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New flavonoids from *Campylotropis hirtella* with immunosuppressive activity

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ABSTRACT

In an effort to identify natural compounds with immunosuppressive activity, nine new flavonoids, including one isoflav-3-ene derivative (**1**), one coumaronochromone (**2**), two isoflavanones (**3**, **4**), one isoflavone derivative (**6**), one isoflavone (**7**), three flavonols (**8**, **9**, **10**), as well as one known compound, hydroisoflavone C (**5**), were isolated from the roots of *Campylotropis hirtella*. The structures of these compounds were elucidated by extensive spectroscopic measurements. All of the compounds were assessed for immunosuppressive activity. Among the isolates, compound **2** showed good inhibitory activity against mitogen-induced splenocyte proliferation with an IC_{50} of 0.28 μM and relatively low cytotoxicity.

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1. Introduction

Campylotropis hirtella (Franch.) Schindl. (family Leguminosae) (Fig. 1) is an undershrub of approximately 1 m in height distributed widely in the subtropical zones of China, such as the Yunnan, Sichuan and Guizhou provinces. The roots of this species have been used in traditional Chinese medicine for the treatment of irregular menstruation, dysmenorrhea, metrorrhagia, metrostaxis, as well as gastric ulcers, either alone or in combinations [1]. The species has previously been reported to contain lignans, sesquilignans, dilignans and coumarins in its roots by Yao et al. and some of the compounds showed inhibitory activity on prostate specific antigen secretion in LNCaP cells [2–4].Our group's earlier efforts have led to the isolation of a bunch of new flavonoids and their derivatives, most of which possess immunosuppressive activity [5–9]. The diversity of flavonoid structures and intriguing bioactivity of the flavonoids from this species attracted our attention and prompted us to perform a further chemical investigation; herein we report the isolation and structure elucidation of nine new flavonoids including one isoflav-3-ene derivative (**1**), one coumaronochromone (**2**), two isoflavanones (**3**, **4**), one isoflavone derivative (**6**), one isoflavone (**7**), three flavonols (**8**, **9**, **10**), as well as one known compound, hydroisoflavone C (**5**) (Fig. 2). All of the isolates were assessed for their cytotoxicity and inhibitory activities against mitogen-induced splenocyte proliferation. Compound **2** was found to be the most active compound with an IC_{50} of 0.28 μM and relatively low cytotoxicity, while compounds **6**, **7** and **8** show moderate activity.

2. Experimental

2.1. General experimental procedures

UV spectra were acquired with a Shimadzu UV–Vis 2201 spectrometer, IR spectra were acquired with a Shimadzu FTIR-8400S Spectrometer, and optical rotations were acquired

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Fig. 1. The aerial part and roots of *C. hirtella*.

with an Autopol IV A2120 automatic polarimeter. CD spectra were acquired with a JASCO DIP-360J-500C polarimeter at 20 °C. ESI-MS was acquired with an Agilent 1100 1946D LC-MS spectrometer, HR-ESIMS was acquired with a Q-TOF Micro LC-MS-MS spectrometer, and NMR spectra were acquired with a Varian INOVA 400 spectrometer using TMS as the internal standard. Column chromatographic separations were carried out using silica gel H60 (300–400 mesh; Qingdao Haiyang Chemical Group Corporation), RP-C_{18} (50 μm; Merck) and Sephadex LH-20 (Amersham Pharmacia Biotech, Canada). Cyclosporine A (CsA, purity 99%, Sandimmun, 50 mg/mL) was manufactured by Novartis Pharma AG, Switzerland. All other chemical reagents were obtained from commercial vendors.

2.2. Plant material

The roots of *C. hirtella* (Franch.) Schindl. were collected from Chuxiong, Yunnan Province, People's Republic of China in November 2009 and authenticated by Professor Xiling Li of Shanghai University of Traditional Chinese Medicine. A voucher specimen (no. 200911013) has been deposited in the herbarium of the Shanghai University of Traditional Chinese Medicine.

2.3. Extraction and isolation

1. The air-dried and comminuted roots of *C. hirtella* (2.0 kg) were extracted twice (each for 7 days) with 95% EtOH (2 × 5 L) at room temperature. The EtOH extract (80.0 g) was suspended in water (0.8 L) and extracted in successful steps using hexane (3 × 1 L) and EtOAc (3 × 1.5 L); the EtOAc portion was evaporated under reduced pressure to afford a crude extract (32.3 g). The crude EtOAc extract was subjected on a silica gel column (5 × 70 cm, 300 g silica gel, 200–300 mesh) eluted with a gradient of hexane/EtOAc (30:1–0:1), to give seven fractions (A–G). Fraction B (4.8 g) was subjected to a silica gel column (4 × 60 cm, 200 g silica gel, 300–400 mesh) eluted with a gradient of hexane/EtOAc (8:2–0:1) to give a subfraction B_{II} (328 mg), which was further applied to preparative HPLC (Merck C_{18} column, 5 μm, 125 × 25 mm; gradient elution with MeCN/H_2O containing 0.1% TFA from 5/3 to 9/1 for 15 min; UV detection at 254 nm; flow rate 14 mL/min;) to give compound **7** (14 mg). Fraction C (4.3 g) was divided into seven subfractions (C_I–C_{VII}) by a silica gel column (4 × 60 cm, 200 g silica gel, 200–300 mesh) eluted with a gradient of hexane/EtOAc (6:1–0:1). Compound **3** (18.0 mg) and compound **4** (13.5 mg) were obtained from subfraction C_I (265 mg) and C_V (396 mg), respectively, with the same method of preparative HPLC as compound **7**. Subfraction C_{VII} (567 mg) was purified by a C_{18} column (3 × 70 cm, 80 g C_{18} material, 50 μm) eluted with a gradient of MeOH/H_2O (3:7–9:1) to give compound **5** (10.2 mg) and compound **6** (3.3 mg). Fraction D (4.0 g) was separated by using a silica gel column (4 × 60 cm, 200 g silica gel, 200–300 mesh) eluted with a gradient of hexane/ethyl acetate (7:1–0:1) to give five subfractions (D_I–D_V). After further purification using a C_{18} column (3 × 50 cm, 60 g C_{18} material, 50 μm) with a gradient of MeOH–H_2O (4:6–9:1), Compounds **1** (4.7 mg) and **8** (13.7 mg) were obtained from subfraction D_I (216 mg) while compound **2** (17.1 mg) was from subfraction D_{IV} (327 mg); Fraction G (3.7 g) was separated using a Sephadex LH-20 column (3 × 60 cm, 100 g; mobile phase: $CHCl_3$/MeOH, 3:1) to give compound **9** (10.0 mg) and compound **10** (7.0 mg).

2.3.1. Compound (1)

Yellow oil; $[a]_D^{25}$ 0 (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ε) 269 (4.13), 383 (5.01) nm; IR (KBr) ν_{max} 3304, 2926, 1639, 1614, 1450, 1379, 1263, 1182, 1163, 1091, 835 cm^{-1}; ^1H and ^{13}C NMR (Table 1); HRESIMS *m/z* 399.1402 $[M + Na]^+$ (calcd for $C_{20}H_{24}O_7Na$, 399.1414); ESIMS *m/z* 375 $[M - H]^-$.

2.3.2. Compound (2)

Colourless needles (MeOH), mp: 274–276 °C; UV (MeOH) λ_{max} (log ε) 258 (4.31), 289 (2.95) nm; IR (KBr) ν_{max} 3517, 3122, 2918, 1623, 1448, 1344, 1286, 1114, 1070, 1037, 823 cm^{-1}; ^1H and ^{13}C NMR (Table 1); HRESIMS *m/z* 397.1277 $[M + H]^+$ (calcd for $C_{22}H_{21}O_7$, 397.1282); ESIMS *m/z* 397 $[M + H]^+$.

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