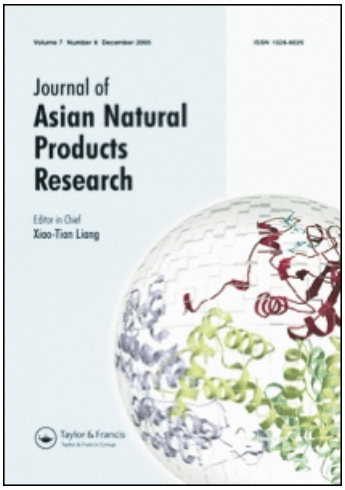

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Benzophenone C-glucosides from *Polygala glomerata* Lour

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Four new benzophenone C-glucosides, glomeratides A (**1**), B (**2**), C (**3**), and D (**4**), along with a known compound arrilanin G (**5**), have been isolated from the whole plant of *Polygala glomerata* Lour. Their structures were determined by extensive analyses of their spectral data. Compounds **1**–**5** showed hepatoprotective activities against D-galactosamine-induced toxicity in WB-F344 rat hepatic epithelial stem-like cells.

Keywords: *Polygala glomerata*; benzophenone; C-glucosides; hepatoprotective

1. Introduction

Polygala glomerata Lour. (Polygalaceae) widely distributed in the south of China has long been used in folk medicine for the treatment of acute tonsillitis, stomatitis and other inflammatory diseases such as myelitis and hepatitis pharyngitis, pulmonary tuberculosis, esophageal cancer and whooping cough.[1] The plant has been investigated and a variety of secondary metabolites such as saponins and saccharide esters have been isolated.[2,3] As a part of ongoing phytochemical investigation on the title plant, four new benzophenone C-glucosides, glomeratides A (**1**), B (**2**), C (**3**), and D (**4**), along with a known compound arrilanin G (**5**)[4] were isolated, and herein, we report their structure elucidation and biological activities.

2. Results and discussion

Compound **1** was obtained as a yellow amorphous powder with the molecular formula $C_{26}H_{32}O_{16}$, established on the basis of HRESI–MS at *m/z* 599.1617 $[M - H]^-$ and ESI–MS, together with ^{1}H NMR and ^{13}C NMR spectra. The ESI–MS in the positive and negative ion mode displayed pseudo-molecular ions at *m/z* 623 $[M + Na]^+$ and 599 $[M - H]^-$ and fragment ions at *m/z* 581 $[M - H - 18]^-$, 479 $[M - H - 120]^-$, and 359 $[M - H - 120 - 120]^-$, corresponding to the typical fragmentation of C-glucosides.[5] The UV absorption maxima at 210, 232 and 320 nm indicated the existence of aromatic rings. The IR spectrum showed absorptions for hydroxyl groups at 3380 cm^{-1} and aromatic groups at 1617 and 1457 cm^{-1}. In the ^{1}H NMR spectrum, four downfield singlets at δ_H 9.87, 8.70 (2H) and 8.56 were assigned to the phenolic hydroxyl groups; a set of aromatic signals at δ_H 6.78 (1H, d, $J = 8.5$ Hz, H-5), 7.19 (1H, dd, $J = 8.5$, 2.0 Hz, H-6) and 7.33 (1H, d, $J = 2.0$ Hz, H-2) were assigned to a 1,3,4-trisubstituted benzene ring. In the aliphatic region, a characteristic doublet with a large coupling constant ($J = 9.5$ Hz) at δ_H 4.70 integrated for two anomeric protons indicated the presence of two glucose units in the molecule and the anomeric configuration of the glucosyl residue was deduced to be β form.[5] A singlet at δ_H 3.78 was assigned to a methoxyl group. The ^{13}C NMR spectrum displayed a signal at δ_C 193.9 attributable to the carbonyl group of benzophenone,[5,6,7] and 12 aromatic carbon signals of which two being overlapped, at δ_C 155.9 (1C), 154.6 (2C), 151.4 (1C), 147.2 (1C), 130.5 (1C), 124.8 (1C), 111.9 (1C),114.6 (1C), 109.1, (1C) and 104.8 (2C), indicating the presence of two aromatic rings in the molecule. The signals at δ_C 81.0, 77.8, 75.3, 72.7, 69.1 and 59.9 were attributed to C-glucosides,[4,5,6] and the superimposition of the data indicated there are two glucoses in the structure of **1**. The existence of two C-glucoses was also supported by ^{1}H NMR and ^{13}C NMR spectra as well as ESI–MS data. The MS data showed a fragment ion at *m/z* 359 $[M - H - 120 - 120]^-$, which suggested that **1** is a benzophenone derivative with a symmetric ally fully substituted aromatic ring (Figure 1). The linkage between the two glucose moieties and the benzophenone was unambiguously demonstrated by the HMBC correlations between H-1″ (δ_H 4.70) and C-3′, 5′ (δ_C 104.8) (Figure 2). The position of the methoxyl group was also determined by the HMBC correlation between OCH_3 (δ_H 3.78) and C-3 (δ_C 147.2). Also in the NOE difference spectrum of **1**, the intensity of H-2 signal was

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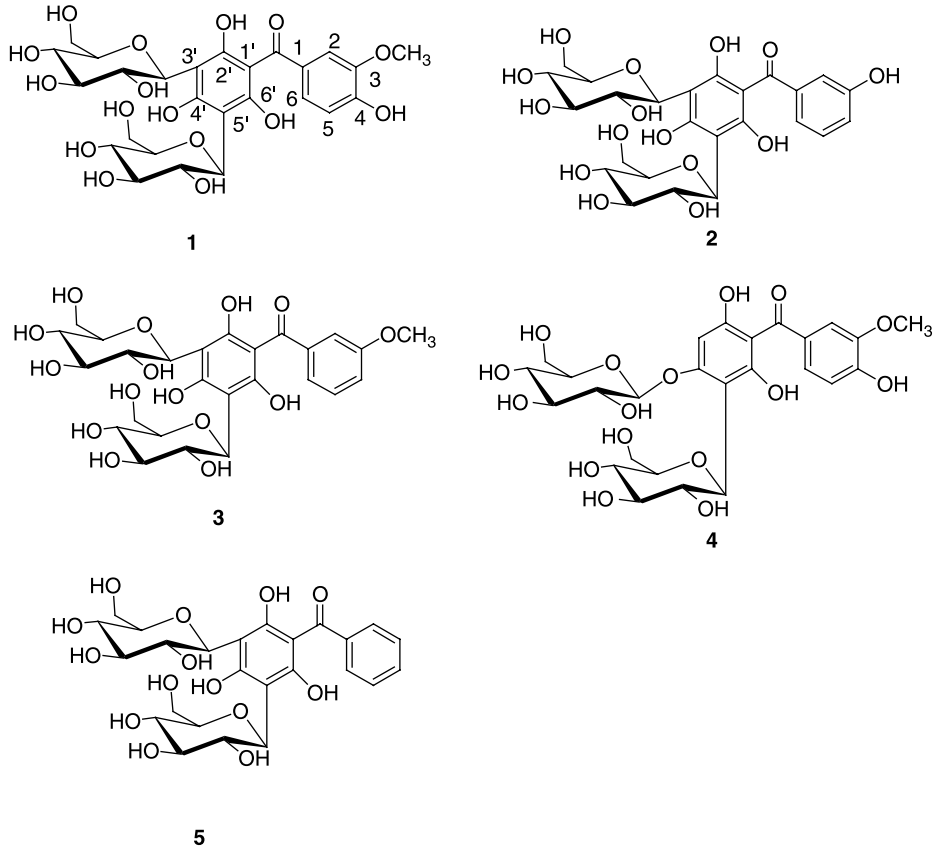

Figure 1. Structures of compounds **1**–**5**.

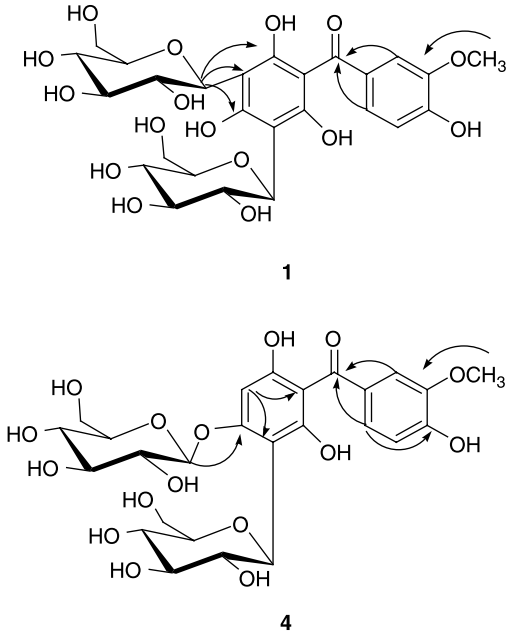

Figure 2. Key HMBC correlations of compounds **1** and **4**.

enhanced by irradiation of the methoxy signal, supporting the above deduction. Thus, compound **1** was identified as 2′,4′,6′,4-tetrahydroxy-3-methoxy-benzophenone-3′,5′-C-β-D-diglucoside, named glomeratide A.

Compound **2** was obtained as a yellow amorphous powder. As shown in the experimental section, the UV, IR, and ^{13}C NMR spectroscopic features of compound **2** were similar to those of **1** except for the absence of a methoxyl group, indicating that it was also a benzophenone C-glycoside. In the positive ESI–MS, **2** gave a quasimolecular ion at *m/z* 593 $[M + Na]^+$, 30 mass units less than that of **1**, implying the absence of a methoxyl group. Meanwhile the ^{1}H NMR spectrum of **2** supported this deduction. The ^{1}H NMR spectrum showed a 1,3-disubstituted benzene moiety due to the proton signals at δ_H 6.94 (1H, dd, $J = 8.0$, 1.5 Hz, H-6), 7.11 (1H, brs, H-2), 7.14 (1H, brd, $J = 8.0$ Hz, H-4) and 7. 24 (1H, t, $J = 8.0$ Hz, H-5) instead of a 1,3,4-trisubstituted benzene unit in **1**. Thus, **2** was identified as 2′,4′,6′,3-tetrahydroxy benzophenone-3′,5′-C-β-D-diglucoside (Figure 1), named glomeratide B.

Compound **3** was obtained as a yellow amorphous powder. The UV, IR, ^{1}H NMR and ^{13}C NMR spectra of compound **3** were almost identical to those of **2**. The HRESI–MS data of **3** showed a quasimolecular ion

at *m/z* 583.1668 $[M - H]^-$, 14 mass units higher than that of **2**, consistent with a molecular formula of $C_{25}H_{29}O_{15}$. Comparing its NMR spectral data with those of **2**, there was a methoxyl group (δ_H 3.78, δ_C 55.2) in **3** attached to C-3 instead of a hydroxyl group in **2**. Thus, the structure of **3** was elucidated as 2′,4′,6′-trihydroxy-3-methoxy-benzophenone-3′,5′-C-β-D-diglucoside (Figure 1), named glomeratide C.

Compound **4** was obtained as a yellow amorphous powder and also determined to be a benzophenone glucoside by comparison of the spectroscopic data with those of compounds **1**–**3**. The ESI–MS in the positive ion mode also displayed the typical fragmentation of C-glucosides at *m/z* 463 $[M + H - 18 - 120]^+$ and 583 $[M + H - 18]^+$ and a fragment ion at *m/z* 461 $[M + Na - 162]^+$ characteristic of the loss of an O-hexose unit. In the ^{1}H NMR spectrum, aromatic signals at δ_H 6.79 (1H, d, J = 8.5 Hz, H-5), 7.12 (1H, dd, J = 8.5, 1.5 Hz, H-6) and 7.34 (1H, d, J = 1.5 Hz, H-2), showed the presence of a 1,3,4-trisubstituted benzene ring, similar to that of compound **1**. A proton singlet at δ_H 6.29 was ascribed to H-3′ of a pentasubstituted benzene ring, due to the HMBC correlations from H-3′ (δ_H 6.29) to C-5′ (δ_C 105.8), C-1′ (δ_C 111.7), C-2′ (δ_C 156.1), C-4′ (δ_C 156.8). In the aliphatic region, a doublet (J = 7.0 Hz) at δ_H 4.67 assignable to the anomeric proton of O-glycoside and another at δ_H 4.76 to the anomeric proton of C-glycoside showed their corresponding carbon signals at δ_C 102.1 and 76.4 in the ^{13}C NMR spectrum, respectively. The position of the 4′-O-glucose was determined by the HMBC correlation from H-1″ of O-glucoside (δ_H 4.67) to C-4′ (δ_C 156.8) (Figure 2). In the NOE difference spectrum of **4**, the intensity of H-1″ was enhanced by irradiation of H-3′, also indicating the existence of 4′-O-glucose. The 5′-C-glucose was confirmed by comparing the chemical shift of C-5′ (δ_C 105.8) with those of compounds **1**–**3**.[5] The position of the methoxyl group was deduced from an analysis of the NOE difference spectrum of **4**, the intensity of H-2 was enhanced by irradiation of the methoxyl group, indicating the presence of 3-methoxy. Thus, **4** was assigned as 2′,6′,4-trihydroxy-3-methoxy-benzophenone-4′-O-β-D-glucoside-5′-C-β-D-glucoside, named glomeratide D.

Compounds **1**–**5** showed hepatoprotective activities against D-galactosamine-induced toxicity in WB-F344 rat hepatic epithelial stem-like cells, using a hepatoprotective activity drug bicyclol as positive control (Table 1).[8] This is the first report of the hepatoprotective activity of benzophenone C-glycoside from *Polygala* species.

Table 1. Hepatoprotective effects of compounds **1**–**5** against D-galactosamine-induced toxicity in WB-F344 cells[a].

Compound	Concentration (mol/ml)	Cell survival rate (% of normal)
Normal	1×10^{-5}	100.0
Control[a]	1×10^{-5}	40.1
Bicyclol[b]	1×10^{-5}	61.9
1	1×10^{-5}	63.1
2	1×10^{-5}	96.0*
Control	1×10^{-5}	29.0
Bicyclol[b]	1×10^{-5}	28.0
3	1×10^{-5}	36.0*
4	1×10^{-5}	30.0*
5	1×10^{-5}	37.0*

[a] Results are expressed as means ± SD (n = 3; for normal and control, n = 6).
[b] Positive control substance.
[c] *$p < 0.05$, significantly different from control by Student's t-test.

3. Experimental

3.1 General experimental procedures

The optical rotations were measured on a Perkin–Elmer 341 digital polarimeter. UV spectra were recorded on a Shimadzu UV-300 spectrophotometer. IR spectra were recorded on a Nicolet Magna FT-IR spectrophotometer. The ^{1}H NMR, ^{13}C NMR, and HMBC spectra were run on an INOVA-500 FT spectrometer at 500 MHz for ^{1}H and 125 MHz for ^{13}C, respectively, using solvent peaks as references. HRFAB–MS were performed on an Auto Spec Ultima-Tof mass spectrometer. HRESI–MS were performed on an Accu-Tof CS mass spectrometer. ESI were obtained using an Agilent 1100 series LC/MSD Trap SL mass spectrometer. Reversed-phase HPLC was carried on a Shimadzu LC-6AD instrument using a SPD-10A detector. A reversed-phase C_{18} column (YMC-Pack ODS-A ∅ 20 × 250 mm, 10 μm) was employed. Column chromatography was carried out on macroporous resin D101 (26–60 mesh, Tianjin Haiguang Chemistry Co., Tianjin, China), silica gel (100–200, 200–300 mesh, Qingdao Marine Chemistry Co., Qingdao, China), and Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden). Kieselgel 60 F_{254} silica gel plates (Merck, Germany) were used for analytical TLC.

3.2 Plant material

The whole plant of *Polygala glomerata* Lour was collected from Jingxiu city of Guangxi Province of China, in November 2002. It was authenticated by Mr Guangri Long (Liuzhou Forestry Bureau, Guangxi, China). A voucher specimen (020322) has been deposited at the herbarium of the Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China.

3.3 Extraction and isolation

The air-dried plants of *Polygala glomerata* (5.0 kg) were extracted with 95% EtOH under reflux. The combined extracts were evaporated under reduced pressure to give a brown green residue (1.7 kg), which was subsequently

chromatographed over silica gel column by eluting with $CHCl_3$, EtOAc, EtOAc/Me_2CO (1:1), Me_2CO, and MeOH to give five fractions. The MeOH extract (540 g) was separated on macroporous resin D101 using water, and 30%, 60%, and 95% EtOH-water in sequence to afford four fractions (A_1–A_4). Fraction A_2 (45 g) was evaporated and separated on a silica gel column by eluting with a gradient of $CHCl_3$/MeOH/H_2O (8:2:0.5 to 7:3:0.5) to give seven fractions (1–7). Fraction 2 (3.35 g) was subjected to a reversed phase MPLC (YMC-ODS-40 μm, 60 × 500 mm, 254 nm, flow rate 20.0 ml/min) in 10:1–1:1 water/methanol to obtain 10 subfractions. Subfraction 2 (350 mg) was subjected to a reversed-phase HPLC (210 nm, water/methanol 6:1, flow rate 7.0 ml/min), to give **1** (57 mg, $t_R = 46.7$ min), **2** (50 mg, $t_R = 57.6$ min), and **3** (13 mg, $t_R = 25.6$ min). Subfraction 4 (250 mg) was also subjected to a reversed-phase HPLC (210 nm, water/methanol 4:1, flow rate 7.0 ml/min), to give **4** (26 mg, $t_R = 55.6$ min) and **5** (10 mg, 5 ml/min, $t_R = 80.0$ min).

3.3.1 *Glomeratide A (1)*

Yellow amorphous powder; $[\alpha]_D^{20}$ +69 (MeOH; *c* 0.10); UV (MeOH) λ_{max}: 210, 232, 320 nm; IR (KBr) ν_{max} (cm^{-1}): 3380, 2925, 1617, 1457, 1285, 1199, 1081, 1028, 801; ^1H NMR and ^{13}C NMR spectral data: see Tables 2 and 3; HRESI–MS *m/z*: 599.1617 $[M - H]^-$ (calcd for $C_{26}H_{31}O_{16}$, 599.1612); ESI–MS *m/z*: 623 $[M + Na]^+$, 601 $[M + H]^+$, 479, 359.

3.3.2 *Glomeratide B (2)*

Yellow amorphous powder; $[\alpha]_D^{20}$ +93 (MeOH; *c* 0.10); UV (MeOH) λ_{max}: 213, 257, 311 nm; IR (KBr) ν_{max} (cm^{-1}): 3376, 2928, 1618, 1456, 1081, 887; ^1H NMR and ^{13}C NMR spectral data: see Tables 2 and 3; HRESI–MS *m/z*: 569.1512 $[M - H]^-$ (calcd for $C_{25}H_{29}O_{15}$, 569.1506); ESI–MS *m/z*: 593 $[M + Na]^+$; 569 $[M - H]^-$, 479, 449.

3.3.3 *Glomeratide C (3)*

Yellow amorphous powder; $[\alpha]_D^{20}$ +57 (MeOH; *c* 0.10); UV (MeOH) λ_{max}: 214, 260, 313 nm; IR (KBr) ν_{max} (cm^{-1}): 3365, 2904, 1618, 1429, 1026, 916; ^1H NMR and ^{13}C NMR spectral data: see Tables 2 and 3; HRESI–MS *m/z*: 583.1668 $[M - H]^-$ (calcd for $C_{26}H_{32}O_{15}$, 583.1663); ESI–MS *m/z*: 607 $[M + Na]^+$, 585 $[M + H]^+$, 583 $[M - H]^-$.

3.3.4 *Glomeratide D (4)*

Yellow amorphous powder; $[\alpha]_D^{20}$ −14 (MeOH; *c* 0.08); UV (MeOH) λ_{max}: 207, 232, 311 nm; IR (KBr) ν_{max} (cm^{-1}): 3341, 2922, 1615, 1426, 1272, 1185, 1070, 1018,

Table 2. ^1H NMR spectral data of compounds **1**–**4** (**1**, **2** and **4** in DMSO-d_6, **3** in CD_3OD, 500 MHz).

Position	1	2	3	4
2	7.33 (d, 2.0)	7.11 (brs)	7.20 (brs)	7.34 (d, 1.5)
3-OH		9.64 (brs)		
4-OH	9.87 (brs)			
4		7.14 (brd, 8.0)	7.25 (brd, 7.5)	
5	6.78 (d, 8.5)	7.24 (t, 8.0)	7.35 (t, 7.5)	6.79 (d, 8.5)
6	7.19 (dd, 8.5, 2.0)	6.94 (dd, 8.0, 1.5)	7.11 (dd, 7.5, 2.0)	7.12 (dd, 8.5, 1.5)
2′-OH	8.70 (brs)	8.81(brs)		
3′				6.29 (s)
4′-OH	8.56 (brs)	8.64 (brs)		
6′-OH	8.70 (brs)	8.81(brs)		
Glc-1″ (1‴)	4.70 (d, 9.5)	4.70 (d, 10.0)	4.70 (d, 10.0)	4.76[a]
Glc-2″ (2‴)	3.27 (m)	3.26 (m)	3.26 (d, 11)	3.26 (m)
Glc-3″ (3‴)	3.24 (m)	3.24 (m)	3.24 (m)	3.24 (m)
Glc-4″ (4‴)	3.43 (t, 8.0)	3.43 (t, 9.0)	3.45 (t, 9.0)	3.43[a]
Glc-5″ (5‴)	3.29[a] m	3.29 (m)	3.29 (m)	3.24[a]
Glc-6″ (6‴)	3.56 (dd, 11.5, 3.5)	3.58 (dd,11.5, 2.5)	3.54 (dd, 11.0, 2.0)	3.56 (dd,11.5, 2.5)
	3.61 (brd, 11.5)	3.61 (brd, 11.5)	3.60 (brd, 11.0)	3.61 (brd, 11.5)
Glc-1‴				4.67 (d, 7.0)
Glc-2‴				3.76 (m)
Glc-3‴				3.35 (m)
Glc-4‴				3.43 (m)
Glc-5‴				3.24[a]
Glc-6‴				3.65 (m)
				3.78 (m)
OMe	3.78 (s)		3.78 (s)	3.76 (s)

[a] Overlap with other signal.

Table 3. ^{13}C NMR spectral data of compounds **1**–**4** (**1**, **2** and **4** in DMSO-d_6, **3** in CD_3OD, 125 Hz).

Position	1	2	3	4
1	130.5	129.2	115.5	130.7
2	111.9	115.1	113.3	111.9
3	147.2	157.2	140.9	148.2
4	151.4	119.9	121.4	152.2
5	114.6	119.5	118.0	115.3
6	124.8	140.3	129.3	125.8
C = O	193.9	195.9	195.8	194.1
1′	109.1	109.0	108.0	111.7
2′	154.6	154.8	159.0	156.1
3′	104.8	104.8	104.6	95.4
4′	155.9	156.4	155.9	156.8
5′	104.8	104.8	104.6	105.8
6′	154.6	154.8	159.0	155.8
Glc-1″ (1‴)	75.3	75.3	75.3	76.4
Glc-2″ (2‴)	72.7	72.7	72.6	74.0
Glc-3″ (3‴)	77.8	77.8	77.8	77.6
Glc-4″ (4‴)	69.1	69.0	69.1	69.9
Glc-5″ (5‴)	81.0	81.0	81.0	81.4
Glc-6″ (6‴)	59.9	59.9	59.9	61.0
Glc-1‴				102.1
Glc-2‴				75.5
Glc-3‴				78.8
Glc-4‴				70.2
Glc-5‴				78.5
Glc-6‴				60.4
OMe	55.6		55.2	56.3

836; ^1H NMR and ^{13}C NMR spectral data: see Tables 2 and 3; HRFAB–MS *m/z*: 623.1595 $[M + Na]^+$ (calcd for $C_{26}H_{32}O_{16}Na$, 623.1588); ESI–MS *m/z*: 623 $[M + Na]^+$, 601 $[M + H]^+$, 461.

3.4 *Protective effect on cytotoxicity induced by D-galactosamine in WB-F344 cells*

The hepatoprotective activity was assayed as previously described.[8] The hepatoprotective effects of compounds **1**–**5** were determined by a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) colorimetric assay in WB-F344 cells, with some modification. Each cell suspension of 1×10^4 cells in 200 μl of Dulbecco's Modified Eagle's Medium containing foetal calf serum (3%), penicillin (100 units/ml), and streptomycin (100 μg/ml) were planted in a 96-well microplate and precultured for 24 h at 37°C under a 5% CO_2 atmosphere. Fresh medium (200 μl) containing bicyclol and test samples were added, and the cells were cultured for 1 h. Then the cultured cells were exposed to 40 mM D-galactosamine for 24 h. Cytotoxic effects of test samples were measured simultaneously in the absence of D-galactosamine. The medium was changed into a fresh one containing 0.5 mg/ml MTT. After 3.5 h incubation, the medium was removed and 150 μl of dimethyl sulphoxide was added to dissolve formazan crystals. The optical density (OD) of the formazan solution was measured on a microplate reader at 492 nm.

All values were expressed as ± SD. The Student *t*-test for unpaired observations between normal or control and tested samples was carried out to identify statistical differences; *p* values less than 0.05 were considered as significantly different.

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