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COMPUTATIONAL VALIDATION OF TACRINE ANALOGS AS ANTI-ALZHEIMER'S AGENTS AGAINST ACETYLCHOLINESTERASES

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ABSTRACT

Globally there are over 48 million people who grieve from Alzheimer's disease (AD), symptomatic treatment exists but there is no cure for it. Due to that, we wish to design suitable Tacrine analogs as anti-Alzheimer molecules for the AChE target using computational tools. AChE was carefully chosen as a target because inhibitors of AChE were effective and proven their efficacy in the management of dementia and mitigation of other symptoms. Extraction of lead molecules for Alzheimer's target (AChE) can be done by ligand - ligand similarity through the PubChem database and performed docking based virtual screening by AutoDock Vina. Based on the binding energy, we prioritized several lead molecules and collected their experimental LD₅₀ from the literature. Later QSAR model was built by applying correlation regression between experimental and predicted LD₅₀ using the EasyQSAR tool. The six designed new analogs (T1-T6) is based on the molecular modification of Tacrine which contain's a planner tricyclic ring system. Pharmacokinetic and toxicity studies were done for all the molecules to find drug likeliness by Mobylye@rpbs portal and Osiris property explorer. Molecular Docking was done with DockThor and AutoDock Vina separately. Acetylcholinesterase (1ACJ) was considered as target and six designed tacrine derivatives were considered as ligands. From the docking results, it was

found that the designed tacrine-like analogs displays better binding affinity and less toxicity than standard tacrine. We, therefore, propose that the above six molecules may act as potent AChE inhibitors.

Keywords: AutoDock Vina, EasyQSAR, PubChem, Molecular docking, Osiris Property Explorer, Virtual screening

INTRODUCTION

Search for new and stable drug with least toxicity is an essential prerequisite for treating Alzheimer's disease. AD, the most common reason of adult-onset dementia is characterized by a progressive decline of cognitive functions go together with behavioural manifestations. It accounts for 60-70% of cases of dementia worldwide, with a global prevalence rate of 24 million cases[1]. The drugs used in the clinical and research stages can delay the course of AD, but there is no drug to cure. Targets like acetylcholinesterase (AChE), N-methyl-d-aspartate (NMDA) receptor, glycogen synthase kinase 3 (GSK3), cyclin-dependent kinase 5 (CDK5), secretase, etc were discovered but the exact drug molecules against these targets are unknown [1].

Conferring to the cholinergic hypothesis, the main cause of AD is the decrease in acetylcholine (ACh) levels. Therefore, one of the potential therapeutic strategies is to intensify cholinergic levels in brain by inhibiting acetylcholinesterase activity. AChE inhibitors can increase the function of neural cells by increasing the concentration of ACh [4].

At present, the enhancement of cholinergic neurotransmission represents as a key approach in the symptomatic treatment of AD. As a result, AChE inhibitors have become a promising therapeutic strategy for treating the symptoms of AD [16]. Food and Drug Administration (FDA) approved only five drugs to treat AD. Among five, four are AChEIs, including Tacrine, Donepezil, Rivastigmine, and Galantamine. Although tacrine is the first generation AChEI, it is restricted in clinical usage due to hepatotoxicity [3]. Tacrine is reversible anticholinesterase binds with and inactivates cholinesterases. However, the use of tacrine is limited due to its many side effects, including nausea, vomiting, loss of appetite, abdominal discomfort, dizziness, headache, anxiety, blurred vision, dry mouth, insomnia, diarrhoea and clumsiness [7]. Patients treated with tacrine need blood monitoring because of hepatotoxicity induced by this drug [2]. The opposed side effects and the inconvenience of the four-times/day administration, as well as the availability of other ChE-Is led to the withdrawal of tacrine from the market in 2013 [5].

Tacrine yet, remained a crucial molecule for research into new AD drugs. It has been widely used in the past and in more recent studies to design hybrid or multi-target compounds in order to combine its potent AChE inhibition with other pharmacological activity [8]. Several tacrine derivatives have been reported so the key and vital point was to demonstrate that the newly-designed tacrine molecules weren't hepatotoxic, while retaining other beneficial cholinergic properties [6, 9].

MATERIALS AND METHODS:

Data Collection:

We have performed a 3D similarity search of tacrine in the PubChem database [13], which resulted in around 900 hits, later by applying Lipinski rule filters like Molecular Weight, Rotatable Bond Count, H-bond Donor Count, H-bond Acceptor Count, Polar Area, and Xlogp, 135 hits were found.

Methodology:

Docking based virtual screening [20] for the filtered hits was performed through AutoDock Vina version 1.1.2 with PERL scripting program. The target i.e., AChE (1ACJ) was downloaded from Protein Data Bank (PDB) and saved in pdb format [19]. The above-filtered ligands along with standard ligand (Tacrine) were saved in sdf format for further process.

Protein Target Preparation: Target preparation was done by deleting water

molecules, ligands, and ions followed by adding polar hydrogens using AutoDock Tools (ADT, v1.5.6).

Docking based screening: Autodock Vina was used for screening studies. Firstly generate a grid around the co-crystallized ligand, using co-ordinates (x = 4.76, y = 70.09, z = 65.94) which were found with the help of MGL Tools & Pharmit (<http://pharmit.csb.pitt.edu/>) [15]. Prepare pdbqt files for both target & ligands. The molecules were analysed after screening and visualized in discovery studio [12, 14].

Combinatorial Design of Tacrine (T1-T6) analogs: Six molecules were designed by molecular modification of tacrine as shown in the **Figure 1**.

QSAR Descriptors: Amongst virtual screening results, ten tacrine analogs were identified which consist of experimental LD₅₀ values, these values further support for building a QSAR model [17]. Descriptors like Molar Volume (MV), Index of Refraction (IR), Surface Tension (ST), Density (Den), Polarizability (Pol), and LogP were calculated for the below designed six analogs by using ChemSketch [11, 21].

ADMETox Screening: ADMETox screening was performed for the designed molecules by using mobile@rpbs online portal [11]. The other drug likeliness properties were screened with Osiris

property explorer [21, 12] to extract best ligands.

QSAR and Activity prediction: The QSAR model built with the help of above ten molecules to predict the toxicity of designed analogs [18]. LD₅₀ values of above ten analogs were taken from literature and the correlation and regression

were generated using EasyQSAR. The activity of selected ligands was predicted with the regression equation and the F statistics was checked for the significance of the correlation and the equation [15, 16]. Multiple regression plot generated for QSAR model (**Figure 2**).

Figure 1: Combinatorial Design of Tacrine (T1-T6) analogs

Figure 2: Multiple regression plot (Linear) of ten compounds against AChE

Molecular Docking studies: Protein-ligand docking helps to predict the binding mode of a ligand and protein of known 3-dimensional structures [10]. The three-dimensional (3D) structure of Acetylcholinesterase (1ACJ) was downloaded from Brookhaven Protein Data Bank (<https://www.rcsb.org>) and saved in pdb format [14]. Molecular docking was performed using DockThor and AutoDock Vina with 6 designed ligands and standard tacrine. Results of Docking were recorded with binding energy, Total energy, Interaction energy, Vanderwalls energy for DockThor (Table 6) and for AutoDock Vina (Table 5).

RESULTS AND DISCUSSION

After performing docking based virtual screening studies for 135 Pubchem tacrine

like molecules, the top ten molecules were taken which consist of experimental LD₅₀ values to build QSAR model by using EasyQSAR tool. The results are shown below (Table 1).

LD₅₀ values for the designed molecules (T1-T6) were predicted on the basis of actual and predicted LD₅₀ values of PubChem screened tacrine analogs by employing the QSAR technique using EasyQSAR tool as shown in (Table 2).

The result was found non-toxic by performing ADME/Tox filter (Table 3) and Osiris property explorer (Table 4) on the designed molecules (T1-T6).

Molecular docking with the target (1ACJ) exhibited that all the six designed molecules have good affinity with highest LD₅₀ score than tacrine (Table 5).

After construction of QSAR model the R^2 (square of correlation coefficient) was 75% and the F statistics value was found to be 78% which were much higher than F critical value 0.314574906 showing the significance of the regression equation.

The equation generated was

$$LD_{50} = -63541.39 + 535.71 + (-43670.21) * (MR) + 217.62 * (MV) + 38665.7 * (RI) + 21.51 * (ST) + (-4078.14) * (D) + 108758.96 * (P)$$

During experimental process flexible docking of all 6 ligands was carried out in the active site of Acetylcholinesterase (1ACJ) using DockThor and AutoDock Vina. Different poses for each ligand were generated out of which one best pose for each ligand was selected on the basis of their binding energy score (Table 5). QSAR model for toxicity i.e., LD_{50} values against top 10 screen molecules were taken from literature [9]. Activities i.e., logarithm

of inverse LD_{50} were correlated with six descriptors viz., molar volume, index of refraction, surface tension, density, polarizability and log P. These descriptors revealed significant correlation with the activities (Table 2).

Analysis of result (Table 5) showed that the designed analogs exhibit the best binding energy for AChE inhibition, with more LD_{50} values. While selecting best ligands, the least score in docking was preferred as it displays more stability in binding [8]. Docking pattern of ligands with the receptor is presented in (Figure 3). In order to have more convincing result, all six ligands were again docked in the same active site of the same target using DockThor [10] (Table 6) and once more found that all six designed molecules, shows best docking scores in two different software.

Table 1: Experimental and Predicted LD_{50} values of 10 PubChem screened molecules

S. No	SMILES	LD_{50} ACTUAL (mg/kg)	LD_{50} PREDICTED (mg/kg)
1	<chem>C1=CC(=CC2=NC3=C(C=CC(=C3)N)C=C21)N</chem>	140	83.16
2	<chem>C1=CC=C2C(=C1)C(=C3C=CC=CC3=N2)N</chem>	80	262.94
3	<chem>Cl=CC1=CC2=CC3=C(C=C(C(=C3)C)N)N=C2C=C1N</chem>	500	438.67
4	<chem>C1=CC=C2C(=C1)C=C3C(=N2)C=CC=C3N</chem>	330	262.94
5	<chem>C1CCC2=NC3=CC=CC=C3C(=C2C1)N</chem>	25	36.23
6	<chem>CC1=CC2=CC3=C(C=C(C(=C3)C)N)N=C2C=C1N</chem>	280	284.85
7	<chem>COC1=C(C=CC(=C1)NS(=O)(=O)C)NC2=C3C=CC=CC3=NC4=CC=CC=C42</chem>	110	110.52
8	<chem>CCOC1=CC2=C(C3=C(C=C(C(=C3)N)N=C2C=C1)N.CC(C(=O)O)O</chem>	75	73.47
9	<chem>COC1=CC2=C(C3=C(C=C(C(=C3)Cl)N=C2C=C1)N</chem>	110	112.22
10	<chem>COC1=CC2=C(C3=CC=CC=C3N=C2C=C1)N</chem>	70	54.99

Table 2: Predicted LD_{50} values of Designed Analogs

Compound Name	Smiles	Predicted LD_{50} (mg/kg)
T1	<chem>o1cc2c(c1)c(c1e(cccc1)n2)N</chem>	189.71
T2	<chem>c12e(cc3c(c1)CCCCC3)N=CC2</chem>	808.21
T3	<chem>c12e(cccc1)cc1c(c2C)CCCCC1</chem>	3898
T4	<chem>c12e(cccc1)cc1c(c2C)CCCCC1</chem>	2639
T5	<chem>Cc1c2e(cccc2)e(c2c1CCCCC2)C</chem>	2872
T6	<chem>c1c2c(cc3c(c2)CCCCC3)sc1</chem>	2647

Table 3: ADME/Tox properties of Ligands (T1 to T6) generated from Mobylye@rpbs portal

Ligand	T1	T2	T3	T4	T5	T6	Tacrine
Molecular Formula	C11H8N2O	C14H17N	C17H20	C16H18	C17H20	C14H16S	C13H14N2
MW (200-600)	184.19	199.29	224.34	210.31	224.34	216.34	198.26
logP ((-2)-6)	1.91	3.49	6	5.46	5.82	5.4	2.71
tPSA (0-150)	53.3	12.36	0	0	0	28.24	40.16
Rotatable Bonds (0-7)	0	0	0	0	0	0	0
Rigid Bonds (0-50)	15	17	18	17	17	17	16
Flexibility (0-15)	0	0	0	0	0	0	0
HBD (0-6)	2	0	0	0	0	0	2
HBA (0-12)	3	1	0	0	0	0	2
Total Charge (0-3)	1	0	0	0	0	0	1
Lipinski_Violation	0	0	1	1	1	1	0
Solubility(mg/l)	13375.36	6564.73	1224.16	1728.42	1371.16	1826.81	9562.9
Solubility Forecast Index	Good	Good	Reduced	Reduced	Reduced	Reduced	Good
Oral_Bioavailability_VEBER	Solubility	Solubility	Solubility	Solubility	Solubility	Solubility	Solubility
Oral_Bioavailability_EGAN	Good	Good	Good	Good	Good	Good	Good

Table 4: Osiris Property Explorer Drug likelinessPredictions of T1-T6 & Tacrine

Compound Name	Druglikeliness	Drug score	Mutagenic	Tumorigenic	Irritant	Reproductive
Tacrine	-7.18	0.16				
T1	0.73	0.73				
T2	-5.73	0.39				
T3	-7.59	0.12				
T4	-7.59	0.14				
T5	-7.69	0.13				
T6	-6.36	0.31				

High risk
 Medium risk
 Safe

Table 5: Binding interactions and docking score (KCal/mol) of designed tacrine analogs by AutoDock Vina

Compound Name	IUPAC Name	Aminoacids involved in binding	Binding Energy (KCal/mol)
T1	furo[3,4- <i>b</i>]quinolin-9-amine	ASP:72, TRP:84, PHE:330, GLY:118, GLY:119, GLU:199, SER:200, HIS:440	-8.8
T2	5,6,7,8,9,10-hexahydro-3 <i>H</i> -cycloocta[<i>f</i>]indole	TRP:84, PHE:330, SER:122, HIS:440, GLU:199	-10.6
T3	5-methyl-6,7,8,9,10,11-hexahydrocycloocta[<i>b</i>]naphthalene	TRP:84, TRP:279, PHE:330, TYR:334,	-12
T4	5-methyl-7,8,9,10-tetrahydro-6 <i>H</i> -cyclohepta[<i>b</i>]naphthalene	TRP:84, PHE:330, HIS:440	-11.5
T5	5,11-dimethyl-7,8,9,10-tetrahydro-6 <i>H</i> -cyclohepta[<i>b</i>]naphthalene	TRP:84, PHE:330, HIS:440	-11.4
T6	5,6,7,8,9,10-hexahydrocycloocta[4,5]benzo[1,2- <i>b</i>]thiophene	TRP:84, PHE: 290, PHE:330, PHE:331, HIS:440, TYR:333, TYR:121, TRP:279, TYR:334	-10.6
Tacrine (Standard)	1,2,3,4-tetrahydroacridin-9-amine	TRP:84, PHE:330, HIS:440, GLU:199, GLY:118, GLY:119,	-9.5

Figure 3: 2D & 3D Docking pattern of the Designed & standard ligands with the receptor active site (1ACJ)

Table 6: Dock Scores of T1-T6 with Dockthron

Compound	Score	Total Energy	Interaction Energy	Vander Wall Energy	Electrostatic Energy
Tacrine	-9.307	15.861	-27.691	-23.712	-3.979
T1	-9.026	2.044	-28.883	-23.233	-5.65
T2	-9.008	53.513	-29.846	-22.207	-7.639
T3	-9.524	71.026	-26.973	-24.781	-2.192
T4	-9.335	50.976	-28.12	-26.377	-1.743
T5	-10.233	70.483	-26.947	-26.522	-0.425
T6	-9.088	60.585	-24.872	-24.254	-0.618

CONCLUSION

Tacrine is the first drug to be widely marketed for Alzheimer's disease. Eventually, it was discontinued because of liver toxicity. Due to that, a virtual screening of tacrine-like analogs was performed in a publicly available chemical database like PubChem, during the study few lead molecules having more affinity, less toxic than tacrine was considered. Therefore, the designed Tacrine analogs show more affinity, less toxicity with permittable ADMET properties towards AChEs than Tacrine was projected. The molecular docking approach with two different software proves the same. Since these molecules are having all the drug-like characters further they can develop as new drugs for the treatment of Alzheimer's disease.

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