2-Arylthiomorpholine derivatives as potent and selective monoamine oxidase B inhibitors

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ABSTRACT

2-Arylthiomorpholine and 2-arylthiomorpholin-5-one derivatives, designed as rigid and/or non-basic phenylethylamine analogues, were evaluated as rat and human monoamine oxidase inhibitors. Molecular docking provided insight into the binding mode of these inhibitors and rationalized their different potencies. Making the phenylethylamine scaffold rigid by fixing the amine chain in an extended six-membered ring conformation increased MAO-B (but not MAO-A) inhibitory activity relative to the more flexible α-methylated derivative. The presence of a basic nitrogen atom is not a prerequisite in either MAO-A or MAO-B. The best K_i values were in the 10^{-8} M range, with selectivities towards human MAO-B exceeding 2000-fold.

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1. Introduction

Functional impairment of the dopamine, serotonin, and noradrenaline neurotransmitter systems has been advocated as an important feature of neurodegenerative diseases, particularly Parkinson's disease, and neuropsychiatric disorders such as depression and anxiety.[1] As the neuronal levels of these neurotransmitters are altered under these conditions, the enzyme monoamine oxidase (MAO, E.C. 1.4.3.4) is an attractive drug target due to its central role in monoaminergic neurotransmitter metabolism.

This FAD dependent enzyme is bound to the mitochondrial outer membrane and catalyzes the oxidative deamination of a range of endogenous and exogenous monoamines. The two mammalian isoforms named MAO-A and MAO-B differ in tissue localization, substrate preference, and inhibitor selectivity.[2] Thus, MAO-A is selectively inhibited by clorgyline and metabolizes serotonin preferentially, whereas MAO-B is inhibited by l-deprenyl and prefers benzylamine and phenylethylamine as substrates. Selective MAO-A inhibitors (MAOAI) are used clinically as antidepressants and anxiolytics, while MAOBI are used for reduction of the progres-

sion of Parkinson's disease and of symptoms associated with Alzheimer's disease.[3–6]

Our group has studied a large number of phenylethylamine derivatives as MAOIs. Modifications include substitutions on the amine nitrogen, alkylation of the α and oxygenation of the β-positions with respect to the amine function, and different substitutions on the aromatic ring including extension of the π system (Fig. 1). Several of these compounds, particularly α-methylphenylethylamines with a single alkoxy or alkylthio substituent at the
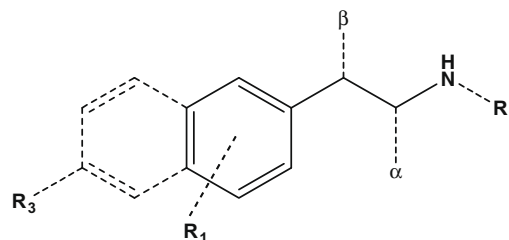

Figure 1. Different modifications (dashed line) to a phenylethylamine scaffold (bold and solid line).

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para position, have proved to be potent and selective MAOAI with negligible MAOBI activity.[7–12]

These compounds have in common a basic amino group linked to a flexible ethyl chain. A number of structurally unrelated selective MAOBI that contain a basic nitrogen atom held in a relatively rigid environment, for example, forming part of a heterocycle, are known.[13,14] Diverse MAOBI have also been described in which either the basicity is reduced or there is no basic group at all.[15] If flexibility around the amino group and its basic character are not prerequisites for potent MAO-B inhibition, it seems reasonable to explore modifications around the ethylamine side chain leading to greater rigidity and including non-basic analogues as possible means of generating a novel series of phenylethylamine-derived MAOBI.

In the present study we tested these hypotheses by evaluating the MAO inhibitory properties of a series of phenylethylamine derivatives in which the nitrogen atom is incorporated into a six-membered sulfur containing heterocycle. The influence of the basic character of the molecule was evaluated by comparing compounds containing a secondary amino group with others in which the nitrogen atom was part of an amide functionality. In addition, as species differences in the pharmacology of MAOIs have been reported,[10,16,17] we studied our compounds as inhibitors of both the human and the rat enzymes. Finally, in order to rationalize our biochemical results, docking experiments were performed using models built on the basis of the crystal structures of human MAO-A and -B.

2. Results and discussion

2.1. Chemistry

The (±)-2-arylthiomorpholin-5-one (**4a–f**) and (±)-2-arylthiomorpholine (**5a–f**) derivatives were synthesized following published methods (Scheme 1), which involved a Henry–Knoevenagel condensation of the appropriately substituted aromatic aldehydes **1a–f** with nitromethane and the subsequent Michael addition of methyl thioglycolate to the nitrostyrenes **2a–f** to generate the corresponding nitroester **3a–f**.[18] Sulfur was selected over other heteroatoms, for example, oxygen, because its greater nucleophilicity ensures better yields of the intermediates. Finally, a one-pot reaction involving a nucleophilic attack on the ester group of the amine, obtained by reduction of the nitro group with Zn^0, afforded the heterocycles **4a–f**.[19] Reduction of the lactams using DIBAL-H, gave the desired thiomorpholine derivatives **5a–f**.[20]
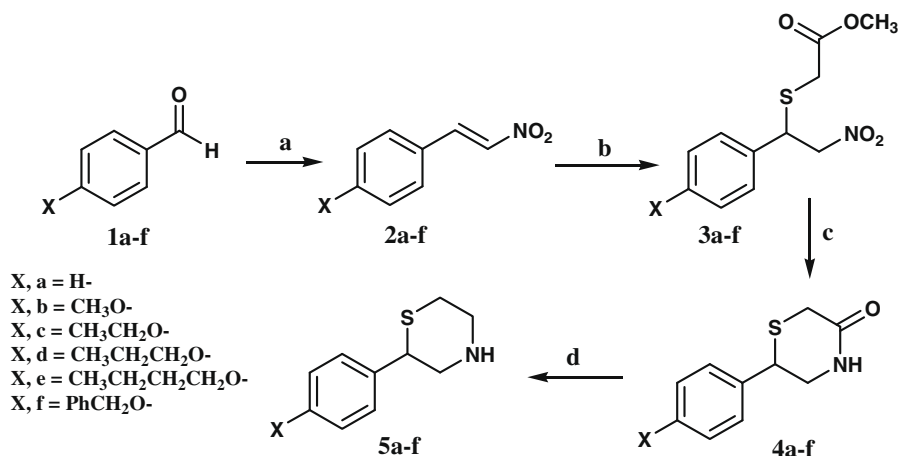
2.2. Biochemistry and molecular docking

Table 1 summarizes the effects and selectivity ratios of the 2-arythiomorpholin-5-one (**4a–f**) and 2-arylthiomorpholine (**5a–f**) derivatives (in all cases as the racemic mixtures), upon human (h) and rat (r) MAO-A and MAO-B. Also included are the reported values for *d*-amphetamine (**6**).[12,21,22]

In contrast to what had been seen in previous studies with phenylethylamine derivatives, most of the compounds exhibited some selectivity for MAO-B with K_i values in the low micromolar and submicromolar range, but only **4f** and **5f** are highly selective for this isoform.
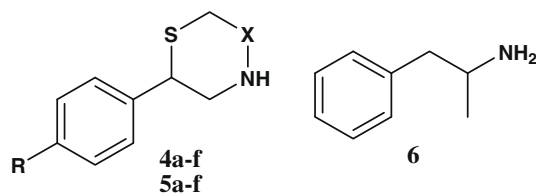
Reversible and competitive inhibition of hMAO-A and hMAO-B was demonstrated for all compounds tested. Figure 2 depicts the results of kinetic measurements (initial velocities) of hMAO-B in the presence of different concentrations of **5f**, the most potent drug described in this paper.

Regarding our first hypothesis, the incorporation of the phenylethylamine amino group into a ring containing a sulfur atom in the β-position (**4a** and **5a**), produced an increase of the rMAO-B inhibitory potency (**4a** K_i = 9.58 μM, **5a** K_i = 2.23 μM) as compared with amphetamine (the α-methylated phenylethylamine analogue **6**, K_i >100 μM), whereas the inhibitory activity of these compounds towards rMAO-A (**4a** K_i = 9.19 μM, **5a** K_i = 10.6 μM) is practically the same as that of amphetamine (K_i = 12.2 μM). Interestingly, neither the benzene ring-unsubstituted thiomorpholin-5-one (**4a**) nor its reduced derivative (**5a**) exhibited any significant inhibition of either human isoform (K_i >100 μM in both cases). These results are in agreement with the observation that striking differences in inhibitory properties can be observed when MAOs from different species are compared.[10,16,17]

Although **4a** and **5a** do not bind with high affinity to the human enzyme isoforms, reducing the mobility of the ethylamine chain by including it in a thiomorpholine ring elicits an increase of the inhibitory potency towards rMAO-B. These results prompted us to further explore the effect of some simple structural modifications of this scaffold. Thus, the introduction of *n*-alkoxyl groups containing 2–4 carbon atoms, at the *para* position of the aromatic ring (**4c–e**, **5c–e**) produces a progressive increase of the inhibitory activity against both human and rat MAO-B, reaching the lowest submicromolar K_i values for the butoxy derivatives (**4e** and **5e**). In the case of hMAO-A, all these compounds showed poor inhibitory potencies and low- to medium selectivity ratios.

Docking experiments suggest that the (*S*)-enantiomers of both thiomorpholine and thiomorpholin-5-one derivatives and their

X, a = H-
X, b = CH₃O-
X, c = CH₃CH₂O-
X, d = CH₃CH₂CH₂O-
X, e = CH₃CH₂CH₂CH₂O-
X, f = PhCH₂O-

Scheme 1. Reagents and conditions: (a) CH_3NO_2/cyclohexylamine/CH_3COOH, 40 °C, 2 h; (b) $HSCH_2COOMe$/N(Et)$_3$/THF, rt, 2 h; (c) Zn/CH_3COOH, 80 °C, 48 h; (d) DIBAL-*n*-hexane/THF, reflux, 2 h.

Table 1
Inhibition constants of 2-arylthiomorpholin-5-one (**4a**–**f**), 2-arylthiomorpholine (**5a**–**5f**) derivatives (racemic mixtures), and *d*-amphetamine (**6**)

Compd	R	X	K_i (μM) human[a]		K_i (μM) rat[b]		Selectivity A/B[c]	
			[d]MAO-A	MAO-B	MAO-A	MAO-B	Human	Rat
4a	H–	–C=O	>100	>100	9.19 ± 1.43	9.58 ± 1.75	–	1
4b	CH₃O–	–C=O	>100	>100	9.28 ± 2.09	13.6 ± 2.16	–	0.68
4c	CH₃CH₂O–	–C=O	60 ± 8.57	16.9 ± 1.73	12.3 ± 1.14	3.40 ± 1.01	3.5	3.6
4d	CH₃(CH₂)₂O–	–C=O	40 ± 1.85	2.4 ± 0.31	8.68 ± 1.12	1.49 ± 0.03	16.7	6
4e	CH₃(CH₂)₃O–	–C=O	10 ± 0.34	0.46 ± 0.18	50.9 ± 6.19	0.16 ± 0.01	22	318
4f	C₆H₅CH₂O–	–C=O	>100	0.048 ± 0.03	27.5 ± 4.62	0.074 ± 0.003	>2000	373
5a	H–	–CH₂–	>100	>100	10.6 ± 0.41	2.23 ± 0.03	–	5
5b	CH₃O–	–CH₂–	16.2 ± 2.29	>100	6.39 ± 0.14	3.85 ± 0.01	–	1.7
5c	CH₃CH₂O–	–CH₂–	9.60 ± 1.63	2.1 ± 0.28	2.17 ± 0.13	2.17 ± 0.31	5	1
5d	CH₃(CH₂)₂O–	–CH₂–	6.62 ± 0.34	0.13 ± 0.011	3.69 ± 0.39	0.98 ± 0.11	51	4
5e	CH₃(CH₂)₃O–	–CH₂–	2.50 ± 0.25	0.068 ± 0.016	14.1 ± 1.22	0.27 ± 0.02	37	52
5f	C₆H₅CH₂O–	–CH₂–	>100	0.038 ± 0.003	19.0 ± 0.36	0.13 ± 0.004	>2600	146
6			14.4 ± 0.5[e]	280 ± 10[f]	12.2 ± 2.72[g]	>100[g]	0.05	>0.12

[a] Calculated directly from the respective full kinetic determinations.
[b] Calculated from IC_{50} values using the Cheng–Prusoff equation: K_m (5-HT, MAO-A) = 100 μM, K_m (DMAPEA, MAO-B) = 5 μM.
[c] Selectivity was expressed as the ratio of the K_i of MAO-A to the K_i of MAO-B.
[d] Standard errors for hMAO-A calculated for a single kinetic analysis based on K_m and V_{max} values at 6–8 concentrations of inhibitor, not comparable with the standard deviation calculated on the average of different assays for hMAO-B, rMAO-A and rMAO-B.
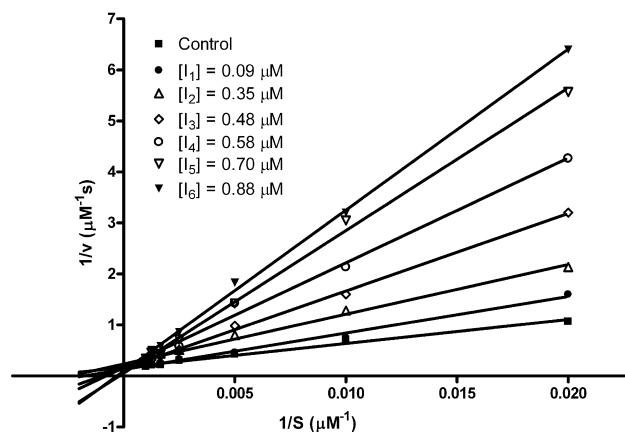[e] Ref. 21.
[f] Ref. 22.
[g] Ref. 12.

Figure 2. Lineweaver–Burk plot for the inhibition by **5f** of human MAO-B assayed with benzylamine as the substrate.

Figure 3. Superimposed structures of compounds **4b** (gray), **4c** (light rose), **4d** (blue) and **4e** (red) docked into the active site of hMAO-B. Main active site amino acid residues (green) and FAD (yellow) are rendered as stick models.

antipodes bind to the hMAO-B active site in very similar orientations (see below), with the heterocycle facing the isoalloxazine ring of the enzyme cofactor, and the aromatic ring located in such a way as to establish interactions with the Tyr326 hydroxyl group, which is at a distance of approximately 3 Å (Fig. 3 illustrates the binding modes obtained for compounds (*S*)-**4b**–**e**; henceforth all figures show the *S*-isomer unless stated otherwise). These types of attractive hydrogen–π interactions, which involve hydroxyl groups and aromatic rings, have been previously reported and although they are weaker than classical hydrogen bonds they can be decisive in determining the preferred pose of the ligand in the binding site
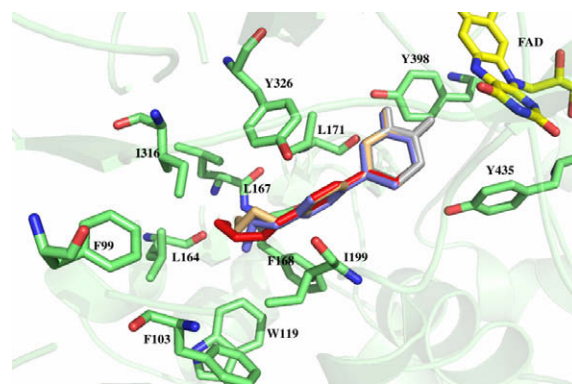
of the enzyme.[23] Additionally, the sulfur atom docks close to Tyr435, suggesting that it might interact with the amino acid's phenol group. Similar interactions between the aromatic cage tyrosines and H bond acceptor heteroatoms, have been described as important for binding of different MAO-B and MAO-A inhibitors.[10,24,25]

The MAO-B active site consists of two cavities, the relatively polar substrate binding site facing the flavin cofactor, and the so-called entrance cavity, which displays a highly hydrophobic environment.[26] Visual inspection of the best poses for compounds belonging to both the amide (**4**) and amine (**5**) series indicated that these drugs can bind in an extended conformation occupying both

cavities and that differences in potencies of alkoxylated derivatives might be attributed to differential hydrophobic interactions of the n-alkoxyl chain and several aromatic and aliphatic residues including Phe99, Phe103, Trp119, Leu164, Leu167, Phe168, Ile199 and Ile316, where the longer substituents interact more strongly with the entrance cavity.

The existence of these aromatic residues in the entrance cavity suggests that replacement of the aliphatic substituents in **4a–e**, **5a–e** by an aromatic entity might lead to stronger interactions. In previous CoMFA studies of indolyl derivatives in rMAO-B it was noted that a benzyloxy group can be placed at a location congruent with the *para* position of our phenylethylamine derivatives.[27] Moreover, the literature records a large number of compounds where the inclusion of a benzyloxy group affords potent and selective MAOBI,[13,25,28,29] thus justifying the synthesis of derivatives with a benzyloxy group at the *para* position of the aromatic ring (**4f** and **5f**). In fact, this substituent resulted in strongly increased potency and selectivity of thiomorpholinone **4f** and thiomorpholine **5f** towards MAO-B of both species (**4f** K_i = 0.048 μM, selectivity >2000; **5f** K_i = 0.038 μM, selectivity >2600), with little or no binding to hMAO-A.

As shown in Figure 4A, docking experiments in human MAO-B revealed that the benzylated compounds **4f** and **5f** exhibit similar binding modes, where their heterocycle points toward the isoalloxazine ring of FAD. In addition, the orientation of both R- and S-isomers of **5f** showed very similar poses into the active site of MAO-B (Fig. 4B). In these cases the sulfur atom in the heterocycle appears to interact with Tyr398 rather than Tyr435 as seems to happen with **4b–e**. These tyrosine residues may show some degree of flexibility and it is reasonable to assume that binding interactions of the inhibitor in the catalytic site may arise from interactions with either amino acid. The position of the central aromatic ring is highly conserved and is the same as in the n-alkoxy derivatives, underlining the likely importance of a phenol–benzene ring interaction with the Tyr326 residue. As expected, **4f** and **5f** dock in extended conformations occupying both cavities. The benzyloxy group is embedded in a hydrophobic environment delimited by Phe99, Phe103, Pro104, Trp119, Leu164, Leu167, Phe168, Ile199 and Ile316. This location is similar to that occupied by a phenyl ring of safinamide, 7-(3-chlorobenzyloxy)-4-(methylamino)-methylcoumarin, 7-(3-chlorobenzyloxy)-4-formylcoumarin, and 1,4-diphenyl-2-butene cocrystallized with hMAO-B,[25,30] and in the docking poses of safinamide and 5- and 6-styrylisatin analogues.[29,31]

To increase our understanding of the main binding interactions at the hMAO active site and explain the high selectivity of **4f** and **5f** toward MAO-B, we superimposed the structures of MAO-B docked with **5f** on the hMAO-A model. Figure 5 shows that the fit of **5f** into the active site of hMAO-A might be hindered by steric interference with the side chain of Phe208, which corresponds to Ile199 of hMAO-B.[32]

The role of the sulfur atom deserves some further comment. As indicated above, this atom seems to interact with the tyrosine residues forming the aromatic cage with the isoalloxazine ring. These tyrosine residues are conserved in the catalytic site of both isoforms, and it may be significant that the hMAO-A-selective 4-alkylthiophenylisopropylamines dock with their sulfur atom between the OH groups of Tyr407 and Tyr444. Although the latter compounds are highly selective for MAO-A, the propyl-, butyl- and higher alkylthio homologues are also weak inhibitors of both rat and human MAO-B.[10,12]

In agreement with our hypothesis that the basic character of the nitrogen is not a prerequisite for potent MAO-B inhibition, Table 1 shows that thiomorpholin-5-one derivatives **4e–f**, lacking a basic group, are potent inhibitors of MAO-B from both species.2

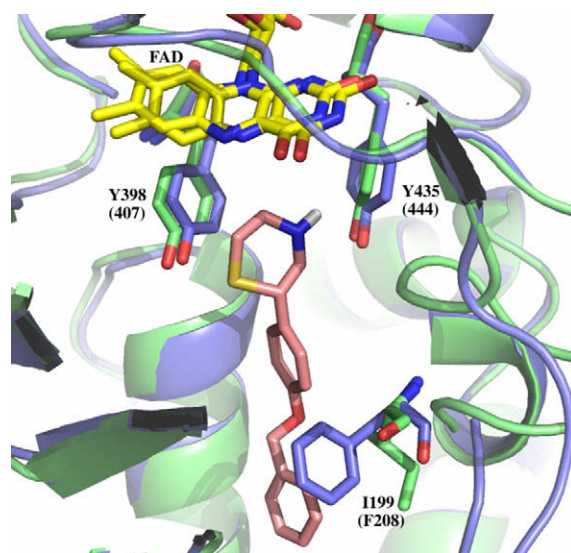**Figure 5.** Superimposition of hMAO-B (green) binding **5f** (rose) onto hMAO-A (blue). Main active site amino acid residues are rendered as stick models.
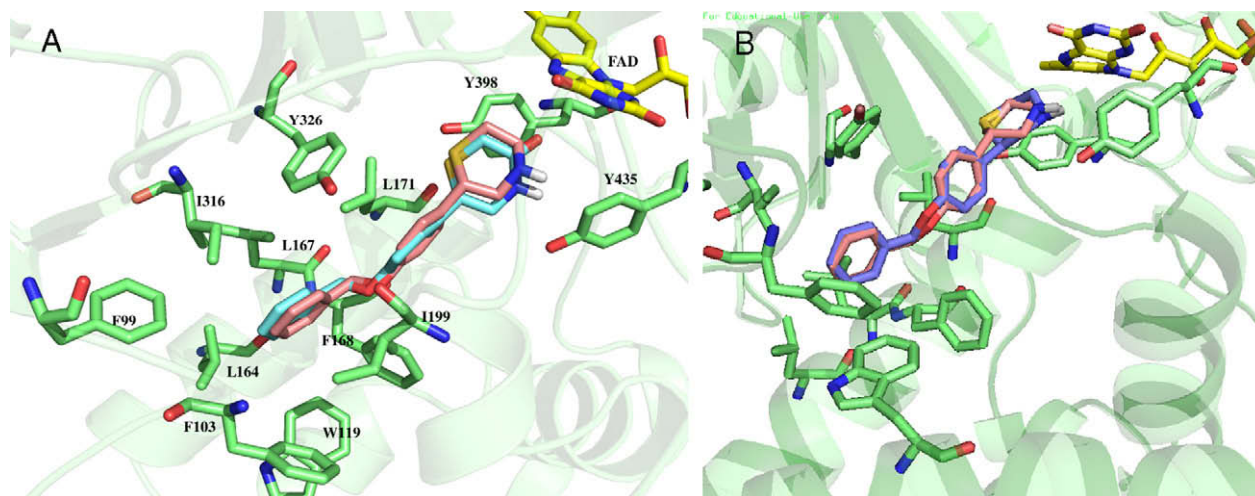

Figure 4. A.- Docking poses of **4f** (light blue) and **5f** (rose) into hMAO-B. Main active site aminoacid residues (green) and FAD (yellow) are rendered as stick models. B.- Superimposition of R- (rose) and S- (blue) **5f** docked into the hMAO-B catalytic site.

3. Conclusions

In this study, two series of rigid phenylethylamine analogues have been synthesized to test the hypotheses that fixing the amine chain in an extended six-membered ring conformation could lead to increased potency as MAO-B inhibitors, and that the basic nitrogen atom is not a prerequisite for this activity. Indeed, the thiomorpholine (**5a**) and thiomorpholinone (**4a**) analogues of phenylethylamine compare favorably with amphetamine (α-methylphenylethylamine) in this regard, retaining very similar activities toward rat MAO-A and showing an increase in MAOBI activity by more than an order of magnitude. Unexpectedly, we found that the sulfur atom in the heterocycle docked into the hMAO-B catalytic site interacts with Tyr398 and/or Tyr435. Furthermore, the docking poses of a series of 4-alkylthiophenylisopropylamines in hMAO-A[10] showed similar interactions with the corresponding Tyr407 and/or 444. This similar binding mode could explain the residual MAO-A inhibitory activity for **4a**–**e** and **5a**–**e**. The 4′-*n*-alkoxy (methoxy to butoxy) derivatives **4b**–**e** and **5b**–**e** exhibit a trend toward progressively higher potencies, and the benzyloxy analogues **4f** and **5f** were the most potent and selective for both rMAO-B and hMAO-B. In the case of the human enzyme, K_i values were in the 10^{-8} M range, and selectivities for MAO-B rather than MAO-A exceed 2000-fold. Docking experiments indicate that the alkoxy groups extend into the entrance cavity of the enzyme, and that the benzyloxy group occupies a similar position to the benzyloxy group of safinamide[25] and appropriately placed aromatic substituents of other MAOBI. The successful design of this series provides information about three specific interactions that could increase the potency and selectivity of MAO inhibitors: an aromatic ring that can participate in a probably hydrogen–π interaction with Tyr326; a heterocycle with a heteroatom that can interact with Tyr398 and/or Tyr435 (in our case, a sulfur atom); and a benzyl substituent at the opposite end of the molecule, establishing interactions with the primarily aromatic residues of the entrance cavity and thus determining selectivity for the B isoform. These structural features are found in previously described MAO-B inhibitors, namely a central phenyl ring flanked by a benzyl substituent and a heterocycle incorporating an atom that might interact with the tyrosine residues in the aromatic cage. The rationalization of these common aspects should contribute to the development of new selective and reversible MAO-B inhibitors with a potential for use in the therapy of neurodegenerative diseases. As mentioned above, all the compounds were evaluated as racemic mixtures. Further experiments are necessary to elucidate which enantiomer is the eutomer. Finally, our data confirm the importance of measuring drug inhibitory properties using the human isoforms of the enzyme, but at the same time support the use of rat MAO as a useful model, since in our case a whole series of potent derivatives might have been disregarded due to the lack of activity of the parent compounds towards the human enzymes, while they showed an interesting activity against both rat MAOs.

4. Experimental section

4.1. Chemistry

Melting points are uncorrected and were determined with a Reichert Galen III hot plate microscope. ^{1}H NMR spectra were recorded using Bruker AMX 400 and AMX 300 spectrometers at 400 or 300 MHz, respectively. Chemical shifts are reported relative to TMS (δ = 0.00) or HDO (δ = 4.79) and coupling constants (J) are given in hertz. The elemental analyses for C, H, N, O and S were performed on a CE Instruments (model EA 1108) analyzer. Reactions and product mixtures were routinely monitored by thin-layer chromatography (TLC) on silica gel-precoated F_{254} Merck plates.

All reagents and solvents were commercially available and were used without further purification.

4.1.1. General procedure for the preparation of 4-alkoxybenzaldehydes (1c–f)

To a stirred solution of 4-hydroxybenzaldehyde (7.00 g, 51 mmol) in CH_3CN (100 mL), K_2CO_3 (14.2 g, 103 mmol) and the appropriate alkyl halide (51 mmol) were added. The reaction mixture was refluxed and stirred for 24 h and then the solvent was removed by rotary evaporation. The product (a white solid) was extracted with EtOAc and 25% NaOH (2×100 mL) and then with H_2O (2×100 mL). The organic layer was dried over Na_2SO_4 and concentrated.

4.1.1.1. 4-Ethoxybenzaldehyde (1c).
Yield 92% (yellow oil), ^{1}H NMR ($CDCl_3$) δ 1.45 (t, J = 7.4 Hz, 3H, CH_3), 4.10 (m, 2H, OCH_2), 6.99 (d, J = 8.6 Hz, 2H, ArH), 7.80 (d, J = 8.6 Hz, 2H, ArH), 9.85 (s, 1H, CHO).

4.1.1.2. 4-Propoxybenzaldehyde (1d).
Yield 97% (orange oil), ^{1}H NMR ($CDCl_3$) δ 1.05 (t, J = 7.5 Hz, 3H, CH_3), 1.75–1.90 (m, 2H, CH_2), 4.00 (t, J = 6.6 Hz, 2H, OCH_2), 6.99 (d, J = 8.8 Hz, 2H, ArH), 7.80 (d, J = 8.8 Hz, 2H, ArH), 9.85 (s, 1H, CHO).

4.1.1.3. 4-Butoxybenzaldehyde (1e).
Yield 94% (orange oil), ^{1}H NMR ($CDCl_3$) δ 0.95 (t, J = 7.4 Hz, 3H, CH_3), 1.40–1.55 (m, 2H, CH_2), 1.70–1.85 (m, 2H, CH_2), 4.05 (t, J = 6.6 Hz, 2H, OCH_2), 7.00 (d, J = 8.6 Hz, 2H, ArH), 7.80 (d, J = 8.6 Hz, 2H, ArH), 9.85 (s, 1H, CHO).

4.1.1.4. 4-Benzyloxybenzaldehyde (1f).
Yield 94%, mp 68.5–69.3 °C (white crystals from MeOH), ^{1}H NMR ($CDCl_3$) δ 5.15 (s, 2H, CH_2), 7.10 (d, J = 8.6 Hz, 2H, ArH), 7.25–7.48 (m, 5H, ArH), 7.85 (d, J = 8.7 Hz, 2H, ArH), 9.90 (s, 1H, CHO).

4.1.2. General procedure for the preparation of arylnitroethenes (2a–f)

The appropriate aldehyde (82 mmol), nitromethane (50 g, 82 mmol) and cyclohexylamine (32.5 g, 328 mmol) were dissolved in acetic acid (100 mL). This reaction mixture was kept at 50 °C for 24 h, after which the solvent was evaporated. The products were recrystallized from MeOH.

4.1.2.1. 2-Phenyl-1-nitroethene (2a).
Yield 46%, mp 49.8–50.7 °C (yellow needles), ^{1}H NMR ($CDCl_3$) δ 7.35–7.57 (m, 5H, ArH), 7.60 (d, J = 13.9 Hz, 1H, CH), 8.00 (d, J = 13.7 Hz, 1H, CH).

4.1.2.2. 2-(4′-Methoxyphenyl)-1-nitroethene (2b).
Yield 83%, mp 85–87 °C (yellow needles), ^{1}H NMR ($CDCl_3$) δ 3.87 (s, 3H, OCH_3), 6.97 (d, J = 8.8 Hz, 2H, ArH), 7.50 (d, J = 8.8 Hz, 2H, ArH), 7.55 (d, J = 13.6 Hz, 1H, Ar*CH*), 7.98 (d, J = 13.7 Hz, 1H, *CH*NO$_2$).

4.1.2.3. 2-(4′-Ethoxyphenyl)-1-nitroethene (2c).
Yield 92%, mp 111–112.8 °C (yellow needles), ^{1}H NMR ($CDCl_3$) δ 1.45 (t, J = 7.4 Hz, 3H, CH_3), 4.08 (m, 2H, OCH_2), 6.93 (d, J = 8.6 Hz, 2H, ArH), 7.50 (d, J = 8.4 Hz, 2H, ArH), 7.52 (d, J = 12.6 Hz, 1H, Ar*CH*), 7.98 (d, J = 13.7 Hz, 1H, *CH*NO$_2$).

4.1.2.4. 2-(4′-Propoxyphenyl)-1-nitroethene (2d).
Yield 60%, mp 93.8–94.2 °C (yellow needles), ^{1}H NMR ($CDCl_3$) δ 1.05 (t, J = 7.3 Hz, 3H, CH_3), 1.75–1.90 (m, 2H, CH_2), 3.98 (t, J = 6,6 Hz, 2H, OCH_2), 6.92 (d, J = 8.7 Hz, 2H, ArH), 7,44 (d, J = 8.2 Hz, 2H, ArH), 7.51 (d, J = 13.3 Hz, 1H, Ar*CH*), 7.94 (d, J = 13.7 Hz, 1H, *CH*NO$_2$).

4.1.2.5. 2-(4′-Butoxyphenyl)-1-nitroethene (2e).
Yield 78%, mp 63.2–63.8 °C (yellow needles), ^{1}H NMR ($CDCl_3$) δ 0.96 (t,

$J = 7.3$ Hz, 3H, CH$_3$), 1.43–1.58 (m, 2H, CH$_2$), 1.73–1.82 (m, 2H, CH$_2$), 4.00 (t, $J = 6,6$ Hz, 2H, OCH$_2$), 6.92 (d, $J = 8.8$ Hz, 2H, ArH), 7.47 (d, $J = 8.5$ Hz, 2H, ArH), 7.52 (d, $J = 13.4$ Hz, 1H, ArCH), 7.95 (d, $J = 13.6$ Hz, 1H, CHNO$_2$).

4.1.2.6. 2-(4′-Benzyloxyphenyl)-1-nitroethene (2f).
Yield 65%, mp 117.4–118.2 °C (yellow needles), ^{1}H NMR (CDCl$_3$) δ 5.13 (s, 2H, OCH$_2$), 7.04 (d, $J = 8.6$ Hz, 2H, ArH), 7.35–7.45 (m, 5H, ArH), 7.50 (d, $J = 8.6$ Hz, 2H, ArH), 7.55 (d, $J = 13.7$ Hz, 1H, ArCH), 7.97 (d, $J = 13.7$ Hz, 1H, CHNO$_2$).

4.1.3. General procedure for the preparation of 1-methylthio-glycolyl-1-aryl-2-nitroethanes (3a–f)
To a stirred solution of the appropriate nitrostyrene (17 mmol) in THF (50 mL) were added methyl thioglycolate (2.02 g, 19 mmol) and Et$_3$N (2.02 g, 20 mmol). The mixture was stirred at room temperature for 2 h. The reaction was quenched with concentrated HCl (5–10 drops), and the volatiles were removed in vacuo. The residue was partitioned between 2 M HCl (2 mL) and CH$_2$Cl$_2$ (3 × 100 mL). The combined organic extracts were dried over Na$_2$SO$_4$, filtered and evaporated.

4.1.3.1. 1-Methylthioglycolyl-1-phenyl-2-nitroethane (3a).
Yield 97% (brown oil), ^{1}H NMR (CDCl$_3$) δ 3.05 (d, $J = 15.1$ Hz, 1H, SCH$_2$), 3.15 (d, $J = 15.1$ Hz, 1H, SCH$_2$), 3.70 (s, 3H, OCH$_3$), 4.76–4.84 (m, 3H, CHCH$_2$NO$_2$), 7.33–7.48 (m, 5H, ArH).

4.1.3.2. 1-Methylthioglycolyl-1-(4′-methoxyphenyl)-2-nitroethane (3b).
Yield 68% (green oil), ^{1}H NMR (CDCl$_3$) δ 3.05 (d, $J = 15.1$ Hz, 1H, SCH$_2$), 3.15 (d, $J = 15.1$ Hz, 1H, SCH$_2$), 3.70 (s, 3H, OCH$_3$), 3.80 (s, 3H, OCH$_3$), 4.75–4.85 (m, 3H, CHCH$_2$NO$_2$), 6.85 (d, $J = 8.6$ Hz, 2H, ArH), 7.25 (d, $J = 8.4$ Hz, 2H, ArH).

4.1.3.3. 1-Methylthioglycolyl-1-(4′-ethoxyphenyl)-2-nitroethane (3c).
Yield 81%, mp 47.8–48.5 °C (white crystals from absolute EtOH), ^{1}H NMR (CDCl$_3$) δ 1.39 (t, $J = 6.9$ Hz, 3H, CH$_3$), 3.05 (d, $J = 15.1$ Hz, 1H, SCH$_2$), 3.15 (d, $J = 15.1$ Hz, 1H, SCH$_2$), 3.70 (s, 3H, OCH$_3$), 4.02 (m, 2H, OCH$_2$), 4.75–4.87 (m, 3H, CHCH$_2$NO$_2$), 6.85 (d, $J = 8.6$ Hz, 2H, ArH), 7.25 (d, $J = 8.8$ Hz, 2H, ArH).

4.1.3.4. 1-Methylthioglycolyl-1-(4′-propoxyphenyl)-2-nitroethane (3d).
Yield 86%, mp 68.6–69.3 °C (white crystals from absolute EtOH), ^{1}H NMR (CDCl$_3$) δ 1.00 (t, $J = 7.4$ Hz, 3H, CH$_3$), 1.72–1.85 (m, 2H, CH$_2$), 3.05 (d, $J = 15.3$ Hz, 1H, CH$_2$), 3.15 (d, $J = 15.3$ Hz, 1H, CH$_2$), 3.70 (s, 3H, OCH$_3$), 3.90 (t, $J = 6.5$ Hz, 2H, OCH$_2$), 4.70–4.85 (m, 3H, CHCH$_2$NO$_2$), 6.85 (d, $J = 8.6$ Hz, 2H, ArH), 7.25 (d, $J = 8.8$ Hz, 2H, ArH).

4.1.3.5. 1-Methylthioglycolyl-1-(4′-butoxyphenyl)-2-nitroethane (3e).
Yield 91%, mp 48.5–49.2 °C (white crystals from absolute EtOH), ^{1}H NMR (CDCl$_3$) δ 0.95 (t, $J = 7.4$ Hz, 3H, CH$_3$), 1.40–1.50 (m, 2H, CH$_2$), 1.70–1.80 (m, 2H, CH$_2$), 3.05 (d, $J = 15.3$ Hz, 1H, CH$_2$), 3.15 (d, $J = 15.3$ Hz, 1H, CH$_2$), 3.68 (s, 3H, OCH$_3$), 3.90 (t, $J = 7.4$ Hz, 2H, OCH$_2$), 4.73–4.85 (m, 3H, CHCH$_2$NO$_2$), 6.84 (d, $J = 8.8$ Hz, 2H, ArH), 7.23 (d, $J = 8.8$ Hz, 2H, ArH).

4.1.3.6. 1-Methylthioglycolyl-1-(4′-benzyloxyphenyl)-2-nitroethane (3f).
Yield 93%, mp 90.0–96.8 °C (golden crystals from absolute EtOH), ^{1}H NMR (CDCl$_3$) δ 3.05 (d, $J = 15.3$ Hz, 1H, CH$_2$), 3.15 (d, $J = 15.3$ Hz, 1H, CH$_2$), 3.65 (s, 3H, OCH$_3$), 4.70–4.85 (m, 3H, CHCH$_2$NO$_2$), 5.03 (s, 2H, OCH$_2$), 6.85 (d, $J = 8.6$ Hz, 2H, ArH), 7.25 (d, $J = 8.8$ Hz, 2H, ArH), 7.28–7.45 (m, 5H, ArH).

4.1.4. General procedure for the preparation of 2-arylthiomorpholin-5-ones (4a–f)
Zinc powder (24.4 g, 400 mmol) was added in portions to a stirred solution of the nitro ester (20 mmol) in glacial acetic acid (500 mL) at room temperature. Stirring was continued at 70 °C for 72 h. After cooling, the mixture was filtered through Celite. The solid was washed with CH$_2$Cl$_2$ (5 × 50 mL) and the filtrate was evaporated. The residue was dissolved in 100 mL of CH$_2$Cl$_2$ and washed with H$_2$O made alkaline with NH$_3$ (2 × 100 mL). The organic phase was dried, filtered and evaporated. The products were recrystallized from absolute EtOH.

4.1.4.1. 2-Phenylthiomorpholin-5-one (4a).
Yield 59%, mp 144.3–145 °C (white needles), ^{1}H NMR (CDCl$_3$) δ 3.45 (d, $J = 16.2$ Hz, 1H, CH$_2$), 3.55 (d, $J = 16.2$ Hz, 1H, CH$_2$), 3.78–3.85 (m, 2H, CH$_2$), 4.35 (dd, $J_1 = 4.6$ Hz, $J_2 = 8.6$ Hz, 1H, SCH), 6.15–6.25 (br s, 1H, NHCO), 7.33–7.50 (m, 5H, ArH). Anal. Calcd for C$_{10}$H$_{11}$NOS·0.5H$_2$O: C, 59.38; H, 5.98; N, 6.92; S, 15.85. Found: C, 58.98; H, 5.96; N, 6.86; S, 16.88.

4.1.4.2. 2-(4′-Methoxyphenyl)thiomorpholin-5-one (4b).
Yield 48%, mp 158.3–159.1 °C (white needles), ^{1}H NMR (CDCl$_3$) δ 3.43 (d, $J = 16.2$ Hz, 1H, CH$_2$), 3.55 (d, $J = 16.2$ Hz, 1H, CH$_2$), 3.70–3.80 (m, 2H, CH$_2$), 3.85 (s, 3H, CH$_3$), 4.,25–4.33 (dd, $J_1 = 4.6$ Hz, $J_2 = 9.1$ Hz, 1H, SCH), 6.25–6.35 (br s, 1H, NHCO), 6.91 (d, $J = 8.6$ Hz, 2H, ArH), 7.36 (d, $J = 8.6$ Hz, 2H, ArH). Anal. Calcd for C$_{11}$H$_{13}$NO$_2$S: C, 59.17; H, 5.87; N, 6.27; S, 14.36. Found: C, 58.91; H, 6.24; N, 6.30; S, 15.53.

4.1.4.3. 2-(4′-Ethoxyphenyl)thiomorpholin-5-one (4c).
Yield 62%, mp 161.5–162.5 °C (white needles), ^{1}H NMR (CDCl$_3$) δ 1.45 (t, $J = 6.9$ Hz, 3H, CH$_3$), 3.45 (d, $J = 15.9$ Hz, 1H, CH$_2$), 3.50 (d, $J = 15.9$ Hz, 1H, CH$_2$), 3.60–3.70 (m, 2H, CH$_2$), 4.00–4.10 (m, 2H, OCH$_2$), 4.25–4.33 (dd, $J_1 = 4.5$ Hz, $J_2 = 8.8$ Hz, 1H, SCH), 6.40–6.50 (br s, 1H, NHCO), 6.90 (d, $J = 8.6$ Hz, 2H, ArH), 7.35 (d, $J = 8.6$ Hz, 2H, ArH). Anal. Calcd for C$_{12}$H$_{15}$NO$_2$S: C, 60.73; H, 6.37; N, 5.90; S, 13.51. Found: C, 60.66; H, 7.13; N, 5.88; S, 14.85.

4.1.4.4. 2-(4′-Propoxyphenyl)thiomorpholin-5-one (4d).
Yield 50%, mp 163.7–164.3 °C (white needles), ^{1}H NMR (CDCl$_3$) δ 1.05 (t, $J = 7.6$ Hz, 3H, CH$_3$), 1.75–1.90 (m, 2H, CH$_2$), 3.35 (d, $J = 16.2$ Hz, 1H, CH$_2$), 3.53 (d, $J = 16.2$ Hz, 1H, CH$_2$), 3.68–3.78 (m, 2H, CH$_2$), 3.95 (t, $J = 6.6$ Hz, 2H, OCH$_2$), 4.25–4.30 (dd, $J_1 = 4.5$ Hz, $J_2 = 8.8$ Hz, 1H, SCH), 6.20–6.30 (br s, 1H, NHCO), 6.90 (d, $J = 8.8$ Hz, 2H, ArH), 7.35 (d, $J = 8.5$ Hz, 2H, ArH). Anal. Calcd for C$_{13}$H$_{17}$NO$_2$S: C, 62.12; H, 6.82; N, 5.57; S, 12.76. Found: C, 62.05; H, 7.29; N, 5.87; S, 15.12.

4.1.4.5. 2-(4′-Butoxyphenyl)thiomorpholin-5-one (4e).
Yield 30%, mp 166.1–167.6 °C (white needles), ^{1}H NMR (CDCl$_3$) δ 0.95 (t, $J = 7.3$ Hz, 3H, CH$_3$), 1.40–1.53 (m, 2H, CH$_2$), 1.68–1.80 (m, 2H, CH$_2$), 3.35 (d, $J = 16.2$ Hz, 1H, CH$_2$), 3.45 (d, $J = 16.2$ Hz, 1H, CH$_2$), 3.60–3.75 (m, 2H, CH$_2$), 3.90 (t, $J = 6.3$ Hz, 2H, OCH$_2$), 4.20–4.25 (dd, $J_1 = 4.6$ Hz, $J_2 = 8.8$ Hz, 1H, SCH), 6.40–6.50 (br s, 1H, NHCO), 6.85 (d, $J = 8.8$ Hz, 2H, ArH), 7.25 (d, $J = 8.6$ Hz, 2H, ArH). Anal. Calcd for C$_{14}$H$_{19}$NO$_2$S: C, 60.36; H, 7.22; N, 5.28; S, 12.08. Found: C, 60.52; H, 8.49; N, 5.33; S, 14.22.

4.1.4.6. 2-(4′-Benzyloxyphenyl)thiomorpholin-5-one (4f).
Yield 62%, mp 193.9–194.6 °C (white needles), ^{1}H NMR (CDCl$_3$) δ 3.35 (d, $J = 16.2$ Hz, 1H, CH$_2$), 3.45 (d, $J = 16.2$ Hz, 1H, CH$_2$), 3.62–3.70 (m, 2H, CH$_2$), 4.25–4.30 (dd, $J_1 = 4.8$ Hz, $J_2 = 8.9$, Hz 1H, SCH), 5.02 (s, 2H, CH$_2$), 6.20–6.30 (br s, 1H, NHCO), 6.92 (d, $J = 8.6$ Hz, 2H, ArH), 7.30 (d, $J = 8.8$ Hz, 2H, ArH), 7.30–7.43 (m, 5H, ArH). Anal. Calcd for C$_{17}$H$_{17}$NO$_2$S: C, 68.20; H, 5.72; N, 4.68; S, 10.71. Found: C, 67.92; H, 6.10; N, 4.73; S, 12.84.

4.1.5. 2-Arylthiomorpholine oxalates (5a–f)
To a refluxing solution of the appropriate lactam (3 mmol) in 10 mL of THF was added 12 mL of 1 M DIBAL-H solution in n-hexane (12 mmol), and the mixture was refluxed for 2 h with stirring.

Then 50 mL of H_2O was added to the reaction mixture and extracted with CH_2Cl_2 (2 × 30 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated to give a yellow oil. The oil was dissolved in EtOH and treated with oxalic acid in Et_2O. The precipitated oxalate salt was collected by filtration and recrystallized from EtOH.

4.1.5.1. 2-Phenylthiomorpholine oxalate (5a). Yield 40%, mp 226.9–227.5 °C (pale yellow crystals), ^{1}H NMR (DMSO-d_6) δ 2.90 (m, 1H, CH_2), 3.05 (m, 1H, CH_2), 3.15 (m, 1H, CH_2), 3.35 (m, 1H, CH_2), 3.60 (m, 2H, CH_2), 4.30 (m, 1H, SCH), 7.25–7.48 (m, 5H, ArH). Anal. Calcd for $C_{20}H_{26}NS \cdot C_2H_2O_4 \cdot H_2O$: C, 56.63; H, 6.48; N, 6.00; S, 13.74. Found: C, 56.67; H, 6.56; N, 5.82; S, 15.83.

4.1.5.2. 2-(4′-Methoxyphenyl)thiomorpholine oxalate (5b). Yield 14%, mp 195.6–196.1 °C (pale yellow crystals), ^{1}H NMR (DMSO-d_6) δ 2.75 (m, 1H, CH_2), 2.95 (m, 1H, CH_2), 3.10 (m, 1H, CH_2), 3.25 (m, 1H, CH_2), 3.48 (m, 2H, CH_2), 3.68 (s, 3H, OCH_3), 4.24 (m, 1H, SCH), 6.86 (d, J = 8.6 Hz, 2H, ArH), 7.22 (d, J = 8.6 Hz, 2H, ArH). Anal. Calcd for $C_{11}H_{15}NOS \cdot C_2H_2O_4 \cdot 0.25H_2O$: C, 51.39; H, 5.81; N, 4.61; S, 10.55. Found: C, 51.35; H, 6.02; N, 4.77; S, 12.56.

4.1.5.3. 2-(4′-Ethoxyphenyl)thiomorpholine oxalate (5c). Yield 29%, mp 204.3–204.9 °C (pale yellow crystals), ^{1}H NMR (DMSO-d_6) δ 1.30 (m, 3H, CH_3), 2.65 (m, 1H, CH_2), 2.90 (m, 1H, CH_2), 3.05 (m, 1H, CH_2), 3.15 (m, 1H, CH_2), 3.40 (m, 2H, CH_2), 3.90 (m, 2H, OCH_2), 4.00 (m, 1H, SCH), 6.90 (d, J = 8.7 Hz, 2H, ArH), 7.25 (d, J = 8.6 Hz, 2H, ArH). Anal. Calcd for $C_{24}H_{34}NOS \cdot C_2H_2O_4$: C, 58.18; H, 6.76; N, 5.22; S, 11.95. Found: C, 57.75; H, 6.91; N, 5.19; S, 13.59.

4.1.5.4. 2-(4′-Propoxyphenyl)thiomorpholine oxalate (5d). Yield 39%, mp 197.3–197.8 °C (pale yellow crystals), ^{1}H NMR (DMSO-d_6) δ 0.93 (m, 3H, CH_3), 1.60–1.75 (m, 2H, CH_2), 2.80 (m, 1H, CH_2), 3.05 (m, 1H, CH_2), 3.15 (m, 1H, CH_2), 3.25 (m, 1H, CH_2), 3.55 (m, 2H, CH_2), 3.90 (m, 2H, OCH_2), 4.28 (m, 1H, SCH), 6.90 (d, J = 8.7 Hz, 2H, ArH), 7.15 (d, J = 8.7 Hz, 2H, ArH). Anal. Calcd for $C_{13}H_{19}NOS \cdot C_2H_2O_4 \cdot 0.5H_2O$: C, 53.55; H, 6.59; N, 4.16; S, 9.53. Found: C, 53.40; H, 6.85; N, 4.18; S, 11.84.

4.1.5.5. 2-(4′-Butoxyphenyl)thiomorpholine oxalate (5e). Yield 33%, mp 202.1–202.9 °C (pale yellow crystals), ^{1}H NMR (DMSO-d_6) δ 0.96 (m, 3H, CH_3), 1.30–1.50 (m, 2H, CH_2), 1.60–1.75 (m, 2H, CH_2), 2.85 (m, 1H, CH_2), 3.05 (m, 1H, CH_2), 3.15 (m, 1H, CH_2), 3.30 (m, 1H, CH_2), 3.55 (m, 2H, CH_2), 3.95 (m, 2H, OCH_2), 4.30 (m, 1H, SCH), 6.90 (d, J = 8.6 Hz, 2H, ArH), 7.25 (d, J = 8.7 Hz, 2H, ArH). Anal. Calcd for $C_{14}H_{21}NOS \cdot C_2H_2O_4 \cdot H_2O$: C, 53.46; H, 7.01; N, 3.90; S, 8.92. Found: C, 53.13; H, 7.05; N, 3.83; S, 10.57.

4.1.5.6. 2-(4′-Benzyloxyphenyl)thiomorpholine oxalate (5f). Yield 14%, mp 201.3–201.6 °C (pale yellow crystals), ^{1}H NMR (DMSO-d_6) δ 2.83 (m, 1H, CH_2), 3.05 (m, 1H, CH_2), 3.15 (m, 1H, CH_2), 3.30 (m, 1H, CH_2), 3.55 (m, 2H, CH_2), 4.25 (m, 1H, SCH), 5.10 (s, 2H, OCH_2), 6.95 (d, J = 8.4 Hz, 2H, ArH), 7.30 (d, J = 8.5 Hz, 2H, ArH), 7.37–7.48 (m, 5H, ArH). Anal. Calcd for $C_{17}H_{19}NOS \cdot C_2H_2O_4 \cdot 0.5H_2O$: C, 59.36; H, 5.77; N, 3.64; S, 8.34. Found: C, 59.72; H, 5.91; N, 3.70; S, 10.88.

4.2. Biochemistry

4.2.1. Rat MAO
The effects of the compounds (the lactams dissolved with the aid of DMSO and the amines, as salts, in buffer) on MAO-A or MAO-B activities were studied following previously reported methodologies, using a crude rat brain mitochondrial suspension as a source of enzyme.[7,8] Serotonin (100 μM) and 4-dimethylaminophenylethylamine (5 μM) were used as selective substrates for MAO-A and -B, respectively, and these compounds and their metabolites were detected by HPLC with electrochemical detection as described previously.[7,8] IC_{50} values (mean ± SD from at least two independent experiments, each in triplicate) were determined using Graph Pad Prism software, from plots of inhibition percentages (calculated in relation to a sample of the enzyme treated under the same conditions without inhibitors) versus −log inhibitor concentration. K_i were determined from the IC_{50} values using the Cheng–Prusoff equation: $K_i = IC_{50}/(1 + [S]/K_m)$,[33] K_m (5-HT, MAO-A) = 100 μM, K_m (DMAPEA, MAO-B) = 5 μM.

4.2.2. Human MAO
4.2.2.1. Mao-A (purified human recombinant).[34] Initial rates of oxidation were measured spectrophotometrically at 30 °C in 50 mM potassium phosphate, pH 7.4, containing 0.05% Triton X-100. K_i values were determined using a substrate range of 0.1–0.9 mM kynuramine (purchased from Sigma)[35] at six different inhibitor concentrations over a 10-fold range. The formation of the product was followed spectrophotometrically at 314 nm (ε = 12,300 M^{-1} cm^{-1}), and the kinetic constants determined using Shimadzu software.

4.2.2.2. Mao-b (purified human recombinant).[36] The activities were determined spectrophotometrically, using benzylamine (purchased from Sigma) as substrate, in 50 mM Hepes buffer, pH 7.5, containing 0.5% (w/v) reduced Triton X-100, at 25 °C. The oxidation of benzylamine by MAO-B was monitored at 250 nm (maximum absorption by benzaldehyde, extinction coefficient = 12,800 M^{-1} cm^{-1}).[37] All spectral data were obtained using a Unicam Helios-*beta* spectrophotometer. Kinetic data were evaluated and plotted using Prism Graph Pad software.

4.3. Molecular simulation

The crystal structures of human MAO-B (PDB: 1S3E) and MAO-A (PDB: 2BXS) were used for all simulations with the Autodock 4.0 suite.[38] All other docking conditions were as previously published.[10,12] Grid maps were calculated using the autogrid4 option and were centered on the putative ligand-binding site. The volumes chosen for the grid maps were made up of 60 × 60 × 60 points, with a grid-point spacing of 0.375 Å. Partial charges of the different compounds were corrected using ESP methodology.[39] The docked compound complexes were built using the lowest docked-energy binding positions. Both enantiomers of the compounds were investigated and the energies and orientations found are very similar with a good correlation with biological results. Therefore in all cases the results obtained for the (S)-isomers were used for the analysis.

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