Supplementary Information

Cytotoxic constituents from the sclerotia of *Poria cocos* against human lung adenocarcinoma cells by inducing mitochondrial apoptosis

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2.1. General experimental procedures

Infrared (IR) spectra were recorded on a Bruker IFS-66/S FT-IR spectrometer. Ultraviolet (UV) spectra were recorded with an Agilent 8453 UV-visible spectrophotometer (Agilent Technologies, CA, USA). Nuclear magnetic resonance (NMR) spectra were obtained from a Bruker AVANCE III 700 NMR spectrometer operating at 700 MHz (1H) and 175 MHz (13C) (Bruker), with chemical shifts given in ppm (δ). LC/MS analysis was performed on an Agilent 1200 Series HPLC system equipped with a diode array detector, a 6130 Series ESI mass spectrometer, and an analytical Kinetex C18 100 Å column (100 mm × 2.1 mm i.d., 5 μm) (Phenomenex, Torrance, CA, USA). Preparative high-performance liquid chromatography (HPLC) was performed with a Waters 1525 Binary HPLC pump with a Waters 996 Photodiode Array (PDA) Detector (Waters Corporation, Milford, CT, USA). Semi-preparative HPLC was conducted with a Shimadzu Prominence HPLC System with SPD-20A/20AV Series Prominence HPLC UV-Vis Detectors (Shimadzu, Tokyo, Japan). Silica gel 60 (Merck, 230-400 mesh) and reversed-phase (RP)-C18 silica gel (Merck, 230-400 mesh) were used for column chromatography. Merck precoated silica gel F254 plates and RP-18 F254s plates were used for thin-layer chromatography (TLC). Spots on the TLC plates were detected under UV light or by heating after spraying with anisaldehyde-sulfuric acid.

2.2. Sample material

Sclerotia of *P. cocos* imported from China were purchased from Kyung-dong Herbal Medicine Market, Seoul, in January 2014. A voucher specimen of the material (PC1308) was identified by one of the authors (K. H. Kim) and deposited in the herbarium of the School of Pharmacy, Sungkyunkwan University, Suwon, Korea.

2.3. Extraction and isolation

Dried sclerotia of *P. cocos* (350.0 g) were extracted with 70% aqueous EtOH (each 2.0 L × 3 days) at room temperature. The extract was evaporated under reduced pressure with a rotavapor to yield the EtOH extract (23.3 g), which was suspended in distilled water (1.8 L) and MeOH (30.0 mL) and successively partitioned with hexane, dichloromethane (CH2Cl2), ethyl acetate (EtOAc), and n-butanol (BuOH). Four layers with increasing polarity were obtained: the hexane-soluble (6.5 g), CH2Cl2-soluble (2.3 g), EtOAc-soluble (2.7 g), and BuOH-soluble fractions (13.7 g). The hexane-soluble fraction (6.5 g) was separated by silica gel column chromatography (200 g, eluted with hexane/EtOAc [2:1]) to afford four fractions (H1-H4). Fraction H1 (4.3 g) was separated on an RP-C18 column eluted with 95% MeOH, yielding 8 subfractions (H11-H18). Subfraction H17 (112.0 mg) was
separated by semi-preparative HPLC (87% MeOH) on a Phenomenex Luna HPLC phenyl-hexyl column (250 × 10 mm; flow rate: 2 mL/min), yielding compound 4 (tR 63.0 min, 1.7 mg). Fraction H2 (1.8 g) was chromatographed on an RP-C18 column (70 g, eluted with 90% MeOH), generating 7 subfractions (H21-H27). Subfraction H26 (46.0 mg) was fractionated by preparative HPLC (95% MeOH) on an Agilent Eclipse C18 column (21.2 × 250 mm; flow rate: 5 mL/min), and 9 subfractions (H261-269) were obtained. Subfraction H265 (10.3 mg) was purified by semi-preparative HPLC (92% MeOH) with a Phenomenex Luna phenyl-hexyl column (250 × 10 mm; flow rate: 2 mL/min) to afford compound 2 (tR 20.5 min, 1.8 mg). On the same column, subfraction H268 (7.0 mg) was also separated by semi-preparative HPLC (87% MeCN), yielding compound 1 (tR 18.0 min, 1.0 mg). Fraction H3 (585.0 mg) was subjected to RP-C18 column chromatography (25 g, eluted with 92% MeOH) to afford 5 subfractions (H31-35). Subfraction H33 (50.3 mg) was separated by semi-preparative HPLC (83% MeOH) with a Phenomenex Luna phenyl-hexyl column (250 × 10 mm; flow rate: 2 mL/min) to yield compound 3 (tR 32.5 min, 3.9 mg).

The EtOH extract of the sclerotia of *P. cocos* and the compounds isolated from it were prepared as stock solutions at concentrations of 100 mg/mL and 20 mM, respectively, in dimethyl sulfoxide (DMSO, Sigma, St. Louis, MO, USA) and stored at -80°C until use.