IN SILICO SCREENING OF POTENTIAL THERAPEUTIC MOLECULE RESCUE FROM ALZHEIMER'S DISEASE TARGETING CDK7

M.Sc. (Biotechnology)

A DISSERTATION

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Submitted by:

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I, **Nishtha Malhotra**, hereby certify that the work which is presented in major work entitled "**In silico screening of potential therapeutic molecule rescue from Alzheimer's disease targeting CDK7**" in fulfilment of the requirement for the reward of the degree of Masters of Science in Biotechnology and submitted to the Department of Biotechnology, Delhi Technological University, Delhi is an authentic record of my own, carried out during a period of January to May 2021, under the supervision of **Prof. Pravir Kumar.**

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Review

Pharmacological intervention in oxidative stress as a therapeutic target in neurological disorders

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Abstract

Objectives Oxidative stress is a major cellular burden that triggers reactive oxygen species (ROS) and antioxidants that modulate signalling mechanisms. Byproducts generated from this process govern the brain pathology and functions in various neurological diseases. As oxidative stress remains the key therapeutic target in neurological disease, it is necessary to explore the multiple routes that can significantly repair the damage caused due to ROS and consequently, neurodegenerative disorders (NDDs). Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase is the critical player of oxidative stress that can also be used as a therapeutic target to combat NDDs.

Key findings Several antioxidants signalling pathways are found to be associated with oxidative stress and show a protective effect against stressors by increasing the release of various cytoprotective enzymes and also exert anti-inflammatory response against this oxidative damage. These pathways along with antioxidants and reactive species can be the defined targets to eliminate or reduce the harmful effects of neurological diseases.

Summary Herein, we discussed the underlying mechanism and crucial role of antioxidants in therapeutics together with natural compounds as a pharmacological tool to combat the cellular deformities cascades caused due to oxidative stress.

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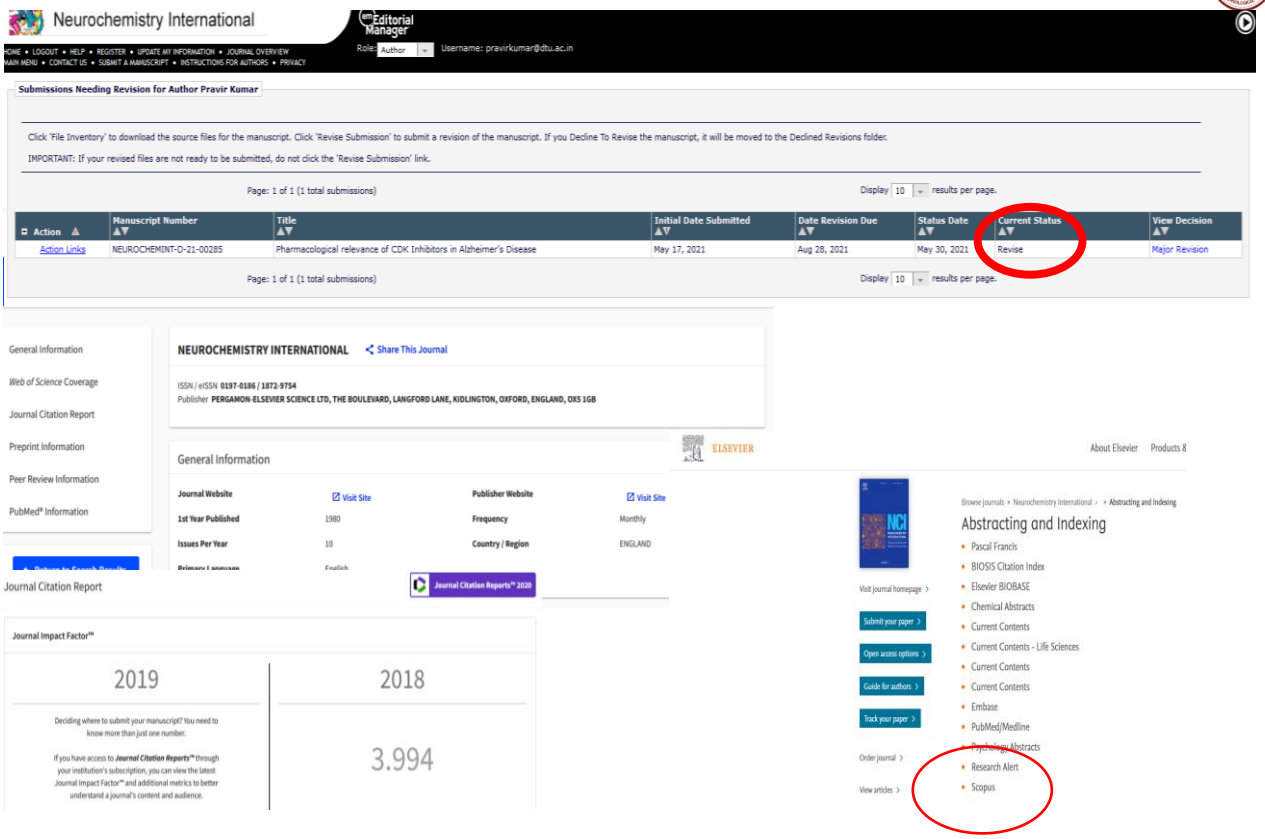

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Last but not the least, I thank the almighty for his blessings.
Thank you,
Nishtha

Abstract

Evidence suggests that cell cycle activation plays a role in the pathophysiology of neurodegenerative diseases. Alzheimer's disease is a progressive, terminal neurodegenerative disease that affects memory and other important mental functions. Intracellular deposition of Tau protein, a hyperphosphorylated form of a microtubule-associated protein, and extracellular aggregation of Amyloid Beta protein, which manifest as neurofibrillary tangles (NFT) and senile plaques, respectively, characterise this condition. In recent years, however, several studies have concluded that cell cycle re-entry is one of the key causes of neuronal death in the pathogenesis of Alzheimer's disease. The eukaryotic cell cycle is well-coordinated machinery that performs critical functions in cell replenishment, such as DNA replication, cell creation, repair, and the birth of new daughter cells from the mother cell. The complex interplay between the levels of various cyclins and cyclin-dependent kinases (CDKs) at different checkpoints is needed for cell cycle synchronisation. CDKIs (cyclin-dependent kinase inhibitors) prevent cyclin degradation and CDK inactivation. Different external and internal factors regulate them differently, and they have different tissue expression and developmental functions. The checkpoints ensure that the previous step is completed correctly before the start of the new cell cycle phase, and they protect against the transfer of defects to the daughter cells. Initially 22 molecules were screened at 75% similarity with CAPE from ChEMBL database. The assessment of drug likeness feature using Lipinski Rule of 5 of these molecules was done using SwissADME. 16 molecules exhibited drug-like characteristics. These 16 molecules were filtered on the basis of their ADME properties and BBB permeability using tools such as pkCSM and cbligand, herein 8 molecules were shortlisted. These 8 compounds were subjected to molecular docking with human CDK7 protein, and lead compounds were screened on the basis of docking scores and interacting residues. A series of in silico experiments were used for virtual screening and ligand-based drug discovery, and then it was calculated that Compound 9(ChEMBL3976811), compound 6 (ChEMBL3597111) and Compound8 (ChEMBL3958339) can act as lead compound against CDK7 protein and can be developed as a therapeutic solution for AD.

Due to the development of more selective and potent ATP-competitive CDK inhibitors, CDK inhibitors appear to be on the verge of having a clinical impact. This avenue is likely to yield new and effective medicines for the treatment of cancer and other neurodegenerative diseases.

These new methods for recognising CDK inhibitors may be used to create non-ATP-competitive agents that target CDK4, CDK5, and other CDKs that have been recognised as important therapeutic targets in Alzheimer's disease treatment.

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AD: Alzheimer’s Disease

CDK: Cyclin Dependant Kinase

CDKI: Cyclin Dependant Kinase Inhibitor

BBB: Blood Brian Barrier

HBD: Hydrogen Bond Donor

HBA: Hydrogen Bond Acceptor

CAPE: Caffeic Acid Phenethyl Ester

CHAPTER 1

INTRODUCTION

The regulation of eukaryotic cell cycle is a cardinal process that governs the homeostasis of mitotic cells [1]. Numerous studies have addressed the role of cell deregulation in the death of adult borne neuronal cells [1]–[3]. Despite being a controversial school of thought, increasing evidence supports the role of aberrant cell cycle activation (CCA) in the etiology of various neurodegenerative disorders [4] [5]–[7], [8], [9], [10], [11], [1], [12], [13]. These landmark discoveries have demonstrated aberrant cell cycle re-entry as a characteristic feature in a multitude of neurodegenerative disorders[14]. Although cell cycle re-entry is more prevalent in tumour cells, the consequences of this event vastly differ between the post-mitotic neurons and tumour cells [15]. CAPE is a phenolic molecule found in nature [16], [17]. It is a compound obtained from honeybee propolis from New Zealand [16], [17]. CAPE's anti-oxidant and anti-inflammatory characteristics have also been used to facilitate neuroprotection in ischemic brain lesions. Several proteins responsible for inducing proliferation such as CDC-20, CDK-7, and BubR1 were shown to be reduced in control and CAPE-induced neurodifferentiated cells; however, the underlying mechanisms at the molecular and cellular levels remain unknown [16]. Some of the existing AD targets are found in the "expanded cell cycle", a term used to describe potential therapeutic targets [14]. It offers a comprehensive view that includes a wide range of molecules that represent potential targets and, as a result, approaches that can be used to treat ADs by inhibiting cell cycle [14].

CHAPTER 2

LITERATURE REVIEW

2.1. Involvement of cell cycle proteins in regulating cell homeostasis

The eukaryotic cell cycle is a well-coordinated system that performs essential functions in cell replenishment. CDKs are a consortium of serine/threonine kinases that yield active heterodimeric complexes upon binding to their regulatory subunits, known as Cyclins [18]. Cyclins comprise of two main families, namely the mitotic cyclins and G_1 cyclins [14], [19]. Numerous CDKs (CDK4, CDK6, CDK2, CDK1, and possibly CDK3) co-operate at various stages to ensure a seamless passage of the cells through the cell cycle [18]. For instance, CDK4 and CDK6 are known to form active complexes with Cyclins D1, D2 and D3 in the early G1 phase of the cell cycle [20]– [23]. Similarly, CDK2 complexes with cyclins E1 and E2 to complete the G1 phase and trigger the S phase [23], [24]. CDK2 also aggregates with cyclin A to oversee the S/G transition [19]. (Figure 1).

Moreover, the degradation of cyclins and consequential CDK inactivation ensures the maintenance of the integrity of the eukaryotic cell cycle [25]. This activity is brought about by a class of proteins known as CDK inhibitors (CDKIs) [25]. The Ink family of CDKIs adhere to CDK4/6 to abrogate its binding with cyclin D, which consequently results in quiescence [25], [26]. The Cip/Kip inhibitors are known to inhibit a wider range of CDKs [27]. As opposed to the INK4a family, the Cip/Kip proteins bind to both the cyclin and the cyclin-dependant kinase thereby reinforcing their function as both a positive and negative regulator of G1-phase progression [19], [27]–[29]. Various extrinsic and intrinsic factors control the activity of Cip/Kip proteins, which have different tissue expression and developmental functions [30]. The activity of cell division cycle 25 (Cdc25) can reverse these phosphorylation events, thereby regulating the activity of CDK1. [25], [31].

Figure 1 Regulators of Cell Cycle

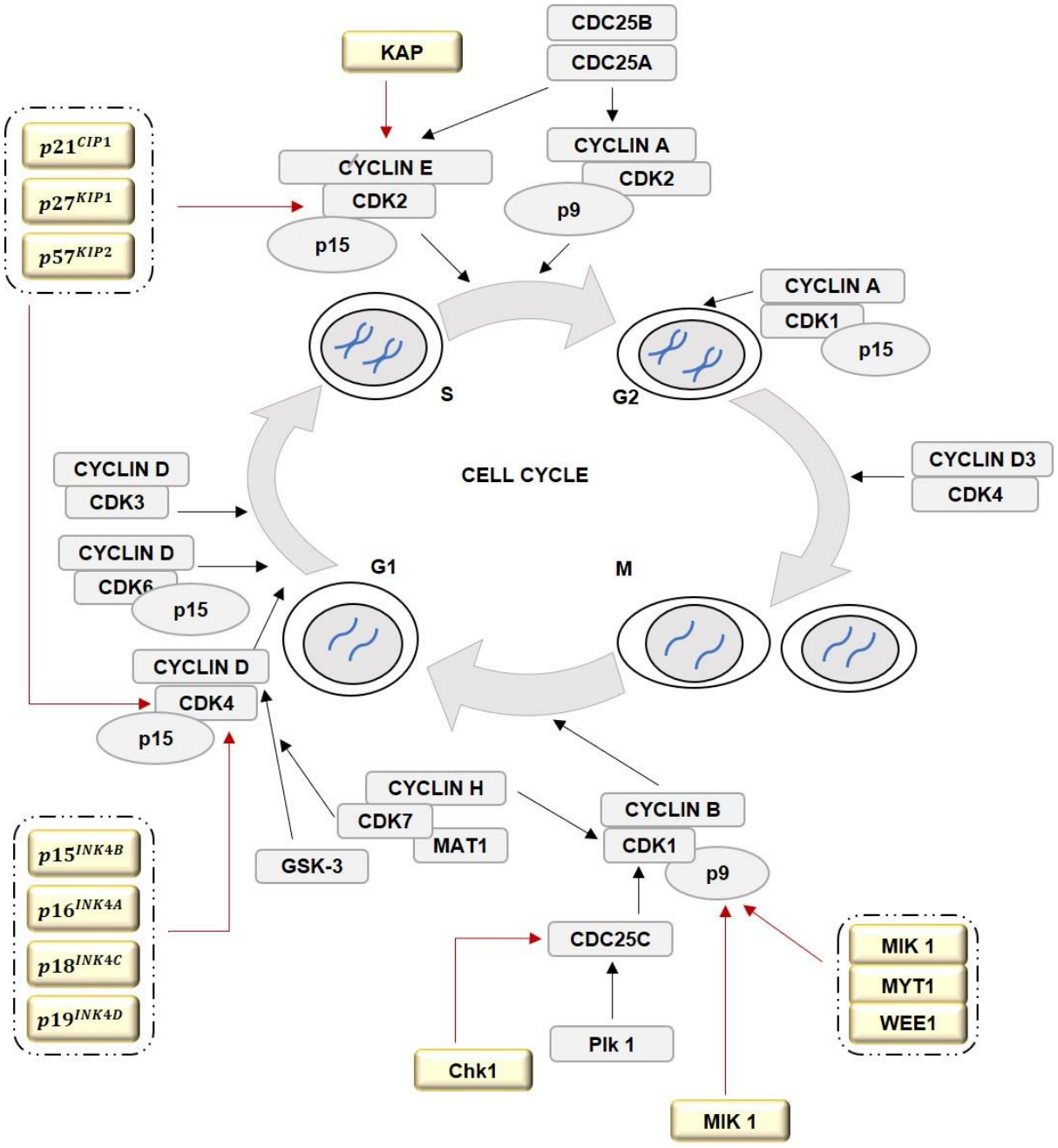


Figure 1. Schematic of cell cycle regulation by means of CDKs and their regulators. The transient association and activation of CDKs controls various stages of the cell division cycle. Small molecular weight proteins (p9CKS and p15CDK-BP) associate with CDKs. They are activated by activating elements (grey) that carry out phosphorylation/dephosphorylation reactions: CDK7/cyclin H/MAT1, Plk1, CDC25 phosphatases (A–C). Several kinases, including wee1, mik1, myt1, chk1, GSK-3, and phosphatases (PP2A, KAP), as well as stoichiometrically interacting inhibitory proteins (Cip/kip and INK4 families), cause the inactivation of CDKs.

2.2. Cell cycle re-entry and its relation to oxidative stress

Multiple demonstrations of oxidative stress biomarkers in several neurodegenerative diseases, along with the presence of cell cycle aberrations in these patients' neurons suggest that both pathways might be linked at the molecular level [32]. Cell cycle arrest has been linked to an increase in DNA damage brought about by the reactive Oxygen Species (ROS). [33]. Conversely, the fate of ROS-exposed cells to transition into growth arrest or apoptosis could be influenced by their position in the cell cycle upon being subjected to insults. Human fibroblasts, for instance, experienced cell cycle arrest or apoptosis after being exposed to H_2O_2. Endogenous free radicals are thought to cause cumulative DNA damage, which has been linked to cancer and neurodegeneration [34], [35]. Furthermore, studies have linked an increased level of this modified base to an increased risk of cancer (and thus cell cycle abnormalities) [36].

Furthermore, an increase in the activity of the enzyme histone deacetylase has been linked to transcriptional repression in standard conditions [37]. The activity of histone deacetylases 1–10 has been shown to be reduced by oxidative stress [38]. This shift in deacetylase activity could result in the global inactivation of transcriptional repressors, resulting in the activation of a large number of genes and cellular death. Furthermore, mitochondrial damage will reduce the amount of NAD^+ available. This could lead to a decrease in SIR2 activity and an increase in transcriptional activation, resulting in abnormal cell cycle re-entry. As a result, decreased SIR2 activity could lead to an increase in p53 activity, which could then signal downstream cell cycle effectors.

In addition, multiple studies have advocated that that hypoxia causes DNA replication in post-mitotic neurons. In response to UPS dysfunction, oxidative stress, in addition to DNA damage, is thought to cause cell cycle re-entry [39]. As a result, oxidative stress, which corresponded to cell cycle re-entry markers, played a major role in the aetiology of neuromuscular degeneration (Figure 2).

Figure 2 **Role of Oxidative Stress in Aberrant Cell-Cycle Re-Entry in Neurodegeneration**

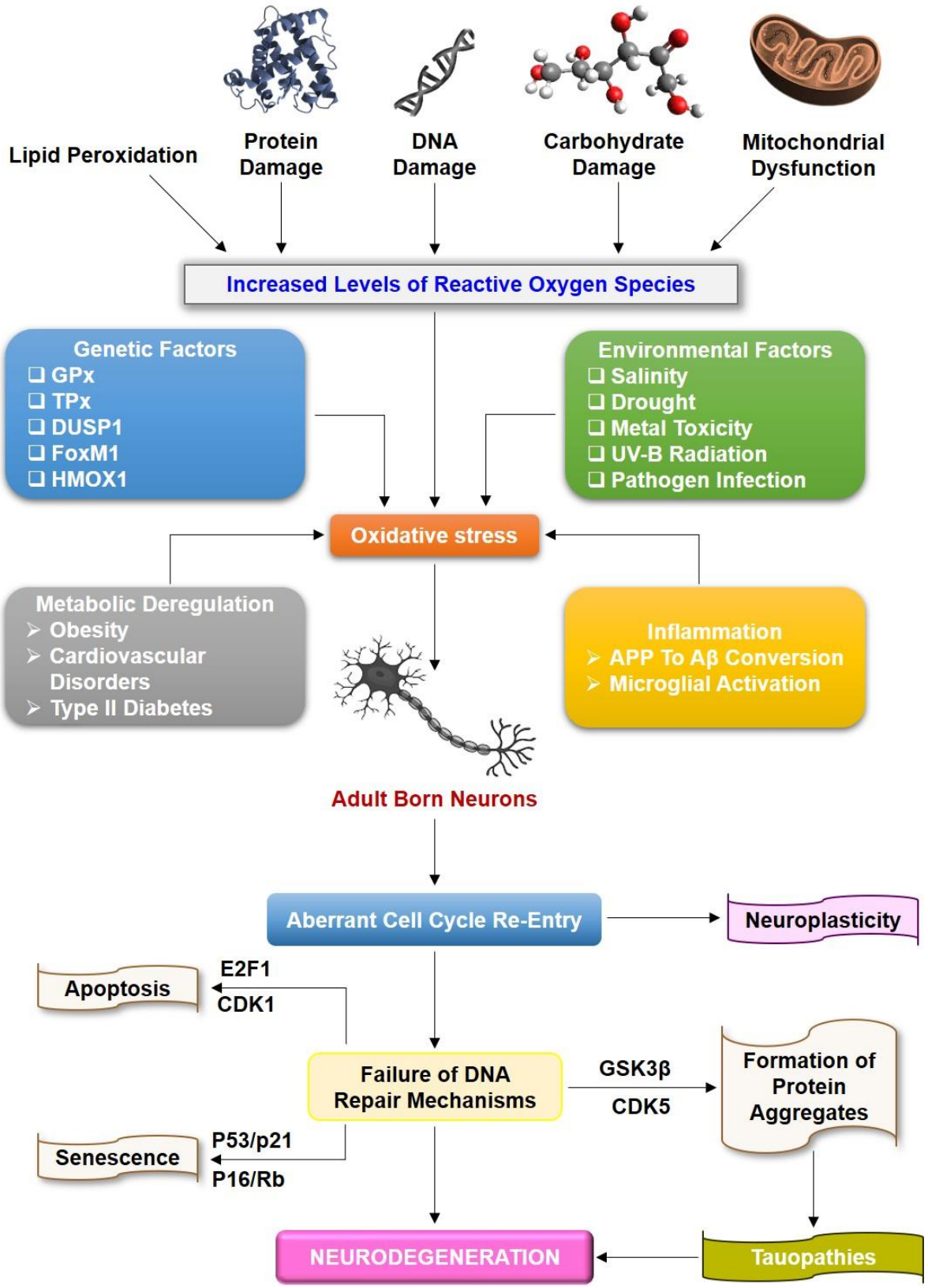


Figure 2. Role of oxidative stress in aberrant cell cycle re-entry in neurodegeneration. Damages in DNA, Proteins and Carbohydrates, and other factors such as mitochondrial dysfunction and lipid peroxidation lead to increased cellular levels of Reactive Oxygen Species. Several processes such as Inflammation and metabolic dysregulation, along with environmental and genetic factors also contribute towards oxidative stress, which leads to aberrant neuronal cell cycle re-entry, resulting in neuroplasticity and failure in DNA repair mechanisms. The latter leads to apoptosis, cellular senescence and formation of protein aggregates, subsequently resulting in the development of age-related neurodegenerative diseases.

2.3. Aberrant cell cycle re-entry in AD

AD is an irrevocable, progressive neurodegenerative disorder that slowly hampers memory and other important mental functions. It is characterised by the intracellular deposition of Tau protein, along with aggregates of Amyloid Beta (Aβ) protein, that usually manifests as neurofibrillary tangles (NFT) and senile plaques respectively. Of late, multiple studies have adjudicated upon the role of aberrant cell cycle re-entry as one of the key phenomena inducing neuronal death in AD patients [40]. Cell cycle insults have been directly correlated with an increase in pathological accumulation of Aβ and tau hyperphosphorylation [25].

The pathogenesis of AD notably involves the fallacious embarkment of G0 quiescent neurons into the G1 phase and beyond [41]–[43]. Despite the lack of the exact reasons for the aberrant re-entry, this theory has been backed up by substantial pathological evidence. Moreover, MCM2, a marker of DNA replication that exemplifies the transition through the S phase is also known to be elevated in AD neurons [44]–[48]. Furthermore, in AD neurons, the mitotic signalling G protein Ras, as well as its downstream mediators MAPK, Raf, and MEK1/2, is activated. [49], [50].

Interestingly, genetic predisposition is also known to be associated with AD and its corresponding mitotic malfunctions. Particularly, genes such as APP and presenilin-1 and presenilin-2 [51]–[53] act as pivotal contributors of cell cycle control, since both of its proteins are mitogenic in vitro [54], [55]. APP-BP1 (*NAE1*) is an adaptor protein involved in the cleavage of APP, and is also responsible for regulating the mitotic transition from S- to M-phase. Overexpression of this protein could result in DNA replication, followed by the expression of the corresponding cell cycle markers CDC2 and cyclin B1 (*CCNB1*). [56]–[58].

Similarly, PS1 and PS2 ensure cell cycle control through the proteolytic cleavage of APP [57]. The deficiency of these genes in transfected HeLa cells results in a hastened transgression from G1 through the S- phase [59], while the overexpression of the same elicits arrest at the G1 phase of the cell cycle [60]–[62].

Such corroborations for mitotic alterations point towards the role of cell-cycle re-entry in the pathogenesis of AD. Henceforth, it is established that cell cycle deregulation is a precursor for AD progression, rather than being an epiphenomenon, implying that it is responsible for triggering neurodegeneration rather than being a result of the same [63].

2.4. Structural basis of CDK Inhibition

The cyclin dependant kinases are a part of the superfamily containing a eukaryotic protein kinase (ePK) catalytic domain that is responsible for regulating the activation of kinases [64]–[68]. A vast majority of protein kinases contain a bi-lobed structure, with the N and C terminal residues roughly containing 85 and 170 amino acids respectively. An ATP binding cleft resides between the two lobes that bind to the γ phosphate of ATP via serine/ threonine/ tyrosine/ hydroxyl groups [68]–[70]. The activation loop consists of roughly 20 amino acid residues located amid the N and C lobe. It acts as a site for facilitating cyclin binding and phosphorylation. The activation and deactivation of kinases are caused by a conformational change in the active loop (T loop) that facilitates the interaction of kinase with the substrate. The reconstruction of the N lobe generates a conformational state that fits the ATP in the active site of the kinase [71]. Moreover, the CDKs are usually activated by binding to the regulatory subunit of cyclins [72]–[75]. Such is also the case with CDK1, CDK2, CDK4 and CDK6 [68]. P25, like cyclins, pushes the C helix to orient Lys33 and Glu51 for proper ATP binding. The mechanism of action of CDK 4/6 inhibitors is based on binding to the ATP pocket of CDK 4 and 6, which results in significant inactivation of CCND-CDK4/6 complexes subsequently increasing the activity of pRb proteins [76]. However, activation of CDK5 differs from the above-mentioned activation mechanisms since the binding of cyclin D and E to CDK5 does not lead to its activation. The activity of CDK5 is brought about by proteins such as p35 and p39, which are usually manifested in neurons as well as in some other cell types [68], [77]–[80].

2.5. Binding mechanisms of CDK Inhibitors

A vast proportion of kinase inhibitors developed so far have been known to target the ATP binding site, wherein the kinase adopts a conformation similar to the one employed in ATP binding [81]. These molecules abrogate the activity of kinase by binding to their active DFG-in conformational state, wherein the Asp-Phe-Gly (DFG) motif assumes a conformation with the Phe residue enfolded within the hydrophobic pocket in the groove that is situated between the two lobes of the kinase protein [68], [82], [83]. To facilitate inhibitor binding, a significant conformational change in the residues of the conserved DFG motif in the kinase's active site is required [68]. The binding of inhibitor proteins leads to the relocation of Phe residues, resulting in DFG-out conformational state [81], [82], [84]. The Type 1 inhibitors bind to the enzyme's "Active Conformation," which is aligned with the loop's DFG-in conformation. Type 2 inhibitors, on the other hand, bind to the protein's "Inactive Conformation," which is associated with a DFG-out conformation [85].

2.6. CDKIs acts as promising therapeutic agents in AD

Substantial evidence indicates that the evidence of AD pathophysiology points towards the re-entry of G0 quiescent neurons into the G1 phase or beyond [68], [86]. Since several molecules are known to be involved in cell apoptosis and neurodegeneration[87], therefore a large number of chemical inhibitors of neuronal apoptosis have been investigated [68].

The methodology of targeting cell cycle inhibition via inhibiting CDK molecules was primarily employed in cancer therapy and recently has been extrapolated against neurodegenerative disorders, therefore, not many drug discovery programs are working on targeting aberrant cell cycle re-entry in AD. Structurally variegated compounds such as indole [88], imidazole, pyrazolopyridine [89], pyridopyrimidines [81], [90]–[94] piperidine [95], and purine [96] derivatives have been tested as CDK inhibitors [97] (Table 1) . Despite multiple CDK inhibitors being reported under clinical trials for tumour inhibition, to date, no CDK inhibitors have been reported under clinical trials for targeting CNS disorders. The CDK inhibitors that have been reported for targeting various neurodegenerative disorders include flavopiridol, a nonselective CDK inhibitor, along with several inhibitors that offer selectivity with CDK1, 2, and 5 such as olocomucine, roscovitine, and butyrolactone 1; GW8510, the inhibitor molecule displaying selectivity towards CDK5 [98], along with inhibition of CDK5 and CDK2, such as Quinazolines [99], 4-aminoimidazole [100], indurubins [100] and 6-oxo-l,6- dihydropyridines [101]. Several CDK inhibitors have been established over the past decade. Flavopiridol, olomoucine, and Roscovitine are the most widely studied CDK inhibitors [102]. Flavopiridol,

a broad spectrum CDK inhibitor, is the first CDK inhibitor that has entered clinical trials in humans. The neuroprotective effect of flavopiridol was proposed particularly due to its inhibitory properties against CDK5 along with CDK2 to some extent [103]. Additionally, studies have shown that at higher concentrations flavopiridol can inhibit other protein kinases as well. Furthermore, flavopiridol is known to disrupt the RNA polymerase II-mediated transcription [96], [104] and may also contribute towards the inhibition of gene expression [105]. Recent shreds of evidence have shown that flavopiridol and Olomoucine attenuate the l-methyl-4-phenylpyridinium (MPP) induced neuronal cell cycle re-entry into the S phase of the cell cycle [106].

Besides Flavopiridol, Roscovitine has also been testified as a neuroprotectant that triggers CDK5 inhibition in colchicines-induced cellular apoptosis [107], as well as in the in-vitro models of HIV neurotoxicity [108]. Furthermore, studies have shown that roscovitine can navigate across the blood-brain barrier and counteract the upsurge of CDK5/p25 in cerebral regions of the focal ischemia models, thereby displaying its neuroprotectant activity in vivo [68], [96]. Butyrolactone-I is another selective CDK inhibitor that predominantly inhibits the activity of CDK5, and has an in vitro IC50 value of 0.491 μM [81]. It is responsible for reducing the baseline activity of CDK5 in the septa-hippocampal regions [109] [75]. Co-incubation with Butyrolactone-I is also known to reduce the neurotoxic effects of AP in primary cultures of hippocampal cells.

Several other 3-substituted indolones have also been found to prevent neuronal death [110]. GW8510 causes in-vitro inhibition of CDKs but has a feeble effect on CDKs present in cultured cells and was found to be a potent inhibitor of CDK2 under in-vitro conditions, with an IC50 value of 60 nM [68], [99]. Moreover, Davis and co-workers have further reported that besides CDK2, GW8510 also has the potency to act as a neuroprotectant agent by causing the inhibition of CDKs 4 and 6, as well as causing CDK5 inhibition both in vitro and in vivo [97] by mechanisms other than the inhibition of cell cycle CDKs [100].

Table 1: IC_{50} values (μM) of selected kinase inhibitors against different member of CDKs.

S.No.	CDKIs	Target IC_{50} values			References
		CDK2/Cyclin A, E	CDK4/Cyclin D	CDK5/p25	
2.	R-Roscovitine	0.7 μM	14.2 μM	0.16 μM	[111]
3.	Olomoucine	7 μM	>1000 μM	3 μM	[112]
4.	N-isopentenyladenine	50 (A) μM	>100 μM	80 μM	[113], [114]

5.	Aminopurvalanol	0.033 μM (A) 0.028 μM (E)	-	0.02 μM	[115]
6.	Purvalanol B	0.006 μM (A), 0.009 μM (E)	>10 μM	0.006 μM	[116], [117]
8.	AT-7519	0.047 μM	0.1 μM	0.13 μM	[115]
9.	TG02	5 nM	-	4nM	[115]
10.	RGB-286638	3 nM	4 nM	-	[115]

CHAPTER 3

MATERIALS AND METHODS

3.1. Compound preparation from biological database

- A cumulative of 22 molecules which bore 75% structural similarity to CAPE were collected from the ChEMBL database as initial dataset compounds.
- The two-dimensional (2D) structures of used to generate various molecular conformations.

3.2. Ligand preparation

- Various features such as number of hydrogen bond acceptor (HBA), hydrogen bond donor (HBD), hydrophobic (HY), hydrophobic aromatic (HY-AR), and positive ionization (PI) were selected as characterising features of the pharmacophore [118].
- 16 molecules were shown to exhibit drug-like characteristics.
- These compounds were primarily screened on the basis of Lipinski's rule of five [118].

3.3. Predicting pharmacokinetic profile of the ligands

- Subsequently, the compounds selected on the basis of Lipinski's rule of five were subjected to ADMET prediction using pkCSM Tool [http://biosig.unimelb.edu.au/pkcsm/] [118]. The predictive ADMET properties are the filtration criteria that are imperative to the drug design process. The various mathematical predictive ADMET pharmacokinetic parameters, such as GI absorption, aqueous solubility, LD50 values and blood-brain-barrier penetration score of the selected ligands were analysed quantitatively by using pkCSM Tool.

3.4. Docking with human CDK7 protein

- The crystal structure of the human CDK7 protein in complex with CAPE (ChEMBL ID: 3983391) was applied for the docking study using AutoDock Tools [http://autodock.scripps.edu/]. The preparation of free protein structures was done by removing all co-crystallized water molecules.
- The prepared protein structure was subsequently provided to define the binding site by using Define and Edit Binding site module. PYMOL [https://pymol.org/2/] and Discovery Studio [https://www.discngine.com/discovery-studio] programs were sequentially utilized in the docking study [118].
- 3 molecules were found to have better docking scores than CAPE, along with presence of similar interacting residues.

CHAPTER 4

RESULTS

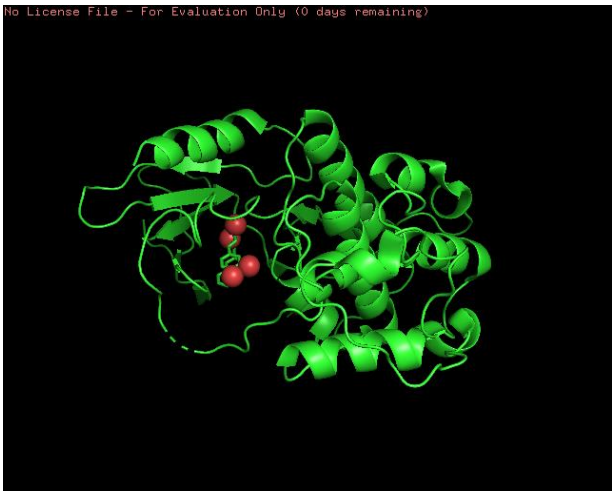
The docking of 1UA2 (Human CDK7) against compounds was graded on the basis of highest score, RMSD value and occupancy of sites as depicted in Table 3.1. The selected compounds displayed binding energy that ranges between -7.6 kcal/mol to -8.2 kcal/mol. The diagrams from LigX option of MOE tool revealed 133492 and 133714 bound with ACE2 receptor with a score of -7.6 kcal/mol and -7.9 kcal/mol forming hydrogen bonds with the side chains of Asn

141 (133492), Gln22 and Leu 144 formed arene-cation interaction (133714), Figure 3.2 (A and B) respectively. The diagram for 319244 complex displayed strong binding with Ser 161, Asp 155 via hydrogen bonds exhibiting a score of -6.8 kcal/mol as shown in Figure 3.2 (C). The compound 442022 was revealed to form hydrogen bonds with residues including Asp 92and Met94 with binding score of -7.7 kcal/mol, Figure 3.2 (D). 2441913 displayed hydrogen bonding with Met 94 with a binding score of -7.6 kcal/mol, Figure 3.2(E). the compound 3597111 formed, hydrogen bonding with residue Ser 161 and score of -7.8 kcal/mol was observed, Figure 3.2 (F). the compound 3915081 revealed displayed hydrogen bonding with Asn 41 with a score of -7.1 kcal/mol as evident in Figure 3.2 (G). The 3958339 revealed to have docking score of -8.3 kcal/mol and bound to the residue Ser 161 and Phe 91 through single hydrogen bond and arene-cation interaction respectively, Figure 3.2 (H). The diagram for 3976811 complex displayed strong binding with Ser 161, Asp 155 via hydrogen bonds exhibiting a score of -8.2 kcal/mol as shown in Figure 3.2 (I)

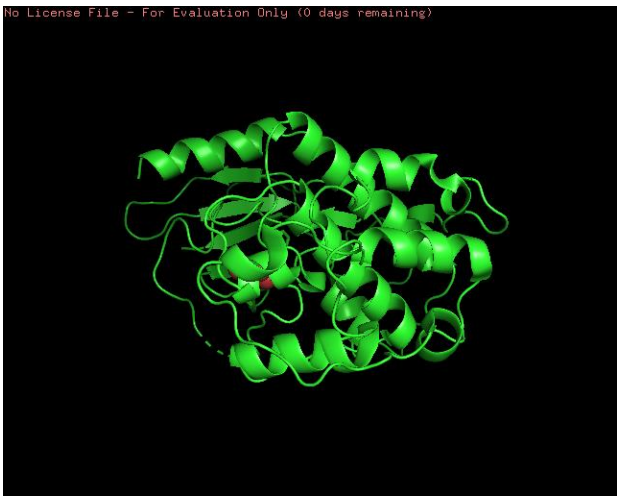
S. no	**CHEMBL ID**	**Docking score (kcal/mol)**	**Interaction detail**	
			Residues	**Interaction**
1	133492	-7.6	Asn 141	H-acceptor
2	133714	-7.9	Gln22 Leu 144	H-acceptor Pi-H
3	319244	-6.8	Ser 161 Asp 155	H- acceptor H- acceptor
4	442022	-7.7	Asp 92 Met 94	H-acceptor H-acceptor
5	2441913	-7.6	Met 94	H-acceptor
6	3597111	-7.8	Ser 161	H-acceptor
7	3915081	-7.1	Asn 141	H-acceptor
8	3958339	-8.3	Ser 161 Phe 91	H-acceptor Pi-H
9	3976811	-8.2	Ser 161 Asp 155	H-acceptor H-acceptor

Table 2: Interaction detail of selected compounds phosphate in the active site of Human CDK2 protein:

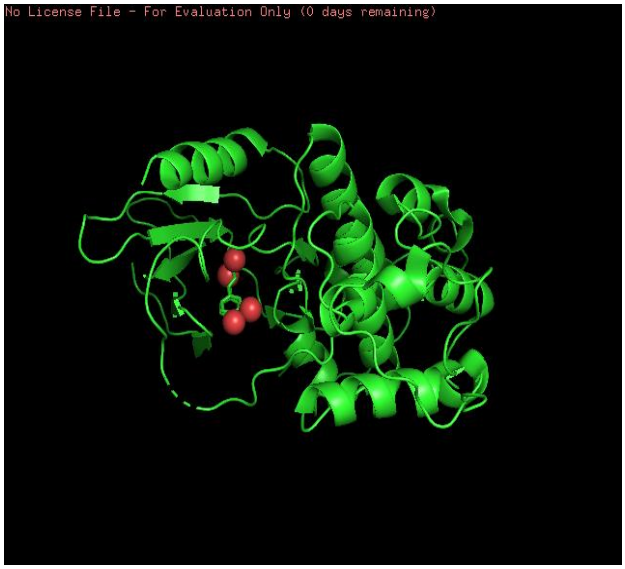
A.



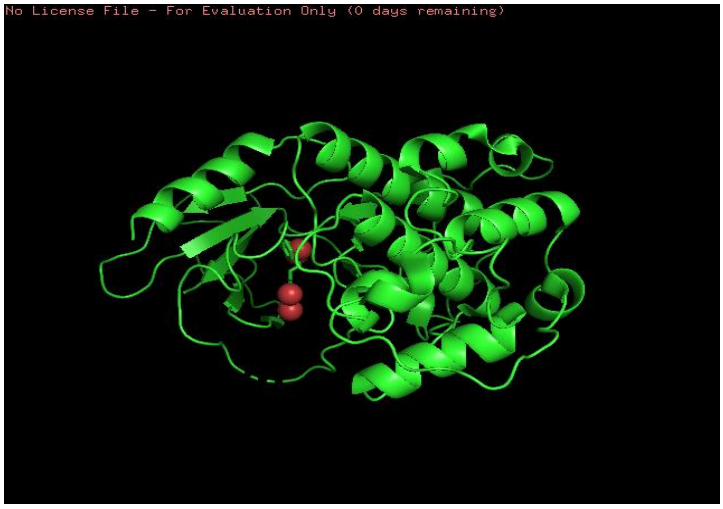
B



C


No License File - For Evaluation Only (0 days remaining)

D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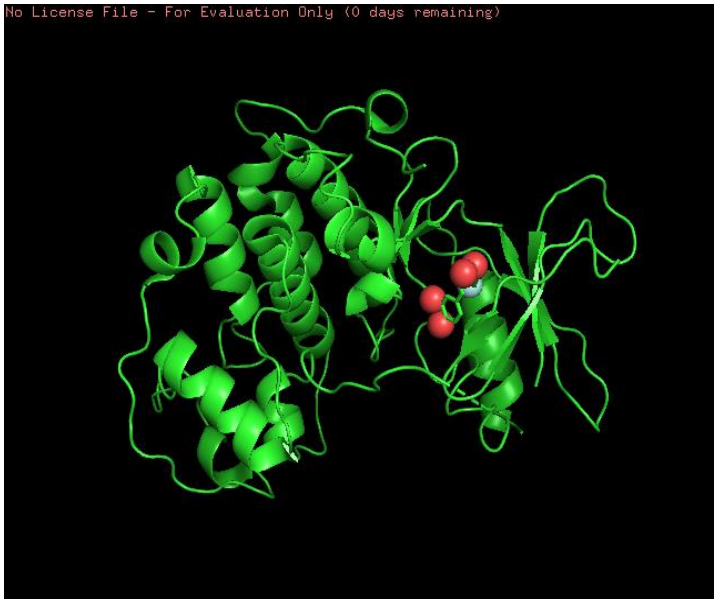
E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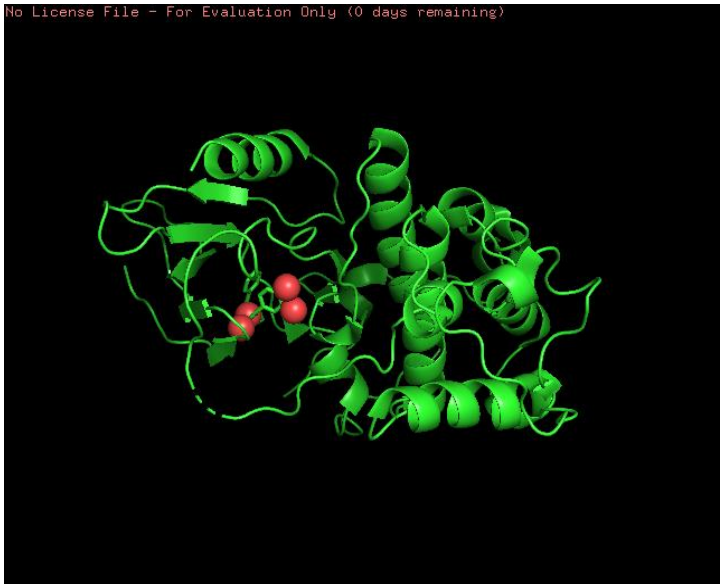
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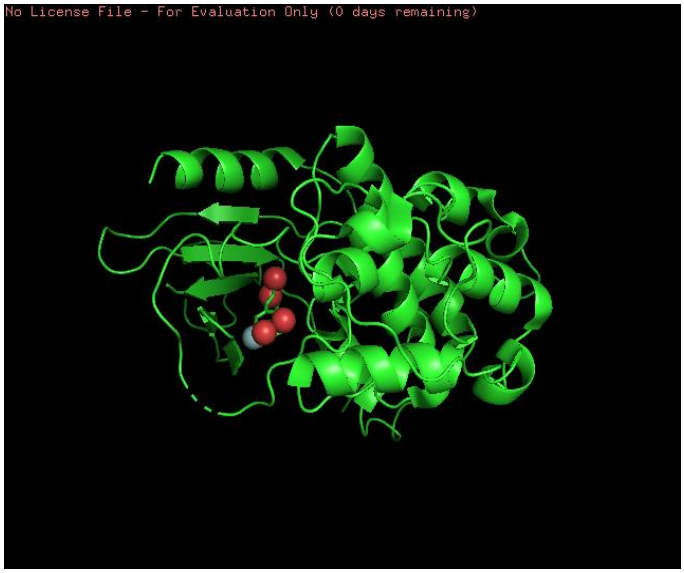
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H


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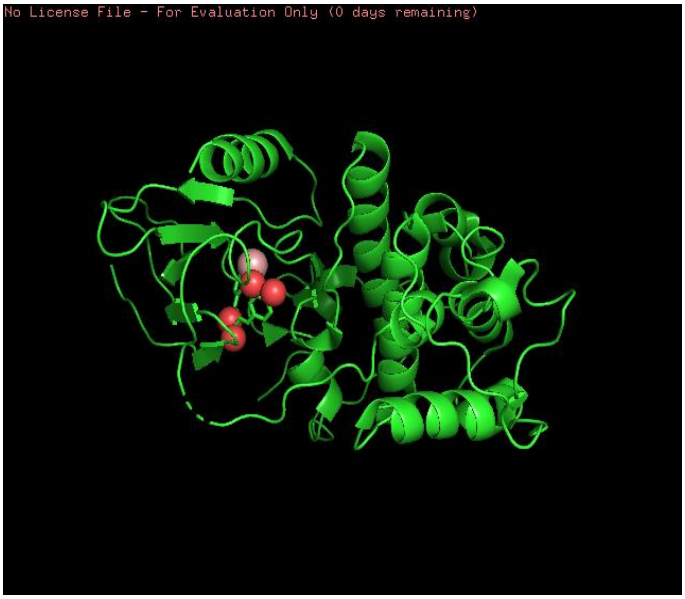
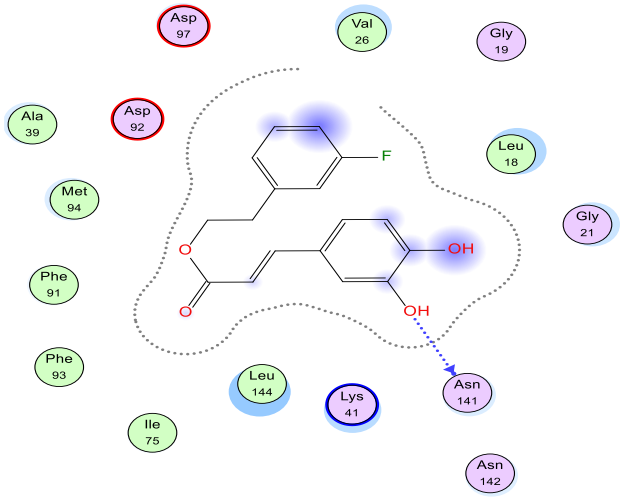
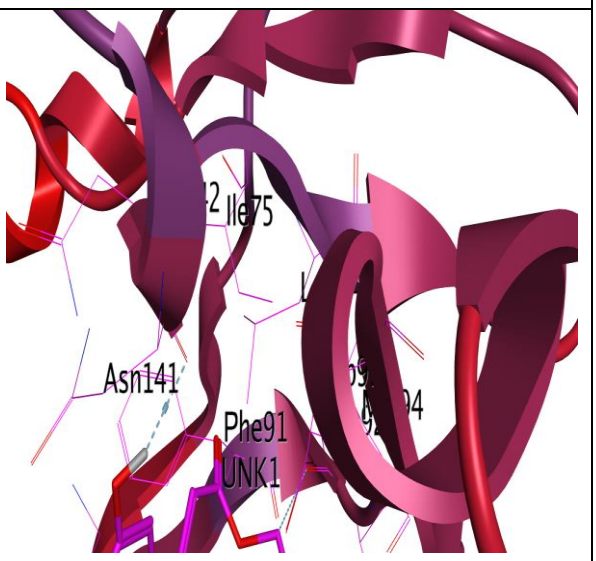
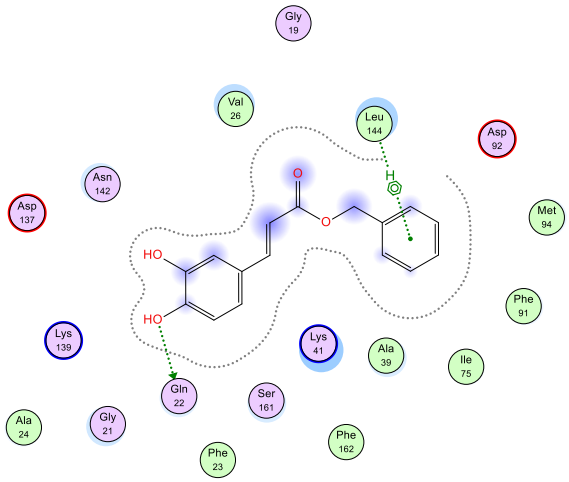
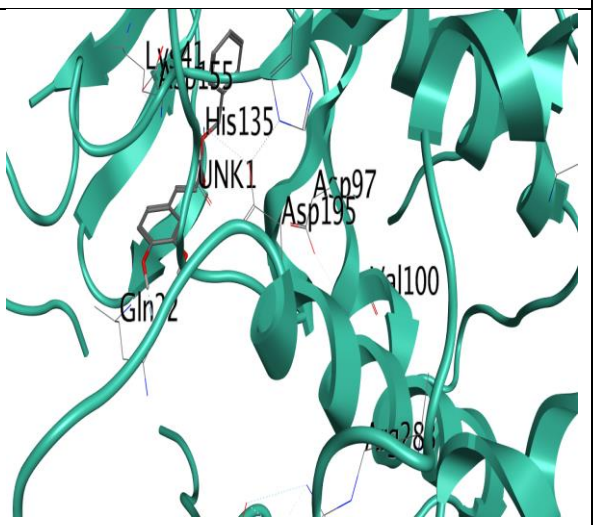
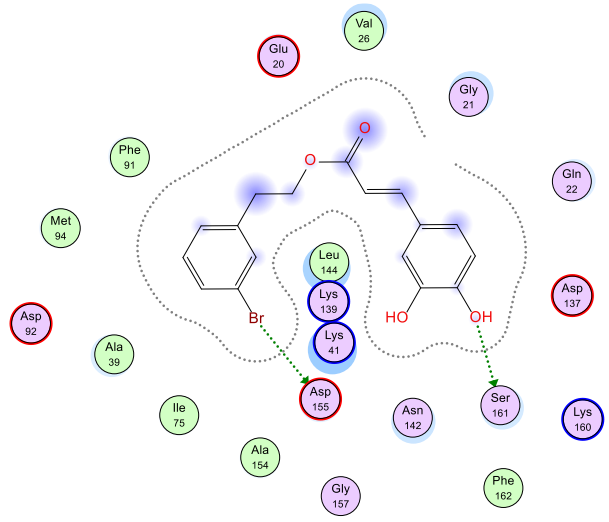
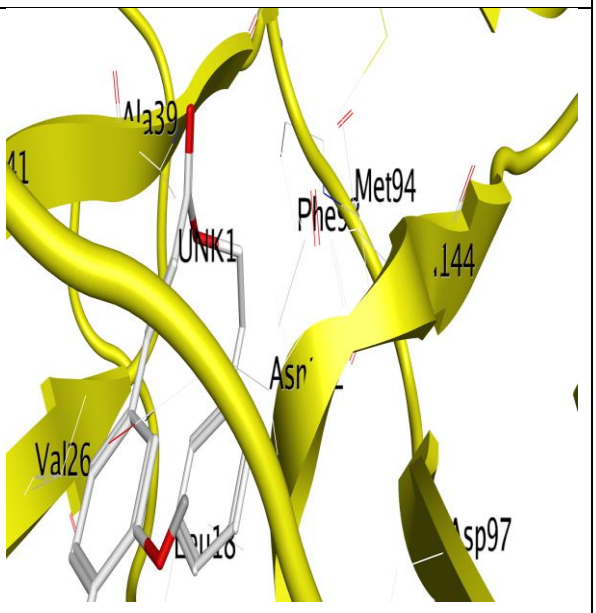
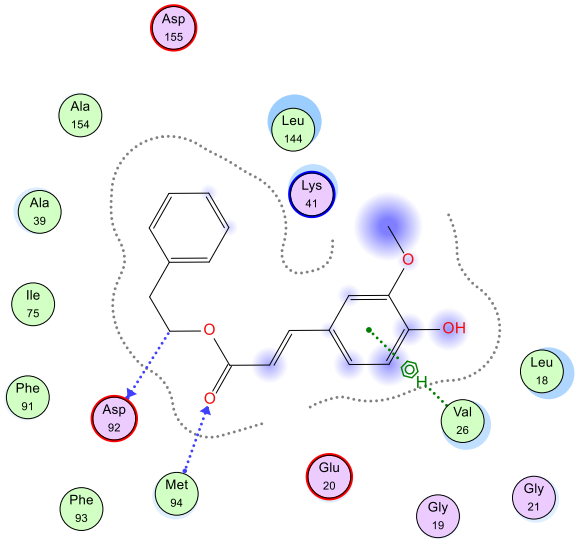
I.



Docked structures of Human CDK7 protein with 133492 (A), 133714 (B), 319244 (C), 442022 (D), 2441913 (E), 3597111 (F), 3915081 (G) 3958339 (H) and 3976811 (I) respectively.

2D	3D
A  Asp 97 Val 26 Gly 19 Ala 39 Asp 92 Leu 18 Met 94 Gly 21 Phe 91 Phe 93 Leu 144 Lys 41 Asn 141 Ile 75 Asn 142 F OH O O OH	 Asn141 Phe91 UNK1
B  Gly 19 Val 26 Leu 144 Asp 92 Asn 142 Asp 137 Met 94 Phe 91 Lys 139 Lys 41 Ala 39 Ile 75 Gln 22 Ser 161 Ala 24 Gly 21 Phe 162 Phe 23 HO HO O O H	 His135 UNK1 Asp97 Asp195
C  Val 26 Glu 20 Gly 21 Phe 91 Gln 22 Met 94 Leu 144 Lys 139 Asp 137 Asp 92 Lys 41 Ala 39 Asp 155 Ile 75 Asn 142 Ser 161 Lys 160 Ala 154 Gly 157 Phe 162 Br HO OH O O	 Met94 UNK1 Val26

D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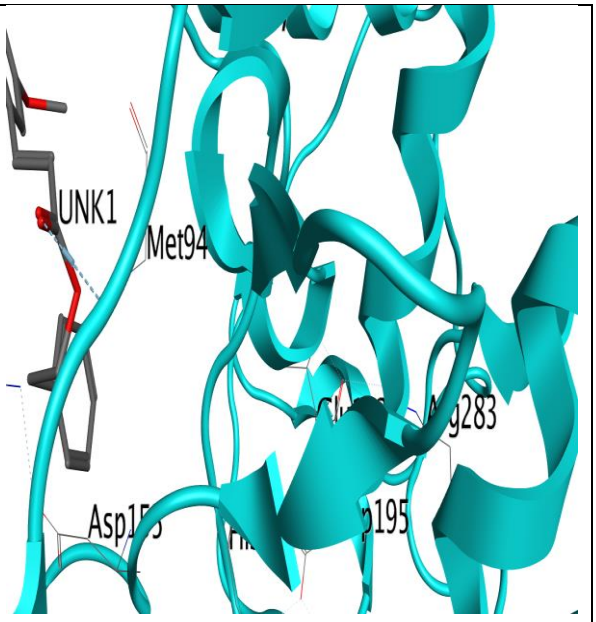
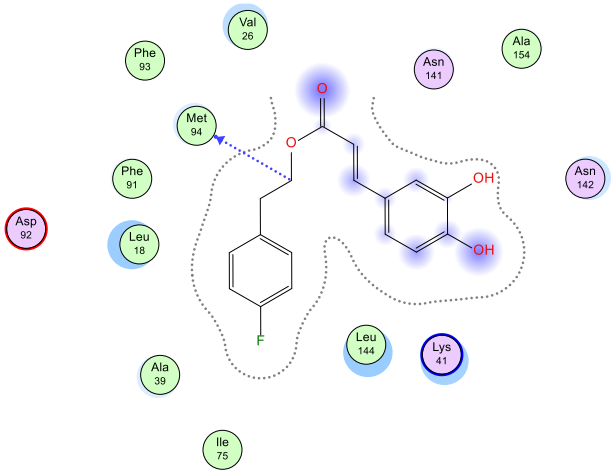
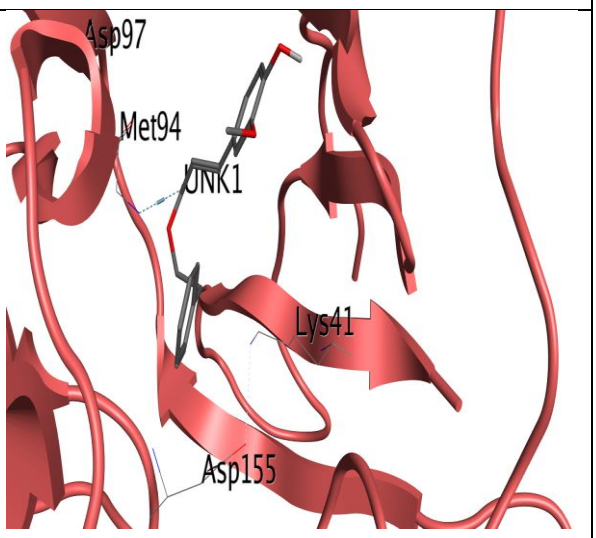
Ala 154
Leu 144
Lys 41
Ala 39
Ile 75
Phe 91
Asp 92
Met 94
Phe 93
Glu 20
Gly 19
Gly 21
Val 26
Leu 18
O
OH
H

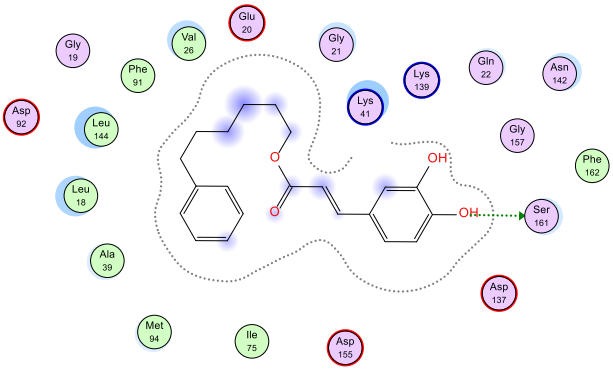

UNK1
Met94

E


Val 26
Phe 93
Asn 141
Ala 154
Met 94
Phe 91
Asn 142
Asp 92
Leu 18
Leu 144
Lys 41
Ala 39
Ile 75
O
OH
F


Asp97
Met94
UNK1
Lys41
Asp155

F


Glu 20
Gly 19
Val 26
Gly 21
Phe 91
Lys 139
Gln 22
Asn 142
Asp 92
Lys 41
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Gly 157
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Ser 161
Ala 39
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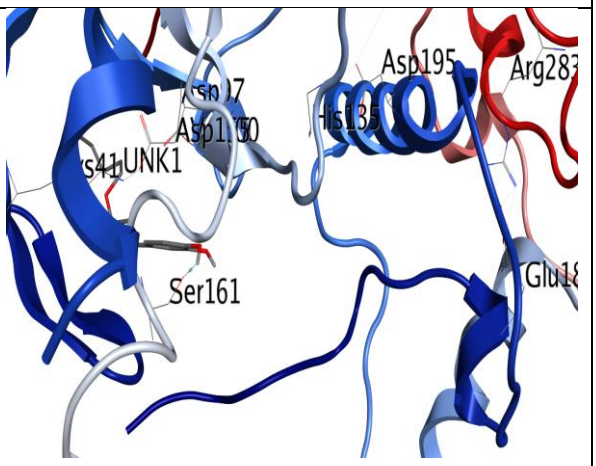
Met 94
Ile 75
Asp 155
O
OH


Asp195
Arg283
His135
UNK1
Ser161

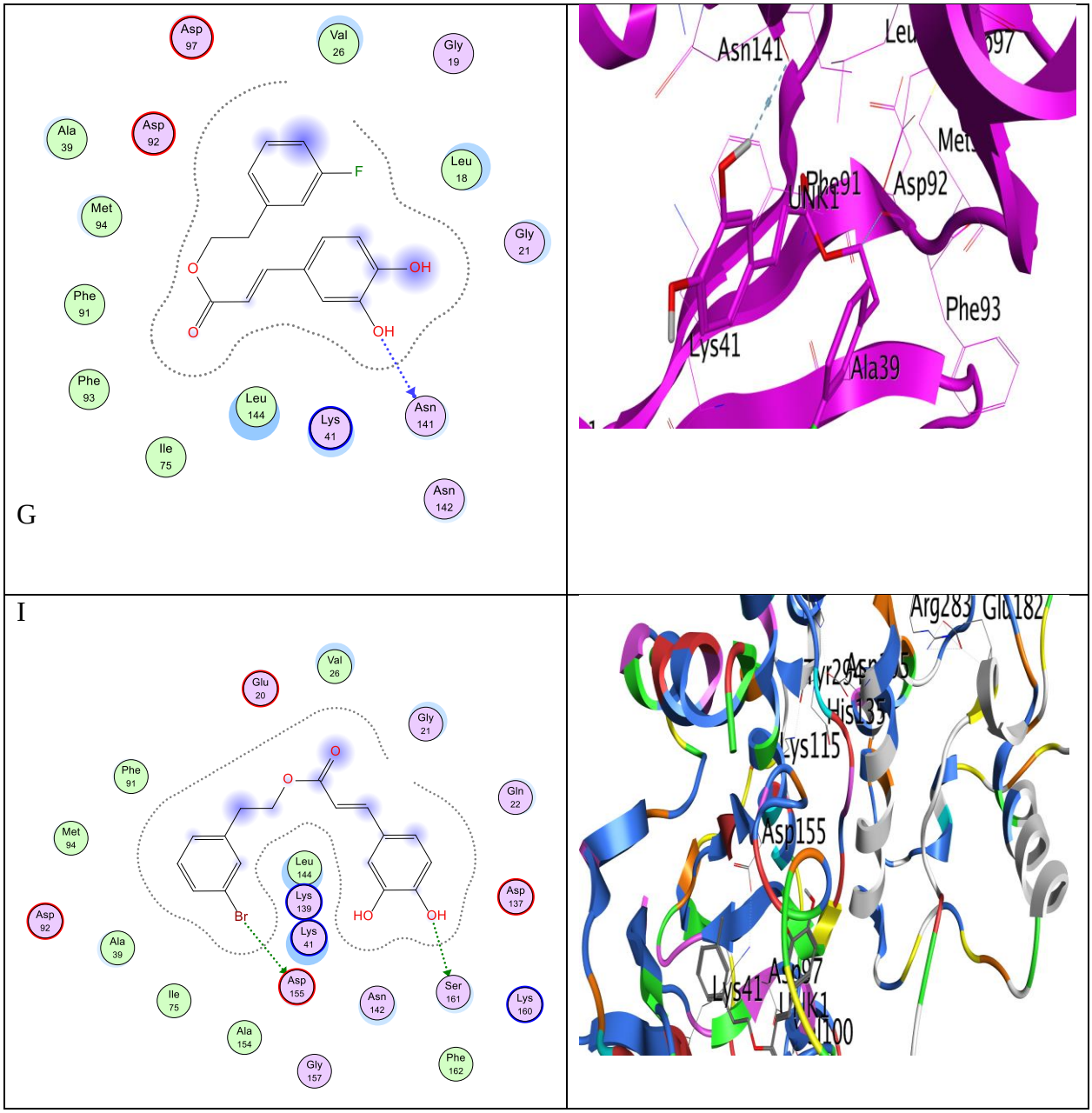


Figure 3.2: 2D and 3D interaction diagram of 1UA2 complex with 133492 (A), 133714 (B), 319244 (C), 442022 (D), 2441913 (E), 3597111 (F), 3915081 (G) 3958339 (H) and 3976811 (I) respectively.

CHAPTER 5

CONCLUSION AND FUTURE PERSPECTIVES

Although several CDK inhibitors have been reported in the literature for the treatment of other diseases such as tumours, few have been thoroughly investigated for their neuroprotective effects. It is still up for debate whether their dual specificity for GSK-3 and CDK5/P25 is harmful or beneficial. There has yet to be discovered a single molecule that inhibits a single kinase. This may be because the CDKs have structural similarities. Various side effects may arise due to the non-specificity of such CDK inhibitors that can act on other cell cycle regulating molecules as well. As a result, achieving high selectivity will be one of the most important goals for new drug development.

From the results, predicted compounds are **non-toxic** and good in the blood-brain barrier **(BBB) permeability**. Compound 9 (**ChEMBL3976811**) can act as a promising lead compound against CDK7 due to better docking score and same interacting residues as that of CAPE (**Ser 161** and **Asp 155**). Compound 6 (**ChEMBL3597111**) and Compound 8 (**ChEMBL3958339**) can also be proposed to be best interacting compounds due to interaction with similar residues (**Ser161**) and can be developed as a therapeutic solution for the AD. The proposed **three lead compounds** would serve as excellent targets to develop new drugs for targeting aberrant cell cycle re-entry, and will enhance the drug discovery process for AD and other neurological disorders.

In conclusion, CDK inhibitors appear to be on the verge of making a clinical effect due to the advent of more selective and potent ATP-competitive CDK inhibitors. New and useful drugs for the treatment of cancer and other proliferative diseases are likely to emerge from this avenue. Additional CDK-selective inhibitors may complement these ATP-competitive inhibitors by disrupting substrate binding to cyclins, blocking CDKs from binding to their cyclin partners, or allosterically abrogating ATP or protein substrate binding to the CDK subunit. These new methods for identifying CDK inhibitors could be used to develop non-ATP-competitive agents that target CDK4, CDK5, CDK6, CDK7, CDK8, CDK9, and other CDKs that have been identified as important therapeutic targets in the treatment of AD.

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