Thieme

C6- and C7-Substituted 3,4-dihydro-2(1H)-quinolinones as Inhibitors of Monoamine Oxidase

Authors
L. Meiring, J. P. Petzer, A. Petzer

Affiliation
Pharmaceutical Chemistry, School of Pharmacy and Centre of Excellence for Pharmaceutical Sciences, North-West University, Potchefstroom, South Africa

Key words
monoamine oxidase, MAO, reversible inhibition, 3,4-dihydro-2(1H)-quinolinone, structure-activity relationship

received 26.08.2016
accepted 31.10.2016

Bibliography
DOI http://dx.doi.org/10.1055/s-0042-120116
Published online: 2016 | Drug Res

ISSN 2194-9379

Correspondence
Prof. A. Petzer, PhD
Pharmaceutical Chemistry
School of Pharmacy
North-West University
Private Bag X6001
Potchefstroom 2520
South Africa
Tel.: +27/18/2994464, Fax: +27/18/2994243
12264954@nwu.ac.za

ABSTRACT

Purpose: Monoamine oxidase (MAO) inhibitors are considered to be useful therapeutic agents and isoform specific inhibitors are employed for the treatment of depression and Parkinson's disease. MAO inhibitors are also under investigation for the treatment of disorders ranging from Alzheimer's disease, prostate cancer and certain cardiomyopathies. While a number of irreversible MAO inhibitors are available in the clinic, reversible inhibitors, particularly of the MAO-B isoform are still being developed. Based on our interest in discovering reversible inhibitors with specificity for MAO-B, we have recently reported that, among a series of 10 3,4-dihydro-2(1H)-quinolinone derivatives, are high potency MAO-B inhibitors, with a number of homologues displaying good selectivities for MAO-B over the MAO-A isoform.

Methods and Findings: To expand on these promising findings and to derive structure-activity relationships, the current study synthesizes a series of 14 3,4-dihydro-2(1H)-quinolinone derivatives. An evaluation of their MAO inhibition properties shows that all derivatives are MAO-B specific with the most potent inhibitor (**3a**) displaying an IC_{50} value of 0.0014 μM. Selectivities for MAO-B ranged from 99 to 40 000-fold.

Conclusions: It may thus be concluded that substitution of 3,4-dihydro-2(1H)-quinolinone on C6 and C7 with a variety of side chains yields highly potent and selective MAO-B inhibitors, compounds with existing and prospective therapeutic applications.

Introduction

The monoamine oxidases (MAOs) are key metabolic enzymes that are expressed in most mammalian tissues. The 2 isoforms, MAO-A and MAO-B, metabolize neurotransmitter amines such as serotonin, noradrenaline, adrenaline and dopamine, thereby terminating their physiological actions in the peripheral tissues and central nervous system [1]. Since the MAOs modulate the levels of key neurotransmitters, inhibitors of these enzymes have been used in the clinic for the treatment of neuropsychiatric and neurodegenerative disorders such as depression and Parkinson's disease [1]. In the central nervous system, serotonin is mostly metabolized by the MAO-A isoform, and MAO-A inhibitors are considered effective treatment of major depression [2, 3]. MAO-B inhibitors are used for the treatment of Parkinson's disease where they prevent the metabolic degradation (by MAO-B) of dopamine in the brain. In this regard, MAO-B inhibitors are often combined with l-dopa, the direct metabolic precursor of dopamine and treatment of choice [4–6]. MAO-B inhibitors thus enhance the therapeutic efficacy of l-dopa and allow for a reduction of the effective l-dopa dosage.

The MAOs also are metabolic barriers, preventing dietary amines from entering the systemic circulation and brain. Thus intestinal (and peripheral) MAO-A metabolizes the sympathomimetic amine, tyramine (present in cheese, wine etc.), and prevents it from entering the systemic circulation in excessive amounts, which could lead to a potentially severe rise in blood pressure. This response, termed the "cheese effect", is often observed when irreversible MAO-A inhibitors are combined with tyramine-containing food, and imposes the restrictive use of these drugs in the clinic [7–9]. MAO-B in the brain microvasculature, in turn, represents a metabolic barrier for β-phenethylamine entry into the brain. β-Phenethylamine is a false neurotransmitter which releases neuronal dopamine and inhibits its active uptake[10, 11]. MAO-B inhibitors drastically increase the brain levels of β-phenethylamine and the resulting enhancement of extracellular dopamine concentrations may, at least in part, be responsible for the symptomatic benefit of these drugs in Parkinson's disease [12]. Paradoxically, the MAOs may also activate xenobiotics to yield metabolites which may be extremely harmful, as exemplified by the activation of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) by MAO-B to

yield 1-methyl-4-phenylpyridinium (MPP^+), a compound that induces a parkinsonian syndrome in experimental animals and humans [13].

The by-products of the MAO catalytic cycle also is of pharmacological and toxicological interest. Hydrogen peroxide and aldehyde intermediates formed by the MAOs may be injurious if not effectively cleared. In the brain these species may damage neuronal cells and contribute to neurodegeneration in disorders such as Parkinson's disease [14, 15]. The MAO-B isoform seems to be of relevance in this regard since MAO-B activity increases as the human brain ages, while MAO-A activity remains largely unchanged [16]. Based on these considerations it has been suggested that inhibitors of MAO-B may be neuroprotective in age-related diseases such as Parkinson's disease. Hydrogen peroxide formed by MAO-A in the heart, in turn, has been implicated in age-related cardiac cellular degeneration in rats [17], thus establishing a rational for MAO-A inhibitors as treatment for certain cardiomyopathies. Recently the role of MAO-A inhibitors in cancer treatment has been investigated. MAO-A levels are found to be elevated in certain types of cancer tissue such as prostate cancer, and MAO-A inhibition may, in synergism with survivin suppressants, inhibit cancer cell growth, migration and invasion [18, 19].

Based on the pharmacological importance of the MAOs, the discovery of potent and isoform specific inhibitors are pursued by a number of research groups [20–22]. As discussed above, inhibitors specific for the MAO-B isoform are appropriate for Parkinson's disease therapy for the following reasons: (a) MAO-B inhibition conserves central dopamine and enhance dopamine levels after l-dopa therapy, (b) MAO-B inhibition increases the brain levels of β-phenethylamine, which leads to the indirect enhancement of extracellular dopamine concentrations, (c) MAO-B inhibition reduces harmful metabolic by-products of MAO-B in the brain, thereby protecting against neurodegeneration and (d) MAO-B inhibition may prevent the activation of proneurotoxins such as MPTP. We have recently reported that a series of 3,4-dihydro-2(1H)-quinolinone derivatives are high potency MAO-B inhibitors with certain homologues displaying good specificity [23]. For example, the most potent MAO-B inhibitor, 7-(3-bromobenzyloxy)-3,4-dihydro-2(1H)-quinolinone (**1a**), exhibits an IC_{50} value of 0.0029 μM with a 2751-fold selectivity for MAO-B over the MAO-A isoform (▶ **Fig. 1**). Another compound, 7-(3-chlorobenzyloxy)-3,4-dihydro-2(1H)-quinolinone (**1b**) inhibits MAO-B with an IC_{50} of 0.0062 μM, while displaying an IC_{50} of >100 μM for MAO-A. Importantly, it was found that substitution on the C7 position of the 3,4-dihydro-2(1H)-quinolinone moiety leads to significantly more potent inhibition compared to substitution on C6, and that a benzyloxy substituent on C7 is more favorable than 2-phenylethoxy and 3-phenylpropoxy substitution. Based on the promising potencies and specificities displayed by some of these 3,4-dihydro-2(1H)-quinolinones, the present study aims to discover additional homologues with high MAO-B inhibition potencies and specificities, and to further derive structure-activity relationships (SARs). For this purpose, the current study synthesizes a series of 14 3,4-dihydro-2(1H)-quinolinone derivatives (▶ **Table 1**). As shown, substitution with the benzyloxy, phenylethoxy and 2-phenoxyethoxy moieties on both C6 (compounds **2**) and C7 (compounds **3**) were considered.

Materials and Methods

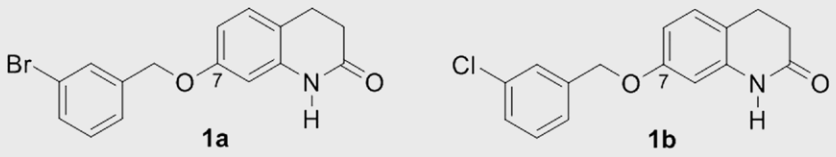
Chemicals and instrumentation

Reagents and solvents required for the chemistry and biology were obtained from Sigma-Aldrich (St. Louis, MO, USA) and were used without further purification. Kynuramine dihydrobromide and insect cell microsomes containing recombinant human MAO-A and MAO-B (5 mg protein/mL) were also obtained from Sigma-Aldrich. ^1H NMR and ^{13}C NMR spectra were recorded with a Bruker Avance III 600 spectrometer (Karlsruhe, Germany) employing DMSO-d_6 as solvent. The chemical shifts are given in parts per million (δ) and were referenced to the residual solvent signal. The spin multiplicities are given as s (singlet), d (doublet), dd (doublet of doublets), t (triplet) or m (multiplet). High resolution mass spectra (HRMS) were recorded with a Bruker micrOTOF-Q II mass spectrometer functioning in atmospheric-pressure chemical ionization (APCI) mode (positive mode). Melting points (mp) were measured with a Buchi B-545 melting point apparatus (Büchi Labortechnik, Flawil, Switzerland) and are uncorrected. The progress of reactions was monitored with silica gel 60 aluminum coated TLC sheets (Merck, Darmstadt, Germany). For this purpose the mobile phase consisted of ethyl acetate, and the developed sheets were visualized under an UV-lamp at a wavelength of 254 nm. Fluorescence spectrophotometry was carried out with a Varian Cary Eclipse fluorescence spectrophotometer (Agilent Technologies, Santa Clara, USA) equipped with a microplate reader.

Chemistry

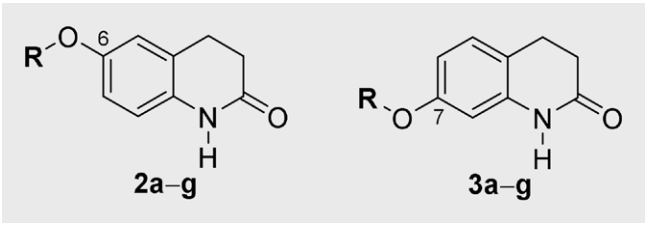
Procedure for the synthesis of 3,4-dihydro-2(1H)-quinolinone derivatives **2a–g** and **3a–g**

The 3,4-dihydro-2(1H)-quinolinone derivatives were prepared according to a previously reported protocol from the key reagents, 6-hydroxy-3,4-dihydro-2(1H)-quinolinone (**4**) and 7-hydroxy-3,4-dihydro-2(1H)-quinolinone (**5**), which are commercially available (Sigma-Aldrich) [23]. Reagents **4** or **5** (1.50 mmol) was suspended in eth-



▶ **Fig. 1** The structures of 7-(3-bromobenzyloxy)-3,4-dihydro-2(1H)-quinolinone (**1a**) and 7-(3-chlorobenzyloxy)-3,4-dihydro-2(1H)-quinolinone (**1b**).

▶ **Table 1** The IC_{50} values for the inhibition of recombinant human MAO-A and MAO-B by compounds **2a–g** and **3a–g**.



	IC_{50} (μM)[a]			
	R	MAO-A	MAO-B	SI[b]
2a	4-$ClC_6H_4CH_2$–	56.4 ± 20.0	0.017 ± 0.0046	3318
2b	4-$BrC_6H_4CH_2$–	>100[c]	0.0054 ± 0.0011	>18519
2c	3-$CH_3C_6H_4CH_2$–	31.0 ± 2.48	0.313 ± 0.028	99
2d	4-$CH_3C_6H_4CH_2$–	>100[c]	0.098 ± 0.024	>1020
2e	3-$CH_3C_6H_4(CH_2)_2$–	39.3 ± 2.47	0.198 ± 0.110	198
2f	4-$CH_3C_6H_4(CH_2)_2$–	>100[c]	0.025 ± 0.0087	>4000
2g	$C_6H_5O(CH_2)_2$–	>100[c]	0.102 ± 0.0019	>980
3a	4-$ClC_6H_4CH_2$–	28.9 ± 4.22	0.0014 ± 0.0003	20643
3b	4-$BrC_6H_4CH_2$–	>100[c]	0.0025 ± 0.0007	>40000
3c	3-$CH_3C_6H_4CH_2$–	26.4 ± 8.20	0.011 ± 0.00065	2400
3d	4-$CH_3C_6H_4CH_2$–	44.1 ± 5.25	0.018 ± 0.0083	2450
3e	4-$CH_3C_6H_4(CH_2)_2$–	34.5 ± 13.1	0.047 ± 0.021	734
3f	$C_6H_5O(CH_2)_2$–	>100[c]	0.047 ± 0.0080	>2128
3g	4-ClC_6H_4O-$(CH_2)_2$–	>100[c]	0.113 ± 0.065	>885

[a] All values are expressed as the mean ± SD of triplicate determinations

[b] The selectivity index is the selectivity for the MAO-B isoform and is given as the ratio IC_{50}(MAO-A)/IC_{50}(MAO-B)

[c] No inhibition observed at a concentration of 100 μM

anol (6 mL) containing KOH (1.66 mmol), and the required alkyl bromide (1.50 mmol) was added. The reaction was heated at reflux for 5 h and, upon completion (as judged by TLC), was poured into aqueous NaOH (1 %). The precipitate that formed was collected by filtration and crystallized from ethanol.

6-(4-Chlorobenzyloxy)-3,4-dihydro-2(1H)-quinolinone (**2a**)

The title compound was prepared in a yield of 6 %: mp 194.3 °C (ethanol). ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.92 (s, 1H), 7.42 (s, 4H), 6.84 (d, J = 2.5 Hz, 1H), 6.80–6.72 (m, 2H), 5.01 (s, 2H), 2.80 (t, J = 7.6 Hz, 2H), 2.38 (t, J = 7.6 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.93, 153.37, 136.39, 132.37, 132.08, 129.47, 128.47, 124.99, 115.85, 114.52, 113.37, 68.67, 30.37, 25.14. APCI-HRMS m/z: calcd for $C_{16}H_{15}ClNO_2$ (MH^+), 288.0786, found 288.0776.

6-(4-Bromobenzyloxy)-3,4-dihydro-2(1H)-quinolinone (**2b**)

The title compound was prepared in a yield of 4 %: mp 185.2–212.7 °C (ethanol). ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.92 (s, 1H), 7.56 (d, J = 8.3 Hz, 2H), 7.37 (d, J = 8.6 Hz, 2H), 6.84 (d, J = 2.5 Hz, 1H), 6.80 – 6.72 (m, 2H), 5.00 (s, 2H), 2.80 (t, J = 7.5 Hz, 2H), 2.38 (t, J = 7.5 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.91, 153.34, 136.82, 132.08, 131.38, 129.77, 124.98, 120.89, 115.84, 114.51, 113.37, 68.68, 30.36, 25.13. APCI-HRMS m/z: calcd for $C_{16}H_{15}BrNO_2$ (MH^+), 332.0281, found 332.0260.

6-(3-Methylbenzyloxy)-3,4-dihydro-2(1H)-quinolinone (**2c**)

The title compound was prepared in a yield of 44 %: mp 163.3 °C (ethanol). ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.92 (s, 1H), 7.25 (t, J = 7.5 Hz, 1H), 7.22 (s, 1H), 7.19 (d, J = 7.6 Hz, 1H), 7.11 (d, J = 7.4 Hz, 1H), 6.85 (d, J = 2.5 Hz, 1H), 6.80 – 6.73 (m, 2H), 4.97 (s, 2H), 2.81 (t, J = 7.5 Hz, 2H), 2.38 (t, J = 7.4 Hz, 2H), 2.30 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.79, 153.58, 137.55, 137.20, 131.92, 128.39, 128.31, 128.18, 124.88, 124.72, 115.76, 114.38, 113.24, 69.50, 30.35, 25.11, 21.02. APCI-HRMS m/z: calcd for $C_{17}H_{18}NO_2$ (MH^+), 268.1332, found 268.1357.

6-(4-Methylbenzyloxy)-3,4-dihydro-2(1H)-quinolinone (**2d**)

The title compound was prepared in a yield of 37 %: mp 173.3–177 °C (ethanol). ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.91 (s, 1H), 7.29 (d, J = 7.9 Hz, 2H), 7.17 (d, J = 7.8 Hz, 2H), 6.84 (d, J = 2.5 Hz, 1H), 6.79 – 6.72 (m, 2H), 4.96 (s, 2H), 2.80 (t, J = 7.5 Hz, 2H), 2.38 (t, J = 7.4 Hz, 2H), 2.28 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.79, 153.55, 136.97, 134.23, 131.88, 128.95, 127.72, 124.85, 115.74, 114.41, 113.29, 69.36, 30.35, 25.11, 20.79. APCI-HRMS m/z: calcd for $C_{17}H_{18}NO_2$ (MH^+), 268.1332, found 268.1324.

6-[2-(3-Methylphenyl)ethoxy]-3,4-dihydro-2(1H)-quinolinone (**2e**)

The title compound was prepared in a yield of 6 %: mp 135.6 °C (ethanol). ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.90 (s, 1H), 7.17 (t, J = 7.5 Hz, 1H), 7.10 (s, 1H), 7.07 (d, J = 7.7 Hz, 1H), 7.01 (d, J = 7.5 Hz, 1H), 6.79 – 6.72 (m, 2H), 6.70 (dd, J = 8.6, 2.7 Hz, 1H), 4.08 (t, J = 6.9 Hz, 2H), 2.94 (t, J = 6.9 Hz, 2H), 2.80 (t, J = 7.6 Hz, 2H), 2.37 (t, J = 7.6 Hz, 2H), 2.27 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.84, 153.63, 138.31, 137.36, 131.76, 129.61, 128.23, 126.92, 126.01, 124.91, 115.82, 114.07, 113.01, 68.48, 35.01, 30.38, 25.09, 21.05. APCI-HRMS m/z: calcd for $C_{18}H_{20}NO_2$ (MH^+), 282.1489, found 282.1473.

6-[2-(4-Methylphenyl)ethoxy]-3,4-dihydro-2(1H)-quinolinone (**2f**)

The title compound was prepared in a yield of 19 %: mp 152.1 °C (ethanol). ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.90 (s, 1H), 7.17 (d, J = 7.8 Hz, 2H), 7.09 (d, J = 7.7 Hz, 2H), 6.77 – 6.71 (m, 2H), 6.69 (dd, J = 8.6, 2.7 Hz, 1H), 4.06 (t, J = 6.9 Hz, 2H), 2.92 (t, J = 6.9 Hz, 2H), 2.79 (t, J = 7.6 Hz, 2H), 2.37 (t, J = 7.4 Hz, 2H), 2.25 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.90, 153.67, 135.35, 135.25, 131.75, 128.93, 128.86, 124.94, 115.85, 114.08, 113.00, 68.57, 34.66, 30.39, 25.10, 20.71. APCI-HRMS m/z: calcd for $C_{18}H_{20}NO_2$ (MH^+), 282.1489, found 282.1484.

6-[2-Phenoxyethoxy]-3,4-dihydro-2(1H)-quinolinone (**2g**)

The title compound was prepared in a yield of 6 %. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.93 (s, 1H), 7.28 (t, J = 7.3 Hz, 2H), 7.01 – 6.88 (m, 3H), 6.82 (s, 1H), 6.79 – 6.72 (m, 2H), 4.29 – 4.24 (m, 2H), 4.24 – 4.18 (m, 2H), 2.81 (t, J = 7.7 Hz, 2H), 2.39 (t, J = 7.7 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.85, 158.32, 153.51, 131.97, 129.58, 124.95, 120.76, 115.84, 114.49, 114.13, 113.12, 66.61, 66.24,

30.37, 25.12. APCI-HRMS m/z: calcd for $C_{17}H_{18}NO_3$ (MH^+), 284.1281, found 284.1273.

7-(4-Chlorobenzyloxy)-3,4-dihydro-2(1H)-quinolinone (**3a**)

The title compound was prepared in a yield of 56 %: mp 162.4–163.3 °C (ethanol). ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.06 (s, 1H), 7.33 (s, 4H), 7.02 (d, J = 8.2 Hz, 1H), 6.55 (dd, J = 8.3, 2.5 Hz, 1H), 6.45 (d, J = 2.4 Hz, 1H), 4.97 (s, 2H), 2.87 (t, J = 7.5 Hz, 2H), 2.60 (t, J = 7.6 Hz, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.28, 158.04, 138.27, 135.25, 133.73, 128.73, 128.71, 128.62, 116.21, 108.94, 102.63, 69.30, 30.95, 24.51. APCI-HRMS m/z: calcd for $C_{16}H_{15}ClNO_2$ (MH^+), 288.0786, found 288.0790.

7-(4-Bromobenzyloxy)-3,4-dihydro-2(1H)-quinolinone (**3b**)

The title compound was prepared in a yield of 25 %: mp 193.4–194.5 °C (ethanol). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.01 (s, 1H), 7.56 (d, J = 8.2 Hz, 2H), 7.36 (d, J = 8.3 Hz, 2H), 7.03 (d, J = 8.3 Hz, 1H), 6.53 (dd, J = 8.3, 2.5 Hz, 1H), 6.48 (d, J = 2.5 Hz, 1H), 5.00 (s, 2H), 2.76 (t, J = 7.5 Hz, 2H), 2.39 (t, J = 7.5 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 170.39, 157.35, 139.26, 136.58, 131.38, 129.75, 128.48, 120.93, 116.06, 107.85, 102.18, 68.40, 30.73, 24.04. APCI-HRMS m/z: calcd for $C_{16}H_{15}BrNO_2$ (MH^+), 332.0281, found 332.0259.

7-(3-Methylbenzyloxy)-3,4-dihydro-2(1H)-quinolinone (**3c**)

The title compound was prepared in a yield of 39 %: mp 151.3 °C (ethanol). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.01 (s, 1H), 7.25 (t, J = 7.5 Hz, 1H), 7.22 (s, 1H), 7.19 (d, J = 7.7 Hz, 1H), 7.12 (d, J = 7.4 Hz, 1H), 7.03 (d, J = 8.2 Hz, 1H), 6.54 (dd, J = 8.2, 2.5 Hz, 1H), 6.50 (d, J = 2.5 Hz, 1H), 4.97 (s, 2H), 2.76 (t, J = 7.6 Hz, 2H), 2.39 (t, J = 7.3 Hz, 2H), 2.30 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 170.33, 157.59, 139.22, 137.59, 136.97, 128.45, 128.42, 128.34, 128.20, 124.74, 115.83, 107.80, 102.08, 69.22, 30.73, 24.02, 21.01. APCI-HRMS m/z: calcd for $C_{17}H_{18}NO_2$ (MH^+), 268.1332, found 268.1328.

7-(4-Methylbenzyloxy)-3,4-dihydro-2(1H)-quinolinone (**3d**)

The title compound was prepared in a yield of 7 %: mp 152.1 °C (ethanol). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.00 (s, 1H), 7.29 (d, J = 8.0 Hz, 2H), 7.17 (d, J = 7.8 Hz, 2H), 7.02 (d, J = 8.2 Hz, 1H), 6.53 (dd, J = 8.3, 2.5 Hz, 1H), 6.49 (d, J = 2.5 Hz, 1H), 4.96 (s, 2H), 2.75 (t, J = 7.5 Hz, 2H), 2.39 (t, J = 7.5 Hz, 2H), 2.28 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 170.32, 157.56, 139.21, 137.04, 134.01, 128.97, 128.40, 127.74, 115.79, 107.85, 102.12, 69.08, 30.73, 24.01, 20.78. APCI-HRMS m/z: calcd for $C_{17}H_{18}NO_2$ (MH^+), 268.1332, found 268.1318.

7-[2-(4-Methylphenyl)ethoxy]-3,4-dihydro-2(1H)-quinolinone (**3e**)

The title compound was prepared in a yield of 9 %: mp 160–161.8 °C (ethanol). ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.96 (s, 1H), 7.17 (d, J = 7.8 Hz, 2H), 7.10 (d, J = 7.7 Hz, 2H), 7.02 (d, J = 8.3 Hz, 1H), 6.47 (dd, J = 8.3, 2.5 Hz, 1H), 6.41 (d, J = 2.5 Hz, 1H), 4.05 (t, J = 6.9 Hz, 2H), 2.93 (t, J = 6.9 Hz, 2H), 2.75 (t, J = 7.5 Hz, 2H), 2.39 (t, J = 7.5 Hz, 2H), 2.25 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 170.34, 157.63, 139.20, 135.26, 135.18, 128.91, 128.82, 128.47, 115.62, 107.62, 101.65, 68.27, 34.47, 30.75, 24.00, 20.68. APCI-HRMS m/z: calcd for $C_{18}H_{20}NO_2$ (MH^+), 282.1489, found 282.1480.

7-[2-Phenoxyethoxy]-3,4-dihydro-2(1H)-quinolinone (**3f**)

The title compound was prepared in a yield of 5 %: mp 180–186.5 °C (ethanol). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.01 (s, 1H), 7.28 (t, J = 7.9 Hz, 2H), 7.05 (d, J = 8.3 Hz, 1H), 7.00 – 6.89 (m, 3H), 6.53 (dd, J = 8.3, 2.5 Hz, 1H), 6.47 (d, J = 2.5 Hz, 1H), 4.31 – 4.24 (m, 2H), 4.24 – 4.16 (m, 2H), 2.77 (t, J = 7.5 Hz, 2H), 2.40 (t, J = 7.6 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 170.41, 158.32, 157.59, 139.28, 129.61, 128.55, 120.82, 115.98, 114.52, 107.55, 101.91, 66.40, 66.17, 30.76, 24.04. APCI-HRMS m/z: calcd for $C_{17}H_{18}NO_3$ (MH^+), 284.1281, found 284.1254.

7-[2-(4-Chlorophenoxy)ethoxy]-3,4-dihydro-2(1H)-quinolinone (**3g**)

The title compound was prepared in a yield of 25 %: mp 153.2 °C (ethanol). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.01 (s, 1H), 7.32 (d, J = 9.0 Hz, 2H), 7.05 (d, J = 8.2 Hz, 1H), 7.00 (d, J = 9.0 Hz, 2H), 6.52 (dd, J = 8.3, 2.6 Hz, 1H), 6.46 (d, J = 2.5 Hz, 1H), 4.31 – 4.24 (m, 2H), 4.23 – 4.16 (m, 2H), 2.77 (t, J = 7.5 Hz, 2H), 2.40 (t, J = 7.6 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 170.32, 157.51, 157.18, 139.26, 129.29, 128.49, 124.46, 116.30, 115.97, 107.49, 101.87, 66.67, 66.26, 30.72, 24.01. APCI-HRMS m/z: calcd for $C_{17}H_{17}ClNO_3$ (MH^+), 318.0891, found 318.0875.

Enzymology

Procedure for the measurement of IC_{50} values for the inhibition of MAO

The protocol for the measurement of IC_{50} values for the inhibition of the MAOs has been reported in literature [24, 25]. In short, the enzyme reactions were carried out to a volume of 200 µL in 96-well microtiter plates and contained kynuramine (50 µM), the test inhibitors (0.003–100 µM), and potassium phosphate buffer (pH 7.4, 100 mM). Each reaction also contained 4 % DMSO as co-solvent, and control reactions, performed in the absence of inhibitor, were included for each inhibitor evaluated. The enzyme reactions were initiated with the addition of recombinant human MAO-A (0.0075 mg protein/mL) or MAO-B (0.015 mg protein/mL) and incubated for 20 min at 37 °C. The reactions were subsequently terminated with the addition of 80 µL sodium hydroxide (2 N) and the oxidation product of kynuramine, 4-hydroxyquinoline, was quantified by fluorescence spectrophotometry (λ_{ex} = 310; λ_{em} = 400 nm) [26]. A linear calibration curve was prepared with authentic 4-hydroxyquinoline (0.047–1.56 µM). The rate data were fitted to the one site competition model of the Prism 5 software package (GraphPad, San Diego, CA, USA) to obtain sigmoidal plots, from which the IC_{50} values were estimated. All measurements were carried out in triplicate and IC_{50} values are given as the mean ± standard deviation (SD).

Procedure for examining the reversibility of inhibition by dialysis

The protocol for investigating the reversibility of MAO inhibition by dialysis has been reported in literature [24, 25]. Recombinant human MAO-B (0.03 mg protein/mL) was combined with the test inhibitor (**2b**) and the mixture (0.8 mL) was preincubated for 15 min at 37 °C. These preincubations were carried out in potassium phosphate buffer (100 mM, pH 7.4) containing 5 % sucrose. Employing this buffer also as dialysis buffer, the mixtures were subsequently dialyzed at

4 °C. For this purpose Slide-A-Lyzer dialysis cassettes with a molecular weight cut-off of 10 000 and a sample volume capacity of 0.5–3 mL were used (Thermo Scientific, Waltham, MA, USA). The dialysis buffer was replaced with fresh buffer at 3 h and 7 h after the start of dialysis. After 24 h of dialysis, the reactions were diluted 2-fold with the addition of kynuramine to give a final substrate concentration of 50 µM and a final inhibitor concentration of $2 \times IC_{50}$. The reactions (500 µL) were incubated for 20 min at 37 °C, and were subsequently terminated with the addition of NaOH (400 µL of 2 N) and 1 000 µL water. The fluorescence emission of 4-hydroxyquinoline in these reactions was measured as described above, employing a 3.5 mL quartz cuvette (pathlength 10 × 10 mm). As negative control, MAO-B was preincubated and dialyzed in the absence of inhibitor. As positive control, MAO-B was preincubated and dialyzed in the presence of a concentration equal to $4 \times IC_{50}$ of the irreversible inhibitor, (R)-deprenyl (IC_{50} = 0.079 µM) [27]. Also included in the study were undialyzed mixtures of MAO-B and **2b**, which were maintained at 4 °C for 24 h and diluted and assayed as above. All reactions were carried out in triplicate and the residual enzyme rates are given as mean ± SD.

Procedure for the construction of Lineweaver-Burk plots

The protocol for constructing Lineweaver-Burk plots has been reported in literature [24, 25]. For the inhibition of MAO-B by **2b**, 6 Lineweaver-Burk plots were constructed. The first plot was constructed in the absence of inhibitor, while the remaining 5 plots were constructed in the presence of the following inhibitor concentrations: $¼ \times IC_{50}$, $½ \times IC_{50}$, $¾ \times IC_{50}$, $1 \times IC_{50}$ and $1¼ \times IC_{50}$. For each plot, kynuramine served as substrate at 8 different concentrations (15–250 µM). After addition of the inhibitor and substrate, the enzymatic reactions (500 µL) were initiated with the addition of MAO-B (0.015 mg/mL). The enzyme reactions and activity measurements were subsequently carried out as described above for the dialysis experiments and the K_i value was estimated by global (shared) fitting of the inhibition data to the Michaelis-Menten equation using the Prism 5 software package. The K_i value may also be estimated from a plot of the slopes of the Lineweaver-Burke plots vs. inhibitor concentration (x-axis intercept equals $-K_i$).

Computer modeling

Procedure for docking with CDOCKER

The procedure for docking with CDOCKER has been reported in literature [24, 28]. For the docking studies, the Windows-based Discovery Studio 3.1 software package (Accelrys, San Diego, CA, USA) was used and the reported X-ray crystal structures of human MAO-A (PDB code 2Z5X) and human MAO-B (PDB code 2V5Z) served as protein models [29, 30]. The protein models were prepared by firstly calculating the pKa values and protonation states of the ionizable amino acids and subsequently adding hydrogen atoms to the models at pH 7.4. The FAD was set to the oxidized state, fixed atom constraints were applied to the protein backbones and the models were energy minimized using the Smart Minimizer algorithm. With the exception of HOH 710, 718 and 739 in MAO-A, and HOH 1 155, 1 170 and 1 351 in the A-chain of MAO-B, all waters and the co-crystallized ligands were removed and docking was carried out with the CDOCKER algorithm. 10 random conformations were generated for each ligand, the heating target temperature was set to 700 K and full potential mode was used. The docking solutions were finally refined using in situ ligand minimization with the Smart Minimizer algorithm. The illustrations were prepared with PyMol (Schrödinger, New York, NY, USA) [31].

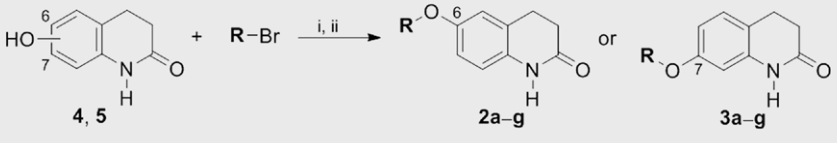
Results and Discussion

Chemistry

The 3,4-dihydro-2(1H)-quinolinone derivatives **2a–g** and **3a–g** were synthesized by nucleophilic substitution according to the reported protocol (▶ **Fig. 2**) [23, 32]. Among the synthesised compounds, **2a–2d** are known [33]. 6-Hydroxy-3,4-dihydro-2(1H)-quinolinone (**4**) or 7-hydroxy-3,4-dihydro-2(1H)-quinolinone (**5**), which are commercially available, were suspended in ethanol and treated with the required arylalkyl bromide. Potassium hydroxide served as base. The reaction mixture was heated at reflux for 5 h and poured into aqueous sodium hydroxide (1 %). A precipitate was obtained which was recrystallized from ethanol to yield the target 3,4-dihydro-2(1H)-quinolinone derivatives in yields of 4–56 %. The structures of **2a–g** and **3a–g** were characterized by ^{1}H NMR, ^{13}C NMR and mass spectrometry as given in the supplementary material. On the ^{1}H NMR spectra, 2 triplets at approximately 2.8 and 2.4 ppm represent the methylene protons of the 3,4-dihydro-2(1H)-quinolinone moiety, while the NH signals are present at 9–10 ppm. On the ^{13}C NMR spectra, the signal of the carbonyl carbon is found at approximately 170 ppm. For **2a–g** the protons on C5 are represented by doublets at approximately 6.85 ppm, while the protons on C7 are represented by a signal at approximately 6.70 ppm (doublet of doublets). For **3a–g** the proton on C8 are represented by doublets at approximately 6.45 ppm, while the protons on C5 and C6 are represented by signals at approximately 7.02 ppm (doublet) and 6.55 ppm (doublet of doublets).

IC_{50} values and SARs for MAO inhibition

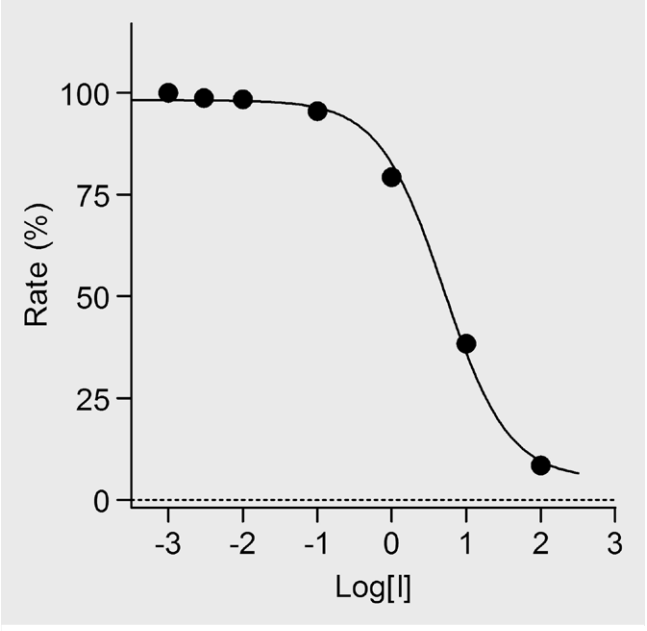
The inhibition of the human MAOs by derivatives **2a–g** and **3a–g** was examined under identical experimental conditions as those of the



▶ **Fig. 2** The synthesis of 3,4-dihydro-2(1H)-quinolinone derivatives **2a–g** and **3a–g**. Reagents and conditions: (i) KOH, ethanol, reflux; (ii) recrystallization from ethanol.

reported 3,4-dihydro-2(1H)-quinolinones [23]. This allows for the direct comparison of IC_{50} values recorded in this study with those cited in the literature report. IC_{50} values were measured by recording the catalytic rates of human MAO-A and MAO-B in the presence of different concentrations of the test inhibitors spanning at least 3 orders of magnitude (0.0003–100 µM). Sigmoidal dose–response curves (catalytic rate vs. logarithm of inhibitor concentration) were constructed in triplicate from which the IC_{50} values were estimated. The recombinant human MAOs served as enzyme sources and kynuramine was used as substrate for both isoforms. Kynuramine is oxidized by the MAOs to yield 4-hydroxyquinoline, a metabolite which may (after alkalization) be measured at the endpoint of the enzymatic reaction using fluorescence spectrophotometry. An example of a sigmoidal dose-response curve obtained in this study is shown in ▶ **Fig. 3**. As shown in ▶ **Table 1**, substitution on both C6 and C7 was considered in order to determine if substitution on C7 of the 3,4-dihydro-2(1H)-quinolinone moiety in all instances lead to significantly more potent inhibition compared to substitution on C6, as proposed in a previous study [23]. This study also explores the effect of meta and para substitution of the benzyloxy side chain with bromine, chlorine and the methyl group for comparison with compounds **1a** and **1b**, previously reported [23]. The effect of the 2-phenoxyethoxy moiety and methyl substituted 2-phenylethoxy side chains were also investigated. The 2-phenoxyethoxy moiety, in particular has been shown to enhance the MAO-B inhibition potency of caffeine derived inhibitors [34].

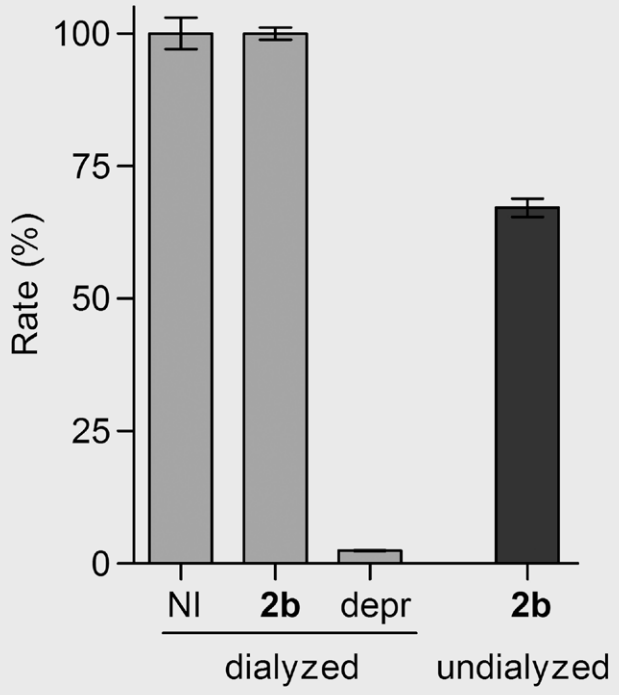
The IC_{50} values show that among both the C6- and C7-substituted 3,4-dihydro-2(1H)-quinolinones high potency MAO-B inhibitors exist. Most notably among these are **2b**, **3a** and **3b** which display IC_{50} values of < 0.006 µM. The MAO-B inhibition potencies of these compounds are comparable to those of **1a** (IC_{50} = 0.0029) and **1b** (IC_{50} = 0.0062), and are significantly more potent than the reference MAO-B inhibitors lazabemide (IC_{50} = 0.091 µM) and safinamide (IC_{50} = 0.048 µM), evaluated under the same experimental conditions [35]. These compounds also display high specificities for the MAO-B enzyme with selectivity index (SI) values > 18 519. In this regard, **3b** is particularly isoform specific since it possesses an IC_{50} for MAO-A inhibition of > 100 µM. Similar to the 3,4-dihydro-2(1H)-quinolinones studied before, **2a–g** and **3a–g** are relatively weak MAO-A inhibitors with IC_{50} > 26.4 µM. From the inhibition data, the following SARs may be derived: (a) Similar to the reported study, C7 substitution yields compounds with higher MAO-B inhibition potencies than C6 substitution. For each pair of homologues, the corresponding C7-substituted compounds display more potent inhibition although differences in inhibition potencies are in many instances very small (compare **2b** vs. **3b**; **2d** vs. **3d**; **2g** vs. **3f**). (b) Chlorine and bromine substitution on the para position of the benzyloxy ring yields similar MAO-B inhibition than meta substitution (compare **1b** vs. **3a**; **1a** vs. **3b**). Methyl substitution on either the meta or para positions of the benzyloxy ring is, however, less suitable for MAO-B inhibition compared to the halogens (compare **2c**/**2d** with **2a**/**2b**; **3c**/**3d** with **3a**/**3b**). (c) Substitution with the 2-phenoxyethoxy moiety on both the C6 (**2g**, IC_{50} = 0.102 µM) and C7 (**3f**, IC_{50} = 0.047 µM) positions of the 3,4-dihydro-2(1H)-quinolinone yields good potency MAO-B inhibition. These compounds are particularly specific inhibitors since they display IC_{50} values for MAO-A inhibition of > 100 µM. Interestingly, chlorine substitution (**3g**, IC_{50} = 0.113 µM) of the phenoxy ring significantly reduces MAO-B inhibition potency compared to the unsubstituted homologue **3f**. (d) Among the 2-phenylethoxy substituted compounds (**2e**, **2f**, **3e**), **2f** (IC_{50} = 0.025 µM) is the most potent MAO-B inhibitor. The good potency of this compound demonstrates that, with the appropriate substitution pattern, 2-phenylethoxy substitution may yield similar potency MAO-B inhibitors compared to benzyloxy substitution (compare **2f** vs. **2d**).



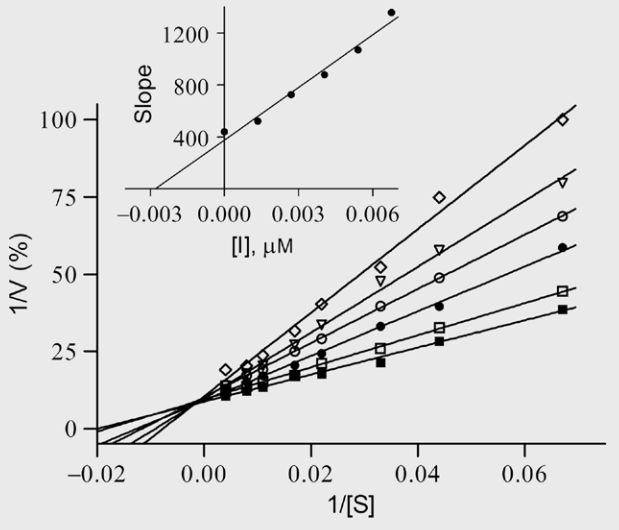
▶ **Fig. 3** Sigmoidal dose–response curve for the inhibition of human MAO-B by **2d**.

Reversibility of MAO-B inhibition

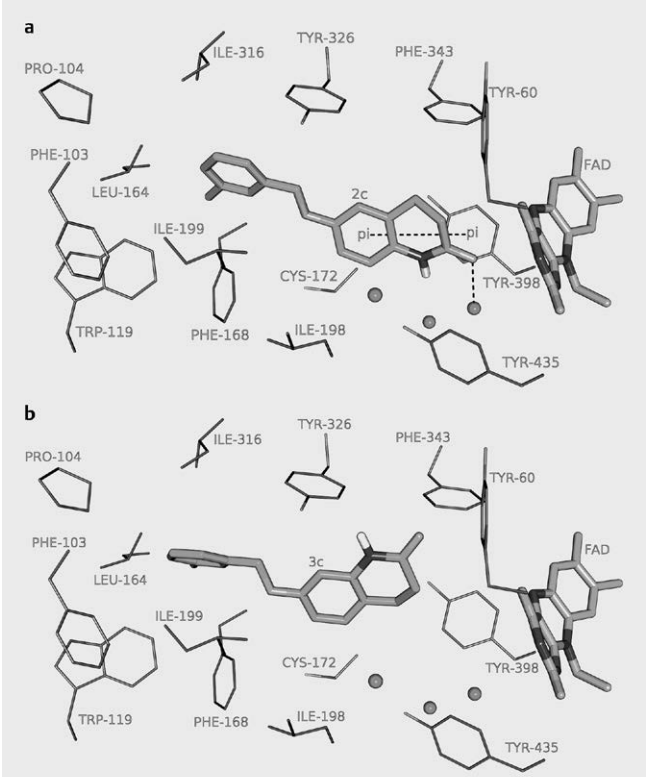
Using compound **1a** as model inhibitor, it has been established that 3,4-dihydro-2(1H)-quinolinones inhibits MAO-B with a reversible mode of action, although some tight-binding of the inhibitor to the enzyme may occur [23]. Since **1a** is a C7-substituted homologue, the present study investigates the reversibility of MAO-B inhibition by a C6-substituted 3,4-dihydro-2(1H)-quinolinone, compound **2b**. Reversibility of inhibition was investigated by dialysis. Compound **2b** (at a concentration of 4 × IC_{50}) was preincubated with MAO-B for 15 min and subsequently dialyzed for 24 h. The incubation mixtures were diluted 2-fold with the addition of kynuramine and the formation of 4-hydroxyquinoline was measured at the endpoint of the enzyme reaction. From the 4-hydroxyquinoline concentrations the residual activities were calculated. Similar dialysis experiments were carried out in the absence of inhibitor (negative control) and presence of the irreversible MAO-B inhibitor, (R)-deprenyl (positive control). The activity (rate of 4-hydroxyquinoline formation) of the negative control represents 100 % residual activity. As third control, the residual MAO-B activity of undialyzed mixtures of MAO-B and **2b** was also recorded. As shown in ▶ **Fig. 4**, following inhibition with **2b**, dialysis restores enzyme activity to 100 % of the negative control (100 %). Following inhibition with (R)-deprenyl, enzyme activity is not restored by dialysis with the residual activity at only 2.4 %. In undialyzed mixtures of MAO-B and **2b**, inhibition persists and the activity is recorded at 67 %. Since dialysis is expected to restore enzyme



▶ **Fig. 4** Compound **2b** inhibits MAO-B with a reversible mode. MAO-B and **2b** (at 4 × IC_{50}) was preincubated for 15 min, dialyzed for 24 h and the residual enzyme activity was measured (**2b** – dialyzed). Similar incubation and dialysis of MAO-B in the absence inhibitor (NI – dialyzed) and presence of the irreversible inhibitor, (R)-deprenyl (depr dialyzed), were also carried out. The residual activity of undialyzed mixtures of MAO-B with **2b** was also recorded (**2b** – undialyzed).



▶ **Fig. 5** Lineweaver-Burk plots for the inhibition of MAO-B by **2b**. The inset is a graph of the slopes of the Lineweaver-Burk plots vs. inhibitor concentration.



▶ **Fig. 6** The proposed binding modes of **2c** and **3c** to MAO-B.

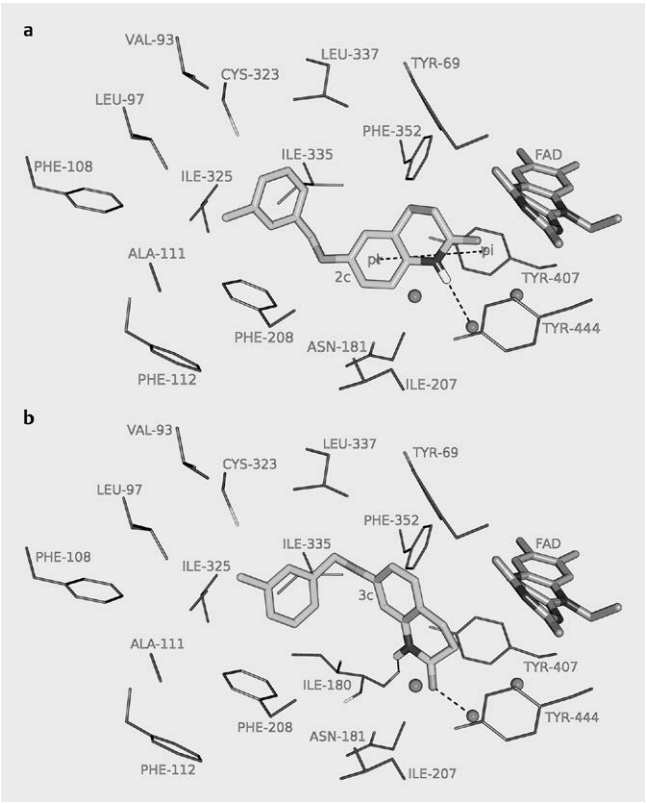
activity to 100 % for reversible inhibitors, it may be concluded that **2b** is a reversible MAO-B inhibitor.

Lineweaver-Burk plots and competitive inhibition

This study also set out to measure the enzyme-inhibitor dissociation constant (K_i value) for the reversible interaction between **2b** and MAO-B. To measure the K_i value a set of 6 Lineweaver-Burk plots was constructed using various inhibitor concentrations (0 µM, ¼ × IC_{50}, ½ × IC_{50}, ¾ × IC_{50}, 1 × IC_{50} and 1¼ × IC_{50}) and 8 concentrations of kynyramine (15–250 µM) for each plot. The set of Lineweaver-Burk plots is shown in ▶ **Fig. 5**. Since the lines are linear and intersect on the y-axis it may be concluded that **2b** is a competitive inhibitor of MAO-B. Global (shared) fitting of the inhibition data directly to the Michaelis-Menten equation yields a K_i value of 0.0026 ± 0.00024 µM (R^2 = 0.98). From a plot of the slopes of the Lineweaver-Burk plots vs. inhibitor concentration a similar value (0.0028 µM) is obtained (x-axis intercept equals – K_i).

Docking studies

An interesting SAR of MAO-B inhibition by 3,4-dihydro-2(1H)-quinolinones is that C7 substitution yields higher potency MAO-B inhibition compared to C6 substitution. In certain instances the differences in MAO-B inhibition are small (e. g. **2b**/**3b**) while for other homologues (e. g. **2c**/**3c**) the C7-substituted homologue is significantly more potent than the corresponding C6-substituted homologue. To determine if differing binding modes to MAO-B may be responsible for this SAR, **2c** and **3c** were docked into the MAO-B active site using



▶ **Fig. 7** The proposed binding modes of **2c** and **3c** to MAO-A.

the CDOCKER docking algorithm of Discovery Studio 3.1 [28]. To propose possible reasons for the weaker MAO-A inhibition of 3,4-dihydro-2(1H)-quinolinones, these compounds were also docked into the MAO-A active site. The docking simulations were carried out according to the literature procedure and the reported crystal structures of human MAO-A (PDB code: 2Z5X) [29] and MAO-B (PDB code: 2V5Z) [30] served as protein models [24]. As shown in ▶ **Figs. 6**, ▶ **2c** and ▶ **3c** exhibit differing binding modes to MAO-B, particularly with respect to the orientation of the 3,4-dihydro-2(1H)-quinolinone moiety. Compared to **2c**, the 3,4-dihydro-2(1H)-quinolinone moiety of **3c** is rotated by approximately 180°. This inversion of orientation allows for the benzyloxy side chain of **3c** to extend into the entrance cavity of the enzyme, similar to **2c**. For **2c**, polar interactions (H-bonding and π–π interaction with Tyr-398) are established in the enzyme's substrate cavity, which are not possible for **3c**. Since **3c** is a more potent MAO-B inhibitor than **2c**, these polar interactions play a lesser role in inhibitor stabilization, and Van der Waals interactions between the benzyloxy side chain and the entrance cavity most likely contribute to a higher degree. This is supported by the observation that **4** and **5**, compounds lacking a C6 or C7 side chain, are very weak MAO-B inhibitors (IC_{50} > 201 μM) [23]. Literature supports the proposal that MAO-B inhibitors (i. e. chalcones) may be stabilised by interaction with the entrance cavity of the enzyme [36, 37]. It may thus be concluded that, although the placements and orientations of these side chains are very similar, for **3c** more productive Van der Waals interactions are possible, perhaps because this compound extends slightly deeper into the entrance cavity enabling it to establish interactions with key residues.

In contrast to MAO-B, compounds **2c** and **3c** exhibit similar binding modes to MAO-A with respect to the orientation of the 3,4-dihydro-2(1H)-quinolinone moiety as well as the placement of the benzyloxy phenyl ring (▶ **Fig. 7**). Both compounds establish polar interactions (H-bonding and π–π interactions) with MAO. The similar binding modes to MAO-A explain their comparable MAO-A inhibition activities. It is noteworthy that **2c** and **3c** bind in folded conformations in MAO-A, while exhibiting extended conformations in MAO-B. In MAO-A restrictions imposed by the side chain of Phe-208 are most likely responsible for the folded conformation. In MAO-B the residue that corresponds to Phe-208 in MAO-A, is Ile-199. For larger inhibitors, the side chain of Ile-199 rotates from the active site cavity, allowing for extension into the entrance cavity. Larger inhibitors are thus, in general, better accommodated in MAO-B than MAO-A, hence more potent inhibition of MAO-B by the C6- and C7-substituted 3,4-dihydro-2(1H)-quinolinones.

Conclusion

In conclusion, the current study shows that 3,4-dihydro-2(1H)-quinolinones, in general, are MAO-B specific inhibitors. 3 compounds, **2b**, **3a** and **3b**, were identified with high potency MAO-B inhibition (IC_{50} < 0.006 μM). Specific MAO-B inhibitors such as these represent potential candidate drugs for the treatment of Parkinson's disease, with a low risk of provoking the side effects associated with MAO-A inhibition (e. g. "cheese effect"). As shown with **2b**, this class acts as reversible and competitive MAO-B inhibitors.

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). 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.

Conflict of Interest

The authors declare that they have no conflicts of interest to disclose.

References

1 Youdim MB, Edmondson D, Tipton KF. The therapeutic potential of monoamine oxidase inhibitors. Nat Rev Neurosci 2006; 7: 295–309

2 Schwartz TL. A neuroscientific update on monoamine oxidase and its inhibitors. CNS Spectr 2013; 18 (Suppl 1): 25–32

3 Lum CT, Stahl SM. Opportunities for reversible inhibitors of monoamine oxidase-A (RIMAs) in the treatment of depression. CNS Spectr 2012; 17: 107–120

4 Shoulson I, Oakes D, Fahn S et al. Parkinson Study Group. Impact of sustained deprenyl (selegiline) in levodopa-treated Parkinson's disease: a randomized placebo-controlled extension of the deprenyl and tocopherol antioxidative therapy of parkinsonism trial. Ann Neurol 2002; 51: 604–612

5 Fernandez HH, Chen JJ. Monoamine oxidase-B inhibition in the treatment of Parkinson's disease. Pharmacotherapy 2007; 27: S174S–S185

6 Finberg JP, Wang J, Bankiewicz K et al. Increased striatal dopamine production from L-DOPA following selective inhibition of monoamine oxidase B by R(+)-N-propargyl-1-aminoindan (rasagiline) in the monkey. J Neural Transm Suppl 1998; 52: 279–285

7 Da Prada M, Zürcher G, Wüthrich I et al. On tyramine, food, beverages and the reversible MAO inhibitor moclobemide. J Neural Transm Suppl 1988; 26: 31–56

8 Flockhart DA. Dietary restrictions and drug interactions with monoamine oxidase inhibitors: an update. J Clin Psychiatry 2012; 73 (Suppl 1): 17–24

9 Finberg JP, Gillman K. Selective inhibitors of monoamine oxidase type B and the "cheese effect". Int Rev Neurobiol 2011; 100: 169–190

10 Lasbennes F, Sercombe R, Seylaz J. Monoamine oxidase activity in brain microvessels determined using natural and artificial substrates: relevance to the blood-brain barrier. J Cereb Blood Flow Metab 1983; 3: 521–528

11 Finberg JP, Lamensdorf I, Armoni T. Modification of dopamine release by selective inhibitors of MAO-B. Neurobiology (Bp) 2000; 8: 137–142

12 Janssen PA, Leysen JE, Megens AA et al. Does phenylethylamine act as an endogenous amphetamine in some patients? Int J Neuropsychopharmacol 1999; 2: 229–240

13 Chiba K, Trevor A, Castagnoli N Jr. Metabolism of the neurotoxic tertiary amine, MPTP, by brain monoamine oxidase. Biochem Biophys Res Commun 1984; 120: 574–578

14 Youdim MB, Bakhle YS. Monoamine oxidase: isoforms and inhibitors in Parkinson's disease and depressive illness. Br J Pharmacol 2006; 147 (Suppl 1): S287–S296

15 Edmondson DE. Hydrogen peroxide produced by mitochondrial monoamine oxidase catalysis: biological implications. Curr Pharm Des 2014; 20: 155–160

16 Fowler JS, Volkow ND, Wang GJ et al. Age-related increases in brain monoamine oxidase B in living healthy human subjects. Neurobiol Aging 1997; 18: 431–435

17 Maurel A, Hernandez C, Kunduzova O et al. Age-dependent increase in hydrogen peroxide production by cardiac monoamine oxidase A in rats. Am J Physiol Heart Circ Physiol 2003; 284: H1460–H1467

18 Xu S, Adisetiyo H, Tamura S et al. Dual inhibition of survivin and MAO A synergistically impairs growth of PTEN-negative prostate cancer. Br J Cancer 2015; 113: 242–251

19 Wu JB, Shao C, Li X et al. Monoamine oxidase A mediates prostate tumorigenesis and cancer metastasis. J Clin Invest 2014; 124: 2891–2908

20 Carradori S, Silvestri R. New frontiers in selective human MAO-B inhibitors. J Med Chem 2015; 58: 6717–6732

21 Carradori S, Petzer JP. Novel monoamine oxidase inhibitors: a patent review (2012–2014). Expert Opin Ther Pat 2015; 25: 91–110

22 Gnerre C, Catto M, Leonetti F et al. Inhibition of monoamine oxidases by functionalized coumarin derivatives: biological activities, QSARs, and 3D-QSARs. J Med Chem 2000; 43: 4747–4758

23 Meiring L, Petzer JP, Petzer A. Inhibition of monoamine oxidase by 3,4-dihydro-2(1H)-quinolinone derivatives. Bioorg Med Chem Lett 2013; 23: 5498–5502

24 Mostert S, Petzer A, Petzer JP. Indanones as high-potency reversible inhibitors of monoamine oxidase. ChemMedChem 2015; 10: 862–873

25 Mostert S, Petzer A, Petzer JP. Inhibition of monoamine oxidase by benzoxathiolone analogues. Bioorg Med Chem Lett 2016; 26: 1200–1204

26 Novaroli L, Reist M, Favre E et al. Human recombinant monoamine oxidase B as reliable and efficient enzyme source for inhibitor screening. Bioorg Med Chem 2005; 13: 6212–6217

27 Petzer A, Harvey BH, Wegener G et al. Azure B, a metabolite of methylene blue, is a high-potency, reversible inhibitor of monoamine oxidase. Toxicol Appl Pharmacol 2012; 258: 403–409

28 Accelrys Discovery Studio 3.1. San Diego, CA, USA: Accelrys Software Inc; 2005 http://www.accelrys.com

29 Son SY, Ma J, Kondou Y et al. Structure of human monoamine oxidase A at 2.2-A resolution: the control of opening the entry for substrates/inhibitors. Proc Natl Acad Sci U S A 2008; 105: 5739–5744

30 Binda C, Wang J, Pisani L et al. Structures of human monoamine oxidase B complexes with selective noncovalent inhibitors: safinamide and coumarin analogs. J Med Chem 2007; 50: 5848–5852

31 DeLano WL. 2002 The PyMOL molecular graphics system. San Carlos, USA: DeLano Scientific

32 Shigematsu N. Studies on the synthetic analgesics. XVI. Synthesis of 1-(2-tert-aminoalkyl)-3,4-dihydrocarbostyrils. Chem Pharm Bull 1961; 9: 970–975

33 Sun XY, Zhang L, Wei CX et al. Design, synthesis of 8-alkoxy-5,6-dihydro-[1,2,4]triazino[4,3-a]quinolin-1-ones with anticonvulsant activity. Eur J Med Chem 2009; 44: 1265–1270

34 Strydom B, Bergh JJ, Petzer JP. The inhibition of monoamine oxidase by 8-(2-phenoxyethoxy)caffeine analogues. Arzneimittel-Forsch 2012; 62: 513–518

35 Petzer A, Pienaar A, Petzer JP. The inhibition of monoamine oxidase by esomeprazole. Arzneimittel-Forsch 2013; 63: 462–467

36 Mathew B, Haridas A, Uçar G et al. Exploration of chlorinated thienyl chalcones: A new class of monoamine oxidase-B inhibitors. Int J Biol Macromol 2016; 91: 680–695

37 Hammuda A, Shalaby R, Rovida S et al. Design and synthesis of novel chalcones as potent selective monoamine oxidase-B inhibitors. Eur J Med Chem 2016; 114: 162–169