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Protein Tyrosine Phosphatase 1B inhibitory Activity of Anthraquinones and Stilbenes

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Abstract – Protein tyrosine phosphatase 1B (PTP1B) is emerging as a potential therapeutic target for the treatment of type-2 diabetes and obesity. To search for new types of PTP1B inhibitors, we have undertaken in vitro enzyme assay for some anthraquinones and stilbenes isolated from plants. Of the anthraquinones tested, physcion (**1**), 1-*O*-methylemodin (**2**), and emodin (**3**) showed high activities, with IC_{50} values of 7.6, 7.0, and 3.8 μg/mL, respectively, while the anthraquinone glycosides, physcion-8-*O*-β-D-glucopyranoside (**4**) and emodin-8-*O*-β-D-glucopyranoside (**5**), were less active than their aglycones. All the stilbenens (**6** - **15**) slightly inhibited PTP1B activity at high concentration of 30 μg/mL. Our findings suggest that the hypoglycemic effect of anthraquinones may be associated with their PTP1B inhibitory activity.
Keywords – Protein tyrosine phosphatase 1B (PTP1B), anthraquinones, stilbenes, in vitro enzyme assay

Introduction

Insulin plays an important role in the regulation of energy metabolism, and metabolic diseases such as diabetes and obesity are associated with insulin resistance (Johnson *et al*., 2002; Issad *et al*., 2003; Bialy and Waldmann, 2005). Although many enzymes and transcription factors in the insulin signaling pathway are potential targets for drug discovery, molecules that specifically increase the action of insulin may be of particular interest (Johnson *et al*., 2002; Issad *et al*., 2003; Bialy and Waldmann, 2005). Protein tyrosine phosphatase 1B (PTP1B) localized predominantly on intracellular membranes inhibits the insulin signaling cascade by means of dephosphorylation of insulin receptor as well as insulin receptor substrates (Johnson *et al*., 2002; Issad *et al*., 2003; Bialy and Waldmann, 2005). Accordingly, PTP1B is emerging as a potential therapeutic target for the treatment of type-2 diabetes and obesity (Johnson *et al*., 2002; Bialy and Waldmann, 2005). To date several types of synthetic PTP1B inhibitors have been developed and applied for clinical trials (Johnson *et al*., 2002; Taylor and Hill, 2004; Bialy and Waldmann, 2005). But, due to the toxicity, side effects, and low bioavailability, new types of PTP1B inhibitors still need to be discovered. In this point of view, natural products are recognized as an attractive source for the development of new PTP1B inhibitors. Since several of plants that belong to Polygonaceae showed the PTP1B inhibitory activity in our preliminary test, we evaluate the PTP1B inhibitory activity of anthraquinones and stilibenes, major constituents of the family Polygonaceae.

Anthraquinones are one of the representative secondary metabolites that are found not only in plants but also in microorganisms. They possess astringent, purgative, anti-inflammatory, anticancer, and antimicrobial activities (Huang *et al*., 2007; Jin *et al*., 2005). Recent studies suggest that various bioactivities of anthraquinones are associated with the inhibition of key enzymes and transcription factors such as tyrosine kinases, phosphoinositol 3-kinase (PI3K), protein kinase C (PKC), NF-κB, and mitogen-activated protein kinase (MAPK) (Huang *et al*., 2007). Those findings indicate that anthraquinones are capable of modulating signaling cascades in cells, which leads us to evaluate the PTP1B inhibitory activity of anthraquinones because PTP1B is also one the intracellular enzymes. Stilbenes are a group of plant polyphenols that are found in many families of higher plants. They have recently attracted a great deal of attention for their biological activities (Na *et al*., 2007a).

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However, there are no reports on the PTP1B inhibitory activity. To find out new types of PTP1B inhibitors, in this study, we evaluate the PTP1B inhibitory activity of anthraquinones and stilbenes using in vitro enzyme assay.

Experimental

Compounds – Anthraquinones, physcion (**1**), 1-*O*-methylemodin (**2**), emodin (**3**), physcion-8-*O*-β-D-glucopyranoside (**4**), and emodin-8-*O*-β-D-glucopyranoside (**5**), were isolated from *Reynoutria sachalinensis* (Jin *et al*., 2005). Stilbenes, resveratrol (**6**), oxyresveratrol (**7**), piceatannol (**8**), rhapontigenin (**9**), deoxyrhapontigenin (**10**), rhaponticin (**11**), deoxyrhaponticin (**12**), piceid (**13**), piceid-2''-gallate (**14**) and piceid-2''-coumarate (**15**), were used the same compounds as those previously described (Na *et al*., 2007a). The purity of compounds tested was > 98%.

Physcion (1) – red brick needle, mp 275 - 278 °C; EIMS *m/z*: 284 $[M]^+$; IR ν_{max} cm^{-1}: 3400 (OH), 1622, 1598 (C = O), 1480 (C = C); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 12.20 (1H, s, OH), 11.99 (1H, s, OH), 7.60 (1H, d, *J* = 2.4 Hz , H-4), 7.33 (1H, d, *J* = 2.4 Hz , H-2), 7.06 (1H, d, *J* = 2.5 Hz, H-5), 6.66 (1H, d, *J* = 2.5 Hz, H-7), 3.94 (3H, s, OCH_3), 2.43 (3H, s, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 161.8 (C-1), 119.5 (C-2), 149.2 (C-3), 120.8 (C-4), 108.0 (C-5), 164.8 (C-6), 106.5 (C-7), 162.1 (C-8), 192.0 (C-9), 181.3 (C-10), 137.2 (C-11), 113.7 (C-12), 115.8 (C-13), 133.4 (C-14), 22.0 (C-15), 56.3 (C-16).

1-*O*-methylemodin (2) – orange solid, mp 257 -260 °C; FABMS *m/z*: 285 $[M + H]^+$; IR ν_{max} cm^{-1}: 3400 (OH), 1622, 1598 (C = O), 1480 (C = C); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 12.28 (1H, s, OH-8), 12.11 (1H, s, OH-6), 7.59 (1H, d, *J* = 2.4 Hz, H-4), 7.33 (1H, d, *J* = 2.5 Hz, H-5), 7.06 (1H, d, *J* = 2.4 Hz, H-2), 6.66 (1H, d, *J* = 2.5 Hz, H-7), 3.93 (3H, s, OCH_3), 2.44 (3H, s, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 162.3 (C-1), 124.4 (C-2), 148.3 (C-3), 121.1 (C-4), 108.2 (C-5), 166.4 (C-6), 106.7 (C-7), 165.0 (C-8), 190.6 (C-9), 181.8 (C-10), 134.9 (C-11), 110.2 (C-12), 113.6 (C-13), 133.1 (C-14), 22.2 (C-15), 65.1 (C-16).

Emodin (3) – orange needle, mp 259 - 260 °C; EIMS *m/z*: 270 $[M]^+$; IR ν_{max} cm^{-1}: 3400 (OH), 1622, 1598 (C = O), 1480 (C = C); ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 12.03 (1H, s, OH), 11.95 (1H, s, OH), 7.40 (1H, d, *J* = 2.4 Hz, H-4), 7.09 (1H, d, *J* = 2.4 Hz, H-2), 7.05 (1H, d, *J* = 2.3 Hz, H-5), 6.55 (1H, d, *J* = 2.3 Hz, H-7), 2.38 (3H, s, CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 161.3 (C-1), 123.9 (C-2), 148.1 (C-3), 120.3 (C-4), 108.7 (C-5), 164.5 (C-6), 107.8 (C-7), 164.3 (C-8), 189.5 (C-9), 181.1 (C-10), 134.9 (C-11), 108.7 (C-12), 113.1 (C-13), 132.6 (C-14), 21.4 (C-15).

Physcion-8-*O*-β-D-glucopyranoside (4) – orange needle, mp 230 - 232 °C; FABMS *m/z*: 469 $[M + Na]^+$; UV λ_{max} nm: 201, 211, 216, 268; IR ν_{max} cm^{-1}: 3400 (OH), 1622, 1598 (C = O), 1088 (glycoside C-O); ^{1}H-NMR (600 MHz, DMSO) δ: 7.48 (1H, s, H-4), 7.36 (1H, d, *J* = 2.4 Hz, H-5), 7.18 (1H, d, *J* = 2.4 Hz, H-7), 7.16 (1H, s, H-2), 5.16 (1H, d, *J* = 7.6 Hz, H-1'), 3.95 (3H, s, OCH_3), 2.40 (3H, s, CH_3); ^{13}C-NMR (150 MHz, DMSO) δ: 161.5 (C-1), 124.1 (C-2), 147.0 (C-3), 119.3 (C-4), 107.2 (C-5), 164.6 (C-6), 106.4 (C-7), 160.5 (C-8), 186.3 (C-9), 181.7 (C-10), 136.2 (C-11), 114.3 (C-12), 114.3 (C-13), 131.9 (C-14), 21.5 (C-15), 56.1 (C-16), 100.6 (C-1'), 73.2 (C-2'), 76.6 (C-3'), 69.7 (C-4'), 77.4 (C-5'), 60.7 (C-6').

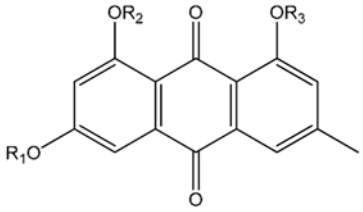
Emodin-8-*O*-β-D-glucopyranoside (5) – orange needle, mp 192 - 193 °C; FABMS *m/z*: 455 $[M + Na]^+$; UV λ_{max} nm: 201, 222, 284; IR ν_{max} cm^{-1}: 3400 (OH), 1622, 1598 (C = O), 1088 (glycosidic C-O); ^{1}H-NMR (600 MHz, CD_3OD) δ: 7.44 (1H, d, *J* = 0.8 Hz, H-4), 7.27 (1H, d, *J* = 2.4 Hz, H-5), 7.14 (1H, d, *J* = 0.8 Hz, H-2), 6.98 (1H, d, *J* = 2.4 Hz, H-7), 5.05 (1H, d, *J* = 7.6 Hz, H-1'), 2.39 (3H, s, CH_3); ^{13}C-NMR (150 MHz, CD_3OD) δ: 161.6 (C-1), 124.1 (C-2), 146.8 (C-3), 119.2 (C-4), 108.2 (C-5), 163.9 (C-6), 108.2 (C-7), 160.9 (C-8), 186.3 (C-9), 181.9 (C-10), 136.4 (C-11), 113.2 (C-12), 114.3 (C-13), 131.9 (C-14), 21.5 (C-15), 100.6 (C-1'), 73.2 (C-2'), 76.4 (C-3'), 69.4 (C-4'), 77.3 (C-5'), 60.5 (C-6').

In vitro 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 (Na *et al*., 2007b). To each of 96 wells in a microtiter plate (final volume: 100 μL) was added 2 mM *p*NPP and PTP1B (0.05 - 0.1 μg) in a buffer containing 50 mM citrate (pH 6.0), 0.1 M NaCl, 1 mM EDTA, and 1 mM dithiothreitol (DTT), with or without test compounds. Following incubation at 37 °C for 30 min, the reaction was terminated with 10 M NaOH. The amount of produced *p*-nitrophenol was estimated by measuring the absorbance at 405 nm. The non-enzymatic hydrolysis of 2 mM *p*NPP was corrected by measuring the increase in absorbance at 405 nm obtained in the absence of PTP1B enzyme.

Results and Discussion

It is well established that insulin signaling, in particular activation of insulin receptor and insulin receptor substrates, is impaired in most patients with Type 2 diabetes (Johnson *et al*., 2002; Issad *et al*., 2003). Protein tyrosine phosphatases (PTPs) are responsible for the

Table 1. Inhibition of PTP1B by anthraquinones **1** - **5**



	R_1	R_2	R_3	IC_{50} (μg/mL)
Physcion (**1**)	CH_3	H	H	7.6
1-*O*-methylemodin (**2**)	H	H	CH_3	7.0
Emodin (**3**)	H	H	H	3.8
Physcion-8-*O*-β-D-glucopyranoside (**4**)	CH_3	Glc[a]	H	25.5
Emodin-8-*O*-β-D-glucopyranoside (**5**)	H	Glc	H	22.7
RK-682				4.5 ± 0.5[b]
Ursolic acid				3.6 ± 0.2[b]

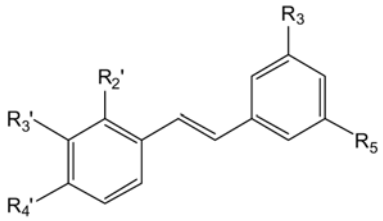
[a] Glc: glucose.
[b] IC_{50} values expressed as μM.

dephosphorylation of tyrosine residues, and are considered 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 (Johnson *et al*., 2002; Issad *et al*., 2003). In cultured cells, overexpression of PTP1B markedly inhibits insulin effect on its receptor phosphorylation (Kenner *et al*., 1996). A study with PTP1B knockout mice has shown that PTP1B knockout resulted in mice with marked increase in insulin sensitivity and which are resistant to diet induced obesity (Elchebly *et al*., 1999). In consequence, PTP1B clearly appears as a potential therapeutic target for the treatment of insulin-resistance. For the development of new types of PTP1B inhibitors, we have tested hundreds of plant extracts and natural compounds, and found out that some species that belongs to Polygonaceae showed the PTP1B inhibitory activity. We thus tried to evaluate the inhibitory activity of anthraquinones and stilibenes major secondary metabolites contained in the species of Polygonaceae.

PTP1B activity was measured using *p*-nitrophenyl phosphate (*p*NPP) as a substrate. Five anthraquinones, physcion (**1**), 1-*O*-methylemodin (**2**), emodin (**3**), physcion-8-*O*-β-D-glucopyranoside (**4**), and emodin-8-*O*-β-D-glucopyranoside (**5**), were assayed for their inhibitory activity against PTP1B, and the results are presented in Table 1. The known PTP1B inhibitors, RK-682 (IC_{50} = 4.5 ± 0.5 μM) and ursolic acid (IC_{50} = 3.6 ± 0.2 μM), were used as positive controls in this assay. Most of the isolates inhibited PTP1B activity in a dose-dependent manner. Of the anthraquinones tested, emodin (**3**), 1-*O*-methylemodin (**2**), and physcion (**1**) exhibited high activities, with IC_{50} values of 3.8, 7.0, and 7.6 μg/mL, respectively. However, the anthraquinone glycosides, physcion-8-*O*-β-D-glucopyranoside (**4**) and emodin-8-*O*-β-D-glucopyranoside (**5**), were less active than their aglycones. This suggests that addition of glucose to anthraquinone skeleton may be responsible for a loss of in vitro activity. Ten stilbenes, resveratrol (**6**), oxyresveratrol (**7**), piceatannol (**8**), rhapontigenin (**9**), deoxyrhapontigenin (**10**), rhaponticin (**11**), deoxyrhaponticin (**12**), piceid (**13**), piceid-2"-gallate (**14**) and piceid-2"-coumarate (**15**), were also tested for their inhibitory activity against PTP1B. As shown in Table 2, all the stilbenens (**6** - **15**) slightly inhibited PTP1B activity at high concentration of 30 μg/mL. Their IC_{50} values were estimated over than 100 μM, which could not be considered to be active in our assay system.

We have investigated whether anthraquinones and stilbenes inhibit PTP1B activity or not, and have identified anthraquinones have the PTP1B inhibitory activity. A recent study demonstrated that the anthraquinones, aloe-emodin-8-*O*-β-D-glucopyranoside, rhein-8-*O*-β-D-glucopyranoside, and chrysophanol, isolated from *Saussrurea lappa* had moderate PTP1B inhibitory activities (Li *et al*., 2006), which is in accordance with our findings. In addition, emodin and chrysophanol isolated from *Rheum undulatum* were reported to inhibit

Table 2. Inhibition of PTP1B by stilbenes **6** - **15**



	R_3	R_5	R_2'	R_3'	R_4'	IC_{50} (μM)
Resveratrol (**6**)	OH	OH	H	H	OH	> 100 (25.2) [d]
Oxyresveratrol (**7**)	OH	OH	OH	H	OH	> 100 (29.5)
Piceatannol (**8**)	OH	OH	H	OH	OH	> 100 (30.5)
Rhapontigenin (**9**)	OH	OH	H	OH	OCH_3	> 100 (18.6)
Desoxyrhapontigenin (**10**)	OH	OH	H	H	OCH_3	> 100 (19.3)
Rhaponticin (**11**)	Oglc[a]	OH	H	OH	OCH_3	> 100 (15.1)
Deoxyrhaponticin (**12**)	OGlc	OH	H	H	OCH_3	> 100 (17.0)
Piceid (**13**)	OGlc	OH	H	H	OH	> 100 (15.6)
Piceid-2"-gallate (**14**)	OglcG[b]	OH	H	H	OH	> 100 (22.0)
Piceid-2"-coumarate (**15**)	OglcC[c]	OH	H	H	OH	> 100 (19.2)
RK-682						4.5 ± 0.5
Ursolic acid						3.6 ± 0.2

[a-c] Glc: glucose, G: galloyl, C: *p*-coumaroyl.
[d] Values in parenthesis represent % inhibition at 30 μg/mL.

postprandial hyperglycemia (Choi *et al*., 2005). Recent studies suggest that some anthraquinones are capable of modulating signaling cascades by means of inhibition of key enzymes and transcription factors in cells. Accordingly, we expect that anthraquinones may enhance the insulin action by inhibition of intracellular PTP1B activity. Therefore, further investigation and optimization of anthraquinones might enable the preparation of new PTP1B inhibitors potentially useful in the treatment of type-2 diabetes and obesity.

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