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RESEARCH ARTICLE

Homology Modeling and *in silico* docking analysis of BDNF in the treatment of Alzheimer's disease

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ABSTRACT:
Alzheimer's disease (AD) is a progressive, degenerative disorder that affects the brain's nerve cells or neurons resulting in memory loss, thinking and behavorial changes. This neurodegenerative disorder affects the neurons of the brain by forming plaques and tangles. There is no effective drug or cure for Alzheimer's disease. There is hope in the potential of stem cells to help us learn and understand about the mechanisms underlying this devastating neurodegenerative disease. From neural stem cell research, it is identified that the protein neurotrophin enhances the level of neurotrophin which will aid in the treatment of neurodegenerative disease. This protein possesses BDNF (brain derived neurotrophic factor) that plays an important role in the process of neurogenesis. The objective of the present study is to evaluate the effect of BDNF and its interaction with the selected ligands in the treatment of Alzheimer's disease. Homology modeling was done for protein BDNF using Modeller v9.13 and the modeled structure was validated using PROCHECK server. A total of 8 ligands such as Citalopram, Ampakines, Fingolimodphosphate, Donepezil, Memantine, Rasogiline, Fluoxetine and Cystamine were taken for docking analysis. The active site prediction was done using CASTP program. Docking analysis was done using Arguslab and the interactions were visualized using PyMol. Fingolimod phosphate showed the least binding energy with the maximum hydrogen bond interactions. The ligand FTY720P had six hydrogen bond interactions where 47ASN binds with 711N….3927O with distance of 2.985Å, 25ASN binds with 391N….3928O with distance of 2.352Å, 4LEU binds with 3906N….56O with distance 2.999Å, 7THR binds with 3906N….114O with distance 2.999 Å, 8MET39290…128O with distance 2.8109Å and 7THR 3929O….114O distance 2.8817Å. Hence, Fingolimodphosphate could be a potent drug and therefore further in vitro studies could be carried out which would play an important role in reducing the effect of AD.

KEYWORDS: Neurotrophin, BDNF, Alzheimer's disease, Homology Modeling, Arguslab, docking.

INTRODUCTION:
Alzheimer's disease (AD) is the most common cause of dementia[1]. The disease is characterized by the presence of neuritic amyloid plaques, cerebrovascular amyloidosis and neurofibrillary tangles (NFTs). The AD brain exhibits extracellular plaques of aggregated amyloid- β -peptide (Aβ) and intracellular NFTs containing hyperphosphorylated TAU protein[2].

Plaques are deposits that surround the neurons, while tangles accumulate within nerve cells, leading to the shrinkage and death of neurons. BDNF could be a breakthrough treatment for Alzheimer's disease and dementia[3]. AD has been regarded as an unsolved, irreversible, and incurable brain neuron degeneration. AD patients normally have difficulties retrieving memory. They exhibit behavioral change as the disease progresses. Eventually, the disease leads to loss of control of body functions[4,5]. There is no cure or available treatments that offer relatively small symptomatic benefit while remaining palliative in nature. In the early stages, it affects short-term memory and thinking ability, and as the disease advances it includes confusion,

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irritability, aggression, mood swings, trouble with language, and long-term memory. Bodily functions are gradually lost, ultimately leading to death[6].

The neurotrophins are a family of proteins that are essential for the development of the vertebrate nervous system[7]. Neurotrophins are dimeric secretory proteins in vertebrate neurons with a broad spectrum of functions. They are generated as pro-proteins with a functionality that is distinct from the proteolytically processed form. Neurotrophins can elicit opposing signals utilising their variable configuration and different receptor types[8]. There is widespread interest in nerve growth factor (NGF) as a potential therapeutic agent in neurodegenerative disorders linked to aging, such as Alzheimer's disease[9]. The neurotrophins are a related family of growth factors presently consisting of nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), NT-4/5, and NT-6[10]. Like other neurotrophins, brain-derived neurotrophic factor (BDNF) was initially identified for its role in neuron proliferation, neurogenesis, differentiation and degeneration. Its function in regulating activity-dependent neuronal modification has also been demonstrated. BDNF regulates both memory formation and long-term potentiation (LTP), an activity-dependent strengthening of synaptic efficacy[11]. Brain-derived neurotrophic factor (BDNF) is a small dimeric protein, structurally related to nerve growth factor, which is abundantly and widely expressed in the adult mammalian brain. BDNF has been found to promote survival of all major neuronal types affected in Alzheimer's disease[12, 13]. BDNF, a member of the neurotrophin family, is neuroprotective in animal models of neurodegenerative diseases[14]. Brain derived neurotrophic factor (BDNF) is a neuro-protective protein that regulates neuronal survival, growth and differentiation[15]. The possibility that neurotrophins are also involved in processes of neuronal plasticity has only recently begun to receive attention[16, 17]. The role of BDNF has been intensively investigated in several clinical situations. As a general rule, a decrease in circulating levels of neurotrophin is harmful[18]. Brain-derived neurotrophic factor (BDNF) was studied initially for its role in sensory neuron development. The functions of neurotrophins in the central nervous system (CNS) appear more diverse than the target-derived paradigm that serves as a useful model in the PNS. BDNF protein is widely distributed in the CNS, beginning early in development and extending throughout the organism's life span[19, 20]. New therapeutic agents that can block the disease-inducing mechanisms are essential[21].

Neurodegenerative diseases result from the gradual and progressive loss of neural cells, leading to nervous system dysfunction. A number of stem and progenitor cell types have been proposed as therapy for neurological disease ranging from neural stem cells (NSCs) to bone marrow-derived stem cells to embryonic stem cells (ESC). Stem cell therapy and cell regenerative approaches to neurological diseases can be divided into a number of categories depending upon the target neurological disease[7]. Both human and animal studies indicate that BDNF signaling is profoundly disrupted in affective disorders, particularly anxiety and depression[22].

MATERIALS AND METHODS:

Homology Modeling:

The prediction of the 3D structure of a protein from its amino acid sequence is achieved using different types of approaches and the first and most accurate approach is "comparative" or "homology" modeling. Homology modeling methods use the fact that evolutionary related proteins share a similar structure[23]. Homology modeling for the protein BDNF has been done using Modeller v9.13. MODELLER is a computer program for comparative protein structure modeling. In the simplest case, the input is an alignment of a sequence to be modeled with the template structures, the atomic coordinates of the templates, and a simple script file. MODELLER then automatically calculates a model containing all non-hydrogen atoms, within minutes on a modern PC and with no user intervention. The prediction process consists of fold assignment, target-template alignment, model building, and model evaluation[24.]

Model Validation:

The PROCHECK suite of programs provides a detailed check on the stereochemistry of a protein structure. Its outputs comprise a number of plots in PostScript format and a comprehensive residue-by-residue listing. These give an assessment of the overall quality of the structure as compared with well refined structures of the same resolution and also highlight regions that may need further investigation. The PROCHECK programs are useful for assessing the quality not only of protein structures in the process of being solved but also of existing structures and of those being modelled on known structures[25.]

Active site prediction:

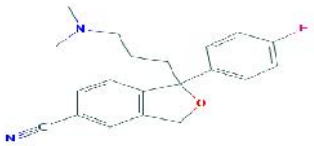
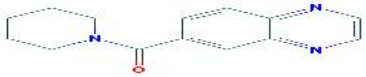
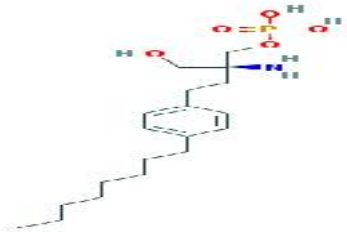
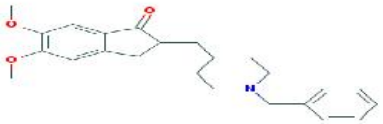
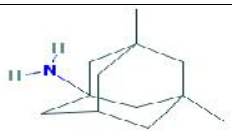
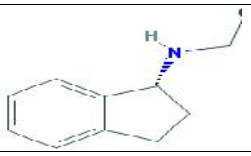
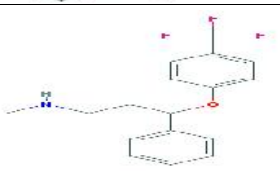
CASTp (http://cast.engr.uic.edu) is an online tool that locates and measures pockets and voids on 3D protein structures. CASTp web server incorporates functional information about a large set of annotated residues on PDB structures obtained from annotations in PDB, Swiss-Prot and Online Mendelian Inheritance in Man (OMIM)[26]. Computed Atlas of Surface Topography of proteins (CASTp) provides an online resource for locating, delineating and measuring concave surface regions on three-dimensional structures of proteins.

These include pockets located on protein surfaces and voids buried in the interior of proteins. The CASTp web server aims to provide a comprehensive and detailed quantitative characterization of interior voids and surface pockets of proteins, which are prominent concave regions of proteins that are frequently associated with binding events. CASTp is based on the alpha shape and the pocket algorithm developed in computational geometry. CASTp identifies all pockets and voids on a protein structure and provides detailed delineation of all atoms participating in their formation[27].

Molecular Docking:

The concept of docking is important in the study of various properties associated with protein-ligand interactions such as binding energy, geometry complementarity, electron distribution, hydrogen bond donor acceptor properties, hydrophobicity and polarizability[28, 29]. Arguslab has been used for docking analysis. Argus lab 4.0.1.version was employed in the docking between protein and ligand using argus dock with a fast and simplified potential of mean force (PMF).

Table 1: List of the Ligands selected with its PubChem ID and structure

S.NO	LIGAND	PUBCHEM ID	STRUCTURE
1	CITALOPRAM	CID 2771	
2	AMPAKINES	CID 148184	
3	FINGOLIMODPHOSPHATE(FTY720-P)	CID 11452022	
4	DONEPEZIL	CID 3152	
5	MEMANTINE	CID 4054	
6	RASOGILINE	CID 3052776	
7	FLUOXETINE	CID 3386	
8	CYSTAMINE	CID 2915	

Dock a ligand in to binding site was done by 'ArgusDock' as the docking engine. 'This programme can generate the amino acid residue around each segment of the final synthesized derivatives and interpreting the different drug-receptor interaction such as hydrogen bonding, hydrophobic interaction and electrostatic force of attraction[30].

Ligand Selection:

Totally 8 drugs were identified from the Pubmed literatures which shows enhancing effects towards protein BDNF. The sdf format of the 3D structure of the ligand was used for docking.

Pymol:

It is a free tool for molecular graphics visualization system for high quality 3D images of small molecules. It has been released under open source software license so that all scientist and software developer can freely use it. All the 3D structure of the drugs was viewed and converted to PDB format which was used for further docking studies.

RESULTS AND DISCUSSION:

The three dimensional structure of the protein BDNF (1BND) was downloaded from PDB and was visualized using Pymol. On visualization, the structure had loops, sheets and only one helix. Hence, the structure of this protein was modeled using Modeller v9.13.

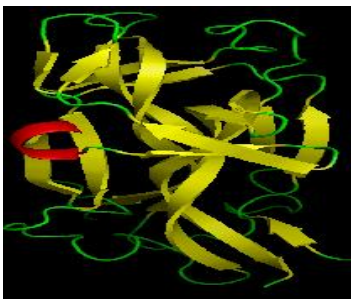


Fig. 1: Protein 1BND from PDB which shows only one helix

The protein BDNF was modeled using Modeller v9.13 with the template PDB ID: 1HCF.

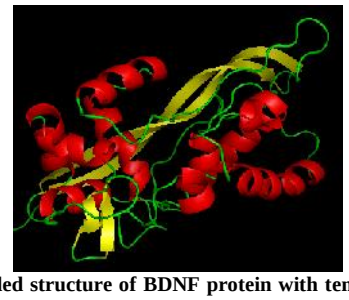
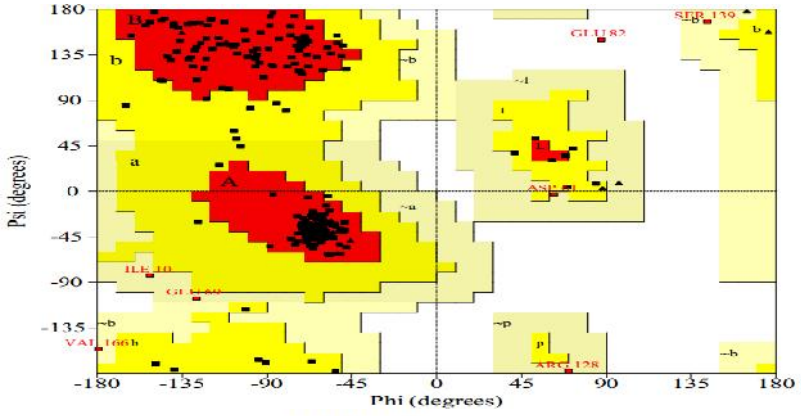


Fig. 2: Modeled structure of BDNF protein with template as PDB ID: 1HCF

Model Validation:



Plot statistics

Residues in most favoured regions [A,B,L]	184	84.8%
Residues in additional allowed regions [a,b,l,p]	26	12.0%
Residues in generously allowed regions [~a,~b,~l,~p]	6	2.8%
Residues in disallowed regions	1	0.5%
Number of non-glycine and non-proline residues	217	100.0%
Number of end-residues (excl. Gly and Pro)	2	
Number of glycine residues (shown as triangles)	18	
Number of proline residues	10	
Total number of residues	247	

Based on an analysis of 118 structures of resolution of at least 2.0 Angstroms and R-factor no greater than 20%, a good quality model would be expected to have over 90% in the most favoured regions.

Fig. 3: Ramachandran plot for the modeled protein structure

The BDNF protein was modeled and the structure was validated using PROCHECK. The number of residues in the most favoured regions [A, B, L] were 184 with 84.8%. The number of residues in the additional allowed regions [a, b, l, p] were 26 with 12% and the number of residues in the generously allowed regions were 6 with 2.8%. The residue in the disallowed regions was 1 with 0.5%.

Active Site Prediction:

The active site prediction using CASTp and Pymol is shown in Fig.4 and 5

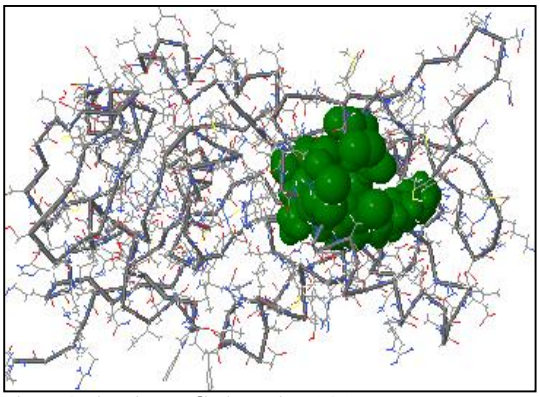


Fig. 4: Active site prediction using CASTp

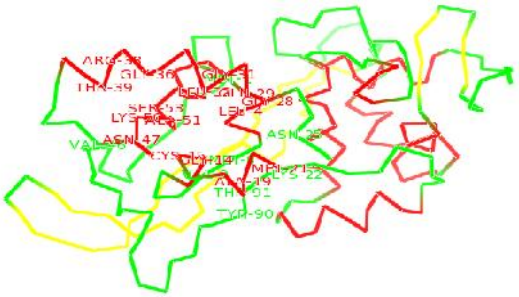


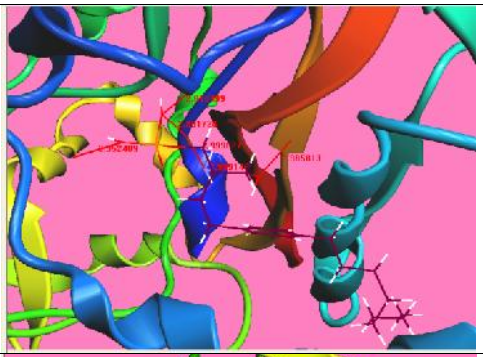
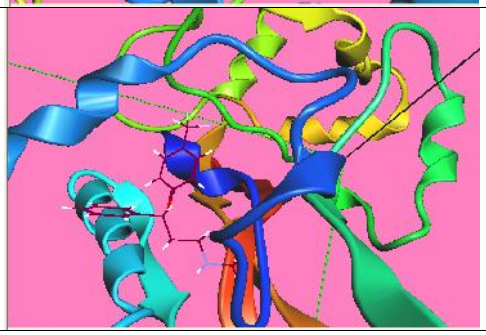
Fig. 5: Active site prediction using Pymol

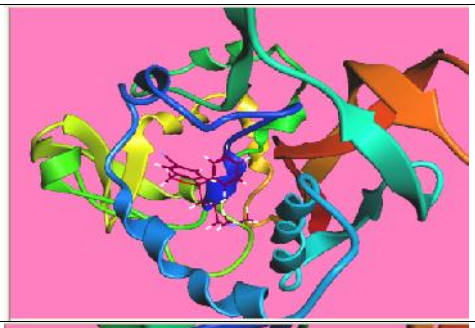
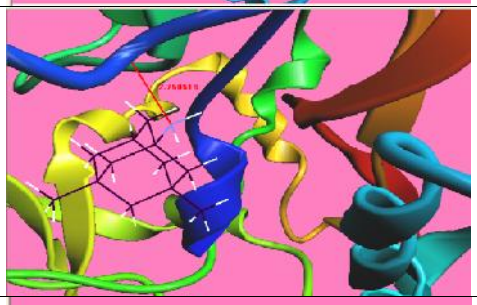
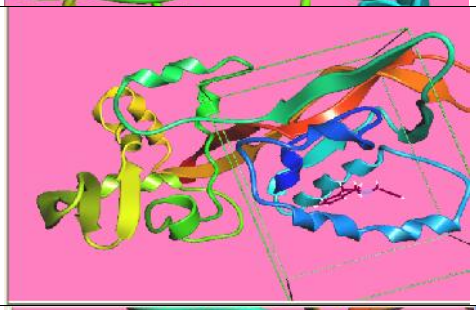
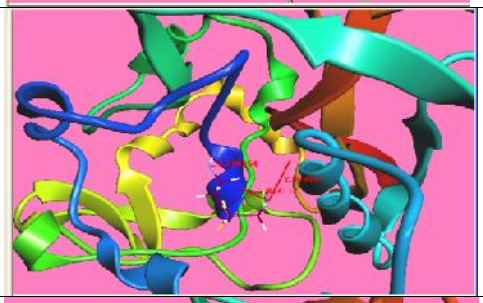
The list of aminoacid residues in the active site is given in Table 2.

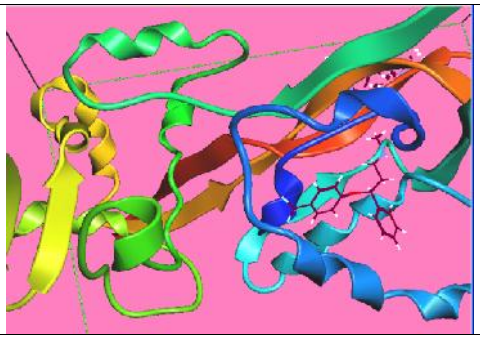
Table 2: Active sites identified from the protein BDNF

S.No	Amino acids	Residues
1	Ala	19,51
2	Arg	38
3	Asn	25,47
4	Cys	15
5	Gln	29
6	Gly	14,28,31,36
7	Leu	4,32
8	Lys	22,50
9	Met	1,8
10	Pro	20,35
11	Ser	45,53
12	Thr	39,91
13	Tyr	90
14	Val	9

Table 3: Docking results of the protein BDNF against the selected ligands

LIGANDS	BINDING ENERGY Kcal/mol	DISTANCE Å / HYDROGEN BOND	INTERACTION
FINGOLIMOD PHOSPHATE (FTY720-P)	-10.9243	2.985813 (47ASN 711N…3927O) 2.352489 (25ASN 391N…3928O) 2.999146 (4LEU 3906N…56O) 2.999626 (7THR 3906N…114O) 2.810998 (8MET 39290…128O) 2.881728 (7THR 39290…114O)	
FLUOXETINE	-10.1377	No hydrogen bond interactions	

CITALOPRAM	**-8.33936**	**No hydrogen bond interactions**	
MEMANTINE	**-8.73327**	**2.750516(14GLY 229N…3906N)**	
RASAGILINE	**-8.07843**	**No hydrogen bond interactions**	
CYSTAMINE	**-6.92278**	**2.790854 (4 leu 3905N…36O)** **2.999993 (47ASN 3906N…707O)** **2.814112 (47ASN 3906N…717O)**	
DONEPEZIL	**-6.91441**	**2.896304 (39THR 595O…3932O)** **2.898497 (39THR 595O…3933O)**	

AMPAKINES	**NLP**	-	

The ligand Fingolimod Phosphate (Fty720-P) had a binding energy of -10.9243kcal/mol with interactions between 47ASN 711N…3927O with a distance of 2.985813Å, 25ASN 391N…3928O with a distance of 2.352489Å, 4LEU 3906N…56O with a distance of 2.999146Å, 7THR 3906N…114O with a distance of 2.999626Å, 8MET 39290…128O with a distance of 2.810998Å, 7THR 39290…114O with a distance of 2.881728Å. The ligand Fluoxetine had a binding energy of -10.1377 kcal/mol, ligand Citalopram had a binding energy of -8.33936 kcal/mol with no hydrogen bond interactions; The ligand Memantine had a binding energy of -8.73327 kcal/mol with interactions between 14GLY 229N…3906N with a distance of 2.750516Å. The ligand Rasagiline had a binding energy of -8.07843 kcal/mol kcal/mol with no hydrogen bond interactions; The ligand Cystamine had a binding energy of -6.92278 kcal/mol with interactions between 4 leu 3905N…36O with a distance of 2.790854Å, 47ASN 3906N…707O with a distance of 2.999993Å, 47ASN 3906N…717O with a distance of 2.814112Å; The ligand Donepezil had a binding energy of -6.91441kcal/mol with interactions between 39THR 595O…3932O with a distance of 2.896304Å and 39THR 595O…3933O with the distance of 2.898497Å; The ligand Ampakines did not produce any binding energy and no hydrogen bonding interactions occurred.

From the results obtained, Fingolimod Phosphate (FTY720-P) had significant interacting poses with the target protein BDNF.

CONCLUSION:

Novel drug with potential biological activity is a need for developing effective drugs. Neurodegenerative diseases result from the gradual and progressive loss of neural cells and lead to nervous system dysfunction. In addition, neural stem cells (NSCs), via brain-derived neurotrophic factor (BDNF), have shown to ameliorate complex behavioral deficits associated with widespread Alzheimer's disease (AD). There are obstacles to use stem cells as a cure for neurodegenerative disease. However, there is hope in the potential of stem cells to help us learn and understand a great deal more about the mechanisms underlying these devastating neurodegenerative diseases. From this study, Fingolimod phosphate could emerge as a potent drug and therefore further invitro studies could be carried out which would play an important role in reducing the effect of AD.

CONFLICT OF INTEREST:

The authors declare they have no competing interests.

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