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Synthesis and in vitro Evaluation of 2-heteroarylidene-1-tetralone Derivatives as Monoamine Oxidase Inhibitors

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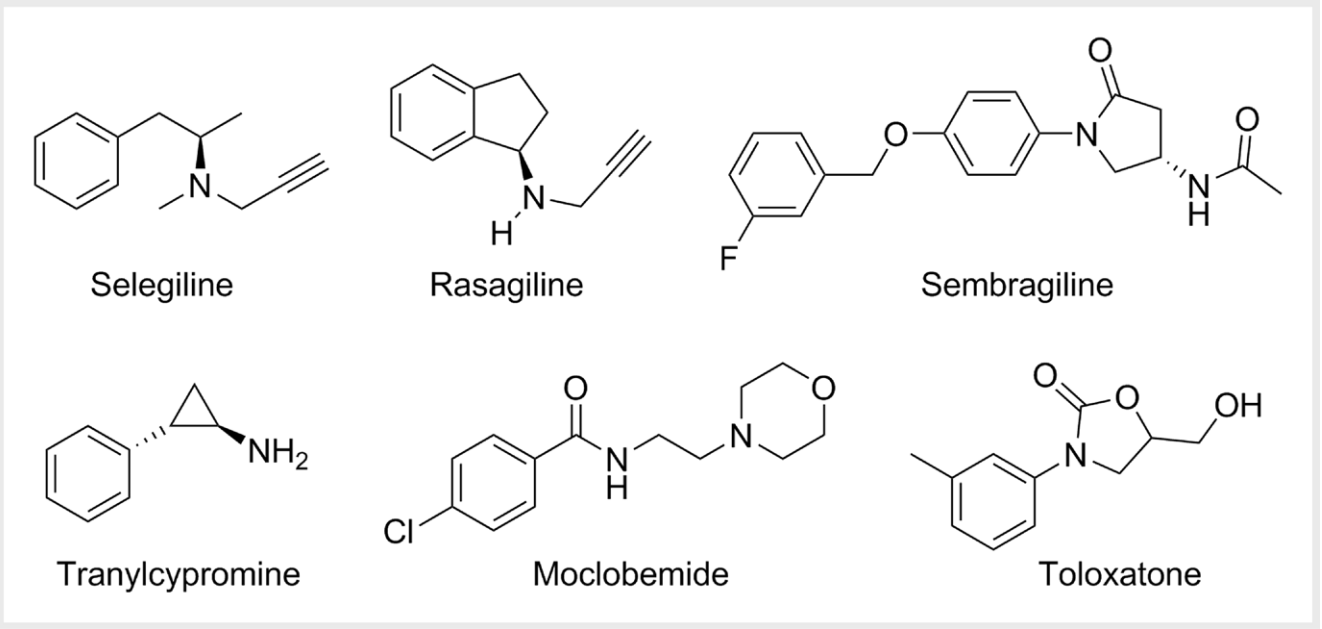
ABSTRACT

The present study investigates the human monoamine oxidase (MAO) inhibition properties of a series of twelve 2-heteroarylidene-1-tetralone derivatives. Also included are related cyclohexylmethylidene, cyclopentylmethylidene and benzylidene substituted 1-tetralones. These compounds are related to the 2-benzylidene-1-indanone class of compounds which has previously been shown to inhibit the MAOs, with specificity for the MAO-B isoform. The target compounds were synthesised by the Claisen-Schmidt condensation between 7-methoxy-1-tetralone or 1-tetralone, and various aldehydes, under acid (hydrochloric acid) or base (potassium hydroxide) catalysis. The results of the MAO inhibition studies showed that the 2-heteroarylidene-1-tetralone and related derivatives are in most instances more selective inhibitors of the MAO-B isoform compared to MAO-A. (2E)-2-Benzylidene-7-methoxy-3,4-dihydronaphthalen-1(2H)-one (IC_{50} = 0.707 μM) was found to be the most potent MAO-B inhibitor, while the most potent MAO-A inhibitor was (2E)-2-[(2-chloropyridin-3-yl) methylidene]-7-methoxy-3,4-dihydronaphthalen-1(2H)-one (IC_{50} = 1.37 μM). The effect of the heteroaromatic substituent on MAO-B inhibition activity, in decreasing order was found to be: cyclohexyl, phenyl > thiophene > pyridine, furane, pyrrole, cyclopentyl. This study concludes that, although some 2-heteroarylidene-1-tetralone derivatives are good potency MAO inhibitors, in general their inhibition potencies, particularly for MAO-B, are lower than structurally related chalcones and 1-indanone derivatives that were previously studied.

Introduction

The monoamine oxidases (MAOs) are enzymes responsible for the metabolism of monoamine neurotransmitters such as serotonin, norepinephrine and dopamine, thereby regulating their concentrations in the central and peripheral tissues [1]. The MAOs exist as two isoforms, MAO-A and MAO-B, which are products of distinct genes. Although the amino acid sequences of the MAO isoforms are 70 % identical they differ significantly in their three-dimensional structures, tissue distributions, and substrate and inhibitor specificities [2–4]. The MAOs also have different physiological roles. MAO-B is a key enzyme for the metabolism of dopamine in the human brain and represents a target for the development of drugs for the treatment of Parkinson's disease and more recently also Alzheimer's disease [5, 6]. Examples of MAO-B inhibitors that are used in the clinic for the treatment of Parkinson's disease are selegiline and rasagiline, while sembragiline is an example of a MAO-B inhibitor that is specifically being developed for the treatment of Alzheimer's disease (▶ **Fig. 1**). MAO-A is a major serotonin metabolising enzyme in the brain and is thus a drug target for the treatment of depression [7]. MAO-A inhibitors that have been used for the treatment of depressive illness include tranylcypromine, moclobemide and toloxatone. An important consideration in MAO inhibitor design is the reversibility of inhibition. Irreversible MAO-A inhibitors are used with caution due to a potentially fatal hypertensive event that may occur when these medications are combined with tyramine-containing food. This adverse event is termed the "cheese



▶ **Fig. 1** The structures of MAO-B specific inhibitors in clinical use for the treatment of parkinson's disease (selegiline and rasagiline) and alzheimer's disease (sembragiline), and the structures of MAO-A inhibitors that have been used for the treatment of depression (tranylcypromine, moclobemide and toloxatone).

reaction" and occurs when MAO-A is irreversibly inhibited [8–10]. Since tyramine is metabolised by MAO-A in the gastrointestinal tract, irreversible inhibition of MAO-A leads to increased systemic concentrations of tyramine. Tyramine is a sympathomimetic amine and causes the release of noradrenaline from peripheral neurons leading to an increase in blood pressure. Reversible MAO-A inhibitors are considered to be safer in this regard and do not potentiate the sympathomimetic effects of tyramine [11, 12]. For the design of MAO inhibitors, a high degree of specificity for MAO-B or a reversible mode of MAO-A inhibition are therefore desirable characteristics [13].

Chalcones are α,β-unsaturated aromatic ketones that are essentially planar due to the presence of carbon atoms with sp^2 hybridization. Traditional medicinal use of chalcone-containing plants led to the discovery of their potential therapeutic applications. Chalcones possess a broad spectrum of pharmacological activities including antimalarial, anticancer, anti-inflammatory, antifungal, antimicrobial, antioxidant and chemopreventive activities [14, 15]. Of interest to us are reports that chalcones act as inhibitors of the MAO enzymes. For example, Chimenti and coworkers (2009) found that, with the appropriate substitution on the phenyl rings, synthetic chalcone derivatives may act as high potency inhibitors of the human MAOs. This is exemplified by chalcone (**1**), which inhibits human MAO-B with an IC_{50} value of 0.0044 μM (▶ **Fig. 2**) [16]. This compound is highly specific for the MAO-B isoform and does not inhibit MAO-A at concentrations up to 50 μM. Another study has recently investigated the human MAO inhibition properties of a series of furanochalcones and discovered potent MAO-B-selective inhibitors with the most active compound (**2**) exhibiting an IC_{50} value of 0.174 μM [17]. Other heterocyclic chalcones have also been found to inhibit MAO-B potently and with high specificity as exemplified by **3**, which possesses an IC_{50} value of 0.067 μM [18].

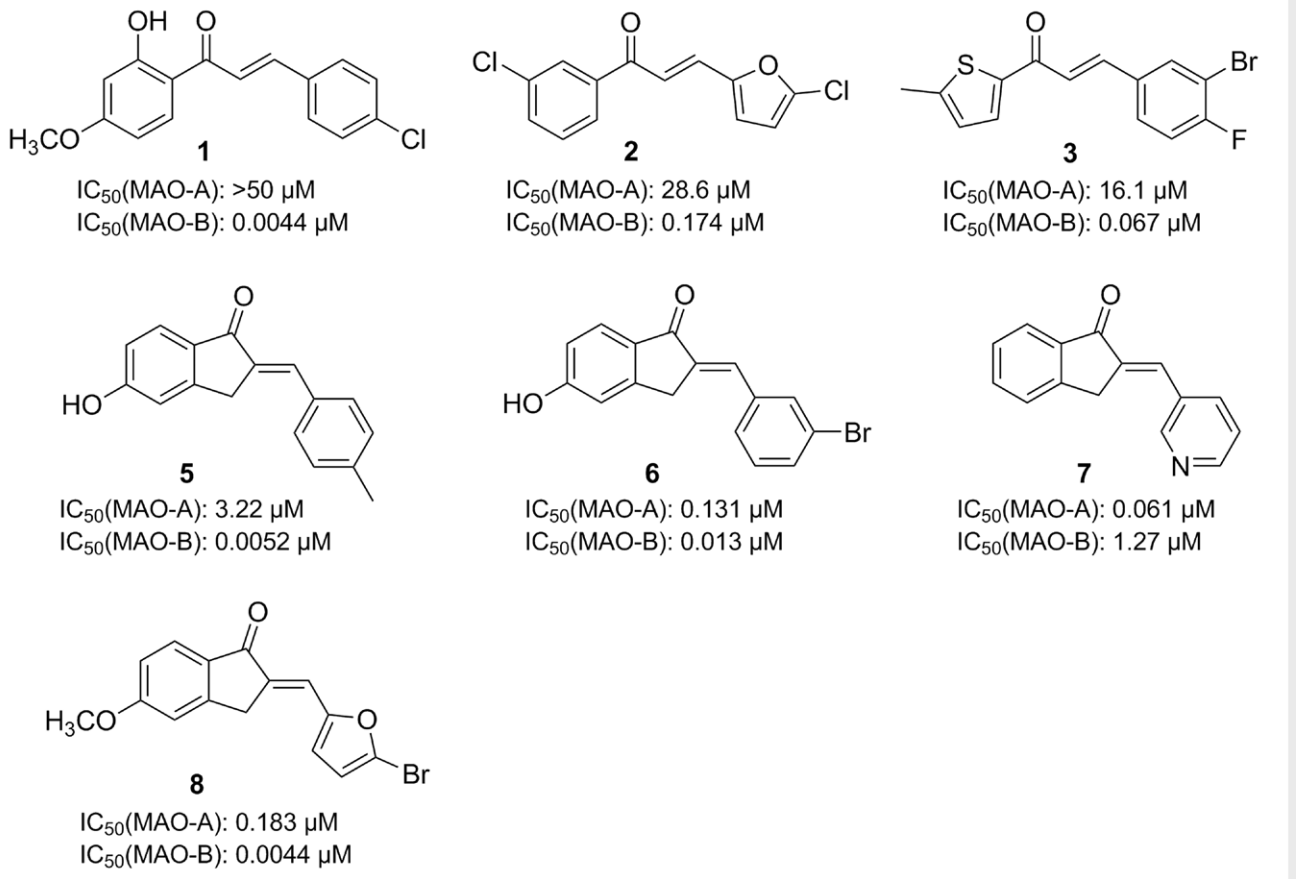
The current study further explores the structure-activity relationships (SARs) for chalcones as MAO inhibitors by introducing conformational restriction and heteroaromatic substitution. This will be done by cyclising the structure of chalcone to yield a series of twelve 2-heteroarylidene-1-tetralone derivatives (**4a**–**l**) (▶ **Fig. 3**). These compounds are not only related to heterocyclic chalcones that have been reported to act as MAO-B specific inhibitors, but are also derivatives of the 2-benzylidene-1-indanone class of compounds which has previously been shown to inhibit the MAOs, also with specificity for the MAO-B isoform [19–21]. For example, series of 2-benzylidene-1-indanones were found to act as specific inhibitors of human MAO-B with compound **5** exhibiting the highest potency MAO-B inhibition [20]. Interestingly, some 2-benzylidene-1-indanone derivatives are also MAO-A inhibitors as exemplified by compound **6** (IC_{50} = 0.131 μM). Similar to the open-chain chalcones, heterocyclic derivatives of 2-benzylidene-1-indanone also exhibit MAO inhibition. In this respect, compounds **7** (IC_{50} = 0.061 μM) and **8** (IC_{50} = 0.0044 μM) were found to be respectively the most potent inhibitors of MAO-A and MAO-B[21].

For the purpose of this study, the 2-heteroarylidene-1-tetralone derivatives (**4a**–**l**) will possess methoxy substitution (except **4d**) on the tetralone phenyl ring (ring A) while a variety of heterocycles (pyridine, furan, pyrrole, thiophene) will be introduced as rings B. For comparison, the effect of non-heteroaromatic systems (cyclohexyl, cyclopentyl) and the phenyl ring as rings B will also be investigated.

Materials and Methods

Chemicals and instrumentation

All reagents and solvents were from Sigma-Aldrich and were used without further purification. Column chromatography was carried



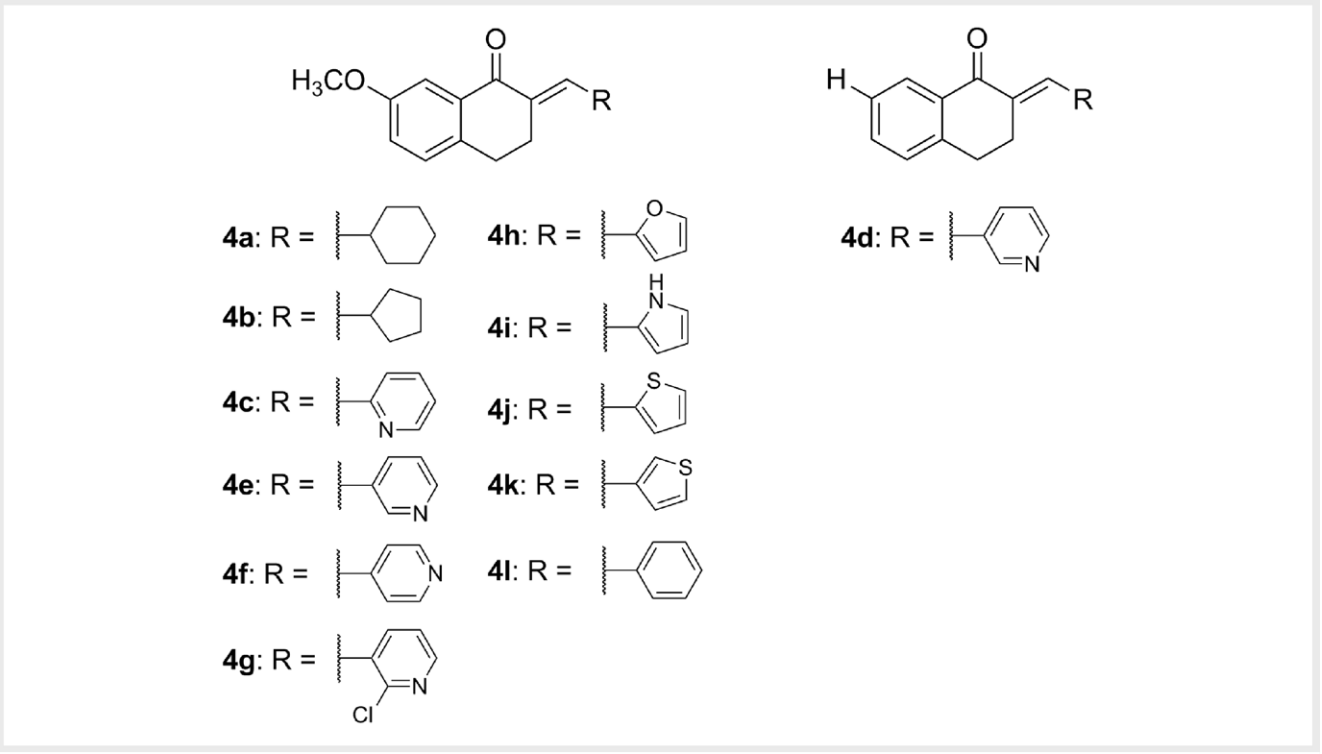
▶ **Fig. 2** Examples of chalcone derivatives that act as MAO inhibitors.

out with silica gel 60 (Fluka, particle size 0.063–0.200 mm) while thin-layer chromatography (TLC) was performed on 0.20 mm thick aluminium sheets coated with silica gel 60 (Macherey-Nagel). ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance III 600 spectrophotometer at 600 MHz and 151 MHz, respectively, and $CDCl_3$ or DMSO-d6 served as NMR solvent. Chemical shifts are reported in parts per million (ð) and were referenced to the residual solvent signal ($CDCl_3$: 7.26 and 77.16 ppm for ^{1}H and ^{13}C, respectively; DMSO-d6: 2.50 and 39.52 ppm for ^{1}H and ^{13}C, respectively). Spin multiplicities are given as s (singlet), d (doublet), t (triplet), dd (doublet of doublets), dt (doublet of triplets), td (triplet of doublets), ddd (doublet of doublet of doublets) and m (multiplet). High resolution mass spectra (HRMS) were recorded on a Bruker micrOTOF-Q II mass spectrometer in atmospheric-pressure chemical ionisation (APCI) mode. Melting points (mp) were determined on a Buchi B-545 melting point apparatus and are uncorrected. Fluorescence measurements were conducted with a Varian Cary Eclipse fluorescence spectrophotometer. Microsomes from insect cells containing recombinant human MAO-A and MAO-B (5 mg/mL), kynuramine dihydrobromide, lazabemide and toloxatone were obtained from Sigma-Aldrich.

Chemical purity was determined by high performance liquid chromatography (HPLC). HPLC analyses were performed using an Agilent 1200 series HPLC system equipped with a quaternary pump and an Agilent 1200 series diode array detector. A Venusil XBP C18 column (4.60 × 150 mm, 5 µm) was used for separation and the mobile phase consisted initially of 30 % acetonitrile and 70 % MilliQ water at a flow rate of 1 mL/min. At the start of each HPLC run a solvent gradient program was initiated by linearly increasing the composition of the acetonitrile in the mobile phase to 85 % acetonitrile over a period of 5 min. Each HPLC run lasted 15 min and a time period of 5 min was allowed for equilibration between runs. A volume of 20 µL of solutions of the test compounds in acetonitrile (1 mM) was injected into the HPLC system and the eluent was monitored at wavelengths of 210, 254 and 300 nm.

Synthesis and characterisation of 4a–l

The 2-heteroarylidene-1-tetralone derivatives (**4a**–**k**) were synthesised via the base-catalysed Claisen-Schmidt condensation reaction of 1-tetralone or 7-methoxy-1-tetralone (2.27 mmol), and the appropriate aldehydes (2.497 mmol). The reactants and potassium hydroxide (4.54 mmol) were dissolved in 5 mL methanol and stirred for 10–24 h at room temperature. Compound **4l** was synthesised via the Claisen-Schmidt condensation reaction using concentrated hydrochloric acid (22 mL) as a catalyst. The products were precipitated by addition of a minimum of 20 mL water and the crude products were collected by filtration and recrystallised from a suitable solvent (e. g. ethanol).



▶ **Fig. 3** The 2-heteroarylidene-1-tetralone derivatives **(4a–l)** investigated in this study.

(2E)-2-(Cyclohexylmethylidene)-7-methoxy-3,4-dihydronaphthalen-1(2 H)-one (4a)

The title compound is a product of 7-methoxy-3,4-dihydronaphthalen-1(2 H)-one and cyclohexanecarbaldehyde in a yield of 10.6 %: light brown crystals, mp 94.0–95.1 °C (ethyl acetate). ^{1}H NMR ($CDCl_3$) δ ppm 7.56 (d, J = 2.7 Hz, 1 H), 7.13 (d, J = 8.3 Hz, 1 H), 7.02 (dd, J = 8.3, 2.8 Hz, 1 H), 6.74 (d, J = 9.7 Hz, 1 H), 3.83 (s, 3 H), 2.89 – 2.82 (m, 2 H), 2.75 (t, J = 6.0 Hz, 2 H), 2.42 – 2.31 (m, 1 H), 1.80 – 1.50 (m, 5 H), 1.37 – 1.16 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ ppm 187.86, 158.49, 145.13, 136.40, 134.50, 133.24, 129.40, 121.14, 110.20, 55.51, 37.27, 32.24, 28.46, 25.95, 25.82, 25.63. APCI-MS (m/z) calcd for $C_{18}H_{23}O_2$. $[MH]^+$, 271.1693, found 271.1723. Purity (HPLC): 99.5 %.

(2E)-2-(Cyclopentylmethylidene)-7-methoxy-3,4-dihydronaphthalen-1(2 H)-one (4b)

The title compound is a product of 7-methoxy-3,4-dihydronaphthalen-1(2 H)-one and cyclopentanecarbaldehyde in a yield of 24.6 %: yellow oil (ethyl acetate). ^{1}H NMR ($CDCl_3$) δ ppm 7.56 (d, J = 2.7 Hz, 1 H), 7.13 (d, J = 8.3 Hz, 1 H), 7.02 (dd, J = 8.3, 2.8 Hz, 1 H), 6.84 (d, J = 9.8 Hz, 1 H), 3.82 (d, J = 4.2 Hz, 3 H), 2.92 – 2.73 (m, 5 H), 1.96 – 1.08 (m, 8 H); ^{13}C NMR ($CDCl_3$) δ ppm 187.67, 158.50, 145.29, 136.40, 134.50, 133.54, 129.40, 121.12, 110.22, 55.51, 38.90, 33.34, 28.36, 25.96, 25.61. APCI-MS (m/z) calcd for $C_{17}H_{21}O_2$ $[MH]^+$, 257.1536, found 257.1552. Purity (HPLC): 97.1 %.

(2E)-7-Methoxy-2-(pyridin-2-ylmethylidene)-3,4-dihydronaphthalen-1(2 H)-one (4c)

The title compound is a product of 7-methoxy-3,4-dihydronaphthalen-1(2 H)-one and picolinaldehyde in a yield of 45.3 %: yellow crystals, mp 86.8–89.1 °C (ethanol). ^{1}H NMR ($CDCl_3$) δ 8.71 – 8.65 (m, 1 H), 7.75 – 7.67 (m, 2 H), 7.60 (d, J = 2.7 Hz, 1 H), 7.42 (d, J = 7.8 Hz, 1 H), 7.22 – 7.14 (m, 2 H), 7.06 (dd, J = 8.3, 2.8 Hz, 1 H), 3.85 (s, 3 H), 3.59 – 3.52 (m, 2 H), 2.95 – 2.88 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ ppm 188.16, 158.54, 155.36, 149.43, 139.18, 136.75, 136.34, 134.01, 133.53, 129.54, 126.96, 122.64, 121.67, 110.21, 55.52, 27.93, 27.01. APCI-MS (m/z) calcd for $C_{17}H_{16}NO_2$ $[MH]^+$, 266.1176, found 266.1166. Purity (HPLC): 100 %.

(2E)-2-(Pyridin-3-ylmethylidene)-3,4-dihydronaphthalen-1(2 H)-one (4d)

The title compound is a product of 3, 4-dihydronaphthalen-1(2 H)-one and nicotinaldehyde in a yield of 29.7 %: yellow crystals, mp 77.5–77.7 °C (petroleum ether). ^{1}H NMR ($CDCl_3$) δ ppm 8.67 (s, 1 H), 8.59 – 8.49 (m, 1 H), 8.11 (d, J = 7.8 Hz, 1 H), 7.81 – 7.66 (m, 2 H), 7.48 (td, J = 7.5, 1.1 Hz, 1 H), 7.30 (ddd, J = 56.7, 10.9, 6.5 Hz, 3 H), 3.08 (dd, J = 9.1, 3.8 Hz, 2 H), 2.95 (t, J = 6.4 Hz, 2 H); ^{13}C NMR ($CDCl_3$) δ ppm 187.27, 150.49, 149.13, 143.08, 137.46, 136.77, 133.54, 133.11, 132.50, 131.70, 128.28, 128.24, 127.14, 123.30, 28.71, 27.15. APCI-MS (m/z) calcd for $C_{16}H_{14}NO$ $[MH]^+$, 236.1070, found 236.1062. Purity (HPLC): 98.5 %

(2E)-7-Methoxy-2-(pyridin-3-ylmethylidene)-3,4-dihydronaphthalen-1(2 H)-one (4e)

The title compound is a product of 7-methoxy-3,4-dihydronaphthalen-1(2 H)-one and nicotinaldehyde in a yield of 72.2 %: peach crystals, mp 105.4–107.1 °C (ethanol). ^{1}H NMR ($CDCl_3$) δ ppm 8.68 (s, 1 H), 8.56 (d, J = 2.9 Hz, 1 H), 7.76 (s, 1 H), 7.71 (d, J = 7.9 Hz, 1 H), 7.59 (d, J = 2.7 Hz, 1 H), 7.34 (dd, J = 7.7, 4.9 Hz, 1 H), 7.15 (d, J = 8.4 Hz, 1 H), 7.07 (dd, J = 8.3, 2.8 Hz, 1 H), 3.85 (s, 3 H), 3.06 (dd,

J = 9.1, 3.7 Hz, 2 H), 2.89 (t, J = 6.4 Hz, 2 H); ^{13}C NMR ($CDCl_3$) δ ppm 187.22, 158.71, 150.50, 149.12, 137.49, 136.76, 135.83, 133.91, 132.54, 129.53, 123.31, 121.83, 110.28, 55.54, 27.92, 27.36. APCI-MS (m/z) calcd for $C_{17}H_{16}NO_2$, $[MH]^+$, 266.1176, found 266.1176. Purity (HPLC): 98.6 %.

(2E)-7-Methoxy-2-(pyridin-4-ylmethylidene)-3,4-dihydronaphthalen-1(2 H)-one (4 f)

The title compound is a product of 7-methoxy-3,4-dihydronaphthalen-1(2 H)-one and isonicotinaldehyde in a yield of 66.9 %: yellow crystals, mp 136.9–137.6 °C (ethanol). ^{1}H NMR ($CDCl_3$) δ ppm 8.64 (d, J = 5.5 Hz, 2 H), 7.68 (s, 1 H), 7.58 (d, J = 2.7 Hz, 1 H), 7.25 (t, J = 5.7 Hz, 2 H), 7.15 (d, J = 8.4 Hz, 1 H), 7.07 (dd, J = 8.3, 2.8 Hz, 1 H), 3.84 (s, 3 H), 3.03 (dd, J = 9.0, 3.7 Hz, 2 H), 2.88 (t, J = 6.4 Hz, 2 H); ^{13}C NMR ($CDCl_3$) δ ppm 187.14, 158.72, 149.97, 143.49, 138.84, 135.92, 133.77, 133.11, 129.57, 123.78, 121.97, 110.25, 55.52, 27.87, 27.32. APCI-MS (m/z) calcd for $C_{17}H_{16}NO_2$ $[MH]^+$, 266.1176, found 266.1189. Purity (HPLC): 100 %.

(2E)-2-[(2-Chloropyridin-3-yl)methylidene]-7-methoxy-3,4-dihydronaphthalen-1(2 H)-one (4 g)

The title compound is a product of 7-methoxy-3,4-dihydronaphthalen-1(2 H)-one and 2-chloronicotinaldehyde in a yield of 75 %: yellow crystals, mp 131.0–132.0 °C (ethanol). ^{1}H NMR ($CDCl_3$) δ ppm 8.36 (dd, J = 4.7, 1.5 Hz, 1 H), 7.75 (s, 1 H), 7.65 – 7.59 (m, 2 H), 7.28 (dd, J = 7.5, 4.8 Hz, 1 H), 7.15 (d, J = 8.4 Hz, 1 H), 7.07 (dd, J = 8.4, 2.8 Hz, 1 H), 3.85 (s, 3 H), 2.89 (s, 4 H); ^{13}C NMR ($CDCl_3$) δ ppm 187.00, 158.73, 151.37, 149.05, 138.76, 138.61, 135.96, 133.77, 131.12, 131.04, 129.62, 122.01, 121.99, 110.25, 55.54, 28.00, 27.49. APCI-MS (m/z) calcd for $C_{17}H_{15}NO_2Cl$ $[MH]^+$, 300.0786, found 300.0803. Purity (HPLC): 99.4 %

(2E)-2-(Furan-2-ylmethylidene)-7-methoxy-3,4-dihydronaphthalen-1(2 H)-one (4 h)

The title compound is a product of 7-methoxy-3,4-dihydronaphthalen-1(2 H)-one and furan-2-carbaldehyde in a yield of 85.5 %: yellow crystals, mp 90.1–90.9 °C (petroleum ether). ^{1}H NMR ($CDCl_3$) δ ppm 7.60 – 7.52 (m, 3 H), 7.15 (d, J = 8.3 Hz, 1 H), 7.04 (dd, J = 8.3, 2.8 Hz, 1 H), 6.69 (d, J = 3.4 Hz, 1 H), 6.50 (dd, J = 3.3, 1.8 Hz, 1 H), 3.84 (s, 3 H), 3.32 – 3.25 (m, 2 H), 2.95 – 2.89 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ ppm 187.27, 158.57, 152.48, 144.30, 136.22, 134.29, 131.80, 129.36, 122.85, 121.27, 116.53, 112.19, 110.20, 55.51, 27.49, 26.84. APCI-MS (m/z) calcd for $C_{16}H_{15}O_3$ $[MH]^+$, 255.1016, found 255.1011. Purity (HPLC): 100 %.

(2E)-7-Methoxy-2-(1 H-pyrrol-2-ylmethylidene)-3,4-dihydronaphthalen-1(2 H)-one (4i)

The title compound is a product of 7-methoxy-3,4-dihydronaphthalen-1(2 H)-one and 1 H-pyrrole-2-carbaldehyde in a yield of 35 %: yellow crystals, mp 186.1–190.2 °C (ethanol). ^{1}H NMR (DMSO) δ ppm 11.53 (s, 1 H), 7.70 (s, 1 H), 7.39 (d, J = 2.7 Hz, 1 H), 7.27 (d, J = 8.4 Hz, 1 H), 7.14 – 7.05 (m, 2 H), 6.64 (d, J = 2.7 Hz, 1 H), 6.27 (s, 1 H), 3.78 (s, 3 H), 3.00 (t, J = 6.2 Hz, 2 H), 2.87 (t, J = 6.6 Hz, 2 H); ^{13}C NMR (DMSO) δ ppm 185.60, 158.10, 135.34, 134.36, 129.59, 128.55, 127.42, 126.95, 122.60, 120.02, 113.46, 110.90, 110.08, 55.23, 26.56, 26.40. APCI-MS (m/z) calcd for $C_{16}H_{15}NO_2$ $[MH]^+$, 254.1176, found 254.1182. Purity (HPLC): 99.1 %.

(2E)-7-Methoxy-2-(thiophen-2-ylmethylidene)-3,4-dihydronaphthalen-1(2 H)-one (4j)

The title compound is a product of 7-methoxy-3,4-dihydronaphthalen-1(2 H)-one and thiophene-2-carbaldehyde in a yield of 86.2 %: yellow crystals, mp 106.5–116.9 °C (ethanol). ^{1}H NMR ($CDCl_3$) δ ppm 8.02 (s, 1 H), 7.58 (d, J = 2.7 Hz, 1 H), 7.49 (d, J = 5.0 Hz, 1 H), 7.38 (d, J = 3.4 Hz, 1 H), 7.19 – 7.08 (m, 2 H), 7.05 (dd, J = 8.3, 2.8 Hz, 1 H), 3.85 (s, 3 H), 3.16 (dd, J = 9.3, 3.8 Hz, 2 H), 2.95 (t, J = 6.5 Hz, 2 H); ^{13}C NMR ($CDCl_3$) δ ppm 187.05, 158.63, 139.13, 135.67, 134.42, 133.23, 131.76, 129.47, 129.33, 129.31, 127.54, 121.26, 110.27, 55.52, 27.27, 27.19. APCI-MS (m/z) calcd for $C_{16}H_{15}O_2S$ $[MH]^+$, 271.0787, found 271.0765. Purity (HPLC): 100 %.

(2E)-7-Methoxy-2-(thiophen-3-ylmethylidene)-3,4-dihydronaphthalen-1(2 H)-one (4k)

The title compound is a product of 3,4-dihydronaphthalen-1(2 H)-one and thiophene-3-carbaldehyde in a yield of 83.0 %: cream crystals, mp 135.4–136.9 °C (ethanol). ^{1}H NMR ($CDCl_3$) δ ppm 7.82 (s, 1 H), 7.58 (d, J = 2.8 Hz, 1 H), 7.50 (d, J = 2.5 Hz, 1 H), 7.36 (dd, J = 4.9, 3.0 Hz, 1 H), 7.28 (dd, J = 5.0, 0.7 Hz, 1 H), 7.15 (d, J = 8.3 Hz, 1 H), 7.05 (dd, J = 8.3, 2.8 Hz, 1 H), 3.85 (s, 3 H), 3.15 – 3.08 (m, 2 H), 2.90 (t, J = 6.5 Hz, 2 H); ^{13}C NMR ($CDCl_3$) δ ppm 187.55, 158.62, 137.36, 135.76, 134.38, 133.76, 130.52, 129.35, 128.96, 127.71, 125.83, 121.35, 110.25, 55.52, 27.59, 27.39. APCI-MS (m/z) calcd for $C_{16}H_{15}O_2S$ $[MH]^+$, 271.0787, found 271.0805. Purity (HPLC): 99.5 %.

(2E)-2-Benzylidene-7-methoxy-3,4-dihydronaphthalen-1(2 H)-one (4 l)

The title compound is a product of 7-methoxy-3,4-dihydronaphthalen-1(2 H)-one and benzaldehyde in a yield of 51.2 %: pale yellow crystals, mp 121.5–122.7 °C (dichloromethane). ^{1}H NMR ($CDCl_3$) δ ppm 7.85 (s, 1 H), 7.61 (d, J = 2.8 Hz, 1 H), 7.41 (dt, J = 15.2, 7.4 Hz, 4 H), 7.33 (t, J = 7.0 Hz, 1 H), 7.15 (d, J = 8.3 Hz, 1 H), 7.06 (dd, J = 8.3, 2.8 Hz, 1 H), 3.85 (s, 3 H), 3.09 (td, J = 6.7, 1.6 Hz, 2 H), 2.90 – 2.84 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ ppm 187.80, 158.62, 136.68, 135.92, 135.85, 135.43, 134.23, 129.84, 129.42, 128.49, 128.40, 121.50, 110.26, 55.52, 28.02, 27.34. APCI-HRMS m/z calcd for $C_{18}H_{17}O_2$ $[MH]^+$, 265.1223, found 265.1212. Purity (HPLC): 99.2 %.

Determination of IC_{50} values

The IC_{50} values for the inhibition of human MAO-A and MAO-B were determined as described in literature [22]. All enzymatic reactions were carried out in 96-well microtiter plates in potassium phosphate buffer (100 mM, pH 7.4) to a final volume of 200 µL. The reactions contained MAO-A (0.0075 mg protein/mL) or MAO-B (0.015 mg protein/mL), the mixed MAO-A/B substrate kynuramine (50 µM) and the test inhibitors (0.003–100 µM). DMSO was added to the reactions to yield a final concentration of 4 % (v/v) DMSO. Control reactions were carried out in the absence of inhibitor and also contained 4 % DMSO. After initiating the reactions with the addition of the MAO enzyme, the reactions were incubated at 37 °C for 20 min and terminated with the addition of 80 µL NaOH (2 N). At endpoint, the concentrations of the MAO generated 4-hydroxyquinoline in the reactions were measured by fluorescence spec-

trophotometry (λ_{ex} = 310 nm, λ_{em} = 400 nm) [23]. To quantify 4-hydroxyquinoline, a linear calibration curve was constructed from solutions of authentic 4-hydroxyquinoline (0.0469–1.5 µM). The rates of kynuramine oxidation were calculated and the rate data were fitted to the one site competition model incorporated into the Prism software package (GraphPad). From the resulting sigmoidal curves (catalytic rate versus the logarithm of the inhibitor concentration), the IC_{50} values were estimated. All experiments were carried out in triplicate and the IC_{50} values are expressed as mean ± standard deviation (SD).

Results

Chemistry

The 2-heteroarylidene-1-tetralone derivatives were synthesised by the Claisen-Schmidt condensation between 7-methoxy-1-tetralone (**9**) or 1-tetralone (**10**) and various aldehydes in reactions catalysed by KOH or HCl with methanol serving as solvent (▶ **Fig. 4**). The structures of the products were verified by ^{1}H NMR, ^{13}C NMR and mass spectrometry while the purities were estimated by HPLC. On the ^{1}H NMR spectra of the 2-heteroarylidene-1-tetralone derivatives the anticipated signal of the vinylic proton of the α,β-unsaturated moiety was observed at approximately 7.6–8.0 ppm. Where appropriate the methoxy CH_3 group was evident as a sharp singlet in the region of δ 3.78–3.85 ppm. On the ^{13}C NMR spectra, signals in the regions of δ 185.6–188.2 ppm and δ 55.2–55.5 ppm represent the carbonyl and methoxy carbons, respectively.

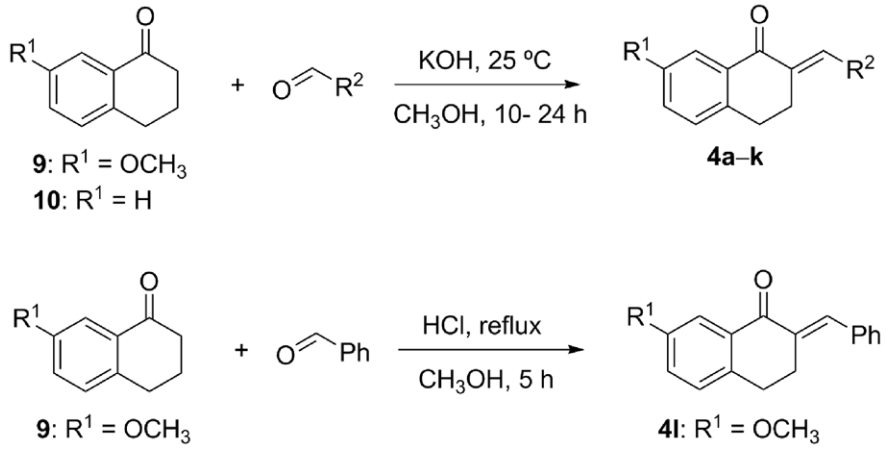
MAO inhibition studies

For the inhibition studies, the catalytic activities of MAO-A and MAO-B were determined by measuring the production of 4-hydroquinoline (4-HQ) from the oxidation of kynuramine by the MAOs. 4-HQ is fluorescent in basic media and can thus be measured by fluorescence spectrophotometry at endpoint, after alkalinisation of the enzyme reactions. Recombinant human MAO-A and MAO-B served as enzyme sources [23]. By measuring MAO activities in the presence of various concentrations of the 2-heteroarylidene-1-tetralone derivatives, sigmoidal graphs (rate of enzyme catalysis versus logarithm of inhibitor concentration) were constructed with the Prism 5 software package (GraphPad), from which IC_{50} values were estimated.

The potencies by which the 2-heteroarylidene-1-tetralone derivatives inhibit MAO-A and MAO-B are expressed as the IC_{50} values and are given in ▶ **Table 1**. The inhibitors exhibit moderate inhibitory activities for MAO-A and MAO-B with the IC_{50} values mostly in the micromolar range. With the exception of **4 g**, the 2-heteroarylidene-1-tetralone derivatives display little isoform selectivity. Compound **4 g** is a MAO-A selective inhibitor and does not inhibit MAO-B, even at a maximal tested concentration of 100 µM. This compound also is the most potent MAO-A inhibitor of the series with an IC_{50} value of 1.37 µM. The most potent MAO-B inhibitor of the series is the phenyl substituted derivative, **4 l** (IC_{50} = 0.707 µM). Although the IC_{50} values are for the most part within a narrow range, some preliminary SARs may be derived: (1) Among the derivatives evaluated, phenyl substitution (**4 l**) is the most optimal for MAO-B inhibition while the 2-chloro-3-pyridine moiety (**4 g**) yields weakest MAO-B inhibition. 2-Chloro-3-pyridine and phenyl substitution, however, are the most optimal for MAO-A inhibition; (2) The thiophene substituted derivatives (**4j**, **4k**) are more potent MAO-B inhibitors than the pyridine (**4c**–**g**) substituted compounds; (3) Substitution with the non-aromatic cyclohexyl ring (**4a**) also yields relatively potent MAO-B inhibition. Interestingly, the cyclopentane derivative (**4b**) is comparatively weaker as a MAO-B inhibitor; (5) In general the effect of substitution on MAO-B inhibition potency in decreasing order is: cyclohexyl, phenyl > thiophene > pyridine, furane, pyrrole, cyclopentyl; (6) Methoxy substitution on ring A (**4e**) yields more potent MAO inhibition compared to the unsubstituted homologue (**4d**).

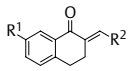
Discussion

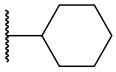
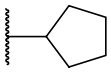
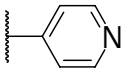
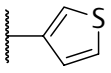
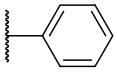
The chalcone class of compounds is well known as inhibitors of the MAOs, with specificity for the MAO-B isoform. This is exemplified by the study of Chimenti et al. (2009) in which a series of chalcone derivatives substituted on both phenyl rings exhibited very good



▶ **Fig. 4** Synthetic routes to the 2-heteroarylidene-1-tetralone derivatives.

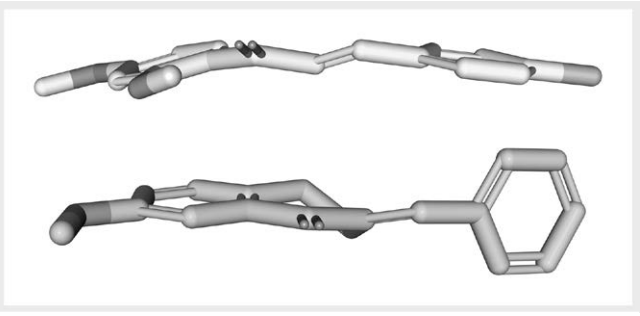
▶ **Table 1** IC_{50} values for the inhibition of human MAO-A and MAO-B by 2-heteroarylidene-1-tetralone derivatives **4a–l** and reference inhibitors.



Compd.	R^1	R^2	IC_{50} (μM ± SD)†		SI‡
			MAO-A	MAO-B	
4a	7-OMe		4.59 ± 2.11	0.895 ± 0.066	5.1
4b	7-OMe		32.3 ± 4.85	5.46 ± 0.978	5.9
4c	7-OMe		8.85 ± 0.981	3.37 ± 0.580	2.6
4d	7-H		23.4 ± 0.055	6.26 ± 0.241	3.7
4e	7-OMe		8.22 ± 0.402	2.69 ± 0.373	3.1
4f	7-OMe		5.82 ± 0.541	6.04 ± 0.355	0.96
4g	7-OMe		1.37 ± 0.209	NI§	–
4h	7-OMe		6.31 ± 0.349	4.53 ± 0.629	1.4
4i	7-OMe		14.5 ± 1.60	4.69 ± 0.245	3.1
4j	7-OMe		6.03 ± 0.062	1.75 ± 0.126	3.4
4k	7-OMe		3.86 ± 0.608	1.03 ± 0.144	3.7
4l	7-OMe		1.96 ± 0.064	0.707 ± 0.088	2.8
Lazabemide			202¶	0.091¶	2220
Toloxatone			3.92¶	–	–
Chrysin			0.770 ± 0.05	0.79 ± 0.09	0.97

† All values are reported as mean ± standard deviation (SD) of triplicate experiments; ‡ Selectivity index (SI) = IC_{50}(MAO-A)/IC_{50}(MAO-B); § No inhibition observed at a maximal tested concentration of 100 μM; ¶ Inhibition values for the reference inhibitors were obtained from literature [23]

specificity for MAO-B with potencies in the micro to nanomolar range. The most potent MAO-B inhibitor, compound (**1**) (IC_{50} = 0.0044 μM) is considerably better than the derivatives of the current study. Furanochalcones such as **2** (IC_{50} = 0.174 μM) and other heterocyclic chalcones such as **3** (IC_{50} = 0.067 μM) [17, 18] also are more potent MAO-B inhibitors than the 2-heteroarylidene-1-tetralone derivatives of this study. This suggests that introducing conformational restriction and heteroaromatic substitution such as with derivatives **4a**–**l** do not improve MAO inhibition compared to open chain chalcones and heterocyclic chalcones. Although the molecular basis for this behaviour is not clear, it may be suggested that conformational freedom and rotation of the α,β-unsaturated ketone moiety of chalcone, although limited, is important for establishing productive interactions with the MAO-B active site. Alternatively, the structures of the 2-heteroarylidene-1-tetralone derivatives are "frozen" in a conformation that is not optimal for MAO-B binding compared to the open chain chalcones. To illustrate this, the calculated three-dimensional structures (MMFF94) of chalcone derivative **1** and a 2-heteroarylidene-1-tetralone derivative (**4l**) are shown in ▶ **Fig. 5**. As shown chalcone **1** only deviates slightly from planarity compared to **4l**, which is frozen in a conformation where the tetralone moiety and phenyl are perpendicular to each other. The flexibility of **1** compared to **4l** may also, in part, explain its ability to better bind to the MAO-B active



▶ **Fig. 5** Three-dimensional structures (MMFF94) of compounds **1** (top) and **4l** (bottom).

site. The observation that heterocyclic derivatives of 2-benzylidene-1-indanone (e. g. **7** and **8**) are good potency MAO-A and MAO-B inhibitors, demonstrates the importance of the ring size of ring C to MAO inhibition, with the 5-membered ring being more favourable than the 6-membered ring [20]. In this respect it may be speculated that although the 1-indanones are also expected to possess limited conformational freedom, the placement of the heterocyclic substituent in the MAO active site cavity is more favourable than with the 1-tetralones of the current study. In addition, the calculated three-dimensional structures of **7** and **8** suggests that these structures are planar and may thus better fit into the MAO active sites.

In conclusion, among the 2-heteroarylidene-1-tetralone derivatives studied here are good potency MAO inhibitors, and although not more potent than chalcones and 1-indanone derivatives, these compounds provide some insight into structural requirements for MAO inhibition, particularly with respect to the effect of deviation from planarity on inhibition potency.

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Conflict of Interest

The authors declare to have no financial/commercial conflict of interest.

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