Human and rat monoamine oxidase-A are differentially inhibited by (S)-4-alkylthioamphetamine derivatives: Insights from molecular modeling studies

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Abstract—Four enantiomerically pure (S)-4-alkylthioamphetamine derivatives were evaluated as monoamine oxidase (MAO) inhibitors using the human and rat isoforms of the enzyme. Molecular dockings were performed in order to gain insights regarding the binding mode of these inhibitors. All compounds were potent and selective MAO-A inhibitors although different rank orders of potencies were observed against the enzymes from different species. This behavior can be rationalized on the basis of different binding modes to each enzyme, as determined in silico. These findings further support the concept that MAO inhibitory activity of novel compounds, determined with enzymes from diverse mammalian species, should be considered with caution if human MAO is the final target to be addressed.

Keywords: Monoamine oxidase; MAO inhibitors; Amphetamine derivatives; Molecular dynamics simulation.

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1. Introduction

Compounds that inhibit monoamine oxidase (MAO, E.C. 1.4.3.4) have shown therapeutic value in a variety of conditions including affective disorders and neurodegenerative diseases. In mammals, two kinds of MAOs (MAO-A and MAO-B) are present in most tissues. Crystal structures of both isoforms of human, and of rat MAO-A (rMAO-A), complexed with pharmacologically relevant inhibitors, have been recently described. The similarities and differences between human MAO-A (hMAO-A) and that from the rat have provided new insights that may be important in drug development. Although hMAO-A and rMAO-A exhibit ~90% sequence identity, both enzymes show important differences in their quaternary structures, as revealed by X-ray diffraction data. Thus, hMAO-A crystallizes and behaves hydrodynamically in solution as a monomer, whereas structural data suggest that rMAO-A forms a dimeric structure in vivo. In addition, the volume of the active site cavity of hMAO-A (~450 Å³) is smaller than that found in hMAO-A (~550 Å³), which is due to a different conformation of the cavity-shaping loop (residues 210–216) present in both enzymes. Even though it has been consistently pointed out, the aforementioned data lend further support to the notion that results obtained with non-human forms of MAO (e.g., the evaluation of the inhibitory properties of a given compound) cannot be unambiguously extrapolated to the situation in humans.

Several phenylisopropylamine derivatives (often referred to in the literature as ‘substituted amphetamines’) have been shown by us and others to be potent MAO inhibitors. Structure-activity relationship (SAR) studies, including quantitative analyses (QSAR), have shown that the presence of electron-donating, unbranched, alkylthio substituents at the para position of the aromatic ring of the amphetamine scaffold generates potent and selective MAO-A inhibitors. In addition, a
few studies have shown that the (S)-isomers of substituted amphetamines (which are always dextrorotatory) are the eutomers. However, little is known about the binding mode of this class of compounds, in spite of their close structural similarity to endogenous monoaminergic neurotransmitters which are the main physiological substrates of MAOs. In this sense, molecular docking is a useful approach since protein–ligand configurations produced by the docking programs allow a visual analysis of protein–ligand interactions and facilitate an intuitive interpretation and understanding of the binding process at the protein-binding site.16 Furthermore, recent studies have shown that molecular modeling yields good correlations between the calculated and experimental $K_i$ values for a series of known and new MAO inhibitors.17,18

In the present study, four enantiomerically pure (S)-4-alkylthiophenylisopropylamine derivatives were prepared and evaluated as MAO inhibitors using the human and rat enzyme isoforms. In addition, using the crystal structures of the proteins, molecular docking was performed in order to gain insights regarding the binding modes of these inhibitors and also to explain observed differences in their inhibitory properties with MAO from different species.

2. Results and discussion

2.1. Chemistry

The synthesis of the (S)-4-alkylthiophenylisopropylamine derivatives 1–4 (Table 1) was carried out by a reaction sequence involving initial Friedel–Crafts acylation of the corresponding alkyl-phenyl thioethers with (S)-N-trifluoroacetylalanyl chloride, followed by reduction of the (S)-2-trifluoroacetamido-1-arylpropan-1-ones with triethylsilane, and deprotection of the amino group by acid hydrolysis, according to a previously reported procedure.19,20 Hydrochloride salts were obtained by adding gaseous HCl (Scheme 1).

This synthetic route proceeded with complete retention of chirality, supporting the versatility of using chiral alanine derivatives,20 previously exploited in the synthesis of amphetamine21 and cathinone analogs.22

Enantiomeric excesses were determined to be >98% in all cases, as evaluated by the comparison of the $^1$H NMR signals of the enantiomer with those of the corresponding racemic mixture, both complexed with europium tris-[3-(heptafluoropropylhydroxymethylene)-(−)-camphorate] (Eu(hfc)$_3$) used as chiral shift reagent.

2.2. Biochemistry

Table 2 summarizes the effects of compounds 1–4 upon MAO-A and -B from human and rat. As previously shown for 1,15 and racemic 2,10 all (S)-4-alkylthioamphetamines selectively inhibited MAO-A from both species with $K_i$ values in the nanomolar range. This inhibition was competitive in all cases, as shown for (S)-4-ethylthioamphetamine 2 in Figure 1. Inhibition of both hMAO-B and rMAO-B was observed only in the case of the derivatives bearing a propylthio (3) or butylthio (4) substituent, but the potency was at least 20-fold lower than that observed for the corresponding MAO-A.

Regarding the MAO-A inhibitory potency, it was similar and followed the same trend for both species in the case of 1 and 2, that is, lengthening of the substituent from a methylthio to an ethylthio group produced a decrease of the $K_i$, which agrees with results obtained with racemic mixtures10 and other alkylthio-substituted

Table 1. Yields and physical properties of (S)-2-amino-1-arylpropane derivatives 1–4

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>Mp (°C)</th>
<th>Yield (%)</th>
<th>$[\alpha]_{D}^{20\text{a}}$</th>
<th>$m\ell$ (free base)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CH$_3$</td>
<td>224–225</td>
<td>47</td>
<td>+7.9</td>
<td>181.09</td>
</tr>
<tr>
<td>2</td>
<td>CH$_3$CH$_2$</td>
<td>211–213</td>
<td>58</td>
<td>+6.4</td>
<td>195.11</td>
</tr>
<tr>
<td>3</td>
<td>CH$_3$CH$_2$CH$_2$</td>
<td>135–137</td>
<td>50</td>
<td>+5.9</td>
<td>209.12</td>
</tr>
<tr>
<td>4</td>
<td>CH$_3$CH$_2$CH$_2$CH$_2$</td>
<td>141–143</td>
<td>38</td>
<td>+6.8</td>
<td>223.13</td>
</tr>
</tbody>
</table>

$^{\text{a}}$ (c 1, MeOH).

\[
\text{Scheme 1. Reagents and conditions: (i) 1,1,3,3-tetramethylguanidine/CF$_3$CO$_2$Et/MEOH; (ii) (CO)$_2$Cl$_2$/Py/CH$_2$Cl$_2$; (iii) AlCl$_3$; (iv) (Et$_3$)$_3$SiH/CF$_3$COOH, reflux; (v) 12 M HCl/2-propanol, 70 °C.}
\]
amphetamine derivatives,\textsuperscript{11} using MAO from rat brain. However, a clear difference between hMAO-A and rMAO-A was observed when the inhibitory properties of compounds 3 and 4 were compared. This different behavior is demonstrated in the plots in Figure 2 where log $K_i$ is plotted versus the van der Waals volume of the alkyl substituent. A linear correlation is observed with hMAO-A, while a strong deviation from linearity is observed for the binding properties of compounds 3 and 4 with rMAO-A.

Thus, while potency against hMAO-A increased for the compounds with longer alkyl chains attached to the sulfur atom at the $para$ position (with the four-carbon derivative, i.e., 4, being the most potent), propyl- and butylthio derivatives 3 and 4 were less potent rMAO-A inhibitors than the ethylthio analog 2. These results are in the same line as a recent study by Novaroli et al.,\textsuperscript{24} which showed that some coumarin and 5$H$-indeno[1,2-$c$]pyridazin-5-one derivatives exhibited striking divergences in their inhibitory potency when evaluated against human or rat MAO-B. As stressed by these authors, species-related differences on inhibitor activity such as that of derivative 4 (more than one order of magnitude between rMAO-A and hMAO-A) not only support the idea that results might greatly vary depending on the enzyme source used, but also have important implications for the selection of hit compounds for lead generation, which is an interesting finding considering that both enzymes are $\approx 90\%$ identical in sequence. Accordingly, the different rank orders of potency shown by the compounds tested here strongly suggest that, even though both hMAO-A and rMAO-A are able to accommodate alkylthio-substituted amphetamines in their active sites, the interactions that account for the binding properties in each enzyme, at the least for compounds 3 and 4, are probably different.

### 2.3. Molecular docking

In order to test the aforementioned hypothesis, compounds 1\textendash}4 were docked into the active sites of rMAO-A and hMAO-A, using the Autodock 3.05 suite,\textsuperscript{25} and the available crystal structures of both enzymes (PDB: 1O5W or 2BXS, respectively). Results are summarized in Figures 3 (rat) and 4 (human), which show the most stable conformations for each drug–enzyme complex.

In rMAO-A, compounds 1 and 2 exhibit binding modes where the amino group points away from the flavin ring forming a hydrogen bond with the carbonyl group of Phe208, whereas the alkylthio chain is located between Tyr407 and Tyr444, a position that would favor interactions between the highly polarizable sulfur atom and either or both aromatic moieties or the hydroxyl groups of the tyrosines (Figs. 3A and B).

In these conformations, the aromatic rings of 1 and 2 seem to be almost identically positioned between the

### Table 2. MAO inhibitory activity of phenylisopropylamine derivatives 1\textendash}4

<table>
<thead>
<tr>
<th>Compound</th>
<th>Rat MAO-A $K_i$ (µM)</th>
<th>Rat MAO-B</th>
<th>Human MAO-A $K_i$ (µM)</th>
<th>Human MAO-B</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.245 ± 0.030</td>
<td>&gt;100</td>
<td>0.133 ± 0.020</td>
<td>NE</td>
</tr>
<tr>
<td>2</td>
<td>0.054 ± 0.009</td>
<td>&gt;100</td>
<td>0.075 ± 0.015</td>
<td>&gt;100</td>
</tr>
<tr>
<td>3</td>
<td>0.115 ± 0.016</td>
<td>15.00 ± 1.430</td>
<td>0.030 ± 0.001</td>
<td>14.40 ± 1.082</td>
</tr>
<tr>
<td>4</td>
<td>0.392 ± 0.033</td>
<td>8.925 ± 1.575</td>
<td>0.022 ± 0.001</td>
<td>4.58 ± 0.850</td>
</tr>
</tbody>
</table>

*NE,* no effect at the highest concentration tested (100 µM).

$K_i$ values for MAOs from rat brain were determined from the IC$_{50}$ values using the Cheng–Prusoff equation (see Section 4).
side chains of Gln215 and Ile180, and perpendicular to the flavin ring. A similar binding mode has been recently described for the naturally occurring eugenol (4-allyl-2-methoxyphenol), which has clear structural similarities with phenylisopropylamines, although in that case the aromatic ring of the ligand was located somewhat closer to the isoalloxazine ring. As previously discussed for similar results obtained with heteroarylisopropylamine derivatives, this binding mode provides a rationale for the observed inhibitory activity, since while blocking the access of any substrate to the active site, these phenylisopropylamines could avoid deamination by adopting a conformation where the amino group is remote from the influence of the flavin ring. In contrast, docking of compounds 3 and 4 yielded binding modes where the inhibitor molecules adopted an almost opposite orientation to derivatives 1 and 2. Thus, the most energetically favorable conformations of 3 and 4 were those where the aminopropyl chain was positioned between Tyr407 and Tyr444, and the alkylthio chain appeared folded into a space surrounded by Ile335, Phe208, Val210 and Thr336, instead of being extended into the hydrophobic channel pointing to the binding site entrance (Figs. 3C and D). The aromatic rings of 3 and 4 were almost in the same position and orientation as those of derivatives 1 and 2. Although different from the foregoing, this binding mode is also consistent with the rMAO-A inhibitory activity of compounds 3 and 4. In spite of an intense debate about the mechanism underlying MAO-catalyzed amineoxidation, the two most accepted schemes, that is, the single electron transfer and the polar nucleophilic mechanisms, involve the abstraction of the pro-R α-proton of the amine by the N5 atom of the flavin ring, which would act as a base. As can be seen in Figures 3C and D, a possible interaction between the sole α-proton of phenylisopropylamine derivatives 3 and 4 and the FAD N5 is made.

Figure 3. Binding modes of phenylisopropylamine derivatives 1–4 to rMAO-A (PDB: 1O5W).
less likely by the presence of the α-methyl group, which prefers to face the re side of the isoalloxazine ring, leaving the pro-R α-proton far from the isoalloxazine N5. Beyond these considerations, the striking differences in the binding modes of derivatives 1-2 and 3-4 to rMAO-A generated by computational simulations can explain the trend of their inhibitory potencies. Thus, no hydrogen bonds between the inhibitor and active site residues were apparent for the best binding conformations of 3 and 4, which agree with a less stable interaction and higher $K_i$ values.

In the case of hMAO-A, the binding modes obtained for compounds 1-4 from docking simulations were similar in all cases and resemble those observed in rMAO-A for 1 and 2, that is, the amino group was oriented away from the flavin ring and the alkylthio chain was positioned in the so-called aromatic cage, between Tyr407 and Tyr444 (Fig. 4).

The aromatic rings of the inhibitors occupy a position close to that found in rMAO-A, but they are located in a clearly less perpendicular orientation to the flavin as compared with that observed in the rat enzyme. This positioning might favor stacking interactions with the aromatic rings of Phe208 and Tyr69 (both about 5 Å from the inhibitors’ aromatic moieties), as shown in Figure 5.

In addition, stabilizing hydrogen bonds between the amino group and the side chain of Ser209, the backbone carbonyl of Val210, or both, were detected for all compounds. The similarities in the binding modes of 1-4 to hMAO-A suggest that the different potencies of these compounds determined in biochemical assays could be correlated with differential interactions at the active site, mainly associated with the different properties of the alkylthio chain attached to the para position. It has been shown that the affinities of 4-substituted benzylamine

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**Figure 4.** Binding modes of phenylisopropylamine derivatives 1-4 to hMAO-A (PDB: 2BX5).
and phenethylamine analogs for hMAO-A are more favorable when the size of the para substituent increases, and QSAR analyses have shown that this effect depends exclusively on steric parameters of the para substituent (van der Waals volume and/or Taft steric parameter $E_s$). The results presented here completely agree with this notion, and an excellent correlation ($r^2 = 0.97$) was found between log $K_i$ and the van der Waals volume of the para substituent of compounds 1–4. In the case of rMAO-A, this SAR does not hold for the compounds tested here, since (as shown by docking experiments) the binding mode is not the same for the whole series. Other examples of arylalkylamine derivatives, in which differential binding modes to MAO from different species have been advocated to explain different inhibitory potencies, include phentermine and benzylamine analogs.

3. Conclusions

The results presented here show that some 4-alkylthiophenethylisopropylamine derivatives are able to differentially inhibit MAO-A from rat and human, a behavior that can be rationalized on the basis of differing binding modes to each enzyme exhibited by the compounds in docking studies. These results might be related to the differences in the volume and shape of the active sites of rMAO-A and hMAO-A demonstrated by structural studies, but further experiments are necessary to fully elucidate how these differences influence the binding of this class of compounds to MAO-A. In addition, we have described two binding modes, consistent with inhibitory properties, for the interaction of substituted amphetamine derivatives with MAO-A. It should therefore be kept in mind that drugs with similar activities may not necessarily bind to their receptor(s) in the same orientation, and that differences of this sort must be considered when analyzing SAR.

Finally, our results further support the concept that MAO inhibitory activity of novel compounds, determined with enzymes from diverse mammalian species, should be considered with caution if human MAO is the final target to be addressed.

4. Experimental

4.1. Chemistry

All reagents and solvents were commercially available and were used without further purification. Melting points are uncorrected and were determined with an Electrothermal apparatus. NMR spectra were recorded using either a Bruker AMX 300 or a Bruker Advance 400 spectrometer at 300 or 400 (1H) and 75 (13C) MHz, employing tetramethylsilane as an internal standard. Chemical shifts are reported relative to TMS (δ = 0.00) and coupling constants ($J$) are given in Hz. Europium tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorate] was purchased from Aldrich. Optical rotation values were obtained with a Perkin-Elmer 341 polarimeter. The elemental analyses for C, H, N, and S were performed on a CE Instruments (model EA 1108) analyzer.

4.1.1. Preparation of (S)-N-trifluoroacetylaniline. (S)-N-Trifluoroacetylaniline was prepared following a procedure described previously from L-alanine (Aldrich), ethyl trifluoroacetate, and 1,1,3,3-tetramethylguanidine. The product (86% yield) had a melting point of 62–64 °C (lit. 70–71 °C) and was sufficiently pure for all subsequent uses.

4.1.2. General procedure for the preparation of (S)-trifluoroacetamido-1-aryl-1-propanones. (S)-Trifluoroacetamido-1-aryl-1-propanones were prepared by Friedel–Crafts acylation of the corresponding arenes with (S)-N-trifluoroacetylanil chloride, obtained in situ by reaction of (S)-N-trifluoroacetylaniline and oxalyl chloride, following previously reported procedures. Final compounds were purified by column chromatography on silica gel 60, eluting with CH$_2$Cl$_2$. (S)-Methylthio and (S)-ethylthio derivatives were recognized by melting points and NMR spectra from the literature, and (S)-propylthio and (S)-butylthio derivatives were identified by comparison with the previously reported NMR signals for the same aromatic substitutions in the racemic compounds.

4.1.3. General procedure for the preparation of (S)-trifluoroacetamido-1-arylanes. The (S)-trifluoroacetamido-1-arylanes were prepared following a previously reported procedure, with some modifications. To a stirred solution of (S)-2-trifluoroacetamido-1-aryl-1-propanone (7 mmol) in trifluoroacetic acid (3 ml) was added triethylsilane (0.55 ml, 35 mmol). The reaction mixture was refluxed and stirred for 6 h. Finally, the mixture was cooled to room temperature and made basic with a saturated solution of NaHCO$_3$. The mixture was extracted with CH$_2$Cl$_2$ (2× 15 ml) and washed with HCl (1 N). The organic layer was dried...
over anhydrous Na$_2$SO$_4$ and concentrated by rotary evaporation at 40°C to afford the crude (S)- trifluoroacetamido-1-arylpropane. The product was purified by column chromatography on silica gel 60, eluting with CH$_2$Cl$_2$.

4.1.3.1. (S)-2-Trifluoroacetamido-1-(4-methylthiophenyl)-propane. Yield 90%. 1H NMR (CDCl$_3$) 1.14 (d, 3H, J = 7.3 Hz, CH$_3$CH$_2$), 1.60 (m, 2H, CH$_3$CH$_2$CH$_2$), 2.75 (m, 4H, CH$_2$CH$_2$, CH$_2$CH$_2$CH$_2$), 6.05; S, 15.07% (calcd: C, 57.00%; H, 7.83%; N, 195.1082. (C$_{11}$H$_{18}$ClNS): C, 57.07%; H, 8.57%; N, 13.8%).

4.1.4. General procedure for the preparation of (S)-2-amino-1-arylpropane hydrochlorides (1–4). The (S)-2-trifuoroacetamido-aryl-1-propane derivatives (0.7 mmol) were dissolved in 2-propanol (16 ml) and concentrated HCl (12 ml). The resulting solutions were then stirred at 70°C for 10 h. The mixtures were allowed to cool to room temperature and the solvent was eliminated by rotary evaporation. The solid mixtures were treated with 1 N NaOH (15 ml) and extracted with CH$_2$Cl$_2$ (2× 15 ml). The organic phases were combined and extracted with 1 N HCl and the aqueous layers were separated and made basic with 2 N NaOH. These solutions were extracted with CH$_2$Cl$_2$ (2× 15 ml) and the organic phases were dried over anhydrous Na$_2$SO$_4$ and concentrated. The residual amines were dissolved in dry ether (10 ml) and the hydrochlorides precipitated with gaseous HCl.
4.1.4.4. (S)-2-Amino-1-(4-butythiophenylethyl)-propane hydrochloride (4). $^1$H NMR (D$_2$O) 0.85 (t, 3H, J = 7.4 Hz, CH$_3$CH$_2$CH$_2$CH$_2$S), 1.26 (d, 3H, J = 6.6 Hz, CHCH$_3$), 1.38 (m, 2H, CH$_2$CH$_2$CH$_2$CH$_2$S), 1.56 (m, 2H, CH$_2$CH$_2$CH$_2$CH$_2$S), 2.86 (dd, 1H, J$_{ab}$ = 13.9 Hz, J$_{ac}$ = 7.4 Hz, CH$_3$CH$_2$CH$_2$S), 2.91 (dd, 1H, J$_{ba}$ = 13.4 Hz, J$_{bc}$ = 7.0 Hz, CH$_3$CH$_2$CH$_2$S), 2.95 (t, 2H, J = 7.2 Hz, CH$_2$CH$_2$CH$_2$CH$_2$S), 3.58 (m, 1H, CH$_2$CH$_3$), 7.22 (d, 2H, J = 8.5 Hz, ArH-3,5), 7.36 (d, 2H, J = 8.5 Hz, ArH-2,6). $^{13}$C NMR (D$_2$O) 15.5 (CH$_3$CH$_2$), 20.0 (CH$_3$CH$_2$CH$_2$S), 32.2 (CH$_3$CH$_2$CH$_2$CH$_2$S), 35.0 (CH$_2$CH$_2$CH$_2$CH$_3$S), 42.2 (CH$_2$CH$_3$), 51.6 (CHCH$_3$), 131.8 (ArC-3,5), 132.7 (ArC-2,6), 136.6 (ArC-4), 137.3 (ArC-1).

HRMS m/z 223.1392, calculated for C$_{13}$H$_{21}$NS (M$^+$/C$_176$0.5% (w/v) reduced Triton X-100, at 25°C. The oxidation of kynuramine catalyzed by MAO-A was monitored at 316 nm (absorption maximum for aldehyde product, extinction coefficient = 11,800 M$^{-1}$ cm$^{-1}$), and that of benzylamine by MAO-B was monitored at 250 nm (maximum absorption by benzaldehyde, extinction coefficient = 12,800 M$^{-1}$ cm$^{-1}$). All spectral data were obtained using a Perkin-Elmer Lambda 2 UV-vis double-beam spectrophotometer with thermostatted cuvette holders. Kinetic data were evaluated and plotted using Microcal Origin or Prism Graph Pad software.

4.3. Molecular simulation

The crystallographic data of rMAO-A or hMAO-A (PDB: 1O5W or 2BXS, respectively) were used for all calculations. The Autodock 3.05 suite was then used to perform the docking simulations and conditions were as previously reported, with some modifications. Briefly, the hydrogen atoms of the protein and the FAD molecule were built using Insight II, and the structures were relaxed following a minimization protocol using Discover_3 and the ESFF force field. The grid maps were calculated using the autogrid3 option and were centered on the putative ligand-binding site. The volumes chosen for the grid maps were made up of 40 x 40 x 40 points, with a grid-point spacing of 0.375 Å. The partial charges of 4-alkylthiophenylisopropylamine derivatives 1-4 were corrected using RESP methodology. The dielectric constant was adjusted either to 10 (hMAO-A) or to 2 (rMAO-A) in the grid parameter file (gpf) of the Autodock suite. This parameter adjustment gave the best correlations between the calculated docking energies and biochemical results. The docked compound complexes were built using the lowest free-energy binding positions.

Acknowledgments

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References and notes