Synthesis and Characterization of Radioiodinated MD-230254: A New Ligand for Potential Imaging of Monoamine Oxidase B Activity by Single Photon Emission Computed Tomography

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A series of iodinated analogues of MD-230254 was synthesized and evaluated for inhibitory potency and selectivity toward monoamine oxidase B (MAO-B). Among them, 5-[4-(2-iodobenzyloxy)phenyl]-3-(cyanoethyl)-1,3,4-oxadiazole-2(3*H*)one (2-IBPO) was found to have high inhibitory potency and selectivity toward MAO-B (IC_{50}=2.0 nM, MAO-A/MAO-B >50000). Analysis of the inhibition kinetics indicated that 2-IBPO acts in a two-step mechanism as a competitive, slow, and tight-binding inhibitor of MAO-B with a *Ki* value of 2.4 nM and an overall *Ki value at an equilibrium of 3.8 nM. The new radioligand for MAO-B, [^{125}I]2-IBPO was conveniently synthesized from a tributylstannyl precursor by an iododestannylation reaction using sodium [^{125}I]iodide and hydrogen peroxide with high radiochemical yield. The *in vivo* tissue distribution studies of [^{125}I]2-IBPO demonstrated its high initial uptake and prolonged retention in the brain. A selective interaction of [^{125}I]2-IBPO with MAO-B was confirmed by the pretreatment experiment with well known MAO specific inhibitors, *l*-deprenyl, Ro-16-6491, clorgyline, and Ro-41-1049. These very desirable characteristics of [^{125}I]2-IBPO suggested that a ^{123}I-labeled counterpart, [^{123}I]2-IBPO, would have great potential in *in vivo* studies of MAO-B in the human brain with single photon emission computed tomography (SPECT).**

Key words monoamine oxidase (MAO); MAO-B; SPECT; MD-230254

Monoamine oxidase (MAO) [E.C. 1.4.3.4.] is a flavin-containing enzyme that catalyzes the oxidative deamination of neurotransmitter amines as well as exogenous amines.[1—4] It has been divided into two subtypes, MAO-A and MAO-B, on the basis of their different specificities toward substrates and inhibitors.[5—10] Recently, determination of the sequence of cloned MAO-A and MAO-B cDNAs has provided the molecular basis for the existence of physically and genetically independent enzymes.[11,12] Both forms appear to be important for neurotransmitter regulation, and fluctuations in functional MAO activity may be associated with human diseases such as Parkinson's disease, depression and certain psychiatric disorders.[13—17] In the human brain, MAO-B predominates (MAO-B : MAO-A=4 : 1) and is associated mainly with glial cells.[18] Unlike most enzyme or neurotransmitter receptors and transporters, MAO-B activity increases with normal aging and in neurodegenerative disease in glial cells in response to age-related or disease-associated neuron loss and gliosis.[19,20] Positron emission tomography (PET) and single photon emission computed tomography (SPECT) have been successfully employed for non-invasive studies of the biochemical transformation and physiological processes in the living human brain, utilizing organic molecules labeled with a positron emitter or a single photon emitter. For the direct and non-invasive mapping and functional studies of the MAO-B activity in the living brain, ^{11}C-labeled pargyline,[21] *l*-deprenyl,[22] ^{123}I-labeled benzamide derivatives[23—25] and ^{123}I-labeled pargyline[26] have been investigated as ligands for PET and SPECT. Recently, a new reversible and highly selective MAO-B inhibitor, 5-[4-(benzyloxy)phenyl]-3-(cyanoethyl)-1,3,4-oxadiazole-2(3*H*)one (MD-230254), was developed.[27] The ^{11}C-labeled MD-230254 has been investigated in living baboon brain with PET, and appears to be as a good candidate for *in vivo* MAO-B specific experiments.[28] Despite attractive features associated with PET techniques, PET studies are still limited, since they usually require on-site cyclotrons. On the other hand, SPECT studies are more commonly used in nuclear medicine clinics. We have explored the feasibility of a [^{123}I]radioiodinated MAO-B inhibitor as an alternative to [^{11}C]MD-230254 for functional MAO-B studies in the brain with SPECT. We report here the synthesis of a novel series of iodinated MD-230254 analogues amenable to radiolabeling with ^{123}I or ^{125}I. *In vitro* and *in vivo* studies on the inhibitory potency and selectivity toward MAO-B were also performed in order to evaluate it as a new ligand for *in vivo* MAO-B studies with SPECT.

Results and Discussion

Chemistry Iodinated derivatives of MD-230254 were synthesized by the reaction outlined in Chart 1, based on the published procedure for MD-230254.[27] Ethyl 4-hydroxybenzoate or its iodinated derivative was converted into esters **2a**—**d** by treatment with sodium hydride in dry *N*,*N*-dimethylformamide (DMF) followed by reaction with appropriate benzyl bromide derivatives. Treatment of **2a**—**d** with hydrazine hydrate afforded hydrazides **3a**—**d**. A Michael reaction between hydrazides **3a**—**d** and acrylonitrile gave **4a**—

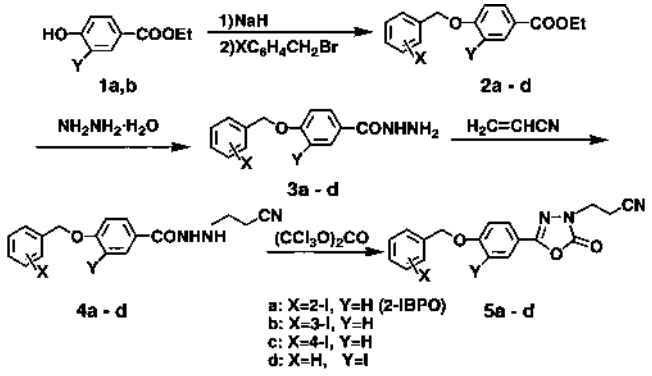

Chart 1

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d. The desired compounds **5a**—**d** were conveniently synthesized from appropriate monosubstituted hydrazines **4a**—**d** by a cyclization reaction using bis(trichloromethyl)carbonate (triphosgene)[29] in a high yield.

***In Vitro* Assay** The inhibitory potency of iodinated derivatives of MD-230254 (**5a**—**d**) and MD-230254 against MAO-A and MAO-B activities in rat liver mitochondoria fraction were measured for selectivity *in vitro* using [^{14}C]serotonin and [^{14}C]phenethylamine, according to a modified radiochemical procedure.[27] These results are summarized in Table 1. Compounds **5a**, **5b**, and **5d** were found to have high inhibitory potency against MAO-B (IC_{50}, 2.0, 3.6, and 2.2 nM, respectively), fully comparable to MD-230254 (IC_{50}, 1.8 nM, lit. 1.4 nM[27]) examined under the same conditions. The selectivity of these inhibitors toward MAO-B were estimated from the ratio of the IC_{50} value (MAO-A/MAO-B). These ratios of IC_{50} value (MAO-A/MAO-B) were >50000 (**5a**), >28000 (**5b**) and >45000 (**5d**), respectively, indicating high selectivity toward MAO-B. However, **5c** was found to be a relatively weak MAO-B inhibitor (IC_{50}, 130 nM). Thus, the most potent compound **5a** (namely 2-IBPO), among the iodinated MD-230254 derivatives tested *in vitro*, was chosen for further evaluations. Kinetic studies of MAO-B inhibition by 2-IBPO are shown as a Lineweaver-Burk plot in Fig. 1 and Fig. 2. The inhibition mechanism of 2-IBPO indicated the same pattern as MD-230254 itself, with a change of Lineweaver–Burk plot from competitive (without preincubation with enzyme, Fig. 1) to "pseudo" noncompetitive patterns, with a parabolic secondary replot (with preincubation with the enzyme, Fig. 2).[27] On the basis of these results, a two-step interaction between 2-IBPO and MAO-B can be assumed according to equilibrum,[27] where the substrate (E) and inhibitor (I) combine rapidly to form a reversible complex EI which is isomerized slowly into a tighter complex EI* (Chart 2). The inhibition constant (*Ki*) value and an overall inhibition constant (*Ki**) value at equilibrium were calculated as 2.4 nM and 3.8 nM, respectively.

Table 1. Inhibition of MAO by Iodinated Analogues of MD-230254

Compound	IC_{50} (nM)		MAO-A/MAO-B
	MAO-B	MAO-A	
5a	2.0	>100000	>50000
5b	3.6	>100000	>28000
5c	130.0	>100000	>770
5d	2.2	>100000	>45000
MD-230254	1.8	>100000	>56000
l-Deprenyl	8.8	920	104

Radiolabeling The electrophilic iododestannylation reaction offers several advantages for radioiodination, since it is performed under very mild conditions and with very high regional selectivity, and also affords a high specific radioactivity. Thus, the preparation of [^{125}I]2-IBPO was performed

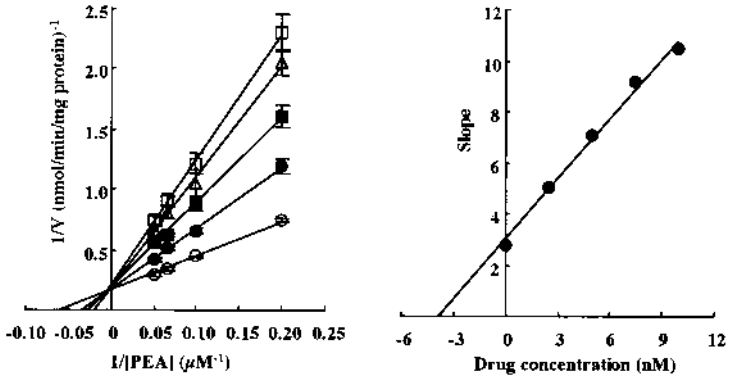

Fig. 1. Lineweaver–Burk Plot (Left) and Lineweaver–Burk Secondary Replot (Right) of the Inhibition of MAO-B by 2-IBPO

MAO-B activity was measured with 2.5, 5, 7.5 and 10 μM [^{14}C]phenethylamine. Final DMSO concentration was 1%. All values represent means of duplicate determinations in three homogenates. Samples were assayed without preincubation in the absence (○) or presence of 2.5 (●), 5 (■), 7.5 (△), and 10 (□) nM of 2-IBPO.

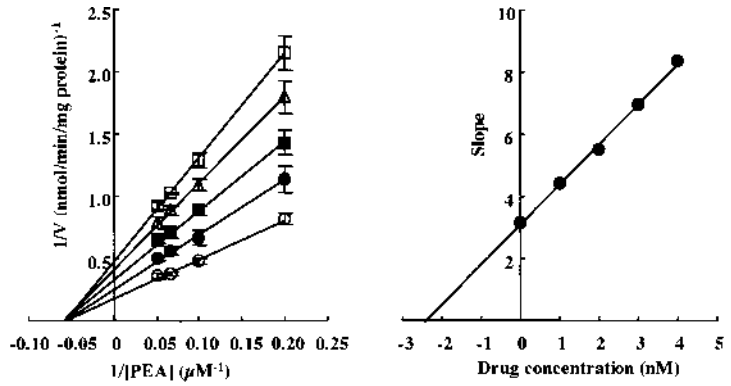

Fig. 2. Lineweaver–Burk Plot (Left) and Lineweaver–Burk Secondary Replot (Right) of the Inhibition of MAO-B by 2-IBPO

MAO-B activity was measured with 2.5, 5, 7.5 and 10 μM [^{14}C]phenethylamine. Final DMSO concentration was 1%. All values represent means of duplicate determinations in three homogenates. Samples were assayed after preincubation of the enzyme for 30 min at 37 °C in the absence (○) or presence of 1 (●), 2 (■), 3 (△), and 4 (□) nM of 2-IBPO.

using an iododestannylation reaction with a tributylstannyl precursor by the reaction outlined in Chart 3. The compound **5a** was synthesized with hexa-*n*-butylditin in the presence of a catalytic amount of tetrakis-(triphenylphosphine)palladium to produce the corresponding tributylstannyl derivative **6** in moderate to high yield (57.7%). Radioiodination of **6** was achieved using hydrogen peroxide as an oxidant and sodium [^{125}I]iodide (specific activity 74 GBq/μmol) in 0.1 M HCl/ethanol solution at room temperature, followed by HPLC purification. The radiochemical yield of the product, [^{125}I]2-IBPO, was 90—93% based on sodium [^{125}I]iodide. The radiochemical purity of [^{125}I]2-IBPO was higher than 99%, as assessed by HPLC analysis, with specific radioactivity of approximately 74 GBq/μmol. This method should be applicable for labeling with ^{123}I, a very suitable radioisotope (half-life 13 h and gamma ray energy of 159 keV) for *in vivo* imaging with SPECT.

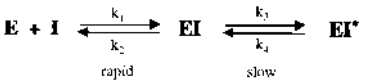

Chart 2

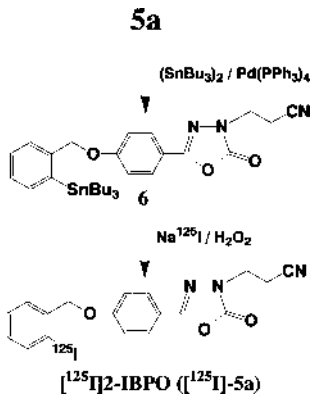

Chart 3

Tissue Distribution *In vivo* tissue distribution of the [^{125}I]2-IBPO was examined in male ddY mice at 5, 15, 30, 60, 120, 180 min after intravenous administration. As summarized in Table 2, [^{125}I]2-IBPO was transported well into various tissues. The maximum uptake of [^{125}I]2-IBPO in the brain was high, 2.44% dose/g at 5 min after injection, then the brain radioactivity level decreased gradually to 1.51% dose/g and 1.17% dose/g at 120 and 180 min after injection, respectively. This retention profile of [^{125}I]2-IBPO in the brain reflected a characteristic inhibition mechanism, 'pseudo' non-competitive inhibition. In contrast to high brain uptake and retention, the blood accumulation was low, resulting in good brain-blood ratios (2.80—4.30). This accumulation of [^{125}I]2-IBPO in the brain and brain-blood ratio at 120 min after injection were the desired retention and contrast for SPECT imaging. Then, further studies on the selective binding of [^{125}I]2-IBPO to MAO-B *in vivo* was demonstrated by the pretreatment study (Fig. 3). The effect of pretreatment with *l*-deprenyl, Ro-16-6491, clorgyline, and Ro-41-1049 on the distribution of [^{125}I]2-IBPO at 120 min after injection are

Table 2. Tissue Distribution of [^{125}I]2-IBPO in Mice[a)]

Tissue	Time after injection					
	5 min	15 min	30 min	60 min	120 min	180 min
Brain	2.44 (0.44)	1.85 (0.13)	1.85 (0.13)	1.74 (0.17)	1.51 (0.12)	1.12 (0.05)
Blood	0.87 (0.07)	0.50 (0.05)	0.43 (0.05)	0.49 (0.04)	0.45 (0.04)	0.40 (0.02)
Pancreas	3.29 (0.37)	2.22 (0.28)	1.85 (0.13)	1.56 (0.26)	1.28 (0.10)	1.09 (0.11)
Spleen	1.49 (0.21)	0.87 (0.11)	0.68 (0.02)	0.74 (0.05)	0.54 (0.03)	0.34 (0.01)
Stomach	2.06 (0.20)	2.97 (0.68)	2.54 (0.22)	3.87 (0.53)	5.42 (0.03)	4.75 (0.24)
Liver	17.9 (0.37)	19.1 (2.73)	20.6 (3.14)	19.1 (0.44)	18.8 (1.58)	13.3 (1.41)
Kidney	3.73 (0.47)	1.85 (0.15)	1.67 (0.08)	1.66 (0.35)	1.68 (0.09)	1.50 (0.09)
Heart	5.92 (0.44)	3.98 (0.45)	3.67 (0.22)	3.59 (0.65)	2.59 (0.34)	2.02 (0.18)
Lung	4.08 (0.33)	3.85 (0.25)	2.52 (0.25)	2.77 (0.13)	2.01 (0.37)	1.80 (0.27)
Brain/Blood	2.80	4.30	4.30	3.55	3.36	2.80

a) Mean % dose (S.D.) per gram tissue of four mice.

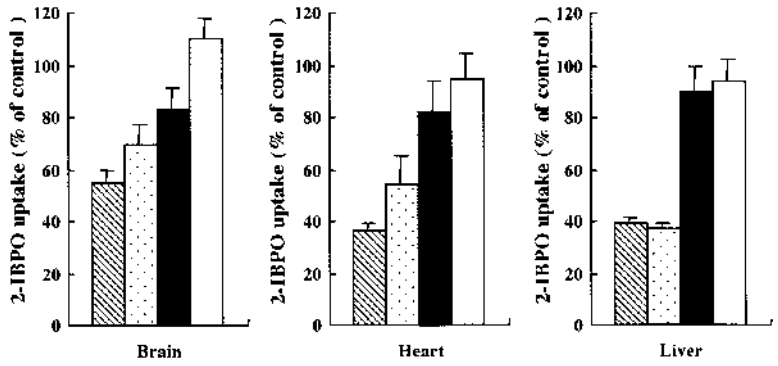

Fig. 3. Effect of *l*-Deprenyl (▨), Ro-16-6491(▩), Clorgyline (■) and Ro-41-1049 (□) on the Uptake of [^{125}I]2-IBPO

Each value represents mean±S.D. of four mice as a percent of the control value.

presented in Fig. 3. Pretreatment with MAO-B selective inhibitors, *l*-deprenyl, and Ro-16-6491, significantly reduced the [^{125}I]2-IBPO uptake in the brain (50% and 30%, respectively). On the other hand, pretreatment with Ro-41-1049 did not indicate a reduction of the [^{125}I]2-IBPO uptake in the brain. Pretreatment with clorgyline indicated about a 20% reduction of [^{125}I]2-IBPO uptake in the brain. This decrease was caused by MAO-B inhibition of clorgyline. The pretreatment studies data suggested that [^{125}I]2-IBPO accumulation reflected MAO-B activity in the brain. Moreover, high uptake in the liver, heart and lung, followed by reduced radioactivity uptake in these organs with MAO-B inhibitor pretreatment, implicated that [^{125}I]2-IBPO was an inhibitor against MAO-B in not only the central nervous system but also other organs.

In conjunction with the tissue distribution, the pretreatment studies data suggested that [^{125}I]2-IBPO is a potentially useful radioligand for *in vivo* studies of MAO-B under both normal and pathological conditions in which alternations of monoamine neurotransmitter metabolism have been reported.[19,30] The ^{123}I-labeled counterpart may be suitable for non-invasive imaging of MAO-B in the living brain with SPECT.

In conclusion, among the iodinated MD-230254 derivatives prepared, **5a** (2-IBPO) was found to have high inhibitory potency and selectivity against the MAO-B. [^{125}I]2-IBPO was conveniently synthesized from a tributylstannyl precursor by an iododestannylation reaction using sodium [^{125}I]iodide and hydrogen peroxide with high radiochemical yield. The *in vivo* tissue distribution studies of [^{125}I]2-IBPO demonstrated its high brain uptake and long retention. The brain-blood radioactivity ratio was high. Pretreatments with MAO inhibitors showed selective binding of [^{125}I]2-IBPO to MAO-B. These very desirable characteristics of [^{125}I]2-IBPO suggest that its ^{123}I-labeled counterpart, [^{123}I]2-IBPO, would have great potential as a SPECT radiopharmaceutical for functional MAO-B studies in the human brain.

Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus and uncorrected. Infrared (IR) spectra were taken on a JASCO IR-700 spectrometer. Proton magnetic resonance (^{1}H-NMR) spectra were recorded on a Varian Gemini-200 (200 MHz) spectrometer, and the chemical shifts are reported in ppm downfield from an internal tetramethylsilane standard. High-resolution mass spectra were obtained on a Hitachi M-80 instrument. The HPLC system used includes a Waters M-600 pump, a Lambada-Max 481 ultravaiolet detector, a Beckman 170 NaI radioactivity detector, and a Cosmosil 5C18-AR column (10×250 mm, Nacalai Tesque). Radioactivity was measured using an Aloka ARC-300 NaI (Tl) gamma scintillation counter.

[^{125}I]NaI was purchased from Amersham Japan. All other chemicals used were of reagent grade and were purchased commercially. Wistar rats and male ddY mice were obtained from Japan SLC Co., Ltd. These animals were kept at least one week before the experiments. Animals chow and drinking water were allowed *ad libitum*. Animals were housed and experiments were performed according to guidelines stipulated by the Osaka University of Pharmaceutical Sciences Animal Care and Use Committee.

Substituted Ethyl 4-(Benzyloxy)benzoate (2a—d) To a stirred and cooled suspension of sodium hydride (2.4 g, 0.10 mol) in dry DMF (20 ml) was added dropwise a solution of ethyl 4-hydroxybenzoate or its derivative (0.10 mol) in dry DMF (40 ml). The stirred mixture was allowed to stand for 30 min at room temperature. Then, a solution of benzyl bromide derivatives in dry DMF (10 ml) was added dropwise. The reaction mixture was stirred for 5 h. The solvent was evaporated and the crude ester was washed with 100 ml ice water and then filtered, dried, and recrystallized from EtOH.

Ethyl 4-(2-Iodobenzyloxy)benzoate (**2a**): Yield 50.5%, mp 69—71 °C. IR (KBr): 1695, 1507, 1121, 861, 36 cm^{-1}. ^{1}H-NMR (DMSO) δ: 1.35 (3H, t, J=6.3 Hz, CH_2CH_3), 4.37 (2H, q, J=6.3 Hz, CH_2CH_3), 5.20 (2H, s, CH_2O), 7.19 (2H, d, J=6.9 Hz, aromatics), 7.34—7.85 (4H, m, aromatics) 8.04 (2H, d, J=6.9 Hz, aromatics). *Anal.* Calcd for $C_{16}H_{15}IO_3$: C, 50.26; H, 3.96. Found: C, 50.29; H, 3.94. HRMS calcd for *m*/*z*: 382.0068. Found: 382.0065.

Ethyl 4-(3-Iodobenzyloxy)benzoate (**2b**): Yield 75.7%, mp 90—91 °C. IR (KBr): 1695, 1505, 1124, 870, 635 cm^{-1}. ^{1}H-NMR (DMSO) δ: 1.38 (3H, t, J=6.3 Hz, CH_2CH_3), 4.45 (2H, q, J=6.3 Hz, CH_2CH_3), 5.31 (2H, s, CH_2O), 7.04 (2H, d, J=6.9 Hz, aromatics), 7.22—7.75 (4H, m, aromatics), 7.89 (2H, d, J=6.9 Hz, aromatics). *Anal.* Calcd for $C_{16}H_{15}IO_3$: C, 50.26; H, 3.99. Found: C, 50.29; H, 3.94. HRMS calcd for *m*/*z*: 382.0068. Found: 382.0071.

Ethyl 4-(4-Iodobenzyloxy)benzoate (**2c**): Yield 50.7%, mp 79—80 °C. IR (KBr): 1696, 1507, 1120, 863, 637 cm^{-1}. ^{1}H-NMR (DMSO) δ: 1.30 (3H, t, J=6.3 Hz, CH_2CH_3), 4.31 (2H, q, J=6.3 Hz, CH_2CH_3), 5.31 (2H, s, CH_2O), 7.11 (2H, d, J=6.9 Hz, aromatics), 7.24 (2H, d, J=6.7 Hz, aromatics), 7.69 (2H, d, J=6.7 Hz, aromatics), 7.78 (2H, d, J=6.9 Hz, aromatics). *Anal.* Calcd for $C_{16}H_{15}IO_3$: C, 50.26; H, 3.96. Found: C, 50.23; H, 3.95. HRMS calcd for *m*/*z*: 382.0068. Found: 382.0069.

Ethyl 4-(Benzyloxy) 3-Iodobenzoate (**2d**): Yield 79.5%, mp 52—56 °C. IR (KBr): 1690, 1509, 1121, 868, 636 cm^{-1}. ^{1}H-NMR (DMSO) δ: 1.29 (3H, t, J=6.3 Hz, CH_2CH_3), 4.42 (2H, q, J=6.3 Hz, CH_2CH_3), 5.23 (2H, s, CH_2O), 7.19 and 7.98 (8H, m, aromatics). *Anal.* Calcd for $C_{16}H_{15}IO_3$: C, 50.26; H, 3.96. Found: C, 50.29; H, 3.94. HRMS calcd for *m*/*z*: 382.0068. Found: 382.0060.

Substituted [4-Benzyloxy)benzoyl]hydrazine (3a—d) A mixture of **2a**—**d** (5 mmol) and hydrazine monohydrate (2.4 ml) in 1-propanol (15 ml) was stirred to reflux for 24 h. After cooling, the solvent was evaporated and the crude compound was resolved in $CHCl_3$. The solution was washed with 100 ml ice water, then the organic layer was dried and evaporated. The obtained solid was recrystallized from MeOH.

[4-(2-Iodobenzyloxy)benzoyl]hydrazine (**3a**): Yield 81.8%, mp 134—136 °C. IR (KBr): 3321, 3201, 1623, 1254, 835 cm^{-1}. ^{1}H-NMR (DMSO) δ: 4.19 (1H, s, $NHNH_2$), 5.10 (2H, s, CH_2O), 7.08 (2H, d, J=6.9 Hz, aromatics), 7.41—7.68 (4H, m, aromatics), 7.84 (2H, d, J=6.9 Hz, aromatics), 9.64 (2H, s, $NHNH_2$). *Anal.* Calcd for $C_{14}H_{13}IN_2O_2$: C, 45.67; H, 3.56; N, 7.61. Found: C, 45.64; H, 3.58; N, 7.62. HRMS calcd for *m*/*z*: 368.0024. Found: 368.0016.

[4-(3-Iodobenzyloxy)benzoyl]hydrazine (**3b**): Yield 55.9%, mp 160—160.5 °C. IR (KBr): 3324, 3200, 1594, 1260, 836 cm^{-1}. ^{1}H-NMR (DMSO) δ: 4.11 (1H, s, $NHNH_2$), 5.14 (2H, s, CH_2O), 7.06 (2H, d, J=6.9 Hz, aromatics), 7.19—7.51 (4H, m, aromatics), 7.80 (2H, d, J=6.9 Hz, aromatics), 9.64 (2H, s, $NHNH_2$). *Anal.* Calcd for $C_{14}H_{13}IN_2O_2$: C, 45.67; H, 3.56; N,7.61. Found: C, 45.66; H, 3.55; N, 7.63. HRMS calcd for *m*/*z*: 368.0024. Found: 368.0027.

[4-(4-Iodobenzyloxy)benzoyl]hydrazine (**3c**): Yield 49.8%, mp 141—143 °C. IR (KBr): 3320, 3193, 1599, 1256, 833 cm^{-1}. ^{1}H-NMR (DMSO) δ: 4.99 (1H, s, $NHNH_2$), 5.05 (2H, s, CH_2O), 7.11 (2H, d, J=6.9 Hz, aromatics), 7.24 (2H, d, J=6.7 Hz, aromatics), 7.61 (2H, d, J=6.7 Hz, aromatics), 7.93 (2H, d, J=6.9 Hz, aromatics), 9.58 (2H, s, $NHNH_2$). *Anal.* Calcd for $C_{14}H_{13}IN_2O_2$: C, 45.67; H, 3.56; N,7.61. Found: C, 45.67; H, 3.55; N, 7.60. HRMS calcd for *m*/*z*: 368.0024. Found: 368.0025.

[4-(Benzyloxy) 3-Iodobenzoyl]hydrazine (**3d**): Yield 49.8%, mp 128—130 °. IR (KBr): 3313, 3199, 1603, 1251, 840 cm^{-1}. ^{1}H-NMR (DMSO) δ: 4.49 (1H, s, $NHNH_2$), 5.30 (2H, s, CH_2O), 7.18—7.79 (8H, m, aromatics), 9.75 (2H, s, $NHNH_2$). *Anal.* Calcd for $C_{14}H_{13}IN_2O_2$: C, 45.67; H, 3.56; N, 7.61. Found: C, 45.68; H, 3.57; N, 7.59. HRMS calcd for *m*/*z*: 368.0024. Found: 368.0022.

Substituted 1-[4-(Benzyloxy)benzoyl]-2-(2-cyanoethyl)hydrazine (4a—d) A mixture of **3a**—**d** (1.01 g) and acrylonitrile (1.83 ml, 27.5 mmol) in EtOH was refluxed for 24 h. The solvent was removed *in vacuo*. The resulting crup was recrystallized from MeOH.

1-[4-(2-Iodobenzyloxy)benzoyl]-2-(2-cyanoethyl)hydrazine (**4a**): Yield 63.5%, mp 117—120 °C. IR (KBr): 3278, 3205, 2252, 1626, 1374 cm^{-1}. ^{1}H-NMR (DMSO) δ: 2.64 (2H, t, J=6.4 Hz, CH_2CN), 3.02 (2H, q, J=6.4 Hz, CH_2CH_2), 5.11 (2H, s, CH_2O), 5.62 (1H, q, J=4.0 Hz, $NHCH_2$), 7.09 (2H, d, J=6.9 Hz, aromatics), 7.42—7.68 (4H, m, aromatics), 7.86 (2H, d, J=6.9 Hz, aromatics), 9.96 (1H, d, J=4.0 Hz, NHNH). *Anal.* Calcd for $C_{17}H_{16}IN_3O_2$: C, 48.45; H, 3.83; N, 9.98. Found: C, 48.44; H, 3.85; N, 9.99. HRMS calcd for *m*/*z*: 442.0367. Found: 442.0364.

1-[4-(3-Iodobenzyloxy)benzoyl]-2-(2-cyanoethyl)hydrazine (**4b**): Yield 25.0%, mp 92—93 °C. IR (KBr): 3268, 3196, 2250, 1606, 1368 cm^{-1}. ^{1}H-NMR (DMSO) δ: 2.63 (2H, t, J=6.4 Hz, CH_2CN), 2.99 (2H, q, J=6.4 Hz, CH_2CH_2), 5.14 (2H, s, CH_2O), 5.44 (1H, q, $NHCH_2$), 7.17 (2H, d, J=6.9 Hz, aromatics), 7.27—7.62 (4H, m, aromatics), 7.86 (2H, d, J=6.9 Hz, aromatics), 9.95 (1H, d, J=4.0 Hz, NHNH). *Anal.* Calcd for $C_{17}H_{16}IN_3O_2$: C, 48.45;

H, 3.83; N, 9.98. Found: C, 48.46; H, 3.83; N, 9.96. HRMS calcd for *m*/*z*: 442.0367. Found: 442.0368.

1-[4-(4-Iodobenzyloxy)benzoyl]-2-(2-cyanoethyl)hydrazine (**4c**): Yield 53.7%, mp 156—157 °C. IR (KBr): 3261, 3208, 2251, 1598, 1365 cm^{-1}. ^{1}H-NMR (DMSO) δ: 2.59 (2H, t, J=6.4 Hz, CH_2CN), 3.04 (2H, q, J=6.4 Hz, CH_2CH_2), 5.21 (2H, s, CH_2O), 5.39 (1H, q, J=4.0 Hz, $NHCH_2$), 7.05 (2H, d, J=6.9 Hz, aromatics), 7.18 (2H, d, J=6.7 Hz, aromatics), 7.57 (2H, d, J=6.7 Hz, aromatics), 7.76 (2H, d, J=6.9 Hz, aromatics), 9.98 (1H, d, J=4.0 Hz, NHNH). *Anal.* Calcd for $C_{17}H_{16}IN_3O_2$: C, 48.45; H, 3.83; N, 9.98. Found: C, 48.43; H, 3.85; N, 9.98. HRMS calcd for *m*/*z*: 442.0367. Found: 442.0370.

1-[4-(Benzyloxy)-3-iodobenzoyl]-2-(2-cyanoethyl)hydrazine (**4d**): Yield 80.7%, mp 143—145 °C. IR (KBr): 3264, 3212, 2248, 1603, 1367 cm^{-1}. ^{1}H-NMR (DMSO) δ: 2.50 (2H, t, J=6.4 Hz, CH_2CN), 2.88 (2H, q, J=6.4 Hz, CH_2CH_2), 5.14 (2H, s, CH_2O), 5.35 (1H, q, J=4.0 Hz, $NHCH_2$), 7.03—7.74 (8H, m, aromatics), 9.90 (1H, d, J=4.0 Hz, NHNH). *Anal.* Calcd for $C_{17}H_{16}IN_3O_2$: C, 48.45; H, 3.83; N, 9.98. Found: C, 48.48; H, 3.81; N, 9.97. HRMS calcd for *m*/*z*: 442.0367. Found: 442.0365.

Substituted 5-[4-(Benzyloxy)phenyl]-3-(2-cyanoethyl)-1,3,4-oxadiazol-2(3*H*)-one (5a—d) Compounds **4a**—**d** were dissolved in dry THF, then triphosgene in dry THF was added dropwise. The reaction mixture was stirred for 3 h at room temperature. The solvent was removed *in vacuo*. The obtained solid was recrystallized from MeOH.

5-[4-(2-Iodobenzyloxy)phenyl]-3-(2-cyanoethyl)-1,3,4-oxadiazol-2(3*H*)-one (**5a**): Yield 82.0%, mp 167—168 °C. IR (MeOH): 2244, 1788, 1614, 1257, 985 cm^{-1}. ^{1}H-NMR (DMSO) δ: 3.01 (2H, t, J=6.3 Hz, CH_2CH_2), 4.02 (2H, t, J=6.3 Hz, CH_2CN), 5.13 (2H, s, CH_2O), 7.21 (2H, d, J=6.9 Hz, aromatics), 7.41—7.95 (4H, m, aromatics), 7.79 (2H, d, J=6.9 Hz, aromatics). *Anal.* Calcd for $C_{18}H_{14}IN_3O_3$: C, 48.34; H, 3.16; N, 9.40. Found: C, 48.34; H, 3.20; N, 9.38. HRMS calcd for *m*/*z*: 447.0082. Found: 447.0074.

5-[4-(3-Iodobenzyloxy)phenyl]-3-(2-cyanoethyl)-1,3,4-oxadiazol-2(3*H*)-one (**5b**): Yield 62.7%, mp 150—151 °C. IR (KBr): 2247, 1771, 1612, 1255, 983 cm^{-1}. ^{1}H-NMR (DMSO) δ: 3.05 (2H, t, J=6.3 Hz, CH_2CH_2), 4.00 (2H, t, J=6.3 Hz, CH_2CN), 5.11 (2H, s, CH_2O), 7.24 (2H, d, J=6.9 Hz, aromatics), 7.32—7.68 (4H, m, aromatics), 7.75 (2H, d, J=6.9 Hz, aromatics). *Anal.* Calcd for $C_{18}H_{14}IN_3O_3$: C, 48.34; H, 3.16; N, 9.40. Found: C, 48.36; H, 3.14; N, 9.37. HRMS calcd for *m*/*z*: 447.0082. Found: 447.0070.

5-[4-(4-Iodobenzyloxy)phenyl]-3-(2-cyanoethyl)-1,3,4-oxadiazol-2(3*H*)-one (**5c**): Yield 69.3%, mp 169—170 °C. IR (KBr): 2249, 1779, 1613, 1248, 986 cm^{-1}. ^{1}H-NMR (DMSO) δ: 2.80 (2H, t, J=6.3 Hz, CH_2CH_2), 4.01 (2H, t, J=6.3 Hz, CH_2CN), 5.01 (2H, s, CH_2O), 6.93 (2H, d, J=6.9 Hz, aromatics), 7.18 (2H, d, J=6.7 Hz, aromatics), 7.65 (2H, d, J=6.7 Hz, aromatics), 7.69 (2H, d, J=6.9 Hz, aromatics). *Anal.* Calcd for $C_{18}H_{14}IN_3O_3$: C, 48.34; H, 3.16; N, 9.40. Found: C, 48.30; H, 3.18; N, 9.43. HRMS calcd for *m*/*z*: 447.0082. Found: 447.0079.

5-[4-(Benzyloxy)3-iodophenyl]-3-(2-cyanoethyl)-1,3,4-oxadiazol-2(3*H*)-one (**5d**): Yield 83.8%, mp 134—135 °C. IR (KBr): 2246, 1784, 1611, 1250, 982 cm^{-1}. ^{1}H-NMR (DMSO) δ: 3.01 (2H, t, J=6.2 Hz, CH_2CH_2), 4.02 (2H, t, J=6.2 Hz, CH_2CN), 5.30 (2H, s, CH_2O), 7.24—8.16 (8H, m, aromatics). *Anal.* Calcd for $C_{18}H_{14}IN_3O_3$: C, 48.34; H, 3.16; N, 9.40. Found: C, 48.36; H, 3.16; N, 9.41. HRMS calcd for *m*/*z*: 447.0082. Found: 447.0071.

Assay of MAO Activity Rat liver was homogenized with 10 volumes of 0.25 M sucrose and 0.05 mM phosphate buffer (pH 7.4) under cooling. The homogenates were centrifuged at 1000 ***g*** for 10 min to remove cell debris. The protein content was measured by the biuret method.[31] The MAO activity was assayed radiochemically using [^{14}C]phenethylamine and [^{14}C]serotonin as substrates, respectively.[27]

5-[4-(Tri-*n*-butylstannylbenzyloxy)phenyl]-1,3,4-oxadiazol-2(3*H*)-one (6) Compound **5a** (0.10 g, 0.22 mmol) and hexa-*n*-butykditin (0.34 ml, 0.67 mmol) were dissolved in dry toluene (5 ml), and a catalytic amount of tetrakis(triphenylphosphine)palladium was added. The mixture was refluxed for 48 h under an argon atmosphere. After cooling, the reaction mixture was filtered through celite. The filtrate was concentrated *in vacuo* and the oily residue was purified by column chromatography on silica gel elution with chloroform–MeOH (40 : 1) affording **6**.

Yield 57.7%, IR ($CHCl_3$): 2250, 1773, 1612, 1510, 1002 cm^{-1}. ^{1}H-NMR (DMSO) δ: 0.83—1.62 (27H, m, Bu_3Sn), 2.92 (2H, t, J=6.3 Hz, CH_2CH_2), 4.13 (2H, t, J=6.3 Hz, CH_2CN), 5.04 (2H, s, CH_2O), 7.06 (2H, d, J=6.9 Hz, aromatics), 7.24—7.68 (4H, m, aromatics), 7.83 (2H, d, J=6.9 Hz, aromatics). HRMS calcd for *m*/*z*: 611.2170. Found: 611.2167.

5-[4-(2-[^{125}I]Iodobenzyloxy)phenyl]-3-(2-cyanoethyl)-1,3,4-oxadiazol-2(3*H*)-one ([^{125}I]2-IBPO) Aqueous hydrogen peroxide (10 μl, 30% w/v) was added to a mixture of **6** (10 μl, 1 mg/ml in ethanol), 0.1 M HCl (0.1 ml), and sodium [^{125}I]iodide (10 μl, 7.4 MBq, specific activity 7.4 TBq/mmol) in a sealed vial. The reaction was allowed to proceed for 30 min at room temperature, after which it was terminated by the addition of sodium bisulfite (0.1 ml, 100 mg/ml in water). [^{125}I]2-IBPO was isolated by HPLC using water–methanol (2 : 8, v/v) as an eluent at a flow rate of 3.0 ml/min. The product fractions were collected and the solvent was removed *in vacuo*. The final product, [^{125}I]2-IBPO, was taken up in an isotonic saline solution and passed through a 0.22 μm filter. The radiochemical yield was 90—95%. The radiochemical purity and specific activity were higher than 99% and 7.4 TBq/mmol, respectively, as determined by HPLC.

***In Vivo* Tissue Biodistribution Studies in Mice** Groups of four male ddY mice (20—25 g) were injected intravenously through a lateral tail vein with [^{125}I]2-IBPO in 0.1 ml of saline solution. At the desired time interval after administration, the animals were sacrificed. Samples of blood and organs of interest were excised and weighed. The radioactivity was measured using a well-type NaI(Tl) gamma scintillation counter. The results were expressed in terms of the percentage of the injected dose per gram of blood or organs.

Effect of Various MAO Inhibitors on Tissue Distribution of [^{125}I]2-IBPO For our pretreatment experiment, mice were given an i.v. injection of *l*-depreny, Ro16-6491, clorgyline or Ro-41-1049 (10 mg/kg, respectively) 1 h before administration of [^{125}I]2-IBPO. At the desirable time after the injection of [^{125}I]2-IBPO, these mice were killed by decapitation. Samples of blood and organs of interest were excised, weighed, and the radioactivity of these samples measured. These results were expressed as a percentage of the injected dose per gram of blood or organs compared with the non-treated control mice, referred to as 100%.

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