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Design of novel nicotinamides as potent and selective monoamine oxidase a inhibitors

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ABSTRACT

A series of *N*-(2-morpholinoethyl)nicotinamide (**1**–**13**) and *N*-(3-morpholinopropyl)nicotinamide derivatives (**14**–**26**) have been designed, synthesized and evaluated in vitro for their monoamine oxidase (MAO) A and B inhibitory activity and selectivity. Most of these synthesized compounds proved to be potent, and selective inhibitors of MAO-A rather than of MAO-B. 5-Chloro-6-hydroxy-*N*-(2-morpholinoethyl)nicotinamide (**13**) displayed the highest MAO-A inhibitory potency (IC_{50} = 0.045 μM) and a good selectivity. 2-Bromo-*N*-(2-morpholinoethyl)nicotinamide (**3**) was the most potent MAO-B inhibitor with the IC_{50} value of 0.32 μM, but it was not selective. Molecular dockings of compound **13** were performed in order to give structural insights regarding the MAO-A selectivity.

1. Introduction

Monoamine oxidases (MAOs; EC 1.4.3.4) are flavin adenine dinucleotide (FAD) containing enzymes, which are localized in the outer mitochondrial membranes of neuronal, glial, and other cells,[1,2] particularly abundant in the liver and brain.[3] They catalyze the oxidation of endogenous and exogenous amines to the corresponding aldehyde while releasing H_2O_2 and ammonia.[4] Two isoforms of MAO (MAO-A and MAO-B) have been demonstrated by Johnston.[5] They can be defined on their differential substrate and inhibitor specificity,[6–8] tissue and cell distribution,[9] and gene expression characteristics,[10,11] are targets for a series of therapeutically valuable drugs. MAO-A is located predominantly in catecholaminergic neurons, while MAO-B is present in serotonergic neurons and glia.[12,13] MAO-A preferentially deaminates aromatic monoamines such as the neurotransmitters serotonin (5-HT), adrenaline (A) and noradrenaline (NA), while MAO-B mainly oxidizes β-phenylethylamines (PEA) and benzylamines. Both isoenzymes deaminate dopamine (DA), tyramine and tryptamine.[14]

Inhibitors of MAO have shown therapeutic value in a variety of neurodegenerative diseases.[15–18] MAO-A inhibitors such as iproniazid, clorgyline and moclobemide are used as antidepressant and antianxiety agents.[19–24] Selective MAO-B inhibitors such as selegiline, rasagiline and lazabemide are useful in the treatment of Parkinson's[25,26] and Alzheimer's diseases (Fig. 1).[27,28]

The recent determination of the crystal structure of the two isoforms of human MAO, by Binda and co-workers elucidates the mechanism underlying the selective interactions between these proteins and their ligands, probes the catalytic mechanism, and provides a better understanding of the pharmacophoric requirements needed for a rational design of potent and selective enzyme inhibitors with a therapeutic potential.[29–33]

Iproniazid was the prototype of potent MAO inhibitor introduced in therapy of depression since the 1957s.[21] However, due to its side interactions with other drugs and certain foods, the therapeutic applications of iproniazid have been diminished.[22] Moclobemide was the first nonhydrazine, reversible MAO-A selective inhibitor approved for use as an antidepressant drug. Although it is a potent MAO-A inhibitor in vivo, it is only a weak inhibitor in vitro. For pursuing more potent and less toxic MAO-A inhibitors as antidepressant agents, we design a series of nicotinamides by combining picolinate and morpholine, which were from different parts of these two antidepressant drugs iproniazid and moclobemide. MAO inhibitory activity and structure–activity relationship of these synthesized nicotinamides were studied. To give structural insights regarding the binding mode of these inhibitors, we carried out docking simulations of the most potent and selective MAO-A inhibitor **13**.

2. Results and discussion

2.1. Chemistry

The synthesis of the title nicotinamides followed the general reaction pathway outlined in Scheme 1. *N*-(2-Morpholinoethyl)

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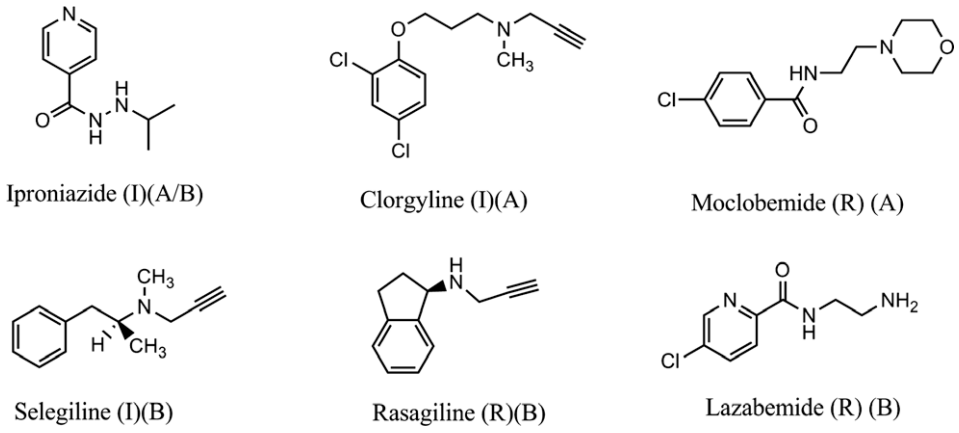
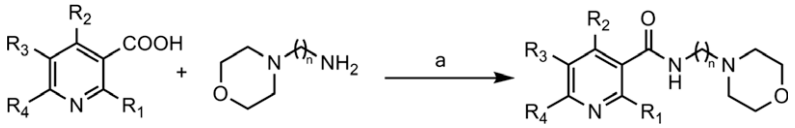


Figure 1. Irreversible (I), reversible (R), and selective MAO-A or MAO-B (A or B) inhibitors.



Scheme 1. General procedure for the synthesis of nicotinamides. Reagents and conditions: (a) EDC·HCl, CH_2Cl_2, reflux, 8–10 h.

nicotinamides **1**–**13** were synthesized by coupling 2-morpholinoethanamine with equimolar quantities of substituted nicotinic acids, using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC·HCl) as condensing agent. The mixture was refluxed in anhydrous CH_2Cl_2 for 8–10 h. *N*-(3-Morpholinopropyl)nicotinamides **14**–**26** were synthesized by 3-morpholinopropan-1-amine and equimolar quantities of substituted nicotinic acids by the same method.

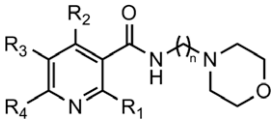
2.2. Biological evaluation

Rat brain mitochondria were used as a source of the two MAO isoforms. MAO-A and MAO-B inhibitory activities of the synthesized nicotinamides were determined by a fluorometric assay, using kinuramine as a substrate, in the presence of their specific inhibitors (selegiline 1 μM for MAO-A and clorgyline 1 μM for MAO-B). The MAO inhibitory activities, expressed as IC_{50} values are summarized in Table 1. Also included was the activity of reference compounds moclobemide and iproniazid.

Most of the synthesized compounds showed potent MAO-A inhibitory activity with the IC_{50} value at low micromolar to sub-micromolar range. Only four compounds (**2**, **3**, **15** and **16**) with *ortho*-chloro or bromo substituent inhibited the B isoform in the low micromolar or sub-micromolar range of concentration but they were poor selective MAO-B inhibitors. The nicotinamides **12** and **13** showed the greatest MAO-A inhibitory activity (IC_{50}(MAO-A) = 0.084 and 0.045 μM, respectively) and good selectivity (IC_{50}-(MAO-B) = 48 and 26 μM, respectively). Compound **3** displayed the most potent MAO-B inhibitory activity (IC_{50}(MAO-B) = 0.32 μM), however, because of its potent MAO-A inhibitory activity (IC_{50} (MAO-A) = 8.5 μM), it was not selective.

Structure–activity relationships (SARs) were inferred from data of enzymatic experiments reported in Table 1. The length of the linker group affected the anti-MAO activity. *N*-(2-Morpholinoethyl)nicotinamide derivatives **1**–**13** ($n = 2$) were more potent than *N*-(3-morpholinopropyl)nicotinamide derivatives **14**–**26** ($n = 3$). This result indicated that elongation of the alkyl chain resulted in the decrease of MAO inhibition.

Table 1
Monoamine oxidase inhibitory activity of nicotinamides **1**–**26**



Compd	R_1	R_2	R_3	R_4	n	MAO-A IC_{50} (μM) ± SD	MAO-B IC_{50} (μM) ± SD
1	H	H	H	H	2	68 ± 12	>100
2	Cl	H	H	H	2	2.6 ± 0.7	1.8 ± 0.3
3	Br	H	H	H	2	8.5 ± 1.3	0.32 ± 0.09
4	OH	H	H	H	2	45 ± 7	>100
5	H	Cl	H	H	2	0.75 ± 0.08	74 ± 6
6	H	H	Br	H	2	26 ± 4	55 ± 10
7	H	H	H	CH_3	2	86 ± 8	>100
8	H	H	H	F	2	15 ± 2	>100
9	H	H	H	Cl	2	0.18 ± 0.10	34 ± 5
10	H	H	H	OH	2	0.43 ± 0.05	>100
11	Cl	H	H	CH_3	2	28 ± 6	>100
12	H	H	Cl	Cl	2	0.084 ± 0.015	48 ± 13
13	H	H	Cl	OH	2	0.045 ± 0.008	26 ± 6
14	H	H	H	H	3	>100	>100
15	Cl	H	H	H	3	9.4 ± 2.5	6.5 ± 0.6
16	Br	H	H	H	3	35 ± 13	1.9 ± 0.2
17	OH	H	H	H	3	>100	>100
18	H	Cl	H	H	3	7.5 ± 2.7	53 ± 8
19	H	H	Br	H	3	23 ± 4	65 ± 16
20	H	H	H	CH_3	3	>100	>100
21	H	H	H	F	3	80 ± 9	>100
22	H	H	H	Cl	3	0.38 ± 0.07	75 ± 6
23	H	H	H	OH	3	56 ± 20	>100
24	Cl	H	H	CH_3	3	>100	>100
25	H	H	Cl	Cl	3	0.10 ± 0.03	45 ± 9
26	H	H	Cl	OH	3	12 ± 3	82 ± 13
Moclobemide						6.8 ± 1.5	>100
Iproniazid						5.0 ± 2.1	7.5 ± 0.4

In the series of *N*-(2-morpholinoethyl)nicotinamide derivatives **1**–**13**, the MAO-A inhibitory activity of nicotinamides with different *para*-substituents increased in the following order: CH_3 (**7**) < H (**1**) < F (**8**) < OH (**10**) < Cl (**9**) (IC_{50} = 86 < 68 < 15 < 0.43 < 0.18 μM).

Compounds **2** and **11** both contained *ortho*-chloro substituent. But the activity of compound **2** (IC_{50} = 2.6 μM) was about 10-fold than compound **11** with methyl substituent (IC_{50} = 28 μM). This result also indicated that the methyl group declined the MAO-A inhibitory activity of the synthesized nicotinamides. Both of compounds **11** and **7** with *para*-methyl showed weak activity. But the activity of compound **11** with chloro substituent (IC_{50} = 28 μM) was more potent than compound **7** (IC_{50} = 86 μM). This result also indicated that the chloro group increased the MAO-A inhibitory activity of the synthesized nicotinamides. Compounds **2**, **5**, **9** with chloro substituent at different position showed distinct MAO-A inhibitory activity. Compound **9** with the *para*-chloro substituent showed more potent activity (IC_{50} = 0.18 μM) than compounds **2** and **5** with the *ortho*-chloro substituent (IC_{50} = 2.6 and 0.75 μM, respectively). Furthermore, compound **3** with *ortho*-bromo substituent (IC_{50} = 8.5 μM) exhibited better anti-MAO-A activity than compound **6** with *meta*-bromo substituent (IC_{50} = 26 μM). Compound **10** with *para*-hydroxyl group (IC_{50} = 0.43 μM) was about 100 times more potent than **4** (IC_{50} = 45 μM) with *ortho*-hydroxyl group as MAO-A inhibitors. This result indicated that *para*-hydroxyl group was benefit for the activity. The most potent MAO-A inhibitory activity of compounds **12** (IC_{50} = 0.084 μM) and **13** (IC_{50} = 0.045 μM) also indicated that the chloro and *para*-hydroxyl substituent were favorable for the activity.

The similar rule was also found in the series of *N*-(3-morpholinopropyl)nicotinamide derivatives **14**–**26**.

2.3. Molecular docking studies

MAO-A and MAO-B are highly similar in primary sequences. Comparison of the two enzymes shows that the main difference between rat MAO-A and human MAO-B is in the structure of their active site cavities in the area opposite to the flavin coenzyme, which is responsible for their differing substrate and inhibitor specificities. Tsukihara and co-workers superimposed the structures of rat MAO-A (PDB code 1O5W)[34] and human MAO-B (PDB code 1GOS)[30] using LSQKAB program to fit the C^{α} atoms of residues 309–499 in MAO-A to the model of residues between 300 and 490 in MAO-B. The major differences in side-chains at the active centers of these two molecules are at two positions; Ile335 in MAO-A becomes Tyr326 in MAO-B, while Phe208 in MAO-A corresponds to Ile119 in MAO-B.[34]

Molecular docking was performed with a focus on compound **13**, which showed the greatest and selective MAO-A inhibitory activity. Figure 2 shows the binding modes of compound **13** (a) into the MAO-A binding cavity and (b) into the MAO-B binding cavity. Visual inspection of the pose of **13** into the MAO-A binding site revealed that the pyridine ring of **13** is placed in the 'aromatic cage' framed by Tyr197, Tyr407, Tyr444, and the FAD aromatic ring and is oriented to establish π–π stacking interactions with Tyr407. The morpholine ring of **13** is embedded in a large hydrophobic pocket formed by Ile180, Phe208, Asn181, Gln215, and Ile335. Moreover, three hydrogen bonds are observable for **13** between the protonated hydroxyl group of **13** and the carbonyl oxygen of Asn181, the protonated hydroxyl group of Tyr 444 and the phenolic oxygen of **13** and the protonated hydroxyl group of Tyr 197 and the phenolic oxygen of **13**. It is observed that the Cl atom of compound **13** is making important van der Waals interactions with the hydroxyl group of the side chain of Tyr197, Tyr444 and FAD.

Visual inspection of the pose of **13** into the MAO-B binding site revealed that compound **13** is inserted into the 'aromatic cage' framed by Tyr60, Tyr326, Tyr398, Tyr435, and the FAD aromatic ring. And the binding is further stabilized by hydrophobic interactions between the morpholine ring and Gln206.

The better binding mode of **13** with MAO-A explains the observation of the higher MAO-A inhibitory potency of this compound.

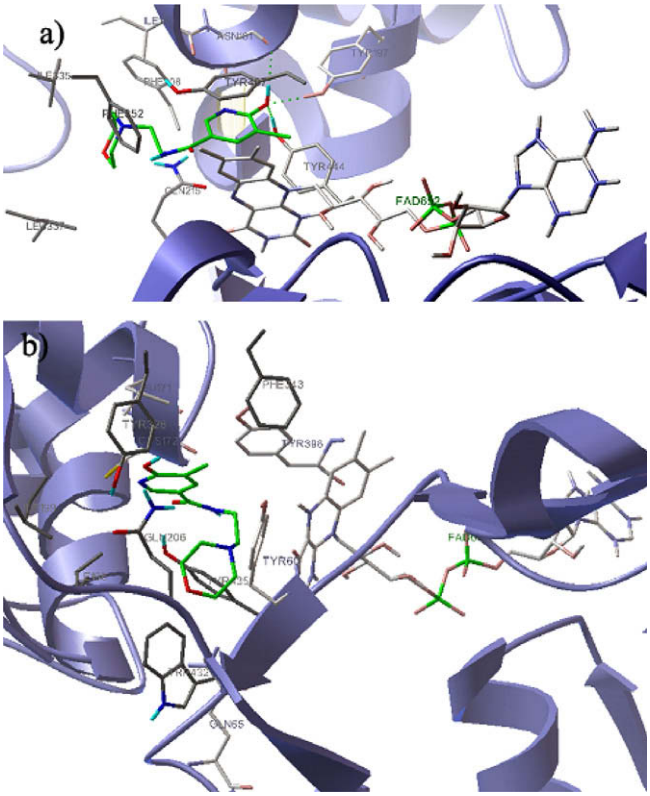


Figure 2. (a) Binding model of **13** in MAO-A active site. (b) Binding model of **13** in MAO-B active site

3. Conclusions

A series of nicotinamide derivatives (**1–26**) were designed and evaluated in vitro for their MAO-A and MAO-B inhibitory activity. Most of the synthesized nicotinamides proved to be potent, and selective inhibitors of MAO-A rather than of MAO-B. Compound **13** showed the greatest MAO-A inhibitory activity (IC_{50}(MAO-A) = 0.045 μM) and good selectivity (IC_{50}(MAO-B) = 26 μM). Molecular dockings of compound **13** into MAO-A and MAO-B were performed. The better binding mode of **13** with MAO-A explains its MAO-A selectivity.

4. Experimental section

4.1. Chemistry

All chemicals (reagent grade) used were purchased from Sigma–Aldrich (USA) and Sinopharm Chemical Reagent Co., Ltd (China). Melting points (uncorrected) were determined on a XT4 MP apparatus (Taike Corp., Beijing, China). ESI-MS spectra were recorded on a Mariner System 5304 Mass spectrometer., and ^{1}H NMR spectra were recorded on a Bruker AV-300 or AV-500 spectrometer at 25 °C with TMS and solvent signals allotted as internal standards. Chemical shifts were reported in ppm (δ). Elemental analyses were performed on a CHN-O-Rapid instrument.

4.2. Synthesis of *N*-(2-morpholinoethyl)nicotinamide and *N*-(3-morpholinopropyl)nicotinamide derivatives

A stirred solution of 2-morpholinoethanamine (0.130 g, 1.0 mmol) or 3-morpholinopropan-1-amine (0.144 g, 1.0 mmol) in CH_2Cl_2 (5 mL) was treated with equimolar quantities (1.0 mmol) of substituted nicotinic acids, using EDC·HCl (0.216 g, 1.2 mmol) as condensing agent. The mixture was refluxed for 8–10 h. Flash chro-

matography (MeOH/CH_3Cl, 1:10) afforded the corresponding compound as white powder.

4.2.1. *N*-(2-Morpholinoethyl)nicotinamide (1)

White powder, yield 84%, mp: 69–70 °C, ^{1}H NMR (500 MHz, $CDCl_3$): 2.52 (s, 4H); 2.63 (t, *J* = 6.0 Hz, 2H); 3.58 (dd, *J* = 5.5, 11.0 Hz, 2H); 3.73 (t, *J* = 4.4 Hz, 4H); 6.83 (s, 1H); 7.40 (dd, *J* = 4.9, 8.0 Hz, 1H); 8.13 (d, *J* = 8.0 Hz, 1H); 8.74 (d, *J* = 4.9 Hz, 1H); 8.98 (s, 1H). MS (ESI$^+$) *m/z* 236 (M+H)$^+$. Anal. Calcd for $C_{12}H_{17}N_3O_2$: C, 61.26; H, 7.28; N, 17.86. Found: C, 61.35; H, 7.24; N, 17.91.

4.2.2. 2-Chloro-*N*-(2-morpholinoethyl)nicotinamide (2)

White powder, yield 82%, mp: 86–87 °C, ^{1}H NMR (300 MHz, $CDCl_3$): 2.51 (s, 4H); 2.61 (t, *J* = 5.9 Hz, 2H); 3.55–3.61 (m, 2H); 3.71 (t, *J* = 4.4 Hz, 4H); 7.33–7.37 (m, 1H); 8.15 (d, *J* = 7.7 Hz, 1H); 8.46 (d, *J* = 2.8 Hz, 1H). MS (ESI$^+$) *m/z* 270 (M+H)$^+$. Anal. Calcd for $C_{12}H_{16}ClN_3O_2$: C, 53.43; H, 5.98; N, 15.58. Found: C, 53.32; H, 6.01; N, 15.54.

4.2.3. 2-Bromo-*N*-(2-morpholinoethyl)nicotinamide (3)

White powder, yield 88%, mp: 127–128 °C, ^{1}H NMR (300 MHz, $CDCl_3$): 2.51 (t, *J* = 4.5 Hz, 4H); 2.62 (t, *J* = 5.9 Hz, 2H); 3.57 (dd, *J* = 5.5, 11.2 Hz, 2H); 3.71 (t, *J* = 4.6 Hz, 4H); 6.95 (s, 1H); 7.34–7.38 (m, 1H); 7.96 (dd, *J* = 2.0, 7.7 Hz, 1H); 8.43 (dd, *J* = 2.0, 4.8 Hz, 1H). MS (ESI$^+$) *m/z* 314 (M+H)$^+$. Anal. Calcd for $C_{12}H_{16}BrN_3O_2$: C, 45.87; H, 5.13; N, 13.37. Found: C, 46.03; H, 5.16; N, 13.34.

4.2.4. 2-Hydroxy-*N*-(2-morpholinoethyl)nicotinamide (4)

White powder, yield 88%, mp: 186–187 °C, ^{1}H NMR (500 MHz, $CDCl_3$): 2.54 (t, *J* = 4.3 Hz, 4H); 2.62 (t, *J* = 6.4 Hz, 2H); 3.60 (dd, *J* = 6.1, 11.9 Hz, 2H); 3.73 (t, *J* = 4.7 Hz, 4H); 6.50 (t, *J* = 6.9 Hz, 1H); 7.49 (dd, *J* = 2.2, 6.1 Hz, 1H); 8.56 (dd, *J* = 2.2, 7.4 Hz, 1H); 9.71 (s, 1H); 12.44 (s, 1H). MS (ESI$^+$) *m/z* 252 (M+H)$^+$. Anal. Calcd for $C_{12}H_{17}N_3O_3$: C, 57.36; H, 6.82; N, 16.72. Found: C, 57.28; H, 6.84; N, 16.76.

4.2.5. 4-Chloro-*N*-(2-morpholinoethyl)nicotinamide (5)

White powder, yield 84%, mp: 92–93 °C, ^{1}H NMR (300 MHz, $CDCl_3$): 2.50 (t, *J* = 4.6 Hz, 4H); 2.61 (t, *J* = 5.9 Hz, 2H); 3.58 (dd, *J* = 5.5, 10.8 Hz, 2H); 3.70 (t, *J* = 4.6 Hz, 4H); 6.94 (s, 1H); 7.36 (d, *J* = 5.3 Hz, 1H); 8.54 (d, *J* = 5.5 Hz, 1H); 8.89 (s, 1H). MS (ESI$^+$) *m/z* 270 (M+H)$^+$. Anal. Calcd for $C_{12}H_{16}ClN_3O_2$: C, 53.43; H, 5.98; N, 15.58. Found: C, 53.61; H, 5.95; N, 15.54.

4.2.6. 5-Bromo-*N*-(2-morpholinoethyl)nicotinamide (6)

White powder, yield 86%, mp: 107–109 °C, ^{1}H NMR (300 MHz, $CDCl_3$): 2.51 (s, 4H); 2.61 (t, *J* = 5.9 Hz, 2H); 3.55 (dd, *J* = 5.5, 11.0 Hz, 2H); 3.73 (t, *J* = 4.5 Hz, 4H); 6.84 (s, 1H); 8.27 (s, 1H); 8.78 (s, 1H); 8.85 (s, 1H). MS (ESI$^+$) *m/z* 314 (M+H)$^+$. Anal. Calcd for $C_{12}H_{16}BrN_3O_2$: C, 45.87; H, 5.13; N, 13.37. Found: C, 45.98; H, 5.09; N, 13.40.

4.2.7. 6-Methyl-*N*-(2-morpholinoethyl)nicotinamide (7)

White powder, yield 85%, mp: 114–115 °C, ^{1}H NMR (500 MHz, $CDCl_3$): 2.51 (t, *J* = 4.6 Hz, 4H); 2.60–2.63 (m, 5H); 3.56 (dd, *J* = 5.8, 11.0 Hz, 2H); 3.72 (t, *J* = 4.6 Hz, 4H); 6.82 (s, 1H); 7.24 (d, *J* = 8.3 Hz, 1H); 8.02 (dd, *J* = 2.5, 8.3 Hz, 1H); 8.85 (d, *J* = 2.1 Hz, 1H). MS (ESI$^+$) *m/z* 250 (M+H)$^+$. Anal. Calcd for $C_{13}H_{19}N_3O_2$: C, 62.63; H, 7.68; N, 16.85. Found: C, 62.77; H, 7.71; N, 16.90.

4.2.8. 6-Fluoro-*N*-(2-morpholinoethyl)nicotinamide (8)

White powder, yield 88%, mp: 99–100 °C, ^{1}H NMR (300 MHz, $CDCl_3$): 2.52 (t, *J* = 4.6 Hz, 4H); 2.62 (t, *J* = 6.0 Hz, 2H); 3.56 (dd, *J* = 5.7, 11.5 Hz, 2H); 3.73 (t, *J* = 4.6 Hz, 4H); 6.80 (s, 1H); 7.02 (dd, *J* = 2.4, 8.4 Hz, 1H); 8.22–8.28 (m, 1H); 8.60 (d, *J* = 2.4 Hz, 1H). MS (ESI$^+$) *m/z* 254 (M+H)$^+$. Anal. Calcd for $C_{12}H_{16}FN_3O_2$: C, 56.91; H, 6.37; N, 16.59. Found: C, 67.05; H, 6.35; N, 16.63.

4.2.9. 6-Chloro-*N*-(2-morpholinoethyl)nicotinamide (9)

White powder, yield 92%, mp: 134–135 °C, ^{1}H NMR (300 MHz, $CDCl_3$): 2.51 (t, *J* = 4.6 Hz, 4H); 2.62 (t, *J* = 5.9 Hz, 2H); 3.55 (dd, *J* = 5.6, 11.3 Hz, 2H); 3.72 (t, *J* = 4.6 Hz, 4H); 6.85 (s, 1H); 7.42 (d, *J* = 8.2 Hz, 1H); 8.08 (dd, *J* = 2.4, 8.2 Hz, 1H); 8.74 (d, *J* = 2.4 Hz, 1H). MS (ESI$^+$) *m/z* 270 (M+H)$^+$. Anal. Calcd for $C_{12}H_{16}ClN_3O_2$: C, 53.43; H, 5.98; N, 15.58. Found: C, 53.56; H, 6.02; N, 15.55.

4.2.10. 6-Hydroxy-*N*-(2-morpholinoethyl)nicotinamide (10)

White powder, yield 92%, mp: 89–90 °C, ^{1}H NMR (300 MHz, $CDCl_3$): 2.51–2.59 (m, 6H); 3.46 (dd, *J* = 6.2, 12.1 Hz, 2H); 3.70 (t, *J* = 4.6 Hz, 4H); 6.44 (d, *J* = 9.5 Hz, 1H); 7.58 (s, 1H); 7.85 (dd, *J* = 2.6, 9.7 Hz, 1H); 8.01 (d, *J* = 2.4 Hz, 1H); 11.87 (s, 1H). MS (ESI$^+$) *m/z* 252 (M+H)$^+$. Anal. Calcd for $C_{12}H_{17}N_3O_3$: C, 57.36; H, 6.82; N, 16.72. Found: C, 57.43; H, 6.85; N, 16.76.

4.2.11. 2-Chloro-6-methyl-*N*-(2-morpholinoethyl)nicotinamide (11)

White powder, yield 86%, mp: 99–101 °C, ^{1}H NMR (500 MHz, $CDCl_3$): 2.52 (d, *J* = 4.0 Hz, 4H); 2.58 (s, 3H); 2.61 (t, *J* = 6.0 Hz, 2H); 3.57 (dd, *J* = 5.5, 11 Hz, 2H); 3.71 (t, *J* = 4.6 Hz, 4H); 7.19 (d, *J* = 8.0 Hz, 1H); 7.29 (s, 1H); 8.08 (d, *J* = 7.7 Hz, 1H). MS (ESI$^+$) *m/z* 284 (M+H)$^+$. Anal. Calcd for $C_{13}H_{18}ClN_3O_2$: C, 55.03; H, 6.39; N, 14.81. Found: C, 55.24; H, 6.41; N, 14.85.

4.2.12. 5,6-Dichloro-*N*-(2-morpholinoethyl)nicotinamide (12)

White powder, yield 90%, mp: 150–152 °C, ^{1}H NMR (500 MHz, $CDCl_3$): 2.51 (t, *J* = 4.3 Hz, 4H); 2.62 (t, *J* = 6.0 Hz, 2H); 3.56 (dd, *J* = 5.6, 11.0 Hz, 2H); 3.73 (t, *J* = 4.5 Hz, 4H); 6.83 (s, 1H); 8.21 (d, *J* = 2.2 Hz, 1H); 8.61 (d, *J* = 1.8 Hz, 1H). MS (ESI$^+$) *m/z* 304 (M+H)$^+$. Anal. Calcd for $C_{12}H_{15}Cl_2N_3O_2$: C, 47.38; H, 4.97; N, 13.81. Found: C, 47.29; H, 4.99; N, 13.84.

4.2.13. 5-Chloro-6-hydroxy-*N*-(2-morpholinoethyl)nicotinamide (13)

White powder, yield 85%, mp: 225–226 °C, ^{1}H NMR (300 MHz, $CDCl_3$): 2.41–2.45 (m, 6H); 3.35–3.37 (m, 2H); 3.58 (t, *J* = 4.6 Hz, 4H); 7.99 (d, *J* = 2.4 Hz, 1H); 8.14 (d, *J* = 2.4 Hz, 1H); 8.27 (s, 1H);. MS (ESI$^+$) *m/z* 286 (M+H)$^+$. Anal. Calcd for $C_{12}H_{16}ClN_3O_3$: C, 50.44; H, 5.64; N, 14.71. Found: C, 50.58; H, 5.68; N, 14.73.

4.2.14. *N*-(3-Morpholinopropyl)nicotinamide (14)

White powder, yield 86%, mp: 77–78 °C, ^{1}H NMR (300 MHz, $CDCl_3$): 1.77–1.85 (m, 2H); 2.52 (t, *J* = 4.4 Hz, 4H); 2.58 (t, *J* = 5.9 Hz, 2H); 3.59 (dd, *J* = 6.0, 11.0 Hz, 2H); 3.70 (t, *J* = 4.6 Hz, 4H); 7.01 (dd, *J* = 2.8, 8.6 Hz, 1H); 8.22–8.31 (m, 2H); 8.63 (d, *J* = 2.4 Hz, 1H). MS (ESI$^+$) *m/z* 250 (M+H)$^+$. Anal. Calcd for $C_{13}H_{19}N_3O_2$: C, 62.63; H, 7.68; N, 16.85. Found: C, 62.73; H, 7.65; N, 16.90.

4.2.15. 2-Chloro-*N*-(3-morpholinopropyl)nicotinamide (15)

White powder, yield 80%, mp: 65–66 °C, ^{1}H NMR (300 MHz, $CDCl_3$): 1.77–1.86 (m, 2H); 2.43–2.64 (m, 6H); 3.56–3.62 (m, 2H); 3.71 (t, *J* = 4.6 Hz, 4H); 7.30–7.36 (m, 1H); 7.71–7.74 (m, 1H); 8.42 (d, *J* = 2.8 Hz, 1H); 9.47 (s, 1H). MS (ESI$^+$) *m/z* 284 (M+H)$^+$. Anal. Calcd for $C_{13}H_{18}ClN_3O_2$: C, 55.03; H, 6.39; N, 14.81. Found: C, 55.26; H, 6.44; N, 14.86.

4.2.16. 2-Bromo-*N*-(3-morpholinopropyl)nicotinamide (16)

White powder, yield 82%, mp: 84–86 °C, ^{1}H NMR (300 MHz, $CDCl_3$): 1.79–1.85 (m, 2H); 2.47 (t, *J* = 4.4 Hz, 4H); 2.56 (t,

J = 6.1 Hz, 2H); 3.56–3.62 (m, 2H); 3.74 (t, *J* = 4.6 Hz, 4H); 7.34–7.38 (m, 1H); 7.81–7.85 (m, 1H); 8.42–8.44 (m, 1H). MS (ESI^+) *m/z* 314 $(M+H)^+$. Anal. Calcd for $C_{13}H_{18}BrN_3O_2$: C, 47.57; H, 5.53; N, 12.80. Found: C, 47.72; H, 5.56; N, 12.75.

4.2.17. 2-Hydroxy-*N*-(3-morpholinopropyl)nicotinamide (17)

White powder, yield 84%, mp: 117–119 °C, ^1H NMR (500 MHz, $CDCl_3$): 1.80–1.86 (m, 2H); 2.44–2.47 (m, 6H); 3.49–3.54 (m, 2H); 3.72 (t, *J* = 4.6 Hz, 4H); 6.54 (t, *J* = 6.7 Hz, 1H); 7.52 (dd, *J* = 2.5, 6.3 Hz, 1H); 8.63 (dd, *J* = 2.2, 7.4 Hz, 1H); 9.67 (s, 1H); 12.21 (s, 1H). MS (ESI^+) *m/z* 266 $(M+H)^+$. Anal. Calcd for $C_{13}H_{19}N_3O_3$: C, 58.85; H, 7.22; N, 15.84. Found: C, 59.01; H, 7.25; N, 15.80.

4.2.18. 4-Chloro-*N*-(3-morpholinopropyl)nicotinamide (18)

White powder, yield 84%, mp: 50–51 °C, ^1H NMR (300 MHz, $CDCl_3$): 1.76–1.85 (m, 2H); 2.46 (t, *J* = 4.4 Hz, 4H); 2.54 (t, *J* = 6.0 Hz, 2H); 3.53–3.63 (m, 2H); 3.74 (t, *J* = 4.6 Hz, 4H); 7.37 (d, *J* = 5.3 Hz, 1H); 7.85 (s, 1H); 8.51 (d, *J* = 5.3 Hz, 1H); 8.77 (s, 1H). MS (ESI^+) *m/z* 284 $(M+H)^+$. Anal. Calcd for $C_{13}H_{18}ClN_3O_2$: C, 55.03; H, 6.39; N, 14.81. Found: C, 55.22; H, 6.42; N, 14.84.

4.2.19. 5-Bromo-*N*-(3-morpholinopropyl)nicotinamide (19)

White powder, yield 85%, mp: 84–86 °C, ^1H NMR (300 MHz, $CDCl_3$): 1.77–1.85 (m, 2H); 2.52 (s, 4H); 2.57–2.61 (m, 2H); 3.58 (dd, *J* = 5.9, 10.8 Hz, 2H); 3.72 (t, *J* = 4.7 Hz, 4H); 8.28 (t, *J* = 2.0 Hz, 1H); 8.45 (s, 1H); 8.78 (d, *J* = 2.2 Hz, 1H); 8.93 (d, *J* = 1.8 Hz, 1H). MS (ESI^+) *m/z* 328 $(M+H)^+$. Anal. Calcd for $C_{13}H_{18}BrN_3O_2$: C, 47.57; H, 5.53; N, 12.80. Found: C, 47.65; H, 5.57; N, 12.84.

4.2.20. 6-Methyl-*N*-(3-morpholinopropyl)nicotinamide (20)

White powder, yield 78%, mp: 87–89 °C, ^1H NMR (300 MHz, $CDCl_3$): 1.77–1.85 (m, 2H); 2.51 (s, 4H); 2.57 (t, J = 5.9 Hz, 2H); 2.61 (s, 3H); 3.58 (dd, *J* = 5.5, 11.1 Hz, 2H); 3.71 (t, *J* = 4.6 Hz, 4H); 7.24 (d, *J* = 8.0 Hz, 1H); 8.04 (dd, *J* = 2.2, 8.0 Hz, 1H); 8.11 (s, 1H); 8.88 (d, *J* = 1.7 Hz, 1H). MS (ESI^+) *m/z* 264 $(M+H)^+$. Anal. Calcd for $C_{14}H_{21}N_3O_2$: C, 63.85; H, 8.04; N, 15.96. Found: C, 64.02; H, 8.06; N, 16.01.

4.2.21. 6-Fluoro-*N*-(3-morpholinopropyl)nicotinamide (21)

White powder, yield 89%, mp: 77–78 °C, ^1H NMR (300 MHz, $CDCl_3$): 1.77–1.85 (m, 2H); 2.52 (t, *J* = 4.4 Hz, 4H); 2.58 (t, *J* = 5.9 Hz, 2H); 3.56 (dd, *J* = 6.0, 11.0 Hz, 2H); 3.70 (t, *J* = 4.7 Hz, 4H); 7.02 (dd, *J* = 2.7, 8.6 Hz, 1H); 8.22–8.31 (m, 2H); 8.63 (d, *J* = 2.4 Hz, 1H). MS (ESI^+) *m/z* 268 $(M+H)^+$. Anal. Calcd for $C_{13}H_{18}FN_3O_2$: C, 58.41; H, 6.79; N, 15.72. Found: C, 58.47; H, 6.82; N, 15.76.

4.2.22. 6-Chloro-*N*-(3-morpholinopropyl)nicotinamide (22)

White powder, yield 85%, mp: 73–74 °C, ^1H NMR (300 MHz, $CDCl_3$): 1.79–1.84 (m, 2H); 2.52 (s, 4H); 2.59 (t, *J* = 5.8 Hz, 2H); 3.59 (dd, *J* = 5.5, 11.3 Hz, 2H); 3.71 (t, *J* = 4.3 Hz, 4H); 7.42 (d, *J* = 8.3 Hz, 1H); 8.13 (d, *J* = 8.3 Hz, 1H); 8.33 (s, 1H); 8.78 (s, 1H). MS (ESI^+) *m/z* 284 $(M+H)^+$. Anal. Calcd for $C_{13}H_{18}ClN_3O_2$: C, 55.03; H, 6.39; N, 14.81. Found: C, 54.89; H, 6.41; N, 14.78.

4.2.23. 6-Hydroxy-*N*-(3-morpholinopropyl)nicotinamide (23)

White powder, yield 90%, mp: 78–80 °C, ^1H NMR (300 MHz, $CDCl_3$): 1.76–1.80 (m, 2H); 2.51–2.57 (m, 6H); 3.52 (dd, *J* = 5.9, 11.2 Hz, 2H); 3.72 (t, *J* = 4.6 Hz, 4H); 6.59 (d, *J* = 9.5 Hz, 1H); 7.79–7.83 (m, 2H); 8.07 (d, *J* = 2.4 Hz, 1H). MS (ESI^+) *m/z* 266 $(M+H)^+$. Anal. Calcd for $C_{13}H_{19}N_3O_3$: C, 58.85; H, 7.22; N, 15.84. Found: C, 58.74; H, 7.24; N, 15.86.

4.2.24. 2-Chloro-6-methyl-*N*-(3-morpholinopropyl)nicotinamide (24)

White powder, yield 81%, mp: 78–79 °C, ^1H NMR (500 MHz, $CDCl_3$): 1.76–1.84 (m, 2H); 2.45 (d, *J* = 4.2 Hz, 4H); 2.57 (s, 3H); 2.63 (t, *J* = 5.9 Hz, 2H); 3.54–3.62 (m, 2H); 3.74 (t, *J* = 4.6 Hz, 4H); 7.17 (d, *J* = 7.9 Hz, 1H); 7.74 (s, 1H); 7.92 (d, *J* = 7.7 Hz, 1H). MS (ESI^+) *m/z* 284 $(M+H)^+$. Anal. Calcd for $C_{14}H_{20}ClN_3O_2$: C, 56.47; H, 6.77; N, 14.11. Found: C, 56.33; H, 6.80; N, 14.07.

4.2.25. 5,6-Dichloro-*N*-(3-morpholinopropyl)nicotinamide (25)

White powder, yield 83%, mp: 72–73 °C, ^1H NMR (300 MHz, $CDCl_3$): 1.76–1.86 (m, 2H); 2.52–2.61 (m, 6H); 3.58 (dd, *J* = 5.7, 10.8 Hz, 2H); 3.72 (t, *J* = 4.7 Hz, 4H); 8.25 (d, *J* = 2.0 Hz, 1H); 8.52 (s, 1H); 8.69 (d, *J* = 2.2 Hz, 1H). MS (ESI^+) *m/z* 318 $(M+H)^+$. Anal. Calcd for $C_{13}H_{17}Cl_2N_3O_2$: C, 49.07; H, 5.38; N, 13.21. Found: C, 49.23; H, 5.37; N, 13.25.

4.2.26. 5-Chloro-6-hydroxy-*N*-(3-morpholinopropyl)nicotinamide (26)

White powder, yield 80%, mp: 197–199 °C, ^1H NMR (300 MHz, $CDCl_3$): 1.73–1.80 (m, 2H); 2.42–2.46 (m, 6H); 3.35–3.40 (m, 2H); 3.69 (t, *J* = 4.6 Hz, 4H); 8.00 (d, *J* = 2.2 Hz, 1H); 8.10 (d, *J* = 2.4 Hz, 1H); 8.18 (s, 1H);. MS (ESI^+) *m/z* 300 $(M+H)^+$. Anal. Calcd for $C_{13}H_{18}ClN_3O_3$: C, 52.09; H, 6.05; N, 14.02. Found: C, 52.26; H, 6.02; N, 14.06.

4.3. Measurement of MAO-A and MAO-B inhibitory activities

Rat brain mitochondria were isolated from Sprague-Dawley rats according to the method of Clark and Nicklas.[35] Protein concentration was determined according to the method of Bradford[36] in which bovine serum albumin was used as standard.

The activity of MAO-A and MAO-B was determined fluorometrically with kynuramine as a substrate by the method of Silvestri and co-workers [37] with modifications. In all assays the incubation mixtures contained: 0.1 mL of 0.1 M potassium phosphate buffer (pH 7.4), mitochondrial suspension (1 mg/mL) and solutions of drugs in DMSO, added to the reaction mixture with a final concentration ranging from 0.1 to 100 μM. The reaction mixture was preincubated at 37 °C for 10 min. Then the substrate, kynuramine, was added with the final concentration of 100 μM, and the reaction mixture was further incubated for 30 min. The inhibitory activities of both MAO-A and MAO-B separately were determined after incubation of the mitochondrial fractions for 30 min at 37 °C, in the presence of the specific inhibitor (selegiline 1 μM to estimate the MAO-A activity and clorgyline 1 μM to assay the MAO-B). The addition of perchloric acid ended the reaction. Then the samples were centrifuged at 10,000 g for 5 min, and the supernatant was added to 2.7 mL of 1 N NaOH. Fluorometric measurements were recorded at 380 nm emission with excitation at 315 nm, using a Perkin–Elmer Victor II spectrofluorometer.

Control experiments were performed without inhibitor, and blanks were run without mitochondrial suspension. In all cases, volume adjustments were made with 0.1 M potassium phosphate buffer. Data are reported as means of three experiments performed in duplicate. The IC_{50} values were determined from plots of inhibition percentage, calculated in relation to a sample of the enzyme treated under the same conditions without inhibitors, versus the logarithm of the inhibitor concentration.

4.4. Molecular docking

The crystal structures of rat MAO-A (PDB code 1O5W)[34] and human MAO-B (PDB code 1GOS)[30] were obtained from the Protein Data Bank (http://www.rcsb.org).

Studies were carried out on only one subunit of the enzymes. The graphical user interface AUTODOCKTOOLS (ADT) was employed to setup the enzymes: all hydrogens were added, Gasteiger charges were calculated and nonpolar hydrogens were merged to carbon atoms. For macromolecules, generated pdbqt files were saved.

The 3D structures of ligand molecules were built, optimized (PM3) level, and saved in mol2 format with the aid of the molecular modeling program Spartan (Wavefunction Inc.). These partial charges of Mol2 files were further modified by using the ADT package (version 1.4.6) so that the charges of the nonpolar hydrogens atoms assigned to the atom to which the hydrogen is attached. The resulting files were saved as pdbqt files.

AutoDock 4.0 was employed for all docking calculations.[38,39] The AUTODOCKTOOLS program was used to generate the docking input files. In all docking a grid box size of $48 \times 48 \times 48$ points in x, y, and z directions was built, the maps were centered on N5 atom of the flavin (FAD) in the catalytic site of the protein. A grid spacing of 0.375 Å (approximately one forth of the length of carbon–carbon covalent bond) and a distances-dependent function of the dielectric constant were used for the calculation of the energetic map. Ten runs were generated by using Lamarckian genetic algorithm searches. Default settings were used with an initial population of 50 randomly placed individuals, a maximum number of 2.5×10^6 energy evaluations, and a maximum number of 2.7×10^4 generations. A mutation rate of 0.02 and a crossover rate of 0.8 were chosen. Results differing by less than 0.5 Å in positional root-mean-square deviation (RMSD) were clustered together and the results of the most favorable free energy of binding were selected as the resultant complex structures.

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