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Bioactive flavones and biflavones from *Selaginella moellendorffii* Hieron

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ABSTRACT

Three new flavones named 5-carboxymethyl-4′,7-dihydroxyflavone (1), its ethyl ester (2) and butyl ester (3) were isolated from the herb *Selaginella moellendorffii* Hieron., together with ten known compounds. Their structures were elucidated on the basis of spectroscopic and chemical analysis. Selected compounds were evaluated for their anti-HBV and cytotoxic activity. Among them, compounds 2 and 3 displayed inhibitory activity *in vitro* on hepatitis B virus (HBV) surface antigen (HBsAg) secretion of the Hep G2.2.15 cell line with IC_{50} values of 0.17 mg/ml and 0.46 mg/ml, and on HBV e antigen (HBeAg) secretion with IC_{50} values of 0.42 mg/ml and 0.42 mg/ml, respectively. Compounds 7, 8, 10 and 12 exhibited selective cytotoxicity against the three human cancer cell lines tested.

1 . Introduction

Selaginella moellendorffii Hieron., a perennial herb of genus *Selaginella* (Selaginellacea), is mainly distributed in the southern area of Changjiang River in China, which is used extensively by the folks for the treatment of gonorrhea, jaundice, hepatitis, and bleeding [1]. Previous investigations of some other *Selaginella* species revealed the genus *Selaginella* to be a rich source of biflavonoids, which exhibited broad activities, including cytotoxic [2,3], antiviral [4], inhibition of nuclear factor-k B activation [5], antiplasmodial and leishmanicidal [6] activities; other types of compounds such as alkaloidal glycosides [7,8], phenylpropanones and lignans [9–11] were also reported from some *Selaginella* species. However, chemical analysis with *S. moellendorffii* has been limited yet [12–16]. Our search for bioactive metabolites of the *S. moellendorffii* herb led to the isolation of three new flavones and ten known compounds (Fig. 1). Selected compounds were evaluated for their anti-HBV activity *in vitro* using the HBV transfected Hep G2.2.15 cell line , as well as for cytotoxic activity against the non-small cell lung cancer (A549), stomach adenocarcinoma (BGC-823) and liver cancer (BEL-7402) human cell lines. This paper presents the details of isolation and structure elucidation of new compounds and results on the anti-HBV and cytotoxic activity.

2. Experimental

2.1 . General

Silica gel (200–300 mesh, Qingdao Marine Chemical Inc.; Qingdao, China), polyamide (100–200 mesh, Sinopharm Chemical Reagent Co., Ltds; Shanghai, China), HPD-100 resin (Changzhou Baoen Chemical Inc.; Hebei, China) and Sephadex LH-20 (Amersham Bioscience, Sweden) were used for column chromatography (CC). UV spectra were carried out on a Shimadzu UV 2401-PC spectrophotometer, λ_{max} in nm. IR spectra were measured on a Bruker Tensor 27 FT-IR spectrometer with KBr pellets, in cm^{-1}. Melting points (m.p.) were determined on a Yanaco MP-S_3 micro-melting point apparatus; uncorrected. MS data were obtained on a VG-Autospec-3000 mass spectrometer; in *m/z* (rel.%). NMR spectra were recorded on a Bruker AV-500

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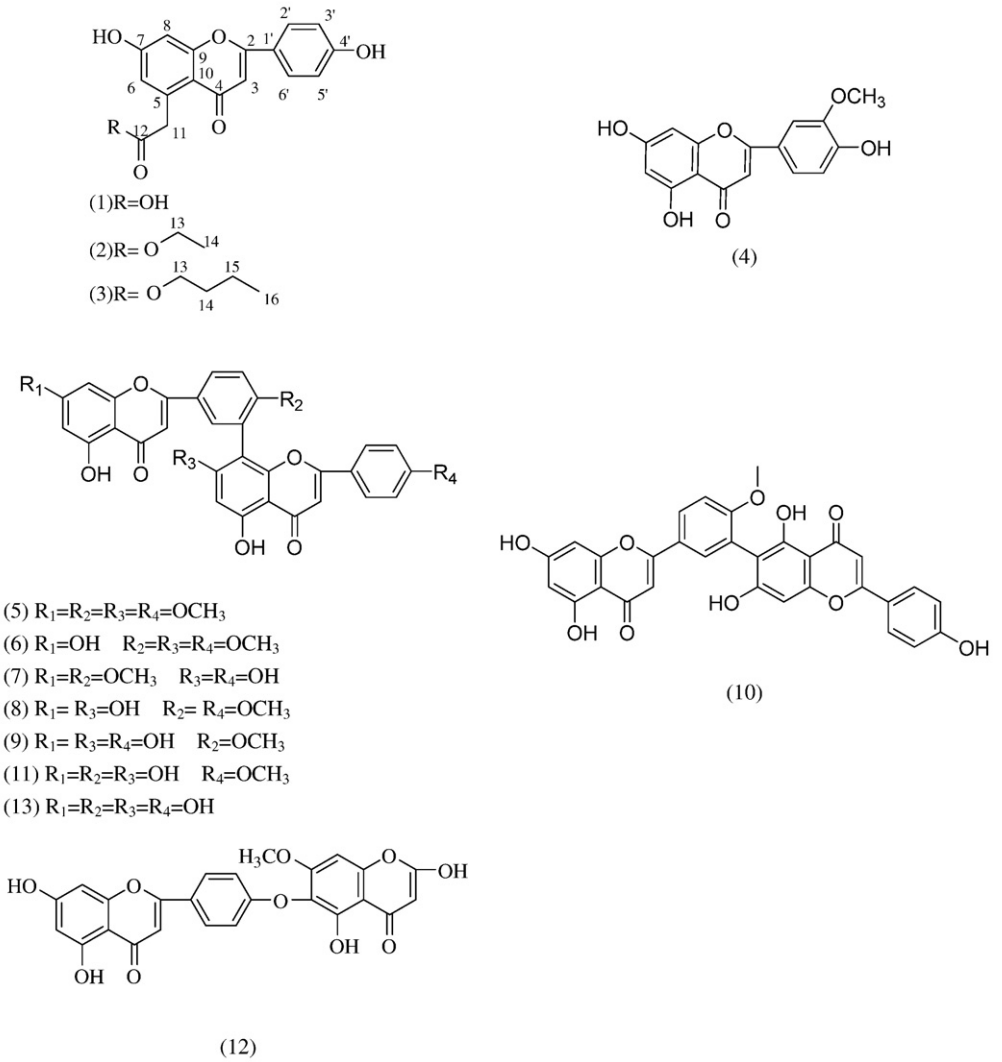

Fig. 1. Structures of compounds 1–13.

($^1H/^{13}C$, 500 MHz/125 MHz) spectrometers and chemical shifts were given in δ with TMS as internal reference.

2.2. Plant materials

The herb *S. moellendorffii* was collected from Zhejiang Province, PR China in October 2007 and identified by Dr. Pro. Qiang Wang, Department of Pharmacognosy, China Pharmaceutical University, where a voucher specimen (2007-10-003) was preserved.

2.3 . Extraction and isolation

The air-dried plant (5 kg) were cut into small pieces and extracted with 95% ethanol (50 L×3) under reflux. The ethanolic extracts were combined and concentrated *in vacuo* to yield a brown residue (250 g) which was defatted with petroleum ether (PE, 60–90 °C; 5 L×3) and then extracted with EtOAc (5 L×3) and n-BuOH (5 L×3), successively, to yield EtOAc fraction (108 g) and n-BuOH fraction (65 g), respectively.

A part of the EtOAc fraction (80 g) was subjected to CC (silica gel; 8×60 cm; 1.2 kg; $CHCl_3$/MeOH 1:0→1:1, and MeOH) to give six subfractions (Fr.). Fr. 2 (2.6 g) was further submitted to repeated CC (silica gel; $CHCl_3$/MeOH 1:0→100:2) to afford compounds 5 (15 mg) and 6 (6.2 mg). Fr. 3 (6.7 g) was separated by repeated CC (silica gel; $CHCl_3$/MeOH 100:1→100:5) to yield compounds 7 (28 mg) and 8 (25 mg). Compounds 9 (7.1 mg), 10 (18.2 mg) and 11 (7.8 mg) were obtained from Fr. 4 (10.5 g) by repeated chromatography over polyamide column (100–200 mesh). Fr. 5 (15.1 g) was subjected to CC (silica gel; 3.5×30 cm; $CHCl_3$/MeOH 96:4→50:50, and then MeOH), and further purified with Sephadex LH-20, produced compounds 12 (9.1 mg) and 13 (105 mg), respectively.

The n-BuOH extract (65 g) was separated by column chromatography over HPD-100 macroporous resin with a gradient from H_2O to 95% EtOH to give five fractions: H_2O