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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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Lutful Haque Saran[1*], Tafsina Haque Aurin[2], Jarin Tasnim[1], Samia Akter[1], Md. Moklesur Rahman Sarker[1*]

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.

[1]Department of Pharmacy, Gono University (Bishwabidyalay), Nolam, Mirzanagar, Savar, Dhaka- 1344, Bangladesh; [2]Department of Life Sciences, Independent University Bangladesh, Plot 16, Block B, Aftabuddin Ahmed Road, Bashundhara Residential Area, Dhaka- 1245, Bangladesh.

***Correspondence:** Lutful Haque Saran, Lecturer, Department of Pharmacy, Gono University, Nolam, Mirzanagar, Savar, Dhaka 1342, Bangladesh; Email: lutful2012@gmail.com; Phone: +8801684147157
Prof. Dr. Md. Moklesur Rahman Sarker, Department of Pharmacy, Gono University, Nolam, Mirzanagar, Savar, Dhaka 1342, Bangladesh; Email: moklesur2002@yahoo.com, dr.moklesur2014@gmail.com

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: a) Inflammation and oxidative damage, and b) 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: i) P/13/Akt/GSK-3Alpha/Beta Pathway (Kitagishi et al., 2014), and ii) 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

Sequence Retrieval and Structure Determination Approach

The three-dimensional (3D) structure of the target protein CDK5R1 (p35) was essential for structure-based drug design. Due to the unavailability of its experimentally determined structure in the Protein Data Bank (PDB), an in silico approach was adopted to model the full-length protein. The primary amino acid sequence of CDK5R1 was retrieved from the UniProt Knowledgebase (UniProtKB) with the accession number Q15078 (Consortium, 2008). (Table 1) The UniProtKB includes both "Reviewed" entries, which are manually curated and supported by literature and computational analysis, and "Unreviewed" entries, which are computationally annotated pending expert evaluation (Schwede et al., 2003).

Homology Modeling

Homology modeling was employed to predict the structure of the protein using the SWISS-MODEL server predicts the 3D structure of protein from the sequence. (Arnold et al., 2006; Guex et al., 2009; Kiefer et al., 2009) This method, also referred to as template-based modeling (Zhang, 2008), involves finding a template protein with a known structure that shares significant sequence identity with the target. A BLAST search was performed via SWISS-MODEL to identify such a template, followed by alignment and model generation based on conserved residues. Partial regions of the sequence could not be modeled due to the lack of appropriate templates.

Ab Initio Modeling

For the unresolved segments, ab initio modeling was performed using the I-TASSER web server. This method constructs 3D structures solely from the protein's primary sequence based on physicochemical principles, such as thermodynamics and statistical mechanics, without the need for a homologous template. (Zhang, 2008).

Structural Assembly and Visualization

The predicted fragments obtained from both homology and ab initio modeling were visualized and merged using UCSF Chimera, an interactive software suite for the visualization and analysis of molecular structures (Pettersen et al., 2004). The joined model was then subjected to optimization procedures to refine the 3D structure and minimize geometrical discrepancies (Mondal et al., 2016).

Structural Validation and Energy Minimization

The quality of the assembled 3D model was validated using a Ramachandran plot generated by PROCHECK (Laskowski et al., 2012, 1993). This tool evaluates the sterically allowed regions of backbone dihedral angles (phi and psi) in protein structures and identifies outliers and structural inaccuracies. Post-validation, energy minimization was performed using Swiss-PDB Viewer to reduce steric clashes and optimize atomic positions, thereby improving the overall structural stability (Guex and Peitsch, 1997).

Pocket Identification and Lead Compound Design

Potential ligand-binding sites were predicted using CASTp 3.0 (Computed Atlas of Structure Topography of Proteins), which identifies surface pockets and internal cavities based on geometric and topological features (Dundas et al., 2006; Tian et al., 2018). The three-dimensional coordinates (x, y, z) of identified pockets were used as input in e-LEA3D, a web server for de novo ligand generation and lead discovery (Douguet, 2010). This tool designs novel, chemically valid structures tailored to the identified binding site, optimizing for pharmacological properties such as binding affinity and ADMET profiles.

Pharmacokinetic Screening

The generated lead compounds were further screened using Mobyle, an integrated web-based platform for pharmacokinetic and drug-likeness evaluation (Néron et al., 2009). Parameters assessed included molecular weight, cLogP, logD, hydrogen bond donors and acceptors, rotatable bonds, ring structures, total charge, PAINS (Pan Assay Interference Compounds) filters, and compliance with Lilly MedChem rules. Each compound was categorized into "Accepted," "Intermediate," or "Rejected" based on a druggability index. Only molecules categorized as "Accepted" were selected as final lead candidates.

Results

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)

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
MGTVLSLSPSYRKATLFEDGAATVGHYTAVQ NSKNAKDKNLKRHSIISVLPWKRIVAVSAKKK NSKKVQPNSSYQNNITHLNNENLKKSLSCANL STFAQPPPAQPPAPPASQLSGSQTGGSSSVKKA PHPAVTSAGTPKRVIVQA
Homology Modeling
STSELLRCLGEFLCRRCYRLKHLSPTDPVLWLR SVDRSLLLQGWQDQGFITPANVVFLYMLCRD VISSEVGSDHELQAVLLTCLYLSYSYMGNEISY PLKPFLVESCKEAFWDRCLSVINLMSSKMLQI NADPHYFTQVFSDLKNES
Ab initio Modeling
GQEDKKRLLLGLDR

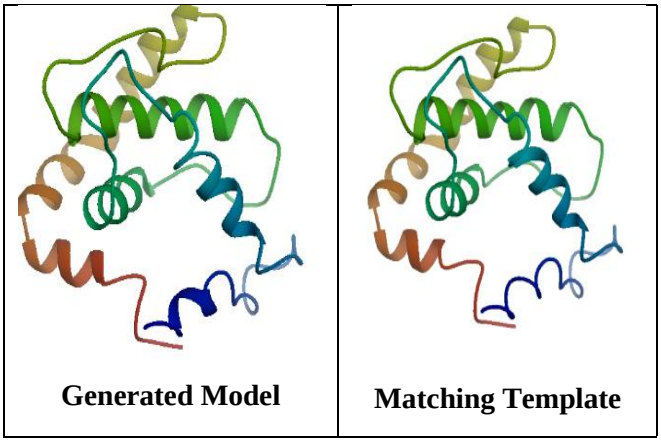

Figure 1. Protein structure generated by homology modelling

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

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).

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

	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

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)

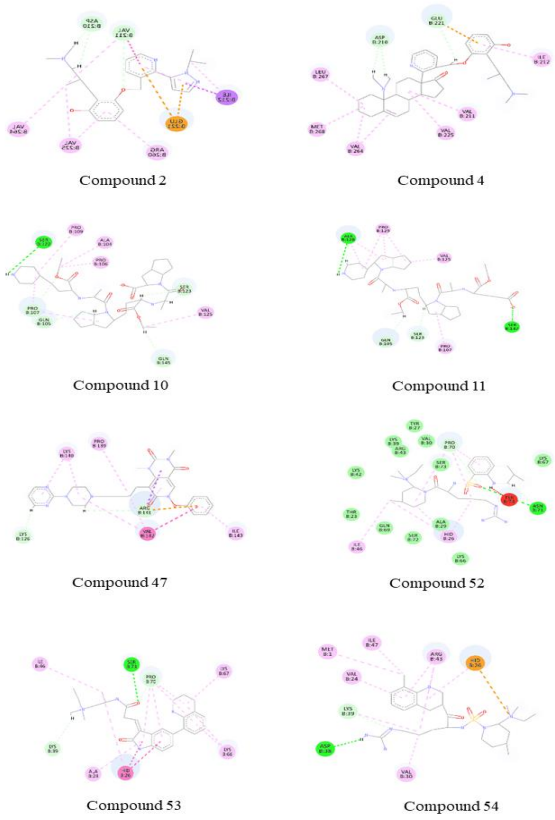

Figure 2. Binding interaction of the selected lead molecules with CDK5R1

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 Å^3).

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 4)

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

Sl. No.	Pocket ID	MS Vol.	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

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 5) These molecules have shown different molecular interaction with the protein.

Table 5. 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

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 6) (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 5) with no Lipinski's violation and significant druglike properties (Table 6). 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.

Table 6. Draggability information of the selected molecules by RPBS Mobyle web server

Ligand	MW	logP	tPSA	Flexibility	Lipinski Violation	Solubility (mg/l)	Solubility Forecast Index	Oral Bioavailability
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

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.

Conclusion and Future Direction

As mentioned earlier, the aim of this study was 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

Data Availability Statement

Data relevant to the study is already included in the article. Raw data will be provided on reasonable request upon contacting with the corresponding author.

Authors Contributions

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, SA and MMRS; supervision: MMRS; editing and final manuscript preparation: LHS. All authors have read and agreed to publish the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest. .

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