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Indole-Substituted Benzothiazoles and Benzoxazoles as Selective and Reversible MAO-B Inhibitors for Treatment of Parkinson's Disease

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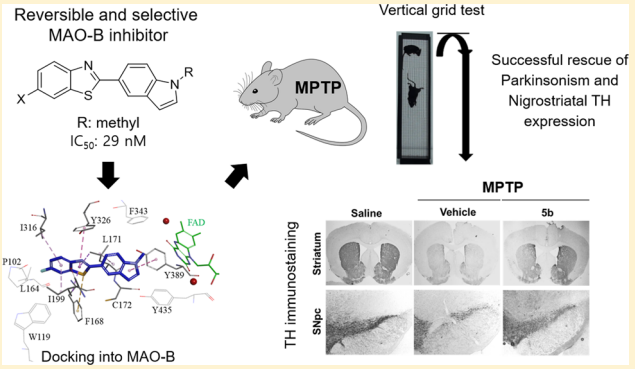
ABSTRACT: To develop novel, selective, and reversible MAO-B inhibitors for safer treatment of Parkinson's disease, benzothiazole and benzoxazole derivatives with indole moiety were designed and synthesized. Most of the synthesized compounds showed inhibitory activities against MAO-B and selectivity over MAO-A. The most active compound was compound **5b**, 6-fluoro-2-(1-methyl-1*H*-indol-5-yl)benzo[*d*]-thiazole with an IC_{50} value of 28 nM with no apparent effect on MAO-A activity at 10 μM. Based on the reversibility assay, compound **5b** turned out to be fully reversible with over 95% of recovery of enzyme activity after washout of the compound. Compound **5b** showed a reasonable stability in human liver microsomes and did not affect the activities of CYP isozymes, suggesting an absence of high-risk drug−drug interaction. In an in vivo MPTP-induced animal model of Parkinson's disease, oral administration of compound **5b** showed neuroprotection of nigrostriatal dopaminergic neurons as revealed by tyrosine hydroxylase staining and prevention of MPTP-induced parkinsonism as revealed by motor behavioral assay of vertical grid test. In summary, the novel, reversible, and selective MAO-B inhibitor compound **5b** was synthesized and characterized. We propose compound **5b** as an effective therapeutic compound for relieving parkinsonism.



KEYWORDS: *MAO-B, MAO-B inhibitor, Parkinson's disease, benzothiazole, benzoxazole, MPTP-induced animal model*

INTRODUCTION

The increase in average life expectancy has brought about the dramatic increase in prevalence of age-related neurodegenerative diseases such as Alzheimer's, Parkinson's, and Huntington's diseases, which has come to the fore as a social issue. Among these neurodegenerative diseases, Parkinson's disease is the second-most common one, which is characterized by distinct motor deficits including bradykinesia, rigidity, tremor at rest, gait dysfunction and postural instability.[1−3] These Parkinsonian motor symptoms are known to be caused by extensive loss of dopaminergic neurons in the substantia nigra resulting in nigrostriatal dopamine deficiency.[4−6]

Although no effective disease-modifying treatment has been discovered yet, various drugs for alleviating the symptoms of Parkinson's disease have been developed and prescribed in clinics.[7,8] These include anticholinergic drugs,[9] dopamine

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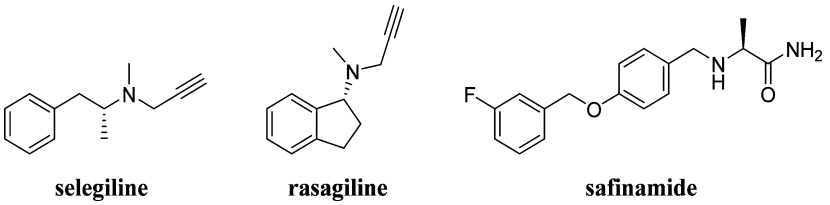
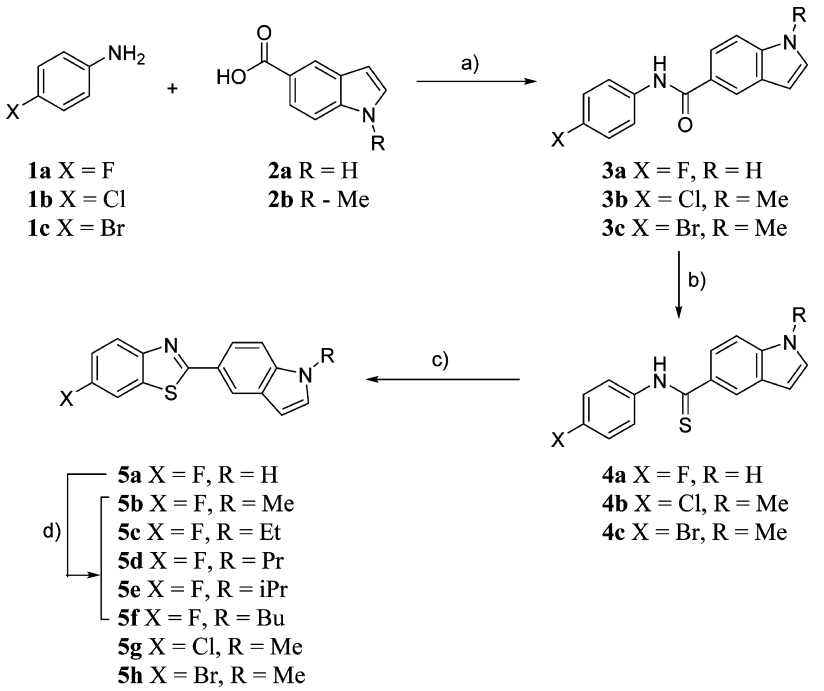


Figure 1. Structures of known MAO-B inhibitors.

Scheme 1. Synthesis of Indol-5-ylbenzothiazole Derivatives 5[a]



[a]Reagents and conditions: (a) HATU, DIPEA, DMF, rt; (b) Lawesson's reagent, toluene, 80 °C; (c) $PdCl_2$, CuI, TBAB, DMSO, NMP, 100 °C; (d) KH, RI, THF, rt.

receptor agonists,[10] dopamine precursors,[11,12] catechol-*O*-methyl transferase (COMT) inhibitors,[13] and monoamine oxidase B (MAO-B) inhibitors.[14,15] Among those drugs, MAO-B inhibitors are one of the most widely used ones for patients with Parkinson's disease due to their effectiveness for alleviating parkinsonism. The therapeutic effect of MAO-B inhibitors is believed to be caused by blocking dopamine degradation in nigrostriatal pathway, because MAO-B, a flavin adenine dinucleotide (FAD)-containing enzyme, catalyzes the oxidative deamination of biogenic and xenobiotic amines including dopamine. Additionally, MAO-B inhibitors have also demonstrated disease-modifying effects in preclinical models, even though these should be confirmed by human studies.[16]

Selegiline and rasagiline,[17−19] selective and irreversible MAO-B inhibitors (Figure 1), have been prescribed to patients with Parkinson's disease. Despite their broad applications, these inhibitors are known to cause undesirable adverse effects such as hallucination and headache.[20] Moreover, neurotoxic or ineffective metabolites are produced through biological actions of these inhibitors, which limit the long-term use of those inhibitors.[21,22] Recent studies demonstrated that short-lived action of selegiline and rasagiline could be attributed to the irreversibility of their action, while a selective and reversible MAO-B inhibitor, safinamide, keeps the effectiveness even in long-term treatment (Figure 1).[23] Safinamide was originally developed for treating epilepsy and is preregistered in United States as an add-on therapy to levodopa alone or in combination with other Parkinson's disease treatments in midto late stage patients.[24] However, due to some undesirable actions as a sodium and calcium channel blocker, safinamide can cause adverse effects.[25] Therefore, there are still pressing needs to develop novel selective and reversible MAO-B inhibitors for more effective and safer treatment of Parkinson's disease.[26]

We previously reported thiazolopyridines and oxazolopyridines as selective MAO-B inhibitors.[27] However, the compounds were not metabolically stable, confirmed by the stability in human liver microsomes.[27] In the current study, we focused on benzothiazoles and benzoxazoles as new selective and reversible MAO-B inhibitor compounds with metabolic stability. We synthesized novel benzothiazole and benzoxazole derivatives of which MAO-B activities and selectivity over MAO-A were investigated in this study. Among the candidate compounds of MAO-B inhibitors, we selected a compound showing the best potency of MAO-B inhibition and we further evaluated the metabolic stability, CYP inhibition effect and finally in vivo therapeutic effect for parkinsonism with MPTP-induced Parkinson's disease animal model.

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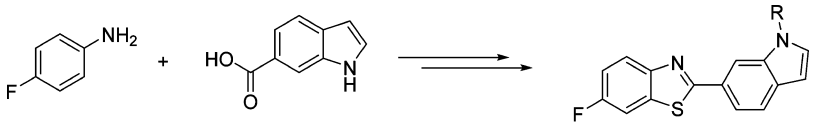
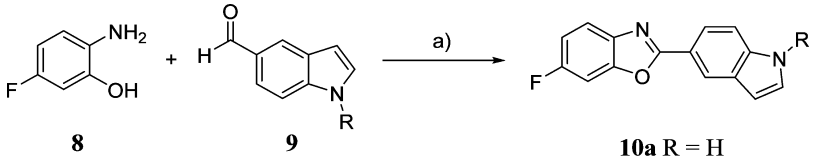
Scheme 2. Synthesis of Indol-6-ylbenzothiazole Derivatives 7



1 **6**

7a R = H
7b R = Me
7c R = Et
7d R = Pr
7e R = iPr
7f R = Bu

Scheme 3. Synthesis of Indol-5-ylbenzoxazole Derivatives 10[a]

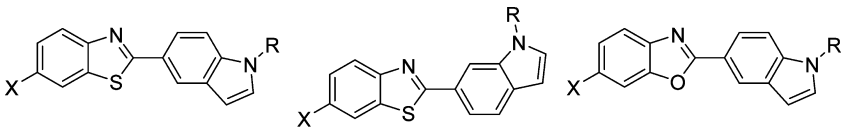


8 **9**

10a R = H
10b R = CH_3

[a]Reagents and conditions: (a) MeOH, rt; DDQ, DCM, rt.

Table 1. IC_{50} Values against hMAO-B of Synthesized Compounds



5 **7** **10**

compd	X	R	pIC_{50}	IC_{50} ± SEM (nM)[a]	selectivity index (SI)[b]
5a	F	H	7.15	72 ± 5	>139
5b	F	methyl	7.55	**28 ± 1**	>357
5c	F	ethyl	6.58	266 ± 14	>38
5d	F	propyl	6.34	461 ± 24	>22
5e	F	isopropyl	6.50	316 ± 8	>32
5f	F	butyl	5.60	3107 ± 1160	>3
5g	Cl	methyl	7.32	**49 ± 4**	>204
5h	Br	methyl	7.47	**34 ± 1**	>294
7a	F	H	6.36	437 ± 11	>23
7b	F	methyl	5.98	1070 ± 39	>9
7c	F	ethyl	5.91	1252 ± 41	>8
7d	F	propyl	5.23	6181 ± 887	>2
7e	F	isopropyl	5.82	1481 ± 213	>7
7f	F	butyl		40.6% inhibition at 10 μM	–[c]
10a	F	H	6.66	217 ± 11	>46
10b	F	methyl	7.31	**49 ± 4**	>204
	safinamide		7.29	**51 ± 1**	>196

[a]All of values were obtained by independent experiments (n = 3). [b]Selectivity index (SI) = IC_{50} (hMAO-A)/IC_{50} (hMAO-B). All of the IC_{50} values against hMAO-A were set as >10 000 nM due to low percent inhibitions (under 23.6% at 10 μM). [c]Not determined

RESULTS AND DISCUSSION

Chemistry. The benzothiazoles **5** with a 5-indole moiety were first designed and synthesized (Scheme 1). In the benzothiazole ring, halogens at the 6-position such as fluorine, chloride, and bromide were introduced, while on the nitrogen of the 5-indol moiety, hydrogen or different alkyl groups as R like methyl, ethyl, propyl, isopropyl, or butyl were substituted. The synthesis of the benzothiazoles **5** was started from 4-haloaniline **1**. 4-Haloaniline **1** with fluorine, chlorine or bromine underwent amide formation with indole-5-carboxylic acid **2a** or 1-methylindole-5-carboxylic acid **2b** by treating HATU (1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo-[4,5-*b*]pyridinium 3-oxid hexafluorophosphate) and DIPEA (diisoproylethylamine) to afford the amides **3a**, **3b**, and **3c** in 52–76% yields. With Lawesson's reagent, the amides **3** were converted to thioamides **4** in 37–58% yields. The benzothiazole ring was formed by C–H direct functionalization reaction with treatment of $PdCl_2$, CuI, and TBAB (tetrabutylammonium bromide) at 100 °C where the benzothiazoles **5a**, **5g**, and **5h** were obtained in 34–44% yields.[28] The benzothiazole **5a** underwent alkylation by treating KH and alkyl iodide to give

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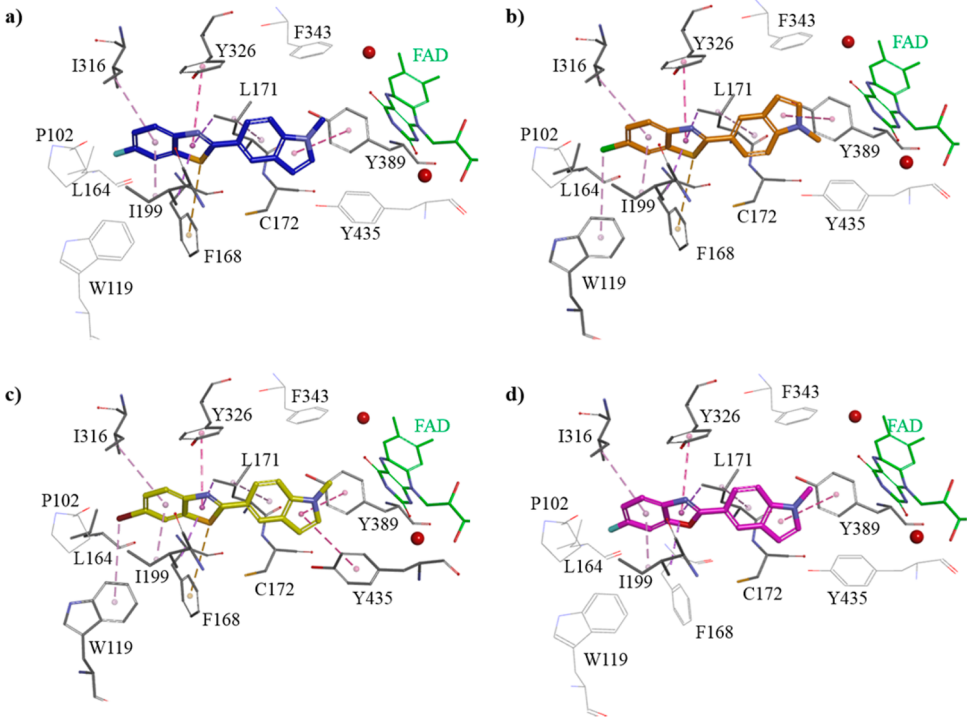

Figure 2. Molecular docking modes of (a) **5b** (blue), (b) **5g** (brown), (c) **5h** (yellow), (d) **10b** (pink) into the binding site of MAO-B. Red dots present the water molecules. FAD: flavin adenine dinucleotide.

alkylated products **5b**−**5f** with methyl, ethyl, propyl, isopropyl, and butyl groups as R in 52−92% yields.

To test the change of MAO-B enzyme activity according to substitution of indole moiety position, the benzothiazoles **7** with a 6-indole moiety were designed and synthesized from 4-fluoroaniline **1** and indole-6-carboxylic acid **6** in exactly the same method as the synthetic method of the compounds **5** with a 5-indole moiety (Scheme 2). We also designed and synthesized benzoxazoles **10a** and **10b**. 2-Amino-5-fluorophenol **8** and indole-5-aldehyde **9** underwent cycloaminal formation followed by oxidation with DDQ (2,3-dichloro-5,6-dicyano-1,4-benzoquinone) to afford benzoxazole products **10a** and **10b** in 15% and 22% yields, respectively (Scheme 3).[29]

MAO-B Inhibition. All the synthesized compounds were tested against human MAO-B (hMAO-B) which converts a hMAO-B substrate like benzylamine to an aldehyde to produce H_2O_2. Inhibitory effect of our test compounds was obtained by measuring the amount of H_2O_2 using the Amplex Red MAO Assay Kit (Thermo Fisher Scientific) after cotreating with benzylamine and each test compound.[30] The compounds were initially screened at 10 μM, and the IC_{50} values were estimated only for the compounds of which percent inhibitions at this concentration were higher than 50%. The inhibitory activities against hMAO-B are listed in Table 1.

The inhibitory activities of the compounds **5a**−**5f** with a 5-indole moiety and fluorine as X were shown, where the activity difference could be seen according to the size or length of the substituted alkyl groups (Rs). The compound **5a** with hydrogen as R showed a good inhibitory activity with an IC_{50} of 72 nM which is comparable with that of a reference compound, safinamide. The compound **5b** with methyl group as R was the best inhibitor among the tested compounds with an IC_{50} of 28 nM, of which the activity is slightly better than that of safinamide with an IC_{50} of 51 nM. In the case of the compounds **5c**−**5f** with bulkier alkyl groups, the order of activities is R = ethyl > isopropyl > propyl > butyl group. Based on these results, the length or size of the alkyl groups is very important in hMAO-B inhibitory activity. The IC_{50}'s of the compounds **5c**−**5f** were between 266 nM to 3,107 nM. Additionally different halide groups were investigated to see how they influence the inhibitory activity against hMAO-B. In the structure of the compounds **5**, chlorine and bromine as X were introduced to give the compounds **5g** and **5h** with methyl group as R and the inhibitory activities were compared with that of the compound **5b** with fluorine as X. The activities of the compounds **5g** and **5h** were similar to that of the compound **5b** with IC_{50}'s of 49 and 34 nM, respectively.

In terms of modification of the indole moiety, 5-indole moiety of the compounds were substituted by 6-indole moiety. The activities of the compounds **7a**−**7f** with 6-indole moiety are listed in Table 1. In this series, activity dependency on size or length was very clear where the compound **7a** with hydrogen as R was the most active with an IC_{50} of 437 nM. The activity order of the other compounds **7b**−**7f** was exactly same as that of the corresponding compounds **6c**−**6f** with 5-indole moiety. The IC_{50}'s of the compounds **7b**−**7e** are between 1070 and 6181 nM, and the compound **7f** with butyl moiety showed only 40.6% inhibition at 10 μM concentration.

The benzothiazole moiety was also modified to a benzoxazole. As benzoxazoles, two compounds **10a** and **10b** with a 5-indole moiety were tested against hMAO-B (Table 1). The compounds **10a** and **10b** showed good inhibitory activities against MAO-B with IC_{50}'s of 217 nM and 49 nM, respectively. Each benzoxazole compound was slightly less active than the corresponding benzothiazole (**6a** or **6b**).

For assessing the selectivity over hMAO-A of the compounds, all the compounds **5**, **7**, and **10** and clorgyline, a known MAO-A inhibitor, were tested against human MAO-A through the same method as hMAO-B assay method. No inhibitory effect against MAO-A was observed at 10 μM

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concentration of the tested compounds which showed less than 23.6% inhibitions, while clorgyline showed inhibitory activity with an IC_{50} of 2.4 nM. Therefore, except the compound **7f**, all other compounds were turned out to be selective MAO-B inhibitors over MAO-A with a selectivity index (SI) from 357-fold (**5b**) to 2-fold (**7d**) (Table 1).

Molecular Modeling. To elucidate the molecular mechanism and the binding affinities of the most active compounds **5b**, **5g**, **5h**, and **10b** against hMAO-B, we performed the molecular docking study by using the CDOCKER docking module in Discovery Studio with the cocrystal structure of hMAO-B with safinamide (PDB: 2V5Z).[31,32] The compound **5b** was smoothly docked to the active site of chain A of hMAO-B and showed $\pi-\pi$ interaction with TYR326 and TYR398, and π-alkyl interaction with LEU171, ILE199 and ILE316 (Figure 2a). Especially, sulfur in the benzothiazole of **5b** formed π-sulfur interaction with PHE168. The compounds **5g** and **5h** were docked to the active site of hMAO-B in similar docking pose with that of the compound **5b**, resulting in having exactly the same interactions such as $\pi-\pi$, π-alkyl and π-sulfur interactions. **5g** and **5h** additively have π-halogen (Cl or Br) interaction with TRP119 (Figure 2b and 2c). However, the compound **10b** with benzoxazole moiety could not form any interaction with PHE168 even though $\pi-\pi$ and π-alkyl interactions were formed as above (Figure 2d). Indole-5-carboxamide analogs structurally similar to our compounds were recently reported to show subnanomolar activities against MAO-B, which have hydrogen bonding interactions as well as hydrophobic interactions in the molecular modeling study.[33] Even though there is no hydrogen bonding interaction in the docking modes of **5b**, **5g**, **5h** and **10b**, the hydrophobic interactions such as $\pi-\pi$, $\pi-$alkyl, $\pi-$sulfur, and/or $\pi-$halogen interactions yield sufficient binding interactions, resulting in the MAO-B potency of the compounds **5b**, **5g**, **5h**, and **10b** which show a little bit better activities than safinamide.

Reversibility of MAO-B Inhibitors. Selegiline is well-known as an irreversible inhibitor of hMAO-B, while safinamide is a reversible hMAO-B inhibitor. When selegiline binds to the active site of hMAO-B enzyme, a covalent bond is formed between the *N*-propargyl moiety of selegiline and FAD, resulting in irreversible inhibition of selegiline to hMAO-B.[34] If the propargyl group or any other reactive groups are absent, the inhibiting action could be reversible. To examine the reversibility of hMAO-B inhibition by the compound **5b**, which is the most potent candidate compound with the lowest IC_{50} of hMAO-B, %-inhibition of the compound **5b** at 1 μM against hMAO-B was compared with %-inhibition after three times washout of the compound **5b** bound to hMAO-B.[35] The compound **5b** was tested for the inhibitory activity at 1 μM, and showed 83%-inhibition against hMAO-B (Table 2). An aliquot of the enzyme solution was washed three times using an effective centrifugation-ultrafiltration method, and then the enzyme was reused for the %-inhibition test of the compound **5b**, resulting in full recovery of percent inhibition of the compound **5b**. By these serial assays, it was confirmed that the compound **5b** is a reversible inhibitor of hMAO-B. As expected, selegiline showed no recovery of hMAO-B activity in the same assay due to the irreversibility.

Table 2. Reversibility and Irreversibility of MAO-B Inhibitors

inhibitor	% inhibition before washout	% inhibition after washout	reversibility
5b	83%	97%	reversible
selegiline	>90%	0%	irreversible

Metabolic Stability and CYP Inhibition of 5b. To test the metabolic stability of the compound **5b**, human liver microsomal stability assay was carried out.[36] The microsomal stability was shown in %-remaining concentration 30 min after incubation with human liver microsomes and 50.8% of the compound **5b** was recovered without change after incubation (Table 3). In order to avoid drug−drug interactions, inhibitory activities of the compound **5b** against CYP isozymes such as CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 were also examined.[37] Except for the percent of remaining activity against CYP3A4, the percent of remaining activities of the compound **5b** against CYP isozymes were not much lower (>40%), implying that the compound **5b** does not have high risk of drug−drug interaction. Because CYP3A4 is one of major CYP isozymes, the compound **5b** should be optimized in due course.

Table 3. Microsomal Stability and CYP Isozyme Activities of the Compound 5b

		5b
human liver microsomal stability of % remaining amount 30 min after incubation		49.4%
% remaining activity at 10 μM	CYP1A2	53.2%
	CYP2C9	89.2%
	CYP2C19	44.9%
	CYP2D6	87.7%
	CYP3A4	15.7%

Therapeutic Effect on Parkinsonian Motor Symptom in the MPTP-Induced Model. To validate the efficacy of the compound **5b**, a novel MAO-B inhibitor, on Parkinson's disease, we applied an MPTP-induced animal model which is one of the widely used animal models for Parkinson's disease.[38] MPTP, 1-methyl-4-phenyl-1,2,3,5-tetrahydropyridine, is converted to neurotoxin MPP^+ (1-methyl-4-phenylpyridinium) by MAO-B of glial cells. MPP+ causes extensive death of dopaminergic neurons in substantia nigra pars compacta (SNpc) of the brain, resulting in permanent motor symptoms of Parkinson's disease. We orally administered the compound **5b** and safinamide, as a positive control, for 3 consecutive days including a day before and after MPTP administration (Figure 3a). First, to see if treatment of the compound **5b** can rescue MPTP-induced Parkinsonian motor symptoms, we performed vertical grid test, which allows a sensitive examination of MPTP-induced motor deficit in mice by assessing motor dysfunction by measuring total time, time to turn, time to climb down, and rate of missed steps as previously described (Figure 3b).[39] We found that oral administration of the compound **5b** significantly reduced the MPTP-induced total time, time to turn and rate of missed steps to the control level and to the level after safinamide treatment (Figure 3c−f). These results imply that compound **5b** alleviates the MPTP-induced Parkinsonian motor dysfunction, similarly as safinamide.

Tyrosine Hydroxylase (TH) Expression in the MPTP-Induced Model. Next, to confirm if the behavioral recovery by compound **5b** comes from the protection of dopaminergic neurons, we tested the expression of TH by performing immunohistochemistry. TH is a key enzyme for dopamine production in dopaminergic neurons of SNpc which project

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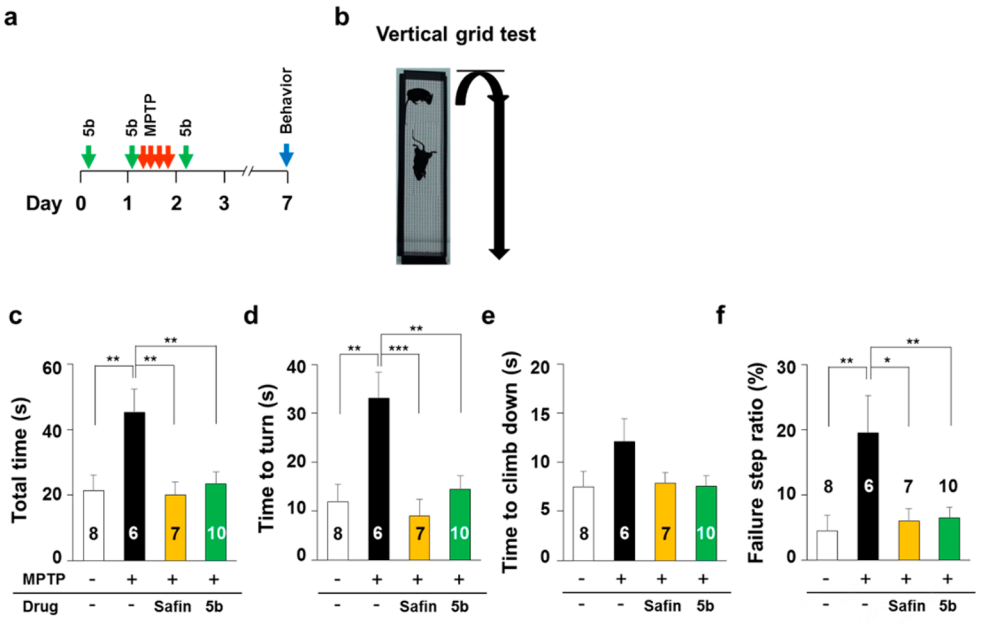

Figure 3. Vertical grid test of **5b** and safinamide as positive control: (a) Experimental timeline of MPTP and MAO-B inhibitor treatment, (b) schematic diagram of vertical grid test, (c) total time to perform the vertical grid test ($F = 5.403$, $p = 0.005$), (d) Time to turn at the top of the grid ($F = 7.272$, $p = 0.001$), (e) time to climb down ($F = 1.941$, $p = 0.147$), and (f) failure step ratio to total step number ($F = 5.407$, $p = 0.007$). Data are represented as mean ± SEM and analyzed with one-way ANOVA with Dunnett's post hoc multiple comparison test.

their fibers to the striatum. Therefore, TH is regarded as a marker for active dopaminergic neurons. We found that TH optical density of striatum was significantly reduced by MPTP administration, which was significantly rescued by treatment of the compound **5b** as well as by safinamide (Figure 4a and b). The number of TH-positive dopaminergic neurons in SNpc was also significantly decreased by MPTP, which was partially, but significantly rescued by compound **5b**. On the other hand, safinamide fully protected the dopaminergic neurons from impairment by MPTP (Figure 4a and c).

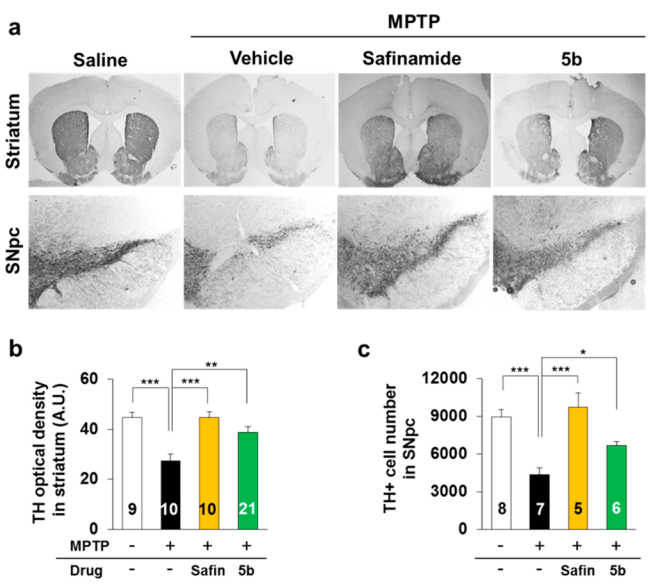

Figure 4. Tyrosine hydroxylase (TH) staining of MPTP-induced animal models after treatment of **5b** and safinamide: (a) Representative images of TH staining, (b) TH optical density in striatum ($F = 8.032$, $p < 0.001$), and (c) TH^+ cell number in SNpc ($F = 13.88$, $p < 0.001$). Data are represented as mean ± SEM and analyzed with one-way ANOVA with Dunnett's post hoc multiple comparison test.

MAO-B is known to convert MPTP to MPP+, which has toxic effect on dopaminergic neurons.[40] Pretreatment of MAO-B inhibitors is expected to block the formation of MPP+, so that dopaminergic neurons can be protected.[41] MAO-B is also believed to be engaged in dopamine degradation.[42] Inhibiting MAO-B might hinder dopamine degradation, therefore striatal dopamine level can be increased. Based on these two aspects, MAO-B inhibitors have been broadly used for patients with Parkinson's disease. Additionally, MAO-B has been recently highlighted as a key enzyme for GABA production in reactive astrocytes,[43] and it has been reported that inhibiting MAO-B activity reduces astrocytic reactivity in brain of Alzheimer's disease.[23] Therefore, MAO-B inhibitor might reduce glia-derived neuroinflammation in the brains of Parkinson's disease patients, which is harmful for neuronal survival. Taken together, the compound **5b**, a newly discovered MAO-B inhibitor, is effective in alleviating MPTP-induced Parkinsonism by restoring TH expression in nigrostriatal pathway.

CONCLUSION

The benzothiazoles and benzoxazoles with indole moiety were synthesized and their inhibition effects against hMAO-B and hMAO-A were biologically evaluated. Most of the compounds showed selectivity over MAO-A and the most active MAO-B inhibitor was **5b** which had an IC_{50} value of 28 nM. Through a reversibility assay, we found the compound **5b** is a reversible and selective MAO-B inhibitor. The compound **5b** was metabolically stable in human liver microsomes with a minimal inhibitory effect on the activities of CYP isozymes, implying a low risk of drug−drug interaction. The compound **5b** showed a robust in vivo therapeutic efficacy on Parkinsonian motor deficits in an MPTP-induced animal model. This behavioral

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rescue coincided with the recovery of TH expression in the SNpc and striatum. In summary, the novel, reversible, and selective MAO-B inhibitor compound **5b** was developed as an effective therapeutic agent for treating parkinsonism in an in vivo MPTP-induced animal model.

METHODS

Chemistry. *General Methods.* All reactions were carried out under dry nitrogen or argon unless otherwise indicated. Commercially available reagents were used without further purification. Solvents and gases were dried according to standard procedures. Organic solvents were evaporated with reduced pressure using a rotary evaporator. Analytical thin layer chromatography (TLC) was performed using glass plates precoated with silica gel (0.25 mm). TLC plates were visualized by exposure to UV light (UV), and then were visualized with a $KMnO_4$ stain followed by brief heating on hot plate. Flash column chromatography was performed using silica gel 60 (230−400 mesh, Merck) with the indicated solvents. A melting point of solid products was measured by using Capillary tubes for the determination of melting point (Marienfeld-Superior) and automated melting point system (OptiMelt, Stanford Research Systems, Inc.). The tubes containing solid compounds were inserted in OptiMelt, and the temperature was slowly raised from 100 to 400 °C. ^{1}H and ^{13}C spectra were recorded on a Bruker 300 or 400 spectrometer. ^{1}H NMR spectra are represented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, m = multiplet, dd = doublet of doublet, td = triplet of doublet, brs = broad singlet, brt = broad triplet), integration, and coupling constant (J) in Hertz (Hz). ^{1}H NMR chemical shifts are reported relative to $CDCl_3$ (7.26 ppm). ^{13}C NMR was recorded relative to the central line of $CDCl_3$ (77.0 ppm). HRMS analyses were performed on Bruker compact ESI$^+$ positive mode. HPLC purifications were performed on Alliance Waters 2489 UV/Visible detector, e2695 Separations module using Waters Xterra prep RP-18 10 μM, 10 × 250 mm column and in a 30−100% acetonitrile in water solvent gradient.

*N-(4-Fluorophenyl)-1H-indole-5-carboxamide (**3a**).* To a solution of 1*H*-indole-5-carboxylic acid (1.00 g, 6.21 mmol) in DMF was added HATU (3.54 g, 9.32 mmol) and DIPEA (1.6 mL, 9.2 mmol) at room temperature. The reaction mixture was stirred at room temperature for 2 h. The resulting mixture was treated with 4-fluoro aniline (0.90 mL, 9.4 mmol), and stirred at room temperature for 1 day. The reaction mixture was diluted with water and extracted with EtOAc. The organic layer was dried over $MgSO_4$ and evaporated. The residue was filtered on short silica gel with an eluent (hexane/EtOAc = 1:1). The filtrate was concentrated and the residue was washed with diethyl ether to give the desired product (1.02 g, 4.01 mmol) as a white solid in 65% yield: ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.42 (s, 1H), 10.20 (s, 1H), 8.28 (s, 1H), 8.64−7.83 (m, 2H), 7.75 (dd, J = 6.3, 1.2 Hz, 1H), 7.52 (s, 1H), 7.49−7.48 (m, 1H), 7.22−7.18 (m, 2H), 6.61−6.60 (m, 1H); ^{13}C NMR (100 MHz, DMSO) δ 167.0, 158.5 (J = 238 Hz), 138.1, 136.6 (J = 2 Hz), 127.4 (J = 7 Hz), 126.1, 122.5, 122.4, 121.1 (J = 49 Hz), 115.6, 115.4, 111.6, 102.7; LC/MS (ESI$^+$): m/z: calcd for $C_{15}H_{11}FN_2O$: 254.26, $[M + H]^+$; found: 255.15

*N-(4-Chlorophenyl)-1-methyl-1H-indole-5-carboxamide (**3b**).* 76% yield: ^{1}H NMR (400 MHz, acetone-d_6) δ 9.57 (brs, 1H), 8.28 (d, J = 1.2 Hz, 1H), 7.95−7.91(m, 2H), 7.85 (dd, J = 8.4, 1.6 Hz, 1H), 7.50 (d, J = 8.8 Hz, 1H), 7.39−7.35 (m, 3H), 6.57 (dd, J = 3.2, 0.8 Hz, 1H), 3.89 (s, 3H); HRMS (ESI$^+$): m/z: calcd for $C_{16}H_{13}ClN_2NaO$: 307.0609, $[M + Na]^+$; found: 307.0610.

*N-(4-Bromoophenyl)-1-methyl-1H-indole-5-carboxamide (**3c**).* 52% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (d, J = 1.6 Hz, 1H), 7.86 (brs, 1H), 7.76 (dd, J = 8.4, 1.6 Hz, 1H), 7.61−7.57 (m, 2H), 7.50−7.47 (m, 2H), 7.40 (d, J = 8.4 Hz, 1H), 7.15 (d, J = 2.8 Hz, 1H), 6.60 (dd, J = 3.2, 0.8 Hz, 1H), 3.85 (s, 3H); HRMS (ESI$^+$): m/z: calcd for $C_{16}H_{13}BrN_2NaO$: 351.0103, $[M + Na]^+$; found: 351.0102.

*N-(4-Fluorophenyl)-1H-indole-5-carbothioamide (**4a**).* To a solution of *N*-(4-fluorophenyl)-1*H*-indole-5-carboxamide (1.00 g, 3.93 mmol) in toluene was added Lawesson's reagent (1.03 g, 2.55 mmol) at room temperature. The mixture was stirred for 4 h at 80 °C. After cooling down to room temperature, the reaction mixture was purified by column chromatography on silica gel (hexane/EtOAc = 3:1) to give the desired product (0.57 g, 2.11 mmol) as a yellowish solid in 53% yield: ^{1}H NMR (400 MHz, DMSO) δ 11.57 (s, 1H), 11.40 (s, 1H), 8.17 (s, 1H), 7.95−7.78 (m, 3H), 7.53−7.39 (m, 2H), 7.28 (t, J = 6.5 Hz, 2H), 6.59 (s, 1H); ^{13}C NMR (100 MHz, DMSO) δ 199.4, 160.1 (J = 238 Hz), 138.0, 137.3 (J = 2 Hz), 134.0, 127.5, 127.2, 127.1 (J = 9 Hz), 122.4, 120.3, 115.5 (J = 23 Hz), 111.1, 103.0; LC/MS (ESI$^+$): m/z: calcd for $C_{15}H_{11}FN_2S$: 270.33, $[M + H]^+$; found: 271.10.

*N-(4-Chlorophenyl)-1-methyl-1H-indole-5-carbothioamide (**4b**).* 37% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.00 (brs, 1H), 8.15 (s, 1H), 7.80−7.63(m, 3H), 7.38−7.30 (m, 3H), 7.11 (d, J = 3.2 Hz, 1H), 6.55 (d, J = 2.8 Hz, 1H), 3.81 (s, 3H); HRMS (ESI$^+$): m/z: calcd for $C_{16}H_{13}ClN_2NaS$: 323.0380, $[M + Na]^+$; found: 323.0382.

*N-(4-Bromophenyl)-1-methyl-1H-indole-5-carbothioamide (**4c**).* 58% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.02 (brs, 1H), 8.16 (s, 1H), 7.81−7.51(m, 5H), 7.33 (d, J = 8.8 Hz, 1H), 7.13 (d, J = 2.8 Hz, 1H), 6.57 (d, J = 3.2 Hz, 1H), 3.83 (s, 3H); HRMS (ESI$^+$): m/z: calcd for $C_{16}H_{13}BrN_2NaS$: 366.9875, $[M + Na]^+$; found: 366.9856.

*6-Fluoro-2-(1H-indol-5-yl)benzo[d]thiazole (**5a**).* To a solution of N-(4-fluorophenyl)-1H-indole-5-carbothioamide (0.50 g, 1.84 mmol) in a mixture of DMSO and NMP (1:1) was added $PdCl_2$ (39 mg, 0.18 mmol), CuI (0.19 g, 0.99 mmol) and TBAB (1.19 g, 3.69 mmol) at room temperature. The reaction mixture was stirred for 2 h at 100 °C. After cooling down to room temperature, the reaction mixture was extracted with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography on slica gel (hexane/EtOAc/MC = 3:1:0.5) to give the desired product (0.22 g, 0.82 mmol) as a white solid in 44% yield: mp 174−179 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.49 (s, 1H), 8.31 (d, J = 0.9 Hz, 1H), 8.09−8.01 (m, 2H), 7.87 (dd, J = 6.3, 1.2 Hz, 1H), 7.57 (d, J = 6.3 Hz, 1H), 7.41 (d, J = 1.8 Hz 1H), 7.39 (td, J = 12.9, 4.4 Hz, 1H), 6.63 (d, J = 7.3 Hz, 1H); ^{13}C NMR (100 MHz, DMSO) δ 169.8 (J = 3 Hz), 159.9 (J = 240 Hz), 151.2, 138.1 135.9 (J = 12 Hz), 128.4, 127.8, 124.5, 123.8 (J = 10 Hz), 120.8, 120.4, 115.1 (J = 24 Hz), 112.7, 109.0 (J = 27 Hz) 102.8; LC/MS (ESI$^+$): m/z: calcd for $C_{15}H_9FN_2S$: 268.31, $[M + H]^+$; found: 269.05.

*6-Fluoro-2-(1-methyl-1H-indol-5-yl)benzo[d]thiazole (**5b**).* To a solution of *N*-(4-fluorophenyl)-1H-indole-5-carboxamide (0.10 g, 0.37 mmol) in THF was added dropwise KH (30 mg, 0.74 mmol) in THF at room temperature. The reaction mixture was treated with iodomethane (0.04 mL, 0.64 mmol) at room temperature, and stirred for 30 min. The resulting mixture was concentrated in vacuo. And diethyl ether was added to the residue and the mixture was filtered. The filtrate was concentrated in vacuo and triturated with hexane to give the desired product (83 mg, 0.29 mmol) as a white solid in 79% yield: mp 176−178 °C; ;^{1}H NMR (400 MHz, DMSO) δ 8.32−8.27 (m, 1H), 8.07−8.00 (m, 2H), 7.25 (dd, J = 8.4, 1.6 Hz, 1H), 7.62 (d, J = 3.2, 1H), 7.48 (d, J = 3.2 Hz, 1H), 7.39 (d, J = 3.2 Hz, 1H), 7.39 (td, J = 9.1, 2.7 Hz, 1H), 6.63 (dd, J = 3.2, 0.8 Hz, 1H) 3.86 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ 169.6 (J = 3 Hz), 160.0 (J = 240 Hz), 151.1, 138.5 135.9 (J = 12 Hz), 132.1, 128.7, 124.6, 123.9 (J = 10 Hz), 120.7 (2C), 115.2 (J = 25 Hz), 111.1, 109.0 (J = 27 Hz), 102.2; LC/MS (ESI$^+$): m/z: calcd for $C_{16}H_{11}FN2_S$: 282.34, $[M + H]^+$; found: 283.10

*6-Fluoro-2-(1-ethyl-1H-indol-5-yl)benzo[d]thiazole (**5c**).* Following the same procedure used for the synthesis of 6-fluoro-2-(1-methyl-1*H*-indol-5-yl)benzo[d]thiazole, the reaction of 6-fluoro-2-(1*H*-indol-5-yl)benzo[*d*]thiazole (70 mg, 0.26 mmol), KH (21 mg, 0.52 mmol), and iodoethane (0.03 mL, 0.37 mmol) gave the title compound (54 mg, 0.18 mmol, 70% yield) as a white solid: mp 150−153 °C; ^{1}H NMR (400 MHz, DMSO) δ 8.31 (d, J = 1.2 Hz, 1H), 8.00−8.09 (m, 2H), 7.90 (dd, J = 8.8, 1.6 Hz, 1H), 7.67 (d, J = 8.8 Hz, 1H), 7.55 (d, J = 3.2 Hz, 1H), 7.39 (td, J = 9.0, 2.7 Hz, 1H), 6.64 (d, J = 2.8, 1H), 4.28 (q, J = 7.3 Hz, 2H), 1.40 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, DMSO) δ 169.6 (J = 3 Hz), 160.0 (J = 240 Hz), 151.2, 137.5, 135.9 (J = 12 Hz), 130.5, 128.9, 124.5, 123.9 (J = 9 Hz), 120.8, 120.7, 115.2 (J = 25 Hz), 111.2, 109.0 (J = 27 Hz), 102.5, 41.0, 16.0; LC/MS (ESI$^+$): m/z: calcd for $C_{17}H_{13}FN_2S$: 296.36, $[M + H]^+$; found: 297.05.

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*6-Fluoro-2-(1-propyl-1H-indol-5-yl)benzo[d]thiazole (**5d**).* Following the same procedure used for the synthesis of 6-fluoro-2-(1-methyl-1*H*-indol-5-yl)benzo[*d*]thiazole, the reaction of 6-fluoro-2-(1*H*-indol-5-yl)benzo[*d*]thiazole (70 mg, 0.26 mmol), KH (21 mg, 0.52 mmol), iodopropane (0.04 mL, 0.42 mmol) gave the title compound (41 mg, 0.13 mmol, 50% yield) as a white solid: mp 133−135 °C; ^{1}H NMR (400 MHz, DMSO) δ 8.30 (d, J = 1.2 Hz, 1H), 8.07−7.99 (m, 2H), 7.89 (dd, J = 8.6, 1.8 Hz, 1H), 7.67 (d, J = 7.3 Hz, 1H), 7.53 (d, J = 3.2 Hz, 1H), 7.39 (td, J = 9.2, 2.8 Hz, 1H), 6.64 (d, J = 3.2, 1H), 4.21 (t, J = 7.0 Hz, 2H), 1.82 (q, J = 7.2 Hz, 2H), 0.86 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, DMSO) δ 169.6 (J = 3 Hz), 160.0 (J = 240 Hz), 151.2, 137.9, 135.9 (J = 12 Hz), 131.2, 128.8, 124.5, 123.9 (J = 9 Hz), 120.8, 120.6, 115.2 (J = 25 Hz), 111.2, 109.0 (J = 27 Hz), 102.3, 47.7, 23.7, 11.6; LC/MS (ESI$^+$): m/z: calcd for $C_{18}H_{15}FN_2S$: 310.39, [M + H]$^+$; found: 311.15

*6-Fluoro-2-(1-isopropyl-1H-indol-5-yl)benzo[d]thiazole (**5e**).* Following the same procedure used for the synthesis of 6-fluoro-2-(1-methyl-1*H*-indol-5-yl)benzo[d]thiazole, the reaction of 6-fluoro-2-(1*H*-indol-5-yl)benzo[*d*]thiazole (70 mg, 0.26 mmol), KH (21 mg, 0.52 mmol), and 2-iodopropane (0.04 mL, 0.41 mmol) gave the title compound (30 mg, 0.10 mmol, 37% yield) as a white solid: mp 124−125 °C ^{1}H NMR (400 MHz, DMSO) δ 8.31 (d, J = 1.6 Hz, 1H), 8.09−8.00 (m, 2H), 7.89 (dd, J = 9.8, 3.6 Hz, 1H), 7.70 (d, J = 8.4 Hz, 1H), 7.65 (d, J = 3.2 Hz, 1H), 7.39 (td, J = 9.1, 2.7 Hz, 1H), 6.67 (d, J = 3.2, 1H), 4.91−4.80 (m, 1H), 1.55−1.47 (m, 6H); ^{13}C NMR (100 MHz, DMSO) δ 169.6 (J = 3 Hz), 160.0 (J = 240 Hz), 151.2, 137.3, 135.9 (J = 12 Hz), 128.8, 127.2, 124.6, 123.9 (J = 9 Hz), 120.8, 120.6, 115.2 (J = 24 Hz), 111.2, 109.0 (J = 27 Hz), 120.8, 47.4, 23.0; LC/MS (ESI$^+$): m/z: calcd for $C_{18}H_{15}FN_2S$: 310.39, [M + H]$^+$; found: 311.15.

*6-Fluoro-2-(1-butyl-1H-indol-5-yl)benzo[d]thiazole (**5f**).* Following the same procedure used for the synthesis of 6-fluoro-2-(1-methyl-1*H*-indol-5-yl)benzo[d]thiazole, the reaction of 6-fluoro-2-(1*H*-indol-5-yl)benzo[*d*]thiazole (70 mg, 0.26 mmol), KH (21 mg, 0.52 mmol), and iodobutane (0.05 mL, 0.40 mmol) gave the title compound (27 mg, 0.09 mmol, 32% yield) as a white solid: mp 105−106 °C; ^{1}H NMR (400 MHz, DMSO) δ 8.30 (d, J = 1.2 Hz, 1H), 8.09−8.00 (m, 2H), 7.89 (dd, J = 8.4, 1.6 Hz, 1H), 7.67 (d, J = 8.4 Hz, 1H), 7.53 (d, J = 3.2 Hz, 1H), 7.39 (td, J = 9.0, 2.7 Hz, 1H), 6.64 (d, J = 2.8, 1H), 4.24 (t, J = 7.0 Hz, 2H), 1.82−1.71 (m, 2H), 1.34−1.21 (m, 2H), 0.91 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, DMSO) δ 169.6 (J = 3 Hz), 160.0 (J = 240 Hz), 151.2, 137.8, 135.9 (J = 11 Hz), 131.1, 128.8, 123.9 (J = 9 Hz), 120.8, 120.7, 115.2 (J = 24 Hz), 111.2, 109.0 (J = 27 Hz), 102.4, 45.9, 32.5, 20.0, 14.0; LC/MS (ESI$^+$): m/z: calcd for $C_{19}H_{17}FN_2S$: 324.42, [M + H]$^+$; found: 325.15.

*6-Chloro-2-(1-methyl-1H-indol-5-yl)benzo[d]thiazole (**5g**).* $PdCl_2$ (2 mg, 0.008 mmol), CuI (8 mg, 0.04 mmol), and Bu_4NBr (51 mg, 0.16 mmol) were added to a solution of *N*-(4-chlorophenyl)-1-methyl-1*H*-indole-5-carbothioamide (23.8 mg, 0.08 mmol) in DMSO (3 mL) and NMP (3 mL). The resulting mixture was stirred at 120 °C for 2 h. After cooling to room temperature, H_2O was added and extracted with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography on silica gel (hexane/CH_2Cl_2/EtOAc = 10:1:1) to obtain the desired product (8.5 mg, 0.03 mmol, 36% yield) as a white solid: mp 196−195 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (dd, J = 1.6, 0.4 Hz, 1H), 7.96 (dd, J = 8.4, 1.6 Hz, 1H), 7.91 (dd, J = 8.4, 0.4 Hz, 1H), 7.83 (dd, J = 1.6, 0.4 Hz, 1H), 7.41−7.37 (m, 2H), 7.10 (d, J = 3.2 Hz, 1H), 6.58 (dd, J = 3.2, 0.8 Hz, 1H), 3.82 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.4, 153.0, 138.4, 136.1, 130.4, 130.3, 128.7, 126.8, 124.9, 123.3, 121.2, 121.1, 121.1, 109.8, 102.3, 33.1; LC/MS (ESI$^+$): m/z: calcd for $C_{16}H_{11}ClN_2S$: 298.79, [M + H]$^+$; found: 299.05.

*6-Bromo-2-(1-methyl-1H-indol-5-yl)benzo[d]thiazole (**5h**).* $PdCl_2$ (2 mg, 0.008 mmol), CuI (9 mg, 0.045 mmol), and Bu_4NBr (58 mg, 0.18 mmol) were added to a solution of *N*-(4-bromophenyl)-1-methyl-1*H*-indole-5-carbothioamide (31 mg, 0.09 mmol) in DMSO (3 mL) and NMP (3 mL). The resulting mixture was stirred at 120 °C for 2 h. After cooling to room temperature, H_2O was added and extracted with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography on silica gel (hexane/CH_2Cl_2/EtOAc = 10:1:1) to obtain the desired product (10.4 mg, 0.03 mmol, 34% yield) as a white solid: mp 205−207 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (dd, J = 1.6, 0.4 Hz, 1H), 8.00 (d, J = 1.6 Hz, 1H), 7.98 (dd, J = 8.8, 1.6 Hz, 1H), 7.55 (dd, J = 8.4, 2.0 Hz, 1H), 7.40 (d, J = 8.8 Hz, 1H), 7.12 (d, J = 3.2 Hz, 1H), 6.58 (dd, J = 2.8, 0.4 Hz, 1H), 3.84 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.4, 153.38, 138.4, 136.6, 130.4, 129.5, 128.7, 124.9, 124.0, 123.7, 121.2, 121.2, 117.9, 109.8, 102.4, 33.1; LC/MS (ESI$^+$): m/z: calcd for $C_{16}H_{11}BrN_2S$: 343.24, [M + H]$^+$; found: 344.25

*6-Fluoro-2-(1H-indol-6-yl)benzo[d]thiazole (**7a**).* Following the same procedure used for the synthesis of 6-fluoro-2-(1*H*-indol-5-yl)benzo[*d*]thiazole, the reaction of *N*-(4-fluorophenyl)-1*H*-indole-6-carbothioamide (0.46 g, 1.7 mmol), $PdCl_2$ (36 mg, 0.17 mmol), CuI (0.16 g, 0.84 mmol) and TBAB (1.10 g, 3.41 mmol) gave the title compound (0.20 g, 0.74 mmol, 44% yield) as a yellowish solid: mp 213-215 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.49 (s, 1H), 8.16 (J = 0.8 Hz, 1H), 8.09−8.02 (m, 2H), 7.77−7.70 (m, 2H), 7.59 (s, 1H), 7.40 (td, J = 9.1, 2.7 Hz, 1H), 6.56 (d, J = 2.8 Hz, 1H); ^{13}C NMR (100 MHz, DMSO) δ 169.5 (J = 3 Hz), 160.0 (J = 241 Hz), 151.2, 136.3 135.9 (J = 11 Hz), 130.7, 129.2, 126.1, 124.0 (J = 9 Hz), 121.3, 118.7, 115.3 (J = 24 Hz), 111.1, 109.1 (J = 27 Hz) 102.2; LC/MS (ESI$^+$): m/z: calcd for $C_{15}H_9FN_2S$: 268.31, [M + H]$^+$; found: 269.10.

*6-Fluoro-2-(1-methyl-1H-indol-6-yl)benzo[d]thiazole (**7b**).* To a solution of 6-fluoro-2-(1*H*-indol-6-yl)benzo[*d*]thiazole (100 mg, 0.37 mmol) in THF was added dropwise KH (30 mg, 0.74 mmol) in THF at room temperature. The reaction mixture was treated with iodomethane (0.04 mL, 0.64 mmol) and stirred for 30 min at room temperature. The reaction mixture was diluted with water and extracted with EtOAc. The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography on silica gel (hexane/EtOAc = 4:1) to give the desired product (64 mg, 0.23 mmol, 87% yield) as a white solid: mp 149−150 °C; ^{1}H NMR (400 MHz, DMSO) δ 8.19 (d, J = 0.4 Hz, 1H), 8.10−8.03 (m, 2H), 7.07−7.69 (m, 2H), 7.41 (td, J = 9.0, 2.7 Hz, 1H), 6.55 (d, J = 2.8, 0.8 Hz, 1H), 3.93 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ 169.5 (J = 3 Hz), 160.0 (J = 241 Hz), 151.1, 136.8, 136.0 (J = 12 Hz), 133.4, 131.1, 124.0 (J = 9 Hz), 121.6, 118.9, 115.3 (J = 24 Hz), 109.3 (J = 27 Hz), 101.4, 15.6; LC/MS (ESI$^+$): m/z: calcd for $C_{16}H_{11}FN_2S$: 282.34, [M + H]$^+$; found: 283.10.

*6-Fluoro-2-(1-ethyl-1H-indol-6-yl)benzo[d]thiazole (**7c**).* Following the same procedure used for the synthesis of 6-fluoro-2-(1-methyl-1H-indol-6-yl)benzo[d]thiazole, the reaction of 6-fluoro-2-(1H-indol-6-yl)benzo[d]thiazole (70 mg, 0.26 mmol), KH (21 mg, 0.52 mmol), iodoethane (0.03 mL, 0.37 mmol) gave the title compound (67 mg, 0.23 mmol, 87% yield) as a yellowish solid: °C dec.; ^{1}H NMR (400 MHz, DMSO) δ 8.22 (d, J = 0.8 Hz, 1H), 8.10−8.03 (m, 2H), 7.73 (s, 2H), 7.63 (d, J = 3.2 Hz, 2H), 7.41 (td, J = 9.1, 2.7 Hz, 1H), 6.57 (dd, J = 3.0, 0.6 Hz, 1H), 4.36 (q, J = 7.2 Hz, 2H), 1.43 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, DMSO) δ 169.5, 160.0 (J = 241 Hz), 151.1, 136.0 (J = 12 Hz), 135.7, 131.8, 131.3, 126.2, 124.0 (J = 9 Hz), 121.7, 118.9, 115.3 (J = 25 Hz), 109.2, 109.0 (J = 19 Hz), 101.7, 41.0, 16.1; LC/MS (ESI$^+$): m/z: calcd for $C_{17}H_{13}FN_2S$: 296.36, [M + H]$^+$; found: 297.15.

*6-Fluoro-2-(1-propyl-1H-indol-6-yl)benzo[d]thiazole (**7d**).* Following the same procedure used for the synthesis of 6-fluoro-2-(1-methyl-1*H*-indol-6-yl)benzo[*d*]thiazole, the reaction of 6-fluoro-2-(1H-indol-6-yl)benzo[d]thiazole (70 mg, 0.26 mmol), KH (21 mg, 0.52 mmol), iodopropane (0.04 mL, 0.42 mmol) gave the title compound (74 mg, 0.24 mmol, 92% yield) as a yellowish solid: mp 116−117 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17−8.14 (m, 1H), 7.99 (q, J = 4.5 Hz, 1H), 7.76−7.67 (m, 2H), 7.57 (dd, J = 8.4, 2.4 Hz, 1H), 7.21 (td, J = 8.9, 2.5 Hz, 2H), 6.54 (dd, J = 2.8, 0.8 Hz, 1H), 4.19 (t, J = 7.2 Hz, 1H), 1.89−2.01 (m, 2H), 0.98 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.9 (J = 3 Hz), 155.5 (J = 243 Hz), 146.2, 131.3, 131.2 (J = 11 Hz), 126.3, 125.9, 122.0, 118.8 (J = 9 Hz), 116.6, 114.5, 109.9 (J = 24 Hz), 103.8, 103.0 (J = 26 Hz), 96.7, 43.5, 18.9, 6.8; LC/MS (ESI$^+$): m/z: calcd for $C_{18}H_{15}FN_2S$: 310.39, [M + H]$^+$; found: 311.15.

*6-Fluoro-2-(1-isopropyl-1H-indol-6-yl)benzo[d]thiazole (**7e**).* Following the same procedure used for the synthesis of 6-fluoro-2-(1-methyl-1*H*-indol-6-yl)benzo[d]thiazole, the reaction of 6-fluoro-2-(1*H*-indol-6-yl)benzo[*d*]thiazole (70 mg, 0.26 mmol), KH (21 mg,

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0.52 mmol), 2-iodopropane (0.04 mL, 0.41 mmol) gave the title compound (42 mg, 0.14 mmol, 52% yield) as a white solid: mp 118−121 °C; ^{1}H NMR (400 MHz, DMSO) δ 8.25 (s, 1H), 8.10−8.02 (m, 2H), 7.77−7.70 (m, 3H), 7.41 (td, J = 9.1, 2.7 Hz, 1H), 6.59 (d, J = 3.2, 1H), 5.02−4.92 (m, 1H), 1.52 (d, J = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, DMSO) δ 169.6 (J = 3 Hz), 160.0 (J = 241 Hz), 151.1, 136.1 (J = 12 Hz), 135.6, 131.1, 128.5, 126.1 124.0 (J = 10 Hz), 121.7, 119.0, 115.3 (J = 25 Hz), 109.2 (J = 26 Hz), 109.0, 102.1, 47.2, 23.1; LC/MS (ESI$^+$): m/z: calcd for $C_{18}H_{15}FN_2S$: 310.39, $[M + H]^+$; found: 311.10.

*6-Fluoro-2-(1-butyl-1H-indol-6-yl)benzo[d]thiazole (**7f**).* Following the same procedure used for the synthesis of 6-fluoro-2-(1-methyl-1*H*-indol-6-yl)benzo[*d*]thiazole, the reaction of 6-fluoro-2-(1*H*-indol-6-yl)benzo[*d*]thiazole (70 mg, 0.26 mmol), KH (21 mg, 0.52 mmol), and iodobutane (0.05 mL, 0.40 mmol) gave the title compound (74 mg, 0.23 mmol, 88% yield) as a yellowish solid: mp 72−73 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16−8.13 (m, 1H), 7.99 (q, J = 4.5 Hz, 1H), 7.76−7.68 (m, 2H), 7.58 (dd, J = 8.0, 2.4 Hz, 1H), 7.26 (s, 1H), 7.25−7.17 (m, 1H), 6.54 (dd, J = 3.2, 0.8 Hz, 1H), 1.94−1.84 (m, 2H), 1.45−1.33 (m, 2H), 0.97 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.6 (J = 3 Hz), 160.2 (J = 243 Hz), 151.0, 136.1, 136.0 (J = 11 Hz), 131.0, 130.5, 126.7, 123.5 (J = 9 Hz), 121.4, 119.3, 114.6 (J = 25 Hz), 108.5, 107.7 (J = 25 Hz), 101.5, 46.3, 32.4, 20.2, 13.7; LC/MS (ESI$^+$): m/z: calcd for $C_{19}H_{17}FN_2S$: 324.42, $[M + H]^+$; found: 325.15.

*6-Fluoro-2-(1H-indol-5-yl)benzo[d]oxazole (**10a**).* To a solution of 2-amino-5-fluorophenol (300 mg, 2.36 mmol) in MeOH was added 1H-indole-5-carbaldehyde (343 mg, 2.55 mmol) at room temperature. The mixture was stirred for 12 h at 80 °C. After cooling down to room temperature, the reaction mixture was concentrated in vacuo. The resulting mixture in DCM was treated with DDQ (589 mg, 2.60 mmol) at room temperature. The reaction mixture was stirred for 4 h at room temperature. After completion of reaction, the resulting mixture was concentrated in vacuo. The residue was diluted with EA and the residue was filtered. The filtrate was washed with saturated Na_2CO_3. The aqueous layer was extracted with EtOAc. The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography on silica gel (DCM/hexane/ether = 40:10:1) to give the desired product (89 mg, 0.35 mmol, 15% yield) as a dark brownish solid: mp 217−218 °C; ^{1}H NMR (400 MHz, DMSO) δ 8.16−8.13 (m, 1H), 7.99 (q, J = 4.5 Hz, 1H), 7.76−7.68 (m, 2H), 7.58 (dd, J = 8.0, 2.4 Hz, 1H), 7.26 (s, 1H), 7.25−7.17 (m, 1H), 6.54 (dd, J = 3.2, 0.8 Hz, 1H), 1.94−1.84 (m, 2H), 1.45−1.33 (m, 2H), 0.97 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, DMSO) δ 165.4 (J = 10 Hz), 160.0 (J = 233 Hz), 150.7 (J = 15 Hz), 139.0, 138.3, 128.3, 127.8, 120.7, 120.7, 120.2 (J = 10 Hz), 117.5, 112.8, 112.6 (J = 24 Hz), 102.9, 99.4 (J = 29 Hz); LC/MS (ESI$^+$): m/z: calcd for $C_{15}H_9FN_2O$: 252.25, $[M + H]^+$; found: 253.10.

*6-Fluoro-2-(1-methyl-1H-indol-5-yl)benzo[d]oxazole (**10b**).* Following the same procedure used for the synthesis of 6-fluoro-2-(1*H*-indol-5-yl)benzo[*d*]oxazole, the reaction of 2-amino-5- fluorophenol (200 mg, 1.57 mmol), 1-methyl-1*H*-indole-5-carbaldehyde (250 mg, 1.57 mmol), and DDQ (463 mg, 2.04 mmol) gave the title compound (93 mg, 0.34 mmol, 22% yield) as a yellowish solid: mp 165−167 °C; ^{1}H NMR (400 MHz, DMSO) δ 8.44 (d, J = 1.2 Hz, 1H), 8.00 (dd, J = 8.8, 1.6 Hz, 1H), 7.82−7.74 (m, 2H), 7.67 (d, J = 8.8 Hz, 1H), 7.50 (s, J = 3.2 Hz, 1H), 7.32−7.24 (m, 1H), 6.66 (dd, J = 3.2, 0.8 Hz, 1H), 3.88 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ 165.3 (J = 3 Hz), 160.1 (J = 239 Hz), 150.7 (J = 15 Hz), 139.0, 138.6, 132.1, 128.6, 120.9, 120.6, 120.2 (J = 10 Hz), 117.5, 112.6 (J = 25 Hz), 111.1, 102.3, 99.4 (J = 28 Hz), 33.18.; LC/MS (ESI$^+$): m/z: calcd for $C_{16}H_{11}FN_2O$: 266.28, $[M + H]^+$; found: 267.15.

MAO-A and -B Inhibition Assay. Human recombinant MAO-A and -B enzyme prepared from insect cells was obtained from Sigma-Aldrich. Tyramine hydrochloride (Sigma) for MAO-A enzyme and benzylamine hydrochloride (Sigma) for MAO-B enzyme were used as substrate. Sodium phosphate buffer (0.05 M, pH 7.4) was used for all the enzyme reactions and dilutions. The final volume of the enzyme reactions was 200 μL, and each reaction contained each substrate, various concentrations of the test inhibitors (0.01−100 μM), and 1% DMSO as cosolvent. The potential effects of the test drugs on hMAO-A and -B activity were investigated by measuring their effects on the production of H_2O_2 from tyramine and benzylamine, respectively, using the Amplex Red MAO assay kit (Invitrogen). Briefly, 0.1 mL of sodium phosphate buffer (0.05 M, 7.4 pH) containing the test drugs (new compounds or reference inhibitors) in various concentrations and adequate amounts of recombinant hMAO-A and B (0.5 μL, 71 U/mg) were incubated for 1 h at 37 °C in a 96-well plate. After this incubation period, the reaction was started by adding (final concentrations) 200 μM Amplex Red reagent, 1 U/mL horseradish peroxidase, and 1 mM tyramine hydrochloride or benzylamine hydrochloride. The production of H_2O_2 and, consequently, of resorufin, was quantified at 37 °C in a multidetection microplate fluorescence reader (TECAN) based on the fluorescence generated (absorbance, 570 nm). Control experiments were carried out simultaneously by replacing the test drugs (new compounds and reference inhibitors) with appropriate dilutions of the vehicles. In addition, the possible capacity of the above test drugs to modify due to nonenzymatic inhibition (e.g., for directly reaction with Amplex Red reagent) was determined by adding these drugs to solutions containing only the Amplex Red reagent in a sodium phosphate buffer. The specific fluorescence absorbance (used to obtain the final results) was calculated after subtraction of the background activity, which was determined from vials containing all components except the hMAO-A and -B, which was replaced by a sodium phosphate buffer solution.

Molecular Docking Studies. The molecular docking study was performed using the cocrtstal structures of human MAO-A (PDB ID: 2Z5X) with harmine and human MAO-B (PDB ID: 2V5Z) with safinamide. The protein structures were prepared under the standard procedure of the Prepare Protein module (CHARMm force field) after adding hydrogens and deleting the water molecules except waters (MAO-A: HOH704, 711, 717, 723, 725−727, 746, 762, 786, 790, 802, 875, and 879; MAO-B: HOH1021, 1025, and 1345−1350) in the active site of both proteins. The binding site was defined and edited by the centroid of cocrystal ligand, safinamide. Ligands were prepared with protonation at pH 7.4 and energy minimization. The molecular docking poses of ligands were obtained using CDOCKER in Discovery Studio 2016 (Accelrys, Inc., v16.1.0.15350) based on the CHARMm docking algorithm. The docking poses of ligands were saved for analysis up to 10 poses.

Reversibility Test. The reversibility of the test compounds was examined by measuring the rate of resorufin formation at 570 nm in spectrophotometer-based assay. The recombinant MAO-B enzymes (50 μL from 5.0 mg/mL solution) were diluted in aqueous 50 mM phosphate buffer (2 mL, pH 7.4). The enzyme solution (441 μL) was incubated with the 0.1 mM MAO-B inhibitor (9 μL, final concentration; 2 μM) for 1 h at room temperature and then divided into two aliquots of reaction enzyme solution (200 μL). An aliquot (200 μL) was examined for inhibitory activity of hMAO-B using the previous method described above. Another aliquot (200 μL) was used for the washout experiment. For the washout experiment, the aliquot was placed in an Amicon Ultra centrifugal filter (3 kDa) (Millipore, Billerica, MA) and centrifuged (14 000g) at 4 °C (10 min). The enzyme retained in the membrane was resuspended in 50 mM phosphate buffer (500 μL) and centrifuged again (under same conditions described above) for three consecutive times. The resulting enzyme was resuspended in aqueous 50 mM phosphate buffer (200 μL, pH 7.4) and an aliquot of this suspension was used for subsequent hMAO-B activity determination. Control experiments without test compounds were performed with the vehicles to define 100% hMAO-B activity. The corresponding values of percent (%) inhibition were separately determined with and without repeated washing.

Production of MPTP Mouse Model of PD and in Vivo Drug Treatment. All mice and rats were kept in a temperature- and humidity-controlled environment with a 12 h light−dark cycle (lights on at 9 a.m.) and had free access to food and water. All animal care and handling was performed according to the directives of the Animal Care and Use Committee of the Institutional Animal Care and Use Committee of KIST (Seoul, Korea). For animal experiments, 10-week-old male C57Bl/6 mice weighing 23−25 g were used. All MPTP experiments used the acute regimen consisting of four times of

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intraperitoneal injection of MPTP-HCl (M0896, Sigma-Aldrich, 2 mg/mL in saline, 20 mg/kg for one injection) in a day with 2 h intervals.

The animals (n = 6−10 per group) were orally administered with safinamide or compound **5b** suspended in *N*-methyl-2-pyrrolidone and 20% Tween 80 in saline at 10 mg/kg body weight/day every 24 h for 3 consecutive days. The first MPTP injection was made 24 h after the first compound **5b** administration.

Behavior Test. The vertical grid test was performed as described in the previous study.[37] A mouse was gently placed inside the apparatus at 3 cm from the top, facing upward, and was allowed to turn around and climb down. All mice underwent habituation to this test three times a day for 2 days prior to MPTP administration. The test was performed 6 days after MPTP administration, and all of the experiment sessions were video recorded to allow the measure the total time of performing the behavior, time to turn, time to climb down, and ratio of failed hindlimb steps.

TH Staining. For histological analysis, mice were deeply anesthetized with 2% avertin (20 μg/g, i.p.) and perfused with 0.9% saline followed by ice cold 4% paraformaldehyde (PFA). Excised brains were postfixed overnight in 4% PFA at 4 °C and immersed in 30% sucrose for over 48 h for cryoprotection. Coronal sections for striatum and SNpc were cut at 30 μm in a cryostat and kept floating in tissue storage solution: Glycerol, ethelene glycol, DW and 0.2 M PB (3:3:3:1, v/v/v/v). After additional washing in PBS, sections were ready to be stained.

The sections were immunostained with a DAB staining kit (TL-060-QHD, Thermo, Waltham, MA). The sections were incubated in hydrogen peroxide block (TA-060-HP, Thermo) for 10 min, washed in PBS three times, incubated for 5 min in Ultravision Block (TA-060-UB, Thermo), and washed in PBS three times again. Then the samples were immunostained with a mixture of primary antibody against TH (1:500, Pel-freez p40101-0) in a blocking solution (0.3% Triton-X, 2% ready-to-use donkey serum (GTX30972, Genetex, San Francisco, CA) in 0.1 M PBS) at 4 °C on a shaker overnight. After washing in PBS three times, sections were incubated in Primary Antibody Amplifier Quanto (TA-060-QPB, Thermo) for 5 min, and washed in PBS again. The sections were incubated in HRP Polymer Quanto for 1 h and washed four times in PBS. DAB+ chromogen and DAB+ substrate buffer (K3468, Dako, Denmark) were mixed in 1:10 ratio and the sections were dipped in the mixture for 30 s and then washed. Finally, sections were mounted with mounting solution and dried. A series of bright field images were obtained with an Olympus microscope.

Image Quantification. For densitometric analysis of the striatum area with TH staining, coronal sections of striatum were observed under a bright field microscope (Olympus, Center Valley, PA) using 10× magnification. The optical density of the TH-stained striatum was determined at an equivalent frame range, with optical density in the corpus callosum used as a reference. Five to eight mice were sacrificed per group, and the results are given as the average of all sections. Cell counting with high magnification images (60× objective lens) were performed using counting tool of Photoshop CS5.

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M.-H.N. and M.P. contributed equally to this work. M.-H.N., M.P., H.P., and J.W.C. performed the experiments. H.C. and C.J.L. designed the studies. H.C., M.-H.N., C.J.L., K.D.P., and S.-J.M. wrote the manuscript. Y.K., S.Y., V.S.S., and J.-H.P. performed the experiments.

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Notes

The authors declare no competing financial interest.

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