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Inhibitory Activity of Isorhamnetin from *Persicaria thunbergii* on Farnesyl Protein Transferase

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The methanolic extract of the aerial parts of *Persicaria thunbergii* was found to show inhibitory activity on Farnesyl Protein Transferase (FPTase). Bioassay-guided fractionation of the methanolic extract resulted in the isolation of isorhamnetin, as an inhibitor on FPTase. This compound inhibited FPTase activity in a dose-dependent manner, and the IC_{50} value of isorhamnetin was 37.5 μM.

Key words: *Persicaria thunbergii*, FPTase, Isorhamnetin

INTRODUCTION

The *ras* oncogenes encode 21 kDa of GTP binding proteins that are involved in the signal transduction pathways regulating cell growth and differentiation and the *ras* oncogene is found mutated in approximately 25 percent of human cancers (Jackson *et al.*, 1993). To become a signal transducer, a series of post-translational modifications has to be carried out in the cytosol prior to localization of Ras to the inner surface of the cell membrane. The first step in this cascade of post-translational modification is the farnesylation onto cysteine 186 at C-terminal of Ras by farnesyl protein transferase (FPTase) (Qian *et al.*, 2000). FPTase inhibitors have been shown to inhibit the growth of Ras-induced tumors in mouse xenograft models and, more dramatically, in transgenic mouse models. 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). Many strategies have been used to develop FPTase inhibitor, including screening of natural products and rational design based upon the substrates of the farnesylation reaction (Head and Johnston, 2003).

In the course of screening for potent inhibitors of FPTase from herbal medicines, a total extract of the aerial parts of *Persicaria thunbergii* (Polygonaceae) was found to show inhibitory activity on FPTase. Subsequent activity-guided fractionation of the methanolic extract led to the isolation of isorhamnetin, as an active principle.

P. thunbergii, as an annual plant is widely distributed in Korea, which has been used as a folk medicine to treat rheumatism, hemorrhage and measles in Korea and China (Yook, 1981). This paper describes the isolation of isorhamnetin from *P.thunbergii* and the inhibitory effect of this compound on FPTase.

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MATERIALS AND METHODS

General procedure

^1H- 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 and Goldstein, 1990) and human FPTase was expressed in bacculovirus, purified by affinity column chromatography.

Plant materials

The aerial parts of *P. thunbergii* 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-03-012).

Extraction and isolation

The shade dried plant material (500 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 (95 g) was followed by successive solvent partitioning to give *n*-hexane (7 g), $CHCl_3$ (15 g), EtOAc (5 g), *n*-BuOH (30 g) and H_2O soluble fractions. Each fraction was tested for inhibitory effects on AChE. Among these fractions, the EtOAc soluble fraction showed the most significant AChE inhibitory activity. Silica gel column chromatography of the EtOAc soluble fraction with $CHCl_3$-EtOAc-MeOH (10:1:1) gave five fractions (fr.1-fr.5). The major fraction fr.2 was rechromatographed on the Sephadex LH-20 column (MeOH) and purified by Lobar-A column chromatography ($CHCl_3$-EtOAc, 7:1) to yield compound **1** (40 mg).

Isorhamnetin (1) yellow powder (MeOH); mp. 303-304 °C; UV λ_{max} nm: (MeOH) 255, 268sh, 306sh, 327sh, 370; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 12.43 (1H, s, 5-OH), 7.73 (1H, d, *J*=1.9 Hz, H-2'), 7.66 (1H, dd, *J*=8.0, 1.9 Hz, H-6'), 6.91 (1H, d, *J*=8.0Hz, H-5'), 6.46 (1H, d, *J*=1.8 Hz, H-8), 6.18 (1H, d, *J*=1.8Hz, H-6), 3.82 (3H, s, OCH_3); ^{13}C-NMR (100 MHz, DMSO-d_6): 176.0 (C-4), 164.1 (C-7), 160.8 (C-5), 156.4 (C-9), 148.9 (C-3'), 147.6 (C-4'), 146.8 (C-2), 136.0 (C-3), 122.1 (C-1'), 121.9 (C-6'), 115.7 (C-5'), 111.8 (C-2'), 103.2 (C-10), 98.4 (C-6), 93.8 (C-8).

In vitro enzyme assay of FPTase

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-KKKSKT-KCVIM. 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).

RESULTS AND DISCUSSION

The methanolic extract of the aerial parts of *P. thunbergii* was found to exhibit inhibitory activity on FPTase (IC_{50} = 19 μg/mL, Table I). To isolate the FPTase inhibitory constituents from *P. thunbergii*, the total methanolic extract was suspended in water and partitioned successively with $CHCl_3$, EtOAc, and *n*-BuOH. As a result, the inhibitory activity was found in the EtOAc soluble fraction. Using several chromatographic techniques, compound **1** was isolated as an active constituent from the EtOAc soluble fraction.

Compound **1** was a yellow amorphous powder from MeOH. The EI-MS of shown an $[M]^+$ ion peak at m/z: 316, which corresponds to the molecular formular $C_{16}H_{12}O_7$. It responded positively to 10% $FeCl_3$(in EtOH) and Mg-HCl test, In the ^{1}H-NMR spectrum of **1**, the typical flavonoid signals, two *meta*-coupled peaks at δ 6.46 (1H, d, *J* = 1.8 Hz, H-8) and 6.18 (1H, d, *J* = 1.8 Hz, H-6), and ABX system peaks δ 7.73 (1H, d, *J* = 1.9 Hz, H-2'), 7.66 (1H, dd, *J* = 8.0, 1.9 Hz, H-6') and 6.91 (1H, d, *J* = 8.0 Hz, H-5'), were observed. Its spectral data including ^{1}H- and ^{13}C-NMR are consistent with those in literature of isorhamnetin (Agrawal, 1989).

Isorhamnetin inhibited FPTase activity in a dose-dependent manner (Fig. 1). It inhibited FPTase with an IC_{50} value of 37.5 μM using arteminolide, which is a FPTase inhibitor isolated from herbal medicine with an an IC_{50} value of 360 nM, as a positive control (Lee *et al.*, 2002).

The antitumor effects of plant flavonoids have been

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

Fraction	Inhibition ratio of FPTase (%, 100 μg/mL)
MeOH ex.	50.0
$CHCl_3$	7.2
EtOAc	2.5
n-BuOH	3.7

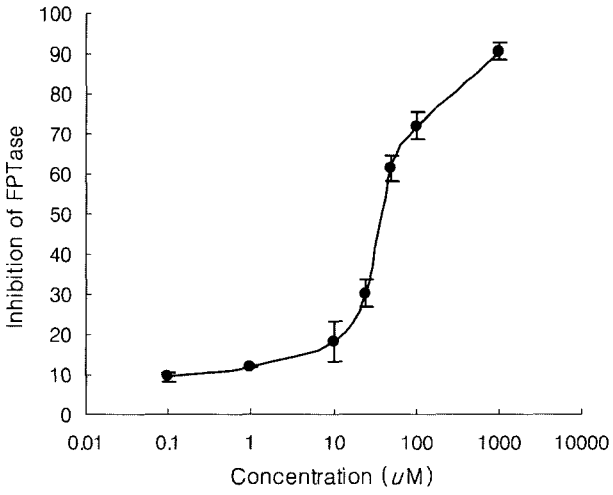

Fig. 1. The inhibitory activity of compound **1** on FPTase

reported to induce cell growth inhibition and apoptosis in a variety of cancer cells (Di Carlo *et al.*, 1999) Potential anticancer or chemopreventive activities of flavonoides may be due in part to a large range of enzyme inhibition including kinases and pro-oxidant enzymes (Agullo *et al.*, 1997). This study showed that a flavonoid compound isolated from *P. thunbergii,* isorhamnetin, 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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