REVOLUTIONIZING CANCER THERAPY: REPURPOSING FDA- APPROVED DRUGS FOR ERK1(MAPK3) INHIBITION VIA INTEGRATED DOCKING AND SIMULATION STRATEGY

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I, **Nishant Kumar**, bearing Roll No. 2K22/MSCBIO/34 hereby certify that the work which is being presented in the thesis entitled "**Revolutionizing Cancer Therapy: Repurposing FDA-Approved Drugs for ERK1(MAPK3) Inhibition Via Integrated Docking and Simulation Strategy**" in partial fulfilment of the requirement for the award of the Degree of Master of Science, submitted in the Department of Biotechnology, Delhi Technological University is an authentic record of my own work carried out during the period from January 2024 to May 2024 under the supervision of Dr. Asmita Das.

The matter presented in the thesis has not been submitted by me for the award of any degree of this or any other Institute.

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Certified that **Nishant Kumar (2k22/MSCBIO/34)** has carried out their search work presented in this thesis entitled **"Revolutionizing Cancer Therapy: Repurposing FDA- Approved Drugs for ERK1(MAPK3) Inhibition Via Integrated Docking and Simulation Strategy"** for the award of the degree of Master of Science and submitted to the Department of Biotechnology, Delhi Technological University, Delhi, under my supervision. This thesis embodies the results of original work, and studies carried out by the student himself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or anybody else from this or any other Institution.

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Revolutionizing Cancer Therapy: Repurposing FDA- Approved Drugs for ERK1(MAPK3) Inhibition Via Integrated Docking and Simulation Strategy

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ABSTRACT

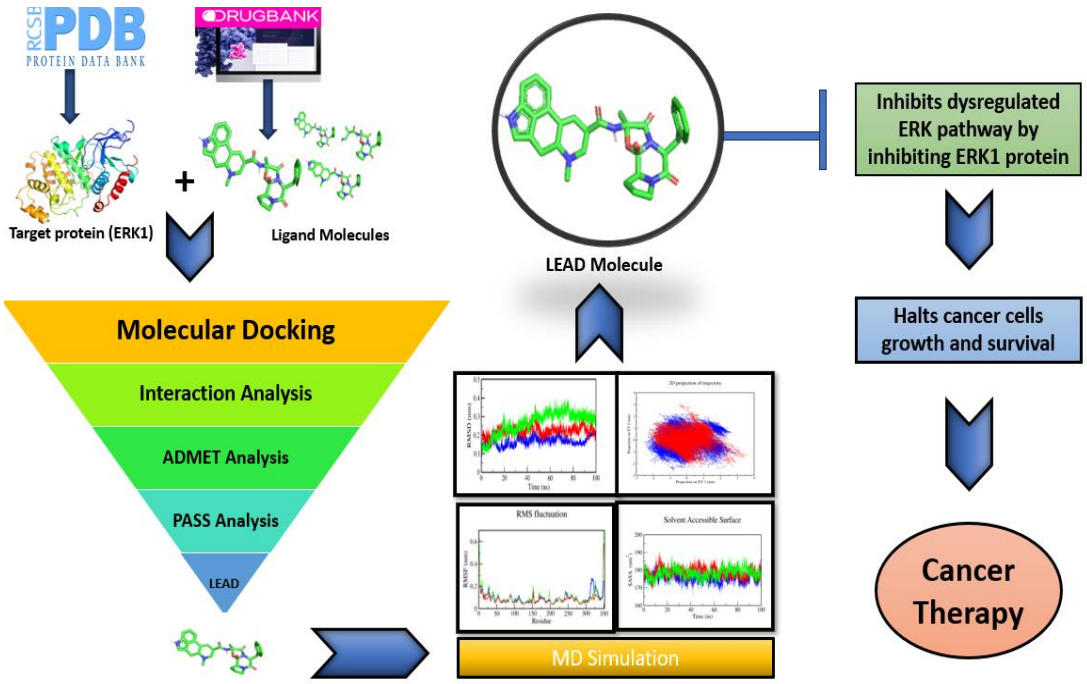
Cancer , a common disease worldwide, defined by the aberrant cells' uncontrollable growth and division, resulting in the development of tumors, which may be harmless, dangerous, or both. Tumors that are cancerous have the ability to infect nearby tissues and spread across other body areas. The rapid growth and dissemination of these aberrant cells are the primary causes of cancer-related deaths Worldwide, cancer is a common disease, and DNA damage is frequently the source of sporadic tumors. A significant contributing element to the onset and spread of cancer because of many genes showing expression which are altered, including kinases. The growth of cancer may be aided by several cellular abnormalities brought on by this abnormal expression. The serine/threonine kinase ERK1(MAPK3) is a member of the MAP kinase family. Because it is an essential part of the ERK/MAPK signaling cascade, ERK1 regulates a number of cellular processes that are disturbed in cancer pathogenesis. When dysregulated, it leads to uncontrollable cell growth because it activates transcription factors that govern gene expression relevant to the progression of the cell cycle, promoting cell proliferation. Numerous malignancies, including those of the liver, pancreas, breast, thyroid, and other organs, are associated with poor prognoses, resistance to therapy, spread, and initiation that are all linked to ERK1 (MAPK3). Thus, it appears that ERK1 (MAPK3) is a viable target for treatment.

In order to determine possible ERK1 inhibitors ERK1, a comprehensive strategy was employed. This included docking simulations on 3,674 FDA-approved drugs to find compounds with higher affinity than existing medications. A detailed screening process narrowed down the candidates. Interaction analysis was then performed on 14 ligands with higher binding affinity than the reference drug. Swiss ADME analysis assessed the physiochemical properties, bioavailability, gastrointestinal absorption, solubility, and Ames toxicity of the selected ligands.
Furthermore, the biological function of the resulting compounds was anticipated by PASS analysis. To further understand the stability and dynamic behaviour of these compounds within the ERK1 binding region, a 100 ns MDS was performed, providing insights into the structural dynamics and stability of the compound-ERK1 complex. Among the analysed drugs, one compound stood out as a promising ERK1 inhibitor. The selection process considered not only binding affinity but also essential

physiochemical properties, toxicity analysis, pharmacokinetic properties, stability, and conformational dynamics. This thorough methodology, integrating molecular docking with a vast library of FDA-approved drugs, advanced interaction analysis, ADME and PASS analysis, and finally MD simulation for studying dynamic and stability behaviour, significantly increases the chances of identifying a viable therapeutic candidate.

Such an integrated approach shows great promise in identifying novel ERK1 (MAPK3) inhibitors, laying a solid foundation for subsequent preclinical and clinical studies

Keywords—ERK1, MAPK3, SWISS ADME, Binding affinity, Molecular Dynamic Simulation



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LIST OF ABBREVIATIONS

ERK	Extracellular Signal-Regulated Kinase
ERK1	Extracellular Signal-Regulated Kinase 1
ERK2	Extracellular Signal-Regulated Kinase 2
MAPK	Mitogen-activated protein kinase
MAPK3	Mitogen-activated protein kinase 3
HCC	Hepatocellular carcinoma
HSC	Hepatic stellate cells
PDAC	Pancreatic ductal adenocarcinoma
PSCs	Pancreatic stellate cells
PTC	Papillary thyroid cancer
PDTC	Poorly differentiated thyroid cancer
ATC	Anaplastic thyroid cancer
MDS	Molecular Dynamic Simulations
PDB	Protein Data Bank
SDF	Structure Data File
ADMET	Absorption, distribution, metabolism, excretion, toxicity
RMSD	Root Mean Square Deviation
RMSF	Root Mean Square Fluctuations
PDF	Probability Distribution Function
SASA	Solvent accessible surface area
Rg	Radius of Gyration
PCA	Principal Component Analysis

CHAPTER 1

INTRODUCTION

Cancer is defined by aberrant cells growing and spreading uncontrollably throughout the body to develop tumors, which can be benign or malignant (cancerous). These malignant tumors have the capacity to spread to other body parts by invading surrounding tissues. Most cancer-related deaths are caused by the fast growth of aberrant cells and the spreading of those cells [1].

Cancer is a leading global health issue. In India, cases were expected to rise from 979,786 in 2010 to 1,148,757 by 2020 [1], [2]

In cancer and in its all kinds ,MAPK/ERK pathway's essential component, ERK1 (MAPK3), which is promisable therapeutic candidate [3]. The carcinogenesis and advancement of various cancers, including cancer of lung, breast, pancreatic and thyroid , liver are significantly influenced by its abnormal activation. Targeting ERK1 signaling slows the growth of cancer by interfering with downstream pathways that encourage tumor cell invasion, division, and metastasis [4]. ERK1 inhibitors offer a targeted approach to overcoming chemoresistance, a common challenge in cancer treatment. However, the efficacy of ERK1-targeted therapies may vary among cancer types, necessitating tailored treatment strategies [5].

ERK1,a part of ERK/MAPK signaling cascade has a vital function in the pathophysiology of cancer by regulating numerous cellular processes disrupted in cancer. It facilitates cell proliferation via turning on transcriptional factors which regulates the gene expression which are related to cell cycle progression, leading to uncontrollable cell growth when dysregulated [3], [6], [7]. Furthermore, ERK1 prevents apoptotic pathways, which allows cancer cells to elude planned death of cells and enhances cell survival and develop resistance to therapies. Furthermore, ERK1 enhances cellular invasion, migration by affecting cytoskeletal dynamics and cellular adhesion, facilitating metastasis. By encouraging VEGF expression, it also controls angiogenesis by guaranteeing tumor vascularization and nutrient delivery. Overactivation of ERK1 is associated with resistance to various cancer treatments, making it a significant therapeutic target [8]. ERK1 (MAPK3), is an essential part of the ERK/MAPK pathway, a basic cell signaling system which is essential for controlling multiple functions such as adhesion, proliferation, migration, and survival. Serine/threonine kinases ERK1 and ERK2, sometimes referred to as ERK1/2 because of their comparable roles, mediate signaling through the Ras-Raf-MEK-ERK pathway, which sends extracellular impulses into the inside of cells [9].

Cellular processes like migration, proliferation, and death are carefully regulated by the ERK/MAPK pathway. Nuclear transcriptional factors such as c-fos, c-Jun are activated by ERK1(MAPK3), which facilitates programmed cell death and division. Liver, thyroid, lung, and stomach malignancies are among the carcinomas whose genesis, development, metastasis have all been linked to aberrant expression or hyperactivity of MAPK3 [10], [11].

In cancer progression ,tumor growth, ERK1 has multiple functions. In the case of breast cancer and colorectal cancer, for example, ERK1 activation promotes tumor development and metastasis in addition to cellular invasion and division. Additionally, in lung cancer , ERK1 activation increases tumor cell survival and resistance to therapy. This is also true for lung cancer. This highlights the importance of ERK1 as a promisable curative target. Also, recent research has emphasized MAPK3's importance in the biology of cancer. For example, studies by Du et al. showed that miR-143 overexpression inhibited the development of cells of cancer of breast by focusing on MAPK3. Similarly, Cao et al. found that in gastric cancerous cells, resistance to cisplatin was linked with elevated MAPK3 levels and reduced miR-129 expression. Through the downregulation of MAPK3 expression, miR-129, able to induce apoptosis as well as reduced cell proliferation [12]. These findings show that MAPK3 is a target which therapeutic with promise in managing cancer, particularly in the battle against drug resistance and carcinogenesis.

The process of discovering new pharmacological applications for FDA-approved pharmaceuticals or prodrugs that are currently on the market, unsuccessful, under development, or commercially commercialized is known as drug repurposing [13], [14]. Repurposing drugs is the procedure of determining if currently FDA-approved medications can be used for therapeutic purposes other than those for which they were designed [15]. Using molecular docking to evaluate licensed medications' interactions with distinct biological targets, researchers can find intriguing candidates for repurposing, which could hasten the creation of cutting-edge remedies for a variety of diseases [16].

Molecular docking analysis in drug discovery is a crucial computational technique that focuses on finding possible inhibitors that target particular biological targets [17]. Molecular docking predicts the ideal conformations and orientations for drug binding by modelling the binding interactions between small molecule drugs and protein targets [18]. The design and optimization of therapeutic agents are aided by this understanding of drug-target interactions, which also offers important information on the intensity and specificity of these interactions to assist in directing the creation of effective remedies for a variety of illnesses. The three main processes of a molecular docking analysis are usually preparation of the structure of small molecule , macromolecule , and assessment of the ligand-receptor binding affinity [19] . In order to obtain a more profound comprehension of the dynamic behaviour and stability of ligands located in the binding site of receptors, like active sites of proteins, after docking studies, like analysis of molecular dynamics (MD), are usually utilized after molecular docking. Moreover, MD makes it easier to evaluate residue flexibility in protein catalytic regions

The dynamic behaviour of biological systems is studied by molecular dynamics (MD) simulations [20]. An illustration of this would be the interactions between ligand and protein. The MD simulations helps in detailed understanding of the structural and functional aspects of drugcible complexes by simulating the movements and interactions of atoms and molecules throughout time. This information is helpful for the development and optimization of medications, which encourages the creation of new drugs with increased efficacy and fewer side effects [21].

In this study, we examined 3,674 FDA-approved medications from the Drug Bank database using a structure-based virtual screening method. By repurposing drugs, it was hoped to find possible inhibitors of MAPK3/ERK1 activity that would block its downstream signaling pathways and, in turn, limit cell migration and proliferation. In order to do this, we assessed the binding affinities of many FDA-approved medications to the MAPK3/ERK1 active site using molecular docking analysis. Through interaction analysis, specific interactions between the chemicals and ERK1 were found and examined.

The chosen drugs'/compounds pharmacokinetic characteristics and safety profiles were predicted using the ADMET Property study, which was done following the docking analysis. The biological activity profiles of the chosen lead and/or compounds were also predicted using PASS analysis, confirming that they fulfilled the requirements for possible therapeutic agents. A 100 ns (MDS) was carried out in order to provide additional insight into the stability and dynamic behaviour of the chemicals that were found within the binding region of ERK1. A deeper comprehension of the interplay is possible thanks to the insights this simulation gave into the conformational dynamics ,stability of the complex formed by selected compound and ERK1
The collective findings of these investigations point to the possibility of using FDA-approved medications as MAPK3 inhibitors in cancer treatment. Via their powerful inhibition of MAPK3, these medications may disrupt important signaling cascade/pathways which are implicated in the migration and growth of cancer cells, providing a potentially viable treatment option.

CHAPTER 2

REVIEW OF LITERATURE

2.1. Cancer

The word "cancer" relates to a wide range of illnesses which affects any area of the body parts. It's often called as malignant tumors or neoplasms. One of the main characteristics of cancer is the fast growth of aberrant cells that can infiltrate neighbouring tissues and migrate to other body parts, via process known as metastasis. These cells develop uncontrollably. The main factor leading to cancer-related deaths is metastasis [1].

Cancer is one of the main culprit of causing major illness in the world. It was determined that the number of cancer cases in India would increase from 979,786 in 2010 to 1,148,757 by 2020 [1], [2]. Globally, it was calculated that there will be 19.3 million cancer cases which are new whereas in 2020 deaths from cancer is 10.0 million . Ten percent of people will die from cancer, which affects 20% of people in their lifetime [22]. The need to improve and extend the present diagnosis as well as treatment facilities—which are not sufficient to bear the present burden—is made more urgent by the rising incidence of cancer. Primary cancer prevention and detection is an essential public health concern because treatment therapies have not been able to significantly lower the mortality rates associated with the majority of malignancies [23].

Cancer encompasses a wide variety of types, including including but not restricted to stomach, liver, lung, and cancer of breast. Each type has unique characteristics and challenges, contributing to the complexity of cancer treatment and the necessity for diverse therapeutic strategies.

An important member in the development and advancement of various cancers is ERK1 [3]. ERK1 belongs to MAPK pathway, which regulates division, differentiation, and survival of cells. Disruptions in this cascade or pathway may culminate in cancer through uncontrollable cell proliferation [4], [24]. Therefore, ERK1 represents a potential curative target in cancer treatment. By inhibiting ERK1, these pathways can be disrupted, potentially slowing down or halting the progression of cancer. This makes ERK1 a promising focus for the creation of novel therapies for cancer.

So, examining how ERK1 functions in different kinds of cancers may improve the efficacy of therapeutic approaches and aid in controlling the rising cancer incidence.

2.2.Types of Cancer

2.2.1. Liver /hepatic cancer

As the second-leading cause of fatalities from cancer worldwide, liver cancer, also known as hepatocellular carcinoma (HCC), presents a significant challenge in healthcare [25]. In China, where the 5-year survival rate stands at a mere 12%, the majority of HCC cases are diagnosed in advanced stages, contributing to a bleak prognosis owing to the limited effectiveness of available treatments[26]. Despite advancements in modern medicine, including radical hepatectomy, the recurrence rate remains high, underscoring the pressing need for innovative therapeutic strategies to improve patient outcomes [27].

Liver fibrosis, a significant contributor to the initiation, progression, and tumor microenvironment (TME) of liver cancer, is prevalent in over 80% of HCC patients [28]. One of the main components of HCC-related fibroblasts, activated (HSCs), is essential for hepatic fibrosis. by producing extracellular matrix (ECM) proteins [29]. Under the influence of cancerous cells and different causes, these HSCs transition from a dormant to an active state. The activated cells release cytokines that enhance the invasiveness and viability of cancer cells, along with extracellular matrix proteins and inflammatory cytokines, all of which contribute to the TME and the advancement of hepatic cancer.The stimulated hepatic stellate cells (HSCs), in the context of hepatic cancer, have a major impact on the tumor microenvironment (TME) and its development [30]. Through the ERK1/2 signaling pathway, HSCs promote processes like the transition from epithelial to mesenchymal (EMT) and the progression of cancer cells cell cycle [31]. ERKs, sometimes referred to as mitogen-activated protein kinases or extracellular signal-regulated kinases, serve as key regulators in various human biochemical signaling pathways, governing crucial functions like transcription, proliferation, and differentiation [32]. Phosphorylation of ERK1/2 is particularly noteworthy in gastrointestinal tumor processes such as autophagy and senescence. Targeted cancer therapies have emerged with the development of ERK1/2 inhibitors, showcasing their potential in cancer treatment [33]. Studies have highlighted that inhibiting ERK1/2 effectively hinders HCC progression, positioning ERK1 as a promising therapeutic target in the quest to improve outcomes for HCC /liver cancer patients [33], [34].

2.2.2.Pancreatic cancer

Pancreatic cancer specially PDAC exhibits an alarming 5-year PDAC which is fourth most repeated cause of death related to cancer globally. The predominant cause of PDAC-associated mortality is liver metastasis, which is found in most cases of pancreatic cancer and has a pitiful 5-year OS rate of 2.7% and a median OS of less than 6 months[35]. To address this terrible situation, novel therapeutic techniques and agents must be pursued, as curative resection is not feasible after liver metastasis [36].

An extensive desmoplastic reaction, indicated by a profusion of tumor stroma in histological investigation, is the hallmark of PDAC. Notably, interactions between the tumor and the surrounding tissue have been linked to the advancement of PDAC and

the development of resistance to traditional chemotherapies [37]. PSCs, major cause of fibrosis in illness of pancreas, are essential to this process. PSCs undergo a change from quiescent to active myofibroblast-like cells when responding to a various stimulus, such as interactions with tumor cells. High frequency of very fibrotic stromal cells in PDAC tumors and metastases implies that focusing on stromal components may be a promising treatment option for PDAC [38], [39].

ERKs (MAPKs), serve as crucial hubs for a diverse array of biochemical signals governing fundamental cellular activities such proliferation, differentiation, transcription, and development [32]. Within the MAPK family, ERK1 and ERK2 have garnered significant attention in breast, hepatocellular, lung, and colorectal malignancies due to their pivotal roles in these diseases [40], [41]. Also new investigations have illuminated the functional importance of ERK1/2 which are phosphorylated in pancreatic cancer, highlighting ERK1 as a promising intended treatment approach target in PDAC.

2.2.3.Thyroid Cancer

Over the last forty years, there has been a consistent increase in the prevalence of endocrine cancer, specifically cancer of the thyroid. Because they are undifferentiated and resistant to radioiodine therapy, aggressive forms of thyroid cancers, like PDTC , ATC and aggressive thyroid cancer in the papillary form (PTC), provide substantial treatment hurdles [42], [43]. Thus, new effective therapeutic techniques are desperately needed to manage these cancers [44].

Thyroid cancer cases frequently include mutations in the MAP kinase (MAPK) pathway, specifically in BRAF [45], [46]. Although pharmaceutical BRAF inhibition has been effective in patients of melanoma with activated BRAF mutations and inconsistent results were produced due to its its use in of patients of thyroid cancer . Intrinsic and acquired resistance pose difficulties for single-agent BRAF inhibition; the latter is frequently linked to the MAPK pathway's downstream reactivation [47], [48].

Selective inhibitors of ERK1/2 have been designed to overcome these restrictions. The idea behind this strategy is to stop the MAPK pathway's most downstream node from activating, as this is the main method that combined BRAF and MEK inhibition causes resistance. Apoptosis may be encouraged and survival signaling may be disrupted by blocking ERK1/2, which is known to induce signaling feedback and be linked to resistance to MEK inhibition [49], [50].
Targeting ERK1/2 is a potentially effective treatment approach for thyroid cancer. For individuals with advanced and refractory forms of thyroid cancer, blocking ERK1/2 may help overcome resistance mechanisms linked to BRAF and MEK inhibitors, thereby enhancing treatment outcomes [51], [52].

2.2.4.Other cancers

A key player in the family of MAPK enzymes, Numerous functions are played by ERK1 in tumors growth as well as cancer progression. For example, in cancer of breast activated ERK1 promotes invasion ,proliferation of cells and tumor spreading and growth in colorectal cancer. The same is true for lung cancer, where ERK1 activation increases tumor cell resistance in cancer of lung to survival and therapy. Another setting in which abnormal ERK1 activation is associated with aggressive tumor activity and chemoresistance is pancreatic cancer, underscoring the importance of this protein as a target for treatment.
Thus, there is considerable therapeutic promise for ERK1 signaling targeting in these cancers. Cancer progression can be impeded by blocking ERK1 activity, by interfering downstream signaling pathways which causes tumor cell invasion, proliferation, and metastasis. Additionally, ERK1 inhibitors provide a focused strategy to address chemoresistance, a frequent problem in the treatment of cancer.

2.3. ERK, or extracellular signal-regulated kinase

ERK, or extracellular signal-regulated kinase functions as the terminal enzyme in a cascade within the MAPK pathway, which governs essential life processes like cell survival, migration, and proliferation [32]. Consequently, dysregulation of ERK signaling often leads to the formation and dissemination of malignancies. In light of this significance, aiming as possible potential treatment of cancer, ERK has become known. ERK1 and ERK2 both, being serine-threonine protein kinases, are widely expressed and evolutionarily conserved, playing pivotal roles in regulating a multitude of critical biological functions.

ERK1/2 refers to the combination of human ERK1 and ERK2, which have an amazing 84% sequence similarity and similar activities [4]. Like many other protein kinases, ERK1/2 has unique C- and N-terminal extensions that confer selectivity for signaling. The kinase insert domain, a 31-amino acid insertion located inside the kinase domain of both ERK1 and ERK2, increases functional selectivity. Additionally, the N-terminal part of ERK1 contains a 17-amino-acid-residue insertion. Despite these structural differences, the rat and mouse ERK2 enzymes have 358 amino acid residues compared to the 360 amino acid residues in the human ERK2 enzyme. In contrast, human ERK1 contains 379 amino acid residues, whereas the rat and mouse ERK1 enzymes have 380. It is noteworthy that there is more divergence between ERK1 and ERK2 within a single species than there is between them [4], [53].

A number of illnesses, including cancer, have been linked to abnormal ERK signaling because of its crucial role in controlling important processes in cells. As a result, ERK1/2 targeting has gained attention as a possible therapy for the treatment of cancer [5]. Thus, targeting this signaling system in disease management may have therapeutic benefits since it may be feasible to stop tumor growth and metastasis by reducing ERK1/2 activation.

2.4. ERK1(MAPK3):- Structure and Function

ERK1, sometimes referred to as MAPK3, a 379-amino acid-containing 43 kDa protein. It shares an impressive 85% identity with its counterpart, ERK2 (MAPK1), and their substrate binding regions exhibit even greater similarity Two DXXD docking sites are present on both ERK1 and ERK2, and these docking sites work as points of interaction with a Kinases Interaction Motif (KIM) that is present on substrates like ELK-1, activators like MAPKK, inhibitors like dual-specificity phosphatase , PTP-SL (PTPRR) [24], [54].

One characteristic that makes ERK1 unique from other proteins is the TEY (Thr-Glu-Tyr) motif that is found inside its activation loop. The tyrosine (Tyr204) and threonine (Thr202) residues of ERK1 (MAPK3) must be dual phosphorylated in order for the protein to fully activate [24], [53]. After activation, ERK1 translocates into the nucleus to carry out its regulatory actions by phosphorylating the transcription factors [55].
In addition to being involved in substrate phosphorylation, ERK1 is essential for several cellular functions, including survival, differentiation, and proliferation [32]. The disruption of ERK1 signaling, which has been connected to several diseases, including carcinoma, presents an attractive target for a carcinoma therapy [56]. Modulating ERK1 activity is one method of regulating cellular responses and maybe reducing the course of the illness.

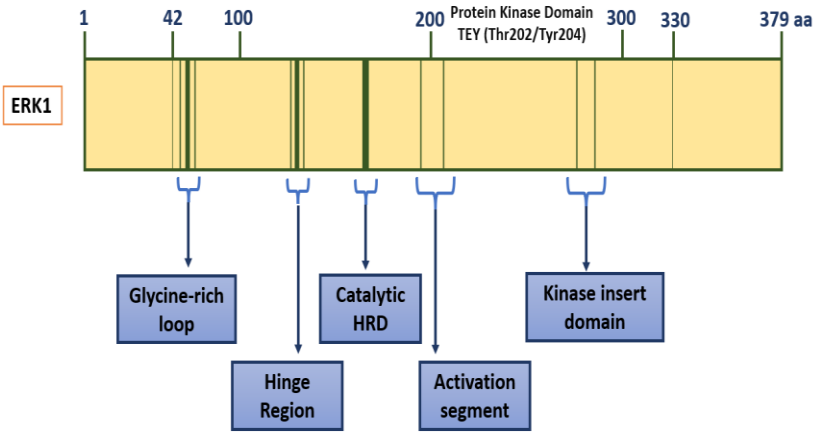

Fig. 2.1 Structural organisation of ERK1

2.5. ERK/MAPK Signaling Pathway/ Cascade

ERK1 (MAPK3), a vital member of the physiological ERK (MAPK) signaling cascade. Because ERK1 and ERK2 have comparable roles in this circuit and share 84% sequence identity, they are frequently referred to as ERK1/2 together. Prominent within the MAPK subfamily, ERK1/2 participates in a tripartite enzymatic cascade

wherein Raf plays the role of a MAPKKK (MAPK kinase kinase), MEK works as a MAPKK (MAPK/ERK kinase), and ERK1/2 acts as a MAPK [57].

ERK1/2 exists within the cytoplasm under normal circumstances. A set of variables, including growth factors, neurotransmitters, inflammation, ischemia, or hypoxia, can stimulate cells intracellularly or extracellularly. Ras is triggered by tyrosine kinases, which are triggered by their corresponding receptors. Translocation of Raf takes place from the cytoplasm to the membrane of cell when activated Ras (Ras-GTP) attaches to it, triggering the signaling process [58], [59].
So, by phosphorylating serine residues, Raf activates the catalytic domain of MEK. ERK1/2 is then activated as a result of MEK's phosphorylation. After being activated, ERK1/2 can control the activity of other protein kinases or phosphorylate target proteins in the cytoplasm. Significantly, phosphorylating several transcription factors like Elk-1, c-Myc, STATs, Jun, Fos, ATF2, and Max can also be accomplished by activated ERK1/2 entering the nucleus [11].

The most studied of the MAPK family of pathways is the ERK1/2 pathway. Tyrosine kinase receptors, such as the EGF receptor (EGFR), bind to and become active when exposed to extracellular growth factors like EGF [60]. This process is known as activation. SOS was drawn protein to the cell membrane when this interaction finds a docking site for the adaptor protein GRB2 . SOS activates Ras by assisting GDP in converting to GTP and generating Ras-GTP. Raf, AF6, phospholipase C, PI3K, are some of the downstream proteins that activated Ras affects [61].
Also, phosphorylation of transcription factors including ELK1,FOS, Jun, ETS, Sp1, and myc can induce genes expression which are in relation to the proliferation and cell cycle . ERK1/2 is activated in order to accomplish this [11]. Moreover, activated ERK1/2 can phosphorylate several kinases which are intracellular, such as MSKs, MNKs and RSKs, influencing cell adhesion and proliferation.

Owing to its crucial role in these processes, ERK1 presents itself as a potentially valuable candidate for therapeutic strategies aimed at managing the advancement and management of cancer.

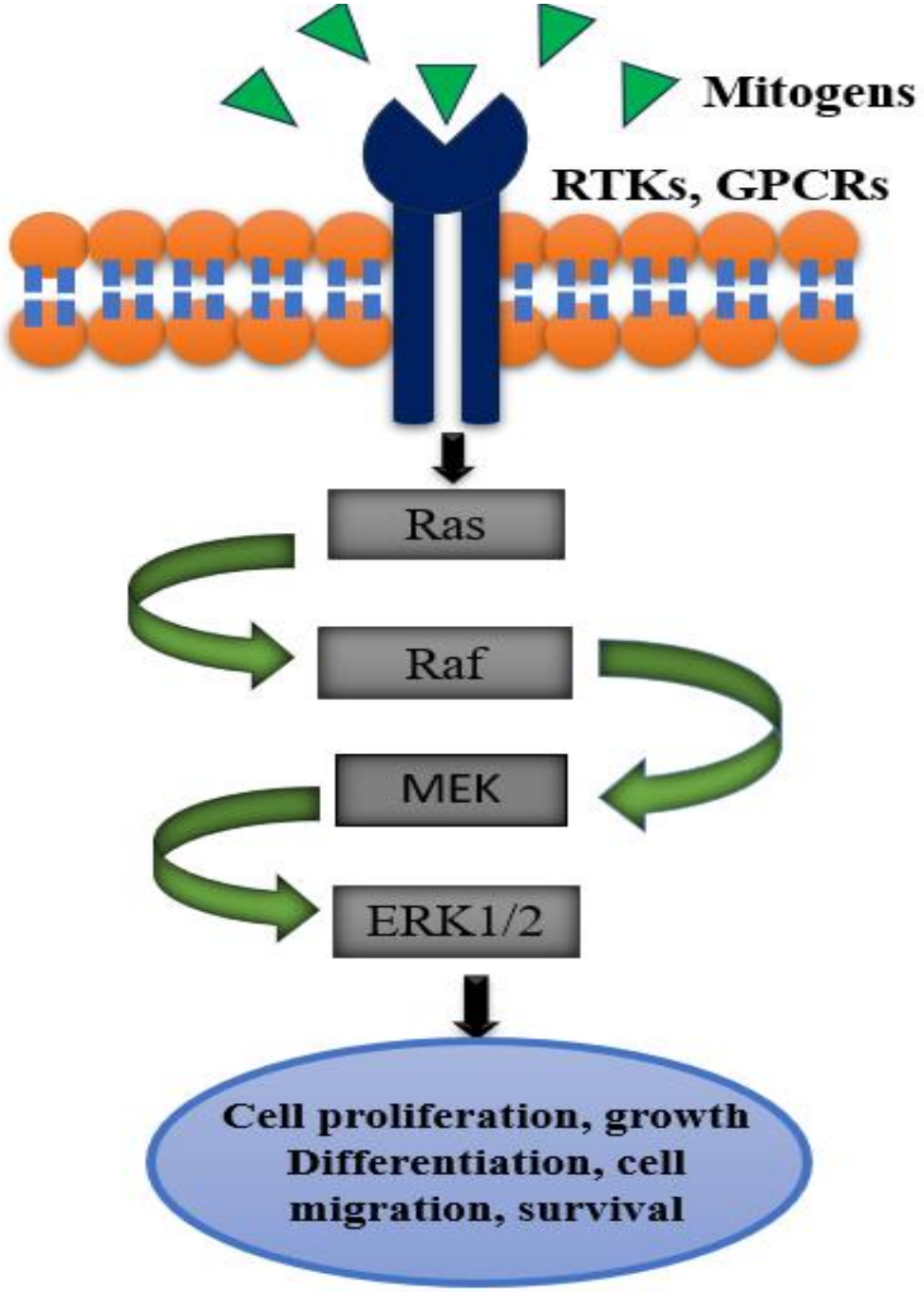

Fig. 2.2 ERK/MAPK Signaling Pathway/ Cascade

2.6. Dysregulation ERK/MAPK Signalling Pathway/ Cascade

Dysregulation of the RAS-ERK cascade is a critical determinant in the development of many forms of cancer [62]. ERK cascades hyperactivation is present in majority of malignancies, and mutations which are activating in this cascade are among the majority of the common carcinogenic drivers of all malignancies. Mutations in various cascade components are frequently observed in cancers affecting humans. For instance, RAS (mostly K-Ras) mutations are the most common, making up 10% of cancer cases or over 30% of all forms of cancer [63]. Roughly 8% of all malignancies have mutations in the RAF proteins, particularly B-Raf. Primary disease-causing mutations in ERK itself are uncommon, despite MEK mutations being less prevalent (around 1%) [64], [65].

The RAS-ERK pathway has a high mutation rate, and oncogenes that are not directly upstream can indirectly activate ERK1, which has led to extensive efforts to create inhibitors that target different parts of the route. These initiatives have produced encouraging outcomes that have advanced cancer treatment [66].
The RAS-ERK pathway has a high mutation rate, and oncogenes that are not directly upstream can indirectly activate ERK1, which has led to extensive efforts to create inhibitors that target different parts of the route. These initiatives have produced encouraging outcomes that have advanced cancer treatment [67].

A promising treatment approach is to target the ERK pathway, given its central role in cancer. With positive outcomes from testing, inhibitors that target the RAS-ERK pathway's many components—including RAS, RAF, and MEK—have been created. By inhibiting hyperactivated ERK1 signaling, these inhibitors seek to slow the growth and spread of tumors [68]. Additionally, a wide range of malignancies may be treated by medicines that target ERK1 or its upstream activators because there are numerous carcinogenic cues that might activate ERK1. The discovery of particular ERK1 inhibitors is particularly notable [67], [69]. By obstructing the final step in the MAPK pathway, these inhibitors' function and which stops ERK1 from becoming phosphorylated and activated. By doing this, they hope to interfere with the feedback loops that frequently result in resistance to other treatments that target upstream elements like as MEK and BRAF. This method improves the effectiveness of cancer therapies while also aiding in the overcoming of resistance.

The attention being paid to ERK1 and the elements of the pathway it is linked to is a strategic step forward in the treatment of cancer. To increase the chances of lifespan and the standard of living for individuals suffering from malignancies brought on by this dysregulation, researchers and physicians aim to block ERK1 signaling and make more effective treatments available to these patients.

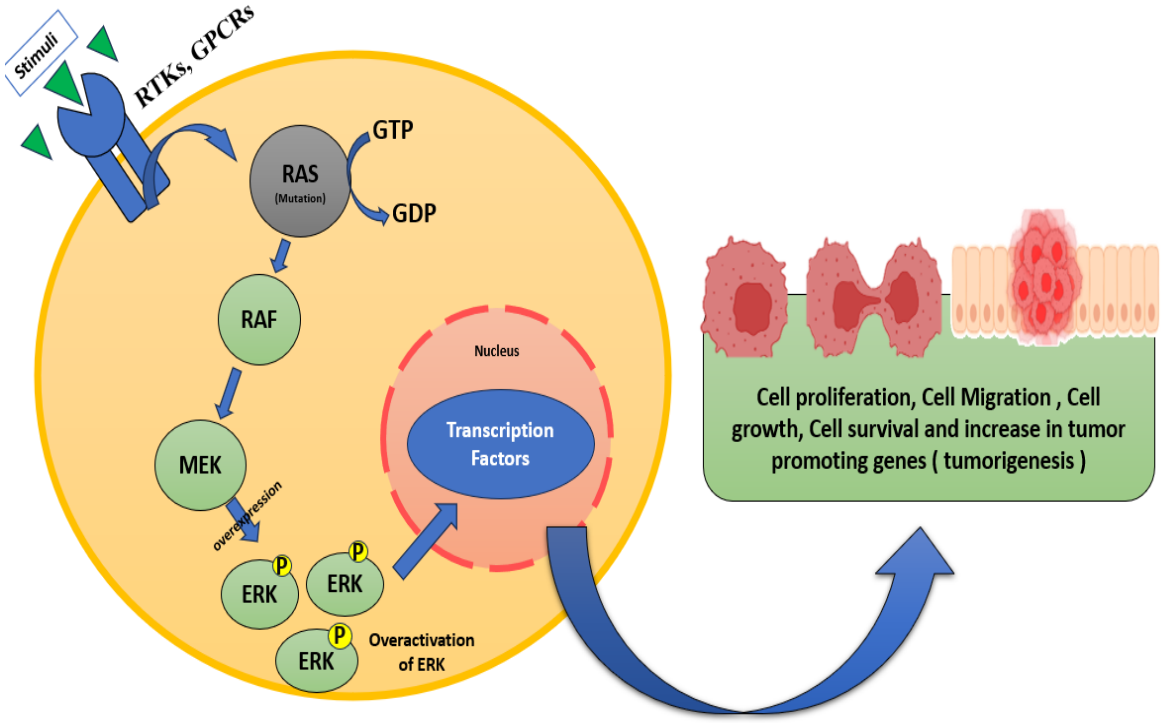

Fig. 2.3 Dysregulation ERK/MAPK Signalling Pathway/ Cascade

2.7. ERK1(MAPK3) as a therapeutical potential

ERK1(MAPK3), is an essential part of the signaling pathway of ERK/MAPK, which serves as a crucial system for cellular communication [3], [69], [70]. [3], [63], [66]. Because of its crucial function in cancer etiology, ERK1 offers itself as a potentially effective therapy candidate. Its significance in cancer biology is highlighted by its involvement in the ERK/MAPK signaling pathway/ cascade which regulates various life processes via transmission of signals from outside of cell to its inside. By phosphorylating multiple downstream cytoplasmic proteins in this pathway nuclear transcriptional factors like c-Fos , c-Jun which are activated by MAPK3., which in turn promotes cell division and death [11], [71].

ERK1 Overexpression or heightened activation are linked to the start, development, and spread of several forms of cancer, including , gall bladder , lung, pancreatic, hepatocellular, and thyroid cancers etc [68], [72]. With its ability to interfere with downstream signaling pathways that promote tumor cell proliferation, invasion, and metastasis, targeting ERK1 provides a versatile strategy for cancer treatment. In instance, when traditional medicines fail to stop the spread of cancer, ERK1 inhibitors may help overcome chemoresistance, a typical problem in cancer treatment.

Furthermore, investigations showed that upregulating miR-143 to target ERK1 (MAPK3) prevented the cellular growth associated with cancer of the breast, while Cao et al. found that gastric cancer cells resistant to cisplatin had a link between reduced expression of miR-129 and increased expression of ERK1 (MAPK3). Apoptosis was increased and cell proliferation was suppressed as a result of the efficient downregulation of ERK1 (MAPK3) caused by miR-129 overexpression [73], [74].

These studies highlights the major role of ERK1(MAPK3) in drug resistance and carcinogenesis across various cancer types, demonstrating its possible use as a candidate for cancer therapy.

2.8.Computational Approaches in Drug Repurposing and Analysis

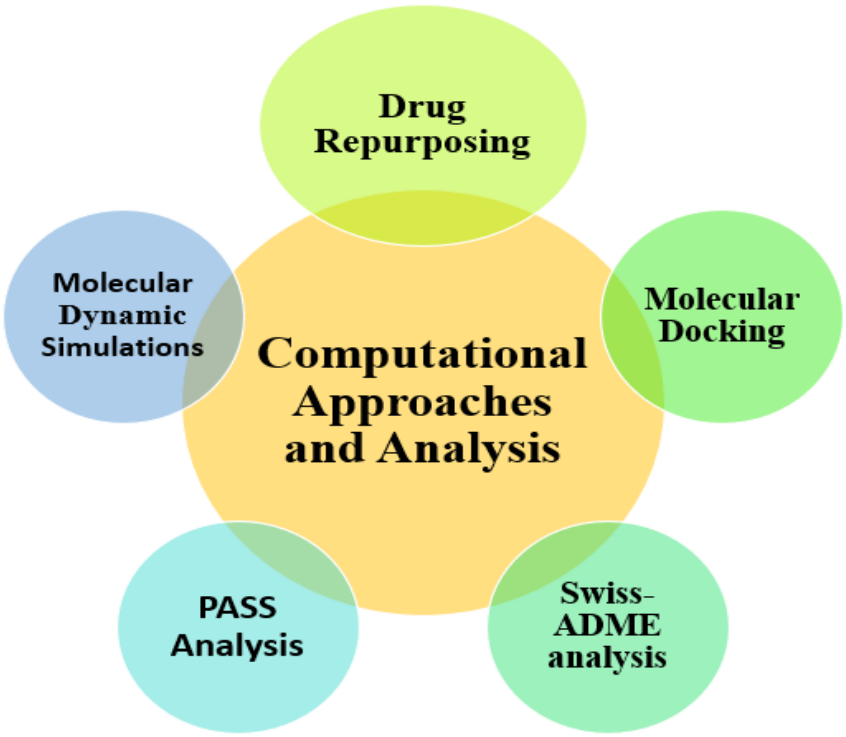

Fig. 2.4: Computational Approaches and analysis

2.8.1. Repurposing drugs Approach

Repurposing drug is the process of identification of novel pharmacological applications for pharmaceuticals or prodrugs approved by FDA, which are already on the market, unsuccessful, in development, or have already been commercialized. Drug repositioning, recycling , re-tasking, rescue , reprofiling, redirection, and therapeutic

switching are some other names for it [75], [76]. Existing drugs will discover new therapeutic applications through this approach, regardless of whether they have been approved, discontinued, abandoned, or are still in the experimental phase. The process of developing a conventional medicine is costly, labour-intensive, time-consuming, and dangerous. . Yet, medication repurposing presents a fresh approach that might be able to get by the exorbitant expenses, protracted development periods, and higher failure rate connected with conventional techniques. Saving an average of 5-7 years, it considerably lowers the probability of failure, which is approximately 45% in traditional drug research because of safety and toxicity issues [77], [78]

Insilico-based and experiment-based methods are the two main categories of drug repurposing techniques. Experimental approach, aka repositioning which is activity-based, looks for new pharmacological uses for the original drugs through experimental testing. This strategy involves screening which are organism/cell , protein target-based in invitro or in-vivo model of disease, without the need of knowledge of target protein structure. Animal models, cell assays, target screening, and clinical procedures are a few examples of the particular experimental repositioning techniques [79], [80]. Conversely, in silico repurposing virtually examines public databases including chemical/drug libraries by utilizing cheminformatics/ bioinformatics computational biology approaches. This method finds potential bioactive chemicals by analysing the chemical interactions between drug molecules and protein targets [81].

Repurposing pharmaceuticals is beginning to plays a role which is important in accelerating the development of drugs process as well as satisfying urgent needs of exhausting healthcare systems. Phase II clinical trials are the next step after selecting potential drugs, assessing their effectiveness with preclinical models, and so on [82].

To find drug candidates, computational and experimental methods are employed, frequently making use of open drug databases. The approach incorporates data from published human studies, anecdotal information on off-label applications, clinical trials, primary and translational research, and other sources. Drug-protein target interactions are methodically identified through the use of bioinformatics tools and artificial intelligence algorithms.

2.8.2. Molecular Docking

An essential bioinformatics-based theoretical modelling technique used to investigate the profiles of ligand-protein interactions, forecast binding conformers, and determine affinity is molecular docking [17]. Computational techniques, which were first developed in the 1980s, have been essential in transforming the drug development process. Because of their limited computational power, early molecular modelling methods provided a strict interpretation of ligand-protein interactions [83], [84]. However, advancements in computer technology over time have made it feasible to model dynamic interactions between proteins and ligands.

This approach entails examining the arrangement and direction of molecules, called "pose," inside a macromolecular target's binding site. Possibilities are generated by a variety of searching algorithms and then assessed and ranked using scoring methods [85]. Among the most notable developments in molecular docking is the creation of scoring functions, which are essential for grading the ligand poses that are created. To assess the binding energy and contact stability between the ligand and the protein target, these scoring functions take into account a number of different factors. Research seeks to raise the predictability of molecular docking simulations via increasing the accuracy and consistency of scoring functions, a task made easier by the continual advancements in computing algorithms and machine learning approaches. A number of software solutions, including well-known systems like GOLD, Auto Dock Vina, Instadock , Molegro Virtual Docker and Pyrx were used for molecular docking [86], [87], [88].

Furthermore, molecular docking has been used for purposes other than conventional drug development [83], [89]. It is being used more and more in various fields, like virtual compound library screening, structure-based medication creation, and comprehending the molecular underpinnings of ligand selectivity and specificity. Furthermore, molecular docking has been included into more extensive computational workflows that include pharmacophore modelling, free energy computations, and molecular dynamics simulations [21], [90]. This has made it possible to investigate ligand-protein interactions in greater detail and in several dimensions.

As a result, molecular docking remains a fundamental component of contemporary computational biology and drug discovery, providing insightful information on the molecular mechanisms behind ligand binding and directing the development of innovative treatments. The relevance of molecular docking is expected to grow as computational techniques and technology progress, spurring innovation and speeding up drug discovery initiatives.

2.8.3. Swiss-ADME analysis (ADMET property analysis)

A drug needs to be at its target concentration in the body and in a bioactive state for a long enough interval of time which causes the desired biological reaction in order to be effective. Absorption, distribution, metabolism, excretion, and toxicity (ADMET) evaluation must be completed early in the drug development process, particularly when there are many candidate compounds and few physical samples [91]. In order to guarantee that only the most promising candidates proceed through the development pipeline, ADMET property analysis is essential for predicting the drug's pharmacokinetic and toxicological profile. Computer simulations provide a good substitute for experimentation in this situation. SwissADME is a free online tool that provides a variety of fast and precise predictive models for drug-like characteristics, pharmacokinetics, physicochemical properties [92]. This tool incorporates cutting-edge internal techniques including Bioavailability Radar, iLOGP, and BOILED-Egg [93], [94]. Key parameters for different molecules can be easily predicted with SwissADME's user-friendly interface, which is available without requiring a login at http://www.swissadme.ch. This makes it simple for both experts and novices in

cheminformatics or computational chemistry to use. By facilitating the assessment of ADMET features, the tool helps scientists spot any problems early during discovery of drugs, which boosts overall effectiveness as well as success rate of creating new drugs for therapy.

2.8.4. Biological Activity Prediction through PASS Analysis

The idea of a biological activity spectrum was established in an attempt to characterize the properties of substances that are biologically active [95]. The PASS program, a helpful resource which helps in discovering novel ligands for specific biological targets [96]. Using substance structural formula, it can forecast so many metabolic , biochemical pathways and pharmaceutical effects. Furthermore, an online user interface has been created for the PASS program [97].

A computational technique method PASS is used to analyse structure-activity relationships (SAR) [97]. Multilevel Neighbourhoods of Atoms (MNA), a type of 2D atom-centric substructural descriptor, are used to depict chemical compounds. Understanding ligand-target interactions is made possible by MNA descriptors, which have shown efficacy in qualitative SAR investigations [98], [99]. When it comes to uncharged, single-component molecules with three or more carbon atoms and a molecular mass less than 1,250 Da, PASS forecasts biological activity profiles. By limiting the number of non-specific or unusual biological activities, these criteria guarantee the inclusion of the majority of drug-like compounds [97], [100].

One of the products of PASS is a ranking of likely biological activities based on Pa-Pi values, in which Pa is the likelihood that a molecule is active and Pi is the likelihood that it is inactive [98]. To separate active molecules from inactive ones, a default cutoff of Pa-Pi > 0 is applied [99], [101]. A list of molecules that are anticipated to be active is produced by PASS virtual screens of chemical libraries, and these molecules can then be suggested for additional biological testing.

2.8.5. MD simulations

MD simulations has come a long way since it was first created in the late 1970s [102], [103]. Today, it can simulate systems with hundreds of atoms and can also model larger macromolecular structures like ribosomes and nucleosomes, as well as with clear solvent representations of whole proteins in solution and membrane-bound proteins [104], [105], [106]. Since high-performance computing (HPC) facilities are available, simulations with fifty thousand to one lakh atoms are routinely performed, and simulations with five lakh atoms are often undertaken. The improved capabilities of HPC and the ease of use of the fundamental MD algorithm are primarily responsible for this advancement [107], [108].

MD simulations helps in predicting the motion of atoms individually over time in a protein as well as in other molecular systems with the help of physics general model which underlies interactions between different atoms. The femtosecond temporal

resolution of these simulations allows for the recording of vital biomolecular processes, like binding of ligand, folding of protein and its conformational changes. Also, it predicts various perturbations of biomolecules at atomic level. [109].

Core idea of MD simulations is to forecast the spatial locations of atoms over time in a biomolecular system. A 3D track of atomic movements is created by computed forces, iterative updates to locations and velocities, and initial atom positions. These simulations allow for exact control over simulation circumstances, including mutations, ligand binding, protein conformation, and environmental variables. They also provide extensive information at atomic level which is very challenging in obtaining on experimental basis. Based on experimental data and quantum mechanical computations, the forces are computed using a molecular mechanic force field [110].

When it comes to drug research, simulations are quite helpful in modifying ligands by improving their properties and efficacy, which helps in optimizing leads. Simulations can provide qualitative data for the guidance of the optimization process of ligand, for example, by emphasizing significant interactions between a ligand and binding pocket, which tells about the binding pocket rearrangement when ligand is bound, or assessing and refining potential poses of ligand [111], [112].

Many important drug discovery targets in brain, including GPCRs, ion channels, and transporters, now have structures thanks to recent developments in structural biology. To fully realize the potential of structure-based drug development, it is imperative to consider the dynamic properties of these targets [113]. MD simulations are therefore an important tool in contemporary biomedical research since they guide studies and offer comprehensive insights that improve the drug discovery process [21].

Also, the dynamic behaviour of proteins which are oncogenic and their possible interactions with possible therapeutic medications are particularly well-understood by researchers because of MD simulations. By providing insight into mutations which occurs due to change in function and structure in cancer-related proteins, this thorough understanding of the atomic level can aid in the development of more focused and effective therapies for cancer [110], [114].

So, MDS helps in the identification of promising drug candidate and the optimization of their efficacy by simulating the binding processes and conformational changes of these proteins. This ultimately led to the creation of focused cancer treatments.

CHAPTER 3

METHODOLOGY

3.1. Computational Resources

Databases utilized: The following databases were employed for literature review, acquisition of protein targets, small molecule (ligand) library acquisition, data retrieval, and evaluation.

- PubMed: PubMed is a free online resource that makes it easier to search , find and retrieve biomedical and life sciences literature with the goal of improving people's and the world's health. https://pubmed.ncbi.nlm.nih.gov
- Drug Bank - Drug Bank is a crucial resource for pharmaceutical research, offering extensive and reliable drug data that is ready for immediate use or seamless software integration. https://go.drugbank.com
- PubChem - PubChem is a National Institutes of Health (NIH) open-access chemical database. Data on both large and small molecules, including carbohydrate, , peptide, lipid modified macromolecule and nucleotide have been made available since 2004. It has been a vital resource for students, researcher, and the general public. https://pubchem.ncbi.nlm.nih.gov
- PDB- Encouraging research in biotechnology, energy, and health, it offers free access to 3-Dimensional structural information for DNA, RNA and proteins. This resource is crucial for understanding molecular roles in health and sustainability. https://www.rcsb.org
- Swiss ADME: An online tool was utilized to help identify potential therapeutic candidates by offering understanding of pharmacokinetic , drug-like property, and other ADME parameters of small compounds. http://www.swissadme.ch/
- pKCSM- It is a computational tool that estimates pharmokinetic, property, ADME and toxicity of small compounds, hence assisting in the design of new drugs. It aids in the identification and improvement of possible therapeutic options by researchers. https://biosig.lab.uq.edu.au/pkcsm
- Pass Webserver- It forecasts a molecule's biological properties based on the relationship between its structure and activity. It makes use of the ratios Pa to Pi, where larger Pa denotes a greater chance that the desired characteristics will be present.

Software Used: The listed bioinformatics tools and softwares were employed for our research study.

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- InstaDock- It is a virtual high-throughput screening and molecular docking graphical user interface (GUI) built on Python. With just one click, users may automatically dock vast compound databases against protein targets. It also offers tools for visualizing and analysing the results, helping to uncover potential lead molecules. It is intended for Windows-powered systems [88].
- Pymol- PyMOL is a widely used open-source model visualization tool in the field of structural biology [115].
- Discovery Studio Visualizer - It is an extensive molecular modelling and visualization tool enabling users to analyze and visualize biological and chemical data. It supports drug discovery by providing detailed 3D representations of molecular structures and interactions[116].
- GROMACS- This is freely available software suite is intended for output analysis and high-performance molecular dynamics application . It enables simulations of proteins, lipids, and nucleic acids in systems with varying particle counts, backed by an active user and developer community [117].

3.2. Work Flow

Based on a comprehensive literature review , it was found that increased MAPK3/ERK1 expression and activity is strongly involved in the beginning, developing, as well as spreading of different cancers, suggesting ERK1(MAPK3) a promising target in cancer therapy. To repurpose FDA-approved drugs for ERK1(MAPK3) targeting, a library of 3,674 drugs was sourced from Drug Bank. Ligand structures were generated from PubChem and Open Babel was used to convert it into .pdbqt format . Further molecular docking was performed with InstaDock, followed by affinity analysis using Biovia Discovery Studio Visualizer. Compounds with high affinity were then evaluated for favourable ADMET properties using SwissADME and pkCSM whereas lead candidate biologically activity were predicted by PASS website. Additionally, in order to evaluate the ligand-receptor complexes' dynamic behaviour and stability, MD simulations were run. This helped identify viable candidates for validation through experimentation.

3.2.1. Data Extraction

The RCSB database (PDB ID: 4QTB) provided the 3-dimensional structure of the ERK1 Molecule/Protein at X-ray resolution of 1.4 Å. DrugBank database, was used for obtaining a collection of 3,674 drugs approved by the FDA. These ligands' 3-D structures were created by PubChem. Additionally, the structure of a well-known ERK1 inhibitor, SCH772984 (reference drug), was also obtained from PubChem and it was also used as a control or for the study of ERK1 .

3.2.2. Target Receptor Preparation

The 3-Dimensional structure of ERK1 was obtained using the RCSB PDB database (PDB ID: 4QTB, 1.4 Å resolution). Chain A, including 351 residues , was analyzed. Heteroatoms, ligands which were co-crystallized, molecules of water were all

eliminated. For virtual screening in InstaDock, structure of ERK1 was prepared . PyMOL software was used to visualize and modify the structure, including the removal of water molecules

3.2.3. Ligand molecules Preparation

3,674 drugs approved by the FDA were obtained from Drug Bank in order to repurpose them against ERK1. PubChem was utilized to generate the three-dimensional structures of the ligands in the SDF format. The Open Babel program was then utilized for conversion of these ligands from the SDF file format to the.pdbqt file format.

3.2.4. Molecular Docking Based Screening/ Studies

Using the free GUI application InstaDock, molecular docking was done following the receptors' preparation as well as structures of ligands. Following docking, the results were recorded in an Excel sheet that noted the binding affinities of each ligand for the receptor protein. Each docked ligand's unique output file, which described interactions and H-bonds forms between protein and ligand , was retained for further 2D interaction analysis. Biovia Discovery Studio Visualizer was used to observe as well in assessing these interactions. Further analysis was performed on compounds whose binding affinities were higher than -10.9 kcal/mol for SCH772984. (Table 4.1)

3.2.5. Interaction Analysis

From the output files of the 14 compounds that were chosen, all potential docking conformations were retrieved in order to study different interactions and then various bonds and forces responsible for binding ERK1 (MAPK3) and the ligands were examined. Each compound's docking conformations were saved for subsequent 2-D interaction analysis, where used specific interactions of the 14 hits with ERK1 (MAPK3) binding site were identified using Discovery Studio Visualizer and PyMOL.

3.2.6. ADMET Property Analysis

ADMET attributes/ characteristics/property were analyzed of the compounds which were having high binding affinity with ERK1 than the reference drug using the different webservers like SwissADME , pkCSM. Further analysis was done for the selected compounds who were not having any hazardous patterns but having favourable ADMET properties as well as drug like characteristics [80].

3.2.7. Biology Activity Prediction (PASS Analysis)

The prediction of biological activity is essential in the search for new drugs. The biological characteristics of the chosen compounds (ergotamine and midostaurin) from the ADMET filter were predicted using the PASS webserver, as indicated in the table.

3.2.8.MDS

MD simulation is a strong technique for examining the motion of atoms of proteins and interactions of protein with ligands [118], [119]. Its experiments validated the results which were taken from docking the interactions between the selected drugs (ergotamine , midostaurin) and ERK1. Using GROMACS v5.5.1, we simulated the coordinates of structures of ERK1 and its complexes with ergotamine and midostaurin [120]. By parameterizing the substances using the CGNF server, a reputable resource for producing topological coordinates of tiny molecules, such as receptor-ligand complexes topological coordinates were created. Using (SPC216) water model, each system was submerged in a cubical box , from edges that was 10 angstroms away. Furthermore, by adding the proper counterions (Na+ and Cl), the simulated systems were rendered neutral.. Under periodic boundary settings for 100 ps, a two-step equilibration process with volume which was constant, heating which was done gradually from 0 to 300K temperature, and pressure of 1 atm was done . For simulations, we used the force field Charmm 36. Also, 100 ns of simulations were run for every system. We used GROMACS technologies, such as QTGRACE software, to analyze the obtained data and create different MD plots in order to assess the stability of the complexes composed of protein-ligand [120], [121], [122], [123], [124].

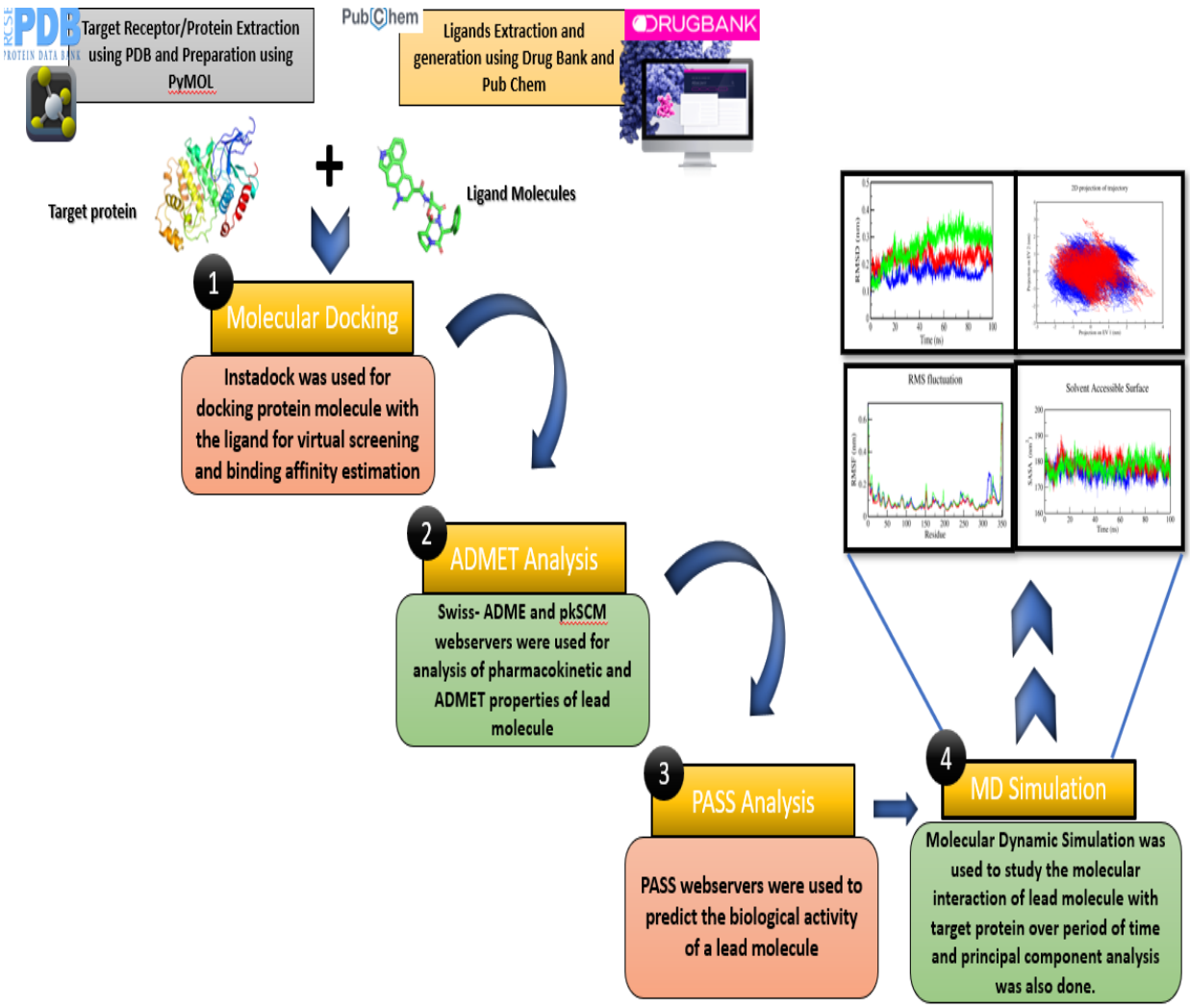


Fig. 2.4 Overview of Methodology

CHAPTER 4

Results and Discussion

4.1. Molecular Docking Based Screening/ Studies

Computational method for identifying possible chemicals against predetermined biological targets is called virtual screening [80]. A molecular docking investigation using InstaDock revealed that 14 of the 3,647 FDA-approved drugs were chosen because of their high binding affinities, which ranged from -11 to -12.3 kcal/mol. These affinities were higher than that of the reference drug, SCH772984 with binding affinity of -10.9 kcal/mol. Table 4.1 includes information about reference drug (SCH772984) in addition to top ligands list with highest negative binding affinities. Discovery Studio Visualizer and PyMOLwere further utilized to conduct a thorough investigation of these 14 hits in order to look at their unique interactions with the ERK1.

Table 4.1 Binding affinities of top 14 ligands and reference drug

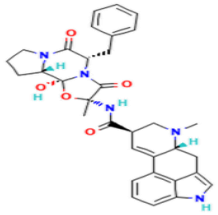
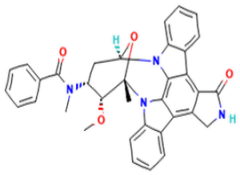
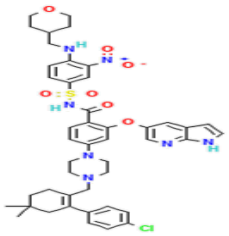
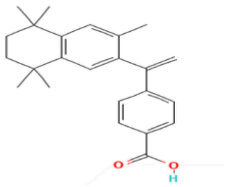
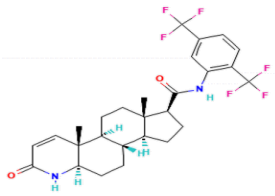
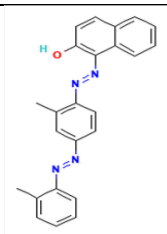
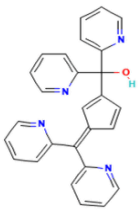
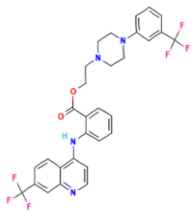
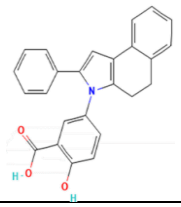
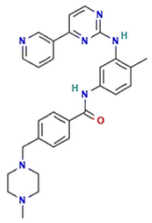
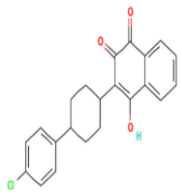
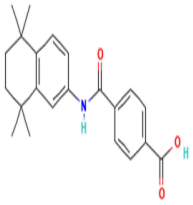
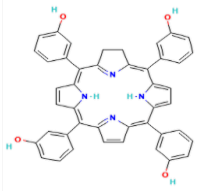
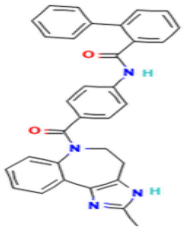
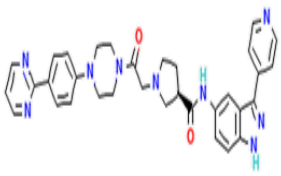
S. No.	Pub-Chem ID	Name of ligands (Drug Molecules)	Structure Of ligands	Binding Affinity (Kcal/mol)
1.	8223	Ergotamine		-12.3
2.	9829523	Midostaurin		-12.1

Table 4.1 (continued)

3.	49846579	Venetoclax		-12
4.	82146	Bexarotene		-11.8
5.	6918296	Dutasteride		-11.7
6.	62330	Scarlet red		-11.6
7.	3052765	Pyrinoline		-11.5
8.	68723	Antrafenine		-11.5

9.	40821	Fendosal		-11.4
10.	5291	Imatinib		-11.4
11.	74989	Atovaquone		-11.3
12.	108143	Tamibarotene		-11.2
13.	60751	Temoporfin		-11.1
14.	151171	Conivaptan		-11
15.	24866313	SCH772984 (reference drug) (Merck/ ScheringPlough)		-10.9

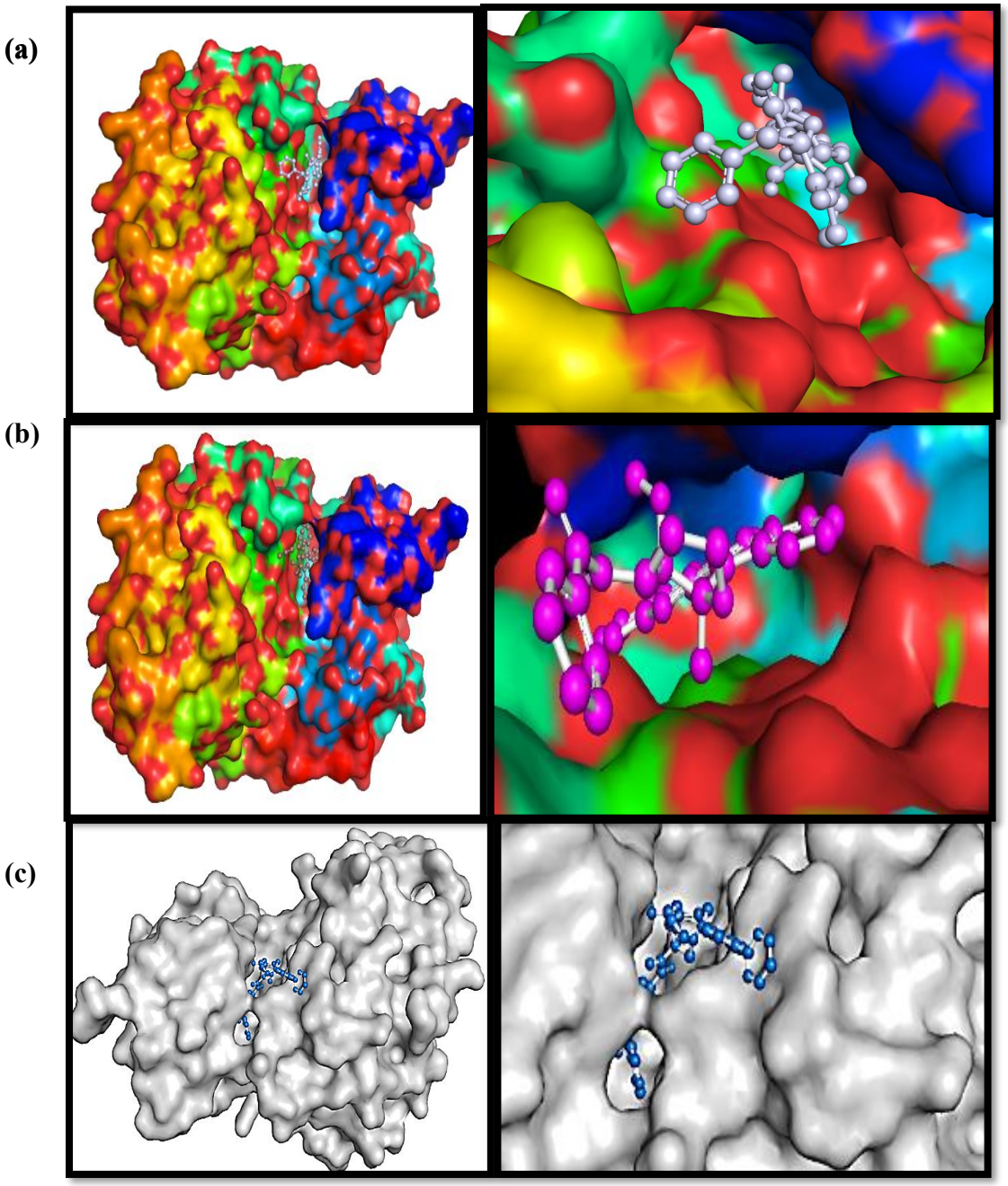


Fig. 4.1: Molecular Docked Complexes: (a)ERK1-Ergotmine (b)ERK1-midostaurin (c) ERK1- SCH772984 (reference drug)

4.2. Interaction analysis

The analysis of the various interactions between the ligands and ERK1 (MAPK3) involved a comprehensive exploration of the various bonds and forces responsible for binding. All conformations of docking of the 14 selected compounds were extracted from the output files for this analysis. Furthermore, individual output files for each docked ligand were stored for subsequent 2-D interaction analysis, highlighting every interaction and H-bond formation between the ligand and the receptor .Utilizing PyMOL and Discovery Studio Visualizer, a detailed investigation was conducted to identify and document the specific interactions of these ten hits with the ERK1 (MAPK3) binding site.

The interactions were meticulously analysed, encompassing H-bonds and other various interactions like pi-pi , electrostatic , van der Waals interaction etc. as shown in Table4.2. This comprehensive investigation provides valuable insights into the binding affinities, patterns, and specific interaction mechanisms that contributing to the overall stability and efficacy of receptor- ligand complexes. These findings are crucial for assessing ligand-protein interactions, prioritizing the most promising candidates for further research, and guiding drug discovery efforts to identify viable therapeutic targets.

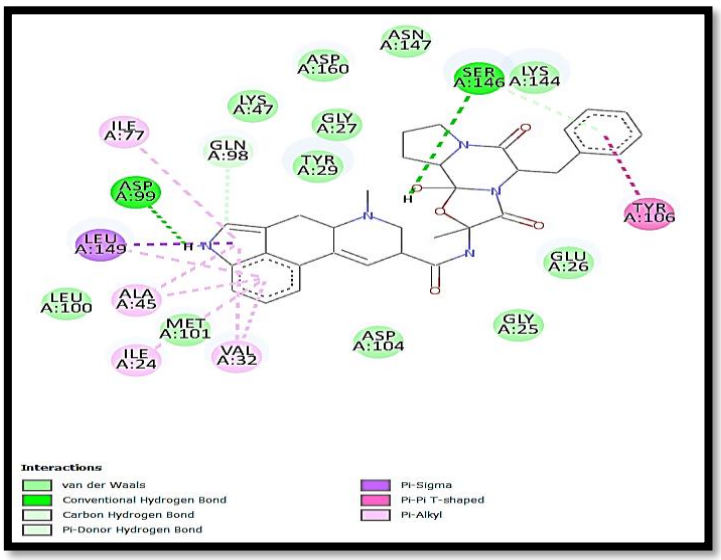
Table 4.2. Interaction analysis showing different amino acids involved in various binding interactions

S.No.	Pub-Chem ID	Name of Ligands (Drug Molecules)	Amino acids are involved in different types of binding interactions	
			Hydrogen bonds	Other interactions
1.	8223	Ergotamine	ASP-99,SER-146,GLN-98,LYS-144	ASP-160, ASN- 147, LYS-144, GLU-26, GLY-25, ASP-104, MET-101, LEU-100, LYS-47, GLY-27, ILE-77, TYR-106, VAL-32, ILE-24, ALA-45, LEU -149
2.	9829523	Midostaurin	ASP-160, SER-146 , GLU 26	LYS- 110, ILE-24 ASP-104, GLY-25, ASN- 147, LYS-144, TYR-106, LEU-149, TYR -29, LYS-107, VAL-32
3.	49846579	Venetoclax	ASP-160, ARG-60	GLU-64, THR-183, GLU-26, GLY-25, LEU-100 MET-101, GLU-102, THR -103, LYS-107, GLN-98, ILE-77 TRP-185, SER -146, LYS-47, TYR-106, PRO-145 , ALA-28, ILE-24, CYS-159, LEU-149, VAL-32, ILE-24, LYS-144

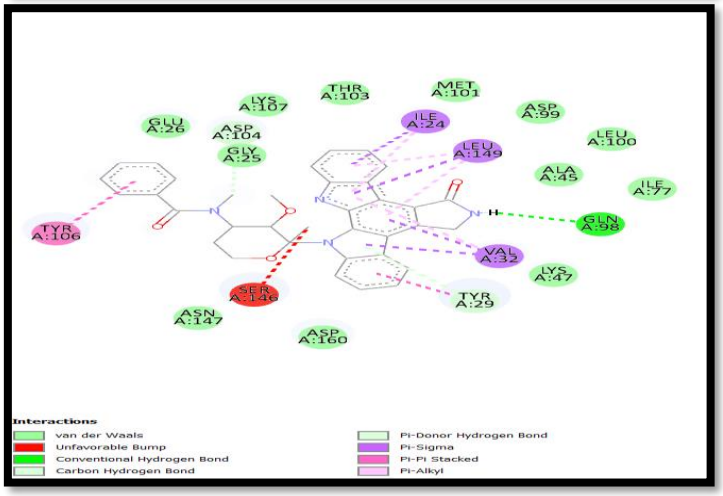
4.	82146	Bexarotene	GLN-59 ,ALA-164	ARG-63, GLY-162, THR-56, MET-326, TYR-180 THR-178, GLU-179, LYS-196, TYR-198, ARG-141, ARG-165, ARG-60, LEU-163, VAL-181
5.	6918296	Dutasteride	THR-103, CYS-159	LEU-100, LYS-107, GLU-102, GLU-26, ASP-104, GLY-27, LYS-144, ASP-160, SER-146, ASN-147, ILE-77, LYS-47, ALA-45, LEU-149, ILE-24, VAL-32, MET-101, TYR-29, GLN-98, ASP-99
6.	62330	Scarlet red	0	LEU-100, MET-101, ASP-99, GLN-98, ASN-147, ILE-96 THR-61, ARG-60, GLY-162 , ASP-160, GLU-64, TYR-29, ILE-77, LYS-47, CYS-159, VAL-32, ALA-45, ILE-24, LEU-149, ILE-49
7.	3052765	Pyrinoline	CYS-159, ASN-147, SER1-46, ASP99	ILE-77, LEU-100, MET-101, GLU-102, THR-103, LYS-107, ASP-104, GLY-27, GLU-26, ASP-160, LYS-47, GLN-98 , ALA-45, ILE-24, LEU-149, VAL-32, TYR-29
8.	68723	Antrafenine	SER-146 ,ASN-147	LYS-47, MET-101, LEU-100, TYR-186, THR-183, GLU-26, GLY-25, TYR-29, CYS-159, ILE-24, VAL-32, LEU-149, GLN-98, ILE-77, ASP-99, ALA-45, TRP185, LYS-144, TYR-106, LYS-107, ASP-104
9.	40821	Fendosal	ASN-147, SER-146, TYR-29	ASP-160, GLY-25, GLY-27, GLU-26, LYS-144, LYS-107, ASP-104, LEU-100, MET-101, ASP-99, LYS-47, CYS-159, ILE-24, ALA-45, LEU-149, VAL-32
10.	5291	Imatinib	ASP-104, ASP-160, ASN-147 ,GLY-25	SER-146, LYS-144, ASP-142, GLY-27, ALA-28, GLU-26, LYS-107, THR-103, MET-101, LEU-100, GLN-98, GLU-64, LYS-47, TYR-29, CYS-159, ALA-45, VAL-32, ILE-77, LEU-149, ILE-24
11.	74989	Atovaquone	0	LYS-107, ASP-104, THR-61, GLY-162, ASP-160, GLN-98, ALA-45,,ILE-24, LEU-149, VAL-32, TYR-29, CYS-159, LYS-47, GLU-64, ILE-49, ARG-60
12.	108143	Tamibarotene	TYR-29	PHE-161, GLU-64, ILE-49, ASP-160, LYS-47, CYS-159, ASN-147, SER-146, GLN-98, ILE-77, ALA-45, ASP-99, MET-101, LEU-100, ILE-24, LEU-149, VAL-32
13.	60751	Temoporfin	GLU-26, TYR-23, ALA-28, SER-146, TYR-106,TYR-29,	ILE-49, ILE-24, GLY-25, LYS-107, ASP-104, LYS-144, ASN-147, ASP-142, GLY-27, LEU149, TRP-185, VAL-32, LYS-47, ASP-160

14.	151171	Conivaptan	TYR-29, SER-146	GLN-98, ILE-24, ASN-147, THR-183, ASP-142, ALA-182, GLY-27, ALA-28, ASP-160, LEU-149, ALA-45, VAL-32, CYS-159, LYS-47, LYS-144, VAL-181, LEU-163
15.	24866313	SCH772984 (reference drug) (Merck/ ScheringPlough)	GLN-98, TYR-29, GLY-25,GLU-26	SER-111, SER-146, ASP-104, ASN-147, MET-101, LEU-100, TYR-106, LYS-47, VAL-32, ALA-45, LEU-149, ILE-24, LYS-107, LYS-110

(a)



(b)



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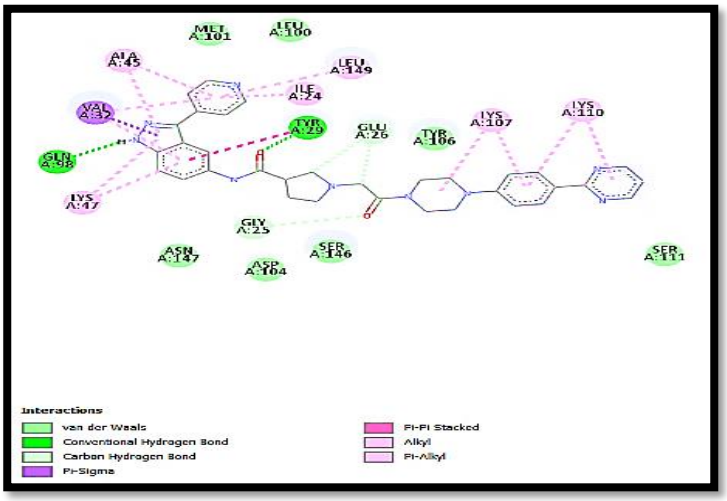


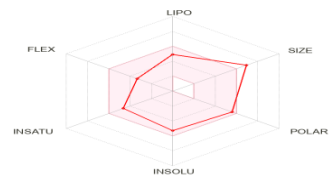
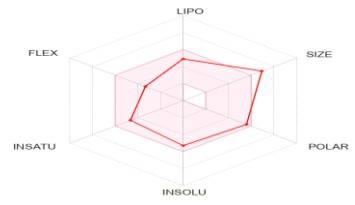
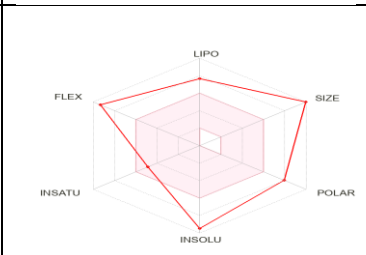
Fig. 4.2 llustratIion of a two-dimensional graphical depiction portraying the diverse interactions between ERK1 protein (a) Ergotamine (b) midostaurin (c) SCH772984 (reference drug)

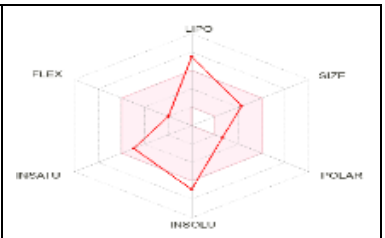
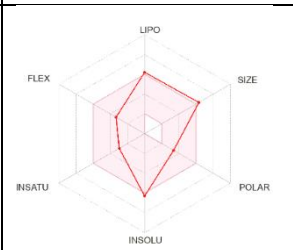
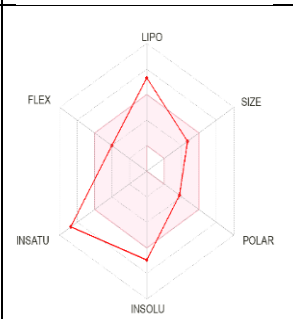
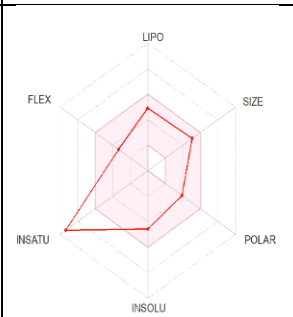
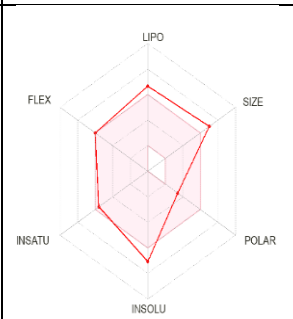
4.3. ADMET Property Analysis

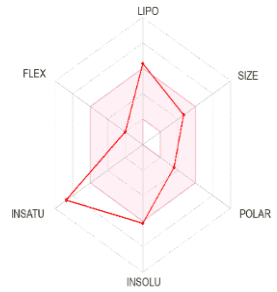
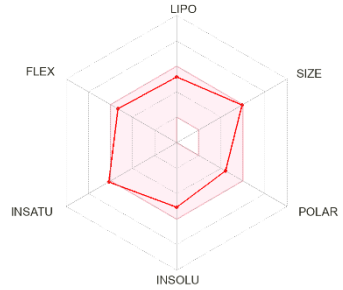
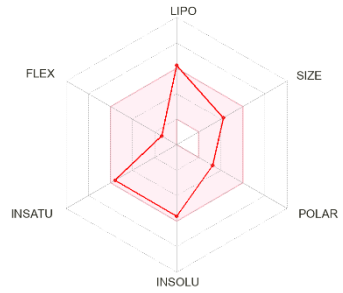
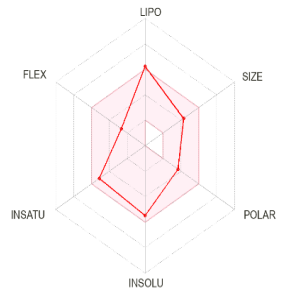
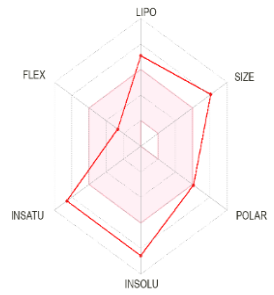
Using the pkCSM and SwissADME web sites, the ADME(T) property analysis commenced by screening those selected hits obtained from the docking research. The aim of this thorough assessment was to evaluate the compounds' pharmacokinetic and physicochemical features in order to identify whether or not they would be suitable candidates for drug development.

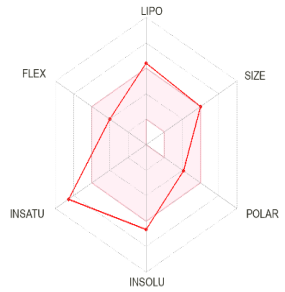
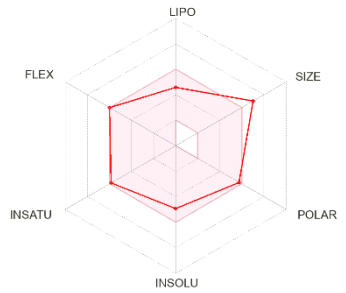
Concurrently, PAINS patterns were scrutinized to ensure the absence of hazardous molecular structures. Subsequently, utilizing the SwissADME and pkCSM web servers, further screening was conducted on the identified hits from the docking research, as detailed in Table 4.3, to predict their ADME(T) characteristics. The process of ADMET prediction involved evaluating physicochemical and pharmacokinetic attributes against predefined thresholds and parameters. Among the fourteen compounds examined, only ergotamine and midostaurin exhibited the requisite physicochemical characteristics and fell within the therapeutic candidacy range. Due to their exceptional binding affinities, both compounds were selected for further analysis

Table 4.3. ADME(T) Properties of high binding affinity ligands

Name of Ligands /Drug molecule	ADMET Properties				Bioavailability Radar Illustration
	Solubility	GI Absorption	Ames Toxicity	Skin permeation LOGKp (cm/s)	
Ergotamine	Moderate	High	No	-7.68	
Midostaurin	Poor	High	Yes	-6.22	
Venetoclax	Poor	Low	No	-5.85	

Bexarotene	Poor	High	No	-3.03	
Dutasteride	Poor	Low	No	-5.71	
Scarlet_red	Poor	High	Yes	-3.49	
Pyrinoline	Moderate	High	No	-6.67	
Antrafenine	Poor	High	No	-5.54	

Fendosal	Poor	High	Yes	-4.61	
Imatinib	Moderate	High	No	-6.81	
Atovaquone	Moderate	High	No	-4.56	
Tamibarotene	Moderate	High	No	-4.6	
Temoporfin	Poor	Low	No	-5.61	

Conivaptan	Poor	High	Yes	-5.32	
SCH772984 (reference drug)	Moderate	High	No	-8.10	

Among the compounds, ergotamine and midostaurin exhibited desirable physicochemical properties and came under the eligibility criteria for drugs. Both of these compounds showed exceptional binding affinities and were further investigated. Ergotamine displayed moderate solubility, enhanced gastrointestinal absorption, and a skin permeation Log Kp of -7.68 cm/s, while midostaurin showed poor solubility, enhanced gastrointestinal absorption, and a skin permeation Log Kp of -6.36 cm/s.

4.4. Biological activity analysis/prediction of selected compounds

Biological activity needs to be thoroughly studied in order to create safe and effective pharmaceuticals. PASS website was used to evaluate the biological activity of the selected compounds, ergotamine and midostaurin, as presented in the table.Compounds with promising therapeutic potential are identified and their selection for further development is guided by the PASS analysis, which takes into account numerous modes of action of drugs, interactions between drugs. Based on their binding studies, particular interactions with ERK1, ADMET property analysis, and PASS analysis, ergotamine and midostaurin were consequently chosen for MD simulation studies with ERK1(MAPK3).

Table 4.4. Biological activities of top 2 selected ligands

S.No.	Name of ligand	Pa	Pi	Biological properties
1.	Ergotamine	0.370	0.020	Antineoplastic alkaloid
		0.327	0.116	Kinase inhibitor
		0.232	0.055	Protein-tyrosine kinase p55(blk) inhibitor
2.	Midostaurin	0.413	0.007	Antineoplastic (SCLC)
		0.180	0.032	Antineoplastic (gastric cancer)
		0.301	0,032	Tyrosine kinase inhibitor

4.5. MD simulations

Protein-ligand complexes' dynamic behaviour and structural features can be studied by employing the widely used molecular dynamics (MD) simulation technique. In this work, ERK1-ergotamine, ERK1-midostaurin, and apo ERK1 were the three systems that were investigated for 100 ns using MD simulations. Further examining the dynamics and stability of ERK1 complexed with ergotamine and midostaurin via means of a closer examination of a number of systematic and structural features.

4.5.1.Structural dynamics and compactness analysis

RMSD and RMSD PDF Analysis

Binding small chemical compounds can cause significant changes in configurations of protein which are structural in nature. RMSD analysis is important for assessing these structural alterations and dynamics over time. ERK1 alone, ERK1-ergotamine, and ERK1-midostaurin complexes were found to have RMSD average values of 0.17, 0.22 , 0.27 nm. Over course of simulation, the binding of ergotamine and midostaurin to ERK1 remained steady, demonstrating consistent RMSD values and fair stability of the docked complexes. Over 100 ns trajectory, both ERK1 alone and ERK1-ergotamine systems displayed notable stability compared to ERK1-midostaurin, as depicted in the RMSD plot (Figure 4.3 ,4.4). However, higher fluctuations were observed in the RMSD of the ERK1-midostaurin complex. Despite this, both ERK1 alone and ERK1-ergotamine systems demonstrated RMSD values which were stable and balanced throughout the entire 100 ns simulation period. The PDF of RMSD dynamics, illustrated in figures 4.5, 4.6, indicates a significant stabilization in ERK1 dynamics upon compound binding, with a higher probability observed consistently throughout

RMSF and RMSF PDF Analysis

During MD simulations, analysing root-mean-square fluctuation (RMSF) aids in evaluating vibrations of residue inside structure of protein . When RMSF of each residue is plotted before and after ergotamine and midostaurin bind to ERK1, persistent fluctuations are seen, which suggests that the protein-ligand complex, especially when ergotamine is present, is significantly consistent. Nonetheless, the binding of midostaurin exhibits marginally elevated residual vibrations, indicating elevated dynamics restricted to loop areas. Overall, RMSF analysis supports the findings from RMSD and RMSF plots by highlighting the ERK1-ergotamine complex's long-term stability in comparison to ERK1-midostaurin (Figure 4.7, 4.8) As compared to midostaurin binding, the PDF of RMSF dynamics, which is illustrated in figures 4.9,4.10 , similarly demonstrates a reduced residual fluctuation over ergotamine binding.

Rg and Rg PDF Analysis

RMS distance between atomic group and their collective centre of mass is called radius of gyration (Rg) and it has a direct correlation with a protein's tertiary structure. By comparing ERK1's Rg before and after ergotamine and midostaurin binding, its compactness was assessed. Rg has a direct relationship with a protein's tertiary structure This property is commonly used to explore how much structure of a protein is folded . Rg of the free ERK1, ERK1-ergotamine, and ERK1-midostaurin complexes were 2.16, 2.20, 2.21 nm. (Figure 4.11). An increase in RMSF values precisely correlates with an increase in ERK1-midostaurin Rg levels. Ergotamine stabilized ERK1 folding, based on the Rg plot (Figure 4.12)
The examination of the Rg PDF indicates that, in comparison to midostaurin and also

no discernible change was found in the average Rg value of the ERK1 following ergotamine binding (Figure 4.13, 4.14).

SASA and SASA PDF Analysis

SASA is the part of a protein which is within reach to its nearby solvent. Protein folding behavior and stability have been investigated using the SASA methodology. Through analysis of plot, it was found that no noteworthy fluctuation in values of SASA over the course of the simulations, indicating more stable ERK1-ergotamine complexes than ERK1-midostaurin complexes. According to the findings, the SASA of the ERK1 and ERK1-ergotamine, ERK1-midostaurin complexes were 176.0 nm2, 178.77 nm2, and 178.32 nm2, respectively (Figure 4.15, 4.16). Across all systems there might be almost same patterns seen in the SASA value distribution. A small rise in an average value of SASA suggests there was loose packing without affecting overall folding of protein.

The SASA PDF indicates that average SASA of ERK1 increases slightly following ergotamine binding and midostaurin binding (Figure 4.17,4.18).

Structural dynamics of ERK1 alone and after complexed with ergotamine and midostaurin as a function of time.

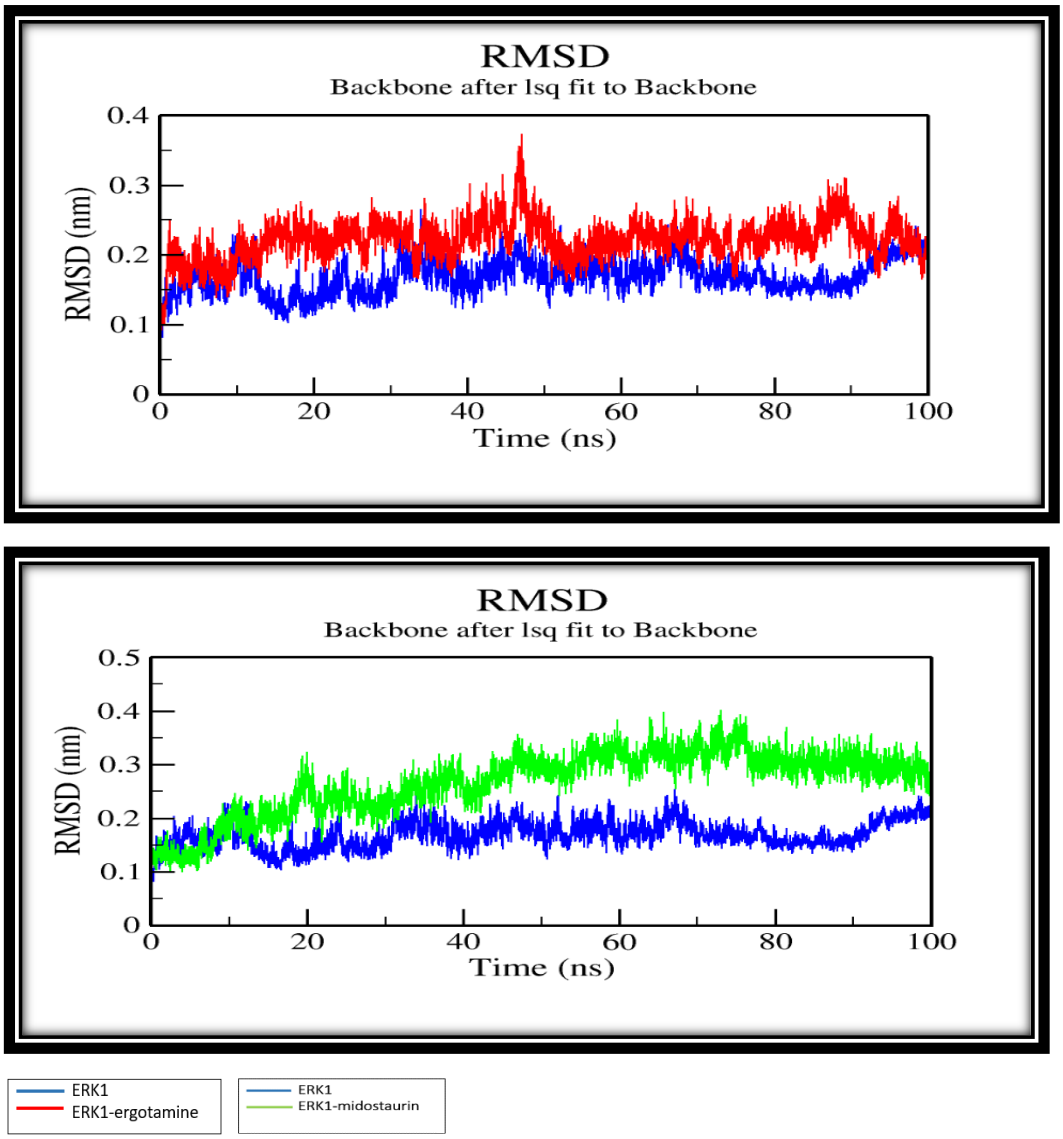


Fig. 4.3 (a) Plot of RMSD values of ERK1, ERK1 complexed with ergotamine. (b) Plot of RMSD values of ERK1 ,ERK1 complexed with midostaurin

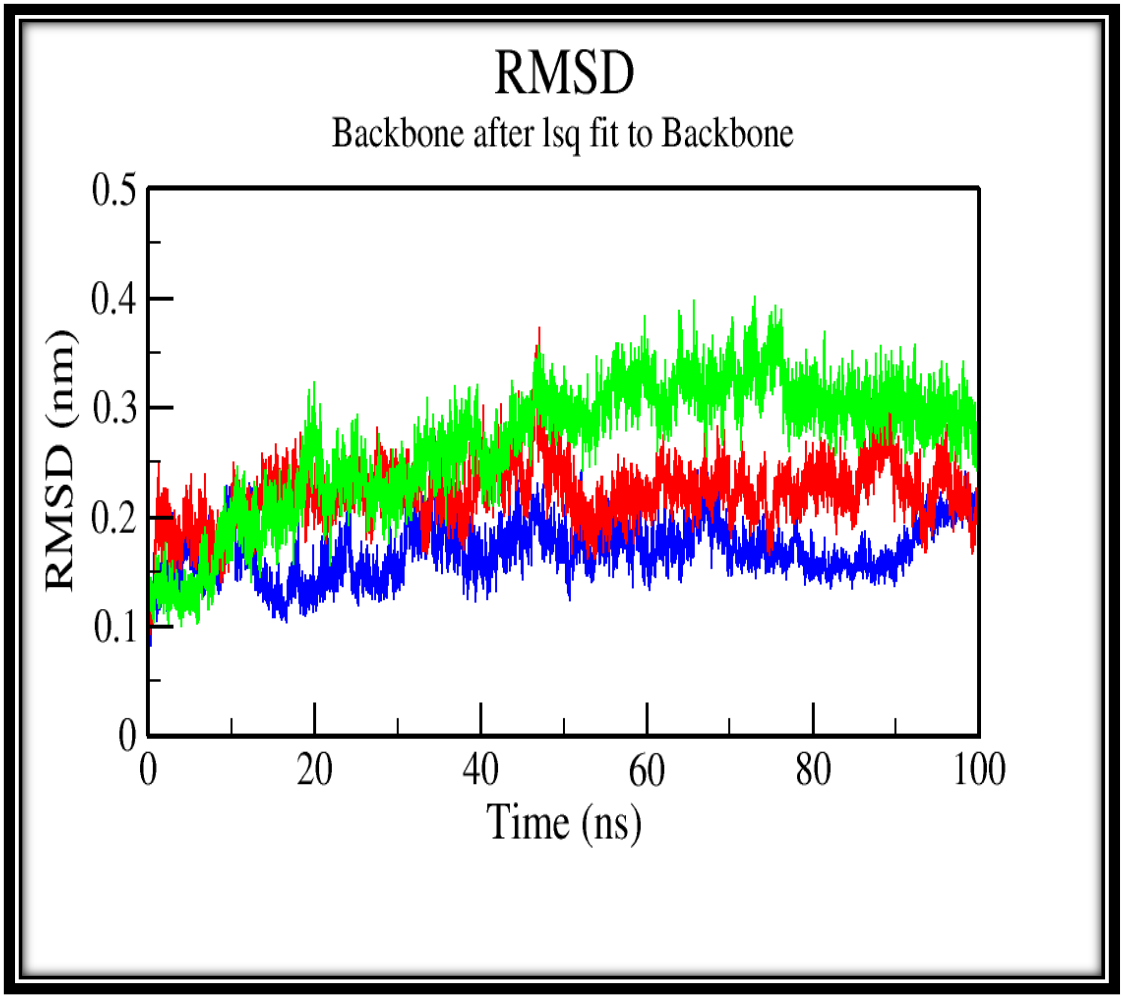


Fig. 4.4 Plot of RMSD values of ERK1 and its complex with ergotamine and midostaurin

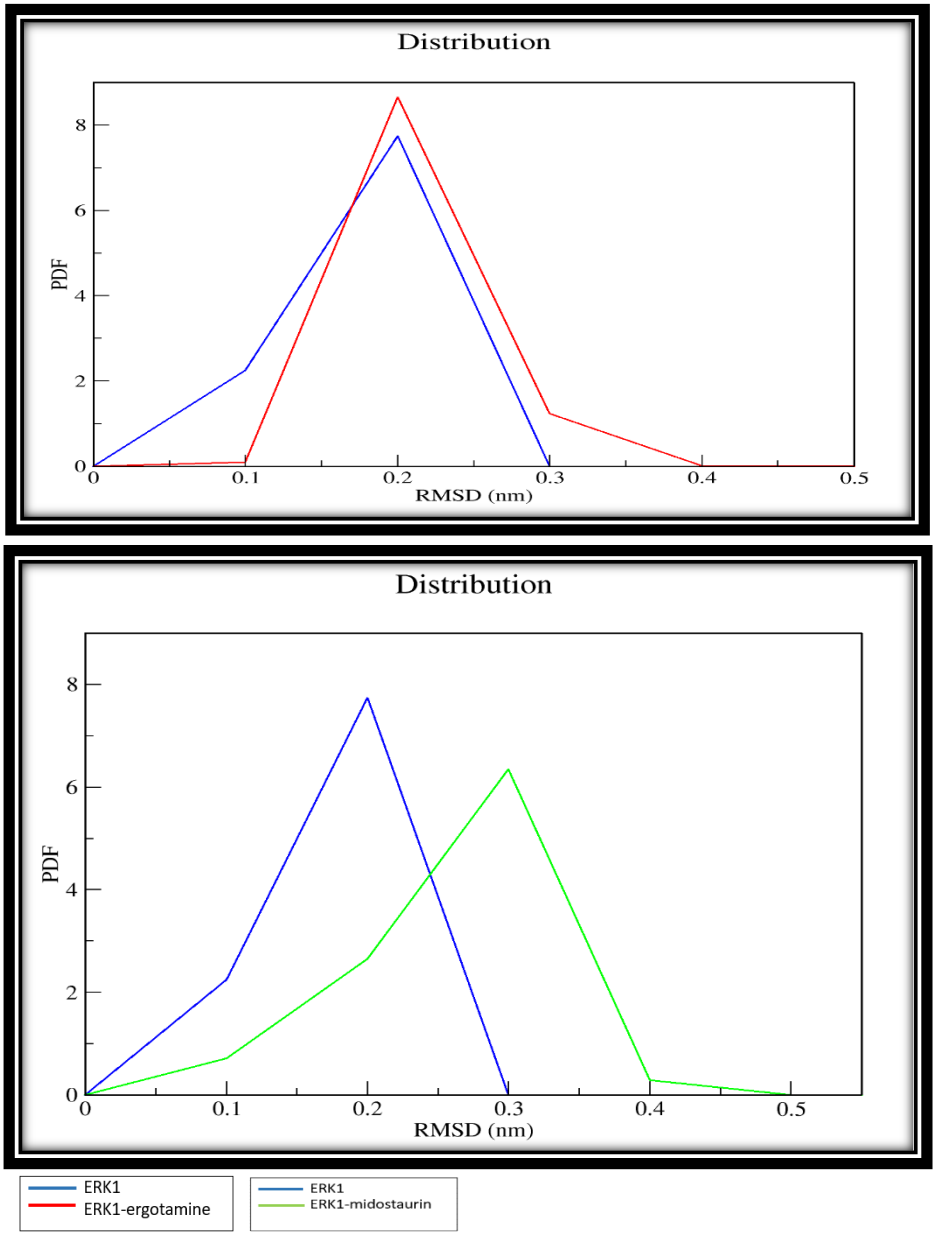


Fig. 4.5 (a) Plot of RMSD PDF values of ERK1, ERK1 complexed with ergotamine. (b) Plot of RMSD of ERK1, ERK1 complexed with midostaurin

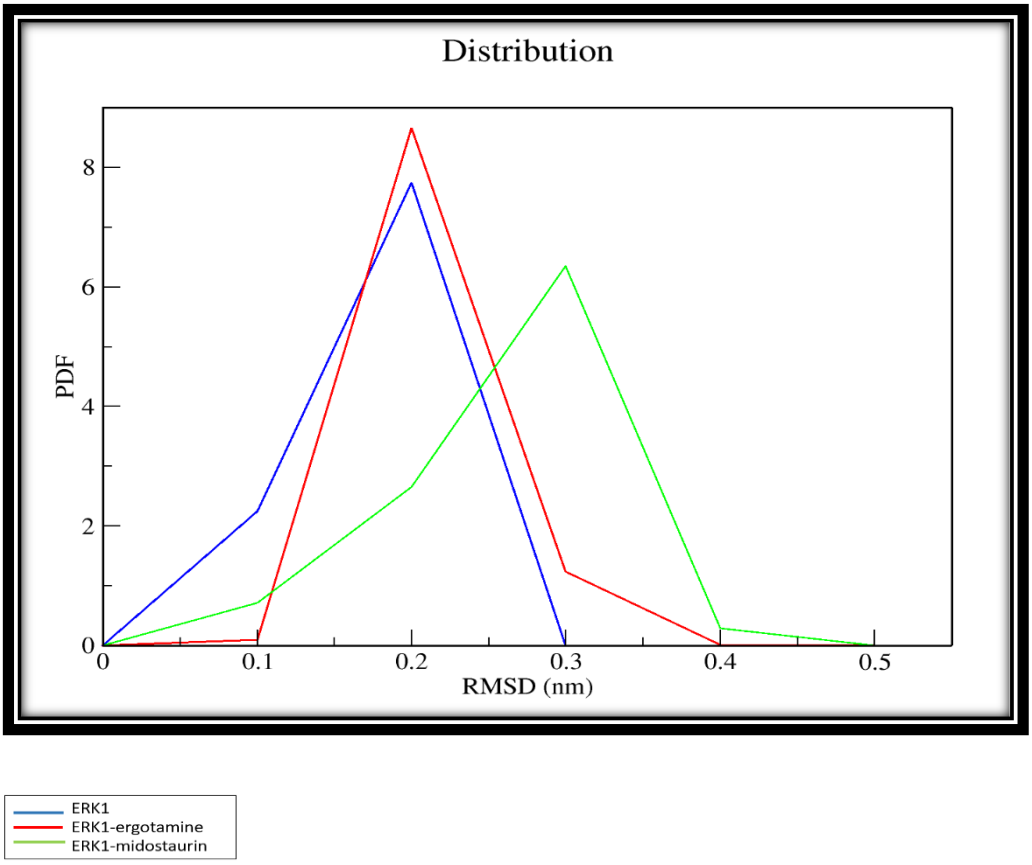


Fig. 4.6 RMSD PDF values plot of ERK1, its complex with ergotamine and midostaurin

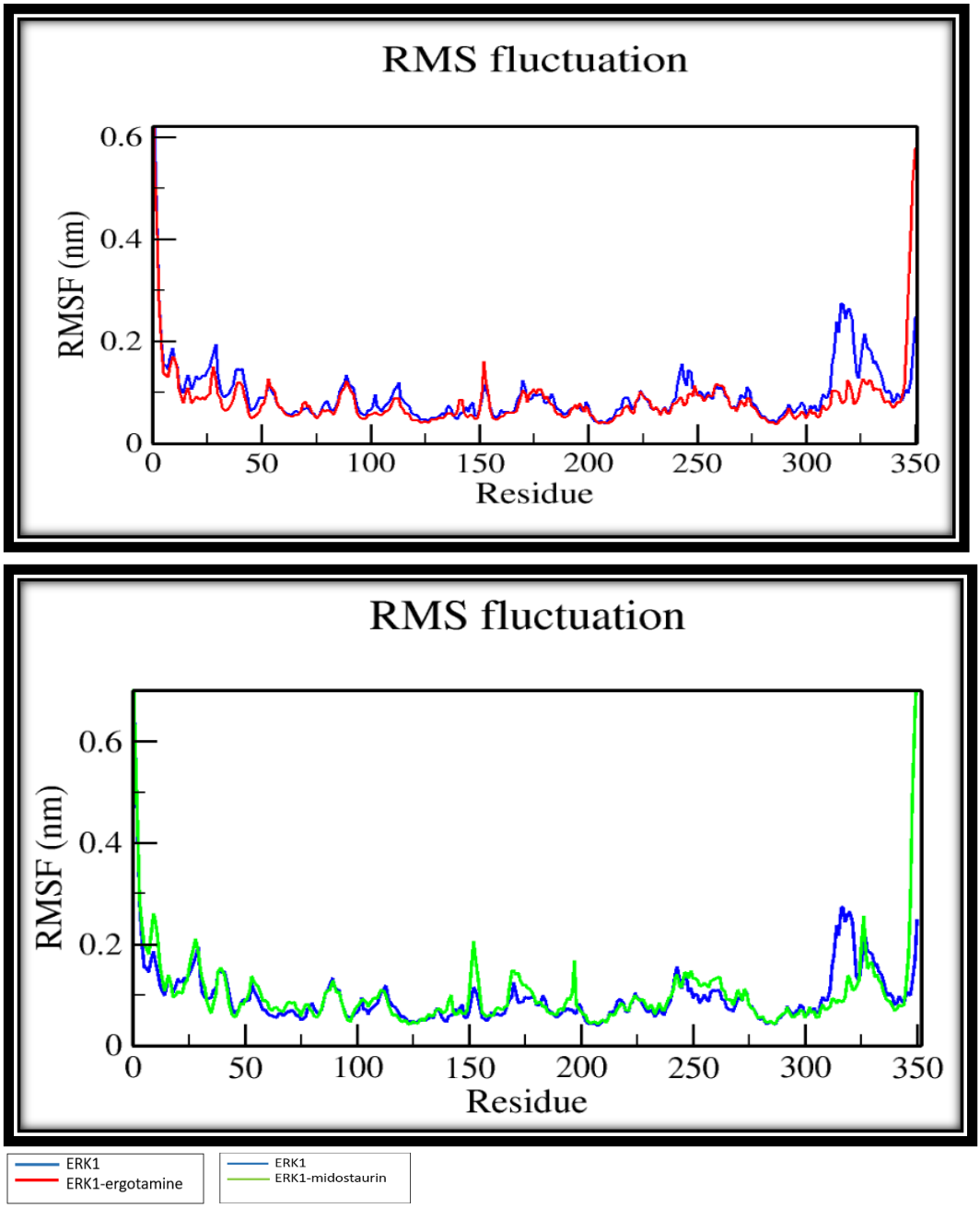


Fig. 4.7 (a) Plot of RMSF values of ERK1 and ERK1 complexed with ergotamine. (b) Plot of RMSF values of ERK1, ERK1 complexed with midostaurin

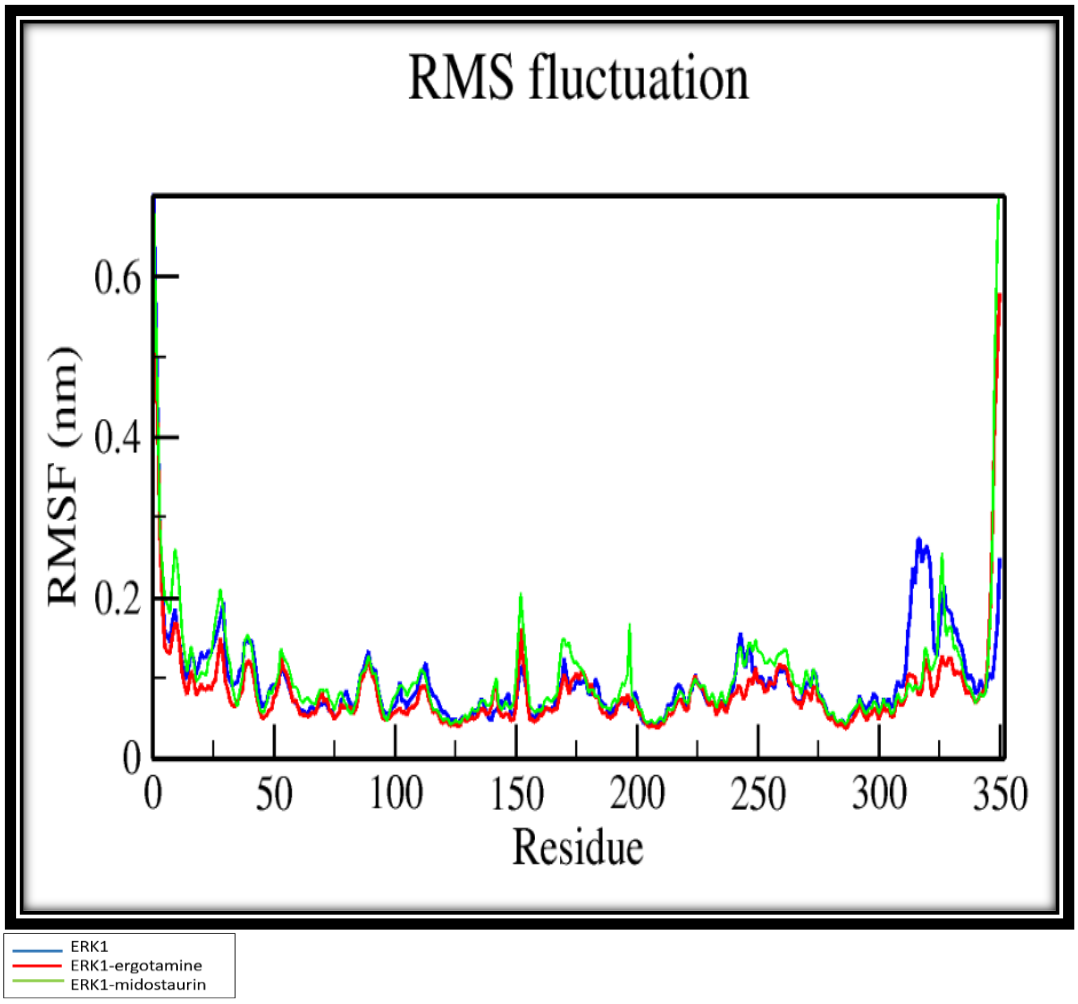


Fig. 4.8 Plot of RMSF values of ERK1, ERK1 complexed with ergotamine and midostaurin

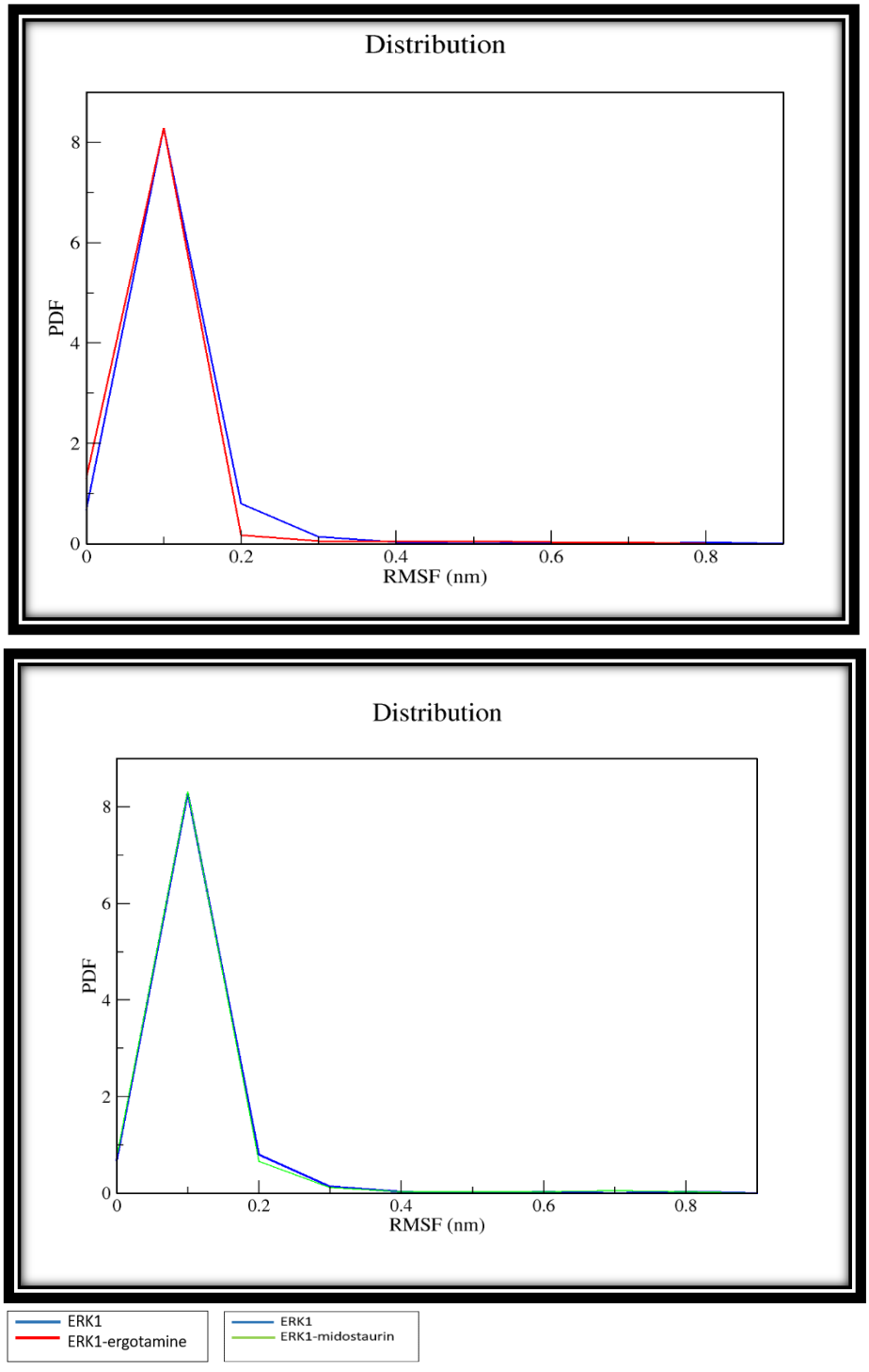


Fig. 4.9 (a) RMSF PDF values plot of ERK1, ERK1 complexed with ergotamine. (b) RMSF PDF values plot of ERK1, ERK1 complexed with midostaurin

.

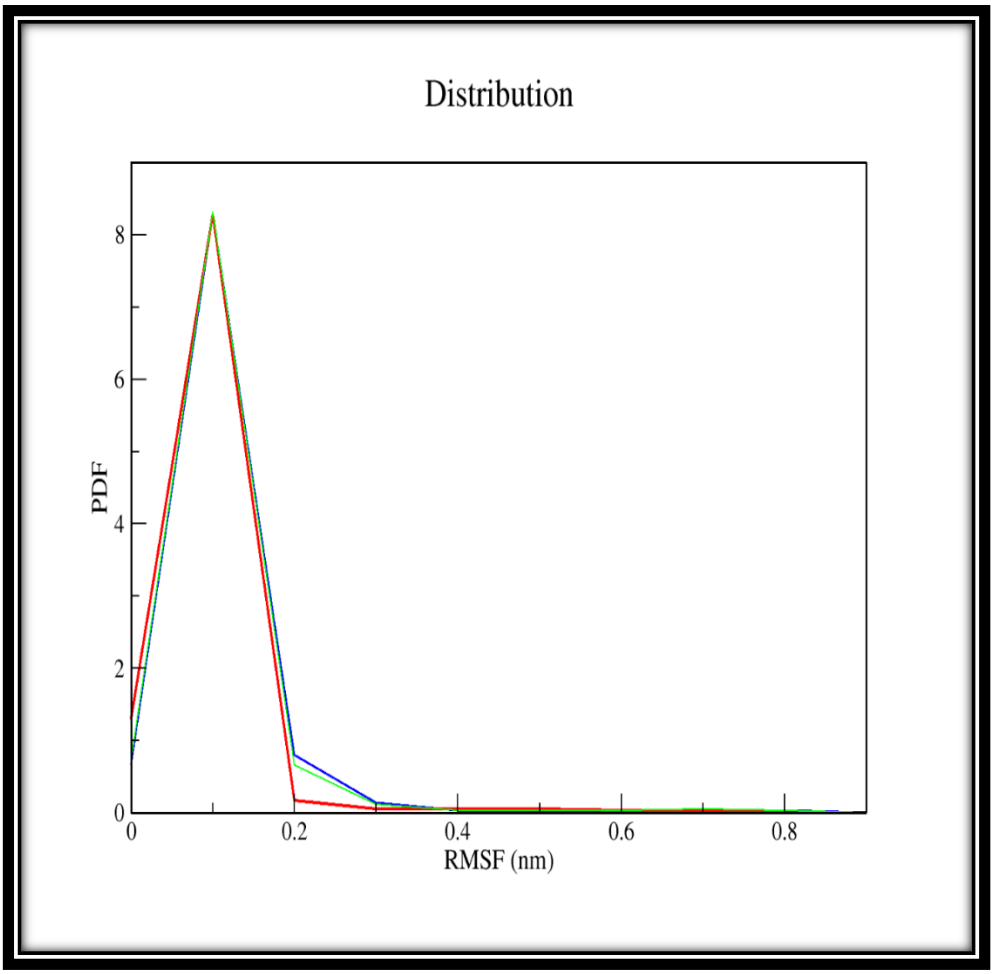


Fig. 4.10 RMSF PDF values plot of ERK1, ERK1 complexed with ergotamine and midostaurin

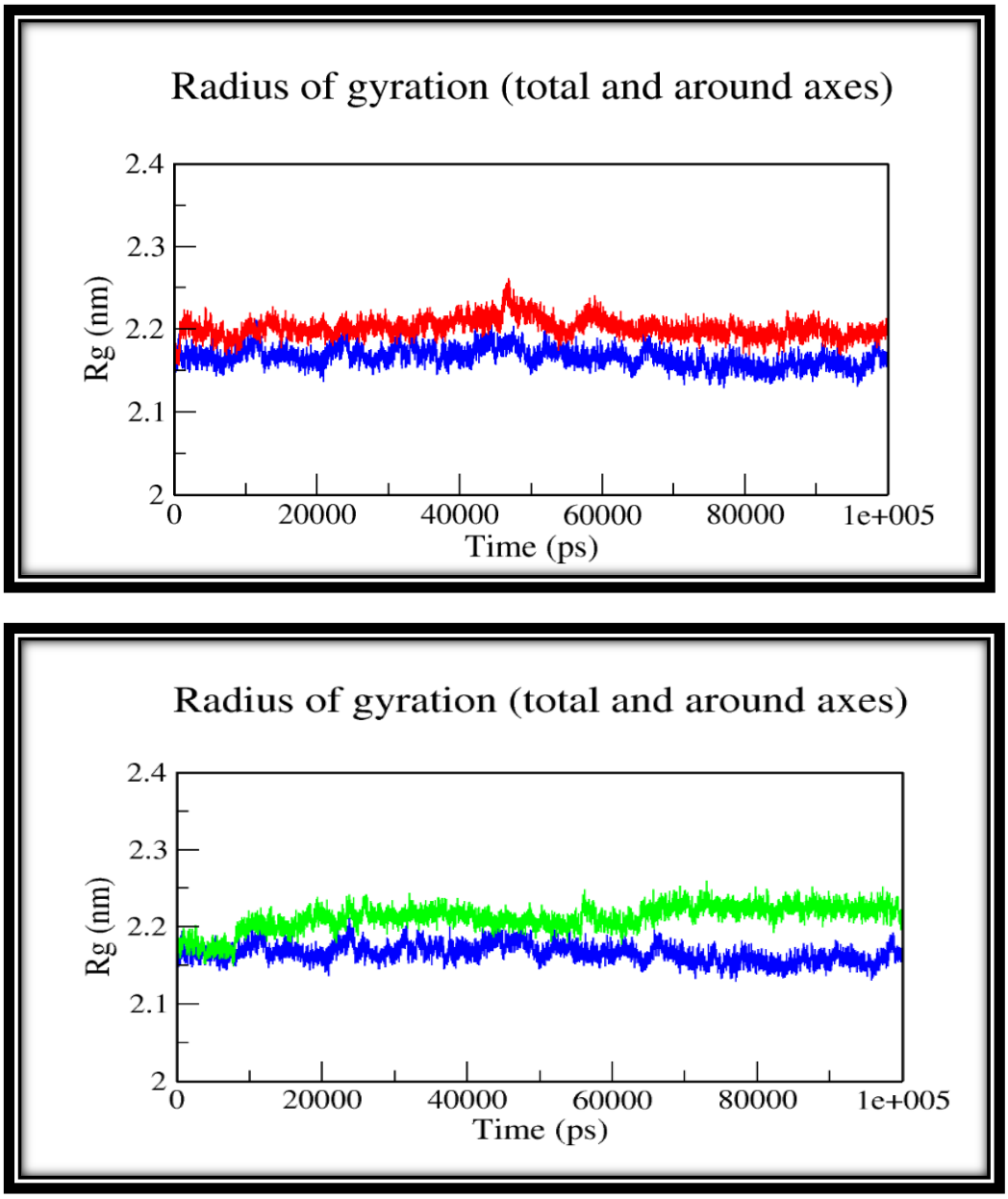


Fig. 4.11 (a) Plot of Rg values of ERK1, ERK1 complexed with ergotamine. (b) Plot of Rg values of ERK1, ERK1 complexed with ergotamine

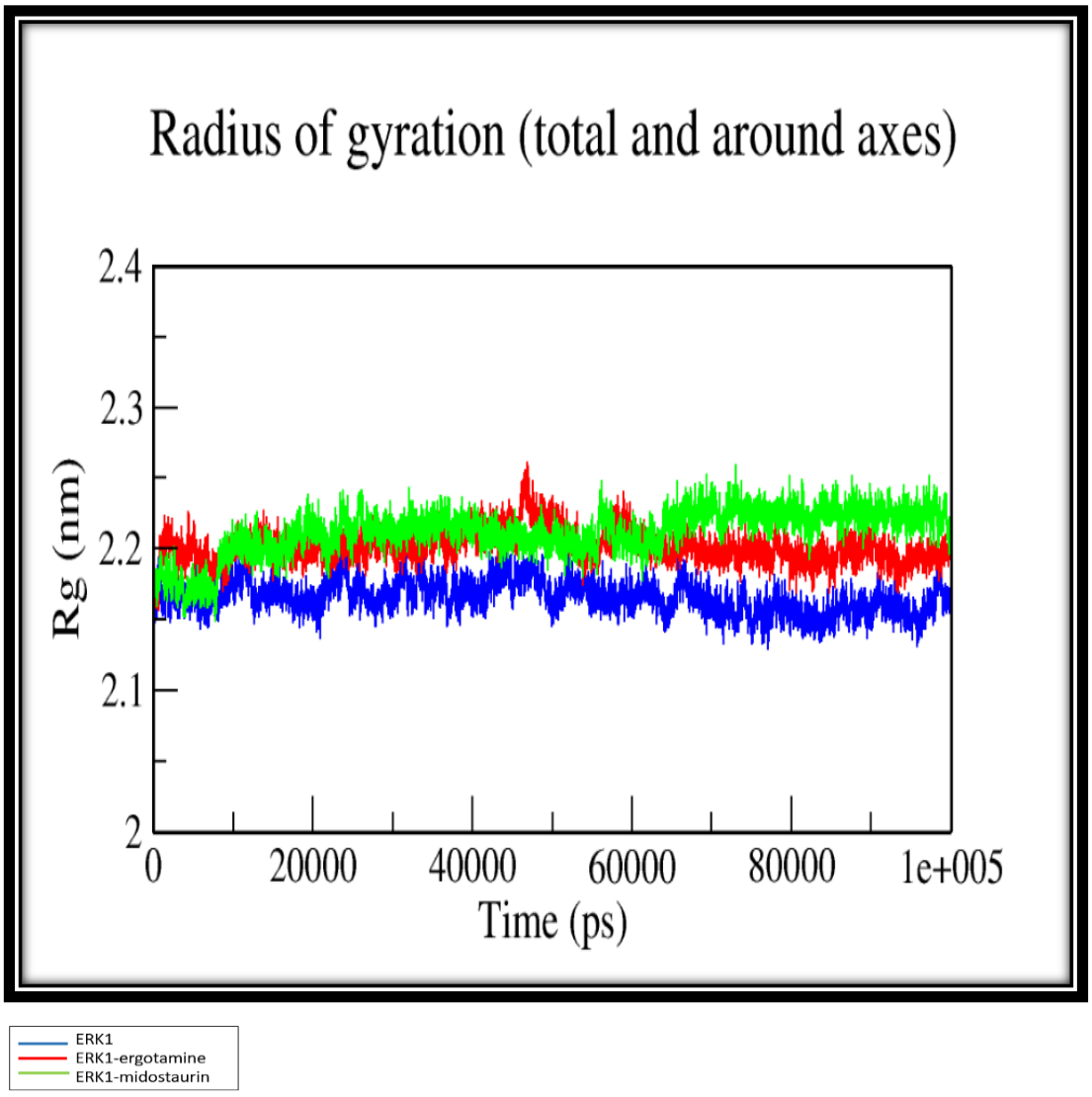


Fig. 4.12 Plot of Rg values of ERK1 and its complex with ergotamine and midostaurin

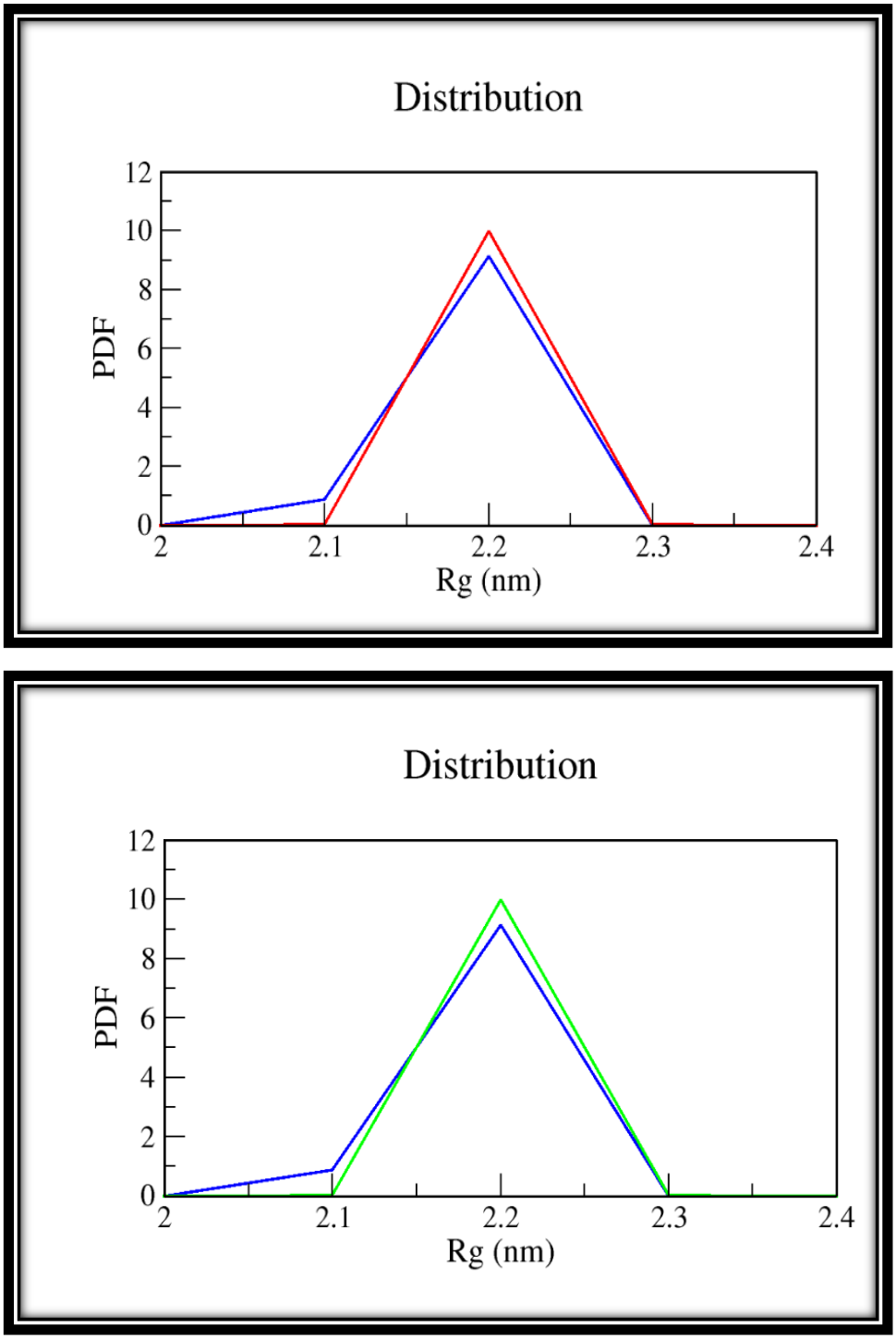


Fig. 4.13 (a) Rg PDF values plot of ERK1, ERK1 complexed with ergotamine. (b) Rg PDF values plot of ERK1, ERK1 complexed with midostaurin.

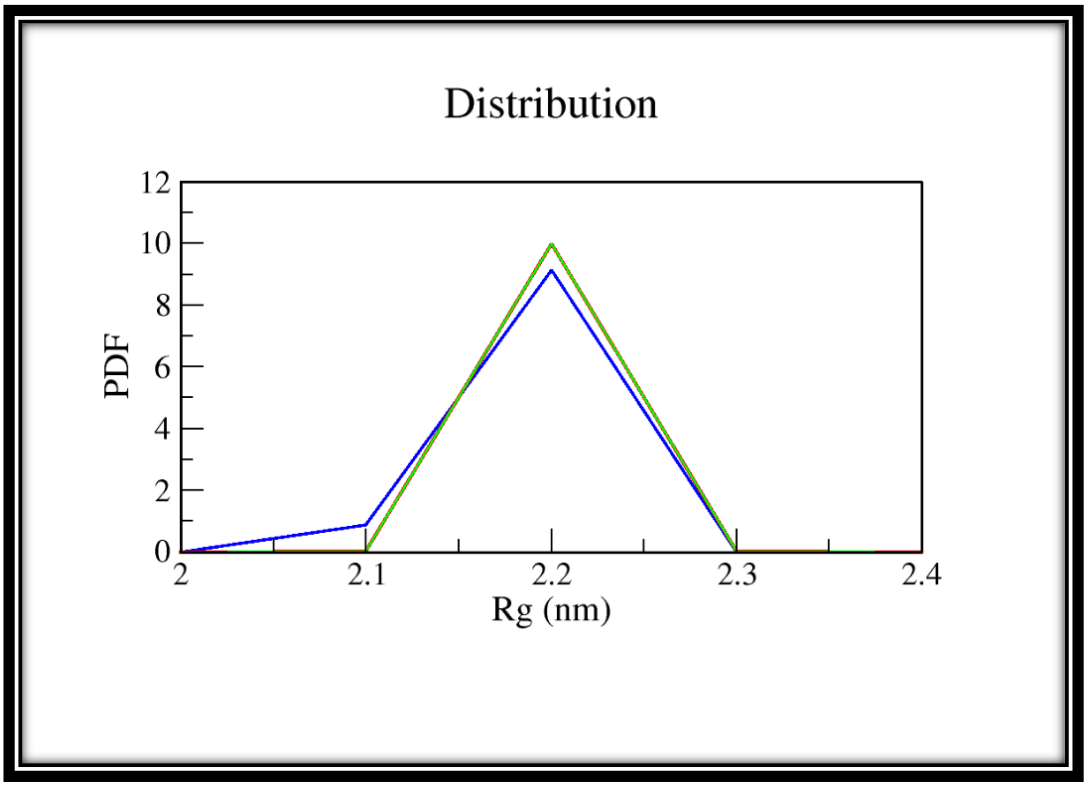


Fig. 4.14 Rg PDF values plot of ERK1, ERK1 complexed with ergotamine and midostaurin

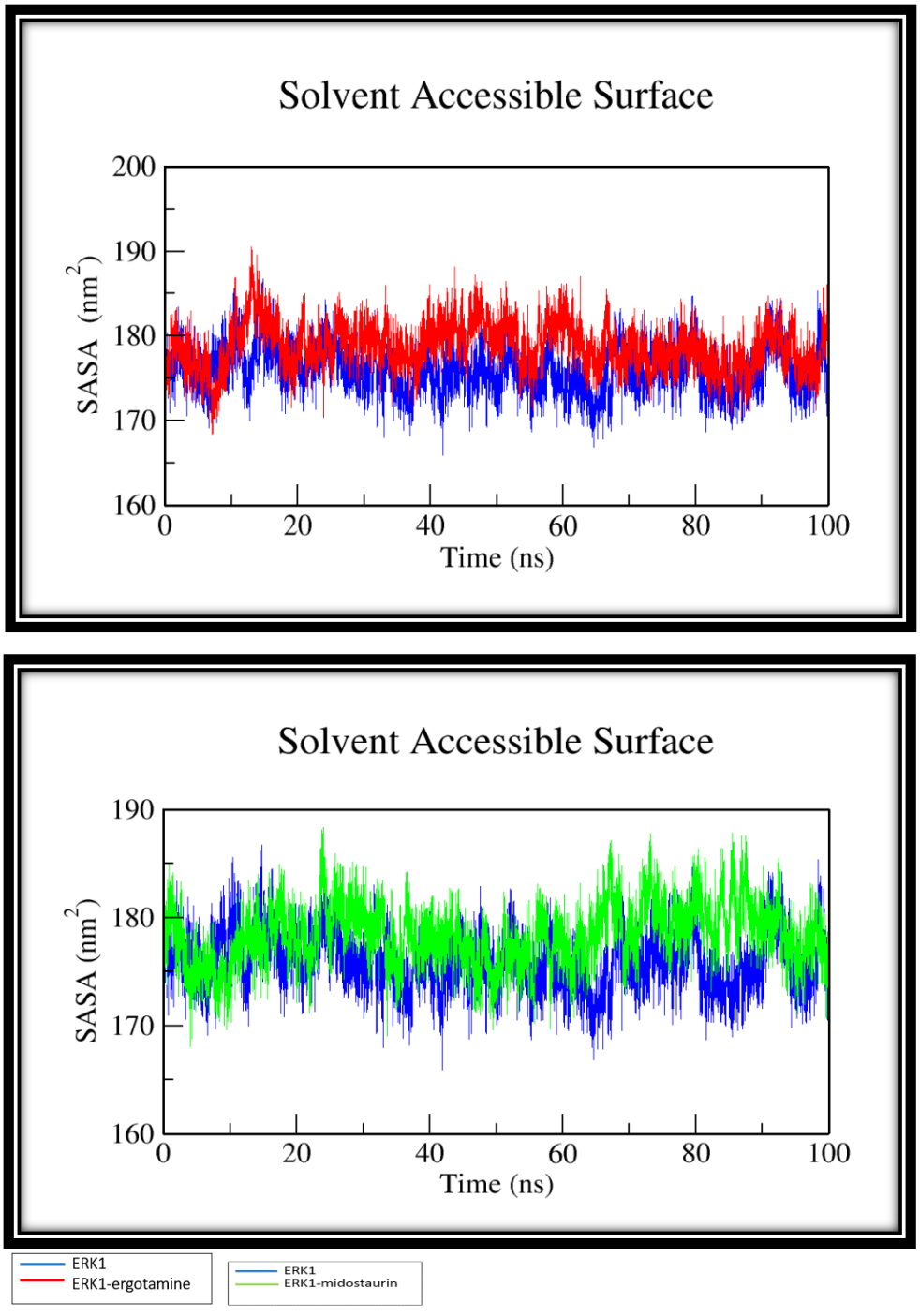


Fig. 4.15 (a) Plot of SASA values of ERK1, ERK1 complexed with ergotamine. (b) Plot of SASA values of ERK1, ERK1 complexed with midostaurin.

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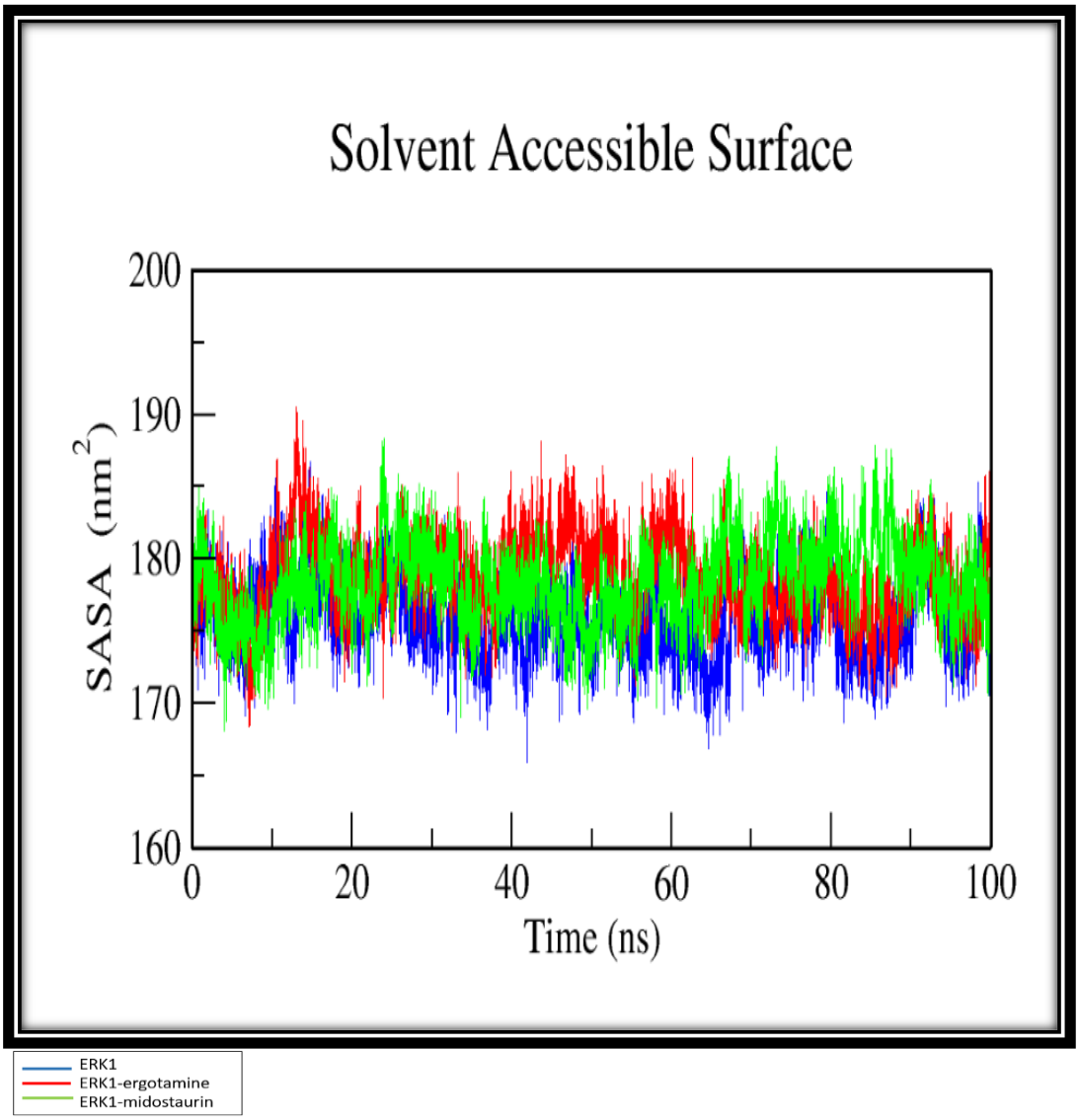


Fig. 4.16 Plot of SASA values of of ERK1, ERK1 complexed with ergotamine and midostaurin

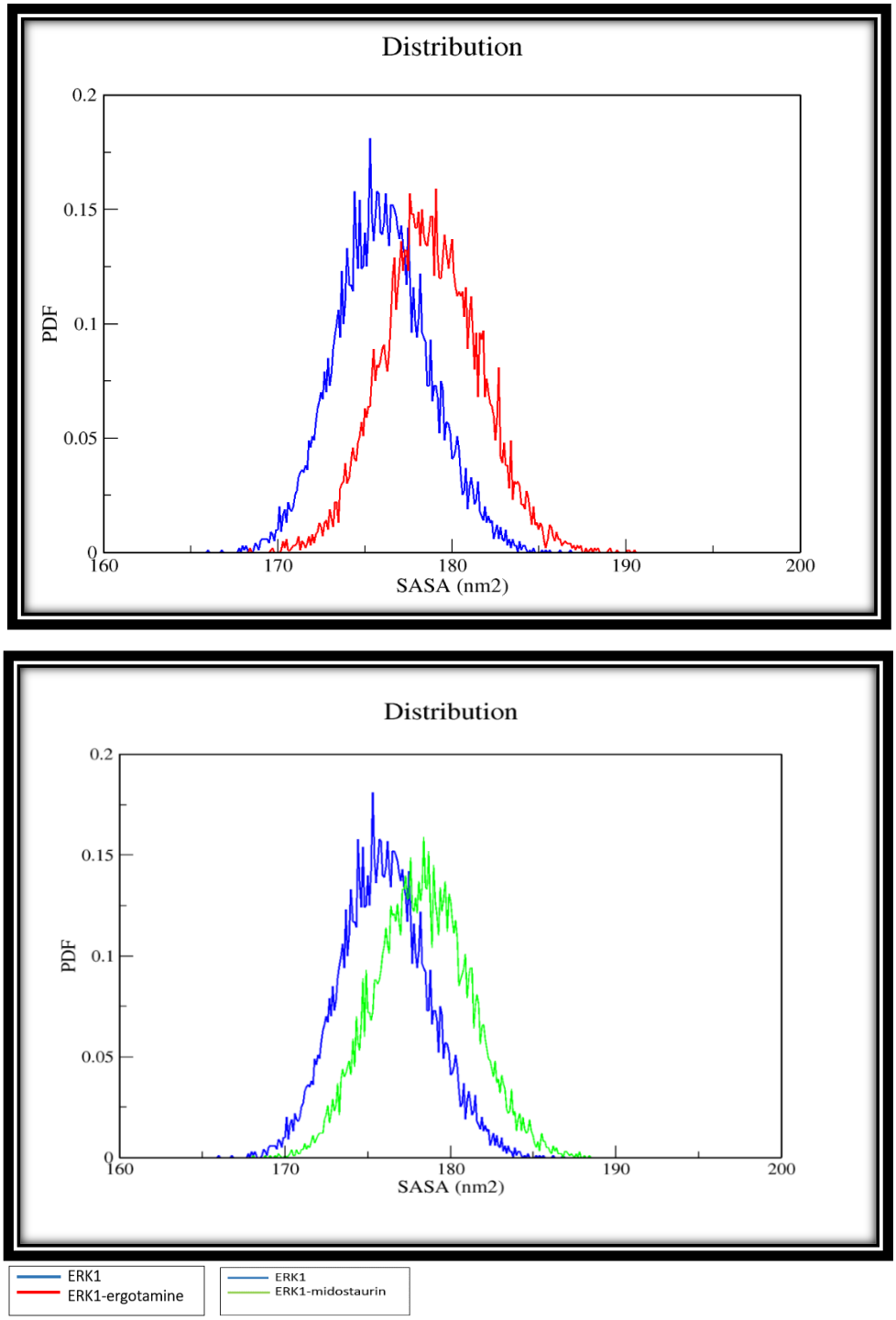


Fig. 4.17 (a) SASA PDF values plot of ERK1, ERK1 complexed with ergotamine. (b)SASA PDF values plot of ERK1, ERK1 complexed with midostaurin

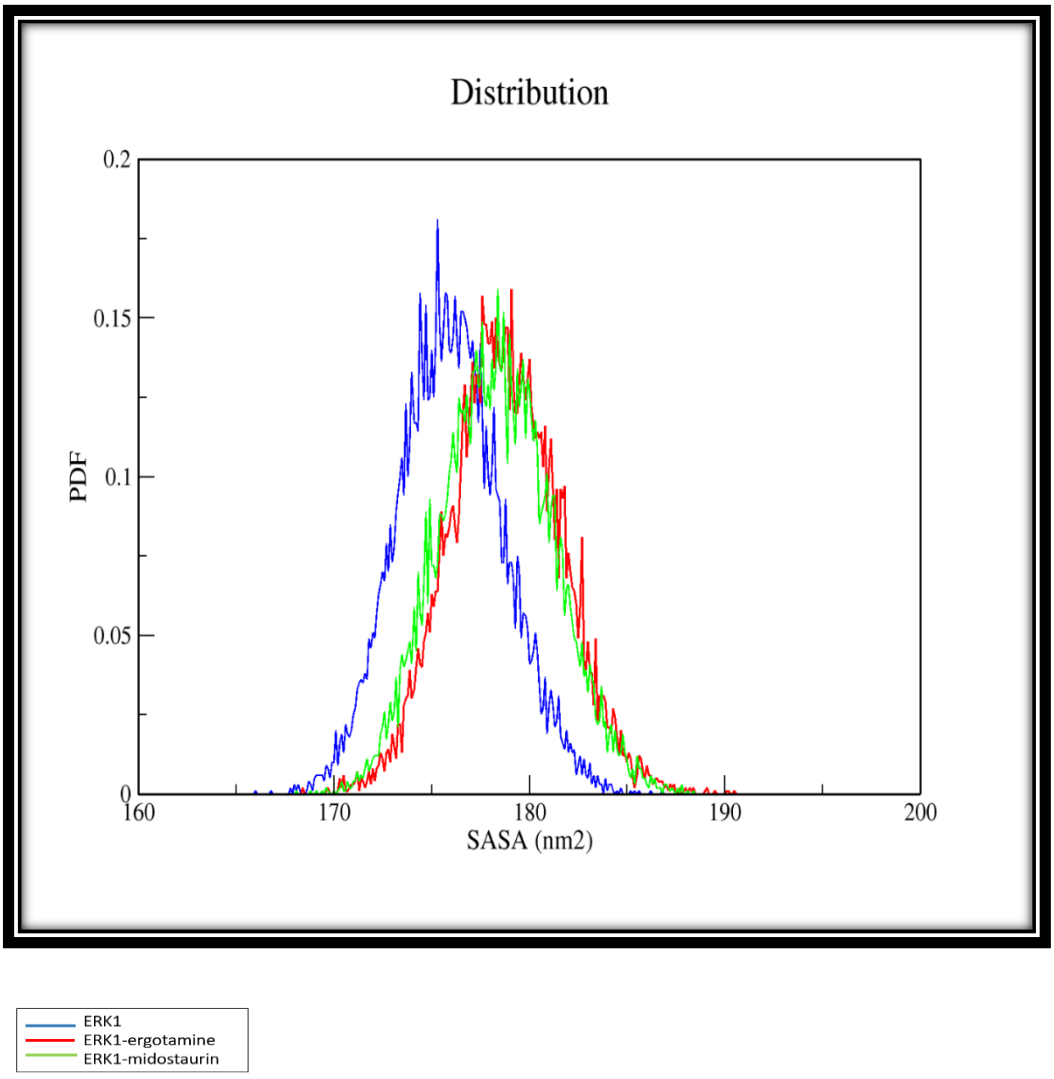


Fig. 4.18 SASA PDF values plot of ERK1, ERK1 complexed with ergotamine and midostaurin

4.5.2. Study of H-bonds

Structural stability is largely dependent on different intramolecular hydrogen bonds, or H-bonds of protein. Before and after ergotamine binding, assessment of the continuity of H-bonding which are intramolecular in nature in ERK1 was required required via analysing H- bonds time evolution . Plot suggests complexing ERK1 with ergotamine and midostaurin but does not significantly alter the number of H-bonds in ERK1.The average number of Hydrogen bonds formed intramolecularly in ERK1 alone, after biding of ergotamine and midostaurin were estimated to be 259, 261, and 260 which were shown in figure 4.19,4.20. There is an increase in H-bonds in correlation with ligand occupancy within the intramolecular space of ERK1. In addition, there is a noticeable stability in the PDF for intramolecular H- bond in each of 3 systems (Figure4.21, 4.22). Also, throughout the simulation this plot validates complexes stability.

Intermolecular H-bond time development was further studied for evaluating H-bonds stability formed between ERK1-ergotamine & ERK1-midostaurin . It was calculated that there were two H-bonds on average generated in each of the ERK1-ergotamine and ERK1-midostaurin complexes (Figure 4.23(a),4.24 (a)). Furthermore, both systems' probability distribution functions (PDFs) for intermolecular H-bonds show a similar pattern, with larger PDF values and an average of two H-bonds (Figure 4.23 (b), 4.24 (b)). Hydrogen bonding which are intermolecular in nature stabilizes the complex structures, suggesting that ergotamine and midostaurin have not shifted from their initial docking sites on ERK1.

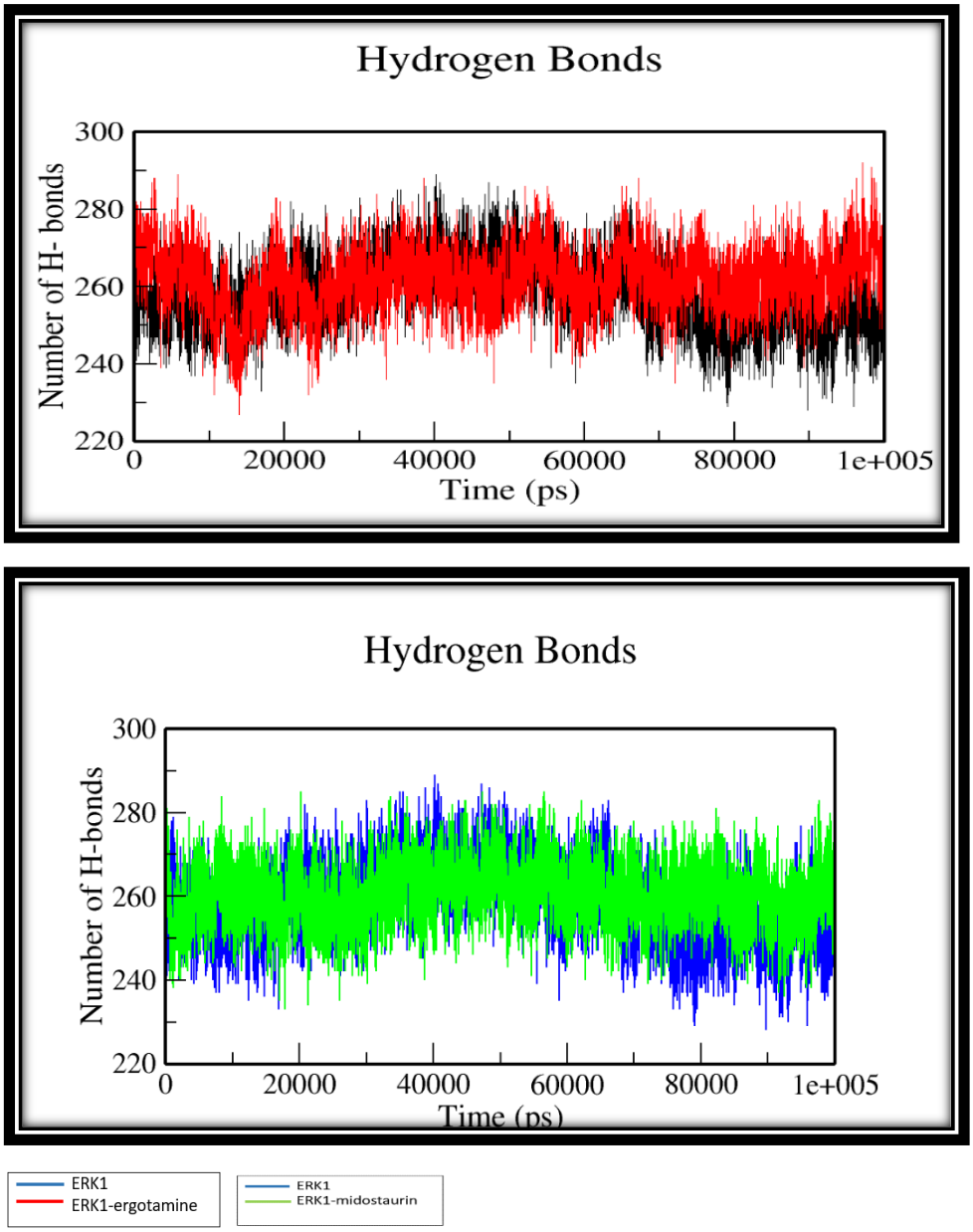


Fig. 4.19 Study of H-Bonds. (a) Plot showing intramolecular H- bonds formed in ERK1 and when it complexed with ergotamine as a function of time. (b)) Plot showing intramolecular H- bonds formed in ERK1 and when it complexed with midostaurin as a function of time.

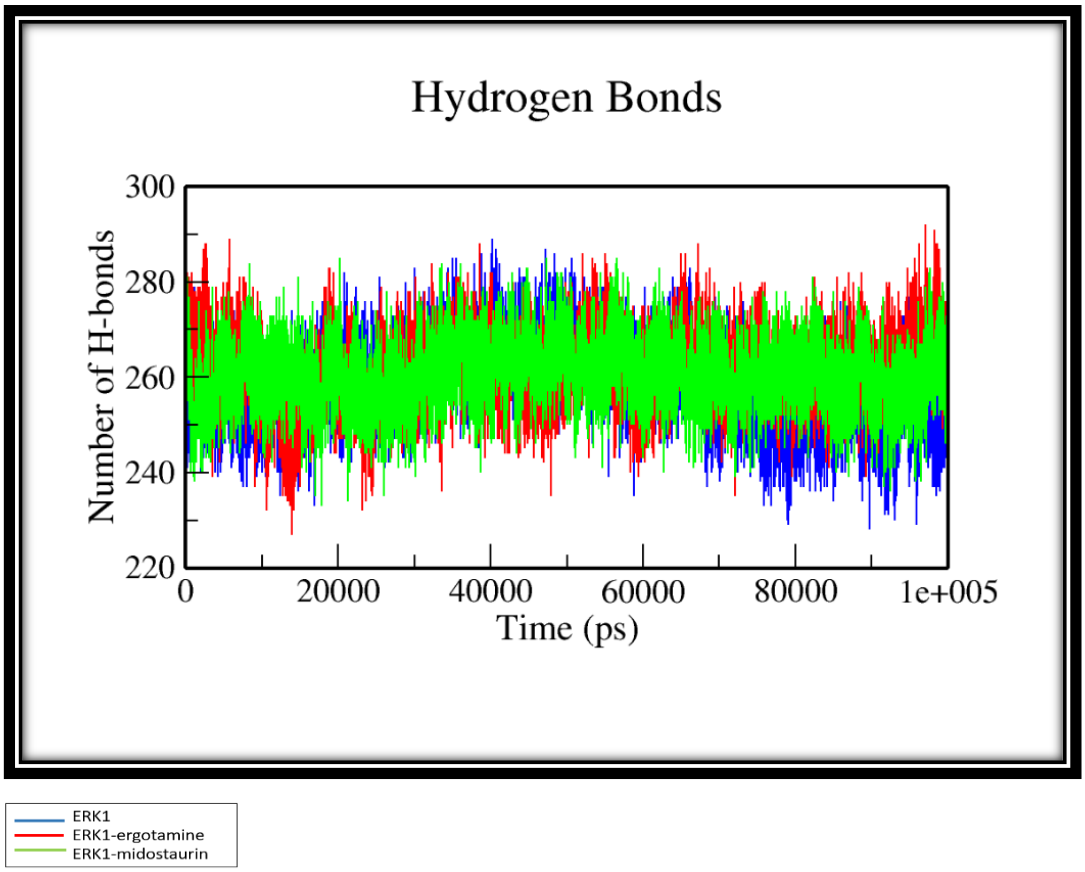
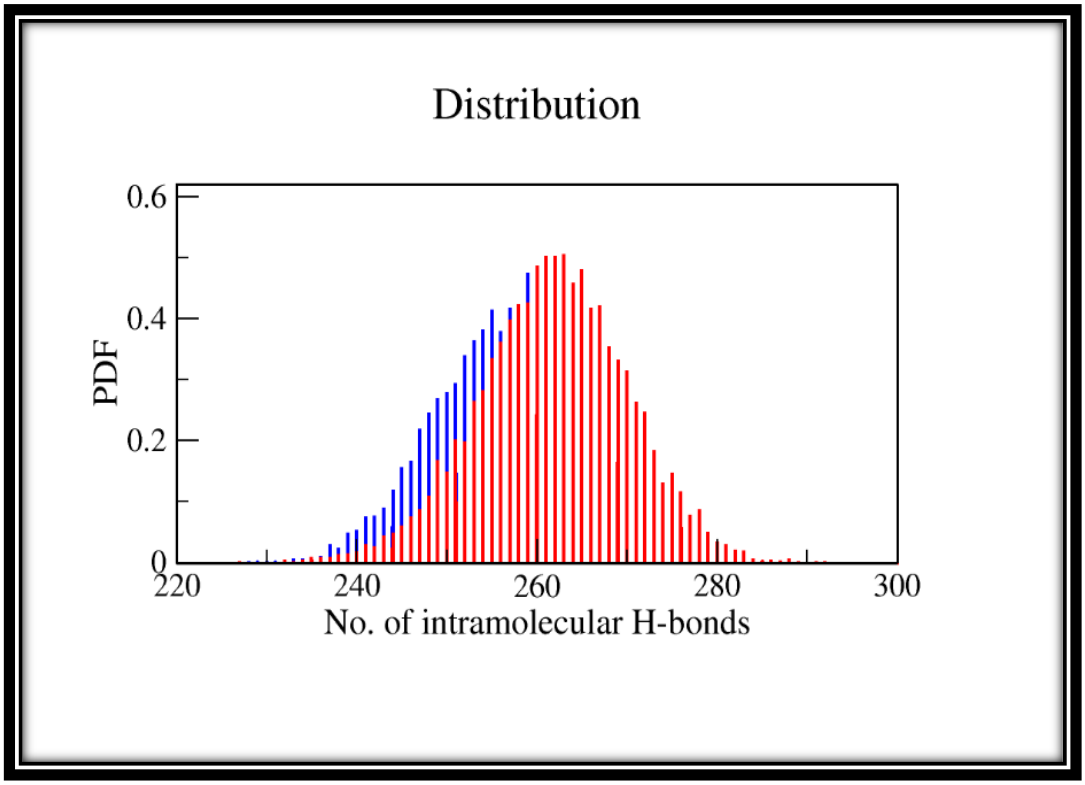


Fig. 4.20 Study of H-Bonds. Plot showing intramolecular H-bonds formed in ERK1 and when it complexed with ergotamine and midostaurin as a function of time.



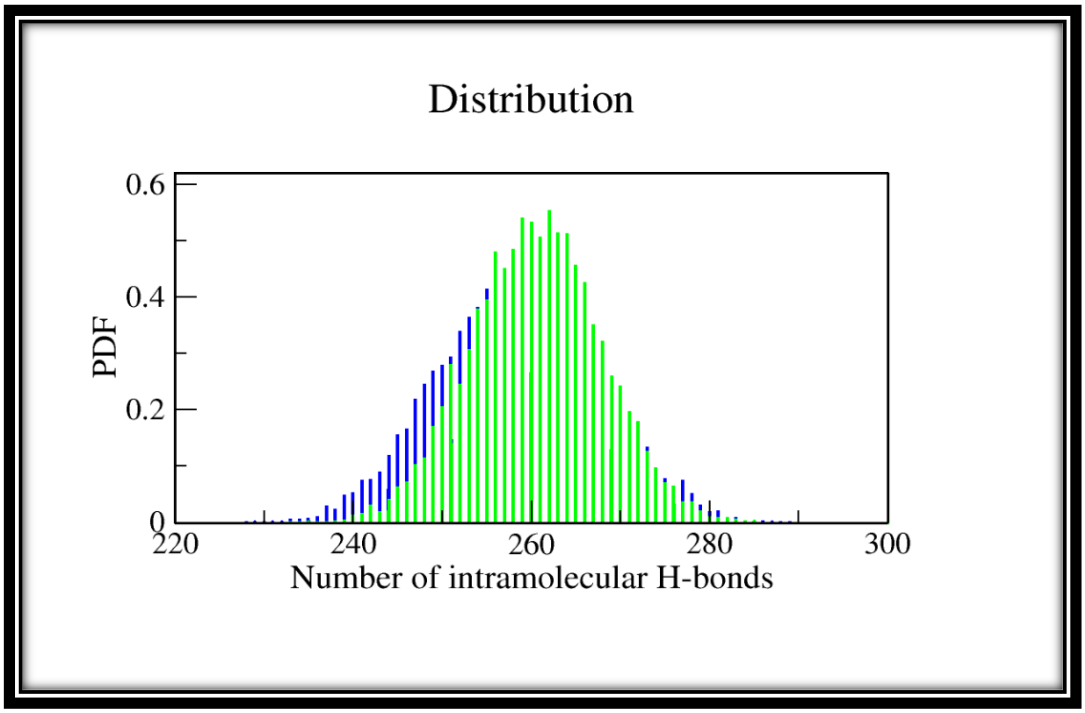


Fig. 4.21 PDF plot of distribution of H-bonds of (a) ERK1 alone ,ERK1 complexed with ergotamine (b) PDF plot of distribution H- bonds of ERK1 alone, ERK1 complexed with midostaurin.

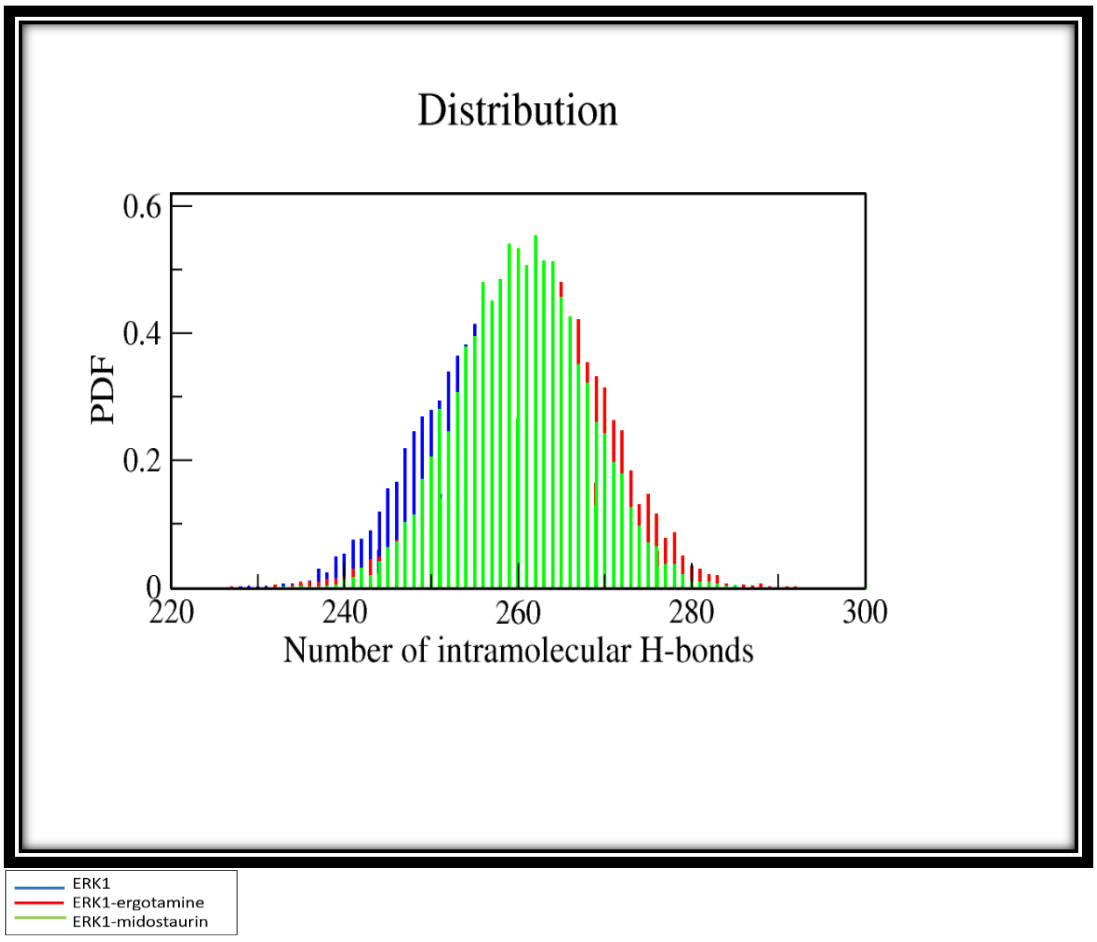
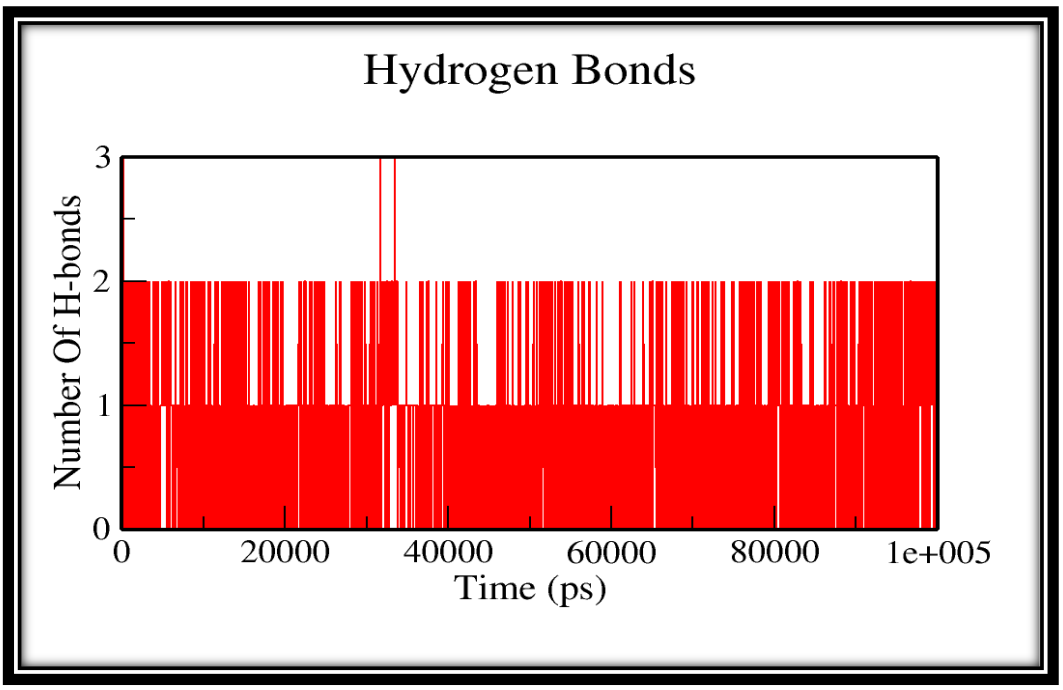
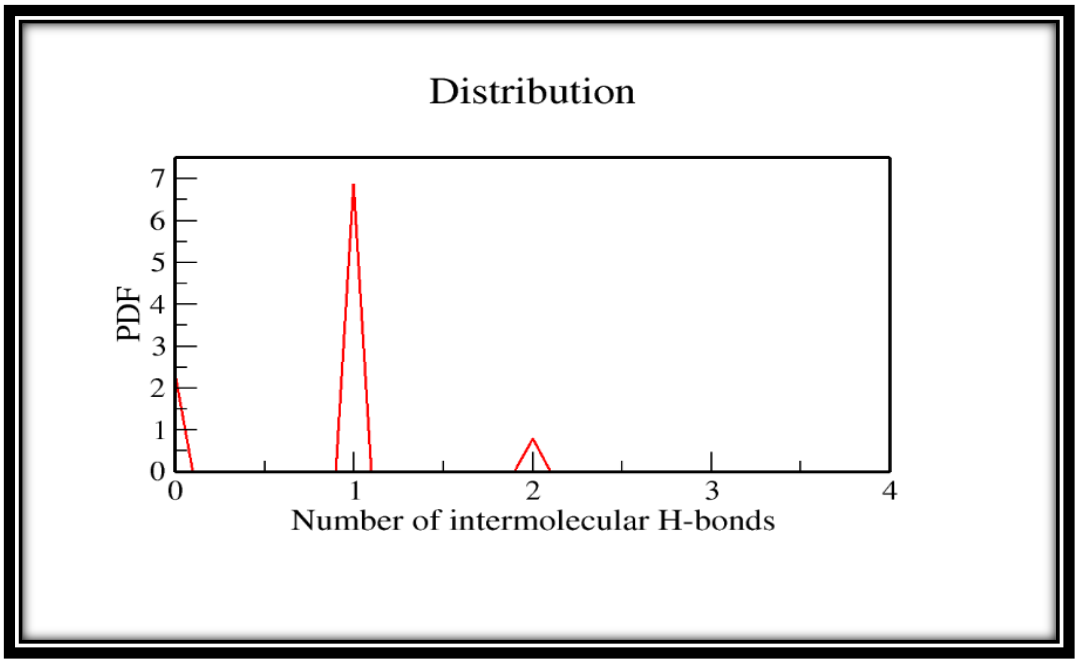


Fig. 4.22 PDF plot of distribution of H-bonds of ERK1 alone, ERK1 complexed with ergotamine and midostaurin





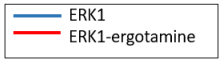


Fig. 4.23 (a) Plot showing intermolecular Hydrogen bonds formed when ERK1 complexed with ergotamine . (b) PDF plot of distribution of H- bonds of ERK1 complexed with ergotamine

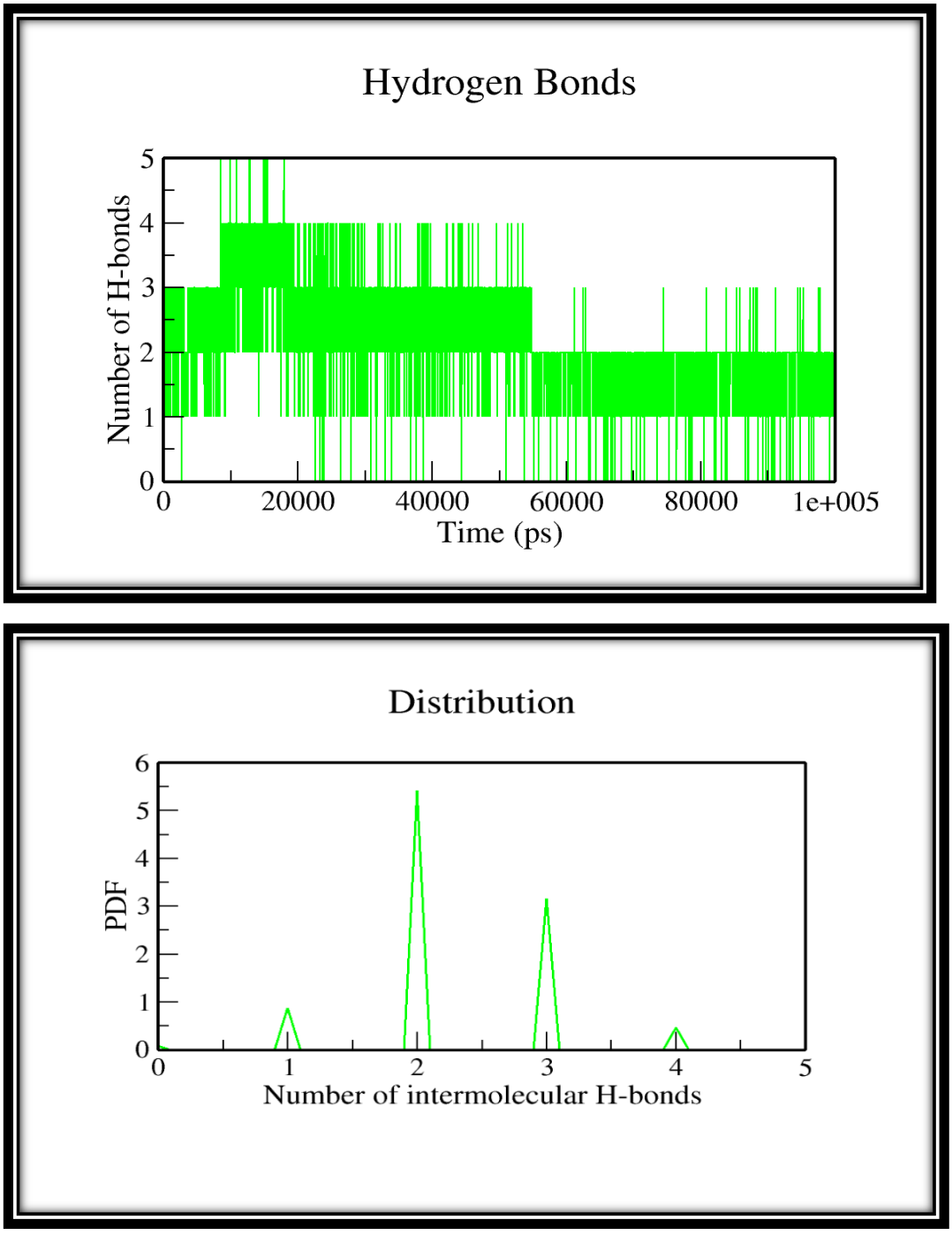


Fig. 4.24 (a) Plot showing intermolecular H- bonds formed when ERK1 complexed with midostaurin. (b) PDF plot of distribution of H- bonds of ERK1 complexed with midostaurin

Analysis of principal component of conformational sampling

PCA was used in the dynamics technique to look into conformational sampling and collectively movements of ERK1, ERK1-ergotamine, and ERK1-midostaurin complexes. Figure shows how these complexes' conformational sampling in subspace is done.

The analysis indicates that both the ERK1-ergotamine and ERK1-midostaurin complexes occupy similar conformational spaces as free ERK1. However, the ERK1-ergotamine complex exhibits lesser flexibility and covers a smaller conformational space compared to the ERK1-midostaurin complex, suggesting greater stability

Analysis of Principal component analysis of conformational sampling

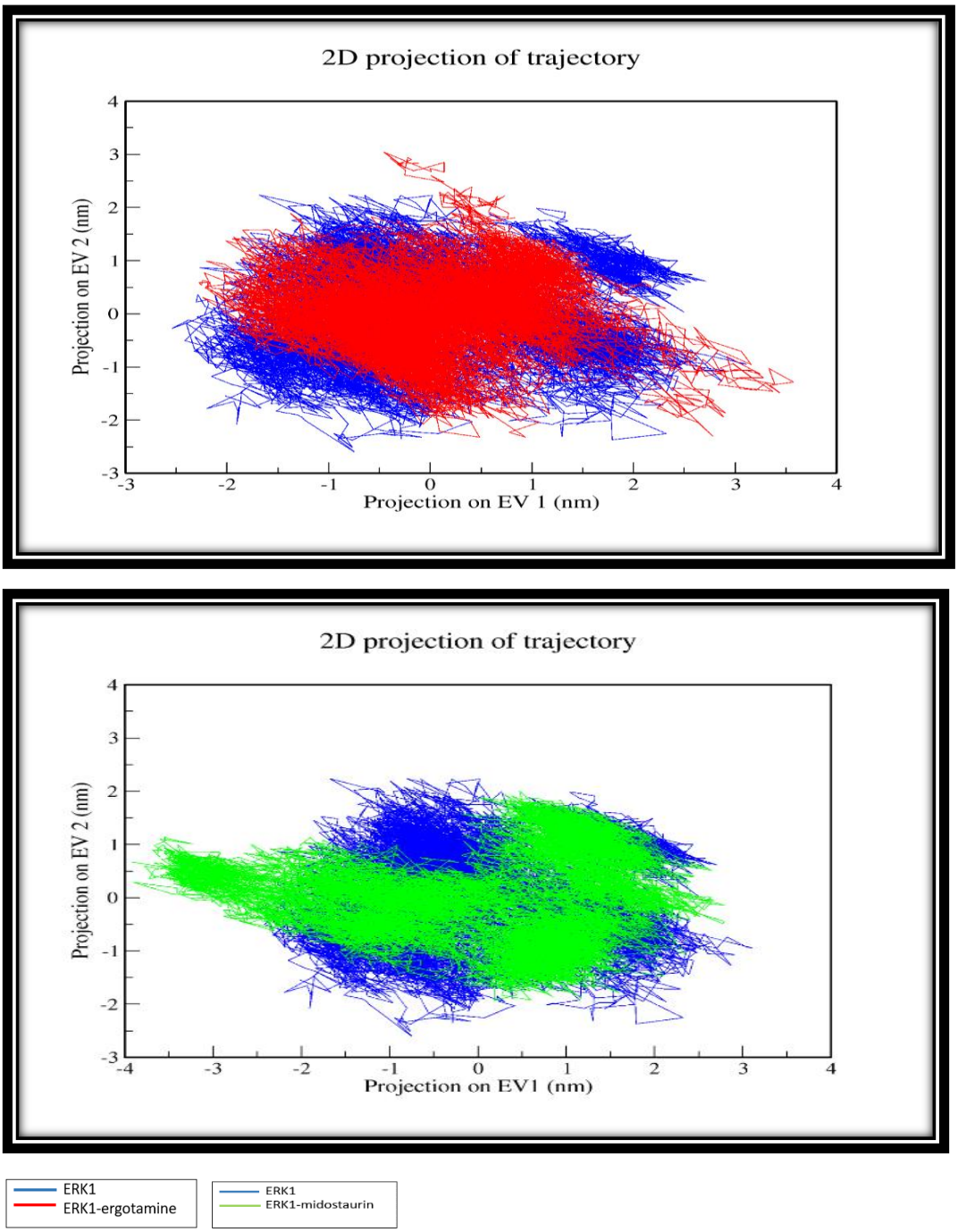


Fig. 4.25 Analysis of Principal component analysis of conformational sampling. Trajectories of 2-Dimensional projections on two (EVs) depicting conformational projections of (a) ERK1,ERK1 ERK1-ergotamine, (b) ERK1,ERK1-midostaurin

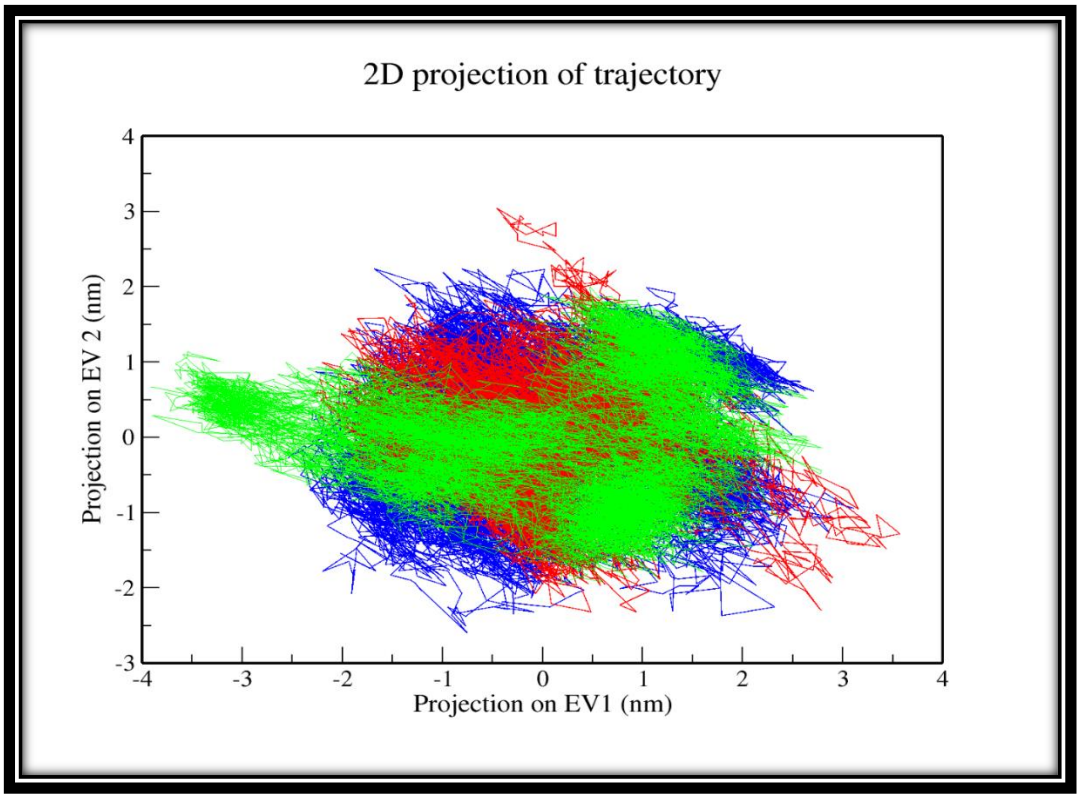


Fig. 4.26 Trajectories of 2-Dimensional projections on two (EVs) depicting conformational projections of ERK1, ERK1-ergotamine, ERK1-midostaurin

CHAPTER 5

Conclusion and Future Directions

Cancer , a predominant illness around the world, defined by the aberrant cells' uncontrollable growth and division. The fundamental cause of sporadic cancers is often DNA damage, but cancer can also result from genomic instability and inherited genetic mutations. Many genes' expressions, like kinases, is changed, which results in a variety of cellular abnormalities and propels the growth and progression of cancer

Serine/threonine kinase ERK1 (MAPK3), is a vital member of ERK/MAPK pathway/cascade. It has demonstrated potential as a treatment option for several cancer kinds.Anomalous ERK1 activation causes carcinogenesis and multiple malignancies advancement, including breast, pancreatic, hepatic, thyroid and lung cancers. Targeting ERK1 signaling can disrupt downstream pathways that promote tumor cell proliferation, invasion, and metastasis, thereby hindering cancer progression. ERK1 inhibitors offer a focused approach to overcoming chemoresistance, a common challenge in cancer treatment. However, the effectiveness of ERK1-targeted therapies may differ among cancer types, necessitating personalized treatment strategies.

In this study, computational methods were used to identify potential bioactive compounds such as ERK1 inhibitors, specifically drugs approved by the FDA. Ergotamine was shown to be a drug approved by the FDA with promise for repurposing against cancer using a structure-based drug-discovery technique. Ergotamine was discovered to have a strong affinity and stability for ERK1 and to inhibit it with noticeable drug-like characteristics. Examining the RMSF, RMSD, SASA, Rg values allowed for the validation of the binding interactions between ERK1 and the discovered drugs. Furthermore, MD simulations showed that ergotamine and ERK1 combine to create extremely stable complexes.
Notably, ergotamine, traditionally used for the treatment of acute migraine headaches and cluster headaches, has shown promise for repurposing in cancer therapy by inhibiting ERK1.

Overall, this study suggests that ergotamine may have significant inhibitory potential for ERK1. These findings open up new avenues for targeting ERK1 activity, pending further in-vivo & in-vitro and experimentation. This study emphasizes the importance of repurposing of already present drugs approved by the FDA for developing new therapies, potentially speeding up the development of effective cancer treatments. Future directions involve conducting detailed mechanistic studies, evaluating the efficacy of ergotamine in various cancer models, and eventually advancing to clinical trials to confirm its therapeutic potential.

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Pharmacoinformatics-Driven Drug Reprofiling Strategy: Identifying Innovative Bioactive Inhibitors Targeting ERK1 (MAPK3) for the advancement in Cancer Treatment

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Abstract*— ERK1, also referred to as Mitogen-activated protein kinase 3 (MAPK3), is part of the MAP kinase family and functions as a serine/threonine kinase. It plays a crucial role in the Ras-Raf-MEK-ERK signaling pathway, which is responsible for regulating a wide range of cellular processes such as cell proliferation, differentiation, and progression through the cell cycle in response to various external signals. ERK1(MAPK3) is implicated in the initiation, advancement, metastasis, drug resistance, and unfavorable prognosis in several types of cancers, including glioma, liver, ovarian, thyroid, lung, breast, gastric, and oral cancers and ERK1 (MAPK3) emerges as a promising therapeutic target. An extensive strategy was employed to identify potential inhibitors targeting ERK1. This involved conducting docking simulations using 3,674 FDA-approved drugs, with the aim of discovering compounds with enhanced affinity compared to current medications. Through a meticulous screening process, candidates were narrowed down. Subsequent Swiss ADME analysis was then conducted to evaluate the physiochemical properties, bioavailability, gastrointestinal absorption, and solubility of the chosen ligands. Among the drugs scrutinized, one particular compound emerged as a promising candidate for ERK1 inhibition. The selection process considered not just binding affinity but also crucial physiochemical characteristics essential for effective drug development. This comprehensive methodology integrates molecular docking with a broad library of FDA-approved drugs and advanced ADME analysis, significantly increasing the chances of discovering a drug with therapeutic potential. Such an integrated approach holds significant promise in identifying novel ERK1(MAPK3) inhibitors, providing a solid foundation for further preclinical and clinical investigations.

Keywords—ERK1, ERK2, MAPK3, SWISS ADME, binding affinity

I. Introduction

Extracellular signal-regulated kinase 1 (ERK1) often referred to as Mitogen-activated protein kinase 3 (MAPK3), is an essential component of the ERK/MAPK pathway, a vital cell signalling system. ERK1 and ERK2, which have an identical sequence of 84%, are often referred to as ERK1/2 due to their comparable roles. Protein-serine/threonine kinases play a major role in the Ras-Raf-MEK-ERK signal transduction cascade, which regulates a multitude of biological processes including cell adhesion, cell cycle progression, migration, survival, differentiation, metabolism, proliferation, and transcription. Through this cascade, signals are transmitted from the cell's exterior to its interior [1].

The ERK/MAPK pathway controls cell migration, proliferation, and apoptosis. In order to activate nuclear transcription factors like c-Jun and c-fos and take part in processes like cell division and death, MAPK3 phosphorylates a number of downstream cytoplasmic proteins as part of this pathway. In several types of carcinomas, such as those of the liver, thyroid, lung, and stomach, the overexpression or hyperactivity of MAPK3 has been linked to the beginning, progression, migration of cancer cells [2].

Prior research by Du et al. found that by focusing on MAPK3, overexpression of miR-143 inhibited the growth of breast cancer cells. Furthermore, Cao et al. showed that in gastric cancer cells, cisplatin resistance was associated with decreased expression of miR-129 and overexpression of MAPK3. By downregulating MAPK3, overexpression of miR-129 also inhibited cell growth and increased cell apoptosis. These results highlight the important role that MAPK3 plays in drug resistance and carcinogenesis in several cancer types. Considering these findings, MAPK3 emerges as a potential target for therapeutic interventions in cancer treatment [3].

Molecular docking analysis is a widely utilized structural bioinformatics method employed in drug discovery to identify potential inhibitors targeting specific biological targets [4]. This approach not only helps in the discovery of potential drugs but also reveals the interaction modes between macromolecules, such as proteins, and drug candidates. By simulating the binding process between a protein target and various small molecule compounds, molecular docking assists in predicting the most favourable orientations and conformations for drug binding. This information is crucial in understanding how potential drugs interact with their target proteins, aiding in the design and optimization of new therapeutic agents. Additionally, molecular docking provides insights into the strength and specificity of these interactions, guiding the development of effective treatments for various diseases.

A. Structure of ERK1 (MAPK3)

The 43 kDa protein known as ERK1 (MAPK3) is made up of 379 amino acids. The MAP kinase ERK2 (MAPK1) and it have 85% identity in common, and their substrate binding areas have even more similarity. Two DXXD docking sites

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on ERK1 (MAPK3) and ERK2 (MAPK1) function as interaction sites with a Kinase Interaction Motif (KIM) present on substrates (ELK-1) and activators (MAPKK), inhibitors (PTP-SL (PTPRR) and dual-specificity phosphatases). The TEY (Thr-Glu-Tyr) motif in the activation loop of ERK1 (MAPK3) separates it from other proteins. ERK1 (MAPK3) requires the dual phosphorylation of tyrosine (Tyr204) and threonine (Thr202) residues in order to become fully active. Activated transcription factors are phosphorylated by ERK1 (MAPK3) as it translocate into the nucleus [5], [6].

B. ERK/MAPK Signaling Cascade

A key component of the ERK/MAPK pathway, an essential physiological signalling cascade, is extracellular signal-regulated kinase 1 (ERK1), sometimes referred to as mitogen-activated protein kinase 3 (MAPK3). Because ERK1 and ERK2 have similar activities within this pathway and have 84% sequence identical, they are referred to as ERK1/2. A prominent subfamily of the MAPK family, ERK1/2 participates in a three-step enzymatic cascade that involves Raf acting as a MAPKKK (MAPK kinase kinase), MAPK/ERK1/2 kinase (MEK) acting as a MAPKK, and ERK1/2 acting as a MAPK [7], [8].

In the normal state, ERK1/2 is found in the cytoplasm. A sequence of events is set in motion upon stimulation by intracellular or extracellular signals, including growth factors, neurotransmitters, inflammation, ischemia, or hypoxia. Ras is activated as a result of tyrosine kinases being activated by their appropriate receptors. Raf is subsequently bound by Ras-GTP, which causes Raf to move from the cytoplasm to the cell membrane and cause a brief signal to be Sent at the membrane. MEK is activated through the phosphorylation of serine residues in the catalytic area by Raf. MEK then phosphorylates ERK1/2 to activate it. Following activation, phosphorylating cytoplasmic target proteins or regulating the actions of other protein kinases is how activated ERK1/2 performs its tasks. Crucially, active ERK1/2 can also go into the nucleus and phosphorylate other transcription factors such as Elk-1, c-Myc, STATs, Jun, Fos, ATF2, and Max [9].

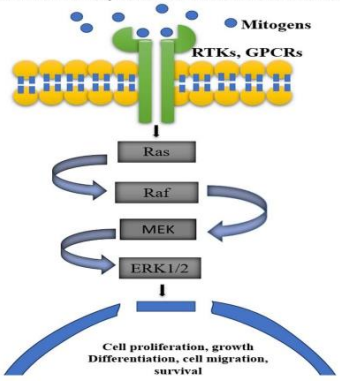

Fig. 1. ERK Signaling Pathway/Cascade

II. Methodology

A. Receptor Preparation

The protein structure of ERK1 was downloaded from the database PDB under the ID (PDB ID:4QTB) (https://www.rcsb.org/structure/4QTB). PyMOL an open source model visualization tool was used for representation and for modification of the protein (receptor) such as removal of water molecules associated with the structure

B. Ligand Preparation

For the Repurposing of drugs against ERK1, a library of 3,674 FDA Approved drugs was used retrieved from Drug Bank. The ligands were initially formatted in SDF (Structure Data File) format. SDF is a standard file format used to represent chemical structures, which can include 2D and 3D coordinates, connectivity information, and other properties of the molecules. To transform the ligands from SDF format to .pdbqt format, the Open Babel software was used.

C. Molecular Docking

InstaDock is a graphical user interface (GUI) tool designed for molecular docking. Its purpose is to simplify the process of conducting docking simulations by offering a user-friendly interface that streamlines setup and execution. InstaDock integrates QuickVina-W, a customized version of AutoDock Vina, for efficient docking calculations. Its key features include high-throughput virtual screening capabilities and the generation of detailed results, such as binding affinity and interaction patterns, for further analysis.[10]. The Excel sheet was generated post-docking which captures the binding affinity of each ligand for the protein, alongside individual output files detailing specific molecular interactions. Also, individual output files for each docked ligand were stored for subsequent 2-D interaction analysis, illustrating every interaction and hydrogen bond formation between the ligand and the macromolecule/protein. These results are crucial for evaluating ligand-protein interactions, prioritizing promising candidates for further study, and guiding drug discovery efforts toward identifying potential drug candidates. Compounds were chosen for further analysis if their binding affinity exceeded -10.9 kcal/mol, using SCH772984 (-10.9 kcal/mol) as the reference compound.

D. ADME Analysis

ADME analysis plays a vital role in drug development by evaluating how a potential drug will be absorbed, distributed, metabolized, and eliminated in the body. Swiss ADME, an online tool, was used which provides insights into pharmacokinetic characteristics, drug-likeness, and other ADME parameters for small molecules, aiding in the identification of promising therapeutic candidates. Lipinski's Rule of Five outlines criteria for assessing the drug-likeness of molecules, particularly for oral administration. These criteria include molecular weight $\leq$ 500 Daltons, LogP $\leq$ 5, and limitations on the number of hydrogen bond donors (less than five) and acceptors (less than ten). Meeting these criteria suggests favorable pharmacokinetic properties, enhancing a molecule's potential as an orally active drug Additional parameters such as water solubility and gastrointestinal absorption are also considered to determine the drug-likeness of a molecule. These guidelines are crucial in identifying

compounds with the potential to become successful orally administered drugs.

III. Result And Discussion

Out of the 3,647 FDA-approved drugs (ligands), 14 ligands were selected based on their varying binding affinities, ranging from -11 to -12.3 kcal/mol, which are higher than the reference drug SCH772984 (-10.9 kcal/mol). The ligands with the most negative binding affinities are presented in TABLE I, along with pertinent information regarding their distinct binding interactions. The details of the reference drug (SCH772984) are also included.

The top three ligands identified through ADME analysis are Ergotamine, Midostaurin, and Venetoclax, showing exceptional binding affinities and they were further investigated. Ergotamine was found to be moderately soluble with enhanced gastrointestinal absorption and exhibited a skin permeation Log Kp value of -7.68 cm/s. Midostaurin was determined to be poorly soluble with enhanced gastrointestinal absorption and had a skin permeation Log Kp value of -6.36 cm/s.

Venetoclax was observed to be insoluble/poorly soluble with low gastrointestinal absorption and showed a skin permeation Log Kp value of -5.79 cm/s.

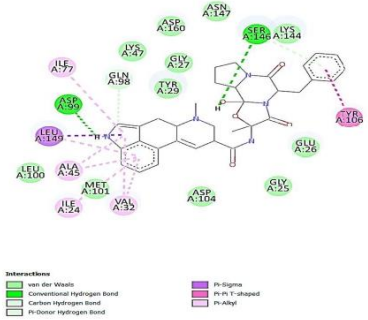

Fig. 2a. Illustrates a two-dimensional graphical depiction portraying the diverse interactions between Ergotamine (ligand with highest binding affinity) and ERK1 (MAPK3) protein.

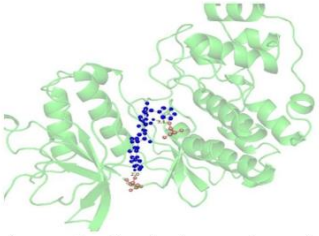

Fig. 2b. Illustrates a three-dimensional structure of Ergotamine (ligand with highest binding affinity) and ERK1 (MAPK3) protein showing H – bonds with distance.

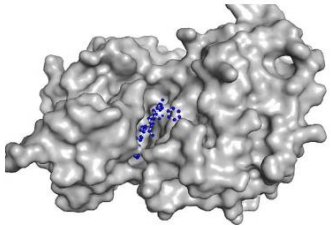

Fig. 2c. Illustrates a three-dimensional structure surface structure showing Ergotamine (ligand with highest binding affinity) in the binding pocket of ERK1 (MAPK3) protein.

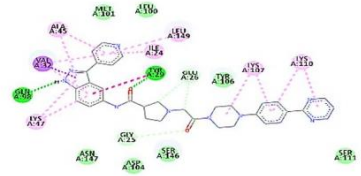

Fig. 3a. Illustrates a two-dimensional graphical depiction portraying the diverse interactions between SCH772984 (reference drug) and ERK1 (MAPK3) protein.

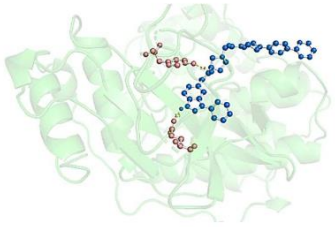

Fig. 3b. Illustrates a three-dimensional structure of SCH772984 (reference drug) and ERK1 (MAPK3) protein showing H – bonds with distance.

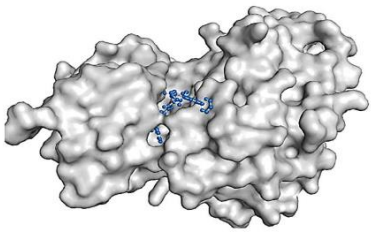

Fig. 3c. Illustrates a three-dimensional structure surface structure showing SCH772984 (reference drug) in the binding pocket of ERK1 (MAPK3) protein.

TABLE I. BINDING AFFINITY AND INTERACTIONS OF LIGANDS AND REFERENCE DRUG

Ligands (Drug Molecules)	Binding Affinity (Kcal/mol)	Amino acids are involved in different types of binding interactions		
		Hydrogen bonds	*Van-der-Waals interactions*	*Other non-polar interactions*
Ergotamine	-12.3	ASP-99,SER-146, GLN-98,LYS-144	ASP-160, ASN- 147, LYS-144, GLU-26, GLY-25, ASP-104, MET-101, LEU-100, LYS-47, GLY-27	ILE-77, TYR-106, VAL-32, ILE-24, ALA-45, LEU -149
Midostaurin	-12.1	ASP-160, SER-146 , GLU 26	LYS- 110, ILE-24 ASP-104, GLY-25, ASN- 147, LYS-144, TYR-106, LEU-149	TYR -29, LYS-107, VAL-32
Venetoclax	-12	ASP-160, ARG-60	GLU-64, THR-183, GLU-26, GLY-25, LEU-100 MET-101, GLU-102, THR -103, LYS-107, GLN-98, ILE-77 TRP-185, SER -146, LYS-47, TYR-106, PRO-145	ALA-28, ILE-24, CYS-159, LEU-149, VAL-32, ILE-24, LYS-144
Bexarotene	-11.8	GLN-59 ,ALA-164	ARG-63, GLY-162, THR-56, MET-326, TYR-180 THR-178, GLU-179, LYS-196, TYR-198, ARG-141	ARG-165, ARG-60, LEU-163, VAL-181
Dutasteride	-11.7	THR-103, CYS-159	LEU-100, LYS-107, GLU-102, GLU-26, ASP-104, GLY-27, LYS-144, ASP-160, SER-146, ASN-147, ILE-77, LYS-47	ALA-45, LEU-149, ILE-24, VAL-32, MET-101, TYR-29, GLN-98, ASP-99
Scarlet_red	-11.6	0	LEU-100, MET-101, ASP-99, GLN-98, ASN-147, ILE-96 THR-61, ARG-60, GLY-162	ASP-160, GLU-64, TYR-29, ILE-77, LYS-47, CYS-159, VAL-32, ALA-45, ILE-24, LEU-149, ILE-49
Pyrinoline	-11.5	CYS-159, ASN-147, SER1-46, ASP99	ILE-77, LEU-100, MET-101, GLU-102, THR-103, LYS-107, ASP-104, GLY-27, GLU-26, ASP-160, LYS-47, GLN-98	ALA-45, ILE-24, LEU-149, VAL-32, TYR-29
Antrafenine	-11.5	SER-146 ,ASN-147	LYS-47, MET-101, LEU-100, TYR-186, THR-183, GLU-26, GLY-25, TYR-29, CYS-159, ILE-24	VAL-32, LEU-149, GLN-98, ILE-77, ASP-99, ALA-45, TRP185, LYS-144, TYR-106, LYS-107, ASP-104
Fendosal	-11.4	ASN-147, SER-146, TYR-29	ASP-160, GLY-25, GLY-27, GLU-26, LYS-144, LYS-107, ASP-104, LEU-100, MET-101, ASP-99	LYS-47, CYS-159, ILE-24, ALA-45, LEU-149, VAL-32
Imatinib	-11.4	ASP-104, ASP-160, ASN-147 ,GLY-25	SER-146, LYS-144, ASP-142, GLY-27, ALA-28, GLU-26 LYS-107, THR-103, MET-101, LEU-100, GLN-98	GLU-64, LYS-47, TYR-29, CYS-159, ALA-45, VAL-32, ILE-77, LEU-149, ILE-24
Atovaquone	-11.3	0	LYS-107, ASP-104, THR-61, GLY-162, ASP-160, GLN-98, ALA-45	ILE-24, LEU-149, VAL-32, TYR-29, CYS-159, LYS-47, GLU-64, ILE-49, ARG-60
Tamibarotene	-11.2	TYR-29	PHE-161, GLU-64, ILE-49, ASP-160, LYS-47, CYS-159, ASN-147, SER-146, GLN-98, ILE-77, ALA-45, ASP-99, MET-101, LEU-100, ILE-24	LEU-149, VAL-32
Temoporfin	-11.1	GLU-26, TYR-23, ALA-28, SER-146, TYR-106,TYR-29,	ILE-49, ILE-24, GLY-25, LYS-107, ASP-104, LYS-144, ASN-147, ASP-142, GLY-27, LEU149, TRP-185	VAL-32, LYS-47, ASP-160
Conivaptan	-11	TYR-29, SER-146	GLN-98, ILE-24, ASN-147, THR-183, ASP-142, ALA-182, GLY-27, ALA-28, ASP-160	LEU-149, ALA-45, VAL-32, CYS-159, LYS-47, LYS-144, VAL-181, LEU-163
SCH772984 (reference drug)	-10.9	GLN-98, TYR-29, GLY-25,GLU-26	SER-111, SER-146, ASP-104, ASN-147, MET-101, LEU-100, TYR-106	LYS-47, VAL-32, ALA-45, LEU-149, ILE-24, LYS-107, LYS-110

IV. CONCLUSION

Based on a comprehensive ADME analysis and binding energy, three top ligands were identified for their outstanding binding affinities and were further investigated. Among these, Midostaurin was noted for its poor solubility with enhanced gastrointestinal absorption and a skin permeation Log Kp value of -6.36 cm/s. Venetoclax was found to be insoluble or poorly soluble with low gastrointestinal absorption and showed a skin permeation Log Kp value of -5.79 cm/s. On the other hand, Ergotamine exhibited moderate solubility with enhanced gastrointestinal absorption and demonstrated a skin permeation Log Kp value of -7.68 cm/s. Therefore, Ergotamine emerges as the most promising candidate based on ADME analysis and binding energy for the target protein ERK1.

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Society for Neurochemistry, India (SNCI), Delhi Local Chapter
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This is to certify that Prof./Dr./Ms./Mr. Nishant Kumar
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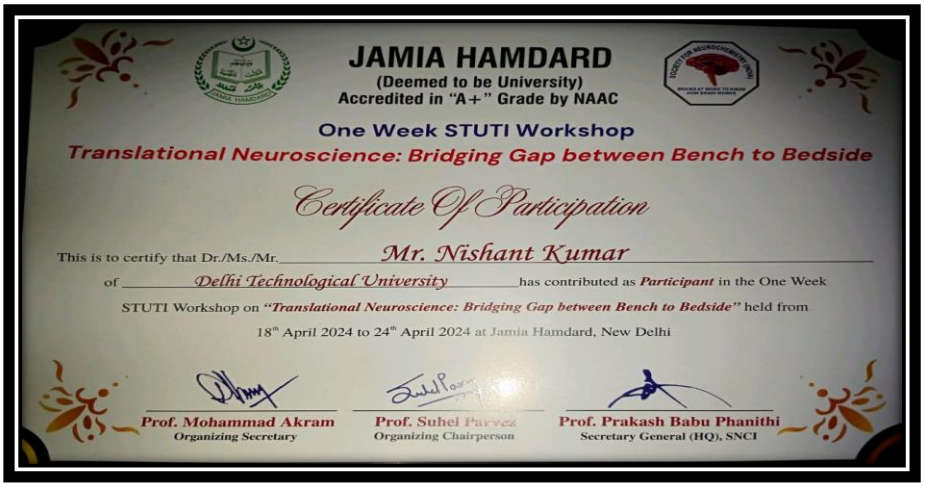

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CBSE(Class XII)	2018	Sahoday Sr. Sec. School	83.8%
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- Worked as intern under summer internship program(SRI-VIPRA 2020) under the supervision Of Dr. Robin Suyesh.
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Health Club Captain ,Sahoday Sr. Sec. School
- Served in the school cabinet

Content and Research Department Head , BIOSOC-DTU
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- Secured third position in Hasya Kavita Vachan competition
- Secured merit certificate in All India Essay Writing Competition
- Participation certificate-NAO(Journey to Nasa Astronomy Olympiad)
- Ex-Member of Anubhuti –Hindi dramatic society of Sri Venkateswara College
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Scientific Technical skills- Microbial Culture techniques, Pipetting, Multichannel Pippetting, SDS PAGE, Gel Electrophoresis, ELISA, Western Blotting , DNA Isolation.