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Potent Selective Inhibition of Monoamine Oxidase A by Alternariol Monomethyl Ether Isolated from *Alternaria brassicae*

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Alternariol monomethyl ether (AME), a dibenzopyrone derivative, was isolated from *Alternaria brassicae* along with altertoxin II (ATX-II). The compounds were tested for the inhibitory activity of monoamine oxidase (MAO), which catalyzes neurotransmitting monoamines. AME was found to be a highly potent and selective inhibitor of human MAO-A with an IC_{50} value of 1.71 μM; however, it was found to be ineffective for MAO-B inhibition. ATX-II was not effective for the inhibition of either MAO-A or MAO-B. The inhibition of MAO-A using AME was apparently instantaneous. MAO-A activity was almost completely recovered after the dilution of the inhibited enzyme with an excess amount of AME, suggesting AME is a reversible inhibitor. AME showed mixed inhibition for MAO-A in Lineweaver-Burk plots with a K_i value of 0.34 μM. The findings of this study suggest that microbial metabolites and dibenzopyrone could be potent MAO inhibitors. In addition, AME could be a useful lead compound for developing reversible MAO-A inhibitors to treat depression, Parkinson's disease, and Alzheimer's disease.

Keywords: Alternariol monomethyl ether, monoamine oxidase, *Alternaria brassicae*, selective inhibitor, mixed inhibition

Monoamine oxidase (MAO; E.C. 1.4.3.4) catalyzes the oxidation of monoamines acting as neurotransmitters and is pharmacologically important, as it works in the brain and peripheral tissues [1]. MAO exists as two isoforms: MAO-A and MAO-B, which are encoded by distinct genes. MAO-A has specific activity in catecholamines and other biogenic amines, such as norepinephrine and epinephrine; conversely, MAO-B has a preference for hydrophobic substrates containing benzylamine and 2-phenylethylamine [2]. Although the two isoforms are related to major depressive and neuropsychiatric disorders, MAO-A is mainly associated with depression and anxiety, whereas MAO-B is a major molecular target to treat Alzheimer's and Parkinson's diseases [3, 4].

MAO inhibitors may be categorized as selective MAO-A, selective MAO-B, or nonselective MAO-A/B inhibitors, and further as reversible or irreversible [5]. It has been reported that the β-carbolines, coumarins, a phenoxathiin derivative, and an amine derivative of furaline are potent and selective reversible inhibitors of MAO-A (RIMAs) [6–10]. Some RIMAs, such as moclobemide, brofaromine, toloxatone, and befloxatone, have been developed as market drugs [11–13].

Natural products have also been explored as candidates for lead compounds in drugs. Natural herbal products have been extensively studied as MAO inhibitors and reviewed in several reports [2, 14–16]. However, very little research has been conducted using microbial metabolites as MAO inhibitors [17, 18]. During our ongoing search for new MAO inhibitors, bacterial and fungal metabolites have been selected as an attractive source to be explored.

Alternariol monomethyl ether (AME) is a mycotoxin produced by the *Alternaria* genus; with alternariol, it has a toxic effect in animals [19, 20]. AME is isolated mainly from *Alternaria alternata* and other species of *Alternaria* [21].

In this study, we examined the inhibition of recombinant

human MAO-A and MAO-B by AME and altertoxin II (ATX-II) isolated from the fungus *A. brassicae*, and we report the selectively potent inhibitory activity of AME for MAO-A.

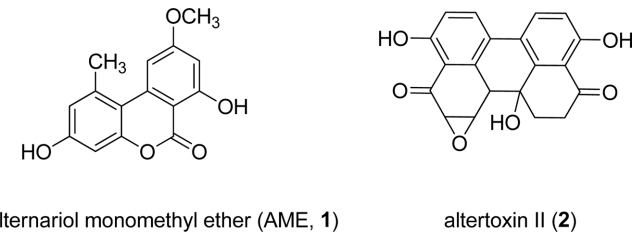
The strain SNF-008 was isolated from the stem of the plant Daehwang (*Rheum palmatum*). It shares 99.6% of its sequence identity with the type strain of *A. brassicae*. This fungal strain was cultivated with 1/10 MEA (2.0 g of malt extract) at 27°C and was shaken at 150 rpm for 7 days. After fermentation, the fungal culture broth was filtered to separate the broth from the mycelia. The mycelium layer was extracted using a mixture of acetone and methanol (1:1), and the solvent was removed under vacuum. Then, the layer was sonicated and filtered from the residue. The mycelium extract and the broth were then mixed, and a crude extract was obtained. The crude extract was fractionated by silica flash column chromatography and eluted with CH_2Cl_2/CH_3OH to obtain four fractions (100:1, 50:1, 20:1, 1:100). Fraction 1 (128.8 mg) was further purified by semi-preparative HPLC with a reversed-phase C18 column (Watchers 120 ODS-BP (250 mm × 10 mm, 5 μm), 2.0 ml/min, UV = 254 nm, and isocratic condition = 55% CH_3CN (0.1% TFA)) to yield AME (**1**, 1.3 mg) and ATX-II (**2**, 1.6 mg).

The ^{1}H NMR spectrum of **1** displayed two sets of meta-coupled aromatic protons (δ 7.21 (d, 1H, *J* = 2.1 Hz), 6.75 (d, 1H, *J* = 2.5 Hz), 6.67 (d, 1H, *J* = 2.1 Hz), and 6.65 (d, 1H, *J* = 2.5 Hz)) and two methyl singlets (δ 3.61 (s, 3H) and 2.72 (s, 3H)). The ^{13}C NMR spectrum of **1** showed 14 carbon signals (δ 137.4 (C), 99.1 (CH), 164.2 (C), 99.9 (CH), 165.7 (CH), 103.0 (CH), 163.7 (C), 152.2 (CH), 101.2 (CH), 158.1 (C), 117.2 (CH), 138.0 (C), 55.4 (CH_3), and 24.5 (CH_3)). Based on the interpretation of the 2D NMR spectroscopic data and on a comparison of the NMR data with the previous data, **1** was identified as AME [22].

The ^{1}H NMR spectrum of **2** displayed two sets of ortho-coupled aromatic protons (δ 8.16 (d, 1H, *J* = 8.8 Hz), 8.08 (d, 1H, *J* = 8.7 Hz), 7.16 (d, 1H, *J* = 8.8 Hz), and 7.07 (d, 1H, *J* = 8.7 Hz)), three down-fielded methine protons (4.38 (d, 1H, *J* = 3.5 Hz), 3.60 (m, 1H), and 3.35 (s, 1H)), and three methine protons (2.99 (m, 1H), 2.78 (m, 1H), and 2.45 (dt, 1H, *J* = 3.5, 2.0 Hz)). By comparing the ^{1}H and ^{13}C NMR spectroscopic data of **2** with the previously reported data in the literature, **2** was identified as ATX-II [23].

Benzylamine, kynuramine, and recombinant human MAO-A and MAO-B were purchased from Sigma-aldrich (USA). The enzyme was stored at −70°C in 100 mM of potassium phosphate (pH 7.4), 0.25 M of sucrose, 0.1 mM of EDTA, and 5% glycerol [17].

The initial rates of oxidation were measured in a 1 ml cuvette containing 50 mM of sodium phosphate (pH 7.4) at 25°C [24]. The activity of MAO-A was assayed with kynuramine as the substrate at 316 nm for 10 min, whereas that of MAO-B was assayed with benzylamine at 250 nm for 15 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 min. By this method, the K_m value for kynuramine was 0.033 mM and the substrate concentration was 6.1 × K_m.



alternariol monomethyl ether (AME, **1**) altertoxin II (**2**)

Fig. 1. Structures of alternariol monomethyl ether and altertoxin II isolated from *A. brassicae*.

The chemical structures of AME and ATX-II are shown in Fig. 1. The kinetics of the inhibition of recombinant human MAO-A and MAO-B by these compounds were studied using a spectrophotometric assay with kynuramine and benzylamine as the substrates, respectively. 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. From the results, it was observed that AME effectively

Table 1. IC_{50} values for the inhibition of recombinant human MAO-A and MAO-B by alternariol monomethyl ether and altertoxin II isolated from *A. brassicae*[a].

Compound	IC_{50} (μM)		SI[b]
	MAO-A	MAO-B	
Alternariol monomethyl ether	1.71 ± 0.13	>80[c]	>46.8
Altertoxin II	>80[d]	>80[e]	-
Toloxatone[f]	3.92	-	-
Clorgyline[g]	0.0049 ± 0.0005	>2.0	-

[a]Inhibitory activity to MAO-A and MAO-B was measured with 0.2 mM of kynuramine and 2.0 mM of benzylamine as the substrate, respectively. Values are the mean ± SE of duplicate experiments.

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

[c]24.7% inhibition at 80 μM.

[d]12.1% inhibition at 80 μM.

[e]32.9% inhibition at 80 μM.

[f]Taken from Ref. [27].

[g]Taken from Ref. [24].

not effective for the inhibition of MAO-B. AME was more selective for MAO-A than for MAO-B, with an SI value of more than 46.8 (Table 1). However, ATX-II was not effective for the inhibition of MAO-A or MAO-B, with an IC_{50} value of more than approximately 80 μM.

In addition, the time dependence of the inhibition of MAO-A by AME was investigated [24, 25]. The remaining activity was determined at 0.2 mM of kynuramine after various periods of preincubation (0, 1, 2, 5, 10, 15, and 30 min) with AME and MAO-A at 25ºC. The compound was used at a concentration about twice as high as its IC_{50} value. It was observed that the activity was almost the same as the preincubation time, although the activity was slightly decreased for up to 5 min (Fig. 2A). This result showed that the inhibition of MAO-A using AME was apparently instantaneous, not time-dependent.

Furthermore, the recovery of enzyme activity was analyzed by the previously described dilution with a slight modification [26]. Excess AME (100 × IC_{50}) was incubated with MAO-A 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. Clorgyline was used as an irreversible MAO-A inhibitor reference. The recovered activity under the diluted condition by AME (51.2%) was almost the same (97.0%) as that under the undiluted condition (52.8%). However, clorgyline showed about half the activity (55.3%), from 45.0% to 24.9% (Fig. 2B). These results suggest that AME is a RIMA rather than an irreversible inhibitor.

The mode of the inhibition of MAO-A by AME was investigated by Lineweaver–Burk plots. The catalytic rates of MAO-A were measured at five different substrate concentrations (0.02–0.5 mM) in the absence or presence of an inhibitor. The lines of the Lineweaver-Burk plots for the inhibition of MAO-A by AME were linear but did not intersect at the y-axis (Fig. 3). This means that AME is a mixed inhibitor of MAO-A. From the secondary plot of the slopes against the inhibitor concentrations, the K_i value for the inhibition of MAO-A was determined to be 0.34 ± 0.04 μM (Fig. 3).

Although many herbal natural products have been reported as MAO inhibitors, information about microbial metabolites as inhibitors is limited; however, 5- methylmellein and nectriapyrone produced from fungal strain 8082 showed inhibitory activity for MAO in mouse brains (IC_{50} = 1.06 and 8.9 μM, respectively) [18]. In addition, anithiactin A from *Streptomyces* sp. was effective for the inhibition of MAO-A (IC_{50} = 13.0 μM) with moderate potency [17].

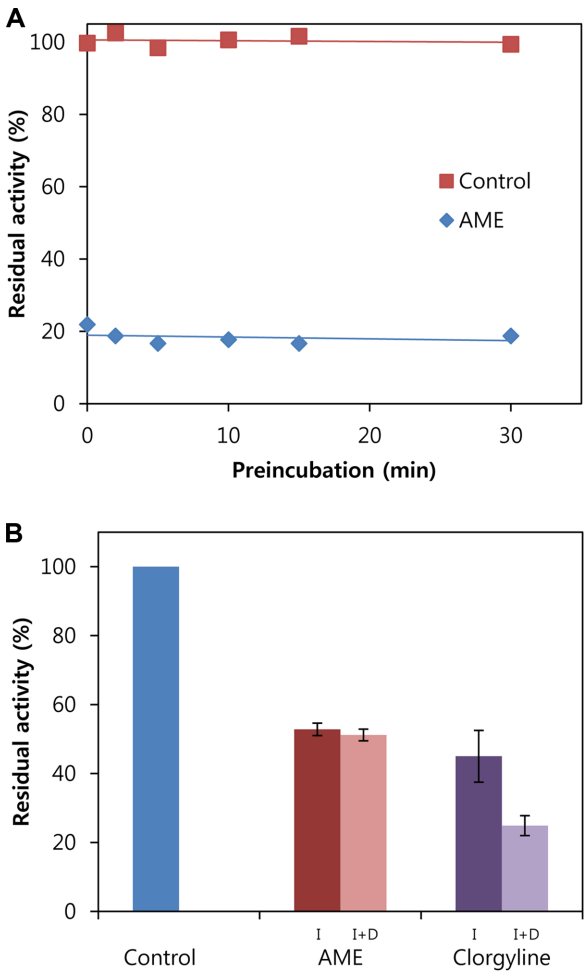


Fig. 2. Time dependency (**A**) and recovery of MAO-A activity with the dilution of the inhibited enzyme by an excess amount of AME (**B**).

Activity was assayed at 316 nm for 10 min in the presence of 0.2 mM kynuramine as the substrate. In panel (**A**), MAO-A was preincubated with 8.0 μM AME (4.7 × IC_{50}). Control: reaction in the presence of 0.2 mM of substrate 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 was used as an irreversible MAO-A inhibitor reference.

Therefore, it might be suggested that AME is the most potent selective inhibitor of MAO-A amongst microbial metabolites.

Benzopyrone constitutes the skeleton of many flavonoids, such as chromone and coumarin, which have strong inhibitory activities for MAO enzymes [2, 15]. However, potent inhibitory activity for MAO-A by dibenzopyrone derivatives has not been reported. It might be suggested that microbial metabolites or dibenzopyrones might be good

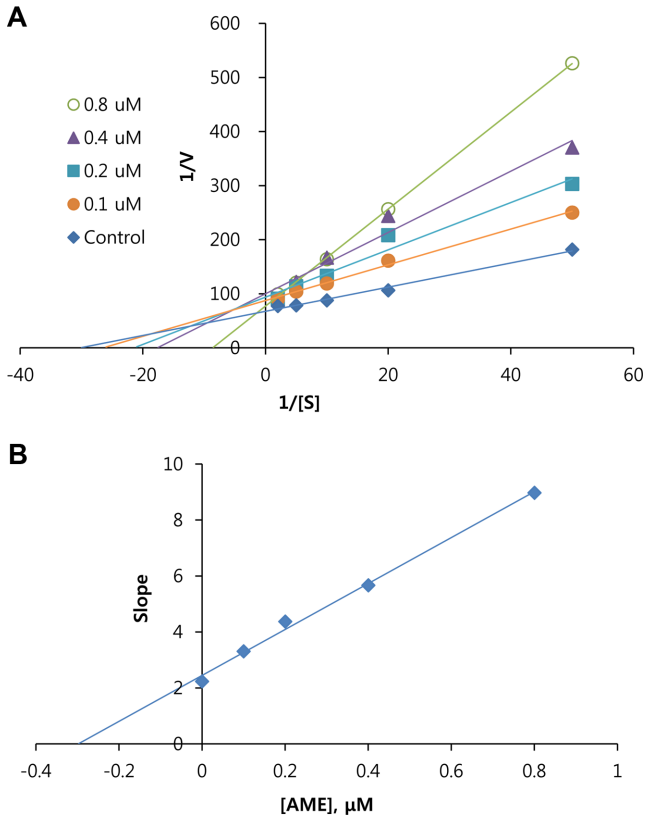


Fig. 3. Lineweaver-Burk plots of MAO-A inhibition by AME (**A**) and a secondary plot of the slopes against the inhibitor concentrations (**B**).

The inhibitor concentrations of AME were 0 (◆), 0.1 (●), 0.2 (■), 0.4 (▲), and 0.8 (○) μM. The initial velocity was expressed as an increased absorbance over 10 min. Kynuramine was used at five different concentrations (0.02–0.5 mM).

sources to discover candidates for antidepressant agents.

The IC_{50} value of AME (1.71 μM) is lower than that of toloxatone (3.92 μM), which is used as an antidepressant drug [27]. The K_i value of AME for MAO-A (0.34 μM) is slightly higher than that of harman (0.26 μM) but lower than those of norharman (3.34 μM) and anithiactin A (1.84 μM) [9, 17].

From all the above results, it is suggested that AME is a selective RIMA with high potency. Furthermore, it can be considered as a new potential lead compound for the further development of novel RIMAs.

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References

1. Ramsay RR. 2012. Monoamine oxidases: the biochemistry of the proteins as targets in medicinal chemistry and drug discovery. *Curr. Top. Med. Chem.* **12:** 2189-2209.
2. Orhan IE. 2016. Potential of natural products of herbal origin as monoamine oxidase inhibitors. *Curr. Pharm. Des.* **22:** 268-276.
3. Yamada M, Yasuhara H. 2004. Clinical pharmacology of MAO inhibitors: safety and future. *Neurotoxicology* **25:** 215-221.
4. Youdim MB, Edmondson D, Tipton KF. 2006. The therapeutic potential of monoamine oxidase inhibitors. *Nat. Rev. Neurosci.* **7:** 295-309.
5. Mostert S, Petzer A, Petzer JP. 2015. Indanones as high-potency reversible inhibitors of monoamine oxidase. *ChemMedChem.* **10:** 862-873.
6. Abdelhafez OM, Amin KM, Ali HI, Abdalla MM, Batran RZ. 2012. Synthesis of new 7-oxycoumarin derivatives as potent and selective monoamine oxidase A inhibitors. *J. Med. Chem.* **55:** 10424-10436.
7. Fowler JS, Logan J, Azzaro AJ, Fielding RM, Zhu W, Poshusta AK, *et al*. 2010. Reversible inhibitors of monoamine oxidase-A (RIMAs): robust, reversible inhibition of human brain MAOA by CX157. *Neuropsychopharmacology* **35:** 623-631.
8. Gentili F, Pizzinat N, Ordener C, Marchal-Victorion S, Maurel A, Hofmann R, *et al*. 2006. 3-[5-(4,5-Dihydro-1H-imidazol-2-yl)-furan-2-yl]phenylamine (amifuraline), a promising reversible and selective peripheral MAO-A inhibitor. *J. Med. Chem.* **49:** 5578-5586.
9. Kim H, Sablin SO, Ramsay RR. 1997. Inhibition of monoamine oxidase A by β-carboline derivatives. *Arch. Biochem. Biophys.* **337:** 137-142.
10. Mattsson C, Svensson P, Sonesson C. 2014. A novel series of 6-substituted 3-(pyrrolidin-1-ylmethyl)chromen-2-ones as selective monoamine oxidase (MAO) A inhibitors. *Eur. J. Med. Chem.* **73:** 177-186.
11. Berlin I, Zimmer R, Thiede HM, Payan C, Hergueta T, Robin L, Puech AJ. 1990. Comparison of the monoamine oxidase inhibiting properties of two reversible and selective monoamine oxidase-A inhibitors moclobemide and toloxatone, and assessment of their effect on psychometric performance in healthy subjects. *Br. J. Clin. Pharmacol.* **30:** 805-816.
12. Dolle F, Valette H, Bramoulle Y, Guenther I, Fuseau C, Coulon C, *et al*. 2003. Synthesis and in vivo imaging properties of [11C]befloxatone: a novel highly potent positron emission tomography ligand for mono-amine oxidase-A. *Bioorg. Med. Chem. Lett*. **13:** 1771-1775
13. Lotufo-Neto F, Trivedi M, Thase ME. 1999. Meta-analysis of the reversible inhibitors of monoamine oxidase type A moclobemide and brofaromine for the treatment of depression. *Neuropsychopharmacology* **20:** 226-247.
14. Carradori S, D'Ascenzio M, Chimenti P, Secci D, Bolasco A. 2014. Selective MAO-B inhibitors: a lesson from natural products. *Mol. Divers.* **18:** 219-243.
15. Mathew B, Suresh J, Mathew GE, Parasuraman R, Abdulla

N. 2014. Plant secondary metabolites – potent inhibitors of monoamine oxidase isoforms. *Cent. Nerv. Syst. Agents Med. Chem.* **14:** 28-33.
16. Viña D, Serra S, Lamela M, Delogu G. 2012. Herbal natural products as a source of monoamine oxidase inhibitors: a review. *Curr. Top. Med. Chem*. **12:** 2131-2144.
17. Lee HW, Jung WK, Kim HJ, Jeong YS, Nam SJ, Kang H, Kim H. 2015. Inhibition of monoamine oxidase by anithiactins from *Streptomyces* sp. *J. Microbiol. Biotechnol.* **25:** 1425-1428.
18. Lee IK, Yun BS, Oh S, Kim YH, Lee MK, Yoo ID. 1999. 5-Methylmellein and nectriapyrone, two new monoamine oxidase inhibitors. *Med. Sci. Res*. **27:** 463-465.
19. Marin S, Ramos AJ, Cano-Sancho G, Sanchis V. 2013. Mycotoxins: occurrence, toxicology, and exposure assessment. *Food Chem. Toxicol*. **60:** 218-237.
20. Ostry V. 2008. *Alternaria* mycotoxins: an overview of chemical characterization, producers, toxicity, analysis and occurrence in foodstuffs. *World Mycotoxin J.* **1:** 175-188.
21. Scott PM, Zhao W, Feng S, Lau BP. 2012. *Alternaria* toxins alternariol and alternariol monomethyl ether in grain foods in Canada. *Mycotoxin Res*. **28:** 261-266.
22. Zhang SY, Li ZL, Bai J, Wang Y, Zhang LM, Wu X, Hua HM. 2012. A new perylenequinone from a halotolerant fungus, *Alternaria* sp. M6. *Chin. J. Nat. Med.* **10:** 68-71.
23. Schwarz C, Tiessen C, Kreutzer M, Stark T, Hofmann T, Marko D. 2012. Characterization of a genetoxic impact compound in *Alternaria alternata* infected rice as altertoxin II. *Arch. Toxicol.* **86:** 1911-1925.
24. Lee HW, Ryu HW, Kang MG, Park D, Oh SR, Kim H. 2016. Potent selective monoamine oxidase B inhibition by maackiain, a pterocarpan from the roots of *Sophora flavescens*. *Bioorg. Med. Chem. Lett*. **26:** 4714-4719
25. Legoabe LJ, Petzer A, Petzer JP. 2012. Inhibition of monoamine oxidase by selected C6-substituted chromone derivatives. *Eur. J. Med. Chem*. **49:** 343-353.
26. Petzer A, Harvey BH, Petzer JP. 2014. The interactions of azure B, a metabolite of methylene blue, with acetylcholinesterase and butyrylcholinesterase. *Toxicol. Appl. Pharmacol*. **274:** 488-493.
27. Petzer A, Pienaar A, Petzer JP. 2013. The inhibition of monoamine oxidase by esomeprazole. *Drug Res*. (*Stuttg*.). **63:** 462-467.