The Inhibition of Monoamine Oxidase by 8-(2-Phenoxyethoxy)Caffeine Analogues

Authors B. Strydom, J. J. Bergh, J. P. Petzer

Affiliation Pharmaceutical Chemistry, School of Pharmacy, North-West University, Potchefstroom, South Africa

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- monoamine oxidase
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- reversible inhibition
- selective inhibition
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Abstract

Previous studies have documented that substituted 8-oxycaffeines act as inhibitors of human monoamine oxidase (MAO) B. A particularly potent inhibitor among the reported compounds was 8-(2-phenoxyethoxy)caffeine with an IC_{50} value of 0.383 µM towards MAO-B. In an attempt to improve on the inhibition potency of this compound and to discover highly potent reversible MAO-B inhibitors, in the present study, a series of 8-(2-phenoxyethoxy)caffeine analogues containing various substituents on C4 of the phenoxy ring, were synthesized and evaluated as inhibitors of human MAO-A and -B. The results show that the 8-(2-phenoxyethoxy)caffeine analogues are selective and reversible MAO-B inhibitors with the most potent homologue, 8-{2-[4-(trifluoromethyl)phenoxy]ethoxy}caffeine, exhibiting an IC_{50} value of 0.061 µM. These highly potent inhibitors are useful leads in the design of therapies for neurodegenerative disorders such as Parkinson's disease.

Introduction

Monoamine oxidase (MAO) is a flavin adenine dinucleotide (FAD) containing enzyme which is bound to the outer mitochondrial membrane. MAO is classified into 2 distinct isoforms, MAO-A and -B, which share a 70% sequence identity and are products of separate genes [1,2]. The principal function of the MAO isozymes is the oxidative deamination of primary amines of an endogenous and dietary nature. In the central and peripheral tissues, MAO is responsible for the termination of the action of neurotransmitter amines such as serotonin, dopamine, epinephrine and norepinephrine [1,3]. The 2 MAO isoforms may be distinguished by their different substrate specificities. MAO-A preferentially catalyzes the oxidation of serotonin while MAO-B favours benzylamine and 2-phenethylamine as substrates [3]. Dopamine, epinephrine, norepinephrine and tyramine are considered to be substrates for both MAO isoforms [3].

MAO has acted as a target for the treatment of central nervous system diseases [3]. Selective MAO-A inhibitors are reported to be effective in the treatment of depression by increasing the levels of serotonin, norepinephrine and dopamine in the brain [4]. An example of such a drug is the reversible MAO-A inhibitor, moclobemide [5]. Since MAO-B is considered to be the major dopamine metabolizing enzyme in the basal ganglia of the brain, MAO-B inhibitors are used in the treatment of Parkinson's disease [6,7]. In the basal ganglia, inhibitors of MAO-B are thought to reduce the depletion of dopamine, and to enhance dopamine levels after treatment with levodopa [8,9]. MAO-B inhibitors may also protect against the neurodegenerative events associated with Parkinson's disease by reducing the levels of potentially neurotoxic aldehydes and H_2O_2, which are generated as by-products in the catalytic cycle of MAO-B [1]. (*R*)-Deprenyl and rasagiline are examples of MAO-B inhibitors currently being used as adjuncts to levodopa therapy in the treatment of Parkinson's disease [10]. While these inhibitors have been widely used, their irreversible mode of inhibition may be associated with certain disadvantages. These include a slow rate of enzyme recovery after drug withdrawal and a loss of selectivity after repeated administration [11,12]. For these reasons, the discovery of new MAO-B inhibitors with a reversible mode of action is being pursued by various research groups.

For the design of reversible MAO inhibitors, C8-substituted caffeine analogues have been previously employed [13–16]. While caffeine is a

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Correspondence
Prof. J. P. Petzer, PhD
Pharmaceutical Chemistry
School of Pharmacy
North-West University
Private Bag X6001
2520, Potchefstroom
South Africa
Tel.: +27/18/2992206
Fax: +27/18/2994243
jacques.petzer@nwu.ac.za

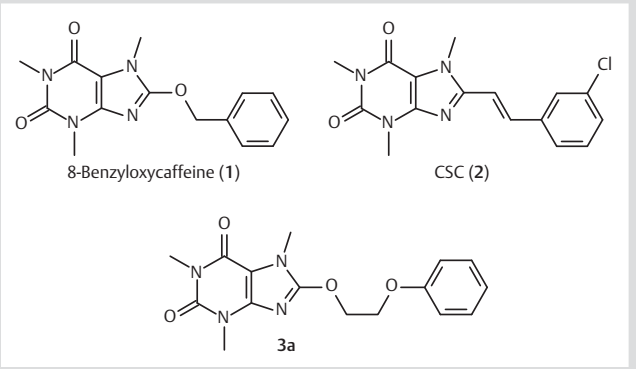


Fig. 1 The structures of 8-benzyloxycaffeine (**1**), CSC (**2**) and 8-(2-phenoxyethoxy)caffeine **3a**.

weak MAO-B inhibitor, substitution with a variety of moieties at C8 often yields compounds that are highly potent and selective MAO-B inhibitors [17]. For example, a series of 8-benzyloxycaffeine analogues (**1**) (**Fig. 1**) has been shown to act as potent inhibitors of human MAO-B with IC_{50} values ranging from 0.068 to 1.77 µM [13]. Interestingly, the 8-benzyloxycaffeine analogues were also found to be potent reversible MAO-A inhibitors with IC_{50} values ranging from 0.397 to 3.72 µM [13]. Modelling studies have indicated that the ability of the 8-benzyloxycaffeine analogues to also bind to the MAO-A active site may depend on the large degree of rotational freedom of the benzyloxy side chain at the carbon-oxygen ether bond [13]. More rigid C8-substituted caffeine analogues such as (*E*)-8-(3-chlorostyryl)caffeine (CSC, **2**) typically do not inhibit MAO-A [13, 17, 18]. Another C8 oxy substituent that leads to potent MAO-B inhibition is the 2-phenoxyethoxy moiety. In fact, 8-(2-phenoxyethoxy)caffeine (**3a**) (IC_{50} = 0.383 µM) is a more potent inhibitor of human MAO-B than is 8-benzyloxycaffeine (IC_{50} = 1.77 µM) [14]. A structure-activity relationship (SAR) study has indicated that for C8-substituted oxycaffeine analogues, a linker consisting of 4 atoms separating the caffeine and the terminal phenyl ring may be particularly suited for MAO-B inhibition [14]. Since this requirement is satisfied by the 8-(2-phenoxyethoxy)caffeine structure, this compound may be considered to be a promising lead compound for the design of highly potent MAO-B inhibitors.

Based on these observations, in the present study the MAO-A and -B inhibition properties of a series of ten 8-(2-phenoxyethoxy)caffeine analogues (**3a**–**j**) were examined. The analogues considered, differed by substitution on the para position of the phenoxy ring. Previous studies reported that halogen substitution at this position leads to a superior enhancement of the MAO-B inhibition potency of 8-benzyloxycaffeine [13, 14] with improved selectivity compared to meta substitution.

Materials and Methods

Materials

All starting materials, unless otherwise noted, were acquired from Sigma-Aldrich®. Proton (^1H) and carbon (^{13}C) NMR spectra were recorded in $CDCl_3$ and DMSO-d_6 with a Bruker® Avance III 600 spectrometer at frequencies of 600 MHz and 150 MHz, respectively. Direct insertion high resolution mass spectra (HRMS) were obtained on a DFS high resolution magnetic sector mass spectrometer (Thermo Electron® Corporation) in electron impact ionization mode. Melting points (mp) were measured with a Buchi® M-545 melting point apparatus and are uncorrected. Fluorescence spectrophotometry was conducted with a Varian® Cary Eclipse fluorescence spectrophotometer. Microsomes from insect cells containing recombinant MAO-A or -B (5 mg/mL) and kynuramine·2HBr were obtained from Sigma-Aldrich®. 8-Chlorocaffeine (**4**) was prepared according to a previously reported procedure [19, 20] by the reaction of caffeine with chlorine in chloroform. The 2-phenoxyethanol analogues (**5**), which were required as reactants for the synthesis of **3**, were prepared by reacting the appropriately substituted phenol (**6**) with bromoethanol in the presence of acetone and potassium carbonate [21, 22]. The purities of previously unreported compounds were determined by HPLC analysis as described previously [15].

Procedures for the synthesis of the 8-(2-phenoxyethoxy)caffeine analogues (3)

For the synthesis of compounds **3a**–**d** a method described in literature was followed with minor modifications [23]. Metallic sodium (1.5 mmol) and the appropriately substituted phenoxyethanol analogue (**5**, 21 mmol) were allowed to react at room temperature. Following consumption of the sodium, 8-chlorocaffeine (**4**, 1.5 mmol) was added and the resulting mixture was stirred for 6 h at 150 °C. The reaction was cooled to room temperature and the 8-(2-phenoxyethoxy)caffeine analogue was recrystallized (at 4 °C) after the addition of ethanol (10–20 mL) to the reaction mixture. The melting points and structure characterizations of compounds **3a**–**c** have been previously reported [14]. The physical data of **3d** are cited below. The 8-(2-phenoxyethoxy)caffeine analogues **3e–j** were synthesized according to the literature procedure by Baumann et al. [24]. Potassium hydroxide (2 mmol) was dissolved in 1 mL distilled water and the appropriately substituted 2-phenoxyethanol analogue (1.85 mmol) was added to yield a solution. 8-Chlorocaffeine (**4**, 1.5 mmol) was added and the resulting reaction mixture was stirred at 150 °C for 6 h. The reaction was cooled to room temperature and ethanol (10 mL) was added. The resulting solution was allowed to recrystallize (at 4 °C).

8-[2-(4-Fluorophenoxy)ethoxy]caffeine (3d): The title compound was prepared from 8-chlorocaffeine (**4**) and 2-(4-fluorophenoxy)ethanol. (63 %) cream coloured solid: mp 177 °C (from ethanol). ^1H NMR (600 MHz, $CDCl_3$, Me_4Si) 3.37 (3 H, s, CH_3), 3.49 (3 H, s, CH_3), 3.68 (3 H, s, CH_3), 4.29 (2 H, t, CH_2, J = 4.52 Hz), 4.77 (2 H, t, CH_2, J = 4.52 Hz), 6.85 (2 H, m, phenyl), 6.97 (2 H, m, phenyl); ^{13}C NMR (150 MHz, $CDCl_3$, Me_4Si) 27.76, 29.75, 29.90, 66.48, 69.20, 103.62, 115.69 (d), 115.90, 116.06, 146.07, 151.67, 154.45, 154.83, 155.27; HRMS *m/z*: calcd for $C_{16}H_{17}FN_4O_4$, 348.1234, found 348.1214; Purity (HPLC): 98 %.

8-[2-[4-(Trifluoromethyl)phenoxy]ethoxy]caffeine (3e): The title compound was prepared from 8-chlorocaffeine (**4**) and 2-[4-(trifluoromethyl)phenoxy]ethanol. (3 %) white solid: mp 130 °C (from ethanol). ^1H NMR (600 MHz, $CDCl_3$, Me_4Si) 3.36 (3 H, s, CH_3), 3.49 (3 H, s, CH_3), 3.67 (3 H, s, CH_3), 4.37 (2 H, t, CH_2, J = 3.39 Hz), 4.81 (2 H, t, CH_2, J = 3.01 Hz), 6.97 (2 H, d, phenyl, J = 7.91 Hz), 7.54 (2 H, d, phenyl, J = 7.91 Hz); ^{13}C NMR (150 MHz, $CDCl_3$, M_4Si) 27.75, 29.74, 29.90, 65.89, 68.86, 103.65, 114.52, 125.14 (q), 127.01, 127.03, 146.03, 151.65, 154.83, 155.16, 160.71; HRMS *m/z*: calcd for $C_{17}H_{17}F_3N_4O_4$, 398.1202, found 398.1198; Purity (HPLC): 93 %.

8-[2-(4-Methylphenoxy)ethoxy]caffeine (3f): The title compound was prepared from 8-chlorocaffeine (**4**) and 2-(4-methylphenoxy)ethanol. (83%) white solid: mp 144 °C (from ethanol). ^{1}H NMR (600 MHz, $CDCl_3$, Me_4Si) 2.27 (3 H, s, CH_3-phenyl), 3.49 (3 H, s, CH_3), 3.64 (3 H, s, CH_3), 3.67 (3 H, s, CH_3), 4.3 (2 H, t, CH_2, J = 4.52 Hz), 4.77 (2 H, t, CH_2, J = 4.52 Hz), 6.81 (2 H, d, phenyl, J = 8.69 Hz), 7.07 (2 H, d, phenyl, J = 8.28 Hz); ^{13}C NMR (150 MHz, $CDCl_3$, Me_4Si) 20.46, 27.75, 29.75, 29.89, 65.92, 69.37, 103.58, 114.51, 130.01, 130.74, 146.11, 151.68, 154.83, 155.37, 156.21; HRMS *m/z*: calcd for $C_{17}H_{20}N_4O_4$, 344.1485, found 344.1480; Purity (HPLC): 98%.

8-[2-(4-Methoxyphenoxy)ethoxy]caffeine (3g): The title compound was prepared from 8-chlorocaffeine (**4**) and 2-(4-methoxyphenoxy)ethanol. (5%) sand coloured solid: mp 138 °C (from ethanol). ^{1}H NMR (600 MHz, $CDCl_3$, Me_4Si) 3.36 (3 H, s, CH_3), 3.49 (3 H, s, CH_3), 3.67 (3 H, s, CH_3), 3.75 (3 H, s, OCH_3), 4.27 (2 H, t, CH_2, J = 4.52 Hz), 4.76 (2 H, t, CH_2, J = 4.52 Hz), 6.83 (2 H, m, phenyl), 6.84 (2 H, m, phenyl); ^{13}C NMR (150 MHz, $CDCl_3$, Me_4Si) 27.73, 29.73, 29.88, 55.70, 66.59, 69.40, 103.56, 114.69, 115.74, 146.09, 151.66, 152.43, 154.31, 154.81, 155.35; HRMS *m/z*: calcd for $C_{17}H_{20}N_4O_5$, 360.1434, found 360.1434; Purity (HPLC): 98%.

8-[2-(4-Iodophenoxy)ethoxy]caffeine (3h): The title compound was prepared from 8-chlorocaffeine (**4**) and 2-(4-iodophenoxy)ethanol. (4%) white solid: mp 160 °C (from ethanol). ^{1}H NMR (600 MHz, $CDCl_3$, Me_4Si) 3.36 (3 H, s, CH_3), 3.49 (3 H, s, CH_3), 3.67 (3 H, s, CH_3), 4.29 (2 H, m, CH_2), 4.77 (2 H, m, CH_2), 6.69 (2 H, d, phenyl, J = 7.91 Hz), 7.55 (2 H, d, phenyl, J = 7.53 Hz); ^{13}C NMR (150 MHz, $CDCl_3$, Me_4Si) 27.74, 29.74, 29.90, 65.85, 68.98, 83.54, 103.61, 116.95, 138.35, 146.03, 151.64, 154.81, 155.19, 158.20; HRMS *m/z*: calcd for $C_{16}H_{17}IN_4O_4$, 456.0294, found 456.0268; Purity (HPLC): 98%.

8-[2-(4-Cyanophenoxy)ethoxy]caffeine (3i): The title compound was prepared from 8-chlorocaffeine (**4**) and 2-(4-cyanophenoxy)ethanol. (8%) white solid: mp > 300 °C (from ethanol). ^{1}H NMR (600 MHz, DMSO-d_6, Me_4Si) 3.18 (3 H, s, CH_3), 3.35 (3 H, s, CH_3), 3.56 (3 H, s, CH_3), 4.43 (2 H, m, CH_2), 4.77 (2 H, m, CH_2), 7.02 (2 H, d, phenyl, J = 9.04 Hz), 7.83 (2 H, d, phenyl, J = 8.66 Hz); ^{13}C NMR (150 MHz, DMSO-d_6, Me_4Si) 27.38, 29.50, 29.64, 65.89, 69.43, 102.69, 114.03, 126.89, 129.39, 145.53, 150.87, 153.85, 154.98, 160.47, 167.31; HRMS *m/z*: calcd for $C_{17}H_{17}N_5O_4$, 355.1281, found 355.1275; Purity (HPLC): 98%.

8-[2-(4-Nitrophenoxy)ethoxy]caffeine (3j): The title compound was prepared from 8-chlorocaffeine (**4**) and 2-(4-nitrophenoxy)ethanol. (63%) white solid: mp 178 °C (from ethanol). ^{1}H NMR (600 MHz, $CDCl_3$, Me_4Si) 3.35 (3 H, s, CH_3), 3.48 (3 H, s, CH_3), 3.68 (3 H, s, CH_3), 4.43 (2 H, t, CH_2, J = 4.52 Hz), 4.83 (2 H, t, CH_2, J = 4.52 Hz), 6.98 (2 H, d, phenyl, J = 9.41 Hz), 8.19 (2 H, d, phenyl, J = 9.04 Hz); ^{13}C NMR (150 MHz, $CDCl_3$, Me_4Si) 27.72, 29.72, 29.91, 66.35, 68.55, 103.64, 114.48, 125.93, 141.92, 145.93, 151.58, 154.76, 154.98, 163.17; HRMS *m/z*: calcd for $C_{16}H_{17}N_5O_6$, 375.1179, found 375.1172; Purity (HPLC): 98%.

IC_{50} determinations

IC_{50} values for the inhibition of MAO-A and -B were determined using the recombinant human enzymes as described previously [25]. Incubations were carried out at pH 7.4 (potassium phosphate 100 mM, made isotonic with KCl) to a final volume of 500 µL. The reactions contained the MAO-A/B mixed substrate kynuramine (45 µM for MAO-A and 30 µM for MAO-B), different inhibitor concentrations (0.003–100 µM) and DMSO as co-solvent (4%). The reactions were initiated with the addition of MAO-A or MAO-B (0.0075 mg protein/mL), incubated for 20 min at 37 ºC and terminated by the addition of 400 µL NaOH (2 N) and 1 000 µL water. The concentrations of the MAO generated 4-hydroxyquinoline were subsequently measured by fluorescence spectrophotometry (λ_{em} = 310; λ_{ex} = 400 nm) [26]. Employing a linear calibration curve (4-hydroxyquinoline: 0.047–1.56 µM), the enzyme catalytic rates were calculated and fitted to the one site competition model incorporated into the Prism software package (GraphPad). The IC_{50} values were determined in triplicate and are expressed as mean ± standard deviation (SD).

Recovery of enzyme activity after dilution

Compound **3h** or pargyline (IC_{50} = 12.97 µM) at concentrations equal to 100 × IC_{50} (92.4 µM and 1.30 mM for the 2 inhibitors, respectively) for the inhibition of MAO-A were preincubated with recombinant human MAO-A (0.75 mg/ml) for 30 min at 37 °C in potassium phosphate buffer (100 mM, pH 7.4, made isotonic with KCl). Compound **3e** or (R)-deprenyl (IC_{50} = 0.079 µM) [27] were similarly preincubated with recombinant human MAO-B (0.75 mg/ml) at concentrations equal to 100 × IC_{50} (6.1 µM and 7.9 µM for the 2 inhibitors, respectively). Control incubations were conducted in the absence of inhibitor, and DMSO (4%) was added as co-solvent to all preincubations. The reactions were diluted 100-fold with the addition of kynuramine to yield final concentrations of the inhibitors equal to 1 × IC_{50}. The final concentration of MAO-A and -B were 0.0075 mg/mL and the concentrations of kynuramine were 45 µM and 30 µM for MAO-A and -B, respectively. The reactions were incubated for a further 20 min at 37 °C, terminated and the residual rates of 4-hydroxyquinoline formation were measured as described above. These reactions were carried out in triplicate and the residual enzyme catalytic rates were expressed as mean ± SD.

Results

Chemistry

The 8-(2-phenoxyethoxy)caffeine analogues (**3a**–**j**) were synthesized according to the protocol previously described for the synthesis of C8 substituted caffeines [14,24]. The target compounds were obtained by reacting 8-chlorocaffeine (**4**) with an appropriately substituted 2-phenoxyethanol (**5**) at high temperatures (150 °C) in the presence of sodium or potassium hydroxide (**Fig. 2**). After recrystallization from ethanol, compounds **3a–j** were obtained in low to high yields (3–83%). The low yields obtained with some of the reactions are most likely the result of incomplete recrystallization after adding ethanol to the reaction mixture. Although inefficient in some instances, this procedure effectively separated the desired product from unreacted 8-chlorocaffeine. 8-Chlorocaffeine was obtained in high yield from a reaction between chlorine and a solution of caffeine in chloroform [19]. In certain instances, the 2-phenoxyethanol analogues that were required for the synthesis of **3a–j** were not commercially available and were thus synthesized according to literature methods [21,22]. For this purpose an appropriately substituted phenol (**6**) was reacted with 2-bromoethanol in the presence of potassium carbonate in acetone. The structures of the 8-(2-phe-

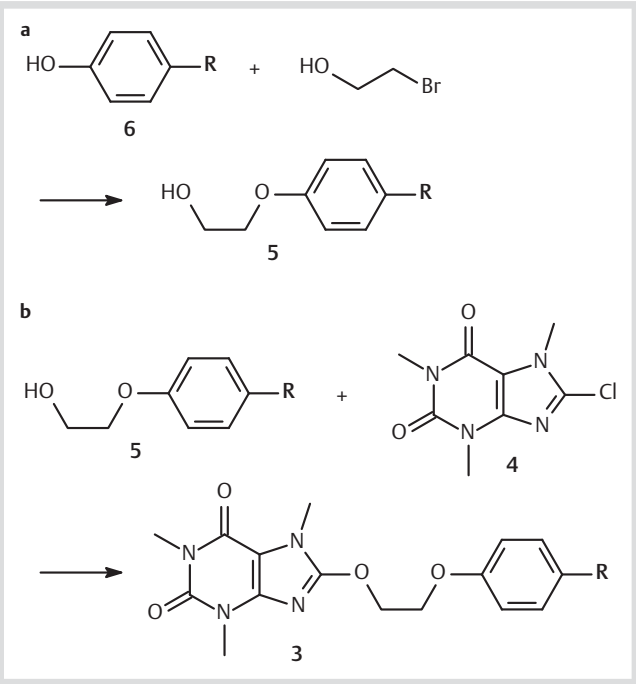
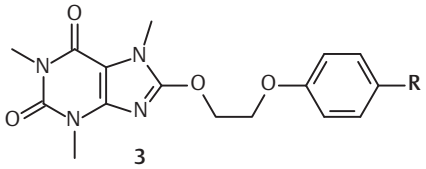


Fig. 2 Synthetic pathway to the 8-(2-phenoxyethoxy)caffeine analogues **3**. Reagents and conditions: **a** Acetone, K_2CO_3 **b** Na or KOH, 150 °C.

Table 1 The IC_{50} values for the inhibition of recombinant human MAO-A and –B by the 8-(2-phenoxyethoxy)caffeine analogues **3a–j** [a].



		IC_{50} values (μM)		
Compd.	**R**	**MAO-A**	**MAO-B**	**SI[b]**
3a	-H	20.4 ± 16.5[c]	0.383 ± 0.021[c]	53
3b	-Cl	1.83 ± 0.013[c]	0.183 ± 0.005[c]	10
3c	-Br	1.65 ± 0.087[c]	0.166 ± 0.003[c]	9.9
3d	-F	9.08 ± 1.38	0.115 ± 0.004	79
3e	$-CF_3$	2.22 ± 0.068	0.061 ± 0.003	36
3f	$-CH_3$	13.4 ± 0.549	1.41 ± 0.068	9.5
3g	$-OCH_3$	7.57 ± 0.187	1.53 ± 0.185	5.0
3h	-I	0.924 ± 0.031	0.128 ± 0.013	7.2
3i	-CN	35.5 ± 3.04	6.98 ± 0.433	5.1
3j	$-NO_2$	4.92 ± 0.286	0.852 ± 0.007	5.8

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

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

[c] Values obtained from literature [14]

noxyethoxy)caffeine analogues (**3a–j**) were verified by mass spectrometry, ^1H NMR and ^{13}C NMR while the purities were estimated by chromatographic analysis.

Enzymology

To examine the MAO-A and -B inhibition properties of the 8-(2-phenoxyethoxy)caffeine analogues (**3a–j**), the commercially available recombinant human enzymes were employed and kynuramine, a MAO-A/B nonselective substrate, served as enzyme substrate. Kynuramine, a non-fluorescent compound, undergoes MAO catalyzed oxidation to yield the fluorescent compound, 4-hydroxyquinoline, as end product. The extent to which kynuramine is oxidized to 4-hydroxyquinoline by MAO was subsequently measured via fluorescence spectrophotometry [26]. None of the inhibitors investigated in this study fluoresced at these excitation/emission wavelengths or quenched the fluorescence of 4-hydroxyquinoline at the inhibitor concentrations used. Sigmoidal dose-response curves were constructed for the inhibition of the MAO isozymes and the inhibition potencies of the test compounds were expressed as the corresponding IC_{50} values.

The results of the MAO inhibition studies are given in **Table 1**. The 8-(2-phenoxyethoxy)caffeine analogues (**3a–j**) were found to act as inhibitors of MAO-B. The most potent inhibitor of the series was the CF_3 substituted homologue, compound **3e**, which exhibited an IC_{50} value of 0.061 μM for the inhibition of MAO-B. Compound **3e** is therefore approximately 6-fold more potent than the corresponding unsubstituted homologue **3a** (IC_{50} = 0.383 μM). The results show that a variety of substituents on C4 of the phenoxy ring lead to enhanced MAO-B inhibition compared to **3a**. These substituents are notably halogens. For example, the Cl (IC_{50} = 0.183 μM), Br (IC_{50} = 0.166 μM), F (IC_{50} = 0.115 μM) and I (IC_{50} = 0.128 μM) substituted homologues inhibited MAO-B with potencies approximately 2–3-fold higher than that of the unsubstituted homologue **3a**. Non-halogen substituents on C4 of the phenoxy ring of the 8-(2-phenoxyethoxy)caffeine analogues resulted in a reduction of MAO-B inhibition potency compared to the unsubstituted homologue **3a**. For example, the CH_3 (IC_{50} = 1.41 μM) and OCH_3 (IC_{50} = 1.53 μM) substituted homologues were 3.5–4-fold weaker inhibitors of MAO-B than the unsubstituted homologue **3a**. Similarly, those homologues containing the CN (IC_{50} = 6.98 μM) and NO_2 (IC_{50} = 0.852 μM) substituents were found to be 18-fold and 2-fold weaker inhibitors than **3a**, respectively.

The 8-(2-phenoxyethoxy)caffeine analogues (**3a–j**) were also found to inhibit MAO-A. As shown by the SI values, which ranged from 5–79, all of the compounds were however selective inhibitors of the MAO-B isoform. The only homologue which exhibited an IC_{50} value in the sub-micromolar range was compound **3h**, which inhibited MAO-A with an IC_{50} value of 0.924 μM. With the exception of the CN substituted homologue **3i** (IC_{50} = 35.5 μM), substitution on C4 of the phenoxy ring led to an enhancement of MAO-A inhibition potency compared to the unsubstituted homologue **3a** (IC_{50} = 20.4 μM). With the exception of the F substituted homologue (IC_{50} = 9.08 μM), those homologues with halogen containing substituents (**3b**, **3c**, **3e** and **3h**) were the most potent MAO-A inhibitors of the series with IC_{50} values ranging from 0.924–2.22 μM.

Reversibility of inhibition

As mentioned in the introduction, literature reports that 8-oxycaffeines interact reversibly with both MAO-A and -B [14]. To verify that the most potent MAO-A inhibitor of the present series, compound **3h**, and the most potent MAO-B inhibitor, compound **3e**, are reversible inhibitors, the recoveries of the enzymatic activities after dilution of the enzyme-inhibitor complexes were evaluated. The MAO enzymes were preincubated with compounds **3h** and **3e** at concentrations of 100 × IC_{50} for 30 min and then diluted to 1 × IC_{50}. The results are presented in **Fig. 3** and show that after diluting **3h** and **3e** to concentrations equal to 1 × IC_{50}, the MAO-A and -B activities were recov-

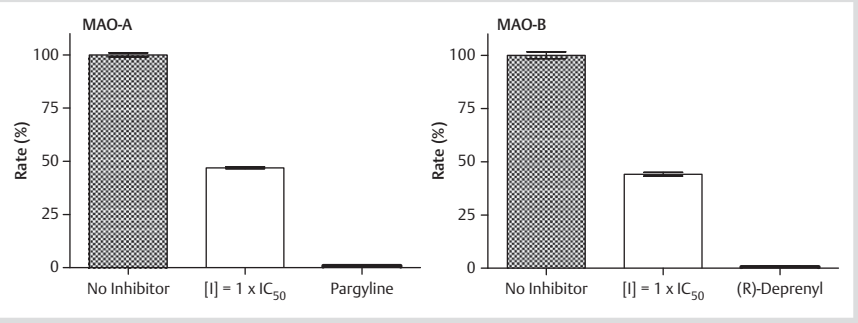


Fig. 3 Reversibility of inhibition of MAO-A and –B by compounds **3h** and **3e**. MAO-A was preincubated with **3h** and pargyline (left panel), and MAO-B was preincubated with **3e** and (R)-deprenyl (right panel), at 100 × IC_{50} for 30 min and then diluted to 1 × IC_{50}. The residual enzyme activities were subsequently measured.

ered to levels of 47 % and 44 % of the control values, respectively. This behavior is consistent with a reversible interaction of **3h** with MAO-A and **3e** with MAO-B. In contrast, after similar treatment of MAO-A and -B with the irreversible inhibitors pargyline and (R)-deprenyl, respectively, the MAO-A and -B activities were not recovered.

Discussion

Based on a previous report that 8-(2-phenoxyethoxy)caffeine (**3a**) is a potent MAO-B inhibitor and potentially suitable lead for the design of reversible MAO-B inhibitors, the present study investigates the SAR for the inhibition of human MAO-A and -B by a series of 8-(2-phenoxyethoxy)caffeine analogues containing different substituents on the para position of the phenoxy ring [14]. The results document that, compared to **3a**, substitution with halogen containing groups leads to an enhancement in MAO-B inhibition potency while non-halogen substituents reduce the MAO-B inhibition potencies of the 8-(2-phenoxyethoxy)caffeine analogues. The enhancement in MAO-B inhibition potency may be attributed to the electron withdrawing properties of the halogen containing substituents, since those substituents that may be considered electron releasing (CH_3 and OCH_3), reduce the MAO-B inhibition potencies of 8-(2-phenoxyethoxy)caffeine. This finding is in accordance with similar studies reported in literature. For example, for the inhibition of MAO-B by a series of 8-benzyloxycaffeine analogues, the inhibition potencies may be improved with electron withdrawing substituents on the benzyloxy ring [13]. Interestingly, although the CN and NO_2 groups are electron withdrawing, substitution with these groups do not result in enhanced MAO-B inhibition. This suggests that additional properties of the C4 substituents also determine binding to MAO-B. The lipophilicity of the para substituents may also play a role since halogen substitution is expected to also result in analogues that are more lipophilic. The active site cavity of the MAO-B is considered to be a mostly hydrophobic space, and it may therefore be expected that C4 substituents with enhanced lipophilicity may interact more favourably via Van der Waals interactions and thus should lead to improved MAO-B inhibition. The finding that OCH_3 CN and NO_2 substitution reduces binding affinity to MAO-B is in agreement with this analysis since these functional groups are expected to reduce the lipophilicity of the caffeine analogues. The observation that the CH_3 group, which is considered to be a lipophilic substituent, does not enhance MAO-B inhibition potency further supports the proposal that a combination of properties of the C4 substituent play a role in the binding to MAO-B.

The 8-(2-phenoxyethoxy)caffeine analogues were also found to be inhibitors of human MAO-A. With the exception of the CN substituted homologue **3i**, substitution on C4 of the phenoxy ring resulted in an enhancement of MAO-A inhibition potency compared to the unsubstituted homologue **3a**. Since the properties of the substituents considered are relatively diverse, this enhancement may, similar to MAO-B, be dependent on a combination of properties of the C4 substituent. In this regard, both electron withdrawing functional groups on C4 of the phenoxy ring, such as the halogen containing groups, and electron releasing substituents (CH_3 and OCH_3) enhance the MAO-A inhibition potency of 8-(2-phenoxyethoxy)caffeine. Similarly, substitution which results in both enhanced (halogens, CF_3 and CH_3) and reduced lipophilicity (OCH_3 and NO_2) leads to higher MAO-A inhibition potency. The 8-(2-phenoxyethoxy)caffeine analogues are, however, MAO-B selective inhibitors. A possible explanation for this observation may be that the active site of MAO-B is more suitable to accommodate relatively larger inhibitors than the active site of MAO-A [28]. In the MAO-B active site, residue Ile-199 may rotate from the active site cavity to allow larger inhibitors to span both the substrate and entrance cavities. In MAO-A, the residue that corresponds to Ile-199 in MAO-B, is Phe-208 [29]. The relatively larger size of the phenyl side chain of this residue may, in certain instances, restrict the binding of larger inhibitors to MAO-A. Although the MAO-B inhibition potencies of the 8-(2-phenoxyethoxy)caffeine analogues are similar to those of the 8-benzyloxycaffeine analogues, they exhibit improved selectivity for the MAO-B isoform [13]. For example, the most potent MAO-B inhibitor among the 8-(2-phenoxyethoxy)caffeines, compound **3e** (IC_{50} = 0.061 µM), exhibits an SI value of 36 while the most potent 8-benzyloxycaffeine, 8-(3-bromobenzyloxy)caffeine (IC_{50} = 0.068 µM) exhibits an SI value of only 1.7 [13]. Considering that MAO-A inhibition may lead to serious side effects such as potentiating the sympathomimetic effects of dietary amines [1], selective MAO-B inhibition is a desirable property.

In conclusion, the present study has shown that, with the appropriate substitution, 8-(2-phenoxyethoxy)caffeine analogues act as highly potent and selective MAO-B inhibitors. In addition, the compounds bind reversibly to the enzyme, which may be considered a desired property for further development as antiparkinsonian therapy.

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Conflict of Interest

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

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