Bioorganic Chemistry 69 (2016) 20-28

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

2-Heteroarylidene-1-indanone derivatives as inhibitors of monoamine oxidase

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ARTICLE INFO

Article history: Received 10 August 2016 Revised 15 September 2016 Accepted 16 September 2016 Available online 17 September 2016

Keywords: Indanone Monoamine oxidase MAO Inhibition Chalcone SAR Heterocyclic

ABSTRACT

In the present study a series of fifteen 2-heteroarylidene-1-indanone derivatives were synthesised and evaluated as inhibitors of recombinant human monoamine oxidase (MAO) A and B. These compounds are structurally related to series of heterocyclic chalcone derivatives which have previously been shown to act as MAO-B specific inhibitors. The results document that the 2-heteroarylidene-1-indanones are in vitro inhibitors of MAO-B, displaying IC_{50} values of 0.0044–1.53 μ M. Although with lower potencies, the derivatives also inhibit the MAO-A isoform with IC_{50} values as low as 0.061 μ M. An analysis of the structure-activity relationships for MAO-B inhibition indicates that substitution with the methoxy group on the A-ring leads to a significant enhancement in MAO-B inhibition compared to the unsubstituted homologues while the effect of the heteroarylidene-1-indanone derivatives are promising leads for the design of MAO inhibitors for the treatment of Parkinson's disease and possibly other neurodegenerative disorders.

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1. Introduction

Monoamine oxidase (MAO) is a mitochondrial-bound flavoenzyme that regulates the levels of amine-containing compounds in the brain and peripheral tissues [1,2]. MAO is expressed as two isoforms, MAO-A and MAO-B. In mammals, these isoforms are found in all tissues but at different levels. The MAOs fulfil important biochemical functions: firstly to metabolise neurotransmitter amines thereby terminating their actions and secondly to act as metabolic barriers for the entry of sympathomimetic amines into the systemic circulation and brain [1]. With respect to the latter, MAO-A present in the gastrointestinal mucosa metabolises dietary tyramine thus limiting its levels in the circulatory system. Excess tyramine may induce the release of norepinephrine from peripheral neurons leading to a dangerous increase in blood-pressure [1,3,4]. MAO-B, in turn, is present in the microvasculature of the brain and metabolises false neurotransmitters such as benzylamine and 2-phenylethylamine, which restricts their entry into the brain [5].

It is, however, their roles in the metabolism of neurotransmitters that makes the MAOs clinically relevant drug targets [6,7].

* Corresponding author. E-mail address: lesetja.legoabe@nwu.ac.za (L.J. Legoabe). MAO-A metabolises serotonin in the brain and MAO-A specific inhibitors are used in the clinic as antidepressants, acting by enhancing serotonin-mediated neurotransmission [8,9]. Examples of antidepressants that act by inhibiting MAO-A are phenelzine (1) and tranylcypromine (2) (Fig. 1). These are irreversible acting compounds. Examples of reversible MAO-A inhibitors for the treatment of depression are moclobemide (3) and toloxatone (4). Reversibility of MAO-A inhibition is an important consideration since irreversible inhibitors may precipitate a serious hypertensive event when combined with tyramine-containing food [3,4]. As a result, irreversible MAO-A inhibitors are used with caution in the clinic and dietary restrictions are imposed. Reversible MAO-A inhibitors, on the other hand, have good safety profiles and are not associated with blood-pressure changes when combined with dietary tyramine [10,11].

The MAO-B isoform, in turn, metabolises dopamine in the brain and specific MAO-B inhibitors are thus used for the treatment of Parkinson's disease in the clinic [6]. MAO-B inhibitors conserve the depleted dopamine supply in the Parkinsonian brain and provide symptomatic relief. In this regard, MAO-B inhibitors are mostly used in combination with L-dopa, and presumably further enhance dopamine levels in response to L-dopa therapy [12,13]. Besides providing symptomatic benefits, MAO-B inhibitors may also protect against neurodegeneration in Parkinson's disease.









Fig. 1. The structures of known MAO inhibitors.

While the mechanism is unclear, MAO-B inhibitors may reduce the formation of injurious by-products of the MAO-B catalytic cycle [1]. These are hydrogen peroxide and aldehyde species, which may be harmful to neuronal cells if not efficiently cleared. Since MAO-B exhibits an age-related increase in the brain, the resulting enhanced formation of hydrogen peroxide and aldehydes in the aged Parkinsonian brain may significantly contribute to the degenerative processes [14]. Two irreversible MAO-B inhibitors are currently used for the treatment of Parkinson's disease, (R)-deprenyl (**5**) and rasagiline (**6**). A reversible inhibitor, safinamide (**7**), has also been developed for this purpose.

Due to their biochemical and clinical importance, the discovery and development of MAO inhibitors are being actively pursued. Recently it has been reported that a series of furanochalcones are potent MAO-B-specific inhibitors with the most active compound (8) exhibiting an IC₅₀ value of $0.174 \,\mu\text{M}$ for the human form of the enzyme (Fig. 2) [15]. In a subsequent paper, a series of heterocyclic chalcone derivatives were examined as human MAO inhibitors [16]. These also were specific MAO-B inhibitors with the most potent inhibitor (**9**) exhibiting an IC₅₀ value of 0.067 μ M. Another inhibitor, compound **10**, also proved to be a potent MAO-B inhibitor an IC₅₀ value of 0.185 μ M. Based on these findings, the present study examines the MAO inhibition properties of a series of fifteen derivatives 2-heteroarylidene-1-indanone (11a-o). The 2-heteroarylidene-1-indanones may be considered to be the cyclic analogues of heterocyclic chalcone analogues such as 8 and 10, and it is thus conceivable that this chemical class may, similar to the chalcones, act as MAO-B inhibitors. In support of this proposal, it was recently shown that 2-benzylidene-1-indanones (e.g. 12) are inhibitors of the MAOs [17]. The present study is to the best of our knowledge the first investigation of the MAO inhibition properties of 2-heteroarylidene-1-indanone derivatives. For the purpose of this study, the following heteroaromatic substituents (ring B) were selected: 2-pyridine, 3-pyridine, 2-chloro-3pyridine, 2-furan, 2-thiophene, 3-thiophene, 2-pyrrole, 5-methyl-2-furan and 5-bromo-2-furan (Table 1). For comparison, derivatives incorporating the cyclohexyl ring were also included. The A-ring was left unsubstituted or substituted with the methoxy group on C5 of the indole. In the reported study on the 2-benzylidene-1-indanones, ring A was disubstituted with the hydroxy and methoxy groups [17].

2. Results and discussion

2.1. Chemistry

The heteroarylidene-1-indanone derivatives, **11a–o**, were synthesised employing basic conditions. 1-Indanone or 5-methoxy-1-indanone was reacted with the appropriate heteroaromatic aldehyde in the presence of potassium hydroxide or sodium hydroxide (Fig. 3). Methanol served as reaction solvent. The reactions were stirred at room temperature for 2–5 h and on completion, the product was isolated by precipitation after the addition of water. Following recrystallisation from ethanol, the target heteroarylidene-1-indanones were obtained in yields of 17–79%. Structures were characterised by ¹H NMR, ¹³C NMR and mass spectrometry as cited in the experimental section.

2.2. Protocol for the inhibition studies

To evaluate the MAO inhibition properties of the heteroarylidene-1-indanone derivatives, recombinant human MAO-A and MAO-B were used [18,19]. For both MAO isoforms, kynuramine served as substrate. The enzymes [0.0075 mg protein/mL (MAO-A) and 0.015 mg protein/mL (MAO-B)], substrate



Fig. 2. The structures of heterocyclic chalcone derivatives (8–10) that exhibit MAO inhibition. The general structures of the 2-heteroarylidene-1-indanone derivatives (11a–0) that will be investigated in this study and that of a 2-benzylidene-1-indanone (12) are also given.

Table 1

The IC₅₀ values for the inhibition of recombinant human MAO-A and MAO-B by heteroarylidene-1-indanone derivatives.



| | R | R′ | IC ₅₀ (μΜ) ^a | | SI ^b |
|-------------------------|------------------|------------------------------|------------------------------------|-------------------|-----------------|
| | | | MAO-A | МАО-В | |
| 11a | Н | | 0.061 ± 0.006 | 1.27 ± 0.596 | 0.05 |
| 11b | OCH ₃ | | 8.55 ± 0.668 | 0.338 ± 0.056 | 25 |
| 11c | Н | | 0.853 ± 0.051 | 1.53 ± 0.346 | 0.56 |
| 11d | OCH ₃ | | 0.302 ± 0.008 | 0.112 ± 0.005 | 2.7 |
| 11e ^c | Н | | 0.471 ± 0.006 | 0.465 ± 0.032 | 1.0 |
| 11f | Н | | 1.20 ± 0.191 | 0.889 ± 0.089 | 1.4 |
| 11g | OCH ₃ | | 2.33 ± 1.23 | 0.394 ± 0.031 | 5.9 |
| 11h | Н | , ∧s | 1.33 ± 0.215 | 0.418 ± 0.053 | 3.2 |
| 11i | OCH ₃ | ∧_s | 5.18 ± 0.628 | 0.114 ± 0.013 | 45 |
| 11j | Н | , [™] z | 0.322 ± 0.061 | 0.379 ± 0.069 | 0.85 |
| 11k | Н | | 1.51 ± 0.212 | 0.114 ± 0.005 | 13 |
| 11I ^c | Н | \sim | 5.21 ± 0.837 | 0.229 ± 0.035 | 23 |
| 11m | OCH ₃ | $\tilde{\langle }$ | No inh ^d | 0.139 ± 0.018 | >719 |
| 11n ^c | OCH ₃ | $\langle \downarrow \rangle$ | 4.73 ± 0.190 | 0.026 ± 0.005 | 182 |
| 110 | OCH ₃ | o Br | 0.183 ± 0.038 | 0.0044 ± 0.0006 | 42 |

All values are expressed as the mean ± standard deviation (SD) of triplicate determinations.

- b Selectivity index (SI) = $IC_{50}(MAO-A)/IC_{50}(MAO-B)$.

^c Low purity samples.

 $^{\rm d}$ No inhibition observed at a maximal tested concentration of 100 μ M.



Fig. 3. The synthetic pathway to heteroarylidene-1-indanone derivatives 11a-o. Reagents and conditions: (a) KOH or NaOH, methanol, room temperature.

 $(50 \,\mu\text{M})$ and test inhibitor (at concentrations of $0.003-100 \,\mu\text{M}$) were incubated for 20 min where after the reactions were terminated with the addition of sodium hydroxide. At this endpoint, the concentration of 4-hydroxyquinoline, the product of kynuramine oxidation, was measured with fluorescence spectrophotometry. The rate of 4-hydroxyquinoline formation in the absence of inhibitor served as 100% MAO activity. From the rate



Fig. 4. Sigmoidal plots for the inhibition of MAO-A and MAO-B by 11n (open circles) and 11o (filled circles). Each measurement was conducted in triplicate and is given as mean ± SD.



Fig. 5. Proposed binding orientations and interactions of 11a (cyan) and 11o (yellow) in MAO-A.

data, sigmoidal plots (rate vs. logarithm of inhibitor concentration) were constructed from which the IC_{50} values were estimated. Provided as an example, the sigmoidal plots for the inhibition of the MAOs by compounds **11n** and **11o** are shown in Fig. 4.

2.3. IC₅₀ values for the inhibition of MAO

The IC₅₀ values for the inhibition of the human MAOs by the heteroarylidene-1-indanone derivatives **11a–o** are given in Table 1. The results show that most of the derivatives are specific inhibitors of MAO-B with only **11a** and **11c** displaying MAO-A specificity. Compounds **11e**, **11f** and **11j** are essentially non-specific MAO inhibitors. High potency MAO inhibitors are present among the compounds evaluated with the most noteworthy being compound **11a** (IC₅₀ = 0.061 μ M), a MAO-A inhibitor, and **11n** (IC₅₀ = 0.026 - μ M) and **11o** (IC₅₀ = 0.0044 μ M), the latter both MAO-B inhibitors. These compounds are more potent MAO inhibitors than the

reference MAO-A inhibitor, toloxatone ($IC_{50} = 3.92 \mu$ M), and the reference MAO-B inhibitors lazabemide ($IC_{50} = 0.091 \mu$ M) and safinamide ($IC_{50} = 0.048 \mu$ M), which were evaluated under identical experimental conditions [20]. It may thus be concluded that heteroarylidene-1-indanone derivatives are, in general, a chemical class that possesses the ability to inhibit the human MAOs. Further support for this is the finding that among the fifteen compounds evaluated, six inhibited MAO-A with $IC_{50} < 1 \mu$ M while thirteen compounds inhibited MAO-B with $IC_{50} < 1 \mu$ M.

An analysis of the structure-activity relationships for MAO inhibition was carried out. The inhibition data reveals the following trends: (1) Substitution with the methoxy group on the A-ring of the indole leads to an enhancement in MAO-B inhibition. Each methoxy-substituted derivative is a more potent MAO-B inhibitor than the corresponding unsubstituted homologue (for example **11a** vs. **11b**). (2) Substitution on the furan ring of **11g** (IC₅₀ = 0.394 μ M) significantly enhances MAO-B inhibition



Fig. 6. Proposed binding orientations and interactions of 11a (cyan) and 11o (yellow) in MAO-B.

potency with methyl- and bromo-substitution yielding 11n $(IC_{50} = 0.026 \,\mu\text{M})$ and **110** $(IC_{50} = 0.0044 \,\mu\text{M})$, the most potent MAO-B inhibitors of the series. (3) Although the differences in the MAO-B inhibition activities of the derivatives are in many instances small, the general effects of the heteroaromatic substituents on activity may be evaluated. When considering the methoxy-substituted homologues, in general, the effect of the heteroaromatic substituent in decreasing order is: 5-bromo-2furan > 5-methyl-2-furan > 2-pyridine \approx 2-thiophene > cyclohexyl > 3-pyridine \approx 2-furan. When considering the unsubstituted homologues, the general, effect of the heteroaromatic substituent in decreasing order is: 3-thiophene > cyclohexyl > 2-pyrrole \approx 2-thiophene \approx 2-chloro-3-pyridine > 2-furan > 3-pyridine \approx 2-pyridine. For both analyses, 3-pyridine and 2-furan substitution yields comparatively weaker MAO-B inhibitors.

2.4. Molecular interactions with MAO via docking studies

In an attempt to characterise the interactions of the heteroarylidene-1-indanone derivatives with the MAOs, compounds 11a (the most potent MAO-A inhibitor) and 11o (the most potent MAO-B inhibitor) were selected and docked into models of the MAO active sites using the CDOCKER docking function of Discovery Studio 3.1 (Accelrys). For this purpose, the crystal structures of human MAO-A in complex with harmine (PDB code: 2Z5X) and MAO-B in complex with safinamide (PDB code: 2V5Z) were used [21,22]. The procedure employed for the docking study has previously been shown to accurately redock the cocrystallised ligands into the MAO models and may thus be deemed suitable for the current study [18]. Figs. 5 and 6 illustrate the binding orientations and selected interactions of **11a** and **110** with MAO-A and MAO-B, respectively. In MAO-A, the inhibitors adopt similar orientations with the heteroaryl moieties (ring B) bound in the rear of the active site in proximity to the FAD. Compound 11a forms a π - π interaction with Tyr-444 while **110** is hydrogen bonded to Cys-323. The orientations of 11a and 11o in MAO-B are virtually superimposable and are reversed compared to those observed in MAO-A. In MAO-B the indanone moiety is thus placed in proximity to the FAD with the heteroaryl ring extending into the entrance cavity. Both inhibitors establish π - π interactions with Tyr-398. The significant difference in the MAO-B inhibition potencies of 11a and 11o may be explained by the possibility that the 5-bromo-2-furan ring of 110 forms more productive Van der Waals interactions with the entrance cavity of MAO-B compared to the pyridyl ring of **11a**. The entrance cavity of MAO-B is lined by hydrophobic residues, which makes Van der Waals interactions the dominant stabilising force for ligands bound to this space. The importance of π - π interactions in MAO-A and MAO-B may be illustrated that the observation that the loss thereof (e.g. 110 in MAO-A) leads to relatively weak inhibition.

3. Conclusion

Chalcones are well-known to inhibit the MAO enzymes with specificity for the MAO-B isoform [23]. Recent reports also show that chalcones incorporating heteroaromatic substituents inhibit the MAOs, with a number of compounds exhibiting specific and potent MAO-B inhibition [15,16]. Furthermore, heterocyclic chalcone derivatives have been shown to inhibit the MAOs reversibly [16]. Based on these reports the present study investigated, for the first time, the MAO inhibitory properties of 2-heteroarylidene-1-indanone derivatives. These may be considered as cyclic analogues of heterocyclic chalcones. Similar to the chalcones, the 2-heteroarylidene-1-indanone derivatives were found to be MAO-B specific inhibitors with two compounds, **11n** and **110** exhibiting particularly potent activities. One inhibitor, **11a**, was found to be high potency MAO-A inhibitor. This compound also exhibits specificity for the MAO-A isoform. Although the reversibility of MAO inhibition was not examined, it is likely that the 2-heteroarylidene-1-indanones are reversible MAO inhibitors since the related 2-benzylidene-1-indanones have been found to act reversibly. Furthermore, none of these structures contain functional groups such as the propargylamine, cyclopropylamine, hydrazine, haloallylamine and N-(2-aminoethyl)carboxamide moieties that are typically associated with irreversible MAO inhibition. Based on these findings, it may be concluded that among 2-heteroarylidene-1-indanone derivatives are compounds with useful MAO inhibition properties [24,25]. MAO inhibitors such as these find application for the treatment of disorders such as depression and Parkinson's disease. Future investigations into the physicochemical, biochemical and pharmacokinetic properties of active compounds are required to determine if these could act as effective inhibitors in vivo.

4. Materials and methods

4.1. Chemicals and instrumentation

Unless otherwise stated, all reagents and solvents were purchased from Sigma-Aldrich and were used without further purification. These include microsomes from insect cells containing recombinant human MAO-A and MAO-B (5 mg protein/mL) and kynuramine dihydrobromide, which were used for the enzymology. A Varian Cary Eclipse instrument was employed for fluorescence spectrophotometry. NMR spectra were recorded on a Bruker Avance III 600 spectrometer in chloroform-d (CDCl₃) or DMSO- d_6 . Chemical shifts are reported in parts per million (∂) and were referenced to the residual solvent signal (CDCl₃: 7.26 and 77.16 ppm for ¹H and ¹³C, respectively; DMSO- d_6 : 2.50 and 39.52 ppm for ¹H and ¹³C, respectively). Spin multiplicities are given as s (singlet), d (doublet), dd (doublet of doublets), dt (doublet of triplets), ddd (doublet of doublet of doublets), t (triplet), td (triplet of doublets) or m (multiplet). High resolution mass spectra (HRMS) were recorded on a Bruker micrOTOF-O II mass spectrometer in atmospheric-pressure chemical ionisation (APCI) mode while melting points (mp) were measured with a Buchi B-545 melting point apparatus. Melting points are given uncorrected. Silica gel 60 aluminium coated thin-layer chromatography (TLC) sheets (Merck) were used to monitor reaction progress. The TLC sheets were visualised under an UV-lamp at a wavelength of 254 nm or by staining with iodine vapour.

4.2. General synthesis of heteroarylidene-1-indanone derivatives **11a**-*k* and **11m-o**

Commercially available 1-indanone or 5-methoxy-1-indanone (3 mmol) and the appropriate heteroaromatic aldehyde (4 mmol) were suspended in methanol (5 mL) containing KOH (4.54 mmol) or NaOH (6.16 mmol). The reaction was stirred at room temperature for 2–5 h and the progress was monitored by TLC. The TLC sheets were developed in a mobile phase consisting of petroleum ether/ethyl acetate (2:1). Upon completion, cold water (20 mL) was added to obtain a precipitate, which was collected by filtration. The crude thus obtained was purified by recrystallisation from ethanol.

4.2.1. (2E)-2-(Pyridin-3-ylmethylidene)-2,3-dihydro-1H-inden-1-one (**11a**)

The title compound was prepared in a yield of 29%: mp 152.9– 153.6 °C (ethanol), white solid. ¹H NMR (600 MHz, CDCl₃) δ 8.88 (s, 1H), 8.58 (d, *J* = 4.3 Hz, 1H), 7.93 (dt, *J* = 8.1, 1.9 Hz, 1H), 7.88 (d, *J* = 7.6 Hz, 1H), 7.64–7.57 (m, 2H), 7.54 (d, *J* = 7.5 Hz, 1H), 7.41 (t, *J* = 7.3 Hz, 1H), 7.37 (dd, *J* = 8.0, 4.8 Hz, 1H), 4.02 (s, 2H); ¹³C NMR (151 MHz, CDCl₃) δ 193.63, 151.54, 149.96, 149.27, 137.61, 136.88, 136.75, 134.95, 131.25, 129.96, 127.85, 126.20, 124.55, 123.72, 32.29; APCI-HRMS *m/z* calcd for C₁₅H₁₂NO (MH⁺), 222.0913, found 222.0911; Purity (HPLC): 95.7%.

4.2.2. (2E)-5-Methoxy-2-(pyridin-3-ylmethylidene)-2,3-dihydro-1H-inden-1-one (**11b**)

The title compound was prepared in a yield of 66%: mp 173.0–174.1 °C (ethanol), beige crystals. ¹H NMR (600 MHz, CDCl₃) δ 8.91 (s, 1H), 8.61 (d, *J* = 4.4 Hz, 1H), 7.94 (d, *J* = 8.1 Hz, 1H), 7.86 (d, *J* = 8.5 Hz, 1H), 7.57 (s, 1H), 7.40 (dd, *J* = 8.0, 4.8 Hz, 1H), 7.00 (s, 1H), 6.97 (dd, *J* = 8.5, 2.2 Hz, 1H), 4.00 (s, 2H), 3.93 (s, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 191.98, 165.46, 152.22, 151.35, 149.72, 137.34, 136.80, 131.43, 131.04, 128.71, 126.36, 123.69, 115.53, 109.65, 55.70, 32.38; APCI-HRMS *m*/*z* calcd for C₁₆H₁₄NO₂ (MH⁺), 252.1019, found 252.0998; Purity (HPLC): 94.3%.

4.2.3. (2E)-2-(Pyridin-2-ylmethylidene)-2,3-dihydro-1H-inden-1-one (**11c**)

The title compound was prepared in a yield of 61%: mp 154.6–155.4 °C (ethanol), white solid. ¹H NMR (600 MHz, CDCl₃) δ 8.73 (d, *J* = 3.9 Hz, 1H), 7.88 (d, *J* = 7.6 Hz, 1H), 7.71 (td, *J* = 7.7, 1.9 Hz, 1H), 7.63–7.56 (m, 2H), 7.55 (d, *J* = 7.6 Hz, 1H), 7.51 (d, *J* = 7.8 Hz, 1H), 7.38 (t, *J* = 7.6 Hz, 1H), 7.21 (ddd, *J* = 7.6, 4.7, 1.2 Hz, 1H), 4.28 (s, 2H); ¹³C NMR (151 MHz, CDCl₃) δ 194.92, 154.75, 151.21, 149.99, 139.03, 137.85, 136.39, 134.81, 130.91, 127.37, 127.35, 126.30, 124.33, 122.98, 33.34; APCI-HRMS *m*/*z* calcd for C₁₅H₁₂NO (MH⁺), 222.0913, found 222.0915; Purity (HPLC): 99.6%.

4.2.4. (2E)-5-Methoxy-2-(pyridin-2-ylmethylidene)-2,3-dihydro-1H-inden-1-one (**11d**)

The title compound was prepared in a yield of 68%: mp 169.7–172.0 °C (ethanol), white solid. ¹H NMR (600 MHz, CDCl₃) δ 8.76 (d, *J* = 4.4 Hz, 1H), 7.86 (d, *J* = 8.5 Hz, 1H), 7.74 (td, *J* = 7.7, 1.9 Hz, 1H), 7.58–7.50 (m, 2H), 7.27–7.20 (m, 1H), 7.02 (s, 1H), 6.96 (dd, *J* = 8.5, 2.2 Hz, 1H), 4.27 (s, 2H), 3.93 (s, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 193.24, 165.39, 154.97, 154.22, 149.93, 139.65, 136.33, 131.38, 129.75, 127.18, 126.15, 122.76, 115.12, 109.63, 55.63, 33.48; APCI-HRMS *m*/*z* calcd for C₁₆H₁₄O₂ (MH⁺), 252.1019, found 252.0997; Purity (HPLC): 98.3%.

4.2.5. (2E)-2-[(2-Chloropyridin-3-yl)methylidene]-2,3-dihydro-1H-inden-1-one (**11e**)

The title compound was prepared in a yield of 78%: mp 178.6– 179.1 °C (ethanol), pale yellow solid. ¹H NMR (600 MHz, CDCl₃) δ 8.36 (dd, *J* = 4.8, 1.9 Hz, 1H), 7.98 (dd, *J* = 7.7, 1.9 Hz, 1H), 7.91– 7.85 (m, 2H), 7.60 (td, *J* = 7.5, 1.2 Hz, 1H), 7.50 (d, *J* = 7.7 Hz, 1H), 7.40 (t, *J* = 7.6 Hz, 1H), 7.33 (dd, *J* = 7.7, 4.7 Hz, 1H), 3.92 (s, 2H); ¹³C NMR (151 MHz, CDCl₃) δ 193.10, 152.45, 149.45, 149.20, 138.83, 137.85, 137.55, 135.08, 130.39, 127.93, 127.90, 126.15, 124.66, 122.42, 31.56; APCI-HRMS *m*/*z* calcd for C₁₅H₁₁ClNO (MH⁺), 256.0524, found 256.0530; Purity (HPLC): 89.0%.

4.2.6. (2E)-2-(Furan-2-ylmethylidene)-2,3-dihydro-1H-inden-1-one (11f)

The title compound was prepared in a yield of 33%: mp 121.0–121.4 °C (ethanol), light yellow crystals. ¹H NMR (600 MHz, CDCl₃) δ 7.86 (d, *J* = 7.6 Hz, 1H), 7.63–7.55 (m, 2H), 7.52 (d, *J* = 7.7 Hz, 1H), 7.43 (t, *J* = 2.1 Hz, 1H), 7.38 (t, *J* = 7.6 Hz, 1H), 6.74 (d, *J* = 3.4 Hz, 1H), 6.53 (dd, *J* = 3.5, 1.8 Hz, 1H), 4.01 (s, 2H); ¹³C NMR (151 MHz, CDCl₃) δ 193.94, 152.23, 149.73, 145.33, 138.42, 134.40, 132.50, 127.41, 126.15, 124.16, 120.00, 116.59, 112.62, 32.30; APCI-HRMS *m*/*z* calcd for C₁₄H₁₁O₂ (MH⁺), 211.0754, found 211.0767; Purity (HPLC): 99.9%.

4.2.7. (2E)-2-(Furan-2-ylmethylidene)-5-methoxy-2,3-dihydro-1Hinden-1-one (**11g**)

The title compound was prepared in a yield of 67%: mp 152.1– 152.3 °C (ethanol), light brown needles. ¹H NMR (600 MHz, CDCl₃) δ 7.78 (d, *J* = 8.5 Hz, 1H), 7.57 (s, 1H), 7.35 (t, *J* = 2.1 Hz, 1H), 6.94 (d, *J* = 2.3 Hz, 1H), 6.90 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.69 (d, *J* = 3.4 Hz, 1H), 6.51 (dd, *J* = 3.4, 1.8 Hz, 1H), 3.95 (s, 2H), 3.87 (s, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 192.38, 165.08, 152.65, 152.33, 145.00, 133.08, 131.83, 125.90, 118.90, 115.94, 115.01, 112.49, 109.67, 55.61, 32.37; APCI-HRMS *m*/*z* calcd for C₁₅H₁₃O₃ (MH⁺), 241.0859, found 241.0850; Purity (HPLC): 94.4%.

4.2.8. (2E)-2-(Thiophen-2-ylmethylidene)-2,3-dihydro-1H-inden-1one e (11h)

The title compound was prepared in a yield of 68%: mp 157.5– 159.8 °C (ethanol), light yellow crystals. ¹H NMR (600 MHz, CDCl₃) δ 7.90–7.82 (m, 2H), 7.58 (td, *J* = 7.4, 1.3 Hz, 1H), 7.56–7.50 (m, 2H), 7.43–7.35 (m, 2H), 7.14 (dd, *J* = 5.1, 3.6 Hz, 1H), 3.89 (s, 2H); ¹³C NMR (151 MHz, CDCl₃) δ 193.74, 148.97, 139.82, 138.46, 134.47, 133.04, 132.67, 130.49, 128.13, 127.59, 126.48, 126.17, 124.22, 32.27; APCI-HRMS *m*/*z* calcd for C₁₄H₁₁OS (MH⁺), 227.0525, found 227.0529; Purity (HPLC): 95.1%.

4.2.9. (2E)-5-Methoxy-2-(thiophen-2-ylmethylidene)-2,3-dihydro-1H-inden-1-one (**11i**)

The title compound was prepared in a yield 78%: mp 145.0–154.4 °C (ethanol), light brown needles. ¹H NMR (600 MHz, CDCl₃) δ 7.82–7.76 (m, 2H), 7.53 (d, *J* = 4.9 Hz, 1H), 7.38 (d, *J* = 3.6 Hz, 1H), 7.13 (dd, *J* = 5.0, 3.6 Hz, 1H), 6.98 (s, 1H), 6.92 (dd, *J* = 8.5, 2.2 Hz, 1H), 3.89 (s, 3H), 3.85 (s, 2H); ¹³C NMR (151 MHz, CDCl₃) δ 192.23, 165.15, 151.89, 139.98, 133.26, 132.59, 131.90, 130.02, 128.06, 126.00, 125.31, 115.22, 109.74, 55.66, 32.36; APCI-HRMS *m/z* calcd for C₁₅H₁₃O₂S (MH⁺), 257.0631, found 257.0635; Purity (HPLC): 95.6%.

4.2.10. (2E)-2-(1H-Pyrrol-2-ylmethylidene)-2,3-dihydro-1H-inden-1one (**11***j*)

The title compound was prepared in a yield of 41%: mp 208.1–209.2 °C (ethanol), yellow crystals. ¹H NMR (600 MHz, DMSO- d_6) δ 11.57 (s, 1H), 7.71 (d, *J* = 7.6 Hz, 1H), 7.68–7.60 (m, 2H), 7.49 (s, 1H), 7.44 (t, *J* = 7.7, 6.4 Hz, 1H), 7.15 (s, 1H), 6.72 (d, *J* = 3.8 Hz, 1H), 6.32 (t, *J* = 3.0 Hz, 1H), 3.86 (s, 2H); ¹³C NMR (151 MHz, DMSO- d_6) δ 192.63, 149.07, 138.45, 134.10, 128.69, 128.28, 127.46, 126.53, 123.68, 123.43, 123.11, 114.34, 111.46, 31.97; APCI-HRMS *m*/*z* calcd for C₁₄H₁₂NO (MH⁺), 210.0913, found 210.0891; Purity (HPLC): 98.1%.

4.2.11. (2E)-2-(Thiophen-3-ylmethylidene)-2,3-dihydro-1H-inden-1one (**11k**)

The title compound was prepared in a yield of 66%: mp 143.0–144.5 °C (ethanol), white crystals. ¹H NMR (600 MHz, CDCl₃) δ 7.87 (d, *J* = 7.6 Hz, 1H), 7.68 (t, *J* = 2.2 Hz, 1H), 7.65 (t, *J* = 2.1 Hz, 1H), 7.59 (td, *J* = 7.4, 1.2 Hz, 1H), 7.53 (d, *J* = 7.7 Hz, 1H), 7.43–7.37 (m, 3H), 3.94 (s, 2H); ¹³C NMR (151 MHz, CDCl₃) δ 194.30, 149.19, 138.36, 137.69, 134.49, 133.34, 129.54, 128.25, 127.62, 127.25, 126.62, 126.15, 124.28, 32.31; APCI-HRMS *m*/*z* calcd for C₁₄H₁₁OS (MH⁺), 227.0525, found 227.0532; Purity (HPLC): 95.4%.

4.2.12. (2E)-2-(Cyclohexylmethylidene)-5-methoxy-2,3-dihydro-1Hinden-1-one (**11m**)

The title compound was prepared in a yield of 61%: mp 187.1– 188.8 °C (ethanol), light yellow crystals. ¹H NMR (600 MHz, CDCl₃) δ 7.77 (d, *J* = 8.8 Hz, 1H), 6.93–6.86 (m, 2H), 6.63 (d, *J* = 9.7 Hz, 1H), 3.86 (s, 3H), 3.61 (s, 2H), 2.35–2.26 (m, 1H), 1.80–1.63 (m, 6H), 1.38–1.15 (m, 6H); ¹³C NMR (151 MHz, CDCl₃) δ 192.49, 164.96, 152.28, 141.54, 134.66, 132.34, 126.02, 114.96, 109.72, 55.60, 39.10, 31.86, 29.85, 25.80, 25.54; APCI-HRMS m/z calcd for $C_{17}H_{21}O_2$ (MH⁺), 257.1536, found 257.1558; Purity (HPLC): 92.6%.

4.2.13. (2E)-5-Methoxy-2-[(5-methylfuran-2-yl)methylidene]-2,3dihydro-1H-inden-1-one (**11n**)

The title compound was prepared in a yield 47%: mp 120.4–121.0 °C (ethanol), light brown solid. ¹H NMR (600 MHz, CDCl₃) δ 7.78 (d, *J* = 8.4 Hz, 1H), 7.30 (t, *J* = 2.0 Hz, 1H), 6.97 (s, 1H), 6.91 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.61 (d, *J* = 3.3 Hz, 1H), 6.16–6.10 (m, 1H), 3.93 (s, 2H), 3.88 (s, 3H), 2.39 (s, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 192.51, 164.92, 155.81, 152.62, 150.94, 132.08, 131.51, 125.78, 119.13, 117.79, 114.91, 109.66, 109.14, 55.61, 32.44, 14.14; APCI-HRMS *m*/*z* calcd for C₁₆H₁₅O₃ (MH⁺), 255.1016, found 255.1009; Purity (HPLC): 70.0%.

4.2.14. (2E)-2-[(5-Bromofuran-2-yl)methylidene]-5-methoxy-2,3dihydro-1H-inden-1-one (**110**)

The title compound was prepared in a yield 79%: mp 157.5–159.8 °C (ethanol), light brown solid. ¹H NMR (600 MHz, CDCl₃) δ 7.74 (d, *J* = 8.5 Hz, 1H), 7.19 (t, *J* = 2.0 Hz, 1H), 6.93 (s, 1H), 6.87 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.59 (d, *J* = 3.5 Hz, 1H), 6.40 (d, *J* = 3.5 Hz, 1H), 3.89 (s, 2H), 3.84 (s, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 192.15, 165.23, 154.20, 152.65, 133.67, 131.70, 125.99, 125.51, 117.96, 117.64, 115.26, 114.38, 109.62, 55.67, 32.26; APCI-HRMS *m/z* calcd for C₁₅H₁₂BrO₃ (MH⁺), 318.9964, found 318.9968; Purity (HPLC): 98.7%.

4.3. General synthesis of heteroarylidene-1-indanone derivative 111

Commercially available 1-indanone (3 mmol) and cyclohexanecarboxaldehyde (3 mmol) were suspended in methanol (5 mL) containing KOH (4.54 mmol). The reaction was stirred at room temperature for 3 h. The reaction progress was monitored by TLC with dichloromethane as mobile phase. Upon completion, cold water (20 mL) was added and the mixture was extracted to 50 mL ethyl acetate. The organic layer was washed with water (50 mL), dried (Na₂SO₄), filtered and evaporated in vacuo. The residue was purified by silica gel column chromatography with petroleum ether/ethyl acetate (7:1) serving as mobile phase.

4.3.1. (2E)-2-(Cyclohexylmethylidene)-2,3-dihydro-1H-inden-1-one (111)

The title compound was prepared in a yield of 17%: mp 70.3–83.0 °C, light yellow crystals. ¹H NMR (600 MHz, CDCl₃) δ 7.84 (d, *J* = 7.6 Hz, 1H), 7.56 (td, *J* = 7.4, 1.2 Hz, 1H), 7.47 (d, *J* = 7.6 Hz, 1H), 7.37 (t, *J* = 7.5 Hz, 1H), 6.72 (dt, *J* = 9.8, 2.1 Hz, 1H), 3.67 (s, 2H), 2.39–2.29 (m, 1H), 1.82–1.63 (m, 5H), 1.39–1.17 (m, 5H); ¹³C NMR (151 MHz, CDCl₃) δ 193.97, 149.45, 142.99, 138.94, 134.34, 127.38, 126.26, 124.29, 39.21, 31.81, 29.76, 25.80, 25.52; APCI-HRMS *m/z* calcd for C₁₆H₁₉O (MH⁺), 227.1430, found 227.1416; Purity (HPLC): 62.7%.

4.4. The determination of IC₅₀ values

IC₅₀ values for the inhibition of MAO-A and MAO-B were measured according to a protocol detailed in the literature [18,26]. Recombinant human MAO-A and MAO-B were used for these studies and kynuramine served as enzyme substrate. Kynuramine is oxidised by both MAO isoforms to yield 4-hydroxyquinoline as ultimate product. For the enzyme reactions MAO-A (0.0075 mg/mL) or MAO-B (0.015 mg/mL), the substrate (50 μ M) and the test inhibitors (0.003–100 μ M) were combined and incubated for 20 min. For this purpose potassium phosphate buffer (pH 7.4, 100 mM) served as solvent. At endpoint the reactions were alkalinised (NaOH) and the fluorescence intensity of 4-hydroxyquinoline was measured ($\lambda_{ex} = 310$ nm; $\lambda_{em} = 400$ nm).

Employing sigmoidal plots (catalytic rate versus logarithm of inhibitor concentration), the IC_{50} values were estimated and are reported as the mean ± SD of triplicate experiments.

4.5. Procedure for molecular docking simulations

Molecular docking was carried out with the Windows-based Discovery Studio 3.1 software package according to a protocol detailed in the literature [18,27]. As MAO-A and MAO-B models, the reported X-ray crystal structures of the human enzymes (PDB codes 2Z5X and 2V5Z, respectively) were used [21,22]. The preparation of the protein models for the docking simulations involved the following steps: (1) The pKa values and protonation states (at pH 7.4) of the ionisable amino acids were calculated and hydrogen atoms were added; (2) The FAD was drawn in the oxidised state and a fixed atom constraint was applied to the protein backbones; (3) Employing the Smart Minimizer algorithm, the protein models were energy minimised; (4) With the exception of HOH 710, 718 and 739 in MAO-A, and HOH 1155, 1170 and 1351 in MAO-B (A-chain), the waters were removed; (5) The co-crystallised ligands were removed. The inhibitors were constructed in Discovery Studio and docked into the MAO models using the CDOCKER algorithm. The solutions were subsequently subjected to in situ ligand minimisation with the Smart Minimizer algorithm. The illustrations were prepared with PyMol [28].

Conflict of interest

The authors have no conflicts of interest to declare.

Acknowledgements

The NMR and MS spectra were recorded by André Joubert and Johan Jordaan of the SASOL Centre for Chemistry, North-West University. This work is based on the research supported in part by the Medical Research Council and National Research Foundation of South Africa (Grant specific unique reference numbers (UID) 85642, 96180, 96135). The Grantholders acknowledge that opinions, findings and conclusions or recommendations expressed in any publication generated by the NRF supported research are that of the authors, and that the NRF accepts no liability whatsoever in this regard.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bioorg.2016.09. 004.

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