Life Science Informatics Publications

Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences

Journal Home page http://www.rjlbpcs.com/

RJLBPCS

ISSN 2454-6348

Original Research Article **DOI - 10.26479/2017.0302.10**

SELEGILINE BINDING TO MONOAMINE OXIDASE ENZYME IN THE REAR VIEW MIRROR

J Nagy [1, 2], H Kalász [2*]

1.Liver Studies Unit and Molecular Biology Division,

Veteran Administration Medical Center, San Francisco, CA, USA

2.Department of Pharmacology and Pharmacotherapy, Semmelweis University,

1089 Budapest, Nagyvárad tér 4, Hungary.

ABSTRACT: The oxidation of various endogenous monoamine compounds is catalyzed by MAO [monoamine oxidase enzyme, EC 1.4.3.4], that exist in multiple forms, such as MAO-A and MAO-B. MAO-B catalizes the breakdown of benzylamine and phenylethylamine, while both MAO-A and MAO-B play an essential role in the oxidative elimination of tyramine, triptamine and dopamine. One therapeutic target of MAO enzymes is to reduce their catalysis of the breakdown of dopamine. Knoll and Magyar discovered that MAO-B is highly sensitive to selegiline [[-]-deprenyl], which is its irreversible inhibitor. Singer, Nagy and Salach identified the amino acid sequence of this peptide and also the active site of flavin-peptide complex, which is the binding site of MAO inhibitors. Flavin-peptide unit of placental MAO was isolated using proteolytic digestion, treatment with trichloroacetic acid, followed by a three-step chromatographic separation using Florisil, Bio-Gel P-2 and Sephadex G-15. MAO binding sites were located using x-ray diffraction, and they were identified using translational neuroimaging; positron emission tomography studies of monoamine oxidase + [^{11}C]-compounds and tracing ligand-binding active sites of irreversible inhibitors: clorgyline, selegiline and their derivatives. [^{14}C]-labeled deprenyl served the use of whole-body autoradiography, while Nag et al synthesized fluoro-labeled ligands, such as deuterated [^{18}F]-fluorodeprenyl and fluororasagilin for positron emission tomography.

KEYWORDS: [-]-Deprenyl; Selegiline; MAO-A; MAO-B; Binding site location; Parkinson's disease.

***Corresponding Author: Dr. H Kalász, Ph.D.**

Department of Pharmacology and Pharmacotherapy, Semmelweis University, 1089 Budapest, Nagyvárad tér 4, Hungary, * Email Address: drkalasz@gmail.com

Peer review under responsibility of Life Science Informatics Publications

1. INTRODUCTION

Certain biogenic amines [such as noradrenaline, tyramine, dopamine, serotonin, etc.] are of basic importance in the living organism. Their local level is given by the rate of their generation and elimination. Dopamine is mainly produced from tyrosine [by tyrosine hydroxylase] through L-DOPA [by aromatic L-amino acid decarboxylase] and it eliminates both monoamine oxidase/aldehyde dehydrogenase and catechol-O-methyltransferase [1]. The equilibrium of rates of dopamine production versus its elimination has vital importance as excessive dopamine levels, i.e. those above the optimum, can lead to the desensitization of its receptors, while levels of dopamine below the required one's results in pathological signs, such as hypokinesia, etc. [2]. A rather frequently occurring problem is a decreased dopamine production in the brain, especially in senescense [3] and certain pathological cases, such as in Parkinson's disease. There are two ways of treatment for these conditions, either the administration of a dopamine agonist, or the restoration of the equilibrium of production/elimination of dopamine. The former possibility is giving DOPA, or MAO inhibitors [4]. Activity of MAO has essential importance in both the periphery [where MAO-A has an essential role]. MAO-B has its function primarily in the central nervous system. Several papers have been published about the controversial values of MAO in the success of promoting pregnancy. Low levels of MAO are preferential in the testis [5], and the treatment with MAO inhibitor significantly increases both the number and motility of sperms as well as the number of off-springs of rats. At the same time, high levels of MAO can contribute to the success of pregnancy [6]. Parkinson's disease is a human neurodegenerative disorder. It starts with a barely noticeable tremor. Progress in Parkinson's disease results in stiffness or slowing movement. Motor symptoms are produced as a consequence of decline in dopamine in the brain [especially in *striatum* and *substantia nigra*] [7]. Decreased dopamine levels in the brain is characteristic of Parkinson's disease [8] and senescence. The monoamine oxidase effect of oxidizing tyramine was first published by Mary Hare as early as in 1928 [9]. Monoamine oxidase is an enzyme of the mitochondrial membrane that catalyses oxidative deamination reactions:

$$\mathbf{RCH_2NH_2 + H_2O + O_2 \rightarrow RCHO + NH_3 + H_2O_2}$$

In the central nervous system, the neurotransmitters of catecholamine-type [adrenaline, dopamine, noradrenaline], serotonin and β-phenylethylamine belong to the most important substrates of MAO. There are two major types of the MAO enzymes, MAO-A [sensitive even to low levels of clorgyline] and MAO-B [relatively insensitive to clorgyline]. MAO-A plays an essential role in the deamination of tyramine and serotonin, while MAO-B plays a principal role in the catabolism of dopamine and β-phenylethylamine in the brain [8]. The administration of L-DOPA temporarily diminishes motor symptoms. Another way of regulation is the inhibition of MAO-B activity, which results in an increased level of dopamine in the brain. In 1971, Knoll and Magyar [10] showed that selegiline selectively inhibits the B-isoform of monoamine oxidase, thereby it is unlikely to cause "cheese effect" [hypertensive crisis]. Both selegiline, and desmethylselegiline are irreversible and selective

inhibitors of monoamine oxidase type B [MAO-B] [11]. A few years later, Birkmayer and Riederer realized that selegiline could be useful in the treatment of Parkinson's disease, the Birkmayer's group published the first paper on the effect of selegiline in Parkinson's disease [12].Pharmacologically active MAO inhibitory compounds are either antidepressant [13] or antiparkinsonian [14] drugs, or are administered in both disorders [depression and parkinsonism]. Generally used MAO-B inhibitory drugs are selegiline [L-deprenyl] and rasagiline [15]. This review outlines the progress of how the binding sites of MAO inhibitors are localized and characterized.

2. MATERIALS AND METHODS

Progress of discovery and precise description of binding sites of MAO-A and MAO-B developed as various new techniques were applied. Autoradiography was used to locate and evaluate the binding of deprenyl to MAO-B. ^{14}C-labeling substituting the carbon of N-methyl group [16] and ^{11}C-labeling [17, 18] were generally used. Grimsby et al [19] cloned chimeric MAO-A and MAO-B; their enzymatic activity and inhibition with clorgyline and deprenyl were determined.Mewies et al [20] used fluorescence emission to identify flavoproteins. They employed sequencing of the protein, and molecular graphics to illustrate flavoproteins. Description of the expression, preparation and characterization of human liver recombinant orthorhombic crystals of MAO-B can be found in the publication of Newton-Vinson et al [21]. Enzyme-inhibitor crystals of MAO-B were produced by incubation [using 2 mM of inhibitor], and then purification, using gel chromatography on a Sephadex G-200 column. Edmondson et al [22] used recombinant human MAO-B [crystallized as a dimer]. The active site cavities were modeled by computer, using a GRID program [23]. Milczek et al [24] crystallized mutant MAO-A and MAO-B enzymes in the presence of methylene blue in a specific solution.

3. RESULTS AND DISCUSSION

Isolation, spectroscopy and sequencing

Nagy et al [25, 26] used spectroscopic methods to prove that the free flavin segment of trimethylamine dehydrogenase is the binding site in its reaction with phenylhydrazine. The flavin binding site of monoamine oxidase was identified using ^{14}C-labeled ligand [^{14}C-phenylhydrazine] [26], and spectrophotometric methods. Nagy and Salach [27] isolated and prove high similarity of the active site of human A-form, and bovine B-form of MAO enzymes. The isolation of the human placental MAO-A included the following steps: the umbilical cord and amniotic membrane from fresh human placenta were removed; the tissue was minced and homogenized in the appropriate buffer, and differential centrifugation at 1600g was applied collecting the supernatant and, at 28,000g, the pellet. The pellet was then washed with the sucrose phosphate suspension medium as well as with KCl-Tris, resulting in the preparation of mitochondria [27]. A Triton X-100 extract was then prepared, treated with phospolopase A and C. The solution also contained 5 mM dithioerythrol, kept at 23 ºC for 30 min, and subjected to centrifugation at 100,000 g for 30 min. Addition of solid

PEG and dextrane was followed by further centrifugation, addition of 25% PEG resulted in the precipitation of the enzyme, that could be dissolved in 0.1 M TEA. Sucrose gradient centrifugation [for 16 hours] resulted in the human MAO-A enzyme of purity that enables it to be structurally analyzed using digestion with trypsin and chymotrypsin. The tryptic-chymotryptic peptide was subjected to chromatography on florisil, followed by successive gel chromatography on Bio-Gel P-2 and Sephadex G-15. Two fractions were resulted by gel chromatography containing FMN- [flavine mononucleotide], and riboflavin forms of the same peptide. Structural elucidation of the peptide gave a pentapeptide [Ser-Gly-Gly-Cys-Tyr] and the flavin was connected to the Cys through its sulphur. Earlier publications of the same research group identified the amino acid sequence of the flavin region of the beef liver MAO-B enzyme, which was a pentapeptide as given above. Nagy and Salach [27] found the same sequence, thereby verifying that it is a conserved region in both human MAO-A and human MAO-B. Active site of monoamine oxidase B enzyme was intensively studied about half a century ago. Singer et al [28] studied irreversible inhibitors of monoamine oxidase by pargyline, clorgyline, deprenyl and certain other amines. The prosthetic group of this enzyme contains FAD [flavin adenine dinucleotide] [29] which is bound to an apoprotein [30]. Isolation of a flavin-containing peptide resulted from the product tryptic-chymotryptic digestion [31], and even the amino acid sequence of this flavin region was successfully identified [32]. Hydrazines were known to inhibit certain metabolisms of biogenic amines [33] and mitochondrial monoamine oxidase. Trimethylamine dehydrogenase and mammalian monoamine oxidase share several characteristics [34]. Nagy et al [25, 26] used spectroscopic methods to prove that the free flavin segment of trimethylamine dehydrogenase is the binding site in its reaction with phenylhydrazine. The flavin binding site of monoamine oxidase was identified using ^{14}C-labeled ligand [[^{14}C]-phenylhydrazine] [26], and spectrophotometric methods.

FAD, the prosthetic group

Mewies et al [20] looked over the five different modes of covalent links of flavin adenine dinucleotide and flavin mononucleotides. Edmondson et al [22] confirmed the results of Nagy et al [25, 26] and gave further details of the FAD binding site of MAO-B. The 1.7 Å structure of recombinant human MAO-A was chrystallized as a dimer, and it contained three functional binding domains, such as flavin b.d. [binding domain], substrate b.d. and a membrane b.d. Fowler et al [17] used positron emission tomography to study the MAO-B binding of [^{11}C]-L-deprenyl. SDS-PAGE [sodium dodecyl sulfate polyacrylamide gel electrophoresis] indicated that the molecular size of the binding protein was 58,000.

Chimeric Experiments

Grimsby et al [19] were searching the binding domains of both MAO-A and MAO-B. They constructed and expressed two chimeric proteins that confer selectivity to the inhibitor and substrate selectivity. Using special replacements, they found that amino acids 152-366 contain substrates and

the inhibitory region of MAO-B.

X-Ray Structural Elucidations

Binda et al [35] gave the 1.7-Å three-dimensional structure of the monomeric form of the human MAO-B together with its bound compound [1,4-diphenyl-2-butene]. Formation of the binding flavin segment depends on the structure of the bound ligand, as it was demonstrated by the high resolution electron density pictures for the complexes of isatin, N[2-aminoethyl]-p-chlorbenzamide and trans-2-phenylcyclopropylamide. Binda et al [36] published a 1.65 Å resolution structure of the wild type recombinant human monoamine oxidase B enzyme. of the three-dimensional structure of the dimer human MAO-B enzyme, which is bound to the membrane. This enzyme has several apolar loops, one of them has the function to either open or close the cavity of the MAO-B active site, thereby regulating substrate binding. Hubalek et al [37] investigated the effect of changes in cysteine residues on NEM (N-ethylmaleimide). Certain cysteine residues are involved in covalent connection either to FAD or FMN, and 7 cysteine residues out of 9 are in the conserved region of either MAO-A or MAO-B. NEM-caused inactivation was much faster [$\tau_{½} \approx 3$ min] in the case of MAO-A than in that of MAO-B [$\tau_{½} \approx 8$ h]. Furthermore, they stated that the inactivation of MAO-A and MAO-B by either clorgyline or pargyline increases their structural stability. Milczek et al [24] verified that MAO-B has a bipartitate cavity where the cavity of the entrance is about 290 $Å^3$ and the substrate cavity is about 400 $Å^3$. Ile199 and Tyr326 side chains separate the two cavities. Milczek et al [24] prepared mutant forms of MAO-B: Ile199Ala and Ile199Ala Tyr326Ala. These mutant forms showed weaker binding affinity compared to the WT [wild type] enzyme when small and reversible inhibitors were used. At the same time, Ile199Ala-Tyr326Ala [i.e. double mutants] exhibited binding properties to inhibitors similar to that of MAO-A rather than to MAO-B.

Dock Program

Toprakci and Yelekci [38, 39] used an AutoDock 3.0.5 program [40]. Dock 6.0.0 program was used by Harkcom and Bevan [41]. Re-docking of 30 MAO inhibitors into the crystal structure was modeled, and good agreement was found between the calculated and experimental [grid code] results. De Colibus et al [33] determined the ligand-binding active site of the single hydrophobic cavity of hMAO-A [human A type monoamine oxidase enzyme] and hMAO-B [human B type monoamine oxidase enzyme], which were about 550 $Å^3$ and 700 $Å^3$, respectively. Both cavities are larger than that of the rat MAO-A [about 450 $Å^3$]. Su et al [42] used a web server for predicting the binding site. Yelekçi et al [39] used computer-aided docking of twelve reversible MAO-B inhibitors at the active binding site of the MAO-B enzyme. They showed the bound form in a three-dimensional picture; calculated the free energy of binding [ΔG_b], and the inhibition constant. Edmondson et al [43] made a structural insight to elucidate the mechanism of amine oxidation by MAO-A and MAO-B. They opened the possibility of two distinct mechanisms. The preferred and more accepted conclusion was that amine oxidation took place using a polar nucleophilic mechanism. Another, less preferred

variation was the single electron transfer.

Usage of PET

Tong et al [18] used [^{11}C]-deprenyl for PET [positron emission tomography] images to see the distribution of MAO-B in the human brain and the results were compared to the distribution of MAO-A, using [^{11}C]-befloxatone, [^{11}C]-clorgyline and [^{11}C]-harmine. MAO-A was abundant in infants' brains, while MAO-B levels increased faster before the age of 1 year. [^{11}C]-Deprenyl, too, was used as a biomarker imaging the up-regulation of MAO-B in neuroinflammation when applying PET by Gulyás et al [44]. Binding of [^{11}C]-deprenyl to post mortem brains of Alzheimer's patients [AD] was compared to that of controls [brains without pathology]. The specific binding of the control brains was higher in various brain regions [thalamus, nucleus subthalamicus] than in the brains of patients with AD. AD brains showed higher specific binding than the controls in certain other brain segments [hippocampus, frontal lobe, temporal lobe, parietal lobe, insula, substantia nigra, white matter]. The authors stated the advantages of [^{11}C]deprenyl as a prospective biomarker in molecular imaging techniques. Further improvements of this biomarker technique were applied by Vasdev et al [45] and Nag [46]. Vasdev [45] used [^{18}F]-labeled compounds for imaging the MAO-B of a rodent brain, while Nag et al [46] utilized [^{18}F]-labeled 4-fluorodeprenyl and [^{18}F]-labeled fluororasagiline *in vivo* binding in a cynomolgus monkey [*Macaca fascicularis*] brain. Nag et al [46, 47] have recently shown that a high density of MAO-B in the brain causes increased levels of [^{18}F]-4-fluorodeprenyl and [^{18}F]-4-fluororasagiline in the brain. Our most recent experimental finding [48, 49] also confirmed the high incorporation of both deprenyl and 4-fluorodeprenyl in the brain relative to the serum. An indirect proof of deprenyl binding to MAO-B was given by Lu et al [50]. A series of selegiline-tacrine hybrids were synthesized, which showed definite binding to the monoamine oxidase B enzyme. One of their compounds became candidate for a potential drug to be used for Alzheimer's disease.

4. CONCLUSION

MAO binding sites were first identified by isolation, using chromatography, spectroscopy and sequencing. A further essential step was made running an Auto Dock program, while the PET image method completed the experimental stereo-chemical location of MAO-A and MAO-B binding sites.

CONFLICT OF INTEREST

The authors have no conflict of interest.

ACKNOWLEDGEMENT

This work was supported by the grant of Hungarian National Granting Agency (OTKA No. 100155). The authors thank Professor Kornélia Tekes for her suggestions and corrections and Mr. János Horváth for his advice. The authors of this paper congratulate Professor József Knoll on his 92nd birthday. Professor Knoll was the inventor of the pharmacology of [-]-deprenyl [selegiline], and his papers led to the characterization of the unique specificity of deprenyl. Professor Knoll has been our

mentor while we were working on the pharmacokinetics and metabolism of [-]-deprenyl, and also throughout our entire practical and scientific activity in pharmacology.

REFERENCES

[1] Damrauser SM, Galanter JM, Cardozo DL: Pharmacology of Dopaminergic Neurotransmission, in Principles of Pharmacology, The Pathophysiologic Basis of Drug Therapy, Golan DE, Tashjian AH, Jr., Armstrong EJ, Galanter JM, Armstrong AW, Arnaout RA, Rose HS (Eds.), Lippincott Williams & Wilkins, Philadelphia, 2004, pp. 162-164.

[2] Lanca AJ: Drugs that Modify Movement Control, in Principles of Medical Pharmacology, Kalant H, Grant DM, Mitchell J (Eds.), Elsevier Canada, Toronto, 2007, pp. 211-222.

[3] Ehringer H, Hornykiewicz O. Verteilung Von Noradrenalin und Dopamin [3-Hydroxytyramin] im Gehirn des Menschen und ihr Verhalten bei Erkrankungen des Extrapyramidalen Systems. Klin Wochenschrift 1960; 38:1236-1239.

[4] Aminoff MJ: Pharmacologic Management of Parkinsonism & Other Movement Disorders, In Basic and Clinical Pharmacology, Katzung BG, Ed., McGraw Hill, New York, 2007, pp. 442-456

[5] Roberge AG, Moufarege A, Lavoie J, Roberge C, Tremblay RR. Biochemical Properties and Kinetic Parameters of Monoamine Oxydase in Human Seminal Plasma. Int J Fertil. 1984; 29:180-185.

[6] Tylden E. Pregnancy and Monoamine Oxidase Inhibitors. Br Med J. 1968; 2:698.

[7] Lanca AJ. Drugs that Modify Movement Control, In Principles of Medical Pharmacology, Kalant H, Grant DM, Mitchell J, Eds., Elsevier, Amsterdam, 2007, p. 211.

[8] Bloom FE. Neurotransmission and the Central Nervous System, In Goodman & Gillman's The Pharmacological Basis of Therapeutics, Brunton LL, Lazo JS, Parker KL, Eds., 11th Ed., McGraw Hill, New York, 2006, pp. 317-340.

[9] Hare ML. Tyramine Oxidase: A New Enzyme System in Liver. Biochem J. 1928; 22:968–979.

[10] Knoll J, Magyar K. Some Puzzling Pharmacological Effects of Monoamine Oxidase Inhibitors. Adv Biochem Psychopharmacol. 1972; 5:393-408.

[11] Heinonen EH, Anttila MI, Karnani HL, Nyman LM, Vuorinen JA, Pyykkö KA, Lammintausta RA: Desmethylselegiline, a Metabolite of Selegiline, is an Irreversible Inhibitor of Monoamine Type B in Humans. J Clin Pharmacol. 1997; 37(7): 602-609.

[12] Birkmayer W, Riederer P, Youdim MB, Linauer W. The Potentiation of the Anti-Akinetic Effect after L-DOPA Treatment by an Inhibitor of MAO-B, Deprenil. J Neural Transm. 1975; 36:303–326.

[13] Mann JJ, Aarons SF, Wilner PJ, Keilp JG, Sweeney JA, Pearlstein T, Frances AJ, Kocsis JH,

Brown RP: A Controlled Study of the Antidepressant Efficacy and Side Effects of (-)-Deprenyl. A Selective Monoamine Oxidase Inhibitor. Arch Gen Psychiatry. 1989; 46:45-50.

[14] Tetrud JW, Langston JW. The Effect of Deprenyl (Selegiline) on the Natural History of Parkinson's Disease. Science 1989; 245(4917):519-522.

[15] Standaert DG, Roberson ED. [2011]: Treatment of Central Nervous System Degenerative Disorders. In: Goodman & Gilman's The Pharmacological Basis of Therapeutics, eds Brunton L, Chabner B, Knollman B, McGraw-Hill, New York, 2011, pp. 609-628

[16] Kalász H, Tekes K, Faigl EB, Postenyi Z, Berekmeri E, Karvaly G, Adeghate E. Monitoring the Level of ^{14}C-Labelled Selegiline Following Oral Administration. Open Med Chem J. 2017; 11:1-8.

[17] Fowler JS, Wolf AP, MacGregor RR, Dewey SL, Logan J, Schlyer DJ, Langstrom B. Mechanistic Positron Emission Tomography Studies: Demonstration of a Deuterium Isotope Effect in the Monoamine Oxidase-Catalyzed Binding of (^{11}C)L-Deprenyl in Living Baboon Brain. J. Neurochem. 1988; 51:1524-1534.

[18] Tong J, Meyer JH, Furukawa Y, Boileau I, Chang LJ, Wilson AA, Houle S, Kish SJ. Distribution of Monoamine Oxidase Proteins in Human Brain: Implications for Brain Imaging Studies. J Cereb Blood Flow Metab. 2013; 33:863-871.

[19] Grimsby J, Zentner M, Shih JC. Identification of a Region Important for Human Monoamine Oxidase B Substrate and Inhibitor Selectivity. Life Sci. 1996; 58: 777-787.

[20] Mewies M, McIntire WS, Scrutton NS. Covalent Attachment of Flavin Adenine Dinucleotide (FAD) and Flavin Mononucleotide (FMN) to Enzymes: The Current State of Affairs. Protein Sci. 1998; 7:7-20.

[21] Newton-Vinson P, Hubalek F, Edmondson DE. High-level expression of liver monoamine oxidase B in *Pichia pastoris*. Protein Expr Purif. 2000; 20:334-345.

[22] Edmondson, DE, Binda C, Mattevi A. The FAD Binding Sites of Human Monoamine Oxidases A and B. Neurotoxicology 2004; 25:63-72.

[23] www.moldiscovery.com, for its use, see: von Itzstein M, Wu WY, Koo GB, Michael S, Pegg MS, Dyason JC, Jin B, Phan TV, Smythe ML, White HF, Oliver SW, Colman PM, Varghese JN, Ryan DM, Woods JM, Bethell RC, Hotham VJ, Cameron JM, Penn CR. Rational Design of Potent Sialidase-Based Inhibitors of Influenza Virus Replication. Nature 1993; 363(6428):418-423.

[24] Milczek EM, Binda C, Rovida S, Mattevi A, Edmondson DE. The 'Gating' Residues Ile199 and Tyr326 in Human Monoamine Oxidase B Function in Substrate and Inhibitor Recognition. FEBS J. 2011; 278:4860-4869.

[25] Nagy J, Kenney WC, Singer TP: The Reaction of Phenylhydrazine with Trimethylamine

Dehydrogenase and with Free Flavins. J Biol Chem. 1979; 254:2684-2688.

[26] Kenney WC, Nagy J, Salach JI, Singer TP, In Monoamine Oxidase: Structure, Function, and Altered Function, (Eds.) Singer TP, Von Korff RW, Murphy DL, Academic Press, New York, 1979, pp. 25-37.

[27] Nagy J, Salach JI. Identity of the Active Site Flavin-Peptide Fragments from Human "A"-Form and Bovine "B"-Form of Monoamine Oxidase. Arch Biochem Biophys. 1981; 2008:388-394.

[28] Singer TP, Von Korff RW, Murphy DL. Monoamine Oxidase: Structure, Function, and Altered Function, Academic Press, New York, 1979.

[29] Igaue I, Gomes B, Yasunobu KT: Beef Mitochondrial Monoamine Oxidase, a Flavin Dinucleotide Enzyme. Biochem Biophys Res Commun. 1967; 29:562-570.

[30] Caldinelli L, Iametti S, Barbiroli A, Fessas D, Bonomi F, Piubelli L, Molla G, Pollegioni L. Relevance of the Flavin Binding to the Stability and Folding of Engineered Cholesterol Oxidase Containing Noncovalently Bound FAD. Protein Sci. 2008; 17:409-419.

[31] Kearney EB, Salach JI, Walker WH, Seng, RL, Kenney W, Zeszotek E, Singer TP. The Covalently-Bound Flavin of Hepatic Monoamine Oxidase. 1. Isolation and Sequence of a Flavin Peptide and Evidence for Binding at the 8Alpha Position. Eur. J. Biochem. 1971; 24: 321-327.

[32] Walker WH, Kearney EB, Seng RL, Singer TP. The Covalently-Bound Flavin of Hepatic Monoamine Oxidase. 2. Identification and Properties of Cysteinyl Riboflavin. Eur J Biochem. 1971; 24:328-331.

[33] De Colibus L, Li M, Binda C, Lustig A, Edmondson DE, 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 USA. 2005; 102:12684-12689.

[34] Barsky J, Pacha WL, Sarkar S, Zeller EA: Amine oxidases. XVII. Mode of Action of 1-Isonicotinyl-2-Isopropylhydrazine on Monoamine Oxidase. J Biol Chem. 1959; 234:389-391.

[35] Binda C, Li M, Hubalek F, Restelli N, Edmondson DE, Mattevi A. Insights into the Mode of Inhibition of Human Mitochondrial Monoamine Oxidase B from High-Resolution Crystal Structures. Proc Natl Acad Sci USA 2003; 100:9750-9755.

[36] Binda C, Hubálek F, Li M, Edmondson DE, 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.

[37] Hubalek F, Pohl J, Edmondson DE. Structural Comparison of Human Monoamine Oxidases A and B: Mass Spectrometry Monitoring of Cysteine Reactivities. J Biol Chem. 2003; 278:28612-28618.

[38] Toprakçí M, Yelekçi K. Docking Studies on Monoamine Oxidase-B Inhibitors: Estimation of Inhibition Constants [K[i]] of a Series of Experimentally Tested Compounds. Bioorg Med Chem Lett. 2005; 15:4438-4446.

[39] Yelekçi K, Karahan O, Toprakçi M. Docking of Novel Reversible Monoamine oxidase-B Inhibitors: Efficient Prediction of Ligand Binding Sites and Estimation of Inhibitors Thermodynamic Properties. J Neural Transm. [Vienna]. 2007; 114:725-732.

[40] www.google.com/AutoDock 3.0.5 program.

[41] Harkcom WT, Bevan DR. Molecular Docking of Inhibitors into Monoamine Oxidase B. Biochem Biophys Res Commun. 2007; 360:401-406.

[42] Su G, Mao B, Wang J. A web Server for Transcription Factor Binding Site Prediction. Bioinformation 2006; 1:156-157.

[43] Edmondson DE, Binda C, Mattevi A. Structural Insight into the Mechanism of Amine Oxidation by Monoamine Oxidases A and B. Arch Biochem Biophys. 2007; 464:269-276.

[44] Gulyás B, Pavlova E, Kasa P, Gulya K, Bakota L, Várszegi S, Keller E, Horváth MC, Nag S, Hermecz I, Magyar K, Halldin C. Activated MAO-B in the Brain of Alzheimer Patients, Demonstrated by [^{11}C]-L-Deprenyl using Whole Hemisphere Autoradiography. Neurochem Int. 2011; 58:60-68.

[45] Vasdev N, Sadovski O, Moran MD, Parkes J, Meyer JH, Houle S, Wilson AA. Development of New Radiopharmaceuticals for Imaging Monoamine Oxidase B. Nucl Med Biol. 2011; 38:933-943.

[46] Nag S, Lehmann L, Kettschau G, Heinrich T, Thiele A, Varrone A, Gulyas B, Halldin C. Synthesis and Evaluation of [^{18}F]Fluororasagiline, a Novel Positron Emission Tomography (PET) Radioligand for Monoamine Oxidase B (MAO-B). Bioorg Med Chem. 2012; 20:3065-3071.

[47] Nag S, Kettschau G, Heinrich T, Varrone A, Lehmann L, Gulyas B, Thiele A, Keller É, Halldin C: Synthesis and Biological Evaluation of Novel Propargyl Amines as Potential Fluorine-18 Labeled Radioligands for Detection of MAO-B Activity. Bioorg Med Chem. 2013; 21:186-195.

[48] Pösténvi Z, Tekes K, Tóth-Molnár E, Kalász H. HPLC Analysis of Blood-Brain Penetration of 4-Fluorodeprenyl. J Pharm Biomed Anal. 2014; 102:529-534.

[49] Kalász H, Tekes K, Pösténvi Z, Vizvári E, Sótonyi P, Szabó D, Tóth-Molnár E.Pharmacokinetics of Selegiline in a Rabbit Model. Lett Drug Design Development 2016; 13:752 -756.

[50] Lu C, Zhou Q, Yan J, Du Z, Huang L, Li X. A Novel Series of Tacrine-Selegiline Hybrids with Cholinesterase and Monoamine Oxidase Inhibition Activities for the Treatment of Alzheimer's Disease. Eur J Med Chem. 2013; 62:745-753.