

## Design and selection of Novel Kinase inhibitors implicated in Alzheimer's disease by High throughput Virtual screening

**Comment [u1]:** Suggestive to change the title appropriate to the study and should be more informative.

### Abstract

Cyclin dependent kinase 5 (CDK5) is an effective target for the treatment of various types of neurodegenerative diseases. Tremendous progress has been made in the development of potent and selective CDK5 inhibitors that engage polar side chains of the ATP-binding pocket as well as forms the specific hydrogen bonds with the kinase. To identify new lead candidates as potential CDK5 inhibitors with better efficacy, ADME properties and wide margin of safety. 2,50,000 molecules from Specs database was screened against CDK5 crystal structure (PDB ID: 1UNL) by high throughput virtual screening using flexible docking. The docking simulation was done first HTVS followed by SP and then XP. Based on GLIDE docking score, interaction pattern to the receptor with new lead candidates have been identified and selected for synthesis. The lead moiety in the co-crystallized roscovitine with CDK5 complex retained the key H-bonding patterns and also adds hydrophobic interaction with Ile10 and Leu133. Selected hits shows hydrophobic interactions within ATP cleft with Asn 144, Gly 13 and Ala143 in a similar manner as shown by reference molecules (Roscovitine). The binding pattern of the lead compound revealed by docking studies using GLIDE indicated that molecules bind into well-conserved catalytic pocket of the kinase.

**Comment [u2]:** Mention the usefulness of the outcome gained in the study.

**Comment [u3]:** Mention why the study was taken up and what is the effective outcome.

**Keywords:** Roscovitine; ADME; Docking; Neurodegenerative

### Introduction

Cyclin dependent kinases are large family of proline directed serine/threonine kinase with significant importance in the regulation of cellular progression by altering multiple events in different phases of cell cycle [1]. The activity of CDK5 is generated is interaction CDK5 subunit with its regulatory subunit cyclin. On the basis of their binding capacity, various number of cdks have been identified and characterized. According to recently proposed nomenclature, the cdk family consists of 20 CDK [2]. Human genome contains about 21 genes encoding Cyclin dependent kinase. CDK1 is essential for cell division and reduces embryonic lethality. CDK2, CDK4 and CDK6 are required for proliferation of specialized cells that show additional activities beyond cell cycle control [3]. CDK5 plays a vital role in

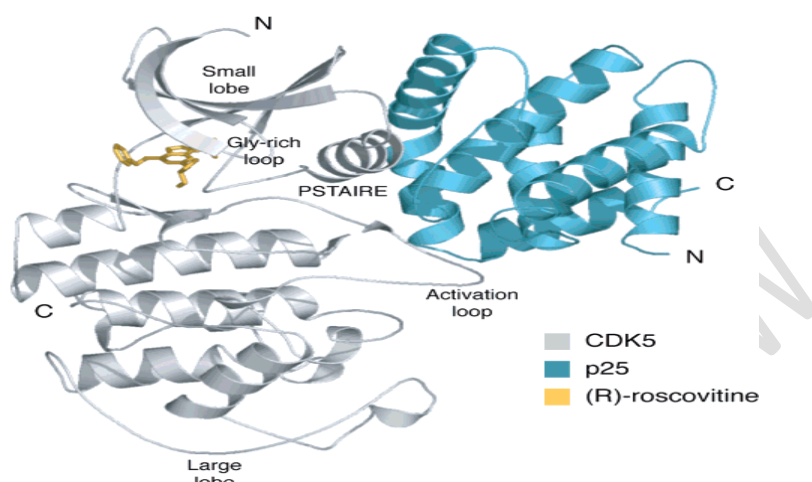
the development of Central nervous system [4]. CDK5 require association with a regulatory partner for kinase activation. There are two regulatory neuronal protein p35 and p39 that activate CDK5 in mammals [5,6]. The growth of the CNS requires the automatic migration, differentiation and association of neurons to form functional circuits capable of expressing synaptic plasticity. Several Studies confirmed that CDK5 is fundamental to all of these steps of CNS development. Ohshima et al. 1996 and Ko et al. 2001 proved that CDK5 null mutant mice, as well as p35/p39 double null mutants shows the development of abnormal cortical laminar architectural disturbances in the cerebellum, brainstem and hippocampus. On the basis of these anatomical findings implicate that CDK5 is very much involved in neuronal migration. CDK5-mediated phosphorylation of a broad range of substrates, including proteins involved in cytoskeletal dynamics and axonal transport, has been linked to neuronal migration. Cicero and Herrup et al. 2005 observed that CDK5 has been associated with neuronal differentiation. Loss of CDK5 during development leads to an inability of neurons to exit the cell cycle, coupled with their incomplete differentiation. Kwon et al. 1999; Hahn et al. 2005 shown that CDK5 has been implicated in the connectivity of developing neurons. Disturbed fasciculation of axonal tracts, such as the corpus callosum in p35 null mutant mice, together with effects of CDK5 on growth cone collapse and neurite actin dynamics, provide evidence for a role of CDK5 in axon guidance. A crucial role for CDK5 in corticogenesis is supported by observations showing that this kinase promotes migration by acting positively on pro-migratory signals, and possibly by antagonizing anti-migratory signals. In fact, CDK5 has been identified as a regulator of neuroblast migration in the postnatal subventricular zone [5]. Incultured primary neurons, the reduction of CDK5 activity by expressing dominant-negative CDK5 mutants, or by using antisense oligonucleotides of Cdk5, p35 or p39, inhibits neurite outgrowth [6]. CDK5 also regulates excitatory ionotropic glutamate receptors that are essential to learning. CDK5 also mediates interactions between calpain and the NR2B subunit of the NMDA receptor. Conditional CDK5 knockout in adult mice improves hippocampal dependent learning and plasticity due to higher levels of NR2B at the synapse caused by the disruption of the calpain/NR2B complex [7]. Interestingly, the enhanced cognition and plasticity area accompanied by elevations in basal excitability and contribute to the development of epileptic form activity and audiogenic seizures [8], supporting CDK5's role in tonal repression and baseline maintenance in neurons.

## Structure of CDK5

**Comment [u4]:** Specify the aim of the study clearly.  
Mention the need for the study

CDK5/p25 was initially crystallized in a monoclinic space group, and the structure was determined at 2.65 Å resolution [9]. The monoclinic crystals structure of the CDK5/p25 has been used for complex molecule, whose biological and modeling implications have been described recently [10,11]. The monoclinic crystals retained a plate like morphology, tended to grow in stacks, and were very fragile, making the handling required for intensive screening of inhibitors very laborious. Now a new versatile protocol to characterize the structure of ATP-competitive inhibitors bound to the CDK5/p25 active site. In particular, a very well diffracting crystal form of this complex, consistently providing X-ray diffraction data to high resolution. This new tool represents a very useful addition to crystals of CDK2 and of the CDK2/cyclin A complex, which serves to a good tool to address the mode of binding of CDK inhibitors to the CDK active site. The CDK5/p25 crystals might provide an alternative to the CDK2 crystals for all those inhibitors whose co crystallization with CDK2 proved impossible due to specific technical limitations [12]. The collection of large datasets of crystallographic models of small molecule inhibitors bound into the active site of different members of the CDK family may eventually provide useful information on structural differences that should be exploited for the design of selective inhibitors. The interaction of the CDK5/p25 complex with (*R*)-roscovitine in the CDK5 active site providing a model for comparison with previously reported structures bound to CDK2 [13-15]. The structure also provide an information that whether CDK5 previously phosphorylated on Tyr15 displayed different susceptibility to inhibition by (*R*)-roscovitine relative to the un phosphorylated counterpart. The structures allow concluding that the phosphorylation of Tyr15, a step that activates CDK5, does not render the CDK5/p25 complex any less susceptible to inhibition by (*R*)-roscovitine. It also suggests the possibility that the conformational space explored by the kinase may be exploited for the development of drugs targeting selectively certain conformations of the kinase (activation states). These may include conformations specifically induced by other protein ligands, such as p35, p39, or Cables in the case of CDK5. The best illustration of this principle is the binding of Gleevec to the active site of Abl [16]. In the complex of Gleevec with Abl, the inhibitor forces the activation loop into an inactive conformation, in which the conserved DFG motif at the entry of this loop is diverted from its usual conformation, so that the phenylalanine points into the ATP-binding site and the aspartate will no longer coordinate the magnesium. The inhibitors described in this study, roscovitine, aloisine, and indirubin, are significantly smaller than Gleevec and are unable to span the distance to the activation segment. As a consequence, this segment (and the DFG

motif in particular) does not change its structure in the presence of these inhibitors relative to the uninhibited structure.



**Figure 1** Ribbon diagram of the CDK5/p25 complex. CDK5 is shown in gray, p25 in blue. The ATP-binding pocket between the N and C terminal lobes of the kinase is occupied by (R)-roscovitine (yellow)[9].

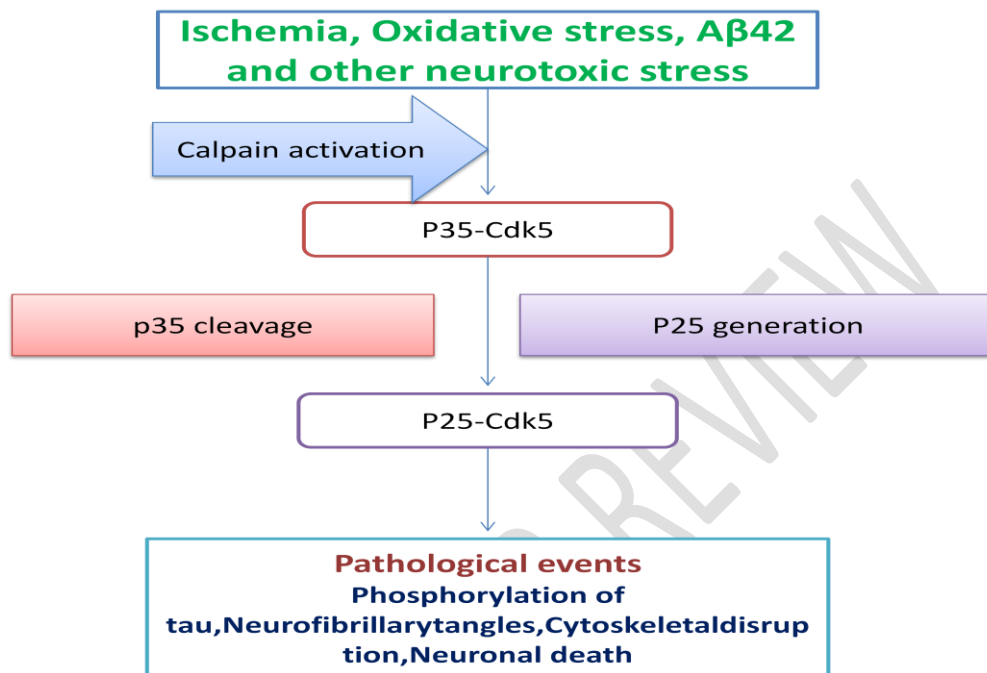
### Regulation of CDK5

P35 is most well known activator for CDK5. It is 307 AA in length and 35 kDa in mass, which is separated into two regions: i.e., N-terminal, contains 98 AA and possesses a myristoylation signal important for membrane targeting of p35 [17], and C-terminal has 209 AA that comprises the p25 region and possesses a proline-rich stretch as well as a Cdk binding and activation domain [18]. The N-terminal also contains a signal for degradation of p35 through the Ubiquitin-Proteasome pathway [19]. P25 presumes a cyclin-like structure, and CDK5 on binding with p25 acquires a conformation which is quite distinguishable from active CDK2. The crystal structure of p25/cdk5 suggests the post-translation phosphorylation and dephosphorylation, which is a distinct feature from CDK1 and CDK2 [20]. The co-crystallized structure also informs that p25 contains residues essential for the substrate specificity of CDK5, and the availability of its regulatory activator is only the rate-limiting step in the CDK5 activation process [21].

### Deregulation of CDK5

CDK5 has been proposed as an attractive candidate to connect A $\beta$  toxicity, tau pathology, and neuro-degeneration [20]. Various neurotoxic conditions, including ischemic brain damage, oxidative stress, excitotoxicity, and  $\beta$  amyloid peptide treatment of primary neurons, lead to

the generation of p25 segment from p35 cleavage by induction of Calpain mediated p35 cleavage [22-24]. It is also evident that phosphorylation of p35 protects its cleavage by Calpain [25-26].



**Figure 2** : The deregulation of CDK5 activity.

### Phosphorylation of Tau by CDK5

Tau is a Micro tubule associated protein (MAP), expressed neurons where it regulates microtubule assembly and stability. Synthesis of Tau is up regulated along with Tubulin during neuronal differentiation in mature neuron particularly in axon [27]. Primary function of MAP Tau is to stabilise microtubule. Structurally tau is characterized by presence of micro tubule binding domain, which is composed of repeats of highly conserved Tubulin binding motifs. It has C- terminal that has basic proline rich region and N-terminal is acidic, known as “Projection Domain”[28]. By using phosphor-epitope specific antibodies ,phosphor-peptide mapping and mass spectrometric analysis shows that Cdk5 phosphorylates Tau on S202, T205, T212, T217, S235, S396, and S404 [29-32].

### Role of CDK5 in Amyloid Precursor Protein

Amyloid Precursor Protein (APP) is a 770 residue type-1 transmembrane glycoprotein with a large hydrophilic amino terminal at extracellular domain and small carboxy terminal at

cytoplasmic domain [33]. APP is coded by a single copy gene located on the mid portion of long arm of human chromosome 21 [34]. Sequential cleavage of APP by  $\beta$ -Secretase (BACE 1) in the ectodomain and  $\gamma$ -Secretase results in the liberation of Fibrillogenic AB peptide [35]. Enhanced AB production and abundant Amyloid plaque due to extracellular deposition of AB peptide represents another salient feature of AD pathology. CDK5 has been to phosphorylate APP on Thr 668 in cytoplasmic domain [36]. Phosphorylation of Thr 668 affects the binding of APP to the cytoplasmic adaptor protein FES [37] that suggests Thr 668 phosphorylation plays avital role in normal APP function. Recently it is reported that Thr 668 phosphorylated APP is up regulated in AD brain tissue where it is enriched in endocytic vesicles. Interestingly inhibition of Thr 668 phosphorylation using CDK5 inhibitors results in marked reduction of amyloid- $\beta$  peptides [38].

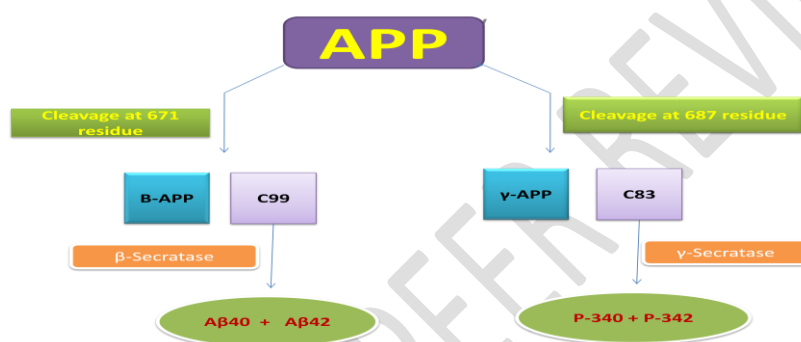


Figure 3: Processing of Amyloid precursor protein.

## Material and methods / experimental details / methodology

### Docking Studies:

Screening of Specs database (library of compounds) was carried by docking against the crystal structure of CDK5 using Glide. Glide searches for favourable interactions between one or more ligand molecules and a receptor molecule, usually a protein. Each ligand acts as single molecule, while the receptor may include more than one molecule, e.g., a protein and a cofactor. Glide was run in rigid or flexible docking modes; the latter automatically generated conformations for each input ligand. The combination of position and orientation of a ligand relative to the receptor, along with its conformation in flexible docking, is referred to as a ligand pose. The ligand poses that Glide generates pass through a series of hierarchical filters that evaluate the ligand's interaction with the receptor. The initial filters test the spatial fit of the ligand to the defined active site, and examine the complementarity of ligand-receptor

interactions using a grid-based method patterned after the empirical Chem Score function. Poses that passed these initial screens entered the final stage of the algorithm, which involves evaluation and minimization of a grid approximation to the OPLS-AA non bonded ligand-receptor interaction energy. Final scoring is then carried out on the energy minimized poses. The docking simulation was carried in following three steps:-

1. HTVS (high-throughput virtual screening)—High-throughput virtual screening docking is intended for the rapid screening of very large numbers of ligands. HTVS has much more restricted conformational sampling than SP docking, and cannot be used with score-in-place or rigid docking. Advanced settings are not available for HTVS, but are fixed at predetermined values.
2. SP (standard precision)—Standard-precision docking is appropriate for screening ligands of unknown quality in large numbers. Standard precision is the default.
3. XP (extra precision)---The extra-precision (XP) mode of Glide combines a powerful sampling protocol with the use of a custom scoring function designed to identify ligand poses that would be expected to have unfavorable energies, based on well-known principles of physical chemistry. The presumption is that only active compounds will have available poses that avoid these penalties and also receive favorable scores for appropriate hydrophobic contact between the protein and the ligand, hydrogen-bonding interactions, and so on. The chief purposes of the XP method are to weed out false positives and to provide a better correlation between good poses and good scores. Extra-precision mode is a refinement tool designed for use only on good ligand poses. Finally, the minimized poses are re-scored using Schrödinger's proprietary Glide Score scoring function. Glide Score is based on Chem Score, but includes a steric-clash term and adds buried polar terms devised by Schrodinger to penalize electrostatic mismatches:

$$\text{Glide Score} = 0.065 * \text{vdW} + 0.130 * \text{Coul} + \text{Lipo} + \text{Hbond} + \text{Metal} + \text{BuryP} + \text{RotB Site}$$

Component	Description
vdW	Van der Waals energy. This term is calculated with reduced net ionic charges on groups with formal charges, such as metals, carboxylates, and guanidiniums.
Coul	Coulomb energy. This term is calculated with reduced net ionic charges on groups with formal charges, such as metals, carboxylates, and guanidiniums.
Lipo	Lipophilic contact term. Rewards favorable hydrophobic

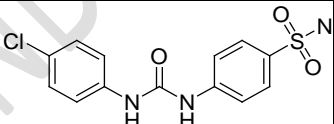
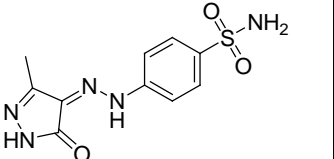
HBond	Hydrogen-bonding term. This term is separated into differently weighted components that depend on whether the donor and acceptor are neutral, one is neutral and the other is charged, or both are charged.
Metal	Metal-binding term. Only the interactions with anionic acceptor atoms are included. If the net metal charge in the apo protein is positive, the preference for anionic ligands is included; if the net charge is zero, the preference is suppressed.
BuryP	Penalty for buried polar groups.
RotB	Penalty for freezing rotatable bonds.
Site	Polar interactions in the active site. Polar but non-hydrogen-bonding atoms in a hydrophobic region are rewarded.

**Table 1:** Glide Score components

### Knowledge based selection of top scoring compounds

On completion of the screening process, the resulting conformations/poses of the ligands in the binding site of 1UNL were studied and per residue H-bond, hydrophobic and interaction pattern with in 15 Å<sup>0</sup> area from center of grid was studied. From results of screening of Specs database ligand-receptor binding interaction pattern and glide docking score was studied (Figure 4)

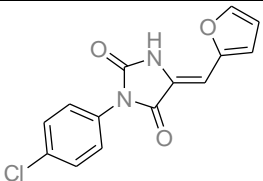
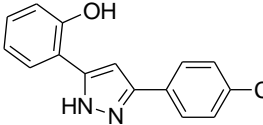
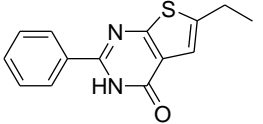
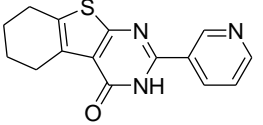
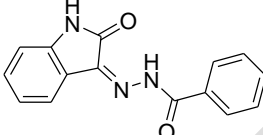
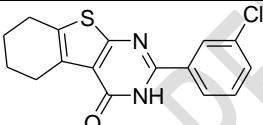
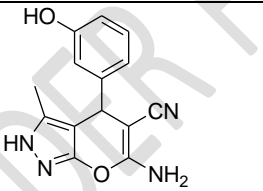
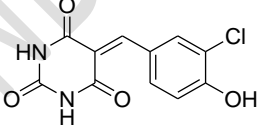
Figure 4: Glide Score components

S.N O	Hit	Structure	IUPAC NAME	Glide score
1	SKD-H1		4-[[4-chloroanilino)carbonyl] amino} benzenesulfonamide	-12.040736
2	SKD-H2		4-[2-(3-methyl-5-oxo-1,5-dihydro-4H-pyrazol-4-ylidene)hydrazino]benzenesulfonamide	-11.229700

**Comment [u5]:** Include the result and discussion section

**Comment [u6]:** 1)In discussion mention the previous studies in this field, available if any.  
2)Need to take up the study.  
3)Add some supportive or contradictory studies.  
4)What is the outcome and its significance.  
5)Mention further scope of the study if any?



3	SKD-H3		3-(4-chlorophenyl)-5-(2-furyl methylene)-2,4-imidazolidinedione	-9.593023
4	SKD-H4		2-[3-(4-chlorophenyl)-1H-pyrazol-5-yl]phenol	-9.286539
5	SKD-H5		6-ethyl-2-phenylthieno[2,3-d]pyrimidin-4(3H)-one	-9.224028
6	SKD-H6		2-(3-pyridinyl)-5,6,7,8-tetrahydro[1]Benzo thieno[2,3-d]pyrimidin-4(3H)-one	-9.194986
7	SKD-H7		N'-(2-oxo-1,2-dihydro-3H-indol-3-ylidene)benzohydrazide	-9.123951
8	SKD-H8		2-(3-chlorophenyl)-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidin-4(3H)-one	-9.077507
9	SKD-H9		6-amino-4-(3-hydroxyphenyl)-3-methyl-2,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile	-8.692094
10	SKD-H10		5-(3-chloro-4-hydroxybenzylidene)-2,4,6-(1H,3H,5H)-pyrimidinetrione	-8.428099

## Conclusion

By developing an active site homology model of CDK5 for further exploration and optimization of potent CDK5 inhibitors such as Benzthiazole, Quinoline, Triazole, Benzimidazole, and Pyridine. Due to its importance in these various neurodegenerative pathways of several brain pathologies, it is valid to assume that CDK5 can be a good

pharmacological target to prevent or even arrest these pathologies. The compounds in this study may serve as molecular probes to better understand CDK5's role in the treatment of neurodegenerative disorders. From this investigation we gained a better understanding of the structural requirements and limitations necessary for the preparation of selective CDK5 inhibitors. In summary, For docking simulation based screening first HTVS screening, followed by SP and XP screening were performed, in order to improve the performance and precision of docking. Initially Specs database library of compounds have been screened by HTVS based flexible docking, so that a quick sorting of ligands having very low affinity to the binding site (active site residues of grid), can be eliminated, followed by SP and XP docking in order to improve the precision of docking performance by optimizing functional group binding interactions to the active site residues.

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