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Potent Inhibition of Monoamine Oxidase B by a Piloquinone from Marine-Derived *Streptomyces* sp. CNQ-027

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Two piloquinone derivatives isolated from *Streptomyces* sp. CNQ-027 were tested for the inhibitory activities of two isoforms of monoamine oxidase (MAO), which catalyzes monoamine neurotransmitters. The piloquinone 4,7-dihydroxy-3-methyl-2-(4-methyl-1-oxopentyl)-6H-dibenzo[b,d]pyran-6-one (**1**) was found to be a highly potent inhibitor of human MAO-B, with an IC_{50} value of 1.21 μM; in addition, it was found to be highly effective against MAO-A, with an IC_{50} value of 6.47 μM. Compound **1** was selective, but not extremely so, for MAO-B compared with MAO-A, with a selectivity index value of 5.35. Compound 1,8-dihydroxy-2-methyl-3-(4-methyl-1-oxopentyl)-9,10-phenanthrenedione (**2**) was moderately effective for the inhibition of MAO-B (IC_{50} = 14.50 μM) but not for MAO-A (IC_{50} > 80 μM). There was no time-dependency in inhibition of MAO-A or -B by compound **1**, and the MAO-A and -B activities were almost completely recovered in the dilution experiments with an excess amount of compound **1**. Compound **1** showed competitive inhibition for MAO-A and -B, with K_i values of 0.573 and 0.248 μM, respectively. These results suggest that piloquinones from a microbial source could be potent reversible MAO inhibitors and may be useful lead compounds for developing MAO enzyme inhibitors to treat related disorders, such as depression, Parkinson's disease, and Alzheimer's disease.

Keywords: Monoamine oxidase, piloquinone, *Streptomyces* sp. CNQ-027, potent selective inhibitor, competitive inhibitor

Monoamine oxidases (MAOs, E.C. 1.4.3.4) exist in most bodily tissues and in the mitochondrial outer membrane; they catalyze the oxidation of pharmacologically important neurotransmitting monoamines [1]. MAOs belong to the flavin-containing amine oxidoreductase family and are divided into two isoforms, MAO-A and MAO-B. Although their substrate specificity overlaps, MAO-A prefers catecholamines and other biogenic amines, such as norepinephrine and epinephrine, whereas MAO-B has a preference for benzylamine and 2-phenylethylamine [2]. MAO-A is related to depression and anxiety, whereas MAO-B is a target in the treatment of Alzheimer's and Parkinson's diseases [3, 4].

MAO inhibitors are categorized as selective MAO-A, selective MAO-B, or nonselective inhibitors; and they are also grouped as reversible versus irreversible [5]. Potent and reversible inhibitors for MAO-A and -B have been reported based on synthetic compounds or natural products [6–9].

Natural products, especially from herbal sources, have been extensively explored for the discovery of novel MAO inhibitors, as described in several reviews [2, 10–12]. From microbial sources, MAO inhibitory activity was first reported for pimprinine, *trans*-cinnamic acid amide, and phenethylamine from three strains of *Streptomyces* with high IC_{50} values (38–760 μM) [13]. Since that time, only

limited information about MAO inhibitors has been reported from microbial metabolites [14–16]. To explore and extend the screening of MAO inhibitors, we selected bacterial and fungal metabolites as attractive sources for investigation, especially marine microorganisms. Marine actinomycetes have been investigated as drug-discovery sources [17]. Recently, MAO-A selective inhibitory activity was reported by a compound anithiactin from a *Streptomyces* sp., isolated from tidal flats or deep-sea sediments [15].

In this study, we examined the inhibition of recombinant human MAO-A and MAO-B by two piloquinone compounds isolated from the bacterium *Streptomyces* sp. CNQ-027, and we describe the potent inhibitory activity of these compounds against MAO enzymes.

Streptomyces sp. CNQ-027 was isolated from sediment gathering up the coast of southern California [18]. A sequence of 16S rRNA from this strain was 97.6% identical to that of a marine-derived *Streptomyces marinus*. The bacterial strain *Streptomyces* sp. CNQ-027 was cultured in thirty-two 2.5 L Ultra Yield Flasks, each containing 1 L (750 ml natural seawater and 250 ml of distilled water) of SYP medium (10 g soluble starch, 4 g yeast extract, 2 g peptone, 10 g $CaCO_3$, 20 g KBr, 8 g $Fe_2(SO_4)_3{\cdot}4H_2O$), and then shaken at 150 rpm at 27°C. After 7 days, the culture medium was extracted with ethyl acetate and the solvent was removed in vacuo to yield 3.6 g of extract. This extract was fractionated by flash silica column chromatography, and eluted with a step gradient of CH_2Cl_2 and MeOH. The CH_2Cl_2/MeOH (100:1) fraction was further purified by reversed-phase HPLC (Phenomenex Luna C18(2), 250 × 100 mm, 2.0 ml/min, 5 μm, UV = 254 nm) using an isocratic solvent system (H_2O:CH_3CN = 20:80) to acquire compounds **1** (6.3 mg) and **2** (12.0 mg), respectively.

The ^{1}H NMR spectrum of **1** showed the signals, δ 7.78 (dd, 1H, *J* = 8.0, 8.0 Hz), 7.73 (s, 1H), 7.59 (d, 1H, *J* = 8.0 Hz), 7.13 (d, 1H, *J* = 8.0 Hz), 2.95 (t, 2H, *J* = 7.7 Hz), 2.45 (s, 3H), 1.65 (t, 2H, *J* = 6.2 Hz), 1.27 (br s, 1H), 0.97 (d, 3H, *J* = 6.2 Hz), 0.88 (d, 3H, *J* = 6.2 Hz), 11.11 (s, OH), and 11.63 (s, OH). The UV and MS spectra of **1** are shown in Fig. 1A. Based on a comparison of the NMR, UV, and MS data with the previous data, **1** was identified as 4,7-dihydroxy-3-methyl-2-(4-methyl-1-oxopentyl)-6H-dibenzo[b,d]pyran-6-one [19, 20]. The ^{1}H NMR spectrum of **2** showed the signals δ 7.64 (dd, 1H, *J* = 7.7, 7.7 Hz), 7.50 (d, 1H, *J* = 7.7 Hz), 7.41 (s, 1H), 7.04 (d, 1H, *J* = 7.7 Hz), 2.87 (t, 2H, *J* = 7.3 Hz), 2.29 (s, 3H), 1.65 (t, 2H, *J* = 6.2 Hz), 1.27 (br s, 1H), 0.97 (d, 3H, *J* = 6.2 Hz), 12.33 (s, OH), and 12.37 (s, OH). The UV and MS spectra of **2** are shown in Fig. 1B. Based on a comparison of the NMR data with the previous data, **2** was identified as 1,8-dihydroxy-2-methyl-3-(4-methyl-1-oxopentyl)-9,10-phenanthrenedione [21].

Benzylamine, kynuramine, toloxatone, lazabemide, and recombinant human MAO-A and MAO-B were purchased from Sigma-Aldrich (USA). Clorgyline and pargyline were from a monoamine oxidase kit supplied by BioAssay Systems (USA). The initial rates of oxidation were measured in a 1 ml cuvette containing 50 mM of sodium phosphate (pH 7.4) at 25°C, as described previously, except for the substrate concentrations and assay times [15, 16]. In this study, the activity of MAO-A was assayed with 0.06 mM of kynuramine as the substrate at 316 nm for 20 min, whereas that of MAO-B was assayed with 0.6 mM of benzylamine at 250 nm for 30 min. The reaction was started by the addition of substrate to the enzyme mixture. The reaction rates were expressed as the changes in absorbance per minute. By this method, the K_m values for kynuramine and benzylamine were 0.025 mM and 0.20 mM, respectively, and thus the substrate concentrations were 2.4 × K_m and 3.0 × K_m, respectively.

The chemical structures of 4,7-dihydroxy-3-methyl-2-(4-methyl-1-oxopentyl)-6H-dibenzo[b,d]pyran-6-one (**1**) and 1,8-dihydroxy-2-methyl-3-(4-methyl-1-oxopentyl)-9,10-phenanthrenedione (**2**) are shown in Fig. 1C. The IC_{50} values were determined by constructing sigmoidal dose-response curves from the residual MAO activities in the presence of various inhibitor concentrations. The IC_{50} values for the inhibition of MAO-A and MAO-B are shown in Table 1. Compound **1** potently inhibited MAO-B (IC_{50} = 1.21 μM), and was effective for the inhibition of MAO-A (IC_{50} = 6.47 μM); **1** was selective, but not extremely so, for

Table 1. IC_{50} values for the inhibition of recombinant human MAO-A and MAO-B by piloquinones **1** and **2** isolated from *Streptomyces* sp. CNQ-027 [a].

Compound	IC_{50} (μM)		SI[b]
	MAO-A	MAO-B	
1	6.47 ± 0.73	1.21 ± 0.071	5.35
2	> 80[c]	14.50 ± 1.29	-
Toloxatone	1.78 ± 0.177	-	-
Lazabemide	-	0.12 ± 0.02	
Clorgyline	0.0042 ± 0.0005	> 2.0	-
Pargyline	> 2.0	0.15 ± 0.041	

[a]Inhibitory activity against MAO-A and MAO-B was measured with 0.06 mM of kynuramine and 0.6 mM of benzylamine as substrates, respectively. Values are reported as the mean ± SE of duplicate experiments.

[b]The selectivity index is given as the ratio of IC_{50} (MAO-A)/IC_{50} (MAO-B).

[c]31.0 ± 1.03% inhibition at 80 μM.

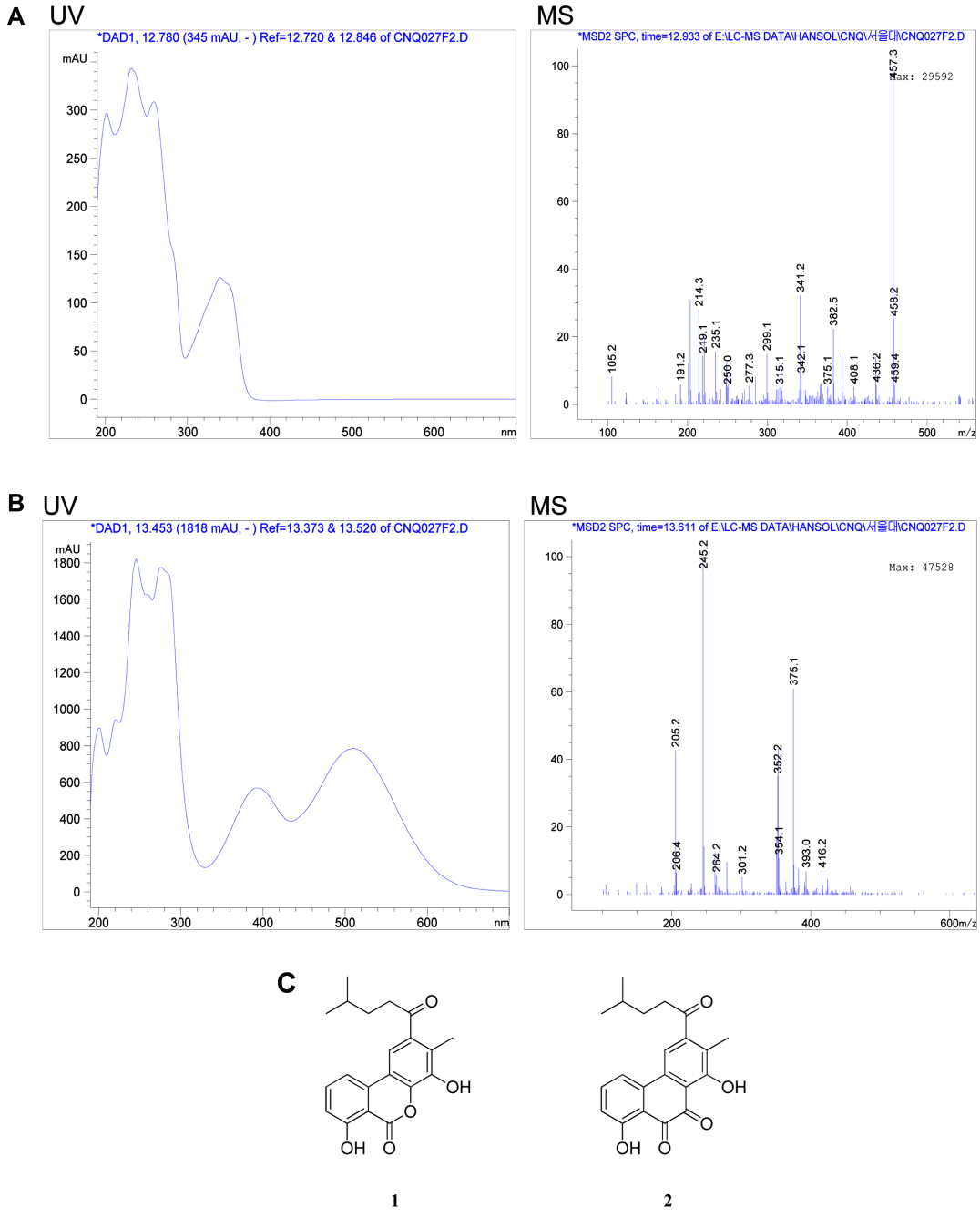

Fig. 1. UV and MS spectra for compounds **1** (**A**) and **2** (**B**), and structures of 4,7-dihydroxy-3-methyl-2-(4-methyl-1-oxopentyl)-6H-dibenzo[b,d]pyran-6-one (**1**) and 1,8-dihydroxy-2-methyl-3-(4-methyl-1-oxopentyl)-9,10-phenanthrenedione (**2**) isolated from *Streptomyces* sp. CNQ-027 (**C**).

MAO-B compared with for MAO-A, with a selectivity index value of 5.35 (Table 1). However, **2** was moderately effective for the inhibition of MAO-B (IC_{50} = 14.50 μM), but not for MAO-A (IC_{50} > 80 μM).

Compound **1** showed more potent inhibitory activity for MAO, compared with that of **2**. This result implies that ester functionality in the ring system is crucial for bioactivity and could be a highly important pharmacophore for MAO inhibitory activity in this class of natural products.

In addition, the time-dependency of the inhibition of MAO-A or -B by **1** and MAO-B by **2** was investigated [16, 22]. The remaining activities of MAO-A and -B were

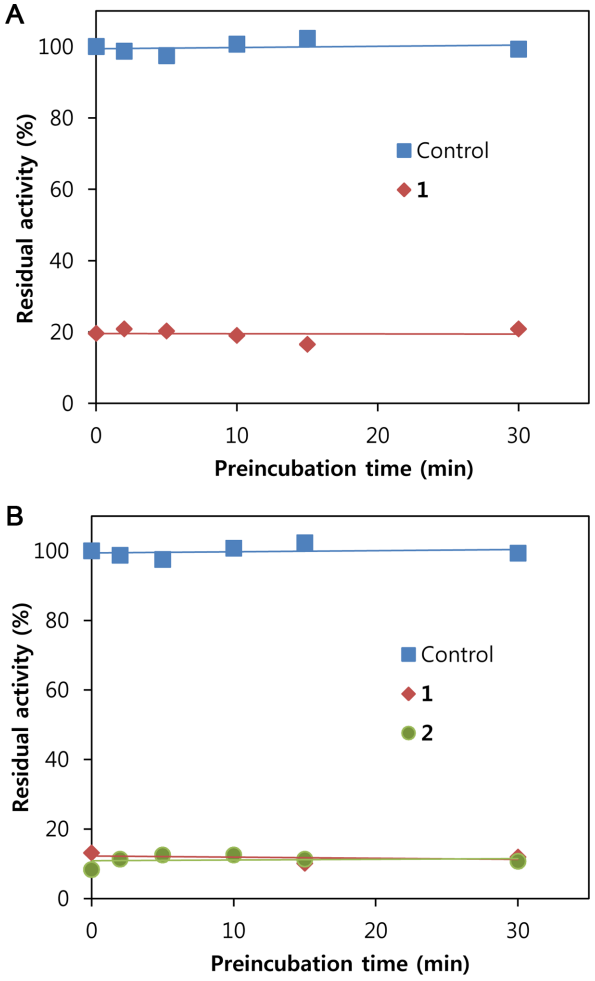

Fig. 2. Time-dependency of MAO-A activity with compound **1** (**A**) and MAO-B activity with compounds **1** and **2** (**B**).
MAO-A was preincubated with 11.8 μM of compound **1** (1.8 × IC_{50}) and MAO-B was preincubated with 5.9 μM of compound **1** (4.9 × IC_{50}) and 23.5 μM of compound **2** (1.6 × IC_{50}).

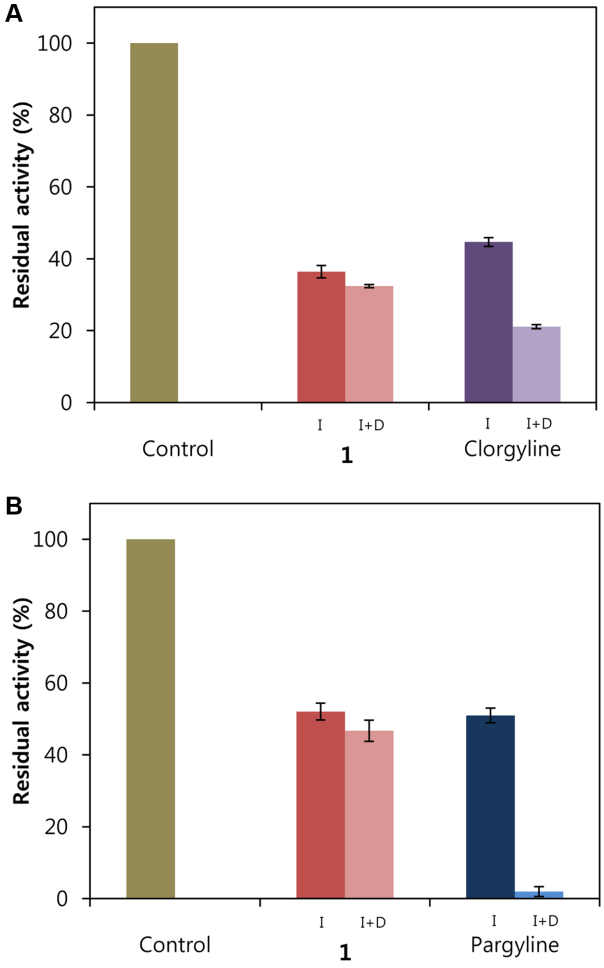

Fig. 3. Recovery of MAO-A (**A**) and MAO-B (**B**) activities with the dilution of the inhibited enzyme by an excess amount of compound **1**.
C: control reaction without an inhibitor; I: reaction with substrate in the presence of an inhibitor (1.0 × IC_{50}); I + D: reaction with enzyme mixture diluted 100 times (final concentration of the inhibitor = 1.0 × IC_{50}). Clorgyline and pargyline were used as irreversible MAO-A and -B inhibitor references, respectively.

determined with 0.06 mM of kynuramine and 0.6 mM of benzylamine, respectively, after various periods of preincubation (up to 30 min) with **1** or **2** at 25°C. It was observed that the activity was almost the same as the preincubation time (Fig. 2), showing that the inhibition of MAO-A and -B using **1** or **2** was not time-dependent.

The recovery of enzyme activity was also analyzed according to the previously described dilution, with a slight modification [16, 23]. Excess **1** (100 × IC_{50}) was incubated with MAO-A and -B for 10 min, and then diluted 100 times (*i.e.*, to be 1.0 × IC_{50}). The residual activity was then compared with that of the undiluted condition (1.0 × IC_{50}), from the commencement of the experiment. Toloxatone and lazabemide were used as reversible inhibitor references for MAO-A and -B, respectively, whereas clorgyline and pargyline were used as irreversible inhibitor references for MAO-A and -B, respectively. The activities of MAO-A and -B by **1** under the diluted condition were almost recovered (89.0% and 89.6%, respectively) to that under the undiluted condition. However, clorgyline showed to about half the original activity level (47.2%) and pargyline showed no activity (Fig. 3). These results suggest that **1** is a reversible rather than an irreversible inhibitor.

The kinetics of the inhibition of recombinant human MAO-A and MAO-B by **1** were studied using a spectrophotometric assay with kynuramine and benzylamine as

the substrates, respectively. The mode of the inhibition of MAO-A and -B by **1** was investigated using Lineweaver–Burk plots. The catalytic rates of MAO-A and -B were measured at five different substrate concentrations (0.006–0.15 and 0.06–1.5 mM, respectively) in the absence or presence of an inhibitor. The lines of the Lineweaver-Burk plots for the inhibition of MAO-A and -B by **1** were linear and intersected at the *y*-axis (Figs. 4A and 4C). This means that **1** is a competitive inhibitor of MAO-A and -B. From the secondary plots of the slopes against the inhibitor concentrations, the K_i values for the inhibition of MAO-A and -B were determined to be 0.573 and 0.248 μM, respectively (Figs. 4B and 4D).

Compared with herbal natural products, very little information about MAO inhibitors from microbial sources is available. MAO inhibitory activities were reported by compounds from three strains of *Streptomyces* with high IC_{50} values (38–760 μM) using a rat liver enzyme preparation [13]. 5-Methylmellein and nectriapyrone from fungal strain 8082 inhibited MAO in mouse brains (IC_{50} = 1.06 and 8.9 μM, respectively) [14]. Anithiactin A from the *Streptomyces* sp. effectively inhibited recombinant human MAO-A (IC_{50} = 13.0 μM) [15]. Alternariol monomethyl ether from a fungus, *Alternaria brassicae*, potently inhibited recombinant human MAO-A (IC_{50} = 1.71 μM) [16]. Therefore, it might be suggested that **1** is the most potent selective inhibitor of MAO-B amongst microbial metabolites.

Several MAO inhibitors among quinone derivatives of natural herbal products have been reviewed [2], including a quinolone derivative showing selective inhibition against MAO-B (IC_{50} = 15.3 μM) [24], an anthraquinone selectively inhibiting MAO-B (IC_{50} = 15.3 μM) [25], and three naphtoquinolones efficiently inhibiting MAO-A (IC_{50} = 10.0–59.1 μM) [26]. Compared with these compounds, the piloquinone **1** shows the best inhibitory activity for MAO-B.

Although the IC_{50} value of **1** for MAO-B (1.21 μM) is higher than that of lazabemide (0.12 μM) (Table 1), which is used as a drug for Parkinson's disease, and is about twice that of maackiain (0.68 μM) [27], it might be suggested that microbial metabolites or piloquinones might be good

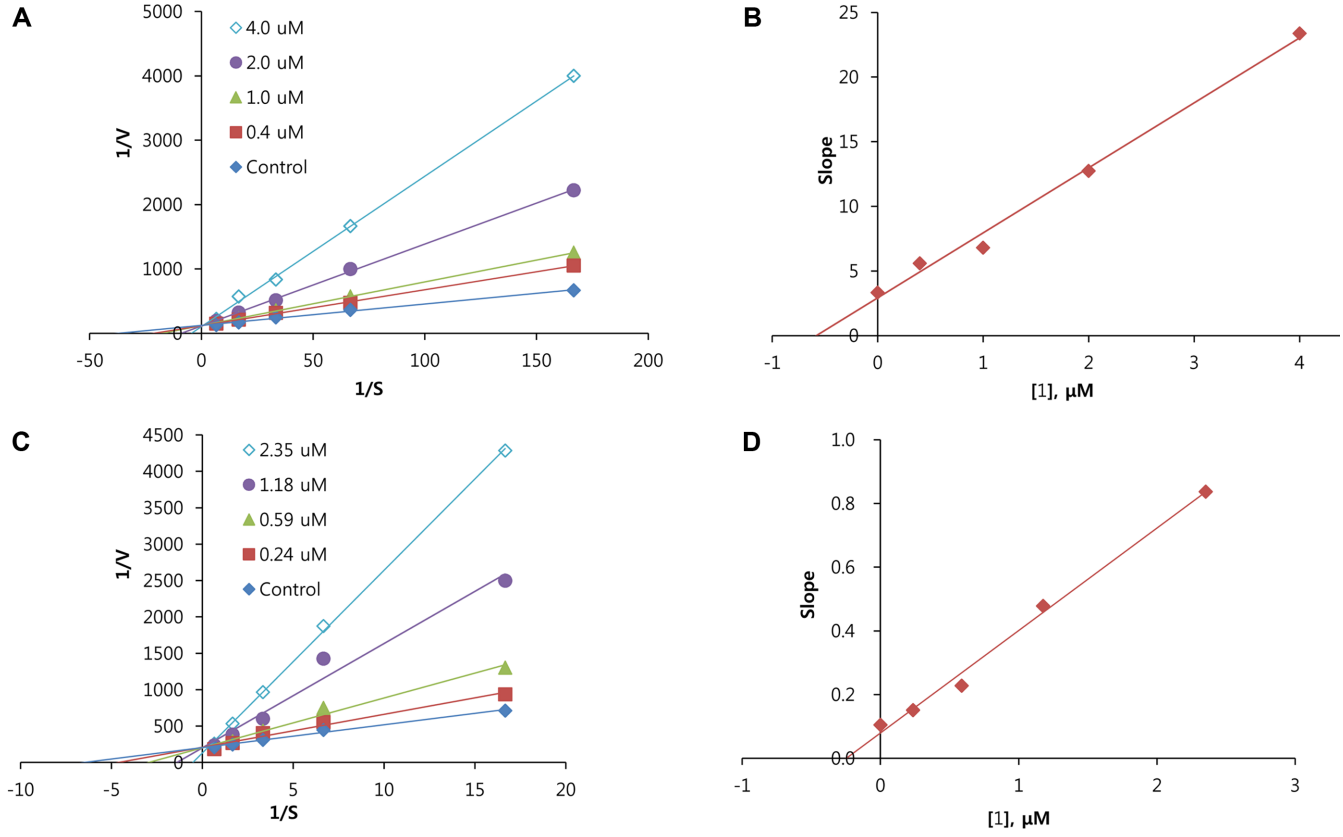

Fig. 4. Lineweaver-Burk plots of MAO-A (**A**) and -B (**C**) inhibition by compound **1** and the secondary plots of the slopes against the inhibitor concentrations of MAO-A (**B**) and -B (**D**).

The initial velocity was expressed as an increased absorbance per minute. Substrates were used at five different concentrations [1] represents the compound **1**.

sources for the discovery of treatment candidates for Parkinson's and Alzheimer's diseases, and antidepressant agents.

The results of the present study suggest that **1** is a potent selective inhibitor of MAO-B and can be considered as a new potential lead compound for the further development of MAO inhibitors.

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