Natural PTP1B Inhibitors From Polygonum cuspidatum and Their 2-NBDG Uptake Stimulation

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Abstract
Ten active principles (compounds 1-10) have been isolated following protein tyrosine phosphatase 1B (PTP1B) assay-guided fractionation of the methanol extract of the root of Polygonum cuspidatum. The chemical structures of the compounds were characterized mainly by nuclear magnetic resonance (NMR) spectroscopic and physicochemical data. This is the first time that 9,10-anthraquinones (compounds 5-6) have been isolated from P. cuspidatum, and this is the first record of compound 9 from the genus Polygonum. Except for compound 4, all the isolates showed potential inhibitory activity against PTP1B with half-maximal inhibitory concentration IC50 values ranging from 6.3 to 28.9 µM. Furthermore, a kinetic study indicated mixed-competitive inhibition with PTP1B for compounds 2 and 9 and noncompetitive inhibition for compounds 3 and 6. In addition, compounds 2, 3, 6, and 9 also induced the 2-deoxy-2-[(7-nitro-2,1,3-benzoxadiazol-4-yl) amino]-d-glucose uptake stimulation in 3T3-L1 adipocytes at concentrations of 10 and 5 µM. Taken together, the results reveal that P. cuspidatum could be a new source of natural compounds for further research and development of antidiabetic agents.

Keywords
Polygonum cuspidatum, Polygonaceae, anthraquinone, PTP1B, glucose-uptake, 2-NBDG, stilbene

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Diabetes is a major disease of the modern world; about 90% of people suffering from this have type 2 diabetes. According to the International Diabetes Federation, an estimated 463 million adults aged 20-79 years worldwide have diabetes, and this number is expected to rise to 574 million by 2030 and 700 million by 2045.1 Glucose-transporter proteins, especially GLUT4, play an important role in glucose uptake into cells. This transport is biologically affected by the PTP1B enzyme, a member of the protein tyrosine phosphatases (PTPs) family. The PTP1B pathway plays a central role as a negative regulator in the insulin signaling pathway implicated in metabolic diseases such as obesity and type 2 diabetes. In insulin signaling, PTP1B dephosphorylates the insulin receptor (IR) and insulin receptor substrate (IRS), whereas, in the leptin pathway, it dephosphorylates the tyrosine kinase Janus kinase 2 (JAK2).2 In the insulin signaling pathway, the recruitment and phosphorylation of the IRS are triggered as a result of the binding of insulin to the IR, which forms a docking site for phosphoinositide 3-kinase (PI3K) at the membrane. Docked PI3K then induces the phosphorylation of both Akt at Thr308 and the 70 kDa ribosomal protein S6 kinase 1 (p70S6K) by activation of phosphoinositide-dependent protein kinase 1 (PDK1).3,5 This facilitates glucose uptake in cells by allowing the translocation of GLUT4 from intracellular storage vesicles to the plasma membrane. In addition, a wealth of evidence from clinical and basic research indicates that the high expression of PTP1B can induce insulin resistance. Therefore, PTP1B is an effective target for the treatment of type 2 diabetes and possibly obesity.6,7

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In Vietnam, the traditional remedies for diabetes often involve the integrated effects of different medicinal plants—some as main ingredients and some as a supplemental factor. *Polygonum cuspidatum* Sieb. et Zucc., family Polygonaceae (known as knotweed in Japan and Huzhang in China), is a small perennial, straight-growing plant that usually grows to between 60 cm and 1 m high (though in some places, it can grow as high as 2.2 m).  

*Polygonum cuspidatum* grows natively in many regions across China and Southeast Asia. It can be harvested year-round, but the best time is in the monsoon season when harvesting occurs continuously for 2-3 months. In Vietnam, *P. cuspidatum* has been used since ancient times for the treatment of liver diseases, rheumatism, arthritis, stomachache, and leg pain, and is used especially for the treatment of hepatitis. Chemically, *P. cuspidatum* is a rich source of several bioactive secondary metabolites with pharmacological effects, including stilbenes, quinones, phenylpropanoid esters of sucrose, anthraquinones, and anthraquinone glucopyranosides. In traditional Chinese medicine (TCM), this plant is used for the treatment of cancer, angiogenesis, human immunodeficiency virus (HIV), hepatitis B, obesity, and oral cancer and is known for its antimicrobial, anti-inflammatory, and neuroprotective qualities. Choi et al reported that *P. cuspidatum* extract showed free radical scavenging activity and prevented obesity-associated disorders in a diabetic rat model in vivo. In addition, the effects of *P. cuspidatum* extract on advanced glycation end products (AGEs), Nε-(carboxymethyl)-l-lysine formation, protein glycation, and diabetes have been investigated. In a diabetic rat model, the levels of blood glucose, serum malondialdehyde, cholesterol, triglycerides, and low-density lipoproteins were reduced after treatment with *P. cuspidatum* extract. However, the bioactive components of Vietnamese *P. cuspidatum* have not yet been identified. Here we describe the purification of active compounds from *P. cuspidatum* and report on their stimulatory effects for glucose uptake and the enzyme inhibitory activities on both PTP1B and α-glucosidase.

### Results and Discussion

The methanol extract of the root of *P. cuspidatum* was partitioned into n-hexane, ethyl acetate (EtOAc), and water-soluble fractions. The EtOAc fraction had the greatest inhibitory activity on PTP1B (Supplemental Material). Chromatographic purification of this fraction led to the isolation of 10 compounds (compounds 1-10) (Figure 1).

Comparison of the 1H-nuclear magnetic resonance (NMR) and 13C-NMR data of the isolated compounds with those published in the literature led to their identification as physcion (1, aloesaponarin), aloesaponarin (2, emodin), 1-hydroxy-2-methyl-6-methoxyanthraquinone (5), soranjiol (6), 2-methoxystypandrone (4), 2-trans-resveratrol (7), 2-trans-piceid (10), which is a derivative compound of 7, 2-dihydroxy-4′-methoxystilbene (8), and tricuspidatin (9) (Figure 1 and Supplemental Material). Isolates 1-10 were evaluated for their inhibitory activity against the PTP1B enzyme using an in vitro assay. The results are presented in Table 1 as half-maximal inhibitory

![Figure 1. Chemical structure of compounds 1-10 isolated from Polygonum cuspidatum.](image)

| Compounds | IC50 (µM)a | Ki values (µM) | Inhibition type |
|-----------|------------|----------------|----------------|
| 1         | 26.1 ± 0.7 | -              | -              |
| 2         | 13.3 ± 0.4 | 27.6 ± 0.9     | Mixed-competitive |
| 3         | 7.6 ± 0.1  | 15.1 ± 0.5     | Noncompetitive |
| 4         | >30        | -              | -              |
| 5         | 28.9 ± 1.7 | -              | -              |
| 6         | 12.4 ± 0.9 | 29.6 ± 0.7     | Noncompetitive |
| 7         | 16.1 ± 1.1 | -              | -              |
| 8         | 28.4 ± 1.5 | -              | -              |
| 9         | 6.3 ± 0.2  | 51.4 ± 1.2     | Mixed-competitive |
| 10        | 27.7 ± 1.4 | -              | -              |
| Ursolic acid | 3.5 ± 0.2      | -              | -              |

**Table 1.** PTP1B Inhibitory Activity of Compounds 1-10.

**Abbreviation:** IC50, half-maximal inhibitory concentration.

**Results**: The IC50 values (µM) are determined by regression analysis and expressed as the means ± SD of 3 replicates.

**Discussion**: The IC50 values (µM) are determined by regression analysis and expressed as the means ± SD of 3 replicates.

**Data not determined.**

**Positive control.**
concentration (IC$_{50}$) values. Of these isolates, compounds 3 and 9 appeared to be the most potent inhibitors of PTP1B with IC$_{50}$ values of 7.6 ± 0.1 and 6.3 ± 0.2 µM, respectively. The next most potent inhibitors were compounds 2, 6, and 7 with IC$_{50}$ values of 13.3 ± 0.4, 12.4 ± 0.9, and 16.1 ± 1.1 µM, respectively. The other compounds showed moderate inhibitory activity with IC$_{50}$ values ranging from 26.1 to 28.9 µM (Table 1). Compound 4, the only stypandrone-type compound, was not active (IC$_{50}$ >30 µM). Among the 9,10-anthraquinone compounds, physcion (compound 1) and emodin (compound 2) were similar in structure; however, compound 2, with a hydroxyl group attached at C-6, possessed stronger activity against PTP1B (IC$_{50}$ 13.3 ± 0.4 µM) than compound 1 (IC$_{50}$ 26.1 ± 0.7 µM), with a 6-methoxy substitution. Similarly, compound 6 (1-hydroxy-2-methyl-6-hydroxyanthraquinone) also showed stronger activity (IC$_{50}$ 16.1 ± 1.1 µM) than 1-hydroxy-2-methyl-6-methoxyanthraquinone (compound 5: IC$_{50}$ 28.9 ± 1.7 µM). This observation suggests that the substitution of a methoxy moiety at the C-6 position in 9,10-anthraquinones could be responsible for a decrease in the PTP1B inhibitory action of these compounds. In stilbene type compounds (compounds 7-10), the 7α-8β configuration and the hydroxylation at these positions may play an important role in the activity against PTP1B. Thus, tricuspidatin B (compound 9), with 7,8-dihydroxylation, showed potent inhibitory activity (IC$_{50}$ 6.3 ± 0.2 µM), while compound 8 (dihydro-R-resveratrol), a bis-dehydroxy analog of compound 9, showed moderate inhibitory activity (IC$_{50}$ 28.4 ± 1.5 µM). In this assay, ursolic acid, used as a positive control, displayed an IC$_{50}$ value of 3.5 ± 0.2 µM. Zhao et al described the antidiabetic activity of P. cuspidatum root by investigating the PTP1B inhibitory effects of individual constituents using PTP1B profiling combined with high-performance liquid chromatography-high resolution mass spectrometry and NMR techniques. In our assay, stypandrone (compound 4) showed weak activity with an IC$_{50}$ value >30 µM. This is in accordance with the value published by Zhao et al (IC$_{50}$ value 83.9 ± 7.45 µM). However, there were meaningful differences in the PTP1B activity for emodin (2) between our study (13.3 ± 0.4 µM) and Zhao’s (171.9 ± 35.9 µM). This discrepancy may be the result of different experimental conditions.

In our kinetic study, ursolic acid was previously determined as a mixed-competitive inhibitor. Compounds with PTP1B inhibitory potency (compounds 2, 3, 6, and 9) were further examined to determine their inhibition type. We produced Lineweaver-Burk and Dixon plot experiments with various concentrations of para-nitrophenylphosphate (p-NPP) substrate in the presence and absence of the inhibitors (Figures 2
and 3); data were analyzed using the Sigma plot program (SPCC Inc., Chicago, IL) and are presented in Table 1. The Lineweaver-Burk plot experiment indicated the inhibition type of active compounds (2, 3, 6, and 9). Of these, compounds 2 and 9, as well as the positive control (ursolic acid), exhibited mixed-competitive inhibition, as evidenced by the fact that the lines intersected at a position in the aerial of the left y-axis and the upper x-axis. Compounds 3 and 6 exhibited noncompetitive inhibition because the lines intersected at a negative value (1/[S]) on the x-axis (1/(intensity/min) = 0). Dixon plot experiments were used to determine the $K_i$ values of compounds 2, 3, 6, and 9 as 27.6 ± 0.9, 15.1 ± 0.5, 29.6 ± 0.7, and 51.4 ± 1.2, respectively (Table 1 and Figure 3).

Glucose transporters are a large group of membrane proteins that facilitate the transport of glucose across the plasma membrane. Among the 14 glucose transporters, GLUT4 is a well-characterized insulin-regulated glucose transporter expressed mainly in adipose tissues and striated muscle (ie, skeletal and cardiac muscle). The translocation of GLUT4 to the plasma membrane in muscle and adipocytes directly facilitates glucose uptake into the cells. This action is dependent mainly on the regulation of 2 physiological pathways: the AMP-activated protein kinase (AMPK) and the insulin signaling pathway. 3 PTP1B negatively regulates the insulin signaling pathway by dephosphorylating the insulin receptor (IR) and insulin receptor substrate (IRS), leading to the inactivation of PDK1 and Akt, the proteins that directly allow the translocation of GLUT4. 3,30 Therefore, the facilitation of the insulin signaling pathway by inhibition of PTP1B activity and/or reduction of its expression level can stimulate glucose uptake in adipocytes. To investigate this possibility, the stimulatory effects of compounds 2, 3, 6, and 9 on glucose uptake were further investigated in 3T3-L1 adipocyte cells using 2-deoxy-2-[(7-nitro-2, 1,3-benzoxadiazol-4-yl) amino]-d-glucose (2-NBDG) as a substrate. Figure 4 illustrates the compounds’ stimulation of 2-NBDG uptake at both 10 and 5 µM concentrations. In comparison with the control (dimethylsulfoxide [DMSO]), compound 9—the most potent inhibitor of PTP1B in this study—stimulated 2-NBDG uptake at concentrations of 5 and 10 µM, respectively. Insulin showed an induction of 1.56-fold at a concentration of 100 nM. Similar to the positive control, aloe-emodin (compound 3) showed an induction of 1.24 (±0.06) fold at a concentration of 10 µM. Emodin (compound 2) and sojanjidiol (compound 6), which had similar PTP1B inhibitory activities, also showed similar stimulatory effects on 2-NBDG uptake with 1.21 (±0.07), 1.38 (±0.06)-fold at concentrations of 5 and 10 µM, respectively. To avoid any cytotoxicity of the tested compounds to their 2-NBDG uptake stimulation result, we used
the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay to measure 3T3-L1 adipocyte cell viability in the presence of compounds 2, 3, 6, and 9. At both 5 and 10 µM, each of these compounds showed minimal cytotoxicity (Supplemental Figure 1, Supplemental Material) indicating that the 2-NBDG uptake effects of these compounds were not affected by cytotoxicity. Our data suggest that compounds 2, 3, 6, and 9 might induce glucose uptake in 3T3-L1 adipocytes cells through the insulin-signaling pathway via inhibition of PTP1B enzyme activity.

**Conclusion**

A study of the chemical constituents of the methanol extract of *P. cuspidatum* root—collected in 2018 from Thai Nguyen Province, Vietnam—has led to the purification and identification of 10 natural bioactive principles. The chemical structures were confirmed by NMR spectroscopic and mass spectrometric analyses and comparison with published data. This is the first time that 9,10-anthraquinones (compounds 5 and 6) have been isolated from *P. cuspidatum*, and compound 9 is identified from a *Polygonum* species. Except for compound 4 (IC₅₀ >30 µM), all isolates showed potential inhibitory activity against PTP1B, with IC₅₀ values ranging from 6.3 to 28.9 µM. A kinetic study to assess the compounds’ potencies showed mixed-competitive inhibition for compounds 2 and 9 and noncompetitive inhibition for compounds 3 and 6. In addition, the PTP1B inhibitors 2, 3, 6, and 9 also induced 2-NBDG uptake stimulation in 3T3-L1 adipocytes at both 10 and 5 mM concentrations. Together, our results suggest potential bioactive components from *P. cuspidatum*—including 9,10-anthraquinones and α,β-dihydroxystilbenes—as new natural products for research and development of antidiabetic agents.

**Materials and Methods**

**General Experimental Procedures**

1H-NMR (400 MHz) and 13C-NMR (100 MHz) were measured on a Varian Unity Inova 400 MHz spectrometer. Electrospray ionization-MS was obtained from a Varian FT-MS spectrometer and MicroQ-TOF III (Bruker Daltonics, Ettlingen, Germany). Other spectroscopic measurements and chromatographic techniques are presented in Supplemental Material.

**Plant Material**

The roots of *P. cuspidatum* were collected in February 2018 from Thai Nguyen province, Vietnam. The sample was identified by Dr. Nguyen Quoc Binh (Vietnam National Museum of Nature, Vietnam Academy of Science and Technology (VAST)). A voucher specimen (CKC-TN01) was deposited at the Institute of Natural Products Chemistry (INPC), VAST.

**Extraction and Isolation**

The roots of *P. cuspidatum* (1.2 kg) were dried and cut into small pieces and then extracted with methanol (MeOH) with sonication for 2 hours, at 45 °C. The extract was then filtered before being evaporated under reduced pressure to give a crude MeOH extract (250 g). This was dissolved in water and further partitioned with n-hexane and EtOAc to give a hexane fraction (20 g) an EtOAc fraction (120.8 g), and a water residue. These fractions were then tested for their 2-NBDG uptake effects on 3T3-L1 adipocytes cells. The detailed isolation and purification of the isolated compounds are presented in the Supplemental Material.
Biological Assay

The PTP1B inhibitory assays, determination of the inhibition mode of active compounds, the 2-NBDG uptake stimulatory, and cell viability assay were performed according to the methods described in Supplemental Material.

Statistical Analysis

Data are represented as mean ± SD of at least 3 independent experiments performed in triplicate assays and determined by regression analysis. Sigma Plot program version 11.0 was used for the analysis of the kinetic data.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Supplemental Material

Supplemental material for this article is available online.

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