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Study and Evaluation of Novel Potent Acetyl Choline Esterase Inhibitors Using Molecular Docking Simulations: An In-silico Approach

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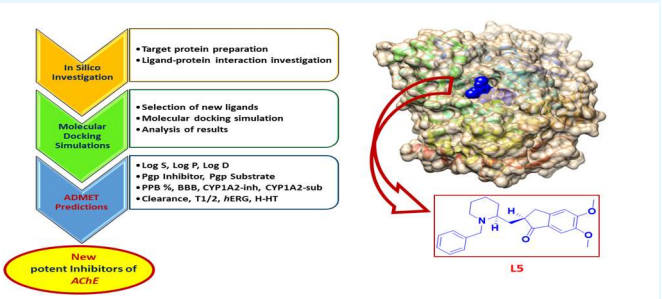
Abstract: Acetyl Choline Esterase (AChE) is one of the most important enzymes in the process of Alzheimer's disease. Inhibition of acetyl choline metabolism using inhibition of AChE is partially successful in improving symptoms of Alzheimer's disease. *In silico* study and evaluation are applied through virtual screening tools such as molecular docking simulations and prediction of ADMET-related properties to investigate novel potent inhibitors of AChE. The molecular docking simulation is performed to achieve the best binding affinity and docking scores. This is done by comparison between the standard inhibitor and high-scoring selected ligands. After evaluation of Molecular docking results and ADMET-related properties, 2-[(1-benzylpiperidin-2-*yl*) methyl]-5,6-dimethoxy-2,3-dihydroinden-1-one was indicated as novel potent AChE inhibitor.



Keywords: in silico, Acetyl Choline Esterase inhibitors, Molecular docking simulations, Novel inhibitors.

1. Introduction

Dementia is currently the seventh leading cause of death among all diseases and one of the major causes of disability and dependency among older people globally. Alzheimer's disease is the most common cause of dementia. The cause of Alzheimer's disease is not known, but it is characterized by marked atrophy of the cerebral cortex and loss of neurons. Histologically, Alzheimer's disease is marked by senile plaques, spherical accumulations of β-amyloid, degenerating neuronal processes, and neurofibrillary tangles. The pharmacotherapy of Alzheimer's disease has focused on increasing cholinergic function in the brain. Acetylcholine precursors, such as choline and lecithin, have not proven beneficial, but inhibition of acetylcholine metabolism using inhibitors of acetylcholinesterase is partially successful in improving symptoms of Alzheimer's disease [1-5]. One of the most popular treatments existing is based on the cholinergic hypothesis of maintaining acetylcholine (ACh) levels[6]. The AChE inhibitors reduce the hydrolysis of acetylcholine (ACh) into acetate and choline and consequently increase the ACh levels at the synaptic cleft which can stimulate cholinergic receptors and further promote memory function[5]. So, cholinesterase inhibitors such as *Donepezil*, *Galantamine*, *Rivastigmine*, and '*Huperzine A'* have been extensively studied as symptomatic treatments for Alzheimer's disease[3,5,7-9]. There were three designed AChE inhibitor groups. Such inhibitors may be classified according to the kinds of occupation sites of AChE enzyme. The active site of AChE consists of the esteratic site at which hydrolysis of the ester occurs and anionic-binding site where the cationic portion of acetylcholine binds. The first group of AChE inhibitors consist of molecules that occupy or interact with anionic site of AChE. The best members of this group are fourth kind amine such as *tetraethyl ammonium*. These compounds act nonspecifically and interact with a variety of enzymes and receptors with anionic sites. *Edrophonium* is the only interested compound in this section[10]. The second group of AChE inhibitors consists of molecules that are occupied just in the esteratic site of the enzyme. These compounds have pseudo-ester carbonyl groups are attack the serine amino acid of enzyme. These compounds are more resistant to acetylcholine to AChE-catalyzed hydrolysis. *Carbaryl* is a reversible, carbamate-derived AChE inhibitor that has a tremendous economic impact as an insecticide for use on houseplants and vegetables as well as for control of fleas and ticks on pets[11-12]. The third group of AChE inhibitors occupy both esteratic and anionic sites and are known as classical AChE inhibitors. These compounds have a positive portion to interact with the anionic site and a pseudo-esteratic portion to interact with the esteratic section of AChE. The *Physostigmine*, *Neostigmine*, *Pyridostigmine,* and *Rivastigmine* are the most important classical AChE inhibitors[13-15]. Furthermore, there are compounds known as nonclassical anti-cholinesterase such as *Donepezil*,

Galantamine, and *Tacrine*. *Donepezil* is a centrally acting, reversible, and noncompetitive AChE inhibitor. Its selectivity for AChE is 570 to 1250-fold than for Butyrylcholinesterase and it also exhibits greater affinity for brain AChE than for peripheral AChE[7-9,16]. In this paper, we report *in silico* study and evaluation of some new structural compounds like *Donepezil* as novel nonclassical anti-cholinesterase. Also, the comparison of binding affinity in active sites was performed to introduce novel AChE inhibitors using molecular docking simulation. The prediction of binding interaction among the protein target and the ligand, the orientation of the ligand in the target's binding pocket, and the scoring of the interaction are achieved by docking programs. Then the ADMET prediction was performed to confirm and evaluate of new introduced candidate compounds. Finally, two compounds were introduced as novel potent AChE inhibitors.

2. Results and Discussion

The three-dimensional structure and crystallographic data of the complex of acetyl choline esterase enzyme (AChE) and standard inhibitor ligand (pdb id code: 4ey7) were received and retrieved from *Protein Data Bank* and used as a target molecule and docking receptor (http://www.rcsb.org/pdb; pdb code: 4ey7). The 2D and 3D structures of selected ligands were received from *PubChem* data server as open chemistry database at *National Institute of Health* (NIH)[17]. All AChE inhibitors were selected and received from *PubChem* data server as compounds in ready-to-dock. *Molegro Virtual Docker* (MVD)[18-19] is a virtual screening software for computational drug discovery that can be used to screen compound libraries against potential drug targets. The *OpenBabel* software[20] used to convert chemical formats to each other and to calculate the minimum energy of the appropriate ligands throughout *PyRx* software. The *Discovery studio Client* (*v16.0.1 Dassault Systems Biovia Corp*) was used to investigate ligand-protein interactions[21-22]. The prediction of binding interaction among the protein target and the ligand, the orientation of the ligand in the target's binding pocket, and the scoring of the interaction are achieved by docking programs.

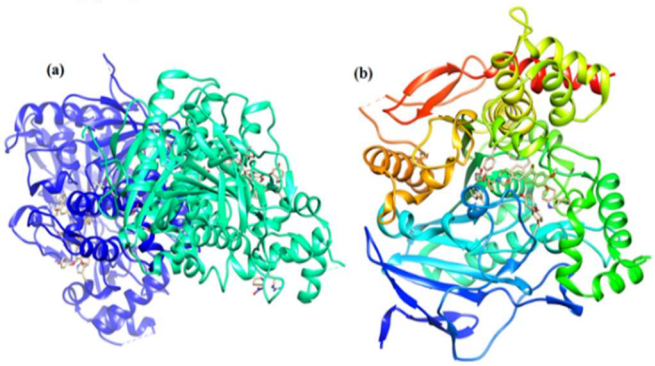


Figure 1. Crystal structure view of target protein retrieved with *Chimera* from protein data bank (pdb ID: 4ey7). (a) depth-cued view of Chain A (blue) and Chain B (green) and (b) position of standard inhibitor ligand (*Donepezil*) in the active site in chain A. Chain B has been removed.

The preparing of target protein

At first, the pdb format of human acetyl choline esterase was received from protein data bank. The crystal structure of human AChE provides an accurate platform for *in silico* study of ligand-target interactions. The pdf file was retrieved using *Chimera 1.8.1* and *AutoDock*. All the basic preparation processes such as removing non-standard molecules (solvent and ion molecules), removing water molecules, selection of basic protein chain, structure analysis, finding Hydrogen Bonds, detection of angles/torsions, and sequence rechecking were performed using *Chimera 1.8.1.* then, the target protein file was resaved as a pdf format for further processes (Figure 1 and S1).

The determination of active site in target molecule

After the preparation of the target protein, all 3D cavities in the crystallographic structure were detected using MVD software. The five expanded Van der Waals cavities were detected and compared with an x-ray diffraction snapshot of the target protein-*Donepezil* as the standard position and active site of ligand docked in the target protein. the active site was superimposable with one of the detected cavities using MVD software (figure 2). The volume of the active site was 172.544 Å^3. The determination of the active site is important to the determination of regions of the docking simulation process.

The preparation of ligands

The selection and preparation of ligands were performed based on a comparison with the 3D structure of *Donepezil* as a standard AChE inhibitor[3,8]. The structures of similar ligands were selected from the PubChem data server using Tanimoto scoring on the data server[23]. The comparison basis was 97% similarity at the Tanimoto threshold. As a result, ten molecules were identified as similar structures. These ten molecules (L1-L10) were considered as new ligands and molecular screening was performed using the *PyRx* platform. The molecular structure of ligands as well as specific code in the PubChem data server and IUPAC names are summarized in Table 1. The structure of *Donepezil* as a standard inhibitor ligand showed because of comparisons between structures[18,24-26].

The molecular docking simulation process

After preparation of target protein and ligands, the docking simulation was performed using *PyRx* as a virtual screening platform for computational drug discovery. All of substance structures were re-checked by *Chem Draw* and *ChemSketch ACD lab*. Then the molecular docking simulation was performed throughout target protein, L1-L10 ligands and *Donepezil* as standard inhibitor. the results and data analysis were obtained. The summary of results was showed in Table 2 and total results were showed in Table S1 in supplementary file.

The results of molecular docking simulations showed that compound L5 (table 2, entry 1) have best reranking score and binding affinity rather than other ligands and *Donepezil*. Furthermore, compounds L8, L10 and L3 have competitive binding affinities rather than *Donepezil* as standard inhibitor. the quantities of some ligands are precisely the same as *Donepezil* but L5 is considerably bigger than standard inhibitor. So, the best proposed ligand to interact and inhibit the AChE is 2-[(1-benzylpiperidin-2-*yl*)methyl]-5,6-dimethoxy-2,3-dihydroinden-1-one (L5, Table 1). Also, the L8, L10 and L3 ligands have good and competitive results in comparison with *Donepezil* as standard inhibitor. these results showed that L5, L8, L10 and L3 would be considered as potent inhibitors as well as *Donepezil* and other reported inhibitors. The 2D diagram of interactions between L5

ligand and active site receptor in target protein has been showed in Figure 3 and S3.

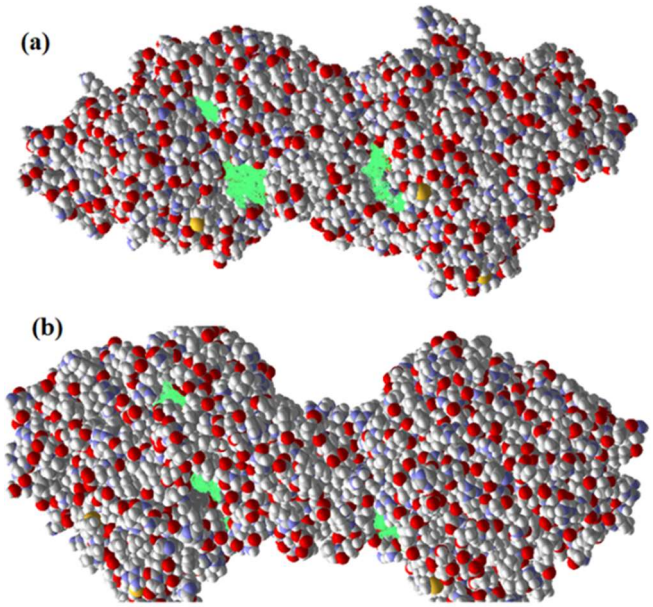


Figure 2. The Van Der Waals cavities detected in Target Protein using Molegro Virtual Docker. (a) side A cavities detected by green (b) side B cavities view from another side.

It can be seen from the figure 3 that there are Pi-Alkyl interactions among TYR341, TYR337, PHE338 and piperidine fragment of ligand. Also, there is conventional hydrogen bond interactions between SER203 and carbonyl group of inden-1-one fragment in L5 ligand. Other kinds of interactions such as van der Waals and carbon hydrogen bonds were indicated in 2D diagram in mentioned figure. For further investigations of active site in target protein and its interactions with L5 as high score selected inhibitor, the solvent accessibility surface (SAS) in $Å^2$ was extracted using *Discovery Studio Client.* The results shown in Figure 4(a). The calculation is done for selected parts of the structure in the context of selected atoms excluding water. Active surface colored by the solvent accessibility of the receptor residues from blue for exposed to green for buried. The interactions distances were indicated in figure 4(b). Also, in H-bonds map shown in Figure 4(c) the active surface colored by hydrogen bond type, with receptor donors colored in cyan and receptor acceptors in green.

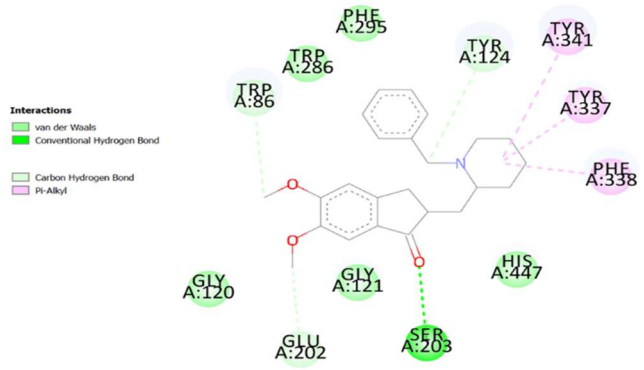


Figure 3. 2D diagram of active site receptor-L5 ligand interactions.

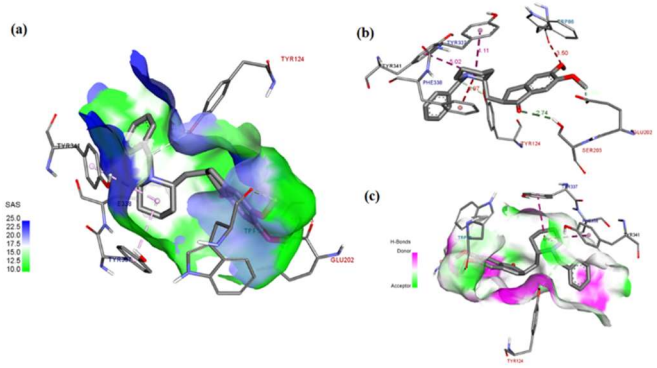


Figure 4. Close view of the active site in target protein (a) solvent accessibility surface of the active site in $Å^2$ (b) the interactions distances in Å (c) the active site surface map of hydrogen bonds.

The scoring functions

Evaluation of docking poses is crucial for the whole docking process. The docking algorithm, before returning the final orientation of a ligand, needs to evaluate the proposed compound position. Among its requirements, despite being accurate in the estimation of particular ligand-protein energy, there is a need for being fast, as the number of ligand-protein configurations can be unlimited. It should also be free of target bias, perform similarly for systems of various types, and display similar performance for different affinity ranges. Ideally, the scoring function should also be interpretable, so that the difference in its values can be explained from the ligand pose. The results of the docking simulations create a list of scoring functions and best binding affinities with a minimum amount of root mean square deviations are listed in Table 2.

4. ADMET-related properties prediction and evaluation

For further evaluation and selection between the ligands with the best binding affinity quantities (L5, L8, L10, and L3 respectively) the *ADMET-related* properties prediction and evaluation were performed. The basic physicochemical properties, absorption, distribution, metabolism, excretion, and toxicity quantities have been extracted from *ADMETLab 2.0*.[27-29]. These evaluations are performed based on high-quality prediction models trained by a multi-task graph attention framework. The summary results of predictions quantities have been showed in Table 3. The pharmacokinetic parameters have an important role in influence the biologic response to drug molecules. So, some parameters from physicochemical properties, absorption, distribution, metabolism and excretion were predicted to comparison between L5, L8, L10, L3 and *Donepezil* as standard inhibitor. Pgp-Inhibitor and Pgp-Substrate are two important parameters in absorption process. The results of these section verify in range 0 to 1. The 0 to 0.3 means excellent result and 0.7 to 1 means poor result. Thus, in Pgp-Inhibitor parameter, L5 and L8 are precisely the same as *Donepezil*. But, in Pgp-Substrate parameter, L5 and L10 are considerably smaller than *Donepezil* and have better results. In protein plasma binding (PPB), a compound is considered to have a proper PPB if it has predicted value < 90%. So, *Donepezil* is rather smaller than L5 and has better result whereas L10 has better result rather than *Donepezil*. In category of metabolism, two parameters were calculated. The output value is the probability of being inhibitor, within the range of 0 to 1. As can be seen in the

Table 1. The chemical structures, IUPAC names and PubChem codes of compounds applied as ligands

Ligand	PubChem CID	IUPAC name	Chemical structure
L1	10762160	(2*E*)-2-[(1-benzylpiperidin-4-yl)methylidene]-5,6-dimethoxy-3*H*-inden-1-one	
L2	91668174	2-[(1-benzylpiperidin-4-yl)methyl]-6-methoxy-5-(1^{11}C)methoxy-2,3-dihydroinden-1-one	
L3	9930479	2-[2-(1-benzylpiperidin-4-yl)ethyl]-5,6-dimethoxy-2,3-dihydroinden-1-one	
L4	137660992	2-(1-benzylpiperidine-4-carbonyl)-5,6-dimethoxy-2,3-dihydroinden-1-one	
L5	137660527	2-[(1-benzylpiperidin-2-yl)methyl]-5,6-dimethoxy-2,3-dihydroinden-1-one	
L6	54097201	2-[[1-[(4-fluorophenyl)methyl]piperidin-4-yl]methyl]-5,6-dimethoxy-2,3-dihydroinden-1-one	
L7	10763156	5,6-dimethoxy-2-[[1-[(4-methylphenyl)methyl]piperidin-4-yl]methyl]-2,3-dihydroinden-1-one	
L8	9822679	2-[3-(1-benzylpiperidin-4-yl)propyl]-5,6-dimethoxy-2,3-dihydroinden-1-one	
L9	25198055	5,6-dimethoxy-2-[[4-(piperidin-1-ylmethyl)phenyl]methyl]-2,3-dihydroinden-1-one	
L10	163563261	2-[(1-benzylpiperidin-4-yl)methyl]-5,6-bis(ethenoxy)-2,3-dihydroinden-1-one	
Donepezil	3152	2-[(1-benzylpiperidin-4-yl)methyl]-5,6-dimethoxy-2,3-dihydroinden-1-one	

results, L5 is almost the same as *Donepezil*. In Clearance and $T_{1/2}$ parameters, have been calculated. As shown in Table 3, L5 and L8 have excellent results just the same as *Donepezil*. Therefore, it can be inferred that L5 is a competitive and comparable inhibitor ligand rather than a standard inhibitor in pharmacokinetic properties. The molecular structures of L5 and L10 are shown in Figure 5.

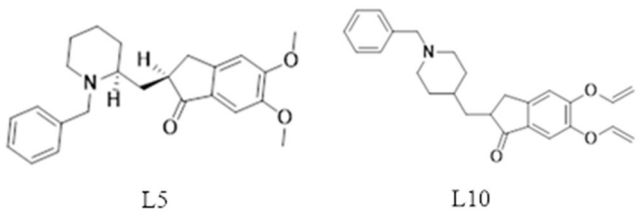


Figure 5. The molecular structure of L5 and L10 as the results of molecular docking simulations and ADMET evaluations.

Table 2. The summary results of molecular docking simulation. The ligand poses, binding affinities and RMSD quantities

Entry	Ligand	Binding Affinity	RMSD (Upper bound/ Lower bound)
1	**L5**	**-8.8**	0/0
2	L8	-7.5	0/0
3	L10	-7.4	0/0
4	L3	-7.4	0/0
5	***Donepezil***	**-7.3**	0/0
6	L1	-7.3	0/0
7	L6	-7.3	0/0
8	L9	-7.3	0/0
9	L2	-7.2	0/0
10	L7	-6.9	0/0
11	L4	-6.7	0/0

Table 3. summary results of prediction of ADMET related properties. Comparison between *Donepezil* and selected ligands

Category	Property	Prediction quantities for Ligands				
		Donepezil	**L5**	**L8**	**L10**	**L3**
Physico-chemical properties	LogS	-4.307	-3.844	-4.999	-5.877	-4.664
	LogP	4.191	4.058	4.928	5.669	4.517
	LogD	3.631	3.606	4.058	4.25	3.854
Absorption	Pgp-Inhibitor	0.998	0.997	0.999	1	0.999
	Pgp-Substrate	0.317	0.008	0.35	0.001	0.526
Distribution	PPB (%)	87.74	93.69	95.90	62.32	94.77
	BBB	0.975	0.943	0.921	0.608	0.956
Metabolism	CYP1A2-inh	0.14	0.145	0.124	0.413	0.123
	CYP1A2-sub	0.927	0.85	0.935	0.224	0.932
Excretion	Clearance	10.635	9.677	9.522	7.911	9.879
	T1/2	0.164	0.347	0.108	0.053	0.133
Toxicity	*h*ERG	0.99	0.965	0.993	0.959	0.992
	H-HT	0.381	0.39	0.38	0.588	0.388

5. Conclusions

In conclusion, the *in-silico* study was performed to investigate and evaluate novel potent inhibitors for Acetylcholine Esterase. The crystallographic structure of AChE and active site pockets were studied using *Chaimera* (PDB code: 4ey7).

Then preparation of the structure of target protein was performed. Some of the reported ligands were studied and one of them was selected as standard inhibitor. Using data servers' libraries as PubChem, some similar compounds selected and simulation molecular docking has occurred. Active site was studied and newly selected ligands were applied to molecular docking simulation in active site and other active cavities in target protein.

The results were evaluated and high quantities in binding affinities were selected as candidate ligands. Then prediction of ADMET related properties occurred using *ADMETLab 2.0* and prediction results were compared with molecular docking results. Binding affinity of L5 in the active site is higher than *Donepezil* and some ADMET-related properties were predicted and indicated better application as AChE inhibitor. Finally, indicated that 2-[(1-benzylpiperidin-2-*yl*) methyl]-5,6-dimethoxy-2,3-dihydroinden-1-one (L5 Table 1) has the best results and must be considered as new potent AChE inhibitor.

Declaration of Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contributions

S. Azimi: Investigation, Methodology, Data curation, Writing-Original draft preparation. E. Vessally: Supervision, Conceptualization. B. Mohammadi: Writing- Reviewing and Editing. K. Jalai-Rad: Data curation.

Supporting Information

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