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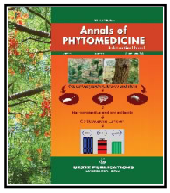

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In silico identification of putative acetylcholinesterase inhibitor in the context of Alzheimer's disease

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Abstract

Neurodegenerative diseases have gained focus in recent years as most of the drugs available for treatment do not provide a complete cure rather, they assist the patient in good living. One of the most common neurodegenerative diseases in old individuals is Alzheimer's disease (AD) against which only limited resources are available to treat the patients such as donepezil. Molecules responsible for the manifestation of disease are the potential targets for the discovery of novel inhibitors. Acetylcholinesterase (AChE) is an enzyme found in the brain and is responsible for the breakdown of acetylcholine. AChE inhibition may lead to slow down or stop the degeneration of neurons, therefore a variety of inhibitors have been discovered but these inhibitors only decrease the rate of neuron degeneracy which in turn provides ample scope for the discovery of new inhibitors. MCULE tool was used to screen out millions of compounds and further various criteria were applied to find out the best plausible therapeutic molecule. A toxicity filter was applied so that only those compounds are selected which are non-toxic. AutoDock-Vina rankings, leaving out ligands having less than four H-bond acceptors, as well as blood-brain barrier impermeability, filtration by ΔG cutoff, rule-of-five (RO5) violation and SWISS ADME profiling, were used to narrow down hits to find out possible binding of selected molecules with human brain AChE. A holistic analysis of the compounds resulted in further screening of the compounds. Various computational tools such as CASTp3.0, MCULE, SWISS ADME, *etc*., were used to examine and screen out millions of compounds to narrow down the search for potential AChE inhibitor which eventually resulted in selecting the 'top molecule', namely; (4Z)-4-[(4-fluorophenyl)hydrazinylidene]-5-methyl-2-phenylpyrazol-3-one with MCULE id MCULE-9685671672, the selected compound was found to display a robust binding with human AChE through 20 amino acid residues (ΔG: – 10.7 kcal/mol) while 7 of these residues were same as those displayed by 'Donepezil binding interactions'. It very easily passed through all major drug screen filters, including the 'toxicity checker'. Post MD analysis depicts MCULE-9685671672 is more stable in comparison to Donepezil with a ΔG of – 10.7 kcal/mol satisfying adequate ADME and molecular dynamic features for further *in vitro* and *in vivo* validation in the context of Alzheimer's disease. The stability of the best ligand hit was assessed through MD simulation of 50 ns duration.

1. Introduction

Alzheimer's disease is a neural abnormality, caused by the improper clustering of normal beta-amyloid protein. Approximately, 15 million people are affected throughout the globe by this disease. Females are more predominant than males and data from Indian statistics 2020 shows that the symptoms of Alzheimer's disease are manifested in the average age group between 66-68 years. This disease is characterized by memory loss/amnesia. Patients can survive up to 8 years after the symptoms of dementia (Das *et al*., 2021). Current medications alleviate symptoms but do not have an overall significant impact on disease progression. Globally, researchers are trying hard to find out the complete cure for the said disease (Kristinsdottir, 2009). Some scientists have also focused on the processing of the amyloid precursor protein to lower the levels of the amyloid deposits. Recently in 2021, ADUCANUMAB (brand name ADUHELM) has been approved by US FDA (US FDA, 2021). It targets aggregated forms of brain of the patients sussering from amyloid beta in the Alzheimer's disease. However, still cholinesterase inhibition remains the major strategy against Alzheimer's disease. Currently, most approved drugs are based on cholinesterase inhibition, although they do not affect the illness's progression (Tomlinson *et al.*, 1970). In the present study, AChE binding interactions with a variety of ligands were explored and analyzed, which may show efficient inhibition of the AChE enzyme by known, speculative or future inhibitors (Summers *et al.*, 1986). The cause of AD is the improper generation of acetylcholine as stated by the cholinergic hypothesis, AChE degrades the acetylcholine. Various AChE inhibitors such as tacrine and donepezil were used, but these inhibitors do slow down the disease's related neurodegeneration, providing temporary comfort to patients without a potential permanent cure in reality. Inhibition of the AChE enzyme, which is accountable for the breakdown of the aforesaid neurotransmitter, is a well-known method for reducing some behavioural and cognitive symptoms linked with Alzheimer's disease (Sugimoto *et al.*, 2000).

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Computational screening has gained much attention because of its ability to contribute to the drug development process. Thousands of available ligand structures with required molecular properties (pharmacophores) can now be investigated to confine to some very effective drug molecule(s); a few of which will be examined *in vitro* and *in vivo*, leading to those ligand hits which may be allowed to proceed to human clinical trials. The *in silico* matching (docking) of enzymes and ligands is an essential part of any structure base virtual screening (SBVS) process (Hughes *et al.*, 2011).

SWISS ADME is the web-based freely available platform for evaluating pharmacokinetic characteristics, drug-likeness, and medicinal chemistry-based friendliness of tiny ligands (Madhavi *et al*., 2013). ADME stands for absorption, distribution, metabolism, and excretion, which are all key aspects of a ligand/drug, while "binding free energy" is represented by ΔG.

There has been a continuous effort by the researcher fraternity to find a solution to the problem of cognitive decline and related ailments among elderly people around the world. Despite these efforts, the number of effective cholinesterase inhibitors accessible for patient usage remains limited (Small *et al*., 2011). Moreover, the present medications available in the market can only give a calmative response rather than a complete treatment of the disease. As a result, the development of novel AChE inhibitors is still a pressing need. The goal of this research was to find new inhibitors of the human brain AChE catalytic site (CAS-site) using SBVS of over 5 million ligand molecules available in the MCULE database, narrow down to a final seed molecule using different criteria as well as to investigate relevant chemical interactions in the bound state for further testing. The basic of any SBVS protocol is enzyme-ligand docking. The decline in cognitive ability has been the target area for researchers worldwide. Patients have very limited options for cholinesterase inhibitors drugs in the market which unlocks the opportunity for researchers worldwide to find out novel potent AChE inhibitors that can be used by the patients suffering from Alzheimer's disease (Ferreira *et al.*, 2015).

2. Materials and Methods

2.1 Study of the AChE binding crevice and preparation of protein

Using the scheme proposed by Tian and his team the three-dimensional (3D) structure of the CAS site of human AChE was investigated using "CASTp," which stands for "computed atlas of surface topography of proteins". This technique, in turn, uses binding crevice analyses by delaunay triangulation, discrete flow computational geometry elements that employ the alpha shape approach to identify important protein characteristics, quantify volume and area, and compute imprint (Cheung *et al*., 2012). The protein data bank PDB ID 4EY7 was thoroughly examined for investigating the binding site using the default probe radius of 1.4 Å. The discovery studio visualizer (BIOVIA/Accelrys) was used to delete the ligand attached with the complex, water molecules were removed except those which were in the vicinity of the CAS-site residues, required H-atoms were added. MAESTRO 9.8 was used to create the optimal protein structure for inclusion in the workflow builder (Trott *et al*., 2010). Minimization of energy was done using computational tool OPLS2005 along with PROPKA utility to achieve a protonation state at pH 7.4 (Kumar Singh *et al*., 2014).

2.2 Computational screening

High throughput computational screening was done to find out the structure of the potential ligand that can serve as a new AChE CAS site inhibitor or be treated as a seed molecule that will lead to the identification of upcoming new inhibitors based on seed design(s). Structure-based virtual screening was done with the drug discovery platform, MCULE, to screen over 5×10^6 potential drug candidates (Daina *et al*., 2017). For the SBVS input, a stepwise query was performed. Primarily, inside the "basic property filter" tab of the MCULE drug discovery platform's SBVS workflow, one RO5 violations was allowed to keep the search broad-ranged as well as flexible; however, maximum of 6 numbers of rotatable bonds were entered along with the sampler size 1000. The criterion of 'the highest number of most-diverse-molecules was given the value of 100. The threshold similarity cut-off was set at 0.7, while the rest of the parameters were left at the 'default' settings given by MCULE. As the screening continued, the 'Open Babel Linear Fingerprint' was used to evaluate molecular descriptors. 3×10^6 was the numerical figure provided to "the greatest number of compounds following sphere-exclusion".

2.3 Molecular docking

Computational tool MAESTRO 9.8 generated pdb file to be used by auto-dock-vina for docking simulation in .pdb file format (Bas *et al*., 2008). A 60 Å ×60 Å ×60 $Å^3$ sized "grid" was provided to completely cover the human brain AChE enzyme CAS site by the auto grid program. To define the "position" of the grid in three-dimensional space, the grid values of x, y and z grid coordinates are required and the same was mined from the complexed crystal structure with the PDB ID 4EY7 available in the protein data bank, this represents the complex of AChE inhibitor drug donepezil and AChE enzyme in the interactive state (Sugimoto *et al*., 2002). The grid position values entered in auto-dock-vina for x was "14.108464 along with "43.832714, and 27.669929 for y and z, respectively (Cheung *et al*., 2012), followed by the default method auto-dock protocol (Bas *et al*., 2008). Ligands hits with significant structures were obtained as result. Following that, the AChE enzyme was allowed to dock with all of the SBVS hits establishing the optimal binding accord and position for individual ligands. Discovery studio 2016 (BIOVIA) along with UCSF chimera 1.9 and PyMol V1.5.0.4 (Schrödinger, New York) were used to do a thorough study of the enzyme-ligand complexes (Pettersen *et al*., 2004).

2.4 Selection of top structure by vina ranking score hits

Stronger binding onto the specified site of the target enzyme is represented by a greater negative vina score which in turn reflects top ligand hits, same is applied to the entire ligand set post-SBVS, and ranking was done according to vina score (Lionta *et al*., 2014). As a result, a refined set of ligand structures comprising higher Vina scores was obtained and ranked accordingly.

2.5 SWISS ADME profiling

SWISS ADME was used to compare the pharmacological profiles of these 99 ligands (Brenk *et al*., 2008). This involved running a variety of experiments on the ligands under investigation. The possibility of the ligand acting as a potential future therapeutic molecule was also projected. Various filters like egan (Pharmacia), ghose (Amgen), veber (GSK), muegge (Bayer) and lipinski (Pfizer), were used to

assess drug similarity. 'PAINS,' a significant medicinal chemistry filter, was also used to evaluate the ligands. All ligands that failed more than two drug-likeness filters were rejected, resulting in a narrowed list of potential medicines.

2.6 MCULE toxicity filtration

The presence of likely substructures existing with the complex structures of harmful and/or multifarious molecules was examined by the "toxicity checker" feature available in the MCULE database and the entire ligand sets were subjected to toxicity check to further narrow down the ligand list (Lipinski *et al*., 2004).

2.7 ΔG cutoff value and zero RO5 violation criterion filtration

The set of ligands previously described was again reduced by assigning a ΔG cutoff value of – 9.9 kcal/mol. As a result, every ligand with an AChE enzyme complex having a ΔG value greater than the – 9.9 kcal/mol was omitted from the final bunch of the ligands. Following that, each ligand with two or more RO5 breaches was deleted, resulting in a smaller set of workable ligands (Shakil, 2019).

2.8 Swiss ADME profiling and molecular interactions for the selected ligands

For additional knowledge enhancement, molecular interactions of the final bucket of usable ligands were analyzed individually using different tools for visualizing molecular interactions such as discovery studio 2016 (BIOVIA), UCSF chimera 1.9 (Alam *et al*., 2012), and PyMol V1.5.0.4. After filtering with above-mentioned visualization applications, swiss ADME profiling was applied to the remaining four ligands. A significant variety of experiments were performed on the candidate drug compounds as part of the SWISS ADME profiling (Schapira *et al*., 2017). There were also assessments for "druglikeness," which included lipinski and muegge filters. Brenk, lead-likeness filters along with PAINS, were also utilized as "medicinal chemistry filters" to uncover the most propitious new seed molecule targeting inhibition of AChE's CAS site.

2.9 Molecular dynamics simulation of MCULE-9685671672 and reference drug donepezil

MD simulation of 50 ns duration was performed on docked complexes of AChE with MCULE-9685671672 and donepezil at 300 K at the MM level using GROMACS 5.1.2 (Van Der Spoel *et al*., 2005).

3. Results

Present work was focused to find out new inhibitors(s) of the CAS site of AChE present human brain, using structure-based virtual screening of more than five million ligand compounds in the MCULE platform as well as investigating relevant chemical interactions in the bounded form as shown in Figure 1. The ligands obtained after SBVS were then ranked according to vina score, MCULE toxicity filtration, ΔG cutoff setting, zero RO5 violation, and SWISS ADME profiling criterion, along with lipinski, ghose, veber, PAINS, BRENK and muegge filters. "PAINS" stands for "Pan assay interference patterns" in this context. Because of possible toxicity issues or adverse pharmacokinetics, the BRENK filter was employed to omit unrequired functionality (Makhouri *et al*., 2018). Apart from these, at each phase of the computer screening, manual screening based on an extensive PubMed literature review was employed to pinpoint potential ligand molecule(s). The final text comprises findings of the present study in the same sequence as in the methodology section for the simplicity of the comprehension.

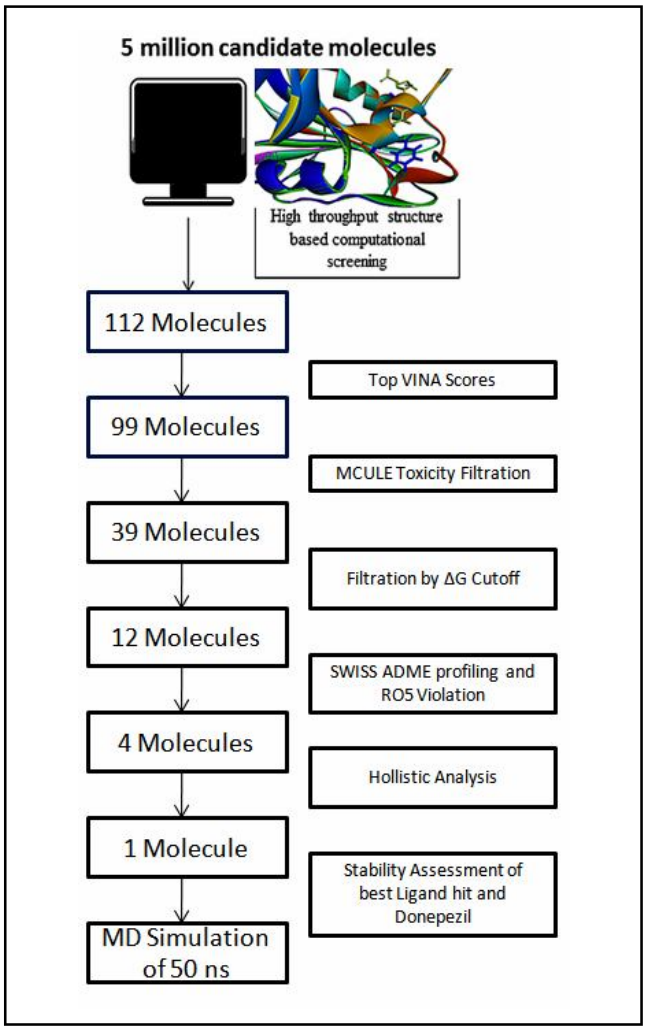


Figure 1: Flow chart of "structure based virtual screening" used to identify plausible ligand hits focusing the human acetylcholinesterase enzyme's catalytic site. Rule-of-five, RO5.

3.1 Binding crevice exploration

The PDB ID: 4EY7 represents the crystal structure of the human brain AChE enzyme's CAS-site, the same was analysed thoroughly using CASTp tool (Trott *et al*., 2010). The 'show pockets' function indicated the interaction between pharmaceutical donepezil and the human brain AChE enzyme's CAS-site *via* 17 amino acid residues, namely Y72, W86, G121, Y124, E202, S203, N265, E268, W286, S293, V294, F295, Y337, F338, Y391, H447 and G448 as resulted in pocket analysis of PDB ID: 4EY7. Filtering of H447 and G448 is done with the BRENK filter. It was also observed that two (S203 and H447) of the three amino acid residues (S203, H447, and E334) make up the "catalytic triad" of human acetylcholinesterase enzyme, in bounded form, were engaged in notable interactivity with the drug molecule.

3.2 MCULE database screening results

Structure based virtual screening is the platform to find out new inhibitors/ligands/drugs against a particular target molecule by searching huge depositories of 3D molecular structures of different molecules to narrow down the hits (ligands) showing significant pharmacological profile(s) which ultimately leads to the compound(s) having finest fit against that particular target.

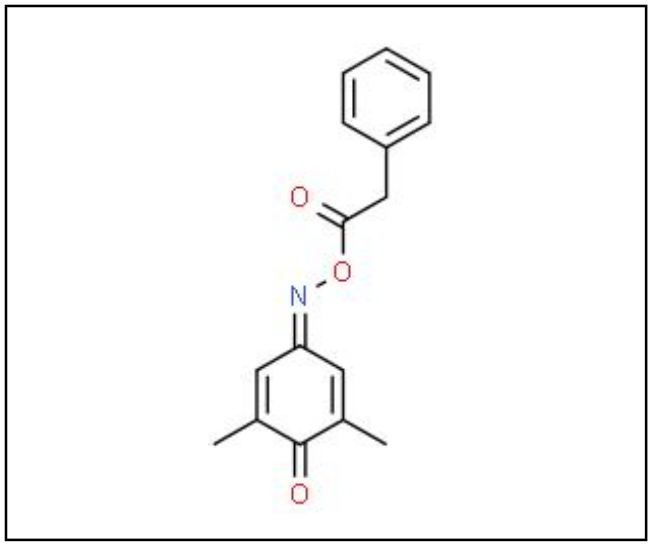
3.3 Results of autodock vina

The Monte Carlo algorithm based auto-dock-vina was used that uses an enhanced gradient optimization protocol which provides more flexibility to docking studies. Furthermore, when compared to other modern docking software, it is quicker and enhances the complete fidelity of the docking posture creation. It is no surprise that it is regarded as one of the best techniques for screening databases with millions of drug-like compounds on a huge scale (Eberhardt *et al*., 2021). The SBVS was able to find 112 ligand hits out of a total of 5×10^6 molecules. The top 99 molecules were then chosen for future research using the matching auto-dock-vina rankings. The calculation of the gib's binding free energy (also known as ΔG) is related to a greater (negative) auto-dock-vina score, is the indicator of a more powerful docking contact (Cosconati *et al*., 2010). The rate of dissociation of ligand/drug from target molecule (can be a protein) decreases with a surge in ΔG value. It is reasonable to expect such ligands to have a long half-life. A weaker binding attracts rapid dissociation rates (Hulme *et al*., 2010). As a result, Vina score rankings were used to choose 99 ligand structures.

3.4 MCULE toxicity filtration

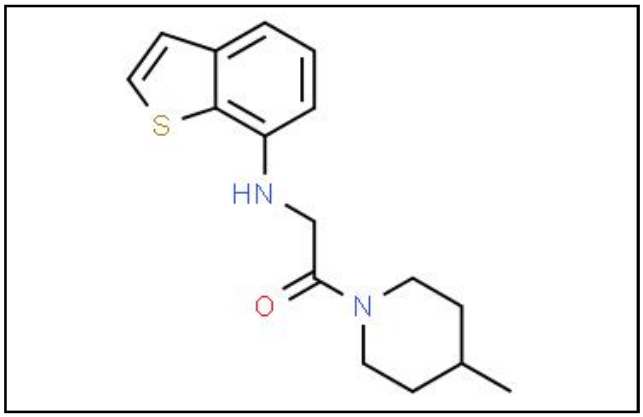
In most computational screening procedures, compounds with substructures that represents the molecular fingerprints of chemical groups/arrangements/interactions prevalent in harmful compounds (complete /part which might be harmful), are usually excluded during the screening process. In a total of 99 ligands, 73 ligands possessing some sort of toxicity were rejected and the remaining 39 passed the mcule toxicity filtration step (Gimeno *et al*., 2019).

(1) MCULE-5994631971



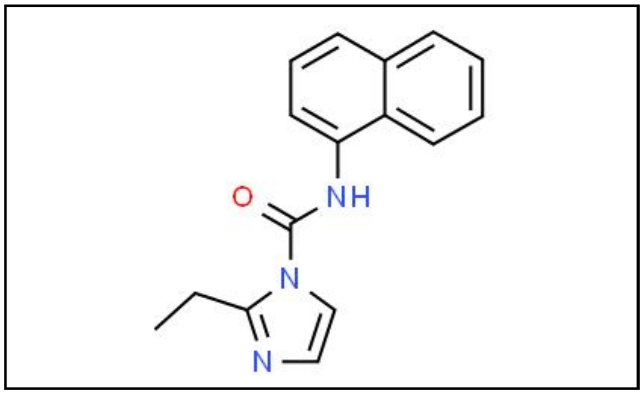
2,6-Dimethyl-4-[(2-phenylacetoxy)imino]-2,5-cyclohexadien-1-one

(2) MCULE-4237926756



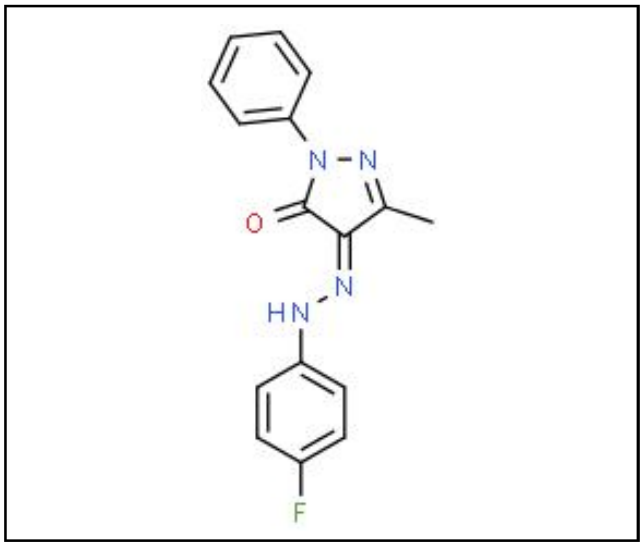
2-(1-Benzothiophen-7-ylamino)-1-(4-methyl-1-piperidinyl) ethanone

(3) MCULE-8113340860



2-Ethyl-N-(1-naphthyl)-1H-imidazole-1-carboxamide

(4) MCULE-9685671672



(4*Z*)-4-[(4-fluorophenyl)hydrazinylidene]-5-methyl-2-phenylpyrazol-3-one

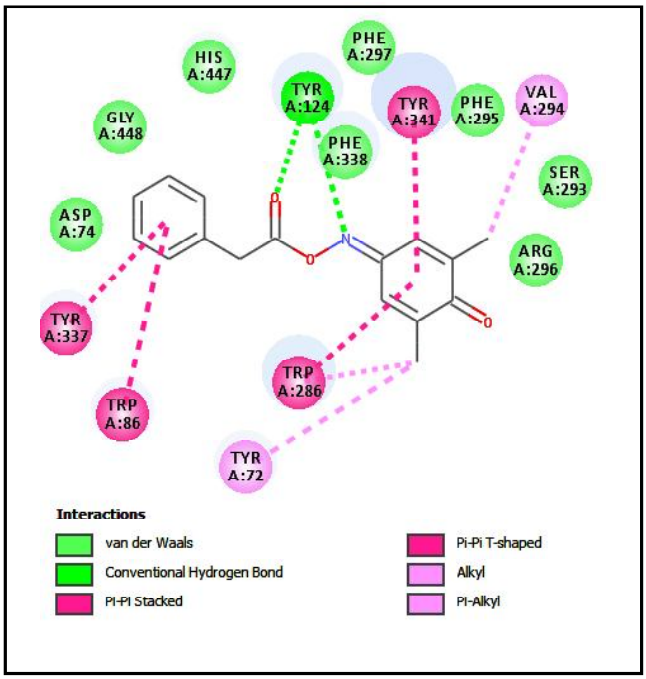
Figure 2: 2D structures of the top 4 ligands hit by SBVS of mcule database using AChE catalytic site as target.

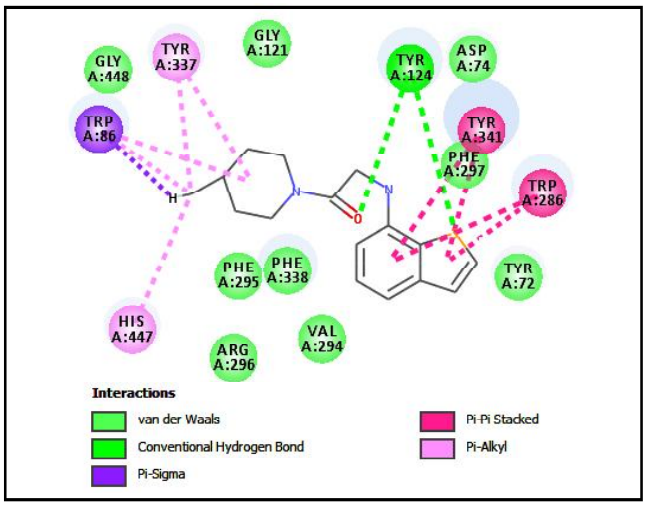
3.5 Screening based on ΔG cut-off and RO5 violation:

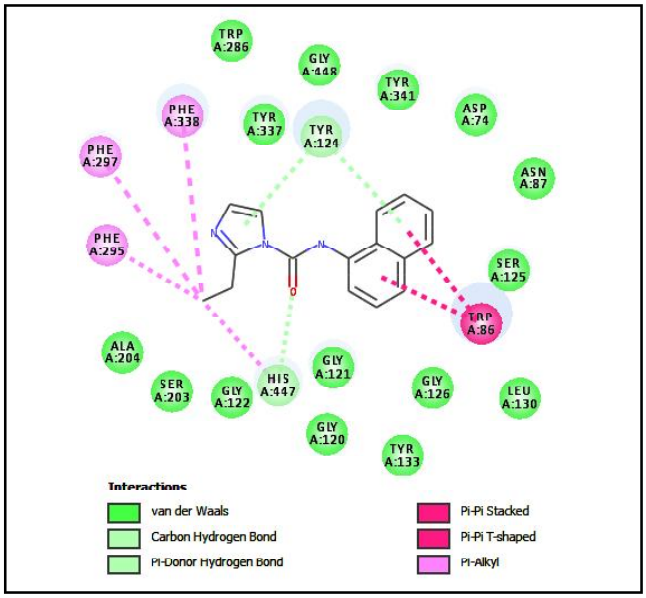
The complexes with AChE, having the ΔG value less than –9.9 kcal/mol were removed; only four complexes were able to pass the filter. All four followed the rule of five leading to the final bunch of total of 4 ligands. These 4 ligands were represented by MCULE-5994631971, MCULE-4237926756, MCULE-8113340860 and MCULE-9685671672 MCULE IDs respectively. The IUPAC names were retrieved from chemspider by the simple input of canonical smile notations. The IUPAC names of the screened ligands are 2,6-dimethyl-4-[(2-phenylacetoxy)imino]-2,5-cyclohexadien-1-one, 2-(1-Benzothiophen-7-ylamino)-1-(4-methyl-1-piperidinyl) ethanone, 2-Ethyl-N-(1-naphthyl)-1H-imidazole-1-carboxamide, (4Z)-4-[(4-fluorophenyl)hydrazinylidene]-5-methyl-2-phenylpyrazol-3-one.

3.6 Swiss ADME profiling for the screened ligands and their molecular interactions

Multiple molecular visualization software, including UCSF chimera 1.9[26] discovery studio 2016 (BIOVIA), and PyMol V1.5.0.4, was used to thoroughly examine the binding of these ligands hits obtained after structure-based virtual screening. The chemical structures of the top 4 ligands chosen from the structure-based virtual screening are shown in Figure 2. The SBVS run resulted in the complex.pdb files of the relevant interacting postures including the top 4 chosen ligands. Figure 3 depicts the binding of the CAS site of AChE with individual top 4 ligands that were filtered out in their docked forms. The interacting amino acid residues that are critical for retaining these potential inhibitors in the AChE enzyme's active centre are indicated. The amino acid interactions for all four ligands were compared with the Acetylcholinesterase- Donepezil complex represented by the PDB ID: 4EY7 and the interacting amino acid residues are Leu A:306, Gly A:310, Leu A:211, Phe A:312, Leu A:210, Asp A:311, Leu A:315, Arg A:216, Gly A:217, Pro A:213, His A:313, His A:220.







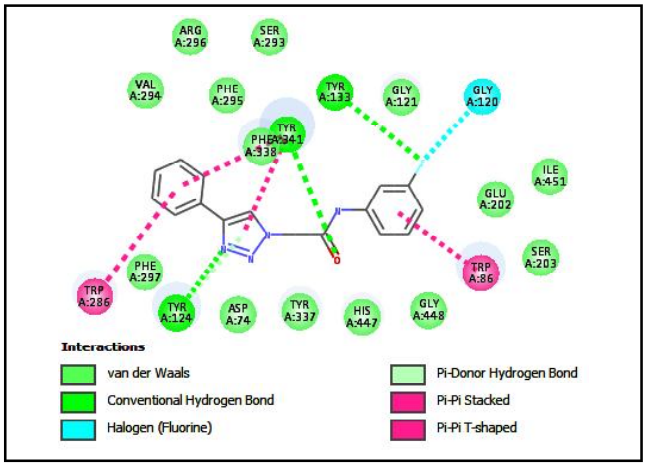


Figure 3: The '2-D-diagram' showing interacting amino acids of the 'top molecules' bound to human acetylcholine-sterase enzyme in their respective docked states: (i) MCULE-5994631971, (ii) MCULE-4237926756, (iii) MCULE-8113340860 (iv) MCULE-9685671672.

Table 1: Top ten AChE catalytic site-targeted screening hits acquired by SWISS ADME and their pharmacokinetic profiles

Ligand name	MW (g/m)	iLOGP	RO5 Violation	RB	TPSA (Å2)	MR	GIA	BBB	LOGS (Ali)	SA
MCULE-8352962011	224.30	2.82	0	3	17.29	74.46	High	Yes	-2.28	3.10
MCULE-5994631971	288.41	3.08	0	4	60.58	89.68	High	Yes	-4.69	2.92
MCULE-7085778427	253.30	1.92	0	4	40.54	78.22	High	Yes	-5.14	2.61
MCULE-9391254060	352.2	2.70	0	2	64.11	83.27	High	Yes	-4.39	3.39
MCULE-3367208738	239.27	1.99	0	1	45.23	76.91	High	Yes	-2.48	2.66
MCULE-7979670775	226.28	2.17	0	1	64.69	67.10	High	Yes	-2.87	2.54
MCULE-1629556661	226.23	2.52	0	1	39.44	66.48	High	Yes	-3.42	2.75
MCULE-4237926756	288.41	3.08	0	4	60.58	89.68	High	Yes	-4.69	2.92
MCULE-8113340860	265.31	2.58	0	4	46.92	80.18	High	Yes	-4.02	2.56
MCULE-9685671672	296.30	2.32	0	5	59.81	80.51	High	Yes	-3.36	2.67

*Abbreviations: MW: Molecular weight, RB: Rotatable bond, TPSA: Topological polar surface area, MR: Molar refractivity, GIA: Gastrointestinal absorption, BBB: Blood brain barrier, LOGS (Ali): Solubility, SA: Synthetic accessibility

Table 2: Interacting amino acid residues and free energy binding values corresponding to the bound complexes of the top four ligands with the acetylcholinesterase catalytic site

MCULE IDs (Ligands)	MCULE-5994631971	MCULE-4237926756	MCULE-8113340860	MCULE-9685671672
Binding free energy	– 9.9 kcal/mol	– 9.8 kcal/mol	– 9.9 kcal/mol	– 10.7 kcal/mol
Interacting amino acid residues corresponding to the bound complexes	TRP :86, HIS A:447,PHE A:338, PHE A:297,TYR A:124,PHE A:295,SER A:293,VAL A:294,ARG A:296,TRP A:286,TYR A:341,TYR A:72 ,ASP A:74,TYR A:337,GLY A:448	GLY A:448,TYR A:337, TYR A:124,TYR A:341,ASP A:74,ASN A:87,SER A:125, GLY A:126, TRP A:86, GLY A:120,TYR A:133,GLY A:120 TYR A:72,PHE A:338, PHE A:295, PHE A:338,ARG A:296,VAL A:294,HIS A:447	TRP A:86,HIS A:447,PHE A:338,PHE A:297,TYR A:124,PHE A:295,SER A:293, VAL A:294, ARG A:296, TRP A:286,TYR A:341,TYR A:72,ASP A:74,TYR A:337,GLY A:448,TRP A:86	GLY A:121,GLY A:120,TYR A:133,ILE A:451,GLU A:202,SER A:203,TRP A:86,GLY A:448,HIS A:447,TYR A:337,ASP A:74,PHE A:138,TYR A:341,PHE A:295,VAL A:294,ARG A:296,SER A:293,TRP A:286, PHE A:297,TYR A:124

Table 3: Calculated parameters for all the systems obtained after 50 ns MD simulations

Complexes	Average potential energy (kJ/mol)	Average RMSD (nm)	Average SASA (nm^2)	ΔGsolv ($kJ/mol/nm^2$)	Rg (nm)	Volume (nm^3)	Density kg/m^3
AChE-Donepezil	– 828836	0.1488	16.626	– 11.3055	2.25496	643.703	1043.0
MCULE-9685671672	– 830093	0.1673	18.298	– 23.8767	2.25633	643.941	1043.1

3.7 Results of molecular dynamics (MD) simulation

3.7.1 Average potential energy of the system

The average potential energy (PE) of AChE-donepezil and AChE-MCULE-9685671672 was estimated to assess the stability of the system. The computed average PE of the above systems (–828836 kJ/mol and–830093 kJ/mol, respectively) was comparable during the entire duration of the 50 ns MD process (Table 3).

3.7.2 Root-mean-square deviation

RMSD measures the protein's stability and resemblance to its native structure. The average RMSD for donepezil (black), and MCULE-9685671672 (red) complexed with AChE was found at 0.1488 nm and 0.1673 nm respectively (Table 3). The RMSD plot reveals that the stability of docked complex of AChE and ligand hit is comparable to known inhibitor donepezil (Figure 4).

3.7.3 Root-mean-square fluctuation

The RMSF graph ensures the stability of protein docked with ligand molecules during the entire period of MD simulation. Residues fluctuations at different sites in the RMSF plot are due to the molecular interaction of reference inhibitor and selected ligand hits (Figure 5).

3.7.4 Solvent accessible surface area

The SASA plot shows the protein's interactable surface area to the solvent molecules. The average value of SASA for donepezil (black) and MCULE-9685671672 (red) docked with AChE was found as 16.626 nm^2 and 18.298 nm^2 (Figure 6, Table 3).

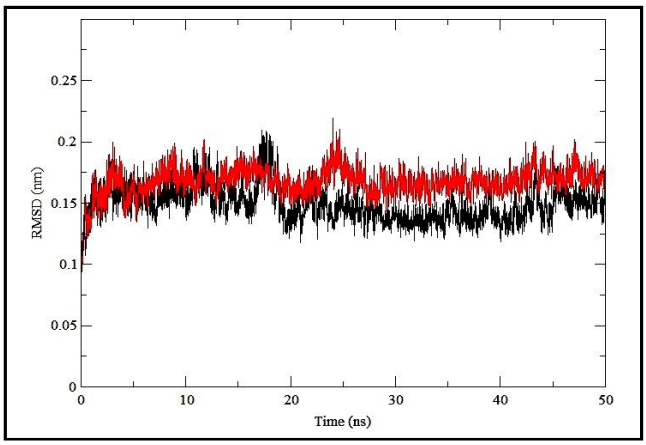


Figure 4: RMSD plot. Black and red colors represent values obtained for AChE-donepezil and AChE-MCULE-9685671672, respectively.

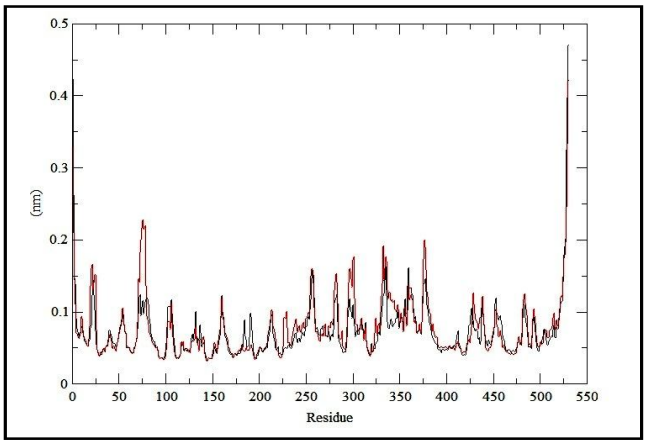


Figure 5: RMSF plot. Black and red colors represent values obtained for AChE-donepezil and AChE-MCULE-9685671672, respectively.

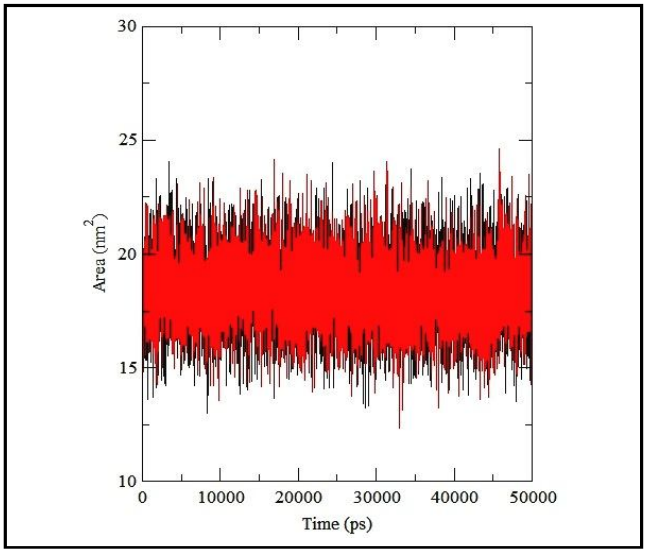


Figure 6: SASA plot. Black and red colors represent values obtained for AChE-donepezil and AChE-MCULE-9685671672, respectively.

3.7.5 Free energy of salvation

The average value of ΔGsolv for donepezil (black) and MCULE-9685671672 (red) docked with AChE was found as – 11.3055 kJ/mol/nm^2 and – 23.8767 kJ/mol/nm^2 (Figure 7, Table 3).

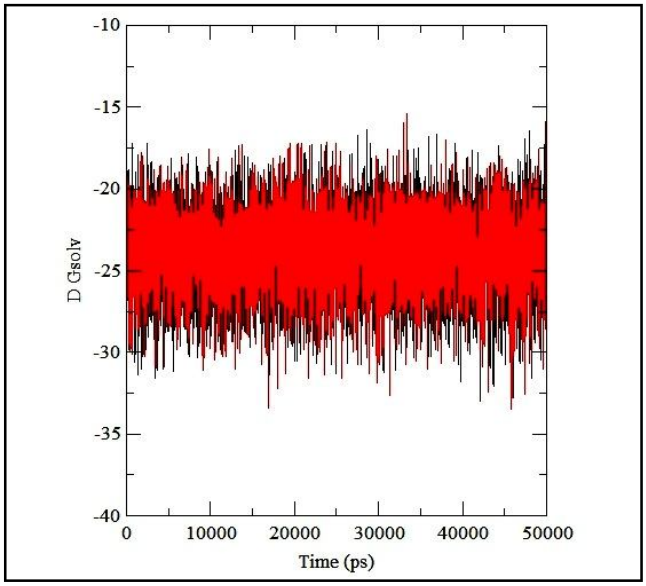


Figure 7: ΔGsolv plot. Black and red colors represent values obtained for AChE-donepezil and AChE-MCULE-9685671672, respectively.

3.7.6 Radius of gyration

The Rg predicts the stability of the target protein in a biological system and is related to the compactness of the protein. The average Rg values of donepezil (2.25496 nm) and MCULE-9685671672 (2.25633 nm) were almost equal during the MD simulation process (Table 3 and Figure 8).

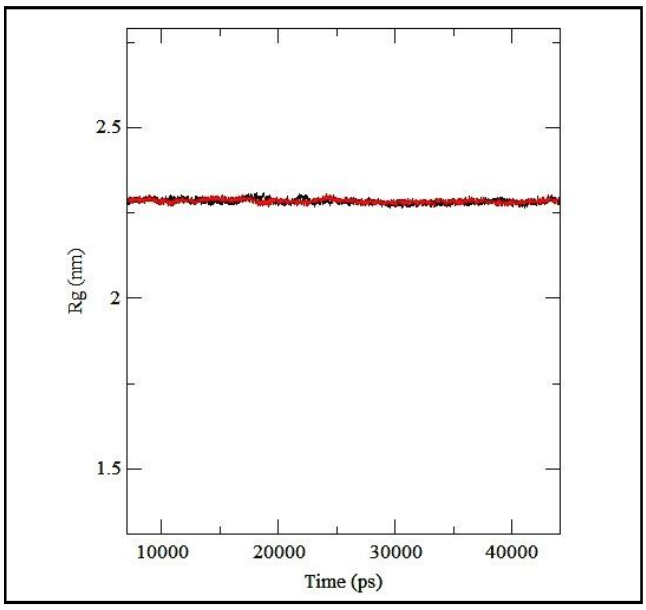


Figure 8: Rg plot. Black and red colors represent values obtained for AChE-donepezil and AChE-MCULE-9685671672, respectively.

3.7.7 Hydrogen bond formation

The HB plot shows the number of hydrogen bond formations and their stability during the entire process of MD simulations (Figure 9a-b).

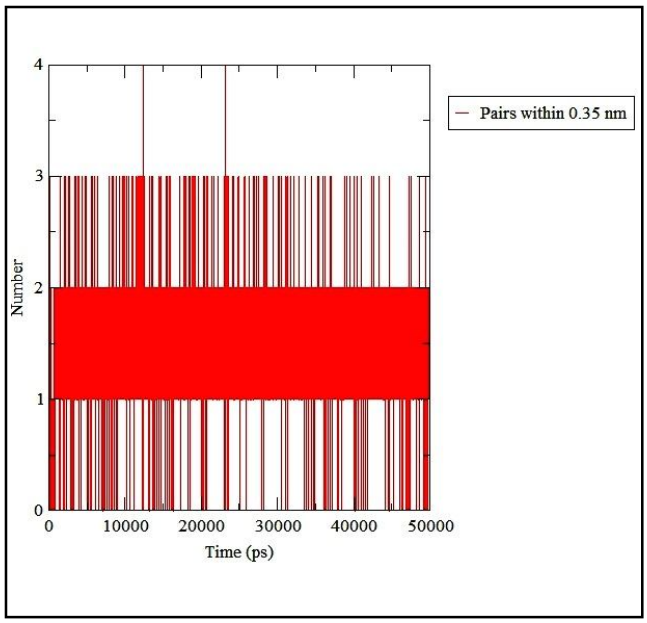


Figure 9(a): HB plot shows the formation and deformation of H-bonds during interaction of donepezil with AChE.

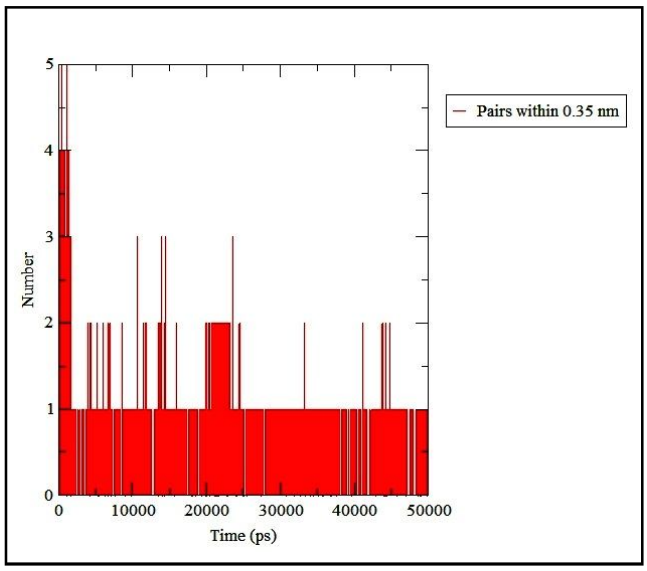


Figure 9b: HB plot shows the formation and deformation of H-bonds during interaction of MCULE-9685671672 with AChE.

During MD simulation of 50 ns duration, RMSD analysis of AChE backbone, MCULE-9685671672, donepezil, and their respective complexes exhibit that both MCULE-9685671672 and donepezil form unstable complexes with AChE protein. (Figure 10a-b).

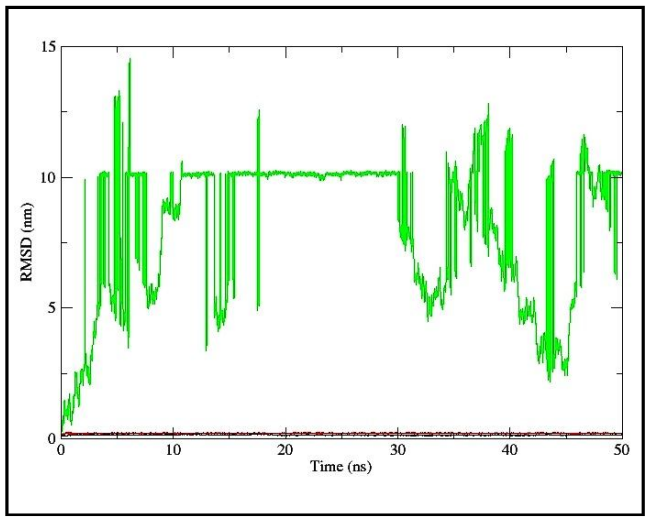


Figure 10a: RMSD plot as a function of time. Black, red, and green colors represent the AChE backbone, donepezil and AChE-donepezil complex, respectively.

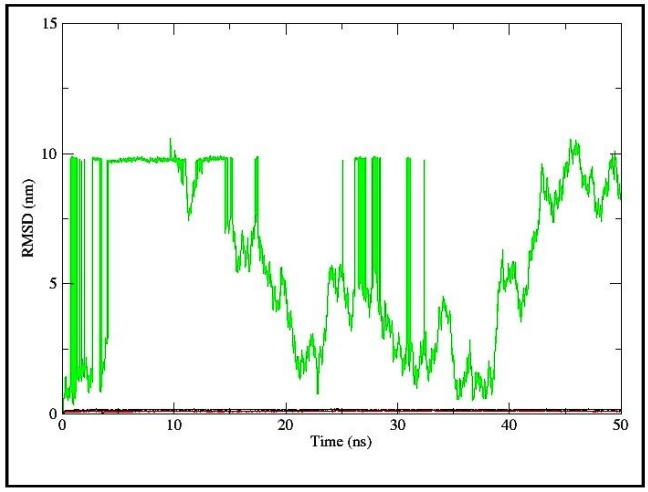


Figure 10b: RMSD plot as a function of time. Black, red, and green colors represent the AChE backbone, MCULE-9685671672 and AChE-MCULE-9685671672 complex, respectively.

4. Discussion

The goal was to identify a new potential AChE CAS site inhibitor. Table 1 shows the chemical profiles generated by the SWISS ADME platform for the top ten screening hits targeted at the AChE CAS site. These predicted ligands obeyed all drug-likeness rules, *e.g.*, RO5, ghose, veber, muegge, egan, and synthetic accessibility (SA). Moreover, medicinal chemistry features like PAINS was also passed by these selected ligands. In general, lead-like compounds should have an XLogP3 value of less than 3.5. All the MCULE IDs have followed the drug-likeness and medicinal chemistry filters as lipinski, ghose, veber, egan, muegge an d PAINS filter. A molecular mass of less than 500 Da, with a log P value of less than 5, are rejected but in the present study, no ligand showed the violation. These ligands had zero "RO5 violations," which is a strong indicator of a potential

therapeutic molecule candidate (Masters *et al*., 2015). These compounds were revealed to be active in the central nervous system (CNS) since they were found to pass through the "blood-brain barrier."

Furthermore, every compound of the working set had a good GI absorption rate, which is a positive sign for neurological medicines or drug candidates taken orally. At last, all the ligands displaying a VINA docking scores greater than – 9.9 were rejected. The selected MCULE IDs are listed in Table 2 along with their interacting amino acids. Following that, the SWISS ADME platform was used to molecularly profile all of the top four screened ligands. This software runs a series of experiments to see if proposed ligands have drug-like qualities. The goal was to identify a new potential AChE CAS site inhibitor. Table 1 shows the chemical profiles generated by the SWISS ADME platform for the top four screening hits targeted at the AChE CAS site. Ligands as potential "candidate medicines" with poor pharmacokinetic characteristics are often ruled out in the early stages of drug development. In general, lead-like compounds should have an XLogP3 value of less than 3.5. As the top ligand can be used as a future inhibitor against AChE, the said ligand should have high gastro-intestinal (GI) absorption as well as the ability to cross the blood-brain barrier (BBB). Blood-brain barrier permeability was also used as important criterion of screening as the seed molecule should be BBB permeable. Twenty-seven compounds were ruled out based upon blood-brain barrier permeability. These twenty-seven compounds were showing inability to cross blood-brain barrier. Only those compounds were selected that were BBB permeable along with high gastrointestinal absorption, resulting in a working set of 12 ligand molecules which was further confined to four ligand hits by screening based on RO5 and drug likeness parameter.

The filtration resulted in remaining 4 ligands with MCULE IDs: MCULE-5994631971; MCULE-4237926756; MCULE-8113340 860; MCULE-9685671672 respectively. Amongst these four ligands, only 1 ligand (proposed lead molecule for future drug design against Alzheimer's disease) with MCULE ID: MCULE-9685671672 showed interaction with at least 2 (S203 and H447) of the 3 catalytic triad residues (these three crucial residues constitute the catalytic triad in AChE structure; these are S203, H447 and E334). Thus, ligand displaying interaction with at least 2 of the 3 catalytic triad residues were retained which are crucial for the proposed lead molecule future drug design against Alzheimer's disease studies. The resulting final seed molecule is the pyrazole derivative; there have been various wet-lab studies as well in which pyrazole derivatives have been investigated for neuroenzyme inhibition (Mohd Faudzi *et al*., 2021; Alam *et al*., 2012).

The selected final seed molecule was then assessed with MD simulation of 50 ns which shows the comparative analysis of the two compounds AChE-donepezil and AChE- MCULE-9685671672 respectively. The results of the MD simulation suggested that the complex AChE-MCULE-9685671672 is behaving almost in the same manner as the reference complex AChE-donepezil, comparing different parameters like average PE, RMSD, HB bond formation, ROG, SASA, free energy salvation and RMSF.

5. Conclusion

Alzheimer's disease is still a major source of discomfort among the elderly around the world. It is always necessary to seek out stronger inhibitors of germane protein targets. A potential therapeutic molecule MCULE-9685671672 has been discovered in the current study. The selected ID has the IUPAC name as (4Z)-4-[(4-fluorophenyl)hydrazinylidene]-5-methyl-2-phenylpyrazol-3-one efficient inhibition of human AChE enzyme's CAS-site. AChE is a prime target for neuropharmaceuticals. The aforementioned molecule had a strong 20-amino-acid-residue contact with the target binding site, resulting in a significant binding free energy (ΔG = -10.7 kcal/mol). Furthermore, SWISS ADME profiling of the top ligand also showed promising results, as it sailed past every important drug screening filter, including lipinski, ghose, veber, egan, muegge, PAINS, and brenk, with no adverse effects in humans. Comparative analysis using MD simulation between the reference complex and the ACE-MCULE-9685671672 complex showed the compound was stable at 50 ns. However, because the work used high-throughput computer screening, furthermore, wet laboratory studies are needed to corroborate the findings.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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