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Potent inhibition of monoamine oxidase A by decursin from *Angelica gigas* Nakai and by wogonin from *Scutellaria baicalensis* Georgi

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ABSTRACT

During the ongoing search for new monoamine oxidase (MAO) inhibitors, five coumarin derivatives and eight flavonoids were isolated from the roots of *Angelica gigas* Nakai and *Scutellaria baicalensis* Georgi, respectively. Of the phytochemicals, decursin (**4**) was found to potently and selectively inhibit human MAO-A (IC_{50} = 1.89 μM). The IC_{50} value of **4** for MAO-A belonged to the lowest group in herbal sources and was similar to that of toloxatone (1.78 μM), a marketed drug. Wogonin (**11**) effectively inhibited MAO-A and MAO-B (IC_{50} = 6.35 and 20.8 μM, respectively). Furthermore, compounds **5** (decursinol angelate) and **10** (baicalein) were observed to selectively and moderately inhibit MAO-A. In addition, compound **4** reversibly and competitively inhibited MAO-A with a K_i of 0.17 μM. Compound **11** also competitively inhibited MAO-A and MAO-B (K_i = 0.56 and 1.96 μM, respectively). Molecular docking simulation revealed that **4** interacts with Asn181 residue of MAO-A or Asn116 residue of MAO-B by formation of hydrogen bond. The findings suggest compounds **4** and **11** be considered as new potent and reversible MAO-A inhibitors or useful lead compounds for the developments of MAO inhibitors for the treatment of disorders like depression, Parkinson's disease and Alzheimer disease.

1. Introduction

Monoamine oxidase (MAO, EC 1.4.3.4) exists as two isoforms, MAO-A and MAO-B, and oxidize, and thus, breaks down neurotransmitter monoamines in the brain and peripheral tissues [1,2]. MAO-A and MAO-B are viewed as drug targets for the treatment of neuropsychiatric disorders such as depression, anxiety, and Alzheimer's and Parkinson's diseases [3]. Although the substrate specificities of MAO-A and MAO-B frequently overlap, for example, for dopamine, tyramine, epinephrine, and norepinephrine, MAO-A selectively deaminates serotonin, and MAO-B selectively deaminates phenylethylamine and benzylamine [4].

MAO inhibitors have been extensively studied and may be categorized as MAO-A selective, MAO-B selective, or MAO-A/B nonselective, and as reversible or irreversible [5–8].

During the ongoing screening of herbal libraries, we found several compounds from *Angelica gigas* Nakai (AG) and *Scutellaria baicalensis* Georgi (SB) showed MAO inhibitory activity, and that decursin and wogonin potently and moderately, respectively, inhibited MAO-A. AG (Dang-Gui) is widely distributed in Korea, China, and Japan, and its roots are viewed in traditional medicine to have anti-inflammatory, anti-cancer, and diuretic properties, and to treat anemia, pain, flu, fever, rheumatism, and infectious diseases [9,10]. Cham-Dang-Gui (the dried root of AG) is cultivated in Korea and used as a medicinal herb. SB is indigenous to Korea, China, Japan, Mongolia and Russia, and also to treat inflammatory, viral and bacterial diseases in the Orient [11,12].

Several compounds have been previously isolated from the roots of AG and SB, such as, decursin and decursinol angelate from SG and several flavonoids, including wogonin, from SB [10,13]. Decursin has been reported to have anti-cancer [10], anti-oxidative [14], and anti-inflammatory [15] effects, and to suppress osteoclast formation [16], and wogonin has been reported to have anti-oxidant, anti-inflammatory, anti-cancer, antiviral and neuroprotective activities [12]. Recently several reviews have been

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published on MAO inhibitors found in herbs and on the inhibitory activities of coumarins and flavonoids [17–21]. However, the inhibitions of MAO enzymes by decursin and wogonin have not been previously described in the literature.

In the present study, we examined the abilities of five coumarin derivatives isolated from AG and eight flavonoids isolated from SB to inhibit recombinant human MAO-A and MAO-B.

2. Materials and methods

2.1. Chemicals and enzymes

Benzylamine, kynuramine, toloxatone, lazabemide and recombinant human MAO-A and MAO-B were purchased from Sigma-Aldrich (St. Louis, MO, USA). The two enzymes were stored at −70 °C in 100 mM potassium phosphate (pH 7.4), 0.25 M sucrose, 0.1 mM EDTA, and 5% glycerol [22]. Clorgyline and pargyline were components of a monoamine oxidase kit supplied by BioAssay Systems (Hayward, CA, USA).

2.2. Instruments and reagents

^{1}H and ^{13}C NMR spectra were recorded on either a JEOL ECX-500 (JEOL, Japan) or a Varian UNITY 400 (Varian, Palo Alto, USA) spectrometer in $CHCl_3$, CD_3OD or DMSO-d_6. All chemical shifts were recorded with respect to TMS. Thin layer chromatography (TLC) was performed on pre-coated silica-gel 60 F_{254} plates or on RP-C18 F_{254s} plates (20 × 20 cm, Merck, Darmstadt, Germany), which were visualized using UV (254 nm) or by spraying with 10% H_2SO_4 in ethanol and heating. Solvents for extraction and isolation were purchased from SK Chemicals (Seoul, Korea) and redistilled before use. Deionized water was prepared using ultrapure water obtained using a Milli-Q system (18.2 MΩ, Millipore, Bedford, MA). The solvents for NMR (DMSO-d_6 and Methanol-d_4) were purchased from the Cambridge Isotope Laboratory (Andover, MA).

2.3. Plant materials and crude extract preparations

AG and SB [permitted by Korea Food and Drug Administration (KFDA)] were purchased from a local market (http://www.omniherb.com/shop/main.ubs) and voucher specimens (PBC449, PBC078-079) were deposited at the herbarium of the Plant Extract Bank of KRIBB (Daejeon, Korea). Dried AG (116 g) and SB (842 g) powders were extracted three times with 3 volumes of absolute (99.8%) methanol at room temperature. Extracts were combined and dried in vacuo at 40 °C to produce extracts (yields AG 9.2% and SB 13.1%).

2.4. Extraction and isolation

Dried powder of AG roots was extracted three times with 3 volumes of absolute (99.8%) methanol at room temperature. The crude extract (1.0 g) so obtained was subjected to MPLC reversed phase silica gel (YMC ODS-AQ, 10 μm, 220 g) using a stepwise MeOH-H_2O gradient (0–20 min 40% MeOH, 20–65 min 40–90% MeOH, 65–75 min 90–100% MeOH, 20 mL/min, 90 min) to give 10 fractions (AG Frs. 1–10). This MPLC procedure was repeated fifty times using the same conditions before further isolation. AG Fr. 4 was recrystallized from *n*-hexane and ethyl acetate to yield compound **1** (1.08 g). AG Frs. 5 and 7, which contained compounds **2** and **3**, were pooled and subjected to prep-HPLC PLC2020 using a reversed-phase column (2.0 × 25 cm, YMC-Pack-ODS-AQ, 5 μm), and eluted with a H_2O-ACN gradient (20–75% ACN, 18 mL/min, 50 min) by repeated injecting 10 mg/ml MeOH dilutions to yield pure **2** (985 mg) and **3** (2.17 g). AG Frs. 8 and 9, which were enriched with compounds **4** and **5** were pooled and isolated using the same method as the pool Frs. 5 + 7 except that a H_2O-ACN gradient (30–75% ACN) to yield pure **4** (870 mg) and **5** (170 mg).

Dried SB root powder was extracted using with essentially the same methods used for AG root powder. Briefly, crude extract (1.0 g) was subjected to MPLC using a stepwise MeOH-H_2O gradient (0–5 min 20% MeOH, 5–65 min 20–60% MeOH, 65–75 min 60–100% MeOH, 20 mL/min, 90 min) to give 11 fractions (SB Frs. 1–11). After repeating the procedure, SB Fr. 3 was recrystallized from MeOH to yield compound **6** (54.0 mg), and Frs. 4, 5, and 6 were pooled and subjected to prep-HPLC using a H_2O-ACN gradient (20–60% ACN, 50 min) to yield pure **7** (1.21 g), **8** (50.0 mg), and **9** (5.8 mg). SB Frs. 7 and 8 were combined and subjected to prep-HPLC using a Waters column (XBridge BEH C18, 20 × 250 mm, 5 μm) eluted with H_2O-ACN gradient (35% ACN isocratic solvents, 10 mL/min, 60 min) to yield pure **10** (1.15 g) and **12** (30.0 mg). SB Frs. 9 and 10 were subjected to prep-HPLC PLC2020 using an Atlantis T3 column eluted with H_2O-MeOH gradient (50–100% MeOH, 18 mL/min, 45 min) to yield pure **11** (21.0 mg) and **13** (10.2 mg).

2.5. UPLC-QTof-MS analysis

This analysis was performed using an ACQUITY UPLC™ system (Waters Corporation, Milford, MA, USA) equipped with a binary solvent delivery manager and a sample manager coupled to a Micromass Q-TOF Premier™ mass spectrometer (Waters Corporation) with an electrospray ionization (ESI) interface and running MassLynex V4.1 software. Chromatographic separation was performed using an ACQUITY HSS T3 chromatography column (2.1 × 100 mm, 1.8 μm). Column temperature was maintained at 35 °C and the mobile phases A and B used were water containing 0.1% formic acid and acetonitrile containing 0.1% formic acid, respectively. The gradient elution programs used were as follows: For AG, 0.0–1.0 min, 30% B; 1.0–3.0 min, 30–60% B; 3.0–11.0 min, 60–75% B; 11.0–12.0 min, 75–100% B; wash for 1.5 min with 100% B; and a 2.0 min recycle time; and for SB; 0.0-1.0 min, 20% B; 1.0–11.0 min, 20–60% B; 11.0–12.0 min, 60–100% B; wash for 1.5 min with 100% B; and a 2.0 min recycle time. The injection volume was 5.0 μL and the flow rate was 0.4 mL/min. The mass spectrometer was operated in negative ion mode. Leu-enkephalin was used as the reference compound (*m*/*z* 556.2771 in positive ion mode). N2 was used as the desolvation gas and the desolvation temperature used was 350 °C, the flow rate was 500 L/h and the source temperature was 100 °C. The capillary and cone voltages were 2700 V and 27 V, respectively. Data were collected for each sample from 200 Da to 1500 Da with a 0.25-s scan time and 0.01-s interscan delay over 25-min.

2.6. Enzyme assays

Initial rates of MAO oxidation were measured in a 1-ml cuvette containing 50 mM sodium phosphate (pH 7.2) at 25 °C, as previously described [22,23]. The activities of MAO-A and MAO-B were assayed for 10 min in the presence of 0.2 mM kynuramine at 316 nm or in the presence of 2.0 mM benzylamine at 250 nm, respectively. Reactions were started by adding substrate to enzyme mixtures. Reaction rates are expressed as changes in absorbance per min. K_m values for kynuramine and benzylamine determined using this method, were 0.029 mM and 0.26 mM, respectively, and substrate concentrations were 6.9 × K_m and 7.7 × K_m, respectively.

2.7. Analysis of inhibitory activities and enzyme kinetics

The MAO inhibitory activities of the extracts, fractions obtained by column chromatography, and isolated compounds were investigated using MAO-A and MAO-B. IC_{50} values were determined by