RESEARCH

An *in-silico* approach to identify potential drug molecules for Alzheimer's disease: a case with four therapeutic targets

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Abstract:

Background: Computational Methods in the 'omics' era has been a boon in the drug discovery field. Bioinformatics and cheminformatics databases and tools complement the successful discovery of promising lead compounds in the treatment of several disease conditions including neurodegenerative diseases such as Alzheimer's Disease (AD). However, commercially available drugs in the market to alleviate the disease progression in AD patients is sparse. The current research aims to apply an *in-silico* approach on multi-therapeutic agents against multi-therapeutic targets through docking studies to explore potential lead compounds for AD clinical trials.

Method: In the proposed research, virtual screening was performed with four US FDA-approved control drugs (Donepezil (DON), Galantamine (GAL), Rivastigmine (RIV), and Tacrine (TAC)) for mild-moderate-severe stages of AD treatment. The panel of compounds identified through virtual screening was subjected to chemical absorption, distribution, metabolism, excretion, and toxicity (ADMET) and Pharmacokinetics (PK). The compound with good ADMET and PK score was investigated further with molecular docking against the four therapeutic targets involved in AD. Ligands showing the highest binding affinity against cholinesterase inhibitors (AChE, BuChE), receptor antagonist (NMDA), and β-amyloid peptide (Aβ) were computed.

Result: It was observed that the compounds Quinazolidinone analogue, 2b, Isoquinoline-pyridine, 1, Benzylmorphine and Coelenteramide are the best lead candidates with the least side effects and better efficacy.

Conclusion: The predicted lead candidates are suitable for further investigation in the drug discovery pipeline.

Keywords: Alzheimer's disease, FDA approved drugs, molecular docking, therapeutic targets, virtual screening.

1. INTRODUCTION

Alzheimer's Disease (AD) is a progressive neurological disorder that affects the function of the brain leading to memory loss, decline in cognitive functions or learning inability. In short, it leads to a situation where patients cannot lead an independent life. Around 50 million people have dementia across the globe, ~10 million new cases every year [1]. Unfortunately, there is no drug available to cure AD and the ones available now only slow down the progression of neurodegeneration [2]. Moreover, the drugs in various phases of clinical trials for AD are much fewer in comparison to various other disease conditions [3].

Computer aided drug design has been popular in recent years in identifying acceptable drugs for various disease conditions. Several strategies adopted through multi-disciplinary approach to drug discovery by the modelers have found applications in the computational biology and chemistry fields for efficient discovery of hit compounds to lead compounds especially in the 'omics' revolution period. Moreover, *in-silico* methods can provide impressive results in terms of assessing toxicity and evaluating drug likeness based on Absorption, Distribution, Metabolism and Excretion (ADME) of the compounds [4]. 'Intelligent drug

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discovery' is increasingly adopted by major biopharma companies [5]. Hence the number of drugs in various stages of pre-clinical or clinical trials have improved significantly over the years. As the size of bioinformatics and cheminformatics databases have been increasing, *in-silico* studies have gained much attention in identifying lead compounds which can eventually be tested *in-vitro* or *in-vivo* for potential drug molecules. However, understanding the key factors involved in AD progression is of great significance for successful drug discovery. The knowledge of potential druggable targets is one such [6]. Around 26 potential Alzheimer's drug targets were reviewed by Chaudhary and co-workers (2018); some of the druggable targets are well known inhibitors, receptor antagonists, amyloid, and neurofibrillary tangles while others are still being studied to test their suitability against ligands [6].

Table **1.** Approved Drugs of choice

S. No	Compound	Chemical Structure	Mode of AChE Inhibition	Application
1.	Donepezil	Piperidine derivative	antagonist	Alzheimer's Disease Autism
2.	Rivastigmine	Carbamate	antagonist	Alzheimer's Disease Lewy bodies Parkinson's disease
3.	Galantamine	Alkaloid	antagonist	Alzheimer's disease
4.	Tacrine	Pyridine derivative	antagonist	Alzheimer's Disease

The enduring process of traditional drug discovery has been superseded by computational studies via virtual screening, docking procedures, molecular dynamics, machine learning algorithms and so on. Virtual screening techniques involving *in-silico* screening of large compounds possessing desirable properties against the targets to optimize the compounds has gained attention in identifying drug leads in contrast to the experimental set up which suffers bottlenecks in terms of cost, time, and effort [7]. This screening method utilizes the molecular docking procedure wherein the small drug molecules are docked with drug targets at the binding sites by finding the correct position and binding affinity prediction [8].

A multi-therapeutic target approach to multi-functional drug candidates yielded two lead compounds for AD treatment against AChE and BACE1 targets which werefurther confirmed experimentally [9]. Yet another study based on molecular docking led to identifying novel lead compounds that were *in-vitro* tested and confirmed the compounds of ethnopharmacological relevance as the therapeutic agents against therapeutic targets including the Cholinesterase and BACE inhibitors [10]. Onoda et al (2019) [11] found a lead compound from Tinospora cordifolia for AD by docking ligands and the active site of enzymes. A machine learning framework called DRIAD has been proposed which identifies potential drug compounds for AD which utilize gene expression profiles and US Food and Drug administration (FDA) approved drugs [12]. Potential lead compounds have been found for the treatment of AD in a study utilizing repurposing approach after considering antipsychotic drugs against five protein targets [3]. However, all of them require wet-lab experiments for final confirmation.

2. MATERIALS AND METHODS

2.1. Virtual screening of ligands

FDA drugs used for AD including Donepezil, Rivastigmine, Galantamine and Tacrine (Table. 1) [13] were used as the control to retrieve similar structures using the Swiss similarity tool [14], for the present investigation. The tool enables the search against a database of 4788 FDA approved experimental drugs. Among the list obtained, the top five ligands were retrieved based on the Tanimoto score (Supplementary Table.1). Finally, 20 test ligands were used for further investigations.

2.2. Pharmacokinetic (PK) Properties

The SMILE structures of the control (4) and test ligands (20) were downloaded from Pubchem database [15]. The pharmacokinetic properties of the ligands were evaluated by swissADME, admetSAR2.0 [16][17][18] and pkCSM online web server ([19]).

2.3. Therapeutic targets

Among the various hypotheses for AD [6][8], four potential targets for anti-Alzheimer's drug discovery are considered in the present study.

Amongst the options to improve the brain functions of AD patients include Cholinesterase inhibitors and N-methyl-D-Aspartate receptor antagonist [20]. Preventing amyloid beta peptide aggregation is yet another significant factor in deteriorating the neuronal loss in AD conditions [21]. Hence beta amyloid protein assembly is a potential therapeutic target of AD [22]. Finally, four potential druggable targets namely acetylcholine esterase (AChE), butyl choline esterase (BuChE), N-methyl-D-aspartate (NMDA) receptors and β-amyloid peptide (Aβ) involved in AD were chosen and their structures were downloaded from PDB database [23].

Table **2**. Target proteins of AD and their Grid box details assigned for molecular docking

S. No	PDB ID	Protein Names	Grid Center	Grid Size
1	1P0M	Human butyryl cholinesterase	x = 136.50 y = 123.28 z = 37.74	x = 39.94 y = 35.47 z = 38.53
2	1QXC	Beta amyloid peptide	x= 0.89 y = -1.28 z = -1.18	x= 16.56 y = 16.75 z = 17.90
3	4EY5	Human Acetylcholine sterase	x= -2.39 y = -40.08 z = 33.92	x= 36.47 y = 42.27 z = 39.56
4	1PBQ	NMDA receptor antagonist	x= 4.16 y = 39.39 z = -17.90	x= 33.40 y = 35.47 z = 29.74

2.4. Docking study

All the ligand structures were downloaded from Pubchem database. The ligands and proteins were minimized and pdbqt files suitable for docking were prepared using AutoDock Tools (ADT) [24]. Further, AutoGrid was used to create the grid box for the target proteins in their respective active site region. The parameters such as addition of hydrogen, Kollman charges and solvation of the protein structures were applied using ADT. The respective grid size, grid center and spacing for all the four target proteins are shown in the Table. 2. AutoDock Vina was employed for docking the ligand structures into the binding pocket of the proteins. The ligand with lowest binding affinity was extracted and aligned with the receptor for further analysis.

3. RESULTS AND DISCUSSION

3.1. Physicochemical and toxicity prediction

The failure of drug candidates to reach the market is due to the unsatisfactory PK property and toxicity prediction. Thus, computer-based prediction methods reduce cost and time of the candidate molecules to reach the drug discovery pipeline.

The physicochemical properties such as topological polar surface area (TPSA) and molecular weight of ligands were determined using the SwissADME web server. All the ligands have shown molecular weight >500 Da. Compounds with low molecular weight and low TPSA are predicted to be orally bioavailable. Oral consumption is the safest route and least expensive way for drug delivery. Also, low TPSA (>75) increases the likelihood of promiscuous binding to off-targets. Almost all the ligands of choice have TPSA below 75 Å which are considered to be suitable for absorption.

Among the twenty test compounds used for prediction, TAC5 resulted with low Gastrointestinal (GI) absorption. Also, the other ligands similar to Tacrine (TAC1-4) have resulted as AMES toxic. Similarly, the test compounds were predicted non-carcinogen except RIV5. Ligands such as GAL4, GAL5, TAC3 and TAC4 were presented under the category II which is moderately toxic and irritating. Apart, the other compounds were resulted with acute toxicity category III which is determined as slightly toxic and slightly irritating. From the log BB predicted values it was evaluated that all the test compounds have log BB values above 0.7. The log BB value greater than 0.3 is considered to cross blood-brain barrier (BBB) and less than -3 are difficult to penetrate the central nervous system (CNS). Thus, it has been observed that all the test compounds used for the study have the potential to cross BBB and CNS (Table. 3). Therefore, the value obtained will be useful to schedule the dose and volume to be used in the initial framework of *in-vivo* animal and human studies.

3.2. Molecular docking

The best ligands as lead molecules were selected based on the highest binding affinity (Table. 4) values (kcal/mol) of the docked complexes. Molecular docking of derivatives with AChE receptor protein of AD revealed DON2, DON4 and GAL2 have highest binding affinity of –11, -10 and -10.5 (kcal/mol) respectively. Compared to the standard drugs used for the study, the clinically approved test ligands have shown highest binding affinity with AChE. DON2 has substantiated its strong binding affinity to protein by establishing four hydrogen bonds with Glu202, Ser203, Tyr133 and His447. Besides, it has pi stacked and pi-alkyl interaction with TRP286 and TYR341respectively (Fig.1A-B). Interestingly, fluorine of DON2 is having interaction with Trp-86 which is an important residue for choline binding. DON2 interacts with the catalytic residues Ser203 and His447 of the active site of AChE. Therefore, it is demonstrated that DON2 can inhibit the catalytic activity of AChE.

Interaction analysis with BuChE has shown the ligands DON4, GAL2, RIV3 and TAC5 have binding energy (>10.8) compared to other derivatives. Among them, DON4 has the highest binding affinity of -11.2 (kcal/mol). DON4 has stabilized the interaction through four hydrogen bond interactions (ASP70, Glu197, Pro285 and His438). It also has Pi-alkyl, Pi-Pi-stacked and amid-Pi stacked interactions with Leu286, Gly116, Phe329, Trp82, and Trp231 (Fig. 1C-D).

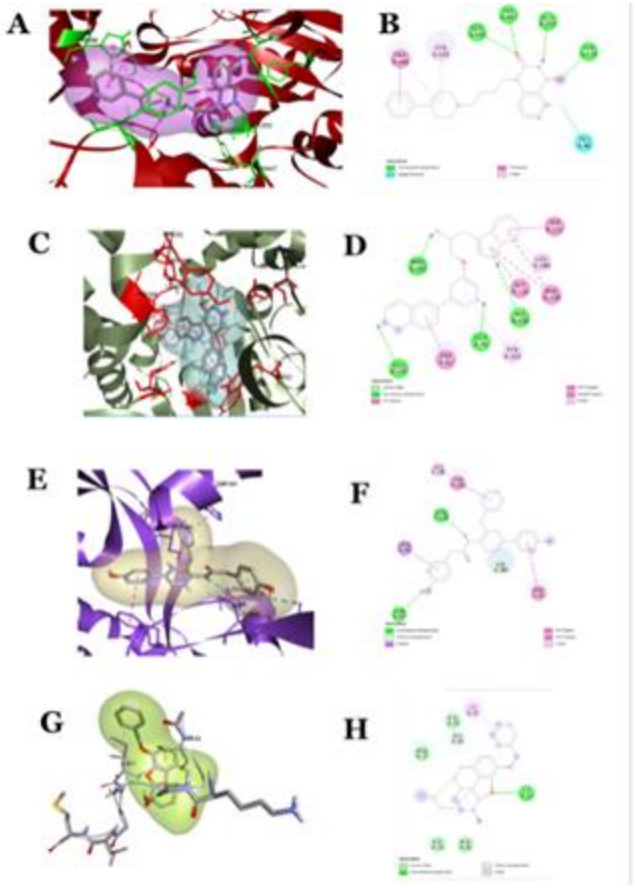

Fig. 1. Interaction map of ligands with the target receptor protein; (A) DON2 interacting with AChE receptor. (C) DON4 interacting with BuChE; (E) Interaction of RIV4 with NMDA receptor 2D structure; (G) Interaction of GAL2 with Aβ-peptide. (B, D, F and H) are the 2D representation of the respective proteins.

Similarly, molecular docking of the ligands with NMDA revealed RIV4 has the highest binding affinity of -10.2 (kcal/mol). DON4 is the second ligand with highest binding affinity of -10.1. The other ligands GAL2 and TAC5 have the binding energy of -9.6 and -9.8 respectively. RIV4 shows two hydrogen bond interactions with ASN123 and GLN95 of NMDA. Different types of pi interactions such as pi-sigma (Thr94), pi-pi (Tyr184, Phe92) and pi- alkyl (Leu146) interactions were observed (Fig. 1E-F).

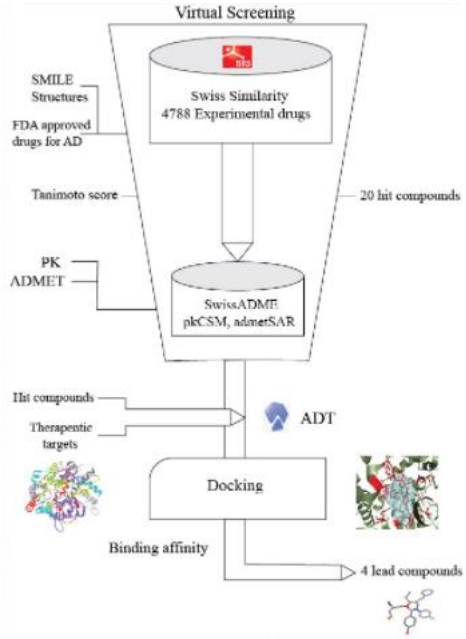

Fig. 2. Workflow of *in-silico* approach

The binding prediction of Aβ-peptide with ligands revealed GAL2 and RIV2 showed the highest binding affinity of -5.4 and -5 (kcal/mol). DON4 and TAC5 have shown binding affinity of -4.7. GAL2 has shown hydrogen bonding interaction with Ile32 in the hydrophobic pocket of Aβ-peptide. Additionally, GAL2 formed a pi- alkyl interaction with Ile31. Eventually, van der Waals interactions were observed with GAL2 and amino acids such as Gly25, Asn27, Ala30 and Gly33 of Aβ-peptide. Thus, the results indicate that such interactions of GAL2 with active residue of Aβ-peptide can hinder the aggregation of the peptide during pathological conditions (Fig. 1G-H).

4. CONCLUSION

Even though the conditions of AD were officially described more than a century ago, there is no cure for it yet and its treatment remains a challenge. Several strategies for AD treatment exist from a molecular druggable target perspective. A wealth of cutting-edge research for drug discovery has been carried out utilizing multiple therapeutic targets based on enzymes/receptors/proteins with drug compounds as inhibitors/receptors. In the current study, four potential targets (PDB ID: 1P0M, 1QXC, 4EY5, 1PBQ) were chosen. Compounds having similar structure of FDA approved drugs for various stages of AD treatment were obtained to perform molecular docking. Overall, from the docking studies following the workflow in Figure 2, it was observed that DON2, DON4, RIV4 and GAL2 (Quinazolidinone analogue, 2b (Pubchem ID: 448890), Isoquinoline-pyridine, 1 (Pubchem ID: 6914611), Coelenteramide (Pubchem ID: 448487) and Benzylmorphine (Pubchem ID: 5362507) respectively) show the highest binding affinity with the target proteins involved in AD. Moreover, based on the PK and toxicity-based studies, it was observed that these ligands are suitable for further investigation in the drug discovery pipeline.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data supporting the findings of the article is available at https://www.evernote.com/shard/s650/sh/8b59950e-e88a-d054-5ea6-7b1ef8a572a9/7f1d18e5a3b669598b03695a811132e4.

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CONFLICT OF INTEREST

None.

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SUPPLEMENTARY MATERIAL

S. Table **1.** Compounds identified based on the similarity with the approved drugs.

Table **3**. ADMET properties of the compounds

Derivatives	TPSA (Å^2)	GI absorption	Log BB	AMES Toxicity	Carcinogens	Acute Oral Toxicity
DON1	38.77	High	0.9953	Non-AMES toxic	Non-carcinogens	III
DON2	58.10	High	0.9592	Non-AMES toxic	Non-carcinogens	III
DON3	117.95	High	0.7450	Non-AMES toxic	Non-carcinogens	III
DON4	76.82	High	0.9845	Non-AMES toxic	Non-carcinogens	III
DON5	78.53	High	0.8336	Non-AMES toxic	Non-carcinogens	III
GAL1	62.16	High	0.9382	Non-AMES toxic	Non-carcinogens	III
GAL2	41.93	High	0.9980	Non-AMES toxic	Non-carcinogens	III
GAL3	68.23	High	0.9723	Non-AMES toxic	Non-carcinogens	III
GAL4	41.93	High	0.9974	Non-AMES toxic	Non-carcinogens	II
GAL5	38.69	High	0.9929	Non-AMES toxic	Non-carcinogens	II
RIV1	23.47	High	0.8664	Non-AMES toxic	Non-carcinogens	II
RIV2	32.78	High	0.9926	Non-AMES toxic	Non-carcinogens	III
RIV3	65.12	High	0.9468	Non-AMES toxic	Non-carcinogens	III
RIV4	95.34	High	0.8769	Non-AMES toxic	Non-carcinogens	III
RIV5	69.64	High	0.7176	Non-AMES toxic	Carcinogens	III
TAC1	38.91	High	0.9217	AMES toxic	Non-carcinogens	III
TAC2	24.92	High	0.9604	AMES toxic	Non-carcinogens	III
TAC3	24.92	High	0.9776	AMES toxic	Non-carcinogens	II
TAC4	50.94	High	0.9279	AMES toxic	Non-carcinogens	II
TAC5	49.84	Low	0.9319	Non-AMES toxic	Non-carcinogens	III

Table **4**. Docking of ligands with therapeutic targets

Compounds	Binding affinity (kcal/mol)				Molecular weight (g/mol)
	AChE	BuChE	NMDA	Beta amyloid peptide	
DON	-6.6	-10.4	-8.7	-4.6	379.5
DON1	-8.7	-10.1	-8.7	-4.6	379.49
DON2	-11	-11.2	-9.4	-4.4	393.45
DON3	-7.2	-10.1	-9.6	-5	478
DON4	-10	-11.2	-10.1	-4.7	394.47
DON5	-6	-9.6	-9	-4.5	592.77
GAL	-6.6	-10.4	-7.8	-4.1	287.35
GAL1	-6	-9.8	-7.1	-4.8	423.54
GAL2	-10.5	-10.9	-9.6	-5.4	375.46
GAL3	-6.2	-10.5	-7.8	-4.1	453.57
GAL4	-6.1	-8.7	-7.8	-4.8	301.38
GAL5	-7.1	-9.6	-8.7	-4.7	314.4
RIV	-6.3	-7.1	-6.9	-3.9	250.34
RIV1	-6.9	-6.2	-6.1	-3.4	165.23
RIV2	-8.3	-10.2	-8.9	-5	441.01
RIV3	-7.4	-10.9	-8.6	-4.8	398.5
RIV4	-7.9	-10.8	-10.2	-4.6	411.45
RIV5	-7.5	-8.5	-7.8	-4.7	302.37

TAC	-8.7	-8.5	-7.7	-4.2	198.26
TAC1	-7.1	-8.5	-9.1	-4.7	298.81
TAC2	-8.8	-10.1	-9.2	-5	414.28
TAC3	-5.3	-8.5	-7.7	-4.1	288.39
TAC4	-8.2	-8.2	-7.7	-4.1	325.49
TAC5	-9.3	-10.8	-9.8	-4.7	452.63

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