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Design, synthesis and evaluation of 2-(indolylmethylidene)-2,3-dihydro-1-benzofuran-3-one and 2-(indolyl)-4*H*-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)-4*H*-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 (IC_{50} 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 R^1 on the A-rings of flavonoids increases MAO-A inhibition whereas methoxy substitution at R^2 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 R^2 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 irreversibly 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].

4*H*-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-(2*H*)-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

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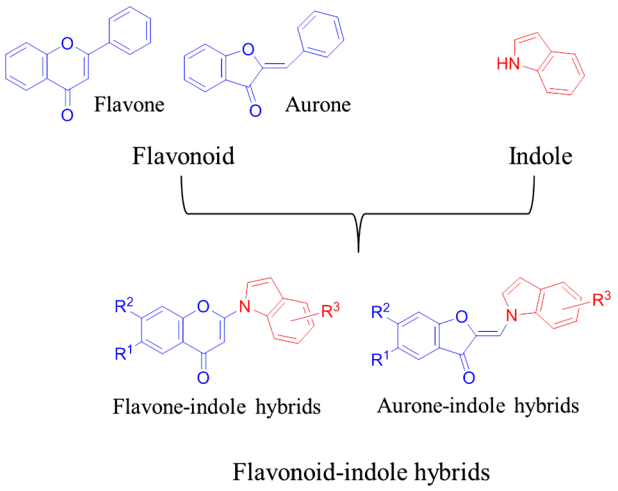

Fig. 1. Design strategy for the flavonoid-indole hybrids.

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 IC_{50} 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 (IC_{50} 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

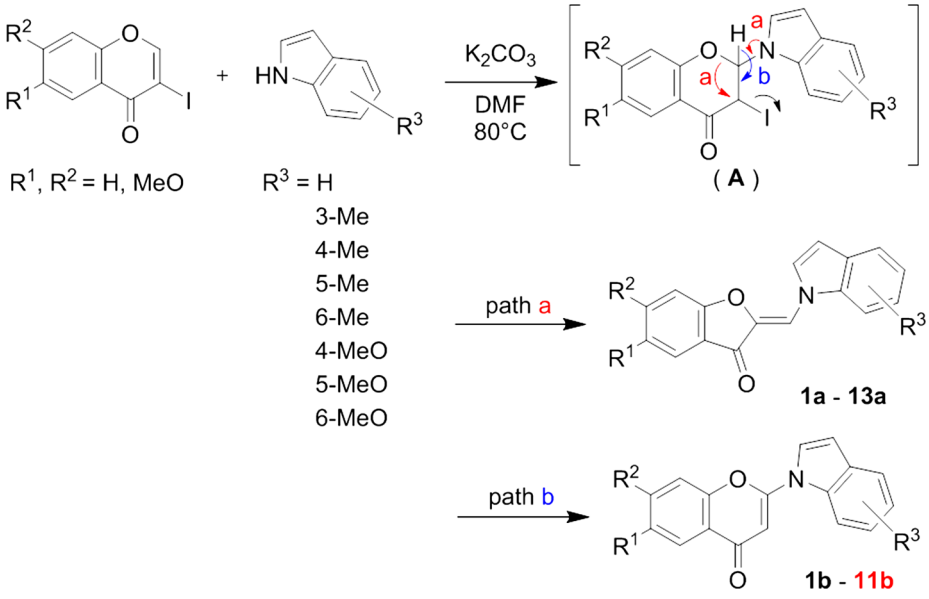

Fig. 2. Synthetic protocol for the flavonoid-indole hybrids. Reagents and conditions: K_2CO_3, DMF, 80 °C.

Table 1
IC_{50} values of flavonoid-indole hybrids **1a–13a** and **1b–11b** for MAO-A and MAO-B.

Compd.	R^1	R^2	Indole (R^3)	IC_{50} (μM)		MAO-B Selectivity (A/B)
				MAO-A	MAO-B	
1a	H	H	H	>100	0.039	>2600
2a			3-Me	>100	>100	-
3a			4-Me	>100	0.066	>1500
4a			5-Me	>100	0.032	>3100
5a			6-Me	>100	0.057	>1800
6a			4-MeO	>100	0.040	>2500
7a			5-MeO	>100	0.090	>1100
8a			6-MeO	>100	0.039	>2600
9a	H	MeO	H	>100	0.0026	>38000
10a			5-Me	>100	0.014	>7100
11a			4-MeO	>100	0.013	>7700
12a			6-MeO	>100	0.12	>830
13a	MeO	H	H	14	0.15	93
Pargyline				4.5	0.22	20

Compd.	R^1	R^2	Indole (R^3)	IC_{50} (μM)		MAO-B Selectivity (A/B)
				MAO-A	MAO-B	
1b	H	H	H	5.6	2.3	2.4
2b			3-Me	>100	24	> 4.1
3b			5-Me	>100	0.78	>130
4b			4-MeO	>100	0.74	> 140
5b			5-MeO	0.60	0.71	-
6b			6-MeO	>100	3.4	> 29
7b	H	MeO	H	>100	0.15	> 670
8b			5-Me	>100	0.15	> 670
9b			4-MeO	>100	0.26	> 380
10b			6-MeO	>100	>100	-
11b	MeO	H	H	0.32	0.63	0.51

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.

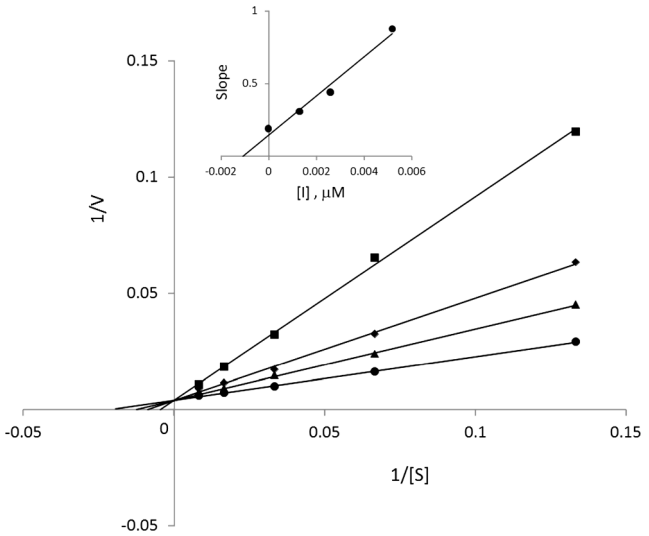

Fig. 3. Lineweaver–Burk plots for the inhibition of MAO-B by **9a**. The plots were constructed in the absence (filled circles) and presence of various concentrations of **9a**. The inset is a graph of the slopes of the Lineweaver–Burk plots versus inhibitor concentration (Ki = 0.0011 μM). The rate (V) is expressed as % of control (Kynuramine was used at 30 μM).

a literature method [21]. 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 R^1 on the A-ring of flavonoids increased MAO-A selectivity whereas methoxy substitution at R^2 on the A-ring increased MAO-B selectivity.

Comparison of **4a** vs. **10a**, **6a** vs. **11a**, **3b** vs. **8b** and **4b** vs. **10b** also showed that methoxy substitution at R^2 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. **4b**, **7a** vs. **5b**, **8a** vs. **6b**, **9a** vs. **7b**, **10a** vs. **8b**, **11a** 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-4*H*-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 R^2 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 ~430 $Å^3$ and the entrance cavity has a

volume of ~290 Å^3. The combined volume of the two cavities is ~700 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 ~550 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 aurone-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-indol-1-ylmethylene)-3(2H)-benzofuranone and 2-(1H-indol-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 K_2CO_3 (20 mmol) in DMF (20 mL) was stirred at 80 °C for 2–6 h. The reaction mixture was extracted with water and $CHCl_3$. The organic layer was dried over Na_2SO_4 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-indol-1-ylmethylene)-3(2H)-benzofuranone derivatives (**1a–13a**) and the 2-(1H-indol-1-yl)-4H-1-benzopyran-4-one derivatives (**1b–11b**).

2-(1H-Indol-1-ylmethylene)-3(2H)-benzofuranone (**1a**) and 2-(1H-indol-1-yl)-4H-1-benzopyran-4-one (**1b**)

1a: Yield 24%. Yellow needles. mp 205–207 °C (lit. 207 °C [20]). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.20 (1H, d, J = 3.6 Hz, H-2′), 7.85 (1H, dd, J = 7.7, 1.7 Hz, H-4), 7.82 (1H, s, =CH), 7.68 (1H, dd, J = 8.3, 1.0 Hz, H-7′), 7.67 (1H, ddd, J = 8.3, 7.3, 1.7 Hz, H-6), 7.65 (1H, dd, J = 7.7, 1.2 Hz, H-4′), 7.38 (1H, ddd, J = 8.3, 7.2, 1.2 Hz, H-6′), 7.36 (1H, dd, J = 8.3, 1.0 Hz, H-7), 7.29 (1H, ddd, J = 7.7, 7.2, 1.0 Hz, H-5′), 7.26 (1H, ddd, J = 7.7, 7.3, 1.0 Hz, H-5), 6.84 (1H, d, J = 3.6 Hz, H-3′). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 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 ($CDCl_3$, 400 MHz) δ 8.27 (1H, dd, J = 7.9, 1.7 Hz, H-5), 8.01 (1H, dd, J = 8.3, 0.9 Hz, H-7′), 7.74 (1H, ddd, J = 8.4, 7.2, 1.7 Hz, H-7), 7.67 (1H, dd, J = 7.8, 1.1 Hz, H-4′), 7.60 (1H, dd, J = 8.4, 1.1 Hz, H-8), 7.57 (1H, d, J = 3.7 Hz, H-2′), 7.48 (1H, ddd, J = 7.9, 7.2, 1.1 Hz, H-6), 7.39 (1H, ddd, J = 8.3, 7.2, 1.1 Hz, H-6′), 7.30 (1H, ddd, J = 7.8, 7.2, 0.9 Hz, H-5′), 6.82 (1H, d, J = 3.7 Hz, H-3′), 6.47 (1H, s, H-3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 178.1, 156.6, 154.5, 134.6, 133.8, 130.7, 126.0, 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].

2-[(3-Methyl-1H-indol-1-yl)methylene]-3(2H)-Benzofuranone (**2a**) and 2-(3-methyl-1H-indol-1-yl)-4H-1-benzopyran-4-one (**2b**)

2a: Yield 54%. Yellow needles; mp 220–221 °C (lit. 219–220 °C [20]). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.98 (1H, br q, J = 1.3 Hz, H-2′), 7.85 (1H, dd, J = 7.7, 1.5 Hz, H-4), 7.79 (1H, s, =CH), 7.68–7.63 (1H, m, H-6), 7.65 (1H, dd, J = 8.3, 1.0 Hz, H-7′), 7.58 (1H, dd, J = 7.7, 1.3 Hz, H-4′), 7.38 (1H, ddd, J = 8.3, 7.3, 1.3 Hz, H-6′), 7.38 (1H, dd, J = 8.3, 1.0 Hz, H-7), 7.31 (1H, ddd, J = 7.7, 7.3, 1.0 Hz, H-5′), 7.31 (1H, ddd, J = 7.7, 7.3, 1.0 Hz, H-5), 2.40 (3H, d, J = 1.3 Hz, CH_3). MS (EI) m/z 275 [M]$^+$. The ^{1}H NMR spectrum was similar to that previously reported [20].

2b: Yield 10%. Pale orange needles. mp 170–173 °C (lit. 170–172 °C [20]). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.26 (1H, dd, J = 7.9, 1.7 Hz, H-5), 8.01 (1H, dd, J = 8.3, 0.9 Hz, H-7′), 7.72 (1H, ddd, J = 8.4, 7.2, 1.7 Hz, H-7), 7.63–7.57 (2H, m, H-8 and H-4′), 7.47 (1H, ddd, J = 7.9, 7.2, 1.1 Hz, H-6), 7.40 (1H, ddd, J = 8.3, 7.2, 1.3 Hz, H-6′), 7.33 (1H, q, J = 1.1 Hz, H-2′), 7.32 (1H, ddd, J = 7.8, 7.2, 0.9 Hz, H-5′), 6.41 (1H, s, H-3), 2.37 (3H, d, J = 1.1 Hz, CH_3). MS (EI) m/z 275 [M]$^+$. The ^{1}H NMR spectrum was similar to that previously reported [20] .

2-[(4-Methyl-1H-indol-1-yl)methylene]-3(2H)-Benzofuranone (**3a**)

3a: Yield 34%. Yellow needles. mp 198–200 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.18 (1H, d, J = 3.6 Hz, H-2′), 7.85 (1H, dd, J = 7.7, 1.5 Hz, H-4), 7.81 (1H, s, =CH), 7.66 (1H, ddd, J = 8.3, 7.2, 1.5 Hz, H-6), 7.51 (1H, br d, J = 8.2 Hz, H-7′), 7.36 (1H, dd, J = 8.3, 1.0 Hz, H-7), 7.31–7.23 (2H, m, H-5 and H-6′), 7.09 (1H, d, J = 7.3 Hz, H-5′), 6.86 (1H, d, J = 3.6 Hz, H-3′), 2.57 (3H, s, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 183.8, 164.8, 136.6, 136.1, 135.5, 131.1, 128.8, 126.6, 124.4, 124.1, 123.5, 123.4, 122.5, 112.8, 111.0, 108.2, 107.7, 18.5. MS (EI) m/z 275 [M]$^+$. *Anal.* Calcd for $C_{18}H_{13}NO_2$: C, 78.53; H, 4.76; N, 5.09. Found: C, 78.38; H 4.79; N 5.15.

2-[(5-Methyl-1H-indol-1-yl)methylene]-3(2H)-Benzofuranone (**4a**) and 2-(5-methyl-1H-indol-1-yl)-4H-1-benzopyran-4-one (**3b**)

4a: Yield 30%. Yellow needles. mp 200–203 °C (lit. 205 °C [20]). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.16 (1H, d, J = 3.6 Hz, H-2′), 7.84 (1H, dd, J = 7.6, 1.4 Hz, H-4), 7.78 (1H, s, =CH), 7.66 (1H, ddd, J = 8.3, 7.3, 1.4 Hz, H-6), 7.56 (1H, d, J = 8.4 Hz, H-7′), 7.42 (1H, br s, H-4′), 7.35 (1H, dd, J = 8.3, 0.8 Hz, H-7), 7.25 (1H, ddd, J = 7.6, 7.3, 0.8 Hz, H-5), 7.19 (1H, d, J = 8.4 Hz, H-6′), 6.77 (1H, d, J = 3.6 Hz, H-3′), 2.47 (3H, s, CH_3). MS (EI) m/z 275 [M]$^+$. The ^{1}H NMR spectrum was similar to that previously reported [20].

3b: Yield 23%. Grayish white needles. mp 187–188 °C (lit. 184–186 °C [20]). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.26 (1H, dd, J = 7.9, 1.7 Hz, H-5), 7.89 (1H, d, J = 8.5 Hz, H-7′), 7.73 (1H, ddd, J = 8.4, 7.2, 1.7 Hz, H-7), 7.58 (1H, dd, J = 8.4, 1.1 Hz, H-8), 7.53 (1H, d, J = 3.6 Hz, H-2′), 7.47 (1H, ddd, J = 7.9, 7.2, 1.1 Hz, H-6), 7.46–7.44 (1H, m, H-4′), 7.20 (1H, dd, J = 8.5, 1.2 Hz, H-6′), 6.73 (1H, d, J = 3.6 Hz, H-3′), 6.47 (1H, s, H-3), 2.48 (3H, s, CH_3). MS (EI) m/z 275 [M]$^+$. The ^{1}H NMR spectrum was similar to that previously reported [20].

2-[(6-Methyl-1H-indol-1-yl)methylene]-3(2H)-Benzofuranone (**5a**)

5a: Yield 27%. Brown solid. mp 212–214 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.12 (1H, d, J = 3.6 Hz, H-2′), 7.85 (1H, dd, J = 7.7, 1.4 Hz, H-4), 7.79 (1H, s, =CH), 7.66 (1H, ddd, J = 8.3, 7.3, 1.4 Hz, H-6), 7.51 (1H, d, J = 8.0 Hz, H-4′), 7.49 (1H, br s, H-7′), 7.35 (1H, dd, J = 8.3, 0.8 Hz, H-7), 7.25 (1H, ddd, J = 7.7, 7.3, 0.8 Hz, H-5), 7.11 (1H, d, J = 8.0 Hz, H-5′), 6.79 (1H, d, J = 3.6 Hz, H-3′), 2.53 (3H, s, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 183.8, 164.8, 137.1, 136.1, 135.4, 134.2, 126.8, 126.5, 124.6, 124.3, 123.4, 122.5, 121.0, 112.8, 110.8, 110.4, 109.6, 21.9. MS (EI) m/z 275 [M]$^+$. *Anal.* Calcd for $C_{18}H_{13}NO_2$: C, 78.53; H, 4.76; N, 5.09. Found: C, 78.35; H 4.88; N 4.81.

2-[(4-Methoxy-1H-indol-1-yl)methylene]-3(2H)-Benzofuranone (**6a**) and 2-(4-methoxy -1H-indol-1-yl)-4H-1-benzopyran-4-one (**4b**)

6a: Yield 32%. Yellow needles. mp 194–201 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.10 (1H, d, J = 3.6 Hz, H-2′), 7.84 (1H, dd, J = 7.7, 1.4 Hz, H-4), 7.78 (1H, s, =CH), 7.66 (1H, ddd, J = 8.3, 7.3, 1.4 Hz, H-6), 7.35 (1H, d, J = 8.3 Hz, H-7), 7.32–7.25 (3H, m, H-5, H-6′, H-7′),

6.94 (1H, d, J = 3.6 Hz, H-3′), 6.74–6.69 (1H, m, H-5′), 3.98 (3H, s, OCH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 183.9, 164.9, 153.5, 138.2, 136.2, 135.6, 125.6, 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.* Calcd for $C_{18}H_{13}NO_3$: C, 74.22; H, 4.50; N, 4.81. Found: C, 74.02; H, 4.59; N, 5.00.

4b: Yield 27%. Brown needles. mp 160–161 °C. ^1H NMR ($CDCl_3$, 400 MHz) δ 8.26 (1H, dd, J = 7.9, 1.8 Hz, H-5), 7.73 (1H, ddd, J = 8.4, 7.2, 1.8 Hz, H-7), 7.62–7.57 (2H, m, H-8 and H-7′), 7.51–7.46 (1H, m, H-6), 7.47 (1H, d, J = 3.7 Hz, H-2′), 7.32 (1H, t, J = 8.1 Hz, H-6′), 6.93 (1H, d, J = 3.7 Hz, H-3′), 6.73 (1H, d, J = 8.1 Hz, H-5′), 6.48 (1H, s, H-3), 3.98 (3H, s, OCH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ-177.9, 156.5, 154.3, 153.3, 135.7, 133.6, 125.8, 125.6, 125.4, 123.3, 122.8, 120.9, 117.2, 106.3, 105.8, 103.0, 97.4, 55.3. MS (EI) m/z 291 $[M]^+$. *Anal.* Calcd for $C_{18}H_{13}NO_3$: C, 74.22; H, 4.50; N, 4.81. Found: C, 73.92; H, 4.45; N, 4.68.

2-[(5-Methoxy-1H-indol-1-yl)methylene]-3(2H)-Benzofuranone (**7a**) and 2-(5-methoxy -1H-indol-1-yl)-4H-1-benzopyran-4-one (**5b**)

7a: Yield 35%. Yellow needles. mp 220–222 °C (lit. 222–223 °C [20]). ^1H NMR ($CDCl_3$, 400 MHz) δ 8.17 (1H, d, J = 3.6 Hz, H-2′), 7.85 (1H, dd, J = 7.6, 1.4 Hz, H-4), 7.75 (1H, s, =CH), 7.65 (1H, ddd, J = 8.3, 7.2, 1.4 Hz, H-6), 7.56 (1H, d, J = 8.9 Hz, H-7′), 7.35 (1H, dd, J = 8.3, 0.8 Hz, H-7), 7.25 (1H, ddd, J = 7.6, 7.2, 0.8 Hz, H-5), 7.10 (1H, d, J = 2.4 Hz, H-4′), 6.99 (1H, dd, J = 8.9, 2.4 Hz, H-6′), 6.77 (1H, d, J = 3.6 Hz, H-3′), 3.88 (3H, s, OCH_3). MS (EI) m/z 291 $[M]^+$. The ^1H NMR spectrum was similar to that previously reported [20].

5b: Yield 24%. Brown needles. mp 173–174 °C (lit. 179–180 °C [20]). ^1H NMR ($CDCl_3$, 400 MHz) δ 8.26 (1H, dd, J = 7.9, 1.7 Hz, H-5), 7.92 (1H, d, J = 9.1 Hz, H-7′), 7.72 (1H, ddd, J = 8.4, 7.2, 1.7 Hz, H-7), 7.58 (1H, dd, J = 8.4, 1.1 Hz, H-8), 7.54 (1H, d, J = 3.6 Hz, H-2′), 7.47 (1H, ddd, J = 7.9, 7.2, 1.1 Hz, H-6), 7.12 (1H, d, J = 2.1 Hz, H-4′), 7.01 (1H, dd, J = 9.1, 2.1 Hz, H-6′), 6.74 (1H, d, J = 3.6 Hz, H-3′), 6.43 (1H, s, H-3), 3.89 (3H, s, OCH_3). MS (EI) m/z 291 $[M]^+$. The ^1H NMR spectrum was similar to that previously reported [20].

2-[(6-Methoxy-1H-indol-1-yl)methylene]-3(2H)-Benzofuranone (**8a**) and 2-(6-methoxy-1H-indol-1-yl)-4H-1-benzopyran-4-one (**6b**)

8a: Yield 23%. Yellow needles. mp 203–211 °C. ^1H NMR ($CDCl_3$, 400 MHz) δ 8.08 (1H, d, J = 3.6 Hz, H-2′), 7.85 (1H, dd, J = 7.7, 1.5 Hz, H-4), 7.75 (1H, s, =CH), 7.67 (1H, ddd, J = 8.3, 7.3, 1.5 Hz, H-6), 7.50 (1H, d, J = 8.5 Hz, H-4′), 7.36 (1H, dd, J = 8.3, 0.8 Hz, H-7), 7.29–7.24 (1H, m, H-5), 7.15 (1H, d, J = 2.2 Hz, H-7′), 6.92 (1H, dd, J = 8.5, 2.2 Hz, H-5′), 6.76 (1H, d, J = 3.6 Hz, H-3′), 3.93 (3H, s, OCH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 183.9, 164.8, 157.9, 137.7, 136.2, 135.4, 126.0, 124.4, 123.4, 122.8, 122.5, 122.0, 112.9, 112.6, 110.9, 109.6, 94.2, 55.9. MS (EI) m/z 291 $[M]^+$. *Anal.* Calcd for $C_{18}H_{13}NO_3$: C, 74.22; H, 4.50, N, 4.81. Found: C, 74.20; H, 4.63; N, 4.83.

6b: Yield 34%. Brown needles. mp 168–169 °C. ^1H NMR ($CDCl_3$, 400 MHz) δ 8.28 (1H, dd, J = 7.9, 1.7 Hz, H-5), 7.73 (1H, ddd, J = 8.4, 7.2, 1.7 Hz, H-7), 7.57 (1H, dd, J = 8.4, 1.1 Hz, H-8), 7.54 (1H, d, J = 8.6 Hz, H-4′), 7.52 (1H, d, J = 2.2 Hz, H-7′), 7.48 (1H, ddd, J = 7.9, 7.2, 1.1 Hz, H-6), 7.44 (1H, d, J = 3.7 Hz, H-2′), 6.95 (1H, dd, J = 8.6, 2.2 Hz, H-5′), 6.73 (1H, d, J = 3.7 Hz, H-3′), 6.44 (1H, s, H-3), 3.93 (3H, s, OCH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 178.1, 157.9, 156.8, 154.4, 135.5, 133.8, 126.0, 125.8, 124.6. 123.5, 123.4, 122.2, 117.3, 111.8, 108.8, 98.3, 97.3, 55.8. MS (EI) m/z 291 $[M]^+$. *Anal.* Calcd for $C_{18}H_{13}NO_3$: C, 74.22; H, 4.50, N, 4.81. Found: C, 73.97; H, 4.42; N, 4.66.

2-(1H-Indol-1-ylmethylene)-6-methoxy-3(2H)-benzofuranone (**9a**) and 2-(1H-indol-1-yl)-7-methoxy-4H-1-benzopyran-4-one (**7b**)

9a: Yield 43%. Pale brown solid. mp 196–197 °C. ^1H NMR ($CDCl_3$, 400 MHz) δ 8.15 (1H, d, J = 3.6 Hz, H-2′), 7.74 (1H, d, J = 9.1 Hz, H-4), 7.72 (1H, s, =CH), 7.66 (1H, dd, J = 8.2, 1.0 Hz, H-7′), 7.64 (1H, dd, J = 7.7, 1.2 Hz, H-4′), 7.36 (1H, ddd, J = 8.2, 7.2, 1.2 Hz, H-6′), 7.27(1H, ddd, J = 7.7, 7.2, 1.0 Hz, H-5′), 6.82 (1H, d, J = 3.6 Hz, H-3′), 6.81–6.77 (2H, m, H-5 and H-7), 3.93 (3H, s, OCH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 182.3, 167.2, 166.9, 136.7, 136.4, 129.0, 127.1, 125.4, 123.8, 122.8, 121.4, 115.6, 112.2, 110.1, 109.6, 109.2, 96.5, 56.0. MS (EI) m/z 291 $[M]^+$. *Anal.* Calcd for $C_{18}H_{13}NO_3$: 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 ($CDCl_3$, 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-8), 6.81 (1H, d, J = 3.6 Hz, H-3′), 6.41 (1H, s, H-3), 3.97 (3H, s, OCH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 177.7, 164.2, 156.3, 156.1, 134.6, 130.6, 127.3, 124.6, 124.4, 122.9, 121.8, 117.1, 114.4, 113.2, 108.6, 100.4, 97.5, 56.0. MS (EI) m/z 291 $[M]^+$. *Anal.* Calcd for $C_{18}H_{13}NO_3$: C, 74.22; H, 4.50; N, 4.81. Found: C, 73.97; 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 ($CDCl_3$, 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, dd, 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, OCH_3), 2.46 (3H, s, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 182.3, 167.1, 166.9, 136.2, 135.0, 132.4, 129.2, 127.2, 125.4, 125.3, 121.3, 115.7, 112.2, 109.9, 109.7, 109.0, 96.5, 56.0, 21.4. MS (EI) m/z 305 $[M]^+$. *Anal.* Calcd for $C_{19}H_{15}NO_3$: C, 74.74; H, 4.95; N, 4.59. Found: C, 74.63; H, 4.90; N, 4.67.

8b: Yield 27%. Brown needles. mp 165–167 °C. ^1H NMR ($CDCl_3$, 400 MHz) δ 8.16 (1H, d, J = 8.8 Hz, H-5), 7.85 (1H, d, J = 8.5 Hz, H-7′), 7.52 (1H, d, J = 3.6 Hz, H-2′), 7.45 (1H, br s, H-4′), 7.20 (1H, dd, J = 8.5, 1.4 Hz, H-6′), 7.03 (1H, dd, J = 8.8, 2.3 Hz, H-6), 6.97 (1H, d, J = 2.3 Hz, H-8), 6.72 (1H, d, J = 3.6 Hz, H-3′), 6.39 (1H, s, H-3), 3.96 (3H, s, OCH_3), 2.48 (3H, s, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 177.7, 164.1, 156.4, 156.1, 132.8, 132.5, 131.0, 127.3, 125.9, 124.6, 121.6, 117.2, 114.3, 113.0, 108.4, 100.4, 97.0, 56.0, 21.3. MS (EI) m/z 305 $[M]^+$. *Anal.* Calcd for $C_{19}H_{15}NO_3$: C, 74.74; H, 4.95; N, 4.59. Found: C, 74.44; H, 4.89; N, 4.61.

6-Methoxy-2-[(4-methoxy-1H-indol-1-yl)methylene]-3(2H)-Benzofuranone (**11a**) and 7-methoxy-2-(4-methoxy-1H-indol-1-yl)4H-1-benzopyran-4-one (**9b**)

11a: Yield 47%. Yellow needles. mp 220–222 °C. ^1H NMR ($CDCl_3$, 400 MHz) δ 8.05 (1H, d, J = 3.6 Hz, H-2′), 7.73 (1H, d, J = 9.2 Hz, H-4), 7.68 (1H, s, =CH), 7.30–7.26 (2H, m, H-6′, H-7′), 6.92 (1H, d, J = 3.6 Hz, H-3′), 6.81–6.76 (2H, m, H-5 and H-7), 6.72–6.68 (1H, m, H-5′), 3.97 (3H, s, OCH_3), 3.93 (3H, s, OCH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 182.2, 167.1, 166.8, 153.3, 138.0, 136.3, 125.5, 125.3, 124.7, 119.1, 115.5, 112.1, 109.8, 106.2, 103.1, 102.9, 96.4, 55.9, 55.4. MS (EI) m/z 321 $[M]^+$. *Anal.* Calcd for $C_{19}H_{15}NO_4$: C, 71.02; H, 4.71; N, 4.36. Found: C, 71.14; H, 4.68; N, 4.59.

9b: Yield 24%. Pale brown needles. mp 193–196 °C. ^1H NMR ($CDCl_3$, 400 MHz) δ 8.16 (1H, d, J = 8.8 Hz, H-5), 7.56 (1H, d, J = 8.4 Hz, H-7′), 7.45 (1H, d, J = 3.7 Hz, H-2′), 7.31 (1H, dd, J = 8.4, 8.0 Hz, H-6′), 7.03 (1H, dd, J = 8.8, 2.3 Hz, H-6), 6.97 (1H, d, J = 2.3 Hz, H-8), 6.92 (1H, d, J = 3.6 Hz, H-3′), 6.72 (1H, d, J = 8.0 Hz, H-5′), 6.41 (1H, s, H-3), 3.98 (3H, s, OCH_3), 3.96 (3H, s, OCH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 177.7, 164.2, 156.4, 156.1, 153.5, 135.8, 127.3, 125.4, 123.1, 121.0, 117.2, 114.4, 106.3, 105.7, 103.0, 100.4, 97.7, 56.0, 55.5. MS (EI) m/z 321 $[M]^+$. *Anal.* Calcd for $C_{19}H_{15}NO_4$: C, 71.02; H, 4.71; N, 4.36. Found: C, 70.78; H, 4.65; N, 4.45.

6-Methoxy-2-[(6-methoxy-1H-indol-1-yl)methylene]-3(2H)-Benzofuranone (**12a**) and 7-Methoxy-2–6-methoxy-1H-indol-1-yl)-4H-1-benzopyran-4-one (**10b**)

12a: Yield 36%. Yellow needles. mp 196–197 °C. ^1H NMR ($CDCl_3$, 400 MHz) δ 8.04 (1H, d, J = 3.6 Hz, H-2′), 7.74 (1H, d, J = 9.2 Hz, H-4), 7.66 (1H, s, =CH), 7.49 (1H, d, J = 8.5 Hz, H-4′), 7.14 (1H, d, J = 2.2 Hz, H-7′), 6.91 (1H, dd, J = 8.5, 2.2 Hz, H-5′), 6.81 (1H, dd, J = 9.2, 2.1 Hz, H-5), 6.80 (1H, d, J = 2.1 Hz, H-7), 6.74 (1H, d,

J = 3.6 Hz, H-3′), 3.94 (3H, s, OCH_3), 3.92 (3H, s, OCH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 182.4, 167.2, 167.0, 157.8, 137.7, 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.* Calcd for $C_{19}H_{15}NO_4$: C, 71.02; H, 4.71; N, 4.36. Found: C, 71.15; H, 4.63; N, 4.29.

10b: Yield 16%. Brown needles. mp 195–196 °C. ^1H NMR ($CDCl_3$, 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-2′), 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, OCH_3), 3.92 (3H, s, OCH_3). ^{13}C NMR ($CDCl_3$, 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, 97.5, 56.0, 55.9. MS (EI) m/z 321 $[M]^+$. *Anal.* Calcd for $C_{19}H_{15}NO_4$: 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**)

13a: Yield 11%. Pale green needles. mp 178 °C. ^1H NMR ($CDCl_3$, 400 MHz) δ: 8.18 (1H, d, J = 3.6 Hz, H-2′), 7.80 (1H, s, =CH), 7.68 (1H, dd, J = 8.3, 0.9 Hz, H-7′), 7.64 (1H, dd, J = 7.7, 1.2 Hz, H-4′), 7.37 (1H, ddd, J = 8.3, 7.2, 1.2 Hz, H-6′), 7.31–7.23 (4H, m, H-4, H-6, H-7 and H-5′), 6.83 (1H, d, J = 3.6 Hz, H-3′), 3.86 (3H, s, OCH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 183.9, 159.9, 156.1, 136.7, 136.3, 129.1, 127.2, 125.6, 123.9, 123.0, 122.6, 121.4, 113.6, 110.7, 110.1, 109.6, 104.8, 56.0. MS (EI) m/z 291 $[M]^+$. *Anal.* Calcd for $C_{18}H_{13}NO_3$: C, 74.22; H, 4.50; N, 4.81. Found: C, 74.22; H, 4.60; N, 4.57.

11b: Yield 9%. Pale brown solid. mp 166 °C. ^1H NMR ($CDCl_3$, 400 MHz) δ: 8.00 (1H, br d, J = 8.4 Hz, H-7′), 7.67 (1H, dd, J = 7.8, 1.2 Hz, H-4′), 7.64 (1H, d, J = 3.1 Hz, H-5), 7.55 (1H, d, J = 3.6 Hz, H-2′), 7.52 (1H, d, J = 9.1 Hz, H-8), 7.39 (1H, ddd, J = 8.4, 7.2, 1.7 Hz, H-6′), 7.33–7.23 (2H, m, H-7 and H-5′), 6.81 (1H, d, J = 3.6 Hz, H-3′), 6.46 (1H, s, H-3), 3.93 (3H, s, OMe). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 178.0, 157.4, 156.5, 149.0, 134.6, 130.7, 124.5, 124.4, 124.1, 123.4, 122.9, 121.8, 118.7, 113.3, 108.8, 105.5, 96.9, 56.0. MS (EI) m/z 291 $[M]^+$. *Anal.* Calcd for $C_{18}H_{13}NO_3$: C, 74.22; H, 4.50; N, 4.81. Found: C, 73.94; H, 4.41; N, 4.70.

3.2. Biological activity

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

3.2.1. MAO inhibitory assay

MAO inhibitory activity was assayed using the method of Novaroli *et al.* [31] with minor modifications. Briefly, 140 µL of 0.1 M potassium phosphate buffer (pH 7.4), 8 µL of 0.75 mM kynuramine, and 2 µL of a dimethyl sulfoxide (DMSO) inhibitor solution (final DMSO concentration of 1% (v/v) and final concentrations of the inhibitors of 0–100 µM), were preincubated at 37 °C for 10 min. Diluted human recombinant enzyme (50 µL) was then added to obtain a final protein concentration of 0.0075 mg/mL (MAO-A) or 0.015 mg/mL (MAO-B) in the assay mixture. The reaction mixture was further incubated at 37 °C and the reaction was stopped after 20 min by the addition of 75 µL of 2 M NaOH. The product generated by MAO, 4-quinolinol, is fluorescent and was measured at Ex 310 nm/Em 400 nm using a microplate reader (Molecular Devices SPECTRA MAX M2). Each data point is the average of triplicate experiments. The sample solution was replaced with DMSO to provide a negative control and pargyline was used as a positive control. The IC_{50} values were calculated from a line through two points which sandwiched the point corresponding to 50% (IC_{50}) by plotting the remaining activity (%) relative to the control (100%) versus the logarithm of the inhibitor concentration to obtain a sigmoidal dose-response curve.

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 × IC_{50}, 1 × IC_{50}, and 2 × IC_{50} (IC_{50} = 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 Auto-Dock 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.bioorg.2019.03.042.

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