ORIGINAL RESEARCH

3-Coumaranone derivatives as inhibitors of monoamine oxidase

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Abstract: The present study examines the monoamine oxidase (MAO) inhibitory properties of a series of 20 3-coumaranone [benzofuran-3(2H)-one] derivatives. The 3-coumaranone derivatives are structurally related to series of α-tetralone and 1-indanone derivatives, which have recently been shown to potently inhibit MAO, with selectivity for MAO-B (in preference to the MAO-A isoform). 3-Coumaranones are similarly found to selectively inhibit human MAO-B with half-maximal inhibitory concentration (IC_{50}) values of 0.004–1.05 μM. Nine compounds exhibited $IC_{50} < 0.05$ μM for the inhibition of MAO-B. For the inhibition of human MAO-A, IC_{50} values ranged from 0.586 to >100 μM, with only one compound possessing an $IC_{50} < 1$ μM. For selected 3-coumaranone derivatives, it is established that MAO-A and MAO-B inhibition are reversible since dialysis of enzyme–inhibitor mixtures almost completely restores enzyme activity. On the basis of the selectivity profiles and potent action, it may be concluded that the 3-coumaranone derivatives are suitable leads for the development of selective MAO-B inhibitors as potential treatment for disorders such as Parkinson's disease and Alzheimer's disease.

Keywords: benzofuran-3(2H)-one, MAO, inhibition, reversible, competitive, Parkinson's disease

Introduction

The enzyme, monoamine oxidase (MAO), exists as two isoforms, MAO-A and MAO-B, which are encoded by two distinct genes.[1,2] The MAOs have been targets in medicinal chemistry and drug discovery for many years since these enzymes metabolize key neurotransmitters in the periphery and in the central nervous system.[3,4] For example, MAO-A metabolizes serotonin in the central nervous system, and inhibitors of this isoform (eg, phenelzine, isocarboxazid, tranylcypromine, and moclobemide) are used in the clinic for the treatment of depression. MAO-B, on the other hand, is the main isoform responsible for central dopamine metabolism, and MAO-B inhibitors (eg, selegiline and rasagiline) are thus used for the treatment of Parkinson's disease.[5] A transdermal delivery system of selegiline at higher doses is now also used for major depression.[6] On the basis of the observation that MAO-B activity is reduced in smokers, MAO-B inhibitors also represent a potential treatment for smoking cessation. MAO-B inhibitors may mimic the neurochemical effects of smoking by increasing synaptic monoamine levels.[7]

Besides modulation of monoamine levels, MAO inhibitors are also of clinical value because they reduce the formation of by-products of the MAO catalytic cycle, hydrogen peroxide and aldehyde species. These products may be harmful, particularly in the brain, if not rapidly metabolized by glutathione peroxidase and aldehyde-metabolizing enzymes, respectively.[8] The possibility that these enzymes may be dysfunctional in conditions such as Parkinson's disease has given rise to the theory that excessive or

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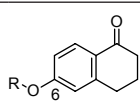Fax +27 18 299 4243
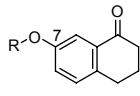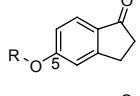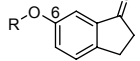Email lesetja.legoabe@nwu.ac.za

even normal MAO activity may result in neurotoxicity and thus contribute to the degenerative process.[8,9] MAO inhibitors have thus been proposed to act as neuroprotective agents in these conditions by reducing the formation of injurious metabolic by-products of the MAO catalytic cycle. MAO-B inhibition seems to be particularly relevant in Parkinson's disease as MAO-B activity and density increases in most brain regions in an age-related manner.[10] Experimental evidence suggests that MAO-generated hydrogen peroxide and aldehyde intermediates in the heart may also be undesirable since these may affect mitochondrial respiration leading to cardiomyocyte death.[11] MAO inhibition may therefore be beneficial for the treatment of certain cardiovascular pathologies. Since MAO-A levels and hydrogen peroxide derived from MAO-A are increased in the hearts of aged rats, MAO-A inhibitors appear to be of particular relevance in this regard.[12]

Although MAO inhibitors have been extensively employed in the clinic, the use of nonselective and selective MAO-A inhibitors declined after the realization that these drugs potentiate the sympathomimetic effects of the dietary amine, tyramine.[13,14] This interaction may lead to a potentially fatal increase in blood pressure termed the "cheese effect". The observation that selective inhibitors of MAO-B do not cause tyramine-induced hypertension and the development of reversible MAO-A inhibitors with low potential for the cheese effect have facilitated the continued use of MAO inhibitors in the clinic.[5] Although the older irreversible MAO-A inhibitors are still available for the treatment of certain types of depression, from a drug design and drug safety point of view, reversible inhibition of MAO-A is a more desirable property and such drugs would not require dietary restrictions.[15,16] Both reversible and irreversible MAO-B inhibitors, on the other hand, have excellent safety profiles and are not associated with changes in blood pressure.[17,18]

Based on the clinical utility of MAO inhibitors, the discovery of novel classes of compounds with potent inhibition activity is of interest. Although a number of MAO inhibitors are used in the clinic, none of these may be considered as having both high potency (IC$_{50}$ [half maximal inhibitory concentration] <0.1 μM) and a reversible mode of action. In this study, a series of twenty 3-coumaranone [benzofuran-3(2H)-one] derivatives have been synthesized and evaluated as inhibitors of human MAO-A and MAO-B. The 3-coumaranone derivatives are structurally related to series of α-tetralone and 1-indanone derivatives, which have recently been shown to potently inhibit MAO-B, and, to a lesser extent, also the MAO-A isoform.[19–21] The MAO
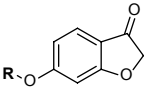
inhibition potencies of the reported α-tetralone (**1**–**2**) and 1-indanone (**3**–**4**) derivatives are summarized in Table 1. Based on the activities of these classes of compounds, it is anticipated that 3-coumaranone–derived compounds would also act as MAO inhibitors, particularly of the MAO-B isoform. Since the α-tetralone and 1-indanone derivatives contain alkyloxy substituents, which greatly enhances MAO inhibition, a 3-coumaranone derivative, compound **5a**, containing the benzyloxy side chain on the C6 position, was considered as the first member of the series of 3-coumaranone derivatives (Table 2). To explore chemical space and to establish structure–activity relationships (SARs), a total of 20 derivatives were synthesized. The benzyloxy-substituted derivative **5a** was substituted on the meta and para positions of the phenyl ring with halogens (F, Cl, Br, I) and alkyl groups (CN, CH$_3$, CF$_3$) to yield 13 derivatives, compounds **5b**–**n**. Derivatives containing the phenylethoxy, phenylpropoxy, and phenoxyethoxy substituents on the C6 position of the 3-coumaranone moiety to yield compounds **5o**–**q** were also synthesized in this study. The phenylethoxy-substituted derivative **5o** was further substituted on the phenyl ring with bromine and the methyl group to yield **5r**–**t**. The goal of this study is therefore to explore the possibility that 3-coumaranone derivatives may act as MAO inhibitors. Among compound classes **1**–**4**, the 1-indanone derivatives, **3**, are most closely related to the 3-coumaranone derivatives of this study, with substitution in analogous positions in compounds **3** (C5)

Table I The reported IC$_{50}$ values for the inhibition of recombinant human MAO-A and MAO-B by α-tetralone (**1** and **2**) and 1-indanone (**3** and **4**) derivatives (R = alkyl/arylalkyl)

	IC$_{50}$ range (μM)	
	MAO-A	**MAO-B**
1	0.024–2.25[a]	0.0045–0.078[a]
2	0.010–1.26[b]	0.00089–0.047[b]
3	0.504–10.75[c]	0.015–0.298[c]
4	0.032–1.348[c]	0.001–0.03[c]

Notes: [a]Data from Legoabe et al.[19] [b]Data from Legoabe et al.[20] [c]Data from Mostert et al.[21]
Abbreviations: MAO-A/B, monoamine oxidase A/B; IC$_{50}$, half maximal inhibitory concentration.

Table 2 The IC$_{50}$ values for the inhibition of recombinant human MAO-A and MAO-B by 3-coumaranone derivatives **5a–t**

	R	IC$_{50}$ (µM)[a]		SI[b]
		MAO-A	**MAO-B**	
5a	C$_6$H$_5$CH$_2$–	26.7±0.351	0.062±0.011	431
5b	4-F-C$_6$H$_4$CH$_2$–	11.4±2.54	0.022±0.003	518
5c	3-F-C$_6$H$_4$CH$_2$–	80.7±9.22	0.136±0.030	593
5d	4-Cl-C$_6$H$_4$CH$_2$–	2.19±0.135	0.019±0.007	115
5e	3-Cl-C$_6$H$_4$CH$_2$–	18.7±7.82	0.012±0.003	1,558
5f	4-Br-C$_6$H$_4$CH$_2$–	2.80±0.079	0.016±0.003	175
5g	3-Br-C$_6$H$_4$CH$_2$–	3.92±0.827	0.004±0.001	980
5h	4-I-C$_6$H$_4$CH$_2$–	>100[c]	0.028±0.006	>3,571
5i	3-I-C$_6$H$_4$CH$_2$–	7.57±1.82	0.007±0.001	1,081
5j	4-CN-C$_6$H$_4$CH$_2$–	5.01±0.919	0.079±0.019	63
5k	3-CN-C$_6$H$_4$CH$_2$–	0.586±0.133	0.166±0.015	3.5
5l	4-CH$_3$-C$_6$H$_4$CH$_2$–	>100[c]	0.054±0.011	>1,852
5m	3-CH$_3$-C$_6$H$_4$CH$_2$–	62.0±16.9	0.096±0.003	646
5n	4-CF$_3$-C$_6$H$_4$CH$_2$–	>100[c]	0.039±0.005	>2,564
5o	C$_6$H$_5$(CH$_2$)$_2$–	13.8±2.11	0.107±0.010	129
5p	C$_6$H$_5$(CH$_2$)$_3$–	11.8±0.930	0.055±0.008	215
5q	C$_6$H$_5$O(CH$_2$)$_2$–	>100[c]	0.050±0.006	>2,000
5r	4-Br-C$_6$H$_4$(CH$_2$)$_2$–	2.65±0.629	0.564±0.049	4.7
5s	4-CH$_3$-C$_6$H$_4$(CH$_2$)$_2$–	11.3±0.683	1.05±0.147	11
5t	3-CH$_3$-C$_6$H$_4$(CH$_2$)$_2$–	25.9±8.72	0.181±0.021	143
6	H–	>100[c]	12.0±4.21	8.3

Notes: [a]All values are expressed as the mean ± standard deviation (SD) of triplicate determinations. [b]The selectivity index is the selectivity for the MAO-B isoform and is given as the ratio of IC$_{50}$ (MAO-A)/IC$_{50}$ (MAO-B). [c]No inhibition observed at maximum tested concentration of 100 µM.
Abbreviations: SI, selectivity index; MAO-A/B, monoamine oxidase A/B; IC$_{50}$, half maximal inhibitory concentration.

and **5** (C6). Since series **3** appears to be, in general, weaker MAO-A inhibitors among the reported classes **1–4**, it may be expected that 3-coumaranones **5** may exhibit a high degree of MAO-B selectivity. In future studies, other positions of the 3-coumaranone moiety (eg, C5) should be substituted and the effect on MAO isoform selectivity evaluated.

Materials and methods
General
Chemicals for the synthetic procedures were acquired from Sigma-Aldrich (St Louis, MO, USA). Chemicals for the enzymatic reactions, including kynuramine 2HBr, and microsomes from insect cells expressing recombinant human MAO-A and MAO-B (5 mg/mL) were from Sigma-Aldrich. The use of the recombinant human proteins in this study were approved by the Ethics Committee of the North-West University. Fluorescence spectrophotometry was carried out with a Varian Cary Eclipse fluorescence spectrophotometer (Agilent Technologies,

Santa Clara, CA, USA). Proton (^{1}H) and carbon (^{13}C) nuclear magnetic resonance (NMR) spectra were recorded in deuterochloroform on a Bruker Avance III 600 spectrometer (Karlsruhe, Germany) at 600 and 151 MHz, respectively. Chemical shifts (Supplementary materials) are given in parts per million (δ) and spin multiplicities as s (singlet), d (doublet), dd (doublet of doublets), t (triplet), td (triplet of doublets), or m (multiplet). High resolution mass spectra were recorded on a Bruker micrOTOF-Q II mass spectrometer in atmospheric pressure chemical ionization mode. Melting points (mps) were determined with a Büchi B-545 melting point apparatus (Büchi Labortechnik, Flawil, Switzerland) and are uncorrected. Thin-layer chromatography was carried out using silica gel 60 (Merck, Darmstadt, Germany), and the sheets were developed in a mobile phase consisting of one part ethyl acetate and four parts petroleum ether. The developed sheets were examined at a wavelength of 254 nm.

Chemistry
Procedure for the synthesis of 3-coumaranone derivatives **5a–t**
To commercially available 6-hydroxy-3-coumaranone (1.998 mmol) in a round bottom flask, 3 mL *N,N*-dimethylformamide and anhydrous potassium carbonate (3.996 mmol) was added. The appropriate arylalkyl bromide (2.197 mmol) was added to the reaction, and the mixture was stirred for 24 hours at room temperature. Thin layer chromatography was performed to determine the completion of the reaction. Upon completion, water (8 mL) was added to the reaction, and the resulting mixture was filtered. The reaction was extracted to dichloromethane (3×20 mL), and the organic phase was dried over anhydrous MgSO$_4$, filtered, and concentrated in vacuo. After crystallization from cyclohexane, the product was collected via filtration, and the crystals were air dried.[22]

Enzymology
Determination of IC$_{50}$ values
The protocol for the determination of IC$_{50}$ values has been reported in detail in recent publications.[19–21] Recombinant human MAO-A and MAO-B served as enzyme sources, and kynuramine was used as substrate for both isoforms.[23] The oxidation of kynuramine by the MAOs ultimately yields 4-hydroxyquinoline, which was measured by fluorescence spectrophotometry (λ$_{ex}$ =310; λ$_{em}$ =400 nm). By measuring MAO activities in the presence of various inhibitor concentrations (spanning at least three orders of magnitude), sigmoidal curves were constructed (enzyme activity versus

the logarithm of inhibitor concentration), from which IC_{50} values were determined. These experiments were carried out in triplicate, and the IC_{50} values are expressed as mean ± standard deviation. We have previously found that the oxidation of kynuramine by the MAO enzymes remains linear throughout the first 20 minutes of the reaction (at 37°C) and that the K_m values for the oxidation of kynuramine by recombinant human MAO-A and MAO-B are 16.1±0.21 and 22.7±0.72 μM for MAO-A and MAO-B, respectively.[24] These are similar to the values of 41 μM for MAO-A and 29 μM for MAO-B obtained in previous studies.[23,25] The conditions of this study, specifically carrying out the reactions at 37°C for 20 minutes, are similar to that reported in the literature.[23]

Dialysis of enzyme/inhibitor mixtures

The protocol for the dialysis experiments has been reported in detail in recent publications.[19,21] The reversibility of MAO-A and MAO-B inhibition by **5k** and **5g**, respectively, was investigated by measuring the recovery of enzyme activity after mixtures containing the enzyme and test inhibitor (at 4× IC_{50}) were dialyzed. These experiments were carried out in triplicate, and the residual enzyme activities (as percentage of the negative control value) were expressed as mean ± standard deviation.

The construction of Lineweaver–Burk plots

The protocol for the construction of Lineweaver–Burk plots has been reported in detail in recent publications.[19,21] A set of six Lineweaver–Burk plots was constructed for the inhibition of MAO-B by **5g**. The first plot was constructed in the absence of inhibitor, and five plots were constructed in the presence of different concentrations of the test inhibitor (0.25 × IC_{50}, 0.5 × IC_{50}, 0.75 × IC_{50}, 1 × IC_{50} and 1.25 × IC_{50}). The K_i value was estimated by global (shared) fitting of the inhibition data directly to the Michaelis–Menten equation using the Prism 5 software package (GraphPad, San Diego, CA, USA) as well as by plotting of the slopes of the Lineweaver–Burke plots versus inhibitor concentration (x-axis intercept equals −K_i).

Results and discussion
Chemistry

The synthesis of the 3-coumaranone derivative **5a** has previously been reported.[22] This protocol was employed in the present study. Commercially available 6-hydroxy-3-coumaranone (**6**; 1 equiv) was reacted with an appropriately substituted arylalkyl bromide (1.1 equiv), with N,N-dimethylformamide serving as solvent and potassium
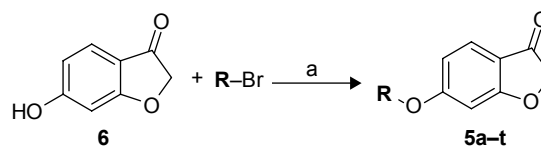

Figure 1 The general synthetic route for the synthesis of 3-coumaranone derivatives, **5a–t**.
Note: Key: (a) DMF and K_2CO_3 at rt.
Abbreviations: DMF, N,N-dimethylformamide; rt, room temperature.

carbonate (2 equiv) as base (Figure 1). After 24 hours reaction at room temperature, water was added and the product was extracted to dichloromethane. Recrystallization of the crude from cyclohexane yielded the target 3-coumaranone derivatives in acceptable yields (45%–66%). The 3-coumaranone derivatives, **5a–t**, were characterized by ^1H NMR, ^{13}C NMR, and mass spectrometry.

IC_{50} values for the inhibition of MAO

The MAO inhibitory properties of the 3-coumaranone derivatives were evaluated by using the recombinant human enzymes. The catalytic activities of both MAO isoforms were measured by using the MAO-A/B mixed substrate, kynuramine, according to the protocol already described in the literature.[21,23] The MAO-catalyzed oxidation product of kynuramine, 4-hydroxyquinoline, may be conveniently measured by fluorescence spectrophotometry. By measuring MAO activities in the presence of various concentrations of the test inhibitor, sigmoidal dose–response curves (catalytic rate versus logarithm of inhibitor concentration) were constructed from which the IC_{50} values, as a measure of the inhibition potencies, were calculated.[24]

The MAO inhibition potencies of the 3-coumaranone derivatives (**5a–t**) are given in Table 2. From these data, the following observations may be made and conclusions drawn:

1) The 3-coumaranone derivatives are selective for MAO-B over the MAO-A isoform. In all instances, the 3-coumaranone derivatives are more potent MAO-B inhibitors than MAO-A inhibitors, with the selectivity index (SI) values >3.5. The most selective inhibitors are compounds **5h**, **5l**, **5n**, and **5q**, which did not inhibit MAO-A, even at a concentration of 100 μM.

2) Although the 3-coumaranone derivatives are selective for the MAO-B isoform, potent MAO-A inhibitors have been discovered. For example, compound **5k** inhibits MAO-A with an IC_{50} value of 0.586 μM. This value is in the submicromolar range and may thus be viewed as potent inhibition. For comparison, toloxatone, a clinically used MAO-A inhibitor exhibits a reported IC_{50} value

of 3.92 µM under identical experimental conditions.[26] Compound **5k** is thus a sixfold more potent MAO-A inhibitor than toloxatone. Other 3-coumaranones with MAO-A inhibition potencies comparable to that of toloxatone are **5d** (IC$_{50}$=2.19 µM), **5f** (IC$_{50}$=2.80 µM), **5g** (IC$_{50}$=3.92 µM), and **5r** (IC$_{50}$=2.65 µM).

3) The 3-coumaranones are highly potent MAO-B inhibitors. With the exception of **5s**, all of the 3-coumaranones display IC$_{50}$ values in the submicromolar range (0.004–0.564 µM). The most potent MAO-B inhibitor discovered is compound **5g**, with an IC$_{50}$ value of 0.004 µM. For comparison, lazabemide and safinamide, reference MAO-B inhibitors, exhibit reported IC$_{50}$ values of 0.091 and 0.048 µM, respectively, under identical experimental conditions.[26] Compound **5g** is thus a 22-fold more potent MAO-B inhibitor than lazabemide. Besides **5g**, four other highly potent MAO-B inhibitors with IC$_{50}$ values <0.020 µM were also discovered, compounds **5d**–**f** and **5i**.

4) Limited SARs for the inhibition of the MAOs by the benzyloxy-substituted derivatives (**5a**–**n**) may be derived. Since derivatives **5a**–**n** are all potent MAO-B inhibitors, few clear SARs are apparent. For the benzyloxy-substituted derivatives, no clear correlation between the position (meta and para) of the substituent on the benzyloxy ring and MAO-B inhibition potency is apparent, and high potency inhibitors are represented by compounds substituted on both the meta and para positions of the benzyloxy ring. Definitive correlations between the nature (alkyl and halogen) of the substituent and MAO-B inhibition potency also are not apparent, and high-potency inhibitors are represented by compounds substituted with halogens (F, Cl, Br, I) and alkyl groups (CN, CH$_3$, CF$_3$) on the benzyloxy ring. It is, however, noticeable that among the five most potent MAO-B inhibitors, the four compounds containing chlorine or bromine as substituents are included. As for MAO-A, no clear correlation between the position (meta and para) or nature (alkyl and halogen) of the substituent and MAO-A inhibition potency is apparent. Since only one of the 20 derivatives, compound **5k**, inhibited MAO-A with an IC$_{50}$ value in the submicromolar range, this particular substitution pattern (the 3-CN group) on the benzyloxy ring is more appropriate for MAO-A inhibition than the others considered in this study.

5) It is interesting that the phenylethoxy-substituted derivatives, **5o** and **5r**–**t**, exhibit reduced MAO-B inhibition potency compared to the benzyloxy-substituted homologues. For example, **5o** (IC$_{50}$=0.107 µM) is a weaker MAO-B inhibitor than its benzyloxy-substituted homologue **5a** (IC$_{50}$=0.062 µM). In each instance, the benzyloxy-substituted homologues are more potent MAO-B inhibitors than the corresponding phenylethoxy-substituted compounds (compare **5r** with **5f**; **5s** with **5l**; **5t** with **5m**). It may thus be concluded that, for 3-coumaranones, phenylethoxy substitution is less appropriate for MAO-B inhibition than benzyloxy substitution.

6) Increasing the length of the C6 substituent from benzyoxy (**5a**) to phenylethoxy (**5o**) to phenylpropoxy (**5p**) enhances MAO-A inhibition potency. This suggests that for 3-coumaranone derivatives, a larger C6 side chain is more appropriate for MAO-A inhibition, at least with respect to chain length. For MAO-B inhibition, a clear trend is not apparent, since the benzyoxy- and phenylpropoxy-substituted derivatives are more potent than the phenylethoxy-substituted derivative. Among these three homologues, the phenylpropoxy-substituted derivative is the most potent MAO-B inhibitor with an IC$_{50}$ value of 0.055 µM.

7) For the inhibition of MAO-B, the phenoxyethoxy-substituted derivative, **5q** (IC$_{50}$=0.050 µM), is approximately equipotent to the phenylpropoxy-substituted derivative **5p** (IC$_{50}$=0.055 µM). For the inhibition of MAO-B, the phenoxy oxygen of **5q** may thus be viewed as isosteric with the CH$_2$ of the phenylpropoxy substituent. The phenoxyethoxy-substituted derivative, in contrast, is not an inhibitor of MAO-A, up to a maximal tested concentration of 100 µM. Since the phenylpropoxy-substituted derivative is a MAO-A inhibitor with an IC$_{50}$ value of 11.8 µM, it may tentatively be concluded that the phenoxy oxygen reduces MAO-A inhibition activity. A possible explanation for this observation is that the phenoxy oxygen confers more rigidity to the inhibitor structure, which may, as a result, be less suited for binding to the smaller MAO-A active site cavity.

8) 6-Hydroxy-3-coumaranone (**6**) proved to be a weak MAO-A and MAO-B inhibitor, with IC$_{50}$ values of >100 and 12.0 µM, respectively. Compared to the benzyloxy-substituted compound, **5a** (IC$_{50}$=0.062 µM), compound **6** is 193-fold weaker as a MAO-B inhibitor. This demonstrates that an appropriate C6 substituent is a requirement for MAO-B inhibition by the 3-coumaranone class of compounds.

MAO inhibition is reversible

Based on the potent MAO-B inhibition activities of the 3-coumaranone derivatives, the reversibility of MAO-B inhibition was investigated. For this purpose, a representative

inhibitor, compound **5g**, was combined with the MAO-B enzyme for 15 minutes and subsequently dialyzed for 24 hours. The concentration of **5g** employed was equal to 4× IC$_{50}$ for the inhibition of MAO-B. After dialysis, the residual MAO-B activity was measured and compared to the activity of MAO-B dialyzed in the absence of inhibitor (negative control). A similar dialysis study was conducted with the irreversible inhibitor, (*R*)-deprenyl, and served as a positive control. For reversible inhibition, enzyme activity is expected to recover to the level of the negative control after dialysis, while for irreversible inhibition, enzyme activity is not recovered. As shown in Figure 2, after dialysis of MAO-B/**5g** mixtures, MAO-B activity is recovered to 87% of the negative control. In contrast, the MAO-B activity of undialyzed mixtures of MAO-B and **5g** is 42% of the negative control. After dialysis of MAO-B/(*R*)-deprenyl mixtures, the residual enzyme activity is only 3.1% of the negative control. These data suggest that **5g** is a reversible MAO-B inhibitor since dialysis partially restores MAO-B inhibition.

Similar dialysis experiments (as for MAO-B) show that MAO-A inhibition by compound **5k** also is reversible. Compound **5k** (at 4× IC$_{50}$) was selected as a representative MAO-A inhibitor and was combined with MAO-A for 15 minutes and subsequently dialyzed for 24 hours. Dialysis experiments with MAO-A in the absence of inhibitor and

in the presence of the irreversible inhibitor, pargyline (at 4× IC$_{50}$), served as negative and positive controls, respectively. After dialysis of MAO-A/**5k** mixtures, MAO-A activity is recovered to 109% of the negative control value (Figure 3). In contrast, the MAO-A activity of undialyzed mixtures of MAO-A and **5k** is 65% of the negative control. After dialysis of MAO-A/pargyline mixtures, the residual enzyme activity is only 1.6% of the negative control. These data are consistent with a reversible interaction between **5k** and MAO-A.

MAO inhibition is competitive

To determine the mode of inhibition (eg, competitive), Lineweaver–Burk plots were constructed for the inhibition of MAO-B by **5g**. For this purpose, MAO-B activities were measured at eight different kynuramine concentrations (15–250 μM) in the absence of inhibitor and in the presence of five different inhibitor concentrations (0.001–0.005 μM). As shown in Figure 4, the set of Lineweaver–Burk plots is indicative of competitive inhibition since the plots intersect on the *y*-axis. Thus, it may be concluded that compound **5g** most likely acts as a competitive inhibitor of human MAO-B. By constructing a plot of the slopes of the Lineweaver–Burk plots versus inhibitor concentration, a K_i value of 0.0045 μM for the inhibition of MAO-B by **5g** is estimated. Global (shared) fitting of the inhibition data directly to the
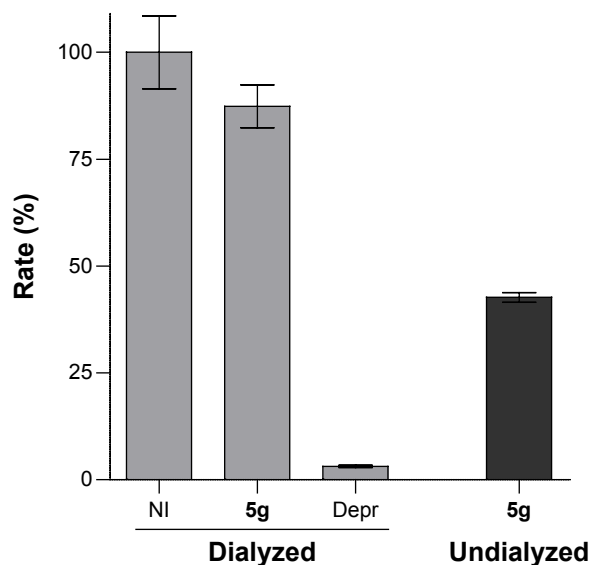

Figure 2 Dialysis of MAO-B/**5g** mixtures demonstrates reversibility.
Notes: MAO-B and **5g** were combined for 15 minutes, dialyzed for 24 hours, and the residual enzyme activity was measured (**5g** dialyzed). Similar dialysis of MAO-B in the absence (NI dialyzed) and in the presence of the irreversible inhibitor (R)-deprenyl (depr dialyzed) served as controls. For comparison, the residual MAO-B activity of undialyzed mixtures of the enzyme and **5g** is also shown (**5g** undialyzed).
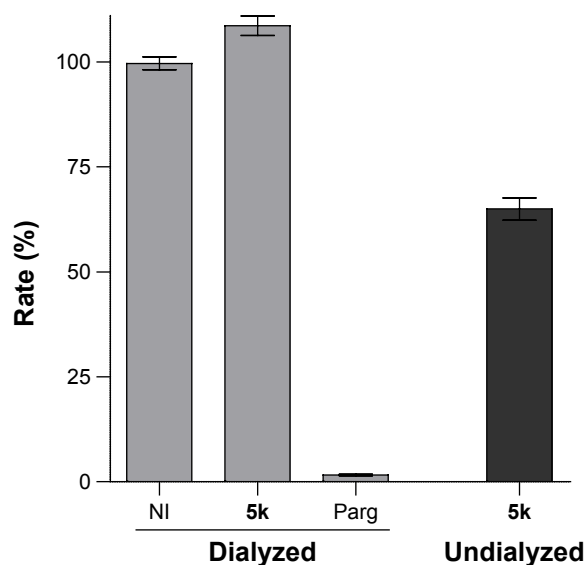**Abbreviations:** MAO-B, monoamine oxidase B; NI, no inhibitor; depr, (*R*)-deprenyl.

Figure 3 Dialysis of MAO-A/**5k** mixtures demonstrates reversibility.
Notes: MAO-A and **5k** were combined for 15 minutes, dialyzed for 24 hours, and the residual enzyme activity was measured (**5k** dialyzed). Similar dialysis of MAO-A in the absence (NI dialyzed) and in the presence of the irreversible inhibitor pargyline (parg dialyzed) served as controls. For comparison, the residual MAO-A activity of undialyzed mixtures of the enzyme and **5k** is also shown (**5k** undialyzed).
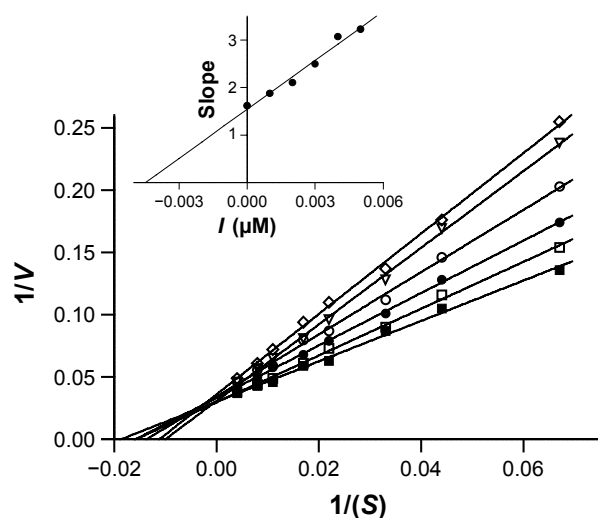**Abbreviations:** MAO-A, monoamine oxidase A; NI, no inhibitor; parg, pargyline.

Figure 4 Lineweaver–Burk plots of the inhibition of human MAO-B by **5g**.
Notes: The plots were constructed in the absence (filled squares) and presence of various concentrations of **5g** ($0.25 \times IC_{50}$ [open squares], $0.5 \times IC_{50}$ [filled circles], $0.75 \times IC_{50}$ [open circles], $1 \times IC_{50}$ [triangles] and $1.25 \times IC_{50}$ [diamonds]). The inset is a graph of the slopes of the Lineweaver–Burk plots versus inhibitor concentration (K_i =0.0045 μM). The units of initial velocity (V) and substrate concentration (S) are nmol/min·mg and μM, respectively.
Abbreviations: MAO-B, monoamine oxidase B; IC_{50}, half maximal inhibitory concentration.

Michaelis–Menten equation yields a similar K_i value of 0.0034±0.0003 μM (r^2 =0.99).

Conclusion

In conclusion, this study shows that 3-coumaranone derivatives are in general potent inhibitors of MAO-B. For example, five (**5d**–**g** and **5i**) of the 20 compounds evaluated exhibited IC_{50} values <0.020 μM. With the exception of one compound (**5s**), all IC_{50} values were <1 μM for the inhibition of MAO-B. These good activities have been anticipated since series of structurally related α-tetralone and 1-indanone derivatives have recently been shown to potently inhibit MAO-B (Table 1).[19–21] It is evident that potent MAO-B inhibitors are present among these reported series as well as among the 3-coumaranone derivatives. This study also finds that the 3-coumaranone derivatives are selective MAO-B inhibitors with comparatively weaker MAO-A inhibition potencies. SI values were >3.5. Only one compound (**5k**) inhibits MAO-A with an IC_{50}<1 μM. Four compounds (**5h**, **5l**, **5n**, and **5q**) did not inhibit MAO-A, even at a concentration of 100 μM (SI >1,852). These compounds are, however, potent MAO-B inhibitors (IC_{50}=0.028–0.054 μM) and may be viewed as particularly appropriate where selective inhibition of MAO-B is required. Since the reported α-tetralone and 1-indanone derivatives exhibit IC_{50}<10.75 μM for the inhibition of MAO-A, compounds **5h**, **5l**, **5n**, and **5q** may

be viewed as superior with respect to isoform selectivity. As mentioned in the "Introduction" section, MAO-A inhibitors may cause tyramine-induced changes in blood pressure, and selective MAO-B inhibition could be a desirable trait. The finding that representative 3-coumaranones are reversible MAO inhibitors further diminishes the possibility that these compounds may provoke the cheese effect. This study thus shows that 3-coumaranone derivatives are potent and selective MAO-B inhibitors with existing clinical utilities (eg, Parkinson's disease) and potential for new application for various disease states (Alzheimer's disease, smoking cessation).[27] Based on the significant MAO inhibitory potencies of 3-coumaranones, future studies should explore the effect of substitution at other positions, C4, C5, and C7 on MAO inhibition.

Disclosure

The authors report no conflicts of interest in this work.

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Supplementary materials
5a: 6-(Benzyloxy)-2H-1-benzofuran-3-one

The title compound was prepared in a yield of 56%. mp 91.4°C–96.6°C. ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.56 (d, J =8.6 Hz, 1H), 7.40 (m, 4H), 7.35 (m, 1H), 6.71 (dd, J =8.6, 2.1 Hz, 1H), 6.60 (d, J =2.0 Hz, 1H), 5.11 (s, 2H), 4.60 (s, 2H). ^{13}C NMR (151 MHz, CDCl$_3$) δ 70.6, 75.5, 97.4, 112.2, 114.5, 125.2, 127.5, 128.4, 128.8, 135.5, 167.2, 176.4, 197.5. APCI-HRMS m/z: calcd for C$_{15}$H$_{13}$O$_3$ (MH$^+$), 241.0859, found 241.0852.

5b: 6-(4-Fluorobenzyloxy)-2H-1-benzofuran-3-one

The title compound was prepared in a yield of 55%. mp 121.6°C–126.5°C. ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.56 (d, J =8.6 Hz, 1H), 7.41–7.35 (m, 2H), 7.08 (t, J =8.7 Hz, 2H), 6.69 (dd, J =8.6, 2.1 Hz, 1H), 6.58 (d, J =2.1 Hz, 1H), 5.06 (s, 2H), 4.60 (s, 2H). ^{13}C NMR (151 MHz, CDCl$_3$) δ 69.9, 75.5, 97.3, 99.9, 112.1, 114.6, 115.8, 125.2, 129.4, 131.3, 161.9, 163.5, 166.9, 176.3, 197.5. APCI-HRMS m/z: calcd for C$_{15}$H$_{12}$FO$_3$ (MH$^+$), 259.0765, found 259.0764.

5c: 6-(3-Fluorobenzyloxy)-2H-1-benzofuran-3-one

The title compound was prepared in a yield of 58%. mp 56.2°C–56.3°C. ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.57 (d, J =8.6 Hz, 1H), 7.35 (td, J =7.9, 5.8 Hz, 1H), 7.17 (d, J =8.1 Hz, 1H), 7.12 (d, J =9.4 Hz, 1H), 7.03 (td, J =8.4, 2.2 Hz, 1H), 6.71 (dd, J =8.6, 2.1 Hz, 1H), 6.58 (d, J =2.0 Hz, 1H), 5.11 (s, 2H), 4.61 (s, 2H). ^{13}C NMR (151 MHz, CDCl$_3$) δ 69.7, 75.5, 97.4, 112.1, 114.2, 114.8, 115.4, 122.7, 125.3, 130.3, 138.1, 162.2, 166.8, 176.3, 197.5. APCI-HRMS m/z: calcd for C$_{15}$H$_{12}$FO$_3$ (MH$^+$), 259.0765, found 259.0752.

5d: 6-(4-Chlorobenzyloxy)-2H-1-benzofuran-3-one

The title compound was prepared in a yield of 51%. mp 137.5°C–141.7°C. ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.56 (d, J =8.6 Hz, 1H), 7.37–7.33 (m, 4H), 6.69 (dd, J =8.6, 2.1 Hz, 1H), 6.57 (d, J =2.1 Hz, 1H), 5.07 (s, 2H), 4.60 (s, 2H). ^{13}C NMR (151 MHz, CDCl$_3$) δ 69.7, 75.5, 97.4, 112.1, 114.7, 125.2, 128.8, 128.9, 134.0, 134.3, 166.9, 176.3, 197.5. APCI-HRMS m/z: calcd for C$_{15}$H$_{12}$ClO$_3$ (MH$^+$), 275.0469, found 275.0452.

5e: 6-(3-Chlorobenzyloxy)-2H-1-benzofuran-3-one

The title compound was prepared in a yield of 57%. mp 108.7°C–112.3°C. ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.47 (d, J =8.6 Hz, 1H), 7.31 (s, 1H), 7.24–7.17 (m, 3H), 6.61 (dd, J =8.6, 2.0 Hz, 1H), 6.48 (d, J =2.0 Hz, 1H), 4.98 (s, 2H), 4.51 (s, 2H). ^{13}C NMR (151 MHz, CDCl$_3$) δ 69.6, 75.5, 97.4, 112.0, 114.8, 125.2, 125.3, 127.4, 128.5, 130.0, 134.7, 137.5, 166.8, 176.3, 197.5. APCI-HRMS m/z: calcd for C$_{15}$H$_{12}$ClO$_3$ (MH$^+$), 275.0469, found 275.0453.

5f: 6-(4-Bromobenzyloxy)-2H-1-benzofuran-3-one

The title compound was prepared in a yield of 51%. mp 140.0°C–142.1°C. ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.56 (d, J =8.6 Hz, 1H), 7.52 (d, J =8.4 Hz, 2H), 7.28 (d, J =8.4 Hz, 2H), 6.69 (dd, J =8.6, 2.1 Hz, 1H), 6.57 (d, J =2.0 Hz, 1H), 5.06 (s, 2H), 4.60 (s, 2H). ^{13}C NMR (151 MHz, CDCl$_3$) δ 69.8, 75.5, 97.4, 112.1, 114.7, 122.4, 125.2, 129.1, 131.9, 134.5, 166.8, 176.3, 197.5. APCI-HRMS m/z: calcd for C$_{15}$H$_{12}$BrO$_3$ (MH$^+$), 318.9964, found 318.9963.

5g: 6-(3-Bromobenzyloxy)-2H-1-benzofuran-3-one

The title compound was prepared in a yield of 50%. mp 120.0°C–122.6°C. ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.59–7.54 (m, 2H), 7.47 (d, J =7.9 Hz, 1H), 7.32 (d, J =7.7 Hz, 1H), 7.26 (d, J =7.8 Hz, 1H), 6.70 (dd, J =8.6, 2.1 Hz, 1H), 6.57 (d, J =2.1 Hz, 1H), 5.07 (s, 2H), 4.61 (s, 2H). ^{13}C NMR (151 MHz, CDCl$_3$) δ 69.5, 75.5, 97.4, 112.0, 114.8, 122.8, 125.3, 125.8, 130.3, 130.3, 131.5, 137.8, 166.8, 176.3, 197.5. APCI-HRMS m/z: calcd for C$_{15}$H$_{12}$BrO$_3$ (MH$^+$), 318.9964, found 318.9955.

5h: 6-(4-Iodobenzyloxy)-2H-1-benzofuran-3-one

The title compound was prepared in a yield of 52%. mp 136.0°C–138.5°C. ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.72 (d, J =8.1 Hz, 2H), 7.56 (d, J =8.6 Hz, 1H), 7.15 (d, J =8.1 Hz, 2H), 6.68 (dd, J =8.6, 1.5 Hz, 1H), 6.56 (d, J =1.6 Hz, 1H), 5.05 (s, 2H), 4.60 (s, 2H). ^{13}C NMR (151 MHz, CDCl$_3$) δ 69.9, 75.5, 94.0, 97.4, 112.1, 114.7, 125.3, 129.2, 135.2, 137.9, 166.8, 176.3, 197.5. APCI-HRMS m/z: calcd for C$_{15}$H$_{12}$IO$_3$ (MH$^+$), 366.9826, found 366.9805.

5i: 6-(3-Iodobenzyloxy)-2H-1-benzofuran-3-one

The title compound was prepared in a yield of 51%. mp 161.2°C–164.5°C. ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.77 (s, 1H), 7.68 (d, J =8.0 Hz, 1H), 7.57 (d, J =8.6 Hz, 1H), 7.36 (d, J =8.1 Hz, 1H), 7.12 (t, J =7.8 Hz, 1H), 6.70 (dd, J =8.6,

2.1 Hz, 1H), 6.57 (d, J =2.0 Hz, 1H), 5.05 (s, 2H), 4.61 (s, 2H).^{13}C NMR (151 MHz, CDCl$_3$) δ 69.5, 75.6, 94.5, 97.4, 112.0, 114.8, 125.3, 126.5, 130.4, 136.3, 137.5, 137.8, 166.8, 176.3, 197.5. APCI-HRMS *m/z*: calcd for C$_{15}$H$_{12}$IO$_3$ (MH$^+$), 366.9826, found 366.9809.

5j: 6-(4-Cyanobenzyloxy)-*2H*-1-benzofuran-3-one

The title compound was prepared in a yield of 59%. mp 185.8°C–187.2°C. ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.68 (d, J =8.2 Hz, 2H), 7.57 (d, J =8.6 Hz, 1H), 7.52 (d, J =8.3 Hz, 2H), 6.70 (dd, J =8.6, 2.1 Hz, 1H), 6.56 (d, J =2.0 Hz, 1H), 5.17 (s, 2H), 4.60 (s, 2H). ^{13}C NMR (151 MHz, CDCl$_3$) δ 69.3, 75.5, 97.5, 111.8, 112.2, 115.0, 118.4, 125.4, 127.5, 132.5, 140.9, 166.4, 176.2, 197.4. APCI-HRMS *m/z*: calcd for C$_{16}$H$_{12}$NO$_3$ (MH$^+$), 266.0812, found 266.0812.

5k: 6-(3-Cyanobenzyloxy)-*2H*-1-benzofuran-3-one

The title compound was prepared in a yield of 59%. mp 148.4°C–155.9°C. ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.72 (s, 1H), 7.67–7.61 (m, 2H), 7.58 (d, J =8.6 Hz, 1H), 7.51 (t, J =7.8 Hz, 1H), 6.71 (dd, J =8.6, 2.1 Hz, 1H), 6.58 (d, J =2.1 Hz, 1H), 5.14 (s, 2H), 4.61 (s, 2H). ^{13}C NMR (151 MHz, CDCl$_3$) δ 69.1, 75.6, 97.5, 111.9, 113.0, 115.0, 118.4, 125.4, 129.6, 130.7, 131.5, 132.0, 137.2, 166.4, 176.2, 197.5. APCI-HRMS *m/z*: calcd for C$_{16}$H$_{12}$NO$_3$ (MH$^+$), 266.0812, found 266.0797.

5l: 6-(4-Methylbenzyloxy)-*2H*-1-benzofuran-3-one

The title compound was prepared in a yield of 52%. mp 104.9°C–109.2°C. ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.55 (d, J =8.6 Hz, 1H), 7.29 (d, J =8.0 Hz, 2H), 7.20 (d, J =7.8 Hz, 2H), 6.69 (dd, J =8.6, 2.1 Hz, 1H), 6.59 (d, J =2.1 Hz, 1H), 5.06 (s, 2H), 4.59 (s, 2H), 2.35 (s, 3H). ^{13}C NMR (151 MHz, CDCl$_3$) δ 21.2, 70.6, 75.5, 97.3, 112.2, 114.4, 125.1, 127.7, 129.4, 132.4, 138.3, 167.3, 176.4, 197.5. APCI-HRMS *m/z*: calcd for C$_{16}$H$_{15}$O$_3$ (MH$^+$), 255.1016, found 255.1003.

5m: 6-(3-Methylbenzyloxy)-*2H*-1-benzofuran-3-one

The title compound was prepared in a yield of 54%. mp 158.4°C–161.7°C. ^{1}H NMR (600 MHz CDCl$_3$,) δ 7.56 (d, J =8.6 Hz, 1H), 7.15 (d, J =7.5 Hz, 1H), 7.23–7.19 (m, 2H), 7.28 (t, J =7.5 Hz, 1H), 6.71 (dd, J =8.6, 2.1 Hz, 1H), 6.60 (d, J =2.0 Hz, 1H), 5.07 (s, 2H), 4.60 (s, 2H), 2.36 (s, 3H).

^{13}C NMR (151 MHz, CDCl$_3$) δ 21.4, 70.7, 75.5, 97.4, 112.2, 114.5, 124.6, 125.1, 128.2, 128.6, 129.2, 135.4, 138.5, 167.3, 176.4, 197.6. APCI-HRMS *m/z*: calcd for C$_{16}$H$_{15}$O$_3$ (MH$^+$), 255.1016, found 255.1020.

5n: 6-[4-(Trifluoromethyl)benzyloxy]-*2H*-1-benzofuran-3-one

The title compound was prepared in a yield of 45%. mp 137.5°C–143.8°C. ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.65 (d, J =8.1 Hz, 2H), 7.57 (d, J =8.6 Hz, 1H), 7.53 (d, J =8.0 Hz, 2H), 6.71 (dd, J =8.6, 2.1 Hz, 1H), 6.58 (d, J =2.1 Hz, 1H), 5.17 (s, 2H), 4.61 (s, 2H). ^{13}C NMR (151 MHz, CDCl$_3$) δ 69.6, 75.5, 97.5, 112.0, 114.9, 123.0, 124.8, 125.3, 125.7 (q), 127.4, 130.5 (q), 139.5, 166.7, 176.3, 197.5. APCI-HRMS *m/z*: calcd for C$_{16}$H$_{12}$F$_3$O$_3$ (MH$^+$), 309.0733, found 309.0720.

5o: 6-(2-Phenylethoxy)-*2H*-1-benzofuran-3-one

The title compound was prepared in a yield of 50%. mp 77.1°C–84.8°C. ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.53 (d, J =8.6 Hz, 1H), 7.33–7.30 (m, 2H), 7.27–7.23 (m, 3H), 6.62 (dd, J =8.6, 2.0 Hz, 1H), 6.51 (d, J =2.0 Hz, 1H), 4.59 (s, 2H), 4.22 (t, J =7.0 Hz, 2H), 3.11 (t, J =7.0 Hz, 2H). ^{13}C NMR (151 MHz, CDCl$_3$) δ 35.4, 69.3, 75.5, 96.9, 112.0, 114.3, 125.1, 126.8, 128.6, 128.9, 137.5, 167.4, 176.5, 197.6. APCI-HRMS *m/z*: calcd for C$_{16}$H$_{15}$O$_3$ (MH$^+$), 255.1016, found 255.1011.

5p: 6-(3-Phenylpropoxy)-*2H*-1-benzofuran-3-one

The title compound was prepared in a yield of 53%. mp 84.4°C–96.7°C. ^{1}H NMR (600 MHz CDCl$_3$,) δ 7.54 (d, J =8.6 Hz, 1H), 7.30–7.27 (m, 2H), 7.21–7.18 (m, 3H), 6.63 (dd, J =8.6, 2.1 Hz, 1H), 6.48 (d, J =2.0 Hz, 1H), 4.59 (s, 2H), 3.99 (t, J =6.3 Hz, 2H), 2.80 (t, J =7.6 Hz, 2H), 2.16–2.09 (m, 2H). ^{13}C NMR (151 MHz, CDCl$_3$) δ 30.3, 31.9, 67.6, 75.5, 96.8, 112.0, 114.2, 125.0, 126.1, 128.4, 128.5, 140.9, 167.6, 176.5, 197.5. APCI-HRMS *m/z*: calcd for C$_{17}$H$_{17}$O$_3$ (MH$^+$), 269.1172, found 269.1180.

5q: 6-(2-Phenoxyethoxy)-*2H*-1-benzofuran-3-one

The title compound was prepared in a yield of 53%. mp 156.2°C–158.2°C. ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.56 (d, J =8.6 Hz, 1H), 7.30 (td, J =7.4, 2.0 Hz, 2H), 6.97 (t, J =7.4 Hz, 1H), 6.94 (d, J =7.9 Hz, 2H), 6.68 (dd, J =8.6, 2.1 Hz, 1H), 6.58 (d, J =2.0 Hz, 1H), 4.62 (s, 2H), 4.39–4.36 (m, 2H), 4.35–4.33 (m, 2H). ^{13}C NMR (151 MHz, CDCl$_3$) δ 65.9, 67.2,

75.5, 97.1, 112.0, 114.6, 114.6, 121.3, 125.2, 129.6, 158.3, 167.1, 176.4, 197.6. APCI-HRMS *m/z*: calcd for $C_{16}H_{15}O_4$ (MH⁺), 271.0965, found 271.0953.

5r: 6-[2-(4-Bromophenyl)ethoxy]-*2H*-1-benzofuran-3-one

The title compound was prepared in a yield of 45%. mp 40.7°C–40.8°C. ¹H NMR (600 MHz, CDCl₃) δ 7.53 (d, *J* =8.6 Hz, 1H), 7.43 (d, *J* =8.4 Hz, 2H), 7.14 (d, *J* =8.4 Hz, 2H), 6.60 (dd, *J* =8.6, 2.1 Hz, 1H), 6.49 (d, *J* =2.0 Hz, 1H), 4.59 (s, 2H), 4.19 (t, *J* =6.7 Hz, 2H), 3.06 (t, *J* =6.7 Hz, 2H). ¹³C NMR (151 MHz, CDCl₃) δ 34.8, 68.9, 75.5, 96.9, 111.9, 114.5, 120.6, 125.1, 130.7, 131.7, 136.6, 167.2, 176.4, 197.5. APCI-HRMS *m/z*: calcd for $C_{16}H_{14}BrO_3$ (MH⁺), 333.0121, found 333.0129.

5s: 6-[2-(4-Methylphenyl)ethoxy]-*2H*-1-benzofuran-3-one

The title compound was prepared in a yield of 56%. mp 98.6°C–104.2°C. ¹H NMR (600 MHz, CDCl₃) δ 7.53 (d,

J =8.6 Hz, 1H), 7.15 (d, *J* =8.1 Hz, 2H), 7.12 (d, *J* =8.0 Hz, 2H), 6.62 (dd, *J* =8.6, 2.1 Hz, 1H), 6.50 (d, *J* =2.0 Hz, 1H), 4.59 (s, 2H), 4.19 (t, *J* =7.1 Hz, 2H), 3.07 (t, *J* =7.0 Hz, 2H), 2.32 (s, 3H). ¹³C NMR (151 MHz, CDCl₃) δ 21.0, 35.0, 69.5, 75.5, 96.8, 112.0, 114.3, 125.1, 128.8, 129.3, 134.3, 136.3, 167.4, 176.5, 197.6. APCI-HRMS *m/z*: calcd for $C_{17}H_{17}O_3$ (MH⁺), 269.1172, found 269.1159.

5t: 6-[2-(3-Methylphenyl)ethoxy]-*2H*-1-benzofuran-3-one

The title compound was prepared in a yield of 66%. mp 209.3°C–212.2°C. ¹H NMR (600 MHz, CDCl₃) δ 7.54 (d, *J* =8.6 Hz, 1H), 7.20 (t, *J* =7.5 Hz, 1H), 7.09–7.04 (m, 3H), 6.63 (dd, *J* =8.6, 2.0 Hz, 1H), 6.51 (d, *J* =2.0 Hz, 1H), 4.60 (s, 2H), 4.20 (t, *J* =7.1 Hz, 2H), 3.07 (t, *J* =7.1 Hz, 2H), 2.33 (s, 3H). ¹³C NMR (151 MHz, CDCl₃) δ 21.4, 35.3, 69.4, 75.5, 96.9, 112.0, 114.3, 125.1, 125.9, 127.5, 128.5, 129.7, 137.3, 138.2, 167.4, 176.5, 197.6. APCI-HRMS *m/z*: calcd for $C_{17}H_{17}O_3$ (MH⁺), 269.1172, found 269.1159.