Selective Inhibitory Activity against MAO and Molecular Modeling Studies of 2-Thiazolylhydrazone Derivatives

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A series of 2-thiazolylhydrazone derivatives have been investigated for the ability to inhibit the activity of the A and B isoforms of monoamine oxidase (MAO) selectively. All of the compounds showed high activity against both the MAO-A and the MAO-B isoforms with pK_i values ranging between 5.92 and 8.14 for the MAO-A and between 4.69 and 9.09 for the MAO-B isoforms. Both the MAO-A and the MAO-B isoforms, deposited in the Protein Data Bank as model 2BXR and 1GOS, respectively, were considered in a computational study performed with docking techniques on the most active and MAO-B-selective inhibitor, **18**.

Introduction

MAO is a flavoprotein present in the outer mitochondrial membranes of neuronal, glial, and other cells. It catalyzes the oxidative deamination of biogenic amines in the brain and the peripheral tissues, regulating their level. MAO exists in two forms, namely, MAO-A and MAO-B.[1] MAO-A is sensitive to the selective inhibitor, clorgyline, whereas MAO-B is irreversibly inhibited by selegiline (Figure 1).

MAO-A and MAO-B metabolize the principal biogenic amines, dopamine (DA), noradrenalin (NA), adrenalin, serotonin (5HT), and 2-phenylethylamine (PEA). MAO plays an important role in neurotransmitters and is a scavenger of various other amines.[2] The typical substrate of MAO-A is 5HT, whereas the substrates of MAO-B are β-phenylethylamine and benzylamine.

Two neurotransmitters that are fundamental in human depression, NA and 5HT, are primarily deaminated by MAO-A. Inhibition of MAO-B increases DA and 5HT levels in mammals and also exhibits neuroprotective effects.[3] MAO-B activity has been shown to increase with age and is particularly high around the senile plaques (SP) in Alzheimer's disease (AD) patients.[3,4] The oxidative deamination of biogenic amines catalyzed by MAO-B produces hydrogen peroxide and other reactive oxygen species (ROS).[3,5−8] Direct evidence for free radical involvement in the etiology of Parkinson's disease (PD) has been reported with increased lipid peroxidation and superoxide dismutase (SOD) activity in the substantia nigra of PD patients.[5−9] CNS degenerative disorders such as AD and PD have been associated with oxidative stress, and increased MAO-B activity, with reduced free radical scavenging capacity.[3,5−12] On the contrary, MAO-A activity does not increase with age, suggesting independent regulation of the expression of these two isoenzymes.[10,12] Recently, Parini et al. have reported that the activity of the MAO-A isoform increases with age in rat hearts.[13]

Furthermore, it has been shown that MAO is responsible for the biotransformation of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) into 1-methyl-4-phenylpyridinium, a Parkinson's-producing neurotoxin[14−16] and may also contribute to the apoptotic process because inhibition of MAO activity suppresses cell death.[17]

For all of these reasons, selective and reversible inhibitors of MAO-A or MAO-B may be useful therapeutic agents devoid of unwanted side effects such as the so-called "cheese effect".[18] In humans MAO-B inhibitors (MAO-B-Is) are useful as coadjuvants in the treatment of Parkinson's disease and perhaps Alzheimer's disease,[2,19,20] whereas MAO-A inhibitors (MAO-A-Is) are antidepressant and antianxiety agents (Figure 1).[21−23]

A recent description of the crystal structure of the two isoforms of human MAO by Binda et al. provides relevant information of the mechanism underlying the selective interactions between these proteins and their ligands. They are helpful in probing the catalytic mechanism and in gaining a better understanding of the pharmacophoric requirements needed for the rational design of potent and selective enzyme inhibitors with a therapeutic potential.[24−29]

Numerous compounds among the great variety of substituted hydrazines behave as MAO inhibitors.[30−32] A common structural feature of substrates and inhibitors is an amino or imino group that is assumed to play an essential role in orientation and complex formation at the active site of the enzyme.[33]

In earlier papers by Cambria and others,[34−37] it was shown that numerous hydrazinothiazole derivatives inhibit MAO-B activity in the range of micromolar concentration. Moreover, benzylidene-hydrazinothiazole derivatives were reported to be the most active, with their activity being further enhanced by the presence of a methyl group at position 4 of the thiazole nucleus. Starting from these studies, we assayed a series of 2-thiazolylhydrazone derivatives (**1**−**18**) for their ability to inhibit MAO activity selectively. Though, except for **15** and **16**, the compounds were already known, their potential inhibitory activity against MAO isoforms had never been de-

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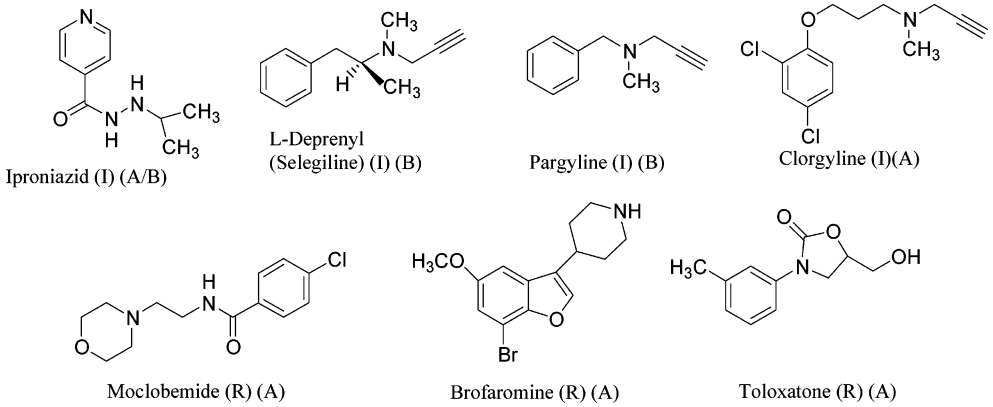


Figure 1. Irreversible (I), reversible (R), and selective MAO-A and/or MAO-B (A or A/B or B) inhibitors.

scribed.[38,39] Furthermore, we considered both the MAO-A and the MAO-B isoforms deposited in the Protein Data Bank as models 1GOS[24] and 2BXR,[26] respectively, in a computational study performed with docking techniques on the most active inhibitor benzaldehyde-4-methyl-(4-phenyl-2-thiazolyl)hydrazone **18**.

Chemistry

2-Thiazolylhydrazone derivatives (**1**−**18**) were synthesized as reported in our previous communications.[38,39]

Cyclic ketones or aryl aldehydes reacted directly with thiosemicarbazide, and the obtained thiosemicarbazones subsequently reacted with α-halogenoketones to yield the 4-substituted thiazole ring derivatives. In the synthesis of all of the compounds, isopropyl alcohol proved to be the best solvent for our purpose. As a matter of fact, the reaction products precipitate upon cooling, and they can be filtered and purified by crystallization from ethanol or ethanol/isopropanol. All of the synthesized products were characterized by analytical methods as reported in the literature.[38,39]

Results and Discussion

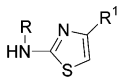
All of the compounds were first evaluated for their ability to inhibit MAO in the presence of kynuramine as a substrate. After the first assessment of their inhibitory effect on the MAO, we tested the compounds to determine their activity toward MAO-A and MAO-B selectively in the presence of the specific substrates, serotonin and benzylamine, respectively (Table 1).

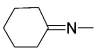
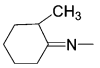
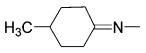
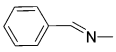
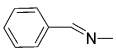
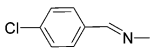
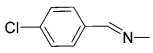
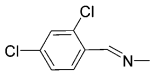
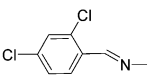
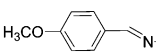
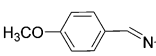
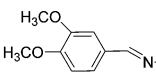
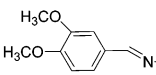
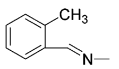
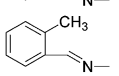
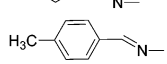
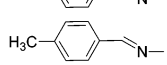
From Table 1, which shows the structures and the MAO inhibition data along with the MAO inhibitory selectivity (pSI: log selectivity index = $pK_{i(MAO-A)} - pK_{i(MAO-B)}$), it can be seen that all of the synthesized compounds show high activity against both the MAO-A and the MAO-B isoforms, with pK_i values in the range of 5.92 to 8.14 and 4.69 to 9.09, respectively.

1−**4**, 2(3H)-thiazolone-4-phenyl-cycloalkylidenehydrazone derivatives, showed selective inhibitory activity against MAO-A with pK_i's in the range of 6.85 to 7.69 and the highest pSI's of 2.7 for **3** and 1.48 for **4**. 4-Phenyl-2-thiazolylhydrazone derivatives showed inhibitory activity against MAO-A with pK_i's in the range of 5.92 to 7.67 and against MAO-B with pK_i's from 7.00 to 9.09. **18** was the most potent of the series, which reached the very interesting inhibitory activity value of pK_i 9.09. **6** and **18** showed significant MAO-B selectivity with higher pSI values of −1.12 and −1.42, respectively. 4-Methyl-2-thiazolylhydrazone derivatives showed interesting inhibitory activity against both MAO-A and MAO-B, without any remarkable selectivity.

The computational work was carried out with the purpose of rationalizing by molecular modeling the selectivity toward MAO isoforms of the most active and selective MAO-B inhibitor **18**. We applied the same docking approach as in other recent communications of ours,[40] that is, performing a flexible docking

Table 1. Structures and Inhibitory Activities of Derivatives **1**−**18**[a]



Compound	R	R^1	pK_i MAO-A	pK_i MAO-B	pSI[b] selectivity
1		C_6H_5	6.85	6.26	0.59
2		C_6H_5	7.69	7.04	0.65
3		C_6H_5	7.39	4.69	2.7
4		C_6H_5	7.00	5.52	1.48
5		CH_3	8.14	8.88	−0.74
6		C_6H_5	5.92	7.04	−1.12
7		CH_3	7.42	8.04	−0.62
8		C_6H_5	7.00	6.77	0.23
9		CH_3	7.92	7.77	0.15
10		C_6H_5	7.03	6.88	0.42
11		CH_3	7.72	8.39	−0.67
12		C_6H_5	7.30	6.88	0.42
13		CH_3	7.88	8.15	−0.27
14		C_6H_5	7.52	7.69	−0.17
15		CH_3	7.45	6.48	0.97
16		C_6H_5	7.18	6.30	0.88
17		CH_3	6.69	6.08	0.61
18		C_6H_5	7.67	9.09	−1.42

[a] The data represent mean values of at least of three separate experiments. [b] pSI: log selectivity index = $pK_{i\ (MAO-A)} - pK_{i\ (MAO-B)}$.

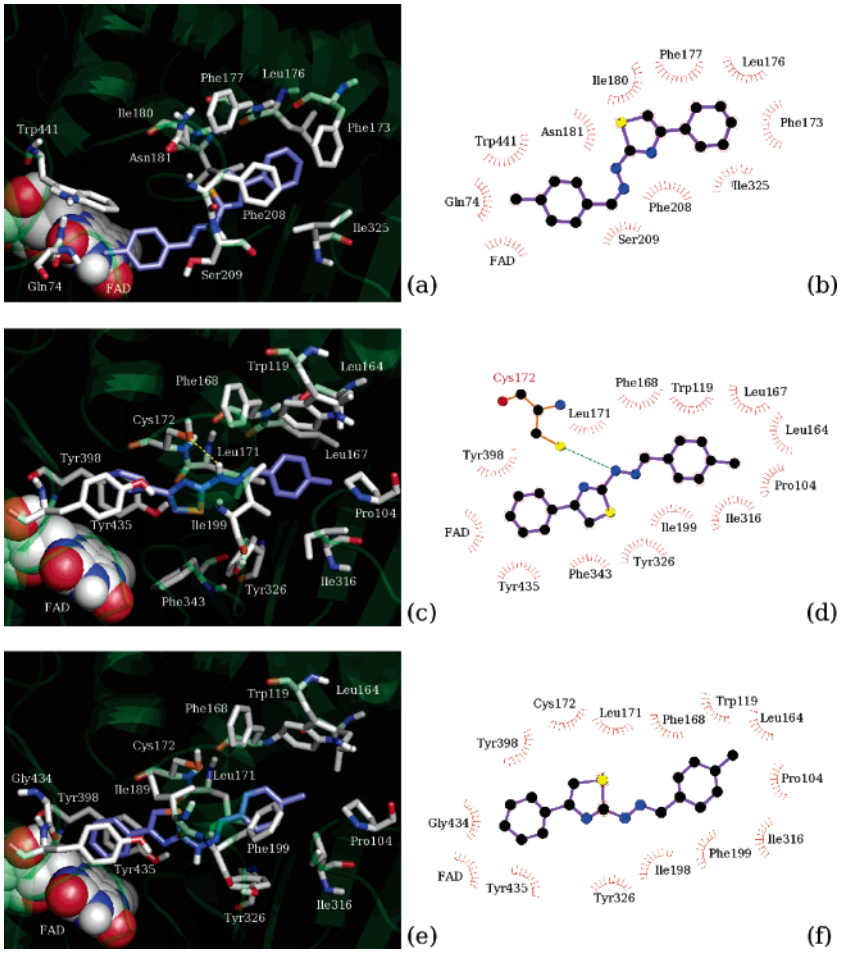


Figure 2. Most stable complexes of **18** and MAO isoforms: A (a, b), B (c, d), and B with Ile199 mutated in Phe199 (e, f). Interacting residues of the active site are shown in labeled polytubes, FAD in the CPK rendering, **18** in the blue carbon polytube, and other aminoacids in the ribbon in MAO-A (a), MAO-B (c), and mutated MAO-B (e) isoforms. LigPlot representation of the same MAO-A (b), MAO-B (d), and mutated MAO-B (f) complexes reported without hydrogens. Hydrogen bonds are reported as dashed lines between heavy atoms.

search with the Monte Carlo (MC) method implemented in the Macromodel software[41] as described in the Experimental Section.

We built and energy-minimized a preliminary model of **18**. We then created a starting configuration of the **18** complex with each enzyme, searching the most stable GLIDE[42] pose with the pretreated enzyme models as reported in the Experimental Section. In agreement with the selectivity data for **18**, in both cases GLIDE energies (GE) and scores (GS) were lower in MAO-B (GE = −43.2 and GS = −8.14) than in MAO-A (GE = −16.5 and GS = −6.36).

These energy-minimum configurations were used for a more accurate and complete docking search with the MC method. As a matter of fact, in the latter search the imine bond was also set to rotate freely, exploring both *E* and *Z* configurations. The generation of the binding modes in the enzyme proved to be quite difficult in the MAO-A isoform, where more than 27 configurations were found with an average number of duplicates of 1.33. Conversely, in MAO-B the MC search was more successful, with 87 configurations and an average number of duplicates of 2.54. These statistical data suggested a more accessible MAO-B cleft for **18**. After full minimization, all of the MC-generated configurations were submitted to a statistical thermodynamic analysis according to the MOLINE method.[43] The free complexation energy ($\Delta\Delta G$) was once in better agreement with the experimental selectivity trend, with values of −41.91 (MAO-A) and −47.05 (MAO-B) kcal/mol. Repeating the calculation with the MAO-B model with Ile199 virtually mutated in Phe, the resulting $\Delta\Delta G$ was equal to −44.87 kcal/mol, still in agreement with the isoform selectivity inhibition.

The analysis of the stereoisomer contributions to the MAO binding showed that in both isoforms the *E* form plays a more important role than the *Z* form, with $\Delta\Delta G_{E-Z}$ differences on the order of 2 to 3 kcal/mol. Moreover, the greater importance of the *E* form has also been highlighted by a Boltzmann population analysis, which was carried out on enantiomer ensembles, and in all simulations for both MAO-A and MAO-B complexes it reported a probability of existence of about 100%.

With the aim of understanding the reason for the selectivity of **18** at the molecular level, using the graphical tools cited in the Experimental Section we inspected the most stable geometries of this compound within the enzymatic clefts of MAO-A and MAO-B. Figure 2 shows pictures of these geometries. In MAO-A, (Figure 2a,b) recognition of **18** is relatively close to the FAD. The phenyl ring of the R^1 substituent interacted with hydrophobic residues such as Phe177, Leu176, Phe173, and Ile325, establishing about 20 contacts computed by the LigPlot[43] analysis. The strongest interaction was found with another hydrophobic residue (Phe208) with 22 contacts. Minor interactions were established with other residues, in particular, by the R substituent. In this energy-minimum configuration, we found no intermolecular hydrogen bonds.

Conversely, recognition of **18** within the MAO-B cleft proved to be more productive (Figure 2c,d). In this case, two hydrophobic sites of the enzyme were implicated in the most stable binding mode. One was based on hydrophobic amino acids

Phe168, Trp119, Leu167, Leu164, Pro104, Ile316, and Ile199 (about 20 contacts). The other was based on FAD cofactors Tyr398 and Tyr435 (about 35 contacts). With these two aromatic residues, a $\pi-\pi$ stacking interaction is established with the R^1 phenyl moiety. This type of interaction was found to be a peculiarity of many potent MAO inhibitors.[44] Moreover, an intermolecular hydrogen bond interaction between the iminic hydrogen and the Cys172 side chain stabilized this configuration.

The same simulation carried out with a MAO-B model obtained; replacing Ile199 with Phe199 led to a similar binding mode. The list of residues involved in the most stable complex was very similar to that of the parent model. The difference in this list was merely qualitative. Instead of Leu167 and Phe343, this model included Gly434 and Ile198. The reason for this different residue recognition should be attributed to an approximately 180° rotation of the binding mode around the thiazole moiety as shown in Figure 2e,f. The most relevant consequence in the interaction pattern was due to an additional hydrophobic contact between Phe199 and the toluene moiety of **18** and the lack of hydrogen bonding with Cys172. Therefore, the ligand−enzyme interaction energy obtained in this case was relatively higher than the binding pose computed with the original 1GOS model. Despite the mutation of the Ile199 residue, which involved a partial destabilization of about 2.18 kcal/mol in the free energy of complexation of **18** within the MAO-B structures, all of the docking models resulted in agreement with the experimental selectivity.

Globally, the number of contacts and the different hydrogen bond network of **18** docked into the two clefts explained the higher selectivity for the MAO-B isoform. Finally, it is interesting that the same extended conformation of compound **18** recognized the MAO-A and MAO-B isoforms in an opposite configuration with respect to the R and R^1 aromatic moieties closer to the cofactor (Figure 2).

This preliminary study, carried out with the most active compound of this series, explained the nature of the selectivity of this inhibitor and can aid the design of new compounds for introducing appropriate substituents onto the 2-thiazolylhydrazone scaffold.

Experimental Section

Chemistry. General Procedure for the Preparation of 2-Thiazolylhydrazone Derivatives (1−18). The appropriate cyclic ketones or aryl aldehydes were dissolved in 2-propanol, which is the most suitable solvent, and were refluxed under magnetic stirring with thiosemicarbazide for 24 h. The obtained thiosemicarbazones subsequently reacted with 2-bromo-acetophenone at room temperature or with 2-chloro-acetone at reflux, with both dissolved in 2-propanol. After cooling to room temperature, the solution was filtered, and the desired derivatives were crystallized from ethanol or ethanol/isopropanol to give compounds **1**−**18**, respectively. Only for compounds **15** and **16** do we report the chemical and physical properties (see Supporting Information).

Biochemistry. All of the chemicals were commercial reagents of analytical grade and were used without purification. Bovine brain mitochondria were used as the enzyme source and were isolated according to the Basford method.[45] In all of the experiments, the AO activities of the beef brain mitochondria were determined by a fluorimetric method according to Matsumoto et al.[46] using kynuramine as a substrate at four different final concentrations ranging from 5 μM to 0.1 mM.

Briefly, the incubation mixtures contained potassium phosphate buffer (0.1 mL, 0.25 M, pH 7.4), mitochondria (6 mg/mL), and drug solutions with final concentrations ranging from 0 to 10^{-3} μM. The solutions were incubated at 38 °C for 30 min. The addition of perchloric acid ended the reaction. The samples were centrifuged at 10 000g for 5 min, and the supernatant was added to NaOH (2.7 mL, 0.1 N). The 2-thiazolylhydrazone derivatives were dissolved in dimethyl sulfoxide (DMSO) and added to the reaction mixture at concentrations ranging from 0 to 10^{-3} mM. To study the effect of the inhibition of 2-thiazolylhydrazone derivatives on the activities of both MAO-A and MAO-B separately, the mitochondrial fractions were preincubated at 38 °C for 30 min with the appropriate specific irreversible inhibitor (0.5 μM L-deprenyl to eliminate MAO-B from the assay of MAO-A activity and 0.05 μM clorgyline to eliminate MAO-A from the assay for the B isoform). Fluorimetric measurements were recorded with a Perkin-Elmer LS 50B spectrofluorimeter. The protein concentration was determined according to Bradford.[47]

Dixon plots were used to estimate the inhibition constant (K_i) of the inhibitors (Table 1). The data are the means of three experiments performed in duplicate. It is interesting that all of the compounds act through the reversible mode, as shown by dialysis performed over 24 h in a cold room against a potassium phosphate buffer (0.1 M, pH 7.2) capable of restoring 90−100% of the activity of the enzyme.

Molecular Modeling. Following the computational work of recent communications of ours,[40] the Protein Data Bank[48] crystallographic models of human MAO-A and MAO-B, respectively known by the pdb codes 2BXR[26] and 1GOS,[24] were considered for the docking experiments knowing that the comparison of the human and bovine sequences at the catalytic site revealed a high level of identity.[49] Both structures were obtained as adducts with two similar compounds, clorgyline for 2BXR and pargyline for 1GOS, covalently linked to the FAD N5 nitrogen. To simulate the active site of the bovine MAO-B, the 1GOS model was virtually mutated, replacing the Ile199 with a Phe residue.

After the covalent bond between the FAD moiety and the cocrystallized inhibitor was removed, the pretreatment of the original pdb models consisted of a 48 kcal/mol constrained energy minimization of those residues within a 15 Å radius of the N5 of the isoalloxazine ring in order to restore the natural planarity of the isoalloxazine FAD ring and relax the active-site amino acids. In the resulting energy-minimum structures, ligands were removed and used as receptor models. This was done with the force field AMBER* united atom notation and the GB/SA water implicit model of solvation as implemented in MacroModel ver 7.2.[41]

Starting geometries of **18** complexes with MAO-A and MAO-B were generated by the GLIDE docking method.[42] Both pretreated enzyme models were submitted to map calculations using a box of about 110 000 $Å^3$ centered on the FAD N5 atom. Flexible docking of the **18** compound was done by generating a maximum number of 5000 configurations. The most stable GLIDE pose was analyzed and used as a starting configuration for a more accurate docking search performed by Monte Carlo using the MOLS directive for rototranslational settings, that is, allowing each ligand to rotate a full ±180° and to translate with a tolerance of ±10 Å. One thousand MC iterations were carried out, and each configuration was energy-minimized with the AMBER* united atom and the GB/SA water implicit model of solvation. The same constrained energy minimization conditions were adopted during the MC search. The obtained MC ensemble was then full energy-minimized, removing any constant force. The same docking route was carried out with the original crystallographic coordinates, that is, with the nonplanar FAD ring conformation, obtaining no significant difference in the results because of the final full energy-minimization procedure.

The interaction energy of all complexes before and after full relaxation were computed according to the MOLINE method[43] and converted to average state equations.

All calculations were made with a Linux cluster of 8 Intel Xeon dual processors at 3.2 GHz with 2 GB of RAM. Graphic manipulations and analysis of the docking experiments were performed by the Maestro Graphical User Interface version 4.1.012 for Linux operating systems.[41] Ligplot version 4.0[50] and Pymol version 0.98[50] were used to create Figure 2.

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Supporting Information Available: Analytical data for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Kalgutkar, A. S.; Castagnoli, N., Jr.; Testa, B. Selective inhibitors of monooxidase (MAO-A and MAO-B) as probes of its catalytic site and mechanism. *Med. Res. Rev.* **1995**, *15*, 325−388.

(2) Wouters, J. Structural aspects of monoamine oxidase and its reversible inhibition. *Curr. Med. Chem.* **1998**, *5*, 137−162.

(3) Volz, H. P.; Gleiter, C. H. Monoamino oxidase inhibitors- a perspective on their use in the elderly. *Drugs Aging* **1998**, *13*, 341−355.

(4) Saura, J.; Luque, J. M.; Cesura, A. M.; Da Prada, M.; Chan-Palay, V.; Huber, G.; Löffler, J.; Richards, J. G. Increased monoamine oxidase B activity in plaque-associated astrocytes of Alzheimer brains revealed by quantitative enzyme radioautography. *Neuroscience* **1994**, *62*, 15−30.

(5) Palmer, A. M.; DeKosky, S. T. Monoamine, neurons in aging and Alzheimer's, disease. *J. Neural. Transm.: Gen. Sect.* **1993**, *91*, 135−159.

(6) Alper, G.; Girgin, F. K.; Ozgönül, M.; Mentes, G.; Ersöz, B. MAO inhibitors and oxidant stress in aging brain tissue. *Eur. Neuropsychopharmacol.* **1999**, *9*, 247−252.

(7) Good, P. F.; Werner, P.; Hsu, A.; Olanow, C. W.; Perl, D. P. Evidence for neuronal oxidative damage in Alzheimer's disease. *Am. J. Pathol.* **1996**, *149*, 21−27.

(8) Cash, A. D.; Perry, G.; Smith, M. A. Therapeutic potential in Alzheimer's disease. *Curr. Med. Chem.* **2002**, *9*, 1605−1610.

(9) Drukarch, B.; Muiswinkel, F. L. Drug treatment of Parkinson's disease. *Biochem. Pharmacol.* **2000**, *59*, 1023−1031.

(10) Sramek, J. J.; Cutler, N. R. Ongoing trials in Alzheimer's disease. *Expert Opin. Invest. Drugs.* **2000**, *9*, 899−915.

(11) Yu, P. H. Pharmacological and clinical implications of MAO-B inhibitors. *Gen. Pharmacol.* **1994**, *25*, 1527−1539.

(12) Sramek, J. J.; Cutler, N. R. Recent developments in the drug treatment of Alzheimer's disease. *Drugs Aging* **1999**, *14*, 359−373.

(13) Maurel, A.; Hernandez, C.; Kunduzova, O.; Bompart, G.; Cambon, C.; Parini, A.; Francés B. Age-dependent increase in hydrogen peroxide production by cardiac monoamine oxidase A in rats. *Am. J. Physiol.: Heart Circ. Physiol.* **2003**, *284*, 1460−1467.

(14) Chiba, K.; Trevor, A.; Castagnoli, N. Metabolism of the neurotoxic tertiary amine, MPTP, by brain monoamine oxidase. *Biochem. Biophys. Res. Commun.* **1984**, *120*, 574−578.

(15) Fritz, R. R.; Abell, C. W.; Patel, N. T.; Gessner, W.; Brossi, A. Metabolism of the neurotoxin in MPTP by human liver monoamine oxidase B. *FEBS Lett.* **1985**, *186*, 224−228.

(16) Grimsby, J.; Toth, M.; Chen, K.; Kumazawa, T.; Klaidman, L.; Adams, J. D.; Karoum, F.; Gal, J.; Shih, J. C. Increased stress response and β-phenylethylamine in MAOB-deficient mice. *Nat. Genet.* **1997**, *17*, 206−210.

(17) De Zutter, G. S.; Davis, R. J. Pro-apoptotic gene expression mediated by the p38 mitogen-activated protein kinase signal transduction pathway. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 6168−6173.

(18) Strolin-Benedetti, M.; Dostert, P. L. Monoamine oxidase: From physiology and pathophysiology to the design and clinical application of reversible inhibitors. *Adv. Drug Res.* **1992**, *23*, 65−125.

(19) Tetrud, J. W.; Langston, J. W. The effect of deprenyl (selegiline) on the natural history of Parkinson's disease. *Neurology* **1989**, *39*, 1483−1487.

(20) Carreiras, M. C.; Marco, J. L. Recent approaches to novel anti-Alzheimer therapy. *Curr. Pharm. Des.* **2004**, *10*, 3167−3175.

(21) Rudorfer, M. V.; Potter, W. Z. Antidepressants. A comparative review of the clinical pharmacology and therapeutic use of the 'newer' versus the 'older' drugs. *Drugs* **1989**, *37*, 713−738.

(22) Pacher, P.; Kohegyi, E.; Keckemeti, V.; Furst, S. Current trends in the development of new antidepressants. *Curr. Med. Chem.* **2001**, *8*, 89−100.

(23) Pacher, P.; Keckemeti, V. Trends in the development of new antidepressants. Is there a light at the end of the tunnel? *Curr. Med. Chem.* **2004**, *11*, 925−943.

(24) Binda, C.; Newton-Vinson, P.; Hubálek, F.; Edmondson, D. E.; Mattevi, A. Structure of human monoamine oxidase B, a drug target for the treatment of neurological disorders. *Nat. Struct. Biol.* **2002**, *9*, 22−26. Data deposition: www.pdb.org (pdb ID code 1GOS).

(25) Edmondson, D. E.; Binda, C.; Mattevi, A. The FAD binding sites of human monoamine oxidases A and B. *NeuroToxicology* **2004**, *25*, 63−72.

(26) De Colibus, L.; Li, M.; Binda, C.; Lustig, A.; Edmondson, D. E.; Mattevi, A. Three-dimensional structure of human monoamine oxidase A (MAO A): Relation to the structures of rat MAO A and human MAO B. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 12684−12689. Data deposition: www.pdb.org (pdb ID codes 2BXR, 2BXS, and 2BYB).

(27) Binda, C.; Hubálek, F.; Li, M.; Edmondson, D. E.; Mattevi, A. Crystal structure of human monoamine oxidase B, a drug target enzyme monotopically inserted into the mitochondrial outer membrane. *FEBS Lett.* **2004**, *564*, 225−228.

(28) Binda, C.; Edmondson, D. E.; Mattevi, A. Structure-function relationships in flavoenzyme-dependent amine oxidations. A comparison of polyamine oxidase and monoamine oxidase. *J. Biol. Chem.* **2002**, *277*, 23973−23976.

(29) Hubálek, F.; Binda, C.; Khalil, A.; Li, M.; Mattevi, A.; Castagnoli, N.; Edmondson, D. E. Demonstration of isoleucine 199 as a structural determinant for the selective inhibition of human monoamine oxidase B by specific reversible inhibitors. *J. Biol. Chem.* **2005**, *280*, 15761−15766.

(30) Tipton, K. F. Inhibition of monoamine oxidase by substituted hydrazines. *Biochem. J.* **1972**, *128*, 913−919.

(31) Mc Kenna, K. F.; Baker, G. B.; Coutta, R. T. N2-acetylphenelzine: Effects on rat brain GABA, alanine and biogenic amines. *Naunyn−Schmiedeberg's Arch. Pharmacol.* **1991** *343*, 478−482.

(32) Yamada, N.; Takahashi, S.; Todd, K. G.; Baker, G. B.; Paetsch, P. R. Effects of two substituted hydrazine monoamine oxidase (MAO) inhibitors on neurotransmitter amines, gamma-aminobutyric acid, and alanine in rat brain. *J. Pharm. Sci.* **1993**, *82*, 934−937.

(33) Belleau, B.; Moran, J. The mechanism of action of the 2-phenylcyclopropylamine type of monoamine oxidase inhibitors. *J. Med. Pharm. Chem.* **1962**, *5*, 215−219.

(34) Pignatello, R.; Mazzone, S.; Castelli, F.; Mazzone, P.; Raciti, G.; Mazzone, G. MAOI activity of thiosemicarbazides and related 2-thiazolylhydrazines. *Pharmazie* **1994**, *49*, 272−276.

(35) Raciti, G.; Mazzone, P.; Raudino, A.; Mazzone, G.; Cambria, A. Inhibition of rat liver mitochondrial monoamine oxidase by hydrazine-thiazole derivatives: Structure-activity relationships. *Bioorg. Med. Chem.* **1995**, *3*, 1485−1491.

(36) Cambria, A.; Raudino, A.; Geronikaki, A.; Buemi, G.; Raciti, G.; Mazzone, P.; Guccione, S.; Ragusa, S. Thiazole derivatives as inhibitors of purified bovine liver mitochondrial monoamine oxidase-B: Structure-activity relationships and theoretical study. *J. Enzyme Inhib.* **1999**, *14*, 307−321.

(37) Gritsch, S.; Guccione, S.; Hoffmann, R.; Cambria, A.; Raciti, G.; Langer, T. A 3D QSAR study of monoamine oxidase-B inhibitors using the chemical function based pharmacophore generation approach. *J. Enzyme Inhib.* **2001**, *16*, 199−215.

(38) Maccioni, E.; Cardia, M. C.; Bonsignore, L.; Plumitallo, A.; Pellerano, M. L.; De Logu, A. Synthesis and anti-microbial activity of isothiosemicarbazones and cyclic analogues. *Il Farmaco* **2002**, *57*, 809−817.

(39) Maccioni, E.; Cardia, M. C.; Distinto, S.; Bonsignore, L.; De Logu, A. An investigation of the biological effect of structural modifications of isothiosemicarbazones and their cyclic analogues. *Il Farmaco* **2003**, *58*, 951−959.

(40) (a) Manna, F.; Chimenti, F.; Bolasco, A.; Secci, D.; Bizzarri, B.; Befani, O.; Turini, P.; Mondovì, B.; Alcaro, S.; Tafi, A. Inhibition of amine oxidases activity by 1-acetyl-3, 5-diphenyl-4,5-dihydro-(1H)-pyrazole derivatives. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3629−3633. (b) Chimenti, F.; Secci, D.; Bolasco, A.; Chimenti, P.; Granese, A.; Befani, O.; Turini, P.; Alcaro, S.; Ortuso, F. Inhibition of monoamine oxidases by coumarin-3-acyl derivatives: Biological activity and computational study. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3697−3703. (c) Chimenti, F.; Maccioni, E.; Secci, D.; Bolasco, A.; Chimenti, P.; Granese, A.; Befani, O.; Turini, P.; Alcaro, S.; Ortuso, F.; Cirilli, R.; La Torre, F.; Cardia, M. C.; Distinto, S. Synthesis, molecular modeling studies and selective inhibitory activity against MAO of 1-thiocarbamoyl-3,5-diaryl-4,5-dihydro-(1H)-pyrazole derivatives *J. Med. Chem.* **2005**, *48*, 7113−7122. (d) Chimenti, F.; Bolasco, A.; Manna, F.; Secci, D.; Chimenti, P.; Granese, A.; Befani, O.; Turini, P.; Alcaro, S.; Ortuso, F. Synthesis and molecular modelling of novel substituted-4,5-dihydro-(1H)-pyrazole derivatives as potent and highly selective monoamine oxidase-A inhibitors. *Chem. Biol. Drug Des.* **2006**, *67*, 206−214. (e) Chimenti, F.; Bolasco, A.; Manna, F.; Secci, D.; Chimenti, P.; Granese, A.; Befani, O.; Turini, P.; Cirilli, R.; La Torre, F.; Alcaro, S.; Ortuso, F.; Langer, T. Synthesis, biological evaluation and 3D-QSAR of 1,3,5-trisubstituted-4,5-dihydro-(1H)-pyrazole derivatives as potent and highly selective monoamine oxidase A inhibitors. *Curr. Med. Chem.* **2006**, *13*, 1411−1428.

(41) (a) MacroModel version 7.2; Schrödinger Inc.: Portland, OR, 1998−2001. (b) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. MacroModel - an integrated software system for modeling organic and bioorganic molecules using molecular mechanics. *J. Comput. Chem.* **1990**, *11*, 440−467.

(42) Eldridge, M. D.; Murray, C. W.; Auton, T. R.; Paolini, G. V.; Mee, R. P. Empirical scoring functions 1. The development of a fast empirical scoring function to estimate the binding affinity of ligands in receptor complexes. *J. Comput.-Aided Mol. Des.* **1997**, *11*, 425−445.

(43) Alcaro, S.; Gasparrini, F.; Incani, O.; Mecucci, S.; Misiti, D.; Pierini, M.; Villani, C. A quasi-flexible automatic docking processing for studying stereoselective recognition mechanisms. Part I. Protocol validation. *J. Comput. Chem.* **2000**, *21*, 515−530.

(44) Moussa, Y. B. H.; Bakhle, Y. S. Monoamine oxidase: Isoforms and inhibitors in Parkinson's disease and depressive illness. *Br. J. Pharmacol.* **2006**, *147*, S287−S296.

(45) Stahl, W. L.; Smith, J. C.; Napolitano, L. M.; Basford, R. E. Brain mitochondria. I. Brain mithocondria: Isolation of bovine brain mitochondria. *J. Cell Biol.* **1963**, *19*, 293−307.

(46) Matsumoto, T.; Suzuki, O.; Furuta, T.; Asai, M.; Kurokawa, Y.; Nimura, Y.; Katsumata, Y.; Takahashi, I. A sensitive fluorometric assay for serum monoamine oxidase with kynuramine as substrate. *Clin. Biochem.* **1985**, *18*, 126−129. Bradford. M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248−254.

(47) Berman, H. M.; Westbrook, J.; Feng, Z.; Gilliland, G.; Bhat, T. N.; Weissig, H.; Shindyalov, I. N.; Bourne, P. E. The Protein Data Bank. *Nucleic Acids Res.* **2000**, *28*, 235−242.

(48) Abell, C. W.; Kwan, S. W. Molecular characterization of monoamine oxidases A and B. *Prog. Nucleic Acid Res. Mol. Biol.* **2001**, *65*, 129−156.

(49) Wallace, A. C.; Laskowski, R. A. and Thornton, J. LIGPLOT: A program to generate schematic diagrams of protein-ligand interactions. *Protein Eng.* **1995**, *8*, 127−134.

(50) DeLano, W. L. *The PyMOL Molecular Graphics System 2002***;** DeLano-Scientific: San Carlos, CA; www.pymol.org.

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