

KADIR HAS UNIVERSITY
GRADUATE SCHOOL OF SCIENCE AND ENGINEERING



IN SILICO DESIGN OF SELECTIVE MONOAMINE OXIDASE B
INHIBITORS USING INDANE RING

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“I, Serkan Altuntaş, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.”

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ABSTRACT

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Master of Science in Computational Biology and Bioinformatics

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May, 2013

Monoamine oxidases (MAOs; EC 1.4.3.4) play significant roles in the control of major monoamine neurotransmitters. MAO has two isoforms; MAO-A and MAO-B. Although they have distinct functionalities their active sites of these enzymes show great similarities.

In order to get specific drug action it is important to design new, more selective and reversible monoamine oxidase inhibitors. Crystallographic structures of MAO-A (pdb code: 2Z5X; human monoamine oxidase in complex with harmine, resolution 2.2 Å) and MAO-B (pdb code : 2V5Z; human MAO-B in complex with inhibitor safinamide, resolution 1.6 Å) isozymes have opened the way for computational modeling and *in silico* design studies.

In the present work, 209952 analogs of an indane scaffold were created using various fragment moieties at different position of indane ring. CHEMPLP module of GOLD program is utilized in the screening process. In order to obtain additional validation of selectivity and scoring values, the first 100 best selective MAO inhibitor candidates are also tested via ChemScore and ASP scoring functions of Gold docking software, AutoDock 4.2 and AutoDock Vina. Inhibition constants and their poses in the active sites of both MAO isozymes are determined. The dispositions of the candidate inhibitors within the organism were checked by ADMET PSA 2D (polar surface area) versus ADMET AlogP98 using Accelrys 3.5 Discovery Studio modeling program.

Keywords: monoamine oxidase, in silico design, docking, modeling

ÖZET

İNDAN İSKELETİ KULLANARAK SEÇİCİ MONOAMİN OKSİDAZ B İNİHİTÖRÜ TASARIMI

Serkan Altuntaş

Hesaplamalı Biyoloji ve Biyoinformatik, Yüksek Lisans

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Mayıs, 2013

Monoamine oxidases (MAOs; EC 1.4.3.4) nörotransmitterlerin kontrolünde önemli rol oynar. MAO iki adet izoforma sahiptir, MAO-A ve MAO-B. Aynı fonksiyonları olmasına rağmen aktif bölgeleri ciddi benzerlik gösterir.

Doğru ilaç etkisini oluşturabilmek için yeni seçici ve geri dönüştürülebilir inhibitörler tasarlamak gerekir. MAO-A (pdb kodu: 2Z5X; harmin ile birlikte insan monoamin oksidazı, resolution 2.2 Å) ve MAO-B'nin (pdb kodu : 2V5Z; safinamide inhibitörü ile birlikte insan monoamin oksidazı, resolution 1.6 Å) kristal yapıları in silico olarak hesaplamalı modellemenin yolunu açmıştır.

Bu çalışmada bir indan iskeletinden farklı fragment parçacıkları farklı pozisyonlarda kullanılarak 209952 analog elde edildi. GOLD docking yazılımının CHEMPLP modülü kullanılarak tarama işlemi gerçekleştirildi. Seçiciliğin ve skor değerlerinin doğruluğunun kontrol edilmesi için en iyi 100 seçici MAO inhibitörü GOLD'un ChemScore, ASP modülleri ve AutoDock 4, AutoDock Vina yazılımları ile yeniden test edildi. İnhibisyon katsayıları ve enzimin aktif bölgelerindeki pozları belirlendi. İnhibitörler ADMET PSA 2D (polar yüzey) ADMET AlogP98'e karşı Accelrys 3.5 Discovery Studio modelleme programı ile test edildi.

Anahtar Kelimeler:monoamin oksidaz, in silico tasarım,docking,modelleme

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Table of Contents

Abstract	
Özet	
Acknowledgements	
List of Tables	ix
List of Figures	ix
Chapter 1: Properties of Monoamine Oxidase Enzyme	1
1.1 Introduction.....	1
1.2 General Properties of Monoamine Oxidase Enzyme.....	4
1.3 Crystallographic and Structural Properties of MAO Isoenzymes.....	5
1.4 Biosynthesis and Biodegradation of Neurotransmitters...	6
1.5 Amine Catalysis Reaction of MAO Isoenzymes.....	7
Chapter 2: Classifications of Monoamine Oxidase Inhibitors	8
Chapter 3: Methods and Procedures Used in Molecular Modeling	9
3.1 Introduction.....	9
3.2 Preparation of Enzymes and Ligands.....	11
3.3 Selecting A Docking Method for Virtual Screening.....	13
3.4 Docking Based Virtual Screening with CHEMPLP.....	14
3.5 Validating CHEMPLP Results With Other Docking Tools	15
3.6 ADMET.....	17
Chapter 4: Results and Discussion	18
4.1 Docking Results.....	18
4.2 3D Structures.....	23
4.3 2D Interaction Diagrams.....	29
4.4 ADMET Results.....	35

Conclusions	36
References	39

List of Tables

Table 4.1	Docking results	23-25
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List of Figures

Figure 1.1 Monoamine Oxidase A.....	2
Figure 1.2 Monoamine Oxidase A.....	2
Figure 1.3 FAD's molecule structure.....	4
Figure 3.1 Indane Ring.....	12
Figure 4.1: Molecule structures of selective ligands.....	21-22
Figure 4.2 3D structure of ligand SA00002	23
Figure 4.3 3D structure of ligand SA00007	23
Figure 4.4 3D structure of ligand SA00012	24
Figure 4.5 3D structure of ligand SA00014	24
Figure 4.6 3D structure of ligand SA00016	25
Figure 4.7 3D structure of ligand SA00028	25
Figure 4.8 3D structure of ligand SA00055	26
Figure 4.9 3D structure of ligand SA00061	26
Figure 4.10 3D structure of ligand SA00070.....	27
Figure 4.11 3D structure of ligand SA00075.....	27
Figure 4.12 3D structure of ligand SA00099.....	28
Figure 4.13 2D Interaction diagram of ligand SA00002 with MAO-B	29
Figure 4.14 2D Interaction diagram of ligand SA00007 with MAO-B	29
Figure 4.15 2D Interaction diagram of ligand SA00012 with MAO-B	30
Figure 4.16 2D Interaction diagram of ligand SA00014 with MAO-B	30
Figure 4.17 2D Interaction diagram of ligand SA00016 with MAO-B	31
Figure 4.18 2D Interaction diagram of ligand SA00028 with MAO-B	31
Figure 4.19 2D Interaction diagram of ligand SA00055 with MAO-B	32
Figure 4.20 2D Interaction diagram of ligand SA00061 with MAO-B	32
Figure 4.21 2D Interaction diagram of ligand SA00070 with MAO-B	33
Figure 4.22 2D Interaction diagram of ligand SA00075 with MAO-B	33
Figure 4.23 2D Interaction diagram of ligand SA00099 with MAO-B	34
Figure 4.24 ADMET Results	35

Chapter 1: Properties of Monoamine Oxidase Enzymes

1.1 Introduction

Monoamine Oxidase (MAO) has two isoenzymes that are related with neurodegenerative diseases and emotional disorders (like Parkinson, Alzheimer diseases and major depression). These diseases affect millions of people all over the world. With the help of better life standards, aging ratio of world population increases the requirement to development of new drugs and powerful methods to treat these diseases [1, 2, and 3].

Monoamine Oxidase (MAO) has two isoforms; Monoamine Oxidase - A (MAO-A) and Monoamine Oxidase – B (MAO-B). Active sites of both enzymes show great similarities. Focus of this study determining the required inhibitors for the treatment of MAO-B isoenzymes related diseases. Because of great similarity between active sites of both enzymes selectivity of the resulting drug is the most important property. Searching new, reversible and selective MAO-B inhibitors via *in silico* drug design methods is the main objective of this study.

Synthesizing new drugs, even with rational drug design techniques, take years to find effective solutions. Also required attention and consumables could be unpredictable. This kind of studies always may result with no solution. Computational modeling studies are cheaper and faster alternative to start with best possible pathway. This creates an ability to start searching via computational technique than follow the pathway which is created with a cheaper and easier way to increase the changes to

find better solutions [1, 2, and 3].

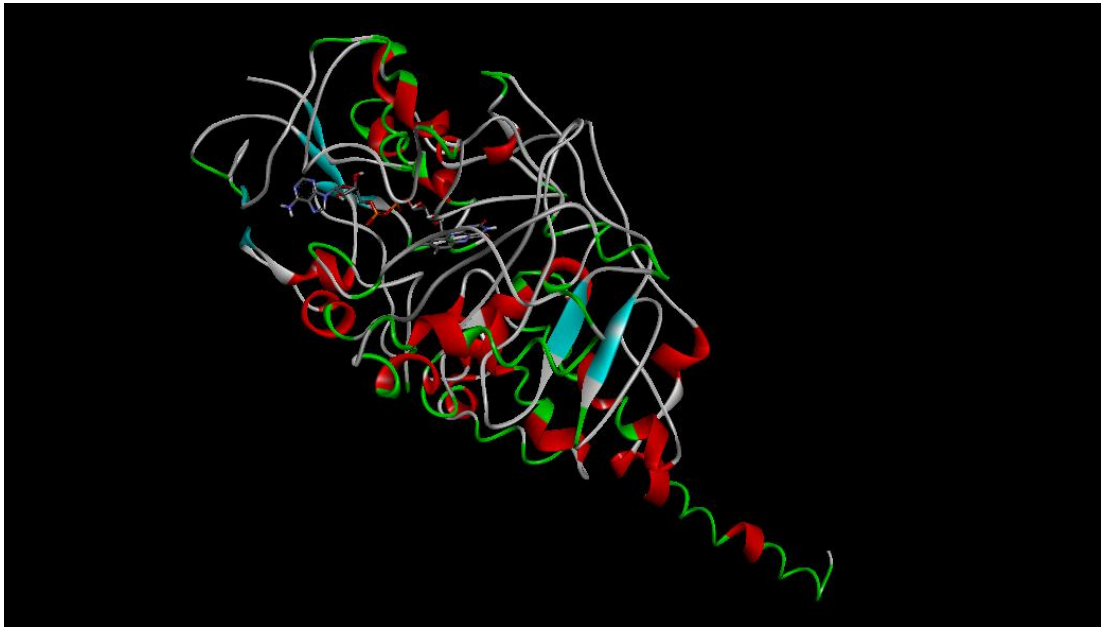


Figure 1.1. Three-dimensional structure of monoamine oxidase A enzyme.



Figure 1.2. Three-dimensional structure of monoamine oxidase B enzyme.

1.2 General Properties of MAO Isoenzymes

Monoamine Oxidase enzymes (EC 1.4.3.4. MAO) includes flavin adenine dinucleotide (FAD) as a cofactor. Catalysis of biological amine oxidative deamination reaction peripheral tissues and inside central nervous system is carried out by MAO isozymes. Two different types of MAO are known as Monoamine Oxidase-A (MAO-A) and Monoamine Oxidase-B (MAO- B). Both of them are placed to the mitochondrial membrane in neural, glial and some other tissues as integral proteins. Placenta, liver, intestine and adrenal medulla are the place that MAO-A shows most of the activity. Brain, platelets, liver and adrenal medulla are the place that MAO-B shows the activity [4-7].

MAO-A consist of 527 amino acids while MAO-B consist of 520 amino acids. Their amino acid sequences have 70% similarity [5].

Cysteine is placed in the active sites of both of these isoenzymes, which has a covalent bond with coenzyme FAD (Ser-Gly-Gly-Cys-FAD-Tyr). Cys406 of MAO-A and Cys497 of MAO-B are making covalent bonds with 8- α -methyl group of FAD via thioether linkage [1,8]. Molecular structure of FAD is shown in Figure-1.3.

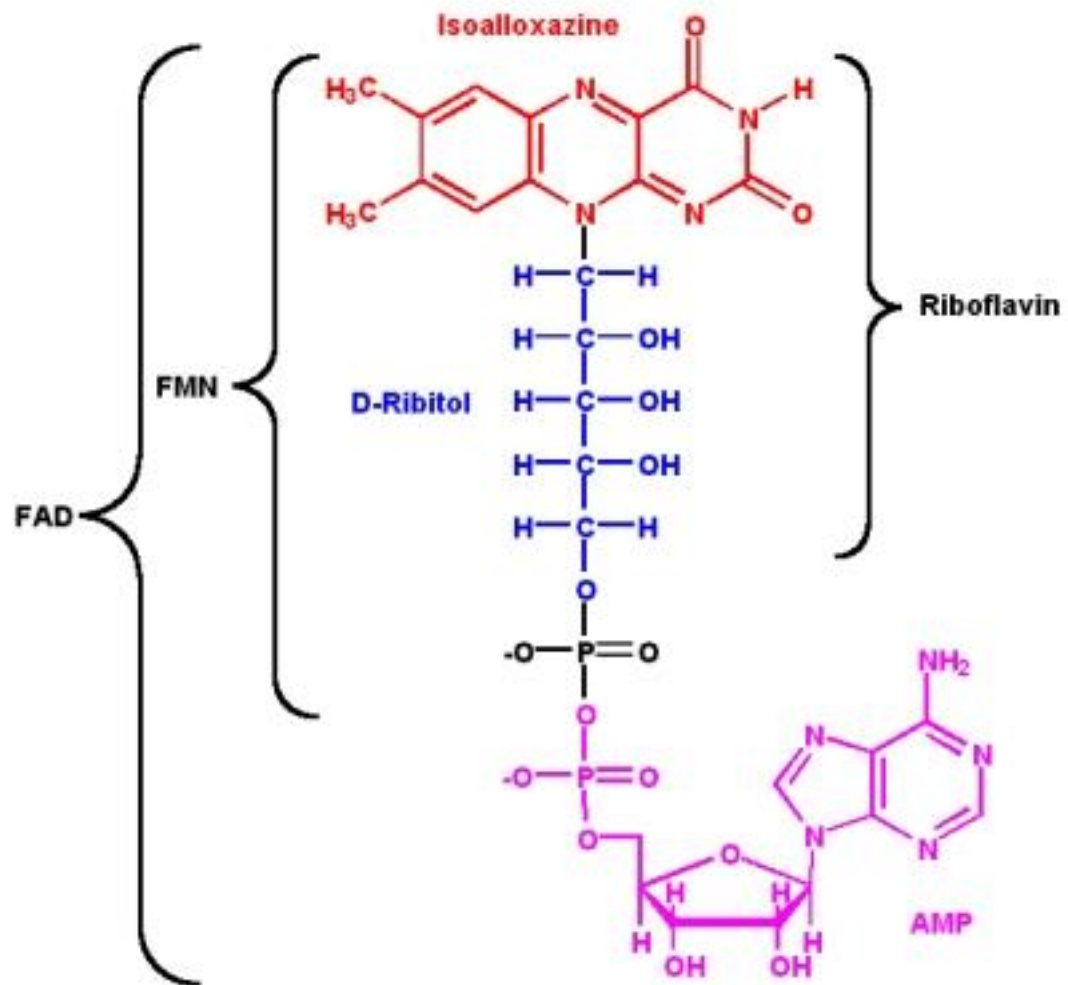


Figure 1.3 FAD's molecular structure¹⁰

1.3 Crystallographic and Structural Properties of MAO Isoenzymes

Many computational simulations and molecular modeling studies exhibit crucial data about MAO isoenzymes. Two enzymes have completely different crystallization forms. While MAO-A crystallizes as monomers, MAO-B crystallizes as dimer. MAO-A has a substrate binding cavity which is the only one cavity inside, MAO-B also includes an additional entrance cavity [1, 4, 5, 11 and 13].

MAO-B entrance cavity volume is about 290\AA^3 . In MAO-B amino acid residues between 99-112 form a cavity-shaping loop. Redirection of substrate into entrance cavity is done by this loop. Hydrophobic entrance cavity is and surrounded by Phe103, Pro104, Trp119, Leu164, Leu167, Phe168, Leu171, Ile199, Ile316 and Tyr326. Ile199 has an important gate like role for entrance of substrate. If the substrate is small enough, just the substrate binding cavity may be enough to be placed inside but if not MAO-B entrance cavity also used for a better placement.

There is no entrance cavity for MAO-A so substrate can directly enter into the single cavity (substrate binding cavity) [1, 4, 5, 11 and 13]. Volume of hydrophobic substrate-binding cavity in MAO-A is $\sim 390\text{\AA}^3$ and in MAO-B is $\sim 550\text{\AA}^3$.

In MAO-A Ile180, Asn181, Phe208, Ile335 residues and in MAO-B Leu171, Cys172, Ile199, Tyr326 residues covers the substrate cavity.

In MAO-A Tyr407 and Tyr444, in MAO-B Tyr398 and Tyr435 are two amino acids which surround the FAD like a cage. Ligand selectivity is mainly based on these cage

structures. This means most important role on selectivity is related with some residues. For MAO-A, amino acid residues between 161 and 375, and for MAO-B, amino acid residues between 152 and 366 are these residues [1, 4, 5, 11 and 13].

1.4 Biosynthesis and Biodegradation of Neurotransmitters

Neurotransmitter oxidative deamination and biogenic amine catalysis are done by MAO isoenzymes.

A group of these neurotransmitters and amines are epinephrine (adrenaline), dopamine, norepinephrine (noradrenalin, NA), serotonin (5-HT), tyramine, Tryptamine, 2-phenylethylamine (PEA) and 1-methyl-4-phenyl-1, 2,3,6-tetrahydropyridine (MPTP).

Degrading the toxic exogenous amines is the cytoprotective role of MAO. This activity helps to keep homeostasis in balance.

Inhibition of norepinephrine and serotonin carried out by MAO-A and inhibition of phenyl ethylamine and benzyl amine is carried out by MAO-B. Both Dopamine and tyramine are oxidized by MAO-A and MAO-B isoenzymes. However dopamine preferentially is oxidized by MAO-A and tyramine by MAO-B are efficiently.

For controlling the depressive and anxiolytic influences, inhibition of MAO-A (for example with clorgiline) could be used and this increases the 5HT and NA levels in brain. Also treatment of neurodegenerative diseases can happen with MAO-B

inhibition (for example with l-deprenyl) which increases the PEA, benzyl amine and MPTP levels.

1.5 Amine Catalysis Reaction of MAO Isoenzymes

FAD is the redox cofactor for the reactions that are catalyzed by MAO. Conversion of the monoamines into corresponding aldehydes is the main objective of reaction [6, 7, 11, 18, 19].

Monoamine substrates include a C α -H bond which is cleaved on the deprotonated form is the starting point of catalytic action. To be reduced to FADH₂, FAD accepts two hydrogens and at this time amine is converted into corresponding imine.

Next step on MAO reaction is re-oxidation of FADH₂ and this happens with the conversion of oxygen into hydrogen peroxide. By the way the imine is also hydrolyzed into the aldehyde and ammonia form.

Although true identification of MAO oxidation mechanisms is not present yet, there are three proposed forms: [5]

- Single Electron Transfer Mechanism (SET)
- Polar Addition-Elimination Pathway
- Hydrogen Atom Transfer (HAT)

Most of the natural substrates are metabolized by two of the MAO isoenzymes, because of having the cofactor part very similar. The main difference comes from structural difference that creates specificity [6, 7, 11, 18, 19, and 20].

Chapter 2: Classifications of Monoamine Oxidase Inhibitors

Monoamine Oxidase inhibitors (MAOIs) are classified inside two different parts: First group is reversible ones which are competitive or slow tight-binding. Second group is irreversible ones which are affinity labeling agents or mechanism-based inactivators. Mechanism-based inhibition was the main role of first MAOIs. They perform covalent binding to the proteins to yield reactive products. In addition to that these kind of MAOIs also had noticeable hepatotoxic side effects, by inactivating the P450, which cannot be tolerated.

“Cheese effect” is the one of the known important side effect. Usage of non-selective MAOI and consumption of tyramine contained foods results an increased blood tyramine level because the inactivation of both MAO isoenzymes unable the regulation of tyramine level.

As a result of that increased tyramine level effects blood pressure and this causes fatal hypertensive crisis. Unknown selectivity of MAOIs is the main reason of these hepatotoxic and hypertensive side effects. This problem requires the development of selective and reversible MAOIs. The increasing functional and structural information about MAO enzyme help to create better selective and reversible inhibitors. Current researches are targeting MAO-B for the treatment of Parkinson and Alzheimer diseases via generating the selective MAO-B inhibitors while selective MAO-A inhibitors are important for the treatment of depression [5].

Chapter 3: Methods and Procedures Used in Molecular Modeling

3.1 Introduction

Enzyme inhibition is the way drugs work on cellular environment. If any drug can inhibit the target enzyme with a low concentration and do not have any effect on other enzymes are known as strong inhibitors. These properties are important to have non-toxic and less side effective drugs.

In this study the main method is Structure Based Drug Design. This method depends on some previously generated inputs like crystallographic structure of the target enzyme. This structural information could be gathered from NMR or X-Ray crystallography. RCSB Protein Data Bank currently includes more than 90000 structures with their 3D information. All these data is available to molecular modeling and computational studies.

In this study 209952 different ligands have been designed and docked into the both MAO enzymes *in silico*. None of these ligands has been synthesized or tested before.

Molecular Docking is the main virtual screening method to test drug candidates. Aim is finding the best matching for ligand and receptor. Also this method predicts the binding energy and possible binding shape in 3D.

Current docking software includes three main parts: system representation, searching conformational space, and the ranking the potential solutions. Having the best results

are based on having an efficient and less faulting search algorithm and a good scoring function. One a days while having a good enough conformational search algorithm is easy but the scoring of the potential result is the main tricky point to identify the best software combination. [36-38] Even for some experiments, using one docking software for only conformational search and then scoring its result with another function can be performed to have better result. One of the best example would be having the AutoDock results (generated docked conformations) than re-ranking it with DrugScore (DSX) [52].

Main job of scoring function is predicting the interaction or binding affinity between two selected molecules. Firstly the system is created which followed by positioning the enzyme and the ligands inside that system. By minimizing the free energy of binding induced fit is achieved. There are two main criteria to be achieved. First is shape cohesion between ligand an enzyme and the second is interaction between them. Without the proper interaction it is useless to have cohesive geometric structures [5].

Main operation o docking is trying to find a good enoygh position to place the ligand inside the active site of enzyme so they are brought together to find the best geometric position. Changing the position and even angles of the ligand molecule is executed by algorithm step by step and hopefully some conformations (poses) of ligands can be placed inside the active side of enzyme. Decision of placement is achieved by calculating the energy of system which uses the molecular mechanics force fields. The lower the energy the better is binding [36-40].

All possible conformations and orientations of structure complex are done by search algorithm. Dealing with a flexible ligand means having millions of possible conformations with the rotations of many angles.

Structural cohesion is observed for both the ligand and the enzyme.

3.2 Preparation of Enzymes and Ligands

Protein Data Bank (PDB) is the source to obtain the structures of MAO enzymes. 2Z5X (MAO-A with inhibitor harmine, resolution 2,2Å) and 2V5Z (MAO-B with inhibitor safinamide, resolution 1,6Å) are the crystal structures that are used.

Accelrys includes a protein preparation tool to make ready the crystal structure dockable. It is used to make more reliable simulation. One monomer was selected for each enzyme because just one of the monomer work on reaction. Already included ligands of protein were deleted. Ionic strength was set to 0.145, dielectric constant was set to 10 and physiological pH values were adjusted.

Then the enzyme structure was minimized and the files were converted to relative formats and saved for the docking procedures [1, 5, 36-40].

Ligand creation and preparation is the most crucial step in this study. Initial point for this operation is an indane ring shown in Figure 3.1.

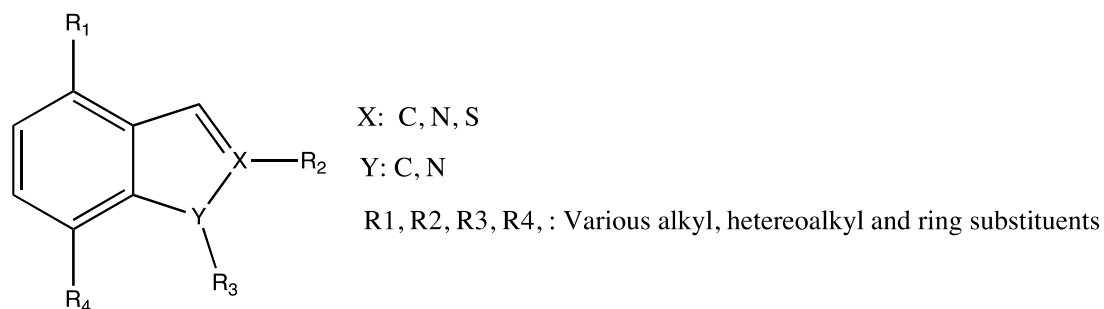


Figure 3.1 Indane Ring

As it is shown in the figure while X atom can be C, N or S, the Y atom only can be C or N. Also for R groups various alkyl, heteroalkyl and ring substituents are used.

Getting the indane ring as a scaffold and creating variations of side chains is actually a permutation problem. Anyone can use a molecular sketching tool draw hundreds of molecules in weeks but this operation is very simple for even a basic computer processor.

To achieve ligand generation goal the handmade method was not efficient and error prone while dealing with hundreds of ligands. As a result of this a Python script is created to execute this part of job.

Python script required the indane ring and the side chains in SMILES format. Do basic permutation generation and generated a simple text file (output.sbl) which included 209952 lines of text, each line represents a brand new ligand in SMILES format.

Permutation generation inside a python script is a fast but memory bound operation so an ordinary desktop computer of the in house HPC cluster was not enough. Any

node did not include required memory available. One of the High Memory Instance of Elastic Compute Cloud solutions from Amazon Web Services is used to do this calculation.

Next step is obtaining 3D structures from SMILES. OpenBabel [8] has been chosen. For compatibility with many different docking software, each ligand was preferred to have its own file in pdb and mol. Creation of 3D shape requires a prediction and default options of Open Babel was used. Having each ligand on different file made the operation I/O bound so termination of this script took two days on a personal computer.

As a result two sets of 209952 ligands has been generated and ready for docking based virtual screening. One set includes pdb files, other set includes mol files of same ligands.

Because of some bugs inside Open Babel, some of the ligands could not be converted into 3D shape perfectly. And it was impossible to detect exact number of defected ligands so it is impossible to determine how many successful ligands were generated. As a result of this operation more than 200000 ligands have been generated and prepared for virtual screening.

3.3 Selecting a Docking Method for Virtual Screening

Many docking tools was tried to get the fastest and best possible result. AutoDock 4, AutoDock Vina, GoldScore, ChemScore, CHEMPLP, ASP and Libdock was tested

with known ligands.

Nature of virtual screening includes false positive and false negative results. This means any screening algorithm can miss a very effective drug or may find a very useless compound as a drug candidate. So selecting the best scoring function and tuning it into the required situation is important.

There were seven different options available also safinamide is the one of the main ligand that is already crystallized with monoamine oxidase B so using its docking solution as an indicator is logical.

ChemScore, CHEMPLP, AutoDock and AutoDock Vina agreed with the known inhibition scores. For any kind of virtual screening the second most important feature is speed so CHEMPLP was chosen to do the screening.

3.4 Docking Based Virtual Screening with CHEMPLP

GOLD (Genetic Optimization for Ligand Docking) is a highly automatized docking optimization tool for flexible docking with different genetic algorithms (Jones and al., 1995, 1997). The scoring function ranking the binding sites consists of terms of hydrogen bonding, pair wise dispersion potential that is able to describe a significant contribution to hydrophobic energy of binding and a molecular mechanics term of internal energy of the ligand [1, 5, 39, 46, and 47].

CHEMPLP is one of the best scoring functions of GOLD docking software. Default

parameter set is used and cavity detection was disabled. A sphere which has 12 Å radius from N5 of FAD is used. This binding site is the one used for both enzymes in many MAO studies.

Docking 209952 ligands into two MAO enzymes has been done simultaneously. Docking calculation take 44 days on at least 75% utilization of single core for each enzyme on HPC cluster.

GOLD software generates bestrankings.lst file which includes output score for each ligand. This file is read by a python script and sorted by the selectivity.

Selectivity means 15% difference between MAO-B and MAO-A dockings for each ligand. Sorting is based on MAO-B / MAO-A ratio. If ratio is lower than 1.15 the ligand considered as non-selective and did not even in the considered group. Best 100 ligands are from this selectively sorted list.

CHEMPLP is not the only method and it should be validated with other scoring functions.

3.5 Validating CHEMPLP Results With Other Docking Tools

Other scoring functions of GOLD docking software are an automated way like it was in CHEMPLP. Using default parameter set and same active site definition lead us to obtain results but AutoDock does not have the efficient virtual screening solution for our study.

Especially Raccoon [9] is a useful script which is highly tied to MGL Tools and can run on PBS based HPC clusters but it runs into many dependency problems. Also it does not parallelize each docking so it is possible to waste some resource.

Having lack of perfect tool for parallel AutoDock runner, a new tool is created as a solution with name YaVST (Yet Another Virtual Screening Tool)

YaVST (Yet Another Virtual Screening Tool) is freely available open-source tool for AutoDock 4 based virtual screening. (<https://github.com/serkanaltuntas/yavst>) Like its predecessors it is also heavily tied to MGL Tools but it is developed as a self-contained box so it is not required to have MGL Tools.

YaVST creates independent workspaces for each screening. Every workspace can include only one enzyme and infinite number of ligands to be tested.

If not provided YaVST generates the PDBQT files from PDB. Then generation of one Docking Parameter File (DPF) and a single Grid Parameter File (GPF) for each ligand follows. These files are generated by scripts of MGL Tools so it produces exactly the same output with AutoDockTools.

In addition to pdbqt, gpf and dpf files, YaVST creates some qsub files which are required for job submission to any kind of Sun Grid Engine based HPC.

3.6. ADMET

Having favorable ADMET characteristics is one of the most daunting hurdles for drug development. ADMET refers to the absorption, distribution, metabolism, excretion, and toxicity properties of a molecule within an organism. All these characteristics are must have properties for any drug. This makes the early optimization important. Unless you reduce the problems that cause ADMET failure early on, later stages may be waste of time. Identification and elimination of unfavorable compounds make the research process more cost effective and efficient [21].

Chapter 4: Results and Discussion

4.1. Docking Results

Best 100 selective monoamine oxidase B inhibitors were docked via ChemScore, CHEMPLP, ASP, AutoDock 4 and AutoDock Vina. Table 1 includes the result for each ligand:

Molecule	ChemScore		ChemPLP		ASP		Vina		AutoDock	
	A	B	A	B	A	B	A	B	A	B
SA00001	21,93	47,35	43,49	55,68	28,95	36,52	-5,9	-6,9	-6,55	-6,48
SA00002	19,94	56,94	45,31	60,22	30,14	40,64	-6,1	-7,2	-6,47	-7,03
SA00003	21,69	51,02	49,79	64,09	32,02	41,2	-6,7	-7,1	-6,53	-6,74
SA00004	21,72	47,34	43,47	55,8	28,42	36,62	-5,9	-6,9	-6,55	-6,46
SA00005	23,93	49,2	48,33	61,15	30,49	38,37	-6,7	-7,1	-6,25	-6,41
SA00006	25,95	53,85	52,94	66,28	30,45	40,16	-6,9	-7,2	-6,3	-6,4
SA00007	27,53	50,63	52,17	76,27	31,7	44,2	-5	-5,7	-6,89	-8,08
SA00008	25,12	61,77	48,34	70,97	28,52	42,52	-5	-5,8	-6,14	-7,16
SA00009	23,71	54,68	51,74	66,43	29,55	38,14	-6,2	-7,5	-6,96	-7,14
SA00010	21,85	54,4	48,7	59,86	29,66	38,02	-6,5	-7,1	-6,87	-7,22
SA00011	23,84	46,88	50,16	67,22	29,09	37,63	-6,8	-7,7	-6,54	-6,62
SA00012	28,59	55,32	55,35	78,16	30,42	40,3	-7	-7,3	-7,46	-8,26
SA00013	23,51	55,84	50,67	65,45	29,53	42,94	-6	-7,1	-6,57	-7,35
SA00014	26,02	50,52	52,9	67,51	27,35	34,95	-7,2	-8	-7,02	-7,13
SA00015	25,91	52,08	52,3	67,09	26,65	34,1	-6,8	-7,6	-7,2	-7,32
SA00016	29,72	54,16	54,76	71,24	29,35	38,78	-6	-7,8	-7,36	-8,31
SA00017	24,18	46,46	47,09	60,03	27,43	35,96	-6,9	-7,2	-6,58	-6,66
SA00018	26,82	44,65	45,93	58,94	24,56	31,7	-7	-7,8	-6,69	-6,89
SA00019	25,02	43,01	43,88	54,28	26,81	33,22	-6,8	-7,6	-6,46	-6,5
SA00020	24,97	39,8	46,22	59,91	25,66	33,33	-7,1	-7,6	-5,92	-6,07
SA00021	23,01	40,61	44,46	56,56	27,01	35,52	-6,9	-7,5	-5,85	-5,8
SA00022	25,78	51,16	53,01	64,89	28,7	36,35	-7,1	-7,4	-6,68	-6,71
SA00023	23,92	49,77	51,22	61,85	28,68	37,16	-6,8	-7,1	-6,3	-6,56
SA00024	26,13	49,41	50,78	65,44	27,07	34,02	-7,4	-7,9	-6,88	-7,12
SA00025	24,6	47,24	48,83	62,1	27,13	36,03	-7,1	-7,5	-6,61	-6,77
SA00026	26,78	45,07	46,13	59,94	24,67	31,72	-7,2	-7,9	-6,44	-6,61
SA00027	25,19	43,9	44,46	55,15	26,27	33,77	-6,8	-7,5	-6,36	-6,38
SA00028	24,81	45,11	46,8	58,25	27,79	34,04	-7,5	-8,3	-7,04	-7,21
SA00029	26,92	45,38	45,87	57,82	24,57	30,98	-6,9	-7,5	-6,95	-7,2
SA00030	24,97	43,64	43,88	54,25	24,98	31,6	-6,8	-7,3	-6,56	-6,83
SA00031	26,67	50,71	51,97	64,47	29,71	35,78	-7,7	-7,8	-7,43	-7,48
SA00032	22,04	46,29	42,8	55,03	24,81	32,13	-6,8	-7,1	-5,9	-6,26
SA00033	24,59	44,61	42,35	51,27	23,4	32,15	-6,7	-7,2	-6,62	-6,64
SA00034	23,02	38,99	41,95	52,14	24,75	32,55	-6,7	-7,1	-6,01	-6,14

SA00035	23,87	50,09	46,08	59,43	25,97	34,19	-6,9	-7	-6,44	-6,57
SA00036	24,74	44,65	42,06	51,55	23,82	32,15	-6,4	-6,8	-5,99	-6,15
SA00037	23,27	44,32	44,24	53,87	26,98	33,23	-7	-7,6	-6,82	-6,92
SA00038	24,79	45,74	42,43	51,14	23,99	32,37	-6,6	-6,9	-6,77	-6,87
SA00039	24,33	51,06	50,78	69,62	30,44	37,8	-7,4	-8,1	-6,41	-6,77
SA00040	23,79	46,52	51,2	64,24	30,47	38,13	-7,2	-7,6	-6,45	-6,66
SA00041	23,27	48,95	50,57	63,22	31,65	41,3	-6,9	-7,4	-6,52	-6,63
SA00042	21,85	45,64	48,04	58,1	27,69	34,01	-6,8	-7,5	-5,78	-6,21
SA00043	21,54	42,64	51,8	65,17	30,83	38,68	-7,3	-7,5	-5,94	-6,07
SA00044	21,69	45,99	51,21	64,53	31,91	42,29	-7,1	-7,5	-5,42	-5,88
SA00045	19,7	46,76	48,14	60,04	27,44	35,61	-7,1	-7,5	-5,13	-5,63
SA00046	20,29	41,53	44,44	57,05	29,46	37,66	-7,5	-7,9	-6,17	-6,32
SA00047	24,16	48,06	51,8	68,08	28,41	36,23	-6,8	-7,5	-6,11	-6,19
SA00048	22,23	53,77	52,94	69,3	30,57	38,61	-6,5	-7,3	-7,08	-7,31
SA00049	21,58	52,43	52,15	67,54	30,1	38,6	-7	-7,6	-6,28	-6,47
SA00050	19,68	50,83	50,63	65,91	31,97	40,52	-6,9	-7,6	-6,04	-6,18
SA00051	20,7	53,05	54,47	70,86	34,13	42,48	-7,1	-7,5	-5,87	-6,33
SA00052	23,82	47,11	51,56	64,38	29,73	38,22	-7,4	-7,7	-6,32	-6,55
SA00053	23,64	50,38	50,09	64,11	30,76	41,98	-7,2	-7,6	-6,02	-6,31
SA00054	21,75	46,86	48,25	59,4	27,44	34,62	-6,9	-7,6	-5,66	-6,03
SA00055	22,18	45,67	44,6	57,31	31,84	38,88	-7,1	-7,8	-6,93	-7,09
SA00056	20,84	44,28	49,16	63,26	30,43	39,39	-7,3	-7,3	-6,25	-6,45
SA00057	21,66	49,02	56,3	66,98	35,42	42,11	-7,5	-7,8	-6,48	-7,27
SA00058	25,83	47,28	54,7	66,36	29,21	38,82	-6,9	-7,3	-6,28	-6,41
SA00059	22,48	42,67	47,06	60,29	28,46	36,26	-7	-7,3	-6,01	-6,04
SA00060	19,1	42,4	45,24	56,83	29,43	38,51	-6,7	-7	-5,61	-5,65
SA00061	25,25	43,15	46,02	59,02	25,62	31,83	-7,1	-7,6	-6,05	-6,21
SA00062	23,3	43,21	44,15	56,38	26,37	35,6	-6,9	-7,3	-6,14	-6,04
SA00063	21,44	40,37	44,08	56,61	28,73	36,71	-6,9	-7,3	-5,39	-5,52
SA00064	23,95	47,33	52,91	65,09	28,48	37,14	-7,3	-7,4	-6,06	-6,02
SA00065	22,46	49,05	51,25	62,06	30,44	39,64	-6,9	-7,1	-5,55	-5,94
SA00066	24,36	45,54	50,79	66,21	28,17	34,97	-7,5	-7,7	-6,29	-6,47
SA00067	22,81	44,69	47,74	61,73	28,56	37,68	-7,2	-7,3	-6,22	-6,3
SA00068	25,07	42,43	46,46	59,76	25,55	32,5	-7,2	-7,8	-5,81	-5,91
SA00069	23,25	46,01	43,85	56,56	26,84	35,84	-6,9	-7,2	-5,96	-5,93
SA00070	23,07	42,22	46,88	58,2	27,51	33,68	-7,7	-8,2	-6,4	-6,51
SA00071	21,53	46,38	44,82	56,33	30,37	38,86	-7,2	-7,6	-6,25	-6,15
SA00072	25,15	44,57	46,1	58,66	25,2	31,92	-7	-7,4	-6,3	-6,51
SA00073	23,33	45,77	43,74	55,85	26,07	34,64	-6,9	-7,2	-6,26	-6,35
SA00074	21,23	47,35	49,74	62,04	29,41	38,59	-7,6	-7,9	-6,6	-7,1
SA00075	26,55	47,67	53,78	69,54	28,34	35,98	-7,9	-8,5	-6,82	-7,1
SA00076	23,25	42,16	41,63	52,86	24,19	30,17	-6,5	-7,1	-5,27	-5,42
SA00077	20,59	41,99	47,11	60,48	26,4	34,46	-6,7	-7	-5,04	-5,45
SA00078	23,06	41,75	45,75	59,39	26,21	32,54	-6,8	-7,2	-5,73	-5,82
SA00079	21,25	39,51	46,02	60,07	27,28	33,77	-6,7	-7,1	-5,16	-5,19
SA00080	22,56	45,17	50,59	66,32	29,22	35,71	-7	-6,9	-5,65	-5,83
SA00081	23,24	40,44	46,04	60,17	26,09	33,22	-6,6	-6,9	-5,18	-5,29
SA00082	21,14	39,71	46,74	58,46	28,41	35,29	-7,2	-7,6	-5,86	-5,95
SA00083	23,23	40,38	45,67	58,77	25,69	32,62	-6,7	-7	-5,83	-6
SA00084	23,26	50,11	45,53	60,75	26,59	36,57	-6,7	-6,6	-5,83	-6,15
SA00085	23,2	52,05	49,43	64,43	31,04	40,8	-6,2	-6,7	-6,44	-6,38
SA00086	21,1	49,28	46,15	59,99	32,39	43,13	-6,3	-7,2	-6,18	-6,14

SA00087	25,38	51,94	49,71	62,93	31,41	41,02	-6,6	-7,4	-7,04	-7,34
SA00088	23,84	48,97	48,73	65,83	30,23	38,86	-6,3	-7,1	-6,81	-6,67
SA00089	21,87	47,13	46,6	61,48	31,16	41,36	-6,7	-7,2	-6,57	-6,49
SA00090	23,33	52,45	46,92	62,59	25,68	34,97	-5,8	-6,6	-7,19	-7,78
SA00091	23,18	49,82	49,39	62,71	32,14	41,32	-7,2	-7,7	-6,43	-6,69
SA00092	23,19	51,35	49,44	62,95	30,87	40,03	-7,3	7,4	-6,47	-6,9
SA00093	21,91	45,08	48,32	65,9	29,97	40,8	-6,3	-7,1	-6,2	-6,02
SA00094	23,36	52,49	53,01	70,73	29,95	37,47	-6,7	-7,5	-6,47	-6,81
SA00095	21,75	46,93	48,4	63,39	28,65	37,16	-6,7	-7,1	-5,61	-5,81
SA00096	19,62	44,55	46,12	61,65	31,41	42,8	-6,8	-7,2	-5,95	-5,8
SA00097	21,64	49,83	48,57	63,05	26,65	35,3	-6	-6,6	-6,49	-7,05
SA00098	23,27	51,81	49,45	66,47	32,96	42,7	-6,7	-7,4	-6,75	-6,72
SA00099	25,35	53,09	50,2	62,93	29,22	41,48	-6,9	-7,5	-6,98	-7,27
SA00100	23,54	49,33	51,06	65,39	32,29	43,44	-7	-7,9	-6,73	-6,52

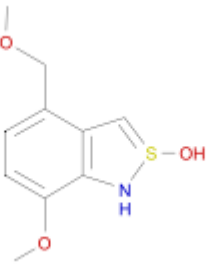
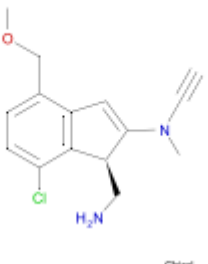
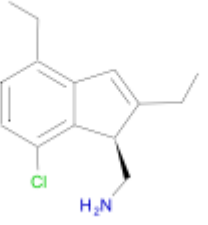
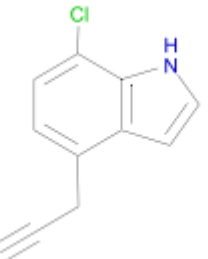
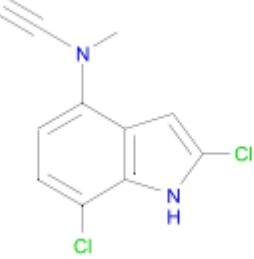
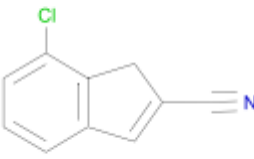
Table 4.1: Docking Results (AutoDock and Vina represents energies (kcal/mol))

Safinamide is the chemical compound which is already included to the crystal structure of MAO-B. For the comparison, safinamide has been tested via CHEMPLP scoring function of GOLD for Monoamine Oxidase B.

CHEMPLP Score of Safinamide: 71.90

As highlighted in the Table 4.1 (SA00002, SA00007, SA00012, SA00014, SA00016, SA00028, SA00055, SA00061, SA00070, SA00075, and SA00099) 11 ligands are highly selective and promising drug candidates. Selection is based on total MAO-B scores of all GOLD scoring functions and its comparison to AutoDock 4. The single conflict is seen between SA00008 and SA00007 and the better AutoDock 4 score is preferred.

2D chemical structures are listed below:

1	 <p>Chemical structure of 5-(hydroxythio)tryptophan, a tryptophan derivative with a hydroxythio group at the 5-position of the indole ring.</p>	SA00002
2	 <p>Chemical structure of 5-(2-amino-3-(prop-1-en-2-ylamino)propyl)tryptophan, a tryptophan derivative with a 2-amino-3-(prop-1-en-2-ylamino)propyl group at the 5-position of the indole ring.</p>	SA00007
3	 <p>Chemical structure of 5-(2-amino-3-propylpropyl)tryptophan, a tryptophan derivative with a 2-amino-3-propylpropyl group at the 5-position of the indole ring.</p>	SA00012
4	 <p>Chemical structure of 5-(3-prop-1-en-1-ynyl)tryptophan, a tryptophan derivative with a 3-prop-1-en-1-ynyl group at the 5-position of the indole ring.</p>	SA00014
5	 <p>Chemical structure of 5-(2-(prop-1-en-2-ylamino)-3,4-dichlorophenyl)tryptophan, a tryptophan derivative with a 2-(prop-1-en-2-ylamino)-3,4-dichlorophenyl group at the 5-position of the indole ring.</p>	SA00016
6	 <p>Chemical structure of 5-(3-chlorophenyl)tryptophan, a tryptophan derivative with a 3-chlorophenyl group at the 5-position of the indole ring.</p>	SA00028

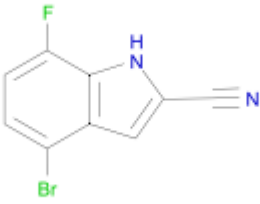
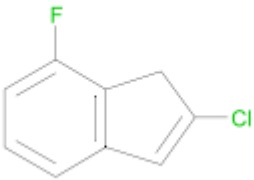
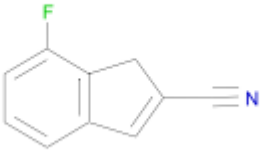
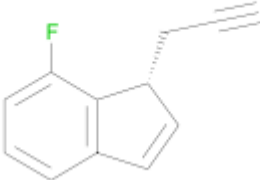
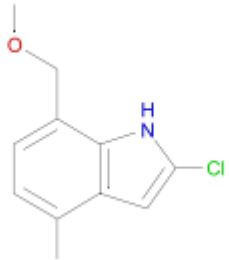
7		SA00055
8		SA00061
9		SA00070
10	<p style="text-align: center;">Chiral</p> 	SA00075
11		SA00099

Figure 4.1: Molecule structures of selective ligands

4.2. 3D Structures

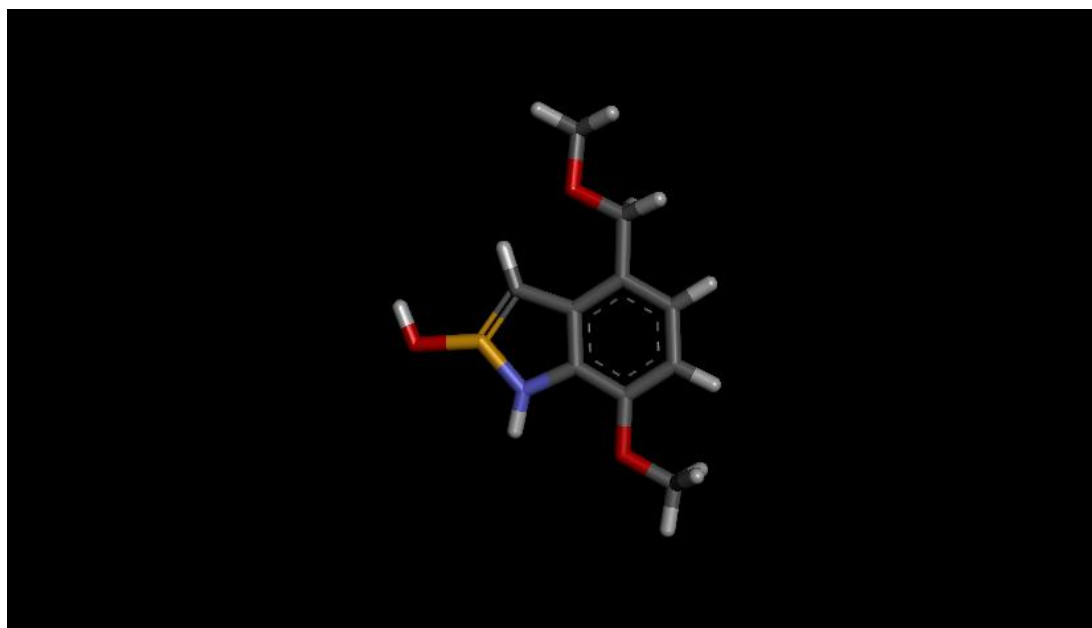


Figure 4.2: 3D structure of ligand SA00002

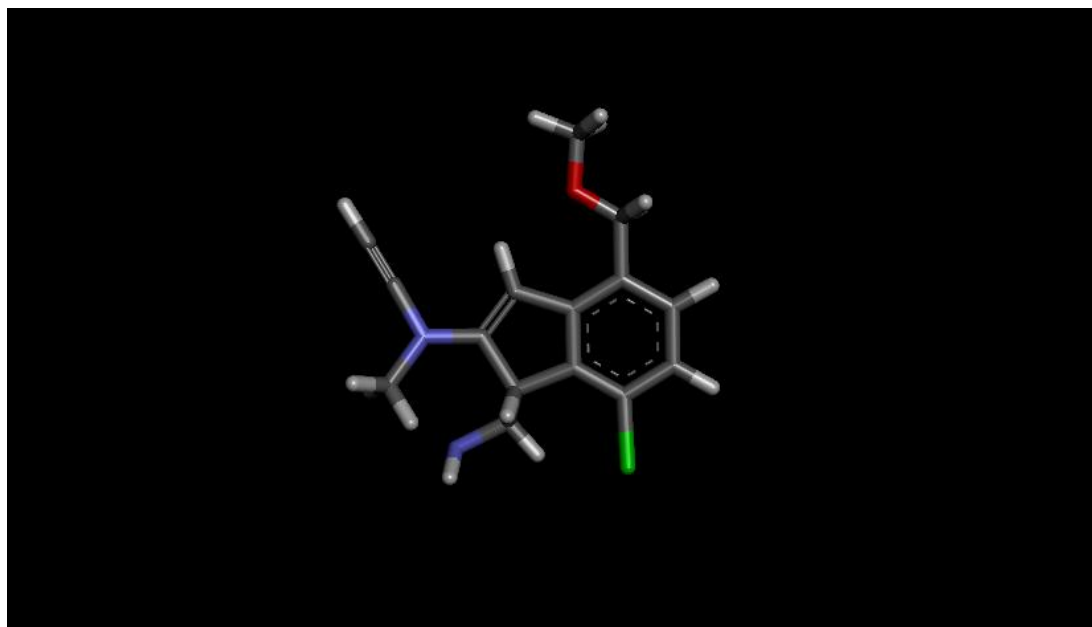


Figure 4.3: 3D structure of ligand SA00007

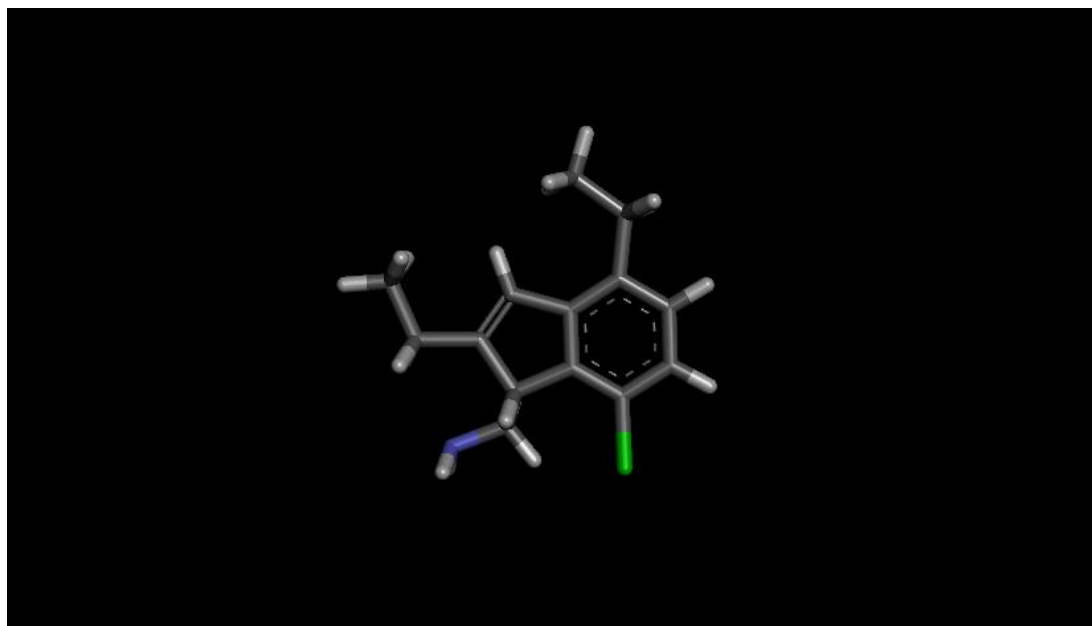


Figure 4.4: 3D structure of ligand SA00012

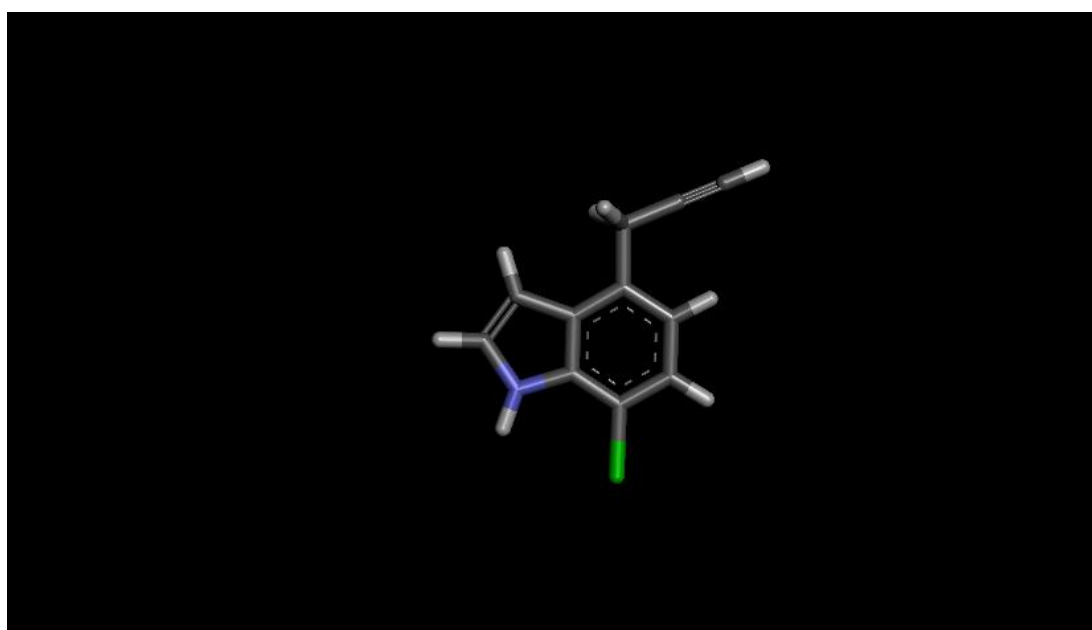


Figure 4.5: 3D structure of ligand SA00014

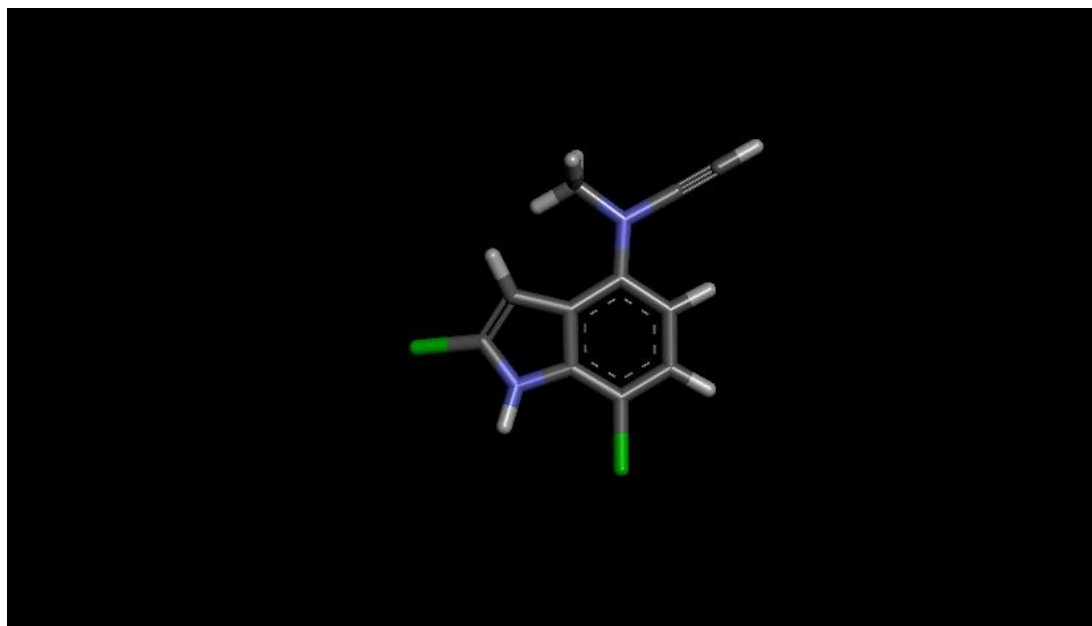


Figure 4.6: 3D structure of ligand SA00016

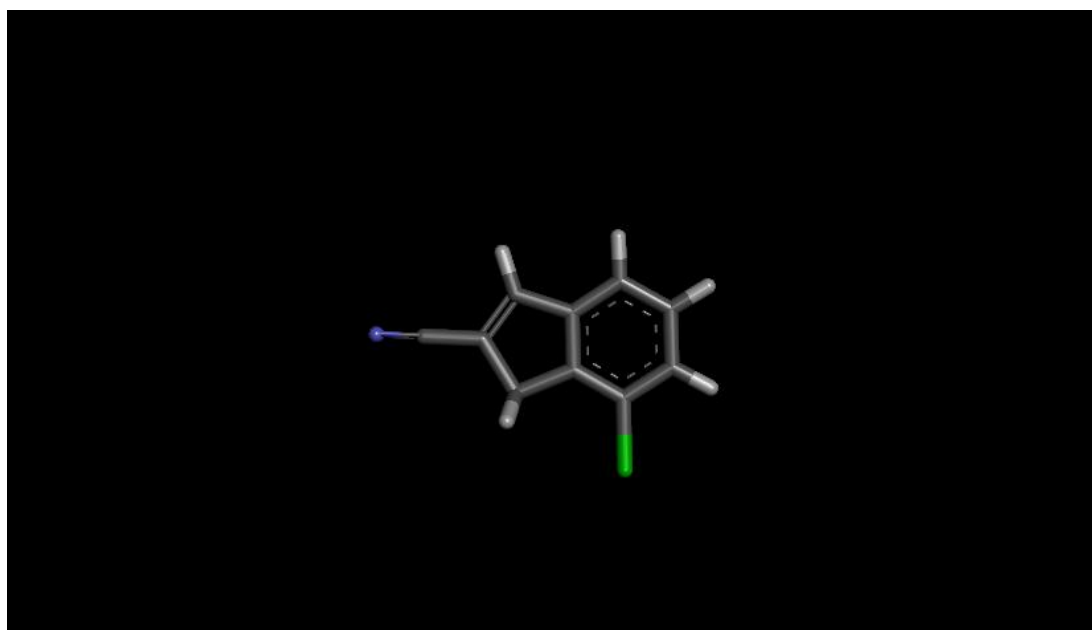


Figure 4.7: 3D structure of ligand SA00028

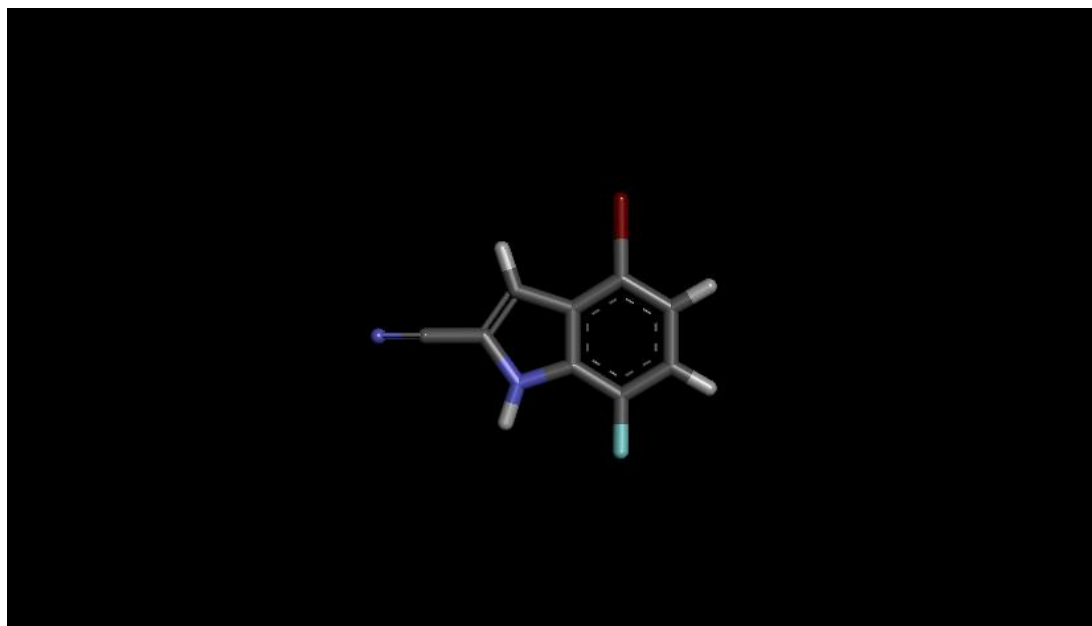


Figure 4.8: 3D structure of ligand SA00055

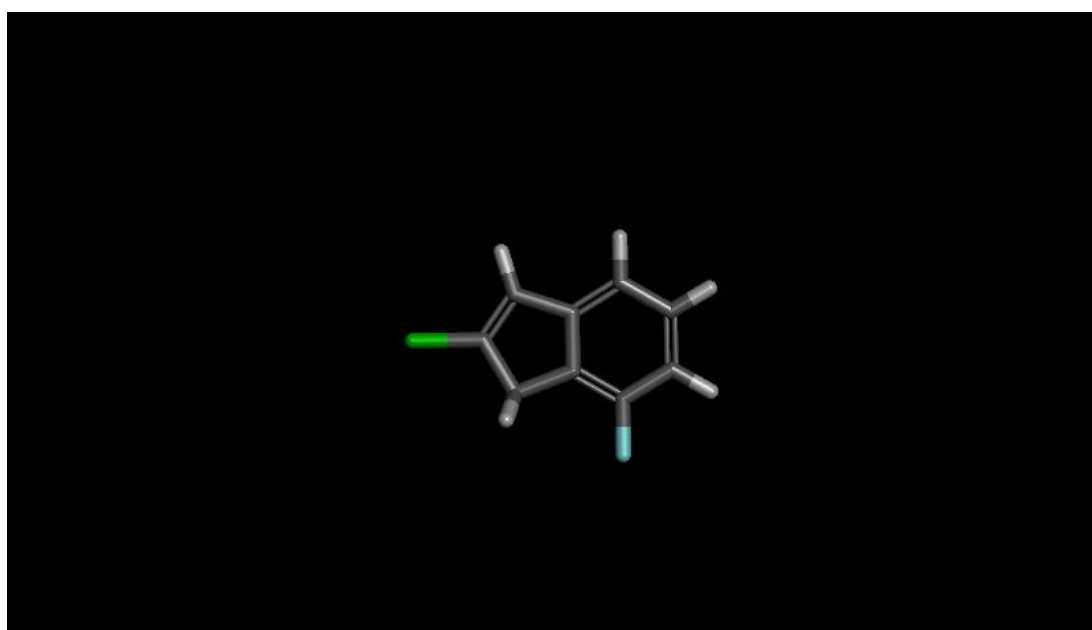


Figure 4.9: 3D structure of ligand SA00061



Figure 4.10: 3D structure of ligand SA00070

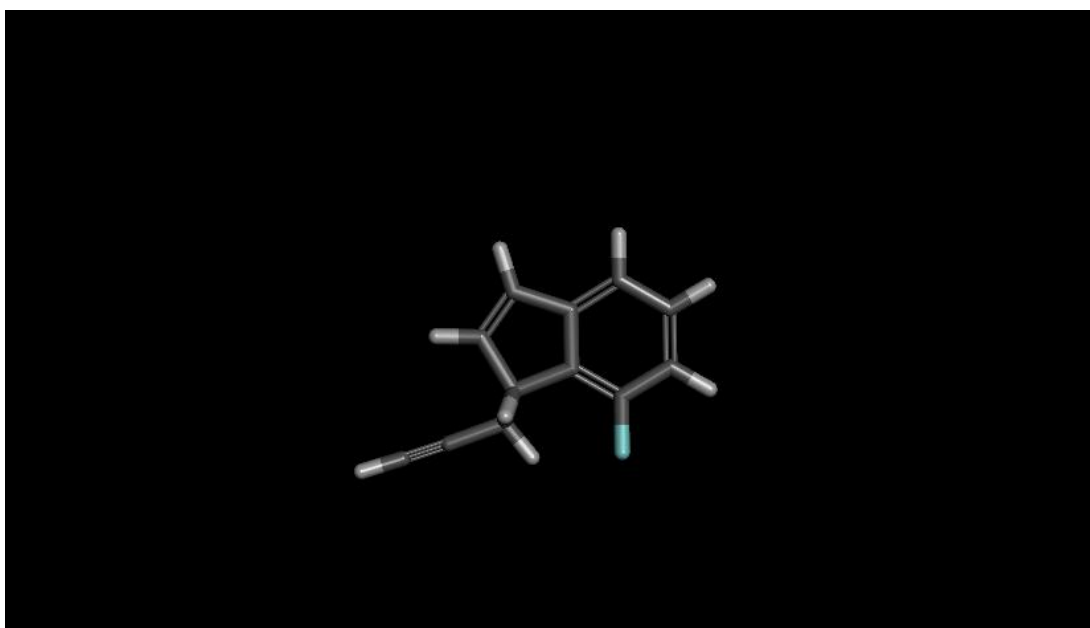


Figure 4.11: 3D structure of ligand SA00075

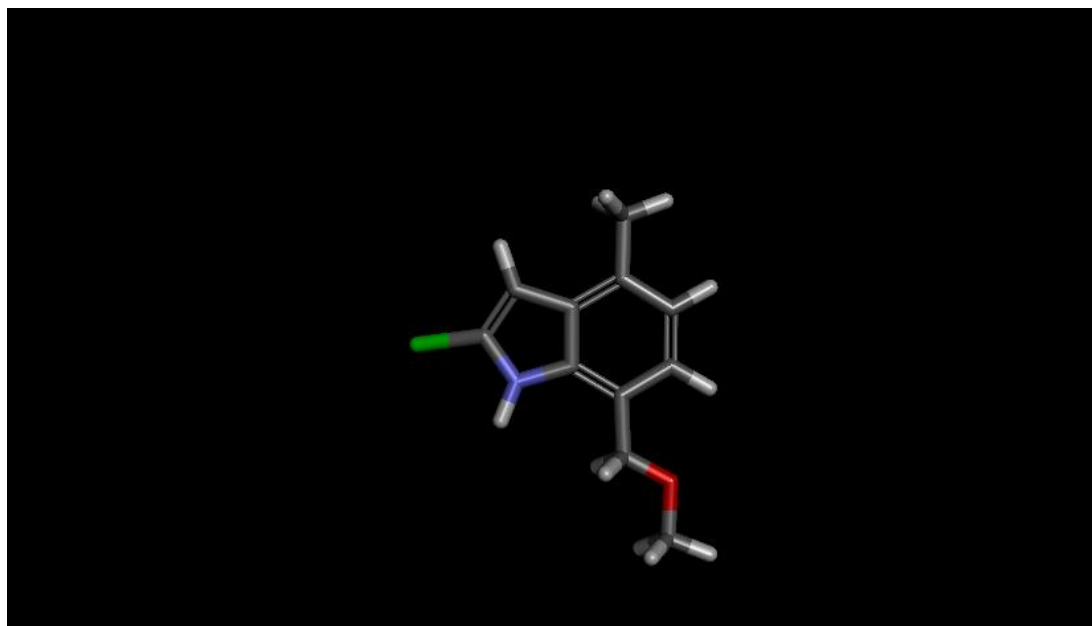


Figure 4.12: 3D structure of ligand SA00099

4.3. 2D Interaction Diagrams

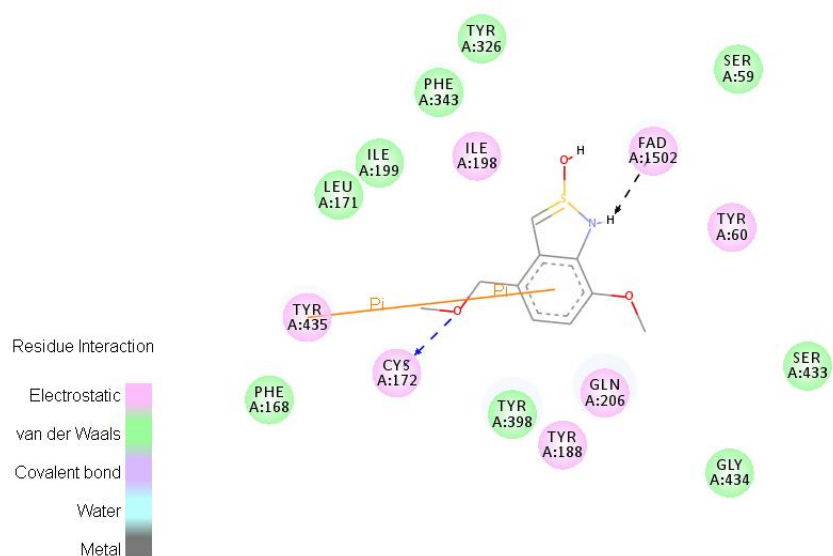


Figure 4.13: 2D Interaction diagram of ligand SA00002 with MAO-B

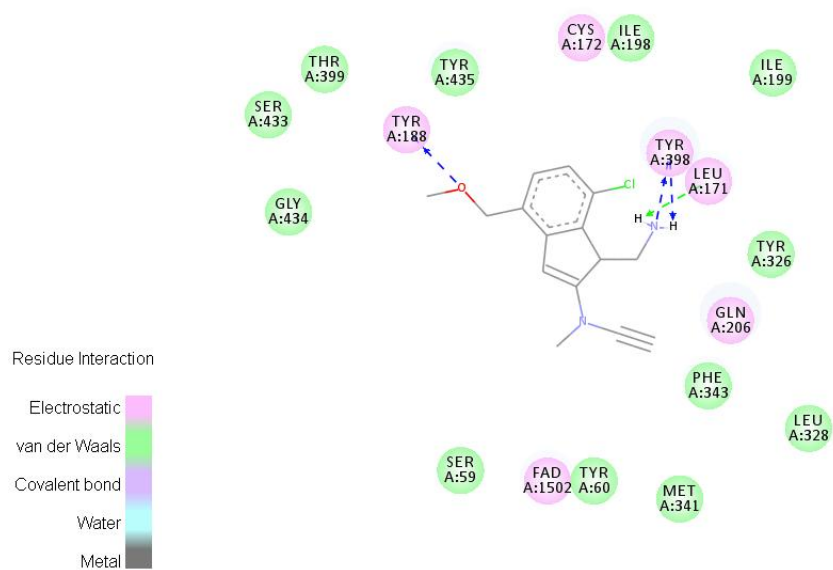


Figure 4.14: 2D Interaction diagram of ligand SA00007 with MAO-B

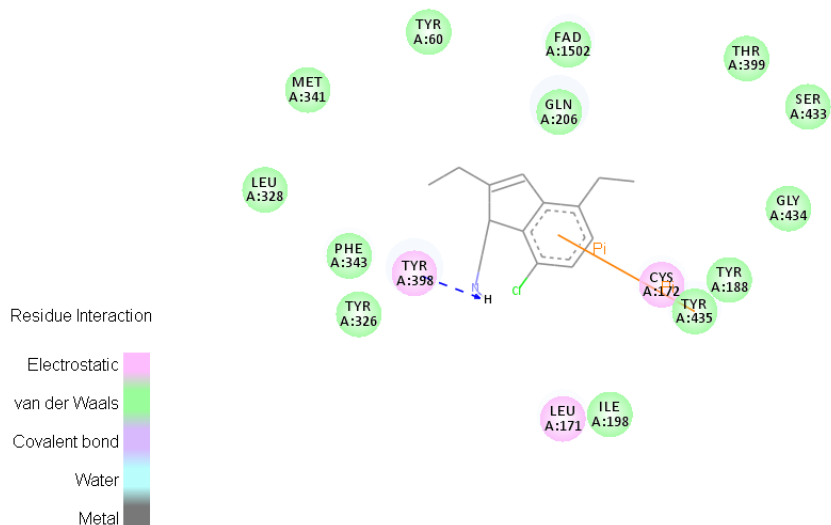


Figure 4.15: 2D Interaction diagram of ligand SA00012 with MAO-B

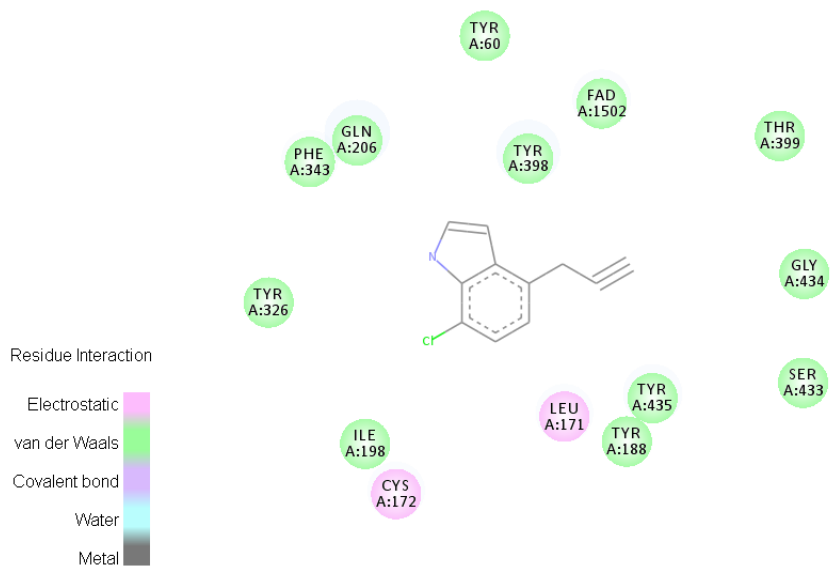


Figure 4.16: 2D Interaction diagram of ligand SA00014 with MAO-B

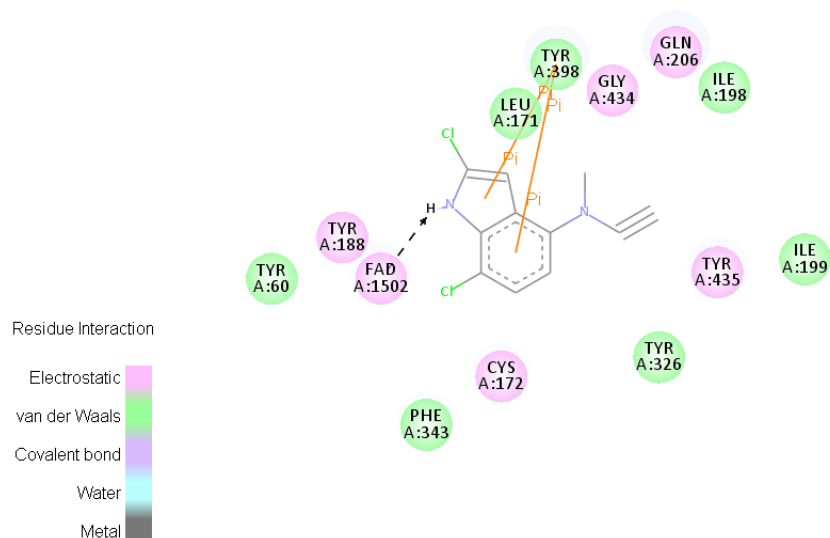


Figure 4.17: 2D Interaction diagram of ligand SA00016 with MAO-B

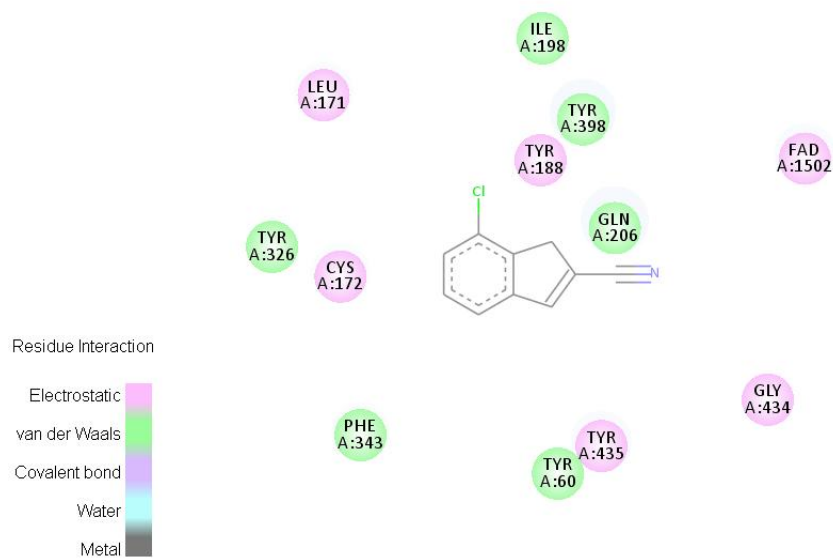


Figure 4.18: 2D Interaction diagram of ligand SA00028 with MAO-B

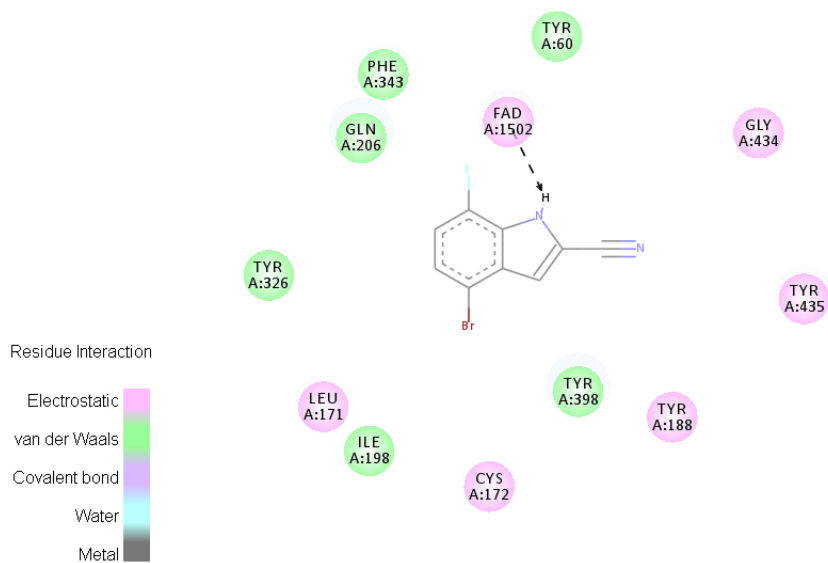


Figure 4.19: 2D Interaction diagram of ligand SA00055 with MAO-B

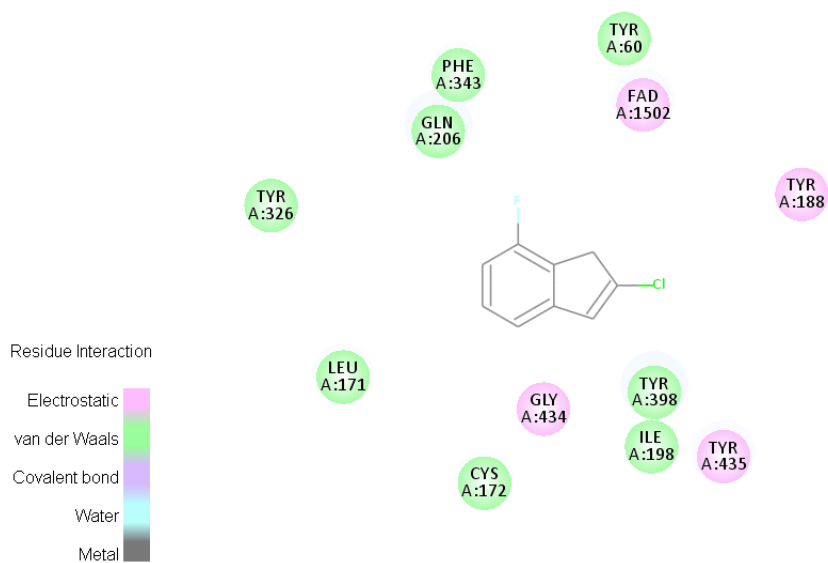


Figure 4.20: 2D Interaction diagram of ligand SA00061 with MAO-B

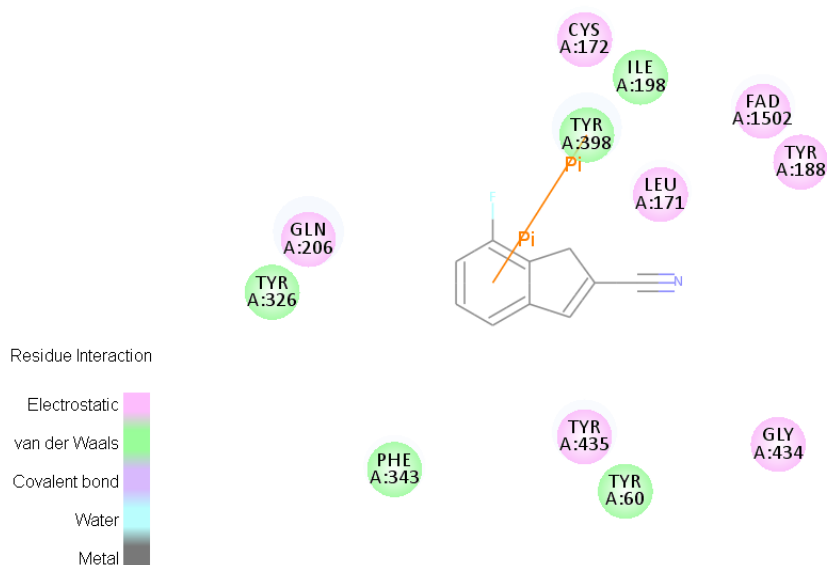


Figure 4.21: 2D Interaction diagram of ligand SA00070 with MAO-B

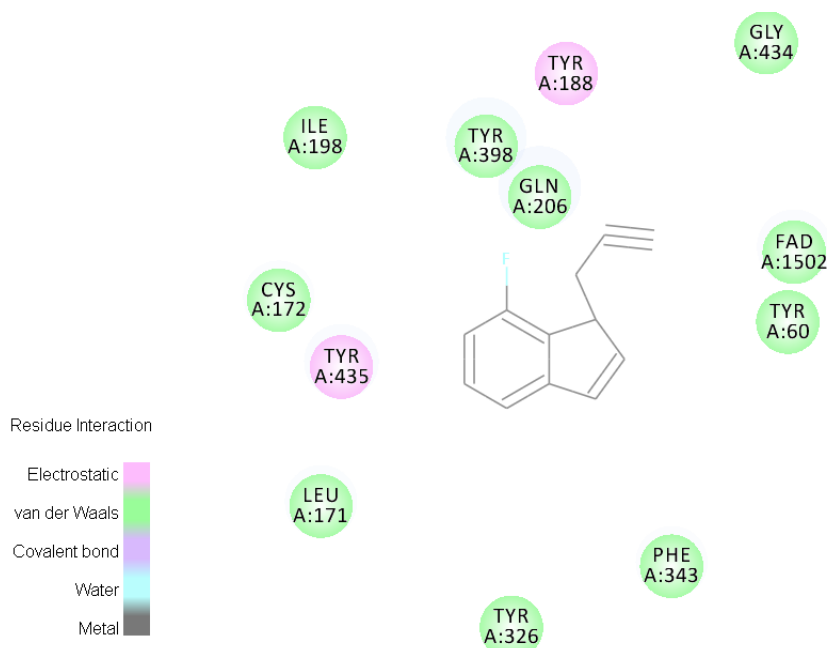


Figure 4.22: 2D Interaction diagram of ligand SA00075 with MAO-B

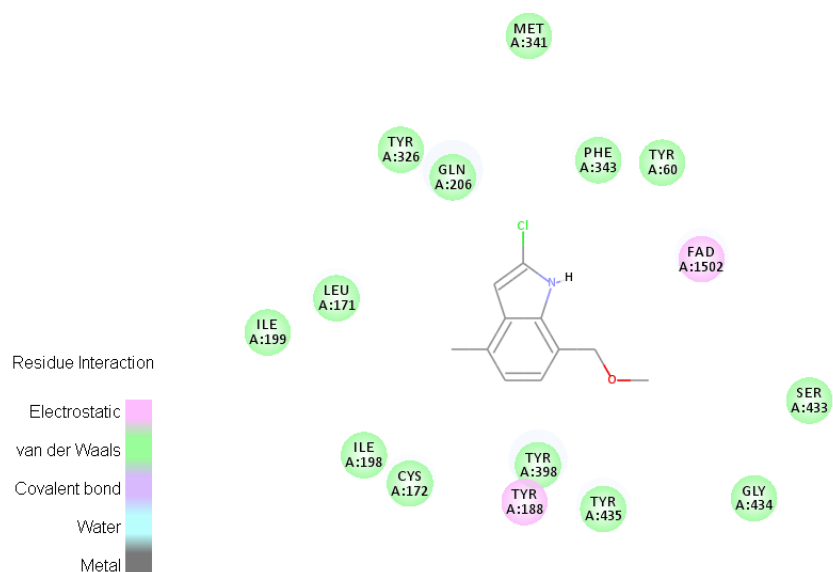


Figure 4.23: 2D Interaction diagram of ligand SA00099 with MAO-B

4.3 ADMET Results:

ADMET experiment is calculated for ADMET_AlogP98, Absorption-95, Absorption-99, BBB-95 and BBB-99. As shown in the Figure 4.20. Only two of the ligand is near the borders of plots. Others are exactly inside safe area. Only SA00061 is very near on the red border. This shows the usability of these ligands as drugs. This means 10 of the selected results are perfect drug candidates according to ADMET.

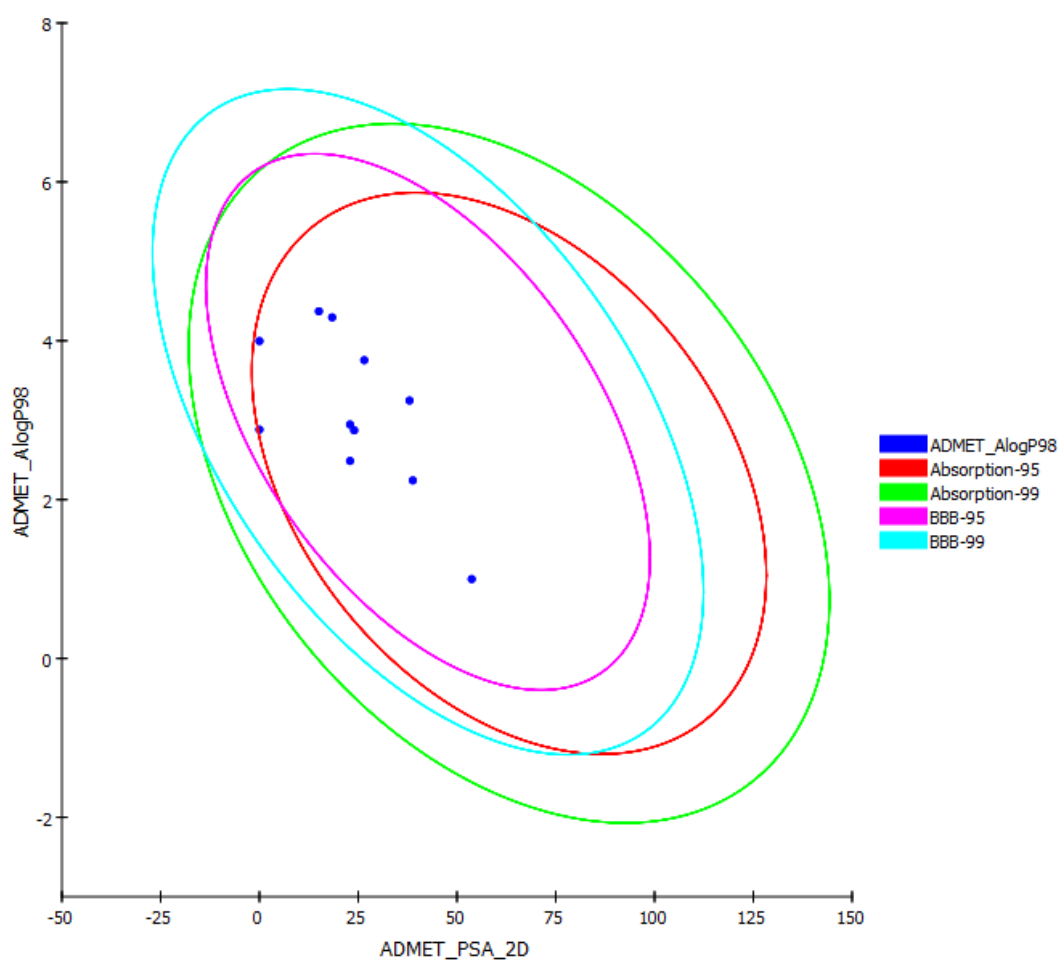


Figure 4.24. ADMET Results

CONCLUSION

Eleven new inhibitors were found to be MAO-B selective inhibitors within the scope of this work.

Safinamide (EMD 1195686) is one of the known MAO-B selective inhibitor which is under clinical study now. For this reason, the inhibition capacity and selectivity of our developed compounds were compared with the inhibition value of safinamide.

The compounds SA00007 (CHEMPLP Score: 76.27) and SA00012 (CHEMPLP Score: 78.16) showed much better inhibition than that of safinamide (CHEMPLP Score: 71.90). Compound SA00016 showed almost the same inhibition value of safinamide. On the other hand the rest of the compounds showed the inhibition score less than safinamide.

However all the compounds that are being developed *in silico* method in our study show much better selectivity to MAO-B in comparison to safinamide.

This compounds that we identified as MAO-B inhibitors also indicated very reasonable ADMET properties (Figure 4.24).

Detailed analysis of 2D interactions show that the compounds having π - π interactions and hydrogen bonding such as SA00012 show much better inhibition.

As a concluding remark, using computational modeling and screening methods are invaluable tools to search for the right compounds. This procedure provides a method

which saves huge amount of money and shortens time drastically.

We believe that the model compounds that we developed in this study worth trying to synthesize in future work.

Curriculum Vitae

Serkan Altuntaş was born in 22 September 1985 in Rize. His BS degree has been received in Biology in 2011 from Karadeniz Technical University. After his undergraduate study, he was accepted to Kadir Has University for a graduate study on Computational Biology and Bioinformatics in September 2011. Combination of life science and computer science is his main interest.

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