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Inhibitory Activity of 6-*O*-Angeloylprenolin from *Centipeda minima* on Farnesyl Protein Transferase

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The methanolic extract of the aerial parts of *Centipeda minima* was found to show inhibitory activity on farnesyl protein transferase (FPTase). Bioassay-guided fractionation of the methanolic extract resulted in the isolation of 6-*O*-angeloylprenolin, as an inhibitor on FPTase. This compound inhibited FPTase activity in a dose-dependent manner, and the IC_{50} value of 6-*O*-angeloylprenolin was 18.8 μM.

Key words: *Centipeda minima*, 6-*O*-Angeloylprenolin, FPTase

INTRODUCTION

Ras proteins play an important role in intracellular signal transduction pathways involved in cell growth and the mutated *ras* genes have been found in thirty percent of human cancers (Gibbs *et al.*, 1993). Farnesyl protein transferase (FPTase), a member of the prenyltransferase enzyme family, is a crucial enzyme which participates in the post-translational modification that the transfer of the farnesyl group from farnesyl pyrophosphate onto cysteine 186 at the C-terminal of the Ras proteins (Qian *et al.*, 1997). This is a mandatory process before anchoring to plasma membrane which is critical for its biological activity, e.g. cell proliferation and tumorigenesis. Recent work has demonstrated that specific inhibitors of the FPTase might be interesting chemical leads to develop effective therapeutic agents for the treatment of cancer (Kohl *et al.*, 1994). Therefore, the discovery of FPTase inhibitors is become an active area for the development of anti-tumor agents.

In the course of our screening for potent inhibitors of FPTase from herbal medicines, a total extract of the aerial parts of *Centipeda minima* (Compositae) was found to show inhibitory activity on FPTase. Subsequent activity-guided fractionation of the methanolic extract led to the isolation of 6-*O*-angeloylprenolin, as an active principle.

C. minima, as an annual plant is widely distributed in Korea, which has been used as a folk medicine to treat headache, cough, expectoration and nasal obstruction in common cold in Korea and China (But *et al.*, 1997). This paper describes the isolation of 6-*O*-angeloylprenolin from *C. minima* and the inhibitory effect of this compound on FPTase.

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MATERIALS AND METHODS

General procedure

^{1}H- and ^{13}C-NMR spectra were determined on a JEOL JMN-EX 400 spectrometer. TLC was carried out on Merck precoated silica gel F_{254} plates, with Kiesel gel 60 (230-400 mesh, Merck) used as the silica gel. Sephadex LH-20 was used for the column chromatography (Pharmacia, 25-100 μm). The column used for LPLC was Lobar-A (Merck Lichroprep Si 60, 240-10 mm). All other chemicals and solvents were analytical grade and used without further purification. Farnesyl transferase was purified from rat brain homogenates by sequential ammonium sulfate fraction and Q-sepharose column chromatography (Reiss *et al.*, 1990) and human FPTase was expressed in bacculovirus, purified by affinity column chromatography.

Plant materials

The aerial parts of *C. minima* were collected and air-dried in October 2003 at Samnye, Jeonbuk, Korea. A voucher specimen was deposited in the herbarium of the college of pharmacy, Woosuk University (WSU-03-014).

Extraction and isolation

The shade dried plant material (300 g) was extracted (three times with MeOH at room temperature) and filtered. The filtrate was evaporated *in vacuo* to give a dark brownish residue. The resultant methanolic extract (63 g) was followed by successive solvent partitioning to give *n*-hexane (8 g), $CHCl_3$ (6 g), EtOAc (3 g), *n*-BuOH (15 g), and H_2O soluble fractions. Each fraction was tested for inhibitory effects on FPTase. Among these fractions, the $CHCl_3$ soluble fraction showed the most significant FPTase inhibitory activity. Silica gel column chromatography of the $CHCl_3$ soluble fraction with *n*-hexane-CH_2Cl_2-EtOAc (5:20:1) gave three fractions (fr.1-fr.3). The major fraction fr.2 was rechromatographed on the Sephadex LH-20 column (MeOH) and purified by Lobar-A column chromatography (*n*-hexane-CH_2Cl_2-EtOAc, 10:20:1) to yield compound **1** (25 mg).

6-*O*-Angeloylprenolin (1)

white powder (MeOH); ^{1}H-NMR (CD_3OD, 400 MHz) δ: 7.88 (1H, d, *J*=7.2 Hz, H-3), 6.06 (1H, m, H-3'), 6.04 (1H, dd, *J*=7.4, 2.8 Hz, H-2), 5.48 (1H, s, H-6), 4.86 (1H, m, H-8), 3.27 (1H, m, H-11), 3.12 (1H, m, H-1), 2.97 (1H, m, H-7), 2.45 (1H, ddd, *J*=15.2, 6.0, 1.8 Hz, H-9α), 2.17 (1H, m, H-10), 1.87 (3H, d, *J*=7.2 Hz, H-4'), 1.72 (3H, s, H-15), 1.45 (3H, d, *J*=7.6 Hz, H-13), 1.25 (3H, d, *J*=7.2 Hz, H-14), 1.01 (3H, s, H-5'); ^{13}C-NMR (100 MHz, CD_3OD): 211.9 (C-4), 181.2 (C-12), 167.4 (C-1'), 164.9 (C-2), 139.3 (C-3'), 129.8 (C-3), 128.6 (C-2'), 81.3 (C-8), 73.0 (C-6), 56.1 (C-5), 55.9 (C-1), 50.0 (C-7), 41.9 (C-9), 41.4 (C-11), 27.2 (C-10), 20.6 (C-5'), 20.1 (C-14), 18.2 (C-15), 16.0 (C-4'), 11.3 (C-13).

In vitro enzyme assay of FPTase (Reiss *et al.*, 1990)

FPTase assays were done with use of a Scintillation Proximity Assay (SPA) kit following the protocol described by the manufacturer except that a biotinylated substrate peptide containing the Ki-Ras carboxyl-terminal sequence was used. The C-terminal peptide of Ki-Ras (Biotin-KKKSKTKCVIM) was synthesized by solid-phase peptide synthesis. FPTase activity was determined by measuring transfer of [^{3}H]-farnesyl pyrophosphate to Biotin-KKK-SKTKCVIM. The inhibitory activity was expressed as the followings; % inhibition of FPTase = [1 – (Sample – B2)/(C – B1)] × 100, Blank 1 (B1) : without sample and enzyme, Blank 2 (B2) : with sample and without enzyme, Control (C) : without sample and with enzyme (Lee *et al.*, 2002, 2003).

RESULTS AND DISCUSSION

FPTase assays were done using a scintillation proximity assay (SPA) kit following the protocol described by the manufacturer except that a biotinylated substrate peptide containing the Ki-Ras carboxyl-terminal sequence was used. The C-terminal peptide of Ki-Ras (Biotin-KKKSK-TKCVIM) was synthesized by solid-phase peptide synthesis. FPT activity was determined by measuring transfer of [^{3}H]-farnesyl from [^{3}H]-farnesyl pyrophosphate to Biotin-KKKSKTKCVIM.

The methanolic extract of the aerial parts of *C. minima* was found to exhibit inhibitory activity on FPTase (Table I). To isolate the FPTase inhibitory constituents from *C. minima*, the total methanolic extract was suspended in water and partitioned successively with *n*-hexane, $CHCl_3$, EtOAc, and *n*-BuOH. As a result, the inhibitory activity was found in the $CHCl_3$ soluble fraction. Using several chromatographic techniques, compound **1** was isolated as an active constituent from the $CHCl_3$ soluble fraction.

Compound **1** was a white amorphous powder from MeOH. The EI-MS of shown an $[M]^+$ ion peak at *m/z*: 346, which corresponds to the molecular formular $C_{20}H_{26}O_5$.

In the ^{13}C- and ^{1}H-^{13}C COSY NMR spectra of **1**, a ketone (δ_C 211.9), two carbonyl (δ_C 181.2, 167.4), and four olefinic (δ_C 164.9, 139.3, 129.8, 128.6) carbon signals were observed. The ^{1}H-NMR spectrum of **1** revealed three olefinic (δ 7.88, 6.06, 6.04), two oxygen bearing (δ 5.48, 4.86), and five methyl (δ 1.87, 1.72, 1.45, 1.25, 1.01) proton signals. The structure of **1** was established by ^{1}H-^{1}H correlation of ^{1}H-^{1}H and ^{1}H-^{13}C COSY spectra. Its spectral data including ^{1}H- and ^{13}C-NMR are consistent with those in literature of 6-*O*-angeloylprenolin (Taylor and Towers, 1998).

6-*O*-Angeloylprenolin, a sesquiterpene, inhibited FPTase activity in a dose-dependent manner (Fig. 2). It inhibited FPTase with an IC_{50} value of 18.8 μM. Arteminolide, which is a FPTase inhibitor isolated from herbal medicine, showed an IC_{50} value of 360 nM, as a positive control (Lee *et al.*, 2002).

Sesquiterpene lactones are one of the class of natural sesquiterpenoids, which are chemically distinct from other members of the group though the presence of a γ-lactone

Table I. FPTase inhibitory activities of solvent fractions on the aerial parts of *Centipeda minima* by scintillation proximity assay

Fraction	Inhibition ratio of FPTase (%, 50 μg/mL)
n-hexane	65.1
$CHCl_3$	89.0
EtOAc	27.4
n-BuOH	10.7

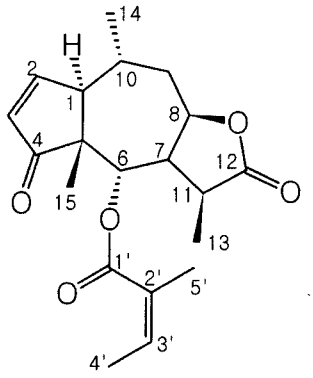

Fig. 1. Structure of **1**

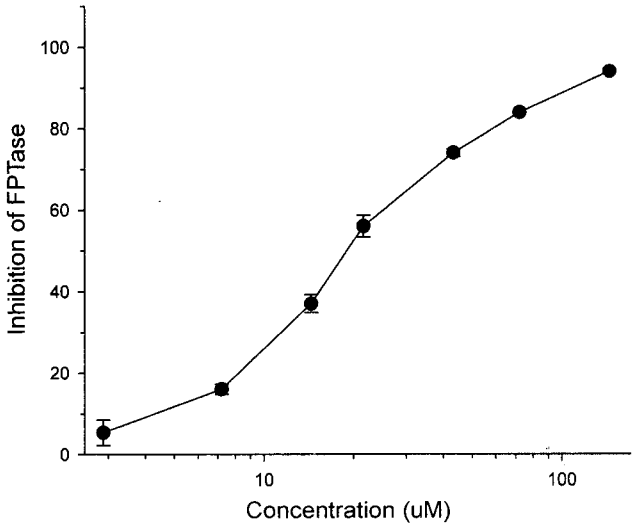

Fig. 2. The inhibitory activity of compound **1** on FPTase

system. 6-*O*-Angeloylprenolin has been reported as an antibacterial sesquiterpene and a platelet activating factor antagonist (Taylor and Towers, 1998; Iwakami *et al.*, 1992). This study showed that a sesquiterpene compound isolated from *C. minimai,* 6-*O*-angeloylprenolin, inhibits FPTase activity. Although it is less effective than that of arteminolide, it may be useful for treatment of tumor because this compound was purified from a natural plant which has been used as a folk medicine in Korea and China.

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