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**Research Article**

**In silico structural determination and validation of Cyclin dependent kinase 5 activator 1, CDK5R1 by homology modeling as a target of anti-Alzheimer’s drug and design of potential lead compounds by de novo synthesis**

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### Abstract

Cyclin dependent kinase 5 activator 1 (CDK5R1), commonly named P35 is an important cellular component which plays a role in the progression of Alzheimer’s disease (AD). P35 is the first component of the process of Tau hyper phosphorylation which produces Amyloid plaques and neurofibrillary tangles (NFT), the two main components responsible for Alzheimer’s disease. It is very much obvious to be hypothesized that blocking of Cdk-5R1 or P35 can be a suitable attempt to stop the progression of the process of Alzheimer’s disease. This study’s aim is to find some leads which can be a good candidate for anti-Alzheimer’s drug. This study is a computational approach based on structure-based drug design techniques to generate some potential lead compounds for targeting P35 protein as an effective treatment approach for AD. A lot of software and webservers are involved in this study protocol. The structure of CDK5R1 has been built from the amino acid sequences by homology modeling, pockets have been searched in the protein and based on pocket information lead molecules have been designed by de novo synthesis techniques. Finally, molecules have been screened based on some druggability assessment. Among the molecules 8 promising leads have been identified.

**Keywords**: CDK5R1, P35, Tau protein, Plaques and NFT, Computational technique, Structure based drug design, Homology modeling, Pockets and leads generation.

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# Introduction

### Alzheimer’s disease (AD) is most common form of Dementia, and still incurable degenerative disease (De La Torre 2010). In AD certain parts of brain are destroyed and it leads to deficits in cognitive functions such as memory, insights, judgment, absorption, language skills and behavior (Karunarathna et al. 2024).The degenerative changes that occur in Alzheimer’s Diseases affect the neurons of those areas which help in thought controlling, memory preservation, and language use, and person’s behavior and regulation of mental function. Often Physical and functions like bowel and bladder control are also affected (Katzman 1989). Typical Alzheimer’s bruise, which is named the presynaptic phase of the disease, begins to develop in the brain 10 to 20 years before the first symptoms have shown up (Prince et al. 2015). Symptoms are started to be visible only when the neuronal injury occurs and spread the decrease rate of cognitive reserve. Dementia affects 5-8% of people of all people above 60 years of age and increasing to around 40% of people older than 90 years. Alzheimer’s disease is more prevalent in women (Barker et al. 2002). The symptoms start from simple forgetfulness and end with severe disorientation of the cognitive functions along with other problems. There is no single test to diagnose Alzheimer’s disease. Diagnosis involved a full assessment of medical and psychiatric history to find out the possible causes. In recent times two type diagnosis is famous: 1. Amyloid brain imaging (Klunk et al. 2004) and 2. Diagnosis based on fluid biomarkers (Georganopoulou et al. 2005). Plaques (extracellular deposits of amyloid beta in gray matter of brain) and NFT or Neurofibrillary Tangles (Aggregates of Hyper Phosphorylated tau protein) are the main causes of Alzheimer’s disease. Amyloid Beta (Denotes, the peptide of 36-43 amino acids that are mainly involved in Alzheimer’s disease) is formed by clipping normal neuron protein amyloid precursor protein or simply APP. Secretase enzyme can cleave APP. Among several kinds of secratases, if APP is cleaved by Beta secratase and followed by gamma secratase then two kinds of amyloid beta are found AB40 and AB42. These proteins form intracellular but exert their damaging effects while transported outside of the cell. AB42 is most highly concentrated Amyloid beta in neurotic plaques; AB40 is more concentrated in cerebrovascular plaques (Lue et al. 1999). AB40 is soluble and innocuous and AB42 is insoluble, most toxic one (Benilova et al. 2012) and clumps together and form insoluble amyloid plaques with excitatory synapse loss (Koffie et al. 2009), because amyloid beta oligomers cause deterioration of synapse (Lacor et al. 2007). Two processes followed by amyloid plaque formation play important role in causing the death of neurons.1. Inflammation and oxidative damage and 2. Neurofibrillary tangles (NFT). Plaques followed by Neurofibrillary tangles are responsible for Synapse deterioration and synapse loss, which will further form Dementia. The formation of Plaques and Neuro fibrillary tangles happened due to hyper phosphorylation of tau protein (Gong and Iqbal 2008). As it said earlier that Tau protein hyper phosphorylation plays the key role in Alzheimer’s Disease progression through Plaques and Neuro Fibrillary Tangles (NFT) formation, So, it is important to identify Tau as a target for AD, moreover tau has some important cellular functions like stabilizing the skeletal scaffolding of neurons or the cytoskeletal micro tubules which can be hampered. For that reason, it is suitable to look at the earlier components in the pathological pathway which are responsible for tau hyper phosphorylation. There are two Pathways, which can progress the Tau Phosphorylation.

### 1. P/13/Akt/GSK-3Alpha/Beta Pathway (Kitagishi et al. 2014).

### 2. P35 cleavage pathway (Patrick et al. 1999).

### For this study, the P35 cleavage pathway has been chosen. This pathway aids the Tau hyper phosphorylation by exploiting four components (Gong and Iqbal 2008). P35 protein, Calpain enzyme, CDK5 and P25 Protein. In Short P35 cleaved by an enzyme named Calpain. Cleavage of P35 produces P25. This P25 form complex with CDK5 (Cycline dependent Kinase 5) and activates it (Lau et al. 2002). This complex Hyper phosphorylates Tau. It is clear that, every single component of this pathway can be a good target for the design of Anti Alzheimer’s Drug, but there is some problem.CDK5 is required for proper development of mammalian CNS (post mitotic development of neurons) (Kusakawa et al. 2000) and P25 is produced from cleavage of P35.As P35 is the first protein of this pathway, so it is the most convenient target. This study has shown a new approach for the development of an Anti-Alzheimer Drug based on this (P35) target. The goal of this study is to find a suitable target and design the leads for that target as a new approach in the field of Alzheimer’s disease treatment.

### Materials and Methods

It is very much needed to determine the 3d structure of target protein to design new lead compounds for that target. It is so good to use the X-ray Crystallographic structure from PDB. If the structure is not solved by X-ray, then NMR solution structures in PDB can be used. If that is also not done and just a sequence is available, then it is better to go for a new method.

To design an effective drug for the selected CDK5R1, PDB structure of the protein was needed but due to the unavailability of PDB structure, homology modeling techniques has been adopted to build the three-dimensional (3D) structure from the sequence of the protein p35 derived from Uniprot Knowledge Base (Uniprot code: Q15078) (Consortium 2008). (Table 1) UniprotKB has two sections “Reviewed” and “Unreviewed”. The section containing manually annotated records with information extracted from literature and curator-evaluated computational analysis is called “Reviewed Section”, and a section with computationally analyzed records that await full manual annotation is the “Unreviewed” (Schwede et al. 2003).

Homology modeling is a computational method used to predict three-dimensional structure of a protein (Krieger et al. 2005). SWISS Model (Arnold et al. 2006; Guex et al. 2009; Kiefer et al. 2009) predicts the 3D structure of protein from the sequence. It is commonly termed as “template-based modeling” (Zhang 2008). The sequence alignment tool BLAST in SWISS Model has been used to find a known structure (template) that shares significant sequence similarity with the target protein P35.Sequence alignment has been done by identifying conserved regions and ensuring accurate similarity between residues. After finishing the structure building by BLAST, it was checked and there were some incomplete parts of the sequence. Homology modeling cannot build all the sequence into 3D structure due to insufficient template. The rest of the sequence has been built with ab initio technique in I-TASSER bioinformatic tool (Zhang 2008). The ab initio technique is an important method for 3D structure prediction where structures of protein from its amino acid sequence can be built alone without depending on homologous template structures. Fundamental, physical and chemical principles like thermodynamics and statistical mechanics have been used in ab initio technique.

**Table 1: Amino acids codes used by Uniprot for FASTA Sequence of Protein CDK5R1 indicating the part-by-part building of 3D structure.**

|  |
| --- |
| Ab initio Modeling  |
| MGTVLSLSPSYRKATLFEDGAATVGHYTAVQNSKNAKDKNLKRHSIISVLPWKRIVAVSAKKKNSKKVQPNSSYQNNITHLNNENLKKSLSCANLSTFAQPPPAQPPAPPASQLSGSQTGGSSSVKKAPHPAVTSAGTPKRVIVQA  |
| Homology Modeling |
| STSELLRCLGEFLCRRCYRLKHLSPTDPVLWLRSVDRSLLLQGWQDQGFITPANVVFLYMLCRDVISSEVGSDHELQAVLLTCLYLSYSYMGNEISYPLKPFLVESCKEAFWDRCLSVINLMSSKMLQINADPHYFTQVFSDLKNES |
| Ab initio Modeling |
| GQEDKKRLLLGLDR |

Protein models obtain from both homology and AB initio are visualized and joined by a software UCSF Chimera (Pettersen et al. 2004). UCSF Chimera is a highly extensible program for interactive visualization and analysis of molecular structures and related data, including density maps, supramolecular assemblies, sequence alignments, docking results, trajectories, and conformational ensembles. High-quality images and animations can be generated. Chimera includes complete documentation. Fragments are then joined and optimized (Mondal et al. 2016). Full 3D structure of P35 has been validated by Ramachandran plot analysis with the help of the server PROCHECK. (Laskowski et al. 2012; Laskowski et al. 1993) Ramachandran (RC) plot is the quality indicator of a protein model which can detect error in the structure. In PROCHECK the different regions in the RC plot are defined based on the density of the data points which are taken from the well-defined database of refined protein structure. The regions are defined as: core, allowed, generously allowed and disallowed. The core regions are particularly important as the points on the plot tend to converge on this area and tightly cluster among themselves. It happened more in the structures solved at high resolution than those of low resolution (Laskowski et al. 2012). Energy minimization of the validated structure has been done by Swiss pdb viewer software. Swiss pdb viewer is a widely used molecular modeling tool for visualizing, analyzing and optimizing protein structures. Swiss-pdb viewer has also the option of energy minimization of protein structure to ensure the refinement of the protein by optimizing atomic coordinates to reduce clashes and improve structural stability (Guex and Peitsch 1997). After the optimization of protein structure, suitable pockets in the protein have been found with the help of CASTp 3.0 server. CASTp 3.0 (Computed Atlas of Structure Topography of Proteins) is a computational tool used to identify and characterize pockets, cavities, grooves and channels in protein structures. These regions have proven to be useful and biologically significant and represent binding sites for micro molecules (Dundas et al. 2006; Tian et al. 2018). Design of new lead compounds have been done based on the pocket’s information from CASTp 3.0. Pockets have been identified by the x, y, z coordinates in three-dimensional space. These coordinates have been specified in e-LEA3D web server to generate lead compounds by de novo synthesis method. The e-LEA3D web server is an online tool for computational lead discovery. The server enables users to design novel molecules from information on its specific biological targets. The de novo synthesis feature in e-LEA3D refers to its ability to design chemically valid molecules starting from basic building blocks or templates. Algorithms generate these molecules with some desired properties like improved binding affinity pharmacokinetics and ADMET properties (Douguet 2010). New lead molecules are brought to screen based on pharmacokinetic criteria by Mobyle (Néron et al. 2009). This web server evaluates logP computation program, retrieve covalent inhibitors checking, PAINS (Filter pan assay interference compounds) filter Lilly Medchem rules etc. 32 profiles including Molecular weight, cLogp, log d, hydrogen bond donor, Hydrogen bond acceptor, flexibility, rings, max sizes, charges have been checked as a druggability index. After checking all the profiles for the lead molecules Mobyle has given results in three accepted categories, rejected and intermediate. Accepted molecules have been selected as promising lead compounds (Néron et al. 2009).

### Results and discussion

### Amino acid sequence and homology modeling

### FASTA sequence of Cyclin dependent kinase 5 activator 1 has 307 amino acid and it has been collected from Uniprot kb. (Table 1) This sequence has subjected to build with homology modeling in SWISS MODEL.The BLAST option in SWISS MODEL has generated the 3D structure from amino acid 147-293 of the sequence of CDK5R1. (Figure 1) BLAST has given a new protein model along with the template used in homology modeling. The structure is done by X-ray method and sequence similarity is 0.61% with coverage of 0.60 while sequence identity is 100. (Table 2)

|  |  |
| --- | --- |
| **Generated** **Model** | **Matching Template** |

**Figure 1:** Protein structure generated by homology modelling

### Ab initio modeling

### For Ab initio modeling I-TASSER web server is used and 5 models for Amino acid sequence 1-147 and 1 model for Amino acid sequence 294-307 are obtained. Those models are then assessed by the PROCHECK (Lovell et al. 2003) server based on Ramachandran plot assessment and three sections of results are found for every model. Favored region, allowed region and outlier. A selection is then taken place based on the result of the total value of Favored and allowed region for each model in tabular form. The model with highest Favored and allowed region (91%) among the models is selected for the further step (Table: 3).

### Model joining and Energy Minimization

### All the models from homology modeling and ab initio modeling have been joined in UCSF Chimera and proceed to energy minimization with SWISS PDB viewer (Guex et al. 2009) and then energy minimized file is obtained from it. (Figure 2)

### Model Validation

### The entire joined structure having 307 amino acids have been validated with PROCHECK based on Ramachandran plot assessment. Energy minimized file is inputted into the web server PROCHECK and after analysis a full assessment is obtained with suitable log file. The file contains the result for the model as follows favored region-82.8%, Allowed region-13.5% and Outlier-3.6%. As most of the amino acid residues are in favored and allowed regions which proves that the final 3D structure of CDK5R1 obtained by homology and ab initio modeling has a refined and optimized structure.

### Pocket detection

### After validation of energy minimized file pockets are tried to be found with CASTp 3.0 and several pockets have been identified out of which 17 pockets have been detected primarily based on MS volume (18.0- 125.4 Å³).

### Pocket Selection

### Among all the 17 pockets, 9 pockets (ID 5,6,7,8,9,10,11,12 and 19) have been selected finally based on the number of openings. Single openings are always preferable because double or more openings can give allosteric binding, and zero openings are not really a pocket (Dundas et al. 2006). The three-dimensional coordinates of the selected pockets have been identified with the help of UCSF Chimera. (Table 3)

### Lead design

### From e-LEA3D (Douguet 2010) 6 (Six) leads have produced for each pocket. All the molecules are divided into 5 generations of lead for each pocket presented as Generation 0 to Generation 5. e-LEA3D used the docking program PLANTS (Protein-Ligand ANT system), where the PLP piecewise linear potential scoring function has been used. In PLP, the most negative results indicate better binding. Best leads are selected for each pocket based on the binding affinity. Binding affinity below -80 Kcal/mol is considered as good binding, whereas binding affinity from -60 to -80 is considered moderate and binding affinity above -60 Kcal/mol is considered as low binding (Korb et al. 2009). (Table 6) These molecules have shown different molecular interaction with the protein.

### Druggability profile check and selection of promising lead molecules and validation challenges

### All the leads of nine pockets are then gone through a checking process of Pharmacokinetic profile through the Mobyle Web Server (Néron et al. 2009). Among the 54 molecules generated by de novo synthesis 8 molecules have shown no violation of the lipinski’s rule of 5. Lipinski’s rule of 5 is a widely used guideline in virtual screening and drug discovery to evaluate the drug-likeness of small molecules. It helps predict whether a compound is likely to have good oral bioavailability. According to Lipinski's Ro5, a molecule is considered drug-like if it has molecular weight ≤ 500 Daltons, Lipophilicity or logP value ≤ 5, Hydrogen bond donors (HBD) ≤ 5 and Hydrogen bond acceptor HBA ≤ 10. If the designed lead compounds violate any of these rules they are not likely to be an drug like compound. (Lipinski et al. 1997) In this study only 8 molecules have these drug-like criteria (Table 5) (Figure 2) So, these molecules have been selected as promising lead compounds. Binding interactions of the selected molecules have been shown on Figure 2 where a significant amount of hydrogen bonds depict the strength of binding between the protein and proposed compounds. Compound 52 shows the highest number of hydrogen bonding with the amino acid residues of CDK5R1. The other molecules also show significant number of hydrogen bonding along with van der Waals interaction, alkyl and pi-alkyl interaction as well. The strength and stability of protein-ligand binding in computational drug discovery are determined by various types of bonds and interactions. Hydrogen bonds contribute specificity and stability through their directional nature, while ionic bonds, formed between oppositely charged groups, add significant strength, especially in polar environments. Hydrophobic interactions play a critical role in stabilizing ligands within hydrophobic protein pockets, while van der Waals forces, though weak individually, provide complementary surface interactions. Pi-pi stacking and cation-pi interactions are essential for binding pockets containing aromatic residues, and metal coordination bonds are crucial for metalloproteins. Covalent bonds, though rare, are used in designing irreversible inhibitors, offering exceptional binding strength.(Chen 2015) Each bond type contributes uniquely to binding affinity, specificity, and drug-likeness. Molecular docking and dynamics simulations incorporate these interactions to predict binding energies, guiding ligand optimization. Enhancing specific interactions improves potency, selectivity, and ADMET properties, minimizing off-target effects. Thus, understanding and accurately modeling these interactions are vital for effective drug design and discovery. (Warren et al. 2006) To validate computer-aided drug designs, molecules should be brought into in vitro studies like binding assays, enzyme inhibition, and cell-based assays to confirm activity and binding affinity. Structural techniques like X-ray crystallography, NMR, or cryo-EM are also used to verify binding modes. In vivo studies in animal models can also be used to assess efficacy, pharmacokinetics, and toxicity. The intention of this study is to present those 8 promising leads based on the binding affinity (Table 6) with no Lipinski’s violation and significant druglike properties (Table 5). The scope of further studies is open where different other ways of chemical drug synthesis and validation process like UV, NMR and cryo-EM will be applied to determine the best lead compounds to develop the anti- Alzheimer’s drug. There are also some scopes for the animal trial of these molecules.

### Limitation and challenges of homology modeling

Homology modeling faces several limitations and challenges, including dependence on high-quality templates, difficulty in modeling low-sequence identity targets, inaccuracies in loop and side-chain modeling, and inability to predict conformational flexibility. Template availability is often limited, and errors in alignment can propagate, reducing model accuracy. Additionally, homology models lack the ability to capture novel folds or accommodate large structural deviations. Overcoming these challenges involves using multiple templates, optimizing sequence alignments with advanced tools, and refining models with molecular dynamics simulations. In this study we have found a significant level of sequence identity with 61% sequence similarity with the template. (Table 2) We have used Ramachandran plot analysis to validate the protein structure as well. Though incorporating experimental data, such as from NMR or X-ray crystallography, can enhance accuracy, this study lacks this experimental validation because the chemical synthesis of these proposed molecules is still on card. Combining computational approaches with experimental validation ensures reliable models for functional and structural studies. It is obvious to extend this study to the ultimate formulation of Anti Alzheimer’s Drug is possible in future, whereas this current study has presented the proper investigation of CDK5R1 as a potential target for Anti Alzheimer’s drug and some promising lead compounds which have all the drug like features to become potent drug for the molecular level inhibition of Alzheimer’s disease.

**Table 2: Structure metrics information of CDK5R1 generated by homology modeling in SWISS MODEL**

|  |  |
| --- | --- |
| **Sequence Identity** | **100** |
| **Method** | **X Ray** |
| **Sequence Similarity** | **0.61** |
| **Coverage** | **0.60** |
| **Range** | **Amino acid 147-293** |

**Table 3: Model information generated by ab initio method from I-TASSER.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Model no.** | **Favored Region (F)** | **Allowed Region (A)** | **Outlier** | **F+A (%)** |
| Model1 | 52.1 | 28.5 | 19.4 | 80.6 |
| Model2 | 58.3 | 27.8 | 13.9 | 86.1 |
| Model3 | 37.5 | 31.9 | 30.6 | 69.4 |
| Model4 | 72.2 | 18.8 | 9 | 91 |
| Model5 | 56.9 | 24.3 | 18.8 | 81.2 |

**Table 4: Selected Pockets generated by CASTp along with three dimensional coordinates**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Sl. No. | Pocket ID | MS Volume | Openings | X | Y | Z |
| 1 | 19 | 23.5 | 1 | 85.661 | 103.504 | 75.785 |
| 2 | 11 | 23.8 | 1 | 107.556 | 72.157 | 72.960 |
| 3 | 12 | 27.6 | 1 | 82.279 | 34.399 | 84.043 |
| 4 | 10 | 35.2 | 1 | 71.184 | 101.835 | 77.681 |
| 5 | 9 | 44.0 | 1 | 113.004 | 41.495 | 54.763 |
| 6 | 6 | 55.1 | 1 | 82.835 | 44.349 | 93.795 |
| 7 | 5 | 86.8 | 1 | 101.551 | 60.283 | 54.743 |
| 8 | 8 | 102.2 | 1 | 78.885 | 92.611 | 74.852 |
| 9 | 7 | 118.2 | 1 | 112.637 | 53.718 | 64.741 |

**Table 5: Draggability information of the selected molecules by RPBS Mobyle web server**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Ligand | MW | logP | tPSA | Flexibility | LipinskiViolation | Solubility(mg/l) | Solubility Forecast Index | OralBioavailability |
| 2 | 447.59 | 3.34 | 70.23 | 0.22 | 0 | 5562.36 | Good | Good |
| 4 | 309.33 | 4.05 | 25.84 | 0.37 | 0 | 5286.07 | Good | Good |
| 10 | 451.56 | 3.09 | 80.07 | 0.24 | 0 | 6819.5 | Good | Good |
| 11 | 375.47 | 1.21 | 91.07 | 0.23 | 0 | 27514.02 | Good | Good |
| 47 | 416.51 | 0.91 | 98.77 | 0.34 | 0 | 37510.97 | Good | Good |
| 52 | 251.33 | 3.06 | 56.32 | 0.16 | 0 | 8088.12 | Good | Good |
| 53 | 408.54 | 3.76 | 64.61 | 0.35 | 0 | 5269.59 | Good | Good |
| 54 | 337.37 | 2.69 | 77.24 | 0.25 | 0 | 10022.87 | Reduced Solubility | Good |

**Table 6: Binding affinity of the selected Lead Compounds**

|  |  |
| --- | --- |
| Compound | Binding affinity (Kcal/mol) |
| Compound 2 | -83.07 |
| Compound 4 | -83.89 |
| Compound 10 | -85.35 |
| Compound 11 | -95.84 |
| Compound 47 | -96.72 |
| Compound 52 | -88.80 |
| Compound 53 | -87.48 |
| Compound 54 | -91.65 |

|  |  |
| --- | --- |
| E:\PAPER\P35,Paper all\band_wagon\Final_Docking Analysis\g1_mol5_conf1_prot0full-Ligand 1_Com_2.png | E:\PAPER\P35,Paper all\band_wagon\Final_Docking Analysis\g3_mol3_conf1_prot0full-Ligand 1_Com_4.png |
| Compound 2 | Compound 4 |
| E:\PAPER\P35,Paper all\band_wagon\Final_Docking Analysis\g3_mol1_conf1_prot0full-Ligand 1_Com_10.png | E:\PAPER\P35,Paper all\band_wagon\Final_Docking Analysis\g4_mol3_conf1_prot0full-Ligand 1_Com_11.png |
| Compound 10 | Compound 11 |
| E:\PAPER\P35,Paper all\band_wagon\Final_Docking Analysis\g4_mol1_conf1_prot0full-Ligand 1_47.png | E:\PAPER\P35,Paper all\band_wagon\Final_Docking Analysis\g3_mol4_conf1_prot0full_com_52.png |
| Compound 47 | Compound 52 |
| E:\PAPER\P35,Paper all\band_wagon\Final_Docking Analysis\g4_mol5_conf1_prot0full-Ligand 1_com_53.png | E:\PAPER\P35,Paper all\band_wagon\Final_Docking Analysis\g5_mol2_conf1_prot0full-Ligand 1_com_54.png |
| Compound 53 | Compound 54 |

**Figure 2:** Binding interaction of the selected lead molecules with CDK5R1

**Conclusion**

As mentioned earlier, the aim of this study is to produce some lead compounds against the target (Cyclin dependent kinase 5 activator 1, P35) that has selected to block the disease progression of Alzheimer’s Disease by using computer techniques. All the regular techniques have applied during the study and a lot of computational data are produced which can be very important for further research in this field. This study also proves the efficiency of computational drug design systems or In silico drug design system, which may help the researchers to find new path in the field of biological research. Along with this a good number of leads have been produced for further development of Alzheimer’s disease treatment.

### List of Abbreviations

CDK5R1: Cyclin dependent kinase 5 activator 1, AD: Alzheimer’s Disease, NFT: Neuro fibrillary tangles.

### Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Author Contributions Statement**

# Conceptualization: LHS; collection of data and software computational work: LHS and THA; manuscript draft writing: LHS, JT and SA; Review, and editing: LHS, THA, JT and SA; editing and final manuscript preparation: LHS, all authors have read and agreed to publish the manuscript.

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# Data relevant to the study is already included in the article or attached in the supplements. Raw data will be provided at reasonable request upon contact with the corresponding.

# Data Availability Statement

Data relevant to the study is already included in the article or attached in the supplements. Raw data will be provided at reasonable request upon contact with the corresponding.

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