Docking and Simulation Studies to Identify Novel Inhibitors for MAO-B Protein for Alzheimer's Disease

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I give my greatest acknowledgement to my **Parents** for all round support during my studies. The endless sacrifices from my parents have made me what I am today.

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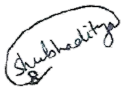
. DECLARATION BY THE STUDENT

I hereby declare that the work reported in the B.Tech project report entitled "**Docking and Simulation Studies to Identify Novel Inhibitors for MAO-B Protein for Alzheimer's Disease**" submitted at Jaypee University of Information Technology, Waknaghat, India, is an authentic record of my work carried out under the supervision of **Dr. Tiratha Raj Singh**. I have not submitted this work elsewhere for any other degree or diploma.



Shubhaditya Bhattacharya (161515)

This is to certify that the above statement made by the candidate is true to the best of my knowledge.

Dr. Tiratha Raj Singh

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Dated:

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ABSTRACT

Alzheimer's Disease has come out as one of the most recognized type of disorder in humans, troubling 45% of humans above 85 years old. This trouble is increasing as the population is increasing. In short, AD is linked with neuronal loss, synaptic dysfunction and functional abnormalities of mitochondrial structures. In order to improve the life of patients suffering from AD, various studies are being proposed or conducted in order to develop more powerful drugs. Monoamine oxidases are enzymes which belong to the flavin adenine dinucleotide (FAD) dependant enzymes group that are available on the external mitochondrial layer. MAOs are solely accountable for the oxidative deamination of numerous xenobiotic and endogenous neurotransmitters and also attune their levels in the brain and peripheral tissues as well. MAO-A isoform is mainly specific to amines like serotonin, epenephrine. On the other hand MAO-B is specific to small amines like benzylamine. The deprotonated form of the amine binds to the active site of the enzyme and is in this way oxidized to imine form. The FAD cofactor which is in the reduced form reacts with oxygen to form oxidized FAD and hydrogen peroxide which generates toxic hydroxyl radicals that are responsible for damage to the neurons and death. A few studies have affirmed that the expression of MAO-B in the brain is more in the case of AD patients and is also linked with the dissipation of cognitive functions. Monoamine Oxidase B (MAO-B) is an enzyme which is encoded by the MAO-B gene which is present in the pathogenesis of Alzheimer's disease (AD) and other neurodegenerative disorders. Increased MAO-B expression in the hippocampus and the cerebral cortex of the brain has been observed in AD patient's brains. This phenomenon leads to increased production of hydrogen peroxide and reactive oxygen species (ROS) which contributes to AD pathology. In this study we planned to design novel inhibitors for MAO-B. Recently, some

MAO-B inhibitors have come to the front because of their neuro-protective properties and the roles they play in the treatment of certain neurodegenerative disorders. This experiment was orchestrated to recognize the potential anti-Alzheimer's compound, a structure-based virtual screening was performed for the natural compounds obtained from the ZINC database (n = 98,072) against the structure of MAO-B. The ligands were docked to the structure of MAO-B protein in two successive docking modes that brought about 510 ligands having better docking score. These 510 compounds were additionally assessed for ADMET analysis. Out of these 510 compounds only 18 compounds were chosen for re-docking examination. After rectifying by molecular docking, four potential inhibitors (ZINC04237106, ZINC32501391, ZINC02147610 and ZINC05818772) were picked that can act as a novel anti-MAOB inhibitor.

INTRODUCTION

Alzheimer's Disease (AD) is an escalating neurodegenerative disorder that currently influence 46 million people around the world and is estimated to upset 131.5 million by 2050. Mental illness due to AD is described by notable reduction of cognitive functions which involves memory impairment, language problems and also problems which includes reasoning or judgment. As the disease advances, there is decrease in daily functioning as well as an increase in neurological symptoms. Pathologically, AD is described by the aggregation of extracellular senile plagues which contains amyloid-B peptides and intracellular neurofibrillary tangles which together with cerebral degeneration and apoptosis of the neuronal cell constitutes the authentic features of the disease. Alzheimer's Disease has come out as one of the most recognized type of disorder in humans, troubling 45% of humans above 85 years old. This trouble is increasing as the population is increasing. In short, AD is linked with neuronal loss, synaptic dysfunction and functional abnormalities of mitochondrial structures. In this paper, different techniques of managing AD have been described, which gives another process of action [1-3]. In order to improve the life of patients suffering from AD, various studies are being proposed or conducted in order to develop more powerful drugs. Monoamine oxidases are enzymes which belong to the flavin adenine dinucleotide (FAD) dependant enzymes group that are available on the external mitochondrial layer. MAO-A isoform is mainly specific to amines like serotonin, epenephrine. On the other hand MAO-B is specific to small amines like benzylamine. The deprotonated form of the amine binds to the active site of the enzyme and is in this way oxidized to imine form. The FAD cofactor which is in the reduced form reacts with oxygen to form oxidized FAD and hydrogen peroxide which generates toxic hydroxyl radicals that are responsible for damage to the neurons and death. A few

studies have affirmed that the expression of MAO-B in the brain is more in the case of AD patients and is also linked with the dissipation of cognitive functions [4].

Monoamine Oxidase B (MAO-B) is an enzyme which is encoded by the MAO-B gene which is present in the pathogenesis of Alzheimer's disease (AD) and other neurodegenerative disorders. Increased MAO-B expression in the hippocampus and the cerebral cortex of the brain has been observed in AD patient's brains. This phenomenon leads to increased production of hydrogen peroxide and reactive oxygen species (ROS) which contributes to AD pathology. Therefore, reduction of ROS-induced oxidative stress with the inhibition of MAO-B activity can delay the progression of the disease further. In this study we planned to design novel inhibitors for MAO-B. Recently, some MAO-B inhibitors have come to the front because of their neuro-protective properties and the roles they play in the treatment of certain neurodegenerative disorders.

MATERIALS AND METHODS

3.1. Recovery of protein and ligand structures

The structure of MAO-B protein (PDB ID: 4A7A) [4] was redeemed from the Protein Data Bank. The primary and secondary metabolites were downloaded from the ZINC database. The library comprised of 98,072 natural compounds, all of which were downloaded in *.mol2* file format.

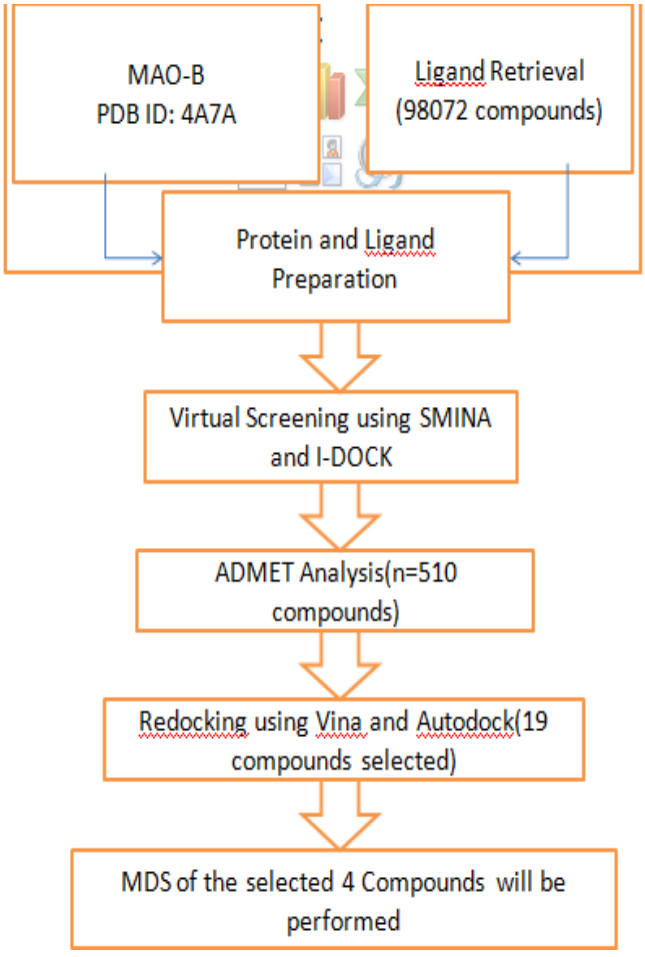


Figure1: Pipeline for virtual screening, ADMET and molecular dynamics simulation

3.2. Virtual screening

It is a computational process which is used in designing of drugs and plays a significant role in the discovery of leads and its subsequent identification. This innovation can screen out ligand databases which contain a huge number of compounds that are utilizing the residues present in the active site of the protein. This expidites the screening of enormous data sets to recognize those structures that are having the viability to bind with the drug target [9-11].

3.3 ADMET Prediction

In the experiment performed, using ZINC database 98,072 natural compounds were obtained and sieved out against the MAO-B protein. From the first round of screening, 510 compounds alongside a reference compound RGZ (also known as competitive inhibitors) were picked for the subsequent stage of screening. The chosen 510 compounds were further assessed for ADMET investigation.

Utilizing the admetSAR1.0 which is a quick, accurate prediction server, examination of the selected compounds based on ADMET properties as possible and conceivable drug candidates was performed [5]. It speculates critical descriptors and pharmaceutically important properties of natural compounds. We have predicted various parameters like BBB, HIA, Caco-2 permeability, Pgp Substrate/Inhibitor, Cyp450 inhibition, toxicity, carcinogenicity and lethal dose etc. On the basis of all these parameter we have selected 18 compounds which displayed key features that were suitable as lead compounds and emulated ADMET properties as well as Lipinski’s Rule.

3.4. Docking analysis

Each of the selected 18 compounds as well as the reference compound were subjected for additional re-docking investigation using the tools Autodock and Autodock Vina [6-7] . Autodock is a freely available tool that is generally utilized for docking. Using Autodock, the receptors and ligand were prepared. Addition of hydrogen molecules to the MAO-B structure, elimination of water molecules as well as allocation of atomic charges. A three-dimensional grid box was generated for docking and the grid points were set to X = 50°, Y = 50°, Z = 50°. Using Lamarckian Genetic Algorithm the binding poses were generated. All the other parameters were kept as default. At last, for redocking studies Autodock Vina was used. For docking of all the 18 compounds as well as for the reference compound, the defined grid for Autodock was considered. Using Autodock Vina, which end up being progressively proficient, showed an exact algorithm that was much faster as compared to Autodock.

RESULTS AND DISCUSSION

4.1. Structure-based virtual screening

For carrying out SBVS, x-ray crystallographic structure of MAO-B was retrieved from Protein Data Bank (PDB ID: 4A7A). To perform SBVS and for docking numerous compounds to the binding site of MAO-B, the idock and Smina were used. The library of 98,072 natural compounds that was collected from the ZINC database is screened against the MAO-B software by using I-dock and Smina. Using I-dock and Smina software, the user can define their own position as well as dimensions of a grid search box but cannot define other settings. Natural compounds are those that contain benign functional groups were removed. A grid box of dimension 50*50*50 was utilized for performing virtual screening with I-dock and Smina software. From the initial step, 510 compounds were picked on the basis of higher binding affinity (>= -11.5 Kcal/mol) than the control ligand RGZ. After that, all the 510 compounds as well as the reference compound were further employed for ADMET analysis.

4.2. ADMET prediction

For designing of drug and its testing ADMET is an important aspect. For determining likeliness of the drug, Lipinski parameters were utilized as well as ADMET properties. From the ZINC database, the Lipinski parameters were fetched. In the study conducted, we discovered 510 suitable compounds from structure-based virtual screening that were further chosen for *in-silico* ADMET examination [7-8].

BBB (Blood Brain Barrier) is a significant criteria for drugs. P-glycoprotein (P-gp) works explicitly as a carrier mediated primary active efflux transporter. Carcinogenicity helps in depicting the capacity of a compound to cause cancer. On the basis of all these parameters like BBB, HIA, Caco-2 permeability, Pgp Substrate/Inhibitor, Cyp450 inhibition, toxicity, carcinogenicity and lethal dose we have selected 18 compounds out of 510 compounds and these compounds were further employed for the redocking studies.

4.3. Molecular docking

From the results of virtual screening and ADMET prediction, we picked 18 compounds that can be seen as potential inhibitors for MAO-B. For predicting the precise outcomes, the re-docking of the hits was performed. Using Autodock and Autodock Vina the best accurate binding conformation and docking energy was predicted. Binding affinity of −8.19 and −8.1 kcal mol^{-1} was displayed by the reference compound RGZ using Autodock and Autodock Vina respectively.

Using Autodock, we got four compounds which are: ZINC04237106, ZINC32501391, ZINC02147610 and ZINC05818772 with propinquity of −12.05, −12.49, −12.41 and −12.16 kcal mol^{-1} respectively. During ligand binding Cys172,

Tyr326, Ile198, Ile199, Leu171 played a major role in all of these compounds as all of them were available to make Hydrogen bonds.

Using Autodock Vina, re-docking of the obtained compounds and predicted those compounds which had an excellent propinquity towards MAO-B, these were: ZINC04237106, ZINC32501391, ZINC02147610 and ZINC05818772 that showed a binding affinity of −12.1, −13.0, −11.5 and −12.2 kcal mol^{-1}, respectively. From the results obtained, we selected only four compounds – ZINC04237106, ZINC32501391, ZINC02147610 and ZINC05818772 for further analysis and molecular dynamics simulation (MDS) of these compounds will be performed.

The detailed result like binding affinity, number of hydrogen bonds, predicted IC_{50} from Autodock of redocking for 18 compounds including RGZ were shown in the Table 1.

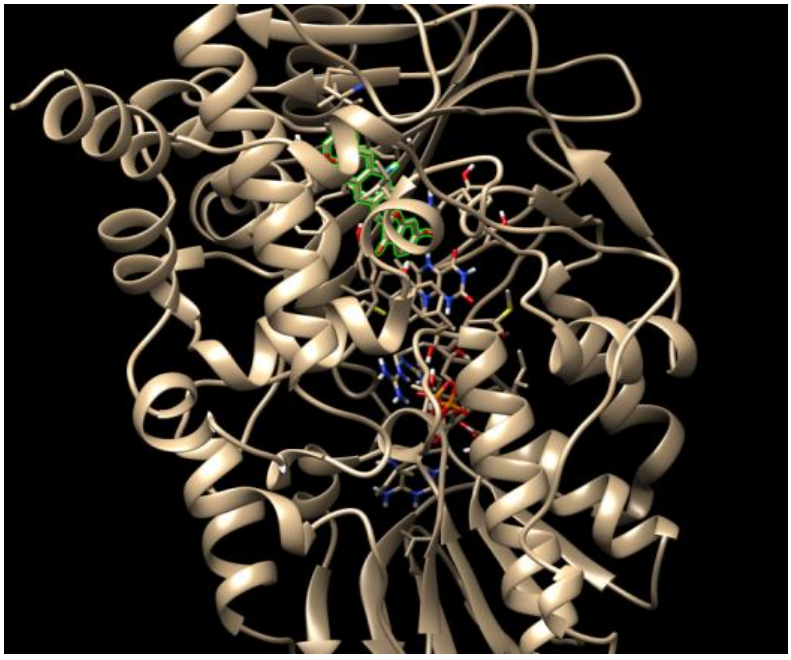
Table 1. The table showing the binding energy detail along with the interacting residues for all the selected 18 hits as well as for control ligand RGZ. The red residues are participating in the formation of hydrogen bonds between protein and ligand. The highlighted bold ZINC_ID showing the good binding affinity and further selected as a lead compound.

Sr. No.	**Compound ID**	**IC_{50}**	**Binding Energy ADT**	**H. bonds**	**Residues**	**Binding energy Vina**	**No. of H bond**	**Residues**
1.	RGZ	995.86nM	-8.19	1	GLN206,LEU171,PHE168,CYS172,TYR398	-8.1	1	PHE343,GLN206,LEU171,ILE199
2.	ZINC03847055	38.48 nM	-10.12	0	GLN206,LEU171,CYS172,ILE199,TRP119,PRO104,ILE316,TYR326	-8.9	0	ILE316,TYR326,LEU171,ILE199,CYS172,PHE168,TRP119
3.	**ZINC04237106**	1.47 nM	-12.05	1	PHE343,GLN206,CYS172,PHE168,LEU167,ILE316,TYR326,ILE199,LEU171	-12.1	1	ILE316,TYR326,ILE199,LEU171,CYS172,PHE168,LEU167,LEU164

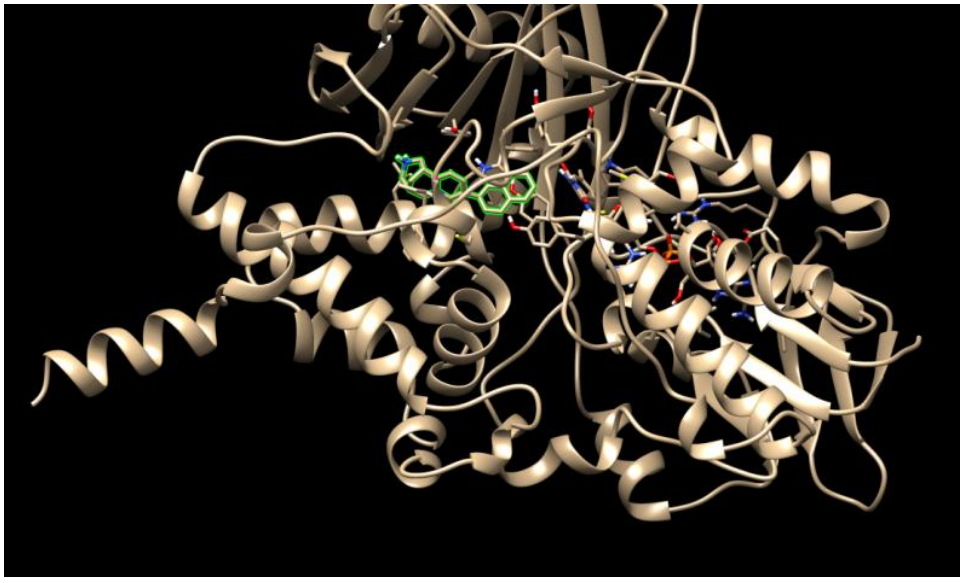
4.	ZINC04701203	2.65 nM	-11.7	3	TYR435,TYR60,SER59,TYR398,CYS397,TRP388,GLN206,CYS172	-11.6	1	TYR326,LEU171,CYS172,PHE168,ILE199,PHE343
5.	**ZINC32501391**	705.02pM	-12.49	0	GLY58,MET436,TYR398,TYR435,SER59,TYR60	-13.0	1	LEU171,PHE168,ILE199,TRP119,LEU164,GLN206,TYR326
6.	ZINC08879410	30.72nM	-10.25	3	GLY58,TRP388,MET436,CYS397,TYR398,TYR435,SER59,TYR60,CYS172,GLN206,FAD600	-11.2	2	GLN206,TYR326,ILE316,LEU171,PHE168,CYS172
7.	ZINC32124479	22.81nM	-10.43	2	TYR326,GLN206,CYS172,ILE198,LEU171,ILE199,PRO104,TRP119,PRO102	-11.7	1	TYR326,GLN206,PHE343,LEU171,ILE199,CYS172,PHE168
8.	ZINC04089530	168.39nM	-9.24	1	GLN206,ILE199,TYR326,LEU171,CYS172,PHE168,LEU167,LEU164,TRP119,PRO104	-8.4	1	TYR326,ILE199,PHE343,LEU171,LEU167,GLN206,CYS172

9.	ZINC68601571	3.52 nM	-11.53	1	TYR435,SER59,TYR60,GLY58,TYR398,CYS397,TRP388,TYR326	-11.3	3	TYR326,GLN206,LEU171,ILE198,ILE199,PHE168,CYS172,TYR435,CYS172
10	ZINC01694578	781.17nM	-8.33	3	ARG100,PHE99,HIS90,PRO105,TYR97,TRP107,PRO98	-10.7	1	TYR326,ILE199,PHE343,LEU171,CYS172,GLN206
11	ZINC06519582	3.43 nM	-11.55	1	TYR60,PHE343,GLN206,LEU171,PHE168,ILE199,TYR326,FAD600	-10.7	1	GLN206,TYR60,LEU171,PHE343,CYS172,PHE168,LEU167
12	ZINC04701595	8.93 nM	-10.98	1	SER59,PHE343,LEU171,PHE168,TYR326,GLN206,TYR60,FAD600	-11.1	0	TYR326,ILE199,LEU171,PHE168,GLN206
13	**ZINC02147610**	801.66pM	-12.41	4	GLN206,TYR326,CYS172,LEU171,PHE168,CYS172,FAD600	-11.5	3	TYR326,PHE343,LEU171,PHE168,GLN206,TYR60,CYS172,TYR435

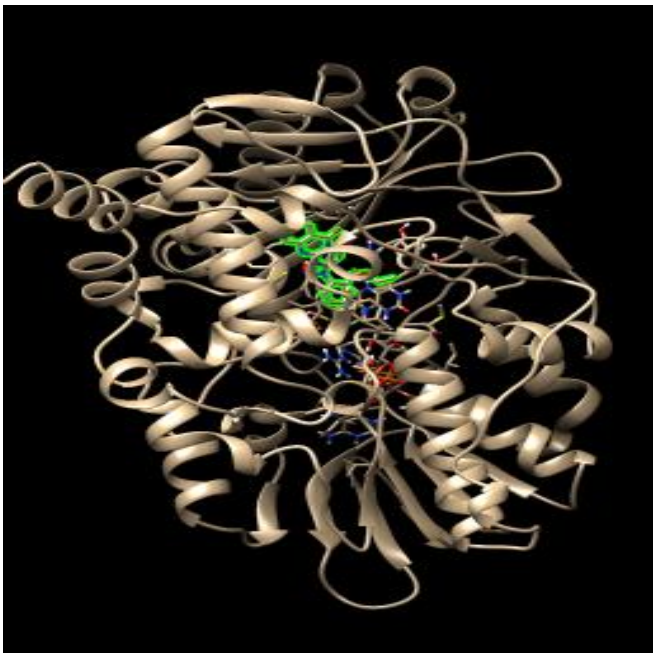
14	ZINC04041686	39.06nM	-10.11	2	GLN206,PHE343,CYS172,LEU171,PHE168,TRP119,ILE199,TYR326,FAD600	-12.1	1	TYR326,ILE199,LEU171,CYS172,GLN206
15	**ZINC05818772**	1.22 nM	-12.16	4	GLN206,PHE343,LEU171,ILE198,CYS172,PHE168,LEU167,ILE199,CYS172	-12.2	2	TYR326,ILE199,PHE343,LEU171,ILE198,CYS172,GLN206
16	ZINC13776773	15.44nM	-10.66	1	PHE343,GLN206,LEU171,CYS172,PHE168,TRP119,LEU164,TYR326	-10.1	2	TYR326,GLN206,PHE343,LEU171,ILE198,PHE168,CYS172,TYR60,TYR435
17	ZINC06493852	15.48nM	-10.66	1	LEU171,GLN206,CYS172,PHE168,ILE199,TRP119	-11.6	3	TYR326,PHE343,ILE198,LEU171,PHE168,CYS172,CYS172,CYS172
18	ZINC11689965	39.19nM	-10.1	1	TYR60,GLN206,PHE343,LEU171,CYS172,PHE168,TYR326,FAD600	-9.8	2	ILE199,TYR326,PHE168,LEU167,LEU171,CYS172,ILE198
19	ZINC11689968	44.99nM	-10.02	0	TYR326,GLN206,LEU171,CYS172,ILE199	-11.3	3	GLN206,PHE343,LEU171,TYR326,TYR435,TYR435



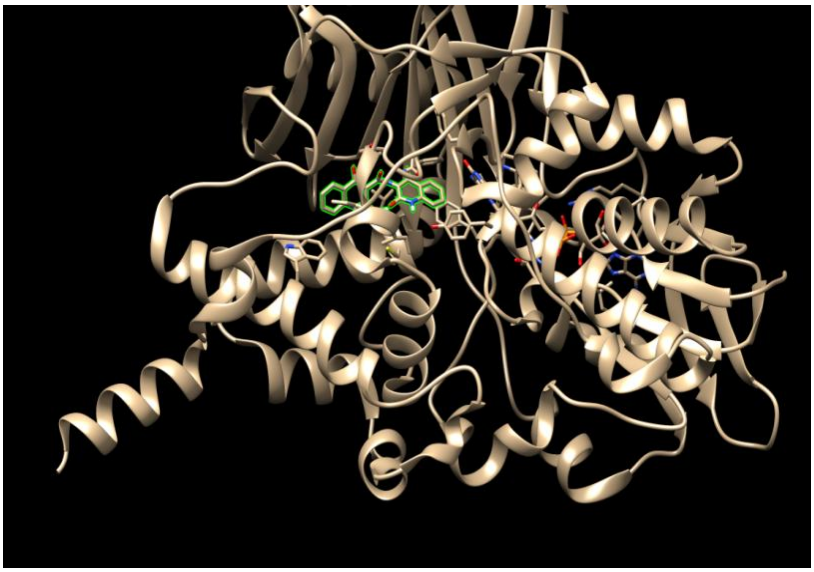
(A). ZINC32501391



(B). ZINC04237106



(C). ZINC02147610



(D). ZINC05818772

Figure 2. The binding poses for selected four hits in the MAOB binding pocket were shown. The ligands were highlighted in green.

CONCLUSION

The Alzheimer's disease is an escalating neurodegenerative disorder that affects millions of people every year and cannot be cured easily. MAO-B protein is accountable for Alzheimer's disease and its expression is elevated in the hippocampus and cerebral cortex areas of the brain. We fetched 98,072 natural compounds from ZINC database. After that we performed virtual screening using SMINA and I-DOCK software and from the virtual screening we obtained 510 compounds which showing the binding energy >=-11.5 Kcal/mol and these compounds were further used for ADMET analysis. We selected only those compounds which were fulfilling our criteria and out of these only 18 compounds were chosen including the reference inhibitor.

After the re-docking of compounds using AutoDock Tools and AutoDock Vina we found that four selected compounds: ZINC04237106, ZINC32501391, ZINC02147610 and ZINC05818772 exhibited excellent binding affinity.

Hence from all these analysis we have proposed these four compounds as a potent inhibitor against MAO-B and can act as a anti-Alzheimer compound in future by further validation using MDS, in vitro and in vivo techniques.

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