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IN SILICO DOCKING AND DYNAMICS OF FLAVONOID 'DIPLACONE' AS A POTENTIAL NATURAL INHIBITOR OF ACETYLCHOLINESTERASE

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Abstract

Acetylcholinesterase (AChE) inhibition is the standard therapy method used for age related disorders. Alzheimer Disease (AD) is the most widespread age related disease, affecting over 20 million individuals around the globe. AD is characterized by senile plaquets and cholinergic deficits. Many drugs currently used for the treatment of AD have severe neuronal side effects. Therefore in this paper we have attempted to identify AChE inhibitor which is natural and has negligible side effects than conventional drugs used in AD treatment. With the development in the cholinergic neurotransmission studies, inhibition of AChE has emerged as the most promising strategy in AD treatment. Compounds which inhibit/delay the hydrolysis of acetylcholine by AChE are known as AChE inhibitors and such compound(s) could become a perfect drug candidate(s). In this study, we have focused on the virtual screening, precision based docking and molecular dynamics studies for the identification and validation of natural inhibitors of AChE. 251 natural inhibitors from plants were identified, screened and docked against the human AChE using Glide module of Schrödinger suites. After screening and docking a natural compound Diplacone showed the superior glide score of -11.49 kcal/mol along with good binding energy -57.17kJ/mol. After analyzing the stability by molecular dynamics simulation techniques, it is convinced that natural compound Diplacone could be a better drug candidate for AD rather than its already established allopathic counterparts. Further in-vitro and clinical studies investigation are required for the validation of these studies.

Key words : Docking, Molecular dynamics simulation, acetylcholinesterase, Alzheimer, natural inhibitor.

Introduction

Alzheimer is age related neurodegenerative disorder that was found by a German researcher Alois Alzheimer in 1906 and named after him (Singhal *et al.*,2012). Age related memory disorders are consistently of key enthusiasm of scientists around the world. Approximately by the year 2050 around 14 million people will be affected by the age related degenerative disorders only in USA (Akhtar *et al.*,2011). Despite extensively studying the mechanism of AD pathogenesis by the researchers it is still not well elucidated till date. Hence there is the need to find novel natural treatment with reducing risk in the elderly.

Cholinergic hypothesis is the most accepted cause of AD. It states that deficits in learning, memory and behavior associated with Alzheimer's caused by the loss of cortical cholinergic neurotransmission (Bartulucci *et al.*, 2006; Contestabile., 2011; Terry and Buccafusco., 2003; Francis *et al.*, 1999). Enzyme that plays a major role in cholinergic transmission belongs to the family of serine hydrolase and known as Acetylcholinesterase (AChE, EC 3.1.1.7). It rapidly hydrolyses the neurotransmitter acetylcholine into choline and acetate leading to termination of neurotransmission at cholinergic synapse (Silman *et al.*,2005). As the duration of termination of impulse decreases with increase in hydrolysis of acetylcholine, symptoms of Alzheimer's start to occur (Francis *et al.*, 1999).

The polypeptide chain of the enzyme has 614 amino acid lengths. Human AChE has an ellipsoidal shape with

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dimensions ~45Å by ~60Å by ~65Å. It is a monomer having 12 stranded central mixed beta sheets surrounded by 14 alpha helix along with a remarkable feature which is a deep and narrow gauge of 20Å long penetrating into the enzyme (Manavalan *et al.*,1985). The active site of AChE lies at the bottom of deep narrow gauge. This enzyme and its inhibitors are the target of many X-ray crystallography studies and various structures are documented in the protein data bank (Kryger *et al.*, 1999).

AChE inhibitors inhibit the enzyme from breaking down acetylcholine thereby increasing its level and duration of action of neurotransmitters (Zimmerman and soreq., 2006). Pesticides such as organophosphates and carbamates act as irreversible inhibitors (Ranjan *et al.*, 2018). Reversible inhibitors are primarily responsible for the pharmacological manipulation of enzyme activity which is helpful in the treatment of neurological diseases (Mehta *et al.*, 2012). So far only a few drugs are approved by Food and Drug Administration (FDA) for Alzheimer treatment such as tacrine, galanthamine, donepezil, rivastigmine (Stefanou *et al.*, 2011; Camps *et al.*, 2010). Several side effects have been observed caused by these drugs such as gastrointestinal disturbances, nausea, dizziness in the elderly because of low bioavailability (Pisoni *et al.*, 2010). Natural compounds having an inhibitory effect on AChE activity can serve as an alternative to these available drugs. Various phytochemicals namely alkaloids (mukherjee *et al.*, 2007), triterpenes (Gurovic *et al.*, 2010), ursanes (Mukherjee *et al.*, 2007) and secondary metabolites such as terpenoids and flavonoids (Eubanks *et al.*, 2006; Campbell *et al.*, 2007) have shown better inhibitory effect than these available drugs (Contestabile., 2009).

With the availability of the bountiful amount of 3D data and structures computational tools such as virtual screening, docking and molecular dynamics are highly useful in discovery and development of new lead compound.

In this study, we have investigated the interaction of natural inhibitors with AChE enzyme using molecular modeling and dynamics and validated its effectiveness as a AChE inhibitor for future drug development.

Materials and Methods

3D structure retrieval of acetylcholinesterase (AChE)

Three Dimensional Structure of acetylcholinesterase (AChE) is retrieved from Protein Data Bank (PDB ID: 1B41) (Contestabile., 2009). It has 539 amino acids, the resolution of the structure obtain is 2.76 Å and it binds with three co-crystal ligands which were Alpha-L-Fucose, N-Acetyl-D-Glucosamine and 2-(Acetylamino)-2-Deoxy-A-D-Glucopyranose and utilized for further studies.

Preparation of protein target structure

The protein structure was subjected to the Protein Preparation Wizard of workflow implemented in Maestro 9.5 Schrodinger software (Sastry *et al.*,2013). It includes the biological unit and zero-order bonds to metals, assigned bond orders, deleted water molecules beyond 5 Å from hetero groups, generated metal-binding states, completed any missing side chains and loops, formed disulfide bonds, added missing hydrogens, and energy minimization using the OPLS_2005 force field (Jorgensen *et al.*,1988).

Binding site analysis and grid generation

The binding site study has an essential role in drug discovery research because of binding site residues of the protein bind to the ligand residues with its hydrogen bond donors and acceptors (Anderson., 2003). The Active site of AChE protein was analyzed by SiteMap (Schrodinger) (Anderson., 2003). SiteMap assigns numerical descriptors to calculate predicted binding sites by a series of physical parameters such as tightness, size, hydrophobic/hydrophilic character, degree of enclosure/ exposure and hydrogen bonding possibilities. A weighted average of these measurements is then assigned to rank possible binding sites. In this analysis, the AChE protein have shown five major sites with site scores and their volume. Among the binding sites, we choose one binding site used for grid generation, which was determined by their site scores and volume of the sites. Afterwards, the suitable site was used to grid generation. The grid generation panel of the Glide module Maestro v 9.5 software tool was placed on the drug target site in the target molecule (Schrodinger Release, 2014). In the receptor grid generation panel, The grid box assisted to fix the drug binding site in the centroid of the target.

Ligand selection and preparation

251 Natural AChE ligand Inhibitors listed were collected from literature and their structures were downloaded from different publicly available databases of ligand such as Pubchem, Zinc database, Chem Spider and some ligands were drawn by Chemsketch. (Dos Santos *et al.*, 2018; Murray *et al.*, 2013; Ahmed., 3013; colovic *et al.*, 2013). All ligands can be retrieved in.sdf format. The set of molecules were prepared using the Ligprep tool available in Schrodinger suite (Schrodinger Release, 2014). It serves the purpose of converting molecules to 3D structures from 2D.sdf files, steric isomers and geometry minimization of ligands and probing for tautomers. All the molecules were

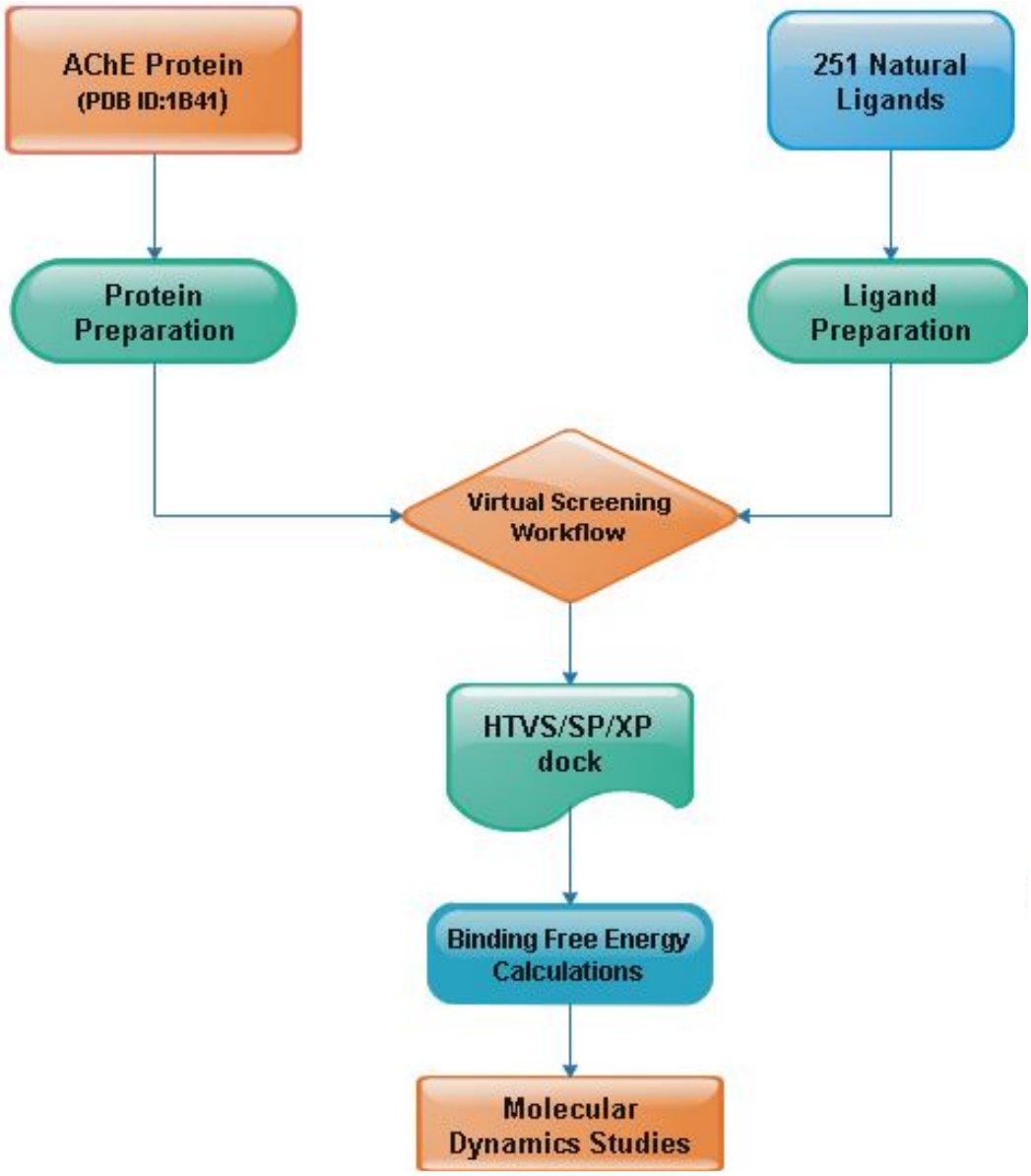

Fig. 1: The screening workflow that was applied to find out AChE inhibitors.

geometrically optimized through Optimized Potentials Liquid Simulations 2005 (OPLS2005) force field. The partial atomic charges were also computed by OPLS2005 force field.

ADME analysis and High throughput virtual screening (HTVS)

The High **t**hroughput virtual screening workflow of Maestro v9.5 was applied to screen the 251 Natural ligand Inhibitors against the binding site of the A ChEprotein target. The virtual screening workflow screening of ligand libraries is a fast and accurate technique to discover novel drugs in the drug discovery process. preliminary virtual screening steps, we skipped the ligand preparation step due to their previous preparation with Ligprep. The further step of virtual screening involves the assessments of drug-likeliness of ligands. Drug-like molecules show positive parameters of absorption, distribution, metabolism and excretion (ADME). If any ligand to be considered a probable drug candidate, it must satisfy these ADME properties profile (Halgren *et al.*, 2009). The Qikprop (Schrodinger Release, 2014) tool was used to examine these parameters. After Qikprop analysis, the screening of ligands initialized using the receptor grid file. Virtual screening of Natural ligand Inhibitors was carried out by HTVS, SP (Standard Precession) and XP (Xtra Precession) modes of filtering to results lead molecules. The final lead molecules were prioritized based on their glide score, glide energy and ADME properties. The steps of virtual screening have been shown through a flowchart diagram (Fig. 1).

Binding free energy calculations

The protein-ligand complex of binding free energies is calculated using the Prime MM-GBSA approach (C.2014-2: QikProp, version 4.0). AChE protein with top lead molecules binding free energies was calculated using Prime- MM/GBSA method with OPLS_2005 force field applied in Schrodinger suite. Binding free energy of complex (Protein to ligand) calculated using the following equation :

ÄGbind = G (ligand-receptor) - (Gprotein + Gligand)

Molecular dynamics simulation studies

Molecular dynamics simulation of AChE protein and docked complexes was carried out using GROMACS 5.1v to identify with the structural behavior of the docked protein and complexes (Abraham *et al.*, 2015). GROMOS 43a1 force field was used for simulations. The systems were solvated using a SPC (Single Point Charge) water model in a cubic box with a distance of 1/ nm from the box to the protein surface. Corresponding ions were additionally added in order to neutralize the systems. The docked complexes energy minimization was carried out by the steepest descent algorithm. For every simulation, 50,000 steps were used for energy minimization. Equilibration of the systems was carried out using NVT and NPT ensembles for 100ps each. The V-rescale thermostat was used for equilibration with 300/ K reference temperature. Finally, the production MD run was carried out for 20ns and is used for further analysis. GROMACS in-built tools were used to compute protein root mean squared deviation (RMSD) and root mean square fluctuation (RMSF). Xmgrace was used for plotting graphs.

Results and Discussion

Binding site Prediction

The active site was predicted on the target AChE protein. In this prediction, the target site displayed five suitable binding sites (Table 1). Out of 5 different sites, site with the highest Site score was selected (Fig. 2). The active site was include by 34 residues, namely Tyr 72, Val 73, Asp 74, Thr 83, Trp 86, Asn 87, Tyr 119, Gly 120, Gly 121, Gly 122, Tyr 124, Ser 125, Gly 126, Leu 130, Tyr 133, Glu 202, Ser 203, Trp 286, His 287, Leu 289, Gln 291, Glu 292, Ser 293, Val 294, Phe 295, Arg 296, Phe 297, Tyr 337, Phe 338, Tyr 341, Trp 439, His 447, Gly 448 And Tyr 449 and used for generation of docking grid

ADME analysis

All 251 ligands were initially screened for their ADME (Absorption, Distribution, Metabolism and Excretion) properties by QikProp tool of Schrodinger suite (C..2014-2: QikProp, version 4.0). This step comes under the Initial steps of the virtual screening workflow and after Qikprop analysis, out of 251 a total of 233 ligands were filtered and the filtered drugs were subjected to virtual screening based docking.

Virtual high throughput screening

For drug discovery research, virtual screening is a broadly used approach that effectively affords new lead ligand compounds. In the current study, virtual screening was done to design effective inhibitors by screening of AChE ligand Inhibitors on the basis of their affinity for the AChE protein. 233 Qikprop filtered compounds subjected to the virtual screening docking. 116 ligands were shortlisted from HTVS ligand docking. This was more followed by SP and XP docking, which filtered the number of remaining compounds to 58 and 29, respectively. Table 2 illustrates the molecular docking output for the XP docked compounds against AChE (only the top 50% compounds with higher dock scores). Docking scores for all 29 compounds have been shown in Supplementary-2.

Table 1: Active binding sites of Ache.

AChE receptor	Site scores
*Site 1	1.069
Site 2	1.014
Site 3	0.948
Site 4	0.874
Site 5	0.849

* Highest site score binding site.

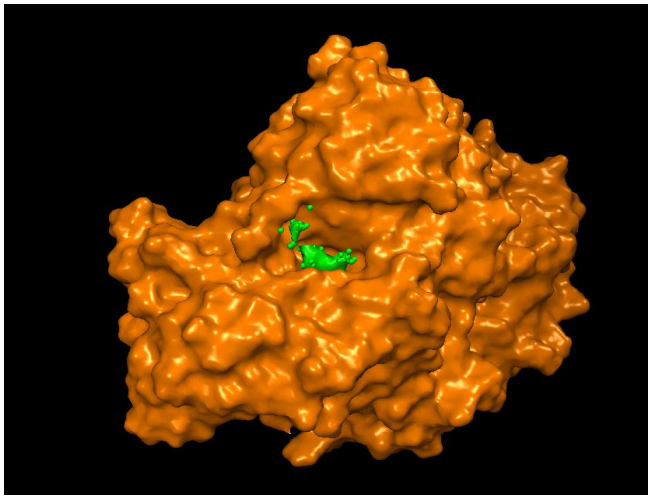

Fig. 2: Top Sitemap (site-1) position in protein surface area.

From the docking interaction profiles as explained in figures 3A-3E and in table 2, diplacone scored a high dock score of -11.49 kcal/mol and shared hydrogen bond interaction with Phe 295 and Gln2 91. kuwanon E scored

Table 2: Docking results of top file lead molecules (Ligands).

Ligands	G score	Glide energy (kcal/mol)
*Diplacone	-11.49	-55.92
Kuwanon E	-11.11	-46.60
Macluraxanthone	-11.00	-43.44
Lycorine	-10.65	-39.55
11alpha-Hydroxy Galantamine	-10.54	-34.30

*Top Docked Score Ligand Complex.

a high dock score of -11.11 kcal/mol and shared hydrogen bond interaction with Tyr72, Tyr74, Trp286 and Ser293. Macluraxanthone scored a high dock score of -11.00 kcal/mol and shared hydrogen bond interaction with Ser293, Phe295 and Arg296. Lycorine Scored a high dock score of -10.65 kcal/mol and shared hydrogen bond interaction with Arg296. 11alpha-Hydroxy Galantamine scored a high dock score of -10.54 kcal/mol and shared hydrogen bond interaction with Arg296 and Tyr341. The ADME properties of identified lead molecules are calculated using Qikprop in Schrodinger suite to estimate drug like activity. All the five identified leads are fulfilling the ADME properties in the allowable range of ADME values (Table

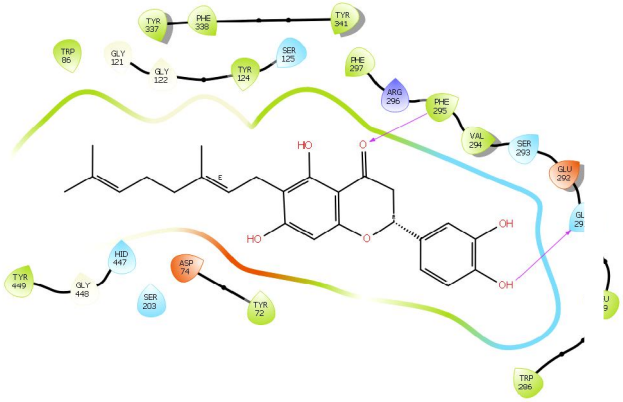

Fig. 3A: Ligand interaction diagram (2D) of the diplacone with AChE.

3).

Binding free energy calculations

Binding free energies of Top five docked ligands are calculated using the in Schrodinger suite prime-MM/GBSA tool. Binding free energies of ligands with AChE protein are calculated using OPLS (2005) force field and GBSA continuum solvent model. Lead molecules with AChE receptors Binding free energies were shown in Table 4.

Based on glide scores and binding free energies, out of five ligands, diplacone has shown the highest docking score after that protein-diplacone complex had undergone

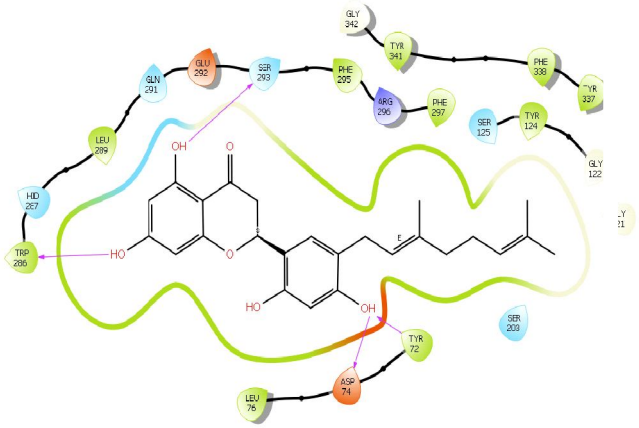

Fig. 3B: Ligand interaction diagram (2D) of the kuwanon E with AChE.

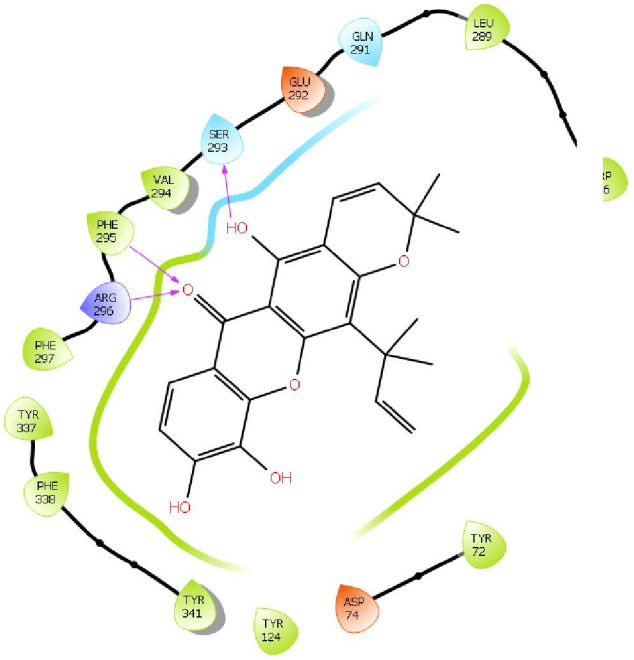

Fig. 3C: Ligand interaction diagram (2D) of the Macluraxanthone with AChE.

Molecular dynamics studies (Fig. 4).

Comparative analysis with Standard Drugs Binding sites

The previous experimental studies reported, Ser-203, His-447, and Glu-334 residues were the most catalytic site residues in the structure of AChE (Shafferman *et al.*, 1992). From the studies it was known that Currently available Ache standard drugs and Previous reported best inhibitors were Donepezil, rivastigmine, galantamine, tacrine, huperzine A, TV-3326 and anseculin which all bind with Asp 74, Thr 75, Leu 76, Gly 82, Thr 83, Trp 84, Trp 86, Asn 87, Ser 200, Glu 202, Ser 203, Trp 279, Trp 286, Phe 295, Phe 297, Glu 327, Phe 330, Glu 334, Tyr 334, Tyr 337, Phe 338, Tyr 341, Trp 439, His 440, His

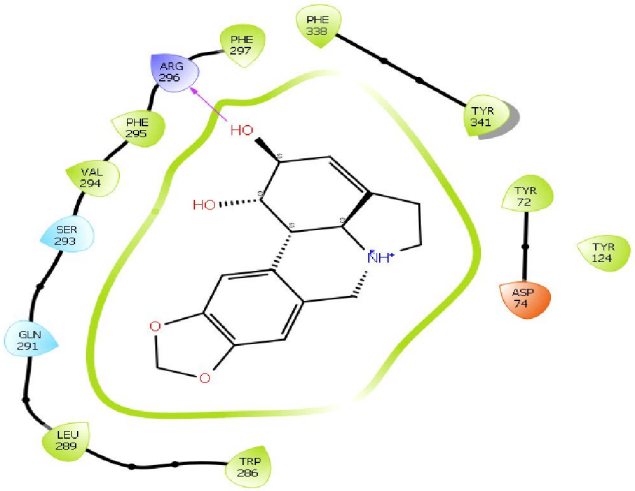

Fig. 3D: Ligand interaction diagram (2D) of the lycorine with AChE.

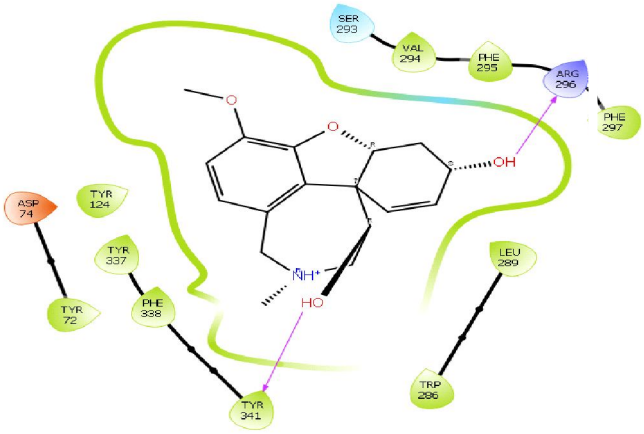

Fig. 3E: Ligand interaction diagram (2D) of the 11alpha-Hydroxy Galantamine with AChE.

447, Gly 448, Tyr 449 (Da Silva *et al.*,2007; Sharma *et al.*,2011). From the comparative analysis, we observed that catalytic site residues and previously reported AChE inhibitors binding residues were present in our site map.

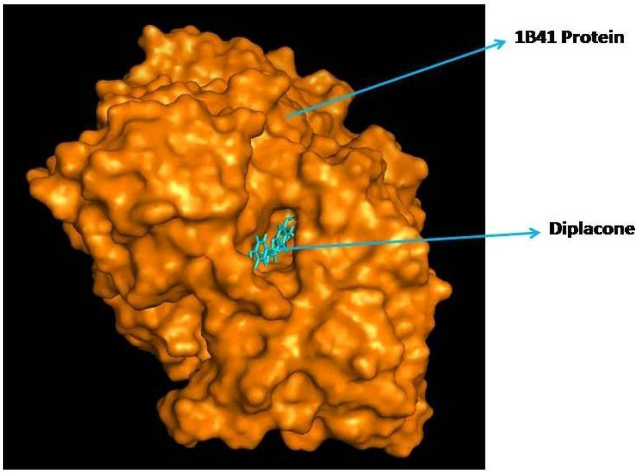

Fig. 4: Surface interaction of Diplacone with Ache Protein.

Approximately more than 95% of the residues were present in our top sitemap (site 1) area. Thus we hope that our current discovered natural inhibitors results may exactly correlate with previously reported drugs.

Molecular dynamics simulation studies

The stability of the AChE protein and selected compound along with AChE (AChE, AChE-Diplacone (14539948)) was assessed using molecular dynamics simulation studies. MD simulation was carried out for 20000 picosecond, for each of the systems. C-alpha atoms were used to compute the RMSD and RMSF in Figure.5-6. RMSD of Protein (AChE) reached equilibrium when it reached 0.19 and after this up to 20ns RMSD will continue stable between the range 0.19 nm to 0.25nm.Similarly, the RMSD of the Protein (AChE) and Protein-Diplacone complex reached 0.24 nm and after this up to 0.30 nm rage, remained stable throughout the course of the simulation. The hydrogen bond plot between the protein and the diplacone is given in Fig. 7. During simulations, the number of hydrogen bonds between the AChE and diplacone varies between 0 and 6.

Table 3: The ADME properties are calculated using QikProp module.

Molecule	CNS	mol_MW	donorHB	accptHB	QPlogPo/w	QPlogS	QPPCaco	QplogBB
Diplacone	-2	424.493	3	4.75	4.351	-6.706	105.641	-2.26
Kuwanon E	-2	424.493	3	4.75	4.223	-6.592	75.15	-2.393
Macluraxanthone	-2	394.423	2	4.5	3.636	-4.961	319.372	-1.146
Lycorine	1	287.315	2	6.9	0.715	-1.456	352.661	0.315
11alpha-Hydroxy Galantamine	1	303.357	2	6.9	1.344	-1.883	430.852	0.1

Table 4: Binding energy calculations of file lead molecules.

S.No.	Ligands	$^{3}\%G_{bind}$ (kJ/mol)
1	Diplacone	-57.1722092
2	Kuwanon E	-59.01897498
3	Macluraxanthone	-53.29212808
4	Lycorine	-58.32483151
5	11alpha-Hydroxy Galantamine	-49.78192344

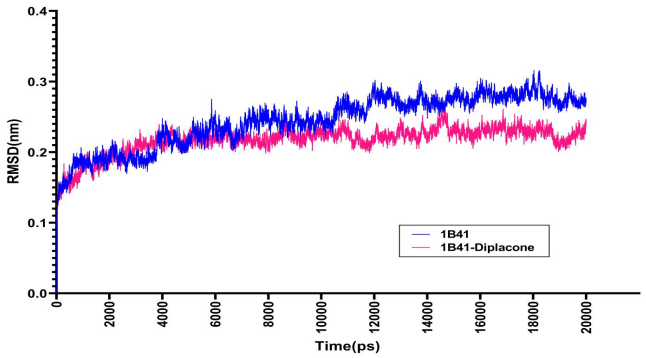

Fig. 5: RMSD of AChE.

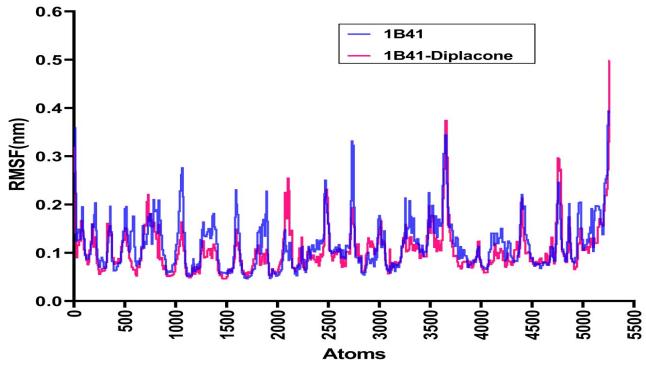

Fig. 6: RMSF of AChE.

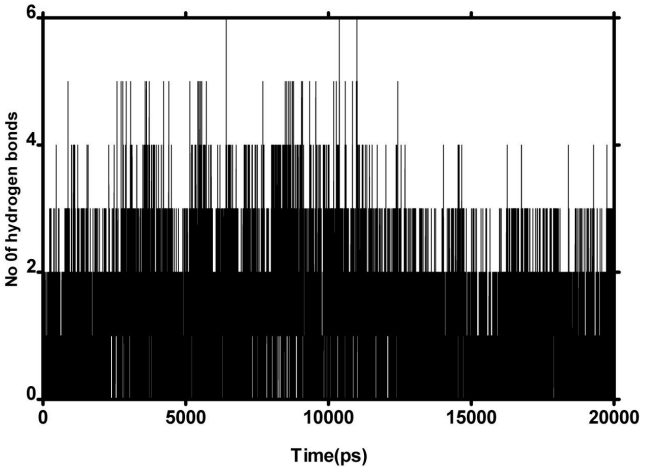

Fig. 7 : Hydrogen bonds between AChE and Diplacone.

Conclusion

ACHE is a serine hydrolase, which is one of the essential neuronal enzyme regulating the nervous transmission. Its uncontrollable hydrolysis is the cause of AD. Currently patients with AD are treated with synthetic drugs with severe side effects in elderly. Therefore this study is directed towards identifying natural compound as an alternative with less harmful effects. In this study, we successfully employed in-silico Virtual Screening, Docking and Molecular Dynamics to identify the potential lead compound that inhibits AChE. Using virtual screening and docking studies, we have screened 251 natural AChE inhibitors against AChE. A total five potential leads Diplacone, Kuwanon E, Macluraxanthone, Lycorine, 11-alpha-HydroxyGalantamine are recognized as novel against target protein which are prioritized based on glide score, glide energy, binding free energies (MM/GBSA) and these molecules are satisfying the drug likeness by showing acceptable ranges of ADME properties. The lead molecule Diplacone is showing maximum glide score, predictable binding free energy from Prime-MM/GBSA and which is more probable to design as potent inhibitor. Through MD simulation of Protein–Diplacone complex analysis, we found that Diplacone has a very strong interaction with AChE. Moreover, the ligand-enzyme complex was quite stable showing it can be a better drug candidate for Alzheimer. We hope that the present computational study will be completely helpful for further in vitro and in silico studies.

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