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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-iodobenzoyloxy)phenyl]-3-(cyanooethyl)-1,3,4-oxadiazole-2(3H)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, [125I]2-IBPO was conveniently synthesized from a tributylstannyl precursor by an iododestannylation reaction using sodium [125I]iodide and hydrogen peroxide with high radiochemical yield. The in vivo tissue distribution studies of [125I]2-IBPO demonstrated its high initial uptake and prolonged retention in the brain. A selective interaction of [125I]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 [125I]2-IBPO suggested that a 125I-labeled counterpart, [125I]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—10 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 appropriate benzyl bromide derivatives.23—25 and 123I-labeled pargyline26) have been investigated as ligands for PET and SPECT. Recently, a new reversible and highly selective MAO-B inhibitor, 5-[4-(benzyloxy)phenyl]-3-(cyanooethyl)-1,3,4-oxadiazole-2(3H)one (MD-230254), was developed.27 The 11C-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 cyclotron. On the other hand, SPECT studies are more commonly used in nuclear medicine clinics. We have explored the feasibility of a [123I]radiiodinated MAO-B inhibitor as an alternative to [11C]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 123I or 125I. 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](image-url)

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The desired compounds 5a–d were conveniently synthesized from appropriate monosubstituted hydrazines 4a–d by a cyclization reaction using bis(trichloromethyl)carbonate (triphosgene) 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 mitochondria fraction were measured for selectivity in vitro using [14C]serotonin and [14C]phenethylamine, according to a modified radiochemical procedure. 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) examined under the same conditions. The selectivity of these inhibitors toward MAO-B was 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). On the basis of these results, a two-step interaction between 2-IBPO and MAO-B can be assumed according to equilibrium, where the substrate (E) and inhibitor (I) combine rapidly to form a reversible complex EI which is isomerized slowly into a tighter complex EI*. The inhibition constant (K_I) value and an overall inhibition constant (K_I*) value at equilibrium were calculated as 2.4 nM and 3.8 nM, respectively.

**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 [125I]2-IBPO was performed.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC_{50} (nM)</th>
<th>MAO-A/MAO-B</th>
</tr>
</thead>
<tbody>
<tr>
<td>5a</td>
<td>2.0</td>
<td>&gt;100000</td>
</tr>
<tr>
<td>5b</td>
<td>3.6</td>
<td>&gt;100000</td>
</tr>
<tr>
<td>5c</td>
<td>13.0</td>
<td>&gt;100000</td>
</tr>
<tr>
<td>5d</td>
<td>2.2</td>
<td>&gt;100000</td>
</tr>
<tr>
<td>MD-230254</td>
<td>1.8</td>
<td>&gt;100000</td>
</tr>
<tr>
<td>L-Deprenyl</td>
<td>8.8</td>
<td>920</td>
</tr>
</tbody>
</table>

**Table 1. Inhibition of MAO by Iodinated Analogues of MD-230254**
using an iododestannylation reaction with a tributylstannyll 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 tributylstannyll derivative 6 in moderate to high yield (57.7%). Radiiodination of 6 was achieved using hydrogen peroxide as an oxidant and sodium \(^{[125\text{I}]}\)iodide (specific activity 74 GBq/\(\mu\)mol) in 0.1 M HCl/ethanol solution at room temperature, followed by HPLC purification. The radiochemical yield of the product, \(^{[125\text{I}]}\)2-IBPO, was 90—93% based on sodium \(^{[125\text{I}]}\)iodide. The radiochemical purity of \(^{[125\text{I}]}\)2-IBPO was higher than 99%, as assessed by HPLC analysis, with specific radioactivity of approximately 74 GBq/\(\mu\)mol. This method should be applicable for labeling with \(^{123}\text{I}\), a very suitable radioisotope (half-life 13 h and gamma ray energy of 159 keV) for \textit{in vivo} imaging with SPECT.

**Tissue Distribution** In \textit{vivo} tissue distribution of the \(^{[125\text{I}]}\)2-IBPO was examined in male ddY mice at 5, 15, 30, 60, 120, 180 min after intravenous administration. As summarized in Table 2, \(^{[125\text{I}]}\)2-IBPO was transported well into various tissues. The maximum uptake of \(^{[125\text{I}]}\)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 \(^{[125\text{I}]}\)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 \(^{[125\text{I}]}\)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 \(^{[125\text{I}]}\)2-IBPO to MAO-B \textit{in vivo} was demonstrated by the pretreatment study (Fig. 3). The effect of pretreatment with \textit{l}-deprenyl, Ro-16-6491, clorgyline, and Ro-41-1049 on the distribution of \(^{[125\text{I}]}\)2-IBPO at 120 min after injection are

### Table 2. Tissue Distribution of \(^{[125\text{I}]}\)2-IBPO in Mice

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Time after injection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 min</td>
</tr>
<tr>
<td>Brain</td>
<td>2.44 (0.44)</td>
</tr>
<tr>
<td>Blood</td>
<td>0.87 (0.07)</td>
</tr>
<tr>
<td>Pancreas</td>
<td>3.29 (0.37)</td>
</tr>
<tr>
<td>Spleen</td>
<td>1.49 (0.21)</td>
</tr>
<tr>
<td>Stomach</td>
<td>2.06 (0.20)</td>
</tr>
<tr>
<td>Liver</td>
<td>17.9 (0.37)</td>
</tr>
<tr>
<td>Kidney</td>
<td>3.73 (0.47)</td>
</tr>
<tr>
<td>Heart</td>
<td>5.92 (0.44)</td>
</tr>
<tr>
<td>Lung</td>
<td>4.08 (0.33)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Brain/Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 min</td>
<td>2.80 (0.33)</td>
</tr>
<tr>
<td>15 min</td>
<td>4.30 (0.33)</td>
</tr>
<tr>
<td>30 min</td>
<td>4.30 (0.33)</td>
</tr>
<tr>
<td>60 min</td>
<td>3.55 (0.33)</td>
</tr>
<tr>
<td>120 min</td>
<td>3.36 (0.33)</td>
</tr>
<tr>
<td>180 min</td>
<td>2.80 (0.33)</td>
</tr>
</tbody>
</table>

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

![Fig. 3. Effect of \textit{l}-Deprenyl ( ), Ro-16-6491 ( ), Clorgyline ( ) and Ro-41-1049 ( ) on the Uptake of \(^{[125\text{I}]}\)2-IBPO](image)

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 [125I]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 [125I]2-IBPO uptake in the brain. Pretreatment with clorgyline indicated about a 20% reduction of [125I]2-IBPO uptake in the brain. This decrease was caused by MAO-B inhibition of clorgyline. The pretreatment studies data suggested that [125I]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 [125I]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 [125I]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.19,20 The 121I-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. [125I]2-IBPO was conveniently synthesized from a tributylstannyl precursor by an iododeesterfication reaction using sodium [125I]iodide and hydrogen peroxide with high radiochemical yield. The in vivo tissue distribution studies of [125I]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 [125I]2-IBPO to MAO-B. These very desirable characteristics of [125I]2-IBPO suggest that its 121I-labeled counterpart, [125I]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-612 V ol. 50, No. 5 apparatus and uncorrected. Infrared (IR) spectra were taken on a JASCO IR-612 apparatus and uncorrected. Infrared (IR) spectra were taken on a JASCO IR-612 apparatus and uncorrected. Infrared (IR) spectra were taken on a JASCO IR-612 apparatus and uncorrected. Infrared (IR) spectra were taken on a JASCO IR-612 apparatus and uncorrected.

**Substituted [4-Benzoyl]benzoylhydrazide (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 obtained solid was recrystallized from MeOH.

4-Benzoyl]benzoylhydrazide (3a): Yield 81.8%, mp 134—136 °C. IR (KBr): 3319, 3199, 1603, 1251, 840 cm⁻¹.

**Substituted [4-Benzoyl]benzoylhydrazide (3b)**: Yield 55.9%, mp 160—160.5 °C. IR (KBr): 3324, 3200, 1594, 1260, 836 cm⁻¹.

**Substituted [4-Benzoyl]benzoylhydrazide (3c)**: Yield 81.8%, mp 134—136 °C. IR (KBr): 3319, 3199, 1603, 1251, 840 cm⁻¹.

**Substituted [4-Benzoyl]benzoylhydrazide (3d)**: Yield 49.8%, mp 128—130 °C. IR (KBr): 3313, 3199, 1603, 1251, 840 cm⁻¹.

**Substituted [4-Benzoyl]benzoylhydrazide (3e)**: Yield 49.8%, mp 128—130 °C. IR (KBr): 3313, 3199, 1603, 1251, 840 cm⁻¹.

**Substituted [4-Benzoyl]benzoylhydrazide (3f)**: Yield 49.8%, mp 128—130 °C. IR (KBr): 3313, 3199, 1603, 1251, 840 cm⁻¹.

**Substituted [4-Benzoyl]benzoylhydrazide (3g)**: Yield 49.8%, mp 128—130 °C. IR (KBr): 3313, 3199, 1603, 1251, 840 cm⁻¹.

**Substituted [4-Benzoyl]benzoylhydrazide (3h)**: Yield 49.8%, mp 128—130 °C. IR (KBr): 3313, 3199, 1603, 1251, 840 cm⁻¹.

**Substituted [4-Benzoyl]benzoylhydrazide (3i)**: Yield 49.8%, mp 128—130 °C. IR (KBr): 3313, 3199, 1603, 1251, 840 cm⁻¹.

**Substituted [4-Benzoyl]benzoylhydrazide (3j)**: Yield 49.8%, mp 128—130 °C. IR (KBr): 3313, 3199, 1603, 1251, 840 cm⁻¹.

**Substituted [4-Benzoyl]benzoylhydrazide (3k)**: Yield 49.8%, mp 128—130 °C. IR (KBr): 3313, 3199, 1603, 1251, 840 cm⁻¹.

**Substituted [4-Benzoyl]benzoylhydrazide (3l)**: Yield 49.8%, mp 128—130 °C. IR (KBr): 3313, 3199, 1603, 1251, 840 cm⁻¹.
983 cm$^2$. IR (KBr): 3261, 3208, 2251, 1598, 1365 cm$^{-1}$. Anal. Calcd for C$_{17}$H$_{16}$I$_3$N$_3$O$_2$: C, 48.45; H, 3.16; N, 9.98. Found: C, 48.45; H, 3.18; N, 9.43. HRMS calcd for C$_{44}$H$_{43}$I$_{11}$N$_{13}$O$_{13}$: 919.6511. Found: 919.6508.

References


1-[(4-Iodobenzyloxy)benzoyl]-2-(2-cyanoethyldihydrazine (4d): Yield 80.7%, mp 134—135 °C. IR (KBr): 2246, 1784, 1611, 1250, 748 cm$^{-1}$. Anal. Calcd for C$_{17}$H$_{16}$I$_3$N$_3$O$_2$: C, 48.45; H, 3.16; N, 9.98. Found: C, 48.45; H, 3.18; N, 9.43. HRMS calcd for C$_{44}$H$_{43}$I$_{11}$N$_{13}$O$_{13}$: 919.6511. Found: 919.6508.

References