jmb

Inhibition of Monoamine Oxidase by Anithiactins from *Streptomyces* sp.

Hyun Woo Lee[1†], Won Kyeong Jung[2†], Hee Jung Kim[1], Yu Seok Jeong[1], Sang-Jip Nam[3], Heonjoong Kang[4], and Hoon Kim[1,2*]

[1]*Department of Pharmacy, Sunchon National University, Suncheon 540-950, Republic of Korea*

[2]*Research Institute of Life Pharmaceutical Sciences, Sunchon National University, Suncheon 540-950, Republic of Korea*

[3]*Department of Chemistry and Nano Science, Ewha Women's University, Seoul 120-750, Republic of Korea*

[4]*Center for Marine Natural Products and Drug Discovery, School of Earth and Environmental Sciences, Seoul National University, NS-80, Seoul 151-747, Republic of Korea*

Monoamine oxidase (MAO) is found in most cell types and catalyzes the oxidation of monoamines. Three anithiactins (A−C, modified 2-phenylthiazoles) isolated from *Streptomyces* sp. were tested for inhibitory activity of two isoforms, MAO-A and MAO-B. Anithiactin A was effective and selective for the inhibition of MAO-A, with an IC_{50} value of 13.0 µM; however, it was not effective for the inhibition of MAO-B. Anithiactins B and C were weaker inhibitors for MAO-A and MAO-B. Anithiactin A was a reversible and competitive inhibitor for MAO-A with a K_i value of 1.84 µM. The hydrophobic methyl substituent in anithiactin A may play an important role in the inhibition of MAO-A. It is suggested that anithiactin A is a selective reversible inhibitor for MAO-A, with moderate potency, and can be considered a new potential lead compound for further development of novel reversible inhibitors for MAO-A.

Keywords: Anithiactin A, monoamine oxidase, *Streptomyces* sp., selective inhibitor, competitive inhibitor

Monoamine oxidase (MAO, E.C. 1.4.3.4) catalyzes the oxidation of monoamines and has been a drug target to treat neuropsychiatric disorders [7, 21, 22]. MAO exists as two isoforms, MAO-A and MAO-B, which are encoded by distinct genes. The substrate specificities of MAO-A and MAO-B are frequently overlapped toward dopamine, tyramine, epinephrine, and norepinephrine. However, MAO-A selectively deaminates serotonin, whereas MAO-B selectively deaminates phenylethylamine and benzylamine [25]. The MAO enzymes are pharmacologically important, as they break down neurotransmitter amines in the brain and peripheral tissues [9]. MAO-A is associated with depression and anxiety, whereas MAO-B is a molecular target to treat Alzheimer's and Parkinson's diseases.

Inhibitors of MAO-A and MAO-B have therefore been extensively studied, including mechanism-based inhibitors, and used in the treatment of the diseases [15]. Based on the various pharmacological roles of MAO inhibitors, the discovery of novel classes and scaffolds is of value. MAO inhibitors may be categorized as MAO-A selective, MAO-B selective, and MAO-A/B nonselective inhibitors, and further as reversible and irreversible [18]. Reversible inhibitors of MAO-A (RIMAs) have been studied and developed, such as moclobemide, brofaromine, toloxatone, amifuraline, and CX157 [3, 9, 10, 14]. It has been reported that the β-carbolines and coumarins are potent and selective RIMAs [1, 11, 16].

Actinomycetes have been investigated as a valuable source of bioactive molecules. Marine environments, especially marine sediments and mudflats, have been focused on for isolating actinomycetes, as well as soil environments [6, 23]. Recently, *Streptomyces* sp., isolated from tidal flats or deep-sea sediments, was found to produce biologically active anithiactins and antibiotics [12, 17, 19]. Three anithiactins, A−C, have been isolated from *Streptomyces* sp. 10A085, and their biological activities in terms of acetylcholine esterase (AChE) inhibition have been described [12]. Recently, a MAO-A and AChE dual inhibitor, MBA236, has been reported [2]. Moreover, no

compounds structurally related to anithiactins have been reported for inhibition of MAO-A and MAO-B so far.

In this study, we have examined the inhibition of recombinant human MAO-A and MAO-B by anithiactins as well as inhibition patterns.

Benzylamine and kynuramine were purchased from Sigma-Aldrich (St. Louis, MO, USA). Brij 35 was sourced from Yakuri Pure Chemicals Co. (Kyoto, Japan). Anithiactins A–C were purified from the extracts of the culture broth of *Streptomyces* sp. 10A085, using silica column chromatography followed by reverse-phase HPLC [12].

Recombinant human MAO-A and MAO-B were purchased from Sigma-Aldrich. The enzyme was stored at –70°C in 100 mM potassium phosphate, pH 7.4, 0.25 M sucrose, 0.1 mM EDTA, and 5% glycerol.

Initial rates of oxidation were measured in a 1 ml cuvette containing 50 mM sodium phosphate, pH 7.2, and 0.2% Brij 35 at 25°C [11]. The activity of MAO-A was assayed with kynuramine as a substrate at 316 nm for 5 min. The activity of MAO-B was followed with benzylamine at 250 nm for 5 min. The reaction was started by the addition of substrate to the enzyme mixture.

The chemical structures for the anithiactins, A (**1**), B (**2**), and C (**3**), used in this study 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 nonionic detergent Brij 35 did not affect the enzyme activity during the assay up to 30 min (data not shown). Upon further analysis, Brij 35 was omitted from the assay. By constructing sigmoidal dose-response curves from the residual MAO activities in the presence of various concentrations of the inhibitors, IC_{50} values were determined. 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 compound **1** effectively inhibited MAO-A with an IC_{50} value of 13.0 μM, but was not effective for the inhibition of MAO-B. Compound **1** was more selective for MAO-A than for MAO-B with an SI

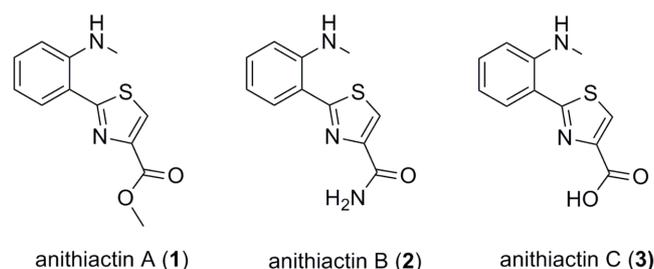**Table 1.** The IC_{50} values for the inhibition of recombinant human MAO-A and MAO-B by anithiactins[a].

	IC$_{50}$ (μM)		SI [b]
	MAO-A	MAO-B	
Anithiactin A (**1**)	13.0 ± 1.4	183.0 ± 7.1	14.1
Anithiactin B (**2**)	>85[c]	Inactive[e]	-
Anithiactin C (**3**)	>170[d]	>170[f]	-

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

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

[c]8.0% inhibition at 85 μM.

[d]15.7% inhibition at 170 μM.

[e]No inhibition at 170 μM.

[f]17.2% inhibition at 170 μM.

value of 14.1 (Table 1). However, compounds **2** and **3** were not effective for the inhibition of the two isoforms MAO-A and MAO-B, with IC_{50} values of more than about 200 μM, respectively.

The reversibility of inhibition of MAO-A was investigated using compounds **1** and **3**. Enzyme activity was measured in the presence of 0.2 mM kynuramine and 32 μM of compound **1** or 85 μM of **3** for 5 min, and then the same amount of the substrate was added to the mixture. After that, the enzyme activity was measured for an additional 5 min. When MAO-A was mixed with compound **1**, the residual activity was 28.0%, and then recovered to 38.5% when the substrate was added (Fig. 2). The inhibition was also reversed by compound **3**, although the degree was much lower than by compound **1** (Fig. 2). From these results, it was found that compounds **1** and **3** were not irreversible inhibitors for MAO-A.

The time dependence of the inhibition of MAO-A by compounds **1** and **3** was investigated. The remaining activity was determined at 0.2 mM kynuramine after various periods of preincubation (0, 1, 2, 3, and 5 min) with compounds **1** or **3** at 25°C. It was observed that the activity was almost the same with preincubation time (data not shown). The inhibition of MAO-A with both compounds was apparently instantaneous. Upon further analysis, the activity was measured without preincubation.

The mode of inhibition of MAO-A by compound **1** was investigated by graphical methods, 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 inhibitor. The lines of the Lineweaver-Burk plots for the inhibition of MAO-A by
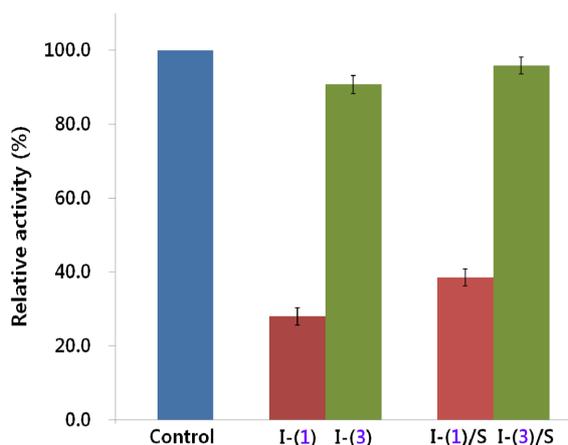

Fig. 1. Structures of anithiactins used in this study.

anithiactin A (**1**)   anithiactin B (**2**)   anithiactin C (**3**)

Fig. 2. Reversibility of MAO-A inhibition by anithiactin A (**1**) and C (**3**).

Activity was assayed for 5 min in the presence of 0.2 mM kynuramine as a substrate at 316 nm. Control, the control reaction in the presence of 0.2 mM substrate without inhibitor; I-(**1**) and I-(**3**), reaction with 32 μM of (**1**) (at 2.5× IC$_{50}$) and with 85 μM of (**3**) (less than IC$_{50}$), respectively; I-(**1**)/S and I-(**3**)/S, reaction with 0.2 mM kynuramine added into I-(**1**) and I-(**3**) at the end of the assay, respectively.

compound **1** was linear and intersected on the y-axis (Fig. 3). This means that compound **1** is a competitive inhibitor of MAO-A. From the secondary plot of the slopes against inhibitor concentrations, the K_i value for the inhibition of MAO-A was determined to be 1.84 μM (Fig. 3).

Anithiactin A (**1**) possesses a methyl ester functionality that is responsible for more hydrophobicity compared with those of anithiactins B (**2**) and C (**3**). With the IC$_{50}$ and K_i values, the corresponding substituent may play an important role in the inhibition of MAO-A.

Anithiactins are microbial secondary metabolites with the 2-phenylthiazole moiety. The known natural products closely related to the anithiactins are aeruginol and the pulicatins [13, 24]. Aeruginoic acid with antihypotensive activity was isolated from the gram-negative strain *Pseudomonas aeruginosa*. The pulicatins, produced by a cone snail (*Conus pulicarius*) associated with *Streptomyces* sp., displayed G protein-coupled receptor 5-HT$_{2B}$ inhibition activity. It might be necessary to investigate the inhibition of 5-HT$_{2B}$ by anithiactin A (**1**), because 5-HT$_{2B}$ is involved in presynaptic inhibition in the central nervous system and regulates serotonin release *via* serotonin transporter [4, 5].

It was reported that anithiactin A (**1**) displayed AChE inhibition with an IC$_{50}$ value of 63 μM [12]. AChE is the most common molecular target for drugs that act on the symptoms of Alzheimer's disease [20]. Compound **1** may act as a dual inhibitor for MAO-A and AChE, whereas the
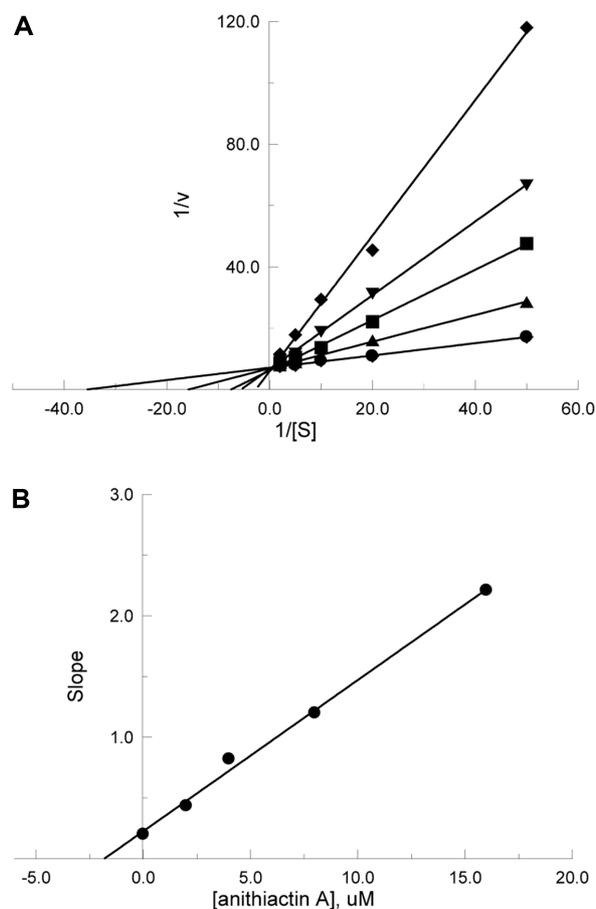

Fig. 3. Lineweaver-Burk plots of the inhibition of MAO-A by (**1**) (**A**) and a secondary plot of the slopes against inhibitor concentrations (**B**).

The inhibitor concentrations of (**1**) were 0 (●), 2.0 (▲), 4.0 (■), 8.0 (▼), and 16.0 (◆) μM. Initial velocity was expressed as increased absorbance for 5 min. Kynuramine was used at five different concentrations (0.02–0.5 mM) as the substrate.

IC$_{50}$ value for AChE was higher than the IC$_{50}$ value of 13.0 μM for MAO-A. Recently, a MAO-A and AChE dual inhibitor, MBA236, was reported during a multitarget-directed ligand approach [2]. The compound MBA236 is *N*-methyl-*N*-((1-methyl-5-(3-(1-(2-methylbenzyl)piperidin-4-yl)propoxy)-1H-indol-2-yl)methyl)prop-2-yn-1-amine, and is not structurally related to compound **1**.

Compound **1** was not found to exhibit any significant cytotoxicity up to 200 μM [12]. The K_i value of compound **1** for MAO-A is similar to that of 2-methyl norharman (1.43 μM), a β-carboline derivative, and 4′-methyl-MPP$^+$ (1.6 μM), an *N*-methyl-4-phenylpyridinium ion (MPP$^+$) derivative [11]. β-Carbolines are heterocyclic, dehydrogenated derivatives of tryptophan and structural analogs of MPP$^+$.

From these results, it is suggested that anithiactin A (**1**) is a selective reversible inhibitor for MAO-A with moderate potency, and that it can be considered a new potential lead compound for the further development of novel RIMAs.

Acknowledgments

References

1. 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.

2. Bautista-Aguilera OM, Samadi A, Chioua M, Nikolic K, Filipic S, Agbaba D, *et al*. 2014. *N*-Methyl-*N*-((1-methyl-5-(3-(1-(2-methylbenzyl)piperidin-4-yl)propoxy)-1H-indol-2-yl)methyl) prop-2-yn-1-amine, a new cholinesterase and monoamine oxidase dual inhibitor. *J. Med. Chem*. **57:** 10455-10463.

3. 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.

4. Callebert J, Esteve JM, Hervé P, Peoc'h K, Tournois C, Drouet L, *et al*. 2006. Evidence for a control of plasma serotonin levels by 5-hydroxytryptamine (2B) receptors in mice. *J. Pharmacol. Exp. Ther.* **317:** 724-731.

5. Doly S, Valjent E, Setola V, Callebert J, Hervé D, Launay JM, Maroteaux L. 2008. Serotonin 5-HT$_{2B}$ receptors are required for 3,4-methylenedioxymethamphetamine-induced hyperlocomotion and 5-HT release *in vivo* and *in vitro*. *J. Neurosci.* **28:** 2933-2940.

6. Fenical W, Jensen PR. 2006. Developing a new resource for drug discovery: marine actinomycete bacteria. *Nat. Chem. Biol.* **2:** 666-673.

7. Ferino G, Vilar S, Matos MJ, Uriarte E, Cadoni E. 2012. Monoamine oxidase inhibitors: ten years of docking studies. *Curr. Top. Med. Chem.* **12:** 2145-2162.

8. Finberg JP. 2014. Update on the pharmacology of selective inhibitors of MAO-A and MAO-B: focus on modulation of CNS monoamine neurotransmitter release. *Pharmacol. Ther.* **143:** 133-152.

9. 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.

10. 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.

11. Kim H, Sablin SO, Ramsay RR. 1997. Inhibition of monoamine oxidase A by β-carboline derivatives. *Arch. Biochem. Biophys.* **337:** 137-142.

12. Kim H, Yang I, Patil RS, Kang S, Lee J, Choi H, *et al*. 2014. Anithiactins AC, modified 2-phenylthiazoles from a mudflat-derived *Streptomyces* sp. *J. Nat. Prod.* **77:** 2716-2719.

13. Lin Z, Antemano RR, Hughen RW, Tianero MD, Peraud O, Haygood MG, *et al*. 2010. Pulicatins A-E, neuroactive thiazoline metabolites from cone snail-associated bacteria. *J. Nat. Prod.* **73:** 1922-1926.

14. 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.

15. Malcomson T, Yelekci K, Borrello MT, Ganesan A, Semina E, De Kimpe N, *et al*. 2015. *cis*-Cyclopropylamines as mechanism-based inhibitors of monoamine oxidases. *FEBS J*. DOI: 10.1111/febs.13260 [Epub ahead of print].

16. 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.

17. Moon K, Chung B, Shin Y, Rheingold AL, Moore CE, Park SJ, *et al*. 2015. Pentacyclic antibiotics from a tidal mud flat-derived actinomycete. *J. Nat. Prod.* **78:** 524-529.

18. Mostert S, Petzer A, Petzer JP. 2015. Indanones as high-potency reversible inhibitors of monoamine oxidase. *ChemMedChem* DOI: 10.1002/cmdc.201500059 [Epub ahead of print].

19. Pan HQ, Yu SY, Song CF, Wang N, Hua HM, Hu JC, Wang SJ. 2015. Identification and characterization of the antifungal substances of a novel *Streptomyces cavourensis* NA4. *J. Microbiol. Biotechnol*. **25:** 353-357.

20. Parsons CG, Danysz W, Dekundy A, Pulte I. 2013. Memantine and cholinesterase inhibitors: complementary mechanisms in the treatment of Alzheimer's disease. *Neurotox. Res.* **24:** 358-369.

21. 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.

22. Ramsay RR. 2013. Inhibitor design for monoamine oxidases. *Curr. Pharm. Des.* **19:** 2529-2539.

23. Stevens H, Brinkhoff T, Rink B, Vollmers J, Simon M. 2007. Diversity and abundance of gram positive bacteria in a tidal flat ecosystem. *Environ. Microbiol.* **9:** 1810-1822.

24. Yang W, Dostal L, Rosazza JP. 1993. Aeruginol [2-(2'-hydroxyphenyl)-4-hydroxymethylthiazole], a new secondary metabolite from *Pseudomonas aeruginosa*. *J. Nat. Prod*. **56:** 1993-1994.

25. Youdim MB, Edmondson D, Tipton KF. 2006. The therapeutic potential of monoamine oxidase inhibitors. *Nat. Rev. Neurosci.* **7:** 295-309.