Virtual Screening of The Cyclin-Dependent Kinase Library for The Identification of Potential CDK11 Inhibitors

By

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A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of
Bachelor of Pharmacy

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BRAC University
August, 2025

Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
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Approval

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Ethics Statement

This study did not involve clinical trials, human participants, or animal subjects, and no animals were harmed during the course of the study.

Abstract

Cancer remains one of the leading causes of death worldwide, emphasizing the need for the development of more specific and potent treatment strategies, as existing therapies are often accompanied by a high toxicity profile, including drug resistance and off-target effects. The cyclin-dependent kinase 11 (CDK11), a crucial regulator of cell cycle progression and transcription, is frequently overexpressed in multiple cancers and is associated with poor prognosis. This study aims to identify potential CDK11 inhibitors through an *in silico* analysis. A ligand-based virtual screening was conducted against 1,000 compounds sourced from a kinase-focused library; the binding affinities of the best compounds were compared with the existing inhibitor OTS964 as the standard. Top candidates were further assessed for pharmacokinetic properties, drug-likeness, and toxicity through ADMET analysis. Several compounds demonstrated stronger binding interactions with the CDK11 active site than the standard and exhibited favorable pharmacological profiles. These findings propose promising hit molecules for subsequent *in vitro* and *in vivo* validation, contributing to the development of more precise and effective targeted anti-cancer therapies.

Keywords: Cyclin Dependent Kinase, Cancer, Cyclin Dependent Kinase 11, Molecular docking, Virtual screening, Targeted therapy.

Table of Contents

List of Tables

List of Figures

List of Acronyms

CDK	Cyclin-dependent kinases
CDK11	Cyclin-dependent kinases 11
IC_{50}	Half Maximal Inhibitory Concentration
P-gp	P-Glycoprotein
AML	Acute Myeloid Leukemia
TNBC	Triple Negative Breast Cancer
TBG	Thyroxine Binding Globulin
NLS	Nuclear Localized Signal
IRES	Internal Ribosomal Entry Site
BBB	Blood Brain Barrier

Chapter 1

Introduction

1.1. Cyclin-Dependent Kinases (CDKs) and Their Role in Cancer

Cyclin Dependent Kinases (CDKs) are a family of serine or threonine protein kinases which impart vital roles in various essential biological functions namely- regulation of cell cycle progression from G1 phase to mitosis (CDK1, CDK2, CDK4 and CDK6) and cellular transcription (CDK7, CDK8, CDK9, CDK11, CDK12, CDK13 and CDK19) (Blazek, 2023). The activity of these kinases is induced by their catalytic partners, cyclins, including Cyclin A, Cyclin B, Cyclin D, and Cyclin E (Ghafouri-Fard et al., 2022; Horton & Templeton, 1997). Each of the kinases of this family is responsible for specific cellular functions and is orchestrated in a coordinated manner by binding with the specific cyclins to phosphorylate essential substrates from their respective aspects to facilitate DNA transcription and cell division (Zhou et al., 2016). Furthermore, they also contribute to the regulation of other cellular processes such as spermatogenesis, metabolism, neural processes, stem cell self-renewal, and epigenetic control (Lim & Kaldis, 2013). Moreover, some of the CDKs are capable of maintaining some biological functions like DNA repair, mRNA processing, and transcriptional processing, independent of the cyclins, by interacting with other proteins and co-factors (Lim & Kaldis, 2013). However, studies have shown that aberrant expression of CDKs plays a significant role in the proliferation of tumor cells by interfering with cell cycles, transcription, or RNA splicing. In continuation of that, Lapenna and Giordano (2009) stated that overexpression of CDK4/CDK6/RB pathways was observed in numerous types of cancers, including glioblastoma, osteosarcoma, lymphomas, breast and cervical cancers, squamous cell carcinoma, and melanoma. Additionally, studies have reported aberrant expression of CDK9 in some of the primary malignancies, notably in lung cancer, prostate cancer, and myeloma, compared to normal healthy cells (Romano & Giordano, 2008). As

malignant diseases can be characterized by uncontrolled cell proliferation and growth, inhibiting these CDKs in malignant cells can be a promising defence approach against cancer (Zhou et al., 2016). This insight has eventually sparked an intensive hunt for CDK inhibitors intended for therapeutic purposes. As a matter of fact, due to their intensive roles in cellular growth and protein transcription, the CDK family is considered an appealing group of targets for anti-cancer drug development and treatment (Shapiro, 2006). Additionally, several studies have suggested that CDK11, one of the less explored members, has a significant influence on the development and proliferation of tumor cells (Malumbres, 2014; Housden et al., 2015; Tiedemann et al., 2009; Bajić et al., 2011). Thus, dysregulation of CDKs attributable to human cancer has made them a compelling target group for discovering novel anti-cancer drugs (Zhou et al., 2016).

1.2. Cyclin-Dependent Kinase 11 (CDK11)

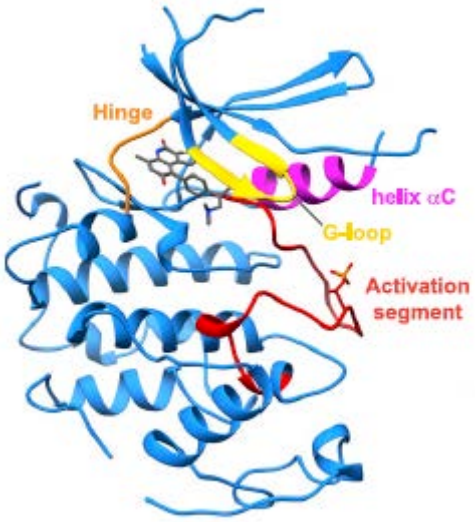


Figure 1: Binding of CDK11 with the co-crystallized ligand OTS964 (Kelso et al., 2022b).

The cyclin-dependent kinase 11 (CDK11) is a member of the transcriptional subclass of the protein kinase-CDK family involved in DNA transcription, pre-mRNA splicing, Mitosis, and apoptosis (Paparidis & Canduri, 2017).

1.2.1. The Structural Features of CDK 11

According to *Figure 1*:

- G-loop: Glue89
- Gly223 (the xDFG residue)
- HelixαC: Pink
- Active segment: 229-244 (Red)
- Hinge residue: Val96 (Orange)
- Gatekeeper residue: Met160 in CDK11 (and also TOPK) is the gatekeeper residue and is located at the back of the ATP-binding pocket (Kelso et al., 2022b).

1.2.2. Backbone Structure

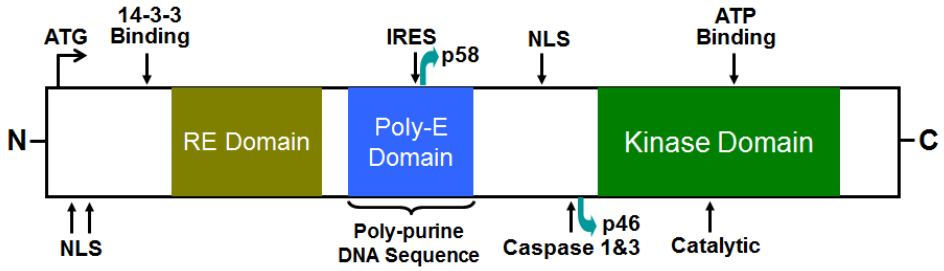


Figure 2: Schematic representation of CDK11 protein kinase backbone. NLS, nuclear localization signal; RE, arginine ® and glutamic (E) acid residues; IRES, internal ribosomal entry site (Zhou et al., 2016).

The backbone structure of CDK11 can be divided into two main lobes or terminals, the N-terminal and the C-terminal (*Figure 2*). The hinge region or the ATP binding site is found between the cleft of these two regions. According to Trembley et al. (2004), two separate domains are located at the center of CDK11, the RE domain and the poly-E domain. Furthermore, the CDK11p110 isoform consists of an IRES and a caspase-3 site, which generates a larger CDK11p58 along with a smaller CDK11p46 isoform (*Figure 2*).

N-terminal: The N-terminal regulatory region comprises multiple nuclear localization signals (NLS) and a 14-3-3 consensus site.

C-terminal: The carboxy-terminal catalytic domain expresses the kinase activity of the protein (Zhou et al., 2016).

1.2.3. The Co-crystalized Ligand: OTS964

The OTS964 is an analogue compound of OTS514, a TOPK inhibitor, and exhibits relatively high bioavailability and an IC_{50} value of 28 nM. It is also co-crystallized with the CDK11 and functions as an orally active and highly selective inhibitor of the CDK11, that binds to CDK11B with a Kd of 40 nM (Matsuo et al., 2014b).

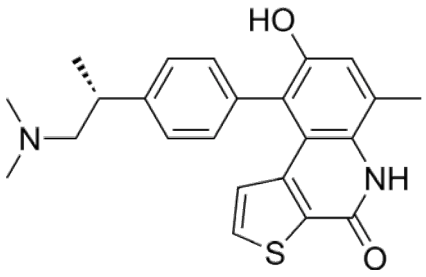


Figure 3: Structure of OTS964; Molecular weight:392.5 g/mol

The small molecule, OTS964 (*Figure 3*), binds at the kinase domain of the CDK11 and anchors at the ATP-binding pocket of CDK11 along the hinge region by interacting with the amino acid residues (*Figure 4*). Moreover, the X-Ray crystal structure provides insight into the selectivity of OTS964 for CDK11 compared to other CDK family members, as well as the explanation of OTS964 resistance due to the Gly223Ser mutation (Kelso et al., 2022b).

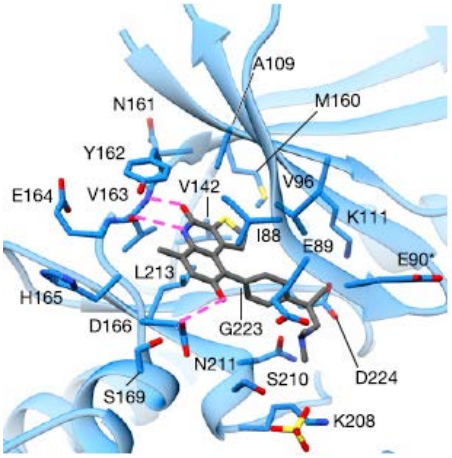


Figure 4: Interaction of OTS964 at the ATP-binding pocket of CDK11. (Kelso et al., 2022b).

1.2.4. Functional Aspects

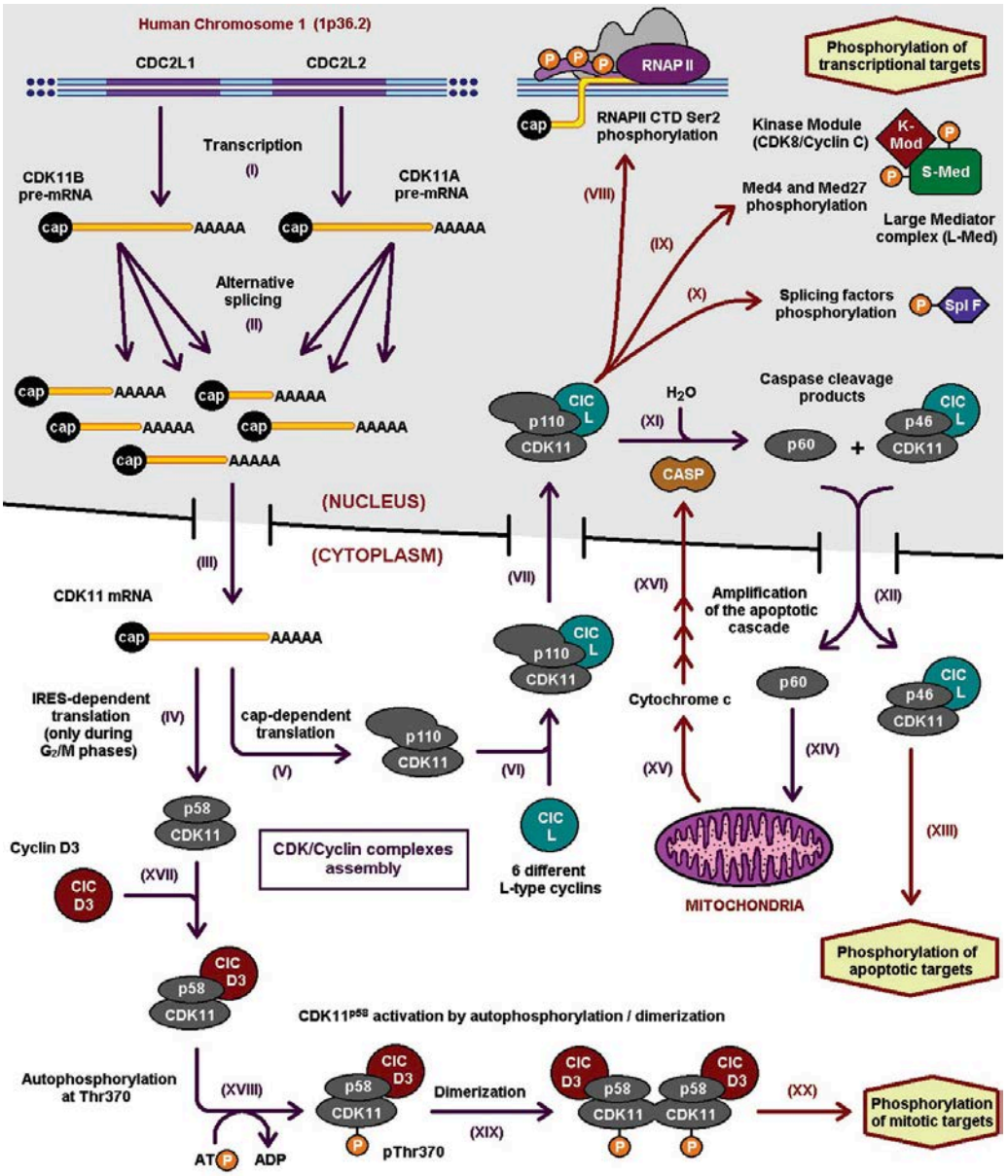


Figure 5: Overview of CDK11 function in cells. The scheme illustrates the origin of different CDK11 isoforms and the specific cellular processes regulated by each of them (Paparidis & Canduri, 2017).

Furthermore, the CDK11 can be classified into three main isoforms depending on the specific substrates responsible for various biological functions (*Figure 5*):

1. Apoptotic- CDK11p46
2. Mitotic- CDK11p58
3. Transcriptional- CDK11p110

1.2.5. Function of CDK11 in Normal Cells: According to (*Figure 5*) the functions of CDK11 discussed by Paparidis and Canduri (2017) are mentioned below:

- **CDK11p110** is synthesized through conventional cap-dependent translation of CDK11 mRNA and works within the nucleus by phosphorylating transcriptional targets, including RNAPII CTD.
- **CDK11p58** is translated only during G2 and M phases of the cell cycle through IRES initiation. It acts in the cytoplasm by phosphorylating the targets involved in mitosis progression.
- **CDK11p46** is the C-terminal fragment derived from the larger CDK11 isoforms through caspase cleavage, and its targets are pro-apoptotic factors.
- **CDK11p60** is the N-terminal fragment complementary to CDK11p46, which lacks the kinase domain but works synergistically in apoptotic signaling (Paparidis & Canduri, 2017).

1.3. Regulation and Expression of CDK11

The mRNA transcription process requires the involvement of several gene-specific factors and a collection of transcriptional factors, and among them, the RNAP II- a large protein mediator complex, plays a significant role in the production and processing of RNA. Remarkably, CDKs with Cyclin members are extensively engaged in the regulation of RNAP II-based transcription, as repeated presence of several heptapeptides at the CTD (Carboxyl Terminal Domain) of RNAP-II (Figure *6*), making it a putative target for the CDK-Cyclin complex (Lim & Kaldis, 2013c). Hence, the CDK11 is a pivotal component of this protein mediator complex, and it has been established that the CDK11/cycL complex is responsible for the coordination of RNAP II with other factors (Zhou et al., 2016). Furthermore, recent studies have revealed that CDK11 is extensively involved in the activation and regulation of

the spliceosome as it phosphorylates SF3B1, a core component of spliceosome and facilitate the action of spliceosomes which are large protein complexes conducting the major control step of co-transcriptional excision of introns of the pre-mRNA and yield catalytically active compounds (Hluchý & Blazek, 2024).

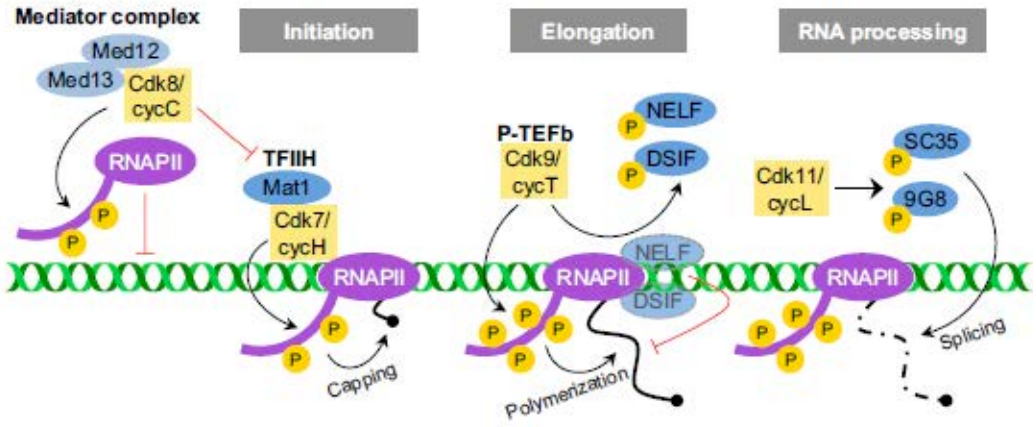


Figure 6: CDKs involved in RNA transcription

Figure *6* illustrates that the Cdk11/cyclin L complex links with multiple elongation factors, including Ell2, TFIIF, TFIIS, and FACT, to promote the elongation phase during mRNA transcription (Trembley et al., 2002). Moreover, the Cdk11/cyclin L complex plays a vital role in RNA processing due to its involvement in co-transcriptional phosphorylation of factors such as SC35 (Srfs2) and 9G8 (Srfs7), which are involved in pre-mRNA splicing (Dickinson et al., 2002; Hu et al., 2003; Loyer et al., 2008).

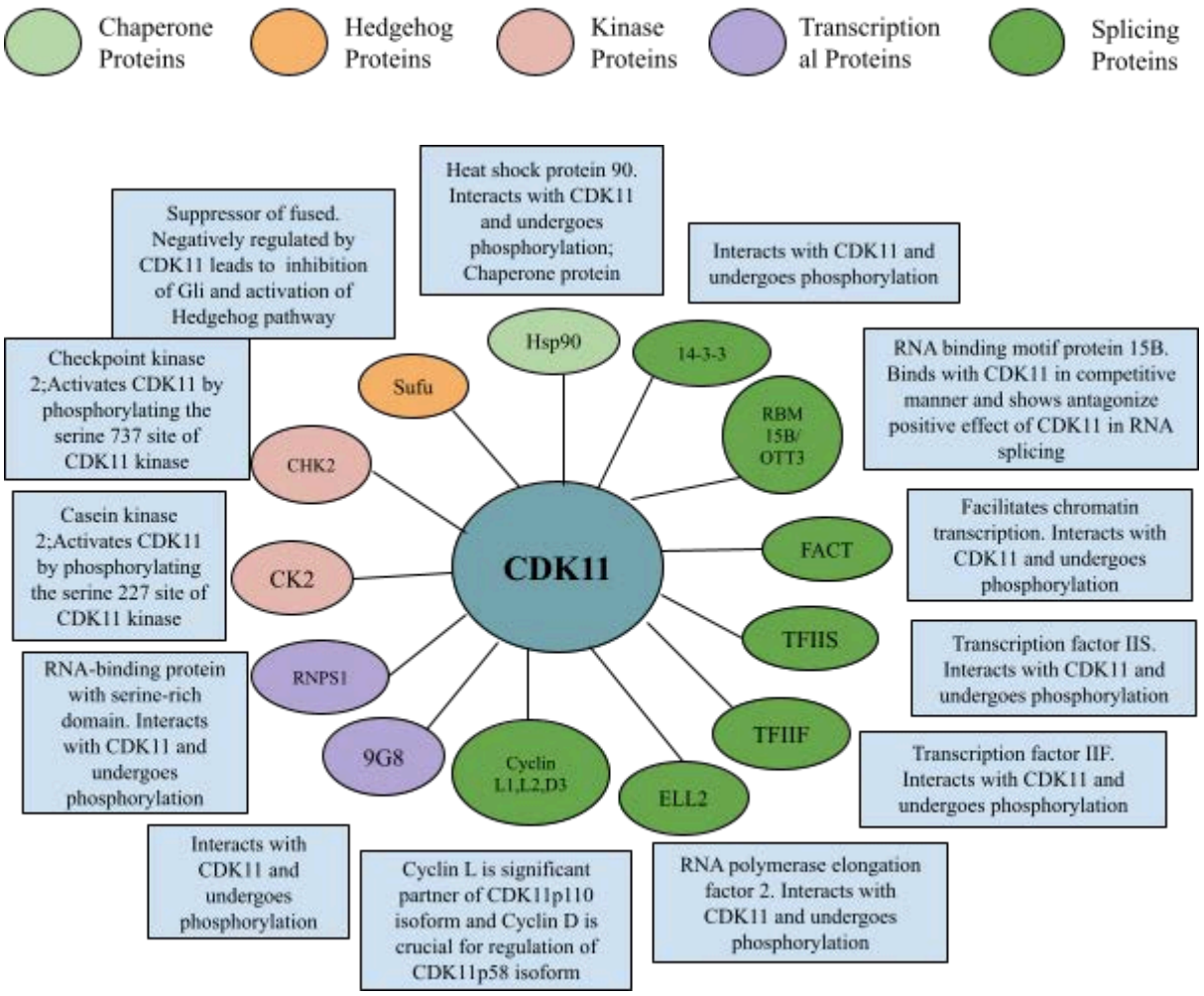


Figure 7: Involvement of CDK11 with other substrates

Furthermore, CDK11 is involved in the Hedgehog signaling pathway and the Wnt/β-catenin signaling cascade, whose deregulation can lead to the emergence of cancers in the human body (Zhou et al., 2016).

1.4. Indirect Targeting of SF3B1 with Existing CDK11 Inhibitor: OTS964

The OTS964 is a potent and selective inhibitor of the CDK11 (Yang et al., 2021) and has been reported to possess anti-cancer effects by suppressing tumor growth and promoting apoptotic cell death in the malignant cells in both in vivo and in vitro cancer models (Matsuo et al., 2014; Ikeda et al., 2016; Pirovano et al., 2018; Yang et al., 2021). As the CDK11 plays a significant role in cellular proliferation, depletion of its expression leads to defective cell cycle, which may result in cell death (Hluchý & Blazek, 2024). Furthermore, according to

Hluchý and Blazek (2024b), the expression of CDK11 is inhibited by OTS964, resulting in an arrested cell cycle in the G2/M phase. The SF3B1, a core spliceosome component, gets rapidly phosphorylated, leading to inhibition of spliceosome assembly and splicing of the pre-mRNA, affecting cellular proliferation **(***Figure 8*).

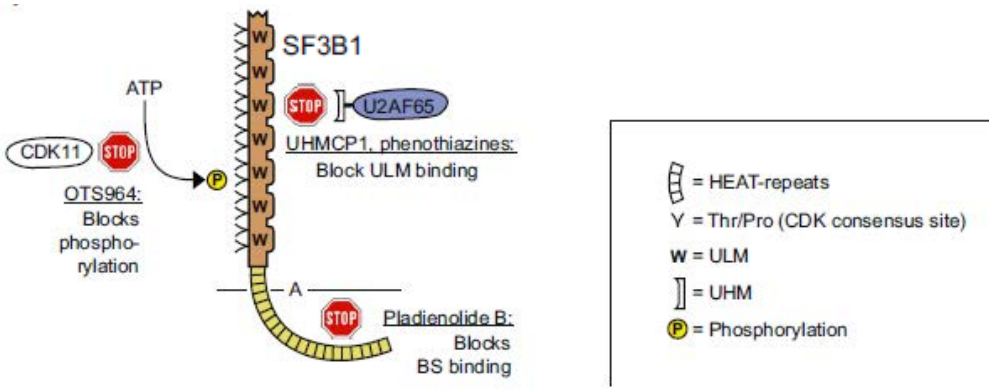


Figure 8: Inhibition of SF3B1 phosphorylation (Hluchý & Blazek, 2024b)

Moreover, the RNAP II and the spliceosomes are functionally coupled and work in a coordinated manner in optimal regulation of cell cycle and gene expression (Das et al., 2006), and any disruption in either of them can lead to impaired cellular processes (Hluchý & Blazek, 2024).

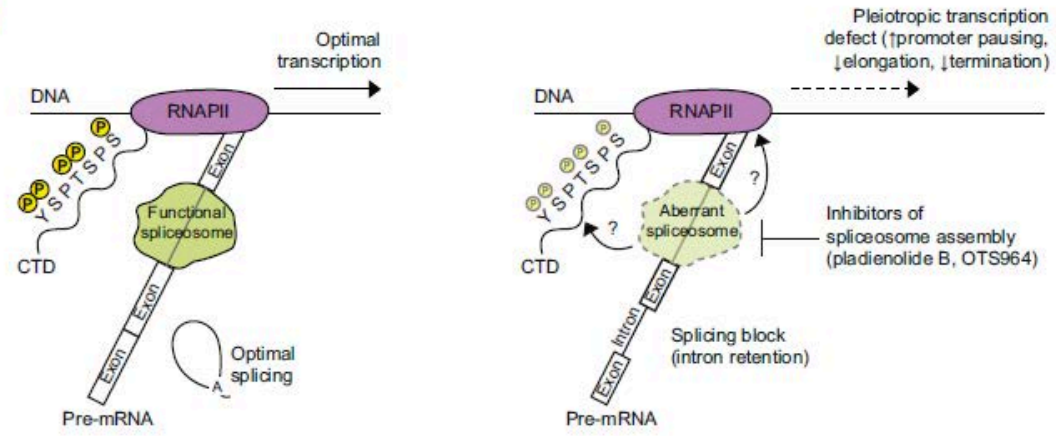


Figure 9: Outcome of CDK11 and its targeting effect on transcription (Hluchý & Blazek, 2024b)

The figure (*Figure 9*) provides a schematic comparison of the outcome of optimal (left) and inhibited (right) pre-mRNA splicing during transcription. In optimal transcription, the CTD-C-terminal domain gets phosphorylated and transcription is completed without any cellular

defect; on the other hand, inhibition of the spliceosome assembly by OTS964 leads to increased promoter pausing and decreased elongation and termination (pleiotropic RNAPII transcription defects) (Hluchý & Blazek, 2024b). Hence, in light of these findings, their study has established that the existing CDK11 inhibitor- OTS964 can indirectly target and block the expression of SF3B1, and its inhibition leads to cellular apoptosis of malignant cells (Zhang et al., 2019).

1.5. Significance of CDK11 Study in Cancer Biology

The study of CDK11 holds major importance as it is involved in essential cellular processes including control of viral transcription and neuronal physiology, bipolar spindle assembly, centromere cohesion, centrosome maturation, apoptotic and androgenic receptor signalling. Even though the CDK11 subclass can be considered as one of the neglected and underexplored members of the CDK family, some extent of research has been conducted on the function of CDK11 in different cancer cells in the human body. Recent studies have suggested that CDK11 has a major influence on the development and proliferation of tumor cells (Malumbres, 2014; Housden et al., 2015; Tiedemann et al., 2009; Bajić et al., 2011). According to Zhou et al. (2016), the following table (Table 01) summarizes the role of CDK11 in various types of human cancers that were collected:

Table 1: Role of CDK11 in Human Cancers

Human Cancers	Functions of CDK11 in tumors
Cervical cancer	Promotion of centrosome maturation and bipolar spindle morphogenesis (Liu et al., 2016).
Breast Cancer	Inhibition of breast cell apoptosis promotes cancer cell proliferation, growth, migration, and cell cycle progression (Zhou et al., 2015).

Table 1: Role of CDK11 in Human Cancers	
Colon Cancer	Functions as a positive modulator of the Wnt/β catenin pathway (Ou et al., 2020).
Osteosarcoma	Inhibition of the apoptosis of osteosarcoma promotes cell proliferation and growth (Feng et al., 2014).
Liposarcoma	Inhibition of liposarcoma cell apoptosis, Desensitization to chemotherapy, and promoting liposarcoma cell growth. Survival (Jia et al., 2013).
Multiple Myeloma	Promotion of myeloma cell proliferation and survival (Mery et al., 2023).
Acute Myeloid Leukemia (AML)	Growth of TSC2-deficient AML cells (Radomska et al., 2012).

Hence, given such involvement of CDK11 in cancer cell development, it has emerged as a potential target in cancer research, undoubtedly.

1.6. Impact of CDK11 knockout in Cancer Cell Progression

The Cyclin-dependent kinases involved in transcriptional regulation carry out exclusive and non-duplicative functions; hence, cell death occurs in case of any knockouts, unlike the cell cycle-regulating CDKs, capable of conducting widespread substitute mechanisms to conduct normal cellular activities (Lim & Kaldis, 2013b). Moreover, recent studies have discovered a groundbreaking approach of RNA interference using siRNA in advanced oncological research, as it targets complementary mRNA for degradation and induces gene silencing in the tumor cells (Dana et al., 2017). Additionally, several studies have established that siRNA-mediated silencing of cellular proteins involved in cancer cell proliferation shows significant apoptotic effects (Pai et al., 2005). Moreover, inhibition of CDK11 with siRNA in

HeLa cells resulted in aberrant spindle assembly, mitotic arrest, and cell death (Franck et al., 2011; Liu et al., 2017; Rakkaa et al., 2014; Petretti et al., 2006; Yokoyama et al., 2008).

1.6.1. Triple Negative Breast Cancer (TNBC)

According to Kren et al. (2015), CDK11 protein expression is strongly found in human breast cancer cells compared to healthy cells, and the tumor cells of TNBC patients showed 100% positive results for higher CDK11 nuclear intensity. Furthermore, a study conducted on mice carrying TNBC tumor cells where TBG capsules designed to target specifically CDK11 with siRNAs had shown significant results in growth inhibition and migration, strikingly in breast tumor cells. Inhibition of CDK11 in TNBC tumor cells results in a reduction of Protein and relevant mRNA expression along with dramatic loss of cell viability and clonal survival as delivering TBG nanocapsules to the targeted genes induces knockdown of CDK11 and provides the outcome of reduced cell expression and proliferation of the tumor cells (Kren et al., 2015).

1.6.2. Liposarcoma

Anti-apoptotic proteins Cyclin D1 and Survivin are found at elevated levels in liposarcoma, and by knocking down CDK11 *via* CDK11 siRNA, cell apoptosis can be induced as it restricts Cyclin D1 and Survivin in both the SW872 and SW982 cell lines in liposarcoma. Additionally, transfection of CDK11 in CDK11 siRNA has reduced the expression of another anti-apoptotic protein, Cytochrome C, in the SW872 cell line of liposarcoma (Jia et al., 2013). Moreover, Cytotoxic effects of chemotherapeutic agents can also be optimized by combining CDK11 knockdown in the cell lines of liposarcoma. According to Jia et al. (2013), a study suggests that the SW872 and SW982 cell lines of liposarcoma transfected with CDK11 siRNA and incubated with the commonly used anti-cancer drug Doxorubicin,

showed downregulation of liposarcoma cells by 30%. Thus, cell proliferation and survival of liposarcoma cells were remarkably inhibited by CDK11 siRNA combined with Doxorubicin.

1.6.3. Osteosarcoma

In osteosarcoma, inhibition of cell growth and instigation of cell apoptosis can be triggered by knocking down CDK11 with lentiviral shRNA, or by synthetic siRNA-independent confirmation (Duan et al., 2012). According to their study, dose-dependent cell death in both KHOS and U2OS osteosarcoma cell lines was observed by transfection of CDK11 with CDK11 siRNA. Besides, a lower level of anti-apoptotic proteins such as MCL-1, Bcl-XL, survivin, cytochrome C, and cyclin D1 was observed by knocking out CDK11 protein in the osteosarcoma cells. The collected data of cell growth downregulation, activation of apoptosis, and reduced expression of anti-apoptotic proteins suggested that CDK11 knockdown plays a significant role in the treatment of osteosarcoma cells (Duan et al., 2012).

1.7. *In Silico* Method

For decades, the *in silico* method has been employed by the pharmaceutical industry as an integral tool for drug discovery. In modern drug discovery, lead compound and pharmacological target discovery are the pragmatic steps to discover and develop appropriate therapeutic interventions with the pharmacological targets, in which the *in silico* approach brings to light the drug likeness of the lead compounds and facilitates drug discovery (Terstappen & Reggiani, 2001). Generally, a drug discovery process consists of several steps to scrutinize a lead compound from all the promising candidates and it starts with high-throughput screening of a large set of compounds using advanced and sophisticated (*In Silico*) tools to distinguish promising lead compounds which later get optimized structurally to enhance the therapeutic activity by performing SAR (Structural Activity Relation) studies. Notably, the *in silico* method has established itself as an indispensable tool for the

fundamental approach of drug discovery due to its rapid and cost-effective screening by using virtual screening only (Roncaglioni et al., 2013). Moreover, this method of study can be carried out at any stage from drug screening to clinical and pre-clinical studies, which remarkably lowers the failure rate and inefficient use of resources during drug discovery (Ahmed, 2013). This rigorous method has made the ligand interaction investigations easy and has opened a revolutionary technique for identifying promising candidates meeting all the required qualities for successful binding with a receptor, along with offering the benefit of a comprehensive molecular study to achieve the intended pharmacological and therapeutic effects (Muthiah et al., 2020). Thus, for effective and efficient study of molecular interactions, the *in silico* method serves as an indispensable tool in modern drug discovery.

1.8. Relevant Study

A recent *in silico* study was conducted by Bhambri and Jha (2025) focusing on discovering natural anti-cancer compounds targeting CDK11 following a combination of computational study with pharmacophore virtual screening, molecular docking, ADMET prediction, and binding free analysis. In their research, they have used 3 different sources of datasets - Specs, UNDP, and DDD as ligands to dock against CDK11 and assessed around 21,923 natural compounds. In order to identify promising candidates from the datasets, pharmacophore-based virtual screening was performed using the most efficient 3D search models- M_1, M_2, and M_3 to identify the compounds that resemble the reference pharmacophore, OTS964. Through the process, they found traces of 561 potent natural compounds, which were later analysed through molecular docking, offering 12 lead compounds with stronger binding affinity than the reference co-crystallized ligand OTS964. Thereafter, H-bond analysis has revealed the four most stable and strongest compounds, UNPD29888, UNPD162537, UNPD217978, and UNPD216468. Furthermore, analysis of binding free energy has identified UNPD29888 as the most promising compound due to its

higher energy value of -51.83 kJ/mol compared to the standard -35.90 kJ/mol of the co-crystallized ligand and other lead compounds. Henceforth, they conducted a structural characteristic-based comparison between UNPD29888 and the co-crystallized ligand OTS964, which has unveiled variations in their functional and characteristic properties.

1.9. Rationale of the Study

This study aims to find potential hit compounds among the listed 1,000 ligands collected from a kinase-focused library and capable of suppressing the cellular expression of CDK11 in the tumor cells by performing *in silico* molecular docking analysis. The cyclin-dependent kinase proteins are widely involved in numerous crucial cellular pathways, making them promising drug targets for a wide range of diseases, including cancer. Hence, extensive studies are carried out on CDK inhibitors in the clinical oncology field (Wu et al., 2015). Despite being the underexplored member of the CDK family, analysis of CDK11 regulation has revealed that inhibition of its overexpression can lay the foundation for potential anti-cancer drug development. Besides, cells lacking CDK11 exhibit impaired cellular division and growth due to mitotic arrest or proliferative defects, which leads to apoptosis (Franck et al., 2011; Liu et al., 2017; Rakkaa et al., 2014; Petretti et al., 2006; Yokoyama et al., 2008). Moreover, several studies have established that inhibition of CDK11 with the existing inhibitor: OTS964 resulted in suppressed growth of cancer cells due to mitotic arrest during the cell cycle (Hluchý & Blazek, 2024b), and such insight may lead to breakthroughs in novel anti-cancer drug discovery. Thus, in light of all these findings regarding the role of CDK11 in the oncological field, future investigation can pave a new way for the development of targeted therapy targeting over-expressed CDK11 in malignant cells, along with improved selectivity and cytotoxic or cytostatic effects to effectively suppress the tumors in the human body.

1.10. Objective of the Study

1. The primary objective of this study is to identify potential CDK11 inhibitors by conducting an extensive *in silico* screening of a kinase-focused chemical library comprising 1,000 carefully selected compounds. Given the critical role of CDK11 in cancer cell progression, survival, and transcriptional regulation, this study aims to screen for hit molecules that are capable of inhibiting the overexpressed CDK11 in cancer cells.
2. Further analysis will be conducted of the top candidates exhibiting the highest binding affinity and a comprehensive pharmacokinetic and toxicity assessment of the shortlisted hit molecules, including evaluations of their absorption, distribution, metabolism, excretion, and toxicity (ADMET) characteristics. This analysis is intended to determine their drug-likeness, safety profile, and therapeutic potential, ultimately supporting the development of targeted anti-cancer agents capable of selectively inhibiting CDK11-driven malignancies.

Chapter 2

Methodology:

Virtual screening is a crucial approach in the screening of promising candidates capable of binding with the specific pharmacological drug target (Islam & Pillay, 2016). In the study, *in silico* techniques have been implemented by following the focused molecular docking method through screening potential molecular libraries against the binding site of cyclin-dependent kinase CDK11 to identify prospective candidates with the assessment of protein-ligand interactions that might suppress the cellular overexpression of CDK11 protein.

2.1 Softwares

In this *in silico* investigation, the curated protein was prepared with the help of *AutoDockTools-1.5.7.* Protein visualization and protein-ligand interaction analysis were carried out using *PyMOL* and *Discovery Studio 2024 Client*. Subsequently, molecular docking was performed by using *PyRx*, and then *Discovery Studio 2024 Client* was again used to visualize the binding profiles of the promising candidates and compare them with the binding orientations of the co-crystallized ligand OTS964. Further data analysis was carried out using the software *DataWarrior* in order to cluster the highest-scoring ligands based on their chemical class. Multiple online databases such as the RCSB-PDB (Protein Data Bank), Life Chemicals, and PubChem were used as chemical and structural information databases, and pharmacokinetic assessments of the promising candidates were done by implementing the datasets of the compounds collected from the website SwissADME.

2.2 Protein Preparation

The 3D protein structure of CDK11 with a high resolution of 2.60Å is collected in PDB format from the Research Collaboratory for Structural Biology (RCSB) Protein Data Bank (PDB), which has only a single detailed structure of CDK11 with the PDB Entry ID - 7UKZ; "CDK11 in complex with small molecule inhibitor OTS964." *Discovery Studio 2024 Client* was used for visualization of protein structure, and a review of protein-ligand binding interaction was carried out to verify the primary binding site amino acid residues for performing focused molecular docking. This method would provide more efficient and accurate data for docking analysis by exploring various ligand interactions at the specific pocket of the protein. The visualization software *PyMOL* was used to eliminate the co-crystallized ligand OTS96 ($C_{23}H_{24}N_2O_2S$), crystallographic water molecules, and all the other chains were removed except for chain A. Further, the protein curation was performed in *AutoDockTools-1.5.7* by the addition of the missing atoms, polar hydrogens, and Kollman

charges to ensure the structural integrity of the final protein. The concluding step was to save the prepared final protein file in PDBQT format, ready for further docking study.

2.3 Ligand Preparation

A library of ligand compounds was developed from a kinase-focused online molecular library, 'Life Chemicals', which offers a wide range of screening libraries consisting of various classifications of the dedicated compound libraries. Approximately 1,000 ligands were listed to construct the ligand library by collecting around 200 ligand compounds from each of the following screening libraries available on this website: Kinase Focused Libraries, Kinase Targeted Libraries by Docking, Lead-like Screening Compound Library, Biologically Active Compound Library, and 3D Compound Libraries for HTS. Notably, the ligand compounds were scrutinized based on Lipinski's Rule of Five to ensure the screened compounds meet the criteria of the standard drug-like compounds (Lipinski, 2004). Thereafter, the 3D structures of those 1000 ligand compounds were downloaded from PubChem in SDF format. *Discovery Studio 2024 Client* was used to conclude ligand preparation by developing a ligand library consisting of all 1000 ligand files of 3D structures, which were compiled into a single file and downloaded as an SDF file. Ligand preparation was concluded by importing the SDF file into *PyRx,* where all ligands underwent energy minimization and were converted into PDBQT format for a subsequent molecular docking study.

2.4 Molecular Docking Analysis

Molecular docking is a crucial step in the drug development process, which provides the opportunity to study each of the candidates' optimal orientation and interaction at the binding sites of the protein (Bhambri & Jha, 2025c). Similarly, in this study, docking analysis was carried out in the software *'PyRx'* to study the binding affinity between the 1000 ligands and the targeted protein CDK11. Moreover, according to Kelso et al. (2022), the binding affinity

between the pre-existing co-crystallized ligand OTS964 and the protein is -9.82 ± 0.16 kcal/mol, and in this study, this value is considered as the standard reference to compare the binding affinity between the protein and the candidate ligands. However, to evaluate any methodological variation, this study was initiated by performing a molecular docking study between the prepared protein and the separately prepared ligand OTS964, which yielded the best binding result of -9.8 kcal/mol. This result clearly shows that the result is consistent with the standard value and indicates that a different methodological approach has a minimal impact on the binding analysis outcomes.

Table 2: Binding Affinity Between the Co-Crystallized Ligand OTS964 and the Prepared Protein CDK11

Ligand	Binding Affinity (kcal/mol)	rmsd/ub	rmsd/lb
OTS964	-9.8	0	0
OTS964	-8.4	7.535	3.363
OTS964	-8.3	7.451	3.112
OTS964	-8.3	2.094	1.141
OTS964	-8.2	8.087	3.302
OTS964	-8.2	3.936	2.386
OTS964	-8.2	8.029	2.975
OTS964	-7.9	3.887	2.202
OTS964	-7.5	8.241	3.394

Furthermore, as a part of focused molecular docking analysis, all the amino acid residues of the binding pocket (*Figure 4)* were selected before performing the docking analysis, and a 3D adjustable gridbox appeared, which was adjusted to envelop all the selected residues at the binding pocket. Afterwards, the software generated a coordinate of X, Y, and Z values indicating centres and dimensions outlining the targeted location of the specific binding pocket in the protein (Faiza, 2020). In this study, the coordinates were:

Table 3: Coordinates of the Docking Study in *Pyrx*			
Coordinates	**X**	**Y**	**Z**
Center	33.3233	- 8.8162	9.4272
Dimensions (Å)	37.5332	36.7476	37.3337

2.5 Protein-Ligand Interaction Analysis

The protein-ligand interaction was studied using *Discovery Studio 2024 Client*. This analysis will provide insight into amino acid residue sequences at the binding pocket, and the interaction distance and type of bonds formed between the ligands and the curated protein. Furthermore, the application offers a 2D diagram of the protein-ligand interaction along with the Hydrogen bond surface area, which confers more comprehensiveness to the analysis of binding interactions.

2.6 Chemical Structure-Based Ligand Clustering

In modern drug discovery, compound clustering improves data organization through efficient selection and prioritization of chemical compounds by minimizing repetitive compound exploration from a large database. Moreover, it facilitates selective and cost-effective experimental validation and accelerates lead identification (Varin et al., 2011). The molecular fingerprints are considered as an integral cheminformatics tool for virtual screenings and chemical mapping, as clustering is achieved by computing the molecular descriptors or fingerprints, and compounds with highly similar scores are grouped into clusters, allowing identification and analysis of structurally related molecules (Capecchi et al., 2020). In this study, the *DataWarrior* application is used for compound clustering as it will calculate the similarities between the selected ligands through its default descriptor, *FragFp* (substructure fragment dictionary based binary fingerprint), and screens out redundant molecules by identifying similar pharmacophores (Sander et al., 2015).

2.7 Pharmacokinetic Parameter Analysis

Optimization of pharmacokinetic properties plays a significant role in safe and effective drug development, as preliminary screening aids in focusing on only promising compounds with favorable characteristics of drug likeliness (Bhambri & Jha, 2025d). Over the recent years, *in silico* ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) studies have become an integral part of the Drug discovery process as they offer insight into how a drug affects the body and several efficient methods can be employed by adopting such data in screening of viable candidates which accelerate the drug development stages to a greater extent (Ntie-Kang et al., 2013). Moreover, the success rate of drug development can be enhanced by employing this approach as it reduces the requirement for thorough experiments and trials (Bhambri & Jha, 2025d). Thus, in this investigation, the pharmacokinetic parameters of the selected ligands were retrieved from SwissADME for further analysis.

Chapter 3

Result

3.1 Docking Result

The binding interaction between the curated protein: CDK11 (7UKZ) and the co-crystallized ligand: OTS964 was re-validated in the *'PyRx'* application by doing a re-docking analysis and the best binding result of -9.8 kcal/mol has been generated; thus, this value is considered as the cut-off value in the evaluation of the docking scores yielded in the same application by doing docking study between the prepared protein and the listed 1,000 ligands.

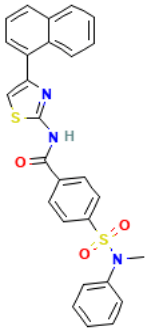
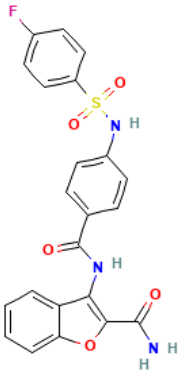
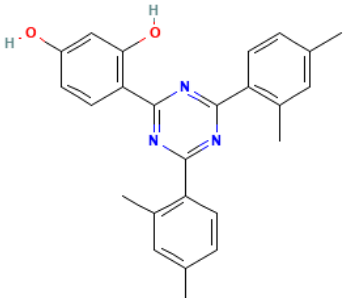
Table 4: Docking Scores of the Ligands (Top 21)				
SL no.	**Ligand Name**	**Chemical Structure**	**Binding Affinity (kcal/mol)**	**Molecular weight (M.W g/mol)**
1	Hit 1 (F0301-0274)		-11	499.60
2	Hit 2 (F2620-1120)		-10.4	453.44
3	Hit 3 (F9995-4301)		-10.2	397.47

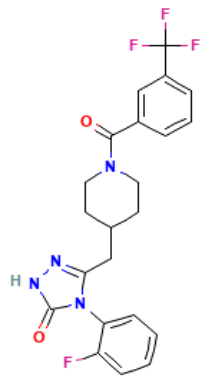
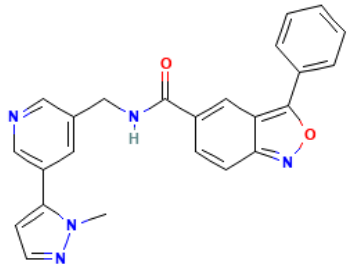
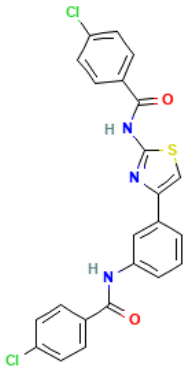
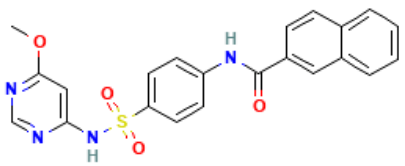
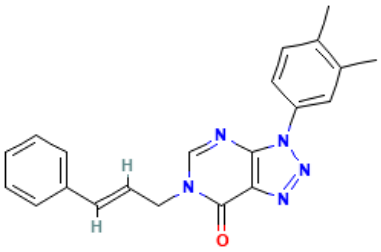
Table 4: Docking Scores of the Ligands (Top 21)				
4	Hit 4 (F6497-2297)		-10.2	448.41
5	Hit 5 (F6560-5940)		-10.2	409.44
6	Hit 6 (F0161-0185)		-10.1	468.36
7	Hit 7 (F3016-0125)		-10.1	434.5
8	Hit 8 (F1876-0824)		-10	357.4

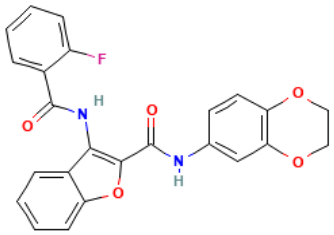
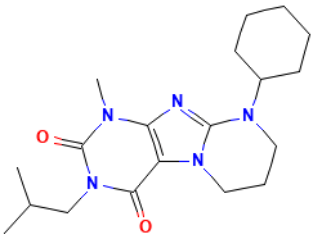
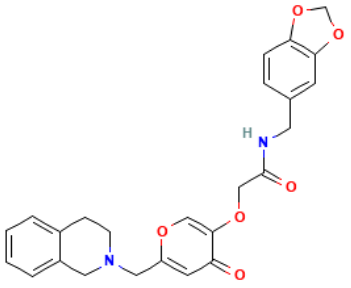
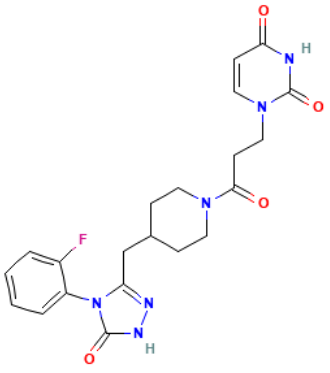
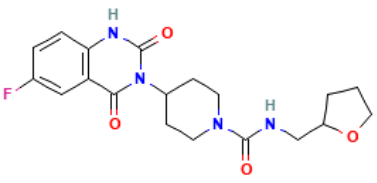
Table 4: Docking Scores of the Ligands (Top 21)				
9	Hit 9 (F1883-1570)		-9.9	432.4
10	Hit 10 (F2013-0276)		-9.9	359.5
11	Hit 11 (F2617-0652)		-9.9	448.5
12	Hit 12 (F6497-2232)		-9.9	442.4
13	Hit 13 (F6523-2294)		-9.9	390.4

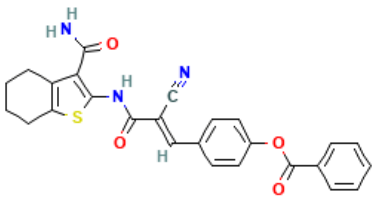
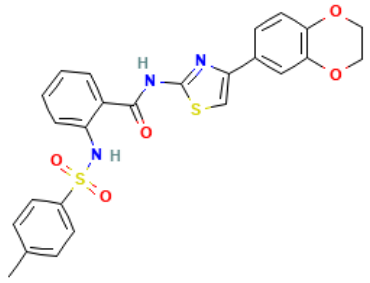
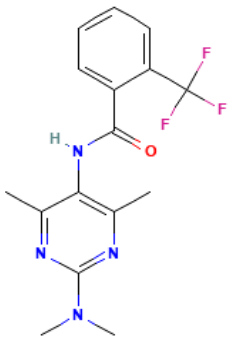
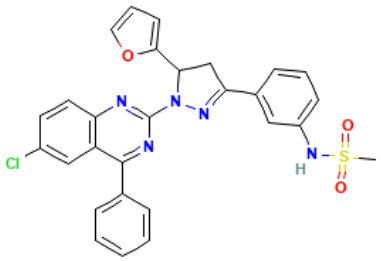
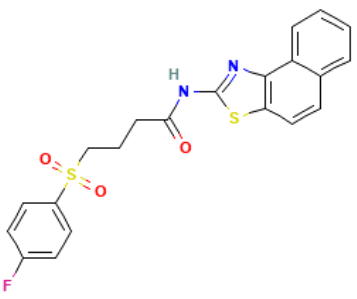
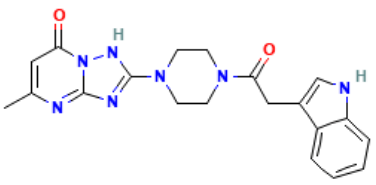
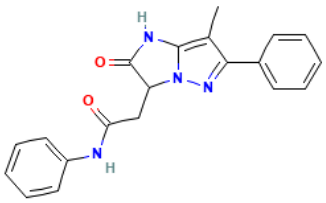
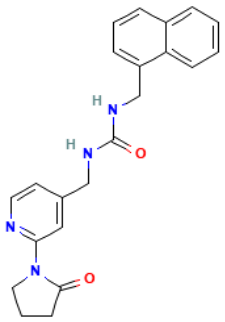
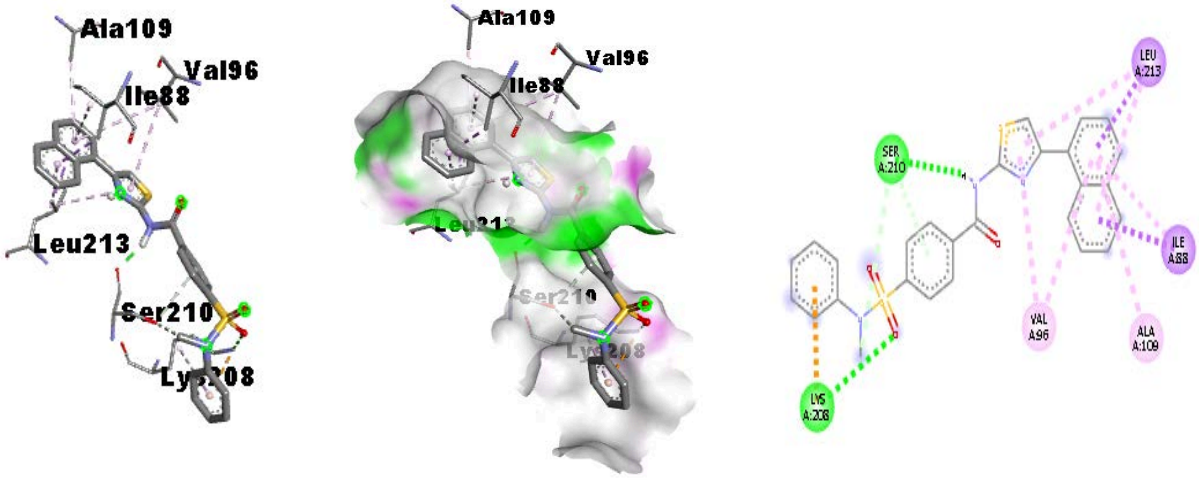
Table 4: Docking Scores of the Ligands (Top 21)				
14	Hit 14 (F0889-0304)		-9.8	471.5
15	Hit 15 (F1300-0376)		-9.8	507.6
16	Hit 16 (F6438-0019)		-9.8	338.33
17	Hit 17 (F1590-0153)		-9.7	544
18	Hit 18 (F2815-0253)		-9.7	428.5

Table 4: Docking Scores of the Ligands (Top 21)				
19	Hit 19 (F6252-6664)		-9.7	391.4
20	Hit 20 (F6278-0028)		-9.7	346.4
21	Hit 21 (F6562-2742)		-9.7	374.4

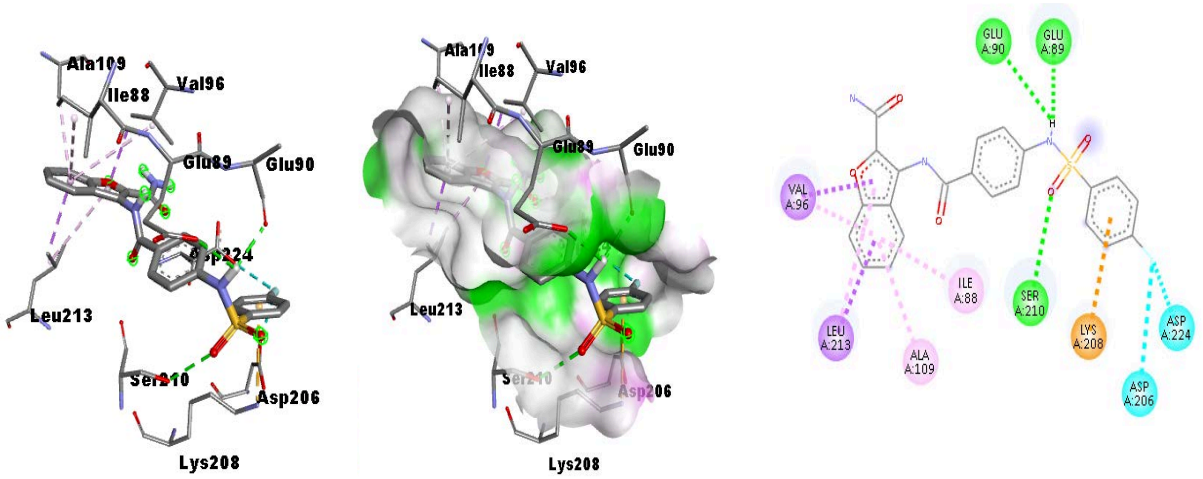
3.2 Protein-Ligand Interaction:

The types of chemical bonds established between the target protein CDK11 and the selected hit compounds- the ten candidates with the highest docking scores were studied comprehensively and visualized using *Discovery Studio 2024 Client* software. The analysis included non-covalent interactions, including hydrogen bond, hydrophobic interactions, pi-related contacts, and the electron cloud distortion around the hydrogen bond. Particularly, the protein amino acid residues that contributed to the interaction with the ligands were identified, and their interaction type was assessed in detail.

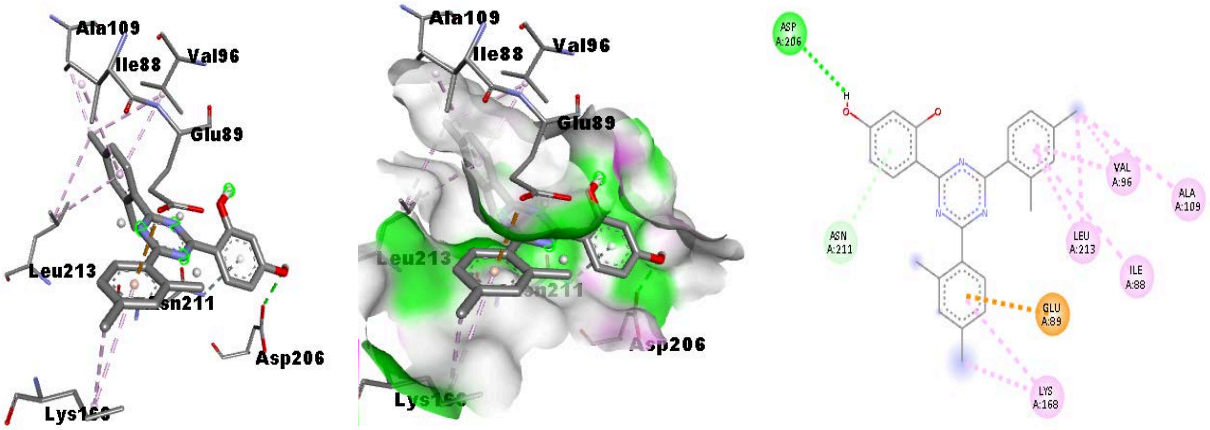
1. F0301-0274



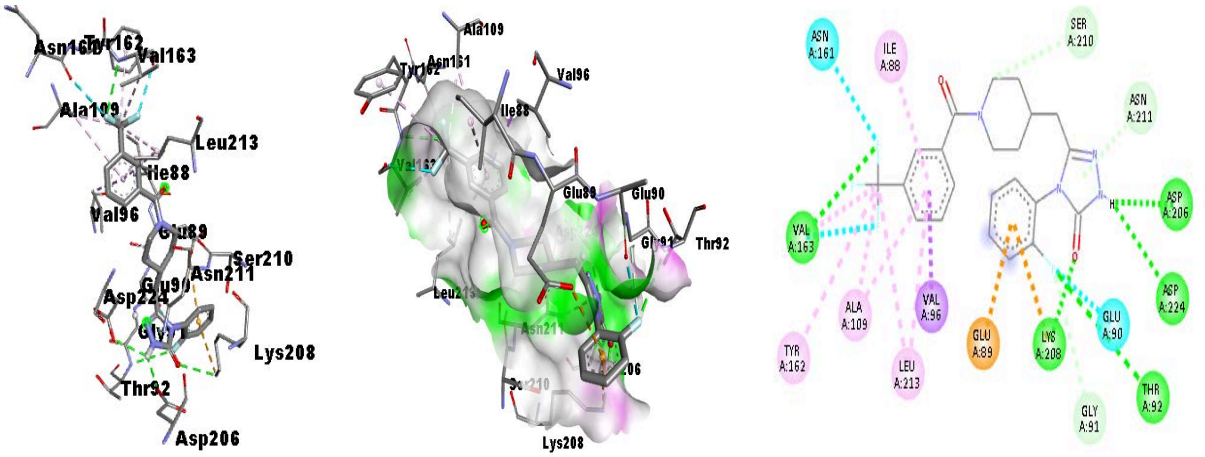
2. F2620-1120



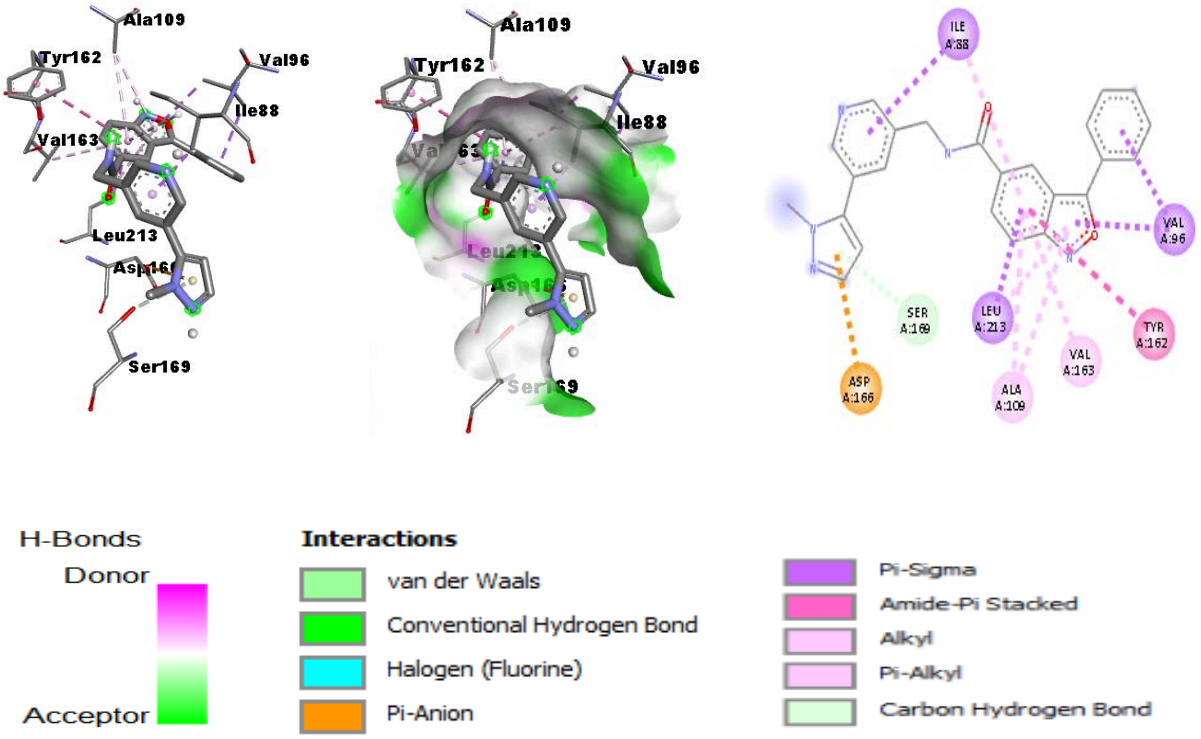
3. F9995-4301



4. F6497-2297



5. F6560-5940



3.3 Ligand Clustering Based on Chemical Class:

Table 5: Chemical Class-Based Compound Clustering

SL no.	Ligand Name	PUBCHEM COMPOUND_CID	Neighbor Cluster No	Neighbor Count	Neighbor Cluster Size	Neighbor Similarity FragFp 80%
1	F6438-0019	71807204	20	0	1	No similarity
2	F6278-0028	136220344	19	0	1	No similarity
3	F6252-6664	121016726	18	0	1	No similarity
4	F3016-0125	1184842	17	0	1	No similarity
5	F2815-0253	18585950	16	0	1	No similarity
6	F2620-1120	16822812	15	0	1	No similarity
7	F2617-0652	16830115	14	0	1	No similarity
8	F2013-0276	7011096	13	0	1	No similarity
9	F1883-1570	16818326	12	0	1	No similarity
10	F1876-0824	7200043	11	0	1	No similarity
11	F1590-0153	4368787	10	0	1	No similarity
12	F1300-0376	5079808	9	0	1	No similarity
13	F0889-0304	1564731	8	0	1	No similarity

Table 5: Chemical Class-Based Compound Clustering						
SL no.	Ligand Name	PUBCHEM COMPOUND_CID	Neighbor Cluster No	Neighbor Count	Neighbor Cluster Size	Neighbor Similarity FragFp 80%
1	F6438-0019	71807204	20	0	1	No similarity
2	F6278-0028	136220344	19	0	1	No similarity
3	F6252-6664	121016726	18	0	1	No similarity
4	F3016-0125	1184842	17	0	1	No similarity
14	F0301-0274	3447056	7	0	1	No similarity
15	F9995-4301	74276	6	0	1	No similarity
16	F6562-2742	126853915	5	0	1	No similarity
17	F6560-5940	121022403	4	0	1	No similarity
18	F6523-2294	91632156	3	0	1	No similarity
19	F0161-0185	5220576	2	0	1	No similarity
20	F6497-2297	91628672	1	1	2	0.82796
21	F6497-2232	91628668	1	1	2	0.82796

The F6497-2297 and F6497-2232 compounds were grouped into a single category (Table 5), as they share nearly 80% FragFp (Fragment Fingerprint) similarity and belong to the same chemical class.

3.4 Pharmacokinetic Parameter Analysis:

According to the login-free website SwissADME, developed by researchers Daina and colleagues in 2017, the following data were collected, and Table 6-8 was generated for further analysis. According to Table 6, among the top five candidates, only Hit-4 and Hit-5 are moderately water soluble; the rest of the three, Hit-1, 2, and 3, are poorly soluble in water, and none of them exhibit reactivity with PAINS or Brenk. Additionally, Table 7 Hit-1,2 shows a relatively lower value, though all the top five hits do not cross the blood-brain barrier, and some of them act as inhibitors of certain CYP enzymes. As shown in Table 8, all top five candidates exhibit similar availability, but differ individually in compliance with the following drug-likeness rules.

Table 6: Physiochemical Properties									
Ligand Name	**Molecular Weight (g/mol)**	**Lipophilicity $LogP_{o/w}$**	**Solubility Class LogS**	**Water Solubility**	**Medicinal Chemistry**				
					Molar Refractivity	**Synthetic Accessibility**	**PAINS (Pan-Assay Interference Compounds)**	**Brenk**	**TPSA (Topological Polar Surface Area) Å²**
F0301-0274	499.60	5.74	-7.94	Poorly Soluble	141.29	3.47	0 Alert	0 Alert	115.99
F2620-1120	453.44	3.54	-6.16	Poorly Soluble	115.59	3.41	0 Alert	0 Alert	139.88
F9995-4301	397.47	5.71	-7.14	Poorly Soluble	120.04	3.02	0 Alert	0 Alert	79.13
F6497-2297	448.41	3.75	-4.93	Moderately Soluble	112.56	3.21	0 Alert	0 Alert	70.99
F6560-5940	409.44	3.04	-4.51	Moderately Soluble	117.21	3.36	0 Alert	0 Alert	85.84
F0161-0185	468.36	5.87	-7.73	Poorly Soluble	126	3.03	0 Alert	0 Alert	99.33
F3016-0125	434.47	3.53	-5.71	Moderately Soluble	117.36	2.99	0 Alert	0 Alert	118.66
F1876-0824	357.41	3.85	-4.92	Moderately Soluble	106.53	3.30	0 Alert	0 Alert	65.60
F1883-1570	432.40	4.70	-6.31	Poorly Soluble	115.47	3.59	0 Alert	0 Alert	89.80
F2013-0276	359.47	3.23	-4.27	Moderately Soluble	108.22	3.42	0 Alert	0 Alert	65.06
F2617-0652	448.47	2.33	-3.86	Soluble	123.37	3.67	0 Alert	0 Alert	90.24
F6497-2232	442.44	0.13	-2.33	Soluble	118.13	3.45	0 Alert	0 Alert	125.85
F6523-2294	390.41	1.01	-2.62	Soluble	105.18	3.75	0 Alert	0 Alert	96.43
F0889-0304	471.53	5.19	-8.10	Poorly Soluble	129.73	3.47	0 Alert	0 Alert	115.99
F1300-0376	507.58	4.94	-7.69	Poorly Soluble	134.71	3.68	0 Alert	0 Alert	143.24
F6438-0019	338.33	3.18	-4.07	Moderately Soluble	85.39	2.57	0 Alert	0 Alert	58.12
F1590-0153	544.02	5.33	-7.37	Poorly Soluble	156.34	4.48	0 Alert	0 Alert	109.07
F2815-0253	428.50	4.46	-6.55	Poorly Soluble	113.63	3.36	0 Alert	0 Alert	112.75
F6252-6664	391.43	0.49	-2.21	Soluble	116.18	3.12	0 Alert	0 Alert	102.39
F6278-0028	346.38	2.52	-3.76	Soluble	102.86	3.58	0 Alert	0 Alert	76.02
F6562-2742	374.44	2.30	-3.50	Soluble	111.84	2.68	0 Alert	0 Alert	74.33

Table 7: Pharmacokinetic Properties										
Ligand Name	**Molecular Weight (g/mol)**	**GI Absorption**	**BBB Permanent**	**$LogK_p$ (Skin permeation) cm/s**	**P-gp substrate**	**CYP1A2 inhibitor**	**CYP2C19 inhibitor**	**CYP2C9 inhibitor**	**CYP2D6 inhibitor**	**CYP3A4 inhibitor**
F0301-0274	499.60	Low	No	-5.27	No	No	Yes	Yes	No	Yes
F2620-1120	453.44	Low	No	-6.55	No	Yes	Yes	Yes	No	Yes
F9995-4301	397.47	High	No	-4.67	Yes	No	Yes	No	Yes	No
F6497-2297	448.41	High	No	-6.37	No	Yes	Yes	Yes	No	No
F6560-5940	409.44	High	No	-6.64	Yes	Yes	Yes	Yes	Yes	Yes
F0161-0185	468.36	Low	No	-4.99	No	No	Yes	Yes	Yes	Yes
F3016-0125	434.47	High	No	-6.44	No	Yes	Yes	Yes	Yes	Yes
F1876-0824	357.41	High	Yes	-5.75	No	Yes	Yes	Yes	No	No
F1883-1570	432.40	High	No	-5.60	No	Yes	Yes	Yes	Yes	Yes
F2013-0276	359.47	High	Yes	-6.20	No	No	Yes	Yes	Yes	No
F2617-0652	448.47	High	No	-7.38	Yes	No	Yes	Yes	Yes	Yes
F6497-2232	442.44	High	No	-8.91	Yes	No	No	No	No	No
F6523-2294	390.41	High	No	-7.96	Yes	No	No	No	No	No
F0889-0304	471.53	Low	No	-5.49	No	No	Yes	Yes	No	Yes
F1300-0376	507.58	Low	No	-5.89	No	No	Yes	Yes	No	Yes
F6438-0019	338.33	High	Yes	-6.11	No	Yes	Yes	No	Yes	No
F1590-0153	544.02	Low	No	-5.83	No	Yes	Yes	Yes	No	Yes
F2815-0253	428.50	Low	No	-5.75	No	Yes	Yes	Yes	No	Yes
F6252-6664	391.43	High	No	-8.34	Yes	No	No	No	No	No
F6278-0028	346.38	High	Yes	-6.62	Yes	No	Yes	Yes	Yes	Yes
F6562-2742	374.44	High	Yes	-6.95	Yes	No	Yes	Yes	Yes	Yes

Table 8: Drug Likeness							
Ligand Name	**Molecular Weight (g/mol)**	**Bioavailability score**	**Lipinski**	**Ghose**	**Veber**	**Egan**	**Muegge**
F0301-0274	499.60	0.55	Yes	No	Yes	No	No
F2620-1120	453.44	0.55	Yes	Yes	Yes	No	Yes
F9995-4301	397.47	0.55	Yes	Yes	Yes	Yes	No
F6497-2297	448.41	0.55	Yes	Yes	Yes	Yes	Yes
F6560-5940	409.44	0.55	Yes	Yes	Yes	Yes	Yes
F0161-0185	468.36	0.55	Yes	No	Yes	No	No
F3016-0125	434.47	0.55	Yes	Yes	Yes	Yes	Yes
F1876-0824	357.41	0.55	Yes	Yes	Yes	Yes	Yes
F1883-1570	432.40	0.55	Yes	Yes	Yes	Yes	Yes
F2013-0276	359.47	0.55	Yes	Yes	Yes	Yes	Yes
F2617-0652	448.47	0.55	Yes	Yes	Yes	Yes	Yes
F6497-2232	442.44	0.55	Yes	Yes	Yes	Yes	Yes
F6523-2294	390.41	0.55	Yes	Yes	Yes	Yes	Yes
F0889-0304	471.53	0.55	Yes	Yes	No	No	No
F1300-0376	507.58	0.55	Yes	No	No	No	Yes
F6438-0019	338.33	0.55	Yes	Yes	Yes	Yes	Yes
F1590-0153	544.02	0.55	Yes	Yes	Yes	Yes	Yes
F2815-0253	428.50	0.55	Yes	Yes	Yes	No	Yes
F6252-6664	391.43	0.55	Yes	Yes	Yes	Yes	Yes
F6278-0028	346.38	0.55	Yes	Yes	Yes	Yes	Yes
F6562-2742	374.44	0.55	Yes	Yes	Yes	Yes	Yes

Chapter 4

Discussion

The *in silico* study of CDK11 in the software *PyRx* was performed against 1,000 ligands selected from a kinase-focused library, 'Life Chemicals'. The focused molecular docking was performed using the same binding pocket and amino acid residues as the existing CDK11 inhibitor OTS964, in order to compare the docking scores of the selected ligands with the standard binding affinity of OTS964 for the protein CDK11. Based on the generated docking scores by *PyRx*, out of the top 21 compounds, the top 5 ligands has exhibited binding affinities exceeding the standard range and the rest of the 16 compounds have shown binding affinity within the range of -9.82 ± 0.16 kcal/mol - the standard binding affinity of the existing inhibitor OTS964 (Kelso et al., 2022b). Following this, the binding interactions, the type of bonds the ligands have formed with the amino acid residues of the protein, and the bond distance of the top-scoring 21 ligands with the protein CDK11 were all analyzed by using the *Discovery Studio 2024 Client* software. Later, using the free application *DataWarrior*, all the screened-out ligands were clustered into 20 different chemical classes based on their structural similarities and presence of pharmacophores (Sander et al., 2015) and organized into Table 05 Chemical class-based compound clustering. According to the table, only F6497-2297 and F6497-2232 have a similarity of FragFp 80% and were clustered into a single group; meanwhile, the rest were clustered individually, given their unique chemical orientation which means the top five hits belong to different chemical classes. Furthermore, the pharmacokinetic parameters of all the screened 21 candidates were retrieved from the website SwissADME, and Table 06-08 was compiled summarizing the comprehensive data collected from the website.

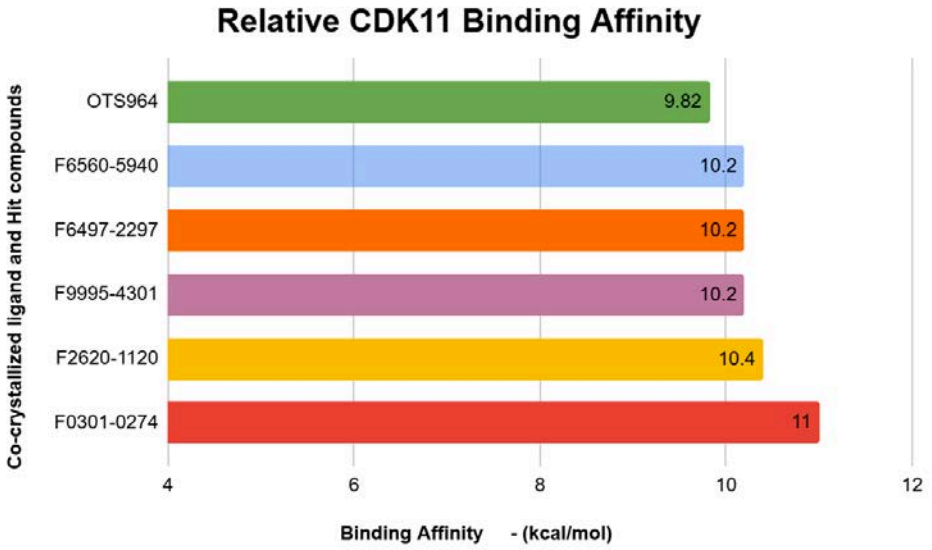


Figure 10: Binding affinity of the selected top five ligands relative to the existing CDK11 inhibitor OTS964.

However, based on the binding affinity, among the shortlisted 21 ligands, only the top 5 scored hits featuring exceeding docking scores (> -9.82 ± 0.16 kcal/mol) comparable to the reference CDK11 inhibitor OTS964 (*Figure 10*) is discussed further, focusing on their interaction profile, pharmacokinetic properties, and their potential as a drug candidate. The interaction profile of the highest-ranking ligand, Hit-1, including its binding residues within the CDK11 active site, is depicted in *Figure 11-12*. *Figure 12* illustrates the docking interaction of the Hit-1 (shown in red) within the active site of the target protein (yellow ribbon structure). The key amino acid residues - Val96, Ala109, Leu213, Ser210, and Lys209 are involved in stabilizing the ligand through Pi-alkyl, Pi-sigma, and hydrogen bonds.

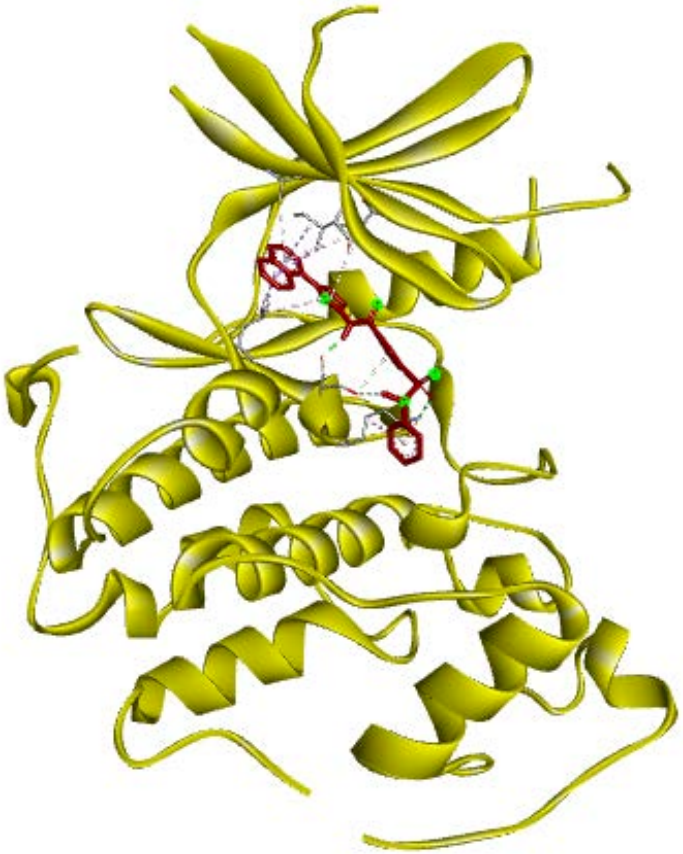


Figure 11: Interaction profile of Hit-1 with CDK11.

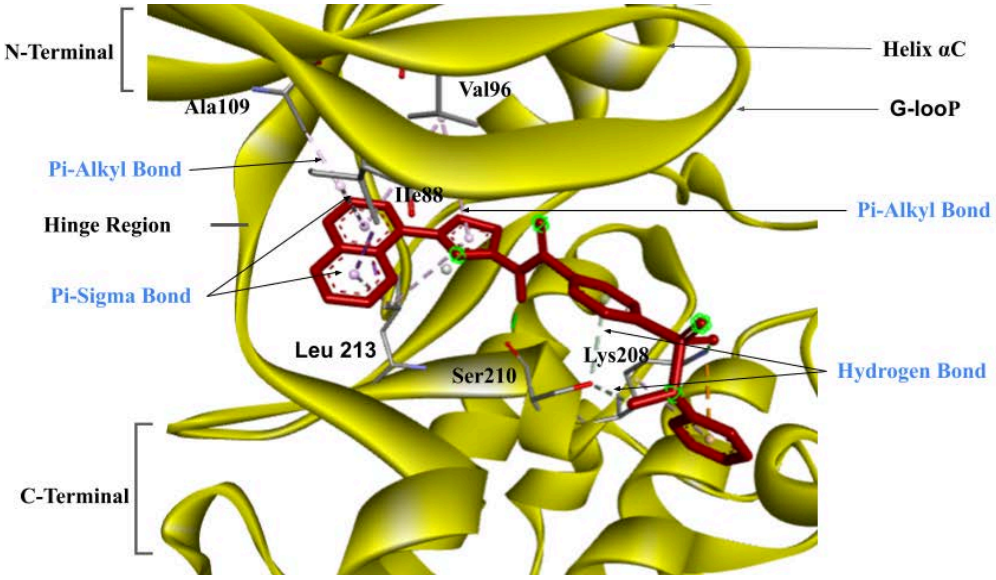


Figure 12: Docked pose of Hit-1 with interacting residues of the CDK11 protein

Further, according to 3.2 Protein-ligand interaction, a summarized table of types of bonds formed between the target protein CDK11 and the top five ligands is compiled below:

Table 9: Possible Bond Analysis Between the Top 5 Hits and CDK11 Protein In Comparison With the Co-Crystallized Ligand OTS964							
	Hydrogen Bond	**Pi-Sigma**	**Pi-Akyl**	**Pi-Anion**	**Halogen**	**Carbon Hydrogen Bond**	**Amide-pi stacking**
							
OTS964	TYR162, VAL163	ILE88, LEU213, VAL96	VAL96, ALA109, MET260	GLU164, ASP166, AS224		ASP224	
Hit 1	SER210, LYS208,	LEU213, ILE88	ALA109, VAL96				
Hit 2	GLU89, GLU90, SER210	VAL96, LEU213	ALA109, ILE88	LYS208	ASP206, AS224		
Hit 3	ASP206,	ILE88, VAL96, ALA109,	ILEU213 , LYS168	GLU89		ASN211	
Hit 4	VAL163, AS224	VAL96	ILE88, LEU213, ALA109	GLU89	ASN261, GLU90	SER210, ASN211	
Hit 5		ILE88, LEU213, VAL96	ALA109, VAL163	ASP166		SER169	TYR162

Moreover, all five candidates have a $LogP_{o/w}$ value ranging from 3-5 (Table 6), indicating higher lipophilicity; none of them crosses the BBB (blood brain barrier) as the logP value ranging from 1-3 is appropriate for BBB permeation (Pajouhesh & Lenz, 2005). Besides, Hit-3, 4, and 5 have higher GI absorption compared to the other two. Even though all of the top five hits pass the PAINS and Brenk filter with 0 alerts and satisfy the Lipinski rule of Five, only the Hit-5 (F6560-5940) complies with all five filters of drug likeness- Lipinski, Ghose, Veber, Egan, and Muegge. However, Hit-5 uniquely exhibits the property of inhibiting all the enzymes - CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4, along with getting transported out by the membrane-bound efflux pump P-gp substrate, making it less potent to become a viable drug candidate. Additionally, all the top five candidates have the $LogK_p$

value ranging from -4.67 to -6.67 mentioned in Table 07, suggesting moderate skin permeability (Potts & Guy, 1992). The final remark regarding the selected five ligands consists of a 0.55 bioavailability score, and can be predicted to have moderate to good bioavailability (Martin, 2005). Lastly, F0301-0274, F2620-1120, F9995-4301, F6497-2297, and F6560-5940 all scored ranging from 3.02-3.47, implying that they are moderately easy to synthesize chemically (Ertl & Schuffenhauer, 2009).

Chapter 5

Conclusion

Globally, cancer is the second leading cause of death worldwide. Although there are several treatment options available, namely chemotherapy, hormone therapy, immunotherapy, radiation therapy, and targeted therapy, most of the treatment strategies are often associated with severe side effects and resistance due to their lack of selectivity. Therefore, these challenges highlight the significance of discovering more specific compounds for developing advanced anti-cancer targeted therapy. Moreover, according to recent studies, overexpression of CDK11 is often observed in various malignancies, including osteosarcoma, liposarcoma, multiple myeloma, breast cancer, cervical cancer, and acute myeloid leukemia, signifying its potency as a target for anti-cancer drug discovery. Considering the prominent role of CDK11 in cancer cell proliferation, survival, and resistance to cell apoptosis, the discovery of CDK11 inhibitors may shed light on advanced targeted therapy against cancer. Hence, identification of potent and specific inhibitory ligands interacting at the active site of the targeted protein, CDK11, can provide a crucial insight into the development of more selective and precise anti-cancer therapy. Supporting this rationale, the objective of this study was to perform virtual screening of potential CDK11 inhibitors from a kinase focused library, using existing inhibitor OTS964 as a standard. Among the 1,000 compounds from the online-based kinase-focused library ‘Life Chemicals’, only the top five candidates were discussed

extensively as they have better binding scores than the standard range. The top five hit compounds are F0301-0274, F2620-1120, F9995-4301, F6497-2297, and F6560-5940, which may have the potential to function as CDK11 inhibitors and play a significant role in targeted cancer treatment. However, their IC_{50} values have not yet been determined, making biological assays essential for validating their inhibitory activity and potential as anti-cancer drugs. Hence, further research may open a new door in cancer therapy, specifically targeting over-expressed CDK11.

Chapter 6

Future work

This study has provided potential CDK11 inhibitors through computational screening and ADME profiling; however, future investigation is essential for experimental validation. Particularly, in order to strengthen and expand the current findings, several aspects need to be studied comprehensively. Most importantly, biological assays - *In Vitro* and *In Vivo* validation is crucial to determine their IC_{50} values against CDK11 and establish potential inhibitors of CDK11. Ideally, the IC_{50} values of these hit candidates should be equivalent to or lower than the standard inhibitor's (OTS964) IC_{50} value of 28 nM to be considered potent. Moreover, the selectivity profile must be analyzed by docking the potential candidates against other CDKs of the kinase family for specificity and off-target analysis. Additionally, the future work should also be focused on hit to Lead optimization by performing molecular dynamic simulations to assess the stability of ligand-protein interactions, structural modification to optimize the therapeutic action also SARs (Structure-Activity Relationships) study, pharmacophore study, and so on. For further lead optimization, candidates must undergo toxicity profiling and animal studies for assessment of drug efficacy, safety, and quality. To advance toward clinical relevance, the efficacy of the lead compounds must be evaluated in

cancer cell lines to establish their potential as anti-cancer drugs. Hence, determination of IC_{50} value by comprehensive biological assay and further lead optimization are the key steps toward the development of CDK11-focused anti-cancer therapy.

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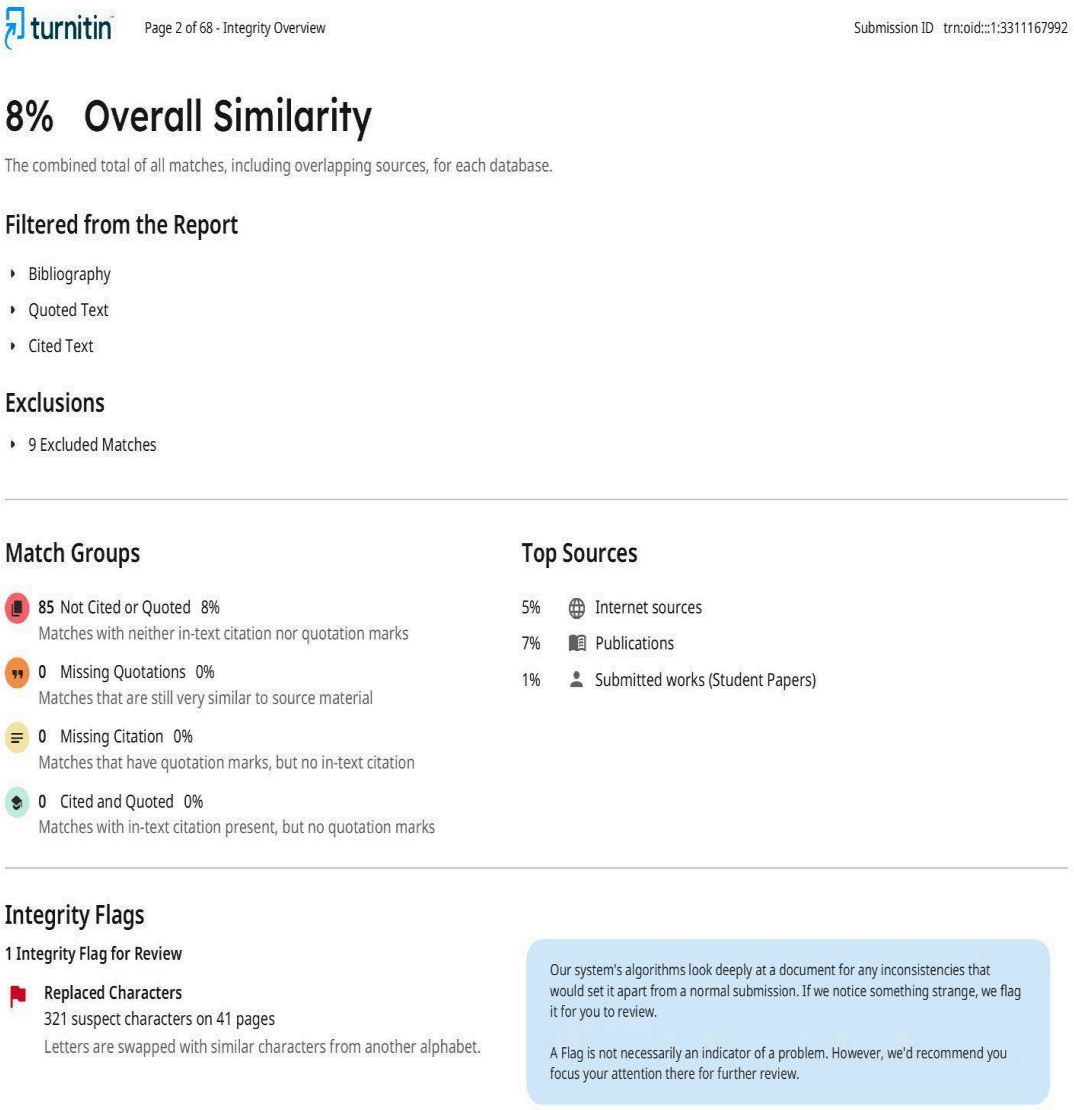
Appendix A.

This academic paper includes statements addressing issues of plagiarism.



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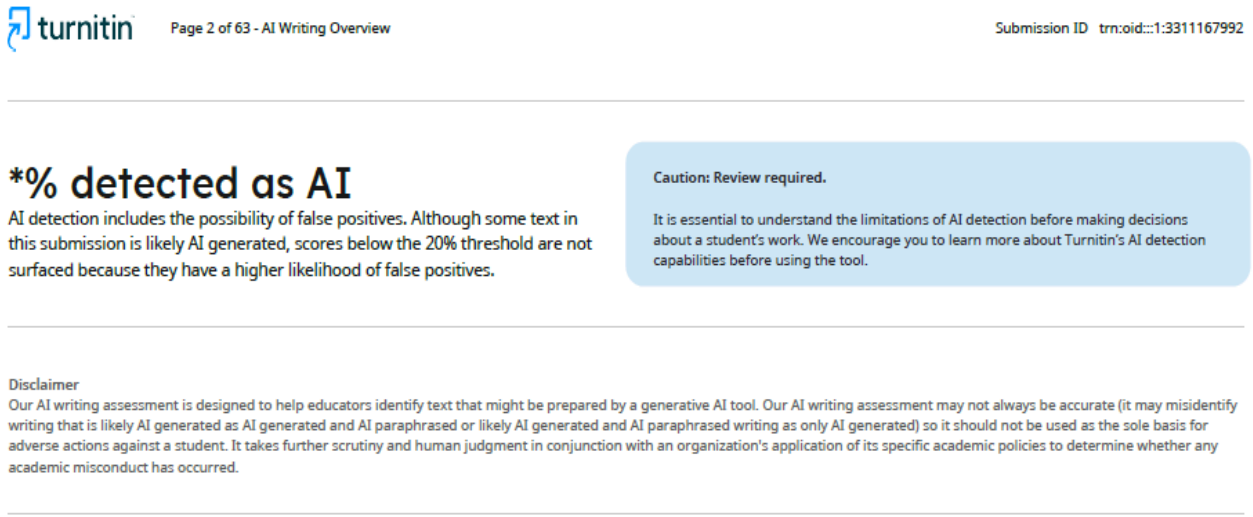

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