for cytotoxic activity with MTT assays on five human tumor cell lines, K562, A549, PC-3M, HCT-8 and SHG-44. Compound **4** showed cytotoxic activity against all the five cell lines.

Keywords: *Polygala japonica*; Polygalaceae; Flavonol glycosides; Xanthone; Guazijinxanthone; Polygalin A

1. Introduction

Polygala japonica HOUTT. (Polygalaceae), locally called ‘gua zi jin’, widely grows in south China. It has been used in folk medicine as expectorant, anti-inflammatory, ataractic and antibacterial agents. Many kinds of constituents have been found in this plant, such as flavones, saponins and steroids [1]. Our further investigations resulted in the isolation of three new flavonol glycosides and a new xanthone with eight known compounds; their structures were established on the basis of spectral data and chemical properties. Previous studies revealed that xanthone with terpenoid side chain such as prenyl and geranyl had significant tumor inhibitor activity [2,3]. So we evaluated the cytotoxic activity of the new xanthone as well as three known compounds, **1**, **7** and **8**, by MTT assay, a short-term *in vitro* test.

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2. Results and discussion

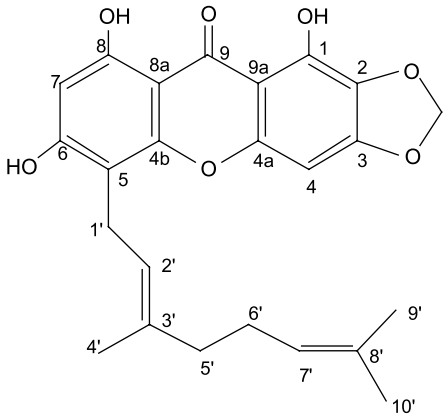
Compound **4** was obtained as light pink amorphous. The ion peak at *m/z* 424 $[M]^+$ of EI-MS is in agreement with the molecular formula $C_{24}H_{24}O_7$, which was confirmed by HRESI-MS, showing the $[M + H]^+$ ion at *m/z* 425.1601. IR spectrum showed the presence of hydroxy (3509 cm^{-1}), hydrogen-bonded hydroxy (3213 cm^{-1}), hydrogen-bonded carbonyl (1650 cm^{-1}), and benzene rings (1604, 1574 and 1472 cm^{-1}). Its UV spectrum is similar to that of the known compound, cudraxanthone D [4]. ^{1}H-NMR spectrum showed three hydroxy protons (δ 11.94, 2H, 9.11, 1H), two aromatic protons (δ 6.47, 1H, 6.27, 1H), a methylenedioxy group (δ 6.08, 2H) and a geranyl moiety. Besides the signals of methylenedioxy and geranyl, ^{13}C-NMR spectrum demonstrated another 13 carbon signals consisted of 12 aromatic carbons and a carbonyl carbon corresponding to a xanthone moiety (see table 1) [3]. By detailed analysis of HMBC spectrum (figure 2), the two aromatic proton singlets at δ 6.47 and 6.27 were assigned to H-4 and H-7, and three hydroxyl groups at δ 11.94, 9.11 and 11.94 were located at C-1, 6 and 8, respectively. The HMBC cross-peaks of H-1′ (δ 3.51) with C-5 (δ 105.6), C-6 (δ 162.6) and C-4b (δ 154.9) indicated that the geranyl group was attached to C-5. The long-range correlations of protons at δ 6.08 (2H, s) with C-2 (δ 130.0) and C-3 (δ 155.5) suggested that the methylenedioxy group was fused at C-2 and C-3. Therefore, compound **4** was identified as 1,6,8-trihydroxy-2,3-methylenedioxy-5-geranyl xanthone, named guazijinxanthone.

ESI-MS of compound **6**, yellow amorphous powder, showed two quasimolecular ions at *m/z* 499 $[M + Na]^+$ and 515 $[M + K]^+$, which were accordant with the molecular formula $C_{23}H_{24}O_{11}$. Hydrolysis of **6** with 0.2 mol/l H_2SO_4 gave aglycone and sugar. While the aglycone was identified as known compound **2** on the basis of its ^{1}H-NMR, ^{13}C-NMR spectral data [5], the sugar was identified as galactopyranose which had the same retention time as authentic sample by GC after complete acetylation. The relatively large *J* values of 8.0 Hz of the anomeric proton indicated β-configuration for the sugar moiety. Comparing the ^{13}C-NMR spectrum of **6** with that of **2**, glycosylation shift at C-2 (+9.5 ppm) and C-3 (−2.4 ppm) of aglycone indicated the sugar was attached to 3-OH [6]. Therefore, **6** was identified as 3,5-dihydroxy-7,4′-dimethoxy-flavone-3-*O*-β-D-galactopyranoside, named polygalin A.

Compound **11** was obtained as yellow amorphous powder. From two quasimolecular ions at *m/z* 631 $[M + Na]^+$, 647 $[M + K]^+$ in ESI-MS spectrum and ^{13}C-NMR spectra, the molecular

Table 1. NMR data of compound **4** ($CDCl_3$; δ, ppm).

	δC	δH		δC	δH
1	143.1		9	184.5	
1-OH		11.94 (s, 1H)	9a	104.1	
2	130.0		1′	22.0	3.51 (d, $J = 7.0$ Hz, 2H)
3	155.5		2′	121.2	5.26 (t-like, 1H)
4	89.9	6.47 (s, 1H)	3′	139.5	
4a	153.7		4′	16.6	1.85 (d, $J = 1.0$ Hz, 3H)
4b	154.9		5′	40.0	2.08 (m, 2H)
5	105.6		6′	26.8	2.10 (m, 2H)
6	162.6		7′	124.0	5.04 (t-like, 1H)
6-OH		9.11 (s, 1H)	8′	132.3	
7	99.5	6.27 (s, 1H)	9′	25.9	1.65 (d, $J = 1.0$ Hz, 3H)
8	161.2		10′	18.0	1.58 (d, $J = 1.0$ Hz, 3H)
8-OH		11.94 (s, 1H)	—OCH_2O—	103.0	6.08 (s, 2H)
8a	102.6				



4

Figure 1. Structure of compound **4**.

formula of **11** was deduced to be $C_{28}H_{32}O_{15}$ which had the same aglycone as **6**, namely, kaempferol-7,4′-dimethyl ether, and confirmed by comparison of ^{1}H-NMR and ^{13}C-NMR spectra of **11** with those of **6**, and TLC analysis of the hydrolysate. Hydrolysis of **11** gave galactopyranose and apifuranose which were identified by the retention time of acetylated sugars by GC. The signals of two anomeric protons (δ 5.64 d, $J = 7.0$ Hz, δ 5.33 s) and the corresponding anomeric carbons (δ 98.9, δ 108.8) revealed the two sugar moiety were both β orientation [7,8]. In the HMBC spectrum, H-1‴ (δ5.33) of the apiose showed long-range correlation with C-2″ (δ75.0) of galactose, exhibiting the apiose was attached to 2″-OH. The location of the sugar moiety was assigned to 3-OH because of the glycosylation shift of C-2 (+8.7 ppm), C-3 (−2.6 ppm) by comparing the ^{13}C-NMR spectrum with that of **2** [6]. Based upon the above evidence, the structure of **11** was characterized as 3,5-dihydroxy-7,4′-dimethoxy-flavone-3-*O*-β-D-apiofuranosyl(1 → 2)-β-D-galactopyranoside, named polygalin B.

The ESI-MS of **12**, obtained as yellow amorphous powder, showed a quasimolecular ion at *m/z* 647 $[M + Na]^+$ and *m/z* 623 $[M - H]^-$ in negative ion mode. The mass and ^{13}C-NMR spectra suggested a molecular formula $C_{28}H_{32}O_{16}$ for **12**. Analysis of ^{1}H-NMR and ^{13}C-NMR spectrum of **12** and **11** revealed that the structures of these two compounds had the

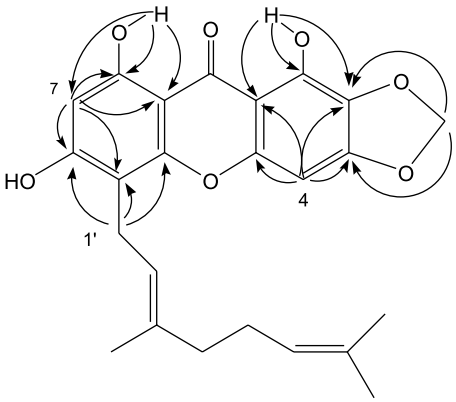
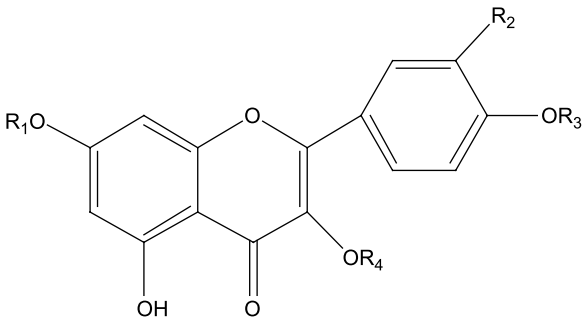


Figure 2. Key HMBC correlations of compound **4**.



	R_1	R_2	R_3	R_4
2	CH_3	H	CH_3	H
5	CH_3	OH	H	H
6	CH_3	H	CH_3	gal
7	H	H	CH_3	gal
8	CH_3	OH	CH_3	gal
9	CH_3	OH	H	gal
10	CH_3	OH	H	glc
11	CH_3	H	CH_3	api(1→2)gal
12	CH_3	OH	CH_3	api(1→2)gal
12a	CH_3	OH	CH_3	H

Figure 3. Structure of flavonoids.

same skeleton except for the substitution in the B ring, the same sugar moiety and both were 3-*O*-desmoside. By acid hydrolysis, **12** afforded 5,3′-dihydroxy-7,4′-dimethoxy-flavonol (**12a)** as aglycone [9]. So, **12** was elucidated as 3,5,3′-trihydroxy-7,4′-dimethoxyflavone-3-*O*-*β*-D-apiofuranosyl(1 → 2)-*β*-D-galactopyranoside, named polygalin C.

The known compounds were characterized as 1,7-dihydroxy-3,4-dimethoxy xanthone **1** [10], kaem-pferol-7,4′-dimethyl ether **2** [5], physcion **3** [11], rhamnetin **5** [12],3,5,7-trihydroxy-4′-methoxy-flavone-3-*O*-*β*-D-galactopyranoside **7** [13], 3,5,3′-trihydoxy-7,4′-dimethoxy-flavone-3-*O*-*β*-D-galac-topyranoside **8** [9], 3,5,3′,4′-tetrahydroxy-7-methoxy-flavone-3-*O*-*β*-D-galactopyranoside **9** [14], 3,5,3′,4′-tetrahydroxy-7-methoxy-flavone-3-*O*-*β*-D-glucopyranoside **10** [15], by comparing with the spectral data reported in literatures.

In the MTT assay (table 4), five human tumor cell lines, including K562, A549, PC-3M, HCT-8 and SHG-44 (human glioma cell), were applied, and Topotecan was used as positive control. **4** showed cytotoxic activities against K562 with IC_{50} value of 0.19 μg/ml, and weak inhibitory effect against HCT-8, SHG-44, A549 and PC-3M with IC_{50} values of 9.31, 14.41, 11.65, 24.61 μg/ml, respectively. Compounds **1**, **7**, **8** showed no activity against all above cell lines with IC_{50} values of more than 30 μg/ml.

3. Experimental

3.1 General experimental procedures

Melting point was measured on an RY-2 melting point apparatus and is uncorrected; NMR spectra were operated on a Bruker DRX-500 spectrometer at 500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR. Chemical shifts were reported with TMS as internal standard; EI-MS

was recorded on a Varian MAT-212 mass spectrometer and HRESI on a Q-TOF micro mass spectrometer; IR was recorded on a Bruker Vector22 spectrometer with KBr pellet; gas chromatography analysis was operated on an HP-5892 II with a FID detector, and a HP-20M (Carbowx 20M) capillary column (25 m × 0.32 mm × 0.3 μm) was used; column chromatography was performed on silica gel (200–300 mesh, Yantai, China), silica gel H (10–40 μm, Yantai, China, macroporous resin (AB-8, Tianjin, China) and sephadex LH-20 (Pharmacia); TLC analysis was run on HSGF254 precoated silica gel plates (10–40 μm, Yantai, China).

3.2 Plant material

Obtained as a commercial sample (purchased from Market for Traditional Material of Bozhou, Anhui province, China) and identified as *Polygala japonica* HOUTT. by Dr. Bao-kang Huang (Dept. of Pharmacognosy, College of Pharmacy, Second Military Medical University, Shanghai, China). A voucher specimen is deposited at Herbarium of School of Pharmacy, Second Military Medical University, Shanghai, China (No. 0211-1).

3.3 Extraction and isolation

Dried and powdered aerial part of *P. japonica* (7 kg) was extracted with 70% aqueous ethanol by infiltration. The solvent was evaporated under vacuum to afford 850 g crude extract. Then the extract was suspended in water and partitioned with petroleum ether, chloroform, ethyl acetate and aqua-saturated n-butol successively. Chloroform extract (65 g) was subjected to chromatography on silica gel column, (200 ~ 300 mesh, 600 g) and eluted with petroleum ether/ethyl acetate (100:1 ~ 2:1) to give 10 fractions (A to J). The fraction C was separated on a silica gel column (petroleum ether/ethyl acetate 15:1, 1000 ml each fraction). The second fraction afforded compound **1** (130 mg) purified by recrystallization. The residue of the third fraction was further chromatographed on silica gel column to give compound **2** (10 mg) and compound **3** (5 mg). The fraction F was passed through a silica gel column to afford compound **4** (50 mg) (petroleum ether/ethyl acetate 10:1). Compound **5** (45 mg) was obtained from fraction J (silica gel column chromatography, petroleum ether/ethyl acetate 5:1). The ethyl acetate extraction (58 g) was separated on a silica column (200 ~ 300 mesh, 600 g, chloroform/methanol 50:1 ~ 1:1) to give six fractions. The third fraction afforded a yellow powder after concentration, which was purified by recrystallization in ethanol to give compound **6** (180 mg). Compounds **7** (30 mg) and **8** (65 mg) were obtained from the fourth part of the ethyl acetate extraction by further chromatographed on silica gel column. Compounds **9** and **10** were obtained as mixture in a ratio of 1:1 (84 mg) from the fifth fraction by silica gel column chromatography. The n-butol extraction (210 g) was passed through a macroporous resin column (1000 g) to afford five fractions by eluting with H_2O, 20%, 40%, 60% and 95% ethanol, successively. The 40% ethanol eluate of n-butol extraction was further subjected to a silica gel column chromatography (500 g) with $CHCl_3/CH_3OH/H_2O$ (65:25:10) as the eluent. The residues of the second and the third 1000 ml eluate were purified on a sephadex LH-20 column (100 g, CH_3OH) to give compounds **11** (45 mg) and **12** (50 mg), respectively. Column separation was companied by TLC analysis on silica gel HSGF254 plates.

3.4 Structure and identification

3.4.1 Guazijinxanthone (1,6,8-trihydroxy-2,3-methylenedioxy-5-geranyl-xanthone, 4). Pink amorphous solid; mp: 178 ~ 180°C. UV (MeOH) λ_{max} (log ϵ): 217 (4.75), 242 (4.58), 263 (4.69), 275 (sh 4.41), 327 (4.56), 373 (br. 4.36). IR (KBr) $\nu_{max.}$ 3509 (—OH), 3213 (—OH), 2968, 2915, 1650 (> C═O), 1604, 1574, 1472 (—Ph—), 1430, 1311, 1269, 1176, 1137, 1098, 1051, 993, 936, 805, 701, 575 cm^{-1}. For ^{1}H- and ^{13}C-NMR (see table 1). EI-MS: *m/z* 424([M]$^{+}$), 355, 301(100), 288, 123. HRESI-MS: *m/z* 425.1601 [M + 1]$^{+}$ (calcd for $C_{24}H_{25}O_7$, 425.1600).

3.4.2 Polygalin A (3,5-dihydroxy-7,4′-dimethoxy-flavone-3-*O*-*β*-D-galactopyranoside, 6). Yellow powder. mp: 221 ~ 223°C $[\alpha]_D^{20}$ +33° (MeOH, *c* 0.22). UV(CH_3OH) λ_{max} (log ϵ): 265 (4.14), 350 (3.99). IR(KBr) υ_{max}: 3405, 2920, 1655 (>C═O), 1600, 1497 (—Ph—), 1345, 1250 cm^{-1}. For ^{1}H- and ^{13}C-NMR (see tables 2 and 3, respectively). ESI-MS: *m/z* 499 [M + Na]$^{+}$, 515 [M + K]$^{+}$. HRESI-MS: *m/z* 499.1215 [M + Na]$^{+}$ (calcd for $C_{23}H_{24}O_{11}Na$, 499.1216).

3.4.3 Polygalin B (3,5-dihydroxy-7,4′-dimethoxyflavone-3-*O*-*β*-D-apiofranosyl(1 → 2)-*β*-D-galacto-pyranoside, 11). Yellow powder. mp: 146 ~ 148°C $[\alpha]_D^{20}$ −47° (MeOH, *c* 0.46). UV(CH_3OH) λ_{max} (log ϵ): 268 (4.19), 348 (4.01). IR(KBr) ν_{max}: 3396, 2939, 1657, 1598, 1498, 1347, 1260, 1212, 1023 cm^{-1}. For ^{1}H- and ^{13}C-NMR (see tables 2 and 3, respectively). ESI-MS: *m/z* 631 [M + Na]$^{+}$, 647 [M + K]$^{+}$. HRESI-MS: *m/z* 631.1643 [M + Na]$^{+}$ (calcd for $C_{28}H_{32}O_{15}Na$, 631.1639).

3.4.4 Polygalin C (3,5,3′-trihydroxy-7,4′-dimethoxy-flavone-3-*O*-*β*-D-apiofranosyl(1 → 2)-*β*-D-galac-topyranoside, 12). Yellow amorphous powder. mp: 151 ~ 153°C $[\alpha]_D^{20}$ −56° (MeOH, *c* 0.51). UV(CH_3OH) λ_{max} (log ϵ): 257 (4.24), 268 (4.11, sh), 355 (3.97). IR(KBr) υ_{max}: 3420, 2941, 1655, 1599, 1499, 1442, 1356, 1213, 1021 cm^{-1}. For ^{1}H- and ^{13}C-NMR (see tables 2 and 3, respectively). ESI-MS: *m/z* 647 [M + Na]$^{+}$, ESI-MS in negative mode: *m/z* 623 [M-H]$^{-}$. HRESI-MS: *m/z* 647.1591 (calcd for $C_{28}H_{32}O_{16}Na$, 647.1588).

3.4.5 Acid hydrolysis of 6, 11, 12 in 0.05 mol/l H_2SO_4. Each compound (10 mg) was heated in 0.05 mol/l H_2SO_4 (50 ml) at 90°C for 4 h. The aglycone was extracted with $CHCl_3$ and identified by ^{1}H-, ^{13}C-NMR in DMSO (see tables 2 and 3).

3.4.6 Acetlytion of sugars. Compounds **6**, **11** and **12** (5 mg each) were heated in a vial for 4 h at 80°C in 2 mol/l HCl (5 ml), respectively. After removal of solvent, the residues were dissolved in 1 ml pyridine, respectively. Then hydroxylammonium hydrochloride was added in them (1 mg each) followed by the solution were kept in a 80°C water bath for 30 min. Finally, the solution was treated with 1 ml Ac_2O and kept in a 90°C water bath for another 40 min. The authentic monosaccharide samples were treated similarly as the production of hydrolysis.

Table 2. ^{1}H-NMR data for compounds **2**, **6**, **11**, **12** and **12a** (DMSO, δ, ppm).

	2	*6*	*11*	*12*	*12a*
Aglycone					
6	6.36 (d, $J = 2.0$ Hz)	6.39 (d, $J = 2.0$ Hz)	6.37 (d, $J = 2.0$ Hz)	6.37 (d, $J = 2.0$ Hz)	6.35 (d, $J = 3.0$ Hz)
8	6.75 (d, $J = 2.0$ Hz)	6.75 (d, $J = 2.0$ Hz)	6.74 (d, $J = 2.0$ Hz)	6.71 (d, $J = 2.0$ Hz)	6.70 (d, $J = 3.0$ Hz)
2′	8.18 (d, $J = 9.0$ Hz)	8.21 (d, $J = 9.0$ Hz)	8.25 (d, $J = 9.0$ Hz)	7.57 (d, $J = 2.0$ Hz)	7.71 (d, $J = 2.0$ Hz)
3′	7.12 (d, $J = 9.0$ Hz)	7.01 (d, $J = 9.0$ Hz)	7.07 (d, $J = 9.0$ Hz)		
5′	7.12 (d, $J = 9.0$ Hz)	7.01 (d, $J = 9.0$ Hz)	7.07 (d, $J = 9.0$ Hz)	6.99 (d, $J = 9.0$ Hz)	7.09 (d, $J = 9.0$ Hz)
6′	8.18 (d, $J = 9.0$ Hz)	8.21 (d, $J = 9.0$ Hz)	8.25 (d, $J = 9.0$ Hz)	7.91 (dd, $J = 9.0$,2.0 Hz)	7.69 (d, $J = 8.0$,2.0 Hz)
OCH_3	3.87 (3H, s)	3.87 (3H, s)	3.87 (3H, s)	3.88 (3H, s)	3.87 (3H, s)
	3.86 (3H, s)	3.86 (3H, s)	3.86 (3H, s)	3.87 (3H, s)	3.86 (3H, s)
Sugars					
Gal-1″		5.46 (d, $J = 8.0$ Hz)	5.64 (d, $J = 7.0$ Hz)	5.62 (d, $J = 8.0$ Hz)	
2″		3.68 (m)	3.78 (t-like)	3.77 (m)	
3″		3.47 (m)	3.59 (m)	3.58 (m)	
4″		3.57 (m)	3.66 (m)	3.65 (m)	
5″		3.37 (m)	3.36 (m)	3.34 (m)	
6″		3.40, 3.33 (m)	3.41, 3.29 (m)	3.43, 3.27 (m)	
Api-1‴			5.33 (s)	5.32 (d, $J = 1.0$ Hz)	
2‴			3.83 (m)	3.80 (m)	
4‴			3.44 (m)	3.45 (d, $J = 6.0$ Hz)	
			3.39 (m)	3.40 (d, $J = 6.0$ Hz)	
5‴			3.85 (d, $J = 9.0$ Hz)	3.84 (d, $J = 9.0$ Hz)	
			3.50 (d, $J = 10.0$ Hz)	3.50 (d, $J = 9.0$ Hz)	

Table 3. ^{13}C-NMR data for compounds **2**, **6**, **11**, **12** and **12a** (DMSO, δ, ppm).

	2	***6***	***11***	***12***	***12a***
2	146.7	156.2	155.4	155.6	146.8
3	136.2	133.8	133.6	133.7	136.2
4	176.0	177.7	177.6	177.5	176.0
5	156.1	160.9	160.9	160.9	156.1
6	97.4	97.9	97.8	97.8	97.4
7	164.9	165.2	165.0	165.0	165.0
8	92.0	92.4	92.2	92.0	91.9
9	160.5	156.3	156.2	156.1	160.4
10	104.0	105.0	104.9	104.9	104.0
1′	123.1	122.4	122.4	122.5	123.3
2′	129.3	130.9	130.7	115.3	114.8
3′	114.0	113.7	113.6	146.0	146.2
4′	160.3	161.3	161.2	150.1	149.5
5′	114.0	113.7	113.6	111.2	111.9
6′	130.8	130.9	130.7	122.1	119.8
7-OCH3	55.9	56.1	56.0	56.0	56.0
4′-OCH3	55.3	55.4	55.3	55.6	55.7
Gal-1″		101.6	98.9	98.9	
2″		71.2	75.0	74.9	
3″		73.1	73.6	73.7	
4″		67.9	68.2	68.2	
5″		75.9	75.7	75.7	
6″		60.2	60.0	59.9	
Api-1‴			108.8	108.7	
2‴			76.1	76.1	
3‴			79.1	79.1	
4‴			64.2	64.3	
5‴			73.8	73.8	

3.4.7 Gas chromatography. 1 μl of the above solutions was injected into a HP-20M capillary column using N_2 as carrier (oven temp. 210°C) and a FID detector (detector temp. 280°C). t_R: D-xylose 3.527 min, D-apiose 4.450 min, L-rhamnose 5.222 min, D-glucose 10.590 min, D-galactose 12.086 min. D-galactose was detected from compounds **6**, **11** and **12**, while D-apiose was detected from compounds **11** and **12**.

3.5 MTT assay

Compounds were tested for inhibitory activity using the microtitre MTT tetrazolium dye assay under conditions of continuous drug exposure as previously reported [16], but with some modifications. Tumor cell lines culture were diluted with fresh medium to 4×10^4 cell/ml and plated in 96-well microplates at 100 μl/well. After 24 hours incubation at 37°C in a 5% CO_2 atmosphere, the test compounds of variety concentrations

Table 4. IC_{50} values (μg/mL) of compounds **1**, **4**, **7**, **8** against human tumor cell lines.

Compd.	*K562*	*SHG-44*	*HCT-8*	*A549*	*PC-3M*
1	ND*	ND	ND	ND	ND
4	0.19	14.41	9.31	11.65	24.61
7	ND	ND	ND	ND	ND
8	ND	ND	ND	ND	ND
Topotecan	0.015	0.18	0.027	0.073	0.064

* Not detected (IC_{50} value >30 μg/mL).

($10^{-2} \sim 10^{2}$ μg/ml) were added to the microplates in 10 μl amounts. Then the tumor cell lines were exposed to the drugs for another 72 h. The absorbance was read on a Wellscan reader (MK-2, Labsystems, Finland) at 570 nm. Topotecan (purchased from Nanjing Tianzun Zezhong Chemical Co. Ltd., China) was used as positive reference substance with concentrations of $10^{-3} \sim 10^{2}$ μg/ml. The human tumor cell lines consisted of K562, HCT-8, A549, PC-3M, and SHG-44 (human glioma cell). The first four cell lines were purchased from ATCC. SHG-44 is preserved in Shanghai Institute for Pharmaceutical Industrial, China.

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