PTP1B Inhibitory Effect of Abietane Diterpenes Isolated from Salvia miltiorrhiza

Yu Mi Han, a,b Hyuncheol Oh, a,b MinKyung Na, a Beom Seok Kim, a Won Keun Oh, a Bo Yeon Kim, a Dae Gwin Jeong, a Seong Eon Ryu, a Dai-Eun Sook, c and Jong Seog Ahn * a

a Korea Research Institute of Bioscience and Biotechnology (KIRIBB); 52 Eoeun-dong, Yusong-gu, Daejeon 305–333, Korea; b College of Natural Science, Silla University; San 1–1, Gwaebop-dong, Sasang-gu, Busan 617–736, Korea; and c College of Pharmacy, Chungnam National University; 220 Gung-dong, Yusong-gu, Daejeon 305–764, Korea.

Received April 11, 2005; accepted May 25, 2005

Protein tyrosine phosphatase 1B (PTP1B) acts as a negative regulator of insulin signaling, and selective inhibition of PTP1B has served as a potential drug target for the treatment of type 2 diabetes. In the course of screening for PTP1B inhibitory natural products, the MeOH extract of the dried root of Salvia miltiorrhiza Bunge (Labiatae) was found to exhibit significant inhibitory effect. Bioassay-guided fractionation and purification afforded three related abietane-type diterpene metabolites 1—3. Compounds 1—3 were identified as isotanshinone IIA (1), dihydroisotanshinone I (2), and isocryptotanshinone (3) mainly by analysis of NMR and MS data. Compounds 1—3 non-competitively inhibited PTP1B activity with 50% inhibitory concentration values of 11.4±0.6 μM, 22.4±0.6 μM and 56.1±6.3 μM, respectively.

Key words Salvia miltiorrhiza; Labiatae; protein tyrosine phosphatase 1B (PTP1B) inhibitor; isotanshinone IIA; dihydroisotanshinone I; isocryptotanshinone

The reversible protein tyrosine phosphorylation by protein tyrosine kinases (PTK) and protein tyrosine phosphatases (PTP) is a key element of the signaling pathways induced by environmental stimuli.1,2) One of the intracellular PTPases, protein tyrosine phosphatase 1B (PTP1B) has been implicated in the negative regulation of insulin signaling by dephosphorylating the insulin receptor (IR) as well as its substrate, insulin receptor substrate-1 (IRS-1) and insulin receptor substrate-2 (IRS-2).3—5) The protein levels are increased in insulin-resistant diabetes patients and the deletion of PTP1B in mice has been shown to increase insulin sensitivity.6—8) Thus, inhibiting PTP1B action by using antisense oligonucleotides or small molecule inhibitors represents one of the novel therapeutic approaches for the treatment of insulin resistance.9,10) There have been a number of reports on the designing and development of synthetic PTP1B inhibitors,11) and several classes of plant-derived secondary metabolites have been described as PTP1B inhibitors.12)

The dried root of Salvia miltiorrhiza Bunge (Labiatae), called ‘Danshen’ in China, is one of the most popular traditional Chinese medicines. This plant has been used for the treatment of various diseases such as menstrual disorder, menorrhagia, insomnia, blood circulation diseases, angina pectoris, and inflammation. Particularly, the drug is valuable in the treatment of coronary heart diseases.13) The major constituents in the root of S. miltiorrhiza are abietane-type diterpene pigments, so called tanshinones, which have ortho- or para-naphthoquinone chromophores.14) They are unique components found in the Salvia genus and many of them exhibit diverse biological activities.15—18)

In the study, the MeOH extract of the dried root of S. miltiorrhiza displayed significant PTP1B inhibitory effect and the further study was conducted to isolate and identify the active principles.

MATERIALS AND METHODS

Plant Materials The dried roots of S. miltiorrhiza were purchased from Il-sin Pharmaceutical Co., Ltd. in Daejeon, Korea. A voucher specimen (no. 162) has been deposited in the author’s laboratory.

Extraction and Isolation The dried roots of S. miltiorrhiza (1 kg) were extracted with MeOH (4 l) for 24 h. A portion (300 ml) of the resulting MeOH extract was concentrated. The dried extract (2.64 g) was subjected to silica gel column chromatography (Merck Kieselgel 60; 100 g; 0.015—0.040 mm particle size; 4.5×18.5 cm). The column was eluted with a gradient consisting of hexane–EtOAc (95 : 5, 1800 ml; 93 : 7, 400 ml; 90 : 10, 400 ml; 80 : 20, 400 ml; 70 : 30, 200 ml; 50 : 50, 200 ml; 40 : 60, 200 ml; 20 : 80, 200 ml; 100% EtOAc 200 ml; collecting 200 ml fraction) and EtOAc–MeOH (90 : 10, 200 ml; 80 : 20, 200 ml; 70 : 30, 200 ml; collecting 200 ml fraction). Fractions of similar composition as determined by TLC analysis were pooled, affording 11 fractions (fr. 1—11). Fr. 2 (23 mg, eluted between 700 to 800 ml) yielded 1 (4.6 mg) by preparative reversed phase HPLC using a gradient from 60 to 100% CH3CN in H2O over 40 min (Shiseido Capcell Pak C18 column; 10×210 mm; 5-μm particle size; 2 ml/mm; UV detection at 254 nm). Fr. 6 (16.1 mg, eluted between 2800 to 3000 ml) was further purified by preparative reversed phase HPLC using a 75% CH3CN in H2O (Shiseido Capcell Pak C18 column; 10×210 mm; 5-μm particle size; 2 ml/mm; UV detection at 254 nm) to yield 2 (1.1 mg) and 3 (3.6 mg).

Enzyme Assays Details of PTP1B, VHR, Ppase1 assays have been described elsewhere.19) The IC50 of the compounds was determined by using linear regression analysis. Three separate determinations were conducted for each compound.

Kinetic Analysis The reaction mixture consisted of different concentrations of pNPP as a PTP1B substrate in the absence or presence of compounds 1, 2 and 3. The Michaelis constant (Km) and maximum velocity (Vmax) of PTP1B were determined by a Lineweaver-Burk plot.
RESULT AND DISCUSSION

In the course of screening for PTP1B inhibitors from natural resources, the MeOH extract of the dried root of S. miltiorrhiza (Danshen) was found to inhibit PTP1B activity at the concentration of 30 μg/ml level and selected for further investigation. To identify the active principles, we performed bioassay and 1H-NMR guided fractionation and purification by using silica gel column chromatography and HPLC, resulting a group of three abietane-type diterpene metabolites (compounds 1—3 shown in Fig. 1). The isolated compounds were identified as isotanshinone IIA (1), dihydroisotanshinone I (2), and isocryptotanshinone (3) by analysis of NMR and MS data, together with comparison of their spectral data with those in the literature.\(^{20–22}\) Compounds 1—3 inhibited the hydrolysis of the pNPP catalyzed by PTP1B in a dose-dependent manner with IC\(_{50}\) values of 11.4±0.6 μM, 22.4±0.6 μM and 56.1±6.3 μM, respectively (Table 1). A known phosphatase inhibitor, RK-682, was employed as a positive control.\(^{23}\) To investigate the inhibition mode of compounds 1—3 on the activity of PTP1B, a kinetic analysis was conducted at various concentrations of 1—3 and pNPP. Using a standard curve showing absorbance versus moles of hydrolyzed pNPP, velocities were calculated and Lineweaver-Burk plots were obtained (Fig. 2). As shown in Fig. 2, all of the compounds decreased the \(V_{\text{max}}\) value, but didn’t alter the \(K_{m}\) value of PTP1B, suggesting that their inhibition modes were non-competitive toward pNPP. The \(K_{i}\) values of compounds 1—3 were determined as 25.2 μM, 31.9 μM, and 67.1 μM, respectively. In addition, the isolated compounds were tested for the inhibitory effects on other types of protein phosphatase. Compounds 1—3 showed no inhibitory effects toward a dual-specificity protein tyrosine phosphatase (VHR) and protein serine/threonine phosphatase (PPase1) with an IC\(_{50}\) value of more than 100 μM or 500 μM, respectively (Table 1). Therefore, it was suggested that compounds 1—3 have a certain degree of specific inhibitory effect on PTP1B. Abietane diterpenes from S. miltiorrhiza, such as isotanshinone IIA, dihydroisotanshinone I, and isocryptotanshinone, have been known to have antibacterial, antifungal, antioxidant, antimutagenic, anti-inflammatory, and antiplatelet aggregation activities.\(^{15–18}\) However, PTP1B inhibitory effects by member of this class have not been previously re-

Table 1. Comparison of the Inhibitory Activity of the Compounds 1—3 Isolated from S. miltiorrhiza against PTP1B, VHR and PPase1

<table>
<thead>
<tr>
<th>Compounds</th>
<th>PTP1B (IC(_{50}) μM)</th>
<th>VHR</th>
<th>PPase1</th>
<th>PTP1B (IC(_{50}) μM)</th>
<th>VHR</th>
<th>PPase1</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11.4±0.6</td>
<td>&gt;100</td>
<td>&gt;500</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>22.4±0.6</td>
<td>&gt;100</td>
<td>&gt;500</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>56.1±6.3</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RK-682(^{20})</td>
<td>5.0±0.4</td>
<td>10.2±1.2</td>
<td>&gt;100</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^{a}\) Positive control.\(^{23}\)
ported.

Acknowledgements  This research was supported in part by the grants from the Plant Diversity Research Center of 21st Frontier Research Program (PF0320903-00), the Molecular BioDiscovery Research Program (M1-0311-00-0023) of the Ministry of Science and Technology of Korea, and the grant from KRICT Research Initiative Program.

REFERENCES