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Selective inhibition of monoamine oxidase A by hispidol

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ABSTRACT

Hispidol, an aurone, isolated from *Glycine max* Merrill, was found to potently and selectively inhibit an isoform of recombinant human monoamine oxidase-A (MAO-A), with an IC_{50} value of 0.26 μM, and to inhibit MAO-B, but with lower potency (IC_{50} = 2.45 μM). Hispidol reversibly and competitively inhibited MAO-A with a K_i value of 0.10 μM with a potency much greater than toloxatone (IC_{50} = 1.10 μM), a marketed drug. It also reversibly and competitively inhibited MAO-B (K_i = 0.51 μM). Sulfuretin, an analog of hispidol, effectively inhibited MAO-A (IC_{50} = 4.16 μM) but not MAO-B (IC_{50} > 80 μM). A comparison of their chemical structures showed that the 3′-hydroxyl group of sulfuretin might reduce its inhibitory activities against MAO-A and MAO-B. Flexible docking simulation revealed that the binding affinity of hispidol for MAO-A (−9.1 kcal/mol) was greater than its affinity for MAO-B (−8.7 kcal/mol). The docking simulation showed hispidol binds to the major pocket of MAO-A or MAO-B. The findings suggest hispidol is a potent, selective, reversible inhibitor of MAO-A, and that it be considered a novel lead compound for development of novel reversible inhibitors of MAO-A.

Monoamine oxidases (MAOs, EC 1.4.3.4) are responsible for the oxidative deamination of monoamine neurotransmitters and other dietary amines.[1] There are two MAO isoforms, MAO-A and MAO-B, which are encoded by separate genes and localize to the outer membranes of mitochondria.[2] Although these two isoforms exhibit high similarity (70%), their active sites, and thus, their substrate specificities, are quite different. For example, MAO-A selectively deaminates serotonin, whereas MAO-B selectively deaminates phenylethylamine and benzylamine (Ramsay and Tipton, 2017; Youdim, et al., 2006).[2,3] Originally, the MAO inhibitors developed were of the irreversible type, and these agents have long been used as antidepressants. Later, reversible inhibitors, such as, moclobemide (selective for MAO-A) and safinamide (selective for MAO-B), were developed to avoid protracted irreversible effects, and are now used as antidepressants and for the treatment of Parkinson's disease (PD), respectively (Ramsay and Tipton, 2017; Fisar, 2016).[2,4]

Considerable research effort has been expended on the identification of natural and synthetic compounds that potently inhibit MAO-A and/or MAO-B, and a number of reviews have been published on the topic (Orhan, 2016; Pathak et al., 2016; Mathew, 2014).[5–7] During the screening of MAO inhibitors in natural herbal products, we found hispidol showed potent inhibitory activity against MAO-A.

Hispidol is a 6,4′-dihydroxyaurone, and was the first aurone derivative found in soybean *Glycine* max (Wong, 1966),[8] and also in the seeds of *Lygos raetam* (El Sherbeiny et al., 1978),[9] and it has also been identified in *Medicago truncatula* cell cultures (Farag et al., 2009).[10] Zwergel et al., and Boucherle et al. have published reviews on the biosyntheses, properties, and biological potentials of aurones, (Zwergel et al., 2012; Boucherle et al., 2017),[11,12] which have been found to have many biological activities, including antioxidant, anti-microbial and anti-cancer effects (Nenadis and Sigalas, 2011; Alsaif et al., 2017).[13,14] Synthesized aurone derivatives have been investigated as probing agents for Alzheimer's disease (AD), due to their high affinities for Aβ aggregates (Maya et al., 2009; Ono et al., 2007; Watanabe et al., 2011),[15–17] and as multifunctional agents for treatment of AD (Li et al., 2016)[18] and as selective MAO-B inhibitors for the treatment of PD (Geldenhuys et al., 2012; Morales-Camilo et al., 2015).[19,20] However, comparatively little information is available on the biological activities of hispidol, although it has been established to have significant antifungal activity (Farag et al., 2009).[10] In this study, we examined the

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inhibitory activities of hispidol isolated from *Glycine* max Merrill against recombinant human MAO-A and MAO-B and its inhibition patterns.

Benzylamine, kynuramine, toloxatone, lazabemide, and recombinant human MAO enzymes A and B were purchased from Sigma-Aldrich (St. Louis, MO, USA). Clorgyline and pargyline were obtained from a monoamine oxidase kit (BioAssay Systems; Hayward, CA, USA), and used as reference inhibitors of MAO-A and MAO-B, respectively. MAO-A and MAO-B were stored at −70 °C in 100 mM potassium phosphate (pH 7.4) containing 0.25 M sucrose, 0.1 mM EDTA, and 5% glycerol**.** Initial rates were measured in 0.5 ml of reaction mixtures containing 50 mM sodium phosphate (pH 7.2) at 25 °C. (Lee et al., 2017a; Note 1).[21,22]

Based on the results of primary screening data in a library of natural products, hispidol and sulfuretin (both aurone derivatives) were isolated from the extracts of *Glycine* max Merrill and *Toxicodendron vernicifluum*, respectively (Fig. 1). (Note 2)[23] The structures of hispidol and sulfuretin were elucidated using 1D and 2D NMR and ESI-MS (Jung et al., 2003; Haudecoeur et al., 2011),[24,25] and are provided in Supplementary data. Samples were analyzed by HPLC in triplicate. The purities of hispidol and sulfuretin used in the experiments mentioned below were 99.8% and 98.8%, respectively.

IC_{50} values were determined by measuring residual MAO activities in the presence of different inhibitor concentrations. IC_{50} values for the inhibitions of MAO-A and MAO-B are shown in Table 1. Hispidol was found to potently inhibit MAO-A (IC_{50} = 0.26 μM), and to inhibit MAO-B to a lesser extent (selectivity index >9.4; Table 1). Sulfuretin was less effective than hispidol at inhibiting MAO-A (IC_{50} = 4.16 μM) and ineffective at inhibiting MAO-B (IC_{50} > 80 μM).

The time-dependences of the inhibition of MAO-A by hispidol and sulfuretin were investigated as previously described (Lee et al., 2016).[26] Remaining activities were determined using 0.06 mM kynuramine as substrate after preincubating kynuramine and MAO-A at different times up to 30 min at 25 °C, but no changes in activity were observed after preincubation. Interactions between MAO-A and hispidol or sulfuretin were nearly instantaneous (data not shown).

The reversibilities of MAO-A and MAO-B inhibitions by hispidol were investigated using DiaEasy dialyzers (0.8 ml, 3–6 kDa cut-off, BioVision Inc., Milpitas, CA, USA). For the undialyzed experiment, MAO-A was preincubated with 0.5 μM (~2 × IC_{50}) of hispidol for 30 min in 100 mM sodium phosphate (pH 7.2), and then residual activity was measured by adding 0.06 mM of kynuramine. Residual activity after the undialyzed experiment (A_U) was compared to control activity measured under the same conditions but without hispidol. To investigate the reversibility of inhibition, a preincubated MAO-A mixture containing hispidol was dialyzed against 100 mM sodium phosphate (pH 7.2) for 6 h with two exchanges of buffer (i.e., three times for 2 h) at 4 °C, prior to measuring residual activity. At the same time, a preincubated enzyme mixture not containing hispidol was dialyzed and enzyme activity was then

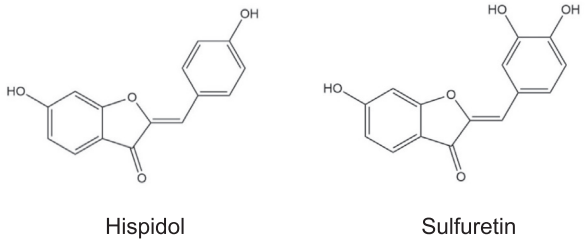

Fig. 1. Structures of hispidol and sulfuretin.

Table 1
IC_{50} values of hispidol and sulfuretin for the inhibitions of recombinant human MAO-A and MAO-B.[a]

	IC_{50} (μM)		SI[b]
	MAO-A	MAO-B	
Hispidol	0.26 ± 0.021	2.45 ± 0.23	9.4
Sulfuretin	4.16 ± 0.0091	>80[c]	>19.2
Toloxatone	1.10 ± 0.085	–	–
Lazabemide	–	0.015 ± 0.0024	–
Clorgyline	0.0062 ± 0.0003	>2.0	–
Pargyline	>2.0	0.12 ± 0.0035	

[a] Inhibitory activities on MAO-A and MAO-B were determined using 0.06 mM kynuramine or 0.6 mM benzylamine as substrates, respectively. Values for toloxatone, lazabemide, clorgyline, and pargyline were determined after preincubation with the enzymes for 30 min. Results are presented as means ± standard errors of at least duplicate experiments.

[b] MAO-A selectivity indices were calculated by dividing IC_{50} values for MAO-B by those for MAO-A.

[c] 20.8% inhibition at 80 μM.

measured. Residual activity after the dialyzed experiment (A_D) was compared to activity measured after dialysis under the same conditions in the absence of hispidol. Finally, reversibility was assessed by comparing A_D and A_U values. For hispidol, these values were 83.7 and 29.8%, respectively (Fig. 2A), which showed the inhibitory activity by hispidol was recovered by dialysis to near the control level. Toloxatone and clorgyline were used as reference reversible and irreversible MAO-A inhibitors, respectively. The reversibilities of toloxatone and clorgyline were carried out at 2.0 and 0.010 μM (~2 × IC_{50}), respectively, and the A_D and A_U values for toloxatone were 75.4 and 30.7%, respectively, and for clorgyline were 18.3 and 17.3%, respectively. MAO-A inhibition by clorgyline was not recovered by dialysis, whereas inhibition by toloxatone was recovered to a great extent. These results showed that hispidol is a reversible inhibitor of MAO-A.

The reversibility for MAO-B was also analyzed according to the method for MAO-A, except using 5.0 μM (~2 × IC_{50}), and measuring by adding 0.6 mM of benzylamine. For hispidol, A_D and A_U values were 87.5 and 28.0%, respectively (Fig. 2B). The A_D and A_U values for lazabemide were 72.9 and 35.0%, respectively, and for pargyline were 17.4 and 24.0%, respectively. Similarly to the MAO-A, MAO-B inhibition by pargyline was not recovered, but inhibition by lazabemide was recovered to a great extent, showing that hispidol is a reversible inhibitor of MAO-B.

The kinetics of recombinant human MAO-A and MAO-B inhibitions by hispidol were studied and the modes of inhibitions were investigated using Lineweaver-Burk plots. Catalytic rates of MAO-A and MAO-B reactions were measured at five different substrate concentrations (0.006–0.15 mM and 0.06–1.5 mM, respectively) in the absence or presence of hispidol. Lineweaver plots for the inhibition of MAO-A by hispidol were linear and intersected the y-axis (Fig. 3A), indicating hispidol is a competitive inhibitor of MAO-A. From secondary plot of slopes against inhibitor concentrations, the K_i value for the inhibition of MAO-A by hispidol was determined to be 0.10 ± 0.011 μM (Fig. 3B). For MAO-B, hispidol is a competitive inhibitor (Fig. 3C) and the K_i value was determined to be 0.51 ± 0.035 μM (Fig. 3D).

Molecular docking simulations of hispidol and MAOs were analyzed using the Autodock Vina program, which has an automated docking facility (Trott and Olson, 2010).[27] The protein structures of MAOs were MAO-A complexed with 7-methoxy-1-methyl-9H-beta-carboline (PDB: 2Z5X) and MAO-B complexed with benzylhydrazine (PDB: 2VRL). Docking and simulation visualizations were performed using Chimera (Pettersen et al. 2004),[28] as described previously (Lee et al., 2016).[26] The major pockets of MAO-A and MAO-B were identified in each complex. The docking affinity of