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Docking studies on monoamine oxidase-B inhibitors: Estimation of inhibition constants (K_i) of a series of experimentally tested compounds

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Abstract—Monoamine oxidase (EC1.4.3.4; MAO) is a mitochondrial outer membrane flavoenzyme that catalyzes the oxidation of biogenic amines. It has two distinct isozymic forms designated MAO-A and MAO-B, each displaying different substrate and inhibitor specificities. They are the well-known targets for antidepressant and neuroprotective drugs. Elucidation of the X-ray crystallographic structure of MAO-B has opened the way for molecular modeling studies. A series of experimentally tested (**1**–**10**) model compounds has been docked computationally to the active site of the MAO-B enzyme. The AutoDock 3.0.5 program was employed to perform automated molecular docking. The free energies of binding (ΔG) and inhibition constants (K_i) of the docked compounds were calculated by the Lamarckian Genetic Algorithm (LGA) of AutoDock 3.0.5. Excellent to good correlations between the calculated and experimental K_i values were obtained.
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1. Introduction

Monoamine oxidase (EC 1.4.3.4; MAO) is a flavoenzyme that is important to the oxidative deamination of a variety of biogenic and diet-derived amines in both the central nervous system (CNS) and in the peripheral tissues.[1,2]

Compounds that inhibit MAO exhibit either antidepressant activity, if they inhibit the A isozyme,[3] or antiparkinsonian activity, if they inhibit the B isozyme.[4] The differentiating role of Ile335 in MAO-A and of Tyr326 in MAO-B in determining substrate and inhibitor specificities in human MAO-A and -B has been experimentally demonstrated and the results have been published.[5]

Dopamine (DA), adrenaline, noradrenaline (NA), serotonin (5-HT), and β-phenylethylamine (PEA) are among the most important substrates for the enzyme in the CNS.

The important function of MAO in the catabolism of neuorogenic amines has attracted the interest of many researchers. The early MAO inhibitors (MAOIs) developed for the treatment of depression were withdrawn from the market because of their severe side effects and irreversible binding mechanism.[6] This problem was alleviated with the discovery and development of selective and reversible MAOIs.[7,8]

Recent findings have shown that MAO-B inhibitors have neuroprotective[9] and antioxidant effects,[10] as well as a role in delaying apoptotic neuronal death.[11]

The development of a new generation of inhibitors has attracted the attention of many researchers working in the design, synthesis, and molecular modeling studies of reversible and selective inhibitors.[12]

The determination of the 3D structure of MAO-B by X-ray crystallography[13] has opened the way for molecular modeling studies.

To get some insight into the oxidation mechanism of MAO-B, a series of amino ethers was synthesized and tested with the enzyme MAO-B.[14] Enzyme–adduct models were also studied using the Self-consistent Field

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theory, using a semi-empirical MP3 method and an ab initio method at the MP2/6-31G*//6-31G* level.[15] The results of these studies and recently published[16] articles about the crystal structure of MAO-B have paved the way for applying computational chemistry to design better inhibitors of this enzyme. In this study, we aim to develop a docking simulation program for the MAO-B enzyme. AutoDock 3.0.5[16] simulation program was employed to determine the binding orientation, free energy of binding, and inhibition constants (K_i) of several experimentally tested MAO-B inhibitors (Table 1). The calculated and experimental inhibition constants of these compounds were then compared.

2. Methods

2.1. Protein setup

For the present study, two different crystal structures of MAO-B (1GOS, 1S3E) were used to test the validity of AutoDock 3.0.5 docking program.

2.2. 1GOS (3.0 Å resolution)

The crystal structure of Monoamine oxidase-B in complex with its inhibitor pargyline was obtained from the Protein Data Bank (PDB entry code 1GOS).[17] MAO-B has two identical subunits (A and B). The study was carried out on only the B subunit of the enzyme protein. The pdb file was edited and the A-chain was removed together with the pargyline group, which was an irreversible inhibitor of MAO-B. Atoms of the FAD cofactor were defined in their oxidized state.

For use with the Autodock docking simulation, all polar hydrogens were added with the GROMACS modeling package.[18,19] The partial charges were placed using the same package keeping FAD in an oxidized sate. The resulting structure was optimized in 400 steps of conjugate gradient minimization, employing the GROMACS87 force field. During minimization, the heavy atoms were kept fixed at their initial crystal coordinates, but added hydrogens were made free to move. Minimization was effected under a vacuum medium. Electrostatic interactions were calculated using the cut-off method. As the acceptable minimal force gradient was reached, the minimization converged and the resultant structure was saved. Finally, solvation parameters were added using the ADDSOL utility of AutoDock 3.0.5. Default values of atomic solvation parameters were used throughout the calculations. The grid maps of the protein used in the docking experiments were calculated using the AutoGrid utility program.

2.3. 1S3E (1.6 Å resolution)

The high-resolution crystal structure of Monoamine oxidase-B, which co-crystalized with its irreversible inhibitor 6-hydroxy-*N*-propargyl-1(*R*)-aminoindan **2**, was obtained from the Protein Data Bank (PDB entry code 1S3E).[17] The same procedure was applied to a 1S3E crystal structure as applied to 1GOS structure above, except that an additional side-chain optimization was performed. This treatment optimized the 1E3S structure further and the conformational changes resulting from binding of the original inhibitor in the crystal structure were partially removed.

2.4. Ligands

For docking experiments with AutoDock 3.0.5, ligand molecules were drawn, optimized, and saved as in mol2 format with the aid of Spartan[20] and VEGA programs.[21] Full hydrogens were added to the ligands and Gasteiger[22] partial atomic charges were computed using the VEGA program and saved in the required format. All possible flexible torsions of the resultant ligand molecules were defined by using AUTOTORS. The prepared ligands were used as input files for AutoDock 3.0.5 in the next step.

2.5. Docking

Docking simulations were performed with AutoDock 3.0.5 using a Lamarckian genetic algorithm.[23] The standard docking procedure was used for a rigid protein and a flexible ligand whose torsion angles were identified (for 10 independent runs per ligand). A grid of 60,60, and 60 points in x, y, and z directions was

Table 1. AutoDock estimated free energies of binding (ΔG_b), calculated [K_i (calculated)] and experimental [K_i (experimental)] inhibition constants of the studied inhibitors (temperature = 298.15 K)

Inhibitors	ΔG_b (kcal/mol) (calculated)		K_i (μM) (calculated)		K_i (μM) (experimental)	Ref.
	1GOS	1S3E	1GOS	1S3E		
1	−8.24	−8.39	0.907	0.708	0.7	26
2	−7.75	−7.90	2.08	1.62	17	26
3	−8.79	−8.45	0.359	0.636	0.6	26
4	−7.29	−7.47	4.55	3.33	1.8 ± 0.20	27
5	−8.38	−8.22	0.717	0.946	0.97 ± 0.13	27
6	−12.04	−10.70	0.436 (nM)	10.2 (nM)	100 (nM)	28
7	−9.63	−9.38	0.0876	0.133	0.600	29
8	−7.39	−7.51	3.84	3.11	3.0	30
9	−12.07	11.97	1.43 (nM)	1.67 (nM)	14 (nM)	31
10	−8.10	−8.33	1.16	0.781	0.084	32

1GOS: 3.0 Å resolution, 1S3E: 1.6 Å resolution.

built, centered on the center of the mass of the flavin (FAD) N5 atom on the catalytic site of the protein. A grid spacing of 0.375 Å and a distance-dependent function of the dielectric constant were used for the calculation of the energetic map. The default settings were used for all other parameters. At the end of docking, ligands with the most favorable free energy of binding were selected as the resultant complex structures. All calculations were carried out on PC-based machines running Linux x86 as operating systems. The resultant structure files were analyzed using Rasmol[24,25] visualization programs.

3. Results

Molecules **1**–**10** were successfully docked onto the active site of MAO-B, according to the above docking protocol. Table 1 shows the results of the docking experiments: calculated free energy of binding, inhibition constants for each complex (with 1GOS and 1S3E), and their corresponding experimental inhibition constants. Rasagiline **1**, *N*-propargyl-1(*R*)-aminoindan, was docked into the active site of the MAO-B enzyme (Fig. 1). A careful inspection of the binding pocket indicated that rasagiline adopted a position in a hydrophobic cage surrounded by Tyr398, Tyr435, Tyr188, Cys172, Tyr60, and Phe 343. The indan ring of rasagiline is perpendicular to the *re* face of the covalent FAD, which itself forms the amine binding site. The aromatic moiety of the rasagiline interacts with the side chains of the residues of Tyr398, Tyr435, and π–π via Tyr188. The propargyl group of rasagiline was aligned on the N5 atom of the FAD. Cys172, Tyr60, and Phe343 also contributed some of the interactions to stabilize the complex. Figure 2 shows 6-hydroxy-*N*-propargyl-1(*R*)-aminoindan **2** in the active site of the MAO-B enzyme. The indan ring was sandwiched between the Tyr398 and Tyr435. The 6-hydroxy group of the indan ring was positioned to make the hydrogen bond to Cys172 and Tyr435. The propargyl group of compound **2** was oriented a little further away compared to compound **1**. The other principal interactions of compound **2** were the same as those of compound **1**. Figure 3 shows the final docked position of *N*-methyl-*N*-propargyl-1(*R*)-aminoindan **3**. In this case, the indan ring was oriented vertically in the hydrophobic cage on the *re* face of FAD. The propargyl group of indan is in close proximity to the N5 atom of the FAD. Pargyline (**4**) was docked, as shown in Figure 4. The phenyl ring of the pargyline was placed between Tyr398 and Tyr188. The propargyl group extends down from the phenyl ring to the *re* face of FAD. Figure 5 shows the binding pattern of selegyline (l-deprenyl) **5**. A similar binding behavior was observed fast as pargyline, except for the phenyl ring, which was bent backwards 90°. Another interesting molecule, 8-(3-chlorostyryl)-caffeine **6** which acts as a potent competitive MAO-B-specific inhibitor[26], was docked into the active site of the MAO-B enzyme, as shown in Figure 6. From the figure, one can see that the caffeine ring was positioned toward the *re* face of FAD. The 3-Chlorostyryl group extended away from the hydrophopic cage and was located between the residues Phe168, Tyr326, and Cys172. The 1,4-diphenyl-2-butene **7**, which is a contaminant of polystyrene, was docked as shown in Figure 7. It was observed that the one of the phenyl moieties of compound **7** was positioned between Tyr398, Tyr435, and Tyr188 in the substrate cavity space. The other phenyl moiety made a strong π–π interaction with the residues of Phe168 and Tyr326. Apparently, the residue Ile199 may contribute to the binding and stabilization of these compounds in the entrance cavity space of MAO-B. The binding mode of reversible MAO-B inhibitor isatin (indol-2,3-dione) **8** is shown in Figure 8. The 1S3E crystal structure picture is visualized here. The indol ring of isatin was positioned between Tyr435 and Tyr398 hydrophobic cage such that the amine group located itself on the *re* face of FAD cofactor. This position satisfied the minimum distance between isatin's nitrogen atom and FAD's N5 atom. Figure 9 shows the optimal binding mode of 3-methyl-8-(4,4,4-trifluoro-butoxy)indeno[1,2-*c*]pyridazin-5-one **9** with MAO-B (1S3E: 1.6 Å crystal structure resolution). The indeno[1,2-*c*]pyridazin-5-one nucleus was sandwiched between Tyr398 and Tyr435. Hydrogen bondings between the carbonyl and pyridazine functional groups of indeno[1,2-*c*]pyridazin-5-one, and Tyr188, Tyr398, and Tyr435 are important, as well as the hydrophobic interaction. Trifluorobutoxy side chain extended itself along the entrance cavity. Finally, we performed docking of the reversible inhibitor

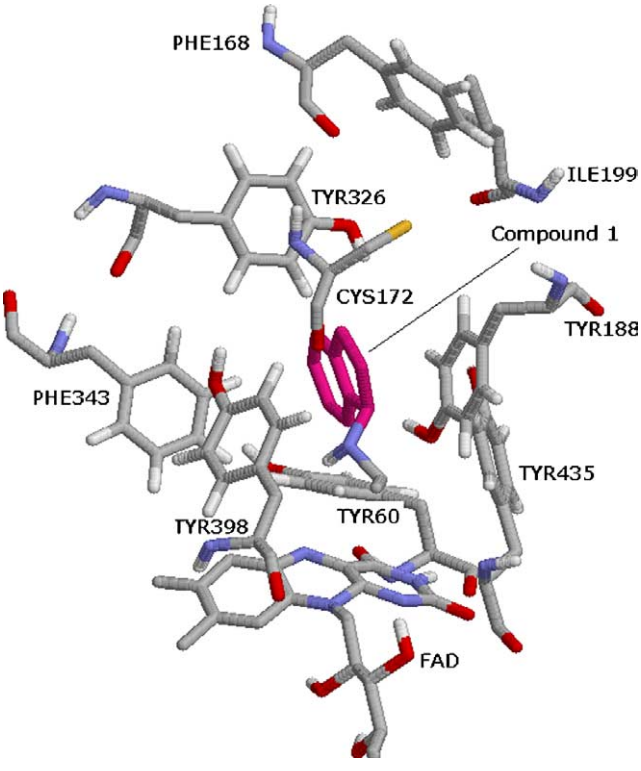


Figure 1. Docking result of *N*-propargyl-1(*R*)-aminoindan **1** (Rasagiline) with MAO-B. The inhibitor, FAD, and the important residues in the active site of the enzyme are presented by stick model.

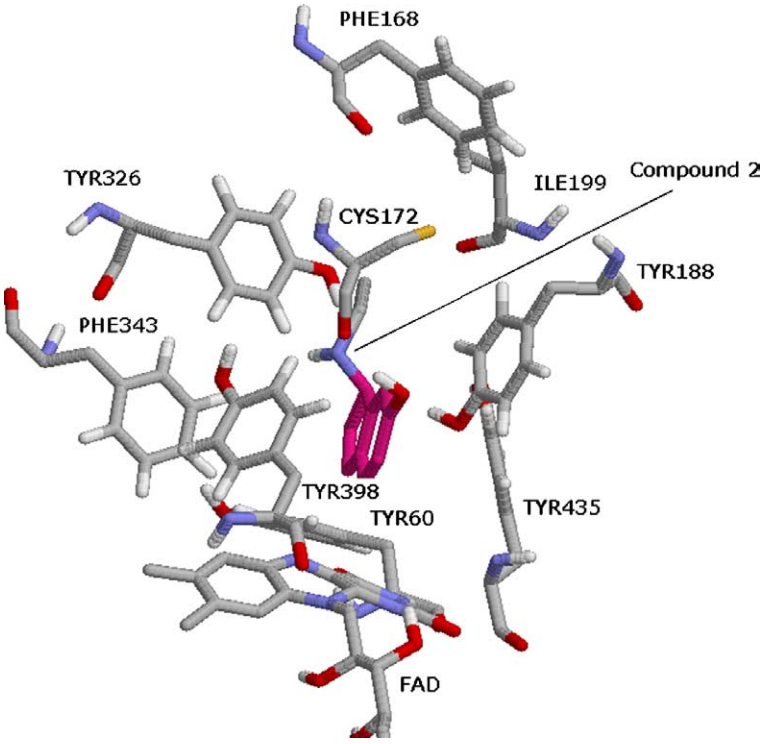


Figure 2. The interacting mode of 6-hydroxy-*N*-propargyl-1(*R*)-aminoindan **2** in the active site of MAO-B enzyme.

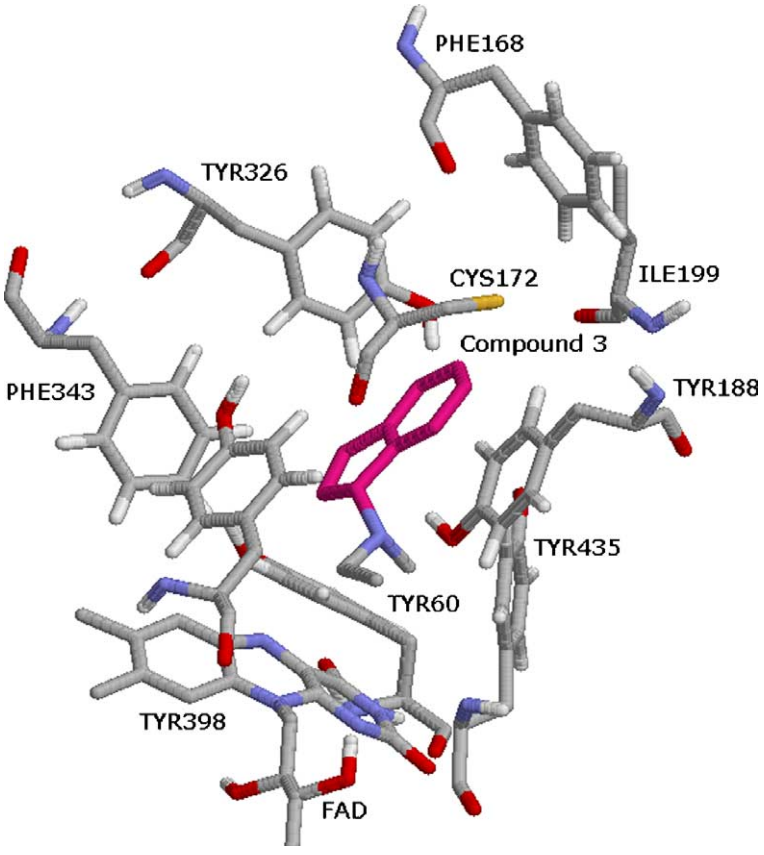


Figure 3. Probable interacting mode of *N*-methyl-*N*-propargyl-1(*R*)-aminoindan **3** with the active site of MAO-B.

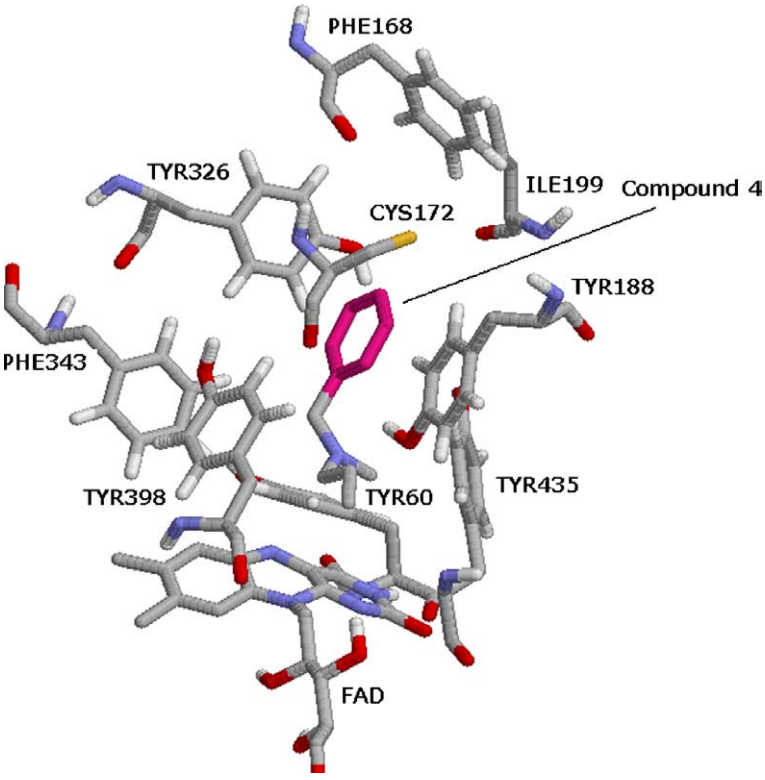


Figure 4. The interacting mode of propargyline **4** and its alignment in the active site of the enzyme.

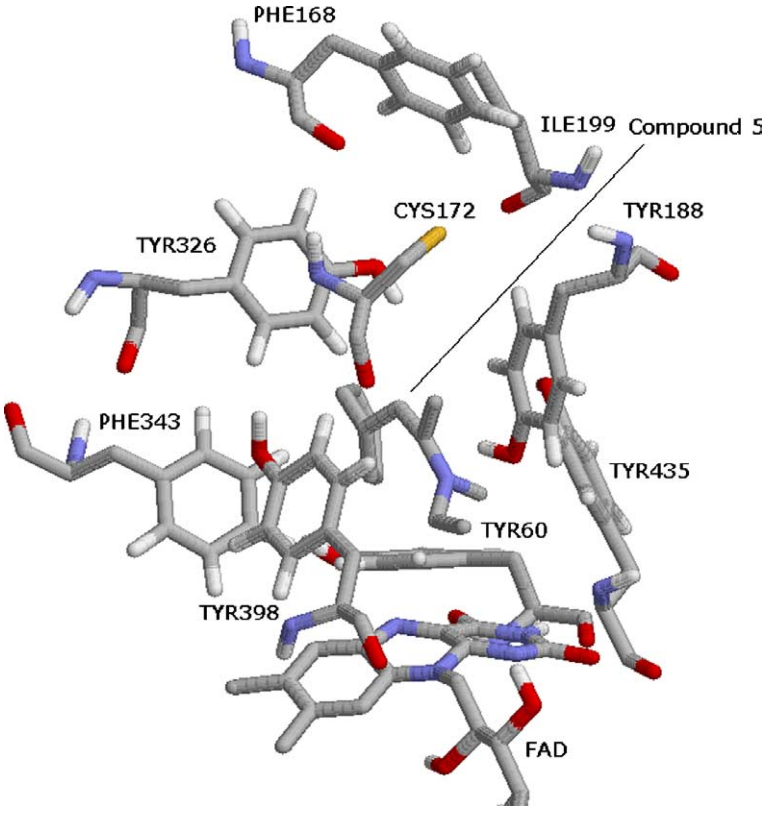


Figure 5. Docking results of selegyline **5** with the active site of the enzyme.

lazabemide **10** into the active site of the MAO-B (1S3E) (Fig. 10). The docking study has revealed that lazebemide **10** localized its interaction with Tyr188. Its π–π aromatic nucleus away from the FAD cofactor making side chain including primary amine and amide moiety was positioned in the vicinity of FAD cofactor

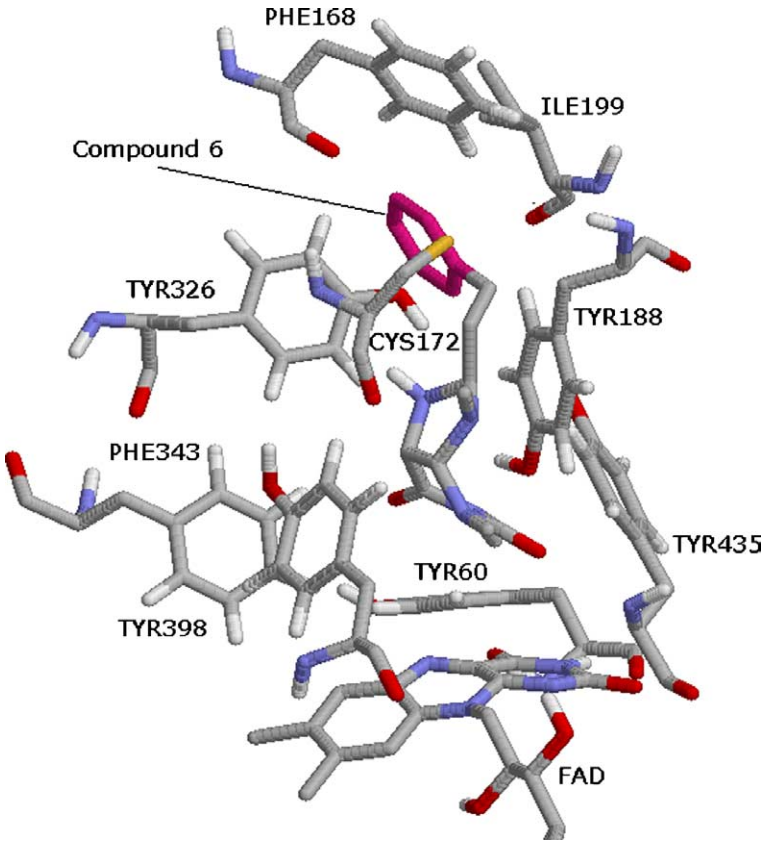


Figure 6. The interacting mode of 8-(3-chlorostyryl)-caffeine **6** with MAO-B. The important residues of the enzyme and the inhibitor are depicted by stick model.

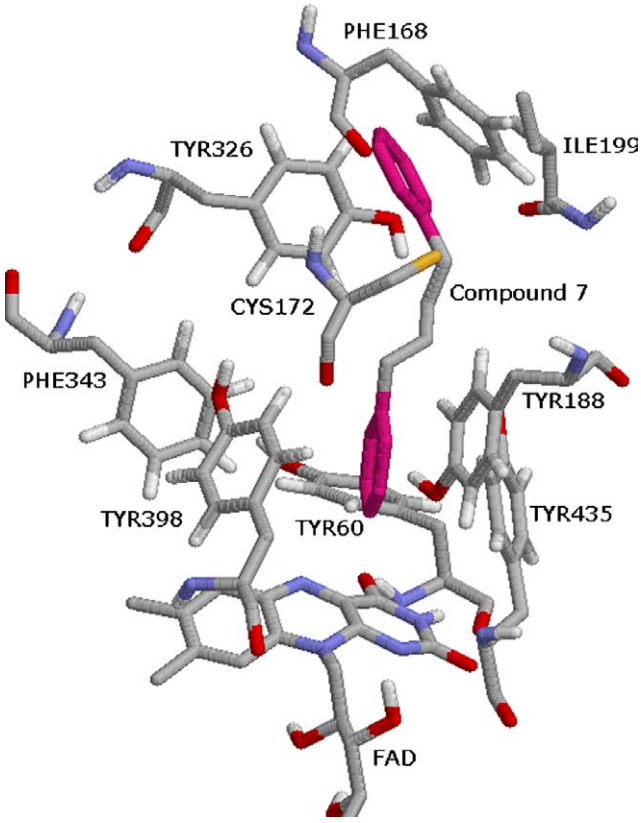


Figure 7. The binding conformation of 1,4-diphenyl-2-butane **7** with MAO-B.

approaching the N5 atom of FAD cofactor as closely as possible (see Chart 1).

4. Discussion

The objective of this study was to implement an AutoDock simulation program to calculate the binding free energies and inhibition constants of experimentally tested MAO-B inhibitors and to compare these computational results with those of the experimentally obtained results. Purified human recombinant MAO-B crystal structure (1GOS), with a 3.00 Å resolution, and crystal structure (1S3E) with a 1.60 Å resolution were used throughout the Autodock simulation study.[13] Experimental inhibition constants were also obtained using this enzyme, [26] except in the case of compounds **4**[27] and **5**,[27] whose experimental inhibition constants were obtained using rat brain MAO-B.[27] Much better results were obtained with a high-resolution crystal structure (1S3E) compared to the results using a 1GOS crystal structure. To check the versatility of the Autodock 3.0.5 docking program, both mechanism-based irreversible and competitive reversible (**7**, **8**, and **9**) inhibitors were used as model compounds. Co-crystallization of mechanism-based irreversible inhibitors within the active site of both enzyme forms may cause some conformational changes of the active site. After the removal of mechanism-based irreversible inhibitors from the active site of both enzyme forms, polar hydrogens were

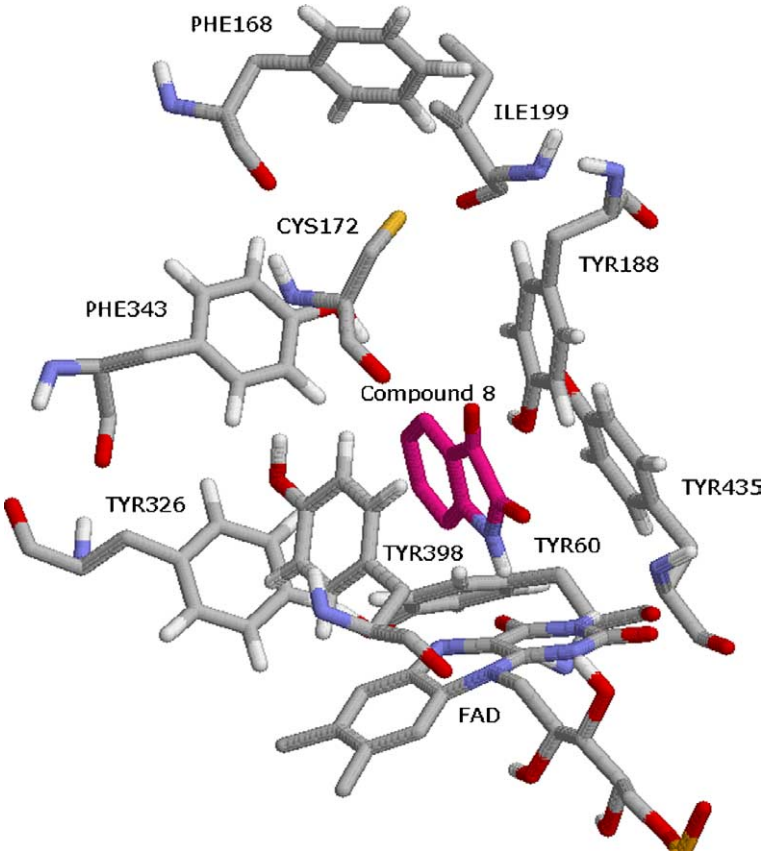


Figure 8. The binding conformation of indol-2,3-dione (isatin) **7** with MAO-B (1S3E: 1.6 Å resolution).

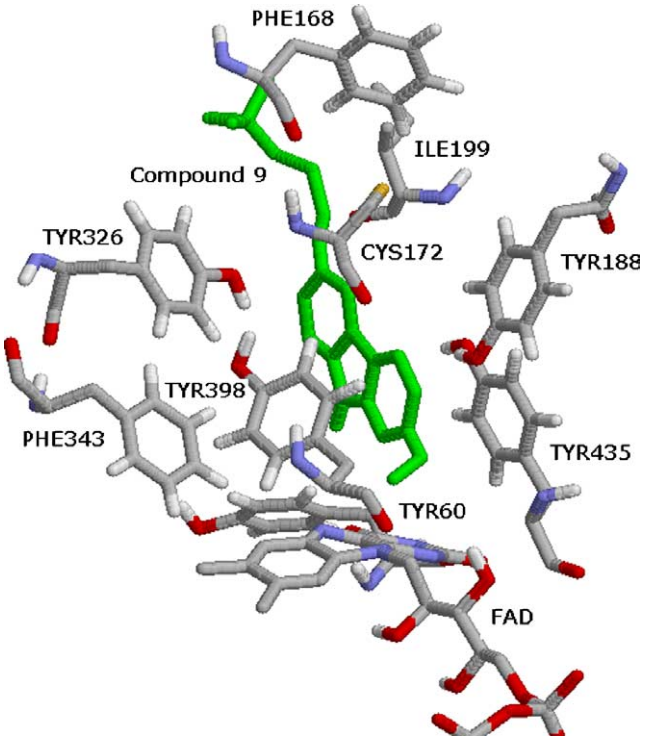


Figure 9. The binding conformation of 3-methyl-8-(4,4,4-trifluorobutoxy)indeno[1,2-*c*]pyridazin-5-one **9** with MAO-B (1S3E: 1.6 Å resolution).

added and optimized. In the case of 1S3E, in addition to the polar hydrogens, the side chains of amino acids were also optimized to imitate the unbound conformation of the active site. The 1GOS results were given only for comparative purposes. Only high-resolution results will be discussed here. As seen from Table 1, an excellent correlation was observed between the estimated K_i values of compound **1**, **3**, **5**, and **8**[30], and their experimental inhibition constants. Reasonable values of estimated inhibition constants were obtained in the case of compounds **2**, **4**, **6**, **7**, **9**[31], and **10**.[32] Compound **2** 10.2-fold, compound **6** 9.8-fold, compound **7** 4.5-fold, and compound **9** 8.4-fold have lower K_i values than that of the experimental values. Compound **4** has a 1.7-fold and compound **10** 9.3-fold have higher K_i values than that of the experimental values. It was experimentally determined that MAO-B from different species does not exhibit the same inhibitor specificities for a particular inhibitor. In this study, we used recombinant human MAO-B crystal structure. However, as some of the available experimental results were obtained on MAO-B isolated from different species. With regard to the results obtained for compounds **1**, **3**, **5**, and **8**, in favorable cases, excellent correlations seem to be possible. However, in some other cases an acceptable agreement with the reported results was obtained. This might be the result of simplifications used in the AutoDock program: no explicit water molecules are considered during docking, and solvation and entropic effects were not taken into account. The orientations of these inhibitors in the

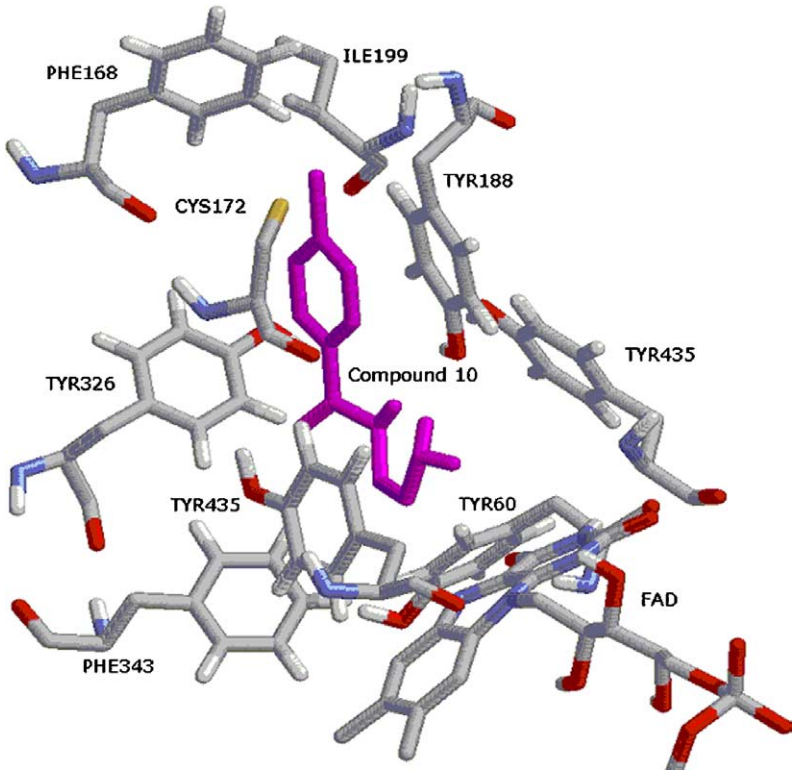
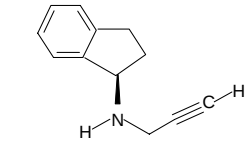
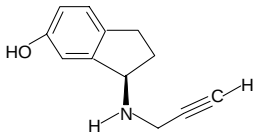


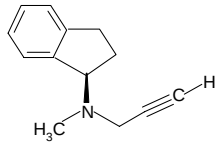
Figure 10. The binding conformation of lazabemide **10** with MAO-B (1S3E: 1.6 Å resolution).



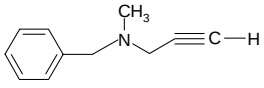
N-propargyl-1(R)-aminoindan (Rasagiline) (**1**)



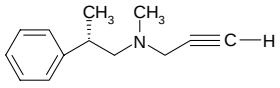
6-hydroxy-N-propargyl-1(R)-aminoindan (**2**)



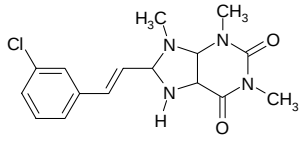
N-methyl-N-propargyl-1(R)-aminoindan (**3**)



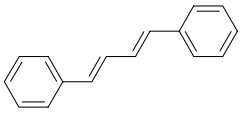
Pargyline [Benzyl(methyl)prop-2-ynylamine] (**4**)



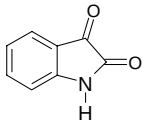
Selegiline (l- deprenyl) (**5**)



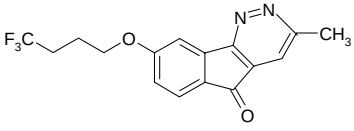
8-(3-Chlorostrylyl)caffeine (**6**)



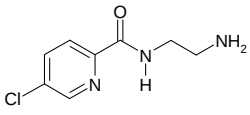
1,4-Diphenyl-1,3-butadiene (**7**)



Indol -2,3-dione (Isatin) (**8**)



3-Methyl-8-(4,4,4-trifluoro-butoxy)indeno[1.2-c]pyridazin-5-one (**9**)



Lazebemide (**10**)

Chart 1. Structures of experimentally tested MAO-B inhibitors used in the study.

active site are also very important, with their Ki values, for rational drug design. Careful observations of the figures reveal that in most of the cases, inhibitor positioning in the active site sits reasonably well. The data obtained by the AutoDock studies here are thought to be important for our continuing research efforts in the design and synthesis of new, selective, and reversible inhibitors for MAO-B.

5. Conclusions

Ten MAO-B inhibitors were successfully docked onto the active site of purified recombinant human MAO-B enzyme. The free energy of binding and the inhibition constant of each complex were calculated using the Autodock 3.0.5 docking program. The obtained K_i values of 10 experimentally tested inhibitors agree reasonably well with previous data in the literature on the inhibition of MAO-B. These studies provide us with an important approach for predicting the inhibition constants of newly designed and previously untested MAO-B inhibitors.

Acknowledgments

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