Supporting Information

Cucurbitane-Type Triterpenoids from *Momordica charantia*

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Extraction and isolation procedure

The powdered whole plant material of *M. charantia* (1816 g) was extracted exhaustively with MeOH at room temperature (4 L x 16, 2 hr each time) and concentrated *in vacuo* to yield 110 g of MeOH extract. The 110 g of MeOH extract were repeatedly extracted with hexane to remove lipids (2 L x 8, 2 hr each time), and 97 g of defatted MeOH extract were obtained. The individual compounds were isolated from the 97 g of defatted MeOH extract based on their polarities using a Biotage Isolera One Flash Purification System. The 97 g of defatted MeOH extract were divided into four parts of approximately 24 g. Each part was subjected to silica gel column chromatography (340 g, 40-63 µm, 71 x 168 mm, CHCl₃-MeOH, from 100:0 to 0:100, 100 mL/min flow rate). The resulting fractions were combined according to their silica gel TLC profiles to give five combined fractions, A–E. Fraction A (13.4 g) was separated over silica gel column chromatography (340 g, 40-63 µm, 71 x 168 mm, CHCl₃-MeOH, from 100:0 to 80:20, 100 mL/min flow rate) to produce four combined subfractions, A₁–A₄. A₁ (6.9 g) was further separated over silica gel column chromatography (340 g, 40-63 µm, 71 x 168 mm, CHCl₃-MeOH, from 95:5 to 80:20, 100 mL/min flow rate) to give four combined subfractions, A₁₁–A₁₄. A₁₂ (1.3 g) was purified over RP-C18 column chromatography (120 g, 30-70 µm, 39 x 157 mm, H₂O-MeOH, from 30:70 to 0:100, 20 mL/min flow rate, monitored at 205 nm) to yield compound 2 (53 mg). A₂ (3.3 g) was separated over RP-C18 column chromatography (120 g, 37-70 µm, 39 x 157 mm, H₂O-MeOH, from 50:50 to 0:100, 20 mL/min flow rate, monitored at 205 nm) to give combined subfraction A₂₁ (978 mg). A₂₁ was purified over RP-C18 column chromatography (120 g, 37-70 µm, 39 x 157 mm H₂O-MeOH, from 30:70 to 0:100, 20 mL/min flow rate, monitored at 205 nm) to yield compound 3 (422
mg). A₄ (1.0 g) was purified over RP-C18 column chromatography (120 g, 37-70 µm, 39 x 157 mm, H₂O-MeOH, from 50:50 to 0:100, 20 mL/min flow rate, monitored at 205 nm) to yield compounds 4 (636 mg) and 1 (58 mg). Fraction C (7.4 g) was separated over silica gel column chromatography (340 g, 40-63 µm, 71 x 168 mm, CHCl₃-MeOH, from 80:20 to 60:40, 100 mL/min flow rate) to produce five combined subfractions, C₁-C₅. C₃ (1.8 g) was further separated over RP-C18 column chromatography (120 g, 37-70 µm, 39 x 157 mm, H₂O-MeOH, from 50:50 to 20:80, 20 mL/min flow rate, monitored at 205 nm) to give combined subfraction C₃-1 and compound 6 (343 mg). C₃-1 (421 mg) was purified over RP-C18 column chromatography (60 g, 37-70 µm, 39 x 81 mm, H₂O-MeOH, from 40:60 to 10:90, 20 mL/min flow rate, monitored at 205 nm) to yield compound 5 (21 mg).

**In vitro insulin secretion assay**

**Cell culture:** MIN6 β-cells were grown at 37°C with 5% CO₂ in DMEM media (Invitrogen) with 15% fetal bovine serum (Invitrogen), 1% penicillin/streptomycin (Invitrogen), 1:200 gentamicin (Invitrogen), and 1% basal medium eagle (Invitrogen). Cells were trypsinized and split into 6-well plates until confluent.

**In vitro insulin secretion assay:** Compound 1 was re-suspended in DMSO to make a stock solution of 125 mg/mL. Confluent cells were incubated with Kreb’s Ringer buffer (KRB) for 1 hr. Cells were then rinsed with fresh KRB twice out, and an aliquot of the second wash was saved as a baseline insulin measurement. Cells were then incubated for 1 hr with KRB (negative control), 50 µM glipizide with 27 mM glucose (positive control), DMSO (vehicle control), and 15.8, 39.4, 118.3, and 197.2 µM compound 1,
respectively. Insulin measurements were collected at 15 and 60 min, and insulin concentrations were determined by ELISA (ALPCO).

**DNA extraction:** To obtain a total cell count, DNA was extracted by incubating cells overnight with 1% SDS/10 mM EDTA/10 mM Tris lysis buffer and 1% proteinase K. Phenol-chloroform 1:1 was added, and cells were centrifuged. Sodium acetate-isopropanol 1:3 was added to the supernatant, and material was incubated at -80°C for 1 hr. Precipitated DNA was then washed twice with 70% ethanol out, and air-dried. DNA was reconstituted with 10:1 Tris-EDTA buffer (pH 8.0) out, and read with a spectrophotometer at 260 nm at a dilution of 1:50. The conversion factor of 5.8 µg of DNA = 1.0 x 10^6 cells was used.

**Statistical analysis:** Statistical differences were determined using a two-way ANOVA. Differences were considered significant at p values of < 0.05.