Supporting Information to:

Three New Acylated Glycosides from the Stems of *Casearia velutina* and their Protective Effect Against H$_2$O$_2$-Induced Impairment in PC12 Cells

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**Materials and methods**

For semi-preparative HPLC, a Waters 600 Controller, a Waters 600 Pump, a Waters 2996 Photodiode Array Detector (UV 254 nm), and a Waters ODS column (i.d. 7.8 × 300 mm)
were used. Optical rotations were recorded on a Perkin-Elmer 243B digital polarimeter. NMR spectra were recorded on Inova 500 MHz NMR spectrometers. HR-ESI-MS spectra were measured on a Bruker APEX IV FTMS mass spectrometer in positive-ion mode. GC were measured on an Agilent 6890N instrument [HP-5 capillary column (28 m × 0.32 mm i.d.); detection, FID; detector temperature, 260 °C; column temperature, 180 °C; carrier gas, N2; flow rate, 40 mL/min]. All solvents used were of analytical grade (Beijing Chemical and Industry Factory). Silica gel (200–300 mesh; Qingdao Mar. Chem. Ind. Co. Ltd.), Sephadex LH-20 gel (Pharmacia), and C$_{18}$ reversed-phase silica gel (150–200 mesh, Merck, performed by applying an N$_2$ pressure of 0.12 Mpa) were used for column chromatography.

The stems of *C. velutina* were collected in Menglun County, Yunnan Province, China, in June 2005. The plant materials were identified by engineer Jing-Yun Cui (Xishuangbanna Botanical Garden). A voucher specimen (CV20050605) is deposited in the herbarium of the Peking University Modern Research Center for Traditional Chinese Medicine.

**Extraction and isolation**

The *n*-BuOH extract (120 g) was subjected to silica gel column chromatography (CC) and eluted with a gradient of CHCl$_3$–MeOH (10:1–2:1) to afford nine fractions (Frs. I–IX).

Fr. IV (25 g) was subjected to silica gel CC (4.0 × 60 cm; 400 g) and eluted with PE–acetone (1:2; 4.5 L) to afford 8 fractions (Frs. IV$_1$–IV$_8$). A separation of Fr. IV$_1$ (2.5 g) by Sephadex LH-20 (1.5 × 100 cm; MeOH) yielded 4 (90 mg), and the impurity was purified by ODS (2.0 × 60 cm; MeOH:H$_2$O, 45:55) to get 5 (5.0 mg). Compound 8 (25 mg) was obtained from Fr. IV$_2$ (3.0 g) by ODS (2.0 × 60 cm; MeOH:H$_2$O, 1:1) isolation and Sephadex LH-20 (1.5 × 100 cm; MeOH) purification. Fr. IV$_3$ (1.8 g) was subjected to Sephadex LH-20 (1.0 × 50 cm; MeOH), followed by semi-preparative HPLC (33% MeOH–H$_2$O, Rt = 14.3 min) to yield 14 (5.0 mg). Fr. IV$_4$ (0.8 g) was isolated by semi-preparative HPLC (30% MeOH–H$_2$O) to provide 2 (10 mg, Rt = 12.4 min) and 3 (7.0 mg, Rt = 13.8 min). Fr. IV$_7$ (3.0 g) was applied to silica gel CC (2.0 × 60 cm; 100 g) and eluted with EtOAc–MeOH–H$_2$O (20:1:0.02; 300 mL). The main fraction was separated by semi-preparative HPLC (35% MeOH–H$_2$O) to afford 12
(16 mg, Rt = 15.9 min) and 13 (80 mg, Rt = 19.7 min). Compound 15 (28 mg) also was obtained from Fr. IV₈ (0.6 g) by semi-preparative HPLC (35% MeOH–H₂O; Rt = 18.2 min).

Fr. VI (10 g) was subjected to silica gel CC (4.0 × 60 cm; 400 g) and eluted with CHCl₃–MeOH (4:1; 2000 mL) to obtain two portions (A and B). Portion B was further subjected to silica gel CC (4.0 × 60 cm; 400 g) with elution of PE–acetone (1:3; 1000 mL), followed by semi-preparative HPLC (25% MeOH–H₂O) separation to afford 1 (13 mg, Rt = 23.7 min) and 6 (203 mg, Rt = 17.8 min).

Fr. VII (10 g) was isolated by silica gel CC (4.0 × 60 cm; 300 g) eluted with a gradient of EtOAc–MeOH (15:1, 10:1 and 7.5:1, each 1500 mL) to get Frs. VII₁–VII₆. A portion of Fr. VII₅ (2.0 g) was applied to Sephadex LH-20 (2.0 × 60 cm; MeOH), followed by semi-preparative HPLC (20% MeOH–H₂O) to afford 7 (25 mg, Rt = 20.1 min), and the reminder of the eluate was subjected to HPLC (12% MeOH–H₂O) again to yield 9 (5.0 mg) and 10 (6.0 mg). Compound 16 (10 mg) was obtained from Fr. VII₆ (1.0 g) by Sephadex LH-20 (2.0 × 60 cm; MeOH) purification.

**Acid hydrolysis and sugar analysis**

Each compound (1–3, 2 mg each) was heated in 5 mL of 10% HCl–dioxane (1:1) at 80 °C for 4 h. After removing the dioxane, the solution was extracted with EtOAc (3 mL × 3). The aqueous layer was neutralized with NaHCO₃ and evaporated until dryness. A small amount of solid residue was dissolved in MeOH and analyzed by TLC (CHCl₃–MeOH–H₂O 8:5:1). The spots were visualized by spraying 95% EtOH–H₂SO₄–anisaldehyde [9:0.5:0.5 (v:v:v)], followed by heating at 120 °C for 10 min. Glucose (Rₚ = 0.30) was detected in compounds 1–3. TLC results were confirmed by GC analyses. The solid residue from the aqueous layer was dissolved in anhydrous pyridine (100 mL). L-cysteine methyl ester hydrochloride (0.1 M, 200 mL) was added, and the mixture was warmed at 60 °C for 1 h. The trimethylsilylation reagent hexamethyldisilazane (HMDS)/chlorotrimethylsilane/pyridine 2:1:10 (Acros Organics) was added, and the mixture was warmed at 60 °C for 30 min. The thiazolidine derivative was analyzed by GC for sugar identification: D-glucose derivative (tᵣ = 12.45 min).
Bioactivity assay

Cell culture and treatment: Rat pheochromocytoma line, undifferentiated PC12 cells were routinely maintained in F12 Kaighn’s medium supplemented with 10% horse serum, 2.5% fetal bovine serum, and streptomycin (100 μg/mL)–penicillin (100 IU/mL) at 37 °C under an atmosphere of 95% air and 5% CO2. After the cells attached (24 h) to the plates, they were pretreated with each compound for 1 h and then incubated with 150 μM/L H2O2 for 24 h. After each treatment, the MTT assay was performed in order to evaluate cell viability. The treated cells were incubated with 0.5 mg/mL MTT at 37 °C for 4 h in the dark, and 200 μL DMSO was added to the culture medium and mixed with a pipette until the blue formazan dissolved completely. The optical density of formazan was measured at 570 nm using a microplated reader. (Sunrise; Tecan). The control consisted of the medium plus DMSO or water with no added compound; its absorbance was set at 100%. Cell viability was determined by MTT assay. PC12 cells were treated with 150 μM H2O2 (model) or distilled water (control) or 150 μM H2O2 in the presence of 10 μM of compound for 24 h. Values are presented as means ± SD from three separate experiments in triplicate.

Enzyme inhibition assay: 33 mM Tris–HCl buffer pH 8.8, 30 mM with 0.00035 U/well final concentration using a microtiter plate assay, and 0.33 mM bis-(p-nitrophenyl) phosphate (Sigma N-3002) as substrate. L-Cysteine (Tianjin BODI Chemical Co., Ltd.) and EDTA (Sigma) were used as positive controls (with inhibitory rates of 5.65% and 72.4%, respectively, at 5 μM). After 30 min pre-incubation of the enzyme with the test samples (all with purity >95%), enzyme activity was monitored spectrophotometrically at 37 °C on a microtiter plate reader (Bio-Rad model 680) by following the rate (change in O.D./min) of release of p-nitrophenol from p-nitrophenyl phosphate at 405 nm. All assays were conducted in triplicate.
Fig. 1S Structures of compounds 4–16.
Fig. 2S $^1$H-NMR spectrum of compound 1 (CD$_3$OD, 500 MHz).
Fig. 3S Expanded $^1$H-NMR spectrum of compound 1.
**Fig. 4S** $^{13}$C-NMR spectrum of compound 1 (CD$_3$OD, 125 MHz).
Fig. 5S DPET spectrum of compound 1 (CD$_3$OD, 125 MHz).
**Fig. 6S** $^1$H-NMR spectrum of compound 2 (DMSO-$d_6$, 500 MHz).
Fig. 7S Expanded $^1$H-NMR spectrum of compound 2 (DMSO-$d_6$, 500 MHz).
Fig. 8S Expanded $^1$H-NMR spectrum of compound 2 (DMSO-$d_6$, 500 MHz).
**Fig. 9** $^{13}$C-NMR spectrum of compound 2 (DMSO-$d_6$, 125 MHz).
Fig. 10S $^1$H-NMR spectrum of compound 3 (DMSO-$d_6$, 500 MHz).
Fig. 11S Expanded $^1$H-NMR spectrum of compound 3 (DMSO-$d_6$, 500 MHz).
Fig. 12S $^{13}$C-NMR spectrum of compound 3 (DMSO-$d_6$, 125 MHz).