Protein Tyrosine Phosphatase 1B Inhibitors from *Plantago asiatica*

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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 IC_{50} 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

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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 phosphotyrosine 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 over-expression 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

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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

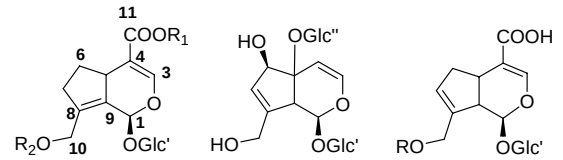
Materials

The whole sample of *Plantago asiatica* L. was purchased from the Herbal Medicine Association of Taejon, Korea and was identified by Prof. BAE Ki-hwan, 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 (^{1}H-NMR: 400 MHz, ^{13}C-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 (75－150 μm, Mitsubishi) and RP-18 (40－63 μm, Merck). TLC was performed on precoated RP-1860 F_{254s} 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 H_2O, 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-C_{18} column chromatography (30 cm × 10 cm, 40－63 μm) using a gradient of H_2O-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 H_2O-MeOH (70:1→50:1), which was separated by column chromatography (30 cm × 5 cm, 75－150 μm) over CHP-20 gel with H_2O-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).



1 R_1=Glc"; R_2=H
5 R_1=H; R_2=Ac

2

3 R=H
4 R=Ac

Fig. 1 Structures of compounds 1－5

PTP1B assay

PTP1B (human, recombinant) was purchased from BIOMOL® International LP (Plymouth Meeting, PA). The enzyme activity was measured using *p*-nitrophenyl phosphate (*p*NPP), 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 *p*NPP 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 ℃ 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 *p*NPP 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 $[M - H]^-$; $[\alpha]_D^{25}$ −38° (*c* 0.6, MeOH). ^{1}H-NMR (400 MHz, D_2O) 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, CH_2-7 and H-6a), 1.48 (m, H-6b); ^{13}C-NMR (100 MHz, D_2O) 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-

4″),61.7 (C-6″), 61.4 (C-10), 61.2 (C-6′), 37.8 (C-5), 35.8 (C-7), 31.5 (C-6). Compound **1** was identified as desacetylhookerioside by comparison of the spectral data with the literature (Jensena, Olsenb, and Rasmussena, 1996).

Compound **2**: white amorphous powder; ESI-MS: *m/z* 523 $[M - H]^-$; $[\alpha]_D^{25}$ −41.9° (*c* 1.03, MeOH). ^{1}H-NMR (400 MHz, D_2O) *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* = 8.0Hz, H-1″), 4.66 (d, *J* = 8.0 Hz, H-1′), 3.35 (1H, d, *J* = 5.4Hz, H-9); ^{13}C-NMR (100 MHz, D_2O) *δ*: 147.2 (C-8), 143.2 (C-3), 128.2 (C-7), 105.8 (C-4), 99.7 (C-1′), 98.2 (C-1″), 94.2 (C-1), 80.1 (C-5), 79.9 (C-6), 78.2 (C-3′), 78.4 (C-3″), 77.2 (C-5′), 78.1 (C-5″), 74.9 (C-2′), 75.1 (C-2″), 70.1 (C-4′), 71.7 (C-4″), 62.1 (C-6′), 62.7 (C-6″), 60.9 (C-10), 50.6 (C-9). Compound **2** was identified as melittoside by comparison of the spectral data with the literature (Swiatek, Salama, and Sticher, 1982).

Compound **3**: white amorphous powder; ESI-MS: *m/z* 373 $[M - H]^-$; $[\alpha]_D^{25}$ +11° (*c* 0.5 MeOH). ^{1}H-NMR (400 MHz, D_2O) *δ*: 7.30 (1H, s, H-3), 5.57 (1H, br s, H-7), 5.00 (1H, d, *J* = 7.6 Hz, H-1), 4.52 (1H, d, *J* = 7.8 Hz, H-1′), 4.07 (1H, d, *J* = 14.4 Hz, H-10b), 3.98 (1H, d, *J* = 14.4 Hz, H-10a), 2.93 (1H, dd, *J* = 16.0, 7.8 Hz, H-6b), 2.57 (1H, t, *J* = 7.6 Hz, H-9), 1.88 (1H, dd, *J* = 16.0, 7.8 Hz, H-6a); ^{13}C-NMR (100 MHz, D_2O) *δ*: 172.6 (C-11), 153.5 (C-3), 142.4 (C-8), 129.8 (C-7), 112.9 (C-4), 99.8 (C-1), 98.0 (C-1′), 77.2 (C-5′), 76.7 (C-3′), 73.8 (C-2′), 70.5 (C-4′), 61.6 (C-10), 60.8 (C-6′), 46.7 (C-9), 39.0 (C-6), 35.3 (C-5). Compound **3** was identified as geniposidic acid by comparison of the spectral data with the literature (Zuhal *et al*, 2006).

Compound **4**: white amorphous powder; ESI-MS: *m/z* 415 $[M - H]^-$; $[\alpha]_D^{25}$ +7° (*c* 0.7 MeOH). ^{1}H-NMR (400 MHz, D_2O) *δ*: 7.12 (s, H-3), 5.89 (m, H-7), 5.28 (d, *J* = 5.0 Hz, H-1), 4.75 (m, 10-CH_2), 4.48 (d, *J* = 7.2 Hz, H-1′), 2.9 (m, H-9), 2.7 (m, H-6a), 2.1 (m, H-6b), 2.05 (s, CH_3CO-); ^{13}C-NMR (100 MHz, D_2O) *δ*: 175.2 (CH_3CO-), 170.6 (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-1), 77.0 (C-5′), 76.5 (C-3′), 73.5 (C-2′), 70.3 (C-4′), 63.6 (C-10), 61.3 (C-6′), 47.0 (C-9), 39.0 (C-6), 35.0 (C-5). Compound **4** was identified as 10-*O*-acetyl-geniposidic acid by comparison of the spectral data with the literature (Jensena, Olsenb, and Rasmussena, 1996).

Compound **5**: white amorphous powder; ESI-MS: *m/z* 415 $[M - H]^-$; $[\alpha]_D^{25}$ −54° (*c* 0.6 MeOH). ^{1}H-NMR (400 MHz, D_2O) *δ*: 7.06 (s, H-3), 6.22 (s, H-1), 4.80 (d, *J* = 8.0 Hz, H-1′), 4.75 (m, 10-CH_2), 2.48 (m, 3H, CH_2-7 and H-6a), 2.06 (s, CH_3CO-), 1.47 (m, H-6b); ^{13}C-NMR (100 MHz, D_2O) *δ*: 174.6 (CH3CO-), 168.5 (C-11), 146.6 (C-3), 137.4 (C-8), 133.4 (C-9), 119.2 (C-4), 99.8 (C-1′), 91.8 (C-1), 77.2 (C-5′), 76.3 (C-3′), 73.4 (C-2′), 70.3 (C-4′), 61.6 (C-10), 61.4 (C-6′), 39.3 (C-5), 34.2 (C-7), 31.2 (C-6). Compound **5** was identified as alpinoside by comparison of the spectral data with the literature (Jensena, Olsenb, and Rasmussena, 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 [IC_{50} = (4.5 ± 0.5) μmol/L] (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 IC_{50} values range from (16.3 ± 1.1) to (19.8 ± 1.2) μmol/L, while compounds **1** and **2** showed very weak inhibitory effects. Compound **1** (IC_{50} > 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 acetyl group substituent at C-10 may indistinctively increase PTP1B activity. Although structure-activity relationships of iridoid glucosides bearing carboxyl 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

Compounds	IC_{50} / (μmol·mL^{-1})[a]
1	> 60
2	> 60
3	17.7 ± 2.5
4	16.3 ± 1.1
5	19.8 ± 1.2
RK-682 [b]	4.5 ± 0.5

a IC_{50} values were determined by regression analyses and expressed a ($\bar{x} \pm s$) 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.

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Introduction of *R* & *D of Kudingcha*

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Chinese version, 233pp. 2010. Science Press: Beijing

Kudingcha, one of the best substitutes of tea, also called other kind of tea in practice and yield, is widely used as health drink with a long history in China. It has been playing an important role in economic development in Chinese rural areas. However, there is no identical quality standard to guide the production and application in China, which significantly affects the development of the Three Rural Issues (rurality, agriculture, and farmers) and China's Western Development.

R & *D of Kudingcha*, edited by Profs. He ZD, Peng Y, and Xiao PG and published by Science Press, is the first monograph on this drink resource. In this book, *Kudingcha* has been overall elaborated in botanical resources, pharmacognosy, pharmacology, pharmaceutical chemistry, and clinical medicine covering the research achievements from 1980 to 2010. Authors not only summarized the botanical origins, chemical constituents, pharmacological effects, and clinical practices of *Kudingcha*, but also provided suggestion for the standards of quality control and development of *Kudingcha* industry. The book is a good reference for the further R & D of resources and bioactivities of *Kudingcha* as well as establishment of reasonable and identical quality standards of *Kudingcha* in Chinese market. It is not only a good assistant for researchers and practitioners working in the field of functional food and drug R & D, but also helpful for institutes and agencies of pharmacy to make decisions in *Kudingcha* trade market.