Supporting Information

New Serratene Triterpenoids from *Palhinhaea cernua* and Their Cytotoxic Activity

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Materials and Methods

General experimental procedures

Optical rotations were measured with a Horiba SEPA-300 polarimeter. IR spectra (KBr) were recorded on a Bruker Tensor 27 spectrophotometer in cm\(^{-1}\). NMR spectra were recorded on a Bruker AV-600 instrument with TMS as an internal standard. ESIMS was recorded on a VG-Autospec-3000 spectrometer, and HREIMS was recorded on a MAT95XP. Silica gel (200-300 mesh; Qingdao Marine Chemical), Lichroprep RP-18 (40-63 um; Merck), and Sephadex LH-20 (Pharmacia Fine Chemical Co. Ltd.) were used for column chromatography (CC). Fractions were monitored by TLC, and spots were visualized by heating TLC sprayed with 10% H\(_2\)SO\(_4\).

Plant material

The plants of *Palhinhaea cernua*, pre-sprayed with coronalon (0.1 mg/mL in water), was collected at Sangzhi County, Hunan Province, China, in October 2009, identified by Prof Fu-Wu Xing, South China Botanical Garden, the Chinese Academy of Sciences (CAS). A voucher specimen (No: 20090522) has been deposited at the Laboratory of Phytochemistry, South China Botanical Garden, CAS.

Extraction and isolation

The powdered material (10 kg) was exhaustively extracted with 90% EtOH at room
temperature, and the ethanol extract was combined and evaporated to near dryness to get a residue, which was re-suspended in 3000 mL of MeOH/H₂O (1:9) and partitioned with EtOAc (1500 mL × 4) to give an EtOAc fraction (230 g). The majority of the EtOAc (230 g) was then absorbed on 300 g silica gel and fractionated by column chromatography over silica gel (75-150 μm, 1300 g, 8 × 100 cm), eluting with CHCl₃:MeOH [100:0 (3 L), 60:1 (2 L), 40:1 (2.5 L), 30:1 (2 L), 10:1 (2.5 L), 5:1 (2 L)] to afford Fr.1-Fr.8 after pooling according to their TLC profiles. Fr.3 (34 g), obtained from the elution of CHCl₃/MeOH of 40:1, was subjected to silica gel CC (75-150 μm, 1000 g, 5 × 80 cm CHCl₃:MeOH 98:2 to 95:5, 6 L) to get ten sub-fractions, of which Fr.3.2 (8 g) was repeatedly purified by silica gel CC (75-150 μm, 300 g, 2 × 60 cm, 99:1 to 97:3, 2 L) to get compounds 1 (12 mg), 2 (9 mg), and 6 (16 mg). Fr. 3.6 (1 g) was further purified by semipreparative reversed-phase HPLC using a Fuji Chromatatorex RP-18 column (Fuji Silysia Chemical Ltd., 250 mm × 10 mm i.d., 45-75 μm) with a solvent system of MeOH in H₂O (from 80 % to 90 %, 1.2 mL, flow rate: 2 mL/min) as the eluent to obtain 3 (7 mg), and further purified by Sephadex LH-20 (CH₃OH, 2.5 L) to yield 4 (10 mg). Fr.5 (19 g), obtained from the elution of CHCl₃/MeOH of 10:1, was subjected to CC [75-150 μm, 600 g, 4 × 80 cm, CHCl₃:MeOH 15:1 (3 L), 12:1 (3.5 L), 9:1 (2.5 L)] to obtain a white precipitate directly from the concentrated sub-elution solutions and further washed with methanol to get pure compound 5 (50 mg).

Cytotoxic assay
The cytotoxic activity of compounds 1-6 against human cancer cell lines K-562, SGC-7901, and SMMC-7721 were assessed using the MTT method as described in literature [17]. Mitomycin C (Kyowa Hakko Kogyo Co.; purity ≥ 98%) was used as reference compound to assess the cytotoxicity of tested compounds. Briefly, cells were plated in 96-well plates 24 h before treatment and continuously exposed to different concentrations of tested and reference compounds. After incubation for 48 h, 20 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (40 μM) was added to each well, which were incubated for a further 4 h. Then, 20% sodium dodecyl sulfate (100 μL) was added to each well. After 12 h at room temperature, the OD values of each well were measured at 595 nm, and the IC\textsubscript{50} values were calculated by the Reed and Muench method. Tested compounds were demonstrated to be pure as evidenced by HPLC and NMR analyses (purity ≥ 95%).
Figures

1S $^1$H-NMR spectrum of compound 1 (pyridine-$d_5$, 600 MHz)

2S $^{13}$C-NMR spectrum of compound 1 (pyridine-$d_5$, 150 MHz)

3S $^1$H-NMR spectrum of compound 2 (pyridine-$d_5$, 600 MHz)

4S $^{13}$C-NMR spectrum of compound 2 (pyridine-$d_5$, 150 MHz)

5S $^1$H-NMR spectrum of compound 3 (pyridine-$d_5$, 600 MHz)

6S $^{13}$C-NMR spectrum of compound 3 (pyridine-$d_5$, 150 MHz)

7S $^1$H-NMR spectrum of compound 4 (pyridine-$d_5$, 600 MHz)

8S $^{13}$C-NMR spectrum of compound 4 (pyridine-$d_5$, 150 MHz)
FIG. 15. The 1H-NMR spectrum of compound 1 (pyridine-d$_5$, 600 MHz).
FIG. 3S. The 1H-NMR spectrum of compound 2 (pyridine-d5, 600 MHz).
FIG. 4. THE 13C-NMR SPECTRUM OF COMPOUND 2 (PYRIDINE-d5, 150 MHz)
Figure 5.5: The 1H-NMR spectrum of compound 3 (pyridine-d5, 600 MHz)
FIG. 7S THE 1H-NMR SPECTRUM OF COMPOUND 4 (PYRIDINE-d5, 600 MHz)
Fig. 8S. The 13C-NMR spectrum of compound 4 (pyridine-d5, 150 MHz).