Notes

Monoterpene Glucosides from *Paeonia lactiflora*

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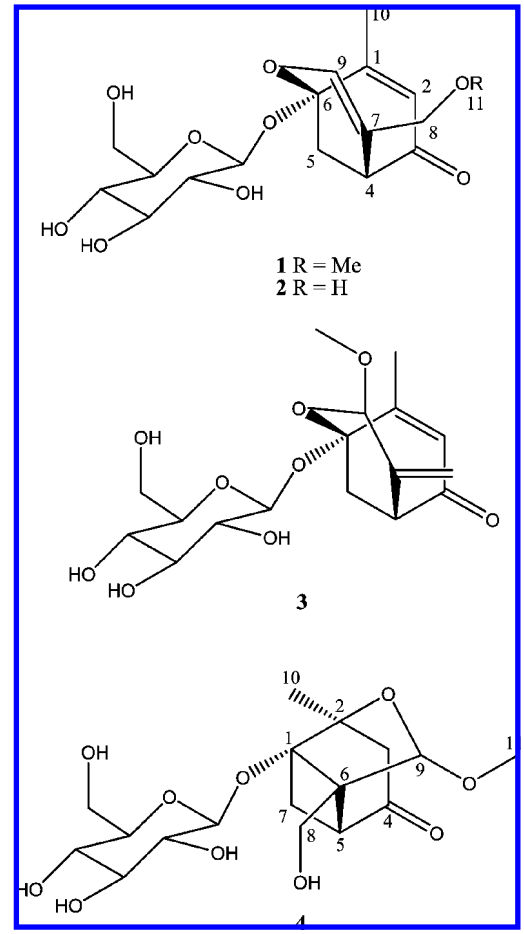
Four new "cage-like" monoterpene glucosides (**1**−**4**) were isolated from *Paeonia lactiflora.* The structures of these compounds were established by spectroscopic methods, mainly 1D and 2D NMR, and mass spectrometric analysis. Compound **4** exhibited moderate cell-protective activity against hydrogen peroxide-induced PC12 cell damage.

Paeonia lactiflora Pall. (Ranunculaceae) is a well-known ornamental plant with ornate flowers blooming in spring in mainland China. Its dried whole roots and skin-free roots are officially listed in the Chinese Pharmacopoeia (2005 edition) as the commonly used traditional Chinese medicines "Chi-Shao" and "Bai-Shao", respectively, used for anti-inflammatory, analgesic, and sedative purposes.[1] The chemical constituents of *P. lactiflora* have been investigated in detail, resulting in the isolation of various monoterpene compounds, among which paeoniflorin, the main constituent of the roots, possesses a "cage-like" skeleton.[2−4] Paeoniflorin and other "cage-like" monoterpene derivatives show antihyperglycemic, anti-inflammatory, antioxidant, and other biological activities.[1,5,6] Paeoniflorin has been patented for the treatment of dementia and is considered as a candidate compound for cognitive enhancement.[7] In the course of a search for nootropic compounds from Chinese medicinal plants, we have investigated the dried roots of *P. lactiflora*. From an ethanol extract of the plant roots, four new monoterpene glucosides were isolated and named paeonins A (**1**), B (**2**), and C (**3**) and 8-debenzoylpaeonidanin (**4**). In this paper, we describe the isolation and structural elucidation of compounds **1**−**4** as well as their in vitro cell-protective activities.

A 95% EtOH extract of the plant roots (5.0 kg) was extracted with petroleum ether, ethyl acetate, and *n*-butanol, successively. From a series of column chromatographic separations, **1** (12 mg), **2** (8 mg), **3** (13 mg), and **4** (5 mg) were obtained from the ethyl acetate extract.

Paeonin A (**1**), a colorless oil, showed the molecular formula $C_{17}H_{24}O_9$, as determined by HRESIMS *m*/*z* 395.1286 $[M + Na]^+$ (calcd for $C_{17}H_{24}O_9Na$, 395.1318), together with the ^1H and ^{13}C NMR data. The UV spectrum revealed an α,β-unsaturated ketone at 252 nm. The IR spectrum displayed absorption bands for a hydroxyl group (3365 cm^{-1}), double bonds (1612, 1640 cm^{-1}), and a conjugated carbonyl group (1679 cm^{-1}). Compound **1** was positive in the Molish test. Hydrolysis of **1** with β-glucosidase yielded glucose as a sugar moiety, as determined by co-HPTLC. The ^1H NMR data (Table 1) contained the signals for an olefinic methyl [δ 2.06 (3H, d, *J* = 1.3 Hz, H-10)], a methoxy [δ 3.14 (3H, s, H-11)], two nonequivalent methylenes [δ 2.48 (1H, dd, *J* = 13.2, 3.4 Hz, H-5α), 2.59 (1H, dd, *J* = 13.2, 2.6 Hz, H-5β) and δ 3.69 (1H, d, *J* = 11.9 Hz, H-8α), 3.94 (1H, d, *J* = 11.9 Hz, H-8β)],



three methines [δ 3.29 (1H, dd, *J* = 3.4, 2.6 Hz, H-4), δ 6.05 (1H, q, *J* = 1.3 Hz, H-2), δ 6.70 (1H, s)], and one glucose unit [δ 5.43 (1H, d, *J* = 7.9 Hz, H-1′), 3.68 (1H, m, H-2′), 3.57 (1H, m, H-3′), 3.43 (1H, m, H-4′), 3.46 (1H, m, H-5′), 3.73 and 3.94 (2H, m, H-6′)]. The ^{13}C NMR data (Table 2) contained signals for two methyls (one olefinic, one oxygenated), two methylenes (one oxygenated), three methines (two olefinic), and four quaternary carbons (one carbonyl, two olefinic, one semiketal), together with a β-glucopyranosyl unit. These spectroscopic features suggested that **1** is a monoterpene glucoside, and its monoterpene moiety

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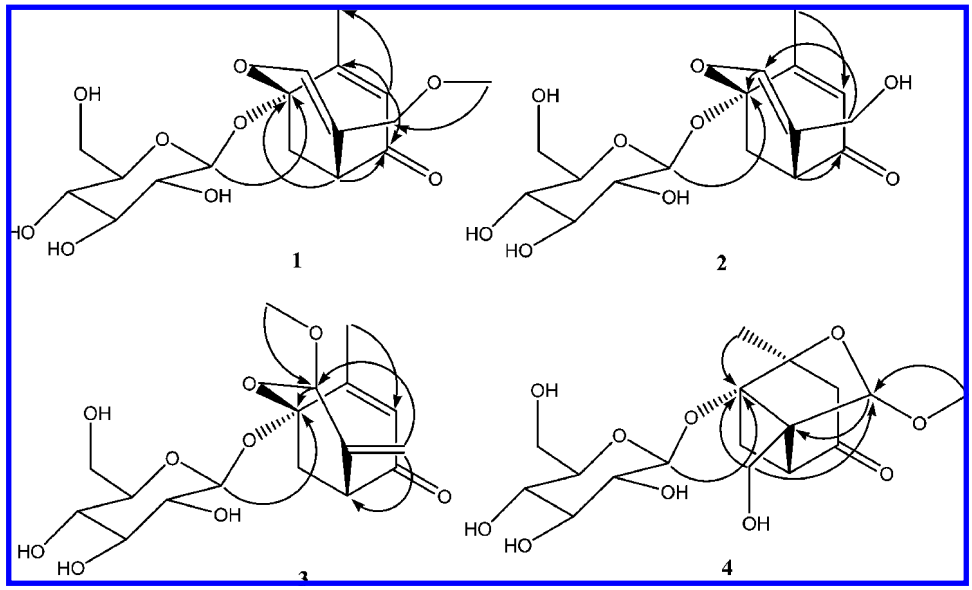

Figure 1. Key HMBC (H→C) correlations of compounds **1**−**4**.

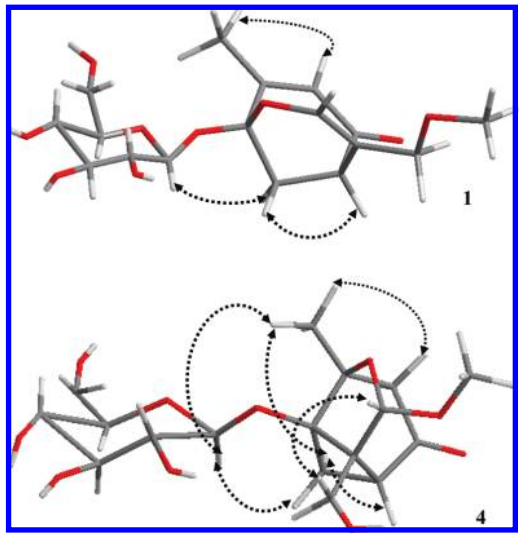

Figure 2. Key ROESY correlations of compounds **1** and **4**.

resembled partly those of paeonisuffral and paeoniflorigenone, isolated from *Paeonia suffruticosa* and *Paeonia albiflora*, respectively.[8,9]

The HMBC correlations (Figure 1) of H-2 with C-1, C-4, and C-10, H-9 with C-4 and C-6, and H-4 with C-3 confirmed the structure for **1**. Additionally, the HMBC correlation of H-1′ with C-6 and H_3-11 with C-8 suggested glucosylation occurred at C-6 and methoxylation at C-8. The β-glucosyl linkage was deduced from the coupling constant of the anomeric proton at δ 5.43 (J = 7.9 Hz). The stereostructure of compound **1** was determined by ROESY correlations of H-1′ with H-5α, H-4 with H-5α and H-5β, and H-2 with Me-10 (Figure 2). Finally, the assignments of all protons and carbons were made unambiguously by ^{1}H−^{1}H COSY, HMQC, HMBC, and ROESY NMR experiments. On the basis of the above evidence, the structure of paeonin A was determined as **1**.

Paeonin B (**2**), an amorphous powder, showed a molecular formula of $C_{16}H_{22}O_9$ by HRESIMS (*m*/*z* 381.1182, [M + Na]$^+$). The ^{1}H and ^{13}C NMR data (Tables 1 and 2) were very similar to those of **1** except for the absence of a methoxy group. On the basis of analysis of the HMBC (Figure 1), HMQC, and ^{1}H−^{1}H COSY spectra, the structure of paeonin B was established as **2**.

Paeonin C (**3**), an amorphous powder, showed the molecular formula $C_{17}H_{24}O_9$, on the basis of its HRESIMS (*m*/*z* 395.1326, [M + Na]$^+$). Hydrolysis of **3** with β-glucosidase yielded glucose as the sugar moiety, as determined by co-HPTLC. The ^{1}H NMR data (Table 1) revealed signals for an olefinic methyl [δ 2.17 (3H, d, J = 1.3 Hz, H-10)], a methoxy [δ 3.66 (3H, s, H-11)], an exocyclic olefinic methylene [δ 5.39 (1H, s, H-8β), 5.45 (1H, s, H-8α)], a nonequivalent methylene [δ 2.73 (1H, br d, J = 11.7 Hz, H-5α), 3.36 (1H, dd, J = 11.7, 3.4 Hz, H-5β)], an olefinic methine [δ 5.88 (1H, br s, H-2)], and two methines [δ 5.32 (1H, s, H-9), 3.61(1H, d, J = 3.4 Hz, H-4)], together with one glucose unit [δ 5.53 (1H, d, J = 7.8 Hz, H-1′), 3.60 (1H, m, H-2′), 3.51 (1H, m, H-3′), 3.45 (1H, m, H-4′), 3.52 (1H, m, H-5′), 3.70 and 3.92 (2H, m, H-6′)]. The ^{13}C NMR data (Table 2) contained signals for one olefinic methyl, one methoxy, two methylenes (one olefinic), three methines (one olefinic, one acetal), and four quaternary carbons (one ketone, two olefinic, one semiketal), together with one glucose unit. This suggested that **3** is a monoterpene glucoside with a structure similar to **1**. However, when compared with **1**, compound **3** exhibited an exocyclic olefinic methylene and an additional hemiacetal methine instead of a trisubstituted double bond and an oxygenated methylene, which suggested the structure shown. The HMBC correlations (Figure 1) of the glucosyl H-1′ with C-6, the methoxy group proton with C-9, H-8 with C-4 and C-9, and H-10 with C-2 confirmed the proposed structure for **3**. Furthermore, the β-glucosyl linkage was deduced from the coupling constant (J = 7.8 Hz) of the anomeric proton at δ 5.53. The ROESY spectrum showed correlations of H-1′ with H-5α, and H-4 with H-5β and H-5α, and indicated that compound **3** has the same stereostructure as compound **1**. Thus, the structure of paeonin C was elucidated as **3**.

Compound **4** was obtained as a colorless oil and gave the molecular formula $C_{17}H_{26}O_{10}$, on the basis of the HRESIMS (*m*/*z* 413.1398, [M + Na]$^+$). The ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) of **4** demonstrated the presence of a ketone carbonyl group, a tertiary methyl group, a methoxy, an oxygenated methylene, two methylenes, one acetal methine, and three quaternary carbons (two oxygenated), besides characteristic glucose signals. Detailed analysis of the NMR data revealed that the signals of **4** were very similar to those of paeonidanin, a monoterpene glucoside isolated from *Paeonia peregrina* and *Paeonia lactiflora*, except for the absence of a benzoyl group linked with the OH at C-8.[10,11] The HMBC spectrum (Figure 1) revealed correlations of the glucosyl H-1′ with C-1, H_3-10 with C-1, H_2-8 with C-1, H_2-8 with C-9, H-9 with C-6,

Table 1. ^{1}H NMR Data of Compounds **1**−**3** (400 MHz, *J* in Hz, pyridine-d_5)

position	1	2	3	4
1				
2	6.05 q (1.3)	6.04 q (1.3)	5.88 br s	
3				2.51 br s
4	3.29 dd (3.4, 2.6)	3.27 dd (3.4, 2.6)	3.61 d (3.4)	
5α	2.48 dd (13.2, 3.4)	2.48 dd (13.1, 3.4)	2.73 br d (11.7)	3.03 d (7.5)
5*β*	2.59 dd (13.2, 2.6)	2.56 dd (13.1, 2.6)	3.36 dd (11.7, 3.4)	
6				
7α				2.21 d (11.0)
7*β*				2.80 dd (11.0, 7.5)
8α	3.69 d (11.9)	3.65 d (11.9)	5.45 s	3.60 s
8*β*	3.94 d (11.9)	3.90 d (11.9)	5.39 s	
9	6.70 s	6.78 s	5.32 s	5.04 s
10	2.06 d (1.3)	2.04 d (1.3)	2.17 d (1.3)	1.60 s
11	3.14 s		3.66 s	3.36 s
1′	5.43 d (7.9)	5.39 d (7.9)	5.53 d (7.8)	5.16 d (7.4)
2′	3.68 m	3.65 m	3.60 m	3.59 m
3′	3.57 m	3.59 m	3.51 m	3.47 m
4′	3.43 m	3.48 m	3.45 m	3.42 m
5′	3.46 m	3.41 m	3.52 m	3.48 m
6′	3.73 m	3.81 dd (12.0, 6.0)	3.70 m	3.69 m
	3.94 m	3.99 dd (12.0, 6.0)	3.92 m	3.91 m

and H_3-11 with C-9. The ROESY spectrum (Figure 2) showed correlations of H-1′ with H-7*β*, H-8 with H-9, H-5 with H-7α, and H-10 with H-1′, H-3, and H-7α and suggested compound **4** has the same relative stereostructure as paeonidanin.[10,11] Further analysis of the HMQC, HMBC, ^{1}H−^{1}H COSY, and ROESY spectra confirmed the structure of compound **4** as 8-debenzoylpaeonidanin.

The monoterpene glucosides **1**−**3** possess new structural features with double bonds and hemiketal units in a "cage-like" skeleton, not found previously from *Paeonia* and other species. The yields of **1**−**3** in the roots were very low. To confirm their natural occurrence, the EtOAc fraction of the ethanol extract of *P. lactiflora* was analyzed by HPLC and resulted in the detection of **1** and **3**. From the viewpoint of biosynthesis, it can be inferred that **1**−**3** are derived from paeonisuffral,[8] a known monoterpene glucoside from *Paeonia suffruticosa*, by cyclic cleavage, dehydration, methylation, and glucosylation. In a cell protection assay on compounds **1**−**4**, compound **4** showed moderate protective activities against H_2O_2-induced cell damage in PC12 cells (Figure 3).

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Horiba Sepa-300 polarimeter. IR spectra were recorded using a Perkin-Elmer 577 spectrometer. NMR spectra were run on a Bruker AM-400 spectrometer with TMS as internal standard. LRESIMS were measured using a Finnigan LCQ-DECA instrument, and HRESIMS data were obtained on a JEOL SX-102 mass spectrometer. HPLC (Agilent 1100 series) was performed on a Zorbax Extend-C_{18} (Agilent Technologies) column (4.6 × 250 mm; mobile phase, MeOH−H_2O, gradient elution; flow rate, 1.0 mL/min; detection, UV absorption at 210 and 254 nm). Column chromatographic separations were carried out on silica gel H-60 (Qingdao Haiyang Chemical Group Corporation, Qingdao, People's Republic of China), Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden), and LiChroprep RP-18 (40−63 *μ*m, Merck). HSGF254 silica gel TLC plates (Yantai Chemical Industrial Institute, Yantai, People's Republic of China) were used for analytical TLC. HPTLC plates (Merck) were used for co-TLC assay. D-Glucopyranose (Chemprosa Holding AG) was used as a standard. Molecular energy-minimized 3D models were calculated by Chemoffice 2004 software.

Table 2. ^{13}C NMR Data of Compounds **1**−**4** (100 MHz, pyridine-d_5)

position	1	2	3	4
1	154.7	154.8	161.1	87.9
2	127.5	127.5	124.3	85.6
3	195.9	196.2	196.4	48.5
4	44.0	44.0	47.9	204.6
5	32.2	32.4	33.8	46.7
6	98.6	98.4	97.6	62.9
7	108.9	112.0	138.3	26.0
8	70.5	60.3	117.2	59.1
9	142.9	141.1	102.8	105.8
10	17.2	17.1	17.1	20.3
11	57.1		55.8	55.8
1′	97.8	98.4	97.6	96.3
2′	74.9	74.8	75.0	74.9
3′	78.4	78.4	78.7	78.3
4′	71.1	71.1	71.6	70.2
5′	78.6	78.7	79.1	79.0
6′	62.5	62.4	62.8	62.1

Plant Material. The roots of *Paeonia lactiflora* were supplied by Shanghai LG Life Company, Shanghai, People's Republic of China, in July 2004. The materials were authenticated by Mr. Liang Wang of Shanghai LG Life Company, where a specimen (No. 040510) is deposited.

Extraction and Isolation. The air-dried and powdered roots of *Paeonia lactiflora* (5.0 kg) were extracted exhaustively with 95% EtOH (10 L × 3) at room temperature. The extract was concentrated in vacuo to yield an EtOH extract (300 g), which was then suspended in distilled water and partitioned successively with petroleum ether, EtOAc, and *n*-BuOH. The EtOAc extract was concentrated in vacuo to give a residue (65 g), which was applied to a silica gel column and eluted with a gradient chloroform−methanol solvent system of increasing polarity (50:1 → 10:1) to afford five fractions (A−E). Fraction B was subjected

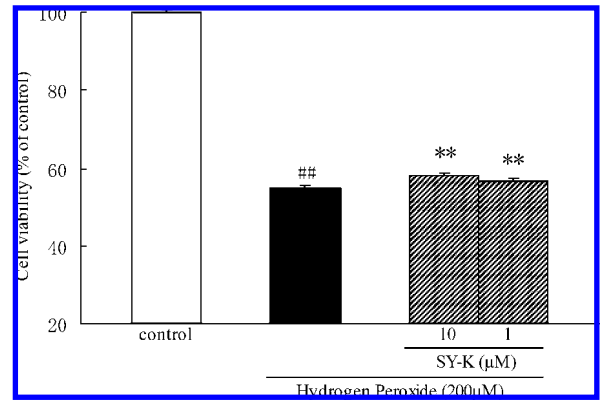

Figure 3. Effects of compound **4** on cell viability. Viability of PC12 cells was obtained by measuring the MTT reduction. Two independent experiments were carried out in triplicate. All data were expressed as percentage of control values. $^{\#\#}p < 0.01$ vs control. $^{**}p < 0.01$ vs H_2O_2 group.

to repeated column chromatography over silica gel ($CHCl_3$−MeOH, 30:1; 10:1) and LiChroprep RP-18 eluted with MeOH−H_2O (3:2) to afford compounds **1** (12 mg, 0.00024%) and **2** (8 mg, 0.00016%). Fraction D was separated by repeated column chromatography over silica gel ($CHCl_3$−MeOH, 10:1; 5:1) and LiChroprep RP-18 eluted with MeOH−H_2O (1:1) and Sephadex LH-20 eluted with MeOH to afford compounds **3** (13 mg, 0.00025%) and **4** (5 mg, 0.00010%).

Paeonin A (1): colorless oil; $[\alpha]^{20}_D$ −36.5 (*c* 0.1, pyridine); UV (MeOH) λ_{max} (log ϵ) 252 (3.84) nm; IR (KBr) ν_{max} 3365, 1679, 1640, 1612 cm^{-1}; ^1H NMR (400 MHz, pyridine-d_5) and ^{13}C NMR data (100 MHz, pyridine-d_5), see Tables 1 and 2; HRESIMS *m*/*z* $[M + Na]^+$ 395.1286 (calcd for $C_{17}H_{24}O_9Na$, 395.1318).

Paeonin B (2): white, amorphous powder; $[\alpha]^{20}_D$ −37.8 (*c* 0.1, pyridine); UV (MeOH) λ_{max} (log ϵ) 254 (3.82) nm; IR (KBr) ν_{max} 3360, 1675, 1638, 1610 cm^{-1}; ^1H NMR (400 MHz, pyridine-d_5) and ^{13}C NMR data (100 MHz, pyridine-d_5), see Tables 1 and 2; HRESIMS *m*/*z* $[M + Na]^+$ 381.1182 (calcd for $C_{16}H_{22}O_9$ Na, 381.1161).

Paeonin C (3): white, amorphous powder; $[\alpha]^{20}_D$ −45.1 (*c* 0.1, pyridine); UV (MeOH) λ_{max} (log ϵ) 256 (3.80) nm; IR (KBr) ν_{max} 3370, 1668, 1650, 1613 cm^{-1}; ^1H NMR (400 MHz, pyridine-d_5) and ^{13}C NMR (100 MHz, pyridine-d_5), see Tables 1 and 2; HRESIMS *m*/*z* $[M + Na]^+$ 395.1326 (calcd for $C_{17}H_{24}O_9Na$, 395.1318).

8-Debenzoylpaeonidanin (4): colorless oil; $[\alpha]^{20}_D$ −30.2 (*c* 0.1, pyridine); IR (KBr) ν_{max} 3430, 1700 cm^{-1}; ^1H NMR (400 MHz, pyridine-d_5) and ^{13}C NMR (100 MHz, pyridine-d_5), see Tables 1 and 2; HRESIMS *m*/*z* $[M + Na]^+$ 413.1398 (calcd for $C_{17}H_{26}O_{10}Na$, 413.1424).

Hydrolysis of Compounds 1 and 3. Compound **1** (4 mg) was dissolved in H_2O (8 mL) and incubated with almond β-glucosidase (20 mg) for 24 h at 37 °C. After general treatment, the sugar moiety was analyzed by silica gel HPTLC (Merck) developed with Me_2CO−2 mM NaOAc (17:3, v/v) and detected by spraying with 0.2% naphthoresorcinol in Me_2CO−3 N H_3PO_4 (5:1, v/v), followed by heating at 105 °C for 5 min (R_f 0.53). D-Glucopyranose was used as standard. The same procedure was used for compound **3**.

HPLC Analysis of the EtOAc Extract of *P. lactiflora*. The EtOAc extract and compounds **1**−**4** were analyzed by HPLC using a Zorbax Extend-C_{18} (Agilent Technologies) column (4.6 × 250 mm) with monitoring of UV absorption at 210 and 254 nm. The mobile phase used was a mixture of MeOH and H_2O in which the proportion of MeOH was increased from 10% to 100% over a 60 min period. The flow rate was 1.0 mL/min. This resulted in the detection of **1** and **3** in the EtOAc extract. The retention times of compounds **1**−**4** were 26.16, 19.21, 17.51, and 16.35 min, respectively.

Assay for Cell-Protective Activities. Evaluation of cell-protective activities of compounds **1**−**4** against H_2O_2-induced PC12 cell damage was performed according to a protocol reported in the literature.[12] Cells were incubated with 200 μM H_2O_2 for 30 min, and the cultures were further developed for another 6 h. Samples were added to the cultures 2 h prior to H_2O_2 addition. Cell viability was obtained by measuring MTT reduction. Two independent experiments were carried out in triplicate. All data were expressed as a percentage of control values. Statistical comparison was made by using one-way ANOVA and followed by Duncan's test. The data were expressed as means ± SEM.

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