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Synthesis of a series of unsaturated ketone derivatives as selective and reversible monoamine oxidase inhibitors

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ABSTRACT

We have synthesized three categories of α,β-unsaturated carbonyl derivatives and evaluated their MAO-A and MAO-B inhibitory activities. Among them, compound **10b** including α,β-unsaturated ketone group showed the most potent and selective MAO-B inhibitory activity (IC_{50} human MAO-B 16 nM, >6000-fold selective vs MAO-A) and compound **10b** exhibited good reversibility compared with selegiline, a well-known irreversible MAO-B inhibitor. However, both α,β-unsaturated amide and ester derivatives exhibited weaker MAO-B inhibition potencies. The docking studies provided insights into the possible binding modes and the key interaction sites of the synthesized MAO-B inhibitors.

1. Introduction

Monoamine oxidase (MAO) is a flavin adenine dinucleotide (FAD) containing enzymes localized on the mitochondrial outer membrane and particularly abundant in the liver and brain.[1] MAOs catalyze the oxidative deamination of monoamine neurotransmitters, such as dopamine, serotonin, and norepinephrine, to their corresponding aldehydes with the production of hydrogen peroxide (H_2O_2).[2,3] There are two isoforms of MAOs, MAO-A and MAO-B, which are differentiated by their substrate and inhibitor sensitivity,[4–6] and tissue/cellular distribution.[7] MAO-A is mainly located in catecholaminergic neurons and selectively inhibited by low concentrations of clorgyline (**1**), whereas MAO-B is primarily found in serotonergic neurons and astrocytes, and selectively inhibited by L-deprenyl (selegiline) (Fig. 1).[8] The activity and the expression levels of MAO-B in the human brain, but not those of MAO-A, increase with age and particularly its activity is significantly increased in the substantia nigra (SN) of patients with Parkinson's diseases (PD). Therefore, selective MAO-B inhibitors (selegiline (**2**) and rasagiline (**3**)) are used solely, or in combination with the dopamine prodrug levodopa to reduce the metabolic degradation of dopamine for the symptomatic treatment of PD.[9,10] However, the potent and selective MAO-B inhibitor selegiline (**2**) shows severe side effects due to its amphetamine metabolites[11] and both selegiline (**2**) and rasagiline (**3**) are irreversible MAO-B inhibitors triggering pharmacological side effects in long term treatment of PD.[12]

Accordingly, the development of selective reversible MAO-B inhibitors may reduce adverse effects of irreversible inhibitors and may be promising target for the treatment of other neurodegenerative diseases. For example, recent study suggested that reversible MAO-B inhibitors might be effective in treatment for Alzheimer's disease (AD) by selective inhibition of astrocytic GABA production.[13] On the basis of the recent patent literatures, novel selective MAO inhibitors have been developed for new therapeutic opportunities such as cancer, hair loss, muscle dystrophies, cocaine addiction and inflammation along with the classical therapeutic window.[14] In the search for selective reversible MAO-B inhibitors,

Abbreviations: AD, Alzheimer's disease; DA, dopamine; DMF, dimethylformamide; DMTMM, 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride; EGCG, epigallocatechin-3-gallate; FAD, flavin adenine dinucleotide; HO-1, heme oxygenase-1; HRP, horseradish peroxidase; IBCF, isobutyl chloroformate; MAOs, monoamine oxidases; NMM, *N*-methyl morpholine; PD, Parkinson's disease; SN, substantia nigra; TEA, triethylamine; THF, tetrahydrofuran; TMS, tetramethylsilane.

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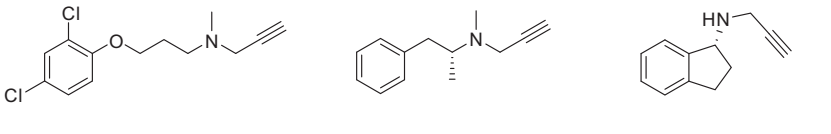

1
Clorgyline
(Selective MAO-A inhibitor)

2
Selegiline
(Selective MAO-B inhibitor)

3
Rasagiline
(Selective MAO-B inhibitor)

Figure 1. Structures of irreversible MAO inhibitors.

chalcones have emerged as a valid scaffold.[15,16] Chalcones (**4**) (1,3-diphenyl-2-propen-1-ones) are considered as the precursor of flavonoids and abundant in edible plants.[17] They consist of open-chain flavonoids including two aromatic rings and an α,β-unsaturated carbonyl system and exhibit a broad spectrum of biological activities.[18,19] Chimenti et al.[15] synthesized a series of chalcones substituted with various functional groups at 2- and 4-position of the A aromatic moiety and 4′-position of the B aromatic moiety of **4** and tested their inhibitory effects against human MAO-A and B. While the synthesized chalcone derivatives exhibited human MAO-B selective inhibitory activity in nM–μM ranges, the most potent compound was disubstituted in the 2- and 4-position of the A aromatic moiety with hydroxyl and methoxy groups and in 4′-position of the B aromatic moiety with a chlorine atom. Recently, Morales-Camilo et al.[20] reported that all synthesized chalcones with hydroxyl and methoxy groups in the 2- and 5 (or 6)-position of the A aromatic showed moderate activities (μM ranges). In this study, we prepared a series of chalcone derivatives substituted with a various functional group at 2-, 3-, 4-position of B aromatic moiety and evaluated inhibitory activities against MAO-A and MAO-B. We also examined whether the potent compounds were reversible inhibitors. Furthermore, we synthesized α,β-unsaturated amide derivatives (**5**) and α,β-unsaturated ester derivatives (**6**), and tested their inhibitory activities to compare with the α,β-unsaturated carbonyl compounds (Fig. 2).

2. Results and discussion

2.1. Chemistry

We prepared the substituted chalcones (**4**) containing an α,β-unsaturated ketone moiety and modified the ketone position according to two categories: amide or ester. Chalcone compounds **8**–**11** were synthesized as previously described.[21] Briefly, the substituted chalcone derivatives were prepared by the Claisen–Schmidt condensation of substituted acetophenones with aromatic aldehydes using lithium hydroxide hydrate ($LiOH{\cdot}H_2O$) as catalyst in ethanol at room temperature (Scheme 1).

To prepare the α,β-unsaturated amide and ester derivatives **12**–**20**, the free acids **7e**–**7g** were coupled with the appropriate substituted aniline using the mixed anhydride coupling (MAC) reaction[22] (i.e., isobutyl chloroformate (IBCF), *N*-methyl morpholine (NMM)) or 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM) amide coupling reaction.[22] For 2′-substituted Cl compounds **12b**, **13b**, **14b** and **15b**, 2-chloro cinnamoyl chloride were directly coupled with the desired aniline with triethylamine in DMF (Scheme 2).

2.2. Inhibitory activities of the synthesized compounds against monoamine oxidase

The synthesized compounds were evaluated for inhibitory activities of human MAO-A and MAO-B using recombinant enzymes. The enzyme inhibition assay on test compounds was performed using Amplex Red reagents. The inhibitory activities were assayed by spectrophotometrically measuring the rate of resorufin formation at 570 nm. The inhibitory potencies (IC_{50} values) for all synthesized compounds are summarized in Table 1 along with similar results obtained by the positive control compounds, selegiline and safinamide.

First, we prepared 18 chalcone derivatives that included various functional groups on both ring A and ring B (Table 1, compounds **8**–**11**). The first set of compounds **10** included a methoxy group attached at the 4-position on ring A. Chimenti et al.[15] reported that the best results were obtained in the presence of chlorine substituent in the 4′-position on ring B. However, only chlorine substituted chalcone derivatives for electron-withdrawing group were evaluated. Thus, we introduced both various electron-withdrawing groups and electron-donating group into ring B (**10a**–**10i**). We observed that substitutions on ring B with an electron-withdrawing group (CF_3, F, or Cl) led to higher inhibitory activities against hMAO-B than an electron-donating group (OMe). For example, the 2′-substituted CF_3, F, and Cl compounds (**10b**, **10e**, **10f**) showed more potent inhibitory activities than the 2′-substituted OMe compound **10g** (**10b**, IC_{50} = 16 nM; **10e**, IC_{50} = 56 nM; **10f**, IC_{50} = 69 nM; **10g**, IC_{50} = 419 nM). For the trifluoromethyl-substituted compounds **10b**–**10d**, we found that the 2′-trifluoromehtyl derivative **10b** was more active than the corresponding 3′- and 4′-trifluoromethyl derivatives **10c** and **10d**. A similar trend on the inhibitory activities of 2′, 3′, and 4′ regioisomers was observed in the methoxy-substituted compounds **10g**–**10i**. These findings suggested that the substitution at 2′-position on ring B in the chalcone derivatives led to increased inhibitory activity.

Next, we placed a methoxy group at the 2- and 3-positions on ring A (**8** and **9**). Introduction of a 2-methoxy or 3-methoxy group on ring A led to decreased inhibitory activities compared with 4-methoxy substituted regioisomers, indicating that *ortho*- or *meta*-methoxy substituents were not well tolerated. For example, among the 2′-substituted CF_3 compounds (**8b**, **9b** and **10b**), **10b** exhibited more potent inhibitory activities against hMAO-B than the corresponding 2- and 3-methoxy derivatives (**10b**, IC_{50} = 16 nM; **8b**, IC_{50} = 253 nM; **9b**, IC_{50} = 2118 nM). Thus, **10b** was the most potent and selective MAO-B inhibitor within the series of the trifluoromethyl-substituted derivatives (**8b**, **9b**, **10b**–**10d**). The introduction of halogen group instead of the CF_3 group at 2′-position on ring B led to slightly decreased inhibitory activities (**10b**, IC_{50} = 16 nM; **10e**, IC_{50} = 56 nM; **10f**, IC_{50} = 69 nM). When the methoxy group on ring A was replaced with hydroxyl group for compounds **11**, the inhibitory activities were similar and little weaker. All chalcone derivatives (**8**–**11**) did not inhibit more than 50% of hMAO-A activity at 100 μM (IC_{50} >100 μM).

We introduced either an α,β-unsaturated amide group or α,β-unsaturated ester group instead of the α,β-unsaturated carbonyl entity of chalcones (**12**–**18** and **19**–**20**, respectively) and tested their inhibitory activities against hMAOs. Most of compounds with an α,β-unsaturated amide group (**12**–**18**) showed lower activities against hMAO-B than the chalcone derivatives. Among them, compound **16c** exerted the best inhibitory activity against hMAO-B (283 nM). Interestingly, the 4-hydroxy derivatives **14a** and **14b** exhibited moderate inhibitory activities against hMAO-A (9.2 μM