Supporting Information to:

Cytotoxic Flavonoids and New Chromenes from *Ficus formosana f.*

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Materials and Methods

General
Uncorrected melting points were determined on a YANACO micromelting apparatus. Optical rotations were measured on a JASCO DIP-370 polarimeter. CD spectra were measured on a JASCO J-810 spectropolarimeter. IR spectra were recorded on a PerkinElmer System 2000 FT-IR spectrophotometer. UV spectra were obtained on a JASCO UV-240 spectrophotometer. EI-MS spectra were measured on a Finnigan/Thermo Quest MAT 95XL spectrometer, and HR-EI-MS spectra were recorded on a JEOL JMS-HX 110 spectrometer. The NMR spectra were measured on a Varian Unity Plus 400 spectrometer. Chemical shifts are given in ppm (δ) with TMS as internal standard. Sephadex™ LH-20 (Amersham Biosciences) and silica gel 60 (Merck 70-230 mesh, 230-400 mesh, ASTM) was used for CC and silica gel 60 F254 (Merck) for TLC.

Plant material
The stems of *F. formosana* f. *formosana* were collected from Pingtung County, Taiwan in July 2001 and identified by Prof. Dr. Ih-Sheng Chen. A voucher sample (No. 4938) is deposited in the Herbarium of the School of Pharmacy, College of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan, R.O.C.

Extraction and isolation
The dried stems (14.3 kg) of *F. formosana* f. *formosana* were exhaustively extracted with cold MeOH and concentrated *in vacuo* to yield a dark residue (350 g). The MeOH extract was partitioned into an *n*-hexane- (fr. A, 60 g), a CHCl₃- (fr. B, 100 g), an *n*-BuOH- (fr. C, 100 g) and a H₂O- (fr. D, 90 g) soluble fraction. Fr. B (100 g) was chromatographed over a silica gel (3 kg) column and eluted with a step gradient of CHCl₃-MeOH (20:1, 10:1, 5:1, 2:1, 1:1, MeOH, 4 l each) to provide 9 frs (B-1 to 9). Fr. B-2 (CHCl₃-MeOH, 10:1, 254 mg) was re-fractionated over a silica gel (8 g) column eluted with a gradient of CH₂Cl₂/EtOAc (CH₂Cl₂, 100:1, 50:1, 20:1, 10:1, EtOAc, 60 ml each) to give 9 frs (B 2-1 to 8). Fr. B-2-3 (CH₂Cl₂/EtOAc 50:1, 36 mg) was purified with preparative TLC (*n*-hexane/EtOAc 5:1) to yield 9 (1.7 mg, Rf = 0.33, *n*-hexane/acetone 10:1) and 11 (26 mg, Rf = 0.67, *n*-hexane/EtOAc 5:1). Fr. B-4 (CHCl₃/MeOH 10:1, 40.8 g) was re-fractionated on silica gel (1.2 kg) and eluted with a gradient of *n*-hexane/EtOAc (5:1, 3:1, 2:1, 1:1, EtOAc, 1.5 l each) to give 11 frs (B-4-1 to 11).
Fr. B-4-2 \((n\text{-hexane/EtOAc 3:1, 136 mg})\) was subjected to a silica gel (4 g) column and eluted with a gradient of \(n\text{-hexane/EtOAc (50:1, 20:1, 10:1, 5:1, EtOAc, 25 ml each)}\) to obtain 7 frs (B-4-2-1 to 7). Crystalline frs of B-4-2-4 \((n\text{-hexane/EtOAc 20:1, 160 mg})\) and B-4-2-5 \((n\text{-hexane/EtOAc 10:1, 15 mg})\) were recrystallized from MeOH to obtain 3 (26 mg, \(R_f = 0.30, n\text{-hexane/EtOH 10:1}\)) and 4 (1 mg, \(R_f = 0.38, n\text{-hexane/acetone 10:1}\)), respectively. Fr. B-4-6 \((n\text{-hexane/EtOAc 3:1, 831 mg})\) was subjected to a Sephadex LH-20 (30 g) column eluted with MeOH (50 ml) to give 7 frs (B-4-6-1 to 7). Crystalline frs of B-4-6-5 \((n\text{-hexane/EtOAc 20:1, 71 mg})\) and B-4-9 \((n\text{-hexane/EtOAc 1:1, 4.43 g})\) were recrystallized from MeOH to yield 5 (2.5 mg, \(R_f = 0.38, n\text{-hexane/acetone 3:1}\)) and 6 (78 mg, \(R_f = 0.50, n\text{-hexane/acetone 1:1}\)), respectively.

The mother liquor (3.68 g) of B-4-9 was subjected to Sephadex LH-20 (120 g) and eluted with MeOH (100 ml) to provide 7 frs (B-4-9-1 to 7). Fr. B-4-9-4 (MeOH, 817 mg) was re-fractionated on silica gel (30 g) eluted with a gradient of \(CH_2Cl_2/MeOH (CH_2Cl_2, 100:1, 50:1, 30:1, 10:1, 5:1, MeOH, 50 ml each)\) to provide 12 frs (B-4-9-4-1 to 12). Fr. B-4-9-4-3 \((CH_2Cl_2/MeOH 100:1, 35.7 mg)\) was purified with preparative TLC \((n\text{-hexane/EtOAc 3:1})\) to obtain 10 (14.5 mg, \(R_f = 0.30, n\text{-hexane/EtOAc 3:1}\)). Fr. B-4-9-4-5 \((CH_2Cl_2/MeOH 50:1, 159 mg)\) was re-subjected to silica gel (6 g) eluted with a gradient of \(n\text{-hexane/acetone (2:1, acetone, 25 ml each)}\) to provide 4 frs (B-4-9-4-5-1 to 4). Fr. B-4-9-4-5-2 \((n\text{-hexane/acetone 2:1, 37.8 mg})\) was purified with preparative TLC \((CHCl_3/EtOAc 10:1)\) to yield 1 (3.2 mg, \(R_f = 0.34, n\text{-hexane/acetone 3:1}\)) and 2 (4.3 mg, \(R_f = 0.36, n\text{-hexane/EtOAc 2:1}\)). Fr. B-6 \((CHCl_3/MeOH 10:3, 2.59 g)\) was subjected to Sephadex LH-20 (50 g) and eluted with MeOH (200 ml) to provide 11 frs (B-6-1 to 11). Fr. B-6-8 (MeOH, 71 mg) was purified with preparative TLC \((CH_2Cl_2/acetone 5:1)\) to give 7 (10 mg, \(R_f = 0.32, CH_2Cl_2/acetone 1:1\)) and 8 (6 mg, \(R_f = 0.38, CH_2Cl_2/acetone 2:1\)).

Spatheliachromen \((3)\): \(C_{13}H_{14}O_4\) [6], [8].

Obovatin \((4)\): \(C_{20}H_{16}O_4\) [9].

Carpachromene \((5)\): \(C_{20}H_{16}O_5\) [10].

Norartocarpetin \((7)\): \(C_{15}H_{10}O_6\) [11].

Steppogenin \((8)\): \(C_{15}H_{12}O_6, [\alpha]_D^{25}: +19.2^\circ (c 0.02, MeOH)\) [12].

6-Prenylnocembrin \((9)\): \(C_{20}H_{20}O_4, [\alpha]_D^{25}: +79.38^\circ (c 0.13, CHCl_3)\) [13], [14].

Chromenylacrylic acid \((10)\): \(C_{15}H_{16}O_4\) [15], [16].

(R)-(−)-Mellein \((11)\): \(C_{10}H_{10}O_3, [\alpha]_D^{25}: −136.9^\circ (c 0.125, CHCl_3)\) [17].
**Cytotoxicity assay**

The cytotoxicity of compounds against HepG2 [ATCC HB-8065; human hepatoma: HBV Ag (-)], PLC/PRF/5 [ATCC HB-8024; human hepatoma: HBV Ag (+)] and Raji (ATCC CCL-86; lymphoma) and (ATCC TIB-202; acute monocytic leukemia) cancer cell lines *in vitro* was assayed with the XTT method [19], [20]. In brief, after incubation at 37°C with 5% CO₂ for 3 days, the XTT mixtures were added to the cells, and incubation was continued for additional 2 hours. Optical densities (OD₄₅₀-OD₆₉₀) were determined with the ELISA reader, and cytotoxicity was expressed as [1-(ODₜ/ODₙₐ) x 1000 %], ODₜ and ODₙₐ indicate the absorbance of the tested compounds and the solvent (DMSO) control, respectively. The concentration of 50% cytotoxicity (IC₅₀) of tested compounds was calculated [19].

**Statistical analysis**

Data were presented as the mean ± SE from three independent experiments. The cytotoxic potency of isolated compounds against human cancer cells was evaluated using the one-way ANOVA and multiple-comparison using Scheffe method. Differences were considered significant when the *p*-value was < 0.05.

**Gibbs test**

Gibbs reagent (2,6-dibromoquinonechloroimide) could react with the phenolic compound (the position *para* to the hydroxy must be un-substituted) to form the blue coloured compound in a buffered solution of pH 9.4 as described by Gibbs. [7]