Design, synthesis and evaluation of 2-(indolylmethylidene)-2,3-dihydro-1-benzofuran-3-one and 2-(indolyl)-4H-chromen-4-one derivatives as novel monoamine oxidases inhibitors

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A B S T R A C T

A series of 2-(indolylmethylidene)-2,3-dihydro-1-benzofuran-3-ones (aurone-indole hybrids) and 2-(indolyl)-4H-chromen-4-ones (flavone-indole hybrids) were designed, synthesized, and their monoamine oxidase (MAO) A and B inhibitory activities were evaluated. Compounds 5b and 11b showed potent inhibitory activities against MAO-A, comparable to that of pargyline used as a positive control, and most of the compounds, except for 2a and 10b, showed potent inhibitory activities against MAO-B. Compound 9a was the most potent and highly selective inhibitor of MAO-B (IC50 value for MAO-B: 0.0026 μM, and MAO-A: >100 μM). Comparison of the inhibitory activities of 1a vs. 9a vs. 13a and 1b vs. 7b vs. 11b suggested that methoxy substitution at R1 on the A-rings of flavonoids increases MAO-A inhibition whereas methoxy substitution at R2 increased MAO-B inhibition. Comparison of 4a vs. 10a, 6a vs. 11a, 3b vs. 8b and 4b vs. 9b showed incremental increases in MAO-B inhibitory activity by R2 substitution on the A ring. Comparison of the MAO-B inhibitory effects of the flavone-indole hybrids and aurone-indole hybrids showed that most of the aurone-indole hybrids were stronger inhibitors than the corresponding flavone-indole hybrids. Molecular docking analysis of compounds 1a and 9a with MAO-B further supported the above structural effects of these compounds on MAO-B inhibitory activity.

This is the first report identifying aurone-indole hybrids as potent MAO-B inhibitors. The results reported here suggest that 2-(1H-indol-1-ylmethylene)-6-methoxy-3(2H)-benzofuranone (9a) might be a useful lead for the design and development of novel MAO-B inhibitors.

1. Introduction

Monoamine oxidases (MAOs, EC 1.4.3.4) are flavoenzymes located on the mitochondrial outer membranes of various mammalian cell types [1]. MAOs consist of two isoforms, MAO-A and MAO-B, and play an important role in the oxidative degradation of neurotransmitters such as dopamine, serotonin, and epinephrine. The MAO-A and MAO-B sequences share approximately 70% identity at the amino acid level and differ in their substrate and inhibitor sensitivities and three-dimensional structures. MAO-A preferentially degrades serotonin, norepinephrine, and epinephrine, and is irreversibly inhibited by clorgyline. On the other hand, MAO-B preferentially degrades dopamine, β-phenethylamine, and benzylamine, and is reversibly inhibited by (R)-(−)-deprenyl. The administration of MAO inhibitors is beneficial in the treatment of several neurodegenerative diseases [2,3]. Selective MAO-A inhibitors are used as anti-depressant and anti-anxiety drugs, and selective MAO-B inhibitors are used alone or in combination with other MAO-B inhibitors to treat Parkinson’s and Alzheimer’s diseases [4–7].

Natural products have long played a significant role in the development of new therapeutic leads. Flavonoids comprise a major category of natural products and represent a large subgroup of phenolic plant secondary metabolites which are widely distributed throughout the plant kingdom. Flavonoids are important sources of bioactive constituents which promote human health. Chemically, flavonoids are divided into subclasses such as flavones, isoflavones, flavanes, isoflavanes and anthocyanins, depending on the oxidation status and saturation level of the heterocyclic ring [8,9].

4H-1-Benzopyran-4-ones (chromones) are an important class of oxygenated heterocyclic compounds. Flavones and isoflavones contain the chromone structure, a pharmacophore found in many natural and synthetic bioactive molecules that have attracted the attention of scientists [10,11]. The biological effects of these bioactive molecules include anti-inflammatory, anti-tumor, and anti-microbial activities, as well as inhibitory activities towards cyclooxygenases, kinases, phosphatases, aromatases, acetylcholinesterases, and MAOs. 2-Azolylchromone derivatives, which are flavone analogues containing heterocycles, were recently reported by our laboratory to be potent and selective MAO inhibitors [12].

2-Benzylidenebenzofuran-3-(2H)-ones, also called aurones, are structural isomers of flavones. This subclass of flavonoids is found in vegetables and flowers [13]. Aurones have recently attracted considerable attention due to their wide range of bioactivities against...
neurological diseases. For example, several groups reported that naturally occurring and chemically synthesized aurones act as MAO inhibitors [14–17].

Indoles are another important class of heterocycles found in a large number of key natural compounds. Indole is a privileged structure found in natural alkaloids and drugs marketed for therapeutic use [18,19].

Taken together, evidence to date suggests that flavones, aurones and indoles could be useful starting compounds in the design of novel MAO inhibitors. Consequently, a series of flavonoid-indole hybrids were designed (Fig. 1), synthesized and investigated for their MAO inhibitory activities to further explore promising lead compounds for developing MAO inhibitors.

2. Results and discussion

2.1. Chemistry

Protocols for synthesizing the flavonoid-indole hybrids are shown in Fig. 2. Synthetic 3-iodochromone derivatives [12] and commercially available indole derivatives were used as the starting materials according to the method of Sugita and Yokoe [20]. Treatment of the appropriate 3-iodochromone with selected aurone-indole or flavone-indole derivatives in the presence of potassium carbonate in DMF while heating at 80 °C afforded the corresponding 2-(1H-indol-1-ylmethylene)-3(2H)-benzofuranone derivative (aurone-indole hybrids, 1a–13a) and 2-(1H-indol-1-yl)-4H-1-benzopyran-4-one derivative (flavone-indole hybrids, 1b–11b), respectively. In these reactions, the first step is the conjugate addition of the indole to the 2-position of the chromone ring to give the intermediate (A), followed by the elimination of H-I to afford the flavonoid-indole hybrid, compounds 1a–13a (aurone-indole hybrids) by path a and compounds 1b–11b (flavone-indole hybrids) by path b, respectively. The compounds 1a–13a might be formed through rearrangement reactions of the initially formed intermediate (A).

2.2. Biological activity

All synthesized aurone-indole hybrids (1a–13a) and flavone-indole hybrids (1b–11b) were evaluated for their MAO-A and MAO-B inhibitory activities (Table 1). Pargyline was used as a positive control, with IC50 values against MAO-A and MAO-B of 4.5 μM and 0.22 μM, respectively.

As shown in Table 1, substitutions on the indole ring and methoxy substitution on the A-rings of flavonoids revealed several interesting structure-activity relationships. Compounds 13a, 1b, 5b and 11b exhibited MAO-A inhibitory activity, with the activities of compounds 5b and 11b being comparable to that of pargyline. Most of the compounds showed potent MAO-B inhibition, except 2a and 10b, and selective MAO-B inhibition with selectivity index ranging between 2.4 and 38000, except 5b: compounds 1b, 2b and 6b were moderate, 12a, 13a, 3b, 4b, 5b, 7b, 8b, 9b and 11b were potent, and 1a 3a, 4a, 5a, 6a, 7a, 8a, 9a, 10a and 11a were much more potent than pargyline. Compound 9a was the most potent and highly selective inhibitor of MAO-B (IC50 value for MAO-B: 0.0026 μM, and for MAO-A: > 100 μM). To determine the mode of inhibition, a set of Lineweaver–Burk plots was constructed for the inhibition of MAO-B by compound 9a (Fig. 3). The lines of the Lineweaver–Burk plots intersected on the y-axis which indicates that the mode of inhibition is competitive and reversible. From a replot of the slopes of the Lineweaver-Burk plots versus the concentration of compound 9a, a Ki value of 0.0011 μM for the inhibition of MAO-B is estimated. To further investigate the reversibility of MAO-B inhibition by compound 9a was evaluated by examining the recovery of enzyme activity after the dilution of the enzyme-inhibitor mixtures according to
The inhibition of MAO-B with \(9a\) was reversible, whereas pargyline inhibited MAO-B irreversibly (data not shown).

The substitution on indole ring of \(9a\) decreased their potency.

Comparison of the inhibitory activities of \(1a\), \(9a\), \(13a\), \(1b\), \(7b\) and \(11b\) suggested that methoxy substitution at R1 on the A-ring of flavonoids increased MAO-A selectivity whereas methoxy substitution at R2 on the A-ring increased MAO-B selectivity.

Comparison of \(1a\) vs. \(1b\), \(4a\) vs. \(3b\), \(6a\) vs. \(5b\), \(7a\) vs. \(6b\), \(8a\) vs. \(7b\), \(9a\) vs. \(8b\), \(10a\) vs. \(9b\), \(12a\) vs. \(10b\) and \(13a\) vs. \(11b\) also showed that methoxy substitution at R2 on the A-ring induced MAO-B inhibitory activity and selectivity, although this correlation was not observed for \(12a\) and \(12b\). These results are consistent with our previous findings with 2-azolylchromone derivatives [12]. Interestingly, Nel et al. [22] reported that 2-benzylidene-1-indanone derivatives (aurone analogues) exhibited inhibitory effects towards MAOs. Their comparison of the inhibitory activities of 5-hydroxy substituted and 6-hydroxy substituted compounds showed reversal of isoform selectivity. Furthermore, 5-methoxy substitution induced MAO-B selectivity. Comparison of the flavone-indole hybrids and the corresponding aurone-indole hybrids (i.e., \(1a\) vs. \(1b\), \(4a\) vs. \(3b\), \(6a\) vs. \(5b\), \(7a\) vs. \(6b\), \(8a\) vs. \(7b\), \(9a\) vs. \(8b\), \(10a\) vs. \(9b\), \(12a\) vs. \(10b\) and \(13a\) vs. \(11b\)) showed that the aurone-indole hybrids inhibited MAO-B more strongly than the corresponding flavone-indole hybrids. Recently, Badavath et al. reported that the Ki values of 2-aryl-4H-chromen-4-ones [23] (flavone analogues) and 2-(arylmethylidene)-2,3-dihydro-1-benzofuran-3-one derivatives [24] (aurone analogues) for MAO inhibition were not significantly different. Other reports also showed weak inhibitory activities by flavones [25,26] and aurones [15–17] against MAOs.

Several indole derivatives have been reported to be potent inhibitors of MAOs [27,28], and indole-flavonoid hybrids, such as indole-based chalcones [29] and 3-indolylcoumarines [30], have been reported to be weak MAO inhibitors. In contrast, the present report is the first to identify aurone-indole hybrids as MAO-B inhibitors.

In an effort to elucidate the mechanism by which compound \(9a\) shows highly potent and selective inhibitory activity against MAO-B, the molecular docking of compound \(9a\) into the ligand binding model site of MAO-B was analyzed using a binding model based on the MAO-B complex structure (PDB code: 4A79) and compared with the molecular docking model obtained using compound \(1a\). Compound \(9a\) interacted with MAO-B through Phe168, Leu171, Cys172, Ile198, Ile199 and Ile316. The calculated free energy of binding of \(9a\) was \(-10.35\) kcal/mol. Compound \(1a\) interacted with MAO-B through Phe168, Leu171, Cys172 and Ile198, and the calculated free energy of binding was \(-10.13\) kcal/mol. These results show that methoxy substitution at R2 induced favorable binding energy and multiple interaction residues and supported the conclusion that methoxy substitution in compound \(9a\) is significantly related to inhibitory activity.

The active site structures of MAO-A and MAO-B were recently reported and show that MAO-B has a bipartite hydrophobic cavity comprising an entrance cavity and a substrate cavity [2,3]. The substrate cavity in MAO-B has a volume of \(\sim 430\) Å³ and the entrance cavity has a

### Table 1

IC\(_{50}\) values of flavonoid-indole hybrids \(1a–13a\) and \(1b–11b\) for MAO-A and MAO-B.

<table>
<thead>
<tr>
<th>Compd.</th>
<th>R(^1)</th>
<th>R(^2)</th>
<th>Indole (R(^3))</th>
<th>IC(_{50}) (µM) MAO-A</th>
<th>IC(_{50}) (µM) MAO-B</th>
<th>MAO-B Selectivity (A/B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>&gt;100</td>
<td>0.039</td>
<td>&gt;2600</td>
</tr>
<tr>
<td>2a</td>
<td>3-Me</td>
<td>H</td>
<td>H</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>-</td>
</tr>
<tr>
<td>3a</td>
<td>4-Me</td>
<td>H</td>
<td>H</td>
<td>&gt;100</td>
<td>0.066</td>
<td>&gt;1500</td>
</tr>
<tr>
<td>4a</td>
<td>5-Me</td>
<td>H</td>
<td>H</td>
<td>&gt;100</td>
<td>0.032</td>
<td>&gt;3100</td>
</tr>
<tr>
<td>5a</td>
<td>6-Me</td>
<td>H</td>
<td>H</td>
<td>&gt;100</td>
<td>0.057</td>
<td>&gt;1800</td>
</tr>
<tr>
<td>6a</td>
<td>4-MeO</td>
<td>H</td>
<td>H</td>
<td>&gt;100</td>
<td>0.040</td>
<td>&gt;2500</td>
</tr>
<tr>
<td>7a</td>
<td>5-MeO</td>
<td>H</td>
<td>H</td>
<td>&gt;100</td>
<td>0.090</td>
<td>&gt;1100</td>
</tr>
<tr>
<td>8a</td>
<td>6-MeO</td>
<td>H</td>
<td>H</td>
<td>&gt;100</td>
<td>0.039</td>
<td>&gt;2600</td>
</tr>
<tr>
<td>9a</td>
<td>H</td>
<td>MeO</td>
<td>H</td>
<td>&gt;100</td>
<td>0.0026</td>
<td>&gt;38000</td>
</tr>
<tr>
<td>10a</td>
<td>5-Me</td>
<td>MeO</td>
<td>H</td>
<td>&gt;100</td>
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<td>&gt;7100</td>
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<tr>
<td>11a</td>
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<td>H</td>
<td>&gt;100</td>
<td>0.013</td>
<td>&gt;7700</td>
</tr>
<tr>
<td>12a</td>
<td>6-MeO</td>
<td>MeO</td>
<td>H</td>
<td>&gt;100</td>
<td>0.12</td>
<td>&gt;830</td>
</tr>
<tr>
<td>13a</td>
<td>MeO</td>
<td>H</td>
<td>H</td>
<td>14</td>
<td>0.15</td>
<td>93</td>
</tr>
<tr>
<td>Pargyline</td>
<td></td>
<td></td>
<td></td>
<td>4.5</td>
<td>0.22</td>
<td>20</td>
</tr>
</tbody>
</table>

MAO-B Selectivity (A/B) is given as the ratio of the IC\(_{50}\) value for MAO-A to the IC\(_{50}\) value for MAO-B.
volume of \(\sim 290\text{Å}^3\). The combined volume of the two cavities is \(\sim 700\text{Å}^3\) when the gating Ile199 is in the open conformation. The active site of MAO-A differs from that of MAO-B in that it has a monopartite cavity with a total volume of \(\sim 550\text{Å}^3\). It therefore appears that MAO-B recognizes larger substrates and less stringently than does MAO-A. Consequently, compound 9a might be more selective for MAO-B. Furthermore, Ile199 is an important residue in MAO-B active site recognition, and thus compound 9a might show stronger inhibitory activity against MAO-B than compound 1a.

This is the first report identifying auorene-indole hybrids as MAO-B inhibitors. 2-(1H-Indol-1-ylmethylene)-6-methoxy-3(2H)-benzofuranone (9a) may be a useful lead compound for the design and development of novel MAO-B inhibitors.

3. Experimental

3.1. Chemistry

All reagents and solvents were purchased from commercial sources. 3-Iodochromone derivatives were synthesized according to previous methods [12]. Analytical thin-layer chromatography was performed on silica-coated plates (silica gel 60 F-254; Merck Ltd., Tokyo, Japan) and visualized under UV light. Column chromatography was carried out using silica gel (Wakogel C-200; Wako Pure Chemical Industry Co., Tokyo, Japan). All melting points were determined using a Yanagimoto micro-hot stage and are uncorrected. \(^1\)H NMR and \(^13\)C NMR spectra were recorded on a Varian 400-MR spectrometer using tetramethylsilane as the internal standard. MS spectra were measured using a JEOL JMS-700 spectrometer. Elemental analyses were carried using a Yanaco CHN MT-6 elemental analyzer.

General procedure for preparation of 2-(1H-1ndol-1-ylmethylene)-3(2H)-benzofuranone and 2-(1H-1ndol-1-yl)-4H-1-benzopyran-4-one derivatives (1–13)

The title compounds were synthesized according to a previous method [20]. A mixture of 3-iodochromone (2 mmol), the appropriate indole (4 mmol) and K₂CO₃ (20 mmol) in DMF (20 mL) was stirred at 80°C for 2–6 h. The reaction mixture was extracted with water and CHCl₃. The organic layer was dried over Na₂SO₄ and the solvent was evaporated in vacuo. The residue was purified by silica gel column chromatography (hexane:AcOEt = 20:1) to give the 2-(1H-1ndol-1-ylmethylene)-3(2H)-benzofuranone derivatives (1a–13a) and the 2-(1H-1ndol-1-yl)-4H-1-benzopyran-4-one derivatives (1b–11b).

2-(1H-1ndol-1-ylmethylene)-3(2H)-benzofuranone derivative (1a)

1a: Yield 24%. Yellow needles. mp 205–207°C (lit. 207–208°C [20]). \(^1\)H NMR (CDCl₃, 400 MHz) \(\delta 8.20 (1H, d, J = 3.6, H-2'), 7.85 (1H, dd, J = 7.7, 1.7, H-4'), 7.69 (1H, s, =CH), 7.68 (1H, dd, J = 8.3, 1.0, H-5'), 7.30–7.25 (2H, m, H-6'), 7.26 (1H, dd, J = 7.7, 1.2, H-6'), 7.36 (1H, dd, J = 8.3, 1.0, H-7'), 7.29 (1H, dd, J = 7.7, 1.2, H-7'), 7.26 (1H, dd, J = 7.7, 7.3, 1.0, H-5'), 6.84 (1H, d, J = 3.6, H-3'). \(^13\)C NMR (CDCl₃, 100 MHz) \(\delta 183.7, 164.8, 136.7, 136.1, 135.5, 129.0, 124.3, 123.9, 123.4, 123.0, 121.4, 112.8, 110.6, 110.0, 109.9, 109.6, 98.9. MS (EI) m/z 261 [M]⁺. The \(^1\)H NMR spectrum was similar to that previously reported [20].

1b: Yield 23%. Brown needles; mp 157–158°C (lit. 157–158°C [20]). \(^1\)H NMR (CDCl₃, 400 MHz) \(\delta 8.27 (1H, d, J = 7.9, 1.7, H-5), 8.01 (1H, dd, J = 8.3, 0.9, H-7'), 7.74 (1H, dd, J = 8.4, 7.2, 1.7, H-7), 7.67 (1H, dd, J = 7.8, 1.1, H-4'), 7.60 (1H, dd, J = 8.4, 1.1, H-8), 7.57 (1H, d, J = 3.7, H-2'), 7.48 (1H, dd, J = 7.9, 7.2, 1.1, H-6), 7.39 (1H, dd, J = 8.3, 7.2, 1.1, H-6'), 7.30 (1H, dd, J = 7.8, 7.2, 0.9, H-5'), 6.82 (1H, d, J = 3.7, H-3'), 6.47 (1H, s, H-3). \(^13\)C NMR (CDCl₃, 100 MHz) \(\delta 178.1, 156.6, 154.5, 134.6, 133.8, 130.7, 130.6, 125.8, 124.6, 124.5, 123.5, 123.0, 121.8, 117.4, 113.4, 108.9, 97.4. MS (EI) m/z 261 [M]⁺. The \(^1\)H NMR spectrum was similar to that previously reported [20].
6.94 (1H, d, J = 3.6 Hz, H-3), 6.74–6.69 (1H, m, H-5), 3.98 (3H, s, OCH₃).

13C NMR (CDCl₃, 100 MHz) δ 183.9, 164.9, 153.5, 138.2, 136.2, 135.6, 125.0, 124.4, 123.4, 122.5, 119.3, 112.9, 111.1, 106.8, 103.3, 103.2, 55.5. MS (EI) m/z 291 [M]⁺. Anal. Calc. for C₂₇H₂₉NO₃: C, 74.22; H, 4.50; N, 4.81. Found: C, 73.92; H, 4.46; N, 4.77.

7b: Yield 17%. Pale brown solid. mp 172–174°C. 1H NMR (CDCl₃, 400 MHz) δ 8.18 (1H, d, J = 8.8 Hz, H-5), 7.98 (1H, dd, J = 8.4, 1.0 Hz, H-7), 7.68 (1H, dd, J = 7.8, 1.2 Hz, H-4), 7.56 (1H, d, J = 3.6 Hz, H-2), 7.40 (1H, ddd, J = 8.4, 7.2, 1.2 Hz, H-6), 7.30 (1H, ddd, J = 7.8, 7.2, 1.0 Hz, H-5), 7.05 (1H, dd, J = 8.8, 2.3 Hz, H-6), 6.99 (1H, d, J = 2.3 Hz, H-3), 6.81 (1H, d, J = 3.6 Hz, H-3), 6.41 (1H, s, H-3), 3.97 (3H, s, OCH₃). 13C NMR (CDCl₃, 100 MHz) δ 177.7, 164.2, 156.3, 156.1, 134.6, 130.6, 123.7, 124.4, 124.9, 122.1, 117.1, 111.4, 113.2, 108.6, 100.4, 97.5, 56.0. MS (EI) m/z 291 [M]⁺. Anal. Calc. for C₂₇H₂₉NO₃: C, 74.22; H, 4.50; N, 4.81. Found: C, 73.79; H, 4.47; N, 4.81.

6-Methoxy-2-(5-methyl-1H-indol-1-yl)-methylene)-3(2H)-Benzofuranone (10a) and 7-methoxy-2-(5-Methyl-1H-indol-1-yl)-4H-1-benzopyran-4-one (8b)

10a: Yield 55%. Yellow needles. mp 179–181°C. 1H NMR (CDCl₃, 400 MHz) δ 8.10 (1H, d, J = 3.6 Hz, H-2), 7.73 (1H, d, J = 9.1 Hz, H-4), 7.68 (1H, s, =CH), 7.54 (1H, d, J = 8.4 Hz, H-7), 7.41 (1H, br s, H-4), 7.18 (1H, d, J = 8.4, 1.6 Hz, H-6), 6.81–6.76 (2H, m, H-5 and H-7), 6.73 (1H, d, J = 3.6 Hz, H-3), 3.93 (3H, s, OCH₃), 2.46 (3H, s, CH₃).

13C NMR (CDCl₃, 100 MHz) δ 182.3, 167.1, 166.9, 136.2, 150.3, 135.2, 129.2, 127.2, 125.4, 125.3, 115.7, 112.2, 109.9, 109.7, 90.9, 96.5, 56.0, 21.4. MS (EI) m/z 305 [M]⁺. Anal. Calc. for C₂₇H₂₉NO₃: C, 74.74; H, 4.95; N, 4.59. Found: C, 74.63; H, 4.90; N, 4.67.

6-Methoxy-2-(5-methyl-1H-indol-1-yl)-methylene)-(2H)-Benzofuranone (10a) and 7-methoxy-2-(5-Methyl-1H-indol-1-yl)-4H-1-benzopyran-4-one (8b)
J = 3.6 Hz, H-3′), 3.94 (3H, s, OCH3), 3.92 (3H, s, OCH3). 13C NMR (CDCl3, 100 MHz) δ 182.4, 167.2, 167.0, 157.8, 137.9, 136.3, 125.9, 125.4, 122.7, 121.9, 115.6, 112.5, 112.3, 109.7, 109.1, 96.5, 94.0, 56.0, 55.9. MS (EI) m/z 321 [M]+. Anal. Calc'd for C19H15NO4: C, 71.02; H, 4.71; N, 4.36. Found: C, 71.03; H, 4.66; N, 4.57.

10b: Yield 16%. Brown needles. mp 195–196 °C. 1H NMR (CDCl3, 400 MHz) δ 8.17 (1H, d, J = 8.8 Hz, H-5), 7.54 (1H, d, J = 8.6 Hz, H-4'), 7.46 (1H, d, J = 2.3 Hz, H-7'), 7.43 (1H, d, J = 3.6 Hz, H-6'). 7.04 (1H, dd, J = 8.8, 2.3 Hz, H-6), 6.97 (1H, d, J = 2.3 Hz, H-8), 6.94 (1H, dd, J = 8.6, 2.3 Hz, H-5'), 6.72 (1H, d, J = 3.6 Hz, H-3'), 6.38 (1H, s, H-3), 3.96 (3H, s, OCH3), 3.92 (3H, s, OCH3). 13C NMR (CDCl3, 100 MHz) δ: 177.7, 164.2, 157.9, 156.4, 156.1, 135.5, 127.3, 124.5, 123.6, 122.1, 117.2, 114.2, 111.6, 108.4, 100.5, 98.1, 75.6, 56.0, 55.9. MS (EI) m/z 321 [M]+. Anal. Calc'd for C19H15NO4: C, 71.02; H, 4.71; N, 4.36. Found: C, 71.03; H, 4.66; N, 4.57.

2-(1H-Indol-1-ylmethylene)-5-methoxy-3(2H)-benzofuranone (13a) and 2-(1H-Indol-1-yl)-6-methoxy-4H-1-benzopyran-4-one (11b)

3.2.1. MAO inhibitory assay

Recombinant human monoamine oxidase A (MAO-A), MAO-B, pargyline and kynuramine were purchased from Sigma-Aldrich Japan Co., Tokyo, Japan.

3.2.2. Lineweaver–burk plots

For the inhibition of MAO-B by 9a, a set consisting of four Lineweaver–Burk plots was constructed. The first plot was constructed in the absence of inhibitor, while the remaining three plots were constructed in the presence of various concentrations of the test inhibitor: 1/2 × IC50, 1 × IC50, and 2 × IC50 (IC50 = 0.0026 μM). The enzyme substrate kynuramine was used at concentrations ranging from 7.5 to 120 μM.

3.2.3. Molecular docking study

The MAO-B crystal structure was retrieved from the Protein Data Bank (PDB code: 4A79) and imported into the AutoDock program (Version 4.2). The structures of compounds 1a and 9a were drawn using ChemBioDrawUltra 11.0 and subjected to energy minimization using molecular mechanics (MM2). AutoGrid was used to calculate the grid maps and the grid was centered on the ligand binding site of MAO-B such that it would totally cover the ligand molecule. The centroid of the grid map was set to X: 17, Y: 125, Z: 29, and the number of grid points was X: 54, Y: 60, Z: 54. The maximum number of energy evaluations was set to 250,000. Ligand and receptor docking was performed using the Lamarckian Genetic Algorithm (Runs 20) after using the default parameter settings generated by Auto Dock Tools for docking.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bios.2019.03.042.

References


