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Synthesis and evaluation of small molecules bearing a benzyloxy substituent as novel and potent monoamine oxidase inhibitors†‡

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A new series of small molecules bearing a benzyloxy substituent have been designed, synthesized and evaluated for hMAO inhibitory activity *in vitro*. Most of the compounds were potent and selective MAO-B inhibitors, and were weak inhibitors of MAO-A. In particular, compounds **9e** (IC₅₀ = 0.35 μM) and **10e** (IC₅₀ = 0.19 μM) were the most potent MAO-B inhibitors, and exhibited the highest selectivity for MAO-B (**9e**, SI > 285.7-fold and **10e**, SI = 146.8-fold). In addition, the structure–activity relationships for MAO-B inhibition indicated that electron-withdrawing groups in the open small molecules were more suitable for MAO-B inhibition, and substitutions at the benzyloxy of the open small molecules, particularly with the halogen substituted benzyloxy, were more favorable for MAO-B inhibition. Molecular docking studies have been done to explain the potent MAO-B inhibition of the open small molecules. Furthermore, the representative compounds **9e** and **10e** showed low neurotoxicity in SH-SY5Y cells *in vitro*. So the small molecules bearing the benzyloxy substituent could be used to develop promising drug candidates for the therapy of neurodegenerative diseases.

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Introduction

Monoamine oxidase (EC 1.4.3.4; MAO), located in the peripheral tissues and the central nervous system (CNS), is a FAD-containing enzyme, which binds tightly to the outer mitochondrial membrane of glial, neuronal and other cells. MAOs could regulate and metabolize biogenic amines by oxidative deamination, such as serotonin, dopamine (DA) and epinephrine.¹

On the account of differences in inhibitor specificity, immunological properties, substrate preference, amino acid sequences and tissue distribution, mammals contain two distinctive MAO enzymes, namely, MAO-A and MAO-B.^{2–6} The MAO-A preferentially metabolizes epinephrine, norepinephrine, and serotonin, and is selectively inhibited by clorgyline.

Whereas the MAO-B is selectively inhibited by selegiline or rasagiline, and specifically deaminates β-phenethylamine.^{7,8} With an increasing number of studies into the X-ray crystal structures of the two MAOs, information about the pharmacophoric requirements and the selective interactions is useful to design potent and selective MAO inhibitors. The active sites of hMAOs are supposed to be the key structural differences because of the different shapes and volumes of the inhibitors/substrate binding pockets. The active site of hMAO-A is a single hydrophobic cavity, while hMAO-B has two distinct cavities: one is the so-called “substrate cavity”, which is connected to the flavin adenine dinucleotide cofactor (FAD) and the other smaller cavity called the “entrance cavity” is located towards the outside of the protein. These cavities of MAOs have narrow pockets, but in hMAO-B the Tyr 326 and Ile 199 residues act as a bottleneck and thus form a gate, which separates the region as two cavities.^{9,10}

Since the MAOs could terminate the actions of neurotransmitter amines in the CNS, they are regarded as attractive targets to treat psychiatric and neurological disorders. Selective MAO-A inhibitors are effective in the therapy of depression,¹¹ while selective MAO-B inhibitors have been applied alone or in combination to treat Alzheimer's and Parkinson's diseases.¹² Thus specific MAO inhibitors are developed for the treatment of neurological disorders.

Hetero-benzofused derivatives bearing a benzyloxy substituent, for novel MAO-B inhibitors, have been selected as

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promising scaffolds with potent inhibition.^{13–20} These MAO-B inhibitors are related to natural compounds, such as coumarins, indoles, chromones, and chromanone analogues (Fig. 1, compounds 1–4).^{13–16} Interestingly, we find that some smaller molecules also exhibit potent MAO-B inhibitory activity, when the heterocycle of hetero-benzofused derivatives is opened (Fig. 1, compounds 5–8).^{17–20} Because these MAO-B inhibitors with benzyloxy substituents are substantially similar, we suppose that the simplified substituents on the benzene ring (A) may also have potential MAO-B inhibitory properties, and the different small substituents may affect the MAO-B inhibitory activity. To obtain smaller MAO-B inhibitors with a novel structure, we modify the molecular structure by introducing different substituents (Br, CHO, and OCH₃) at the *meta*-position of the benzene ring (A) (Fig. 2). To further examine the structure–activity relationships (SAR) against MAO, different substitutions (F, Br and CH₃) were introduced to the benzyloxy ring, and the effects on MAO inhibition were examined.

Results and discussion

Chemistry

The target compounds (9a–g, 10a–g and 11a–g) were efficiently synthesized as shown in Scheme 1. The commercially available 3-bromophenol (9), 3-hydroxybenzaldehyde (10) and 3-methoxyphenol (11) were reacted with the appropriate benzyl bromides in the presence of K₂CO₃ to give the target compounds in good yields (85–97%).

Inhibition of hMAO activity

For compounds 9a–g, 10a–g and 11a–g, the MAO inhibitory activities were tested with iproniazid as a reference.²¹ The corresponding IC₅₀ values with MAO and the selectivity ratios (IC₅₀ of MAO-A/IC₅₀ of MAO-B) are shown in Table 1. It could be seen that most of the tested compounds (except compounds 11a–g) were selective MAO-B inhibitors with IC₅₀

Fig. 2 Design strategy for the novel series of heterocycle-opened derivatives as MAO-B inhibitors.

values in the low micromolar range. Among the synthesized compounds, compound 9e (IC₅₀ = 0.35 μM, SI > 285.7) and compound 10d (IC₅₀ = 0.19 μM, SI = 206.3) were the most potent and selective inhibitors against MAO-B, being about 23.5-fold more and 43.4-fold more active than iproniazid, respectively.

Initially, by introducing benzyloxy groups to compounds 9–11, compounds 9a–11a were synthesized. As shown in Table 1, compound 11a, with an OCH₃ substitution at the *meta*-position of the benzene ring (A), exhibited an IC₅₀ value of 15.8 μM for MAO-B which was less active than the Br-substituted compound 9a (IC₅₀ = 2.73 μM) and the CHO-substituted compound 10a (IC₅₀ = 1.96 μM). From this result, it might be concluded that the electron-withdrawing groups at the *meta*-position of the benzene ring (A) were more suitable for MAO-B inhibition.

Then, we thought of introducing substituents with varying positions and electronic properties with respect to benzyloxy substitution to study the possible effects on MAO-B inhibition potency. It was noteworthy that those substituted at the *para*-position (compounds 9c, 9e and 9g) were more potent than those substituted at the corresponding *meta*-position (compounds 9b, 9d and 9f). Compared to compound 9a,

Fig. 1 Structures of known MAO inhibitors bearing a benzyloxy substituent.

Scheme 1 Syntheses of target compounds **9a-g**, **10a-g** and **11a-g**. Reagents and conditions: (a) K₂CO₃, CH₃CN, reflux, 12 h.

Table 1 hMAO inhibitory activities of the synthesized compounds

Compounds	R	MAO-A inhibition ^a (%)	MAO-B IC ₅₀ ^b (μM)	Selectivity index ^c
9a	—	23	2.73 ± 0.08	>36.6
9b	3-F	37	1.06 ± 0.18	>94.3
9c	4-F	31	0.58 ± 0.12	>172.4
9d	3-Br	27	0.76 ± 0.03	>131.6
9e	4-Br	30	0.35 ± 0.06	>285.7
9f	3-CH ₃	43	1.98 ± 0.09	>50.5
9g	4-CH ₃	24	1.53 ± 0.06	>65.4
10a	—	45.6 ± 0.87 μM ^b	1.96 ± 0.11	23.3
10b	3-F	64.2 ± 0.59 μM	1.21 ± 0.08	53.1
10c	4-F	42.5 ± 1.06 μM	0.93 ± 0.05	45.7
10d	3-Br	28.2 ± 0.85 μM	0.53 ± 0.04	53.2
10e	4-Br	27.9 ± 0.49 μM	0.19 ± 0.07	146.8
10f	3-CH ₃	51.6 ± 0.53 μM	2.91 ± 0.08	17.7
10g	4-CH ₃	64.8 ± 0.73 μM	2.53 ± 0.16	25.6
11a	—	25	15.8 ± 0.23	>6.3
11b	3-F	19	12.3 ± 0.79	>8.1
11c	4-F	22	15.6 ± 0.28	>6.4
11d	3-Br	22	19.3 ± 0.51	>5.2
11e	4-Br	25	8.63 ± 0.24	>11.6
11f	3-CH ₃	23	10.9 ± 0.17	>9.2
11g	4-CH ₃	26	14.6 ± 0.35	>6.8
Ipreniazid	—	6.78 ± 0.35 μM	8.24 ± 0.18	0.82

^a Test concentration is 100 μM. ^b IC₅₀: 50% inhibitory concentration (means ± SEM of three experiments). ^c Selectivity index = IC₅₀ (MAO-A)/IC₅₀ (MAO-B).

compounds **9b-e** bearing electron-withdrawing groups exhibited large enhancement in MAO-B inhibition. However, compounds **9f** and **9g** with an electron-donating group showed a slight increase in MAO-B inhibition. For example, compound **9e** (IC₅₀ = 0.35 μM for MAO-B) possessing the electron-withdrawing substituent Br was about 8-fold more active than compound **9a**, while compound **9f** (IC₅₀ = 1.98 μM for MAO-B) substituted with CH₃, increased the MAO-B inhibition potency of compound **9a** by 1.4-fold. Furthermore, among compounds **9b-g**, compounds with *para*-substitution of the benzyloxy phenyl ring were more potent for MAO-B inhibition than those with *meta*-substitution.

Moreover, by introducing different substituents to benzyloxy of compound **10a** to study the possible effects on MAO

inhibition, compounds **10b-g** were synthesized. Interestingly, those substituted at the *para*-position were more potent than those substituted at the corresponding *meta*-position, which was consistent with compounds **9b-g**. Additionally, compared to compound **10a**, compounds **10f** and **10g** with an electron-donating group showed a slight decrease in MAO-B inhibition.

Overall, these results demonstrated that substitution with a wide variety of benzyloxy side chains of small molecules led to structures with potent MAO-B inhibition. Meanwhile, MAO-A inhibition was very weak and no apparent SARs existed.

Reversibility of hMAO-B inhibition

As we know, MAO-B inhibitors could be classified as irreversible and reversible. To examine whether the small molecules bearing a benzyloxy substituent were reversible or irreversible MAO-B inhibitors, the time dependencies of inhibition were evaluated.¹⁴ Compounds **9e** and **10e** were selected as representative inhibitors since they displayed the most potent MAO-B inhibitory activity. For a reversible inhibitor, the MAO-B activity would be almost the same, when the enzyme was preincubated with a reversible inhibitor over different time periods. In contrast, for an irreversible inhibitor, the MAO-B activity would show a time-dependent reduction. Compounds **9e** and **10e** were preincubated with MAO-B over different time periods (0–60 min) at a concentration of two-fold the IC₅₀. As shown in Fig. 3, we could observe that MAO-B activities were almost the same (compound **9e**: 44.8% at 0 min, 44.9% at 15 min, 48.6% at 30 min and 49.2% at 60 min; compound **10e**: 46.4% at 0 min, 45.8% at 15 min, 49.9% at 30 min and 49.5% at 60 min), and the results demonstrated that compounds **9e** and **10e** were not time-dependent inhibitors of MAO-B. So these experiments clearly indicated that the small molecules bearing a benzyloxy substituent were reversible MAO-B inhibitors.

Kinetic study of hMAO-B inhibition

Compound **9e** was also used to further investigate the mode of MAO-B inhibition. The type of MAO-B inhibition was

Fig. 3 The time-dependent inhibition of hMAO-B by compounds **9e** and **10e**. The compounds were preincubated for various periods of time (0–60 min) with hMAO-B at concentrations equal to twofold the IC_{50} values for the inhibition of the enzyme. After dilution to concentrations equal to the IC_{50} , the inhibitory rates were recorded.

determined by Michaelis–Menten kinetic experiments.²² The catalytic rates were measured at five different *p*-tyramine concentrations (50–500 μ M), and each plot was constructed at four different concentrations of compound **10e** (0, 0.095, 0.19 and 0.38 μ M). The overlaid reciprocal Lineweaver–Burk plots (Fig. 4) showed that the plots for different concentrations of compound **9e** were linear and intersected at the *y*-axis. This pattern indicated that compound **9e** was a competitive MAO-B inhibitor, and these results further proved that the small molecules bearing a benzyloxy substituent were reversible MAO-B inhibitors.

Molecular modeling studies

In order to explain the difference in MAO-B inhibition, we have carried out a structure-based molecular modeling study using hMAO cocrystals deposited with the PDB. Crystallographic structures of MAO-B (PDB code 2V61) were used to

Fig. 4 Kinetic study on the mechanism of hMAO-B inhibition by compound **10e**. Overlaid Lineweaver–Burk reciprocal plots of the MAO-B initial velocity at an increasing substrate concentration (50–500 μ M) in the absence of the inhibitor and in the presence of **10e** are shown. Lines were derived from a weighted least-squares analysis of the data points.

dock the derivatives under study.¹⁷ And a molecular docking study was performed using the software package MOE 2008.10.²³ According to the inhibition results, compounds **9e**, **10e** and **11e** with different small substituents (Br, CHO, and OCH_3) at the *meta*-position of the benzene ring (A) were selected as typical ligands. The 3D and 2D pictures of binding are illustrated in Fig. 5. As shown in Fig. 5A and B, compound **9e** is located in the well-known binding pocket of MAO-B,²⁴ with the Br-substituted benzene ring (A) interacting with Tyr 398 *via* aromatic π – π stacking interactions at the bottom of the substrate cavity; the Br-substituted benzyloxy group occupied the entrance cavity *via* π – π interactions with Tyr 326. From Fig. 5C and D, compound **10e**, with the CHO-substituted benzene ring (A), not only had aromatic π – π stacking interactions with Tyr 398, but also had a hydrogen bond interaction with Tyr 188 at the bottom of the substrate cavity; the Br-substituted benzyloxy group occupied the entrance cavity which is a hydrophobic subpocket existing only in the MAO-B isoform and is composed of Leu 171, Ile 316, Tyr 326, Ile 199, Phe 99, Pro 104 and Phe 168. However, no interaction between the ligand **11e** with the OCH_3 -substituted benzene ring (A) and the MAO-B was observed in Fig. 5E and F. So these results might explain why the MAO-B inhibitory activities of compounds **9e** and **10e** were more potent than that of compound **11e**, and the reason could be ascribed to the different interactions between compounds and the MAO-B. Moreover, the modelling studies between compound **10e** and the standard compound 7-((3-chlorobenzyl)oxy)-4-((methylamino)methyl)-coumarin (C. Binda, *J. Med. Chem.*, 2007, **50**, 5848–5852) are compared in the ESI.†

Cell toxicity

Based on the screening results above, compounds **9e** and **10e** as the most potent inhibitors against MAO-B were selected to further examine their potential toxicity effect on SH-SY5Y cells.²⁵ After incubating the cells with compound **9e** or **10e** for 48 h, the cell viability was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assay. As shown in the Fig. 6, the results revealed that compounds **9e** and **10e** at 3–50 μ M did not have neurotoxicity. This suggested that compounds **9e** and **10e** might be used to develop promising drug candidates for the therapy of neurodegenerative diseases.

Prediction of BBB penetration of compounds **9a–g**, **10a–g** and **11a–g**

High pharmacological activity and low toxicological effects are not enough for a compound to become a drug candidate. Molecules should meet the limiting terms of Lipinski's rules: a molecular weight (MW) less than 500, the number of hydrogen bond acceptor atoms (HBA) should be less than 10, the number of hydrogen bond donor atoms (HBD) should be less than 5, the calculated logarithm of the octanol–water partition coefficient ($\log P$) should be less than 5, and a small

Fig. 5 (A) A 3D docking model of compound **9e** with hMAO-B. Atom colors: yellow – carbon atoms of compound **9e**, gray – carbon atoms of residues of hMAO-B, dark blue – nitrogen atoms, and red – oxygen atoms. The dashed lines represent the interactions between the protein and the ligand. (B) A 2D schematic diagram of a docking model of compound **9e** with hMAO-B. (C) A 3D docking model of compound **10e** with hMAO-B. (D) A 2D schematic diagram of a docking model of compound **10e** with hMAO-B. (E) A 3D docking model of compound **11e** with hMAO-B. (F) A 2D schematic diagram of a docking model of compound **11e** with hMAO-B. The figure was prepared using the ligand interactions application in MOE.

polar surface area less than $90 \text{ \AA}^2$. With the aim of developing CNS drugs, the ability of compounds to cross the blood-brain barrier (BBB) is very important.²⁶ $\log BB$ is calculated as: $\log BB = 0.0148 \times \text{PSA} + 0.152 \times \log P + 0.139$.²⁷ By calculating their $\log BB$ for their potential applications as CNS drugs and with their properties fitting the definition of the restrictive terms of Lipinski's rules, as shown in Table 2,

compounds **9a-g**, **10a-g** and **11a-g** satisfied possible brain penetration and drug-like standards.

Conclusions

In conclusion, we have synthesized a series of small molecules bearing a benzyloxy substituent and evaluated their

Fig. 6 Effects of compounds on cell viability in SH-SY5Y cells. The cell viability was determined by the MTT assay after 48 h of incubation with various concentrations of **9e** and **10e**. The results were expressed as a percentage of control cells. Values are reported as the mean $\pm$ SD of three independent experiments.

MAO inhibitory activity and toxicity *in vitro*. The results showed that most of the studied compounds were remarkably competitive and reversible inhibitors of MAO-B rather than of MAO-A. The SAR of the synthesized compounds showed that the electron-withdrawing groups at the *meta*-position of the benzene ring (A) were more suitable for MAO-B inhibition; halogen substituents on the benzyloxy ring further increased MAO-B inhibition. So different electron-withdrawing groups will be introduced at different positions of the benzene ring (A) and the MAO-B inhibition will be tested, which is an on-going further study. Moreover, molecular docking studies of **9e** and **10e** suggested that the potent MAO-B inhibition might be ascribed to the π - π stacking/

cation- π interactions and the larger set of residues interacting with MAO-B. Due to the possible BBB permeability and low neurotoxicity in SH-SY5Y cells *in vitro*, these compounds could be used to develop promising drug candidates for the therapy of neurodegenerative diseases.

Experimental section

Materials and methods

All chemicals (reagent grade) used were purchased from Sinopharm Chemical Reagent Co., Ltd. (China). The reaction progress was monitored using analytical thin layer chromatography (TLC) on precoated silica gel GF254 (Qingdao Haiyang Chemical Plant, Qing-Dao, China) plates and the spots were detected under UV light (254 nm). ^1H NMR and ^{13}C NMR spectra were measured on a BRUKER AVANCE III spectrometer at 25 °C and referenced to TMS. Chemical shifts are reported in ppm (δ) using the residual solvent line as an internal standard. Splitting patterns are designed as s, singlet; d, doublet; t, triplet; and m, multiplet. The purity of all compounds was confirmed to be higher than 95% through analytical HPLC performed with an Agilent 1200 HPLC System, with a photodiode array detector (DAD), 55% (v/v) of CH_3OH gradient, and a flow rate of 1.0 mL min^{-1} . Mass spectra were obtained on a MS Agilent 1100 Series LC/MSD Trap mass spectrometer (ESI-MS).

General procedure for the preparation of compounds **9a-g**, **10a-g** and **11a-g**

Compounds **9**, **10** or **11** (1.85 mmol) was suspended in acetonitrile (15 mL) containing K_2CO_3 (3.70 mmol). The reaction was treated with an appropriately substituted arylalkyl bromide (2.04 mmol) and heated under reflux for 12 h. The reaction progress was monitored using silica gel TLC with hexanes/EtOAc as the mobile phase. Upon completion, the acetonitrile was evaporated *in vacuo* and the mixture was then poured into water, which was extracted with 3×200 mL of EtOAc, washed with brine, dried over anhydrous Na_2SO_4 and purified by chromatography (hexanes/EtOAc) on silica gel.

1-(Benzyloxy)-3-bromobenzene (9a). Yield 89%; light yellow oil; ESI/MS m/z : 264.1 $[\text{M} + \text{H}]^+$; ^1H -NMR (400 MHz, CDCl_3): δ 7.45–7.38 (m, 4H), 7.37–7.31 (m, 1H), 7.18–7.07 (m, 3H), 6.95–6.87 (m, $J = 8.0, 2.4, 1.2$ Hz, 1H), 5.05 (s, 2H). ^{13}C -NMR (100 MHz, CDCl_3): δ 159.57, 136.42, 130.59, 128.68, 128.18, 127.51, 124.10, 122.84, 118.22, 113.86, 70.24.

1-Bromo-3-((3-fluorobenzyl)oxy)benzene (9b). Yield 92%; light yellow oil; ESI/MS m/z : 282.1 $[\text{M} + \text{H}]^+$; ^1H NMR (400 MHz, CDCl_3) δ 7.36–7.25 (m, 1H), 7.20–7.09 (m, 5H), 7.03–6.95 (m, 1H), 6.89–6.81 (m, 1H), 5.04 (s, 2H). ^{13}C -NMR (100 MHz, CDCl_3) δ 162.93 (d, $^1J_{\text{CF}} = 287.43$ Hz), 159.24, 139.01 (d, $^3J_{\text{CF}} = 8.27$ Hz), 130.64, 130.22 (d, $^3J_{\text{CF}} = 8.48$ Hz), 124.35, 122.88, 122.71, 118.21, 115.01 (d, $^2J_{\text{CF}} = 21.68$ Hz), 114.23 (d, $^2J_{\text{CF}} = 22.25$ Hz), 113.80, 69.37.

1-Bromo-3-((4-fluorobenzyl)oxy)benzene (9c). Yield 93%; light yellow oil; ESI/MS m/z : 282.1 $[\text{M} + \text{H}]^+$; ^1H NMR (400

Table 2 Physical properties of compounds **9a-g**, **10a-g** and **11a-g**

Compounds	MW ^a	clog P ^a	HBA ^a	HBD ^a	PAS ^a	log BB ^a
9a	261.99	4.832	1	0	9.23	1.010
9b	279.99	4.975	1	0	9.23	1.031
9c	279.99	4.975	1	0	9.23	1.031
9d	339.91	5.695	1	0	9.23	1.141
9e	339.91	5.695	1	0	9.23	1.141
9f	276.02	5.311	1	0	9.23	0.953
9g	276.02	5.311	1	0	9.23	0.953
10a	212.08	3.546	2	0	26.30	1.067
10b	230.07	3.690	2	0	26.30	1.089
10c	230.07	3.690	2	0	26.30	1.089
10d	289.99	4.410	2	0	26.30	1.199
10e	289.99	4.410	2	0	26.30	1.199
10f	226.10	4.046	2	0	26.30	1.143
10g	226.10	4.046	2	0	26.30	1.143
11a	214.10	3.918	2	0	18.46	0.878
11b	232.09	4.061	2	0	18.46	1.029
11c	232.09	4.061	2	0	18.46	1.029
11d	292.01	4.781	2	0	18.46	1.139
11e	292.01	4.781	2	0	18.46	1.139
11f	228.12	4.417	2	0	18.46	1.084
11g	228.12	4.417	2	0	18.46	1.084
Rules	≤ 450	≤ 5.0	≤ 10	≤ 5	≤ 90	≥ -1.0

^a MW: molecular weight; clog P: calculated logarithm of the octanol-water partition coefficient; HBA: hydrogen-bond acceptor atoms; HBD: hydrogen-bond donor atoms; PSA: polar surface area; log BB = $0.0148 \times \text{PAS} + 0.152 \times \text{clog P} + 0.139$.

MHz, CDCl₃) δ 7.43–7.35 (m, 2H), 7.18–7.03 (m, 5H), 6.89–6.79 (m, 1H), 5.00 (s, 2H). ¹³C-NMR (100 MHz, CDCl₃) δ 162.62 (d, ¹J_{CF} = 245.43 Hz), 159.37, 132.18, 132.15, 130.62, 129.38 (d, ³J_{CF} = 8.36 Hz), 124.24, 122.87, 118.19, 115.59, 115.59 (d, ²J_{CF} = 21.59 Hz), 113.83, 69.56.

1-Bromo-3-((3-bromobenzyl)oxy)benzene (9d). Yield 89%; light yellow oil; ESI/MS *m/z*: 343.0 [M + H]⁺; ¹H NMR (400 MHz, CDCl₃) δ 7.58 (s, 1H), 7.47 (d, *J* = 7.9 Hz, 1H), 7.34 (d, *J* = 7.7 Hz, 1H), 7.29–7.23 (m, 1H), 7.18–7.08 (m, 3H), 6.89–6.80 (m, 1H), 5.01 (s, 2H). ¹³C-NMR (100 MHz, CDCl₃) δ 159.22, 138.73, 131.23, 130.66, 130.35, 130.22, 125.84, 124.39, 122.90, 122.76, 118.21, 113.76, 69.28.

1-Bromo-3-((4-bromobenzyl)oxy)benzene (9e). Yield 90%; light yellow oil; ESI/MS *m/z*: 343.1 [M + H]⁺; ¹H NMR (400 MHz, DMSO-d₆) δ 7.59 (d, *J* = 8.2 Hz, 2H), 7.40 (d, *J* = 8.2 Hz, 2H), 7.28–7.20 (m, 2H), 7.14 (d, *J* = 7.9 Hz, 1H), 7.02–6.95 (m, 1H), 5.11 (s, 2H). ¹³C NMR (101 MHz, DMSO-d₆) δ 159.58, 136.55, 131.86, 131.86, 131.69, 130.30, 130.30, 124.23, 122.57, 121.55, 118.16, 114.80, 69.16.

1-Bromo-3-((3-methylbenzyl)oxy)benzene (9f). Yield 87%; light yellow oil; ESI/MS *m/z*: 278.2 [M + H]⁺; ¹H NMR (400 MHz, CDCl₃) δ 7.29 (t, *J* = 7.5 Hz, 1H), 7.25–7.20 (m, 2H), 7.18–7.08 (m, 4H), 6.91–6.86 (m, 1H), 5.00 (d, *J* = 6.0 Hz, 2H), 2.38 (s, 3H). ¹³C-NMR (100 MHz, CDCl₃) δ 159.63, 138.39, 136.31, 130.57, 128.95, 128.57, 128.27, 124.63, 124.04, 122.83, 118.21, 113.83, 70.31, 21.43.

1-Bromo-3-((4-methylbenzyl)oxy)benzene (9g). Yield 86%; light yellow oil; ESI/MS *m/z*: 278.2 [M + H]⁺; ¹H NMR (400 MHz, CDCl₃) δ 7.31 (d, *J* = 8.0 Hz, 2H), 7.21 (d, *J* = 7.8 Hz, 2H), 7.17–7.05 (m, 3H), 6.90–6.83 (m, 1H), 5.00 (s, 2H), 2.37 (s, 3H). ¹³C-NMR (100 MHz, CDCl₃) δ 159.64, 138.00, 133.36, 130.55, 129.35, 129.35, 127.66, 127.66, 123.99, 122.81, 118.21, 113.87, 70.18, 21.22.

3-(Benzyl)oxybenzaldehyde (10a). Yield 88%; light yellow oil; ESI/MS *m/z*: 213.2 [M + H]⁺; ¹H NMR (400 MHz, CDCl₃) δ 9.98 (s, 1H), 7.50–7.32 (m, 8H), 7.26–6.17 (m, 1H), 5.13 (s, 2H). ¹³C-NMR (100 MHz, CDCl₃) δ 192.07, 159.35, 137.86, 136.33, 130.13, 128.70, 128.70, 128.22, 127.55, 127.55, 123.69, 122.21, 113.32, 70.26.

3-((3-Fluorobenzyl)oxy)benzaldehyde (10b). Yield 93%; light yellow oil; ESI/MS *m/z*: 231.2 [M + H]⁺; ¹H NMR (400 MHz, CDCl₃) δ 9.98 (s, 1H), 7.52–7.44 (m, 3H), 7.36–7.24 (m, 1H), 7.27–7.14 (m, 3H), 7.03 (td, *J* = 8.3, 2.4 Hz, 1H), 5.12 (s, 2H). ¹³C-NMR (100 MHz, CDCl₃) δ 191.97, 163.02 (d, ¹J_{CF} = 245.48 Hz), 159.05, 138.93 (d, ³J_{CF} = 7.83 Hz), 137.89, 130.30, 130.22, 123.99, 122.79, 122.17, 115.06 (d, ²J_{CF} = 21.32 Hz), 114.27 (d, ²J_{CF} = 22.14 Hz), 113.14, 69.38.

3-((4-Fluorobenzyl)oxy)benzaldehyde (10c). Yield 90%; light yellow oil; ESI/MS *m/z*: 231.2 [M + H]⁺; ¹H NMR (400 MHz, CDCl₃) δ 9.98 (s, 1H), 7.50–7.45 (m, 3H), 7.45–7.39 (m, 2H), 7.27–7.22 (m, 1H), 7.12–7.06 (m, 2H), 5.08 (s, 2H). ¹³C-NMR (100 MHz, CDCl₃) δ 192.02, 162.64 (d, ¹J_{CF} = 245.58 Hz), 159.17, 137.87, 132.08, 130.18, 129.43, 129.43 (d, ³J_{CF} = 8.27 Hz), 123.92, 122.22, 115.62, 115.62 (d, ²J_{CF} = 21.84 Hz), 113.11, 69.57.

3-((3-Bromobenzyl)oxy)benzaldehyde (10d). Yield 90%; light yellow oil; ESI/MS *m/z*: 292.1 [M + H]⁺; ¹H NMR (400

MHz, CDCl₃) δ 9.95 (s, 1H), 7.59 (s, 1H), 7.49–7.45 (m, 2H), 7.45–7.42 (m, 2H), 7.34 (d, *J* = 7.7 Hz, 1H), 7.25–7.20 (m, 2H), 5.06 (s, 2H). ¹³C-NMR (100 MHz, CDCl₃) δ 191.95, 159.03, 138.65, 137.90, 131.28, 130.39, 130.24, 130.24, 125.90, 124.01, 122.79, 122.14, 113.15, 69.29.

3-((4-Bromobenzyl)oxy)benzaldehyde (10e). Yield 91%; light yellow oil; ESI/MS *m/z*: 292.1 [M + H]⁺; ¹H NMR (400 MHz, DMSO-d₆) δ 9.98 (s, 1H), 7.61 (s, 1H), 7.50–7.47 (m, 2H), 7.45–7.41 (m, 2H), 7.35 (d, *J* = 7.7 Hz, 1H), 7.26–7.21 (m, 2H), 5.21 (s, 2H). ¹³C NMR (100 MHz, DMSO-d₆) δ 193.35, 159.09, 139.97, 138.15, 131.25, 131.17, 130.94, 130.72, 130.27, 127.09, 123.43, 122.15, 114.38, 68.95.

3-((3-Methylbenzyl)oxy)benzaldehyde (10f). Yield 89%; light yellow oil; ESI/MS *m/z*: 227.2 [M + H]⁺; ¹H NMR (400 MHz, CDCl₃) δ 9.98 (s, 1H), 7.51–7.45 (m, 3H), 7.30–7.22 (m, 4H), 7.16 (d, *J* = 7.3 Hz, 1H), 5.09 (s, 2H), 2.39 (s, 3H). ¹³C-NMR (100 MHz, CDCl₃) δ 192.10, 159.41, 138.43, 137.86, 136.23, 130.12, 129.00, 128.60, 128.32, 124.68, 123.62, 122.18, 113.36, 70.33, 21.43.

3-((4-Methylbenzyl)oxy)benzaldehyde (10g). Yield 91%; light yellow oil; ESI/MS *m/z*: 227.1 [M + H]⁺; ¹H NMR (400 MHz, CDCl₃) δ 9.97 (s, 1H), 7.51–7.43 (m, 3H), 7.34 (d, *J* = 8.0 Hz, 2H), 7.27–7.19 (m, 3H), 5.09 (s, 2H), 2.37 (s, 3H). ¹³C-NMR (100 MHz, CDCl₃) δ 192.10, 159.41, 138.05, 137.84, 133.29, 130.10, 130.10, 129.38, 129.38, 127.71, 123.57, 122.23, 113.37, 70.21, 21.22.

1-(Benzyl)oxy-3-methoxybenzene (11a). Yield 91%; light yellow oil; ESI/MS *m/z*: 215.2 [M + H]⁺; ¹H NMR (400 MHz, CDCl₃) δ 7.60 (s, 1H), 7.46 (d, *J* = 7.9 Hz, 1H), 7.35 (d, *J* = 7.7 Hz, 1H), 7.29–7.16 (m, 3H), 6.56–6.43 (m, 3H), 5.02 (s, 2H), 3.80 (s, 3H). ¹³C-NMR (100 MHz, CDCl₃) δ 160.91, 159.75, 139.36, 131.03, 130.38, 130.15, 130.00, 125.88, 122.70, 106.90, 106.87, 101.44, 69.13, 55.31.

1-Fluoro-3-((3-methoxyphenoxy)methyl)benzene (11b). Yield 93%; light yellow oil; ESI/MS *m/z*: 233.2 [M + H]⁺; ¹H NMR (400 MHz, CDCl₃) δ 7.60 (s, 1H), 7.46 (d, *J* = 7.9 Hz, 1H), 7.35 (d, *J* = 7.7 Hz, 1H), 7.29–7.16 (m, 2H), 6.56–6.41 (m, 3H), 5.02 (s, 2H), 3.80 (s, 3H). ¹³C-NMR (100 MHz, CDCl₃) δ 163.02 (d, ¹J_{CF} = 245.35 Hz), 160.91, 159.78, 139.65 (d, ³J_{CF} = 8.27 Hz), 130.13 (d, ³J_{CF} = 8.24 Hz), 129.99, 122.76, 114.80 (d, ²J_{CF} = 21.33 Hz), 114.26 (d, ²J_{CF} = 22.26 Hz), 106.93, 106.81, 101.44, 69.21, 55.30.

1-((4-Fluorobenzyl)oxy)-3-methoxybenzene (11c). Yield 91%; light yellow oil; ESI/MS *m/z*: 233.2 [M + H]⁺; ¹H NMR (400 MHz, CDCl₃) δ 7.45–7.36 (m, 2H), 7.23–7.16 (m, 1H), 7.12–7.02 (m, 2H), 6.60–6.50 (m, 3H), 5.01 (s, 2H), 3.79 (s, 3H). ¹³C-NMR (100 MHz, CDCl₃) δ 162.54 (d, ¹J_{CF} = 245.19 Hz), 160.89, 159.89, 132.73, 129.96, 129.38, 129.38 (d, ³J_{CF} = 8.95 Hz), 115.50, 115.50 (d, ³J_{CF} = 22.11 Hz), 106.94, 106.71, 101.43, 69.37, 55.29.

1-Bromo-3-((3-methoxyphenoxy)methyl)benzene (11d). Yield 90%; light yellow oil; ESI/MS *m/z*: 294.1 [M + H]⁺; ¹H NMR (400 MHz, CDCl₃) δ 7.45 (m, 2H), 7.42–7.37 (m, 2H), 7.20 (t, *J* = 8.1 Hz, 1H), 6.62–6.51 (m, 3H), 5.06 (s, 2H), 3.80 (s, 3H). ¹³C-NMR (100 MHz, CDCl₃) δ 160.88, 160.10, 137.01, 129.93, 128.62, 128.62, 128.00, 127.55, 127.55, 106.99, 106.64, 101.43, 70.08, 55.30.

1-((4-Bromobenzyl)oxy)-3-methoxybenzene (11e). Yield 94%; light yellow oil; ESI/MS m/z : 294.1 $[M + H]^+$; 1H NMR (400 MHz, $CDCl_3$) δ 7.58 (d, J = 8.3 Hz, 2H), 7.40 (d, J = 8.3 Hz, 2H), 7.18 (t, J = 8.1 Hz, 1H), 6.60–6.54 (m, 2H), 6.53 (dd, J = 8.2, 2.1 Hz, 1H), 5.07 (s, 2H), 3.72 (s, 3H). ^{13}C NMR (100 MHz, DMSO- d_6) δ 160.97, 159.83, 137.07, 131.81, 131.81, 130.46, 130.22, 130.22, 121.35, 107.46, 107.04, 101.60, 68.84, 55.56.

1-Methoxy-3-((3-methylbenzyl)oxy)benzene (11f). Yield 91%; light yellow oil; ESI/MS m/z : 229.2 $[M + H]^+$; 1H NMR (400 MHz, $CDCl_3$) δ 7.36–7.13 (m, 5H), 6.59–6.47 (m, 3H), 5.04 (s, 2H), 3.82 (s, 3H), 2.41 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.87, 160.16, 138.30, 136.89, 129.90, 128.77, 128.51, 128.31, 124.67, 106.95, 106.60, 101.40, 70.14, 55.29, 21.43.

1-Methoxy-3-((4-methylbenzyl)oxy)benzene (11g). Yield 91%; light yellow oil; ESI/MS m/z : 229.3 $[M + H]^+$; 1H NMR (400 MHz, $CDCl_3$) δ 7.36 (d, J = 8.0 Hz, 2H), 7.22 (t, J = 8.2 Hz, 3H), 6.67–6.52 (m, 3H), 5.04 (s, 2H), 3.82 (s, 3H), 2.40 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.85, 160.16, 137.76, 133.94, 129.88, 129.28, 129.28, 129.28, 129.28, 106.98, 106.55, 101.40, 69.99, 55.28, 21.21.

Abbreviations

5-HT	5-Hydroxy-tryptamine
BBB	Blood–brain barrier
CNS	Central nervous system
DA	Dopamine
FAD	Flavin adenine dinucleotide
MAO	Monoamine oxidase
MTT	Methyl thiazolyl tetrazolium
NE	Norepinephrine
SI	Selectivity index

Conflicts of interest

The authors declare no conflict of interest.

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