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Abstract: Objective To identify the active compounds for protein tyrosine phosphatase 1B (PTP1B) from the seeds of Plantago asiatica. Methods Bioassay-guided fractionation resulted in the isolation of iridoid glucosides (1–5) with PTP1B inhibitory activity. Results Five compounds were identified as desacetylhookerioside (1), melittoside (2), geniposidic acid (3), 10-O-acetyl-geniposidic acid (4), and alpinoside (5). Conclusion Isolated compounds 3–5 inhibit PTP1B with IC₅₀ values ranged from (16.3 ± 1.1) to (19.8 ± 1.2) μmol/L.

Key words: alpinoside; geniposidic acid; iridoid glucosides; Plantago asiatica; protein tyrosine phosphatase 1B

Introduction

Binding insulin to the extracellular α-subunit of insulin receptor (IR) triggers a conformational change that activates the intrinsic tyrosine kinase activity of the β-subunit via autophosphorylation of specific tyrosine residues. These results in the phosphorylation of IR substrates (IRS) 1–4, which then activate several signaling cascades, lead to biological responses, such as glucose transport into the cell and glycogen synthesis (Johnson, Ermolieff, and Jirousek, 2002). Protein tyrosine phosphatases (PTPs) are responsible for the dephosphorylation of tyrosine residues, and are considered as negative regulators of insulin signaling. Although several PTPs such as PTP-α, leukocyte antigen-related tyrosine phosphatase (LAR), and SH2-domain-containing phosphorysine phosphatase (SHP2) have been implicated in the regulation of insulin signaling, there is substantial evidence supporting protein tyrosine phosphatase 1B (PTP1B) as the critical PTP-controlling insulin signaling pathway (Bialy and Waldmann, 2005). PTP1B can interact with and dephosphorylate the activated IR as well as IRS proteins (Bialy and Waldmann, 2005). Its overexpression has been shown to inhibit the IR signaling cascade and increased expression of PTP1B occurs in insulin-resistant states (Ahmad et al., 1997). Furthermore, recent genetic evidence has shown that PTP1B gene variants are associated with changes in insulin sensitivity (Elchebly et al., 1999). As with the insulin signaling pathway, the leptin signaling pathway can be attenuated by PTPs and there is compelling evidence that PTP1B is also involved in this process (Johnson, Ermolieff, and Jirousek, 2002). Therefore, it has been suggested that compounds that reduce PTP1B activity or expression levels can be used not only for treating type 2 diabetes but also obesity (Han, Liang, and Chen, 2005). Although there have been a number of reports on the design and development of PTP1B inhibitors (Taylor and Hill, 2004), new types of PTP1B inhibitors with suitable pharmacological properties remain to be discovered. Since plants are a promising source for the development of new PTP1B inhibitors (Cui et al., 2006; Zhu et al., 2010), we have undertaken a screen of hundreds of plant extracts against this biological target.

During this screening effort we found that a BuOH-soluble extract of Plantago asiatica L. inhibited PTP1B activity (> 70% inhibition at 30 μg/mL). The ispaghula (P. ovate Forssk.) is an indigenous product of South Asia and is a widely used herbal product both in traditional and modern medicines. Seed husk of ispaghula has a long history of use as a dietary fiber supplement to promote the regulation of large bowel
function (Cummings and Stephen, 1980). It has been shown to lower blood cholesterol level in recent studies (Anderson et al., 2000). Moreover, it is used in folk medicines as demulcent, emollient, and laxative. It is used in the treatment of dysentery, constipation, catarrhal conditions of the genito-urinary tract, and inflamed membranes of the intestinal canal, etc. A number of chemical constituents including phenyl-ethanoid glycosides, flavonoids, and iridoid glucosides have been isolated from this genus, some of which exhibited antimicrobial and anti-oxidative activities (Gohel et al. 2000; Gohel, Patel, and Amin, 2003). But the biologically active principles are still unknown. Further investigation on the PTP1B inhibitory compounds from this plant has led to the isolation of a series of iridoid glucosides. The isolation, structure elucidation, and evaluation of PTP1B inhibitory properties of these compounds are described in this paper.

Materials and methods

Materials

The whole sample of *Plantago asiatica* L. was purchased from the Herbal Medicine Association of Taejon, Korea and was identified by Prof. BAE Kihwan, College of Pharmacy, Chungnam National University, and the voucher specimen (No. 06103) was deposited at the College of Pharmacy, Beihua University, Jilin, China.

NMR spectra were recorded at ACF—400 NMR instrument (1H-NMR: 400 MHz, 13C-NMR: 100 MHz), with TMS as internal standard. All accurate mass experiments were performed on a Micromass QTOF2 (Micromass, Wythenshawe, UK) Mass Spectrometer. Column chromatography was conducted using MCI gel CHP-20P (75 μm, Mitsubishi) and RP-18 (40 μm, Merck). TLC was performed on precoated RP-18F254s plates (Merck). Spots were visualized using UV light or 10% sulfuric acid.

Extraction and isolation

The seeds of *P. asiatica* (500 g) were extracted with MeOH (5.0 L) for 24 h. The MeOH extract was concentrated, suspended in H2O, and sequentially partitioned with hexane, EtOAc, and BuOH. The BuOH-soluble extract (105.0 g, IC-50 = 30.2 μg/mL) was separated by RP-C18 column chromatography (30 cm × 10 cm, 40–63 μm) using a gradient of H2O-MeOH (100:1→1:1), to yield five fractions (Fr. 1–5) according to their TLC profiles. The PTP1B inhibitory activity was concentrated in Fr. 2 (9.5 g, IC-50 = 21.2 μg/mL), eluted with H2O-MeOH (70:1→50:1), which was separated by column chromatography (30 cm × 5 cm, 75–150 μm) over CHP-20 gel with H2O-MeOH (50:1→1:1); this yielded pure compounds 1 (16.0 mg, 0.03%), 2 (11.0 mg, 0.03%), 3 (15.0 mg, 0.05%), 4 (7.0 mg, 0.09%), and 5 (9.0 mg, 0.1%), respectively (Fig. 1).

PTP1B assay

PTP1B (human, recombinant) was purchased from BIOMOL® International LP (Plymouth Meeting, PA). The enzyme activity was measured using p-nitrophenyl phosphate (pNPP), as described previously (Cui et al., 2006). To each of 96 well in a microtiter plate (final volume: 100 μL) was added 2 mmol/L pNPP and PTP1B (0.05 – 0.1 μg) in a buffer containing 50 mmol/L citrate (pH 6.0), 0.1 mol/L NaCl, 1 mmol/L EDTA, and 1 mmol/L dithiothreitol (DTT), with or without test compounds. Following incubation at 37 °C for 30 min, the reaction was terminated with 1 mol/L NaOH. The amount of produced p-nitrophenol was estimated by measuring the absorbance at 405 nm. The nonenzymatic hydrolysis of 2 mmol/L pNPP was corrected by measuring the increase in absorbance at 405 nm obtained in the absence of PTP1B enzyme.

Results and discussion

Compound 1: white amorphous powder; ESI-MS: m/z 535 [M – H]+; [α]D25′′−38° (c 0.6, MeOH). 1H-NMR (400 MHz, D2O) δ: 7.60 (s, H-3), 6.30 (s, H-1), 5.59 (d, J = 8.0 Hz, H-1″), 4.85 (d, J = 8.0 Hz, H-1″), 4.32 (d, J = 12.0 Hz, H-10a), 4.20 (d, J = 12.0 Hz, H-10b), 2.49 (m, 3H, CH2-7 and H-6a), 1.48 (m, H-6b); 13C-NMR (100 MHz, D2O) δ: 168.2 (C-11), 154.2 (C-3), 143.8 (C-8), 130.0 (C-9), 113.2 (C-4), 99.3 (C-1′), 94.6 (C-1″), 92.6 (C-1), 77.5 (C-5″), 77.0 (C-5″), 76.5 (C-3″), 76.3 (C-3″), 73.5 (C-2″), 72.9 (C-2″), 70.2 (C-4′), 70.1 (C-
Compound 2: white amorphous powder; ESI-MS: m/z 523 [M – H]−; [α]D−41.9° (c 1.03, MeOH). 1H-NMR (400 MHz, D2O) δ: 6.52 (1H, d, J = 6.5 Hz, H-3), 5.86 (1H, dd, J = 1.80, 2.0 Hz, H-7), 5.46 (1H, d, J = 5.4 Hz, H-1), 5.16 (1H, d, J = 6.5 Hz, H-4), 4.58 (1H, br s, H-6), 4.28, 4.26 (2H, J = 15.2 Hz, 2H-10), 5.36 (d, J = 8.0 Hz, H-1″), 4.66 (d, J = 8.0 Hz, H-1′), 3.35 (1H, d, J = 5.4 Hz, H-9); 13C-NMR (100 MHz, D2O) δ: 172.6 (C-11), 151.5 (C-3), 138.4 (C-8), 132.8 (C-7), 113.9 (C-4), 99.6 (C-1′), 97.8 (C-9), 77.2 (C-5′), 76.5 (C-5′), 73.4 (C-3′), 70.3 (C-4′), 66.1 (C-10), 61.8 (C-6′), 35.3 (C-5). Compound 3 was identified as geniposidic acid by comparison of the spectral data with the literature (Jensen, Olsen, and Rasmussen, 1996).

All the isolates were assayed for their inhibitory activity against PTP1B, and the results are presented in Table 1. The known PTP1B inhibitors, such as RK-682 (Cui et al., 2006), were used as positive controls in this assay. Among the isolates, compounds 3–5 inhibited PTP1B activity in a dose-dependent manner with IC50 values ranging from (16.3 ± 1.0) to (19.8 ± 1.2) μmol/L, while compounds 1 and 2 showed very weak inhibitory effects. Compound 1 (IC50 > 60 μmol/L), with glucosyl moiety substituent at C-11 exhibited a significantly lower PTP1B inhibitory activity than that of compounds 3–5 on which similar positions were attached as carboxyl groups. Furthermore, compounds 3 and 4 showed higher activities, indicating that acyl group substituent at C-10 may indistinctively increase PTP1B activity. Although structure-activity relationships of iridoid glucosides bearing carboxylic groups were not thoroughly investigated, our results indicated that substitution of carboxyl groups on iridoid glucosides may be important for in vitro PTP1B inhibitory activity. Therefore, further investigation and optimization of these

### Table 1: Comparison of the inhibitory activities of the compounds isolated from P. asiatica against PTP1B

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC50 / (μmol·mL−1) ± (μ)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>&gt; 60</td>
</tr>
<tr>
<td>2</td>
<td>&gt; 60</td>
</tr>
<tr>
<td>3</td>
<td>17.7 ± 2.5</td>
</tr>
<tr>
<td>4</td>
<td>16.3 ± 1.1</td>
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<tr>
<td>5</td>
<td>19.8 ± 1.2</td>
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<tr>
<td>RK-682</td>
<td>4.5 ± 0.5</td>
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</table>

a IC50 values were determined by regression analyses and expressed as mean ± (μ) of three replicates.
b: positive control (Cui et al., 2006)
derivatives might enable the finding of new PTP1B inhibitors that are potentially useful in the treatment of type 2 diabetes as well as obesity.

References