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Lignan Glycosides from *Gentianella acuta* (Michx.) Hulten. and Their Protective Effects Against H_2O_2-Induced Apoptosis in H9c2 Cardiomyoblasts

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Abstract: Two new lignan glycosides named acutosides A (**1**) and B (**2**) have been isolated from the whole plant of *Gentianella acute* (Michx) Hulten. and their structures were determined as (-)-(7*R*,8*S*,7'*E*)-4-hydroxy-3,3'-dimethoxy-7,4'-epoxy-8,5'-neolign-7'-ene-9,9'-diol 9'-ethyl ether-4-*O*-*β*-D-glucopyranoside and (-)-(7*R*,8*S*,7'*E*)-4-hydroxy-3,3'-dimethoxy-7,4'-epoxy-8,5'-neolign-7'-ene-9,9'-diol 9-ethyl ether-9'-*O*-*β*-D-glucopyranoside by means of 1D-, 2D-NMR, HR-ESI-MS, CD techniques and chemical reactions. Along with the above two new compounds we have obtained six known ones: (7*R*,8*S*)-dehydrodiconiferyl alcohol-4,9'-di-*O*-*β*-D-glucopyranoside (**3**), alaschanisoside A (**4**), citrusin A (**5**), 8,4',9'-trihydroxy-3,3'-dimethoxy-9,7'-epoxylignan 4-*O*-*β*-D-glucopyranoside (**6**), leptolepisol D (**7**) and acanthoside D (**8**). The cardioprotective effects of compounds **1**-**8** were evaluated by measuring the viability of the compounds **1**-**8** pretreated H9c2 cardiomyoblasts after exposure to H_2O_2-induced apoptosis, the results showed that compounds **1, 3, 6** and **8** which could increase cell viability >15% at 200 μM had remarkably protective influence against H_2O_2-induced apoptosis. All the compounds were tested for cytotoxicity against two human tumor cell lines: gastric cancer SGC-7901 and breast cancer MCF-7. But they were inactive (IC_{50}>40 μM).

Keywords: Lignan glycosides; *Gentianella acute* (Michx.) Hulten.; H9c2 cardiomyoblasts; cardioprotective activity.

1. Introduction

About one hundred and twenty species of *Gentianella Moench* genus (*Gentianaceae*) were distributed in Americas, Asia and Europe. In China, about 8 species of this genus are being used for the treatment of hepatic, choleric and inflammatory diseases [1, 2]. The plant *G*entianella *acuta* (Michx.) Hulten., distributed in northeast of China, is used by the local people as a Mongolian Medicine for the treatment of arrhythmias and coronary disease. A previous chemical investigation of this plant revealed that the major chemical constituents are xanthones, flavonoids, seco-iridoid glycosides and triterpenoids [3-5]. And the report about the cardioprotective activity indicated that bellidifolin which was contained in this plant slowed heart rates of rats [6]. In the course of our chemical and pharmacological studies on *G. acuta* plants, we tried to examine the chemical

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constituents from this plant and isolated two new neolignan glucosides, **1** and **2**, together with six known lignans (**3**-**8**) (Fig. **1**). This paper mainly deals with the structure elucidation of these two new compounds and the activities of all the eight lignans, including the protective effects against H_2O_2-induced apoptosis in H9c2 cardiomyoblasts and the cytotoxicity against SGC-7901 and MCF-7.

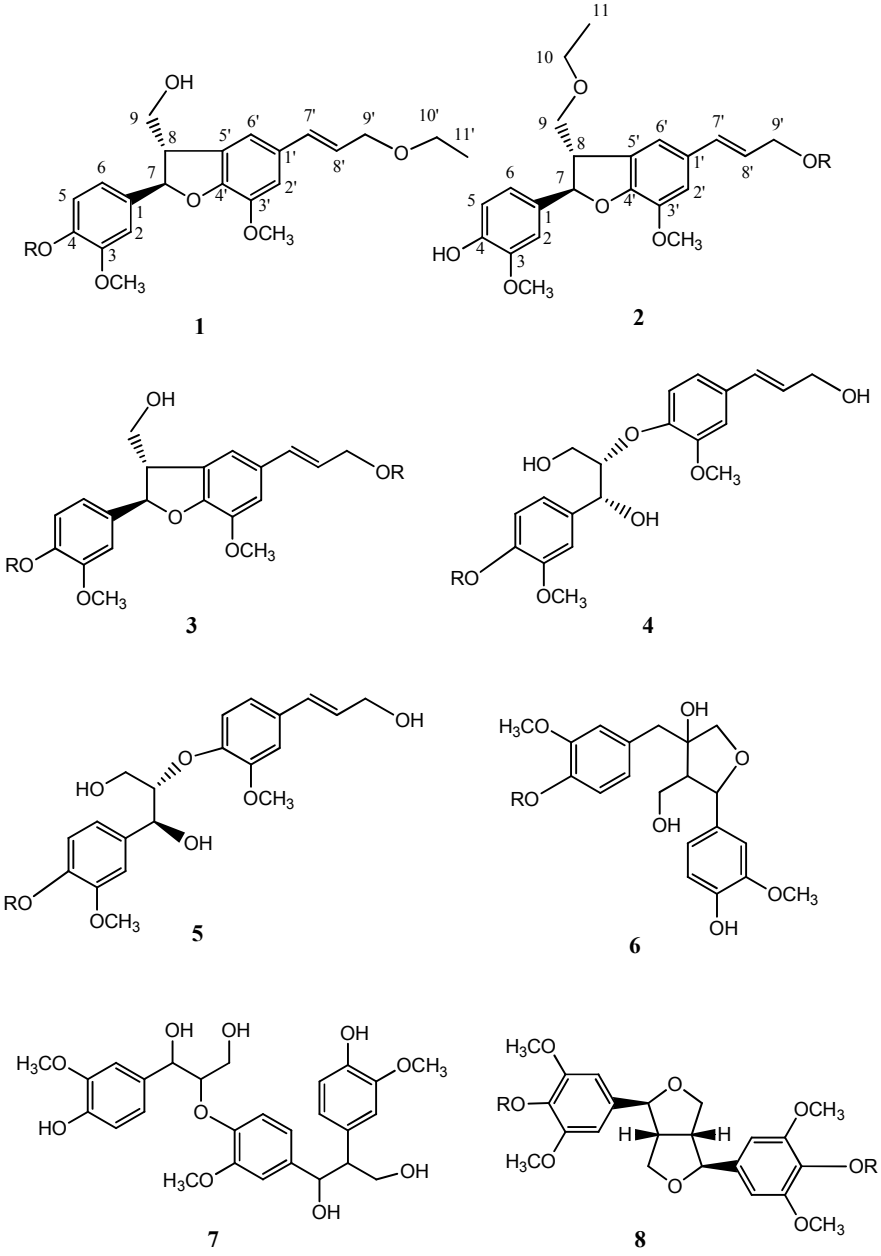

Figure 1. Structures of compounds **1-8** isolated from *Gentianella acuta* (Michx.) Hulten.

2. Materials and Methods

2.1. General

Open column chromatography was carried out using silica gel (200-300 mesh, Qingdao Marine Chemical Co., Qingdao, China) or octadecyl silica gel (ODS, 25-40 μm, Fuji, Tokyo, Japan) as stationary phase. TLC employed precoated silica gel plates (5-7 μm, Qingdao Marine, Qingdao, China). Preparative HPLC was carried out on a Waters 600 instrument equipped with a Waters 2414 refractive index detector. A Waters Sunfire prep C_{18} OBD (19 × 250 mm i.d.; Milford, PA, USA) column was used for preparative purpose. IR spectra were recorded on a Shimadzu FTIR- 8400S

spectrometer. The UV spectra were recorded on a Shimadzu UV-1601instrument and GC analyses were carried out using a Fuli 9790 instrument, DM-5 column (0.25 μm, 30 m × 0.25 mm, Dikma, Beijing, China). NMR spectra were recorded on a Bruker DPX 500 instrument (500MHz for ^{1}H NMR and 125MHz for ^{13}C NMR). Chemical shifts were given as values with reference to tetramethylsilane (TMS) as internal standard, and coupling constants were given in Hz. HR-ESI-MS were carried out using IonSpec Ultima 7.0 T FTICR.

2.2. Plant Material

The plants of *Gentianella acuta* (Michx.) Hulten.were collected from Great Khingan Range Mountains of China, on September 2010, and identified by Prof. Zhenyue Wang, of Heilongjiang University of Chinese Medicine. The voucher specimen (20100079) was deposited at the Herbarium of Heilongjiang University of Chinese Medicine, Harbin, China.

2.3. Extraction and Isolation

The dried plant of *Gentianella acuta* (Michx.) Hulten. (7.0 kg) were powdered and extracted 3 times with 75%EtOH (3×35 L) under reflux for 6 h. Evaporation of the solvent under reduced pressure provided a 75%EtOH extract (986 g), and through AB-8 macroporous resin, the extract was partitioned into an EtOH-H_2O (3:7; 6:4; 9:1, *v/v*) mixture to furnish three fractions: A (210 g), B (200 g) and C (230 g). Fr. A, was subjected to ordinary-phase silica gel column chromatography [3.0 kg, CH_2Cl_2-MeOH (10:1; 9:1; 8:1; 7:1; 6:1; 5:1; 4:1; 3:1; 2:1; 1:1, *v/v*)] to give six fractions (1-6). Fr. 1 (12.1 g) was subjected to reversed-phase silica gel column chromatography [(MeOH-H_2O) 1:9; 2:8; 3:7;4:6; 5:5; 6:4, *v/v*] to afford six fractions [Fr. 1-1 (236 mg), Fr. 1-2 (1.25 g), Fr. 1-3 (3.86 g), Fr. 1-4 (868 mg), Fr. 1-5 (902 mg), Fr. 1-6 (338 mg)]. Fr. 1-2 was separated by preparative HPLC using MeOH-H_2O (2:3, *v/v*) into **7** (47.5 mg, t_R = 16.4 min). Fr. 1-4 was separated by preparative HPLC using MeOH-H_2O (55:45, *v/v*) into **1** (43.5 mg, t_R = 33.2 min), Fr. 1-5 was separated by preparative HPLC using MeOH-H_2O (1:1, *v/v*) into **2** (26.1 mg, t_R = 34.1 min). Fr. 3 (8.05 g) was subjected to reversed-phase silica gel column chromatography (1:9; 2:8; 3:7; 4:6; 5: 5; 6: 4, *v/v*) to afford five fractions [Fr. 3-1 (820 mg), Fr.3-2 (1.61 g), Fr. 3-3 (2.10 g), Fr. 3-4 (907 mg), Fr. 3-5(512 mg)]. Fr. 3-2 was separated by preparative HPLC using MeOH-H_2O (28:72, *v/v*) into **4** (35.5 mg, t_R = 38.7 min), **5** (35.6 mg, t_R = 40.45 min), **6** (18.6 mg, t_R = 41.8 min). Compound **8** (568 mg) crystalized in Fr. 3-2. Fraction 5 (9.75 g) was treated in the same way as Fr. 1 and Fr. 3 to get six fractions [Fr. 5-1 (620 mg), Fr.5-2 (1.71 g), Fr. 5-3 (2.25 g), Fr. 5-4 (869 mg), Fr. 5-5(982 mg) , Fr. 5-6(502 mg)], and we got compound **3** (43.2 mg, t_R = 53.0 min) from Fr. 5-4 by preparative HPLC using MeOH-H_2O (3:7, *v/v*).

2.4. Acid hycrolysis

Acid hydrolysis was performed by a previously described method [7]. For this purpose, compounds **1**, **2** (each 3.0 mg) was hydrolyzed with 1 M HCl (1.0 mL) for 2 h at 85 ℃. The reaction mixture was cooled and partitioned between $CHCl_3$ (2.0 mL) and H_2O (2.0 mL). The aqueous layer was washed with $CHCl_3$ (3.0 mL × 3), neutralized, filtered, and evaporated under reduced pressure. The residue was dissolved in pyridine (1.0 mL) and 0.1 M L-cysteine methyl ester hydrochloride in pyridine (2.0 mL) was added. The mixture was heated at 60 ℃ for 1 h. An equal volume of Ac_2O was added with heating continued 1 h. The acetylated thiazolidine derivatives were analyzed by GC using a DM-5 Column (30 m × 0.25 mm, 0.25 μm). Temperatures of injector and detector were 280 ℃ for both. A temperature gradient system was used for the oven; starting at 160 ℃ and increasing up to 195 ℃ at a rate of 5 ℃/min. Peaks of the hydrolysate were detected by comparison with retention time of authentic samples of D-glucose (10.08 min) after treatment with L-cysteine methyl ester hydrochloride in pyridine.

2.5. The effects on H_2O_2-induced cytotoxicity in H9c2 cells

H9c2 cells (Cell Bank of the Chinese Academy of Science Shanghai, China) were grown in the high-glucose Dulbecco's modified Eagle's medium (Hyclone, USA) supplemented with 10% (*v/v*) fetal bovine serum (Hyclone, USA), 1% (*v/v*) L-glutamine, penicillin G (100 U/mL), and streptomycin (100 μg/mL) in a humidified 5% CO_2 atmosphere at 37℃. Cells at exponential growth were dissociated with 0.25% trypsin-0.02% EDTA and were seeded in the 96-well plate at a seeding density of 1×10^4/well before being incubated for 36 h. Cells were then treated with either compounds **1-8** to figure out their safe concentrations to be used in the bioassay. Cell viability was determined colorimetrically by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium (MTT) assay [8]. Quercetin (40 μM), which significantly inhibited cytotoxicity induced by H_2O_2 treatment, served as positive control in this study [9]. H9c2 cells were seeded at 1×10^4/well in the 96-well plates for 36 h. After 24 h of treatment with different safe concentrations of compounds **1-8** followed by incubation with 150 μM H_2O_2 for 6 h [9], 20 μL of 5 mg/mL MTT solution was added to each well (0.1 mg/well), and incubated for 4h.The supernates were aspirated, and the formazan crystals in each well were dissolved in 100 mL of DMSO. The absorbance was measured at 490 nm on a Microplate reader (Safire2, Tecan Group Ltd., Maennedorf, Switzerland).

2.6. Test for cytotoxicity in vitro

Two human cancer cell lines, gastric cancer SGC-7901 and breast cancer MCF-7 (purchased from Harbin Medical University Cancer Hospital), were used in the cytotoxicity assay. Cell viability was determined colorimetrically by MTT assay. All the cells were cultured in RPMI-1640 (Hyclone, USA), supplemented with 10% fetal bovine serum (Hyclone, USA). Briefly, cells were seeded in 96-well plate at a seeding density of 0.5×10^4/well and allowed to adhere for 24 h before drug addition. Each tumor cell line was exposed to the test compound dissolved in DMSO in triplicates for 24 h at 37℃, with DDP (Sigma, 99%) as positive controls. Then, 20 μL of 5 mg/mL MTT solution were added to each well (0.1 mg/well) and the tumor cells were incubated for another 4 h at 37℃. After the supernatant liquor was removed, 100 mL DMSO was added to each well. The absorbance was measured at 490 nm on a Microplate reader. Results were expressed as the percentages of reduced MTT, assuming the absorbance of control as 100%. Cell viability was detected and a cell growth curve was graphed. IC_{50} values were calculated using the Reed and Muench method [10].

3. Results and Discussion

3.1. Structure elucidation

Investigation of the whole plant of *Gentianella acuta* (Michx.) Hulten. led to the isolation of two new compound named acutoside A (**1**) and acutoside B (**2**), (7*R*, 8*S*)- dehydrodiconiferyl alcohol-4,9'-di-*O*-*β*-D-glucopyranoside (**3**) [11], alaschanisoside A (**4**), citrusin A (**5**) [12-13], 8,4',9'-trihydroxy-3,3'-dimethoxy-9,7'-epoxylignan 4-*O*-*β*-D-glucopyranoside (**6**) [14], leptolepisol D (**7**) [15], acanthoside D (**8**) [16]. All of them were isolated for the first time from this plant.

Compound **1** was obtained as a pale yellow amorphous solid, $[\alpha]^{25}_{,D}$= -1.40° (c = 0.9, MeOH). The HR-ESI-MS gave a molecular ion at *m/z* 571.2251 (calc. 571.2255, $[M+Na]^+$), and the molecular formula was deduced to be $C_{28}H_{36}O_{11}$. IR absorptions were observed at 3354 cm^{-1}, 1683 cm^{-1}, 1600 cm^{-1}, 1558 cm^{-1}, 1488 cm^{-1}, which indicated the presence of hydroxyl group and benzyl moiety. Its UV absorption showed two bands at λ_{max} 221, 278 nm. After acid hydrolysis, the gas chromatography (GC) analysis revealed the presence of D-glucose, and the glucose linkage was assigned as *β* from the observation of the anomeric proton coupling constant at *δ* 4.88 (1H, *d*, *J* = 7.5 Hz). The ^{1}H NMR (CD_3OD) (Tab. **1**) spectra of **1** also indicated the presence of two methoxy groups [*δ* 3.82 (3H, *s*), *δ* 3.87 (3H, *s*)] and an ABX-type aromatic ring [*δ* 7.02 (1H, *d*, *J* = 2.0 Hz), 7.13 (1H, *d*, *J* = 8.5 Hz), 6.92 (1H, *dd*, *J* = 8.5, 2.0 Hz)]. ^{13}C NMR (CD_3OD) (Tab. **1**) spectra of **1** revealed the existence of a glucosyl group at *δ* (C) 102.7, 74.9, 78.2, 71.3, 77.8, 62.5. As shown in Fig. **2**, the gross structure of **1**

was determined by analysis of NMR data including ^{1}H-^{1}H COSY, ^{1}H-^{13}C COSY, HMBC and NOESY experiments, and by referring to the data [17], we deduced **1** was a benzofuran lignan, the correlation in HMBC between C-4 (δ 147.7) and Glc-H_1 (δ 4.88) proved the attachment of the β-glucopyranolsyl to C-4 of the skeleton, and the correlation in HMBC between C-10' (δ 66.5) and H-11' [δ 1.20 (3H, *t*, *J* = 7.0 Hz)] indicated the presence of an ethyl. Furthermore, according to the signal between C-9' (δ 72.4) and H-10' [δ 3.54 (2H, *q*, *J* = 7.0 Hz)], this ethyl was connected to C-9'. Therefore ,**1** is shown to be 4-hydroxy-3,3'-dimethoxy-7,4'-epoxy-8,5'-neolign-7'-ene-9,9'-diol 9'-ethyl ether-4-*O*-β-D-glucopyranoside. The identification of the relative stereostructure of compound **1** is based on the very week NOE between H-7 and H-8, which indicated trans 7/8 configuration, it could also be confirmed from their coupling constant (*J* = 5.8 Hz) [18]. The absolute configuration of the dihydrofuran ring was determined to be 7*R* and 8*S* from the positive cotton effect at 283nm ($\Delta\varepsilon$ - 4.714) and 270nm ($\Delta\varepsilon$ - 4.654) in the CD spectrum, with reference to the CD data ($\Delta\varepsilon$ - 5.462 at 286nm and ($\Delta\varepsilon$ - 5.275 at 271nm) for (-)-(7*R*,8*S*,7'E)-4-Hydroxy-3,5'-dimethoxy-7,4'-epoxy-8,3'-neolign-7'-ene-9,9'-diol 9'-ethyl ether [19], Simultaneously, the configuration of H-7' and H-8' could be confirmed as trans-isomer from their large coupling constant (*J* = 15.5 Hz). Therefore, **1** was shown to be (-)-(7*R*,8*S*,7'*E*)-4-hydroxy-3,3'-dimethoxy-7,4'-epoxy-8,5'-neolign-7'-ene-9,9'-diol 9'-ethyl ether-4-*O*-β-D-glucopyranoside (**1**).

Compound **2** $[\alpha]^{25}_{,D}$= -8.26° (c=0.5, MeOH), which was as a pale yellow amorphous solid, had the molecular formula $C_{28}H_{36}O_{11}$. HR-ESI-MS, *m/z* 571.2251 (calc. 571.2255, $[M+Na]^+$). The IR spectra showed these absorptions: 3745 cm^{-1} 2921 cm^{-1}, 1665 cm^{-1}, 1558 cm^{-1}, 1505 cm^{-1},1450 cm^{-1}, 1054 cm^{-1}, 1016 cm^{-1}. Its UV absorption showed two bands at λ_{max} 222, 278 nm, similar to those of compound **1**. Comparison of the ^{1}H NMR and ^{13}C NMR (Tab. **1**) spectral data of **2** with **1** showed that the aglycone of **2** was similar to that of **1**, except for the signals of the sugar moiety and the ethyl moiety. This suggested that **2** had the same aglycone as **1**. The ^{1}H NMR spectra of **2** indicated the presence of one β-glucopyranolsyl unit [Glc-H_1: δ 4.24 (1H, *d*, *J* = 7.5 Hz), Glc-C_1: δ 103.0], and the acid hydrolysis of **2** only gave D-glucose. Furthermore, the planar structure of **2** was unambiguously confirmed by means of ^{1}H-^{1}H COSY and HMBC experiments, a long-range correlation was observed between Glc-H_1 (δ 4.24) and C-9' (δ 70.0) of the aglycone, and the correlation in HMBC between C-9 (δ 70.4) and H-10 [δ 3.44 (2H, *q*, *J* = 7.0 Hz)] showed that the ethyl was connected to C-9. Important HMBC interactions of **2** are shown in Fig. **2**. The relative stereochemistry of the dihydrofuran ring was elucidated by an NOE experiment, the very week NOE between H-7 and H-8 indicated trans 7/8 configuration [17], it could also be confirmed from their coupling constant (*J* = 6.0 Hz) [18]. The absolute configuration of the dihydrofuran ring was determined to be 7*R* and 8*S* from the positive cotton effect at 275nm ($\Delta\varepsilon$ – 1.818) and 235nm ($\Delta\varepsilon$ + 0.364) in the CD spectrum, with reference to the CD data ($\Delta\varepsilon$ – 1.797 at 275nm and ($\Delta\varepsilon$ + 1.369 at 239nm) for (-)-(7*R*,8*S*,7'*E*)-4-hydroxy-3,5,5'-trimethoxy-7,4'-epoxy-8,3'-neolign-7'-ene-9,9'-diol 9'-ethyl ether [19]. The configuration of H-7' and H-8' could be confirmed as trans-isomer from their large coupling constant (*J* = 15.5 Hz). Thus, the structure was represented as (-)-(7*R*,8*S*,7'*E*)-4-hydroxy-3,3'-dimethoxy-7,4'-epoxy-8,5'-neolign-7'-ene-9,9'-diol 9-ethyl ether-9'-*O*-β-D-glucopyranoside (**2**).

The new neolignan glycosides were isolated from the EtOH extraction of *G. acuta*. Are these new compounds with ethoxy group the artifacts since EtOH is used as extraction solvent? In order to figure out this problem, we exacted the dried plant with MeOH, and analysed the MeOH extraction of *G. acuta* with UPLC-MS/MS. The molecular ions (*m/z* 571.2252 $[M+Na]^+$, *m/z* 571.2254 $[M+Na]^+$) of the two new compounds were found in the total ion chromatogram (TIC). Finally, we concluded that these compounds existed in this plant, and they were new natural products.

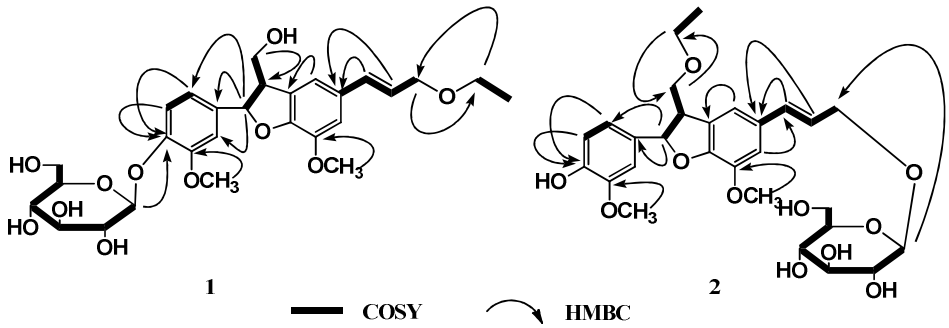

Figure. 2. ^{1}H-^{1}H COSY correlations and the selected HMBC correlations of compounds **1** and **2**.

Table. 1. ^{1}H NMR and ^{13}C NMR data of **1** and **2** in CD_3OD (δ in ppm, *J* in Hz, recorded at 500 MHz and 125 MHz, respectively).

Position	**1**		**2**	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1	—	138.0	—	132.1
2	7.02 (1H, *d*, *J* = 2.0 Hz)	111.2	6.93 (1H, *d*, *J* = 2.0 Hz)	110.6
3	—	151.0	—	147.5
4	—	147.7	—	146.3
5	7.13 (1H, *d*, *J* = 8.5 Hz)	118.0	6.73 (1H, *d*, *J* = 8.5 Hz)	115.3
6	6.92 (1H, *dd*, *J* = 8.5, 2.0 Hz)	119.4	6.77 (1H, *dd*, *J* = 8.5, 2.0 Hz)	118.3
7	5.57 (1H, *d*, *J* = 5.8 Hz)	88.8	5.52 (1H, *d*, *J* =6.0 Hz)	87.0
8	3.45 (1H, *m*)	55.4	3.58 (1H, *m*)	50.6
9	3.77 (1H, *dd*, *J* = 11.5, 7.5 Hz) 3.84 (1H, *m*)	64.9	4.03 (2H, *dd*, *J* = 6.5,1.5 Hz)	70.4
10	—	—	3.44 (2H, *q*, *J* = 7.0 Hz)	64.6
11	—	—	1.13 (3H, *t*, *J* = 7.0 Hz)	15.1
1'	—	132.4	—	130.2
2'	6.95 (1H, *d*, *J* = 1.0 Hz)	112.2	6.97 (1H, *d*, *J* = 1.5 Hz)	110.4
3'	—	145.6	—	143.7
4'	—	149.4	—	147.3
5'	—	130.1	—	128.8
6'	6.95 (1H, *d*, *J* = 1.0 Hz)	116.6	7.03 (1H, *s*)	115.6
7'	6.54 (1H, *d*, *J* = 15.5 Hz)	133.9	6.51 (1H, *d*, *J* = 16.0 Hz)	131.5
8'	6.17 (1H, *dt*, *J* = 15.5, 6.0, 6.5 Hz)	124.7	6.20 (1H, *dt*, *J* = 16.0, 6.0, 5.5Hz)	124.2
9'	4.10 (2H, *dd*, *J* = 6.5, 6.0 Hz)	72.4	3.03 (2H, *m*)	70.0
10'	3.54 (2H, *q*, *J* = 7.0 Hz)	66.5	—	—
11'	1.20 (3H, *t*, *J* = 7.0 Hz)	15.4	—	—
Glc-1	4.88 (1H, *d*, *J* = 7.5 Hz)	102.7	4.24 (1H, *d*, *J* = 7.5 Hz)	103.0
Glc-2	3.44 (1H, *m*)	74.9	3.01 (1H, *m*)	73.5
Glc-3	3.37 (1H, *m*)	78.2	3.13 (1H, *m*)	77.0
Glc-4	3.37 (1H, *m*)	71.3	3.66 (1H, *m*)	70.4
Glc-5	3.46 (1H, *m*)	77.8	3.09 (1H, *m*)	76.8
Glc-6	3.62 (1H, *m*) 3.82 (1H, *m*)	62.5	3.64 (1H, *m*) 3.42 (1H, *m*)	61.1
3-OCH_3	3.82 (3H, *s*)	56.7	3.73 (3H, *s*)	55.6
3'-OCH_3	3.87 (3H, *s*)	56.8	3.80 (3H, *s*)	55.7

3.2. Protective effects on H_2O_2-induced cytotoxicity in H9c2 cells

Cytotoxicity evaluation showed that compounds **1-8** were all non-cytotoxic below 200 μM. As can be seen in Fig. **3**, cell viability significantly reduced after exposure to 150 μM H_2O_2 for 6 h as compared to the control group. The positive control Quercetin markedly increased the cell viability at 40 μM. The cell viability of compounds **1-8** were shown in Tab. **2**, and these observations suggested

that compounds **1-8** could attenuate H_2O_2-induced injury to some extent. Especially, compounds **1**,**3**,**6**,**8** which could increase cell viability >15% at 200 μM had remarkably protective influence against H_2O_2-induced apoptosis.

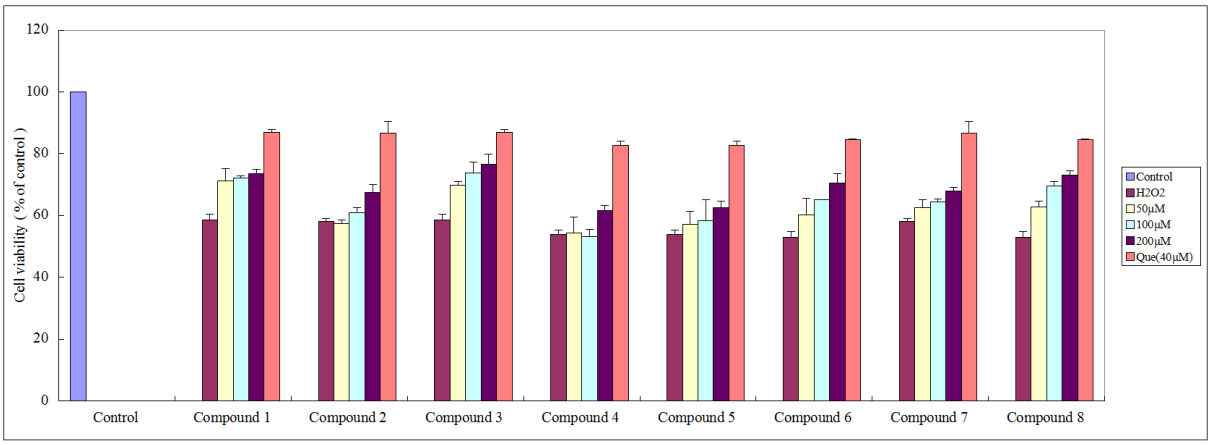

Figure. 3. Effects of compounds **1-8** on H_2O_2-induced H9c2 cells injury

Cells were pretreated with safe concentrations of compounds **1-8** for 24 hours before exposed to 150 μM H_2O_2 for 6 hours .Cell viability were measured by MTT assay. The data are expressed as means ± SD from three independent experiments.

Table. 2. The protective effects of different concentration of compounds **1-8** on H_2O_2-induced H9c2 cell injury (Means ± SD, n = 3)

Concentration	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
(μM)	Cell Viability (%)							
H_2O_2	58.44±2.04$^{\#}$	58.00±0.99$^{\#}$	58.40±2.04$^{\#}$	53.94±1.41$^{\#}$	53.94±1.41$^{\#}$	52.90±1.89$^{\#}$	58.00±0.99$^{\#}$	52.90±1.89$^{\#}$
50	71.11±4.07*	57.49±0.98	69.89±1.07**	54.45±5.09	57.11±4.29	60.18±5.44	62.48±2.56*	62.83±1.87**
100	72.11±0.84**	60.91±1.50*	73.78±3.56**	53.13±2.31	58.26±6.97	65.00±0.20**	64.41±0.96**	69.62±1.24**
200	73.56±1.39**	67.39±2.64**	76.56±3.36**	61.55±1.59**	62.55±2.21**	70.59±3.07**	67.94±1.22**	73.02±1.54**
Quercetin	86.89±0.84**	86.69±3.65**	86.89±0.84**	82.76±1.41**	82.76±1.41**	84.43±0.39**	86.69±3.65**	84.43±0.39**

$^{\#}$, P<0.01 vs. control, **, P<0.01 vs. H_2O_2 model group, and *, P<0.05 vs. H_2O_2 model group.

3.3. Cytotoxicity

Cytotoxicity of compounds **1-8** was evaluated against human gastric cancer SGC-7901 and breast cancer MCF-7 by the MTT assay. But all the compounds showed no cytotoxicity against the two cancer cell lines with IC_{50} values over 40 μM.

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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