MASTER'S PROJECT

EXPLORING ISO-INDOLINONES AND ARISTOLACTAMS AS POTENTIAL CDK7 INHIBITORS USING CHEMINFORMATICS TOOLS

A PROJECT REPORT
SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE
OF
MASTER OF SCIENCE
IN
CHEMISTRY

Submitted by

Ms. Chahat

(2K21/MSCCHE/12)

Under the supervision of

Prof. Ram Singh **Dr. Saurabh Mehta**

DEPARTMENT OF APPLIED CHEMISTRY

DELHI TECHNOLOGICAL UNIVERSITY

Bawana Road, Delhi-110042

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CANDIDATE'S DECLARATION

I, **Chahat**, Roll No. 2K21/MSCCHE/12 student of M.Sc. (Chemistry), hereby declare that the project Dissertation titled "**EXPLORING ISO-INDOLINONES AND ARISTOLACTAMS AS POTENTIAL CDK7 INHIBITORS USING CHEMINFORMATICS TOOLS**" which is submitted by me to the Department of Applied Chemistry, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Science (Chemistry), is original and not copied from any source without proper citation. This is an authentic record of my own work carried out by me under the supervision of Prof. Ram Singh (Professor, Department of Applied Chemistry, DTU) and Dr. Saurabh Mehta (Ex-Assistant Professor, Department of Applied Chemistry, DTU).

I, further declare that this work has not previously formed the basis for the award of any Degree, Diploma Associateship, Fellowship or other similar title or recognition.

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CERTIFICATE

I, hereby certify that the Project Dissertation titled "**EXPLORING ISO-INDOLINONES AND ARISTOLACTAMS AS POTENTIAL CDK7 INHIBITORS USING CHEMINFORMATICS TOOLS**" which is submitted by Ms. Chahat, Roll No. 2K21/MSCCHE/12, Department of Applied Chemistry, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Science (Chemistry), is a record of the project work carried out by the students under my supervision.

To the best of my knowledge this work has not been submitted in part or full for any degree or diploma to this university or elsewhere.

Place: Delhi

Prof. Ram Singh
Department of Applied Chemistry
Delhi Technological University
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Place: USA

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Chahat

Abstract

Isoindolin-1-ones are heterocyclic molecules with useful therapeutic properties. Synthetic and medicinal research groups have designed many methodologies for these compounds due to their intriguing biological activity for these compounds. The development of small molecular scaffolds as specific CDK inhibitors is a promising strategy in the discovery of anticancer drugs. *In silico* evaluation of the isoindolin-1-ones and aristolactam skeleton is done using Cheminformatics tools to evaluate the bioactivity of the virtually synthesized library of 48 compounds. The protein target (CDK7) and ligand 7 had the maximum obtained docking energy of -10.1 kcal/mol, which revealed four hydrogen bonding interactions with the amino acid residues LYS139, ASN141, ASN142, and MET94. The hydrogen bonding interactions in the docked complexes of CDK7 (1UA2.pdb) and active ligands were analysed.

Out of 48 ligands, 41 ligands exhibited hydrogen bonding Interactions. With the help of MD simulations, the molecular docking results were further verified. Examining the root mean square deviation, the stability of the simulated complexes was thoroughly tested during the Molecular Dynamics analysis. Our hits exhibit superior qualities to known CDK7 inhibitors, according to the comprehensive pharmacokinetic parameters that were predicted. Both ligands 7 and 14 showed good scores in pKCSM characteristics such as intestinal absorption in humans, P-glycoprotein I and II inhibitors, central nervous system permeability, blood-brain barrier permeability, etc. The results indicate that Isoindolin-1-ones and phenanthrene lactam moieties are showing anti-cancer action because they have remarkable drug-like features and could serve as effective Cyclin-Dependent kinase inhibitors 7 (CDK7).

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LIST OF ABBREVATIONS

MDS: Molecular Dynamic Simulation

MD: Molecular Dynamic

CAK: Cyclin Activating Kinase

CDK: Cyclin Dependent Kinase

Kcal/mol: Kilocalories per mole

mmff: Merck Molecular Force Field

RMSD: Root Mean Square Deviation

B.E.: Binding Energy

CDK7: Cyclin Dependent Kinase 7

pKCSM: Prediction of Kinase Specific Cyclin Dependent Kinase Substrate Motifs

CHAPTER 1

INTRODUCTION

1.1 INTRODUCTION OF CANCER

1.1.1 Basic understanding of Cancer

Uncontrolled growth and spread of cells throughout the body define the group of health issues known as cancer. Genetic alterations that interfere with the normal regulation systems for cell growth and division are the main cause of this disorder [1]. The masses of altered cells that make up tumors can be classified as benign and malignant tumors. Benign tumors do not spread to other sections of the body. Whereas, malignant tumors have the ability to grow and spread to adjacent tissues [2]. Breast cancer is the leading fatal disease in women's cancer-related fatalities. The diverse and complex biomolecular profiles of breast cancer and the development of drug resistance have reduced the therapeutic effectiveness of existing anti-breast cancer drugs. When an enzyme is hyperactive, a medicine called an inhibitor can block substrate binding or catalysis. When an enzyme is underactive, a drug called an activator can increase the activity of the active site and treat the disorder. Adopting a healthy lifestyle by reducing exposure to carcinogens, becoming immunized against cancer-causing illnesses, and going through prescribed cancer screenings are all cancer prevention techniques [3].

Cancer research is always being conducted in an effort to further our understanding of this serious condition, create better preventive plans, advance early detection techniques, and find novel treatment approaches. For a precise cancer diagnosis, course of treatment, and management, it is crucial to consult with medical professionals and specialists.

1.1.2 Impact of Cancer Worldwide

Chemotherapeutic treatments for breast cancer mainly comprise cytotoxic medications such as tamoxifen, paclitaxel, and docetaxel, but these often come with severe side effects. Every year, cancer causes a significant number of new cases and fatalities. The global cancer burden is influenced by a number of variables, including ageing of the population, changes in lifestyle, and environmental exposures. Each year, millions of individuals are thought to get a cancer diagnosis, and millions more are thought to pass away from the condition. Individuals who are diagnosed with cancer suffer an enormous amount of quality-of-life loss [4]. Surgery, chemotherapy,

radiation therapy, and targeted therapies are just a few of the cancer treatments that can have serious emotional and physical side effects. These negative effects might include of discomfort, exhaustion, nausea, hair loss, and emotional anguish. Patients and their relatives may also suffer emotionally and with a great deal of worry of recurrence [5]. Cancer has a significant effect on people, families, healthcare systems, economies, and social well-being throughout the world. Comprehensive approaches that include prevention, early detection, and continuous research to enhance outcomes and reduce the global impact are needed to address the difficulties, confronts by cancer [6].

1.1.3 Advancing Frontier in Drug Discovery

Tolerability and controllable toxicity profile of cancer, cyclin-dependent kinase inhibitors have recently emerged as a valuable therapeutic option in hormone-dependent breast cancer. Chemotherapeutic treatments for breast cancer mainly comprise cytotoxic medications such as tamoxifen, paclitaxel, and docetaxel, but these often come with severe side effects. SY-1365, a CDK7 inhibitor with an IC_{50} of 84 nM is under undergoing Phase I clinical trials, was created by Syros Pharmaceuticals [7]. Only a few CDK7 inhibitors including SY-1365 and THZ1 with good selectivity have been discovered, and they are now in different phases of clinical trials. Many studies have discovered CDK7 inhibitors thus far; nevertheless, the use of first-generation inhibitors in clinical trials has been constrained due to their significant side effect. The direct involvement of CDK7 in many malignancies has made it a desirable target for cancer treatment [8].

Several CDK7 inhibitors, including roscovitine, are now entering clinical trials, indicating the biological potential for anticancer treatment. Due to their crucial functions in regulating some critical cellular activities, kinases have become one of the most extensively researched families of therapeutic targets [9]. Comprehensive approaches that include prevention, early detection, and continuous research to enhance outcomes and reduce the global impact are needed to address the difficulties, confronts by cancer. Advancing the frontier in drug discovery is essential for developing new and effective strategies for various diseases and improving patient outcomes [10]. The field of drug discovery encompasses the identification, design, development, and optimization of novel compounds that can selectively target specific disease processes.

Target Identification and Validation:

Advancements in drug discovery include identifying and validating new therapeutic targets. This process involves understanding the involved molecular mechanisms of diseases and identifying unique molecules that can be targeted to interfere with disease action. Novel techniques, such as genomics, proteomics, and systems biology, have contributed to the identification and validation of new drug targets [10].

High-Throughput Screening:

This allows for the rapid testing of large libraries of compounds against specific drug targets. Automation and robotics have enhanced the efficiency and speed of screening processes, enabling the evaluation of thousands of compounds for their potential therapeutic activity. This strategy accelerates the identification of potential lead compounds for further optimization.

Computational Approaches:

Computational methods play a vital role in advancing drug discovery. This strategy uses computational modeling and simulations to predict the interactions between drugs and target molecules [11].

Artificial Intelligence and Machine Learning:

This technique can analyze data, including genomic and proteomic data, chemical structures, and clinical information, to identify patterns, predict drug-target interactions, and optimize drug design. These technologies enable more efficient screening of ligand libraries and the discovery of novel drug scaffolds.

Drug Repurposing:

Repurposing existing drugs can accelerate the drug discovery process by leveraging the vast knowledge of pharmacokinetics. Several computational and experimental approaches are used to identify novel solutions for approved drugs, potentially reducing costs and timelines associated with clinical development [12].

1.2 KINASES

1.2.1 Cyclin Dependent Kinases

Cyclin-Dependent Kinases (CDK) control every phase of the cell cycle. Among the transcription-associated kinases, CDK7 is a remarkable member of the CDK family, because of its unique role in cell division, regulation and transcription. The Ribo Nucleic Acid polymerase-based transcription is strictly regulated by the CDK7 [13]. In comparison to surrounding normal breast tissues, CDK7 protein and mRNA levels are increased during malignancy. CDKs are the unique target for new clinical interventions that play a crucial role in cancer growth and progression. Many CDK7 inhibitors, including flavopiridol and roscovitine, are currently undergoing clinical investigations, which disclose that the therapeutic significance of CDK7 makes it a potential target for chemotherapy treatment. The direct involvement of CDK7 in many malignancies has made it a desirable target for cancer treatment [14].

Cyclin-dependent kinases (CDKs) are cell cycle regulators whose activity relies on a specific protein partner called cyclin. All CDKs must bind to the cyclins, a non-catalytic regulatory protein component, in order to function accurately. A heterodimer CDK-Cyclin complex is produced as a result of their interaction, as it is essential to regulate cellular functions [15]. A group of 20 hetero-dimeric serine-threonine protein kinases called CDKs has been found which regulates the transcription of cells during their entire existence cycle. The lack of control over the cell cycle, which results in unregulated cell proliferation, is a well-known indicator of cancer growth and metastatic potential [16].

1.2.2 Cyclin Dependent Kinase 7 (CDK7)

Tumor cells possess intrusive, drug-resistant, metastatic and anti-apoptotic traits through an abnormal expression of cell-cycle-related proteins. The genetic disruption of the communicating pathways that control the cells' proliferation, progression of cell cycle, and apoptosis are characteristics of the disorder's beginning. Inhibition of CDK7 results in inhibiting transcription and cell proliferation, which has a specific anti-cancer effect [17]. The activity of kinases is controlled by several factors, including the systematic formation and decomposition of regulators, cyclins, hostile regulators, and, cyclin kinase inhibitors. Through its involvement in the phosphorylation of RNA polymerase II, CDK7 also contributes significantly to transcription. It forms complexes with cyclin H, forming CDK-activating-kinase (CAK), which phosphorylates other CDKs that regulate cell cycle progression [18].

To function appropriately, CDKs have to bind to a cyclin with their T-loops phosphorylated by a CDK-activating kinase (CAK). By phosphorylating the T-loop inside the activation section, CDK7 participates in cell-cycle regulation to control the activation of other CDKs [19]. A constituent of several chromatin remodelling complexes, MAT1, interacts with CDK7 (Figure 1.1) to construct a dimeric complex**.** CDK-activating kinase with extra cyclin H regulates the cell cycle by phosphorylating the T-loop CDKs [20]. This complex causes phosphorylation of the T loop, activating several CDK/Cyclin. Cell-cycle CDKs and transcriptional CDKs are the two non-overlapping subgroups of CDKs that are further separated based on their functional characteristics [21]. They play an essential role in phase separation and regulating gene transcription. Due to its unique roles in transcription regulation and cell-division control, CDK7 is a special member of the Cyclin Dependent Kinase family [22].

DNA synthesis occurred in the S phase and the onsets of mitosis are the two main phases of cell division that CDKs regulate. Due to CDK7's unique function, there is a need for research in the creation of specific inhibitors with limited success in inhibiting CDK7. CDK7 contributes to transcription as an integral part of the more significant transcription factor II H (TFIIH) complex [23].

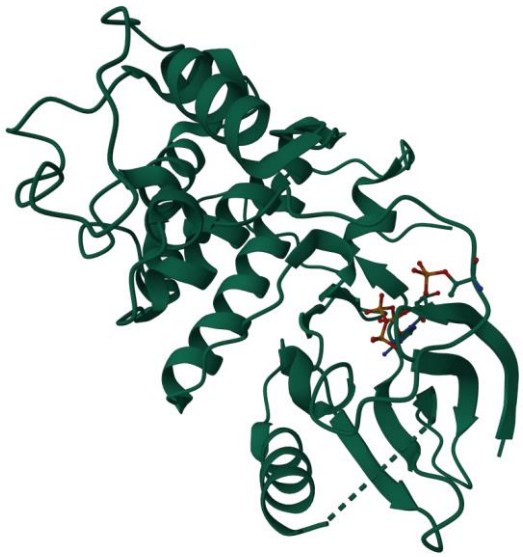


Figure1.1 Crystal Structure of Human Cdk7

1.2.3. Need of CDK7 Inhibitors

With an inhibitory half-maximal dosage (IC_{50}) of 21 nM, BS-181 is discovered as the first reversible small-molecule CDK7 inhibitor. The fundamental regulators' cyclin-dependent kinase (CDKs) recreates an essential function in the initiation and progression of cancer, making them an attractive target for cutting-edge therapeutic strategies [24]. Due to their crucial functions in regulating some critical cellular activities, kinases have become one of the most extensively researched families of therapeutic targets. Additionally, new research has shown that blocking CDK7 has anticancer benefits, stimulating the creation of innovative, more affordable CDK7 inhibitors, with improved selectivity [25].

There is just one known crystal structure of ATP-bound human CDK7 at this time (1UA2.pdb). The protein's kinase domain, which has a length of 12 to 295 amino acids, is located at both the N- and C-termini of CDK7, and Residues 90 to 170 comprise most of the protein's ATP-binding region [26]. Researchers have previously used information about CDK7's structure and function to design inhibitors that can interact with the enzyme's ATP-binding site. The structural-based method utilized THZ1-bound structure (PDB ID: 6DX3), the sole covalent inhibitor also available. Hence, a complete analysis of the molecular mechanisms behind the special recognition of CDK7 by ligands and the kinetics of ligand-receptor interactions is crucial to the sound understanding of isoform-specific inhibitors [27].

Interestingly, CDK7 and CDK2 have a 44% sequence identity with an estimated root mean square deviation of 1.25Å. Cell cycle progression and transcription may be interrupted by CDK7 inhibition [27, 28]. To be a member of the CDK family, CDK7 and CDK2 exhibit considerable structural similarity. Hence, CDK7 is an important therapeutic target in cancer studies [28].

Toxicity is often observed earlier than the desired anticancer effects, thus becoming a crucial endpoint for pharmacodynamics studies. For effective modelling for pharmacodynamics, it is crucial to have prior knowledge of the toxicity of anticancer lead molecules [29]. As a result, researchers are directing their efforts toward creating novel chemical structures with superior cytotoxic profiles, focusing on targeting specific biological targets. Additionally, the narrow therapeutic index of cytotoxic agents, which means that there is a minimal difference between the dose required for effective antitumor response and the amount that causes unacceptable toxicity, poses a major clinical issue [30]. The research demonstrates that tremendous

progress has been made in the last several years in the discovery and development of CDK inhibitors.

1.3 ISO-INDOLINONES

1.3.1 Definition and Chemical Structure

A family of chemical compounds with an indolinone core structure is known as iso-indolinone (Figure 1.2). A ketone group (C=O) is present on the five-membered ring of the indolinone scaffold, which is made up of two rings that are fused together. The isomeric character of these substances is indicated by the prefix "ISO" in ISO-indolinones [31].

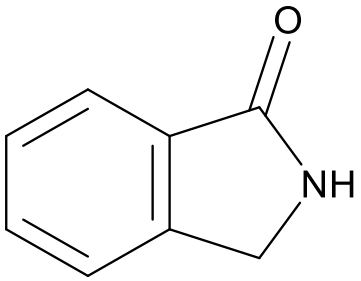


Figure 1.2 Chemical structures of iso-indolinones

1.3.2 Common Synthetic Routes

Substances found in several alkaloids, such as aristolactams, etc., and other significant natural chemicals have a fused lactam scaffold [31]. Due to these compounds' broad range of remarkable biological activities, organic chemists have pioneered various methodologies for synthesizing Isoindolin-1-ones using various chemical processes, such as base-catalyzed cyclization and transition metal-catalyzed cyclization. Iodo-cyclization of functionalized alkynes is a well-known approach for creating an extensive collection of heterocyclic molecules (Figure 1.3). It is vital to consider that attempts to prepare isoindolin-1-ones by the iodocyclization of alkynyl amides have already been made in the past [32].

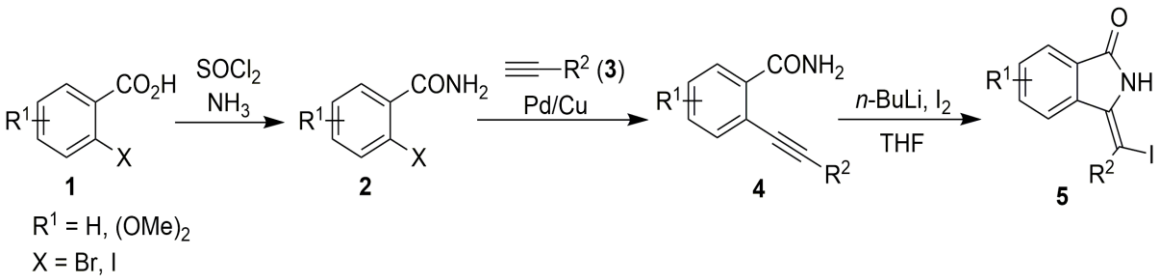


Figure 1.3 Synthetic routes of iso-indolinones [33]

1.3.3 Pharmacological Properties

Many literature studies reporting several isoindolin-1-one analogs as kinase inhibitors supported this hypothesis. Most of the iso-indolinoes in our library had kinases as their primary target family, according to the "**Swiss Target Prediction,**" which identified kinases as the significant target family. Many literature papers that list several isoindolin-1-ones analogs as kinase inhibitors validated this observation [34]. Due to their numerous biological functions and possible therapeutic applications, Iso-indolinones have generated substantial attention in medicinal chemistry. They show a variety of pharmacological characteristics, including analgesic, anti-inflammatory, anticancer, antibacterial, and neuroprotective actions.

Anticancer properties have been investigated, and several derivatives of Iso-indolinone have demonstrated astonishing results in preventing the proliferation of cancer cells and triggering apoptosis. Moreover, blocking important enzymes and signaling pathways involved in the inflammatory response, Isoindoline has shown anti-inflammatory benefits [35].

1.4 INTRODUCTION OF ARISTOLACTAMS

1.4.1 Definition and Chemical Structure

Aristolactams are important alkaloid natural products and contain a phenanthrene lactam scaffold (Figure1.4). Aristolactams are an essential sub-class of aporphinoids. These plant extracts have been used in obstetrics since old times, yet they are still used as folk medicines in some areas of India, Argentina, Turkey, and China [36]. These compounds are found in various plant families, such as Aristolochiaceae and Piperacea. A variety of natural aristolactams as well as their

synthetic derivatives have been studied and evaluated for their biological properties [37].

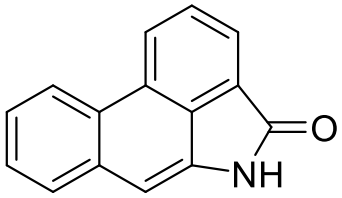


Figure 1.4 Chemical structure of phenanthrene lactam scaffolds

1.4.2 Common Synthetic Routes

The important synthetic methods include –

- Suzuki-Miyaura coupling/Aldol condensation reactions
- C-H bond activation
- Dehydro-Diels-Alder reaction
- Radical cyclization, *etc.*

Diversification has been carried out on phenanthrene lactam skeleton to get an excellent leading drug development from natural products [38].

1.4.3 Pharmacological Properties

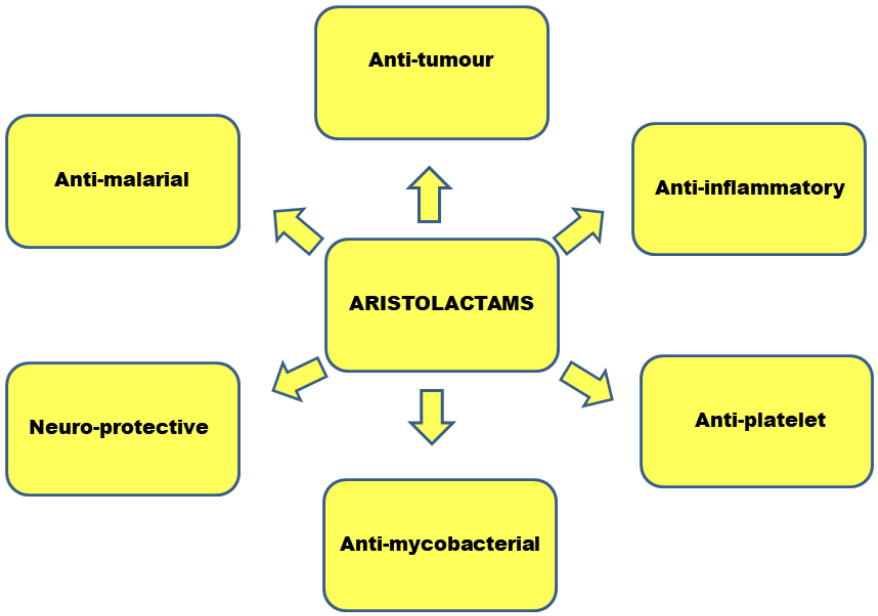


Figure 1.6 Pharmacological Properties of Aristolactams

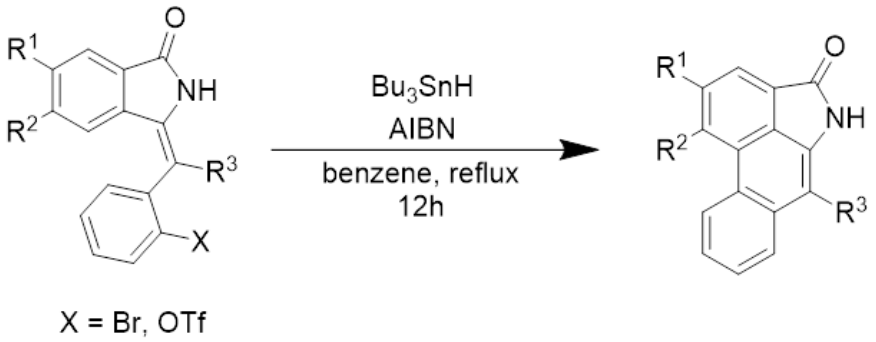


Figure 1.5 Free radical cyclization reactions to synthesize aristolactams [32]

1.5 MOLECULAR DOCKING

A simple, effective, and rapid molecular modelling technique called molecular docking predicts a ligand's active conformation in the active site of its target protein and utilizes a docking score to calculate the binding energy [39]. The term "hinge region residue" in biochemistry and molecular biology refers to a particular amino acid residue or a group of residues situated in the flexible hinge region of a protein. For instance, in enzymes, substrate binding causes a conformational change in the hinge region, allowing the active site residues to be positioned for catalysis [40]. The expression "active site" is used in bioinformatics to describe a particular area of a protein where a substrate molecule interacts and performs a chemical reaction that is catalyzed by the protein. The active site is often a tiny, well-defined area inside the broader protein structure, and it comprises amino acid residues that are specially organized to assist the chemical interaction [41]. The active site of a protein can bind to particular substrates and catalyse particular chemical processes because of the precise arrangement of amino acid residues there.

1.6 MOLECULAR DYNAMIC SIMULATIONS

Molecular Dynamics (MD) simulations handle the ligand-receptor combination flexibly and have typically been used to post-process docking data to accurately represent the dynamical behaviour of the complex at the atomic level [42]. The Groningen Machine for Chemical Simulations (GROMACS v5.1.5) was used to perform molecular dynamics (MD) simulations to better comprehend how proteins and possible substances interact in physiological circumstances [43].

CHAPTER 2

EXPERIMENTAL

2.1 PREPARATION OF VIRTUAL LIBRARY

In silico evaluation of the isoindolin-1-ones and Phenanthrene lactam scaffolds are done using cheminformatics tools to comprehend the bioactivity of the virtually synthesized library of 48 moieties. Developing a variable library of multi-substituted isoindolin-1-ones and Phenanthrene lactam scaffolds and evaluating their biological profile, particularly their anticancer properties using cheminformatics tools.

PerkinElmer ChemDraw Professional version 19.0.0.22 was employed to create two-dimensional molecular structures and SMILES notations for the Isoindolin-1-ones, its substituents, and aristolactams. Isoindolin-1-ones and phenanthrene lactam scaffolds are generated virtually by studying the literature and analyzing the biological importance of isoindolin-1-ones and phenanthrene lactam scaffolds. For performing these Experiments using computational methods, SMILES notations have been converted to three-dimensional structures using Open Bable software.

2.2 VIRTUAL SCREENING USING PyRx

The crystal structure of the protein kinase 7 associated with cell division in humans (PDB code: 1UA2) has been downloaded (www.rcsb.org). The protein structure prepared while preserving the protein's existing hydrogen. The current research focuses on molecular docking studies to determine how enzymes and ligands interact.

In the ongoing study, CDK7 (1UA2.pdb) has been docked with active Isoindolin-1-ones, its substituents, and aristolactams using Pyrx virtual screening tool, in which AUTODOCK VINA is the software used for docking. PyRx is a high-throughput screening program that can be used in computational drug development to assess libraries of compounds against potential therapeutic targets [44]. The energy of all the ligands has minimized in the PyRx software itself using mmff94 force-field, followed by the conversion of compounds to auto dock ligands (.pdbqt) for docking. Studies involving molecular docking are highly helpful in understanding how an enzyme and a ligand interact [45].

2.2.1 Analysing Binding Energy

The Ligand 1-48 for CDK7 calculated binding energies ranging from -6.8 to -10.1 kcal/mol, evidencing the preferential binding towards CDK7. According to the docking experiment data, ligand 7 has the lowest binding energy, strong interactions,

& forms stable complex with CDK7 compared to known inhibitors, i.e., Ligand 51, 52&50. Hence, it is indicated by investigations utilizing molecular docking and molecular dynamics simulations that the Ligand (7) with CDK7 has a strong and stable hydrogen bonding interaction, indicating that it is a promising therapeutic candidate to inhibit CDK7. Moreover, ligand 14 also has the lowest binding energy (-9.3 kcal/mol), strong hydrogen bonding interactions, and forms a stable complex with CDK7 compared to other known inhibitors, i.e., ligands 51 and 52.

2.3 STUDY OF HYDROGEN BONDING INTERACTION

2.3.1 Biovia Discovery Studio Visualizer

The hydrogen bonding interactions in the docked complexes of CDK7 (1UA2.pdb) and active ligands were analysed using BIOVIA Discovery Studio Visualizer**.** The generated compounds then docked into the receptor's active site. We then examined interactions between the designed molecules and H-bonds, Pi-stacks, hydrophobic molecules, and van der Waals forces [46].

We have identified 20 active amino acid protein targets. We can design better inhibitors by analysing the docking experiments and identifying the crucial amino acid residues involved in ligand binding.

2.4 MOLECULAR DYNAMIC SIMULATIONS

In order to understand how atoms and molecules behave and interact over time, scientists utilize programming methodology called molecular dynamics (MD) simulations. The CHARMM36-jul2021 force field was used in GROMACS and SwissParam to produce the parameters. The mobility of the molecules is described in an MD simulation using any classical or quantum mechanical equations of motion [47]. The equilibration process was performed under NVT and NPT ensembles, using a V-rescale thermostat & Parrinello-Rahman barostat. Using BIOVIA Discovery Studio and visual molecular dynamics (VMD), the MD simulation trajectory is analysed. These equations may be numerically solved to simulate the system's behaviour over time and provide information about the physical and chemical characteristics of the system. For 50 ns, all simulations were done using periodic boundary conditions [48].

2.5 PHARMACOKINETICS CHARACTERISTICS

The pharmacokinetic (PK) characteristics were studied. We contend that our identified hits will aid the newly developed CDK7 medicines. An essential stage in the drug development process to find new inhibitors is to predict the pharmacokinetic characteristics [49]. For additional optimisation of chosen hit as a successful lead compound, a thorough prediction of the Pharmacokinetics characteristics is significant [50]. As a result, PK characteristics were predicted in the current investigation utilizing an open webserver called pkCSM .In order to evaluate the attributes of the chosen hits, PerkinElmer ChemDraw Professional version 19.0.0.22 was utilized (http://biosig.unimelb.edu.au/pkcsm/)(viewed on March 2, 2023).

CHAPTER 3

RESULTS AND DISCUSSION

3.1 OBSERVATION AND DISCUSSIONS

The results indicate that the Isoindolinones moieties are crucial for anticancer action because they have remarkable drug-like features and could serve as effective cyclin-dependent kinase inhibitors 7. For observing the interactions, the ligand conformation with the most favourable binding interactions and the docking score were taken into account [49]. These docked complexes exhibited negative binding energies, indicating the structure's stability. Out of 48 ligands, 41 ligands exhibited Hydrogen Bonding Interactions. We have determined the essential interactions that impact the predicted binding affinity, i.e., hydrogen bonding interactions among hydrophobic interactions and electrostatic interactions (Figure 3.1-3.13) [51].

The Ligand 1-48 for CDK7 calculated binding energies ranging from -6.8 to -10.1 kcal/mol, evidencing the preferential binding towards CDK7. Few results of molecular docking between protein ligand are displayed (Figure 3.1 - 3.13). According to the docking experiment data, one ligand has the lowest binding energy that is -10.1 Kcal/mol. Hence, it is indicated by investigations utilizing molecular docking and molecular dynamics simulations that the isoindolinones with CDK7 has a strong and stable hydrogen bonding interaction, indicating that it is a promising therapeutic candidate to inhibit CDK7. Moreover, other ligand also has the lowest binding energy (-9.3 kcal/mol), strong hydrogen bonding interactions, and forms a stable complex with CDK7 compared to other known inhibitors, i.e., ligands 51 and 52. Hence, results showed that the generated isoindolinones had an excellent binding affinity for CDK7 (Figure 3.1-3.13) [52].

We can design better inhibitors by analysing the docking experiments and identifying the crucial amino acid residues involved in ligand binding. Ligands 1, 3, 4, 5, 8, 10, and 11 exhibited a good binding affinity with CDK7, forming three hydrogen bonds with LYS139, ASN142, and ASN141. Ligands 2, 23, 24, 26, 28, 29, 34, 35, 36, 39, 40, 41, 42, 43, 44, and 48 exhibited a good binding affinity with CDK7, forming one hydrogen bond with MET94. The ligands 6, 12, 20, 30, 38, and 45 did not exhibit good binding affinity with CDK7 because none form hydrogen bonds. The Ligand 46 did not exhibit an excellent binding affinity with CDK7 because of the formation of unfavourable donor bonds. Four hydrogen bond interactions have formed with the target protein's amino acid residues LYS139, ASN141, ASN142, and MET94 in the ligand 7's generated docking pose. Ligand 14 also exhibited an excellent binding

affinity (-9.3 kcal/mol) with CDK7, forming three hydrogen bonds with the GLN22, LYS41 & SER161. Only two ligands exhibited hydrogen bonding with SER161.

Covalent inhibitors are a specific class of enzyme inhibitors that create a covalent binding to the target enzyme, rendering it permanently inactive [53]. In contrast, non-covalent inhibitors can disassociate from the enzyme after reversibly binding. The advantages of covalent inhibitors versus non-covalent inhibitors are numerous [54]. As a result, they are particularly advantageous for targeting high-turnover enzymes that can quickly take the place of non-covalently bound inhibitors. Due to the covalent bond's ability to precisely target an enzyme's active site or specific amino acid residue, covalent inhibitors are additionally very selective [55]. Covalent inhibitors, however, are also susceptible to specific adverse effects. Covalent inhibitors must be carefully designed and optimized to reduce these hazards and increase their therapeutic potential [56].

In this particular investigation, two Kinase bound inhibitor complexes were simulated independently. An illustration of hydrogen bonds between particular protein residues and ligand molecules over time is called a hydrogen bonding graph (figure 3.16) [57]. The hydrogen bonding graph displays the H-bonds formed between particular residues in the protein and ligand molecule over time. The thickness or colour of the edges can be used to determine the strength or occupancy of the hydrogen bonds [58]. The backbone RMSD of the ligand docked with CDK7 was investigated to comprehend the stability of complexes (figure 3.15). The RMSD of the protein-ligand complex were investigated to study the stability of complex (figure 3.14). The resolution of experimental structure, size and flexibility of the Ligand, and the kind of interaction between the protein and Ligand can all affect the typical value of RMSD for a protein-ligand complex [59].

Generally, a low RMSD value indicates a good fit between the protein and the Ligand [60]. In the case of a protein-ligand complex, RMSD is determined by superimposing the protein structures in both conformations and comparing the locations of the Ligand's atoms in its bound conformation to the positions of the same atoms in its unbound conformation. We have obtained the backbone RMSD of protein-ligand (7) complex in the range of 0.2 to 0.6 nm for 50ns. For protein –Ligand (14) complex, the backbone RMSD ranges between 0.2 to 0.5 nm for 50ns. We have obtained the backbone RMSD of Ligand (7) in the range of 0.5 to 2.5 nm for 50ns. For ligand (14), the backbone RMSD ranges between 0.2 to 1.0 nm for 50ns. It is

significant to highlight those additional criteria, including shape complementarity, electrostatic interactions, and hydrogen bonding, must be considered to assess the strength of a protein-ligand complex in addition to RMSD [61].

A computational approach called pKCSM (Prediction of Kinase-specific Cyclin-dependent Kinase Substrate Motifs) forecasts the potential phosphorylation sites of kinase-specific substrates. According to the threshold and expected values available - https://biosig.lab.uq.edu.au/pkcsm/theory , the output data were evaluated for two ligands (7 & 14). According to PK characteristics results analysis, ligands 14 & 7 can be potential CDk7 inhibitors. Both ligands showed good scores in pKCSM characteristics such as human intestinal absorption, P-glycoprotein I, and II inhibitors, central nervous system permeability, and blood-brain barrier permeability The Pharmacokinetics Characteristics of one ligand is shown in the Table 3.1. [62].

As we know, cytotoxic agents' toxicity is prioritized over the selected scaffold's anticancer activity. We have conducted the PK analysis to check the little difference between the toxicity and therapeutic effects of the desired drug. We can find the potential anticancer drug molecule with the fewest side effects and the known difference between toxicity concentrations and therapeutic effects.

Even if a hypothesized substrate motif gets a high score, it may not be physiologically relevant. Ultimately, it is crucial to evaluate other aspects, such as the conservation of the pattern across species, experimental evidence of phosphorylation at the motif, and potential functional repercussions of phosphorylation [63]. Experimentally confirming pKCSM predictions is crucial. In vitro phosphorylation tests, mutagenesis studies, and in vivo phosphorylation level measurements are all examples of experimental validation [64]. Before moving a medication candidate to clinical trials, testing the predictions using experimental techniques is crucial [65]. In conclusion, pKCSM can be a helpful tool for identifying probable substrate sites for a kinase of interest, but the findings should be carefully understood and experimentally verified [66].

Binding Energy = **-9.4 Kcal/mol**

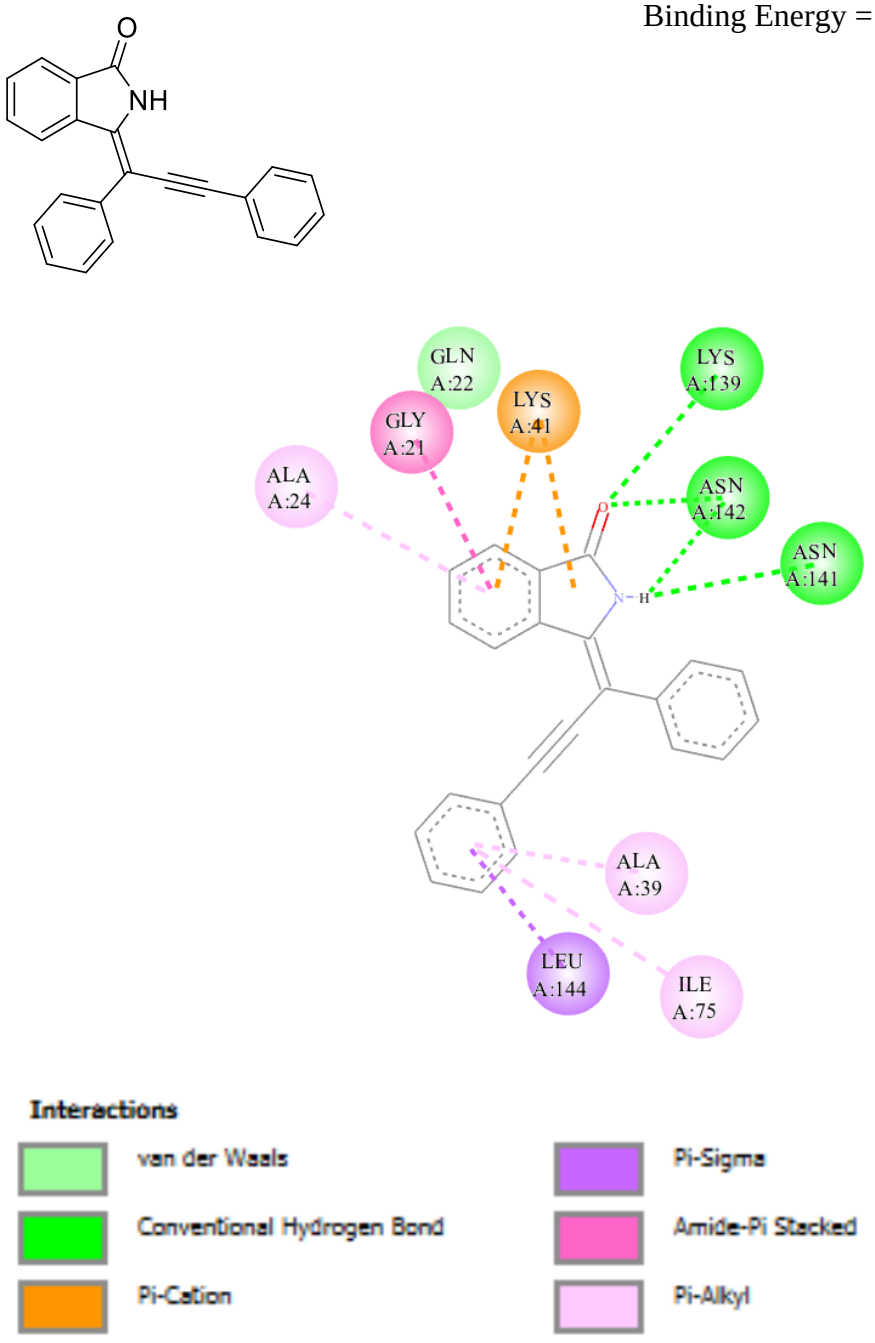
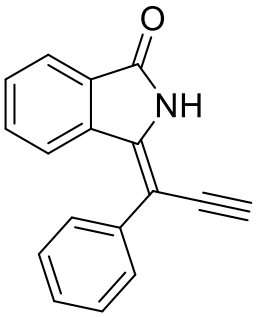


Figure 3.1 Hydrogen Bonding Interactions in (Z)-3-(1, 3-diphenyl prop-2-yn-1-ylidene) Isoindolin-1-one

Binding Energy = **-8.3 Kcal/mol**



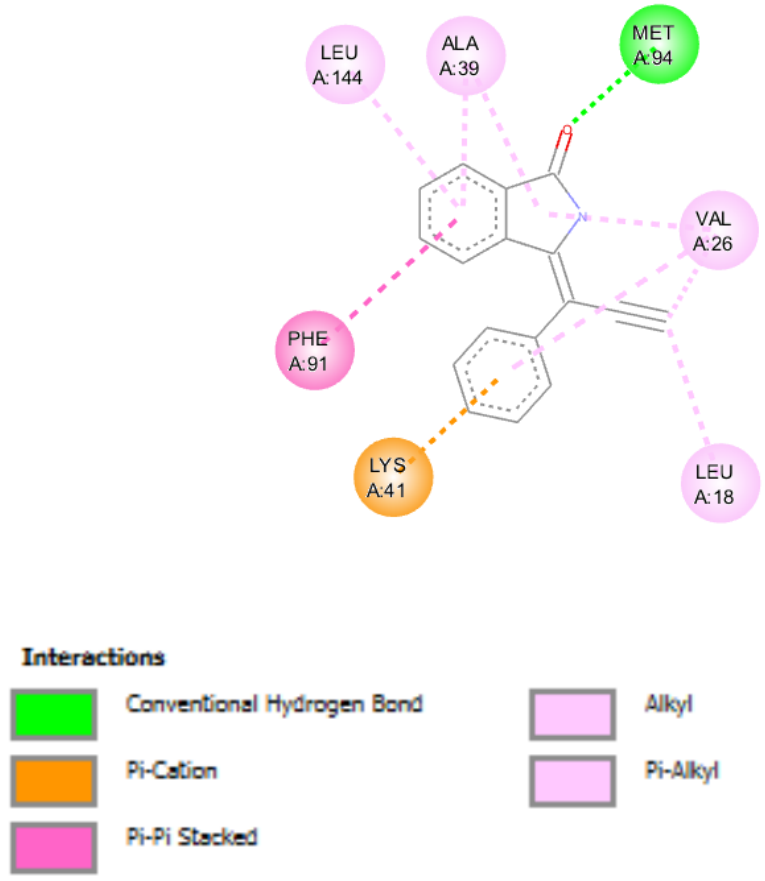
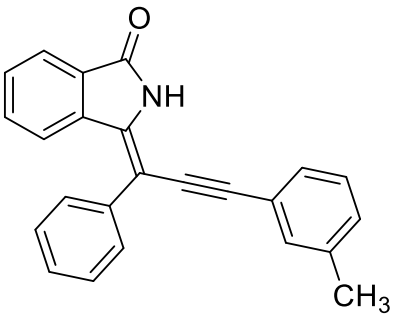


Figure 3.2 Hydrogen Bonding Interactions in (E)-3-(1-phenylprop-2-yn-1-ylidene) Isoindolin-1-one



Binding Energy = **-9.7 Kcal/mol**

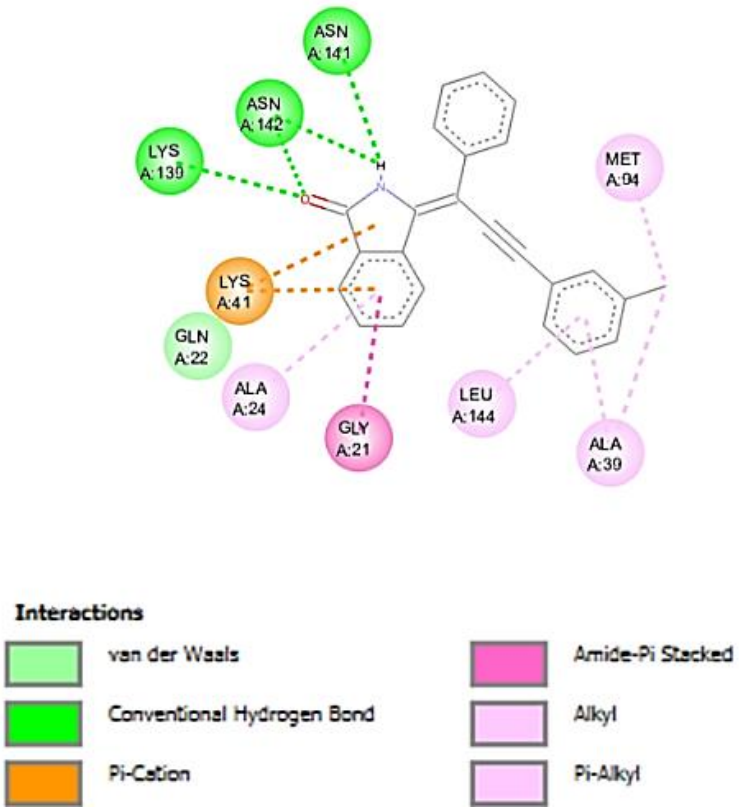
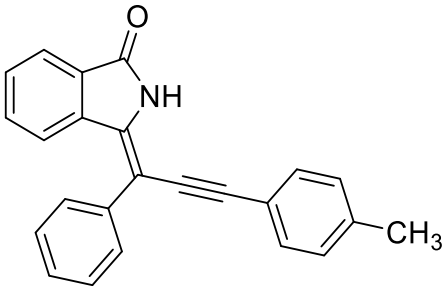


Figure 3.3 Hydrogen Bonding Interactions in (Z)-3-(1-Phenyl-3-(m-tolyl) Prop-2-yn-1-ylidene) Isoindolin-1-one



Binding Energy = **-9.5 Kcal/mol**

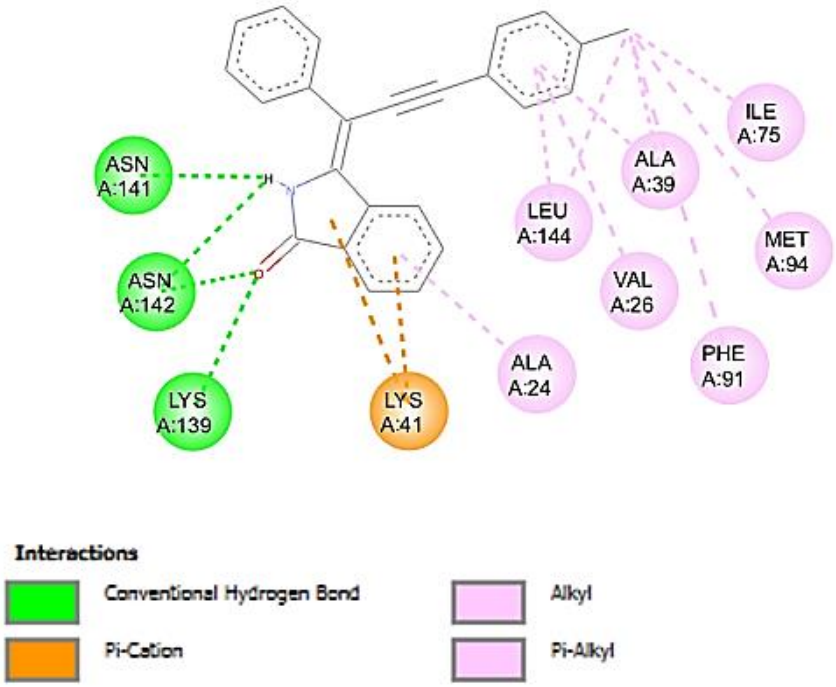
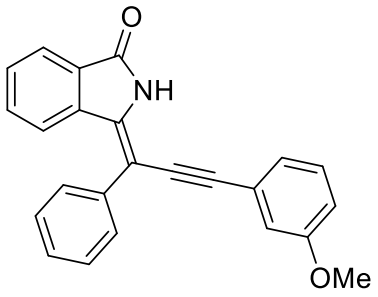


Figure 3.4 Hydrogen bonding interactions in (Z)-3-(1-phenyl-3-(p-tolyl) prop-2-yn-1-ylidene) isoindolin-1-one



Binding Energy = **-9.3 Kcal/mol**

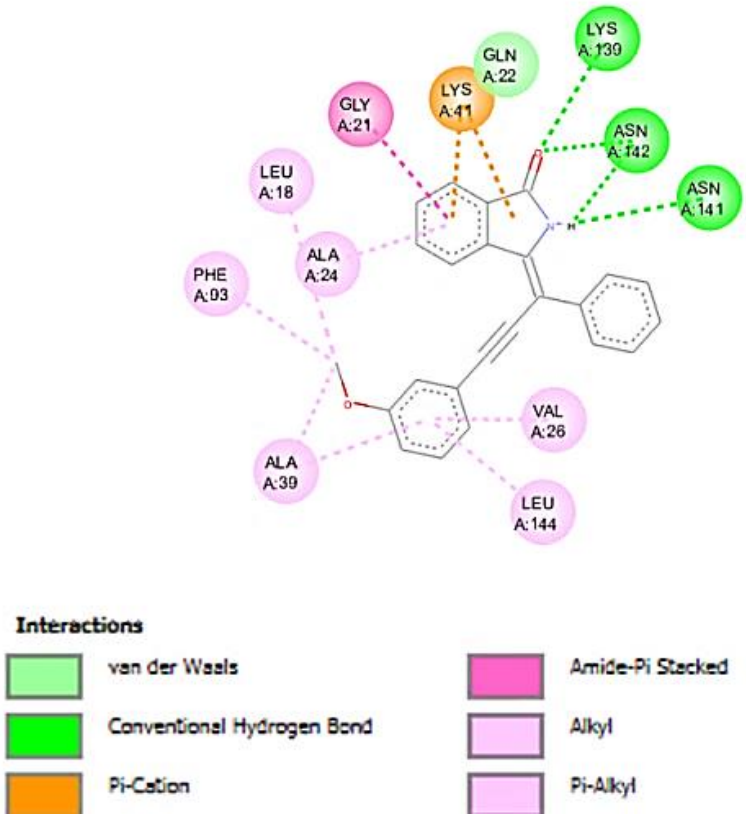
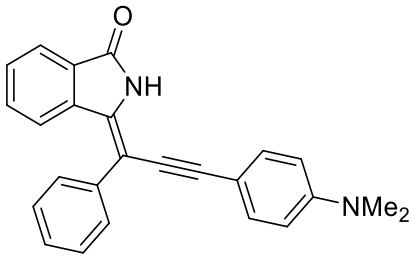


Figure 3.5 Hydrogen bonding interactions in (Z)-3-(3-(3-methoxyphenyl)-1-phenylprop-2-yn-1-ylidene) isoindolin-1-one

Binding Energy = **-9.8 Kcal/mol**



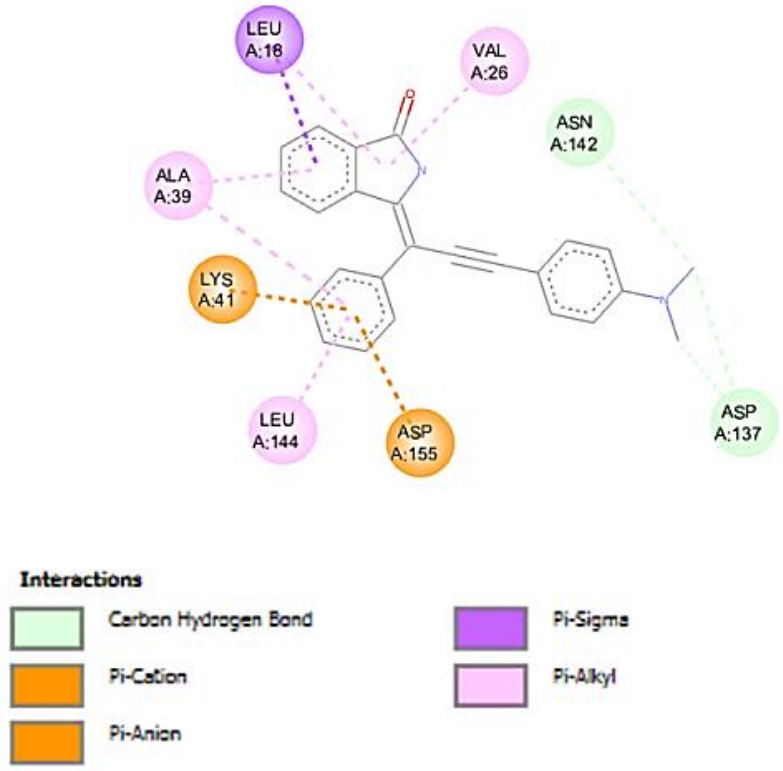
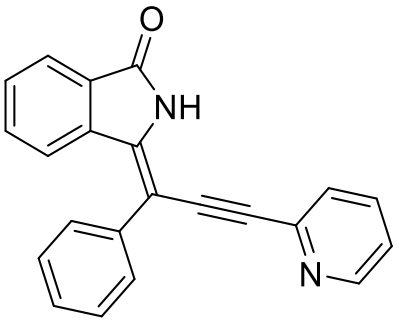


Figure 3.6 Hydrogen bonding interactions in (Z)-3-(3-(4-(dimethylamino)phenyl)-1-phenylprop-2-yn-1-ylidene)isoindolin-1-one



Binding Energy = **-9.4 Kcal/mol**

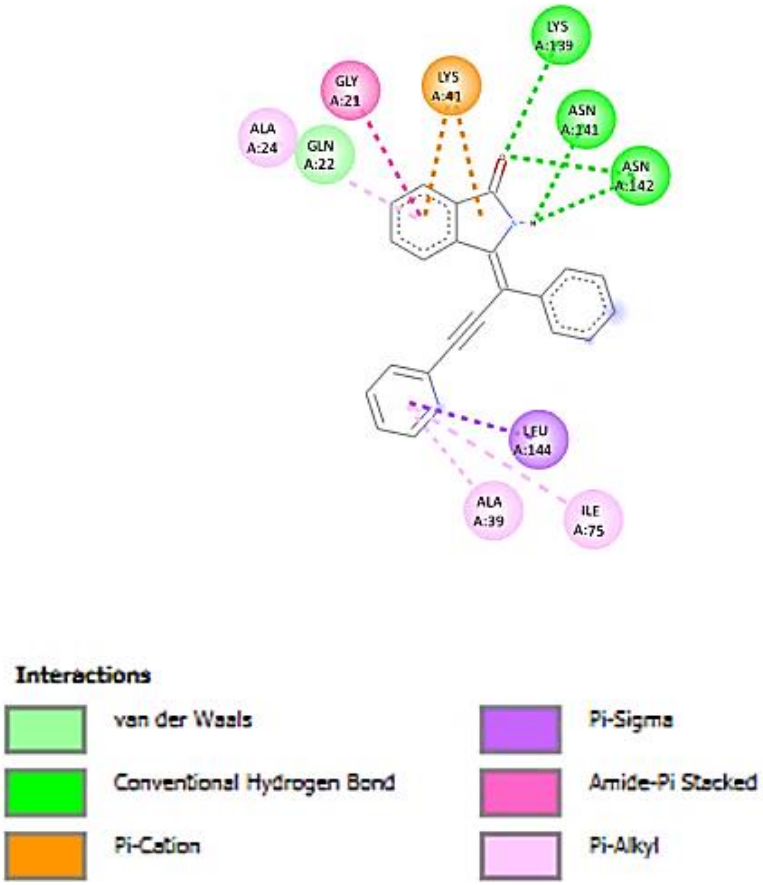
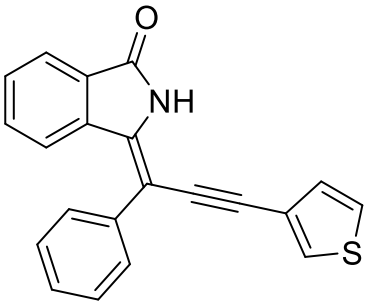


Figure 3.7 Hydrogen bonding interactions in (Z)-3-(1-phenyl-3-(pyridin-2-yl) prop-2-yn-1-ylidene)isoindolin-1-one



Binding Energy = **-8.5 Kcal/mol**

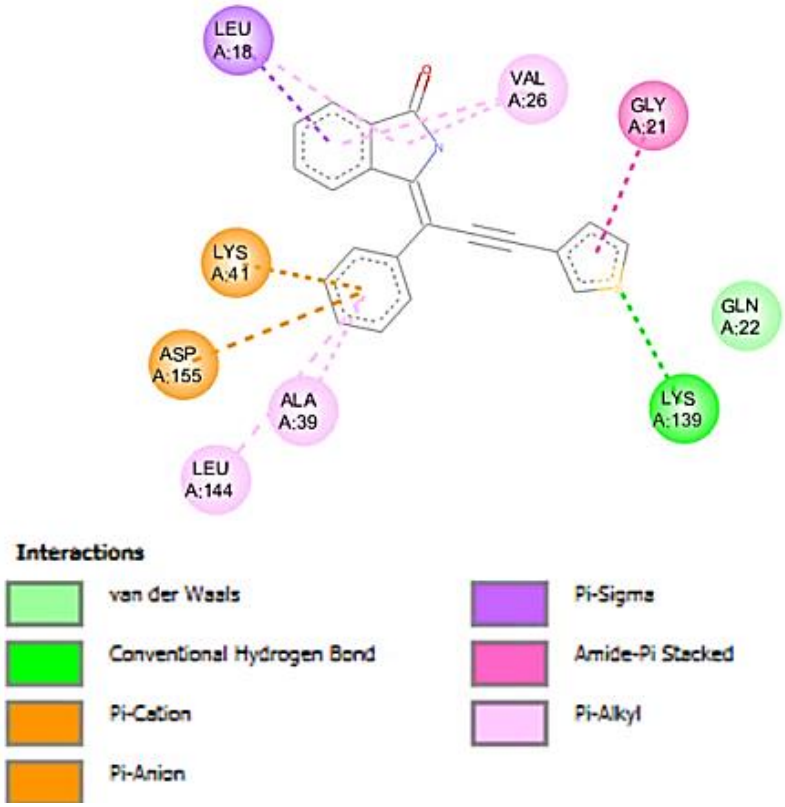
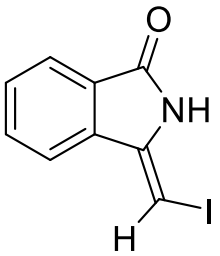


Figure 3.8 Hydrogen bonding interactions in (Z)-3-(1-phenyl-3-(thiophen-3-yl) prop-2-yn-1-ylidene) isoindolin-1-one



Binding Energy = **-6.8 Kcal/mol**

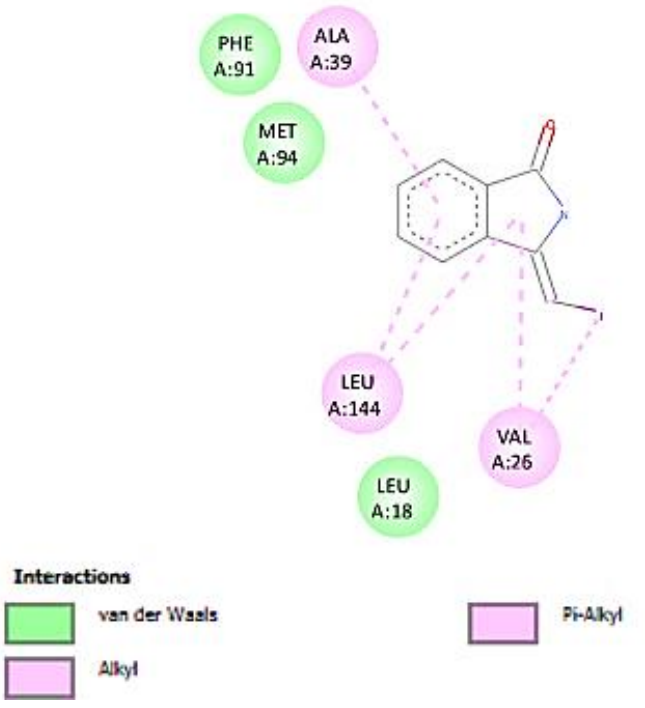
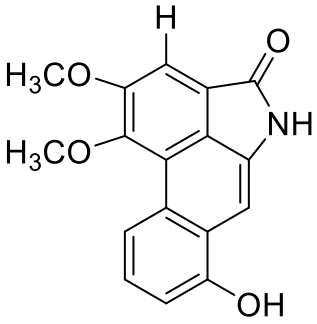


Figure 3.9 Hydrogen bonding interactions in (Z)-3-(iodomethylene) isoindolin-1-one



Binding Energy = **-9.0 Kcal/mol**

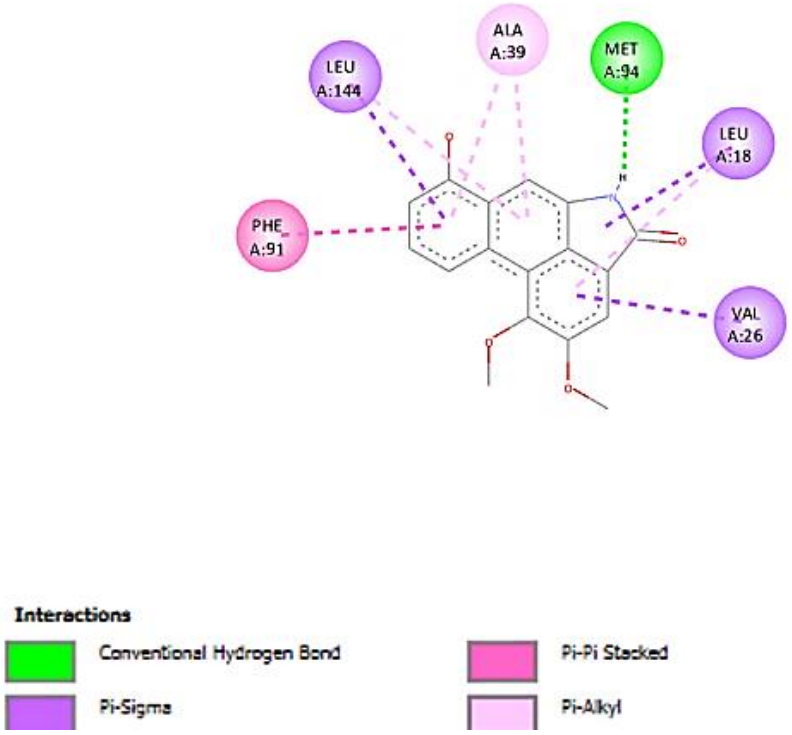
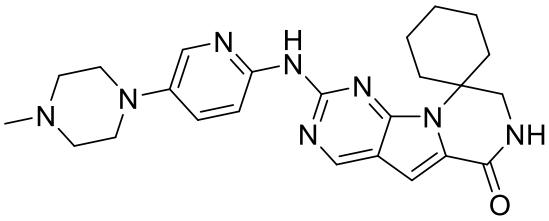


Figure 3.10 Hydrogen bonding interactions in 7-hydroxy-1,2-dimethoxy dibenzo[cd,f]indol-4(5h)-one



Binding Energy = **-10.9 Kcal/mol**

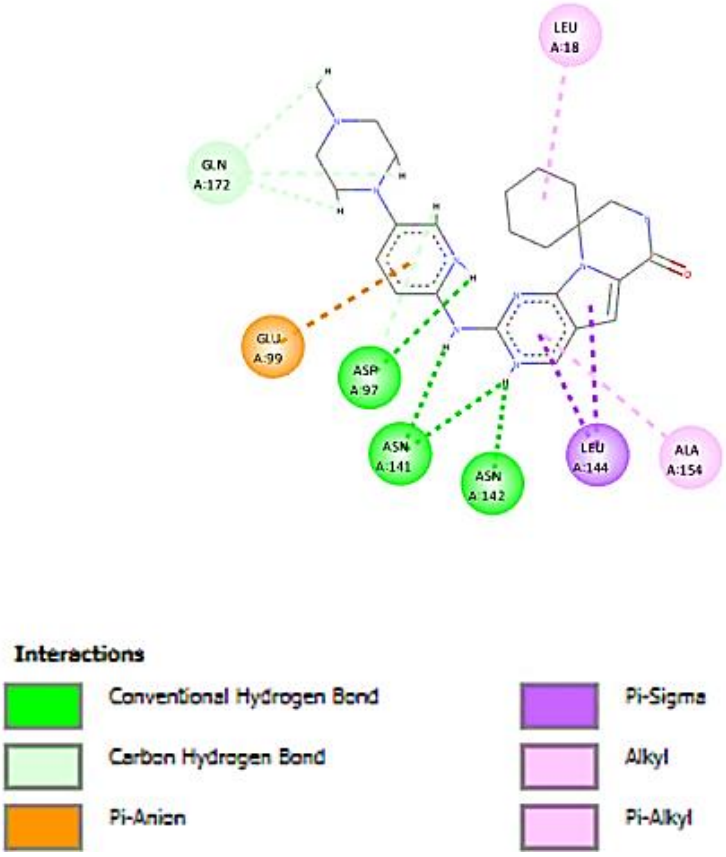
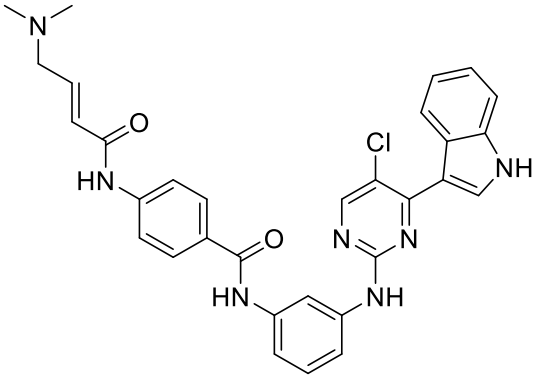


Figure 3.11 Hydrogen bonding interactions in trilaciclib (known drug)



Binding Energy = **-9.5 Kcal/mol**

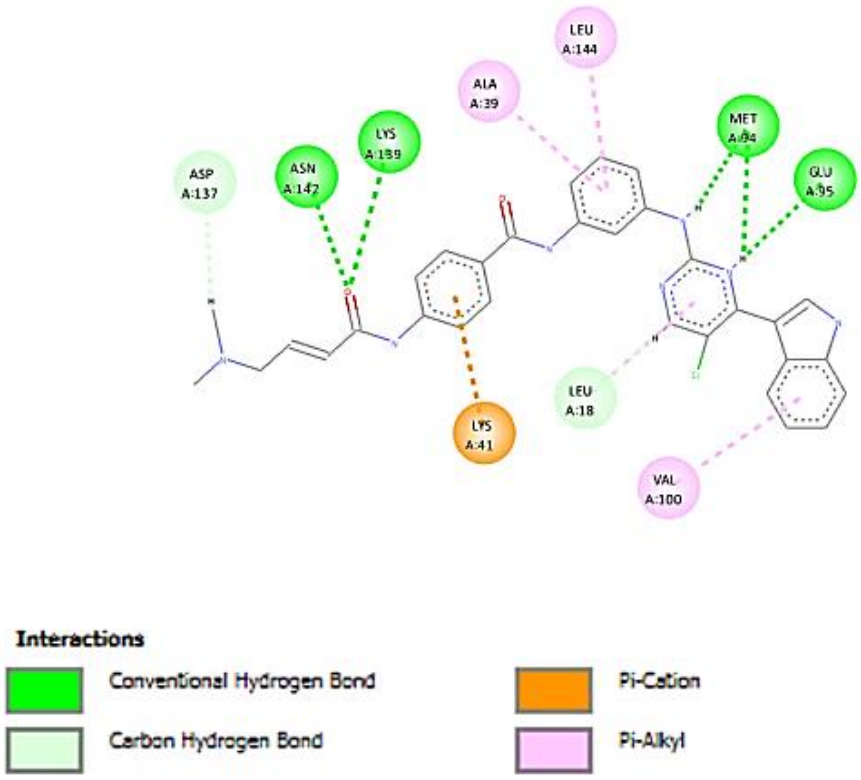
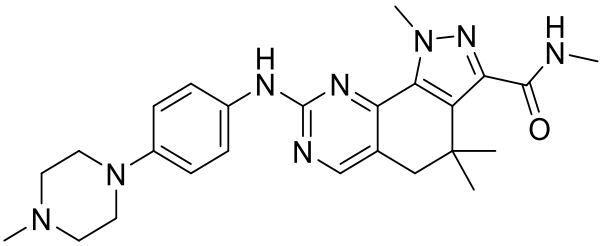


Figure 3.12 Hydrogen bonding interactions in THZ1 (known inhibitor)



Binding Energy = **-9.1 Kcal/mol**

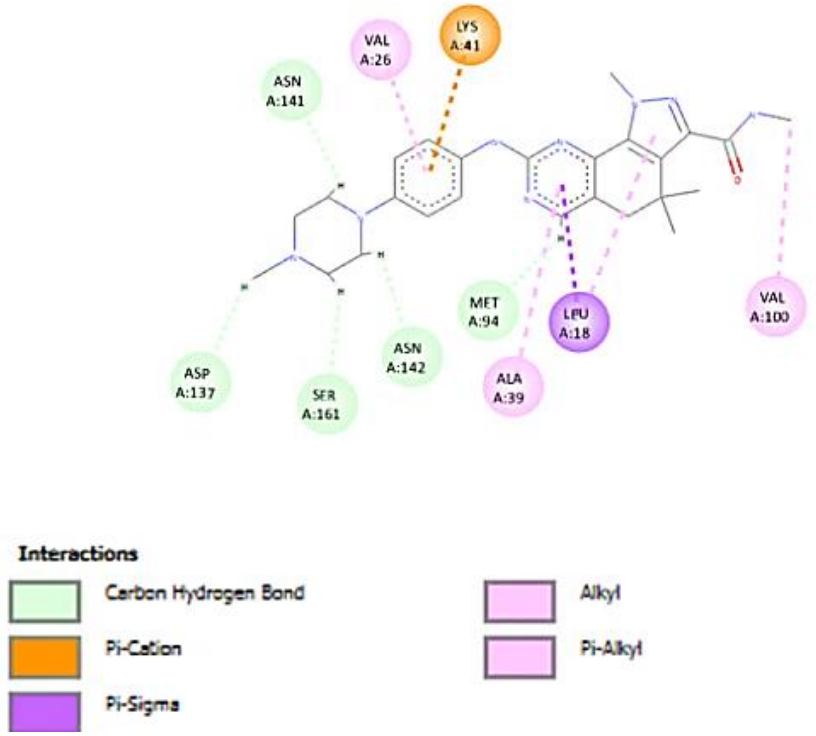


Figure 3.13 Hydrogen bonding interactions in Milciclib (known inhibitor)

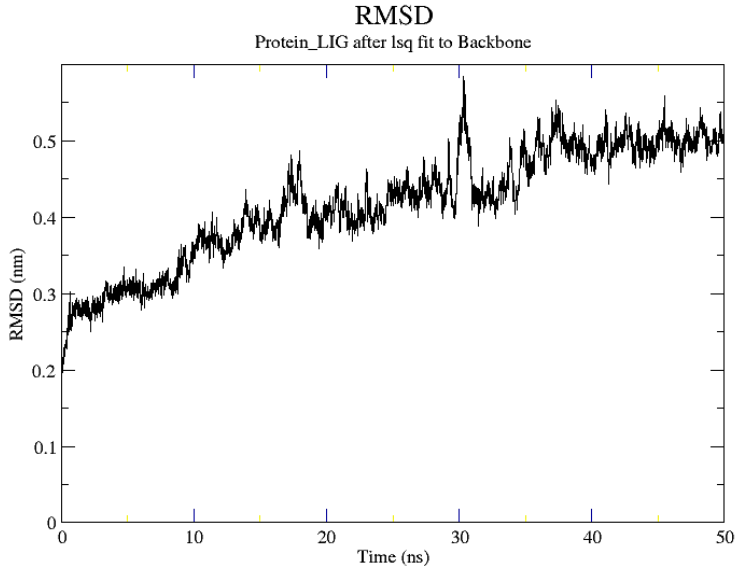


Figure 3.14 Root mean square deviation of protein ligand complex over 50 nanoseconds

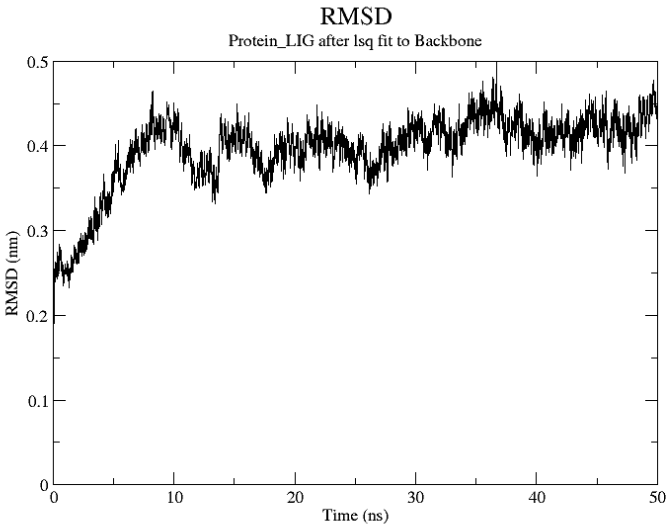


Figure 3.15 Root mean square deviation of protein ligand complex over 50 nanoseconds

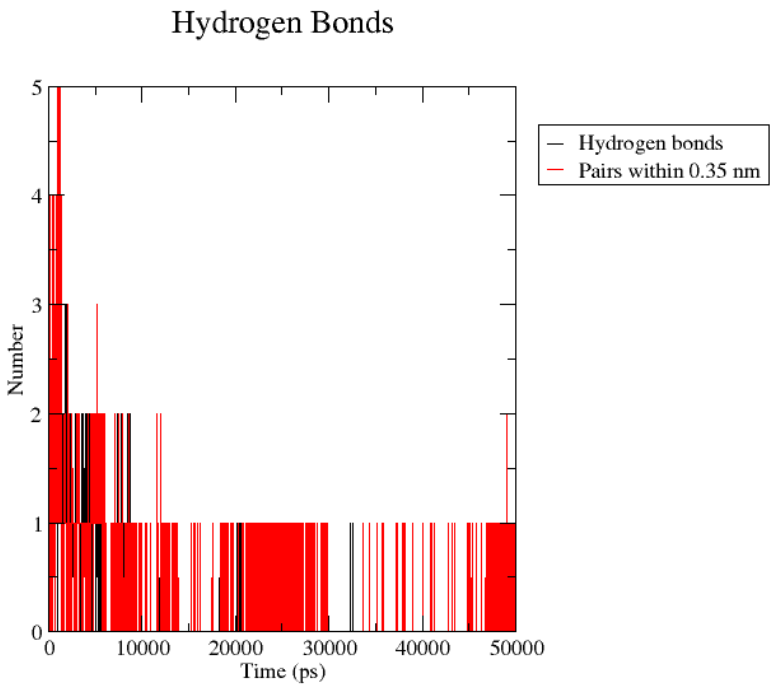


Figure 3.16 Hydrogen bonding graph – hydrogen bonds formed between specific residues in protein and ligand over 50 nanoseconds

Table 3.1 Pharmacokinetics Characteristics

Molecular Weight	**355.824g/mol**
Log P	**5.0034**
Rotatable Bonds	**1**
Acceptors	**1**
Donors	**1**
Surface Area	**156.689**
Water solubility(log mol/L)	**-4.346**
$CaCO_2$ permeability(log Papp in 10^{-6} cm/s)	**0.98**
Intestinal absorption (human)	**94.518%**
Skin Permeability(log Kp)	**-2.76**
P-glycoprotein substrate	**Yes**
P-glycoprotein I inhibitor	**Yes**
P-glycoprotein II inhibitor	**Yes**
VDss (human) (log L/kg)	**0.23**
Fraction unbound (human)	**0.103**
BBB permeability(log BB)	**0.582**
CNS permeability(log PS)	**-0.175**
CYP2D6 substrate	**No**
CYP3A4 substrate	**Yes**
CYP1A2 inhibitor	**Yes**
CYP2C19 inhibitor	**Yes**
CYP2C9 inhibitor	**No**
CYP2D6 Inhibitor	**Yes**
CYP3A4 Inhibitor	**Yes**
Total Clearance (log ml/min/kg)	**-0.047**
Renal OCT2 substrate	**No**
AMES toxicity	**Yes**
Max. tolerated dose (human)(log mg/kg/day)	**0.543**
hERG I inhibitor	**No**
hERG II inhibitor	**Yes**
Oral rat acute toxicity (LD50)	**2.813 mol/kg**
Oral rat chronic toxicity (log mg/kg_bw/day)	**1.602**
Hepatotoxicity	**Yes**
Skin sensitization	**No**
T.Pyriformis toxicity	**0.314 (log ug/L)**
Minnow Toxicity (log mM)	**-0.193**

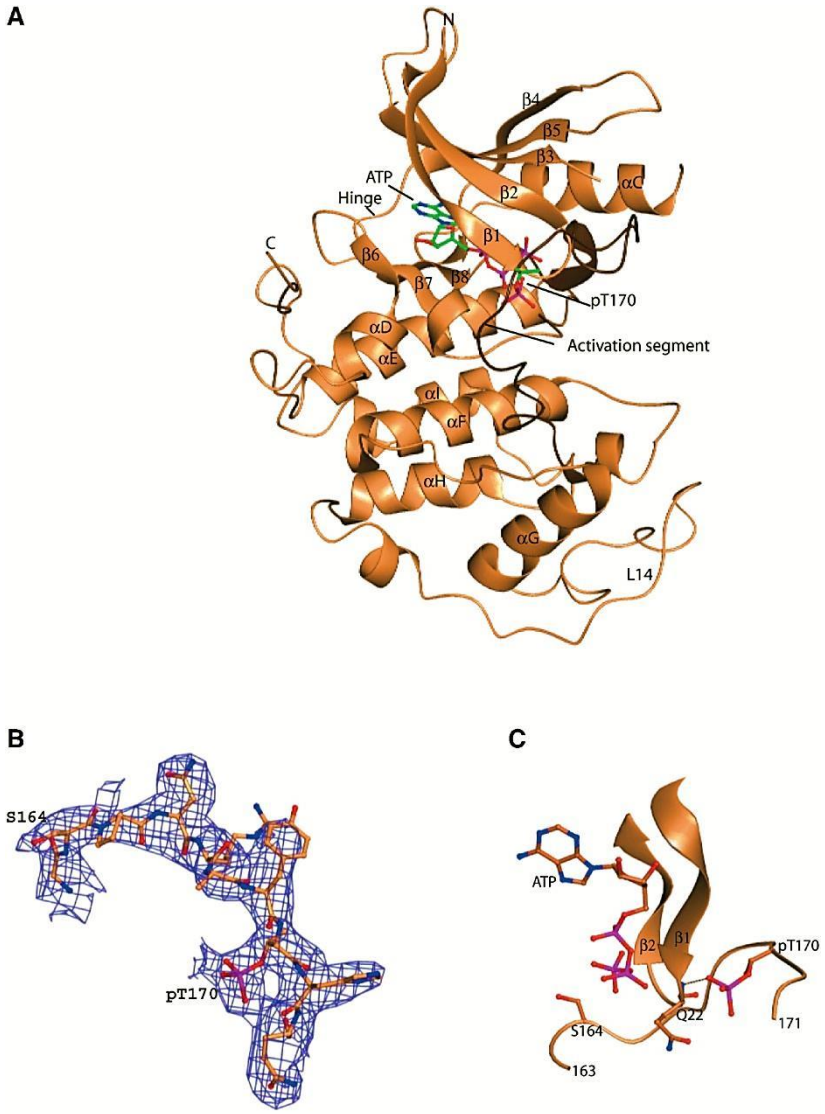


Figure 3.17 The Structure of CDK7/ATP Complex [41]

(A) A schematic diagram of CDK7 with ATP.

(B) The activation segment is phosphorylated on Thr170 and not on Ser164.

(C) The activation segment of CDK7 (residues 163–171).

CHAPTER 4

CONCLUSIONS

Virtual construction of the medicinally relevant scaffold of isoindolin-1-ones and aristolactams was done. According to docking studies, library members may be potential kinase drug target inhibitors. Isoindolin-1-ones, can suppress CDK7 via strong hydrogen bonding interactions, according to molecular docking studies. This research has provided fresh opportunities to create more specific CDK7 inhibitors in the future and may help develop more potent and widely available anti-cancer treatments. In our lab, more enzymatic research is being conducted to support these early findings. Hence, the inhibitors that may specifically block CDK7 are crucial. According to molecular docking analysis, simulation studies, and PK characteristics, two ligands can be potential CDK7 inhibitors. Research is always needed to identify efficient inhibitors that can limit overexpression and counteract the emergence of cancer resistance since CDK7 is so crucial for cell proliferation and transcription. In order to effectively target CDK7 inhibition, we have done a structure-based pharmacophore modelling technique with several additional computational tools. Computational evaluation of unique analogs and conducting more research to increase bioactivity will be undertaken, and results will be communicated as soon as possible.

REFERENCES

1. el Saghir, N. S., Khalil, M. K., Eid, T., el Kinge, A. R., Charafeddine, M., Geara, F., Seoud, M., & Shamseddine, A. I. (**2007**). Trends in epidemiology and Management of Breast Cancer in developing Arab countries: A literature and registry analysis. International Journal of Surgery 5(4), 225–233.
2. Bansode P, Anantacharya R, Dhanavade M, Kamble S, Barale S, Sonawane K, Satyanarayan ND, Rashinkar G, (**2019**), Evaluation of drug candidature: In silico ADMET, binding interactions with CDK7 and normal cell line studies of potentially
3. Chatelut, E., Delord, J. P., & Canal, P. (**2003**). Toxicity patterns of cytotoxic drugs. *Investigational New Drugs*, *21*(2), 141–148.
4. Cui, W., Aouidate, A., Wang, S., Yu, Q., Li, Y., & Yuan, S. (**2020**). Discovering Anti-Cancer Drugs via Computational Methods. *Frontiers in Pharmacology* (Vol. 11). Frontiers Media S.A.
5. Bansode, P., Jadhav, J., Kurane, R., Choudhari, P., Bhatia, M., Khanapure, S., Salunkhe, R., & Rashinkar, G. (**2016**). Potentially antibreast cancer enamidines: Via azide-alkyne-amine coupling and their molecular docking studies. *RSC Advances*, *6*(93), 90597–90606.
6. Ajit Kumar Saxena, G. C. (**2014**). Targetting Cdks in Cancer: An Overview and New Insights. *Journal of Cancer Science & Therapy*, *6*(12).
7. Peyressatre, M., Prével, C., Pellerano, M., & Morris, M. C. (**2015**). Targeting Cyclin-Dependent kinases in human cancers: from small molecules to Peptide inhibitors. *Cancers*, *7*(1), 179–237.
8. Galbraith, M.D.; Bender, H.; Espinosa, J.M. Therapeutic targeting of transcriptional cyclin dependent kinases, *Transcription **2019***, 10, 118–136
9. Diab, S., Yu, M., & Wang, S. (**2020**). CDK7 Inhibitors in Cancer Therapy: The Sweet Smell of Success? *Journal of Medicinal Chemistry* (Vol. 63, Issue 14, pp. 7458–7474).
10. Fisher R. P. (**2019**). Cdk7: a kinase at the core of transcription and in the crosshairs of cancer drug discovery. *Transcription*, *10*(2), 47–56.
11. Lapenna, S., & Giordano, A. (**2009**). Cell cycle kinases as therapeutic targets for cancer. *Nature Reviews. Drug Discovery*, *8*(7), 547–566.

12. Leitch, A. E., Lucas, C. D., Marwick, J. A., Duffin, R., Haslett, C., & Rossi, A. G. (**2012**). Cyclin-dependent kinases 7 and 9 specifically regulate neutrophil transcription and their inhibition drives apoptosis to promote resolution of inflammation. *Cell Death and Differentiation*, *19*(12), 1950–1961.

13. Mayer E. L. (2015). Targeting breast cancer with CDK inhibitors. *Current Oncology Reports*, *17*(5), 443.

14. Sánchez-Martínez, C.; Lallena, M.J.; Sanfeliciano, S.G.; de Dios, A. Cyclin dependent kinase (CDK) inhibitors as anticancer drugs: Recent Advances (2015–2019). *Bioorganic Med. Chem. Lett.* ***2019***, 29, 126637

15. Wang, M., Wang, T., Zhang, X., Wu, X., & Jiang, S. (**2020**). Cyclin-dependent kinase 7 inhibitors in cancer therapy. *Future Medicinal Chemistry*, *12*(9), 813–833.

16. Asghar, U., Witkiewicz, A. K., Turner, N. C., & Knudsen, E. S. (**2015**). The history and future of targeting cyclin-dependent kinases in cancer therapy. *Nature Reviews Drug Discovery* (Vol. 14, Issue 2, pp. 130–146). Nature Publishing Group.

17. Kim, J., Cho, Y. J., Ryu, J. Y., Hwang, I., Han, H. D., Ahn, H. J., Kim, W. Y., Cho, H., Chung, J. Y., Hewitt, S. M., Kim, J. H., Kim, B. G., Bae, D. S., Choi, C. H., & Lee, J. W. (**2020**). CDK7 is a reliable prognostic factor and novel therapeutic target in epithelial ovarian cancer. *Gynecologic Oncology*, *156*(1), 211–221.

18. Zhang, Z., Peng, H., Wang, X., Yin, X., Ma, P., Jing, Y., Cai, M. C., Liu, J., Zhang, M., Zhang, S., Shi, K., Gao, W. Q., Di, W., & Zhuang, G. (**2017**). Preclinical Efficacy and Molecular Mechanism of Targeting CDK7-Dependent Transcriptional Addiction in Ovarian Cancer. *Molecular Cancer Therapeutics*, *16*(9), 1739–1750.

19. B.N. Marak, J. Dowarah, L. Khiangte, V.P. Singh (**2020**). A comprehensive insight on the recent development of Cyclic Dependent Kinase inhibitors as anticancer agents. European Journal of Medicinal Chemistry, 203, 112571.

20. Chohan, T. A., Qian, H. Y., Pan, Y. L., & Chen, J. Z. (**2015**). Molecular simulation studies on the binding selectivity of 2-anilino-4-(thiazol-5-yl)-pyrimidines in complexes with CDK2 and CDK7. *Molecular BioSystems*, *12*(1), 145–161.

21. Lim, S., & Kaldis, P. (**2013**). Cdks, cyclins and CKIs: Roles beyond cell cycle regulation. *Development (Cambridge)* (Vol. 140, Issue 15, pp. 3079–3093).
22. Barnum, K. J., & O'Connell, M. J. (**2014**). Cell cycle regulation by checkpoints. *Methods in Molecular Biology*, *1170*, 29–40.
23. Deshpande, A., Sicinski, P., & Hinds, P. W. (**2005**). Cyclins and cdks in development and cancer: a perspective. *Oncogene*, *24*(17), 2909–2915.
24. Pisu, M., Concas, A., & Cao, G. (**2015**). A novel quantitative model of cell cycle progression based on cyclin-dependent kinases activity and population balances. *Computational Biology and Chemistry*, *55*, 1–13.
25. Liu, K., Zheng, M., Lu, R., Du, J., Zhao, Q., Li, Z., Li, Y., & Zhang, S. (**2020**). The role of CDC25C in cell cycle regulation and clinical cancer therapy: a systematic review. *Cancer Cell International*, *20*, 213.
26. Sanchez-Vega, F., Mina, M., Armenia, J., Chatila, W. K., Luna, A., La, K. C., Dimitriadoy, S., Liu, D. L., Kantheti, H. S., Saghafinia, S., Chakravarty, D., Daian, F., Gao, Q., Bailey, M. H., Liang, W. W., Foltz, S. M., Shmulevich, I., Ding, L., Heins, Z., Ochoa, A., … Schultz, N. (**2018**). Oncogenic Signaling Pathways In The Cancer Genome Atlas. *Cell*, *173*(2), 321–337.e10.
27. Khanapure, S., Jagadale, M., Bansode, P., Choudhari, P., & Rashinkar, G. (**2018**). Anticancer activity of ruthenocenyl chalcones and their molecular docking studies. *Journal of Molecular Structure*, *1173*, 142–147.
28. Knockaert M, Greengard P, Meijer L (**2002**) Pharmacological inhibitors of cyclin dependent kinases. Trends Pharmacol Sci 23:417-425
29. Malumbres, M., Harlow, E., Hunt, T., Hunter, T., Lahti, J. M., Manning, G., Morgan, D. O., Tsai, L. H., & Wolgemuth, D. J. (**2009**). Cyclin-dependent kinases: a family portrait. *Nature Cell Biology*, *11*(11), 1275–1276.
30. Fisher R. P. (**2005**). Secrets of a double agent: CDK7 in cell-cycle control and transcription. *Journal of Cell Science*, *118*(Pt 22), 5171–5180.
31. Kumar, V., Parate, S., Thakur, G., Lee, G., Ro, H. S., Kim, Y., Kim, H. J., Kim, M. O., & Lee, K.W. (**2021**). Identification of CDK7 Inhibitors from Natural Sources Using Pharmacoinformatics and Molecular Dynamics Simulations. *Biomedicines*, *9*(9), 1197.
32. Tassan, J. P., Jaquenoud, M., Fry, A. M., Frutiger, S., Hughes, G. J., & Nigg, E. A. (**1995**). In vitro assembly of a functional human CDK7-cyclin H

complex requires MAT1, a novel 36 kDa RING finger protein. *The EMBO Journal*, *14*(22), 5608–5617.

33. Mehta, S., & Brahmchari, D. (**2019**). Phosphazene Superbase-Mediated Regio- and Stereoselective Iodoaminocyclization of 2-(1-Alkynyl)benzamides for the Synthesis of Isoindolin-1-ones. *Journal of Organic Chemistry*, *84*(9), 5492–5503.
34. Morgan, D. O. (**1997**). Cyclin-Dependent Kinases: Engines, Clocks, and Microprocessors. *Annu. Rev. Cell Dev. Biol* (Vol. 13).
35. Singla, P., Luxami, V., & Paul, K. (**2014**). Benzimidazole-biologically attractive scaffold for protein kinase inhibitors. *RSC Advances* (Vol. 4, Issue 24, pp. 12422–12440).
36. Wang S, Wood G, Meades C, et al. Synthesis and biological activity of 2-anilino-4-(1H-pyrrol-3-yl) pyrimidine CDK inhibitors. Bioorganic & Medicinal Chemistry Letters. **2004** Aug;14(16):4237-4240.
37. Paul McGillan, Neil G. Berry, Gemma L. Nixon, Suet C. Leung, Peter J. H. Webborn, Mark C. Wenlock, Stefan Kavanagh, Andrew Cassidy, Rachel H. Clare, Darren A. Cook, Kelly L. Johnston, Louise Ford, Stephen A. Ward, Mark J. Taylor, W. David Hong, and Paul M. O'Neill, *ACS Medicinal Chemistry Letters* **2021** *12* (9), 1421-1426.
38. Ramanathan, Y.; Rajpara, S.M.; Reza, S.M.; Lees, E.; Shuman, S.; Mathews, M.B.; Pe'Ery, T. Three RNA Polymerase II Carboxylterminal Domain Kinases Display Distinct Substrate Preferences. J. Biol. Chem. **2001**, 276, 10913–10920
39. Kaliszczak, M., Patel, H., Kroll, S. H., Carroll, L., Smith, G., Delaney, S., Heathcote, D. A., Bondke, A., Fuchter, M. J., Coombes, R. C., Barrett, A. G., Ali, S., & Aboagye, E. O. (**2013**). Development of a cyclin-dependent kinase inhibitor devoid of ABC transporter-dependent drug resistance. *British Journal of Cancer*, *109*(9), 2356–2367.
40. Kwiatkowski, N., Zhang, T., Rahl, P. B., Abraham, B. J., Reddy, J., Ficarro, S. B., Dastur, A., Amzallag, A., Ramaswamy, S., Tesar, B., Jenkins, C. E., Hannett, N. M., McMillin, D., Sanda, T., Sim, T., Kim, N. D., Look, T., Mitsiades, C. S., Weng, A. P., Brown, J. R., … Gray, N. S. (**2014**). Targeting transcription regulation in cancer with a covalent CDK7 inhibitor. *Nature*, *511*(7511), 616–620.

41. Lolli, G., Lowe, E. D., Brown, N. R., & Johnson, L. N. (**2004**). The crystal structure of human CDK7 and its protein recognition properties. *Structure (London, England : 1993)*, *12*(11), 2067–2079.

42. Greber, B. J., Perez-Bertoldi, J. M., Lim, K., Iavarone, A. T., Toso, D. B., & Nogales, E. (**2020**). The cryoelectron microscopy structure of the human CDK-activating kinase. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(37), 22849–22857.

43. Clare, P. M., Poorman, R. A., Kelley, L. C., Watenpaugh, K. D., Bannow, C. A., & Leach, K. L. (**2001**). The cyclin-dependent kinases cdk2 and cdk5 act by a random, anticooperative kinetic mechanism. *The Journal of Biological Chemistry*, *276*(51), 48292–48299.

44. Hazel, P., Kroll, S. H., Bondke, A., Barbazanges, M., Patel, H., Fuchter, M. J., Coombes, R. C., Ali, S., Barrett, A. G., & Freemont, P. S. (**2017**). Inhibitor Selectivity for Cyclin-Dependent Kinase 7: A Structural, Thermodynamic, and Modelling Study. *ChemMedChem*, *12*(5), 372–380.

45. Norman, R. A., Toader, D., & Ferguson, A. D. (**2012**). Structural approaches to obtain kinase selectivity. *Trends in Pharmacological Sciences*, *33*(5), 273–278.

46. Kumar, J. S., Reddy, G. S., Medishetti, R., Ray, A., Bele, S. D., Hossain, K. A., Thirupataiah, B., Edwin, R. K., Behera, P., Joseph, A., Shenoy, G. G., Rao, C. M., & Pal, M. (**2021**). Sonochemical synthesis of rosuvastatin based novel 3-methyleneisoindolin-1-one derivatives as potential anticancer agents. *Journal of Molecular Structure*, *1240*.

47. Curtin, M. L., Frey, R. R., Heyman, H. R., Sarris, K. A., Steinman, D. H., Holmes, J. H., Bousquet, P. F., Cunha, G. A., Moskey, M. D., Ahmed, A. A., Pease, L. J., Glaser, K. B., Stewart, K. D., Davidsen, S. K., & Michaelides, M. R. (**2004**). Isoindolinone ureas: a novel class of KDR kinase inhibitors. *Bioorganic & Medicinal Chemistry Letters*, *14*(17), 4505–4509.

48. Reddy, M. C., & Jeganmohan, M. (**2017**). Total synthesis of aristolactam alkaloids: Via synergistic C-H bond activation and dehydro-Diels-Alder reactions. *Chemical Science*,*8*(5), 4130–4135.

49. Savela, R., & Méndez-Gálvez, C. (**2021**). Isoindolinone Synthesis via One-Pot Type Transition Metal Catalyzed C-C Bond Forming Reactions. *Chemistry (Weinheim an der Bergstrasse, Germany)*, *27*(17), 5344–5378.

50. Yao, T., & Larock, R. C. (**2005**). Regio- and stereoselective synthesis of isoindolin-1-ones via electrophilic cyclization. *The Journal of Organic Chemistry*, *70*(4), 1432–1437.

51. Martinez, A. M., Afshar, M., Martin, F., Cavadore, J. C., Labbé, J. C., & Dorée, M. (**1997**). Dual phosphorylation of the T-loop in cdk7: its role in controlling cyclin H binding and CAK activity. *The EMBO Journal*, *16*(2), 343–354.

52. Brahmchari, D., Verma, A. K., & Mehta, S. (**2018**). Regio- and Stereoselective Synthesis of Isoindolin-1-ones through BuLi-Mediated Iodoaminocyclization of 2-(1-Alkynyl)benzamides. *The Journal of organic chemistry*, *83*(6), 3339–3347.

53. Mehta S, Mangyan M, Brahmchari D. Design, Synthesis and Anti-cancer Activity Evaluation of a 3-methyleneisoindolin-1-one Library. Combinatorial Chemistry & High Throughput Screening. **2022** Oct.

54. Rys, V., Couture, A., Deniau, E., Lebrun, S., & Grandclaudon, P. (**2005**). A concise benzotriazolyl-mediated synthesis of 9-methoxycepharanone A. *Tetrahedron*, *61*(3), 665–671.

55. Kumar, V., Poonam, Prasad, A. K., & Parmar, V. S. (**2003)**. Naturally occurring aristolactams, aristolochic acids and dioxoaporphines and their biological activities. *Natural Product Reports* (Vol. 20, Issue 6, pp. 565–583).

56. Meng, X. Y., Zhang, H. X., Mezei, M., & Cui, M. (2011). Molecular docking: a powerful approach for structure-based drug discovery. *Current Computer-Aided Drug Design*, *7*(2), 146–157.

57. Pronk, S., Páll, S., Schulz, R., Larsson, P., Bjelkmar, P., Apostolov, R., Shirts, M. R., Smith, J. C., Kasson, P. M., van der Spoel, D., Hess, B., & Lindahl, E. (**2013**). GROMACS 4.5: a high-throughput and highly parallel open source molecular simulation toolkit. *Bioinformatics (Oxford, England)*, *29*(7), 845–854.

58. O'Boyle, N. M., Banck, M., James, C. A., Morley, C., Vandermeersch, T., & Hutchison, G. R. (**2011**). Open Babel: An open chemical toolbox. *Journal of Cheminformatics*, *3*, 33.

59. Sonawane K, Barale SS, Dhanavade MJ, et al. Homology Modeling and Docking Studies of TMPRSS2 with Experimentally Known Inhibitors

Camostat Mesylate, Nafamostat and Bromhexine Hydrochloride to Control SARS-Coronavirus-2. ChemRxiv; **2020.**

60. Dallakyan, S., & Olson, A. J. (**2015**). Small-molecule library screening by docking with PyRx. *Methods in Molecular Biology (Clifton, N.J.)*, *1263*, 243–250.
61. Halgren T. A. (**1999**). MMFF VI. MMFF94s option for energy minimization studies. *Journal of Computational Chemistry*, *20*(7), 720–729.
62. Mark Abraham, Andrey Alekseenko, Cathrine Bergh, Christian Blau, Eliane Briand, Mahesh Doijade, Stefan Fleischmann, Vytautas Gapsys, Gaurav Garg, Sergey Gorelov, Gilles Gouaillardet, Alan Gray, M. Eric Irrgang, Farzaneh Jalalypour, Joe Jordan, Christoph Junghans, Prashanth Kanduri, Sebastian Keller, Carsten Kutzner, … Erik Lindahl. (2023). GROMACS 2023 Manual (Version 2023). Zenodo.
63. Zoete, V., Cuendet, M. A., Grosdidier, A., & Michielin, O. (**2011**). SwissParam: a fast force field generation tool for small organic molecules. *Journal of Computational Chemistry*, *32*(11), 2359–2368.
64. Sapay, N., & Tieleman, D. P. (**2011**). Combination of the CHARMM27 force field with united-atom lipid force fields. *Journal of Computational Chemistry*, *32*(7), 1400–1410.
65. Parrinello, M., & Rahman, A. (**1981**). Polymorphic transitions in single crystals: A new molecular dynamics method. *Journal of Applied Physics*, *52*(12), 7182–7190.
66. Humphrey, W., Dalke, A., & Schulten, K. (**1996**). VMD: visual molecular dynamics. *Journal of Molecular Graphics*, *14*(1), 33–28.
67. Pires, D. E. V., Blundell, T. L., & Ascher, D. B. **(2015**). pkCSM: Predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures. *Journal of Medicinal Chemistry*, *58*(9), 4066–4072.
68. Lagorce, D., Douguet, D., Miteva, M. A., & Villoutreix, B. O. (**2017**). Computational analysis of calculated physicochemical and ADMET properties of protein-protein interaction inhibitors. *Scientific Reports*, *7*, 46277.
69. National Center for Biotechnology Information **(2023**). PubChem Compound Summary for CID 68029831, Trilaciclib. Retrieved March 7, 2023 https://pubchem.ncbi.nlm.nih.gov/compound/Trilaciclib.

70. National Center for Biotechnology Information (**2023**). PubChem Compound Summary for CID 73602827. Retrieved March 7, 2023 from https://pubchem.ncbi.nlm.nih.gov/compound/thz1.
71. National Center for Biotechnology Information (**2023**). PubChem Compound Summary for CID 49867929. Retrieved March 7, 2023 from https://pubchem.ncbi.nlm.nih.gov/compound/bs-181.
72. National Center for Biotechnology Information (**2023**). PubChem Compound Summary for CID 16718576, Milciclib. Retrieved March 7, 2023 from https://pubchem.ncbi.nlm.nih.gov/compound/Milciclib.
73. BIOVIA, Dassault Systèmes, [Discovery Studio 2021 client], [Discovery Studio Visualizer v21.1.0.20298], San Diego: Dassault Systèmes, [**2021**]. https://www.3ds.com/products-services/biovia/products/molecular-modeling-simulation/biovia-discovery-studio/
74. Jamal, Q. M., Dhasmana, A., Lohani, M., Firdaus, S., Ansari, M. Y., Sahoo, G. C., & Haque, S. (**2015**). Binding Pattern Elucidation of NNK and NNAL Cigarette Smoke Carcinogens with NER Pathway Enzymes: an Onco-Informatics Study. *Asian Pacific Journal of Cancer Prevention: APJCP*, *16*(13), 5311–5317.

Presentation in Conferences

1. Poster Presentation on "Chemical Synthesis and Biological Importance of Aristolactams" at International Symposium "Chemical Wisdom by Her" Organized by Department of Chemistry, Deshbandhu College, University of Delhi (2022)
2. Poster Presentation on "Recent Advances in Chemical Synthesis and Biological Studies of Aristolactams" at International Conference on "Advances in Chemical Sciences and Nano composites" Organized by Zakir Hussain Delhi College, University of Delhi and ISAS. (2022)
3. Oral Presentation on "*In-Silico* Exploration of Small Molecules as Kinase Inhibitor" at International Conference on "Chemical and Allied Sciences and their Applications" Organized by Delhi Technological University. (2023)
4. Poster Presentation on "Computational Studies of Heterocyclic Compounds towards Anticancer Properties" at International Conference on "Recent Trends in Chemical Sciences and Sustainable Energy" Organized by NIT, Delhi and University of Delhi. (2023)

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