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Coumarins from *Angelica decursiva* inhibit α-glucosidase activity and protein tyrosine phosphatase 1B

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ABSTRACT

In the present study, we investigated the anti-diabetic potential of six natural coumarins, 4-hydroxy Pd-C-III (**1**), 4′-methoxy Pd-C-I (**2**), decursinol (**3**), decursidin (**4**), umbelliferone 6-carboxylic acid (**5**), and 2′-isopropyl psoralene (**6**) isolated from *Angelica decursiva* and evaluated their inhibitory activities against protein tyrosine phosphatase 1B (PTP1B), α-glucosidase, and $ONOO^-$-mediated protein tyrosine nitration. Coumarins **1**–**6** showed potent PTP1B and α-glucosidase inhibitory activities with ranges of IC_{50} values of 5.39–58.90 μM and 65.29–172.10 μM, respectively. In the kinetic study for PTP1B enzyme inhibition, compounds **1**, **5**, and **6** were competitive, whereas **2** and **4** showed mixed type, and **3** displayed noncompetitive type inhibition. For α-glucosidase enzyme inhibition, compounds **1** and **3** exhibited good mixed-type, while **2**, **5**, and **6** showed noncompetitive and **4** displayed competitive type inhibition. Furthermore, these coumarins also effectively suppressed $ONOO^-$-mediated tyrosine nitration in a dose-dependent manner. To further investigate PTP1B inhibition, we generated a 3D structure of PTP1B using Autodock 4.2 and simulated the binding of compounds **1**–**6**. Docking simulations showed that different residues of PTP1B interacted with different functional groups of compounds **1**–**6** through hydrogen and hydrophobic interactions. In addition, the binding energies of compounds **1**–**6** were negative, suggesting that hydrogen bonding may stabilize the open form of the enzyme and potentiate tight binding of the active site of PTP1B, thereby resulting in more effective PTP1B inhibition. These results demonstrate that the whole plant of *A. decursiva* and its coumarins are useful as potential functional food ingredients for the prevention and treatment of type 2 diabetes.

1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disease characterized by hyperglycemia resulting from reduced insulin secretion and/or insulin resistance [1]. It is widely known that elevation of blood glucose caused by disruption of carbohydrate, protein, and fat metabolism can lead to diabetic complications in several organs and tissues, including eyes, kidneys, nerves, and blood vessels [2]. Several therapeutic approaches have been proposed, including inhibitors of PTP1B and α-glucosidase. Protein tyrosine phosphatases (PTPs) play a critical role in the regulation of a variety of cellular processes, such as growth, proliferation, differentiation, metabolism, immune response, cell-cell adhesion, and cell-matrix contacts [3]. PTP1B is a major non-transmembrane phosphotyrosine phosphatase in human tissues and is a known negative regulator of the insulin-stimulated signal transduction pathway [4]. Despite the identification of many potent compounds, a PTP1B-inhibiting drug has yet to reach the clinic, because of, these molecules still lack efficacy *in vivo* because they have weak oral bioavailability, poor membrane permeability and weak selectivity [5]. Therefore, it is still essential to search for new inhibitors with better safety and high efficacy. Modern medicine indicates that one of the most effective therapeutic approaches for controlling blood glucose level

Abbreviations: DMSO, dimethyl sulfoxide; IC_{50}, inhibitory concentrations 50%; (PTP1B), protein tyrosine phosphatase 1B; DM, diabetes mellitus; $ONOO^-$, nitrotyrosine; $CDCl_3$, chloroform; pNPP, *p*-nitrophenyl phosphate; EDTA, ethylenediaminetetraacetic acid; pNPG, *p*-nitrophenyl α-D-glucopyranoside; DTT, dithiothreitol; TLC, thin layer chromatography.

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is to inhibit absorption of glucose by suppressing carbohydrate-hydrolyzing enzymes such as α-glucosidase [6–8]. In addition, nitrotyrosine is a product of $ONOO^-$ action and thus, the production of $ONOO^-$ can be indirectly inferred by the presence of nitrotyrosine residues [9]. Recently, much attention has been paid to the role of nitrotyrosine as a possible risk factor in diabetes, as increased levels of nitrotyrosine have been reported in the plasma of diabetic patients [10], and there is evidence that an acute increase in glycemia induces an increase in nitrotyrosine [11]. It has also been reported that postprandial hyperglycemia is accompanied by nitrotyrosine generation [12]. So, PTP1B, α-glucosidase, and nitrotyrosine are therefore attractive targets in the development of new treatments for DM and other related metabolic syndromes.

Angelica decursiva Fr. et Sav (Umbelliferae) is a perennial herb and it is widely distributed in China, Japan, and Korea. This plant has been long used in traditional Korean medicine as an antitussive, analgesic, antipyretic, tumor suppressor, and cough remedy [13,14], while in traditional Chinese medicine, it is used as a remedy for thick phlegm, asthma, and upper respiratory tract infections [15–17]. This plant is a rich source of different types of coumarin derivatives which include nodakenin, nodakenetin, isorutarine, umbelliferone, umbelliferone 6-carboxylic acid, 2′-isopropyl psoralene, *cis*-3′-acetyl-4′-angeloylkhellactone, 3′(*R*)-*O*-acetyl-4′(*S*)-*O*-tigloylkhellactone, columbianadin, Pd-C-I, Pd-C-II, Pd-C-III, 4-hydroxy Pd-C-III, (+)-decursidinol, decursin, and decursidin [13,18–22], which have been reported to possess a wide range of biological activities, including anti-inflammatory, antioxidant, neuroprotective, anti-diabetic, and anti-Alzheimer activities [13,18–20,22–24].

Despite the potentiality of different Angelica species including *A. decursiva* and its constituents (nodakenin, nodakenetin, 3′(*R*)-*O*-acetyl-4′(*S*)-*O*-tigloylkhellactone, isorutarine, umbelliferone, and *cis*-3′-acetyl-4′-angeloylkhellactone) as PTP1B and α-glucosidase weak inhibitors [18], there has been no detailed investigation into the possibility of developing anti-diabetic drugs via enzyme kinetic and molecular docking evaluation. Therefore, as a part of our continuous research to identify potent anti-diabetic agents from *A. decursiva*, we isolated and investigated the activity of six coumarins against PTP1B, α-glucosidase, and nitrotyrosine. Enzyme kinetic analyses of the compounds **1**–**6** were also performed by using Dixon and Lineweaver-Burk plots in order to confirm the type of enzymatic inhibition and to propose guidelines for coumarins as anti-diabetic agents in discovering new drugs. Since there is currently no detailed information regarding the molecular interactions between compounds **1**–**6** and PTP1B, we performed molecular docking analysis and detailed enzyme kinetic analysis in order to investigate the possibility of using compounds **1**–**6** as anti-diabetic drug candidates.

2. Materials and methods

2.1. General experimental procedures

The ^1H and ^{13}C NMR spectra were acquired using a JEOL JNM ECP-400 spectrometer at 400 and 100 MHz, respectively, in deuterated solvents chloroform ($CDCl_3$). Column chromatography was conducted using silica gel 60 (70–230 mesh, Merck, Darmstadt, Germany), sephadex LH20 (20–100 μm, Sigma, St. Louis, MO, USA), and LiChroprep® RP-18 (40–63 lm, Merck). All TLC was conducted on pre-coated Merck Kieselgel 60 F254 plates (20 × 20 cm, 0.25 mm, Merck) and using 50% H_2SO_4 as a spray reagent.

2.2. Chemicals and reagents

Yeast α-glucosidase, acarbose, *p*-nitrophenyl phosphate (pNPP), *p*-nitrophenyl α-D-glucopyranoside (pNPG), and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma-Aldrich. PTP1B (human recombinant) was purchased from Biomol International LP (Plymouth Meeting, PA, USA), and dithiothreitol (DTT) was purchased from Bio-Rad Laboratories (Hercules, CA, USA). All other chemicals and solvents used were purchased from E. Merck, Fluka, and Sigma-Aldrich, unless otherwise stated.

2.3. Isolation of coumarins from A. decursiva

The coumarins 4-hydroxy Pd-C-III, 2′-isopropyl psoralene, decursidin and umbelliferone 6-carboxylic acid were previously isolated and identified in our laboratory [13,20,22]. 4′-Methoxy Pd-C-I and decursinol was isolated from subfraction-4, and 5 respectively of dichloromethane fraction from *A. decursiva*. 4′-Methoxy Pd-C-I and decursinol were identified by spectroscopic evidence including ^1H and ^{13}C NMR, as well as by comparison with spectral published data [25–27]. The structures of all isolated compounds are shown in Fig. 1.

2.4. Assay for PTP1B inhibition

The PTP1B inhibitory activity was evaluated using pNPP Cui et al. [28]. In each well of a 96-well plate (each with a final volume of 100 μL), 40 μL of PTP1B enzyme [0.5 units diluted with a PTP1B reaction buffer containing 50 mM citrate (pH 6.0), 0.1 M NaCl, 1 mM EDTA, and 1 mM DTT] were added with or without sample dissolved in 10% DMSO. The plate was preincubated at 37 °C for 10 min and then 50 μL of 2 mM pNPP in PTP1B reaction buffer was added. Following incubation at 37 °C for 20 min, the reaction was terminated by the addition of 10 M NaOH. The amount of *p*-nitrophenyl produced after enzymatic dephosphorylation of pNPP was estimated by measuring the absorbance at 405 nm using a microplate spectrophotometer (Molecular Devices). The nonenzymatic hydrolysis of 2 mM pNPP was corrected for the measured increase in absorbance at 405 nm obtained in the absence of PTP1B enzyme. The inhibition percentage was obtained using the following equation: % inhibition = (Ac − As)/Ac × 100, where Ac is the absorbance of the control and As is the absorbance of the sample. Ursolic acid was used as a positive control.

2.5. Assay for α-glucosidase inhibition

The enzyme inhibition study was performed spectrophotometrically using the procedure reported by Li et al. [29]. A total of 60 μL of reaction mixture containing 20 μL of 100 mM phosphate buffer (pH 6.8), 20 μL of 2.5 mM pNPG, and 20 μL of the sample dissolved in 10% DMSO, was added to each well followed by 20 μL of α-glucosidase [0.2 U/mL in 10 mM phosphate buffer (pH 6.8)]. The plate was incubated at 37 °C for 15 min, and 80 μL of 0.2 M sodium carbonate solution was then added to stop the reaction. Immediately thereafter, the absorbance was recorded at 405 nm using a microplate spectrophotometer (Molecular Devices, Sunnyvale, CA, USA). The control contained the same reaction mixture except with an equivalent volume of phosphate buffer instead of any sample solution. Acarbose dissolved in 10% DMSO was used as a positive control. The inhibition percentage (%) was obtained using the same equation as in the PTP1B enzymatic assay.

2.6. Inhibition of $ONOO^-$-mediated protein tyrosine nitration

$ONOO^-$-mediated protein tyrosine nitration was evaluated