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HIGH AFFINITY SMALL MOLECULE CDK1 INHIBITORS FOR THE TREATMENT OF GLIOBLASTOMA

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PREFACE

This undergraduate thesis represents the culmination of my academic journey in the fields of molecular biology, molecular modelling and drug designing, and bioinformatics. The study of high affinity small molecule inhibitors of Cyclin-Dependent Kinase 1 (CDK1) for the treatment of glioblastoma captures the intersection of my academic interests and aspirations to contribute to the fight against cancer. Glioblastoma, known for its aggressiveness and resistance to conventional therapies, necessitates innovative research and novel treatment approaches.

Embarking on this research project has been both a challenging and rewarding experience. It required a comprehensive review of the existing literature, meticulous experimental design, and detailed data analysis. Throughout this process, I have been fortunate to receive guidance and support from a network of dedicated mentors and colleagues who have significantly shaped my understanding and approach to scientific inquiry.

The completion of this thesis would not have been possible without the encouragement and expertise of many individuals. Their contributions have been invaluable, and their passion for research has been a constant source of inspiration. This thesis is dedicated to all those affected by glioblastoma and to the scientific community's relentless pursuit of a cure.

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To my family and friends, thank you for your continuous encouragement, understanding, and patience during the demanding phases of this thesis. Your support has been a pillar of strength throughout this journey.

Lastly, I dedicate this work to the individuals and families affected by glioblastoma. It is my hope that this research will contribute to the development of more effective treatments and bring us closer to finding a cure.

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ABBREVIATIONS

- GBM - Glioblastoma
- IDH - Isocitrate Dehydrogenase
- TERTp - Telomerase Reverse Transcriptase Promoter
- EGFR - Epidermal Growth Factor Receptor
- CDK1 - Cyclin-Dependent Kinase 1
- FDR - False Discovery Rate
- |log2FC| - Absolute Log2 Fold Change
- GEPIA2 - Gene Expression Profiling Interactive Analysis 2
- STRING - Search Tool for the Retrieval of Interacting Genes
- ADMET - Absorption, Distribution, Metabolism, Excretion, and Toxicity
- PDB - Protein Data Bank
- NCBI - National Center for Biotechnology Information
- CASP - Critical Assessment of Methods of Protein Structure Prediction
- UniProt - Universal Protein Resource
- EMBOSS - European Molecular Biology Open Software Suite
- BLAST - Basic Local Alignment Search Tool
- SOPMA - Self-Optimized Prediction Method with Alignment
- CNS - Central Nervous System
- GCN2 - General Control Nonderepressible 2
- Akt - Protein Kinase B

CHAPTER 1 – INTRODUCTION

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1. INTRODUCTION

1.1. INTRODUCTION TO GLIOBLASTOMA

Glioblastoma or glioblastoma multiforme (GBM) is a type of cancer that affects the head, neck, and central nervous system, making it a significant health concern globally **[1]**. Within the category of cancers of the central nervous system, gliomas constitute approximately 26% of all CNS tumours **[2]** and 78% of all malignant CNS tumours, with glioblastoma constituting 14.2% of all CNS tumours and 50.9% of all malignant CNS tumours **[1]**. An incidence rate of 3.27 per 100,000 population, makes glioblastoma one of the most prevalent and aggressive subtypes **[2]**. According to WHO, it is classified as a grade 4 cancer, indicating its highly malignant nature and rapid progression **[3]**.

Among cancers affecting the head, neck, and central nervous system, glioblastoma stands out as particularly lethal, causing a disproportionate number of deaths. It is associated with a median overall survival (OS) of only 10 months **[4]**, underscoring its grave prognosis and the urgent need for effective treatment strategies. Despite advancements in medical science, there is currently no cure for glioblastoma. Even with treatment, which typically involves a combination of surgery, radiation, and chemotherapy, the likelihood of recurrence remains high, further complicating management and prognosis **[5]**.

Moreover, glioblastoma not only manifests with severe physiological symptoms but also imposes significant psychological effects on patients and their families. The aggressive nature of the disease, coupled with its poor prognosis, often leads to feelings of anxiety, depression, and distress among affected individuals **[6]**. This highlights the multifaceted burden of glioblastoma on both physical and mental well-being, emphasizing the importance of holistic patient care and support services.

Given the grim outlook associated with glioblastoma, there is an urgent imperative to develop more effective treatments. Current therapeutic options provide limited benefits and often come with substantial side effects, necessitating the exploration of novel approaches to combat this formidable cancer **[7]**. Research efforts are focused on identifying molecular targets, immunotherapeutic strategies, and personalized treatment regimens that can improve outcomes and prolong survival for patients with glioblastoma.

1.2. ORIGIN AND CLASSIFICATION OF GLIOBLASTOMA

Glioblastoma is classified as a type 4 malignancy, indicative of its severe nature and discouraging prognostic outlook **[3]**. However, the bleak prognosis associated with this condition can potentially be improved by developing a comprehensive understanding of the origin of glioblastoma cells. Such knowledge would significantly enhance diagnostic accuracy and enable the selection of more tailored and effective therapeutic interventions.

Prior to the 1960's, prevailing scientific belief held that the brain no longer retained proliferative capacity postnatally. However, this notion was disproved with the discovery of neural stem cells (NSC's). These NSC's were found to reside in the subventricular zone (SVZ) and sub-granular zone (SGZ) regions of the dentate gyrus (DG) of the hippocampus, also referred to as neurogenic niches **[8]**. NSC's are able to give rise to glial cells such as astrocytes and oligodendrocytes as well as neurons. Postnatally these differentiated cells will lose their proliferative capacity with the exception of oligodendrocyte progenitor cells (OPC's). Additionally, astrocytes are also thought to maintain a degree of replicative capability, particularly in response to brain trauma. Through their transformation and acquisition of tumour traits, these three cell types -NSC's, NSC's-derived astrocytes and OPC's- are thought to be the key contributors to the development of glioblastoma **[9-16]**. Moreover, the stem-cell like attributes possessed by these cells contribute to the significant heterogenicity displayed by glioblastoma tumour as well as the high rate of reoccurrence.

Glioblastoma is commonly categorised into two primary classifications based on the tumour's developmental pathway: de novo, indicating the formation of a primary glioblastoma tumour, or secondary glioblastoma, arising from a less aggressive precursor lesion such as low-grade or anaplastic astrocytoma. **[17]**. However, the categorization of these tumour types has remained a difficult task as histologically they were almost indistinguishable **[17,18]**. The latest 2021 WHO update incorporates recommendations from cIMPACT-NOW (Consortium to Inform Molecular and Practical Approaches to CNS Tumour Taxonomy – Not Official WHO). These recommendations advocate for the inclusion of molecular parameters as biomarkers for tumour grading and prognosis purposes. Hence, contemporary classification systems entail a layered approach that combines molecular characteristics with tumour histology and morphology. **[3]**.

One particular gene dictating the diagnosis of glioblastoma is the IDH gene (isocitrate dehydrogenase). Glioblastoma diagnosis is exclusively reserved for cases where the IDH gene is wild-type (IDH-WT). Furthermore, in the latest update, WHO has discontinued the

terminology “glioblastoma multiforme”. Instead, all tumours falling into this category will now be referred to as “glioblastoma”. This decision stems from the recognition that the term “multiforme” inadequately represents the heterogenous nature of these tumours. Consequently, the focus has shifted towards identifying the various subtypes of glioblastoma like gliosarcoma, giant cell glioblastoma, and epithelioid cell glioblastoma, each characterized by unique genotypic and morphological features **[19,20]**.

Additionally, lower grade diffuse astrocytic IDH wildtype gliomas that exhibit any one of the following characteristics- microvascular proliferation, necrosis, TERTp mutation, EGFR gene amplification, and combined +7/-10 chromosomal abnormalities – are classified as “glioblastoma IDH-WT” **[19-23]**.

The implementation of layered classification schemes is not only crucial for simplifying diagnostic categorization but also for informing prognosis and guiding treatment decisions **[24]**. Accurate classification allows clinicians to tailor treatment strategies based on the specific molecular characteristics of the tumour, maximizing therapeutic efficacy and minimizing adverse effects. Additionally, understanding the molecular mechanisms underlying glioblastoma development and progression may lead to the identification of novel therapeutic targets and the development of more effective treatment approaches.

1.3. SIGNS AND SYMPTOMS OF GLIOBLASTOMA

Glioblastoma is a type of aggressive brain tumour that can present with a variety of signs and symptoms. Symptoms can vary depending upon the location, size of the tumour, and the rate of growth of the tumour, as well as the individual patient. Glioblastoma can have physiological and psychological effects, which can manifest as either transient or permanent in nature. Given the brain’s high sensitivity, any tumour growth within this vital organ also poses a grave risk of permanent cognitive deficit, severely impairing the patient’s quality of life, even after cancer remission **[25]**.

Headaches are often the first and most common symptoms of glioblastoma. Unlike normal headaches, those induced by brain tumours tend to increase in frequency and severity as the disease advances. Moreover, they often prove to be resistant to conventional over-the-counter headache medications **[26]**. These headaches stem primarily from the increased intracranial pressure (ICP) caused by tumour expansion within the confined skull cavity. The pain is often

persistent and may worsen when bending down, exercising or coughing. Headaches may also cause nausea or vomiting **[27]**. Headaches are very rarely standalone symptoms; patients may also experience general pain and fatigue.

Glioblastoma tumours, depending on their location, can exert pressure on specific regions critical for movement, speech, or other vital functions. This pressure can lead to significant impairments, such as motor deficits, communication difficulties and can present themselves as signs and symptoms. For example, interruptions of the optic pathways, such as the optic nerve, may lead to blurred vision, diplopia or other visual deficits **[28]**. Similarly, compression of the motor strip or cerebellum by tumour growth may result in gait impairment, ataxia, hemiparesis, myopathy, proximal bilateral leg weakness or overall motor weakness **[29, 30]**.

Elevated ICP significantly contributes to the cognitive decline observed in glioblastoma patients. This decline manifests as a diverse range of neurological impairments and language disorders, including memory loss, confusion, mental dulling, dysphasia (difficulty speaking), aphasia (impaired language comprehension) or drowsiness. Beyond these cognitive deficits, patients may experience focal or generalized seizures. In more severe and progressed cases, seizures may lead to a rapid decline into states of stupor or coma **[31-37]**.

Glioblastoma may also manifest as psychiatric symptoms. Studies have demonstrated a surprisingly high prevalence of psychiatric symptoms in glioblastoma patients, with one study reporting a staggering 78% prevalence across a cohort of 530 individuals **[38]**. Depression emerges as the most common psychiatric manifestation, affecting nearly half (44%) of individuals with brain tumours **[39]**. Other psychiatric symptoms include apathy, mania, anxiety disorders, psychosis, eating disorders, hallucinations and personality changes. In rare cases, these psychiatric symptoms can serve as the initial clinical presentation, preceding the emergence of traditional neurological signs associated with primary brain tumours **[6,40-42]**. It is hence imperative for healthcare professionals to maintain a high degree of suspicion for neurological etiologies in individuals presenting with psychiatric disturbances. Recognising the potential for glioblastoma to manifest though psychiatric symptoms will ensure timely and accurate diagnosis and treatment.

1.4. CAUSES AND RISK FACTORS OF GLIOBLASTOMA

Glioblastoma, a lethal form of malignant brain cancer, has several identified causes and risk factors, although its exact etiology remains complex and multifactorial. Some commonly studied and debated causes and risk factors are as follows.

Exposure to ionizing radiation is one of the most well known and established risk factors not just for glioblastoma but for various other cancers as well. Studies have repeatedly shown that individuals who have undergone radiotherapy for other cancers, particularly those involving the head, are at a significantly higher risk for developing gliomas later in life **[43]**. The carcinogenic effects of ionizing radiation are due to its ability to cause direct DNA damage, often leading to mutations and genomic instability, which can further progress into cancer **[44]**.

Head injuries are a debated risk factor for glioblastoma. While epidemiological studies have generally not uncovered any correlation, some experimental studies suggest otherwise. Upon examining these discrepancies, many researches have proposed that there may be additional factors that are involved in post-trauma glioma formation, which epidemiological studies might not have considered **[45]**. These factors may include, but not limited to, genetic predispositions or environmental exposures that interact with the injury to promote tumour development.

Obesity, especially in early adulthood, has been linked to an increase risk of gliomas. Studies found that individuals who were obsessed at the age of 18 were nearly four times as much at risk of developing gliomas compared to those with a normal body-mass index (BMI) **[46].** Furthermore, a lower BMI, (BMI < 18.5 kg/m²) at the age of 21, was also associated with a lower risk of developing gliomas, particularly in women **[47].** However, the relationship between diet, metabolism and risk of development of glioblastoma remains blurry and researchers are actively seeking to establish a clear relationship. It is hypothesized by some studies that there is an inverse relationship between high fat/cholesterol intake and glioma incidence **[48].**

Similar to other cancers, an increase in height is often associated with greater risk of glioblastoma development. Studies report that individuals with heights over 190 cm have more than twice the risk of developing gliomas compared to those under 160 cm. However, there are some studies that oppose this claim and oppose that there is any significant link between height and glioma incidence, and instead, is caused by other environmental factors **[44]**.

Another disputed risk factor is alcohol consumption. While alcohol can cross the blood brain barrier and potentially effect glial cells, studies have not established a consistent and significant

association between alcohol consumption and glioma incidence **[49]**. Studies have reported no link, with some evidence suggesting hat low to moderate alcohol consumption may even lower the risk of glioma **[48**].

Sex hormones are found to influence men and women vert differently. Some studies suggesting that female sex hormones may have protective effects against glioblastoma. Researchers have observed a decrease risk in women using hormonal contraception or hormone replacement therapy **[50]**. However, other studies have not found any significant effect of estrogen on the likelihood of glioma development **[51]**. It is interesting to note that men are at a higher risk of glioblastoma development compared to women.

Nonsteroidal Anti-Inflammatory Drugs (NSAIDs), particularly aspirin, may reduce the risk of glioblastoma, owing to their anti-inflammatory properties as well as inhibition of prostaglandin E2 synthesis. Studies have conflicting opinions with some indicating potential protective effect, while others report no significant correlation **[52-54]**. The heterogenicity of glioblastoma may be the root of these discrepancies.

Another drug class that has been investigated for its correlation and potential protective effects against glioblastoma is anti-histamines **[55]**. Many studies state that individuals with allergies or asthma, who take antihistamines, are at a reduced risk of glioma development. However, at this time there is no concrete evidence to support these claims **[56]**.

Statins, widely used for their cholesterol lowering effects, have also been examined for their potential anti-cancer properties. Some researchers suggest that statins may reduce glioma risk, although the exact cause and mechanisms of this correlation are not known **[57]**. Due to this, many claim that there is no actual statistically significance.

Similar to other cancers, a family history of glioblastoma is a significant risk factor, indicating a genetic predisposition to the disease. Additionally, glioblastoma has an established correlation with age, with the majority of cases occurring in individuals over 50 years old **[58]**.

1.5. TREATMENT OPTIONS FOR GLIOBLASTOMA

One of the major challenges of glioblastoma which contribute to its lethality is its resistance to standard therapies. The current standard of therapy is chemotherapy with temozolomide (TMZ) along with radiotherapy, after maximal surgically resection of tumour tissue from the brain. Even

after this highly aggressive treatment modality, the median survival of patients is only 15 months, with a five-year survival rate of less than 5% **[60]**. Due to this, many researchers are focusing their effort to develop innovative treatments aimed to enhance patient outcomes.

Temozolomide (TMZ), an oral alkylating agent, is the primary chemotherapy drug used for glioblastoma. It is usually given alongside radiotherapy and subsequently for 6 maintenance cycles. This regime has been shown to enhance the survival compared to standalone radiotherapy. Nevertheless, the development of resistance to TMZ is a major challenge, often leading to tumour recurrence **[61]**.

Researchers are therefore investigating various innovative treatment strategies to overcome these limitations and improve patient outcomes. These strategies include immunotherapy, targeted therapy, gene therapy and nanomedicine.

Immunotherapy aims to boost the body's immune system's ability to identify and attack tumour cells. One promising immunotherapy approach is chimeric antigen receptor T-cell (CAR-T) therapy, which involved genetically modifying a patients T-cells to express receptors targeting tumour antigens. A phase I clinical trial targeting the protein EGFRvIII in patients with recurrent glioblastoma showed a median overall survival of 11.1 months, indicating potential effectiveness **[62]**.

Immune checkpoint inhibitors, an alternative form of immunotherapy, work by blocking portions that prevent the bodies immune cells from attacking cancer cells. One example is the drug prembrolizumab, an anti-PD-1 antibody. However, this drug was evaluated till phase II trials and did not significantly improve overall survival in patients with recurrent glioblastoma **[63]**. Regardless, research continues into the potential benefits of combining these checkpoint inhibitors with other therapies so as to potentially enhance patient prognosis.

Targeted therapies are another area of active research. These therapies aim to inhibit specific molecular pathways crucial for tumour growth and progression. Some examples include proteasome inhibitors such as bortezomib, have shown promise when combined with standard care, achieving a median overall survival of 21.4 months in recently diagnoses glioblastoma patients **[64]**. EGFR inhibitors like erlotinib, have also been studied, though a phase II clinical trial reported no significant improvement in survival **[65]**. Angiogenesis inhibitors, such as bevacizumab, aim to disrupt the formation of new blood vessels that are essential for tumours to grow. Despite promising preclinical trials, this drug also did not improve patient survival **[66]**.

Cyclin-dependent kinase (CDK) inhibitors, particularly those targeting CDK1 are now emerging as potential therapeutics for glioblastoma. CDK1 is essential for cell cycle progression and its inhibition can lead to cell cycle arrest and eventual cell death in tumour cells. Early-phase pre-clinical and clinical trials have ongoing **[67].**

Gene therapy offers another innovative approach by introducing genetic material into cancer cells to modify their behaviour. For instance, viral gene therapy uses engineered viruses to deliver therapeutic genes directly into tumour cells. Toa 511, a retroviral vector that delivers a suicide gene, showed a median overall survival of 14.4 months in combination with standard care **[68]**. Another example is cell therapies like ICT-107, which is a dendritic cell vaccine that targets multiple glioblastoma-associated antigens. This treatment method demonstrated an overall survival of 38.4 months in a phase I trial for newly diagnosed glioblastoma patients **[69]**.

Nanomedicine is another promising avenue for innovation. Rather than drug discover, nanomedicine aims to focus on drug deliver. Nanoparticles are used to deliver drugs directly into cancer cells. This would increase drug concentrations at the target site, minimize off-target toxicity and improve efficacy. Liposomal formulations of chemotherapeutic agents such as irinotecan, have shown promise in early clinical trials **[70]**.

1.6. GENETICS OF GLIOBLASTOMA

Glioblastoma displays a complex genetic landscape, due to this the disease is often difficult to cure. However, advancements in genomic technology have enabled to to more accurately undertint the molecular changes that drive glioblastoma. Identifying key mutations not only provides us with prognostic and predictive information but is also useful in the development of targeted therapies. Some of the major genetic alterations associated with glioblastoma are as follows.

1.6.1. ATRX MUTATION

The ATRX (α-thalassemia/mental retardation syndrome X-linked) gene, which is located on Xq21.1, encodes for a protein n which is involved in chromatin remodelling, allowing histone H3 to be incorporated into heterochromatin **[71]**. ATRX mutations occur in 57% of secondary glioblastomas and are more common in IDH-mutant than in wild-type **[72]**. These mutations often occur alongside IDH1 and TP3 mutations **[72,73]**. Interestingly, ATRX mutations are actually associated with better prognostic outlooks. This was demonstrated as patients with

astrocytic tumours and ATRX mutations exhibited between prognosis than those with ATRX-wild type **[74]**.

1.6.2. TERT PROMOTER MUTATION

The TERT (Telomerase Reverse Transcriptase) gene encodes for telomerase, an enzyme that is critical for maintain the telomere length during DNA replication **[75]**. Mutations in this gene, notably, C228T and C250T, results in increased telomerase activity and telomere elongation, thus facilitating uncontrolled cell proliferation **[76]**. The mutations are present in up to 80% of all glioblastoma cases and occur more frequently in IDH-wt than in IDH-mutant **[77]**. However, the relationship between TERT promoter mutation and patient outcome remains largely unclear **[78]**.

1.6.3. TP53 MUTATION

The TP53 gene, encodes for the p53 protein, a critical regulator of cellular proliferation, survival and genomic stability **[79]**. IDH-wildtype glioblastoma exhibit a greater occurrence of TP53 mutations when compared to IDH-mutant **[80]**. In glioblastoma, the inactivation of the p53 protein is associated with increased invasiveness, decreased cell death and increased cell proliferation **[81]**. While TP53 mutations are often associated with poor prognosis in tother cancer, there is no such relationship in the case of glioblastoma **[82]**. Gain-of-function TP53 mutations increase malignancy by enhancing cell proliferation, migration, invasiveness, and drug resistance **[83]**.

1.6.4. B-RAF V600E MUTATION

B-RAF is part of the ERK MAP kinase pathway and plays a crucial role in cell growth. The V600E mutation results in the substitution of Valine for Glutamate at the 600^{th} position, this leads to continuous kinase activity and in turn uncontrolled cell proliferation **[84]**. This mutation is however, rare in glioblastoma, only occurring in about 2-6% of cases **[85]**.

1.6.5. GATA4 MUTATION

GATA4, a transcription factor of the GATA family is considered a TSG (tumour suppressor gene). In a knockout gene study on mice with null-p53 status, the absence of GATA4 induces transformation that is associated with increased proliferation as well as resistance to chemotherapy and radiotherapy induced apoptosis **[85]**. The loss of the gene expression in

glioblastoma cells results in reduced sensitivity to TMZ. This characteristic can make GATA4 useful as a predictive biomarker **[87]**.

1.6.6. FGFR1 MUTATION

Tyrosine kinase-functioning transmembrane receptors are members of the FGFR protein family. FGFR1 mutations are linked to enhanced radiotherapy resistance, invasiveness, and stemness in GBM and lead to a poor prognosis **[88]**. FGFR1 signalling is a possible therapeutic target since it is essential to the pathobiology of GBM **[89]**.

1.6.7. EGFR MUTATION

Epidermal growth factor (EGF) activates the tyrosine kinase-activating receptor known as EGFR. About 40% of GBM patients had EGFR gene amplification, which stimulates cell proliferation through the PI3K-Akt and MAPK pathways **[90]**. The most prevalent mutation in EGFR, known as EGFRvIII, is a loss that causes constitutive activation of EGFR and related signalling pathways. Although data are conflicting, EGFR amplification and mutations are more prevalent in GBM of the IDH wildtype and have been linked to a poor prognosis **[91]**.

1.6.8. MGMT PROTMOTER METHYLATION

The MGMT gene is one that encodes for a DNA repair protein. Methylation of the gene effectively silences it, in turn reducing its ability to repair DNA as well as enhancing the efficacy of alkylating agents like TMZ **[92]**. Due to this fact, individuals with this mutation often have between overall survival than those of wildtype **[60]**.

1.6.9. WT1 MUTATION

First discovered in Wilms tumours, the WT1 gene, which is found on chromosome 11p13, is a transcription factor. WT1 was originally identified as a tumour suppressor, but because it is overexpressed in a number of malignancies, including GBM, it has since been shown to be an oncogene **[93]**.WT1 protein is positively detected in 94% of GBM samples, indicating its involvement in the progression of the cancer **[94]**.

1.6.10. PTEN ALTERATION

The tumour suppressor gene PTEN, which is found on chromosome 10q23, is linked to a poor prognosis in GBM when its function is lost as a result of mutation or loss of heterozygosity (LOH) **[95]**. PTEN controls cell growth, proliferation, and survival by blocking the PI3K/Akt

pathway. Reduced PTEN expression is associated with poorer outcomes and is a sign of GBM development **[96]**.

1.7. INTRODUCTION TO CDK1

As part of the family of cyclin-dependant kinases (CDKs), CDK1 plays an essential role in the highly regulated process of cellular division. The cell cycle, a tightly monitored sequence of events, depends heavily on CDKs and their associated cyclins for smooth cell cycle progression and prevention of abnormal cell growth **[97]**. Among the various members of the CDK family and their associate cyclins, one pair particularly stands out. This is the cyclin B-CDK1 complex. This is because this complex is responsible for the transition of cells from the G2 phase to the M (mitotic) phase of the cell cycle **[98]**.

CDK1 functions in tandem with various cyclins, one of which is cyclin B, to properly regulate the cell cycle. Since this complex is of immense importance, it is also highly regulated by specific upstream modulators such as WEE1 and CDC25. WEE1 functions by inhibiting CDK1 while CDC25 is an activator of CDK1. A delicate balance has to be achieved between inhibition and activation to ensure smooth functioning of the cell cycle events **[99]**.

Initially, CDK1 associates with cyclin A. This cyclin A-CDK1 complex is responsible for the transition of the cell from S phase (synthesis) to the G2 phase. However, its main role is in the cyclin B-CDK1 complex that is influenced by WEE1 and CDC25 to promote G2/M phase transition. This transition involves the regulation of events such as the nuclear envelope breakdown, spindle assembly, and chromosome condensation **[100]**.

CDK1 is a kinase, which means it functions by phosphorylating various checkpoint proteins that help regulate the cell cycle. These checkpoint proteins, once activated, are responsible for maintaining genomic stability.

In case the body detects any DNA damage or other stress, CDK1 will be inhibited and thereby cause cell cycle arrest in the G2 phase. This will prevent cells with damaged DNA from replicating, thus protecting our body from accumulating genetic mutations. This is an essential anti-cancer mechanism of our body, protecting us from the uncontrolled proliferation of cancer cells. This crucial protein can therefore serve as a promising target during cancer treatment **[102,103]**.

1.8. ROLE OF CDK1 IN GLIOBLASTOMA

Glioblastoma is not only characterized by its histopathological heterogenicity but also by its complex genetic landscape. Several efforts have been made by researchers to identify key regulatory genes, proteins, and pathways that can serve as potential therapeutic targets. One such protein, is CDK1, which plays a crucial role in the regulation of the cell cycle. CDK1 is often found to be overexpressed in many cancer cells, including glioblastoma. In fact, CDK1, in particular, has been identified as an oncogenic signature in glioblastoma, suggesting that it could play a pivotal role in the progression of this type of cancer.

CDK1, also known as Cdc2, is a type of serine/theorine kinase. Over-expression of this gene has been associated with the promotion of oncogenesis as well as the progression oh human gliomas while its downregulation has shown to have the opposite effect, inhibiting the proliferation of human gliomas **[104]**. This upregulation of CDK1 can thus be interpreted to contribute to the aggressive characteristic of this type of cancer **[105]**. Furthermore, studies have shown that CDK1 is up-regulated by some chemotherapeutic agents, such as TMZ, in glioblastoma. Knockdown experiments proved that the absence of CDK1 increases the sensitivity of glioblastoma cell to TMZ **[106]**. These studies further highlight the potential of CDK1 to serve as a therapeutic target.

Several CDK inhibitors have been develop and tested in pre-clinical as well as clinical studies, and show positive results in inhibiting glioblastoma cell growth and inducing apoptosis. One such example is Dinaciclib, a potent inhibitor of CDK1, CDK2, cDK5, and CDK9 **[108]**. Another study demonstrated the potential of second-generation CDK inhibitors such as AT7519 to inhibit GBM cell growth **[109]**.

CHAPTER 2 – AIMS & OBJECTIVES

CHAPTER 2 – AIMS AND OBJECTIVES

- Analyse mRNA expression profiles from glioblastoma patients and normal individuals to identify differential gene expression patterns.

- Conduct gene ontology and pathway enrichment analysis of the differentially expressed genes to elucidate underlying biological processes and molecular pathways associated with glioblastoma.

- Construct an interaction network to delineate the relationships between these genes and identify key regulatory nodes.

- Compile a comprehensive list of known CDK1 inhibitors from scientific literature.

- Conduct molecular docking studies to assess the binding affinity of established CDK1 inhibitors.

- Perform virtual screening of best-established compound against the NCBI PubChem database to identify potential CDK1 inhibitors.

- Conduct molecular docking studies to assess the binding affinity of virtual screening compounds.

- Study pharmacophore of the top-ranked virtual screening compound.

- Compare and evaluate the ADMET profiles of the top-ranked established compound against the top-ranked virtual screening compound

CHAPTER 3 – REVIEW OF LITERATURE

HAPTER 3 – REVIEW OF LITERATURE

3.1. INTRODUCTION

Glioblastoma, commonly referred to as GBM, is one of the deadliest and prevalent forms of primary malignant brain tumors in adults. This type of brain tumor is often characterized by its rapid growth rate as well as extensive amount of infiltration into the surrounding brain tissue. The substantial amount of infiltration makes surgical resection immensely complicated and also contributes to its poor prognosis as well as high chance of reoccurrence. Despite multiple advancements in glioblastoma treatment modalities, this type of cancer remains highly incurable, making it a significant challenge to both patients and healthcare providers. The current standard of treatment includes chemotherapy or radiotherapy post-surgical resection of the tumor tissue if possible. However, due to its extensive infiltration as well as resistance to many chemotherapeutic agents, many patients are either unable to be cured or experience cancer remission. The discovery of effective therapeutic targets is therefore crucial for developing treatments that can improve patient outcomes. Cyclin-dependent kinase 1 (CDK1 or Cdc2) has emerged as a potential target due to its essential role in cell cycle regulation and its dysregulation in various forms of cancers, including glioblastoma. This literature review will explore the definition and characteristics of GBM, its epidemiology and incidence rates, current treatment options along with their limitations, and explore the role of CDK1 in cell cycle regulation, its dysregulation in cancer and the potential of small molecule CDK1 inhibitors in the treatment of glioblastoma.

3.1.1. DEFINITION AND CHARACTERISTICS OF GLIOBLASTOMA

Glioblastoma is marked by its highly aggressive nature, high tumor heterogenicity as well as distinct histopathological features. As noted by **Germano I. M. et al. (1989)** and **Russell et al. (1989),** histopathological features such as necrosis, microvascular proliferation are used to identify glioblastoma and differentiate it from other gliomas such as astrocytoma. **Wippold et al. (2006)** mentioned another distinguishing histopathological feature of glioblastoma to be psuedopalisades, which are associated with necrosis. The composition of glioblastoma tumor tissues is often extremely heterogenous in nature as demonstrated by **Molina D. et al. (2016)** through the use of MRI texture analysis. Their analysis not only revealed the complex nature of tumor composition but also how these heterogenous regions can correlate to patient prognosis

and survival. **Dunn et al. (2012)** addresses how tumor heterogenicity not only complicates the choice of treatment modality but also makes tumor classification cumbersome. According to the latest **World Health Organization (WHO) Classification of Central Nervous System Tumors (2021)**, glioblastoma is classified as grade 4 tumor, the highest grade, indicative of its severe malignancy.

As noted by **Friedmann-Morvinski et al. (2014)** and **Seoane et al. (2012)**, glioblastoma not only displays high histopathological heterogenicity, but also displays a variety of genetic and epigenetic alterations that contribute to tumor progression. These include mutations in the EGFR, PTEN and TP54 genes, as well as alterations in the RTK/RAS/PI3K, p53 and retinoblastoma signaling pathways (**Gusyatiner et al., 2017**). This molecular heterogenicity necessitates the discovery of personalized treatment approaches.

Lathia et al. (2015) describes another factor contributing to the high reoccurrence rate of glioblastoma; the presence of self-renewing, tumorigenic cancer stem cells. These stem cells play a part in tumor initiation, maintenance and resistance to conventional therapies**.** Due to the presence of these cancer stem cells**, Yulin Zhang et al. (2020)** quotes a post-operative recurrence rate of glioblastoma to be greater than 60%.

3.1.2. EPIDEMILOGY AND INCIDENCE RATES

Glioblastoma is the most prevalent primary brain tumors in adults. **Ostrom et al. (2018)** quotes an incidence rate of approximately 3.2 per 100,000 population per year in the United States. It represents about 15% of all intracranial neoplasms and 60-70% of all astrocytic tumors. A statistical study conducted by **Tian et al. (2018)** found that approximately 55.2% of glioblastoma cases were diagnosed between the ages of 41-60. This work was further supported by **Gittleman et al. (2018)** which found a correlation between the patient age and occurrence of glioblastoma. **Louis et al. (2017)** also highlighted a slight male predominance in incidence rates, with a male-to-female ratio of approximately 1.6:1.

Glioblastoma is described as one of the deadliest cancers due to the fact that along with its high incidence rate, it also has a poor median survival time of only 14-16momnts despite aggressive treatment (**Stupp et al., 2005**). In fact, according to the **Central Brain Tumor Registry of the United States** (2007-2011), the 5-year survival rate for glioblastoma was a mere 5%. This dismal

prognosis is attributed to the tumour's aggressive nature, rapid growth, and diffuse infiltration adjacent to the brain tissues, which makes complete surgical resection nearly impossible.

3.1.3. CURRENT TREATMENT OPTIONS AND THEIR LIMITATIONS

M. Mehta et al. (2017) mentions that the current standard of care for glioblastoma involves a multimodal approach, including maximal safe surgical resection, followed by radiotherapy and concurrent chemotherapy with temozolomide. This regimen, known as the Stupp protocol, has been show to significantly improve survival rates compared to therapy alone (**Stupp et al., 2005**). However, despite this highly aggressive treatment approach, the efficacy is often limited by several factors

Mason et al. (2015) and **Harder et al. (2018)** address one major limitation in the treatment of glioblastoma is the intrinsic resistance mechanisms of glioblastoma, including the presence of the blood-brain-barrier (BBB), which restricts the entry of many therapeutic agents into the brain. Additionally, **Notch et al. (2018)** highlights that even with similar treatment modalities, patients often exhibit variable therapeutic responses.

Due to these reasons, new therapeutic approaches are being explored to overcome all these obstacles. A study by **Thomas et al. (2014)** shows targeting tumour growth factor receptors such as EGFR and PDGFR, have shown promised in preclinical studies, though clinical outcomes have been mixed. A research paper by **Zondor et al. (2004)** highlighted how angiogenesis inhibitors, such as bevacizumab, which aim to disrupt the blood supply to the tumour, have shown promise, although clinical information is limited. **Yang et al. (2020)** address how cancer stem cells are another promising target, with therapies aimed at eliminating these resilient cells to prevent tumour recurrence.

Immunotherapy has emerged as another promising avenue for glioblastoma treatment. This approach utilizes checkpoint inhibitors and vaccines to potentially improve the body's immune response against cancer cells. **Lim et al. (2018)** describes how immunotherapy has variable efficacy as it has to overcome the challenge of the unique immune environment of the central nervous system. **Zanders et al. (2019)** proposes a multimodal therapeutic intervention by combining immunotherapy with the existing standard of care treatments to potentially improve patient outcomes. They also propose the development of personalized strategies to better address tumour heterogenicity.

Recent advancements in drug discovery and development methods are also being investigated to further improve patient outcomes. **Sheraglis et al. (2018)** talks about nanoparticles and prodrug development to facilitate the delivery of the drug across the BBB and increase the availability of therapeutic agents within the tumour. These novel delivery methods have the potential to improve the efficacy of existing as well as facilitate the development of new drugs.

However, despite these advancements, the treatment of glioblastoma still remains a significant challenge in the field of neuronocolgy due to the tumour's complex biology and adaptive resistance mechanisms. Ongoing research is focused on identifying new molecular targets, improving drug delivery systems and the development of new combination therapies. All of this is aimed at enhancing treatment efficacy and patient outcome. Integrating genetic, epigenetic and metabolic data into personalized treatment plans could further assist in overcoming the diverse and adaptive nature of glioblastoma **(Lathia et al., 2015)**.

3.2. CDK1: A CRUICAL TARGET IN CANCER THERAPY

3.2.1. ROLE OF CDK1 IN CELL CYCLE REGULATION

As stated by **Enserink et al. (2022),** CDK1 is a key regulator of the cell cycle, particularly in the transition from the G2 phase to the M phase. **Schafer (1998)** gives us a comprehensive overview of all the events of the cell cycle. The cell cycle is comprised of 4 main stages, namely, G1 (gap 1), S (synthesis), G2 (gap 2), and M (mitosis). CDK1 forms a complex with cyclin B and together this unit is known as the maturation promoting factor (MPF), which is essential for the onset of mitosis.

CDK1's activation is tightly regulated by phosphorylation. **Ding et al. (1960)** describes the complex interplay between various molecules for the regulation of CDK1 activity which in turn regulates the cell cycle. CDK1 is inhibited by kinases such as Wee1 and Myt1, which phosphorylate CDK1 on specific tyrosine residues. On the other hand, phosphates such as Cdc25 activate CDK1 by dephosphorylating these residues. Once activated, the CDK1-Cyclin B complex phosphorylates various substrates to promote mitotic entry, including nuclear Lamins (which leads to nuclear envelope breakdown) and microtubule-associated proteins (for mitotic spindle formation) (**Morgan, 2007; Nurse 1990**).

3.2.2. IMPORTANCE OF CDK1 IN CELL PROLIFERATION

CDK1's role is essential for cell proliferation. It ensure that the cells progress though mitosis, thus facilitating cell division. **Nigg (2001)**, notes how the absence of CDK1 halts the cell cycle, thus leading to cell death, highlighting its critical role. This critical role, if not properly regulated, can lead uncontrolled cell division, and eventually can progress into cancer. **Meyer & Penn (2008)** describe CDK1 as a "central hub" in the vast network of cell cycle regulators, maintaining genomic stability and proper cell division.

3.2.3. CDK1 DYSREGULATION IN CANCER WITH A FOCUS ON GLIOBLASTOMA

CDK1 is often found to be overexpressed in various cancers, one of which is glioblastoma, a particularly aggressive brain tumour. This overexpression leads to increased cell proliferation and tumour growth. Studies by **Aldea et al. (2016)** and **Kuntz et al. (2012),** show that elevated levels of CDK1 correlate with poor prognosis and higher tumour grade in several cancers such as breast, liver and lung cancers. In glioblastoma, CDK1 overexpression promotes rapid and uncontrolled cell division, which can be deadly in a confined space such as the brain and can lead to increased intracranial pressure and eventually death (**Wen, 2008**).

Glioblastoma cells exploit CDK1 overexpression to bypass normal cell cycle checkpoints, enabling uncontrolled proliferation. This aberrant proliferation is a hallmark of glioblastoma's resistance to treatment and its tendency to reoccur. **Sherr et al. (1999)** highlighted how alterations in the expression of CDK1's regulatory partners, for example, Cyclin B or CDK inhibitors p21 and p27, can further exacerbate the dysregulation, and lead to tumour growth. Additionally, mutations or deletions in genes encoding these regulatory proteins can also disrupt normal cell cycle control and contribute to tumour progression.

3.2.4. IMPLICATIONS OF CDK1 AS A THERAPEUTIC TARGET

Due to CDK1's central role in cell cycle regulation as well its dysregulation in various cancers, it has potential to be a therapeutic target. According to **Van Arsdale et al. (2015)**, inhibiting CDK1 could effectively halt cancer cell proliferation by inducing cell cycle arrest. This approach could selectively target tumour cells while sparing normal cells, which proliferate at lower rates,

or, in the case of cancers such as glioblastoma, where normal tissue cells have lost replicative capability.

Thus, several small molecule inhibitors have been developed that target CDK1 and are now undergoing preclinical and clinical trials. These inhibitors work by blocking CDK1's kinase activity. By doing this, they prevent the phosphorylation and subsequent activation of its substrates and in turn halt cell cycle progression. By inducing this G2/M check point arrest, CDK1 inhibitors can trigger apoptotic cell death in cancer cells (**Malumbres & Barbacid, 2009**).

Preclinical studies in glioblastoma have shown positive results with CDK1 inhibitors. These inhibitors reduce tumour growth as well as increase the efficacy of existing therapies such as radiation and chemotherapy. **Michaund et al. (2010)** discussed how CDK1 inhibitors can sensitize glioblastoma cells to treatments and help therapeutic agents overcome resistance mechanisms that usually limit their effectiveness.

Benson et al. (2010) urges for the development of selective CDK1 inhibitors which could potentially minimize off-target effects. Designing inhibitors that target CDK1's ATP-binding site can help reduce interactions with other CDK's or kinases effectively reducing toxicity.

Multimodal therapeutic approaches, such as combining CDK1 inhibitors with other targets therapies or immune checkpoint inhibitors was discussed by **Polivika & Janku (2014)**. This would allow therapeutic agents to target, multiple pathways associated with tumour growth and immune evasion, which would enhance the overall anti-cancer response and potentially improve patient outcome. One example is combining CDK1 inhibitors with inhibitors of the PI3K/AKT/mTOR pathway, frequently activated in glioblastoma.

3.2.5. HISTORICAL CONTEXT AND FOUNDATIONAL STUDIES

The importance of CDKs in cell cycle regulation was first highlighted by **Nurse et al. (1985)**, who discovered a protein kinase regulated by phosphorylation involved in major cell cycle controls in yeast. This work laid the foundation for understanding the role of CDKs in call cycle progression.

Further studies by **Sherr et al. (1996)** revealed that cancer cells often acquire damage or mutations to genes that directly regulate their cell cycle, with frequent genetic alterations in the

retinoblastoma protein (RB) pathways being potentially necessary for tumour development. **Irma Sanchez et al. (1996)** expanded on this by showing that critical call cycle regulators, like the retinoblastoma family of tumour suppressor proteins and CDKs, play pivotal roles in maintaining normal cell cycle control.

3.2.6. CDK1'S UNIQUE ROLE

David Santamaria et al. (2007) provide crucial insights by demonstrating that CDK1 is the only essential CDK required for cell cycle progression, with his experiment on mice. This challenged the previous belief that multiple CDK's were necessary. This study underscored CDK1's unique and indispensable role in cell cycle regulation, further highlighting its potential as a therapeutic target.

3.2.7. THERAPEUTIC POTENTIAL OF TARGETING CDKS

Vermeulen et al. (2003) discussed the potential of targeting CDKs for drug discovery, emphasizing that alterations/mutations in the cell cycle led to aberrant cell proliferation and cancer development. Targeting CDKs, specifically CDK1, offers unique opportunities for developing novel cancer therapies.

3.3. USE OF SMALL MOLECULE INHIBITORS

3.3.1. DEFINTION AND GENERAL MECHANISM OF ACTION

Small molecule inhibitors are low-molecular-weight compounds that can affect biological processes by binding to specific proteins, often enzymes or receptors, and changing their activity. These inhibitors are typically less than 500 Daltons in size, allowing them to easily diffuse across cell membranes and reach intracellular targets. The fundamental mechanism of action for small molecule inhibitors involves binding to the active or allosteric sites of target proteins, thereby modulating their biological activity. This can result in the inhibition of enzymatic activity, disruption of protein-protein interactions, or modulation of signaling pathways.

The specificity and potency of small molecule inhibitors come from their ability to fit precisely into the binding pockets of target proteins, a concept often described as the "lock and key" model. This precise fit is essential for the effective inhibition of target proteins, which often play pivotal roles in disease processes, particularly in cancer. As **McCormick (2000)** notes, protein kinases, a popular target for small molecule inhibitors, are integral to numerous signaling pathways involved in cell growth and survival, making them attractive targets for drug discovery.

3.3.2. TARGETING PROTEIN KINASES

Protein kinases have been an attractive target for small molecule inhibitors due to their biological importance, abundance, and availability of structural information on various kinase classes **(McCormick, 2000)**. Kinases have many functions in the body, especially in regulating various cellular processes by phosphorylating specific substrate and their dysregulation is a hallmark of many cancers. These small molecule inhibitors function by targeting kinases and therefore preventing the phosphorylation of their substrates, thereby interrupting the signaling pathways necessary for tumor growth and survival.

Furthermore, specific inhibitors for numerous kinases have been identified despite the structural similarities between the closely related enzymes. This facilitates the development of highly selective small molecule inhibitors which is crucial for minimizing off-target effects, lowering toxicity as well as enhancing the therapeutic index of these compounds.

3.3.3. ADVANTAGES OF SMALL MOLECULE INHIBITORS OVER OTHER THERAPEUTIC AGENTS

3.3.3.1. RAPID ONSET AND REVERSIBILITY

One of the main advantages of small molecule inhibitors is their quick onset of action. Due to their small size and ability to disuse though cellular membranes, these inhibitors can reach the target site quickly and also increase therapeutic concentration, leading to a quick pharmacological response. **Chu et al. (2018),** highlights how this rapid action mechanism is particularly helpful in acute settings where immediate intervention is required.

Furthermore, the effects of these small molecule inhibitors are also often irreversible. This reversibility enables healthcare providers to fine-tune therapeutic interventions, with dose adjustments based on patient response and minimize potential adverse effects. This offers benefits over larger biologics such as monoclonal antibodies, which often have prolonged effects that aren't easily reversible, thus making managing side effects a much more complicated process.

3.3.3.2. IMPROVED PATIENT TOLERABILITY AND EFFICACY

A study by **Khera et al. (2017)** points out that in comparison to conventional cytotoxic drugs, small molecule inhibitors generally offer better patient tolerability and higher efficacy. This is because traditional chemotherapy agents often lack specificity, and target rapidly dividing cells indiscriminately thus leading to significant toxicity. However, small molecule inhibitors are able to target specific aberrant protein or pathways involved in disease, reducing collateral damage to normal cells.

For example, targeted therapies using these small molecule inhibitors have shown remarkable clinical efficacy in treating cancer with specific genetic mutations or dysregulated signaling pathways. **Bédard et al. (2020)** emphasizes how small molecule therapies can deliver personalized treatments, tailored to the molecular profile of the patient's tumor, thereby improving patient outcomes while reducing adverse effects.

3.3.3.3. POTENTIAL FOR OVERCOMING DRUG RESISTANCE

Another significant advantage of small molecule inhibitors is their potential to overcome drug resistance, a major obstacle for conventional chemotherapeutic agents. Resistance to treatment often arises due to mutation in the target protein or compensatory activation of alternate signaling pathways. As noted by **Wu et al. (2015)**, allosteric small-molecule inhibitors overcome this issue by binding to the non-ATP sites, thereby reducing the likelihood of resistance development to the mutation in the ATP-binding pocket.

Moreover, the combination of small molecule inhibitors with other therapeutic agents can synergistically enhance the anti-tumor effects and prevent the development of resistance. **Reed at al. (2009)** gives an example of this, where small-molecule inhibitors of DNA repair proteins

can enhance the efficacy of existing cancer treatments by sensitizing tumor cells to DNA-damaging agents, therefore overcoming resistance while also allowing highlight specific and individualized cancer treatments.

3.3.3.4. ENHANCED SELECTIVITY AND POTENCY

Small-molecule inhibitors exhibit tremendous selectivity and potency. This offers the potential for high therapeutic specificity. Extreme specificity is achieved by structure-based drug design, where molecular interactions between the inhibitor and the target protein are optimized to enhance the binding affinity and specificity (**Lugovskoy et al., 2002**). A study by **McCormick et al. (2000)** on small molecule inhibitors targeting the anti-apoptotic protein Bcl-xL has shown that the development of small molecule inhibitors has benefited from structural and computational approaches which are aimed at improving binding affinity and rationalize the binding mechanism.

This precise targeting by small molecule inhibitors can lead to more effective treatments with fewer side effects as the inhibitors are designed to interact specifically with pathological protein while sparing normal cellular functions. This characteristic is particularly helpful in the field of oncology where the aim is to target tumour-specific proteins.

3.3.3.5. FLEXIBILITY IN DRUG DESIGN AND DEVELOPMENT

Small molecule inhibitors exhibit a great amount of chemical diversity. This allows for significant flexibility in drug design and development. **Spring (2000)** discusses how advances in chemical screening technologies have enabled the identification of compounds that can induce specific cellular states and regulate the activity of target proteins. This chemical genetic approach can be systematically applied to various biological problems and disease processes, facilitating the discovery of novel therapeutic agents **(Stockwell, 2000)**.

Additionally, small molecule inhibitors have a vast range of possible targets/biological molecules, like enzymes, receptors, protein-protein interactions. This versatility offers an advantage over biologics which are often limited to extracellular targets due to their larger size and inability to penetrate cellular membranes. Hence, this ability of small molecule inhibitors to

target intracellular proteins, such as, kinases and transcription factors, widens the therapeutic potential of small molecule inhibitors.

3.3.4. CLINICAL APPLICATIONS AND FUTURE DIRECTIONS

The clinical success of small molecules inhibitors in treating various cancers underscores their therapeutic potential. Drugs like imatinib (Gleevec) for chronic myeloid leukaemia and gefitinib (Iressa) for non-small cell lung cancer have revolutionized cancer treatment by targeting specific oncogenic kinases, leading to substantial improvements in patient outcomes. These successes have encouraged ongoing research and development efforts to identify new targets and develop novel small molecule inhibitors for other malignancies.

Emerging technologies, such as dynamic clinical trials and liquid biopsies are predicted to further enhance the application of small-molecule inhibitors in precision medicine. Dynamic clinical trials allow for real-time adjustments to treatment regimens based on patient responses. Liquid biopsies enable non-invasive monitoring of tumour dynamics and drug resistance, providing critical insights into the effectiveness of small molecule inhibitors and guiding treatment decisions (**Bédard et al., 2020**).

Furthermore, the development of small-molecule inhibitors that target protein-protein interactions offers another promising avenue for drug discovery and development. However, this approach comes with its own challenges such as the fact that often the interactions surface of protein-protein interfaces is very flat. To overcome this, innovative solutions such as the use of covalent inhibitors have shown promise in this area (**Lugovoskoy et al., 2002**). By targeting key protein-protein interactions involved in disease processes, small molecule inhibitors can disrupt critical signaling pathways.

3.3.5. KNOWN CDK1 INHIBITORS

3.3.5.1.BEY1107

Wang et al. (2023) discusses BEY1107 (avotaciclib) is orally active CDK1 inhibitors currently under investigation for potential use in the treatment of various cancers. A phase 1/2 clinical trial assessed the maximum tolerated dose, safety, and efficacy of BEY1107, particularly focusing

on patients with locally advanced or metastatic pancreatic cancer. This trial explored the use of BEY1107 both as a monotherapy as well as in combination with gemcitabine, a standard chemotherapeutic agent for pancreatic cancer. The findings from this study suggest that BEY1107 has potential for improving outcomes in cancer by targeting CDK1, a crucial regulator of the cell cycle.

3.3.5.2.FLAVOPIRIDOL

Flavopiridol (alvocidib) is a well-known pan CDK inhibitor that suppresses multiple CDK's including CDK1, CDK2, CDK4, CDK6, CDK7, and CDK9 with varying degrees of potency (**Wang et al., 2023**). Although flavopiridol is a pan CDK inhibitor, it has been extensively studied for its effects on CDK1 and has undergone sever clinical trials for various cancers including leukemia, multiple myeloma, sarcoma, and gastrointestinal stromal tumors. However, flavopiridol has shown limited efficacy in human clinical trials as well as notable side effects.

3.3.5.3. RONICICLIB

Roniciclib (BAY100394) is another notable pan-CDK inhibitor that targets CDK1. It has been testes in multiple clinical trials for various malignancies. While roniciclib showed promised when combined with chemotherapy for treating extensive-disease small-cell lung cancer (ED-SCLC), further development was discontinued due to safety concerns observed in a related phase 2 study (**Wang et al., 2023**).

3.3.5.4. P276-00

P276-00 (Riviciclib) is another potent inhibitor of CDK1/4/9 with observed preclinical and clinical anti-cancer activity. While phase 1 studied aimed at determining the maximum tolerated dose, toxicity profiles, pharmacokinetics and anti-cancer activity in patients with advanced refractory neoplasms showed promising results, phase 2 studies on patients with relapsed or refractory mantle cell lymphoma resulted in patients with disease progression (**Wang et al., 2023**).

3.3.5.5.AT7519

AT7519 (AT7519M) is a potent inhibitor of multiple CDK's, one of which is CDK1. This drug has shown encouraging anti-cancer activity in multiple cancer cell lines and tumor xenografts. Clinical trials of AT7519 were aimed at evaluating its efficacy in treating lymphoma, leukemia and more. A phase I study demonstrated the safety and preliminary anti-cancer activity of AT7519, with promising results when combined with the HSP90 inhibitor onalespib. These findings support further investigation into AT7519 as a viable CDK1-associated cancer therapy (**Wang et al., 2023**).

3.3.5.6.OTHER CDK1 INHIBITORS

Wang et al. (2023) listed other CDK1 inhibitors such as Seliciclib (Roscovitine), AG-024322, PHA-793887, AZD-5438 and Indirubin, which have all shown potential for clinical application however, further studied are required to properly assess their safety as well as clinical efficacy (**Wang et al., 2023**).

3.4. BIOINFORMATICS APPROACHES IN DRUG DISCOVERY

3.4.1. ROLE OF BIOINFORMATICS IN IDENTIFYING DRUG TARGETS

Bioinformatics has become the cornerstone in modern drug discovery, utilizing computational power to uncover potential therapeutic targets and streamline the drug development process. The identification and selection of drug targets is a crucial step, involving the discovery of biomolecules that play critical roles in disease mechanisms. **Wishart et al. (2018)** addresses how bioinformatics tools and techniques facilitate this process by analyzing vast amounts of biological data to pinpoint these targets more efficiently and accurately.

One major advancement in the role of bioinformatics in identifying drug targets is the integration of various types of omics data, such as genomics, proteomics and transcriptomics. This type of data provides comprehensive insights into the molecular landscape of diseases. **Rabbani et al. (2015)** give us an example how the analysis of gene expression profiles can reveal upregulated genes in cancer cells, which can serve as potential targets for drug discovery. Bioinformatics

tools help identify genetic mutations that drive disease progression herby highlighting key molecular targets for drug intervention.

Additionally, bioinformatics assists in the function annotation of genes and proteins, helping to uncover their roles in cellular pathways and networks. This particularly important in understanding complex diseases like cancer, where multiple signaling pathways are often dysregulated. **Yu et al. (2016)** highlights how mapping these pathways can help to identify critical nodes interactions that may serve as drug targets.

3.4.2. USE OF COMPUTATIONAL TOOLS IN TARGET IDENTIFICATION

Computational tools are indispensable in the bioinformatics-driven identification of drug targets. Tools range from sequence analysis software to advanced algorithms for data integration and interpretation. One of the most fundamental tools, is BLAST (Basic Local Alignment Search Tool), which enables users to compare nucleotide or protein sequences against databases to find regions of similarity that may indicate functional or evolutionary relationships **(Altschul et al., 1990).**

Other advanced computational tools include network analysis software such as Cytoscape. This software allows users to visualize and analyze molecular interaction networks. Examining these networks leads to the discovery of key regulatory genes or proteins that may serve as potential drug targets (**Shannon et al., 2003**). **Larrañaga et al. (2006)** pointed out that machine learning algorithms are increasingly being used to predict potential drug targets by analyzing complex biological data and identifying patterns that may have not been detected through traditional methods.

Databases like The Cancer Genome Atlas (TCGA) and the Human Protein Atlas are essential to bioinformatics research. They serve as rich repositories of genomic, transcriptomic and proteomic data that is essential for target identification. **Uhlén et al. (2015)** emphasizes that these resources, combined with computational tools, enable a more comprehensive and systematic approach to discovering novel drug targets.

Along with drug identification, new approaches, such as Next Generation Sequencing (NGS), can be used to identify potential drug targets involved in disease processes. NGS helps in the identification of significantly upregulated or downregulated genes compared to normal patients.

These are referred to as differentially expressed genes, and can be identified for specific diseases like glioblastoma, (**Lathia et al., 2015**).

Protein-protein interaction networks can be constructed using tools like STRING and Cytoscape, revealing critical nodes and pathways involved in glioblastoma pathogenesis and progression (**Szklarczyk et al., 2019**). **Gao et al. (2013)** also proposes the use of survival analysis asked on available clinical data to identify those key genes with specific prognostic value, such as CDK1. The use of an integrative bioinformatic approach promotes the discovery of a more suitable and promising drug candidate as well as the appropriate selection of drug target.

3.4.3. DATABASES AND RESOURCES FOR BIOINFORMATICS RESEARCH

Bioinformatics research relies immensely on the availability of various databases and resources containing data necessary for target identification and drug discovery. These databases store a wealth of biological information, including genomic sequences, protein structures and biochemical pathways.

One such database is the UniProtKB/SwissProt database. This database is one of the most comprehensive repositories of protein sequence and functional information. It provides detailed annotations on protein function, structure and interactions, which are crucial for understanding potential drug targets (**UniProt Consortium, 2019**). **Berman et al. (2000)** describes Protein Data Bank (PDB) as another significant database to the field of bioinformatics. This database offers a repository of structural data for proteins and nucleic acids, which is indispensable for molecular docking and drug discovery.

Other important databases include PubChem – which contains information on chemical molecules and their biological activity, and DrugBank – which integrates comprehensive drug data with detailed drug target information **(Wishart et al., 2018).** These databases facilitate the identification of small molecules that may potentially interact with drug targets, thus laying the groundwork for subsequent drug development efforts.

3.4.4. MOLECULAR DOCKING AND VIRTUAL SCREENING

Molecular docking and virtual screening are two techniques critical for bioinformatics-assisted drug discovery. These methods allow researchers to predict how small molecules, such as

potential drug candidates, will interact with the drug target at molecular levels. This facilitates researchers to narrow down potential candidates from millions to a couple hundred compounds which have the highest probability of interacting with the target protein.

3.4.5. EXPLANATION OF MOLECULAR DOCKING TECHNIQUES

Morris & Lim-Wilby (2008) describe molecular docking as a computational technique that simulates the interaction between a small molecule (the ligand) and a target protein. The aim of docking studies it to predict the preferred orientation of the ligand when bound to the protein, as well as the strength of the interaction, which can be quantified as a binding affinity score.

Docking involved multiple steps: preparation of the ligand and protein structures, definition of the active/binding site, the actual docking simulation and the final scoring of the binding poses. The preparation stage is essential to ensure that the structures are in the correct and compatible format and include all the necessary components. The binding site is defined based on either previously known binding sites (such as from other ligand drug complexes) or predicted regions of interest. The docking simulation comprises of placing the ligand in various orientations and conformations relative to the protein (usually involving a flexible ligand and rigid protein) followed by scoring each orientation based on how well it fits and interacts with the binding site **(Trott & Olson, 2010)**.

Commonly available molecular docking software include AutoDock, DOCK and Glide. These tools utilize different algorithms and scoring function to calculate binding affinity and predict the strength of the interaction. **Morris & Lim-Wilby (2008)** emphasize that the accuracy of molecular docking results depends on the quality of the protein and ligand structures, the appropriateness of the scoring function and the ability to account for protein flexibility.

3.4.6. ROLE OF VIRTUAL SCREENING IN DRUG DISCOVERY

Shoichet (2004) describes virtual screening as a computational tool to identify potential drug candidates from large library of small molecules (such as PubChem or DrugBank). It involves the systematic evolution of compound libraries to predict which molecules are the most likely to bind to the target protein with the highest affinity.

There are two main types of virtual screening – ligand-based and structure-based. **Lagger et al. (2009)** elucidates the different virtual screening methods. Ligand-based virtual screening relies in known active compounds to identify new candidates with similar chemical properties. This approach uses techniques such as quantitative structure-activity relationships (QSAR) modelling and pharmacophore mapping to predict the activity of new compounds.

On the other hand, structure-based virtual screening uses the 3D structure of the target protein to identify potential binding ligands. This method involved docking a large number of compounds into the binding site of the protein and scoring their interactions to identify the best candidate. **Irwin & Schoichet (2005)**, highlight how structure-based virtual screening can be particularly powerful when high-resolution 3D structures of the target protein/molecule are available.

As mentioned by **Kitchen et al. (2004)**, the integration of molecular docking with virtual screening, enables the efficient identification of promising drug candidates. By prioritizing the compounds based on their predicting binding affinities and interactions, researchers can direct their experimental efforts on the most promising candidates, thereby accelerating the drug discovery process.

3.4.7. DEVELOPMENT OF PHARMACOPHORE MODELS FOR CDK1 INHIBITORS

Pharmacophore modelling is another essential step in the bioinformatics-assisted drug discovery pipeline. Pharmacophore modelling is a technique which is used to identify the essential features required for a molecule (such as a ligand) to interact with the target (the target protein). **Wolber and Langer (2005)** emphasized on the importance of pharmacophore modelling in drug discovery. Software like Discovery Studio, can be used to develop pharmacophore models for CDK1 inhibitors. These models allow researchers to identify key features such as hydrogen bond donors (HBD) and hydrogen bond acceptors (HBA), hydrophobic regions, and aromatic rings, which may be essential for effective binding to CDK1.

3.4.8. ADMET PROFILING

ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) profiling is a crucial step for evaluating the drug-likeness as well as the safety of potential inhibitors. Cheng et al.

(2012) discussed the importance of ADMET profiling in drug development Online tools, such as ADMETsar, can be used by researchers to predict the ADMET properties of the chemical compounds being studied. This enables researcher to further prioritize compounds that have favorable pharmacokinetic and toxicity profiles, ensuring they are viable candidates for further development and clinical research.

3.5. CONCLUSION

The comprehensive approaches described in this literature review underscore the importance of integrating various bioinformatics tools and techniques in the drug discovery process. By leveraging databases for sequence and structural information, employing molecular docking and virtual screening to identify potential inhibitors and validating these findings through pharmacophore modelling and ADMET profiling, we can hope to identify promising CDK1 inhibitors for glioblastoma treatment.

CHAPTER 4 – METHODOLOGY

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4.1. DIFFERENTIAL GENE ANALYSIS OF GLIOBLASTOMA CELLS USING GEPIA2

GEPIA2 which is an updated version of GEPIA stands for "Gene Expression Profiling Interactive Analysis", is a web-based bioinformatics tool that was developed to simplify gene expression analysis in a variety of cancers and normal tissues. The data used by GEPIA is from Genotype-Tissue Expression (TEx) and The Cancer Genome Atlas (TGCA). GPEIA offers various features including comparable gene detection, patient survival analysis, correlation analysis and differential expression analysis **[150]**. In this research, we will be using the differential analysis feature under the section "Differential Genes" to identify differentially expressed genes between normal brain tissue and glioblastoma samples. We have used the cut-off values of "2" and "0.01" for |Log2FC| and q-values respectively. The differential method used is "ANNOVA" and Chromosomal Distribution is selected as "Over-expressed" as we are looking for upregulated genes to serve as potential drug targets for small molecule inhibitors [Fig. 4.1].

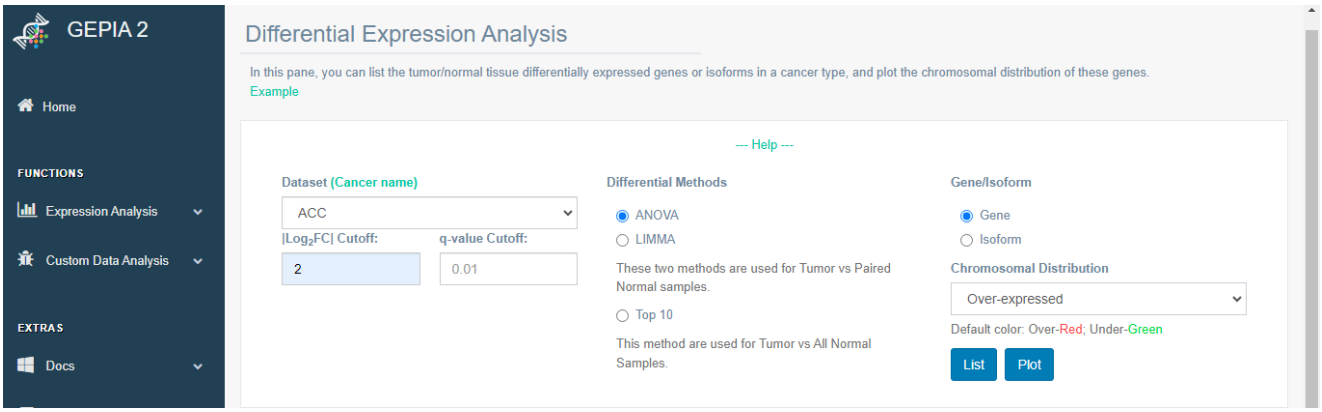


Figure 4.1. Values Set for Differential Expression Analysis using GEPIA2

4.2. CONSTRUCTION OF INTERACTION NETWORK OF KEY DEG's

STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) is a comprehensive database and web resource created for the visualization of protein-protein interaction networks. STRING creates this network by integrating information from various publicly available databases. This network helps researchers understand the functional and biological relationships

between various genes specified by the user. One advanced feature offered by STRING is known as gene enrichment analysis, this basically identified significant pathways and molecular interactions that are associated with the particular interaction network. Using STRING, scientists can understand the complex relationship between various proteins and better select proteins that can be targeted in disease pathways **[152]**. In this research, we used STRING to create an interaction network of the top 50 differentially expressed genes. The top 20 genes were found by sorting the differentially expressed genes information by |log2FC|. Under the "Multiple Proteins" section the top 50 gene list was entered into the "List of Names" input box and "Homo sapiens" was selected under organism [Fig 4.2.].

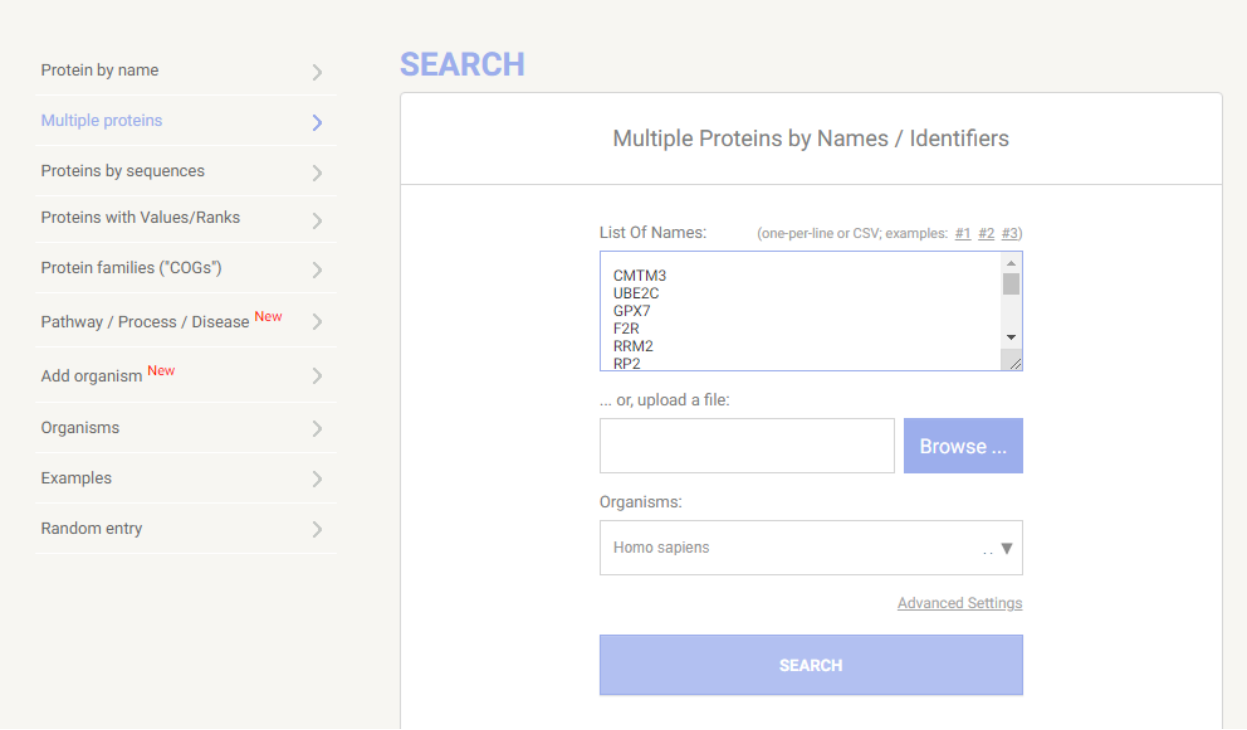


Figure 4.2. Generation of Protein-Protein Interaction Network of Top DEG's using STRING

4.3. VISUALIZATION OF CDK1 EXPRESSION ACROSS VARIOUS CANCERS

University of Alabama at Birmingham Cancer Data Analysis Portal or UACLAN, is another bioinformatics web-based tool that aims to provide easy analysis of clinical and genetic data. Genetic sequences, clinical records and expression profile data are all integrated in the analysis. UCLAN enables user to make data driven decisions in precision medicine and customize treatment plans by bridging the gap between raw data and actionable information, eventually

aiming to improve patient outcomes **[152,153]**. In this research paper we have used UACLAN to visualize how CDK1 is expressed across various cancers. In the "TGCA" tab, the gene symbol "CDK1" was entered and "Glioblastoma multiforme" dataset was chosen to view a pan-cancer CDK1 expression analysis [Fig. 4.3.].

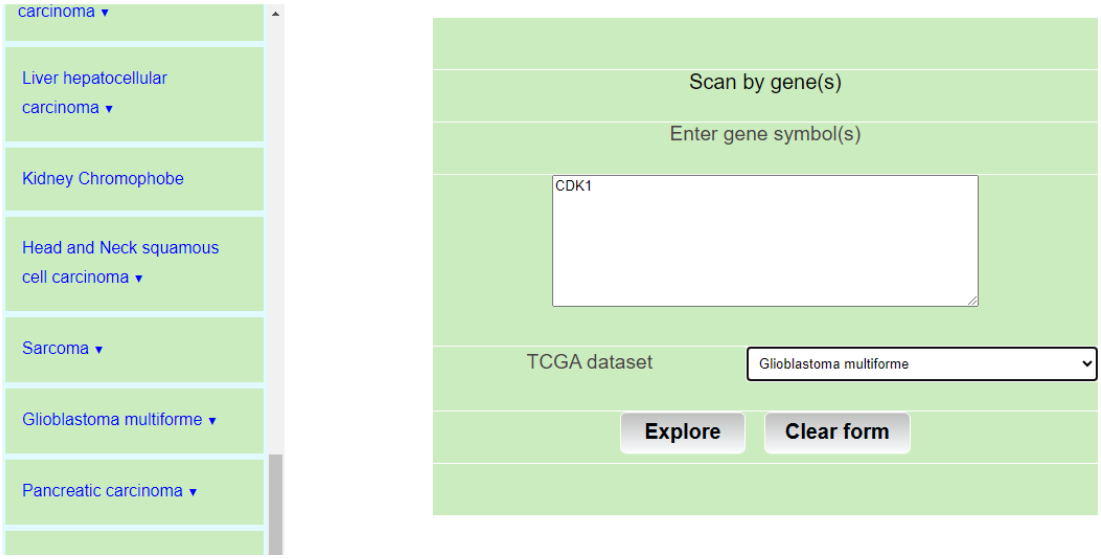


Figure 4.3. Visualization of CDK1 Expression Across Various Cancers using UACLAN

4.4. RETRIEVAL OF PROTEIN SEQUENCE OF CDK1 FROM SWISSPROT/UNIPROT

UniprotKB/Swiss-Prot is one of the most used databases of biological information. UniprotKB/Swiss-Prot is maintained in collaboration by the Swiss Institute of Bioinformatics and the European Bioinformatics Institute. This database contains protein sequence records that is annotated with more information such as protein function, structure, post-translational modifications, and disease information. This database is widely used by researchers in the fields of biochemistry, genetics, molecular biology as well as pharmaceutical companies and other organizations involved in drug discovery and development. UniProtKB/Swiss-Prot is updated regularly and is freely available to the scientific community **[154-157]**. In this research we used UniProtKB/Swiss-Prot for the retrieval of CDK1 protein sequence of Homo sapiens [Fig 4.4].

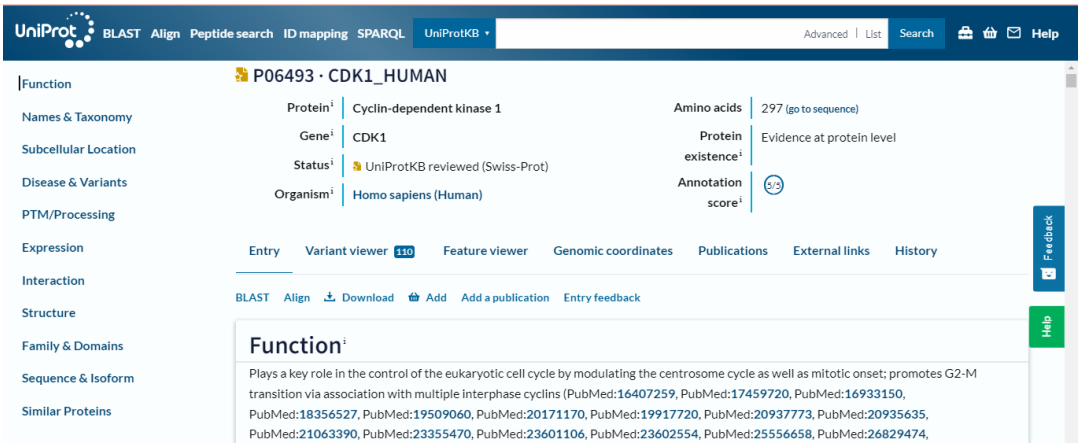


Fig 4.3- Retrieval of protein sequence of CDK1 from UniprotKB

4.5. PROTEIN SEQUENCE ANALYSIS OF CDK1 USING BIOEDIT

BioEdit, which was initially developed as a biological sequence alignment editor for Windows, has now become a popular software used in molecular biology research. This software has a wide range of features for alignment such as easy hand alignment, split window view, user defined colour, information-based shading, and integration with other sequence alignment programs such as BLAST and ClustalW. While initially being developed for only sequence alignment, it has now been updated with a wide range of new features. Some of the newer features include plasmid drawing, annotation, restriction mapping, and more tools that are crucial for research in molecular biology. BioEdit is also free and produces quick response and has a very user friendly interface **[158,159]**. In this research, we used BioEdit for the protein sequence analysis of CDK1 of Homo sapiens. [Fig 4.4]. After opening the sequence file downloaded from Uniprot in BioEdt, the "Sequence" tab is selected, under which "Amino acid analysis" is selected.

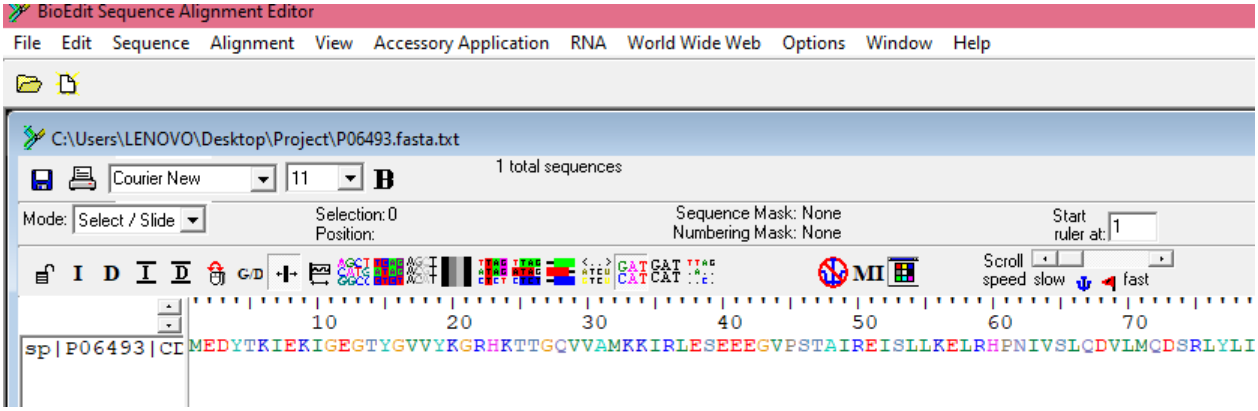


Fig 4.4 - Protein sequence analysis of CDK1 using BioEdit

4.6. PROTEIN BIOCHEMICAL PROPERTIES ANALYSIS USING EMBOSS

EMBOSS or "The European Molecular Biology Open Software Suite", is a free and open-source software analysis package. It is able to handle biological data in various formats and also has features to retrieve data from the web. With over 100 features, EMBOSS can be used by experts and practicing scientists as well. EMBOSS is a command line based software. Some features offered by EMBOSS include sequence analysis, alignment, and more, for various structural formats. The software is free and is widely used in production environments **[160-163]**. In this research, we used EMBOSS for the analysis of the protein biochemical properties of CDK1 of Homo sapiens [Fig 4.5]. This was done by using the "pepsats" command, with the input file being the CDK1 protein sequence.

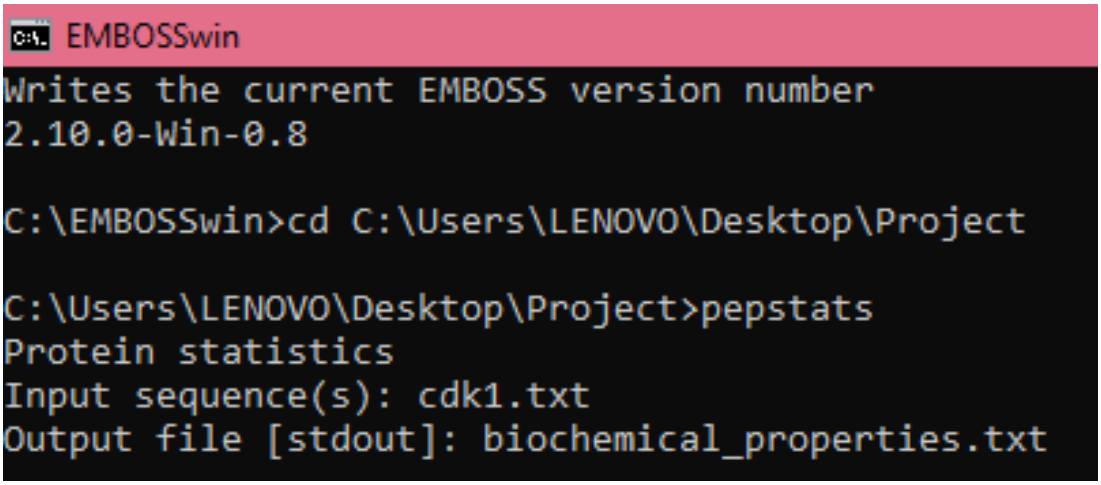


Fig 4.5 - Protein Biochemical Properties analysis of CDK1 using EMBOSS

4.7. PROTEIN PRIMARY STRUCTURE ANALYSIS USING PROTPARAM

ProtParam is a tool used for primary structure analysis. This web-based tool maintained by the Swiss Institute of Bioinformatics, is used to compute various physiochemical properties that can be deduced from the protein sequence. The tool can be used either by entering the Swiss-Prot/TrEMBL accession number or ID, or by providing the raw sequence. The properties computed by ProtParam include molecular weight, theoretical pI, amino acid composition, atomic composition, extinction coefficient, estimated half-life, instability index aliphatic index, and grand average of hydropathicity (GRAVY). ProtParam can also be accessed through the

command line. It is a great tool for researchers as it can help in the prediction of the stability of any molecule **[164,165]**. In this research we used ProtParam for analysing the physiochemical properties of the primary structure of CDK1 of *Homo sapiens* [Fig 4.7]. The raw amino acid sequence of CDK1 (retrieved from UniProt) was entered into the input box.

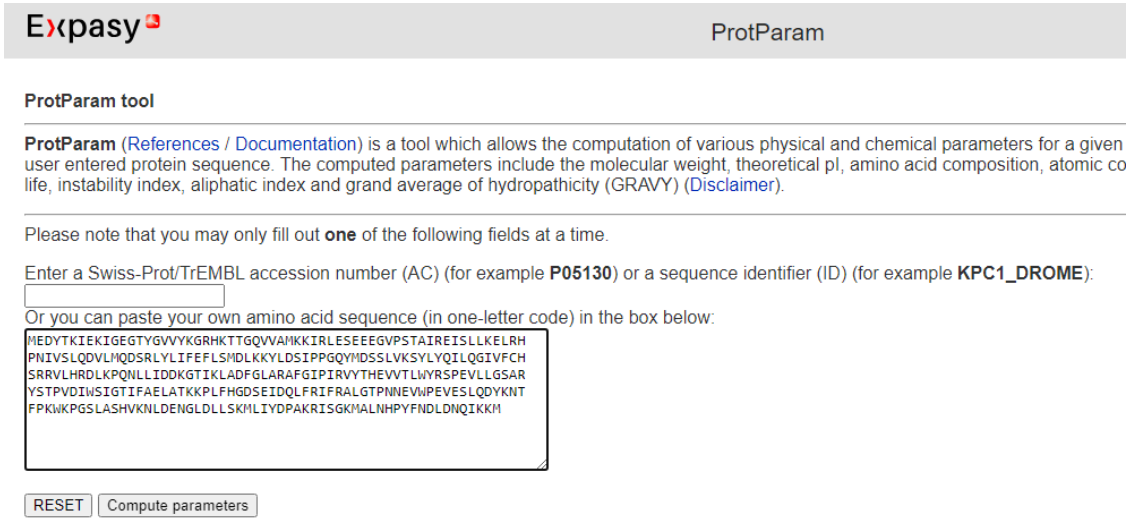


Fig 4.7. Protein Primary structure of CDK1 using ProtParam

4.8. PROTEIN SECONDARY STRUCTURE PREDICTION USING SOPMA

Self-Optimized Prediction Method with Alignment, also known as SOPMA, is a widely used computational tool for predicting the secondary structure of proteins based on their amino acid sequences. It does this by using a sophisticated algorithm that combines multiple information like, sequence alignment, and optimizes them to improve the accuracy of its predictions. SOPMA analyses the input protein sequence and predicts the probability of each residue being a part of a particular secondary structure (like alpha-helix, beta-strand, turn or random coil). SOPMA's predictions are useful in understanding protein folding, functions and interactions, making them insanely valuable in the fields of structural biology and bioinformatics **[166,167]**. In this research we will be using SOPMA for secondary structure prediction of CDK1 [Fig. 4.8.]. The protein sequence of CDK1 was entered into the input box.

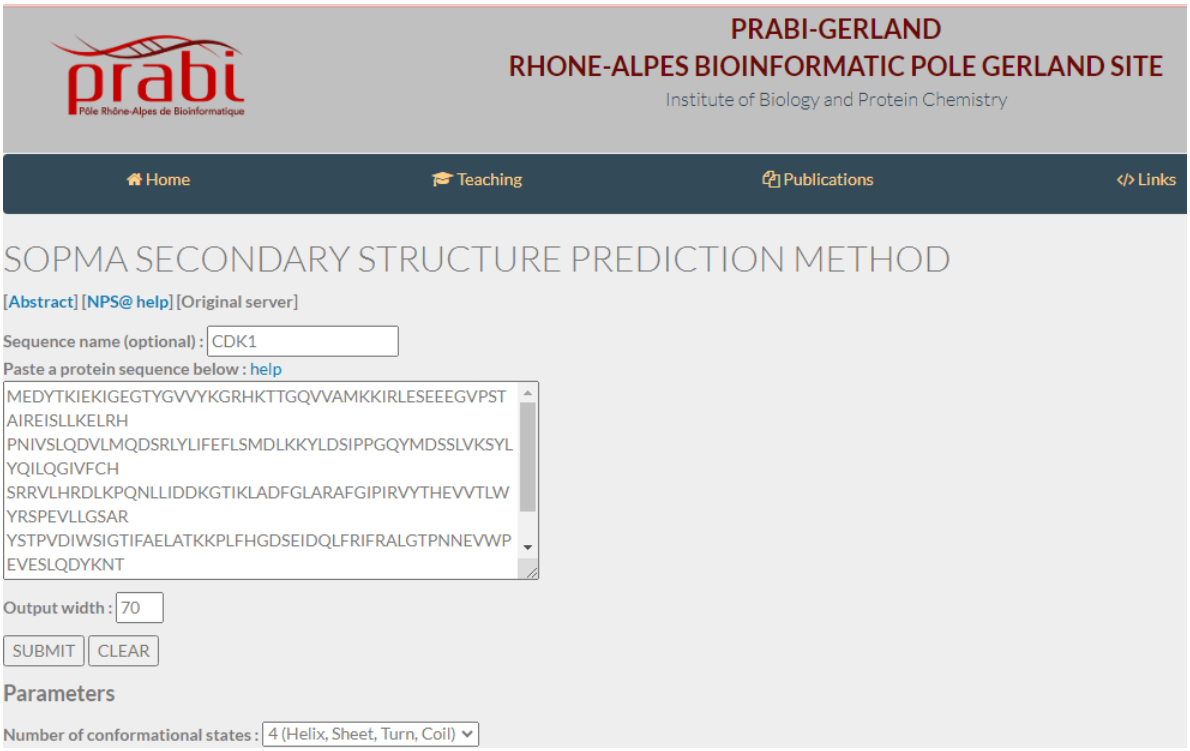


Fig 4.8 - Protein Secondary Structure prediction of CDK1 using SOPMA

4.9. PROTEIN 3D STRUCTURE SEARCHING FROM PDB USING BLAST

BLAST, an acronym for Basic Local Alignment Search Tool, is a pivotal algorithm and program used in genomic research for the comparison of a query DNA sequence with a database of sequences. One of its variations blastp, is used for comparing protein sequences. The algorithms functions by identifying short sequences in the query sequence and then aligning them with the database sequences, allowing for the efficient identification of homologous sequences and the inference of functional and evolutionary relationships. BLASTp, in particular is deigned to compare the protein query sequence to the various sequences present in the database **[168-170]**. In this research we used BLASTp against the PDB database to search for the 3D structure of CDK1 of *Homo sapiens* [Fig 4.9]. The fasta sequence of CDK1 was entered in the input box and the database was selected as “PDB”.

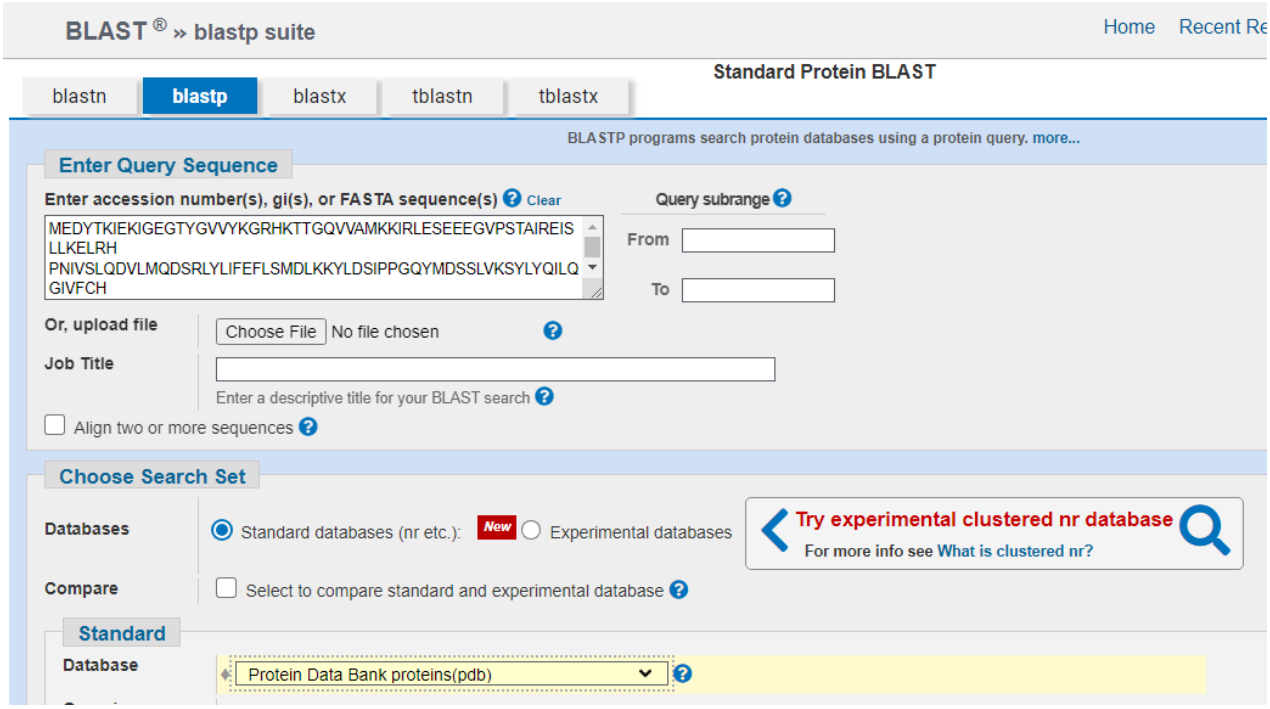


Fig 4.9. - Protein 3D structure searching from PDB using Blastp

4.10. PROTEIN 3D STRUCTURE RETRIEVAL FROM PDB

The Protein Data Bank (PDB) is a global repository of 3D structural data of large biological molecules such as proteins and nucleic acids. It contains information such as the coordinate files for biological macromolecules, listing the atoms in each protein and their 3D spatial locations. This structural information is obtained through methods like X-ray crystallography, NMR spectroscopy, and cyo-microscopy. This database is freely accessible and regularly maintained **[171-173]**. In this research we used PDB for retrieving the 3D structure of CDK1 of *Homo sapiens* using the accession id **4YC6** [Fig 4.10].

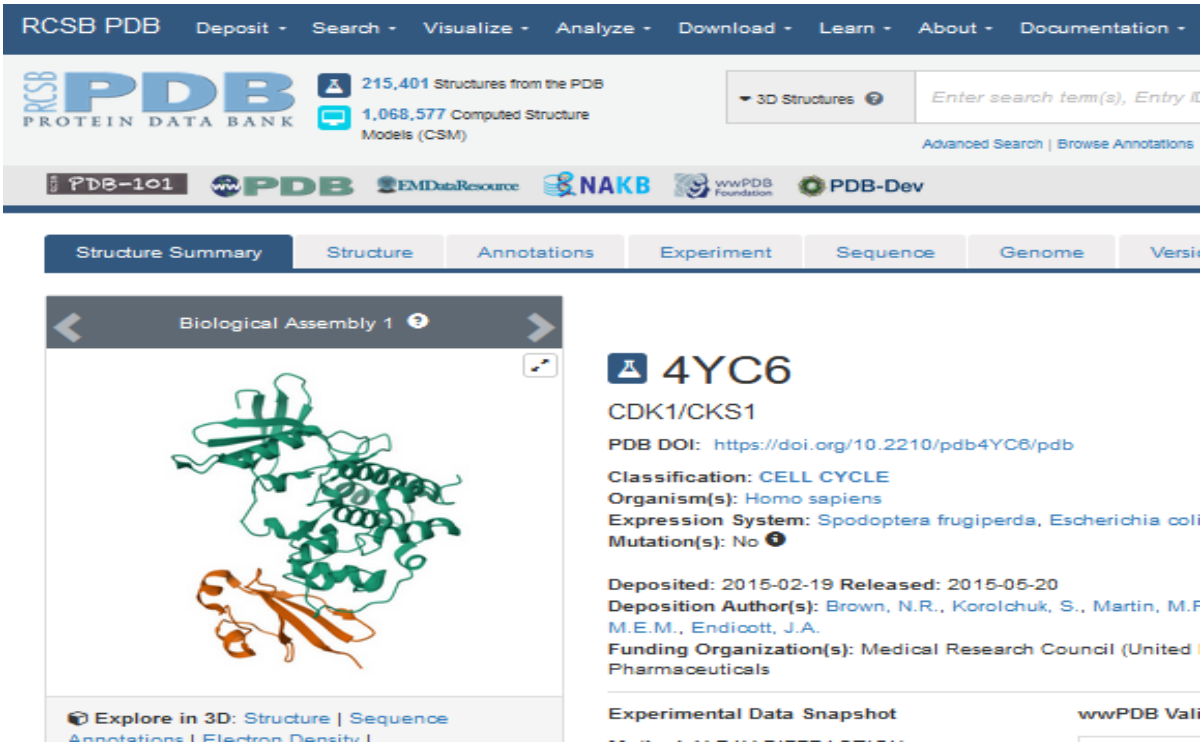
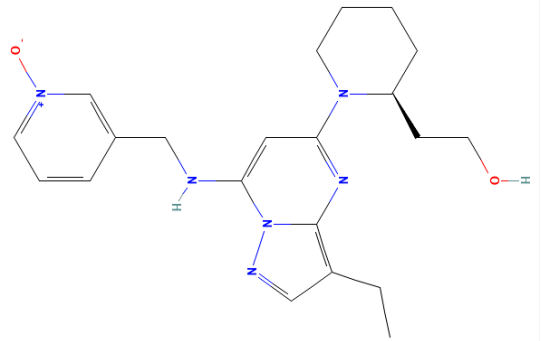
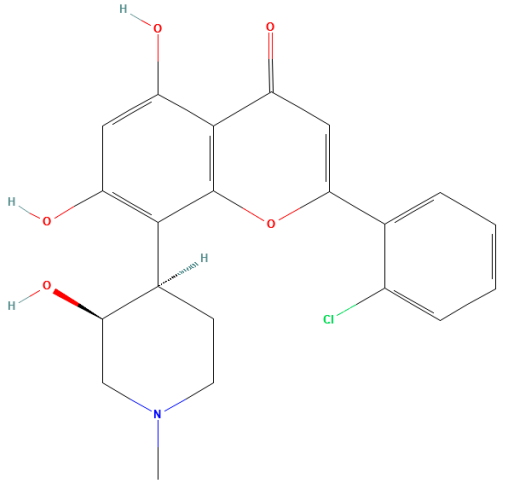


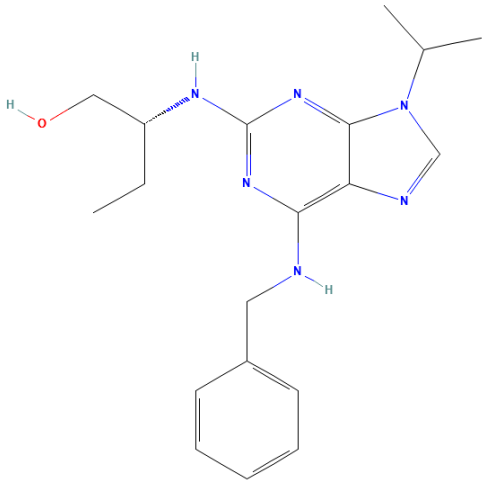
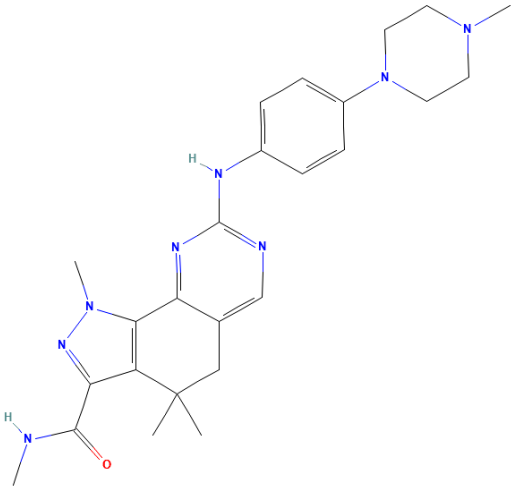
Fig. 4.10. - Protein 3D structure retrieval CDK1 from PDB Database

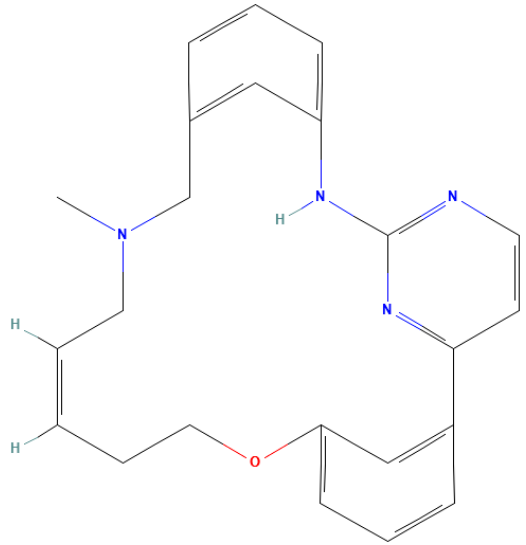
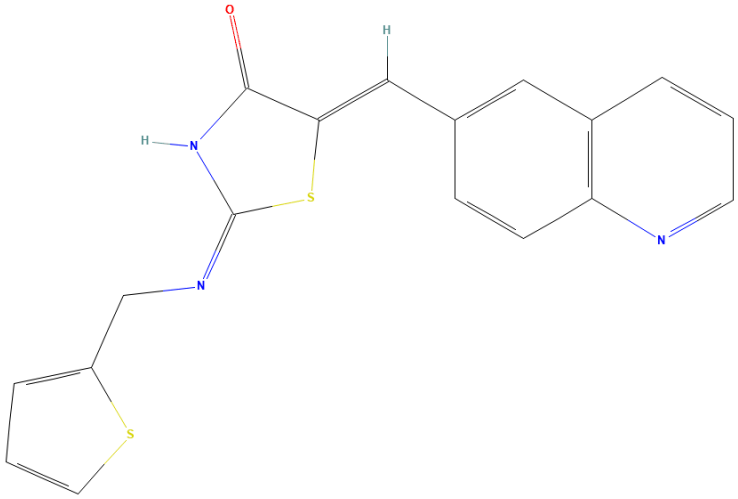
4.11. COLLECTING LIST OF ESTABLISHED INHIBITORS OF CDK1 FROM VARIOUS LITERATURE

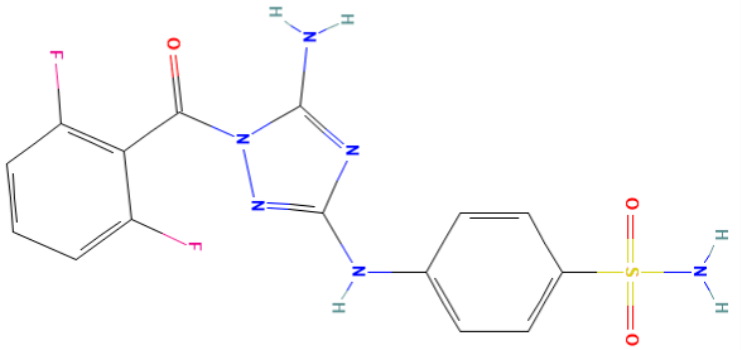
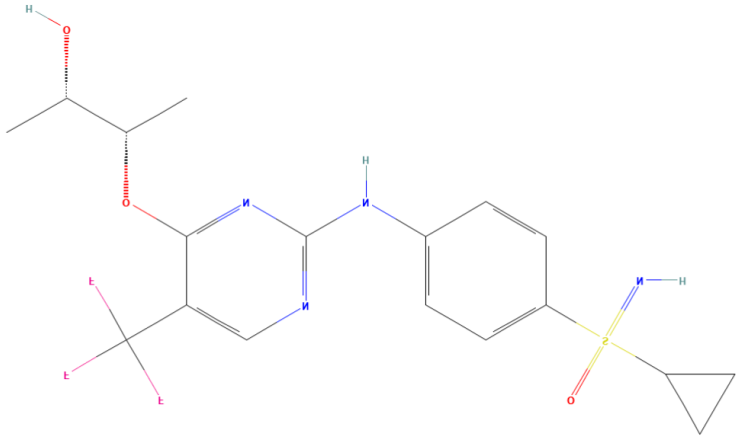
To compile a comprehensive collection of CDK1 inhibitors (both chemical and phytochemical compounds) tested on gliomas, an extensive literature search was conducted using several scientific databases, including PubMed, Web of Science, and Google Scholar. Keywords such as "CDK1 inhibitors," "gliomas," "glioblastoma," and "clinical trials" were used to identify relevant studies. The search was refined using Boolean operators to include combinations like "CDK1 AND glioma," "CDK1 inhibitors AND glioblastoma," and "CDK1 inhibition AND cancer therapy." Abstracts and full texts of the articles were reviewed to ensure relevance and quality. Additionally, references within the selected papers were cross-checked to uncover further pertinent studies. Data on the inhibitors, including their molecular mechanisms, efficacy, and clinical outcomes, were systematically extracted from PubChem for analysis.

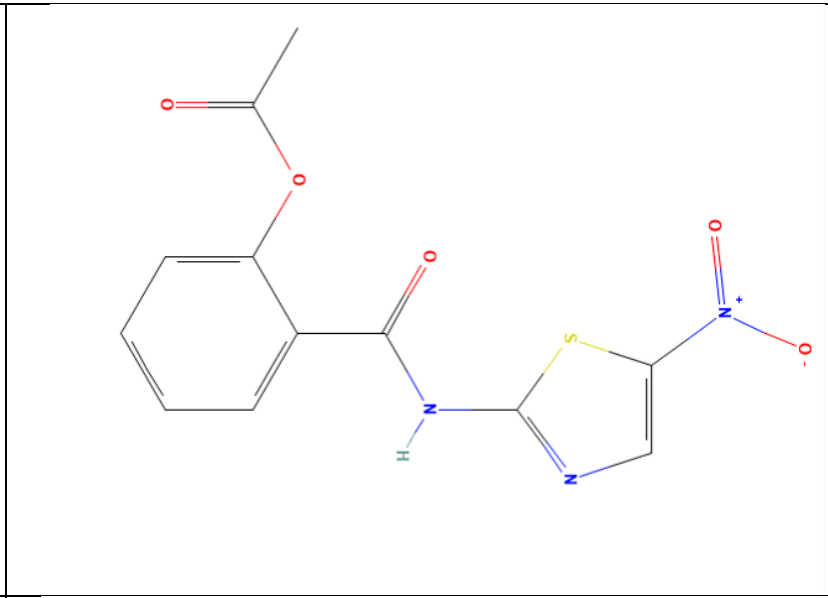
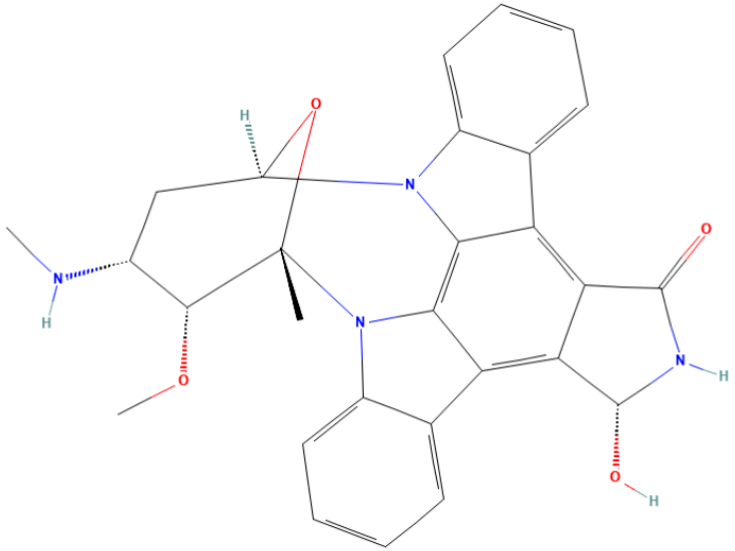
Table 4.11.1. Chemical CDK1 Inhibitors

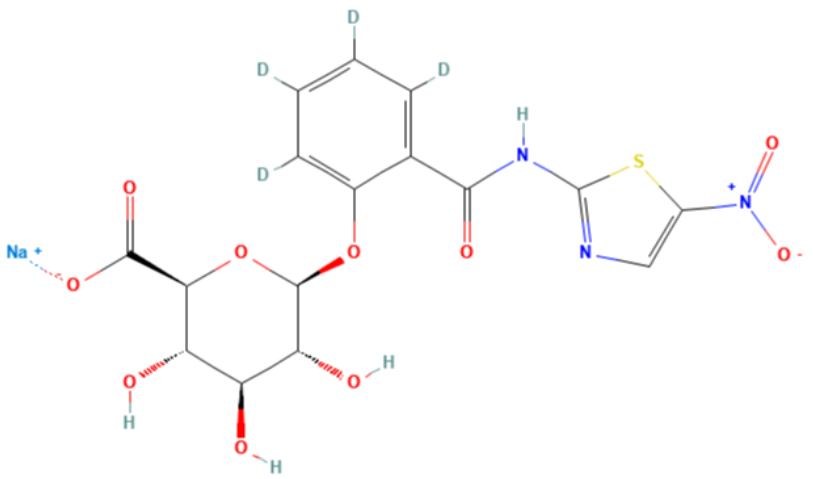
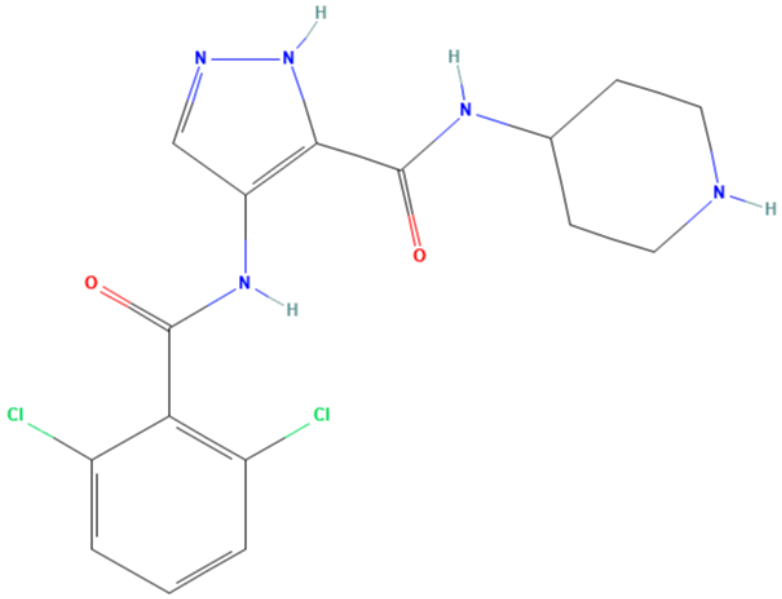
S. No.	Name	PubChem ID	HBD	HBA	MW	Structure	Ref
01	Dinaciclib	46926350	2	6	396.5 g/ mol		[174]
02	Flavopiridol	5287969	3	6	401.8 g/ mol		[175]

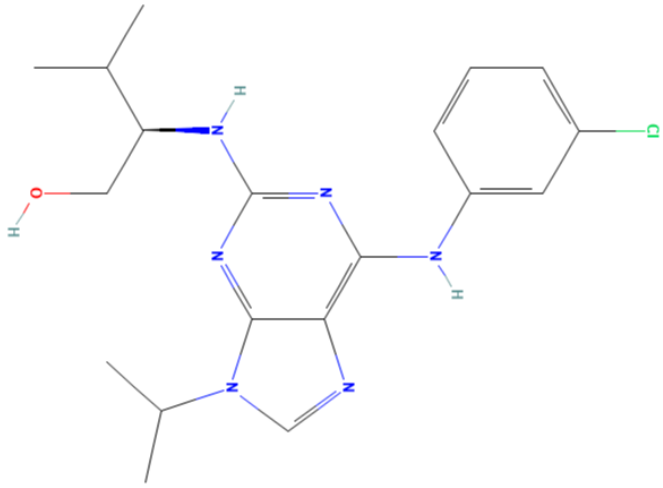
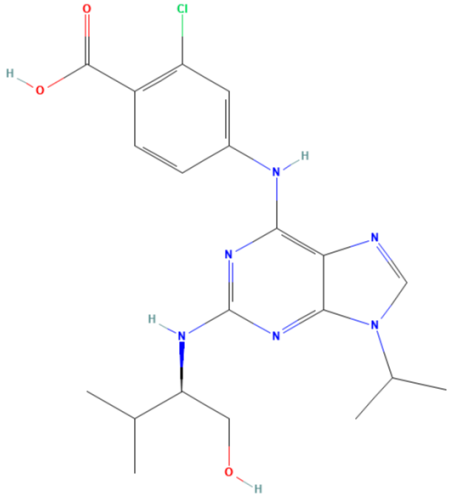
03	Roscovitine	160355	3	6	354.4 g/ mol		[175]
04	Milciclib / PHA-848125	16718576	2	7	460.6 g/ mol		[175] [176]

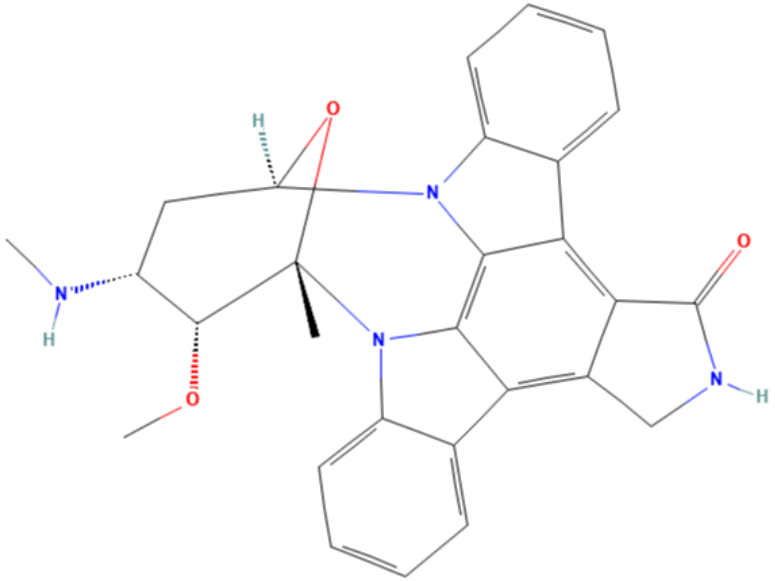
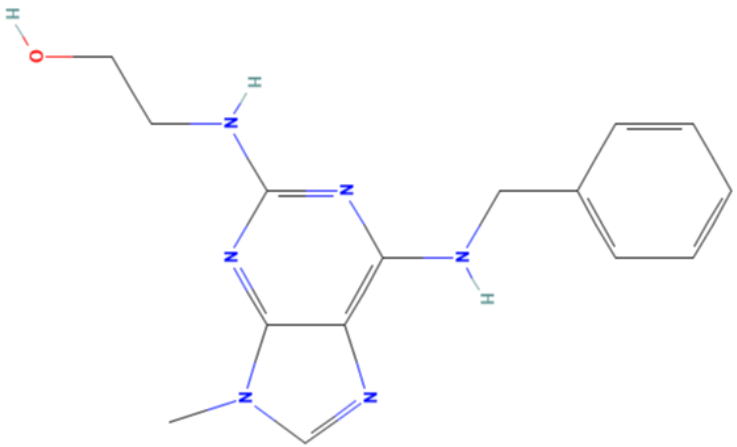
05	SB1317 / TG02	16739650	1	5	372.5 g/ mol		[175] [177]
06	RO-3306	135400873	1	5	351.4 g/ mol		[179]

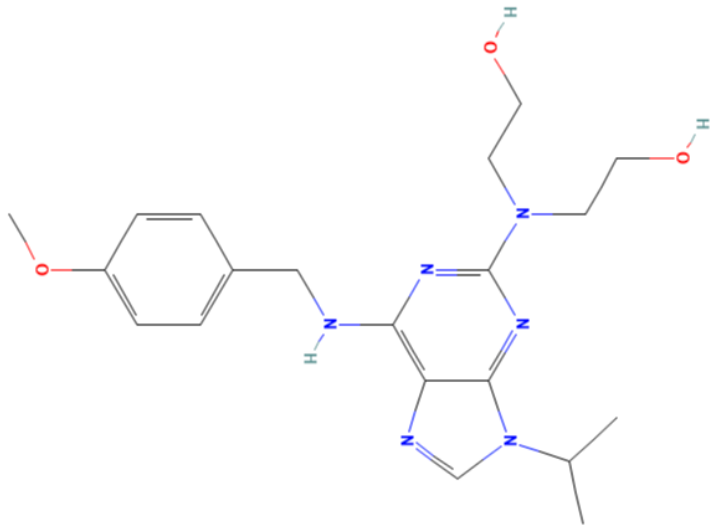
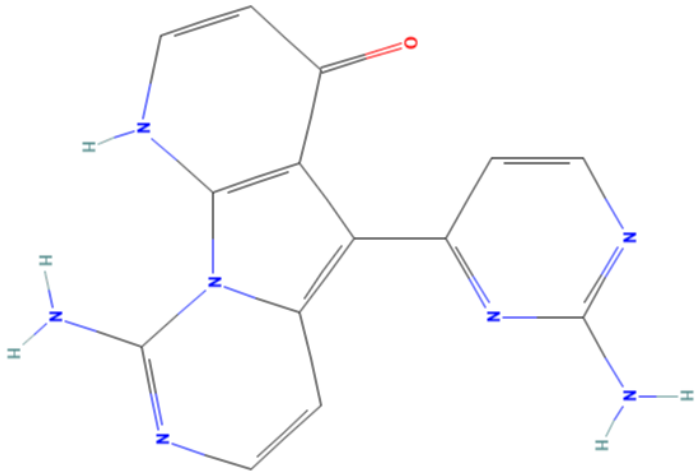
07	JNJ-7706621	5330790	3	10	394.4 g/ mol		[179] [178]
08	Roniciclib	45380979	3	10	430.4 g/ mol		[179]

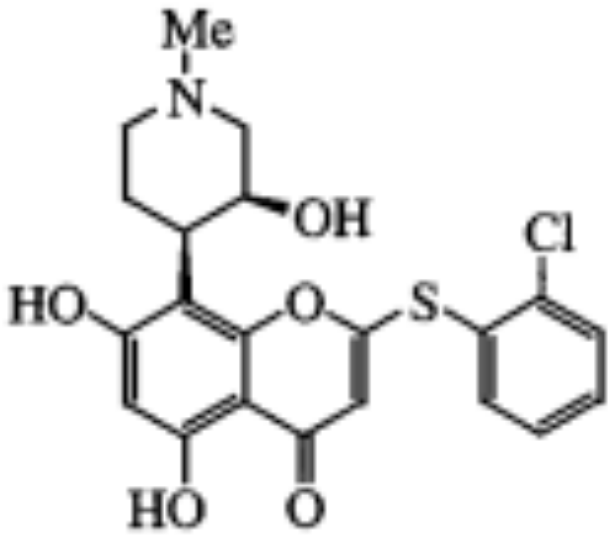
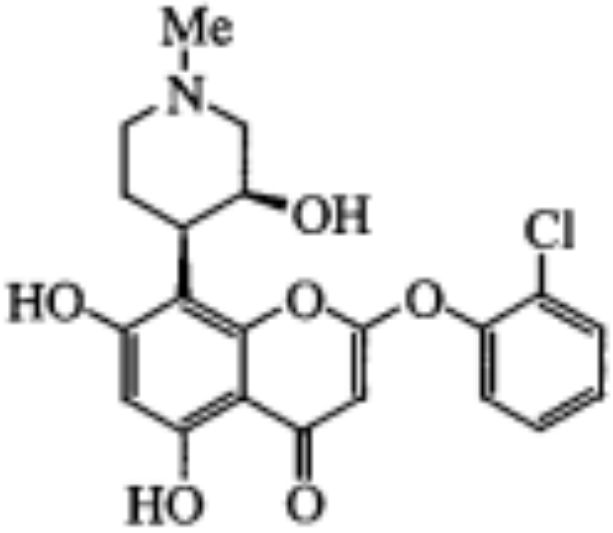
09	Nitazoxanide	41684	1	7	307.28 g/ mol		[180]
10	UCN-01	72271	3	5	482.5 g/ mol		[181]

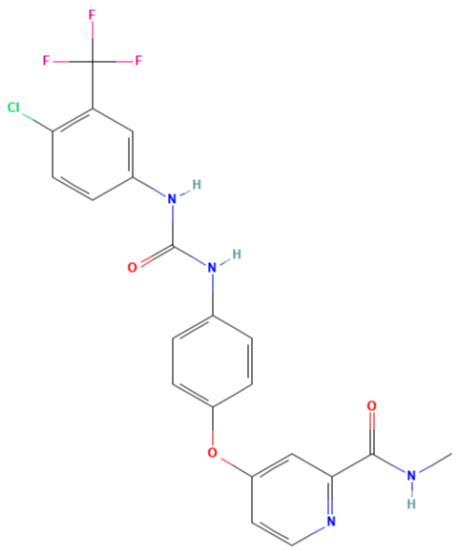
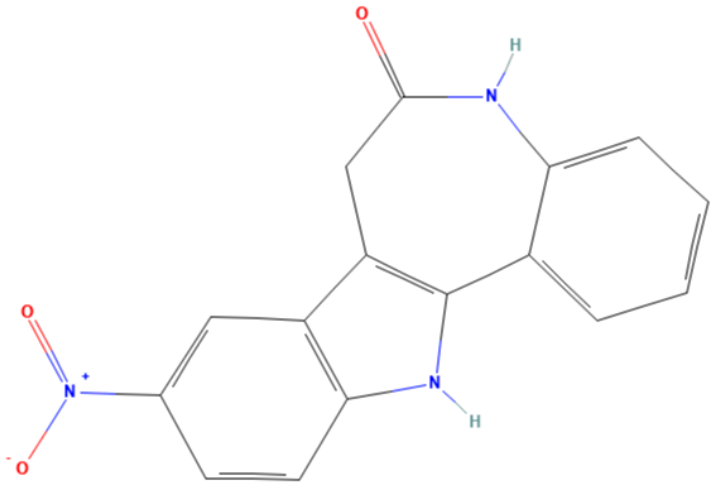
11	Tizoxanide	162641226 /139025169	4	12	467.4 g/mol		[182]
12	AT7519	11338033	4	4	382.2 g/mol		[183]

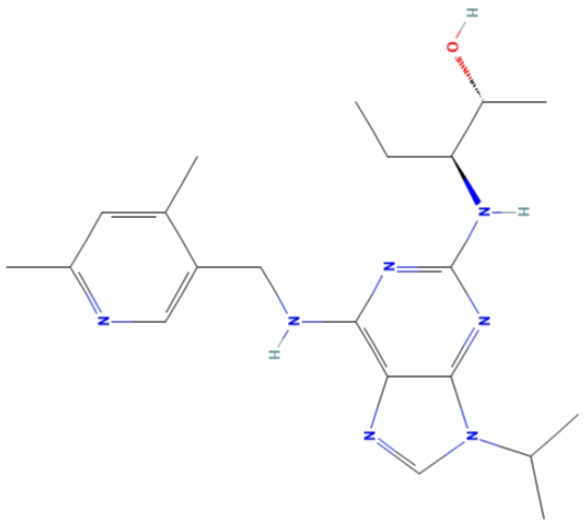
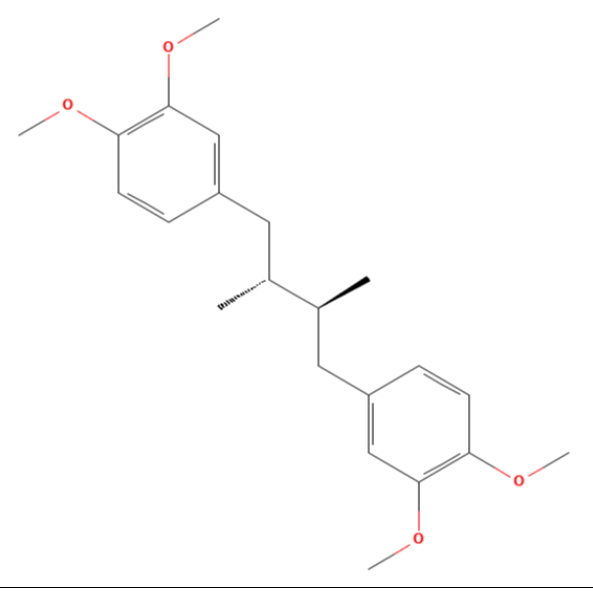
13	Purvalanol A	456214	3	6	388.9 g/ mol		[184] [185]
14	Purvalanol B	448991	4	8	432.9 g/ mol		[184]

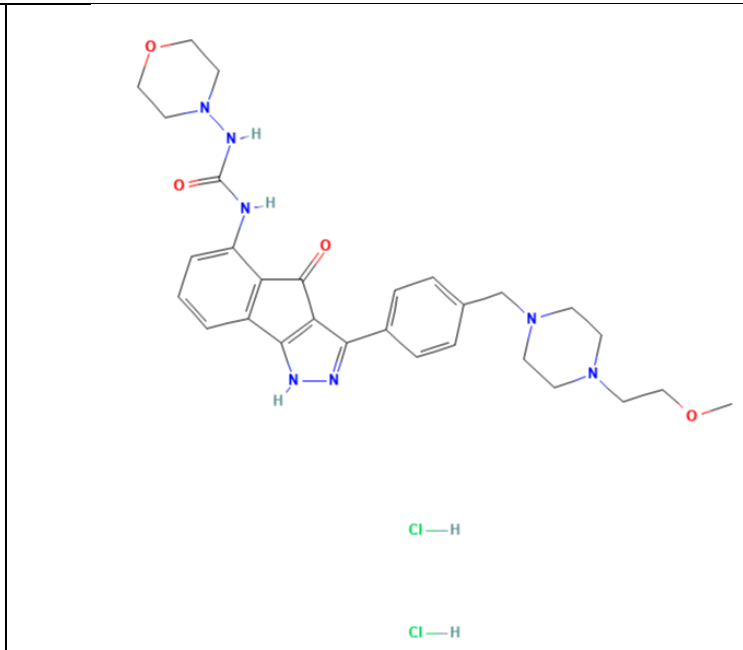
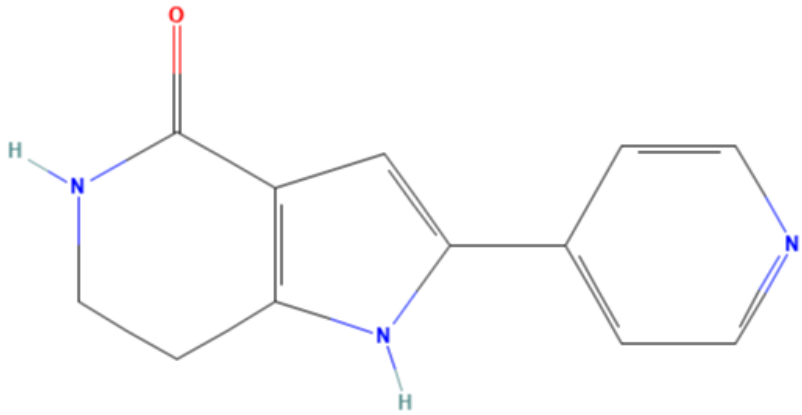
15	Staurosporine	44259	2	4	466.5 g/ mol		[186]
16	Olomoucine	4592	3	6	298.34 g/ mol		[187]

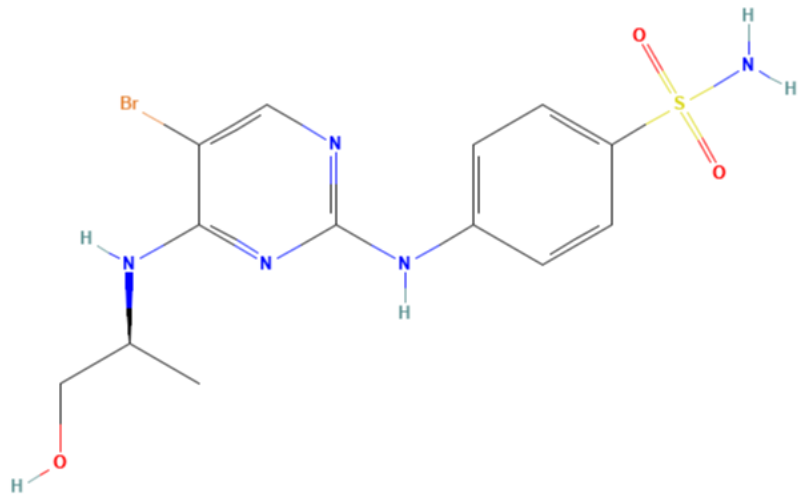
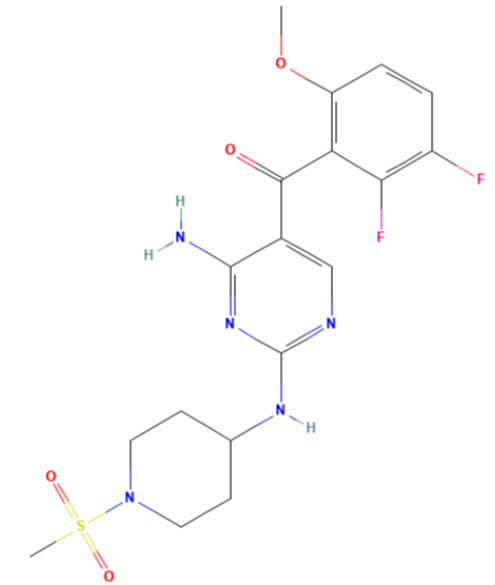
17	CVT-313	6918386	3	8	400.5 g/ mol		[188]
18	Variolin B	9817550	3	7	293.28 g/ mol		[189]

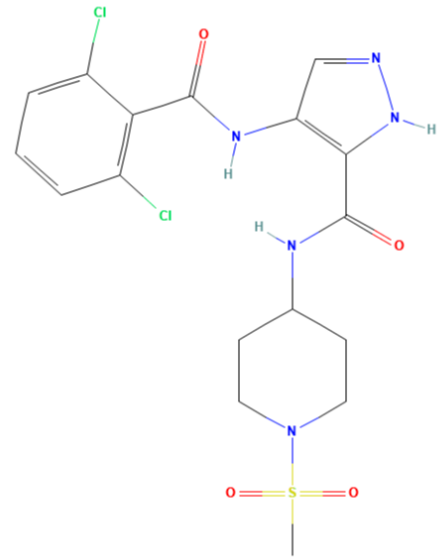
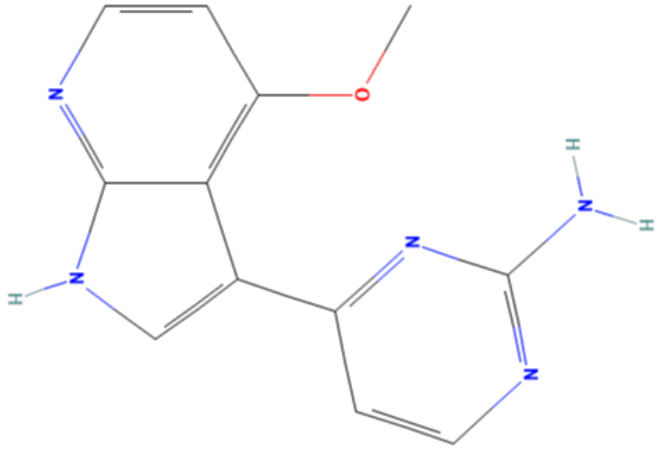
19	Thioflavopiridol	-	-	-	-		[190] [191]
20	Oxoflavopiridol	-	-	-	-		[190] [191]

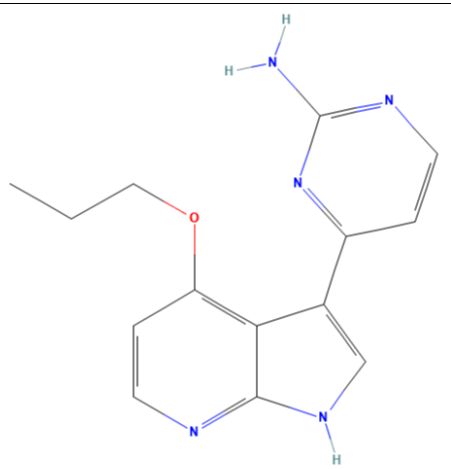
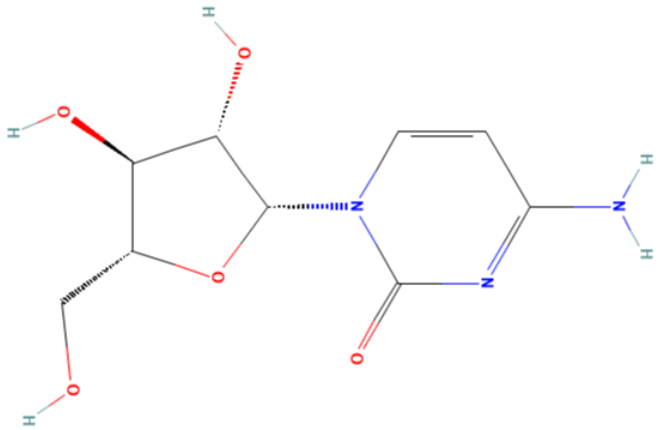
21	Sorafenib	216239	3	7	464.8 g/ mol		[192] [193]
22	Alsterpaullone	5005498	2	3	293.28 g/ mol		[194]

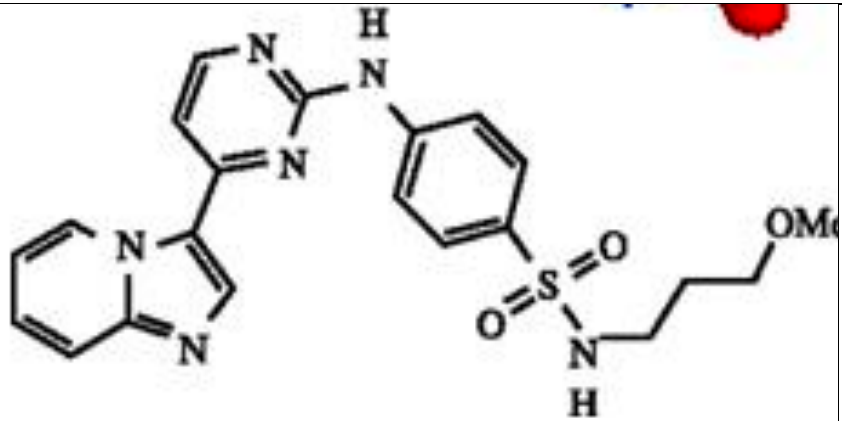
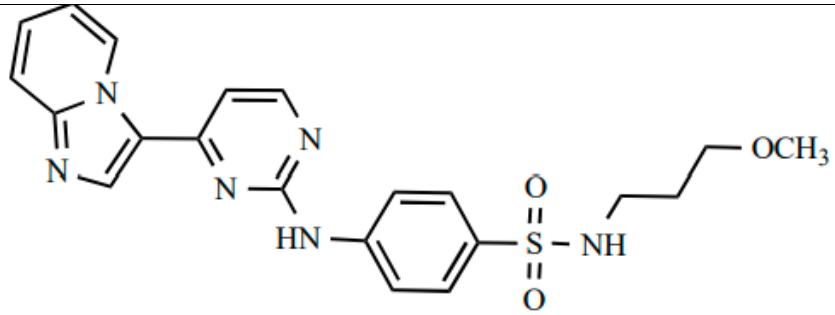
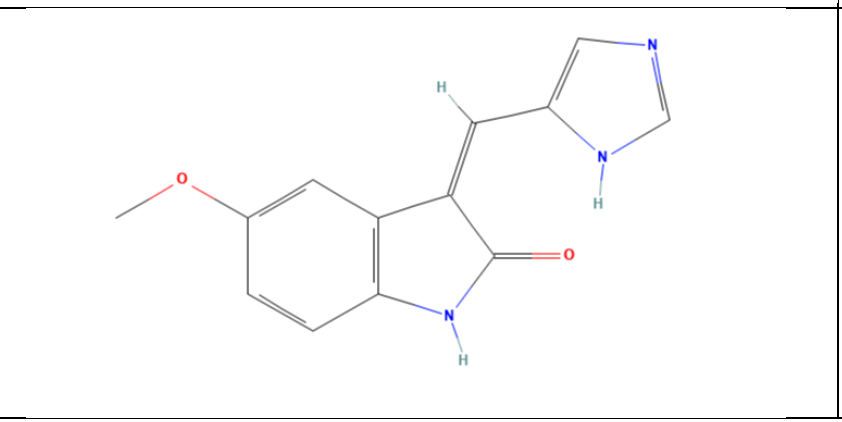
23	CYC065/ Fadraciclib	24983461	3	7	397.5 g/ mol		[195]
24	Teramperprocol	476861	0	4	358.5 g/ mol		[196]

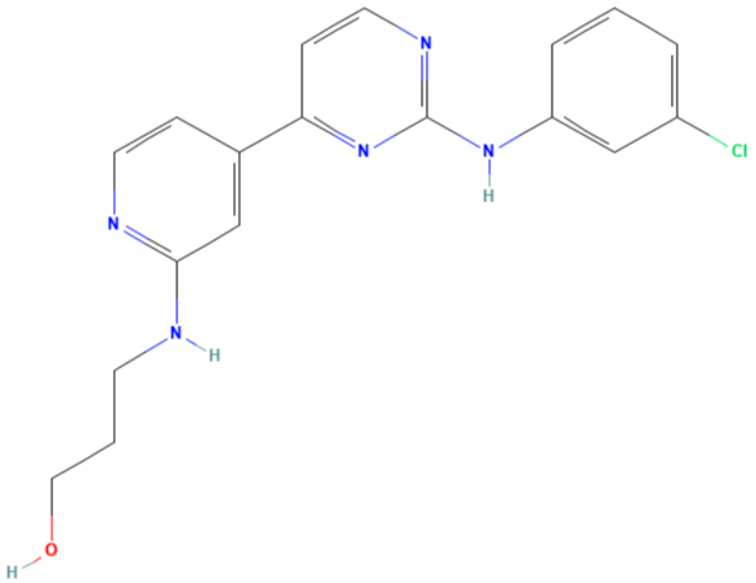
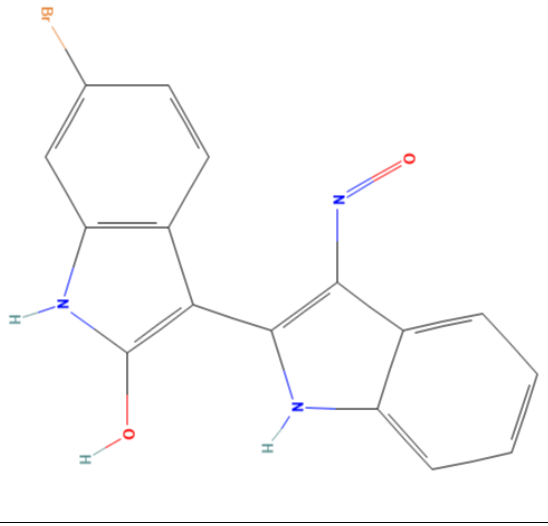
25	RGB-286638	11285001	5	8	618.6 g/ mol		[197]
26	PHA-767491	11715767	2	2	213.23 g/ mol		[198] [199]

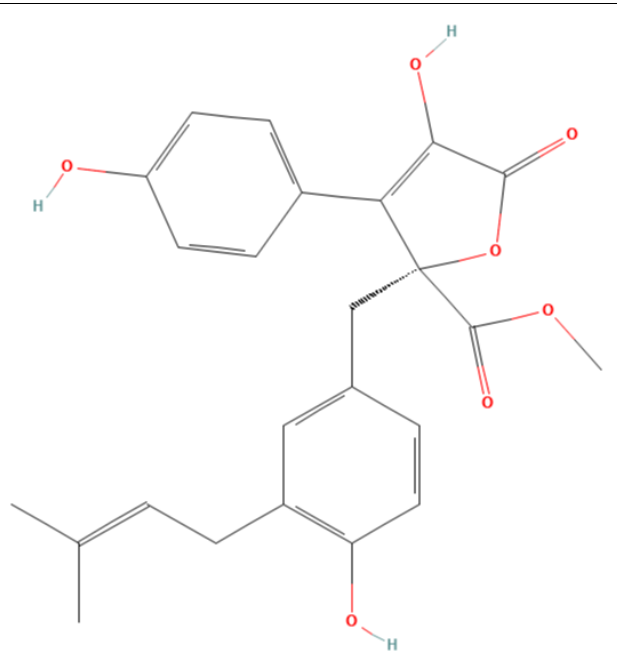
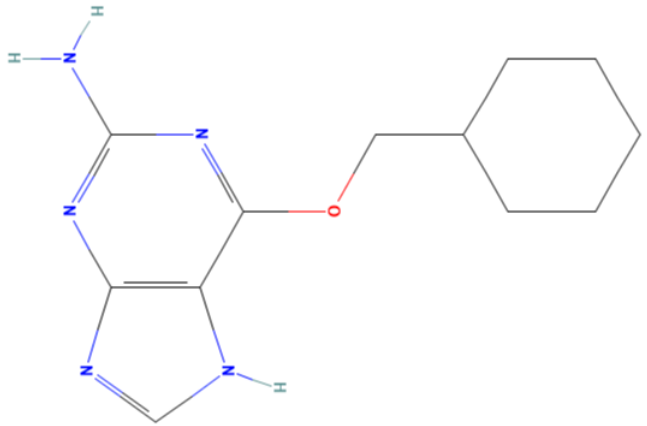
27	ZK304709	58891018	4	8	402.27 g/ mol		[200]
28	R-547	6918852	2	11	441.5 g/ mol		[201]

29	NVP-LCQ195	11655534	3	6	460.3 g/ mol		[202]
30	Meriolin 3	23727981	2	5	241.25 g/ mol		[203]

31	Meriolin 5	23727982	2	5	269.30 g/ mol		[203]
32	Cytarabine	6253	4	5	243.22 g/ mol		[204][205]
33	Meriolin 15	-	-	-	-	-	[206]
34	Meriolin 16	-	-	-	-	-	[206]
35	Meriolin 25	-	-	-	-	-	[206]
36	Meriolin 28	-	-	-	-	-	[206]
37	Meriolin 35	-	-	-	-	-	[206]

38	Olomoucine II	-	-	-	-		[207]
39	AZ703	-	-	-	-		[208] [209]
40	SU9516	5289419	2	3	241.24 g/ mol		[209] [210]

41	CGP-60474	644215	3	6	355.8 g/ mol		[209] [211]
42	6-Bromoindirubin-3'-oxime	448949	3	3	356.17 g/ mol		[212]

43	Butyrolactone I	123740	3	7	424.4 g/ mol		[209] [213]
44	NU-2058	4564	2	5	247.30 g/ mol		[214]

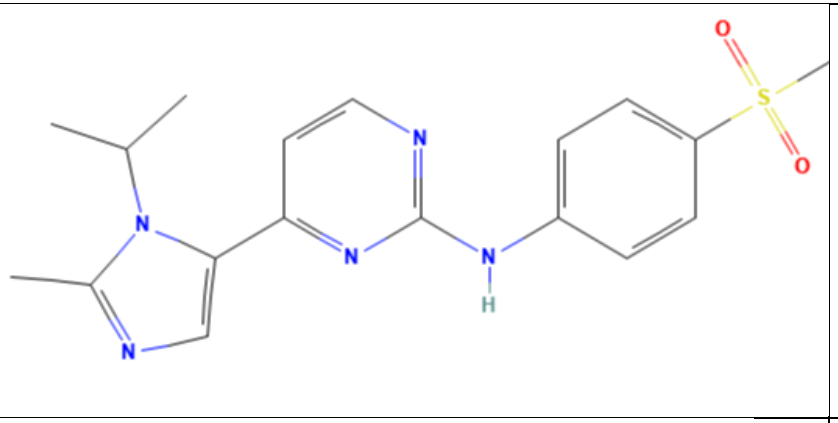
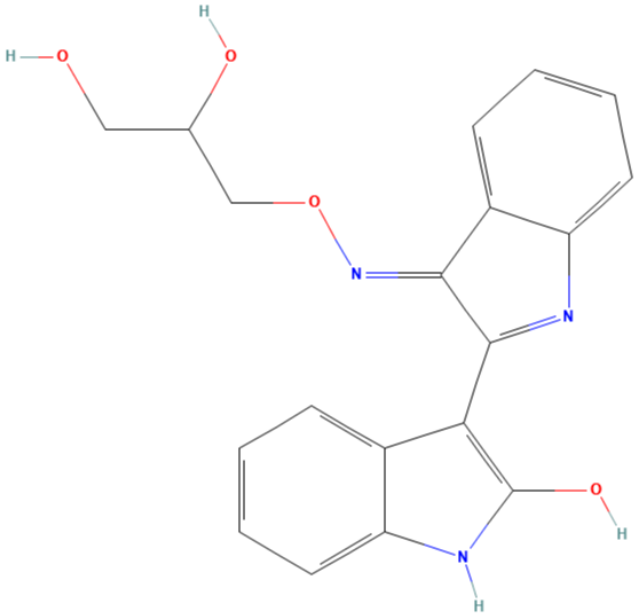
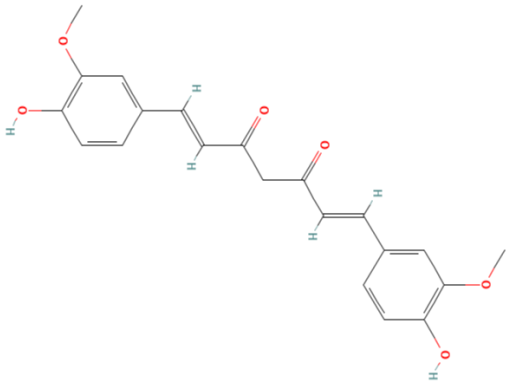
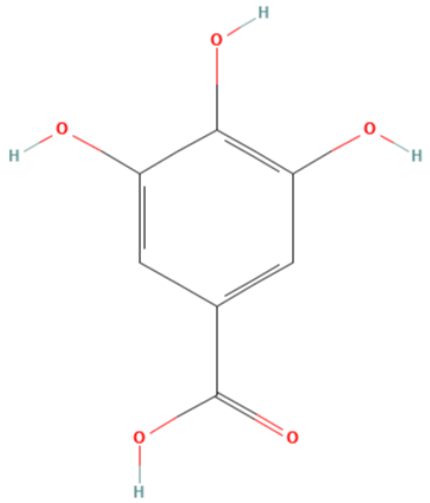
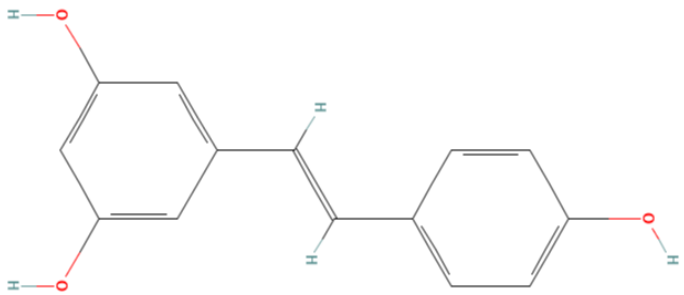
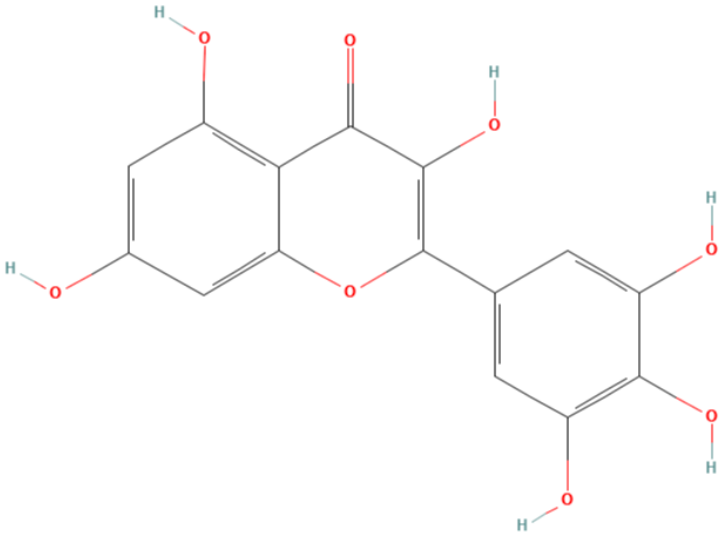
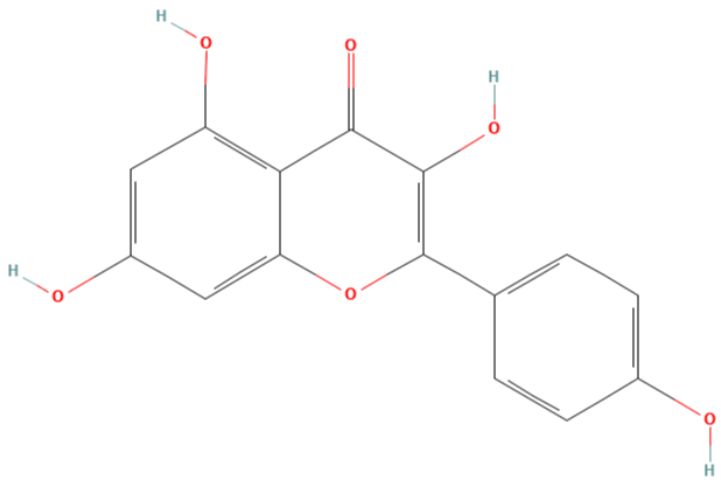
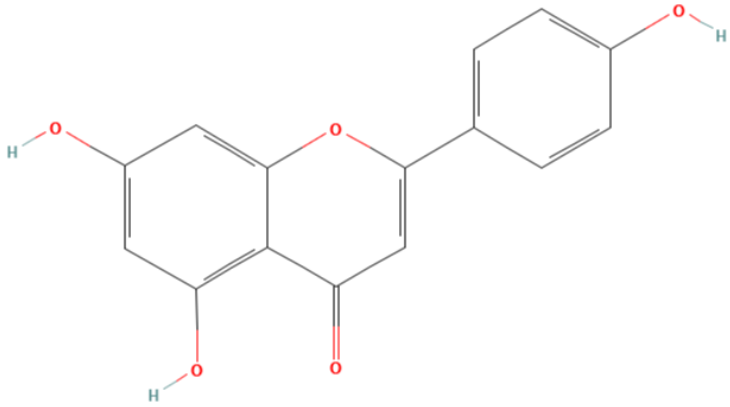
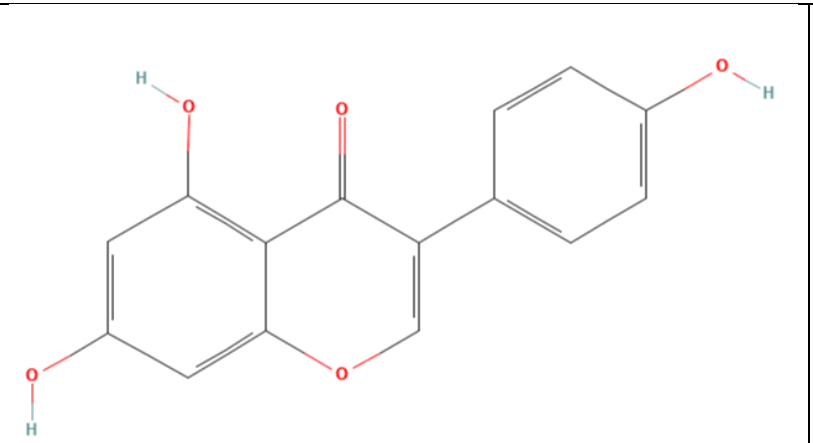
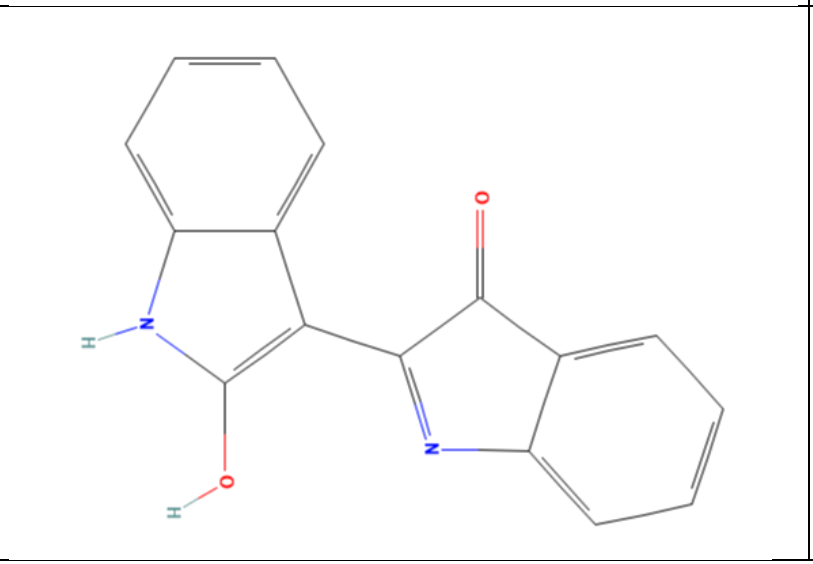
45	AZD5438	16747683	1	6	371.5 g/ mol		[215] [216]
46	E804	135510708	4	6	351.4 g/ mol		[217]

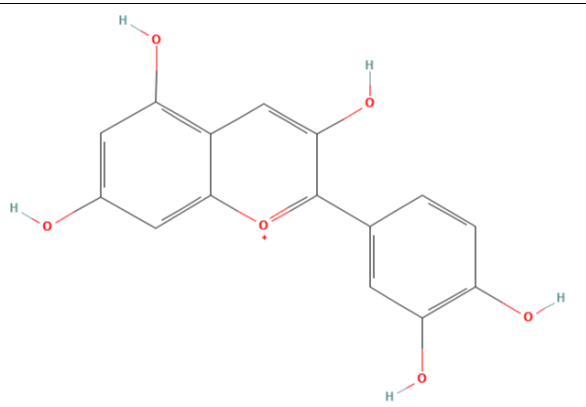
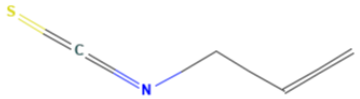
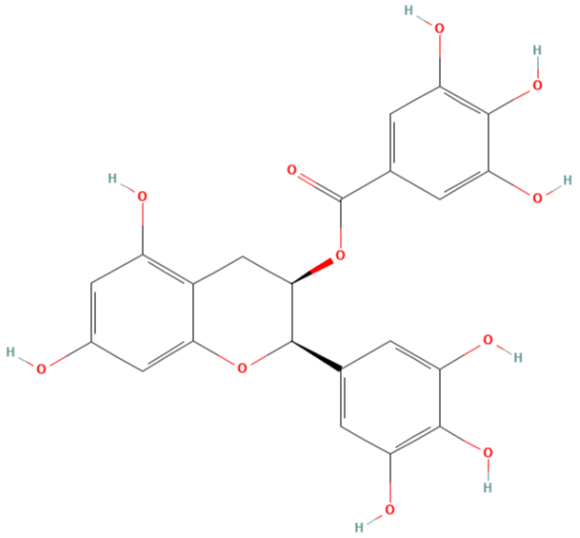
Table 4.11.2. Phytochemical CDK1 Inhibitors

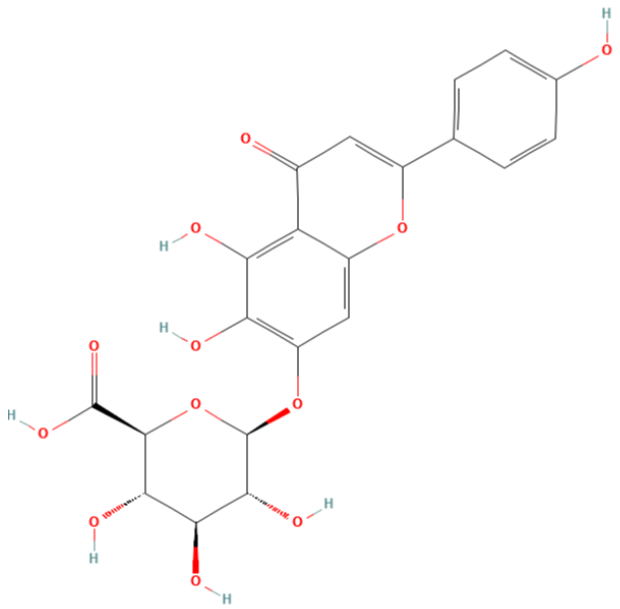
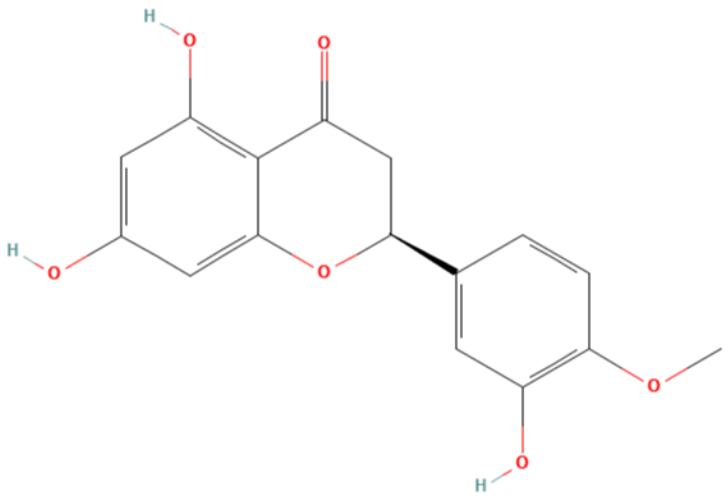
S. No.	Name	PubChem ID	Source	HBD	HBA	MW	Structure	Ref
1	Curcumin	969516	Curcuma longa	2	6	368.4 g/ mol		[218]
2	Gallic Acid	370	-	4	5	170.12 g/ mol		[219] [220]

3	Resveratrol	445154	-	3	3	228.24 g/mol		[221] [222]
4	Myricetin	5281672	-	6	8	318.23 g/mol		[223] [224]

5	Kaempferol	5280863	-	4	6	286.24 g/mol		[225] [226] [227]
6	Apigenin	5280443	-	3	5	270.24 g/mol		[228]

7	Genistein	5280961	-	3	5	270.24 g/mol		[229]
8	Indirubin	10177	Baphicacanthus cusia	2	3	262.26 g/mol		[230]

9	Cyanidin – in lung cancer cells	128861	*Crataegus*	5	5	287.24 g/ mol		[231]
14	Allyl isothiocyanate	5971	*Brassica juncea*	0	2	99.16 g/ mol		[231] [232]
15	Epigallocatechin Gallate	65064	Camellia sinensis	8	11	458.4 g/ mol		[233] [234]

16	Scutellarin	185617	*Scutellaria barbata/ Scutellaria lateriflora*	7	12	462.4 g/ mol		[235] [236]
17	Hesperetin	72281		3	6	302.28 g/ mol		[237]

4.12. MOLECULAR DOCKING OF ESTABLISHED INHIBITORS OF CDK1

Molecular docking using Molegro Virtual Docker (MVD) is an advanced computational technique employed to predict the interaction between small molecules inhibitors (in this case our established inhibitors) and their protein targets (in this case CDK1). MVD utilizes sophisticated algorithms and scoring functions to identify and rank the optimal binding poses of inhibitors within the active site of the proteins **[238,239]**. In this research, all of the established inhibitors were collected and their 3D sdf files were imported into MVD along with the CDK1 protein structure. 5 binding poses were calculated for each ligand. There were 5 cavities detected in the CDK1 structure, out of which cavity 1 was the biggest and the one chosen for binding. [Fig. 4.12.2]

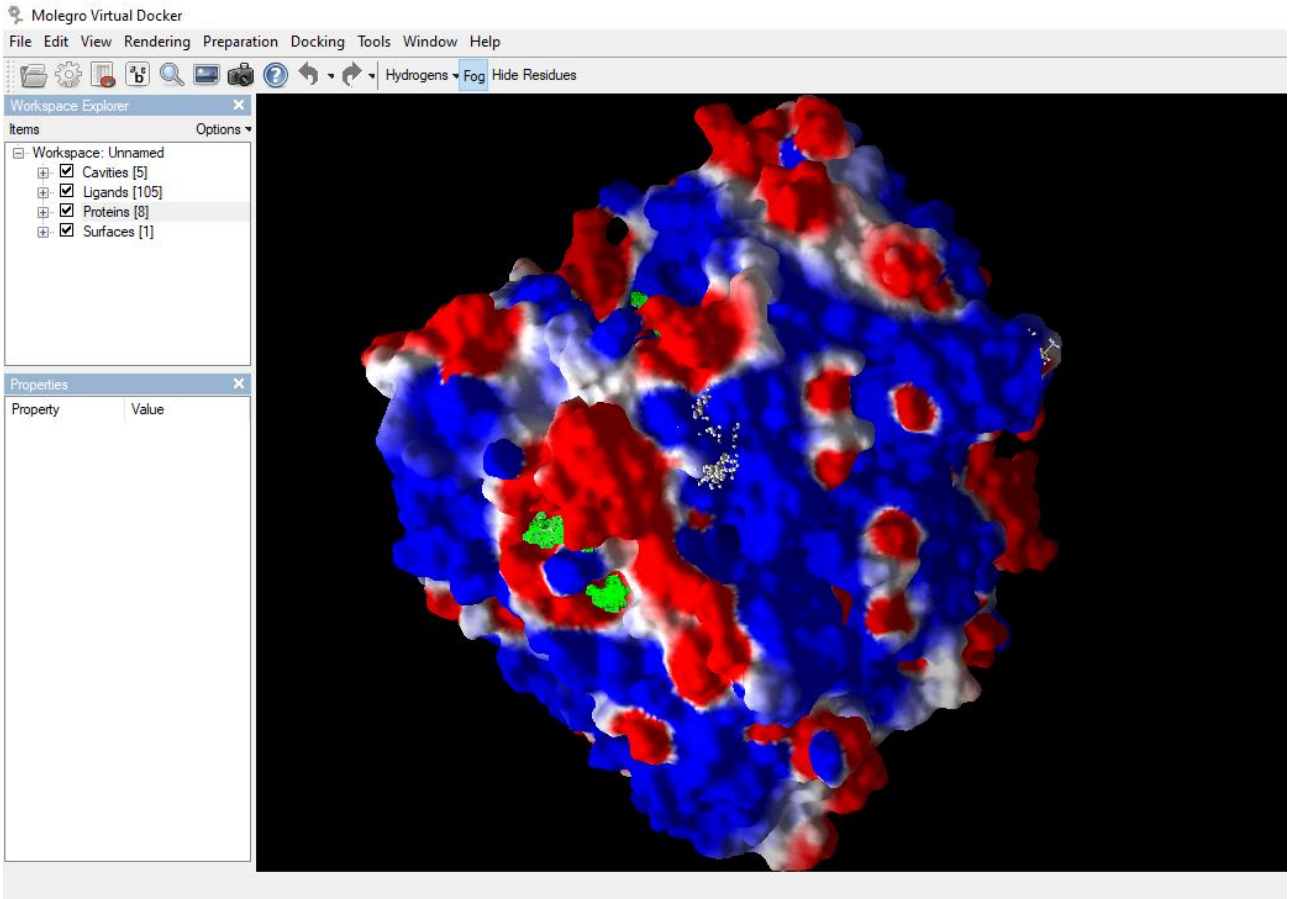


Fig. 4.12.1- CDK1 Protein Structure Prepared for Docking

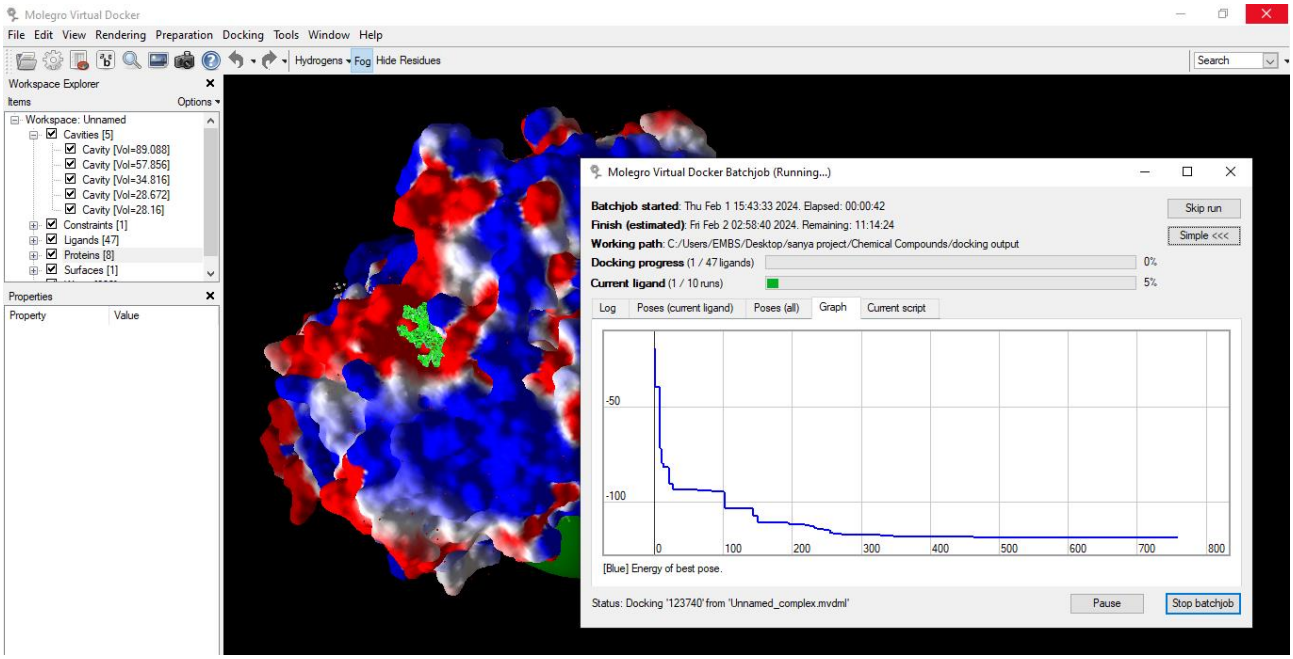


Fig. 4.12.2- Molecular Docking of Established CDK1 Inhibitors

4.13. ANALYSING DOCKING RESULTS OF PHYTOCHEMICALS AND CHEMICAL COMPOUNDS

In the analysis of docking results of established inhibitors, the rerank score computed by MVD plays a crucial role in evaluating the binding affinity and stability of the compounds within the active site of the target protein. After docking both the chemical and phytochemical compounds, the results were analysed. These poses were assessed and sorted based on their re-rank scores, which combine several factors, including van der Waals forces, electrostatic interactions, hydrogen bonding, and solvation effects, to provide a comprehensive binding affinity estimation. The compound with the lowest re-rank score among the phytochemicals and chemicals would be considered as the most promising candidate, as a lower re-rank score indicates a stronger and more favourable interaction with the target protein.

	A	B	C	D	E	F	G	H	I	J	K	L
1	Name	Ligand	Filename	MolDock Score	Rerank Score	HBond	MW	SMILES				
2	[01] 4564	4564	[01] 4564.mol2	-88.3824	-70.6225	-8.87816	247.296	NC(N=1)=NC=2N=CNC=2C=1OCC3CCCCC3				
3	[00] 4564	4564	[00] 4564.mol2	-86.8049	-72.4871	-1.10691	247.296	NC(N=1)=NC=2N=CNC=2C=1OCC3CCCCC3				
4	[02] 4564	4564	[02] 4564.mol2	-85.8155	-68.9648	-3.89651	247.296	NC(N=1)=NC=2N=CNC=2C=1OCC3CCCCC3				
5	[03] 4564	4564	[03] 4564.mol2	-84.2561	-68.3519	-2.64064	247.296	NC(N=1)=NC=2N=CNC=2C=1OCC3CCCCC3				
6	[04] 4564	4564	[04] 4564.mol2	-81.025	-65.5548	-2.29397	247.296	NC(N=1)=NC=2N=CNC=2C=1OCC3CCCCC3				
7	[00] 4592	4592	[00] 4592.mol2	-105.378	-36.7496	-12.3209	298.343	OCCNC(=N1)N=C2N(C)C=NC2=C1NCC=3C=CC=CC=3				
8	[02] 4592	4592	[02] 4592.mol2	-101.432	-70.6937	-8.73224	298.343	OCCNC(=N1)N=C2N(C)C=NC2=C1NCC=3C=CC=CC=3				
9	[01] 4592	4592	[01] 4592.mol2	-100.526	-65.2923	-6.8934	298.343	OCCNC(=N1)N=C2N(C)C=NC2=C1NCC=3C=CC=CC=3				
10	[04] 4592	4592	[04] 4592.mol2	-100.207	-76.7845	0	298.343	OCCNC(=N1)N=C2N(C)C=NC2=C1NCC=3C=CC=CC=3				
11	[03] 4592	4592	[03] 4592.mol2	-99.7795	-79.108	-3.99948	298.343	OCCNC(=N1)N=C2N(C)C=NC2=C1NCC=3C=CC=CC=3				
12	[02] 6253	6253	[02] 6253.mol2	-89.723	-70.5206	-13.3546	243.217	OC1C(O)C(CO)OC1N2C=CC(N)=NC2=O				
13	[00] 6253	6253	[00] 6253.mol2	-87.8236	-61.9892	-11.2296	243.217	OC1C(O)C(CO)OC1N2C=CC(N)=NC2=O				
14	[01] 6253	6253	[01] 6253.mol2	-84.0534	-56.8457	-10.7589	243.217	OC1C(O)C(CO)OC1N2C=CC(N)=NC2=O				
15	[03] 6253	6253	[03] 6253.mol2	-80.0228	-62.2691	-4.89871	243.217	OC1C(O)C(CO)OC1N2C=CC(N)=NC2=O				
16	[04] 6253	6253	[04] 6253.mol2	-79.5514	-61.6285	-5.19073	243.217	OC1C(O)C(CO)OC1N2C=CC(N)=NC2=O				
17	[00] 41684	41684	[00] 41684.mol2	-92.943	-76.2432	-8.14995	307.282	O=C(NC=1SC(=CN=1)N(=O)O)C2=CC=CC=C2OC(=O)C				
18	[03] 41684	41684	[03] 41684.mol2	-89.6847	-72.9897	-2.9358	307.282	O=C(NC=1SC(=CN=1)N(=O)O)C2=CC=CC=C2OC(=O)C				
19	[02] 41684	41684	[02] 41684.mol2	-89.0637	-69.6289	-3.57935	307.282	O=C(NC=1SC(=CN=1)N(=O)O)C2=CC=CC=C2OC(=O)C				
20	[01] 41684	41684	[01] 41684.mol2	-81.6896	-55.3863	-10.6268	307.282	O=C(NC=1SC(=CN=1)N(=O)O)C2=CC=CC=C2OC(=O)C				
21	[04] 41684	41684	[04] 41684.mol2	-81.4355	-61.7108	-3.56567	307.282	O=C(NC=1SC(=CN=1)N(=O)O)C2=CC=CC=C2OC(=O)C				
22	[01] 44259	44259	[01] 44259.mol2	135.896	82.8457	0.44487	466.531	O=C1NCC=2C1=C3C4=CC=CC=C4N5C3=C6C				

Fig. 4.13.1- Molecular Docking of Established CDK1 Chemical Inhibitors

	A	B	C	D	E	F	G	H	I	J	K	L
1	Name	Ligand	Filename	MolDock Score	Rerank Score	HBond	MW	SMILES				
2	[00] 370	370	[00] 370.mol2	-67.5088	-50.76	-12.2686	170.12	OC=1C(O)=CC(C(=O)O)=CC=1O				
3	[02] 370	370	[02] 370.mol2	-67.1902	-50.382	-9.78371	170.12	OC=1C(O)=CC(C(=O)O)=CC=1O				
4	[01] 370	370	[01] 370.mol2	-64.4747	-49.1695	-11.3407	170.12	OC=1C(O)=CC(C(=O)O)=CC=1O				
5	[04] 370	370	[04] 370.mol2	-62.9325	-44.4164	-12.7102	170.12	OC=1C(O)=CC(C(=O)O)=CC=1O				
6	[03] 370	370	[03] 370.mol2	-62.8087	-46.4992	-10.4159	170.12	OC=1C(O)=CC(C(=O)O)=CC=1O				
7	[00] 5971	5971	[00] 5971.mol2	-39.7097	-32.9739	-2.5	99.1542	S=C=NCC=C				
8	[01] 5971	5971	[01] 5971.mol2	-39.3956	-32.6338	-2.5	99.1542	S=C=NCC=C				
9	[03] 5971	5971	[03] 5971.mol2	-38.8722	-32.5094	-2.5	99.1542	S=C=NCC=C				
10	[02] 5971	5971	[02] 5971.mol2	-37.8648	-31.7975	-2.01725	99.1542	S=C=NCC=C				
11	[04] 5971	5971	[04] 5971.mol2	-37.4567	-31.5243	-2.05122	99.1542	S=C=NCC=C				
12	[00] 10177	10177	[00] 10177.mol2	-94.27	-71.1504	-3.15385	262.263	OC=1NC2=CC=CC=C2C=1C3=NC4=CC=CC=C4C3=O				
13	[02] 10177	10177	[02] 10177.mol2	-92.6729	-69.7763	-4.19703	262.263	OC=1NC2=CC=CC=C2C=1C3=NC4=CC=CC=C4C3=O				
14	[03] 10177	10177	[03] 10177.mol2	-89.288	-68.0876	-2.45815	262.263	OC=1NC2=CC=CC=C2C=1C3=NC4=CC=CC=C4C3=O				
15	[04] 10177	10177	[04] 10177.mol2	-88.7429	-63.5996	-1.63586	262.263	OC=1NC2=CC=CC=C2C=1C3=NC4=CC=CC=C4C3=O				
16	[01] 10177	10177	[01] 10177.mol2	-88.5121	-65.6763	-3.59135	262.263	OC=1NC2=CC=CC=C2C=1C3=NC4=CC=CC=C4C3=O				
17	[01] 65064	65064	[01] 65064.mol2	-134.656	-72.379	-8.85181	458.372	OC=1C=C(O)C=C(OC2(C3=CC(=C(C(=C3)O)O)O))C=1C				
18	[02] 65064	65064	[02] 65064.mol2	-133.249	-95.1963	-10.8867	458.372	OC=1C=C(O)C=C(OC2(C3=CC(=C(C(=C3)O)O)O))C=1C				
19	[00] 65064	65064	[00] 65064.mol2	-128.295	-79.0009	-9.98125	458.372	OC=1C=C(O)C=C(OC2(C3=CC(=C(C(=C3)O)O)O))C=1C				
20	[03] 65064	65064	[03] 65064.mol2	-128.035	-82.3139	-10.9209	458.372	OC=1C=C(O)C=C(OC2(C3=CC(=C(C(=C3)O)O)O))C=1C				
21	[04] 65064	65064	[04] 65064.mol2	-123.225	-69.3898	-9.99351	458.372	OC=1C=C(O)C=C(OC2(C3=CC(=C(C(=C3)O)O)O))C=1C				
22	[03] 72281	72281	[03] 72281.mol2	92.8361	68.7428	12.7931	302.279	O=C(C1)C2=C(O)C=C(O)C=C2OC1C=3C=CC(OC)=C(O)C				

Fig. 4.13.2- Molecular Docking of Established CDK1 Phytochemical Inhibitors

4.14. SIMILARITY SEARCH AGAINST PUBCHEM OF BEST-ESTABLISHED COMPOUND

PubChem is a comprehensive and freely accessible chemical database that is maintained by the National Center for Biotechnology Information (NCBI). It provides detailed information on the chemical properties, biological activities and more, of various chemical compounds. Researchers and scientists use PubChem to find chemical structure, biological test results and links to the related literature. Hence, PubChem is an invaluable resource for drug discovery and chemical research**[240,241]**.

PubChem offers an advanced feature known as similarity searching. This powerful tool allows users to find compounds that have similar structures or properties to the query compound. It does this by utilizing algorithms that compare various molecular features such as fingerprints, descriptors, etc. and then ranks the compound based on similarity. This enables researchers to quickly and easily find compounds that share similar structure and in turn may share similar function **[240,241]**. In this research, PubChem similarity search was used against the PubChem ID of the best established Inhibitor (24983461) with a threshold value of 95% [Fig. 4.14.].

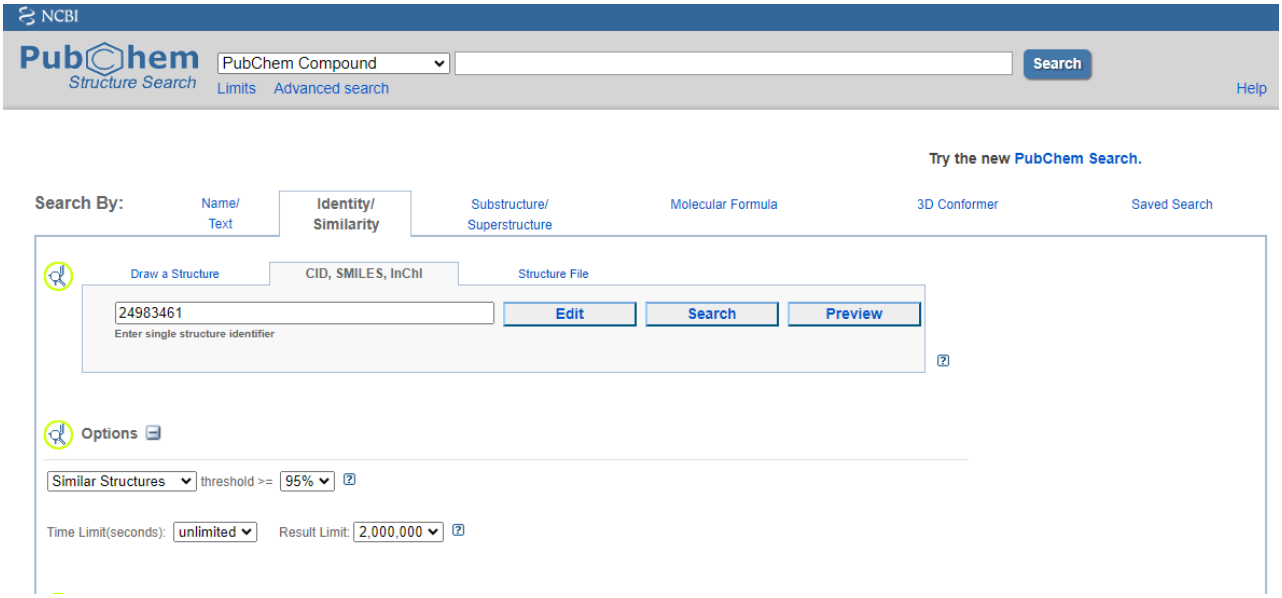


Figure 4.14. - PubChem Similarity Search

4.15. MOLECULAR DOCKING OF VIRTUAL SCREENING COMPOUNDS

Molecular docking was once again conducted against the CDK1 protein. This time the ligands used were the results of the PubChem similarity search conducted previously. Docking was performed on Molegro Virtual Docker. All parameters were kept the same as in the previous docking [Fig. 14.15.].

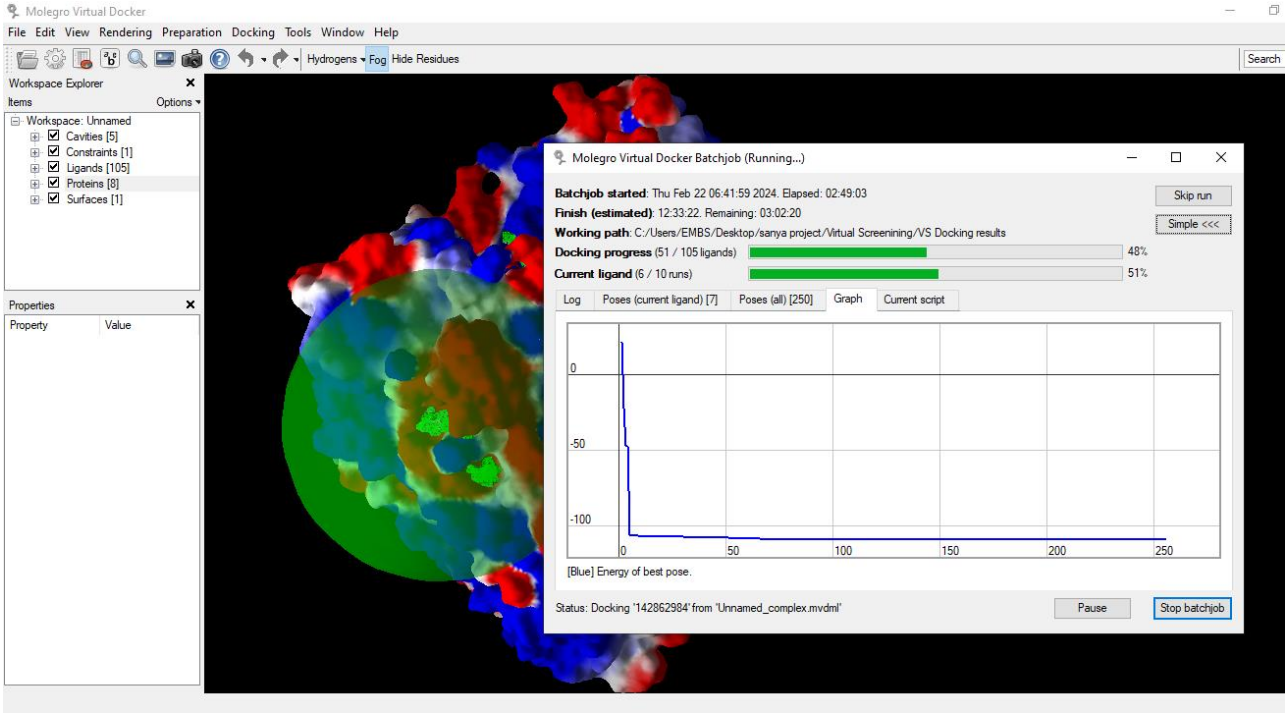


Fig. 4.15.- Molecular Docking of Virtual Screening Compounds

4.16. ANALYSING DOCKING RESULTS OF VIRTUAL SCREENING COMPOUNDS

The compound with the lowest re-rank score among the virtual screening compounds was identified as the most promising candidate, as a lower re-rank score indicates a stronger and more favourable interaction with the target protein. The rerank score of the best virtual screening compound was then compared to the rerank score of the best established inhibitor.

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	Name	Ligand	Filename	MolDock Score	Rerank Score	HBond	SMILES						
2	[03] 9842276	9842276	[03] 9842276.m	-119.879	-76.5359	-10.2357	OC(C)C(CC)NC(=N1)N=C2N(C(C)C)C=NC2=C1NCC3=CN=CC=C3						
3	[01] 9842276	9842276	[01] 9842276.m	-117.664	-73.8323	-2.03143	OC(C)C(CC)NC(=N1)N=C2N(C(C)C)C=NC2=C1NCC3=CN=CC=C3						
4	[00] 9842276	9842276	[00] 9842276.m	-117.303	-89.3186	-2.96392	OC(C)C(CC)NC(=N1)N=C2N(C(C)C)C=NC2=C1NCC3=CN=CC=C3						
5	[02] 9842276	9842276	[02] 9842276.m	-115.734	-77.8026	-4.18309	OC(C)C(CC)NC(=N1)N=C2N(C(C)C)C=NC2=C1NCC3=CN=CC=C3						
6	[04] 9842276	9842276	[04] 9842276.m	-114.745	-84.3009	-3.09865	OC(C)C(CC)NC(=N1)N=C2N(C(C)C)C=NC2=C1NCC3=CN=CC=C3						
7	[04] 10000883	10000883	[04] 10000883.r	-137.344	-93.0432	-7.0699	OC(C(C)C)C(CC)NC(=N1)N=C2N(C(C)C)C=NC2=C1NCC3=CC=CC=N3						
8	[00] 10000883	10000883	[00] 10000883.r	-135.858	-89.7361	-4.96882	OC(C(C)C)C(CC)NC(=N1)N=C2N(C(C)C)C=NC2=C1NCC3=CC=CC=N3						
9	[03] 10000883	10000883	[03] 10000883.r	-131.165	-86.2243	-4.91771	OC(C(C)C)C(CC)NC(=N1)N=C2N(C(C)C)C=NC2=C1NCC3=CC=CC=N3						
10	[01] 10000883	10000883	[01] 10000883.r	-128.848	-93.1933	-10.1575	OC(C(C)C)C(CC)NC(=N1)N=C2N(C(C)C)C=NC2=C1NCC3=CC=CC=N3						
11	[02] 10000883	10000883	[02] 10000883.r	-126.886	-69.8465	-4.50983	OC(C(C)C)C(CC)NC(=N1)N=C2N(C(C)C)C=NC2=C1NCC3=CC=CC=N3						
12	[00] 10001665	10001665	[00] 10001665.r	-142.919	-102.224	-3.18666	OC(C(C)(C)C)C(CC)NC(=N1)N=C2N(C(C)C)C=NC2=C1NCC3=CC=CC=N3						
13	[03] 10001665	10001665	[03] 10001665.r	-136.126	-90.2595	-2.68999	OC(C(C)(C)C)C(CC)NC(=N1)N=C2N(C(C)C)C=NC2=C1NCC3=CC=CC=N3						
14	[01] 10001665	10001665	[01] 10001665.r	-134.744	-76.0783	-4.90906	OC(C(C)(C)C)C(CC)NC(=N1)N=C2N(C(C)C)C=NC2=C1NCC3=CC=CC=N3						
15	[02] 10001665	10001665	[02] 10001665.r	-131.731	-93.3107	-5.81748	OC(C(C)(C)C)C(CC)NC(=N1)N=C2N(C(C)C)C=NC2=C1NCC3=CC=CC=N3						
16	[04] 10001665	10001665	[04] 10001665.r	-130.475	-96.6038	-13.6165	OC(C(C)(C)C)C(CC)NC(=N1)N=C2N(C(C)C)C=NC2=C1NCC3=CC=CC=N3						
17	[00] 10001666	10001666	[00] 10001666.r	-142.483	-95.3604	-3.78294	OC(C(C)(C)C)C(CC)NC(=N1)N=C2N(C(C)C)C=NC2=C1NCC3=CC=CC=N3						
18	[01] 10001666	10001666	[01] 10001666.r	-136.351	-92.9505	-2.88649	OC(C(C)(C)C)C(CC)NC(=N1)N=C2N(C(C)C)C=NC2=C1NCC3=CC=CC=N3						
19	[02] 10001666	10001666	[02] 10001666.r	-136.05	-89.1687	-7.3783	OC(C(C)(C)C)C(CC)NC(=N1)N=C2N(C(C)C)C=NC2=C1NCC3=CC=CC=N3						
20	[03] 10001666	10001666	[03] 10001666.r	-130.034	-85.3035	-2.25695	OC(C(C)(C)C)C(CC)NC(=N1)N=C2N(C(C)C)C=NC2=C1NCC3=CC=CC=N3						
21	[04] 10001666	10001666	[04] 10001666.r	-129.295	-80.1106	-1.56337	OC(C(C)(C)C)C(CC)NC(=N1)N=C2N(C(C)C)C=NC2=C1NCC3=CC=CC=N3						
22	[01] 10155152	10155152	[01] 10155152.r	120.823	81.3301	3.99365	OC(CC)NC(=N1)N=C2N(C(C)C)C=NC2=C1NCC=3C=CN=CC=3						

Fig. 4.16.- Molecular Docking Results of Virtual Screening Compounds

4.17. PHARMACOPHORE STUDIES OF BEST VIRTUAL SCREENING COMPOUND

Discovery Studio was employed for pharmacophore modelling to identify and characterize the essential features required for optimal ligand-receptor interactions. This advanced software tool facilitated the creation of a pharmacophore model by analysing key parameters such as hydrogen bonds, electrostatic interactions, receptor-ligand interactions, electrostatic surface, aromatic surface, and ionic surface **[242]**.

4.18. ADMET STUDIES

ADMETsar is a freely accessible bioinformatics web-tool that helps researchers conduct ADMET properties prediction. ADMET stands for Absorption, Distribution, Metabolism, Excretion and Toxicity. ADMET prediction involves correlating specific molecular structures with their observed ADMET properties, enabling researchers to predict and improve the behaviour of new compounds. By integrating ADMET analysis in the drug design process, the probability of success in subsequent steps is greatly increased, ultimately accelerating the path to market for promising drug candidates **[243]**. In this research, we used ADMETsar [Fig. 4.18.] by entering the canonical smiles of the best virtual screening compound. We then compared these results to the ADMET of the best established compound.

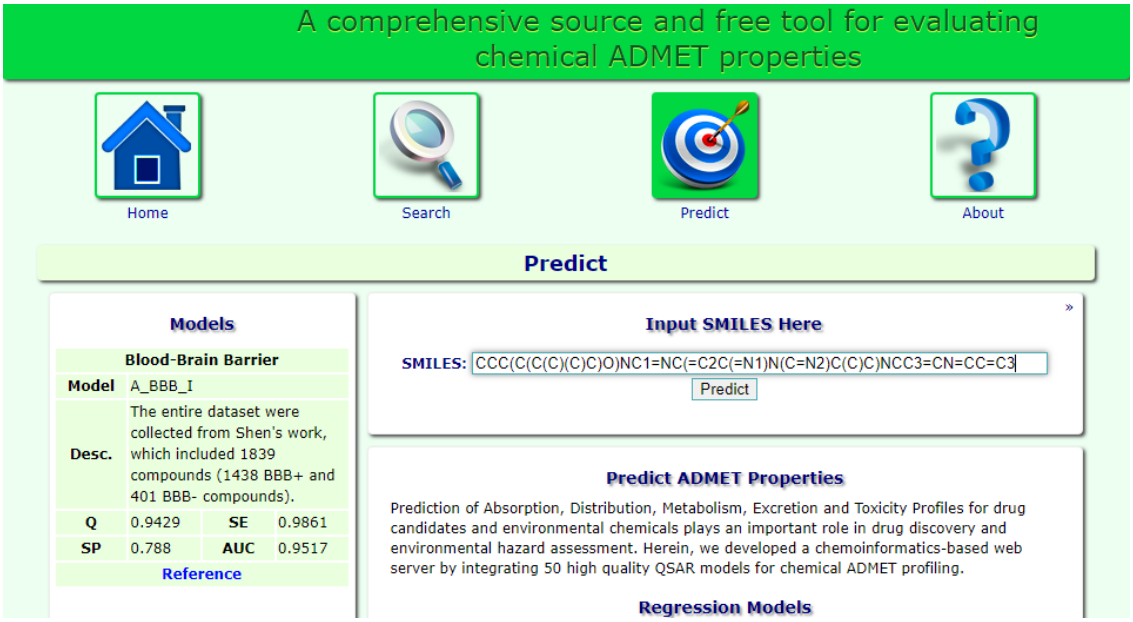


Fig. 4.18. – ADMET Prediction using ADMETsar

CHAPTER 5 – RESULTS

CHAPTER 5 – RESULTS

5.1. DIFFERENTIAL EXPREESSION ANALYSIS USING GEPIA2

Differentially expressed genes sorted from lowest to highest adjusted p-value. Top 20 entries out of 2283 gene ids are displayed below [Table 5.1.].

Table 5.1. Top 20 Results After Filtering

Gene Symbol	Gene ID	Median (Tumor)	Median (Normal)	Log2(Fold Change)	adjp
CMTM3	ENSG00000140931.19	74.099	3.4	7.285	1.22E-93
UBE2C	ENSG00000175063.16	60.698	0.12	6.749	1.23E-89
GPX7	ENSG00000116157.5	28.44	2.34	6.419	9.26E-106
F2R	ENSG00000181104.6	33.511	0.56	6.222	3.83E-176
RRM2	ENSG00000171848.13	27.751	0.1	6.055	1.64E-125
RP2	ENSG00000102218.5	11.32	0.91	5.937	7.49E-79
PRDX4	ENSG00000123131.12	173.032	11.99	5.863	2.2E-138
RPLP0P6	ENSG00000213553.4	30.619	1.84	5.784	1.24E-199
NUP37	ENSG00000075188.8	22.659	2.71	5.781	4.87E-105
EIF4EBP1	ENSG00000187840.4	68.588	3.71	5.571	1.57E-83
TOP2A	ENSG00000131747.14	35.341	0.09	5.528	4.04E-142
CKLF	ENSG00000217555.12	92.25	9.28	5.511	4.77E-139
OLFML3	ENSG00000116774.11	55.31	1.83	5.46	7.78E-135
CHST14	ENSG00000169105.7	24.609	2.13	5.408	1.37E-126
WEE1	ENSG00000166483.10	30.091	0.8	5.388	7.83E-137
TNC	ENSG00000041982.14	133.342	0.8	5.372	3.9E-123
CDK1	ENSG00000170312.15	21.43	0.54	5.314	5.39E-115
SOHLH1	ENSG00000165643.10	0.12	28.281	5.275	7.97E-155

SPARC	ENSG00000113140.10	3495.298	106.122	5.247	1.76E-134

5.2. PROTEIN-PROTEIN INTERACTION NETWORK OF TOP DEG'S

Protein-protein interaction network of the top 20 differentially expressed genes (up-regulated) is displayed below [Fig. 5.2].

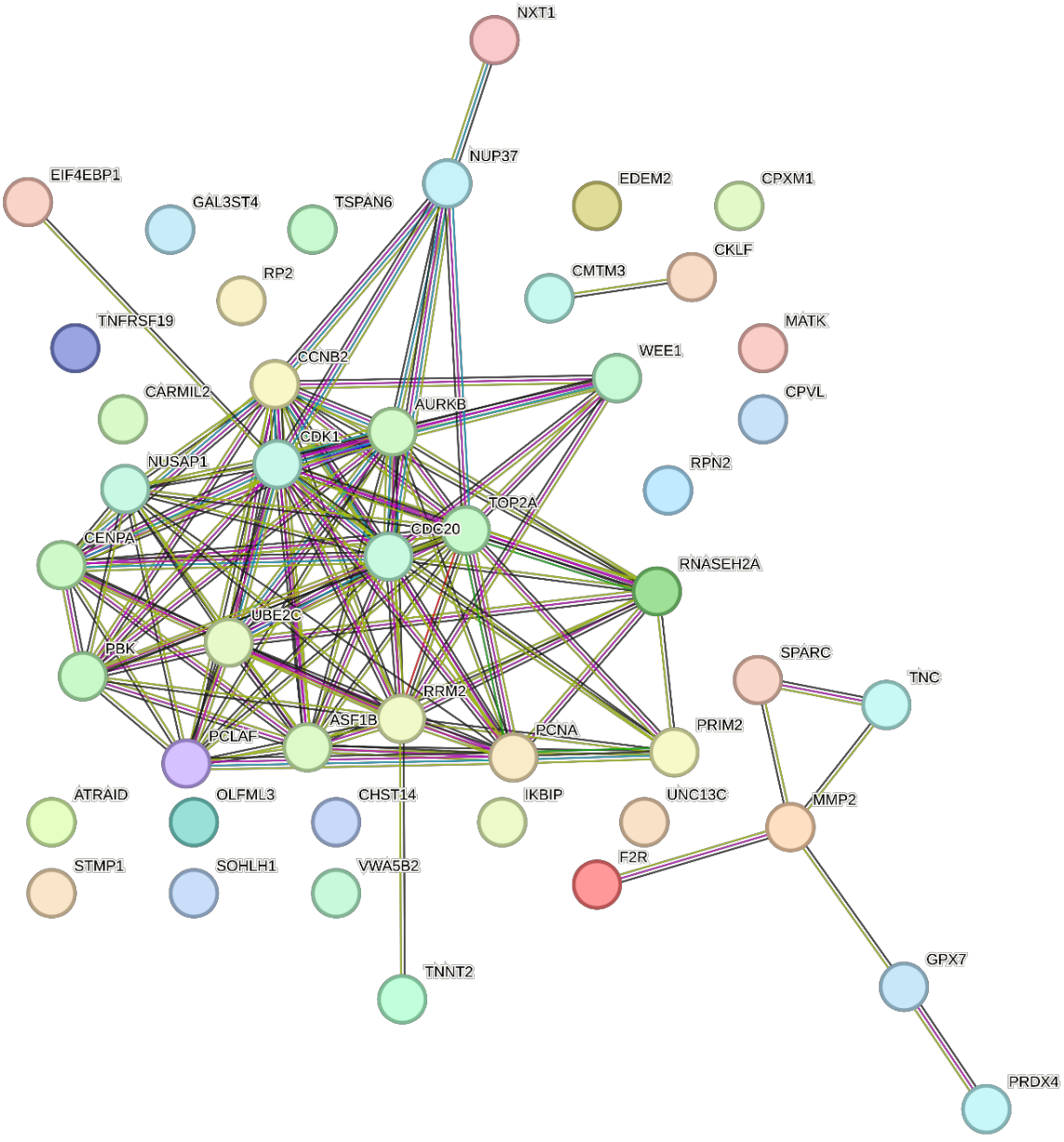


Figure 5.2. Protein-Protein Interaction Network Generated using STRING

5.3. CDK1 EXPRESSION BOXPLOTS USING UACLAN

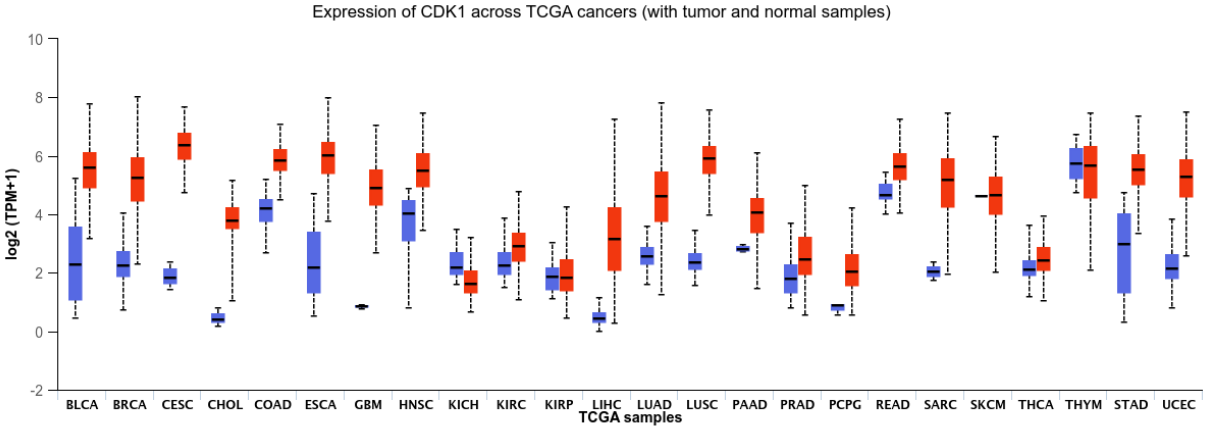


Fig. 5.3.1. CDK1 expression between normal tissue (blue) and disease (normal)

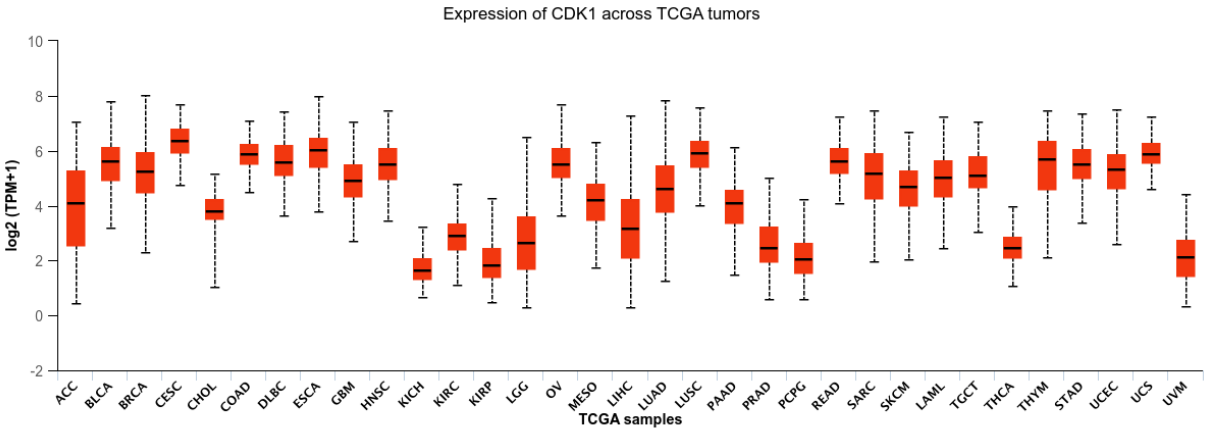


Fig. 5.3.2. CDK1 expression in disease state across different cancers

5.4. RETRIVAL OF CDK1 SEQUENCE FROM UNIPROTKB

```
>sp|P06493|CDK1_HUMAN Cyclin-dependent kinase 1 OS=Homo sapiens OX=9606 GN=CDK1 PE=1 SV=3
MEDYTKIEKIGEGTYGVVYKGRHKTTGQVVAMKKIRLESEEEGVPSTAIREISLLKELRH
PNIVSLQDVLMQDSRLYLIFEFLSMDLKKYLDSIPPGQYMDSSLVKSYLYQILQGIVFCH
SRRVLHRDLKPQNLLIDDKGTIKLADFGLARAFGIPIRVYTHEVVTLWYRSPEVLLGSAR
YSTPVDIWSIGTIFAELATKKPLFHGDSEIDQLFRIFRALGTPNNEVWPEVESLQDYKNT
FPKWKPGSLASHVKNLDENGLDLLSKMLIYDPAKRISGKMALNHPYFNDLDNQIKKM
```

5.2 PROTEIN SEQUENCE ANALYSIS OF BACE1 USING BIOEDIT

Protein: sp|P06493|CDK1_HUMAN Cyclin-dependent kinase 1 OS=Homo sapiens OX=9606 GN=CDK1 PE=1 SV=3

Length = 297 amino acids
Molecular Weight = 34093.63 Daltons

Amino Acid		Number	Mol%
Ala	A	12	4.04
Cys	C	1	0.34
Asp	D	19	6.40
Glu	E	18	6.06
Phe	F	11	3.70
Gly	G	18	6.06
His	H	8	2.69
Ile	I	22	7.41
Lys	K	24	8.08
Leu	L	36	12.12
Met	M	8	2.69
Asn	N	10	3.37
Pro	P	15	5.05
Gln	Q	10	3.37
Arg	R	15	5.05
Ser	S	21	7.07
Thr	T	13	4.38
Val	V	18	6.06
Trp	W	4	1.35
Tyr	Y	14	4.71

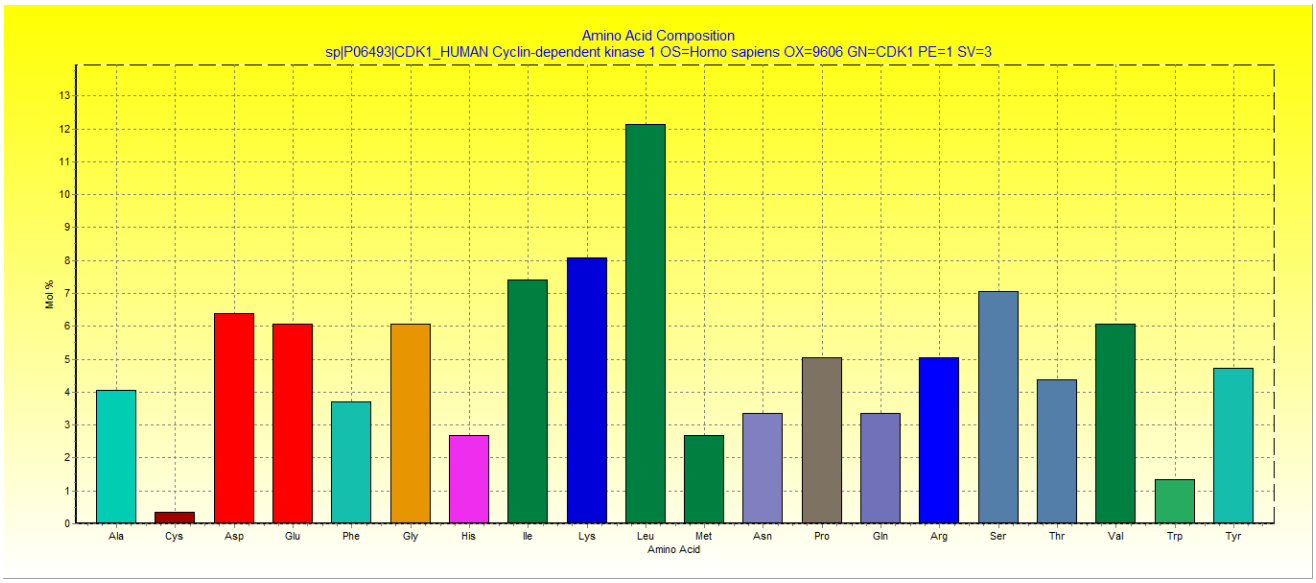


Fig 5.2: Histogram shows the amino acid composition of CDK1

5.3 PROTEIN BIOCHEMICAL PROPERTIES ANALYSIS OF CDK1 USING EMBOSS

PEPSTATS of CDK1_HUMAN from 1 to 297

Molecular weight = 34095.35
Residues = 297
Average Residue Weight = 114.799
Charge = 6.0
Isoelectric Point = 8.7522
A280 Molar Extinction Coefficient = 40680
A280 Extinction Coefficient 1mg/ml = 1.19
Improbability of expression in inclusion bodies = 0.716

Residue	Number	Mole%		DayhoffStat
A = Ala	12	4.040	0.470	
C = Cys	1	0.337	0.116	
D = Asp	19	6.397	1.163	
E = Glu	18	6.061	1.010	
F = Phe	11	3.704	1.029	
G = Gly	18	6.061	0.722	
H = His	8	2.694	1.347	
I = Ile	22	7.407	1.646	
K = Lys	24	8.081	1.224	
L = Leu	36	12.121		1.638
M = Met	8	2.694	1.584	
N = Asn	10	3.367	0.783	
P = Pro	15	5.051	0.971	
Q = Gln	10	3.367	0.863	
R = Arg	15	5.051	1.031	
S = Ser	21	7.071	1.010	
T = Thr	13	4.377	0.718	
V = Val	18	6.061	0.918	
W = Trp	4	1.347	1.036	
Y = Tyr	14	4.714	1.386	

Table 5.1: Biochemical properties of CDK1 of *Homo sapiens*

Property	Residues	Number	Mole%
Tiny	(A+C+G+S+T)	65	21.886
Small	(A+C+D+G+N+P+S+T+V)	127	42.761
Aliphatic	(I+L+V)	76	25.589
Aromatic	(F+H+W+Y)	37	12.458
Non-polar	(A+C+F+G+I+L+M+P+V+W+Y)	159	53.535
Polar	(D+E+H+K+N+Q+R+S+T)	138	46.465

Charged	(D+E+H+K+R)	84	28.283
Basic	(H+K+R)	47	15.825
Acidic	(D+E)	37	12.458

5.4 PROTEIN PRIMARY STRUCTURE OF CDK1 USING PROTPARAM

ProtParam

User-provided sequence:

```
        10         20         30         40         50         60
MEDYTKIEKI GEGTYGVVYK GRHKTTGQVV AMKKIRLESE EEGVPSTAIR EISLLKELRH

        70         80         90        100        110        120
PNIVSLQDVL MQDSRLYLIF EFLSMDLKKY LDSIPPGQYM DSSLVKSYLY QILQGIVFCH

       130        140        150        160        170        180
SRRVLHRDLK PQNLLIDDKG TIKLADFGLA RAFGIPIRVY THEVVTLWYR SPEVLLGSAR

       190        200        210        220        230        240
YSTPVDIWSI GTIFAELATK KPLFHGDSEI DQLFRIFRAL GTPNNEVWPE VESLQDYKNT

       250        260        270        280        290
FPKWKPGSLA SHVKNLDENG LDLLSKMLIY DPAKRISGKM ALNHPYFNDL DNQIKKM
```

Number of amino acids: 297

Molecular weight: 34095.45

Theoretical pI: 8.38

Amino acid composition:

Ala (A) 12 4.0%
Arg (R) 15 5.1%
Asn (N) 10 3.4%
Asp (D) 19 6.4%
Cys (C) 1 0.3%
Gln (Q) 10 3.4%
Glu (E) 18 6.1%
Gly (G) 18 6.1%
His (H) 8 2.7%
Ile (I) 22 7.4%
Leu (L) 36 12.1%
Lys (K) 24 8.1%
Met (M) 8 2.7%
Phe (F) 11 3.7%
Pro (P) 15 5.1%

Ser (S)	21	7.1%
Thr (T)	13	4.4%
Trp (W)	4	1.3%
Tyr (Y)	14	4.7%
Val (V)	18	6.1%
Pyl (O)	0	0.0%
Sec (U)	0	0.0%

(B)	0	0.0%
(Z)	0	0.0%
(X)	0	0.0%

Total number of negatively charged residues (Asp + Glu): 37
Total number of positively charged residues (Arg + Lys): 39

Atomic composition:

Carbon	C	1550
Hydrogen	H	2444
Nitrogen	N	406
Oxygen	O	440
Sulfur	S	9

Formula: $C_{1550}H_{2444}N_{406}O_{440}S_9$
Total number of atoms: 4849

Extinction coefficients:

Extinction coefficients are in units of M^{-1} cm^{-1}, at 280 nm measured in water.

Ext. coefficient 42860
Abs 0.1% (=1 g/l) 1.257, assuming all pairs of Cys residues form cystines

Ext. coefficient 42860
Abs 0.1% (=1 g/l) 1.257, assuming all Cys residues are reduced

Estimated half-life:

The N-terminal of the sequence considered is M (Met).

The estimated half-life is: 30 hours (mammalian reticulocytes, in vitro).
>20 hours (yeast, in vivo).
>10 hours (Escherichia coli, in vivo).

Instability index:

The instability index (II) is computed to be 39.26

This classifies the protein as stable.

Aliphatic index: 97.78

Grand average of hydropathicity (GRAVY): -0.281

5.5 PROTEIN SECONDARY STRUCTURE PREDICTION OF CDK1 USING SOPMA

Fig. 5.5. SOPMA results

5.6 PROTEIN 3D STRUCTURE OF CDK1 SEARCHING FROM PDB USING BLASTP

Table 5.6. Top 100 Blastp results

query acc	subj acc	% ident	align len	q. start	q. end	s. start	s. end	e value	bit score	% position
5076164	4YC6_A	100	297	0	0	1	297	0	612	100
5076164	4Y72_A	100	297	0	0	1	297	0	611	100
5076164	7NJ0_B	99.663	297	1	0	1	297	0	610	99.66
5076164	4EON_A	64.901	302	95	2	1	297	6.72E-144	407	78.48
5076164	4EOM_A	64.901	302	95	2	1	297	6.89E-144	407	78.48
5076164	5K4J_A	64.901	302	95	2	1	297	1.72E-143	407	78.15
5076164	7NVQ_A	64.901	302	95	2	1	297	1.78E-143	407	78.15
5076164	6OQI_A	64.901	302	95	2	1	297	1.92E-143	406	78.15
5076164	6INL_A	64.901	302	95	2	1	297	1.92E-143	406	78.15
5076164	4EOK_A	64.901	302	95	2	1	297	2.01E-143	406	78.15
5076164	1AQ1_A	64.901	302	95	2	1	297	2.20E-143	406	78.15
5076164	1B38_A	64.901	302	95	2	1	297	2.28E-143	406	78.15
5076164	3EZR_A	64.901	302	95	2	1	297	2.32E-143	406	78.15
5076164	3PJ8_A	64.901	302	95	2	1	297	2.33E-143	406	78.15
5076164	5UQ1_A	64.901	302	95	2	1	297	2.56E-143	406	78.15
5076164	5OO0_A	64.901	302	95	2	1	297	2.75E-143	406	78.15
5076164	4EOJ_A	64.901	302	95	2	1	297	2.93E-143	406	78.15
5076164	3PXF_A	64.901	302	95	2	1	297	3.23E-143	406	78.15
5076164	1GZ8_A	64.901	302	95	2	1	297	3.54E-143	406	78.15
5076164	6Q4G_A	64.901	302	95	2	1	297	3.56E-143	406	78.15
5076164	1OIT_A	64.901	302	95	2	1	297	4.65E-143	405	78.15

5076164	4ERW_A	64.901	302	95	2	1	297	4.78E-143	405	78.15
5076164	1VYW_A	64.901	302	95	2	1	297	5.87E-143	405	78.15
5076164	1H01_A	64.57	302	96	2	1	297	1.39E-142	404	77.81
5076164	7VDU_A	64.57	302	96	2	1	297	1.41E-142	404	77.81
5076164	4I3Z_A	64.57	302	96	2	1	297	1.41E-142	404	77.81
5076164	1OIR_A	64.57	302	96	2	1	297	1.44E-142	404	77.81
5076164	1E9H_A	64.57	302	96	2	1	297	1.48E-142	404	77.81
5076164	8BZO_A	64.57	302	96	2	1	297	1.49E-142	404	77.81
5076164	3BHT_A	64.57	302	96	2	1	297	1.57E-142	404	77.81
5076164	1GY3_A	64.57	302	96	2	1	297	1.60E-142	404	77.81
5076164	7E34_A	64.784	301	95	2	2	297	1.76E-142	404	78.07
5076164	1FQ1_B	64.57	302	96	2	1	297	1.78E-142	404	77.81
5076164	1W98_A	64.57	302	96	2	1	297	1.80E-142	404	77.81
5076164	8H6P_A	64.57	302	96	2	1	297	1.82E-142	404	77.81
5076164	2JGZ_A	66.327	294	88	2	1	289	2.01E-142	404	78.91
5076164	4BCM_A	64.57	302	96	2	1	297	2.12E-142	404	77.81
5076164	4EOQ_A	64.57	302	96	2	1	297	2.12E-142	404	77.81
5076164	3QHR_A	64.57	302	96	2	1	297	2.15E-142	404	77.81
5076164	5OO1_A	64.57	302	96	2	1	297	2.32E-142	404	78.15
5076164	4EOS_A	64.57	302	96	2	1	297	2.43E-142	404	77.81
5076164	4CFU_A	64.57	302	96	2	1	297	2.53E-142	404	77.81
5076164	6GUE_A	64.57	302	96	2	1	297	2.56E-142	404	77.81
5076164	1OGU_A	64.57	302	96	2	1	297	2.61E-142	404	77.81
5076164	1H1P_A	64.57	302	96	2	1	297	2.80E-142	404	77.81
5076164	8BYA_A	64.57	302	96	2	1	297	2.86E-142	404	77.81

5076164	5MHQ_A	64.57	302	96	2	1	297	3.01E-142	404	77.81
5076164	4EOO_A	64.238	302	97	2	1	297	7.58E-142	402	77.81
5076164	2BHH_A	64.238	302	97	2	1	297	8.25E-142	402	77.48
5076164	6Q4I_A	64.57	302	96	2	1	297	8.79E-142	402	77.81
5076164	4EOP_A	64.238	302	97	2	1	297	9.46E-142	402	77.81
5076164	2IW6_A	64.238	302	97	2	1	297	1.20E-141	402	77.48
5076164	2CJM_A	64.238	302	97	2	1	297	2.16E-141	401	77.48
5076164	4EOI_A	63.907	302	98	2	1	297	4.00E-141	400	77.48
5076164	1GII_A	63.907	302	98	2	1	297	6.46E-141	400	77.48
5076164	2IW8_A	63.907	302	98	2	1	297	9.26E-141	400	77.48
5076164	7XQK_A	66.667	288	95	1	1	288	4.07E-138	402	80.56
5076164	3NIZ_A	60.339	295	113	3	1	294	7.67E-125	360	75.59
5076164	2QKR_A	60.339	295	113	3	1	294	1.07E-124	359	75.59
5076164	1V0B_A	60.554	289	111	2	1	289	3.78E-123	355	73.7
5076164	1V0O_A	60.554	289	111	2	1	289	4.36E-123	354	73.7
5076164	1OB3_A	60.208	289	112	2	1	289	7.20E-123	354	73.7
5076164	1H4L_A	56.849	292	121	4	1	290	4.34E-116	337	72.6
5076164	1UNG_A	56.507	292	122	4	1	290	2.24E-115	335	72.6
5076164	7VDP_A	56.701	291	121	4	2	290	3.90E-115	334	72.51
5076164	4AU8_A	56.701	291	121	4	2	290	4.15E-115	335	72.51
5076164	3GBZ_A	57.191	299	117	4	1	291	8.52E-112	327	72.24
5076164	3MTL_A	52.013	298	139	3	1	297	2.67E-110	323	72.15
5076164	2PK9_A	52.041	294	132	4	4	289	2.94E-103	305	69.73
5076164	1JOW_B	46.179	301	146	8	2	292	1.84E-87	265	65.78
5076164	1BI7_A	46.333	300	145	8	2	291	4.27E-87	264	66

5076164	6OQL_A	46.128	297	144	8	2	288	7.13E-86	260	65.66
5076164	3NUP_A	46.128	297	144	8	2	288	1.08E-85	260	65.66
5076164	8P4Z_A	44.828	290	152	5	4	289	4.96E-84	256	63.1
5076164	6XD3_J	44.828	290	152	5	4	289	6.26E-84	257	63.1
5076164	6O9L_8	44.828	290	152	5	4	289	1.01E-83	256	63.1
5076164	8ORM_J	44.828	290	152	5	4	289	1.02E-83	256	63.1
5076164	6XBZ_J	44.828	290	152	5	4	289	4.53E-83	256	63.1
5076164	8GXQ_HI	44.483	290	153	5	4	289	5.06E-83	254	62.76
5076164	1UA2_A	44.483	290	153	5	4	289	6.78E-83	254	62.76
5076164	7B5O_J	44.828	290	152	5	4	289	7.46E-83	256	63.1
5076164	7UKZ_A	41.414	297	160	5	1	289	1.32E-81	250	65.99
5076164	6P8E_B	44.369	293	150	6	4	288	9.35E-81	247	63.48
5076164	2W9F_B	44.027	293	151	6	4	288	1.23E-80	247	63.48
5076164	2W99_B	44.027	293	151	6	4	288	4.25E-80	246	63.14
5076164	2W96_B	44.027	293	151	6	4	288	9.61E-80	245	63.14
5076164	3G33_A	43.243	296	152	7	4	288	7.15E-76	235	62.16
5076164	5FWK_K	43.243	296	152	7	4	288	7.36E-76	235	62.16
5076164	6XI8_A	40.345	290	169	2	3	290	4.25E-75	233	57.93
5076164	7SJ3_A	42.905	296	153	7	4	288	4.44E-75	233	61.82
5076164	7KUE_A	40.345	290	169	2	3	290	5.64E-75	233	57.93
5076164	5EFQ_A	40.816	294	160	4	10	291	8.45E-75	234	61.9
5076164	4UN0_C	41.156	294	159	5	10	291	5.29E-74	231	61.9
5076164	4CXA_A	41.156	294	159	5	10	291	6.97E-74	231	61.9
5076164	6B3E_A	41.156	294	159	5	10	291	7.44E-74	230	61.9
5076164	6TD3_B	41.156	294	159	5	10	291	1.63E-73	230	61.9

5076164	4NST_A	41.156	294	159	5	10	291	1.98E-73	230	61.9
5076164	4AGU_A	43.243	296	156	6	1	290	2.27E-73	229	60.47
5076164	4AAA_A	40.066	302	153	4	1	288	6.16E-73	228	57.62

5.7 RETRIEVAL OF PROTEIN 3D STRUCTURE OF CDK1 FROM PDB DATABASE

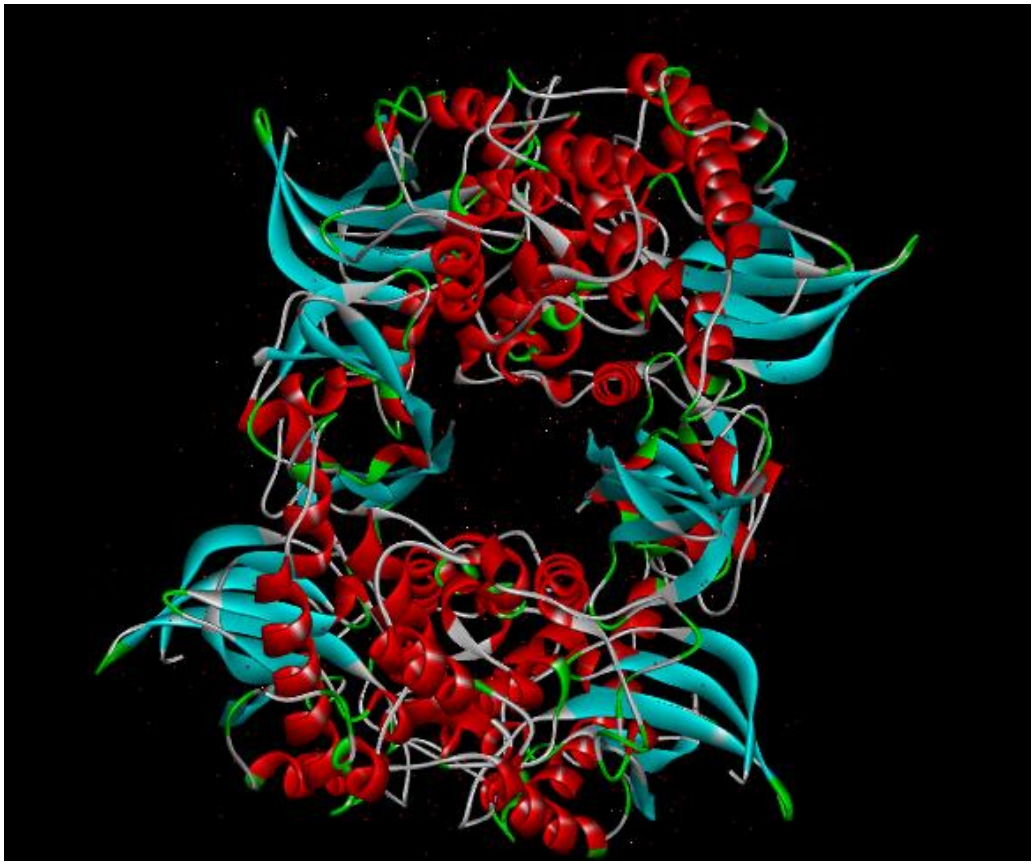


Fig 5.7: Protein 3D structure CDK1 from PDB database

5.8 MOLECULAR DOCKING OF ESTABLISHED INHIBITORS OF CDK1

Table 5.8.1 Molecular Docking Results of Chemical Compounds

Name	Ligand	MolDock Score	Rerank Score	HBond	MW
[00] 24983461	24983461	-131.682	-106.185	-4.64954	397.517
[00] 11655534	11655534	-112.198	-105.665	-2.5	460.335

[02] 16718576	16718576	-131.733	-104.545	-3.11053	460.575
[02] 11285002	11285002	-141.668	-103.249	-3.30168	545.633
[00] 135510708	135510708	-128.538	-99.5939	-2.15661	351.356
[00] 11285002	11285002	-142.224	-99.3748	-3.56302	545.633
[01] 11285002	11285002	-150.243	-98.4998	-4.38423	545.633
[00] 448991	448991	-130.424	-96.0877	-6.64744	432.904
[01] 16718576	16718576	-130.217	-94.8638	-2.74627	460.575
[02] 123740	123740	-126.69	-94.7271	-10.3212	424.443
[01] 24983461	24983461	-126.312	-94.6687	-2.38128	397.517
[00] 6918852	6918852	-116.219	-94.3946	-2.71115	441.452
[02] 24983461	24983461	-123.971	-92.8933	-2.70373	397.517
[00] 16718576	16718576	-135.939	-91.9692	-6.26002	460.575
[02] 46926350	46926350	-121.037	-91.5676	-4.50792	396.486
[01] 72271	72271	-143.079	-91.4391	-3.53664	482.53
[03] 46926350	46926350	-123.593	-91.2781	-2.9731	396.486
[00] 456214	456214	-125.807	-91.192	-2.60241	388.894
[03] 135510708	135510708	-121.821	-91.0327	-2.41183	351.356
[02] 135510708	135510708	-119.8	-90.0527	-4.94452	351.356
[02] 5330790	5330790	-105.52	-89.9721	-2.88258	394.356
[01] 5330790	5330790	-111.745	-89.6508	-7.49658	394.356
[04] 46926350	46926350	-120.369	-89.2036	-2.5	396.486
[03] 456214	456214	-116.599	-88.9271	-3.45723	388.894
[01] 216239	216239	-107.67	-88.2892	-1.39594	464.825
[00] 139025169	139025169	-134.792	-87.9581	-10.0069	441.369
[03] 6918386	6918386	-123.217	-87.133	-9.08352	400.475
[04] 644215	644215	-104.515	-86.6504	-4.7631	355.821
[01] 456214	456214	-124.207	-86.4654	-4.81931	388.894
[02] 45380979	45380979	-112.875	-86.3104	-4.7514	430.445
[01] 139025169	139025169	-135.962	-86.0855	-9.60225	441.369
[01] 6918386	6918386	-125.426	-86.0736	-2.468	400.475
[03] 72271	72271	-139.279	-85.8704	-2.44598	482.53
[00] 45380979	45380979	-110.716	-85.6599	-1.56369	430.445
[02] 135400873	135400873	-104.09	-85.5099	0	351.445
[03] 135400873	135400873	-102.621	-84.256	-2.5	351.445
[00] 123740	123740	-129.433	-84.0814	-8.16352	424.443
[01] 644215	644215	-104.323	-83.9331	-4.21388	355.821
[02] 456214	456214	-115.412	-83.914	-4.06112	388.894
[03] 24983461	24983461	-117.666	-83.6887	-1.59404	397.517
[03] Unknown 1_8	Unknown 1_8	-109.689	-83.555	-1.74722	431.889
0	Un	-79.4332	-83.5259	-3.37733	
[00] 448949	448949	-108.234	-83.2627	-3.9796	356.173
[00] 44259	44259	-134.518	-83.2284	-0.99749	466.531
[02] 644215	644215	-102.952	-82.8676	-4.95526	355.821
[01] 44259	44259	-135.996	-82.8457	-0.44487	466.531

[00] 160355	160355	-119.07	-82.7586	-4.27622	354.449
[04] 24983461	24983461	-119.763	-82.7483	-2.89105	397.517
[00] 476861	476861	-101.447	-82.1889	-0.77067	358.471
[02] 160355	160355	-107.708	-82.128	-0.39299	354.449
[01] 45380979	45380979	-104.683	-81.8314	-4.18696	430.445
[00] 216239	216239	-110.418	-81.8009	-7.24688	464.825
[01] 448949	448949	-110.157	-81.7327	-6.90025	356.173
[04] 135400873	135400873	-103.253	-80.8204	-1.93334	351.445
[01] 135510708	135510708	-120.504	-80.26	-1.27179	351.356
[03] 216239	216239	-104.378	-80.0621	-5.14333	464.825
[01] 16747683	16747683	-104.141	-80.0614	-2.5	371.457
[02] 5005498	5005498	-95.5799	-79.9095	0	293.277
[03] 123740	123740	-114.983	-79.8653	-6.07606	424.443
[00] Unknown 1_1	Unknown 1_1	-105.768	-79.407	-3.51014	327.424
[04] 5330790	5330790	-101.246	-79.3444	-6.22499	394.356
[00] 135400873	135400873	-107.822	-79.3391	-8.76476	351.445
[00] Unknown 1_6	Unknown 1_6	-105.674	-79.1517	-4.71433	415.824
[03] 4592	4592	-99.7795	-79.108	-3.99948	298.343
[03] 11655534	11655534	-106.525	-78.9032	-4.69735	460.335
[04] 11655534	11655534	-106.287	-78.188	-4.38284	460.335
[01] 46926350	46926350	-129.684	-78.0891	-6.0929	396.486
[04] 448991	448991	-106.9	-77.9196	-3.80987	432.904
[04] 11285002	11285002	-130.554	-77.6867	-4.78211	545.633
[04] 160355	160355	-107.258	-77.5775	-2.12347	354.449
[00] 6918386	6918386	-124.555	-77.4702	-0.16256	400.475
[00] 58891018	58891018	-98.491	-77.3092	-6.86547	402.267
[03] 139025169	139025169	-129.033	-77.0417	-5.18284	441.369
[01] 135400873	135400873	-101.347	-77.0316	0	351.445
[01] Unknown 1_8	Unknown 1_8	-107.292	-76.9921	-4.32831	431.889
[04] 4592	4592	-100.207	-76.7845	0	298.343
[01] 11655534	11655534	-100.645	-76.754	-9.38241	460.335
[00] 5005498	5005498	-96.3251	-76.669	-10.577	293.277
[00] 46926350	46926350	-122.374	-76.6342	-5.83896	396.486
[01] 11338033	11338033	-103.909	-76.2975	-3.6886	382.244
[00] 41684	41684	-92.943	-76.2432	-8.14995	307.282
[03] 5330790	5330790	-97.478	-76.1023	-2.5	394.356
[04] Unknown 1_3	Unknown 1_3	-101.025	-75.9866	-4.08176	306.365
[01] 58891018	58891018	-98.3643	-75.9371	-4.44955	402.267
[04] Unknown 1_6	Unknown 1_6	-99.7151	-75.8393	-5.33694	415.824
[02] Unknown 1_1	Unknown 1_1	-103.321	-75.7469	-3.47178	327.424
[00] Unknown 1_8	Unknown 1_8	-106.016	-75.6457	-4.81474	431.889
[03] 160355	160355	-107.312	-75.6297	-6.37704	354.449
[03] 45380979	45380979	-106.489	-75.5711	-5.08349	430.445
[04] Unknown 1_8	Unknown 1_8	-102.772	-75.417	-3.26631	431.889

[04] 216239	216239	-105.527	-75.3544	-7.75691	464.825
[00] 5330790	5330790	-100.425	-75.2537	-2.73218	394.356
[03] Unknown 1_1	Unknown 1_1	-106.228	-75.1249	-1.20351	327.424
[00] 11338033	11338033	-106.394	-74.9153	-6.11924	382.244
[02] Unknown 1	Unknown 1	-97.3522	-74.813	-3.10308	307.35
[04] 45380979	45380979	-98.8981	-74.4377	-0.32944	430.445
[04] 135510708	135510708	-116.725	-74.2673	-12.0073	351.356
[03] Unknown 1_3	Unknown 1_3	-97.7204	-74.0527	-4.14819	306.365
[01] Unknown 1_1	Unknown 1_1	-104.615	-73.2835	-4.40906	327.424
[01] 476861	476861	-98.1212	-73.1994	-3.058	358.471
[02] 6918386	6918386	-118.453	-73.1266	-6.42417	400.475
[03] 41684	41684	-89.6847	-72.9897	-2.9358	307.282
[04] 139025169	139025169	-118.928	-72.7875	-15.399	441.369
[03] 5005498	5005498	-88.4554	-72.7253	-0.39339	293.277
[00] 4564	4564	-86.8049	-72.4871	-1.10691	247.296
[01] 123740	123740	-119.333	-72.4656	-6.34953	424.443
[00] 9817550	9817550	-88.5642	-72.378	-7.50942	293.283
[01] 5005498	5005498	-91.4317	-72.3295	-7.47204	293.277
[03] 9817550	9817550	-88.2496	-72.305	-3.03611	293.283
[02] 5287969	5287969	-94.5935	-72.2506	-9.72447	401.84
[02] 16747683	16747683	-97.07	-72.1883	-1.34664	371.457
[03] 5287969	5287969	-91.4807	-72.0687	-4.91429	401.84
[01] 5287969	5287969	-97.8211	-72.0525	-9.60409	401.84
[00] 72271	72271	-138.808	-71.8104	-6.6474	482.53
[02] 216239	216239	-104.005	-71.7814	-3.214	464.825
[02] 9817550	9817550	-89.3713	-71.6011	-6.23048	293.283
[02] 448991	448991	-118.605	-71.5335	-5.22135	432.904
[04] 5005498	5005498	-87.9992	-71.3948	0	293.277
[04] 72271	72271	-136.403	-71.2701	0	482.53
[01] Unknown 1_3	Unknown 1_3	-101.572	-71.0674	-4.20884	306.365
[04] 58891018	58891018	-91.1074	-71.0341	-11.4088	402.267
[04] 9817550	9817550	-81.8934	-70.8956	-7.42628	293.283
[03] Unknown 1	Unknown 1	-95.4365	-70.7323	-1.21774	307.35
[02] 4592	4592	-101.432	-70.6937	-8.73224	298.343
[01] 4564	4564	-88.3824	-70.6225	-8.87816	247.296
[02] 6253	6253	-89.723	-70.5206	-13.3546	243.217
[02] Unknown 1_6	Unknown 1_6	-92.3084	-70.2767	-1.62236	415.824
[02] Unknown 1_8	Unknown 1_8	-103.275	-70.1873	-4.8855	431.889
[04] 448949	448949	-95.4495	-69.9166	-3.27901	356.173
[02] 41684	41684	-89.0637	-69.6289	-3.57935	307.282
[03] 23727982	23727982	-90.3162	-69.0369	-6.25893	269.302
[02] 4564	4564	-85.8155	-68.9648	-3.89651	247.296
[00] 16739650	16739650	-98.3513	-68.7721	0	372.463
[04] 456214	456214	-116.538	-68.7592	-1.63577	388.894

[00] 11715767	11715767	-80.0073	-68.6576	-1.89554	213.235
[00] 644215	644215	-100.286	-68.4659	-1.61398	355.821
[01] 9817550	9817550	-84.2565	-68.3865	-4.66724	293.283
[03] 4564	4564	-84.2561	-68.3519	-2.64064	247.296
[03] 644215	644215	-99.4879	-68.293	-7.13092	355.821
[02] 476861	476861	-92.3185	-68.1964	-2.17285	358.471
[00] 5287969	5287969	-94.1344	-68.0164	-5.7802	401.84
[00] 16747683	16747683	-97.7171	-67.8525	-3.9661	371.457
[01] 160355	160355	-109.808	-67.7903	-5	354.449
[02] Unknown 1_3	Unknown 1_3	-97.8506	-67.7548	-4.57073	306.365
[01] Unknown 1	Unknown 1	-98.8208	-67.5062	-2.17691	307.35
[01] 16739650	16739650	-95.7064	-67.4959	0	372.463
[02] 6918852	6918852	-89.0328	-67.454	-3.04333	441.452
[03] 11285002	11285002	-146.134	-67.3383	-0.69645	545.633
[03] 16747683	16747683	-94.0626	-67.3099	0	371.457
[03] 6918852	6918852	-95.9377	-67.3064	-2.68896	441.452
[01] 6918852	6918852	-102.602	-66.5337	-5.22991	441.452
[01] 23727982	23727982	-89.6385	-66.195	-6.93293	269.302
[04] 4564	4564	-81.025	-65.5548	-2.29397	247.296
[00] 23727982	23727982	-92.8863	-65.3667	-3.16589	269.302
[01] 4592	4592	-100.526	-65.2923	-6.8934	298.343
[04] 123740	123740	-122.06	-65.2369	-2.83465	424.443
[00] Unknown 1_3	Unknown 1_3	-98.2525	-65.0338	-3.08261	306.365
[01] Unknown 1_6	Unknown 1_6	-98.1681	-64.953	-5.72797	415.824
[00] Unknown 1	Unknown 1	-97.8632	-64.8472	-1.28596	307.35
[04] 23727981	23727981	-78.755	-64.7063	-3.0445	241.249
[03] 58891018	58891018	-96.8338	-64.4691	-3.45558	402.267
[01] 448991	448991	-117.498	-64.4153	-8.25358	432.904
[02] 23727982	23727982	-89.2007	-64.0543	-4.16325	269.302
[04] 5287969	5287969	-92.7564	-63.4283	-3.40875	401.84
[02] Unknown 1_2	Unknown 1_2	-81.1583	-63.3934	-4.8293	258.322
[04] 16747683	16747683	-90.4537	-63.2876	-4.09232	371.457
[02] 58891018	58891018	-82.7789	-63.276	-2.78417	402.267
[04] Unknown 1_4	Unknown 1_4	-79.6711	-63.2303	-5.37009	244.296
[04] 476861	476861	-96.6436	-62.8455	-1.92936	358.471
[02] 72271	72271	-139.169	-62.6922	-4.92983	482.53
[00] 23727981	23727981	-82.2774	-62.6077	-4.29794	241.249
[03] 6253	6253	-80.0228	-62.2691	-4.89871	243.217
[00] 6253	6253	-87.8236	-61.9892	-11.2296	243.217
[01] 11715767	11715767	-71.4586	-61.9181	-2.40843	213.235
[02] 448949	448949	-91.8572	-61.7344	-10.0639	356.173
[04] 41684	41684	-81.4355	-61.7108	-3.56567	307.282
[04] 6253	6253	-79.5514	-61.6285	-5.19073	243.217
[01] 23727981	23727981	-81.8541	-61.3505	-8.74787	241.249

[04] Unknown 1_1	Unknown 1_1	-101.592	-61.2323	-3.98776	327.424
[00] Unknown 1_2	Unknown 1_2	-82.9188	-61.1685	-3.76708	258.322
[04] Unknown 1	Unknown 1	-90.8479	-61.0162	-4.45902	307.35
[04] 11715767	11715767	-71.1148	-60.8601	-2.39308	213.235
[04] 16739650	16739650	-93.0623	-60.8546	0	372.463
[02] 23727981	23727981	-80.2277	-59.5587	-4.90973	241.249
[01] Unknown 1_4	Unknown 1_4	-79.6984	-58.8782	-4.35554	244.296
[04] 16718576	16718576	-118.593	-58.4992	-5.75689	460.575
[03] 476861	476861	-91.0151	-58.3748	-4.67189	358.471
[03] 11715767	11715767	-69.423	-57.872	-4.14206	213.235
[03] 23727981	23727981	-78.4776	-57.6936	-4.43888	241.249
[02] 11715767	11715767	-69.7166	-57.4096	-8.47822	213.235
[04] 11338033	11338033	-88.2441	-57.3133	-5	382.244
[01] 6253	6253	-84.0534	-56.8457	-10.7589	243.217
[03] 11338033	11338033	-88.5625	-56.6475	-2.5	382.244
[03] 448949	448949	-101.485	-56.0728	-7.44503	356.173
[02] 16739650	16739650	-92.236	-55.7604	-3.47898	372.463
[03] Unknown 1_4	Unknown 1_4	-76.5852	-55.5238	-5.6241	244.296
[01] 41684	41684	-81.6896	-55.3863	-10.6268	307.282
[00] Unknown 1_4	Unknown 1_4	-79.4616	-55.3088	-3.34112	244.296
[04] Unknown 1_2	Unknown 1_2	-82.6306	-55.225	-4.88046	258.322
[04] 23727982	23727982	-84.8502	-54.9447	-6.87943	269.302
[03] 16739650	16739650	-94.0735	-53.8062	-1.37061	372.463
[04] 6918852	6918852	-92.3066	-52.5424	-0.04347	441.452
[02] Unknown 1_4	Unknown 1_4	-77.2429	-50.8924	-1.35869	244.296
[03] Unknown 1_2	Unknown 1_2	-75.5893	-50.0049	-1.39196	258.322
[01] Unknown 1_2	Unknown 1_2	-73.5613	-49.7158	-2.82569	258.322
[02] 11655534	11655534	-97.5053	-48.1493	-5.92461	460.335
[03] 448991	448991	-110.891	-46.9317	-5.85141	432.904
[02] 11338033	11338033	-89.7664	-42.6698	-3.41638	382.244
[03] 16718576	16718576	-130.358	-38.7368	-2.72336	460.575
[00] 4592	4592	-105.378	-36.7496	-12.3209	298.343
[02] 139025169	139025169	-120.172	-31.1225	-13.146	441.369
[04] 6918386	6918386	-115.837	-21.5669	-3.74659	400.475
[03] Unknown 1_6	Unknown 1_6	-95.3329	-0.979106	-7.01841	415.824

Table 5.8.2 Molecular docking results of photochemical compounds

Name	Ligand	MolDock Score	Rerank Score	HBond	MW
[00] 185617	185617	-144.878	-105.299	-12.3074	462.36
[01] 185617	185617	-148.856	-105.187	-10.2104	462.36
[03] 185617	185617	-133.905	-95.6865	-11.115	462.36
[02] 65064	65064	-133.249	-95.1963	-10.8867	458.372

[02] 185617	185617	-138.749	-94.1881	-17.0373	462.36
[03] 65064	65064	-128.035	-82.3139	-10.9209	458.372
[04] 185617	185617	-132.443	-81.8695	-8.26744	462.36
[02] 969516	969516	-114.676	-81.4033	-4.5963	368.38
[00] 65064	65064	-128.295	-79.0009	-9.98125	458.372
[04] 969516	969516	-111.605	-76.1296	-2.38242	368.38
[03] 969516	969516	-112.734	-75.9351	-2.79329	368.38
[01] 72281	72281	-92.7573	-73.9247	-6.39611	302.279
[04] 72281	72281	-91.816	-72.9049	-6.26917	302.279
[00] 72281	72281	-91.809	-72.4285	-5.43793	302.279
[01] 65064	65064	-134.656	-72.379	-8.85181	458.372
[02] 72281	72281	-92.2674	-72.3223	-6.13046	302.279
[01] 969516	969516	-109.002	-71.7622	-6.46701	368.38
[00] 10177	10177	-94.27	-71.1504	-3.15385	262.263
[04] 5281672	5281672	-98.905	-70.2048	-10.5984	318.235
[02] 10177	10177	-92.6729	-69.7763	-4.19703	262.263
[01] 5281672	5281672	-96.0967	-69.6492	-9.09511	318.235
[00] 969516	969516	-112.586	-69.4717	-10.7733	368.38
[04] 65064	65064	-123.225	-69.3898	-9.99351	458.372
[03] 72281	72281	-92.8361	-68.7428	-12.7931	302.279
[03] 10177	10177	-89.288	-68.0876	-2.45815	262.263
[01] 128861	128861	-89.1065	-67.6582	-8.76362	287.244
[00] 128861	128861	-89.376	-67.4545	-8.78223	287.244
[04] 128861	128861	-81.2714	-67.2086	-11.4077	287.244
[01] 10177	10177	-88.5121	-65.6763	-3.59135	262.263
[03] 128861	128861	-84.3995	-65.2595	-11.3189	287.244
[04] 5280863	5280863	-79.273	-64.8188	-5.06857	286.236
[02] 5280863	5280863	-81.6505	-64.5353	-7.52257	286.236
[00] 5280443	5280443	-77.7278	-63.7994	-5.09234	270.237
[04] 10177	10177	-88.7429	-63.5996	-1.63586	262.263
[00] 445154	445154	-78.2611	-63.42	-7.28785	228.243
[02] 5280443	5280443	-79.0568	-63.3022	-8.42989	270.237
[03] 5280443	5280443	-77.0223	-63.0602	-4.04997	270.237
[03] 5280863	5280863	-77.1507	-62.929	-5.9557	286.236
[02] 5280961	5280961	-79.9344	-62.8261	-6.77187	270.237
[01] 5280961	5280961	-78.7446	-62.6829	-8.24278	270.237
[01] 445154	445154	-75.262	-62.2358	-5.43126	228.243
[02] 128861	128861	-78.0311	-61.7505	-7.58042	287.244
[01] 5280863	5280863	-78.542	-61.7345	-7.17355	286.236
[00] 5280961	5280961	-80.9961	-61.4776	-5.96635	270.237
[02] 445154	445154	-72.5606	-60.0978	-6.05944	228.243
[00] 5280863	5280863	-85.989	-59.1358	-12.9897	286.236
[01] 5280443	5280443	-74.9533	-59.0681	-6.35237	270.237
[04] 445154	445154	-70.5305	-58.3851	-5	228.243

[04] 5280443	5280443	-73.8149	-57.8338	-4.01994	270.237
[00] 5281672	5281672	-92.0686	-56.9553	-9.38014	318.235
[03] 445154	445154	-71.428	-55.4391	-6.00449	228.243
[03] 5280961	5280961	-71.6451	-54.6841	-5.28692	270.237
[00] 370	370	-67.5088	-50.76	-12.2686	170.12
[02] 370	370	-67.1902	-50.382	-9.78371	170.12
[04] 5280961	5280961	-75.4984	-49.8403	-7.58993	270.237
[01] 370	370	-64.4747	-49.1695	-11.3407	170.12
[03] 370	370	-62.8087	-46.4992	-10.4159	170.12
[04] 370	370	-62.9325	-44.4164	-12.7102	170.12
[02] 5281672	5281672	-88.4944	-42.9717	-9.72209	318.235
[00] 5971	5971	-39.7097	-32.9739	-2.5	99.1542
[01] 5971	5971	-39.3956	-32.6338	-2.5	99.1542
[03] 5971	5971	-38.8722	-32.5094	-2.5	99.1542
[02] 5971	5971	-37.8648	-31.7975	-2.01725	99.1542
[04] 5971	5971	-37.4567	-31.5243	-2.05122	99.1542
[03] 5281672	5281672	-90.9114	-2.98877	-12.8891	318.235

5.9. MOLECULAR DOCKING OF VIRTUAL SCREENING COMPOUNDS

Table 5.9. Molecular Docking Results of Virtual Screened Compounds

Name	Ligand	MolDock Score	Rerank Score	HBond	MW
[02] 16718337	16718337	-141.516	-106.722	-4.63079	411.544
[00] 170074997	170074997	-137.199	-104.984	-10.235	411.544
[00] 142862973	142862973	-141.214	-104.832	-3.72652	383.491
[00] 17966266	17966266	-148.791	-104.322	-4.26419	411.544
[01] 16718381	16718381	-135.51	-103.739	-2.7049	411.544
[00] 68849059	68849059	-141.563	-103.28	-5.94074	411.544
[02] 16718333	16718333	-136.227	-102.856	-3.99309	397.517
[02] 134468483	134468483	-139.533	-102.794	-3.6108	394.513
[00] 10001665	10001665	-142.919	-102.224	-3.18666	411.544
[00] 167264145	167264145	-135.872	-101.638	-4.84366	407.512
[01] 142862973	142862973	-133.152	-101.078	-1.32078	383.491
[00] 16718282	16718282	-136.933	-100.8	-5.03471	383.491
[01] 57433124	57433124	-129.694	-100.565	-6.3509	383.491
[01] 17966260	17966260	-137.302	-100.551	-5.70358	397.517
[02] 142862973	142862973	-132.531	-100.528	-4.71687	383.491
[01] 16718283	16718283	-135.159	-100.186	-4.2055	383.491
[01] 16718337	16718337	-145.86	-100.154	-5.14469	411.544
[00] 69301979	69301979	-140.742	-100.126	-9.13476	397.517

[01] 16718332	16718332	-134.774	-100.065	-2.51062	397.517
[02] 69301979	69301979	-136.49	-100.056	-7.72382	397.517
[03] 69301979	69301979	-138.161	-100.029	-9.42463	397.517
[01] 170074997	170074997	-130.461	-99.8272	-13.837	411.544
[01] 16718382	16718382	-130.504	-99.6694	-3.4247	411.544
[00] 91004632	91004632	-134.478	-99.6545	-3.74391	383.491
[00] 73309219	73309219	-143.875	-99.5501	-4.2366	383.491
[01] 11617621	11617621	-128.554	-99.1946	-3.93788	369.464
[01] 126746752	126746752	-131.013	-98.84	-1.54508	397.517
[00] 134468483	134468483	-137.919	-98.8314	-7.91602	394.513
[02] 142862972	142862972	-134.769	-98.7728	-8.11883	383.491
[02] 142405659	142405659	-126.625	-98.7666	-11.0665	383.491
[01] 25020438	25020438	-134.784	-98.6199	-2.26009	423.435
[01] 142862972	142862972	-139.379	-98.6124	-4.85793	383.491
[00] 25071575	25071575	-139.387	-98.4363	-6.1536	369.464
[03] 154684505	154684505	-128.441	-98.3631	-6.23631	369.464
[01] 142862956	142862956	-137.027	-98.3288	-5.65513	411.544
[03] 142862981	142862981	-134.162	-98.1844	-5.60572	369.464
[01] 167264145	167264145	-129.78	-98.1741	-10.3965	407.512
[02] 57391639	57391639	-130.784	-97.8326	-4.76749	369.464
[00] 25020436	25020436	-133.981	-97.7797	-3.57452	437.462
[04] 69301979	69301979	-127.114	-97.6718	-10.1754	397.517
[00] 12111410	12111410	-128.09	-97.5582	-2.70437	383.491
[02] 57395048	57395048	-126.38	-97.1447	-4.48126	369.464
[02] 12111409	12111409	-132.26	-96.982	-8.82585	383.491
[00] 16718381	16718381	-136.133	-96.806	-3.59992	411.544
[03] 16718337	16718337	-130.107	-96.7102	-4.506	411.544
[04] 10001665	10001665	-130.475	-96.6038	-13.6165	411.544
[00] 69301816	69301816	-134.94	-96.5167	-1.77689	369.464
[03] 142862973	142862973	-133.675	-96.4885	-7.703	383.491
[04] 71053736	71053736	-123.538	-96.2918	-4.75406	437.462
[01] 69301979	69301979	-140.65	-96.2843	-6.05368	397.517
[02] 16718382	16718382	-129.157	-96.1133	-3.80457	411.544
[02] 16718332	16718332	-134.811	-96.1076	-4.74602	397.517
[02] 16718335	16718335	-124.178	-96.0762	-1.0711	397.517
[01] 134468483	134468483	-131.514	-95.9823	-2.00359	394.513
[01] 57395048	57395048	-130.538	-95.932	-4.17773	369.464
[02] 16718381	16718381	-125.347	-95.7828	-2.46142	411.544
[02] 126746752	126746752	-133.125	-95.7823	-11.7305	397.517
[02] 25020438	25020438	-120.468	-95.739	-7.58408	423.435
[00] 12111413	12111413	-137.398	-95.7053	-3.60666	411.544
[04] 16718332	16718332	-121.197	-95.4367	-7.3001	397.517
[00] 126746752	126746752	-136.657	-95.3994	-3.50621	397.517
[00] 25020438	25020438	-134.855	-95.3641	-1.13024	423.435

[00] 10001666	10001666	-142.483	-95.3604	-3.78294	411.544
[02] 170074997	170074997	-133.144	-95.2698	-3.82694	411.544
[00] 57463052	57463052	-132.944	-95.1711	-5.62141	381.475
[03] 12111409	12111409	-129.97	-95.0795	-3.04298	383.491
[02] 57463052	57463052	-125.961	-95.0685	-4.91903	381.475
[00] 12111414	12111414	-133.401	-94.9835	-5.09851	411.544
[00] 12111411	12111411	-141.19	-94.9687	-6.00817	397.517
[00] 16718331	16718331	-130.757	-94.9435	-3.38806	383.491
[01] 91004632	91004632	-132.156	-94.9157	-2.06544	383.491
[03] 168499477	168499477	-125.627	-94.9042	-8.26911	383.491
[00] 12111409	12111409	-137.609	-94.8313	-5.17054	383.491
[00] 25052919	25052919	-127.761	-94.6926	-5.30692	355.437
[01] 24782039	24782039	-117.382	-94.6204	-12.2827	355.437
[01] 156808296	156808296	-127.345	-94.5013	-11.3297	383.491
[04] 170074997	170074997	-124.765	-94.4454	-12.7979	411.544
[03] 16718175	16718175	-118.246	-94.3771	-2.39604	369.464
[00] 24983461	24983461	-131.085	-94.3631	-4.64575	397.517
[04] 24782039	24782039	-120.794	-94.3123	-5.40645	355.437
[00] 16718333	16718333	-136.715	-94.2323	-3.83262	397.517
[00] 16718382	16718382	-135.027	-94.2174	-1.96057	411.544
[00] 78073451	78073451	-127.237	-93.921	-3.3403	327.384
[01] 53327148	53327148	-116.848	-93.7692	-2.80579	397.517
[04] 12111409	12111409	-127.149	-93.7125	-4.06518	383.491
[02] 12111410	12111410	-127.958	-93.7091	-5.13789	383.491
[02] 69069109	69069109	-127.78	-93.671	-9.07533	355.437
[00] 10475938	10475938	-134.279	-93.6627	-4.09984	397.517
[00] 71161249	71161249	-129.023	-93.6449	-5.57433	355.437
[02] 142862981	142862981	-131.608	-93.6069	-6.62329	369.464
[01] 16718284	16718284	-123.552	-93.4981	-1.31688	383.491
[04] 12111411	12111411	-131.161	-93.4306	-2.42395	397.517
[02] 154684505	154684505	-123.27	-93.4208	-9.01734	369.464
[00] 69301819	69301819	-128.125	-93.3524	-3.27516	369.464
[01] 16718175	16718175	-129.89	-93.3247	-8.77196	369.464
[02] 10001665	10001665	-131.731	-93.3107	-5.81748	411.544
[01] 10000883	10000883	-128.848	-93.1933	-10.1575	397.517
[00] 142862981	142862981	-142.473	-93.1092	-8.63339	369.464
[04] 16718381	16718381	-124.967	-93.1035	-8.85118	411.544
[04] 10000883	10000883	-137.344	-93.0432	-7.0699	397.517
[01] 12111414	12111414	-132.169	-92.984	-2.64803	411.544
[00] 142862980	142862980	-130.986	-92.9793	-6.36535	369.464
[01] 10001666	10001666	-136.351	-92.9505	-2.88649	411.544
[02] 69301816	69301816	-128.571	-92.922	-2.7199	369.464
[00] 16718337	16718337	-141.177	-92.9039	-4.29731	411.544
[02] 57433122	57433122	-125.758	-92.851	-9.50989	341.411

[01] 16718333	16718333	-136.098	-92.7943	-3.98486	397.517
[02] 12111408	12111408	-126.158	-92.6868	-5.95154	369.464
[02] 16718331	16718331	-121.772	-92.5862	-2.90988	383.491
[00] 16718335	16718335	-130.996	-92.4359	-4.29515	397.517
[03] 142405659	142405659	-119.65	-92.4101	-4.50122	383.491
[01] 16718336	16718336	-134.428	-92.3689	-8.40602	411.544
[00] 21362562	21362562	-125.987	-92.2018	-5.70544	355.437
[02] 143547214	143547214	-126.707	-92.0467	-6.4914	355.437
[04] 16718283	16718283	-126.749	-92.0154	-4.57866	383.491
[04] 16718336	16718336	-118.652	-91.9104	-2.88471	411.544
[01] 24983461	24983461	-125.107	-91.9017	-2.67189	397.517
[00] 91175802	91175802	-144.469	-91.8469	-7.89466	411.544
[03] 135278505	135278505	-121.75	-91.8178	-4.22525	397.537
[04] 142862972	142862972	-126.087	-91.7771	0	383.491
[01] 25071575	25071575	-126.211	-91.7726	-4.77103	369.464
[00] 71455943	71455943	-125.58	-91.7721	-3.17392	327.384
[01] 71455942	71455942	-125.54	-91.7371	-6.13434	355.437
[03] 91004632	91004632	-119.484	-91.6474	-1.84835	383.491
[02] 12111414	12111414	-127.52	-91.6036	-1.16399	411.544
[00] 16718336	16718336	-133.356	-91.5876	-2.52498	411.544
[00] 25020435	25020435	-138.581	-91.5165	-1.87874	398.525
[02] 142862984	142862984	-119.01	-91.4357	-1.53625	355.437
[03] 135278508	135278508	-126.869	-91.3199	-5.3486	411.544
[04] 70899058	70899058	-123.622	-91.1784	-5.29891	455.452
[04] 16718337	16718337	-132.053	-91.164	-2.13328	411.544
[04] 25020439	25020439	-131.406	-91.0338	-5.91977	491.433
[01] 16718334	16718334	-131.696	-91.0248	-3.52624	397.517
[02] 70899058	70899058	-123.958	-90.941	-4.97147	455.452
[03] 91175802	91175802	-123.134	-90.9232	-2.00362	411.544
[03] 16718331	16718331	-120.751	-90.8327	-3.40743	383.491
[04] 135278505	135278505	-120.26	-90.8284	-1.9492	397.537
[03] 69069109	69069109	-114.777	-90.7724	-4.94403	355.437
[03] 10475938	10475938	-129.553	-90.7305	-4.1564	397.517
[04] 69301819	69301819	-123.189	-90.7178	-4.71545	369.464
[01] 69301816	69301816	-136.813	-90.667	-5.58358	369.464
[00] 12111408	12111408	-128.174	-90.5896	-5.0484	369.464
[02] 25052919	25052919	-122.593	-90.3841	-5.6954	355.437
[00] 57395048	57395048	-125.836	-90.3209	-4.58182	369.464
[04] 134468483	134468483	-125.694	-90.2833	-7.29536	394.513
[03] 10001665	10001665	-136.126	-90.2595	-2.68999	411.544
[04] 168285854	168285854	-127.719	-90.2289	-3.7853	397.517
[00] 71161248	71161248	-131.138	-90.1049	-6.44342	355.437
[02] 71161249	71161249	-114.592	-90.0109	-1.73999	355.437
[02] 73309219	73309219	-129.203	-89.9924	-5.10339	383.491

[04] 142862981	142862981	-117.045	-89.8944	-5.62902	369.464
[01] 57391639	57391639	-123.601	-89.7718	-4.09467	369.464
[00] 156808296	156808296	-128.452	-89.7505	-3.70897	383.491
[00] 10000883	10000883	-135.858	-89.7361	-4.96882	397.517
[03] 16718282	16718282	-124.071	-89.5422	-6.04372	383.491
[01] 164925828	164925828	-125.936	-89.5119	-11.5249	395.501
[01] 25020384	25020384	-121.83	-89.448	-8.55548	369.464
[01] 74379282	74379282	-133.467	-89.3675	-3.67974	397.517
[00] 9842276	9842276	-117.303	-89.3186	-2.96392	369.464
[00] 57391639	57391639	-125.115	-89.2405	-4.13878	369.464
[03] 24983461	24983461	-119.951	-89.2088	-0.98779	397.517
[02] 57433123	57433123	-123.474	-89.177	-5.5732	383.491
[02] 10001666	10001666	-136.05	-89.1687	-7.3783	411.544
[02] 16718282	16718282	-130.456	-89.1646	-5.67923	383.491
[03] 12111411	12111411	-129.042	-88.9555	-3.49364	397.517
[01] 21362562	21362562	-125.28	-88.9152	-1.3686	355.437
[00] 155565350	155565350	-121.004	-88.8708	-9.02161	383.491
[02] 74379282	74379282	-129.18	-88.672	-6.67294	397.517
[03] 25020435	25020435	-125.48	-88.5861	-1.55863	398.525
[00] 68586983	68586983	-119.702	-88.5382	-7.22667	341.411
[01] 16718282	16718282	-131.951	-88.4763	-5.50968	383.491
[04] 91004632	91004632	-114.167	-88.3814	-8.25227	383.491
[04] 142862978	142862978	-113.53	-88.3751	-8.72331	341.411
[02] 168285854	168285854	-126.319	-88.3734	-3.72466	397.517
[02] 17966260	17966260	-131.672	-88.3492	-2.5	397.517
[01] 71455943	71455943	-122.254	-88.3197	-5.39963	327.384
[00] 143189927	143189927	-129.541	-88.2855	-1.61076	355.437
[01] 68849059	68849059	-131.07	-88.1982	-4.52813	411.544
[01] 17966266	17966266	-143.427	-88.1551	-8.38243	411.544
[03] 142405658	142405658	-121.59	-88.1505	-3.15239	383.491
[00] 10155152	10155152	-120.215	-88.0727	-2.90162	355.437
[00] 142862978	142862978	-118.084	-88.0645	-4.73486	341.411
[00] 11617621	11617621	-128.696	-88.0402	-7.70134	369.464
[00] 168499477	168499477	-137.863	-87.9877	-8.72116	383.491
[00] 57433122	57433122	-128.59	-87.9762	-5.07188	341.411
[03] 78073451	78073451	-117.931	-87.9649	-10.7214	327.384
[04] 78073450	78073450	-118.22	-87.9242	-1.459	355.437
[02] 16718284	16718284	-121.012	-87.9226	-4.65336	383.491
[04] 12111412	12111412	-121.434	-87.891	-2.59535	397.517
[02] 12111411	12111411	-132.643	-87.769	-12.6742	397.517
[04] 57433122	57433122	-117.959	-87.7576	-6.71758	341.411
[04] 78073451	78073451	-113.477	-87.6159	-2.91559	327.384
[01] 69301819	69301819	-121.817	-87.5743	-3.13099	369.464
[01] 16718331	16718331	-122.6	-87.5579	-1.36369	383.491

[03] 71053736	71053736	-114.539	-87.5127	-3.05555	437.462
[04] 69301816	69301816	-128.422	-87.4926	-4.52166	369.464
[03] 53327149	53327149	-121.748	-87.4916	-8.82073	397.517
[02] 156808296	156808296	-121.376	-87.4632	-3.68959	383.491
[00] 24782039	24782039	-121.895	-87.3588	-4.69474	355.437
[00] 16718334	16718334	-131.223	-87.3376	-8.42522	397.517
[03] 69301819	69301819	-121.291	-87.2777	-2.97969	369.464
[02] 142405658	142405658	-123.399	-87.0573	-6.91242	383.491
[02] 10247627	10247627	-112.766	-87.0293	-6.85275	355.437
[00] 25020439	25020439	-125.155	-86.91	-1.68181	491.433
[01] 57433123	57433123	-129.685	-86.8762	-4.05189	383.491
[04] 25020384	25020384	-113.87	-86.8664	-3.56806	369.464
[03] 57433124	57433124	-123.482	-86.8158	-3.34627	383.491
[01] 16718335	16718335	-126.595	-86.8018	-3.63169	397.517
[04] 74379282	74379282	-125.805	-86.5836	-9.57271	397.517
[03] 68586983	68586983	-110.174	-86.5673	-2.62726	341.411
[04] 71161249	71161249	-113.838	-86.5164	-4.96658	355.437
[03] 78073450	78073450	-114.74	-86.502	-6.34819	355.437
[02] 21362562	21362562	-120.894	-86.4364	-4.70911	355.437
[03] 17966266	17966266	-132.959	-86.3854	-4.15891	411.544
[00] 78073450	78073450	-126.541	-86.2616	-7.81999	355.437
[01] 25020436	25020436	-134.451	-86.2389	-0.43629	437.462
[03] 10000883	10000883	-131.165	-86.2243	-4.91771	397.517
[01] 12111408	12111408	-122.358	-86.2174	-4.40785	369.464
[04] 25052919	25052919	-119.601	-86.1044	-9.89121	355.437
[00] 16718332	16718332	-143.589	-86.0968	-8.00677	397.517
[01] 71053736	71053736	-124.806	-86.0801	-4.46591	437.462
[04] 71161248	71161248	-117.572	-85.9499	-11.6241	355.437
[04] 16718331	16718331	-118.84	-85.9314	-8.50142	383.491
[03] 16718284	16718284	-118.079	-85.8832	-2.5	383.491
[02] 25071575	25071575	-116.986	-85.8705	-1.86802	369.464
[04] 71455942	71455942	-115.621	-85.8541	-4.04802	355.437
[04] 71161250	71161250	-109.04	-85.7409	-3.14969	355.437
[01] 73309219	73309219	-135.555	-85.6619	-3.10296	383.491
[01] 12111413	12111413	-138.742	-85.6348	-5.85634	411.544
[01] 12111409	12111409	-136.683	-85.4887	-4.0117	383.491
[03] 142862978	142862978	-113.57	-85.469	-3.43334	341.411
[02] 68586983	68586983	-115.664	-85.4543	-9.06775	341.411
[02] 53327147	53327147	-126.402	-85.3091	-3.57922	397.517
[03] 10001666	10001666	-130.034	-85.3035	-2.25695	411.544
[00] 53327149	53327149	-140.367	-85.2092	-5.18301	397.517
[01] 25052919	25052919	-130.802	-85.1072	-2.09665	355.437
[02] 143189926	143189926	-118.117	-85.0865	-2.5	355.437
[01] 142862981	142862981	-133.02	-84.9658	-6.15369	369.464

[03] 16718335	16718335	-120.546	-84.935	-4.04538	397.517
[00] 16718175	16718175	-124.444	-84.9273	-4.40493	369.464
[03] 126746752	126746752	-131.074	-84.8859	-8.64757	397.517
[01] 70899058	70899058	-127.682	-84.8658	-0.62592	455.452
[00] 16718283	16718283	-131.629	-84.8055	-3.87019	383.491
[02] 25020384	25020384	-122.191	-84.7287	-1.40262	369.464
[01] 142405658	142405658	-123.915	-84.6991	-6.52138	383.491
[03] 155565350	155565350	-123.523	-84.6882	-5.93851	383.491
[00] 143547214	143547214	-119.925	-84.6619	-3.30408	355.437
[03] 25020438	25020438	-125.16	-84.6328	-5.98892	423.435
[04] 142862973	142862973	-130.31	-84.506	-2.24768	383.491
[00] 142862972	142862972	-142.888	-84.3728	-7.79971	383.491
[04] 164621561	164621561	-124.13	-84.324	-2.63597	411.544
[04] 9842276	9842276	-114.745	-84.3009	-3.09865	369.464
[01] 68586983	68586983	-122.257	-84.2554	-4.58209	341.411
[03] 21362562	21362562	-115.72	-84.2428	-1.34714	355.437
[03] 17966260	17966260	-132.17	-84.2371	-4.1629	397.517
[01] 71161248	71161248	-118.574	-84.1719	-5.04376	355.437
[04] 158338454	158338454	-116.094	-84.0709	-5.16211	355.437
[02] 10475938	10475938	-126.019	-84.011	-2.86306	397.517
[01] 142405659	142405659	-128.587	-83.8106	-6.20489	383.491
[03] 71161249	71161249	-118.298	-83.8067	-3.64326	355.437
[02] 71455942	71455942	-119.748	-83.7479	-1.15685	355.437
[01] 57433122	57433122	-132.077	-83.7282	-6.56417	341.411
[02] 69301819	69301819	-120.026	-83.7279	-4.92787	369.464
[01] 153494105	153494105	-125.984	-83.6524	-3.8235	443.609
[03] 170074997	170074997	-122.831	-83.4771	-6.8603	411.544
[01] 57463052	57463052	-123.64	-83.4358	-0.9943	381.475
[01] 154684506	154684506	-123.121	-83.1962	-3.74706	369.464
[01] 12111412	12111412	-125.82	-83.1784	-1.46767	397.517
[02] 25020436	25020436	-128.546	-83.176	-6.27148	437.462
[00] 70899058	70899058	-124.394	-83.1613	-2.76941	455.452
[03] 143189926	143189926	-118.926	-83.1436	-5.06056	355.437
[04] 21362562	21362562	-121.503	-83.1413	-3.0379	355.437
[04] 142862980	142862980	-116.344	-82.9952	-6.15145	369.464
[00] 158338454	158338454	-129.572	-82.9818	-4.61013	355.437
[02] 167264145	167264145	-124.317	-82.9385	-4.35698	407.512
[01] 164621561	164621561	-128.818	-82.8899	-4.45448	411.544
[00] 143189926	143189926	-123.54	-82.8617	-2.89004	355.437
[04] 11617621	11617621	-114.716	-82.7487	-6.29785	369.464
[00] 17966260	17966260	-142.725	-82.7415	-5.66179	397.517
[04] 143189927	143189927	-116.129	-82.704	-3.27007	355.437
[00] 16718284	16718284	-126.597	-82.5438	-5.82247	383.491
[04] 156808296	156808296	-118.959	-82.5256	-4.34693	383.491

[02] 53327148	53327148	-115.954	-82.5008	-4.51048	397.517
[02] 155565350	155565350	-121.232	-82.4519	-5.36827	383.491
[01] 154684505	154684505	-125.638	-82.4375	-6.78015	369.464
[01] 143189927	143189927	-125.339	-82.3578	-8.6664	355.437
[03] 164925828	164925828	-119.482	-82.3518	-3.21203	395.501
[04] 73309219	73309219	-130.245	-82.3506	-3.03733	383.491
[02] 16718283	16718283	-122.629	-82.3221	-10.1684	383.491
[02] 142862978	142862978	-118.914	-82.1036	-6.16609	341.411
[02] 57433124	57433124	-126.689	-82.024	-6.44055	383.491
[01] 143189926	143189926	-116.672	-82.0145	-6.43375	355.437
[02] 91175802	91175802	-138.08	-81.9461	-5.75389	411.544
[04] 12111408	12111408	-121.763	-81.8472	-3.3915	369.464
[00] 57433124	57433124	-127.598	-81.7636	-5.62821	383.491
[04] 16718333	16718333	-123.239	-81.739	-1.97722	397.517
[03] 57433122	57433122	-122.189	-81.381	-2.93281	341.411
[02] 25053272	25053272	-104.473	-81.3784	-3.01389	326.396
[03] 156808296	156808296	-118.828	-81.3679	-5.95491	383.491
[01] 10155152	10155152	-120.923	-81.3301	-3.99365	355.437
[03] 134468483	134468483	-132.209	-81.243	-5.72277	394.513
[02] 135278508	135278508	-125.647	-81.2134	-1.09196	411.544
[02] 10155152	10155152	-116.568	-81.1116	-4.8423	355.437
[03] 53327147	53327147	-126.345	-81.0374	-5.29613	397.517
[01] 25020435	25020435	-124.02	-80.9776	-4.40298	398.525
[04] 12111410	12111410	-109.916	-80.8568	-4.41005	383.491
[03] 11617621	11617621	-116.213	-80.7488	-3.51987	369.464
[01] 78073451	78073451	-114.57	-80.697	-1.50932	327.384
[00] 57433123	57433123	-129.54	-80.6854	-1.42826	383.491
[03] 16718334	16718334	-125.517	-80.6763	-4.97248	397.517
[03] 142862956	142862956	-133.899	-80.5954	-6.74406	411.544
[04] 142405659	142405659	-122.422	-80.4456	-8.18138	383.491
[00] 71053736	71053736	-119.003	-80.263	-3.48316	437.462
[04] 10001666	10001666	-129.295	-80.1106	-1.56337	411.544
[01] 53327149	53327149	-127.254	-80.0518	-1.62645	397.517
[03] 142862972	142862972	-126.987	-79.8865	-6.87731	383.491
[01] 53327147	53327147	-127.498	-79.8666	-3.59378	397.517
[00] 87784230	87784230	-129.541	-79.7486	-4.89804	429.371
[00] 68776785	68776785	-124.238	-79.6479	-8.65508	445.56
[01] 78073450	78073450	-122.457	-79.6056	-4.78528	355.437
[02] 168499477	168499477	-124.888	-79.6022	-6.8239	383.491
[03] 10247627	10247627	-108.031	-79.5809	-7.19387	355.437
[02] 24782039	24782039	-115.89	-79.4088	-4.67746	355.437
[03] 25071575	25071575	-111.852	-79.1766	-5.13644	369.464
[03] 71455943	71455943	-114.389	-79.0347	-9.40746	327.384
[02] 25053204	25053204	-104.595	-78.8307	-3.34591	326.396

[03] 16718382	16718382	-119.826	-78.7879	-0.63905	411.544
[03] 57391639	57391639	-124.267	-78.7765	-6.1399	369.464
[02] 16718336	16718336	-118.207	-78.7666	-11.8807	411.544
[01] 142862984	142862984	-121.78	-78.7366	-4.57846	355.437
[01] 71161249	71161249	-122.754	-78.3993	-6.61508	355.437
[00] 68776787	68776787	-156.749	-78.1	-6.95097	445.56
[01] 168499477	168499477	-124.765	-78.0588	-7.14301	383.491
[03] 12111414	12111414	-127.277	-78.0508	-4.41617	411.544
[03] 16718283	16718283	-124.078	-77.8758	-6.08911	383.491
[00] 12111412	12111412	-131.499	-77.8211	-6.72507	397.517
[02] 9842276	9842276	-115.734	-77.8026	-4.18309	369.464
[02] 143189927	143189927	-116.082	-77.7936	-0.54034	355.437
[01] 71161250	71161250	-112.274	-77.6971	-2.5759	355.437
[00] 71455942	71455942	-123.951	-77.6583	-3.46023	355.437
[04] 126746752	126746752	-128.811	-77.6043	-0.33033	397.517
[01] 25053272	25053272	-106.313	-77.5712	-8.67438	326.396
[03] 153494105	153494105	-116.694	-77.5145	-1.12649	443.609
[02] 16718175	16718175	-118.568	-77.435	-0.48358	369.464
[03] 68776785	68776785	-114.452	-77.3969	-7.55717	445.56
[04] 12111414	12111414	-119.97	-77.1719	-5.15278	411.544
[04] 69069109	69069109	-117.301	-77.1494	-6.97908	355.437
[03] 167264145	167264145	-126.573	-77.0772	-4.94489	407.512
[02] 154684506	154684506	-119.424	-77.0441	-3.69916	369.464
[03] 57395048	57395048	-116.153	-77.0164	-6.13535	369.464
[01] 155565350	155565350	-122.352	-76.9233	-2.9087	383.491
[04] 53327148	53327148	-117.658	-76.9207	-4.36244	397.517
[04] 25020438	25020438	-112.345	-76.8523	-2.2165	423.435
[04] 25053272	25053272	-102.139	-76.8412	-2.609	326.396
[03] 57463052	57463052	-123.95	-76.7384	-3.25474	381.475
[04] 143189926	143189926	-117.142	-76.7141	-1.5303	355.437
[00] 142862956	142862956	-136.445	-76.6623	-4.09749	411.544
[04] 10247627	10247627	-111.929	-76.6153	-3.28722	355.437
[01] 158338454	158338454	-119.771	-76.5869	-5.4814	355.437
[03] 9842276	9842276	-119.879	-76.5359	-10.2357	369.464
[01] 143547214	143547214	-121.283	-76.4636	-5.61824	355.437
[03] 24782039	24782039	-114.106	-76.4009	-5.57246	355.437
[00] 25053204	25053204	-115.456	-76.3412	-6.35292	326.396
[00] 69069109	69069109	-124.578	-76.3248	-5.8646	355.437
[03] 12111412	12111412	-115.648	-76.2677	-5.21446	397.517
[03] 168285854	168285854	-123.386	-76.235	-3.08703	397.517
[03] 16718332	16718332	-128.476	-76.1197	-2.73846	397.517
[00] 135278508	135278508	-147.257	-76.0957	0	411.544
[01] 10001665	10001665	-134.744	-76.0783	-4.90906	411.544
[04] 25020435	25020435	-125.211	-75.962	-3.32618	398.525

[04] 16718175	16718175	-114.832	-75.9569	-2.98608	369.464
[03] 143547214	143547214	-116.812	-75.7717	-4.02883	355.437
[04] 57391639	57391639	-125.555	-75.74	-3.67603	369.464
[04] 57433124	57433124	-120.616	-75.2046	-1.11692	383.491
[04] 16718284	16718284	-120.718	-75.0461	-5.77799	383.491
[04] 154684505	154684505	-126.827	-75.0378	-7.21487	369.464
[03] 25072803	25072803	-111.954	-74.9339	-2.071	397.517
[04] 142405658	142405658	-120.16	-74.8912	-2.94129	383.491
[04] 71455943	71455943	-112.809	-74.7675	-10.0274	327.384
[01] 68587957	68587957	-99.4207	-74.5567	-4.61547	383.491
[03] 25053204	25053204	-104.435	-74.3789	-8.28235	326.396
[00] 154684505	154684505	-140.079	-73.9108	-6.96165	369.464
[04] 25071575	25071575	-104.833	-73.8703	-4.14218	369.464
[01] 9842276	9842276	-117.664	-73.8323	-2.03143	369.464
[03] 25052919	25052919	-125.226	-73.8277	-8.25074	355.437
[02] 25020439	25020439	-118.62	-73.6913	-5.42822	491.433
[00] 154684506	154684506	-133.931	-73.5512	-6.77946	369.464
[02] 68849059	68849059	-137.065	-73.3634	-4.4179	411.544
[04] 68586983	68586983	-107.926	-73.3403	-4.12026	341.411
[01] 87784230	87784230	-125.434	-73.339	-5.49966	429.371
[03] 143189927	143189927	-120.862	-73.2484	-3.12649	355.437
[03] 12111410	12111410	-118.299	-73.2387	-3.28794	383.491
[04] 142862984	142862984	-116.631	-73.2197	-5.69936	355.437
[03] 164926106	164926106	-117.509	-72.9842	-3.53451	426.578
[03] 142862984	142862984	-117.749	-72.9094	-0.79164	355.437
[03] 70899058	70899058	-121.144	-72.7973	-6.46627	455.452
[04] 17966260	17966260	-124.8	-72.7771	-1.60332	397.517
[04] 10475938	10475938	-121.486	-72.717	-1.66222	397.517
[04] 12111413	12111413	-114.593	-72.5883	-7.72056	411.544
[04] 16718334	16718334	-122.746	-72.5819	-4.89962	397.517
[03] 71161250	71161250	-107.264	-72.483	-3.46271	355.437
[04] 25020436	25020436	-126.702	-71.9558	-3.19073	437.462
[02] 71455943	71455943	-115.381	-71.7367	-4.65172	327.384
[02] 126604381	126604381	-128.211	-71.5291	-7.26475	411.544
[03] 53327148	53327148	-114.59	-71.4804	-5.02017	397.517
[02] 12111413	12111413	-128.223	-71.282	-2.05336	411.544
[03] 71161248	71161248	-118.222	-71.1969	-6.53181	355.437
[02] 87784230	87784230	-116.212	-71.1814	-3.91706	429.371
[04] 87784230	87784230	-109.456	-71.0844	-5.47778	429.371
[01] 25072803	25072803	-117.123	-71.0408	-5.21609	397.517
[02] 68776785	68776785	-123.937	-70.8606	-2.09017	445.56
[00] 68587957	68587957	-107.045	-70.7742	-5.35557	383.491
[03] 68849059	68849059	-131.969	-70.608	-3.15935	411.544
[03] 25020384	25020384	-116.093	-70.5143	-1.58913	369.464

[02] 142862980	142862980	-125.449	-70.4595	-3.90478	369.464
[04] 57395048	57395048	-110.52	-70.424	-1.79581	369.464
[04] 164925828	164925828	-120.041	-70.1555	-7.92156	395.501
[00] 71161250	71161250	-117.082	-70.1095	-6.85188	355.437
[02] 10000883	10000883	-126.886	-69.8465	-4.50983	397.517
[00] 142405658	142405658	-127.471	-69.7426	-4.69674	383.491
[01] 25020439	25020439	-125.098	-69.3414	-2.62484	491.433
[01] 142862980	142862980	-132.496	-69.0462	-7.80189	369.464
[03] 10155152	10155152	-109.711	-68.9423	-8.55458	355.437
[01] 25053204	25053204	-107.284	-68.9061	-4.63066	326.396
[03] 12111413	12111413	-124.595	-68.7036	-3.95952	411.544
[04] 143547214	143547214	-113.712	-68.3271	-3.68854	355.437
[02] 71161250	71161250	-112.77	-68.2069	-3.2549	355.437
[02] 16718334	16718334	-125.272	-68.0233	-8.05478	397.517
[00] 168285854	168285854	-123.927	-67.823	-2.59757	397.517
[01] 164926106	164926106	-120.097	-67.7623	-2.03822	426.578
[02] 53327149	53327149	-125.335	-67.6811	-2.69445	397.517
[03] 16718333	16718333	-123.924	-67.6799	-3.61888	397.517
[04] 16718282	16718282	-121.942	-67.4714	-1.43061	383.491
[01] 68776785	68776785	-125.865	-67.3991	-1.45089	445.56
[04] 25053204	25053204	-95.7872	-67.1749	-5.24099	326.396
[00] 142862984	142862984	-120.52	-67.094	-4.59202	355.437
[02] 25020435	25020435	-124.628	-66.713	-4.00962	398.525
[02] 78073450	78073450	-113.416	-66.5858	-2.84106	355.437
[02] 11617621	11617621	-120.448	-66.2345	-1.45636	369.464
[02] 17966266	17966266	-140.933	-66.1285	-4.15852	411.544
[04] 68849059	68849059	-126.127	-66.0059	-4.55609	411.544
[00] 25053272	25053272	-103.984	-65.9889	-3.30778	326.396
[02] 71161248	71161248	-117.26	-65.9174	-6.31441	355.437
[04] 10155152	10155152	-115.907	-65.2742	-4.44622	355.437
[00] 153494105	153494105	-129.229	-64.2541	-1.03984	443.609
[02] 68776787	68776787	-141.982	-63.9027	-2.57009	445.56
[00] 126604381	126604381	-134.191	-63.7946	-4.93095	411.544
[02] 164925828	164925828	-122.062	-62.6956	-1.16632	395.501
[04] 164926106	164926106	-118.999	-62.5059	-1.21409	426.578
[01] 91175802	91175802	-121.276	-62.4696	-1.02413	411.544
[01] 10475938	10475938	-131.409	-61.2926	-5	397.517
[00] 53327148	53327148	-120.891	-60.4473	-1.75301	397.517
[00] 135278505	135278505	-128.712	-60.0915	-11.4483	397.537
[04] 25072803	25072803	-104.902	-60.0655	-10.9038	397.517
[02] 68587957	68587957	-100.965	-59.6955	-3.60349	383.491
[04] 126604381	126604381	-118.856	-59.6176	-0.65264	411.544
[01] 69069109	69069109	-122.664	-59.2792	-4.59245	355.437
[03] 25053272	25053272	-104.058	-58.7064	-7.53463	326.396

[00] 25020384	25020384	-129.413	-58.4202	-4.98056	369.464
[04] 154684506	154684506	-120.41	-58.2389	-3.06855	369.464
[00] 164926106	164926106	-126.714	-58.0147	0	426.578
[03] 74379282	74379282	-128.856	-57.9217	-4.53703	397.517
[03] 57433123	57433123	-121.93	-57.564	-4.52414	383.491
[02] 78073451	78073451	-117.875	-57.1602	-10.8492	327.384
[04] 168499477	168499477	-117.585	-56.9141	-2.98492	383.491
[04] 153494105	153494105	-120.017	-56.7283	-0.82114	443.609
[02] 25072803	25072803	-110.876	-55.0366	-6.80013	397.517
[03] 142862980	142862980	-124.636	-54.8973	-6.61191	369.464
[00] 25072803	25072803	-120.537	-53.173	-2.72518	397.517
[02] 12111412	12111412	-119.318	-53.1671	-9.67224	397.517
[01] 135278508	135278508	-134.223	-52.1695	-2.25313	411.544
[04] 53327149	53327149	-119.866	-51.5713	-9.6307	397.517
[03] 71455942	71455942	-119.23	-51.036	-5.30601	355.437
[04] 16718382	16718382	-110.31	-50.6963	-2.43606	411.544
[01] 126604381	126604381	-130.706	-50.6644	-2.44412	411.544
[00] 53327147	53327147	-140.341	-50.2735	-6.23653	397.517
[01] 142862978	142862978	-120.82	-50.2479	-5.04396	341.411
[03] 73309219	73309219	-124.171	-50.2375	-1.55992	383.491
[03] 126604381	126604381	-121.849	-49.8917	-0.85812	411.544
[01] 12111411	12111411	-140.265	-49.7905	-3.83811	397.517
[02] 158338454	158338454	-124.415	-49.7666	-2.77924	355.437
[04] 68776785	68776785	-117.812	-49.408	-2.5	445.56
[03] 25020436	25020436	-117.176	-49.0599	-3.09511	437.462
[00] 74379282	74379282	-134.64	-46.5969	-0.18033	397.517
[04] 142862956	142862956	-137.512	-45.0232	-4.33703	411.544
[02] 91004632	91004632	-116.566	-44.9445	-5.04946	383.491
[00] 164925828	164925828	-132.482	-44.1421	-3.13837	395.501
[02] 142862956	142862956	-131.368	-43.7493	-1.74474	411.544
[03] 69301816	69301816	-128.383	-43.7135	-6.45786	369.464
[04] 155565350	155565350	-119.009	-39.9036	-3.84644	383.491
[01] 135278505	135278505	-130.019	-39.5446	-6.06038	397.537
[02] 71053736	71053736	-118.7	-39.5443	-5.05429	437.462
[01] 12111410	12111410	-123.296	-38.667	-3.39808	383.491
[04] 24983461	24983461	-118.417	-37.7187	-9.75361	397.517
[03] 16718381	16718381	-130.722	-36.9898	-3.38264	411.544
[03] 25020439	25020439	-135.354	-36.4568	-3.69162	491.433
[04] 53327147	53327147	-122.223	-34.8288	-4.48025	397.517
[01] 168285854	168285854	-125.126	-33.5329	-2.36734	397.517
[02] 164926106	164926106	-115.319	-32.6497	-2.08105	426.578
[03] 158338454	158338454	-116.587	-32.0903	-3.41656	355.437
[02] 153494105	153494105	-128.013	-31.5849	-3.84726	443.609
[00] 142405659	142405659	-125.202	-30.8335	-4.38795	383.491

[02] 24983461	24983461	-121.818	-30.3624	-4.41875	397.517
[03] 12111408	12111408	-117.178	-29.8028	-2.99331	369.464
[04] 135278508	135278508	-124.993	-29.1328	-1.30778	411.544
[01] 10247627	10247627	-118.128	-28.3909	-9.39366	355.437
[04] 16718335	16718335	-116.985	-28.0246	-2.02194	397.517
[04] 57463052	57463052	-120.86	-27.8932	-4.2236	381.475
[04] 17966266	17966266	-134.822	-27.7105	-4.24453	411.544
[03] 68776787	68776787	-133.921	-27.5364	-0.18495	445.56
[00] 10247627	10247627	-115.073	-26.3575	-0.85616	355.437
[03] 87784230	87784230	-117.99	-26.2061	-2.39926	429.371
[00] 164621561	164621561	-130.163	-24.1424	-5.11642	411.544
[04] 167264145	167264145	-121.537	-24.0346	-3.2634	407.512
[01] 68776787	68776787	-145.542	-23.9915	-2.55658	445.56
[03] 68587957	68587957	-105.187	-21.3937	-7.1399	383.491
[03] 154684506	154684506	-120.942	-19.4304	-2.53063	369.464
[04] 57433123	57433123	-119.474	-17.2515	-0.91804	383.491
[04] 91175802	91175802	-123.049	-17.1616	-3.69487	411.544
[02] 164621561	164621561	-128.68	-14.223	-2.43468	411.544
[04] 68587957	68587957	-97.6175	-12.0046	-1.90359	383.491
[02] 135278505	135278505	-123.792	-10.6535	-2.02379	397.537
[04] 68776787	68776787	-139.845	-5.91329	-0.55794	445.56
[03] 16718336	16718336	-121.325	8.19745	-1.29875	411.544
[03] 164621561	164621561	-120.274	51.7706	-4.39751	411.544

5.10. PHARMACOPHORE MODELING

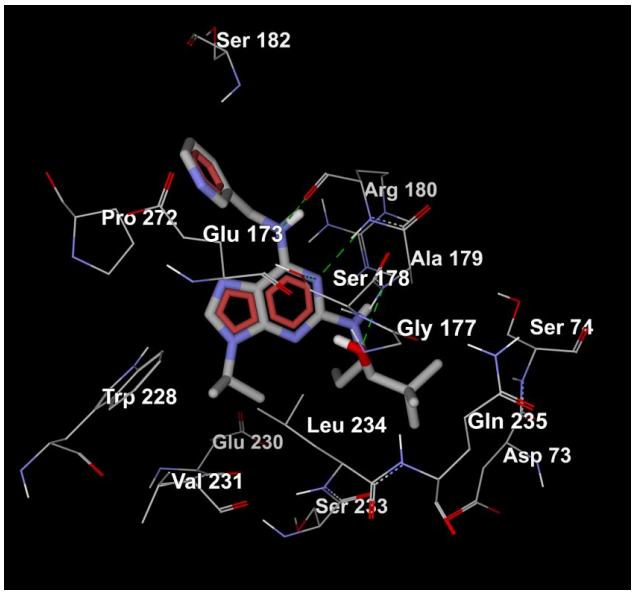


Fig.10.1. The most effective compound obtained from virtual screening shows h-bond interaction with CDK1

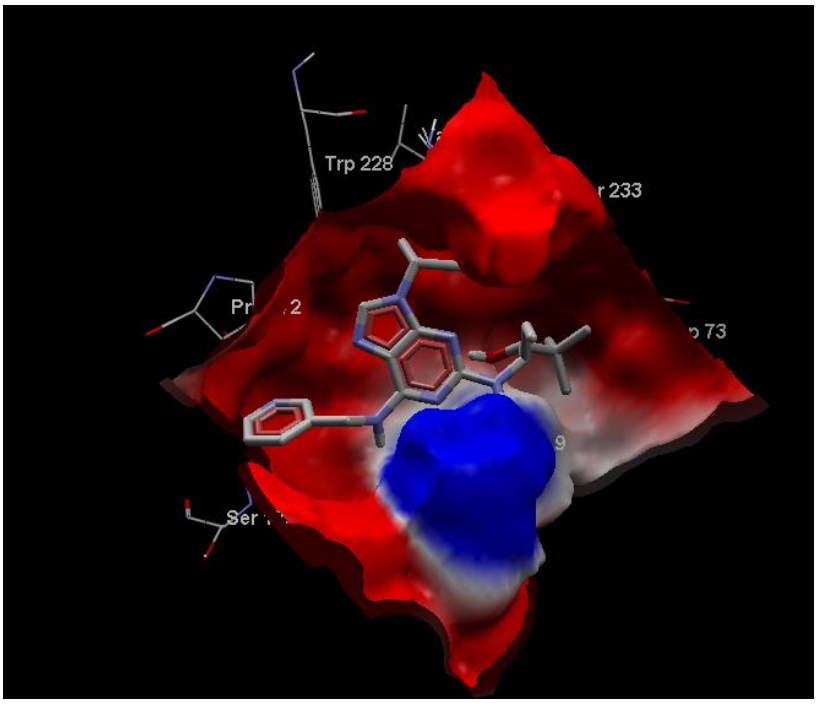


Fig.10.2. The most effective compound obtained from virtual screening shows electrostatic interaction with CDK1

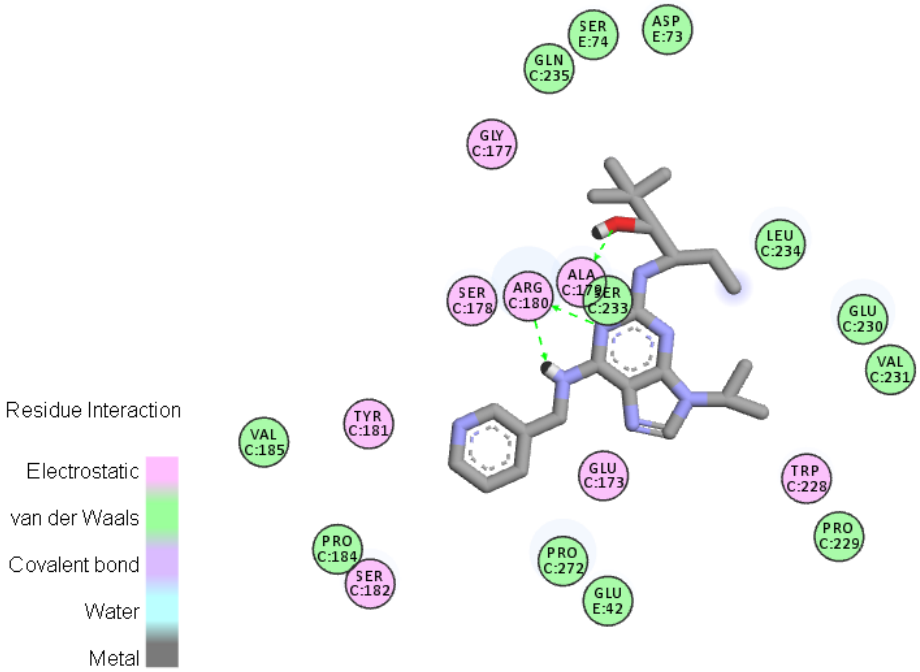


Fig.10.3. The most effective compound obtained from virtual screening shows van der Waals interaction with CDK1

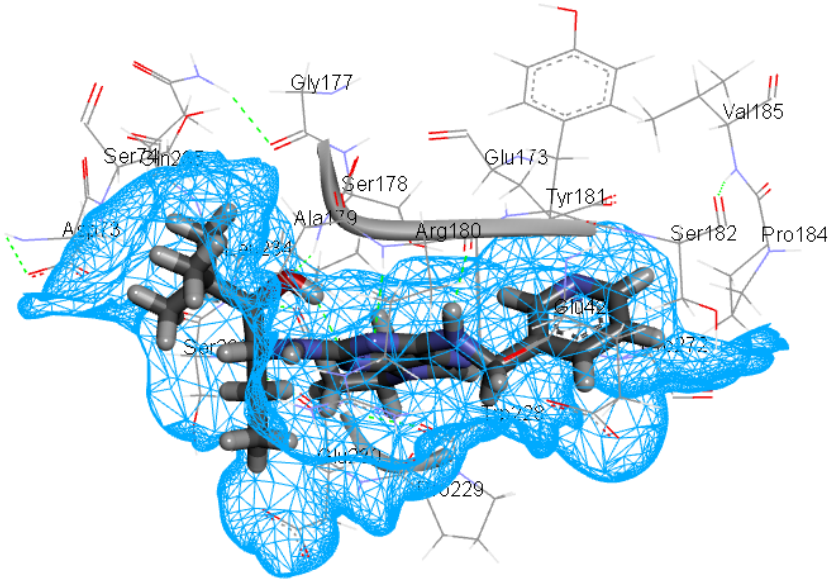


Fig.10.4 The most effective compound obtained from virtual screening shows receptor ligand interaction with CDK1

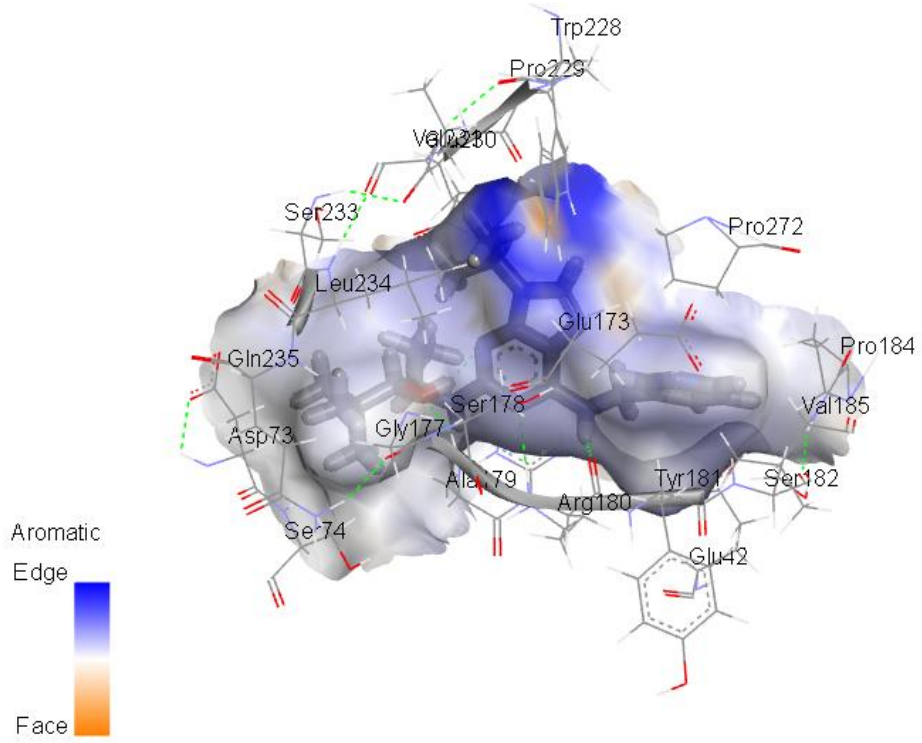


Fig. 10.5. The most effective compound obtained from virtual screening shows receptor ligand interaction with CDK1

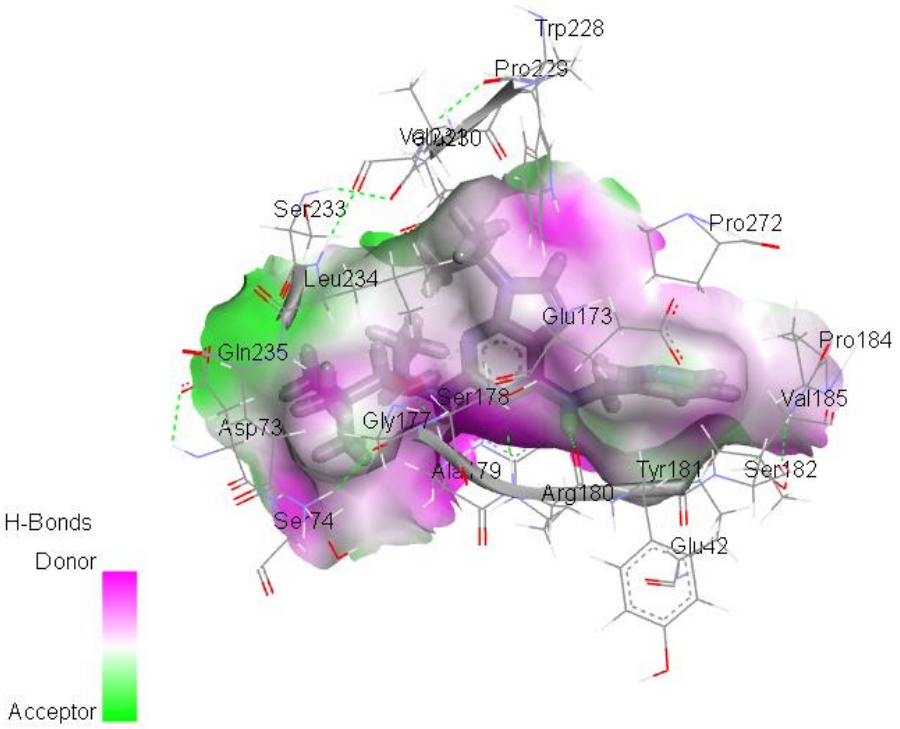


Fig. 10.6. The most effective compound obtained from virtual screening shows h-bond interaction with CDK1

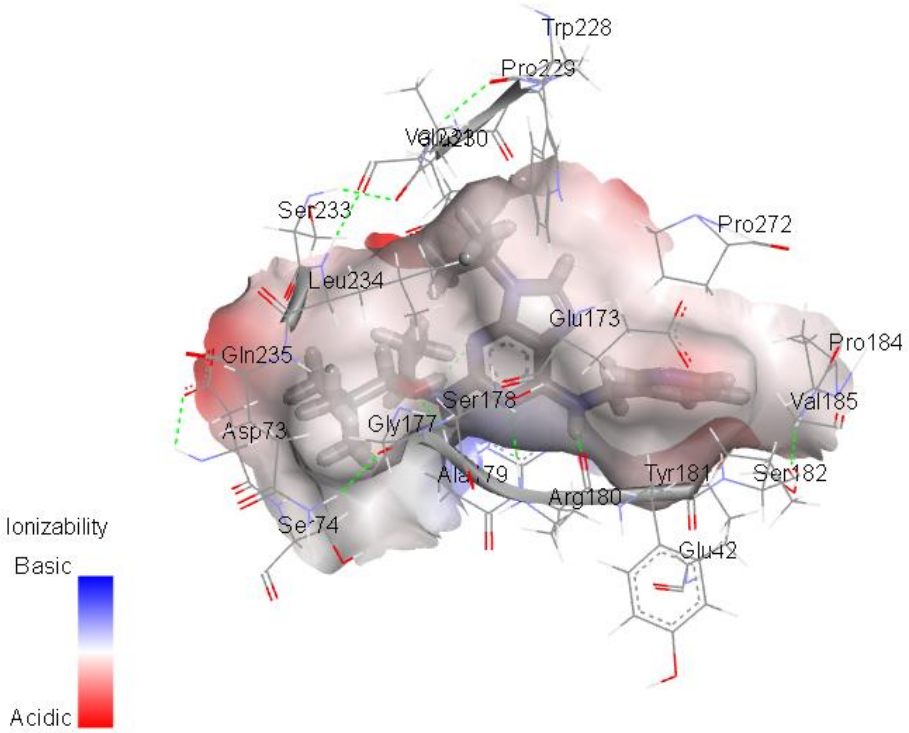


Fig.10.7. The most effective compound obtained from virtual screening shows ionizability interaction with CDK1

5.11. ADMET PREDICTION

Model	Result	Best VS Compound	Best Established Compound
Absorption			
Blood-Brain Barrier	BBB+	0.5762	0.6069
Human Intestinal Absorption	HIA+	1	1
Caco-2 Permeability	Caco2-	0.6012	0.5439
P-glycoprotein Substrate	Substrate	0.8027	0.8026
P-glycoprotein Inhibitor	Inhibitor	0.5326	0.5593
	Inhibitor	0.6804	0.5
Renal Organic Cation Transporter	Non-inhibitor	0.8054	0.7887
Distribution			
Subcellular localization	Lysosome	0.5373	0.3495
Metabolism			
CYP450 2C9 Substrate	Non-substrate	0.8419	0.808
CYP450 2D6 Substrate	Non-substrate	0.7825	0.7742
CYP450 3A4 Substrate	Substrate	0.5613	0.5249
CYP450 1A2 Inhibitor	Inhibitor	0.6975	0.6605
CYP450 2C9 Inhibitor	Inhibitor	0.618	0.6154
CYP450 2D6 Inhibitor	Inhibitor	0.629	0.7859
CYP450 2C19 Inhibitor	Non-inhibitor	0.7686	0.8099
CYP450 3A4 Inhibitor	Inhibitor	0.5858	0.6405
CYP Inhibitory Promiscuity	Low CYP Inhibitory Promiscuity	0.5278	0.6031
Excretion			
Toxicity			
Human Ether-a-go-go-Related Gene Inhibition	Weak inhibitor	0.8884	0.8168
	Inhibitor	0.5791	0.5318
AMES Toxicity	Non AMES toxic	0.6062	0.6267
Carcinogens	Non-carcinogens	0.8217	0.8331
Fish Toxicity	Low FHMT	0.6629	0.8258
Tetrahymena Pyriformis Toxicity	High TPT	0.9649	0.9563
Honey Bee Toxicity	Low HBT	0.7207	0.7458
Biodegradation	Not readily biodegradable	0.9944	0.9797
Acute Oral Toxicity	III	0.5913	0.5996
Carcinogenicity (Three-class)	Non-required	0.4888	0.5224
ADMET Predicted Profile --- Regression			
Model	Unit	Value	Value
Absorption			
Aqueous solubility	LogS	-3.4362	-3.2427
Caco-2 Permeability	LogPapp, cm/s	0.3817	0.6628
Toxicity			

Rat Acute Toxicity	LD50, mol/kg	2.7359	2.6272
Fish Toxicity	pLC50, mg/L	1.6169	1.4946
Tetrahymena Pyriformis Toxicity	pIGC50, ug/L	0.3531	0.5517

CHAPTER 6 – DICUSSION

CHAPTER 6 – DISCUSSION

After performing differential gene expression analysis using GEPIA2, a list of 2,289 over-expressed/up-regulated genes was generated. All of these genes had an FDR adjusted p-value of less than 0.01 and a log2 fold change (|log2FC|) greater than 2. The substantial amount of data gives us insights into just how extensive the genetic alterations are in glioblastoma cells. Among these 2,289 genes, some were found to be significantly upregulated in comparison to levels found in normal tissues. These genes were identified by sorting the results from the largest to the smallest fold change.

Focusing on the top 20 most significantly upregulated DEG's, a protein-protein interaction network was constructed using STRING. This network analysis revealed a complex web of interactions, with CDK1 emerging as one of the key nodes. The fact that CDK1 was a key node underscores its critical involvement in cellular and molecular processes, particularly the cell cycle. Given its central role as well as significant upregulation in glioblastoma, CDK1 was selected for further detailed research and analysis.

Using UACLAN, the expression levels of CDK1 were observed across various cancers. The analysis revealed that CDK1 is over expressed in a wide array of malignancies, reinforcing its role as a critical regulator in cancer biology. CDK1 was found to be significantly upregulated in comparison to normal brain tissue. This marked overexpression in glioblastoma in comparison to non-cancerous tissues, emphasizes the importance of CDK1 in the oncogenic processes driving cancer progression. This finding not only corroborates the findings from the differential gene expression analysis but also reveals how CDK1 can be a universal cancer marker. Due to these reasons, CDK1 was selected as the drug target of choice in this study.

The protein sequence of CDK1 from Homo sapiens was successfully retrieved from the UniprotKB/Swissprot database using the query sequence ["CDK1" AND "Homo sapiens"]. The FASTA file of the sequence was downloaded and analysed using BioEdit.

BioEdit protein sequence analysis revealed that CDK1 consists of 297 amino acids and has a molecular weight of 34093.63 Daltons. The amino acid composition histogram indicated a high occurrence of Leucine, Lysine, and Isoleucine (present 36, 24, and 22 times respectively), while

Cysteine and Tryptophan were the least occurring amino acids (present 1 and 4 times respectively).

The “pepstats” command in EMBOSS was employed to analyse the protein's biochemical properties. It was found that the protein contains more non-polar amino acids than polar amino acids (53.535% vs 46.465%), with a higher proportion of aliphatic amino acids compared to aromatic ones (42.761% vs 25%), and slightly more basic amino acids than acidic ones (15.825% vs 12.458%).

Protein primary structure analysis using ProtParam revealed a theoretical pI of 8.38, indicating electric neutrality at pH 8.38. The instability index of 39.26 classifies the protein as stable (<40.0), while the aliphatic index of 97.78 suggests higher thermostability. The Grand Average of Hydropathicity (GRAVY) score of -0.281 indicates the protein's hydrophilic nature.

Secondary structure prediction using SOPMA revealed that the protein comprises of majority (43.77%) alpha helix followed by 34.68% random coil, 14.4% extended strand, and 7.07% beta turns.

The search for the protein's 3D structure involved a Blastp search against the PDB database, yielding accession 4YC6 with a perfect match (e-value of 0), 100% identity, and a high bit score of 612. The corresponding 3D structure was downloaded in PDB format from the PDB database.

Furthermore, 46 chemical and 13 phytochemical compounds inhibiting CDK1 and effective against glioma or glioblastoma cell lines were identified through an extensive search of literature sources and databases. Their 3D structures were obtained from PubChem in 3D sdf format, supplemented with manually drawn or converted 2D to 3D sdf formats using Marvin Sketch, ensuring a comprehensive dataset for further docking studies.

Docking of both chemical as well as phytochemical established CDK1 inhibitors was conducted and the data was sorted by smallest to largest rereank score. Compound ID – 24983461 also known as CYC065, had the lowest rerank score of both groups (-106.185). A lower rerank score

indicated higher binding affinity. Due to this reason, 24983461 was determined to be the best established compound.

Virtual screening was conducted using the compound id 24983461 using PubChem similarity search (95% similarity threshold) and a total of 103 compounds were identified as structurally similar to the query molecule. Given that function is often correlated and derived from structure, these structurally similar compounds were hypothesized to potentially exhibit similar CDK1 inhibitory activity. To test this theory, all 103 compounds were subject to docking against the CDK1 molecule. Among these compounds, compound ID 16718337 stood out, achieving a rerank score of -106.77. This score was slightly better than the best established CDK1 inhibitor (24983461) which had a rerank score of -106.185. The lower rerank score of 16718337 indicates an even stronger binding affinity and potential efficacy in inhibiting CDK1. Therefore, this compound was selected for further analysis.

Pharmacophore studies of 16718337 revealed the presence of 4 hydrogen bonds between the ligand and amino acid residues of CDK1. These interactions were Ser178 (1 h-bond), Ala179(1 h-bond) and Arg180 (2 h-bonds). The electrostatic surface analysis indicated that the ligand had higher attraction towards negatively charged amino acids, suggesting electrostatic interaction between CDK1 and the ligand. Van der waals interaction was also observed.

Looking at the different molecular surfaces, the aromatic surface showed an edge near the ligand, indicating close proximity to aromatic residues, while the rest of the surface remained generally neutral. This suggests potential π-π interactions which could further stabilize the ligand binding. Examining the h-bond surface, there were equal areas of h-bond donors and h-bond acceptors. Ionizability surface was generally neutral, with some areas of acidity. These acidic patches correspond to negatively charged regions that could interact with the positively charged areas of the ligand, thus enhancing binding interaction.

Comparing the ADMET profiles of the best established compound (24983461) vs the best virtual screening compound (16718337), the following things were observed

1. Absorption
    a. Blood-Brain Barrier Penetration : The best established compound showed a slightly better score (0.5762 vs 0.609) than the best virtual screening compound.

b. Human Intestinal Absorption: Both compounds show a complete absorption score of 1.

c. CaCo-2 Permeability: The best virtual screening compound shows a slightly better score (0.6012 vs 0.5439) compared to the best established compound, indicating that the best virtual screening (VS) compound may have better intestinal permeability.

d. P-glycoprotein Interaction:

    i. Substrate: Both compounds are identified as P-gp substrates with similar scores (0.8027 for VS and 0.8026 for established). This suggests they might be subject to efflux by P-gp, affecting their bioavailability.

    ii. Inhibitor: The VS compound shows dual P-gp inhibition activity with scores of 0.5326 and 0.6804, compared to 0.5593 and 0.5 for the established compound. This dual inhibitory action might influence drug-drug interactions and pharmacokinetics.

e. Both compounds are non-inhibitors of the renal organic cation transporter, with scores of 0.8054 (VS) and 0.7887 (established), suggesting they are unlikely to interfere with renal excretion processes.

2. Distribution:

    a. Subcellular localization: The best VS compound is more likely to localize in the lysosome (0.5373) compared to the established compound (0.3495). This localization could impact the intracellular distribution and efficacy of the drug

3. Metabolism:

    a. Cytochrome P450 (CYP) Interactions:

        i. Substrate: Both compounds are non-substrates for CYP450 2C9 and 2D6, with the VS compound showing scores of 0.8419 and 0.7825, respectively. They are substrates for CYP450 3A4, with the VS compound at 0.5613, slightly higher than the established compound at 0.5249.

        ii. Inhibitor: Both compounds inhibit several CYP enzymes (CYP450 1A2, 2C9, 2D6, and 3A4) with varying scores, suggesting potential drug-drug interactions. The VS compound generally shows comparable or slightly

lower inhibition scores than the established compound, indicating a potentially better safety profile concerning CYP-mediated metabolism.

   b. CYP Inhibitory Promiscuity: The VS compound exhibits lower CYP inhibitory promiscuity (0.5278) compared to the established compound (0.6031), suggesting it may have fewer off-target effects on CYP enzymes.

4. Excretion:
   a. Human Ether-a-go-go-Related Gene (hERG) Inhibition: Both compounds are weak inhibitors of the hERG channel, with the VS compound scoring 0.8884 compared to 0.8168 for the established compound. This suggests a low risk of cardiotoxicity.
   b. AMES Toxicity and Carcinogenicity: Both compounds are non-AMES toxic and non-carcinogenic, indicating a favourable toxicity profile.
5. Toxicity:
   a. Acute Oral Toxicity: Both compounds fall into toxicity class III, with scores of 0.5913 (VS) and 0.5996 (established), indicating moderate acute oral toxicity.

In Regression Models:

1. Aqueous Solubility and Permeability: The best VS compound has lower aqueous solubility (LogS: -3.4362) compared to the established compound (-3.2427). However, it shows lower Caco-2 permeability (LogPapp: 0.3817) than the established compound (0.6628), suggesting differences in absorption and bioavailability.
2. Rat Acute Toxicity and Fish Toxicity: The VS compound shows a higher LD50 in rats (2.7359 mol/kg) compared to the established compound (2.6272 mol/kg), indicating lower acute toxicity. The fish toxicity is also lower for the VS compound (pLC50: 1.6169) compared to the established compound (1.4946), suggesting a better safety profile in aquatic environments.

The ADMET profile suggests that the best VS compound, while having a slightly lower BBB penetration and aqueous solubility, shows better Caco-2 permeability and a lower environmental toxicity profile compared to the best-established compound. Its lower CYP inhibitory promiscuity and weaker hERG inhibition further suggest it might be a safer and more effective candidate for targeting CDK1 in glioblastoma. These findings highlight the importance of comprehensive ADMET analysis in the selection and optimization of potential drug candidates.

CHAPTER 7 – CONCLUSIONS

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CDK1 was found to exhibit significant upregulation across various cancer types, notably in glioblastoma. It's pivotal role in various protein-protein interaction networks, along with its crucial role in the cell cycle, led to its selection as a promising candidate for both biomarker identification and a potential drug target for the treatment of glioblastoma. The discovery of compound 16718337 as a potential CDK1 inhibitor with a superior docking score underscores the efficacy of our integrated computational approach. This novel compound not only demonstrates better predicted binding affinity but also shows promising pharmacokinetic properties, making it a strong candidate for further development. The next steps involve conducting rigorous preclinical studies to validate its efficacy and safety in biological systems, followed by clinical trials to evaluate its therapeutic potential in patients.

In summary, this study underscores the power of advanced computational techniques in identifying and optimizing potential therapeutic agents. The integration of molecular docking, pharmacophore modelling, and ADMET analysis has led to the discovery of a promising new CDK1 inhibitor, paving the way for preclinical and clinical studies. These findings represent a significant step towards developing effective treatments for glioblastoma and potentially other cancers, ultimately enhancing patient outcomes.

CHAPTER 8 – BIBLIOGRAPHY

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