

IN SILICO DISCOVERY OF DUAL HSP90 INHIBITORS TARGETING HDAC6 AND ALK FOR CANCER THERAPY

M.Sc. THESIS
SUBMITTED TO THE DEPARTMENT OF BIOENGINEERING
AND THE GRADUATE SCHOOL OF ENGINEERING AND SCIENCE
OF ABDULLAH GUL UNIVERSITY
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
MASTER OF SCIENCE

By
Muhsin Samet YÜCEL
August 2025

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SCIENTIFIC ETHICS COMPLIANCE

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ABSTRACT

IN SILICO DISCOVERY OF DUAL HSP90 INHIBITORS TARGETING HDAC6 AND ALK FOR CANCER THERAPY

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August 2025

Heat shock protein 90 (Hsp90), histone deacetylase 6 (HDAC6), and anaplastic lymphoma kinase (ALK) are crucial therapeutic targets in cancer research with their interconnected roles in regulating protein homeostasis and cellular processes. Interaction of these proteins within the cytosolic complex plays a critical role in regulating cancer cell survival and progression. Notably, current studies highlight that the simultaneous inhibition of Hsp90-HDAC6 or Hsp90-ALK can produce synergistic effects and offer a promising therapeutic potential for combating malignant cancers. The objective of this thesis was to explore potential compounds that can inhibit both Hsp90-HDAC6 and Hsp90-ALK proteins. Therefore, a number of in-silico computational techniques were employed. For the Hsp90-HDAC6 part, 791 molecules with similarity filtration from the ZINC database and 361,179 compounds with Lipinski rule of 5 criteria from the COCONUT database were selected for the Hsp90-ALK part. All of them were subjected to docking on responsible protein structures. The top ligands demonstrating the best binding scores against both targets, along with their references, were selected for further analysis. Subsequently, ADME prediction and molecular dynamics simulations were conducted on the selected ligands. Upon completion of all analyses, a detailed in-silico evaluation revealed that ZINC27653366 and CNP0264442.1 exhibited the highest inhibitory potential for Hsp90-HDAC6 and Hsp90-ALK, respectively, making them the most promising inhibitors.

Keywords: Molecular Docking, Molecular Dynamics, Dual Inhibitor, Cancer, ADME Analysis

ÖZET

KANSER TEDAVİSİ İÇİN HDAC6 VE ALK'Yİ HEDEF ALAN İKİLİ HSP90 İNHİBİTÖRLERİNİN İN SİLİKO KEŞFİ

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Isı şoku proteini 90 (Hsp90), histon deasetilaz 6 (HDAC6) ve anaplastik lenfoma kinaz (ALK), protein homeostazını ve hücrel süreçleri düzenlemedeki birbiriyle bağlantılı rolleriyle kanser araştırmalarında önemli terapötik hedeflerdir. Bu proteinlerin sitozolik kompleks içindeki etkileşimi, kanser hücrelerinin hayatta kalmasını ve ilerlemesini düzenlemede kritik bir rol oynar. Özellikle, güncel çalışmalar, Hsp90-HDAC6 veya Hsp90-ALK'nin eş zamanlı inhibisyonunun sinerjik etkiler üretebileceğini ve kötü huylu kanserlerle mücadele için umut verici bir terapötik potansiyel sunabileceğini vurgulamaktadır. Bu tezin amacı, hem Hsp90-HDAC6 hem de Hsp90-ALK proteinlerini inhibe edebilen potansiyel bileşikler keşfetmektir. Bu amaçla, bir dizi in-silico hesaplama tekniği kullanıldı. Hsp90-HDAC6 bölümü için, ZINC veri tabanından benzerlik filtresi ile 791 molekül ve COCONUT veri tabanından 5 kriterli Lipinski kuralı ile 361.179 bileşik Hsp90-ALK bölümü için seçildi. Seçilen ligandlar sorumlu protein yapıları üzerinde yerleştirmeye tabi tutuldu. Her iki hedefe karşı en iyi bağlanma skorlarını gösteren en iyi ligandlar, referanslarıyla birlikte daha ileri analizler için seçildi. Daha sonra, seçilen ligandlar üzerinde ADME tahmini ve moleküler dinamik simülasyonları gerçekleştirildi. Tüm analizlerin tamamlanmasının ardından, ayrıntılı bir in-silico değerlendirmesi, Hsp90-HDAC6 bölümünde ZINC27653366'nın ve Hsp90-ALK bölümünde CNP0264442.1'in en yüksek inhibitör potansiyelini gösterdiğini ve bunları en umut verici inhibitörler haline getirdiğini ortaya koydu.

Anahtar kelimeler: Moleküler Doking, Moleküler Dinamik, İkili İnhibitör, Kanser, ADME analizi

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LIST OF ABBREVIATIONS

CHE	Catastrophic Health Expenditures
GDP	Gross Domestic Product
CT	Computed Tomography
MRI	Magnetic Resonance Imaging
PET	Positron Emission Tomography
HDAC	Histone Deacetylase
DNA	Deoxyribonucleic Acid
RNA	Ribonucleic Acid
HAT	Histone Acetyltransferase
AC	Acetyl Group
PKM2	Pyruvate Kinase M2
PDB	Protein Data Bank
HSP90	Heat Shock Protein 90
ZnF-UBP	Zinc Finger Ubiquitin-Binding Domain
HDACi	Histone Deacetylase Inhibitors
ZBG	Zinc Binding Group
TSA	Trichostatin A
SAHA	Suberoylanilide Hydroxamic Acid
FDA	United States Food and Drug Administration
CTCL	Cutaneous T-cell Lymphoma
PTCL	Peripheral T-cell Lymphoma
HSF-1	Heat Shock Transcription Factor 1
ALS	Amyotrophic Lateral Sclerosis
HSP	Heat shock protein
sHSPs	Small Heat Shock Proteins
ER	Endoplasmic Reticulum
HtpG	High-temperature protein G
Hhsp90	Human Hsp90
NTD	N-terminal domain
MTD	Middle domain
CTD	C-terminal domain

Pi	Inorganic Phosphate
RTK	Receptor Tyrosine Kinase
ALK	Anaplastic Lymphoma Kinase
IR	Insulin Receptor
STAT	Signal Transducer and Activator of Transcription
NSCLC	Non-small Cell Lung Cancer
IMT	Inflammatory Myofibroblastic Tumor
ALCL	Anaplastic Large-cell Lymphoma
CNS	Central Nervous System
TKI	Tyrosine Kinase Inhibitor
CADD	Computer-aided Drug Discovery
MD	Molecular Dynamics
UCSF	University of California, San Francisco
VS	Virtual Screening
SBVS	Structure-based Virtual Screening
cryo-EM	Cryo-electron Microscopy
LBVS	Ligand-based Virtual Screening
ADME	Absorption, Distribution, Metabolism Excretion
RMSD	Root Mean Square Deviation
RMSF	Root Mean Square Fluctuation
Rg	Radius of Gyration
MM/PBSA	Molecular Mechanics/Poisson–Boltzmann Surface Area
ΔG_{bind}	Binding Free Energy
SASA	Solvent Accessible Surface Area
RCSB	Research Collaboratory for Structural Bioinformatics
COCONUT	Collection of Open Natural Products
CGenFF	CHARMM General Force Field
GROMACS	Groningen Machine for Chemical Simulations
NVT	Constant number of Particles, Volume, and Temperature
NPT	Constant number of Particles, Pressure, and Temperature
SMILES	Simplified Molecular Input Line Entry System
NS	Nanosecond
Å	Angstrom

NM	Nanometer
MW	Molecular Weight
HBD	Hydrogen Bond Donor
HBA	Hydrogen Bond Acceptor
RB	Rotatable Bond



Chapter 1

Introduction

1.1 Cancer

Cancer is a complex group of diseases marked by the uncontrolled growth of abnormal cells [1], [2]. These cells can invade nearby tissues and spread to other body parts. This unregulated cell growth is primarily caused by genetic mutations that impact cell cycle regulation, the process of apoptosis (programmed cell death), and DNA repair mechanisms [3], [4]. The pathogenesis of cancer development involves a series of genetic changes that disrupt normal cellular functions [5]. These mutations may be acquired or inherited from environmental factors such as UV light, tobacco smoke, and certain chemicals [6]. In addition, lifestyle factors such as poor diet, excess body weight, and lack of physical activity have been linked to an increased risk of developing cancer. [6], [7]. By 2022, cancer continued to pose a major global health issue, with around 20 million new cases identified and nearly 9.7 million deaths recorded across the world. [8]. Projections indicate that by 2050, the total number of cancer cases will rise to 35.3 million, a 76.6% increase from 2022 figures, with cancer deaths reaching 18.5 million, an 89.7% increase [9]. In addition to direct medical expenses, cancer has a substantial financial impact due to indirect costs including interrupted work and unpaid caregiving. [10]. According to a recent systematic review and meta-analysis, 56.1% of cancer patients globally experience catastrophic health expenditures (CHE), which are medical bills that surpass a specific threshold of household income [11]. Countries with low and moderate incomes are most affected financially. The prevalence of CHE among cancer patients may reach 67.9% in these kinds of nations, compared to 23.4% in high-income nations. [12], [13]. Between 2020 and 2050, the global economic cost of cancer is projected to reach \$25.2 trillion in international dollars, which is equal to an annual tax of 0.55% on global gross domestic product (GDP) [14]. In the United States, the financial impact of cancer is similarly

profound. In 2017, cancer-related healthcare spending was approximately \$161.2 billion. This amounted to a financial burden of roughly 1.8% of GDP [15] .

1.1.1 Classification of Cancer

The classification of cancer is critical for accurate diagnosis, prognosis, and treatment planning. Traditionally, cancers are classified based on the tissue or organ of origin (e.g., lung, breast, prostate) and the histological types, such as carcinoma, sarcoma, lymphoma, leukemia, and melanoma [3]. Carcinomas, originating from epithelial cells, represent the majority of human cancers, including breast cancer, colon cancer, and lung cancer [16]. Sarcomas arise from mesenchymal tissues like bone or muscle, while lymphomas and leukemias affect the lymphatic and blood-forming tissues [17]. In recent years, molecular and genetic profiling has revolutionized cancer classification by introducing subtypes based on gene expression patterns, chromosomal aberrations, and epigenetic modifications [18]. For example, breast cancer is divided into four subtypes: triple-negative, HER2-positive, luminal A, and luminal B. Each has unique molecular characteristics and treatment outcomes. [19], [20]. In addition to improving our knowledge of tumor biology, this molecular stratification makes precision medicine techniques easier to implement, enabling therapies to be personalized to the genetic profile of each patient's tumor [21]. Furthermore, recent advances in multi-omics technologies such as genomics, transcriptomics, and proteomics have enabled researchers to analyze cancer from multiple biological layers. These developments are helping to create a more comprehensive classification system that integrates traditional histopathology with molecular features, and these developments are allowing for more precise and personalized cancer diagnosis and treatment [22].

1.1.2 Diagnosis of Cancer

A timely and precise cancer diagnosis is necessary for better patient outcomes and efficient planning of therapy. [23], [24], [25] . The diagnosis process typically includes imaging techniques, biopsy procedures, and laboratory tests to confirm the existence and degree of cancer [26]. Imaging techniques such as computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET), and X-rays are essential for detecting and staging malignancies. [25], [27]. Imaging methods can be useful in detecting lesions, but biopsy and histological analysis are frequently necessary for a

conclusive diagnosis. [28]. The development in liquid biopsy and gene expression profiling has led to important changes in cancer diagnostics [29]. These approaches make it possible to detect genetic mutations, epigenetic alterations, and circulating tumor DNA more accurately and efficiently [30].

1.1.3 Current treatment of Cancer

In the past, cancer treatment mainly focused on surgery and radiation therapy. These approaches were successful in targeting tumors restricted to one location, but they were less effective in managing cancer that had spread beyond the original site. [31]. A breakthrough was the development of chemotherapy in the middle of the 20th century, which offered a systemic approach but frequently lacked specificity and caused significant toxicity. In recent years, treatment has shifted toward precision oncology, with immunotherapies, targeted agents, and combination strategies at the forefront. [31], [32]. Immunotherapy has transformed the treatment of melanoma, lung cancer, and renal cell carcinoma. However, challenges such as immune-related side effects and inherent resistance continue to limit its overall effectiveness. [33], [34]. Targeted therapies, such as next-generation tyrosine kinase inhibitors and antibody-drug conjugates, provide better selectivity, but they are often compromised by acquired resistance and intratumoral heterogeneity [35]. However, chemotherapy remains a central modality, especially for hematological malignancies and solid tumors where molecular targets are unknown or resistance mechanisms have emerged [36]. Since it can cause tumor regression in a variety of cancer types due to its broad cytotoxic activity, it is useful in aggressive or advanced malignancies. Additionally, to increase therapeutic effectiveness and lower the risk of resistance, chemotherapy is frequently used in combination regimens with targeted medicines or radiation therapy [37]. From a drug discovery perspective, chemotherapy has significantly contributed to our understanding of the key molecular mechanisms that allow cancer cells to survive, including DNA replication, cell division control, and stress response pathways. These insights have led to the rational design of more targeted treatments, such as dual inhibitors that simultaneously target multiple oncogenic drivers [38], [39]. Chemotherapy is still relevant in this context because of its conceptual contribution to the development of modern oncology drug research as well as its therapeutic utility. For example, dual inhibition techniques frequently attempt to decrease systemic toxicity by concentrating on cancer-specific targets while reproducing or

improving the broad-spectrum effects of conventional chemotherapy [40]. While numerous molecular targets have been identified in cancer research, only a limited subset has demonstrated consistent therapeutic relevance across multiple cancer types. These validated targets often play central roles in maintaining hallmark processes of cancer, such as sustained proliferative signaling, resistance to cell death, and genome instability [3]. In this context, histone deacetylase 6 (HDAC6), heat shock protein 90 (Hsp90), and anaplastic lymphoma kinase (ALK) have emerged as highly promising targets due to their involvement in multiple cancer-related cellular processes. Together, these targets offer an ideal equilibrium between therapeutic tractability and functional relevance, which makes them appealing candidates for the creation of dual inhibitors that can interfere with several cancer-enabling networks simultaneously. The following sections will explore each of these targets in greater detail, outlining their biological roles, clinical relevance, and potential for dual-target drug discovery.

1.2 Histone Deacetylases

Histone deacetylases (HDACs) are a class of enzymes responsible for catalyzing the deacetylation of lysine residues on both histone and non-histone proteins [41], [42]. In the human genome, 18 distinct isoforms of HDAC have been identified, which function through either zinc-dependent or NAD⁺-dependent catalytic mechanisms. While the removal of acetyl groups from histones by HDACs serves as a key epigenetic regulatory mechanism that influences chromatin remodeling and gene expression, the deacetylation of non-histone proteins extends the functional repertoire of HDACs by modulating a wide range of cellular processes [41], [43]. Figure 1.1 illustrates the epigenetic regulation of gene expression through histone acetylation and deacetylation. DNA is tightly wrapped around histone proteins, forming condensed nucleosomes. This compact structure restricts access to transcriptional machinery, such as RNA polymerase II, and inhibits gene expression. Histone acetyltransferases (HATs) catalyze the addition of acetyl groups (AC) to lysine residues on histone tails. This acetylation neutralizes the positive charge of histones, weakening their interaction with the negatively charged DNA. As a result, chromatin adopts a more relaxed, open conformation, allowing RNA polymerase II to access DNA and initiate transcription. On the other hand, histone deacetylases (HDACs) remove acetyl groups from histones. Histone-DNA interactions are strengthened, and

chromatin structure is compressed. Therefore, RNA polymerase II accessibility is limited, and gene transcription is suppressed. This reversible process of histone acetylation and deacetylation plays a crucial role in the dynamic regulation of gene expression and is a key mechanism of epigenetic control in eukaryotic cells [44], [45], [46].

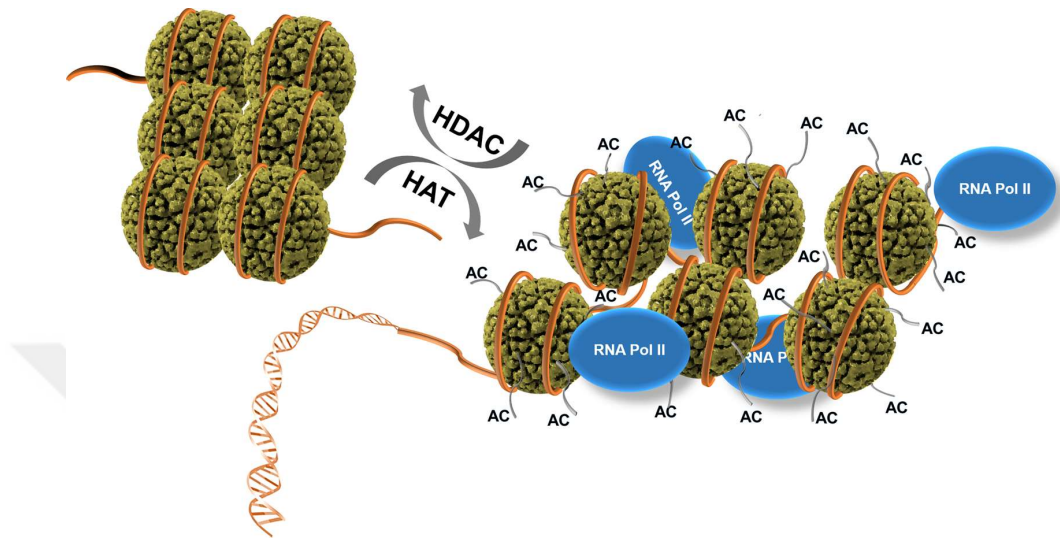


Figure 1.1 Schematic representation of histone acetylation and deacetylation in the regulation of gene expression. In the compact chromatin state (left), tight histone–DNA interactions restrict RNA polymerase II access, repressing transcription. Histone acetyltransferases (HATs) catalyze the addition of acetyl groups (AC) to lysine residues on histone tails, loosening chromatin and allowing transcriptional activation. Conversely, histone deacetylases (HDACs) remove acetyl groups, restoring chromatin compaction and repressing gene expression [44].

1.2.1 Classification of Histone Deacetylases

To date, 18 distinct histone deacetylases (HDACs) have been identified in humans, and based on their structural features, functional properties, and homology to yeast counterparts, they are classified into four major classes: I, II, III, and IV [47] (Figure 1.2). Histone deacetylases are grouped into four major classes based on structural and functional characteristics [41], [48]. Class I HDACs, which include HDAC1, 2, 3, and 8, are primarily localized in the nucleus and play key roles in transcriptional repression [48]. Class II HDACs consist of HDAC4, 5, 6, 7, 9, and 10, and are further subdivided into Class IIa (HDAC4, 5, 7, and 9) and Class IIb (HDAC6 and 10), with the latter characterized by the presence of two catalytic domains [49], [50]. Class III HDACs, also known as sirtuins (SIRT1–SIRT7), are NAD^+ -dependent enzymes with diverse regulatory functions [44]. Class IV is represented solely by HDAC11, which exhibits structural and functional features of both Class I and Class II HDACs [51].

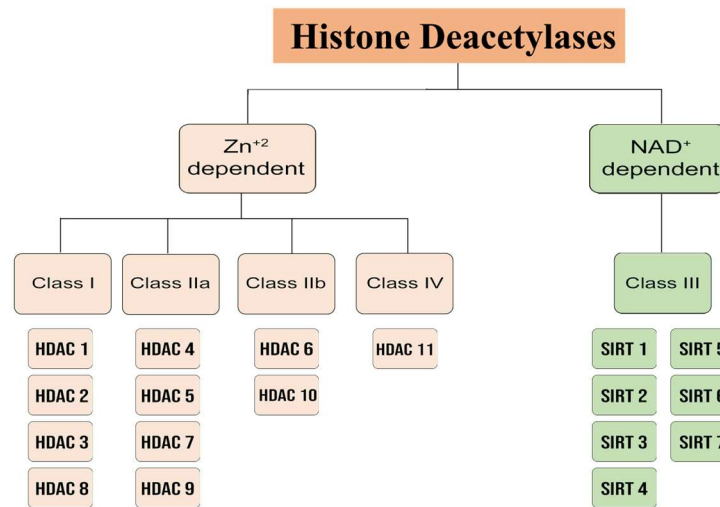


Figure 1.2 Classification of HDACs.

1.2.2 Role of Histone Deacetylases in Cancer

In cancer, abnormal HDAC activity has been linked to the silencing of tumor suppressor genes and the promotion of oncogenic pathways, which contribute to tumor initiation and progression [52]. As explained in the above section, HDACs are categorized into four classes based on their homology and function. They are found to be expressed across a variety of tumor types. Specifically, HDACs belonging to classes I and II are widely recognized as oncoproteins due to their ability to modulate gene expression through interactions with various substrates. These enzymes play a role in starting and developing tumors. They can work on their own or together with transcriptional co-repressors [53], [54], [55]. When HDACs remove acetyl groups from transcription factors including p53, E2F, and c-Myc, it changes how these proteins work. This affects important cell processes such as the cell cycle, apoptosis, and DNA repair mechanism. In addition to their role in gene regulation, HDACs also exert significant influence over cellular metabolism. Notably, HDAC3 has been shown to regulate the activity of pyruvate kinase M2 (PKM2), which is a critical enzyme in the glycolytic pathway. With this interaction, HDAC3 contributes to metabolic reprogramming which is also another characteristic of cancer cells [53], [56], [57]. In general, HDACs play a multifaceted role in cancer development and progression by regulating gene expression, protein function, and cellular metabolism. Understanding the specific mechanisms by which HDACs contribute to oncogenesis has an important role in the development of targeted therapies that aimed at modulating their activity in cancer treatment.

1.2.3 Histone Deacetylases 6

Histone deacetylase 6 (HDAC6) is a distinctive member of the class IIb histone deacetylase family. They are characterized by their predominant cytoplasmic localization, dual catalytic domains, and a unique ubiquitin-binding zinc finger domain. These properties set them apart from other HDACs that are primarily nuclear and focus on histone substrates [58]. The crystal structure of selected HDAC6 (PDB ID: 7UK2) for the experiments and its interaction with the ligand can be seen in Figures 1.3 and 1.4, respectively.

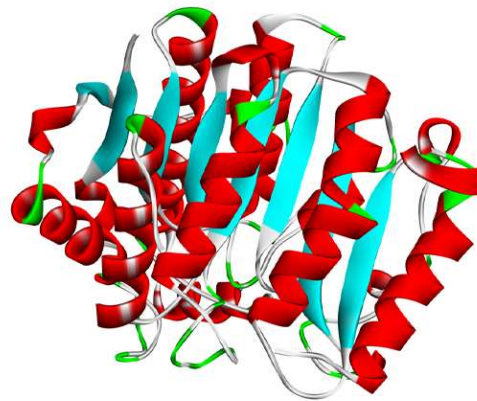


Figure 1.3 Selected HDAC6 structure, namely 7UK2 retrieved from protein data bank (PDB).

Also, unlike classical nuclear HDACs that regulate gene expression through histone deacetylation, HDAC6 primarily targets non-histone substrates, including α -tubulin, heat shock protein 90 (Hsp90), cortactin, and peroxiredoxins [59], [60]. HDAC6 is essential for the cellular stress response because it facilitates the removal of misfolded proteins by the aggresomal pathway and autophagy [61], [62]. This multifunctional protein possesses two catalytic domains and a zinc finger ubiquitin-binding domain (ZnF-UBP), which enables it to regulate various cellular processes, including cytoskeletal organization, protein degradation, autophagy, and stress granule dynamics [62]. It is essential for immune cell function and cancer metastasis because HDAC6 regulates microtubule stability by deacetylating α -tubulin, which in turn affects cell motility, intracellular trafficking, and immunological synapse formation [63].

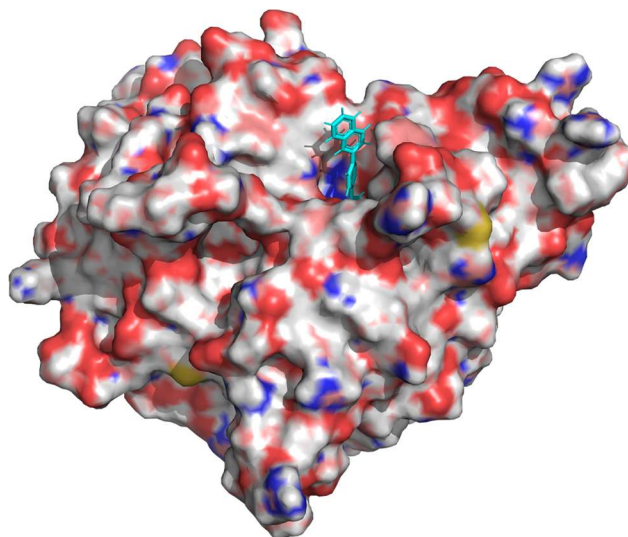


Figure 1.4 Structure of HDAC6 (7UK2) and its respective binding ligand.

Furthermore, the function of the chaperone is modulated by HDAC6's capacity to deacetylate Hsp90, which affects the stability and maturation of oncogenic client proteins like AKT, HER2, and BCR-AB [64]. Its overexpression has been implicated in various cancers, including breast, ovarian, and multiple myeloma, where it contributes to enhanced cell survival and resistance to proteotoxic stress [65], [66]. With a number of selective inhibitors presently undergoing clinical research, HDAC6 has become a desirable target for cancer treatment with its non-nuclear targets and selective functions [67].

1.2.4 Histone Deacetylases 6 Inhibitors

HDAC inhibitors (HDACis) typically consist of three main structural elements: a cap group that interacts with the outer surface of the enzyme, a zinc-binding group (ZBG) responsible for coordinating with the catalytic zinc ion, and a linker that connects these two regions. The linker spans the distance between the cap and the ZBG and ensures that the molecule adopts a conformation suitable for effective binding to the enzyme. [68]. Among the vast array of histone deacetylase inhibitors, several candidates have progressed from experimental evaluation to clinical application (Figure 1.5). Trichostatin A (TSA), a potential HDAC inhibitor, remains confined to preclinical research due to its significant toxicity, limiting its therapeutic potential [69]. In contrast, vorinostat (suberoylanilide hydroxamic acid, SAHA) emerged as the first HDAC inhibitor approved by the United States Food and Drug Administration (FDA), specifically for the treatment of relapsed and refractory cutaneous T-cell lymphoma (CTCL) [70]. Since then,

additional HDACi have gained regulatory approval to manage hematologic malignancies. Notable examples are romidepsin, belinostat (PXD-101), which is approved for peripheral T-cell lymphoma (PTCL), and panobinostat (LBH589), which is approved for multiple myeloma. Together with vorinostat, these substances work as pan-HDAC inhibitors, and they focus on several HDAC isoforms simultaneously. Broad-spectrum HDAC inhibition for cancer treatment is still of interest, as seen by the several pan-HDACi that are currently undergoing clinical examination, including givinostat (ITF2357), resminostat (4SC201), abexinostat (PCI-24781), and quisinostat (JNJ-26481585). In parallel, selective HDAC inhibitors have also been developed, primarily within the hydroxamic acid class. Rocilinostat (ACY-1215), a selective inhibitor of class II HDACs; pracinostat (SB939), which exerts inhibitory effects across classical HDAC classes I, II, and IV; and CHR-3996, a class I-selective HDAC inhibitor, are all under active investigation in clinical trials. These developments highlight the therapeutic promise of both pan- and isoform-selective HDAC inhibitors in the treatment of various malignancies, particularly lymphomas and multiple myeloma [69].

Although numerous histone deacetylase isoforms have been extensively investigated and their corresponding inhibitors thoroughly characterized, HDAC6 has attracted considerable scientific interest since its initial identification [71]. HDAC6 is a structurally and functionally distinct cytoplasmic deacetylase recognized for its selective deacetylation of a range of non-histone cytosolic substrates. Notably, it targets key proteins such as heat shock protein 90 (Hsp90), cortactin, peroxiredoxin, α -tubulin, and heat shock transcription factor 1 (HSF-1) [72]. It means that HDAC6 plays critical roles in various cellular processes. Numerous studies have demonstrated that selective inhibition of HDAC6 represents a promising therapeutic strategy. This approach has shown considerable potential in the context of neurodegenerative disorders, including Alzheimer's disease, Huntington's disease, and Parkinson's disease [73], as well as in the treatment of various malignancies such as bladder cancer, malignant melanoma, and lung cancer [74]. Furthermore, the broad therapeutic importance of targeting HDAC6 has also been shown by recent studies that have extended the scope of HDAC6 inhibitors to include rare neurological conditions like Rett syndrome, Charcot-Marie-Tooth disease, and amyotrophic lateral sclerosis (ALS) [75], [76].

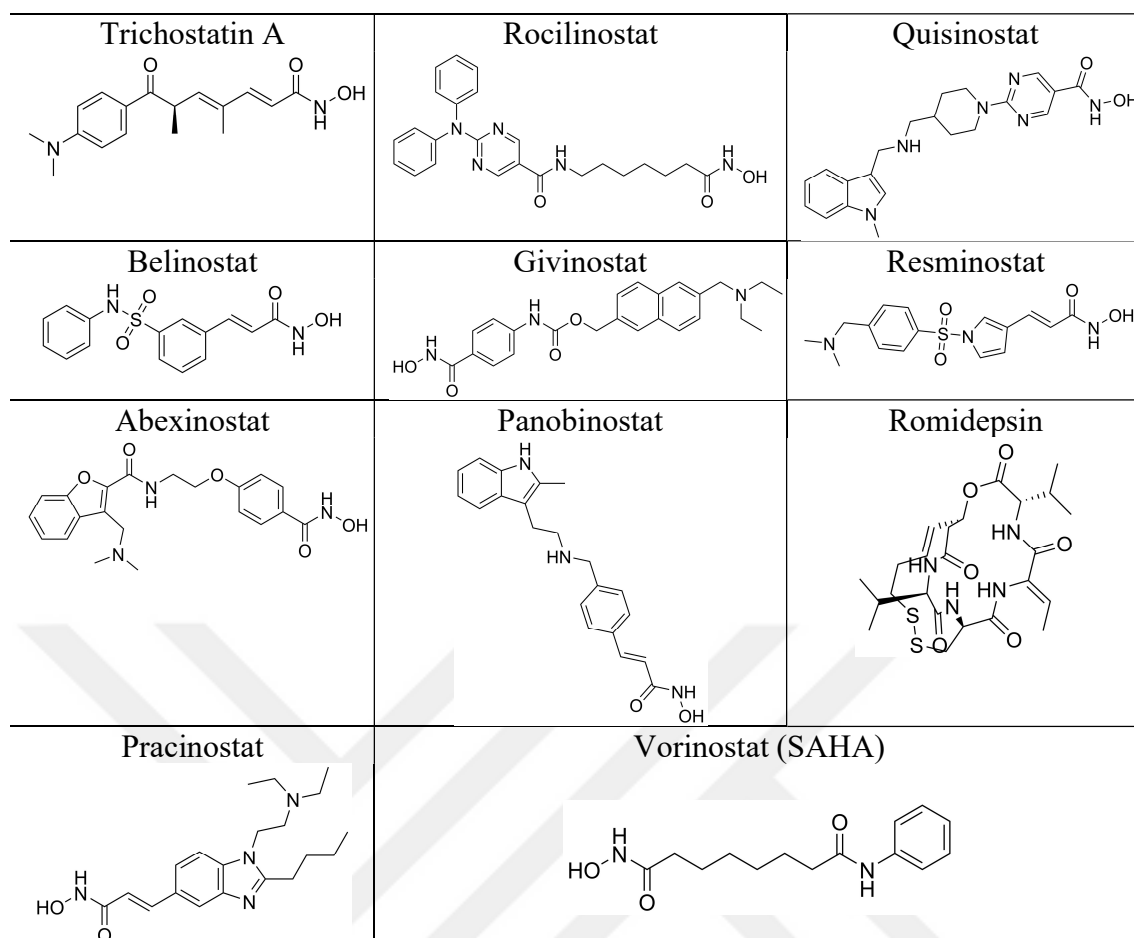


Figure 1.5 Structures of known HDAC inhibitors.

Computational research on HDAC6 inhibitors has increased recently, with a number of noteworthy *in silico* studies using molecular docking, pharmacophore modeling, virtual screening, and molecular dynamics (MD) simulations. For example, Poonia et al. (2022) conducted a HypoGen 3D QSAR pharmacophore model to screen more than 8 million compounds from the ZINC database. This technique has been followed by molecular docking, ADMET filtering, and MD analysis. In the end, they identified a lead “N1” molecule with binding properties comparable to rocinostat [77]. In another study, Sixto-López et al. (2021) designed a set of 485 compounds with hydroxamic and bulky-hydrophobic moieties and conducted docking and MD simulations on all these hydroxamic derivatives. They found that several compounds have displayed significant HDAC6 affinity and *in vitro* neuroprotective activity, among which the most potent compound was FH-27 [78]. Additionally, Shamsi et al. (2024) performed virtual screening of FDA-approved drugs from DrugBank against HDAC6 and highlighted that penfluridol and pimozone showed stable docking profiles and favorable 500 ns MD and

binding free energy results [79]. The structures of these compounds can be seen in Figure 1.6.

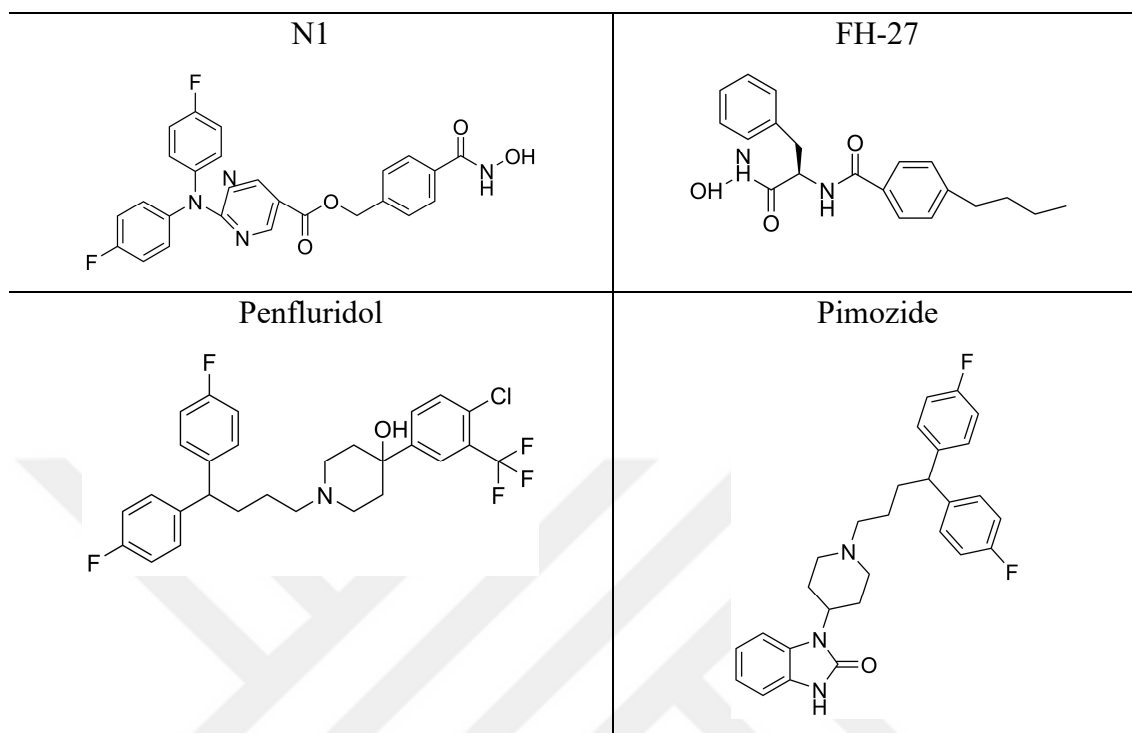


Figure 1.6 Structures of lead compounds identified in previous studies with potent HDAC6 inhibitory activity.

1.3 Heat Shock Protein

Heat shock proteins (HSPs) are a family of highly conserved molecular chaperones that are synthesized in response to various forms of cellular stress, including elevated temperatures, oxidative damage, hypoxia, and exposure to toxins [80], [81]. Molecular chaperones are indispensable for fundamental cellular maintenance processes, including folding newly synthesized polypeptide chains, translocating proteins across intracellular membranes, quality control mechanisms within the endoplasmic reticulum, and regulating protein degradation and turnover. Beyond these core functions, chaperones are also critically involved in more complex cellular activities, such as the post-translational modulation of signaling proteins, the dynamic assembly and disassembly of transcriptional complexes, and the processing of antigenic peptides essential for immune system function. [82]. The fundamental role of HSP is to maintain proteostasis by assisting in the proper folding of nascent proteins, preventing the aggregation of

misfolded proteins, and facilitating the refolding or degradation of denatured polypeptides [83]. HSPs are ubiquitously expressed in both prokaryotic and eukaryotic organisms and are essential for cellular survival under physiological and pathological conditions [84]. HSPs also play critical roles in development, aging, and immune response. Notably, their aberrant expression and function have been implicated in the pathogenesis of various diseases, including neurodegenerative disorders and cancer. Because of their involvement in protein quality control and cellular signaling pathways, HSPs are increasingly recognized as potential therapeutic targets [82].

1.3.1 Classification of Heat Shock Protein

Heat shock proteins (HSPs) are classified into several families based on their molecular weight and functional characteristics (Figure 1.7). The primary classes include large HSPs, HSP90, HSP70, HSP60, HSP40, and small HSPs (sHSPs). Each class plays a distinct role in the maintenance of protein homeostasis [81], [85], [86]. Large heat shock proteins such as HSP110 and Grp170 are high-molecular-weight chaperones predominantly found in eukaryotic organisms. HSP110 is broadly expressed in both the cytoplasm and the nucleus, while Grp170 is specifically localized in the endoplasmic reticulum (ER) [87]. Due to their structural and functional homology with the HSP70 family, they are frequently classified within the HSP70 superfamily. These chaperones are constitutively expressed under normal physiological conditions, but their levels can be rapidly upregulated in response to cellular stress to support proteostasis. Functionally, both HSP110 and Grp170 act as molecular holdases and they stabilize unfolded or misfolded proteins and prevent their aggregation during proteotoxic stress [87], [88].

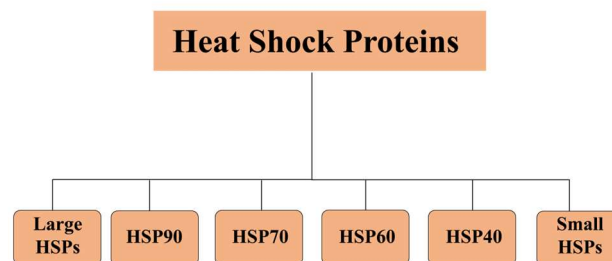


Figure 1.7 Classification of Heat Shock Protein.

1.3.2 Role of Heat Shock Protein in Cancer

Heat shock proteins (HSPs) are of significant interest in cancer research due to their roles in maintaining cellular homeostasis under stress. This condition is frequently encountered in the tumor microenvironment [89]. HSPs allow malignant cells to survive under situations that might be lethal, such as genotoxic stress, hypoxia, and nutrition restriction, by stabilizing various oncogenic proteins and inhibiting apoptosis [82], [90], [91]. Their ability to assist in properly folding, trafficking, and functional regulation of proteins involved in proliferation and survival makes them crucial facilitators of tumor growth and metastasis [92]. HSPs are frequently overexpressed in different types of cancer and are linked to aggressive tumor behavior, resistance to treatment, and poor clinical outcomes. These characteristics not only emphasize their significance in tumor biology but also suggest their potential as biomarkers for diagnosis and prognosis [92]. Among the HSP family members, HSP90 has become the most extensively studied and therapeutically targeted chaperone in oncology due to its unique ability to stabilize and activate a broad range of client proteins that drive tumor progression [93]. The role of HSP90 in cancer and its potential as a target for treatment will be covered in more detail in the following section.

1.3.3 Heat Shock Protein 90

Heat shock protein 90 (HSP90) is a highly conserved molecular chaperone that plays a crucial role in maintaining cellular proteostasis by assisting in the proper folding, stabilization, and functional regulation of various client proteins [94]. The crystal structure of selected Hsp90 for the experiments and its interaction with the ligand can be seen in Figures 1.8 and 1.9, respectively.

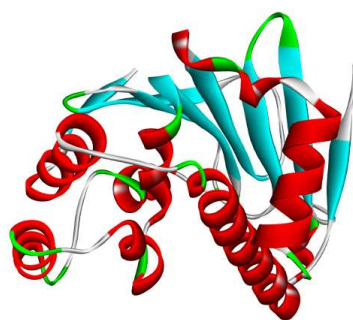


Figure 1.8 Selected Hsp90 structure, namely 3OWD retrieved from protein data bank (PDB).

Hsp90 is among the most prevalent proteins within the cell and demonstrates a high degree of evolutionary conservation across species, from bacteria to humans. The Hsp90 homolog in *Escherichia coli*, known as high-temperature protein G (HtpG), exhibits around 50% sequence similarity with the human Hsp90 (hHsp90) [95]. Hsp90 is crucial for the folding, stabilization, and functional regulation of various client proteins and many of which play significant roles in signal transduction, cell cycle regulation, and transcription [96]. The structurally conserved chaperone Hsp90 is composed of three distinct domains: the N-terminal domain (N domain), which harbors the ATP-binding site, a drug-binding pocket, and motifs for co-chaperone interaction; the middle domain (M domain), which facilitates binding to client proteins and co-chaperones and contributes to the formation of the functional ATPase; and the C-terminal domain (C domain), which contains the dimerization motif, an additional drug-binding site, and interfaces for interactions with various co-chaperones [93], [94], [97].

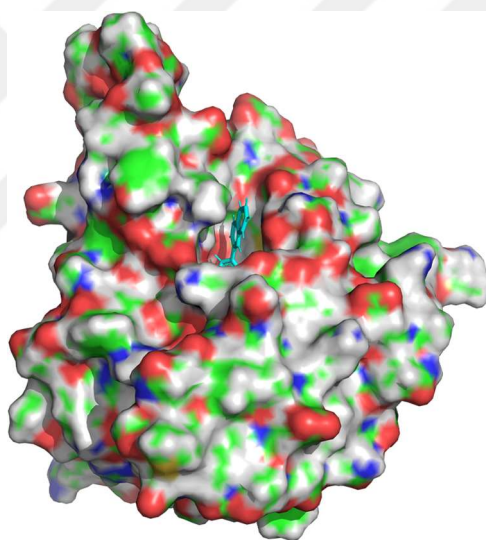


Figure 1.9 Structure of Hsp90 (3OWD) and its respective binding ligand.

Figure 1.10 illustrates that in the absence of nucleotides, Hsp90 exists in an open conformation where C-terminal domain-driven dimerization allows client binding at the middle domain [81]. When ATP binds, the N-terminal domains of the chaperone come together, resulting in a partially closed state (Close I) that helps stabilize the client protein. After ATP is hydrolyzed, the chaperone shifts into a fully closed conformation (Close II), which supports proper folding and maturation of the client. Finally, the release of ADP and inorganic phosphate (Pi) resets Hsp90 to its open conformation, allowing the client protein to be released and a new cycle to begin. This process is finely regulated by cochaperones, which modulate ATPase activity, client binding, and protein quality

control [81]. Hsp90 works in conjunction with a network of co-chaperones, including HOP, p23, Aha1, and CDC37, to regulate its activity, client specificity, and ATPase cycle. These co-chaperones play a critical role in regulating the activity of Hsp90. They modulate its function in a context-dependent manner. This regulation becomes important in cells exposed to environmental stress or oncogenic signals. [98], [99]. Collectively, the structural intricacy of HSP90, its broad functional capacity, and its tendency to associate with oncogenic client proteins emphasize its fundamental importance in maintaining cancer cell viability and promoting adaptive responses under stress.

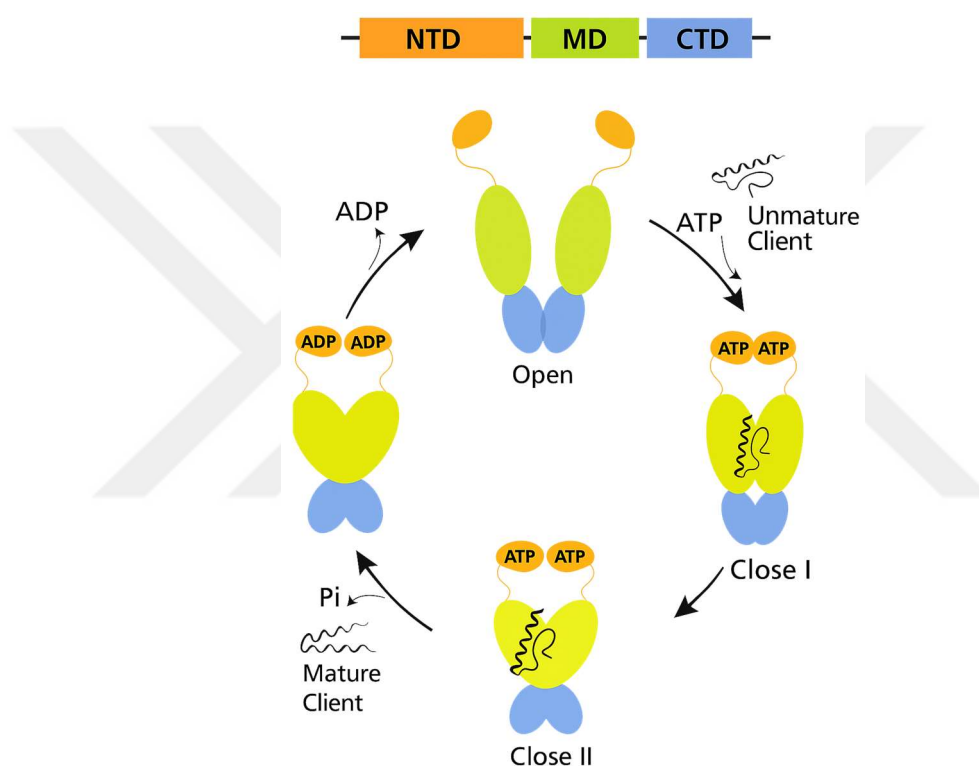


Figure 1.10 A schematic representation of Hsp90 domain and its ATP-dependent chaperone cycle [81].

1.3.4 Heat Shock Protein 90 Inhibitors

The therapeutic targeting of Hsp90 has garnered considerable attention due to its central role in maintaining the stability and function of numerous oncogenic client proteins [93]. Hsp90 inhibitors (Hsp90i) are generally categorized into three main classes based on their binding sites within the Hsp90 structure: (1) inhibitors that target the ATP-binding pocket within the N-terminal domain (NTD), (2) inhibitors that disrupt interactions with co-chaperones, and (3) inhibitors that bind to the middle domain (MD) or the C-terminal domain (CTD) [100], [101]. Extensive efforts have been directed

toward developing inhibitors that specifically target the N-terminal domain (NTD) of Hsp90, as this region is responsible for ATP binding and hydrolysis—key processes required for the activation of its chaperone function. Among the various classes of Hsp90 inhibitors, those targeting the NTD ATPase site have been the most widely explored in clinical trials, with over 100 studies conducted to date; however, none have yet received FDA approval (Figure 1.11) [101], [102].

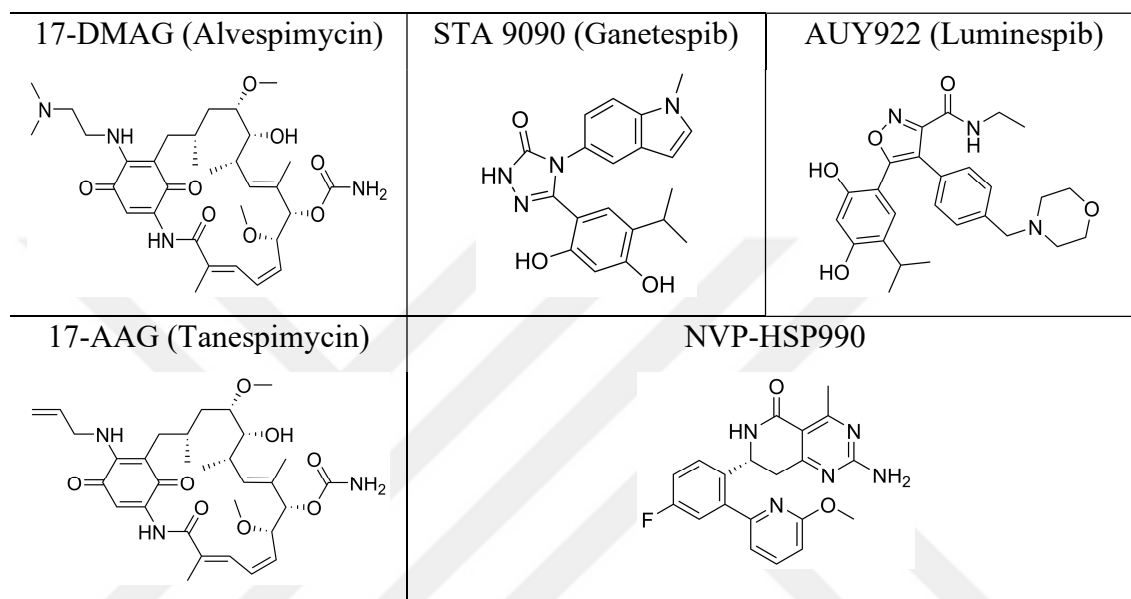


Figure 1.11 Structures of known Hsp90 inhibitors.

AUY922 (luminespib) is a resorcinol-based inhibitor that targets the N-terminal ATP-binding domain of Hsp90 [103]. This binding leads to the degradation of client proteins like JAK2 and suppresses downstream proliferative signaling pathways. In a phase II clinical study, AUY922 showed therapeutic potential in treating myeloproliferative neoplasms, particularly by stabilizing the disease and alleviating anemia [104]. Similarly, ganetespib (STA-9090), a second-generation triazolone-containing inhibitor, has shown superior potency and safety compared to the first-generation compound 17-AAG. Preclinical studies have revealed its effectiveness in inducing rapid degradation of HSP90 client proteins and suppressing tumor progression [105]. Another example for HSP90i is NVP-HSP990, a highly selective oral that has demonstrated potent antitumor activity across various cancer models [106]. NVP-HSP990 exhibits strong antiproliferative effects across a range of tumor cell lines and primary patient samples *in vitro*, as well as therapeutic efficacy in several human tumor xenograft models *in vivo*. These preclinical findings, combined with favorable

pharmaceutical characteristics, support continued investigation of NVP-HSP990 in clinical trials [107].

In recent years, *in silico* research studies that target Hsp90 have gained importance by utilizing a combination of structure-based virtual screening, pharmacophore modeling, molecular docking, and molecular dynamics simulations to identify novel and multitarget Hsp90 inhibitors. Ouassaf et al. (2023) focused on marine-derived molecules by applying pharmacophore modeling, virtual screening, *in silico* ADMET assessment, and molecular dynamics simulations. A comprehensive investigation was carried out on a dataset of 31,488 chemicals obtained from the CMNPD database during the virtual screening stage. Following the studies, they highlight the six new marine chemicals as potential candidates, the two best of them can be seen in Figure 1.12 for the development of antimicrobial and anticancer drug development [108]. In another study, Chandrasekaran et al. (2025) synthesized thieno[2,3-c]pyridine derivatives to find a suitable Hsp90 inhibitor. The results of molecular docking analysis showed that the synthesized compounds had critical molecular interactions with the target Hsp90. Finally, compound 6i demonstrated potent anticancer activity *in vitro* (e.g., $IC_{50} \approx 10\text{--}12\ \mu\text{M}$), suggesting that thieno[2,3-c]pyridine derivatives are promising scaffolds for the development of effective Hsp90-targeting anticancer agents (Figure 1.12) [109].

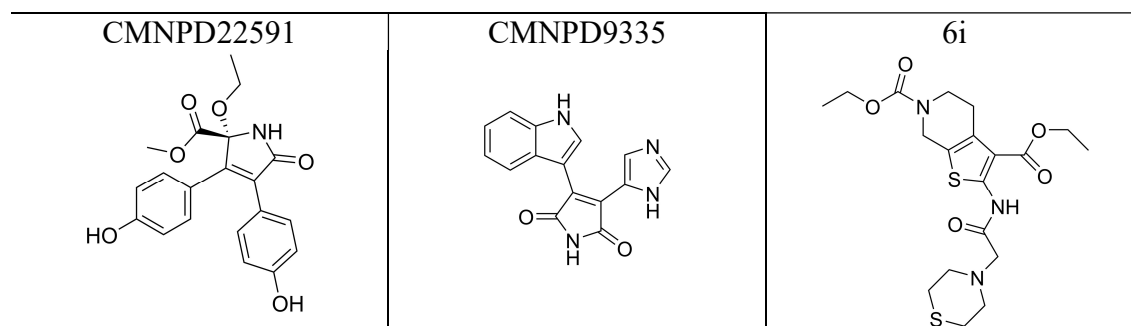


Figure 1.12 Structures of lead compounds identified in previous studies with potent Hsp90 inhibitory activity.

1.4 Anaplastic Lymphoma Kinase

Receptor tyrosine kinases (RTKs) are essential for mediating extracellular signals that regulate key cellular processes such as proliferation, survival, and differentiation

[110]. Anaplastic lymphoma kinase (ALK), a member of the insulin receptor (IR) superfamily is an example of RTK with dual roles in both normal physiological functions and oncogenesis [111]. The crystal structure of selected ALK for the experiments can be seen in Figures 1.13.

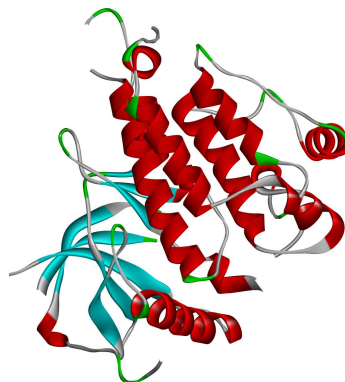


Figure 1.13 Selected ALK structure, namely 8ARJ retrieved from protein data bank (PDB).

The mature ALK protein is a typical receptor tyrosine kinase consisting of three main regions: an extracellular domain of 1030 amino acids responsible for ligand binding, a 28-amino acid transmembrane segment, and a 561-amino acid intracellular domain with tyrosine kinase activity. The kinase domain of ALK contains a conserved three-tyrosine motif, namely Tyr1278, Tyr1282, and Tyr1283, within its activation loop. This motif serves as the primary site for autophosphorylation, which is essential for activating the kinase function [112], [113] (Figure 1.14). ALK can only be activated by ligand-induced homodimerization. In the absence of ligand, the receptor is inactivated by dephosphorylation, mediated by the receptor protein tyrosine phosphatase beta/zeta complex [114]. Although its endogenous ligands are not fully characterized, pleiotrophin and midkine have been proposed as potential ligands [115]. Genetic alterations or pathological conditions that cause ALK hyperactivation can drive oncogenic transformation by excessively stimulating downstream signaling pathways, particularly the STAT (signal transducer and activator of transcription) pathway [116]. Under normal conditions, ALK is mainly expressed in the nervous system during embryogenesis, highlighting its role in brain and neural development [111].

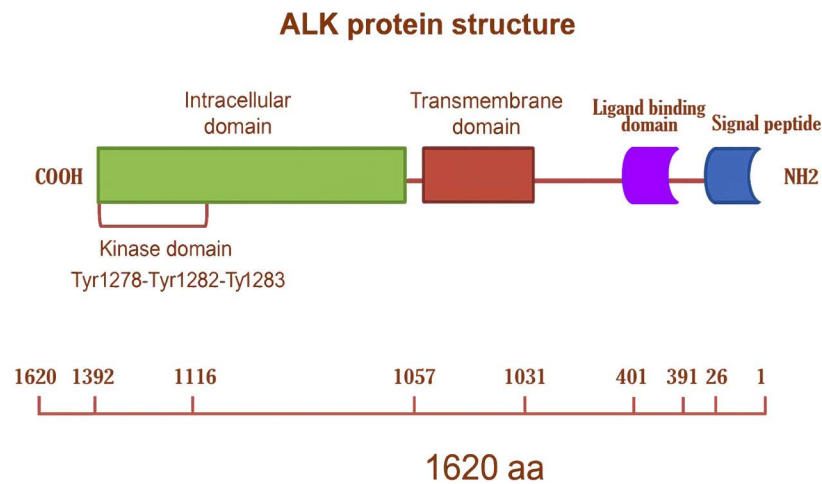


Figure 1.14 Structure of the ALK protein [113].

1.4.1 Role of ALK in Cancer

Abnormal ALK activation, caused by gene fusions, mutations, or amplifications, has been linked to the development of various cancers. Among these, the most recognized case is the formation of fusion proteins like EML4-ALK which is frequently detected in non-small cell lung cancer (NSCLC) [117]. These fusion proteins result in continuous activation of ALK's kinase function, promoting uncontrolled cell proliferation and survival by stimulating key downstream signaling pathways such as RAS-ERK, PI3K-AKT, and JAK-STAT [115], [118]. In addition to non-small cell lung cancer, ALK rearrangements have also been seen in inflammatory myofibroblastic tumors (IMTs), anaplastic large-cell lymphoma (ALCL), and a subset of neuroblastomas, where ALK gene amplifications or point mutations are common [111]. As aggressive tumor behavior and poor outcomes are correlated with these changes, ALK is a useful target for oncology diagnosis and treatment.

1.4.2 ALK Inhibitors

The discovery of anaplastic lymphoma kinase as a significant factor in cancer development has led to the development of targeted drugs that specifically inhibit its kinase activity. These treatments are designed to slow or stop tumor growth caused by changes in the ALK gene. They work by blocking the abnormal signals from ALK. Crizotinib was the first ALK inhibitor approved by the U.S. Food and Drug Administration (FDA) in 2011 for treating ALK-positive non-small cell lung cancer (NSCLC). [119]. Crizotinib works by competitively binding to the ATP-binding pocket

of the ALK protein, which prevents its phosphorylation and disrupts downstream signaling pathways essential for cancer cell survival and proliferation. Although it shows strong initial effectiveness, a number of patients eventually develop resistance. This resistance is frequently caused by secondary mutations in the ALK gene, such as the L1196M mutation, or by the activation of other signaling pathways that allow cancer cells to bypass ALK inhibition [120]. Another drawback of crizotinib is poor central nervous system (CNS) penetration. To be able to solve these problems, next generation ALK inhibitors such as ceritinib, alectinib, and brigatinib were developed. These agents are designed to be effective against a broader range of ALK mutations and exhibit enhanced ability to cross the blood–brain barrier. Also, they offer improved therapeutic outcomes, especially in patients with brain metastases [121], [122]. Further, a third-generation inhibitor called lorlatinib has also improved efficacy against resistant ALK mutations and CNS metastases [123]. The structures of these inhibitors can be seen in Figure 1.15. Ongoing research continues to focus on the discovery and development of more effective ALK inhibitors because current therapies need to improve in this area. These efforts aim to overcome current limitations, such as drug resistance and limited efficacy.

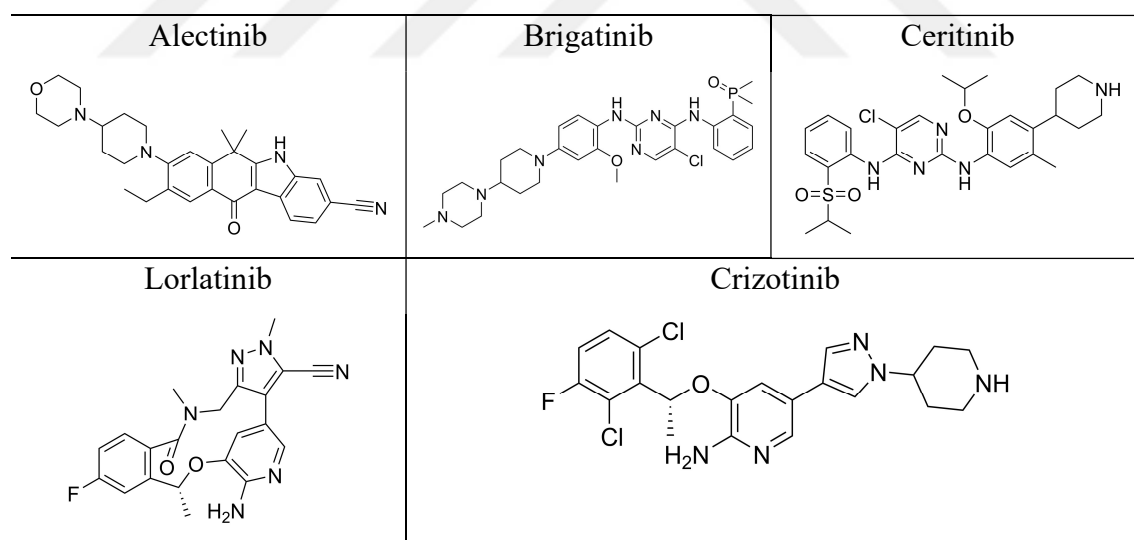


Figure 1.15 Structures of known ALK inhibitors.

In recent years, *in silico* research targeting ALK has expanded significantly, employing virtual screening, molecular docking, ADMET prediction, pharmacophore modeling, and molecular dynamics (MD) simulations to identify both novel and repurposed ALK inhibitors. For example, Bouone et al. (2023) synthesized a novel 4-hydroxyquinolone compound and performed docking against ALK. They found a

−8.05 kcal/mol docking score with key interactions at Met1199 and Glu1197, with favorable ADMET predictions [124]. In a pharmacological repurposing approach, Castillo et al. (2024) used homology modeling, docking, MD and pharmacophore modeling on the ALK to screen FDA-approved drugs. They identified mitoxantrone, riboflavin, and abacavir as high-affinity binders with superior interaction energies (Figure 1.16). Moreover, the targeted design of piperidin-4-one derivatives, modeled after second-generation inhibitors like ceritinib, showcased promising docking poses and binding modes via comprehensive *in silico* analysis [125].

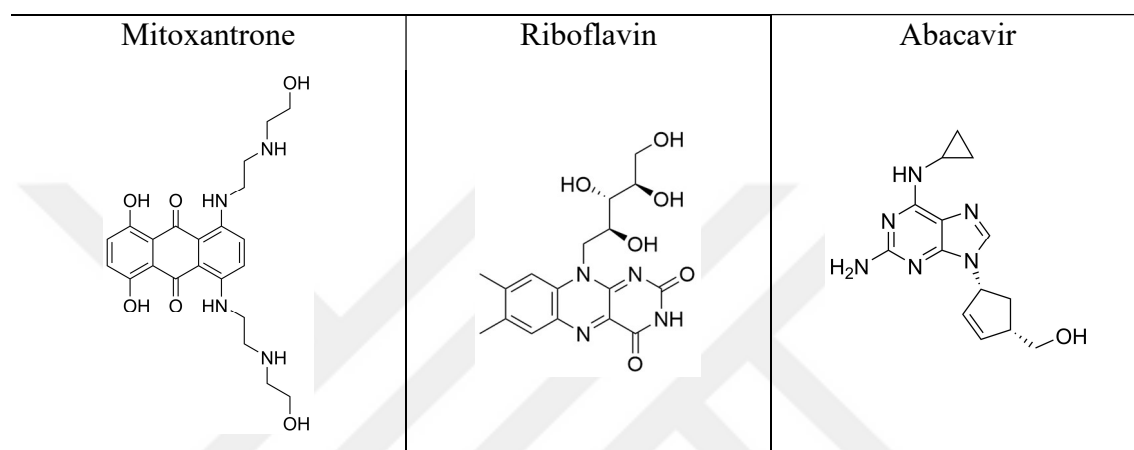


Figure 1.16 Structures of lead compounds identified in previous study with potent Hsp90 inhibitory activity.

1.5 Dual Inhibitors

The use of combination therapy involving two or more anticancer drugs may result in unforeseen drug–drug interactions and complex pharmacokinetic or pharmacological challenges, which may lead to unexpected side effects or reduced treatment effectiveness [126]. A single chemical that interacts with several targets may have a safer profile in comparison to medications that are highly selective for a single target [127]. Identifying a single drug capable of targeting multiple molecular pathways offers the potential to enhance the balance between therapeutic efficacy and safety [128].

Multiple dual inhibition strategies have been developed involving Hsp90 as a common target, one of which includes the co-inhibition of HDAC6. In this context, the interaction between HDAC6 and Hsp90 in cancer has gained significant interest, with studies exploring how HDAC6 inhibitors synergistically enhance *in vitro* anticancer

activity when combined with Hsp90 [129], [130], [131]. Hsp90 is regulated by HDAC6 through both transcriptional control and modifications that occur after protein synthesis [132]. Conversely, HDAC6 is a client protein of Hsp90, which plays a crucial role in maintaining HDAC6's protein stability [133]. This reciprocal interaction supports the concept of targeting both proteins simultaneously. Previous studies have shown that combining HDAC6 and Hsp90 inhibitors in cancer therapy can produce synergistic effects, enhancing the overall therapeutic outcome (Figure 1.17) [134], [135], [136], [137]. On the other hand, numerous studies have explored the simultaneous targeting of ALK and Hsp90, and their combined inhibition has been shown to exert synergistic effects, particularly in overcoming drug resistance in ALK-driven cancers. Hsp90 is a molecular chaperone that is essential for the stability and function of numerous oncogenic client proteins, including ALK fusion proteins such as EML4-ALK, which are commonly found in non-small cell lung cancer (NSCLC) [138]. The inhibition of Hsp90 causes the degradation of ALK fusion oncoproteins and suppresses the downstream signaling pathways that promote tumor growth and survival [93]. Moreover, resistance to ALK tyrosine kinase inhibitors (TKIs), such as crizotinib, often involves secondary mutations in ALK or bypass signaling mechanisms, which can be mitigated by Hsp90 inhibition due to its broader impact on multiple oncogenic pathways [139], [140]. Significant tumor regression and the removal of resistance mechanisms in ALK-driven non-small cell lung cancer have been shown in both *in-vitro* and *in-vivo* models where dual inhibition of Hsp90 and ALK has shown synergistic antitumor benefits (Figure 1.17) [140]. Hsp90 inhibitors, including ganetespib and IPI-504, have been shown to promote the degradation of the EML4-ALK fusion protein, which leads to reduced oncogenic signaling and the induction of apoptosis in cancer cells [141], [142]. These results demonstrate the therapeutic potential of targeting ALK and Hsp90 simultaneously in order to improve clinical outcomes and treatment efficacy in patients with cancers that have been rearranged by ALK.

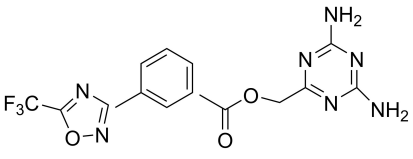
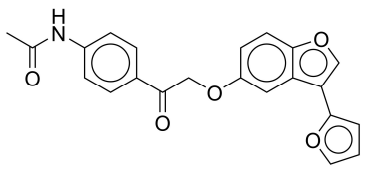
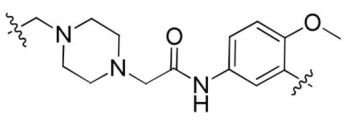
Targets	Lead Compound	Reference
HDAC6-Hsp90	ZINC96116556	[131]
		
HDAC6-Hsp90	Compound 10	[137]
		
Hsp90-ALK	10h	[143]
		

Figure 1.17 Examples of dual Hsp90 inhibitors investigated in literature with secondary targets HDAC6 or ALK.

1.6 Computer Aided Drug Discovery

Computer-aided drug Discovery (CADD) uses computational methods and tools to facilitate and accelerate the discovery, identification, and design of new candidates with a desired biological activity. CADD is an important tool that helps optimize the drug discovery development process by choosing compounds with the greatest probability of success and reducing the time and expenses thorough chemical and pharmacological testing. In addition to identifying promising candidates, CADD provides insights into the molecular mechanisms underlying biological activity and facilitates the prediction of new derivatives with improved therapeutic efficacy [144].

A number of methods are included in computer-aided drug discovery, such as virtual screening, molecular docking, druglikeness properties, molecular dynamics (MD) simulation, and free energy calculations. To carry out these studies, a range of specialized softwares are employed, such as Autodock Vina, University of California, San Francisco (UCSF) Chimera, PyMol, Discover Studio, and GROMACS. Each of these tools serves a specific function; for instance, docking studies are typically performed using AutoDock Vina, while MD simulations are widely conducted through GROMACS.

Visualization and protein – ligand preparations are often facilitated by UCSF Chimera or PyMOL, and Discovery Studio provides a comprehensive interface for multiple stages of the modeling workflow. Molecular docking, molecular dynamics, and energy calculations require the correct execution of a series of scripts and commands to successfully produce results. Additionally, due to the computational requirements of these simulations, the efficiency of these tasks and how quickly they reach their conclusions depend on the computer's hardware parameters, such as processor speed, number of cores, and available memory.

As a result, access to high-performance computers is very important for making CADD studies faster and more practical. Generally, researchers use remote access to powerful computational servers to conduct extensive analyses and simulations that are not feasible on standard personal computers. One of these, which was also used in this project, is the TRUBA system operated by ULAKBİM in Türkiye, which provides researchers with access to advanced high-performance computing resources. Studies with necessary preparations and parameters are sent to TRUBA's queue system and when their turn comes, the simulation automatically starts running on the remote computer. This enables tasks to be executed quickly and efficiently on multiple high-performance nodes. In summary, such systems significantly accelerate data processing and simulation workflows and thus support high-throughput and complex computational drug discovery projects.

1.6.1 Virtual Screening

Virtual screening (VS) is a computational method used to search through large libraries of chemical compounds in order to identify those most likely to bind to a specific biological target. The main goal of virtual screening is to prioritize compounds for experimental testing by predicting their binding potential using computer simulations. This process significantly reduces the number of candidates and lowers the costs associated with wet-lab screening [145]. There are two main approaches to virtual screening: structure-based and ligand-based. Structure-based virtual screening (SBVS) uses the three-dimensional structure of the target protein, which is typically obtained through X-ray crystallography or cryo-electron microscopy (cryo-EM). This method evaluates how well each compound fits into the active site of the protein using a molecular docking strategy [146]. On the other hand, ligand-based virtual screening (LBVS)

identifies potential hits based on the similarity of compounds to known active molecules using methods such as pharmacophore modeling or machine learning techniques [147]. Using VS, millions of compounds can be evaluated and analyzed, far more than traditional experimental methods could ever accomplish. This high-throughput capability allows researchers to rapidly narrow down large chemical libraries to a manageable number of high-potential candidates [148]. As a result, virtual screening serves as an essential filter that reduces experimental effort and increases the likelihood of success in the later stages of drug development.

1.6.2 Molecular Docking

The terms of virtual screening and molecular docking are generally used in the same sense. However, they represent a bit different idea. Virtual screening refers to the overall process of computationally filtering large chemical libraries to identify potential bioactive compounds. In contrast, molecular docking is a specific technique used within this process to predict the preferred orientation and binding affinity of a ligand toward a target protein [146]. Basically, Molecular docking is employed more rigorously. It involves flexible ligand conformations, more accurate scoring functions, and sometimes even flexible receptor models. At this stage, the focus is on analyzing the binding poses, estimating binding affinities, and interpreting specific molecular interactions in detail [149].

Some pre-processing steps need to be taken for correct and trouble-free molecular docking results. First, both the target protein and the ligand molecules must be prepared in a way that is suitable for docking. Protein structures, usually obtained from the Protein Data Bank (PDB), are often pre-processed to remove co-crystallized ligands, water molecules, or heteroatom if they are not crucial for docking. Hydrogen atoms must be added, and missing side chains or loops must be reconstructed [150]. These steps are usually performed using tools such as AutoDock Tools, Chimera, or Schrödinger's Protein Preparation Wizard. Once the docking process is complete, each ligand is ranked according to a scoring function which is expressed in kcal/mol and estimates its binding affinity. In these results, the more negative the value, the stronger the predicted interaction between the ligand and the target [151], [152]. However, binding affinity scores should be interpreted with caution. Although they indicate binding affinity, they are relative indicators, not absolute predictors of biological activity. Therefore, the binding positions,

hydrogen bonds, hydrophobic interactions, π - π stacking, and salt bridges of the selected docking results are analyzed. These qualitative analyses are followed by further validation studies on drug-likeness properties, as well as molecular dynamics analysis [153].

1.6.3 Drug-likeness and ADME Properties

Another critical factor that should be checked in the early stages of the study is to evaluate whether the candidate compound has physicochemical properties that make it suitable for development as an orally active drug. This concept, known as drug-likeness, refers to the degree to which a molecule exhibits properties consistent with known drugs, particularly in terms of absorption, distribution, metabolism, and excretion (ADME). The most widely recognized criteria for evaluating drug-likeness is known as Lipinski's Rule of Five. This rule suggests that, generally, an orally active drug should not violate more than one of the following criteria: it should have no more than 5 hydrogen bond donors, no more than 10 hydrogen bond acceptors, a molecular weight under 500 Da, and a logP (partition coefficient) value not exceeding 5. Compounds that comply with these guidelines are more likely to exhibit favorable oral bioavailability [154]. In addition to drug similarity, evaluation of ADME properties is also important for assessing the pharmacokinetic suitability of a compound. Absorption involves estimating how well a molecule can cross biological barriers such as the intestinal epithelium. Distribution examines the ability of a compound to reach various tissues, including the central nervous system. Metabolism focuses on enzymatic processing, particularly by cytochrome P450 enzymes, which affects drug stability and interactions. Finally, excretion evaluates how efficiently a drug is excreted via the kidney or biliary tract [155], [156].

1.6.4 Molecular Dynamics Simulations

The first molecular dynamics (MD) simulation of a biologically important macromolecule was published 48 years ago by McCammon, Gelin, and Karplus in 1977 [157]. Since that seminal study, MD simulations have become an indispensable tool in drug discovery and development. This technique is widely used in modern drug discovery to investigate structural flexibility, conformational changes, and the dynamic behavior of proteins and ligands at the atomic level. As previously explained, molecular docking provides only a single snapshot of how a ligand binds to its target. However, molecular dynamics simulations allow researchers to observe how this interaction evolves over time

under physiologically relevant temperature, pressure, and solvent conditions. Rather than assuming that a suitable docking position remains fixed, MD allows the temporal stability and adaptability of the ligand-protein complex to be assessed. A ligand that binds very well to the target site as a result of molecular docking may dissociate from the protein over time and shift to a different site by showing instability and alternative binding modes. Therefore, MD simulations are critical for supporting and validating docking results because they provide a more comprehensive and realistic assessment of binding interactions and ligand behavior in dynamic biological environments [158], [159]. Simulations need a carefully prepared input structure. This preparation involves adding any missing atoms, assigning appropriate charges, solving the system in an explicit water box, and neutralizing the overall charge. To describe the potential energy of the system, force fields such as AMBER, CHARMM, or GROMOS are utilized [160]. A number of critical parameters can be calculated from the results obtained from MD simulations. The most commonly used of these include root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration (Rg), and hydrogen bond occupancy. These measurements help evaluate the ligand's structural integrity and interaction consistency with respect to its target. The duration of MD simulations may vary from nanoseconds to microseconds. This time scale depends on the research question and the available computational resources because, as we have explained, the computer's power significantly impacts the duration of the simulations. Common software platforms for MD simulations include GROMACS, AMBER, and NAMD. GROMACS was used in this thesis [161]. To sum up, MD simulations are a valuable computational tool that can significantly contribute to the early stages of modern drug discovery and development. Computational technologies continue to advance day by day, and we expect these developments to increase the significance of MD simulations in the future of drug discovery and development. [162].

1.6.5 Binding Free Energy Calculations

Calculations of binding free energy evaluate the energy difference between a protein-ligand complex's bound and unbound states. Two widely used end-point methods are Molecular Mechanics/Poisson–Boltzmann Surface Area (MM/PBSA) and Molecular Mechanics/Generalized Born Surface Area (MM/GBSA). In the MM/PBSA method, the polar solvation term is calculated by solving the Poisson–Boltzmann equation. This

approach is more physically rigorous but also more computationally demanding. On the other hand, MM/GBSA utilizes the Generalized Born model to approximate polar solvation, which allows for significantly faster calculations, although it sacrifices some accuracy in the process. The MM/PBSA technique was preferred in this thesis study to estimate the binding free energy of a ligand-receptor complex based on molecular dynamics simulation trajectories. This method extracts snapshots from MD simulations and calculates the binding energy by combining molecular mechanics and solvation-free energies [163], [164]. A more negative binding free energy (ΔG_{bind}) value indicates a stronger and more stable interaction between the ligand and its target. This type of binding free energy shows a higher binding affinity and suggests that the ligand may be a promising candidate for further optimization and drug development [165]. A typical MM/PBSA analysis yields several energy components that approximate a protein-ligand complex's binding free energy. These components are usually reported in kilojoules per mole (kJ/mol) and represent different physical interactions such as electrostatic energy, van der Waals interactions, Polar solvation energy, and SASA (Solvent Accessible Surface Area) energy [166]. Due to its balance between computational cost and accuracy, MM/PBSA has become an essential approach in computer-aided drug discovery.

Chapter 2

Materials and Methods

2.1 Optimizing Target Proteins' Crystal Structures

The crystal structures of target proteins for HDAC6 (PDB ID: 7UK2), Hsp90 (PDB ID: 3OWD, 4W7T), and ALK (PDB ID: 8ARJ) were retrieved from The Research Collaboratory for Structural Bioinformatics (RCSB) protein data bank, with the resolution of 1.60 Å, 1.63 Å, 1.80 Å, and 1.65 Å, respectively. In the first study, which aimed at dual inhibition of HDAC6 and Hsp90, 7UK2 and 3OWD crystal structures were used as target proteins. The HDAC6 inhibitor binding site was co-crystallized with the ligand N-hydroxy-4-[propan-2-yl] (2,3,4,5-tetrafluorobenzene-1-sulfonyl) amino methyl benzamide (O2L) and Hsp90 with the N-{[1-(5-chloro-2,4-dihydroxyphenyl)-2-oxo-2,3-dihydro-1H-benzimidazol-5-yl] methyl} naphthalene-1-sulfonamide (MEY) [167], [168]. In the second study, which aimed at dual inhibition of Hsp90 and ALK, 4W7T and 8ARJ crystal structures were used as target proteins. The Hsp90 inhibitor binding site was co-crystallized with the ligand (7S)-2-amino-4-methyl-7-phenyl-7,8-dihydroquinazolin-5(6H)-one (3JC) and ALK with the 3-(dimethylamino)-1-[3-[4-(4-methylpiperazin-1-yl)phenyl]-9~{H}-pyrido[2,3-b]indol-6-yl]prop-2-en-1-one (NRR) [169], [170]. These ligands are bound to the active site of protein structures, and they are recognized for their ability to inhibit HDAC6 (for O2L), Hsp90 (for MEY and 3JC), and ALK (NRR) enzymatic activity. The coordinates of these ligands were used as references, and grid box studies were performed accordingly. The structure of proteins was refined by using UCSF Chimera, which is a visualization system for exploratory research and analysis. In this refinement process, unwanted molecules like heteroatoms and water were removed, and also missing hydrogen atoms and charges were added, thereby contributing to a purified protein structure [171].

2.2 Preparation of Ligands

For ligand selection to investigate potential dual inhibitors of HDAC6 and Hsp90, ZINC15 database (<https://zinc15.docking.org/>) were screened with compounds structurally similar bio actives to four known HDAC inhibitors (belinostat, panobinostat, givinostat, and vorinostat) were individually identified, with a minimum similarity threshold of 30% [172]. After this similarity filtration, we get 173 compounds from belinostat (similarity ratios between 59% - 37%), 49 compounds from givinostat (similarity ratios between 59% - 31%), 95 compounds from panobinostat (similarity ratios between 80% - 35%) and 474 compounds from vorinostat (similarity ratios were between 83% - 35%). Further 791 molecules downloaded from ZINC database, and they were considered as ligands for screening.

For ligand selection to investigate potential dual inhibitors of Hsp90 and ALK, the COCONUT database (<https://coconut.naturalproducts.net/>) were screened with filtering by applying Lipinski's Rule of Five [173]. After this similarity filtration, we get 361,179 total natural products, and they were downloaded from COCONUT database and considered as ligands for screening.

In both two studies, ligands were organized to contain unique compounds in a ready-to-dock format, with 3D coordinates generated for each molecule. All compounds were energy minimized using MMFF94 forcefield at maximum 1000 steps making use of the OpenBabel version 3.1.1 [174]. The CHARMM General Force Field (CGenFF) tool was employed to automatically generate the CHARMM force field parameters for the ligands [175].

2.3 Molecular Docking

Smina, a version of AutoDock Vina, which is designed and optimized to support high-throughput scoring and user-specified custom scoring functions, was used for molecular docking studies [176], [177], [178]. In the HDAC6 – Hsp90 part, for the HDAC6 protein, center coordinates of 19.57, 2.69, 18.15 with the sizes of 9.83, 13.64, 14.03 and for the Hsp90 protein, center coordinates of -0.78, 7.44, 22.47 with the sizes of 8.72, 10.52, 8.50 were used as their grid box. In the Hsp90 – ALK part, for Hsp90 protein, center coordinates of -0.24, -10.78, -24.63 with the sizes of 12.28, 11.95, 8.47, and for

the ALK protein, center coordinates of 31.44, 48.19, 9.07 with the sizes of 14.57, 17.18, 14.64 were used as their grid box. For the Hsp90-ALK dual inhibitors part, initial molecular dynamics simulations were performed for a period of 500 ns on the apo forms of the proteins. The final equilibrated structure obtained from this simulation was then used for molecular docking studies. The first docking poses of each interaction were selected, and the results were re-ordered according to binding energies. Interactions of docking results were visualized by using BIOVIA, D.S. 2024 Discovery Studio Visualizer.

2.4 Molecular Dynamics (MD) Simulations

Molecular dynamics simulations to check the stability of protein-ligand complexes partially performed hosted on TUBITAK ULAKBIM, High Performance and Grid Computing Center (TRUBA resources) by using CHARMM36 force field and CHARMM-modified TIP3P3 as the explicit water models in GRONingen Machine for Chemical Simulations (GROMACS) ver. 2023.1 [161], [175], [179]. Protein complexes were cleaned and prepared for simulation steps by using UCSF Chimera package (version 1.73.3) [171]. Sodium (Na^+) and chloride (Cl^-) ions were subsequently introduced for neutralization and energy minimization was performed using the steepest descent algorithm, at a maximum of 50,000 steps.

Once the system reached its minimum energy state, molecular dynamic process started. Equilibration steps were performed under two distinct conditions: NVT (constant number of particles, volume, and temperature) and NPT (constant number of particles, pressure set to 1 atm, and temperature). Each equilibration step was carried out for a duration of 100 picoseconds. The V-rescale method was employed for temperature regulation throughout all simulations, allowing the system's temperature to be gradually and systematically adjusted, thereby it enhances the stability of the simulated dynamics. Initially, the complex was simulated within an NVT ensemble, with a time constant of 0.1 picoseconds to establish velocities consistent with the Maxwell–Boltzmann distribution. Subsequently, the complex simulated under NPT conditions, with an additional mechanism to control system pressure. For this purpose, Berendsen pressure coupling was implemented with a time constant of 2.0 picoseconds. This strategy ensures pressure stability by proportionally adjusting atomic velocities in response to deviations from the desired pressure. The integration of the V-rescale thermostat for temperature

regulation in both NVT and NPT ensembles, together with the Berendsen pressure coupling in the NPT ensemble, was designed to achieve a balanced and accurate representation of the system's thermodynamic properties during the molecular dynamics simulations.

In the HDAC6 – Hsp90 studies, all simulations were conducted for a duration of 300 nanoseconds, while in the Hsp90 - ALK dual inhibition part, all simulations were conducted for a period of 500 nanoseconds and utilized the leap-frog integration method with a time interval of 2 femtoseconds. The stability of each complex was analysed using tools available in the GROMACS software [180].

2.5 MM/PBSA Binding Free Energy Calculation

To estimate the strength of binding interactions between ligands and receptors, MM/PBSA binding free energy calculations were performed [181]. The calculations were conducted using the GROMACS-compatible free energy software g_mmpbsa [182]. A grid spacing of 0.5 Å and a salt concentration of 0.150 M were applied. In the HDAC6 protein MM/PBSA calculations, the Zn²⁺ and K ions were considered as components of the protein. The binding free energy of the protein-ligand complex is calculated using:

$$\Delta G_{\text{bind}} = \Delta G_{\text{complex}} - (\Delta G_{\text{protein}} + \Delta G_{\text{ligand}}) \quad (2.1)$$

In this formula; $\Delta G_{\text{complex}}$ represents the total free energy of the complex, $\Delta G_{\text{protein}}$ represents total free energy of the protein, and the ΔG_{ligand} is represent the total free energy of the ligand.

2.6 Drug-likeness Properties

The pharmacokinetic properties of absorption, distribution, metabolism, and excretion (ADME) for the selected compounds were predicted using the Swiss-ADME online platform (accessible at <http://www.swissadme.ch/>) [183]. In this process, each molecule's Simplified Molecular Input Line Entry System (SMILES) names were taken and submitted to the Swiss-ADME server. Then, the generated results were collected used to construct result tables.

Chapter 3

Investigation of Potential Dual Inhibitors of HDAC6 – HSP90

3.1 Molecular Docking Evaluation

Molecular docking, which is a commonly used technique in computational biology for successfully identifying and characterizing receptor-ligand interactions, is an important part of drug discovery process [149]. In the study, the binding interaction of complexes were analyzed by using this strategy.

A total of 791 molecules received from ZINC database according to similarity threshold of four known HDAC inhibitors were docked into the HDAC6 (7UK2) and Hsp90 (3OWD) active site separately. This step was carried out on SMINA docking program to get the lowest energy conformers of the compounds at the binding site of HDAC6 and Hsp90 proteins. All results were ordered from the lowest binding energy to highest binding energy. A total of 8 compounds with average binding energies of -8.20 and lower were selected as possible candidates for further studies (Table 3.1). These compounds can be listed as follows for HDAC6 and Hsp90 respectively: ZINC36349281 with -8.4 kcal/mol and -8.5 kcal/mol; ZINC28110230 with -9.1 kcal/mol and -8.2 kcal/mol; ZINC28092696 with -9.7 kcal/mol and -7.9 kcal/mol; ZINC27653366 with -9.0 kcal/mol and -8.0 kcal/mol; ZINC16249755 with -9.0 kcal/mol and -8.8 kcal/mol; ZINC16249757 with -9.0 kcal/mol and -8.5 kcal/mol; ZINC3626741 with -8.5 kcal/mol and -7.9 kcal/mol; and finally ZINC43020382 with -8.7 kcal/mol and -7.7 kcal/mol.

Histone deacetylases 6 is a metalloenzyme protein and they have a zinc ion which is responsible for stability of the catalytic center [184]. HDAC6 inhibitors make an interaction with this ion for inhibition of the protein [185]. In this respect, ZINC28110230, ZINC27653366, ZINC362674, ZINC43020382, and our reference molecule panobinostat showed metal-acceptor binding with the distance of 2.84 Å, 2.18

Å, 2.55 Å, 2.26 Å, and 2.20 Å, respectively. Belinostat, which is an FDA approved HDAC6 inhibitor exhibited interactions through Pro464, His574, Phe583, Cys584, Asp612, His614, Phe643, Asp705, Leu712, Tyr745 and key interactions for binding appear as π - π interactions [186]. In this study, our reference molecule, panobinostat, which is another FDA-approved HDAC6 inhibitor has interacted with His463, Pro464, Gly582, Phe583, Cys584, Phe643.

We can say that these residues have a critical role for successfully inhibiting HDAC6 protein. Table 3.2 summarizes the main interacting residues, distances and interaction types between HDAC6 protein and the top eight candidate molecules and reference, with corresponding visual representations provided in Figure 3.1.

All 8 docked compounds showed relatively similar interactions in the active site of the HDAC6 protein. The five of eight molecules (except ZINC16249755 and ZINC36349281: no Phe643 and ZINC28110230: no Phe583), revealed π - π stacking interactions between the central aromatic group and Phe583 and Phe643. In a previous study by Nguyen et al., a number of key residues were emphasized as critical for binding and inhibiting the activity of HDAC6 protein in the active site, namely Pro464, Ser531, His573, His574, Phe583, Asp612, His614, Phe643, Asp705, Leu712, Tyr745 [186]. Remarkably, we observed that all eight compounds participated with most of these critical residues. For instance, ZINC43020382 exhibited interactions with Pro464, Ser531, Phe583, Phe643, Leu712 and Tyr745.

In another study conducted by Miyake et al. (2016), it is explained that the active site of HDAC6 is well-conserved and characterized by a distinct narrow hydrophobic channel, which is composed of key residues including Pro464, Gly582, Phe583, Phe643, and Leu712 [187]. All compounds exhibited interactions with a minimum of two or more residues within the critical residues. ZINC36349281 with Pro464, Ser531, Phe583 and Leu712; ZINC28110230 with Gly582, Phe583, His614, Phe643, and Leu712; ZINC28092693 with Gly582, Phe583, His614, and Phe643; ZINC27653366 with Gly582, Phe583 and Phe643; ZINC16249755 with Ser531 and Phe583; ZINC16249757 with Phe583, His614, Phe643, and Leu712; ZINC3626741 with Pro464, Ser531, His573, His574, Phe583, His614, and Phe643; ZINC43020382 with Pro464, Ser531, Phe583, Pro463, Leu712, and Tyr745 (Table 3.2).

Table 3.1 Molecular docking results of selected compounds on both HDAC6 (7UK2) and Hsp90 (3OWD) proteins and their zinc IDs.

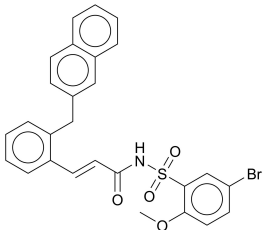
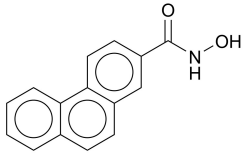
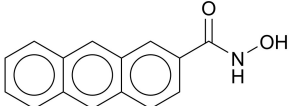
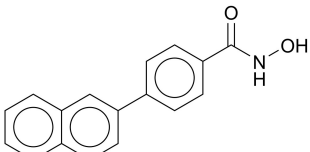
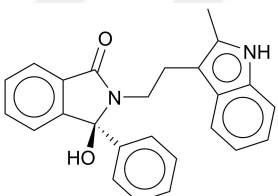
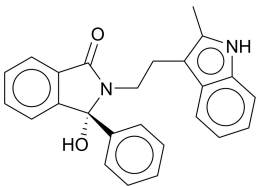
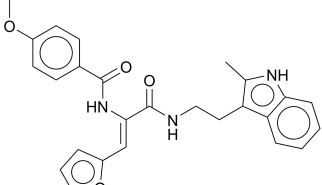
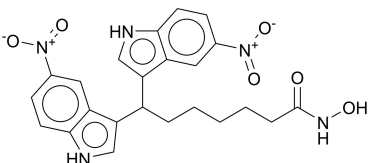
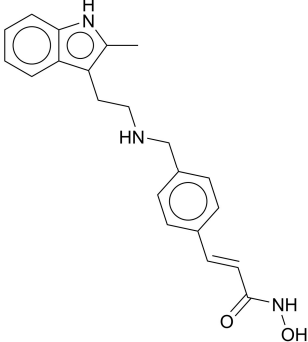
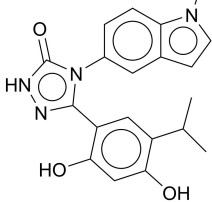
ZINC ID	Binding Affinity (kcal/mol)	
	HDAC6	HSP90
ZINC36349281 	-8.4	-8.5
ZINC28110230 	-9.1	-8.2
ZINC28092696 	-9.7	-7.9
ZINC27653366 	-9.0	-8.0
ZINC16249755 	-9.0	-8.8
ZINC16249757 	-9.0	-8.5
ZINC3626741 	-8.5	-7.9
ZINC43020382 	-8.7	-7.7

Table 3.1 (continued).

ZINC ID	Binding Affinity (kcal/mol)	
	HDAC6	HSP90
ZINC220106498 (Panobinostat) 	-8.5	-
ZINC43130413 (Ganetespib) 	-	-8.3

In the other part of the experiment, we analyzed the interaction profiles of the selected compounds and ganetespib as a reference molecule targeting the Hsp90 protein's binding pocket. Consistent with this, Table 3.2 summarizes the main interacting residues, distances and interaction types between Hsp90 protein and the top eight candidate molecules and reference, with corresponding visual representations provided in Figure 3.1. In previous studies, Asn51 has been found as a key residue for Hsp90 inhibition [188], [189]. We observed this residue in the binding interaction of compounds ZINC36349281, ZINC28110230, ZINC27653366, ZINC16249755, and ZINC3626741. Reference molecule, ganetespib, exhibited interactions with Ser52, Asp54, Gly97, Met98, Leu107, Phe138, and Val150.

Notably, most of these amino acids were also present within the complex of candidate compounds (Table 3.2). These similar binding interactions also support the conserved binding mode and shared interaction pattern between the candidate compounds and the reference compound. This data also increases the reliability of docking results. These results emphasize the potential of our candidate compounds to effectively target the functional residues which were identified in prior studies and support their potential as promising dual inhibitors of Hsp90 and HDAC6.

Table 3.2 Molecular docking results and interaction details of top 8 hit molecules and references against HDAC6 and Hsp90 protein. (Panobinostat for HDAC6 reference and Ganetespib for Hsp90 reference).

ZINC ID	HDAC6			Hsp90		
	Main interacting residues	Distance (Å)	Interaction types	Main interacting residues	Distance (Å)	Interaction types
ZINC36349281	His463	4.33	Pi-pi stacked	Asn51	2.82	Pi-sigma
	Pro464	5.40	Pi-alkyl	Ala55	4.98	Pi-alkyl
	Ser531	2.39	Carbon hydrogen bond	Ala55	4.67	Pi-alkyl
	Phe533	5.27	Pi-alkyl	Ile96	5.35	Pi-alkyl
	Phe583	4.01	Pi-pi stacked	Met98	5.22	Pi-alkyl
	Phe583	4.69	Pi-pi stacked	Met98	5.24	Pi-alkyl
	Leu712	5.42	Pi-alkyl	Met98	5.38	Pi-sulfur
ZINC28110230	Gly582	1.90	Conventional H. bond	Leu107	5.20	Pi-alkyl
	Phe583	4.32	Pi-pi stacked	Asn51	4.45	Amide-pi stacked
	Phe583	5.87	Pi-pi stacked	Ala55	4.79	Pi-alkyl
	His614	4.46	Pi-pi stacked	Asp93	2.17	Conventional H. bond
	Phe643	4.44	Pi-pi stacked	Met98	3.67	Pi-sulfur
	Phe643	4.93	Pi-pi stacked	Met98	3.83	Pi-sulfur
	Phe643	4.82	Pi-pi stacked	Met98	5.19	Pi-alkyl
	Leu712	4.89	Pi-alkyl			
	Zn802	2.84	Metal-acceptor			
	Gly582	1.84	Conventional H. bond	Asp54	5.02	Amide-pi stacked
ZINC28092696	Phe583	5.95	Pi-pi stacked	Asp54	4.90	Amide-pi stacked
	Phe583	4.25	Pi-pi stacked	Asp54	5.89	Amide-pi stacked
	His614	4.75	Pi-pi stacked	Ala55	3.64	Pi-alkyl
	Phe643	5.14	Pi-pi stacked	Ala55	3.97	Pi-alkyl
	Phe643	3.95	Pi-pi stacked	Lys58	5.04	Pi-alkyl
	Phe643	4.08	Pi-pi stacked	Asp93	2.33	Conventional H. bond
				Met98	4.74	Pi-alkyl
ZINC27653366	Gly582	1.91	Conventional H. bond	Asn51	2.72	Pi-sigma
	Phe583	3.97	Pi-pi stacked	Ala55	5.03	Pi-alkyl
	Phe643	4.67	Pi-pi stacked	Asp93	2.09	Conventional H. bond
	Zn802	2.18	Metal-acceptor	Met98	4.51	Pi-alkyl
				Met98	5.34	Pi-alkyl
ZINC16249755	Ser531	4.26	Pi-pi stacked	Leu48	5.44	Pi-alkyl
	Ser531	3.21	Carbon hydrogen bond	Asn51	3.33	Pi-donor H. bond
	Phe583	4.05	Pi-pi stacked	Ala55	4.99	Pi-alkyl
	Phe642	5.73	Pi-pi stacked	Ala55	5.23	Pi-alkyl
				Asp93	2.52	Conventional H. bond
				Met98	5.47	Pi-alkyl
				Val186	4.92	Pi-alkyl
ZINC16249757	Phe583	4.16	Pi-pi stacked	Lys58	4.43	Pi-cation
	Phe583	4.96	Pi-pi stacked	Lys58	4.54	Pi-cation
	His614	2.49	Conventional H. bond	Met98	2.09	Conventional H. bond
	His614	4.95	Pi-pi stacked	Met98	5.10	Pi-alkyl
	Phe642	3.77	Pi-sigma	Leu107	5.12	Pi-alkyl
	Phe643	4.13	Pi-pi stacked	Thr184	2.99	Carbon hydrogen bond
	Leu712	2.81	Conventional H. bond			
	Leu712	5.28	Pi-alkyl			
	Leu712	5.26	Pi-alkyl			
	His463	5.06	Pi-alkyl	Leu48	5.33	Pi-alkyl
ZINC3626741	Pro464	4.17	Pi-alkyl	Asn51	3.27	Pi-donor H. bond
	Pro464	5.34	Pi-alkyl	Lys58	4.72	Pi-alkyl
	Ser531	3.06	Carbon hydrogen bond	Gly97	3.00	Carbon hydrogen bond
	His573	3.54	Carbon hydrogen bond	Thr184	2.89	Conventional H. bond
	His574	4.45	Pi-alkyl	Val186	4.82	Pi-alkyl
	Phe583	4.16	Pi-pi stacked			
	His614	5.17	Pi-alkyl			
	Phe642	5.20	Pi-pi stacked			
	Phe643	4.97	Pi-pi stacked			
	Zn802	2.55	Metal-acceptor			
	His463	2.48	Carbon hydrogen bond	Ser52	2.67	Conventional H. bond
	Pro464	4.34	Pi-alkyl	Asp54	3.91	Pi-anion
	Pro464	4.53	Pi-alkyl	Ala55	5.37	Pi-alkyl
ZINC43020382	Ser531	4.65	Amide-pi stacked	Asp93	2.02	Conventional H. bond
	Phe583	4.11	Pi-alkyl	Gly97	2.37	Conventional H. bond
	Phe643	4.95	Pi-alkyl	Met98	3.88	Pi-sulfur
	Leu712	5.00	Pi-alkyl	Met98	5.35	Pi-alkyl
	Tyr745	2.00	Conventional H. bond	Leu107	4.75	Alkyl
	Zn802	2.26	Metal-acceptor	Gly135	2.12	Conventional H. bond
	His463	4.63	Pi-pi T-shaped	Ser52	2.53	Carbon hydrogen bond
	Pro464	4.80	Pi-alkyl	Asp54	3.80	Carbon hydrogen bond
	Gly582	2.41	Conventional H. bond	Ala55	5.08	Pi-alkyl
	Phe583	5.23	Pi-pi stacked	Ala55	3.62	Pi-alkyl
Reference	Cys584	2.88	Conventional H. bond	Gly97	2.09	Conventional H. bond
	Phe643	3.90	Pi-pi stacked	Met98	4.49	Pi-sulfur
	Zn802	2.20	Metal-acceptor	Met98	5.40	Alkyl
				Leu107	3.75	Alkyl
				Phe138	4.00	Pi-alkyl
				Val150	4.51	Alkyl

HDAC6		HSP90	
ZINC ID & 3D interactions	2D interactions	ZINC ID & 3D interactions	2D interactions
ZINC36349281			
A	B	C	D
ZINC28110230			
A	B	C	D
ZINC28092696			
A	B	C	D
ZINC27653366			
A	B	C	D

Figure 3.1 Binding modes of the top eight candidate and reference molecules: 3D and 2D interaction diagrams with HDAC6 (A, B) and Hsp90 (C, D).

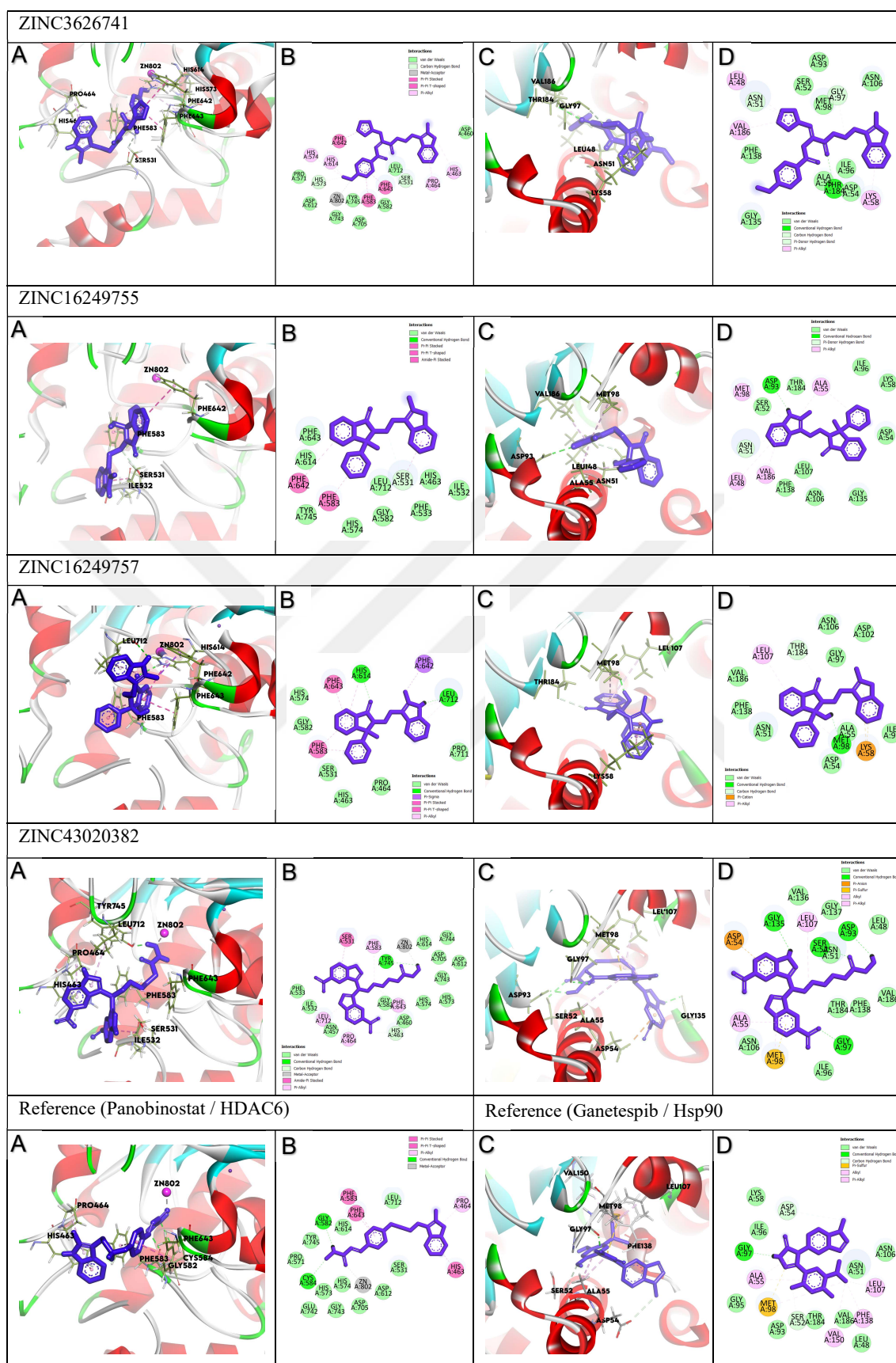


Figure 3.1 (continued).

3.2 Molecular Dynamics Analysis

In the in-silico drug discovery process, good docking results are not sufficient alone to evaluate the potential drug candidates. The insights gained from docking studies need to be supported with molecular dynamics simulations to identify the stability and behavior of ligand-protein interactions under dynamic conditions. This strategy improves the accuracy of computational analyses and helps to determine promising drug candidates. Molecular dynamics (MD) simulations were performed using Gromacs software to evaluate the stability and molecular behavior of the 8 candidate compounds that were examined for both HDAC6 and Hsp90. They were analyzed for a period of 300 nanoseconds for a comprehensive evaluation of the complexes. Gromacs is used to perform MD simulations from nanoseconds to microseconds at high and efficient computational speeds with accurate representations. At the end of each simulation run, a number of different results were obtained.

Table 3.3 Investigation of Hsp90 complexes stability average values through molecular dynamics simulations.

Complexes	RMSD (Å)	RMSF (Å)	Rg (nm)	SASA (nm ²)
ZINC000036349281	3,495105248	1,078410628	1,724361927	112.839
ZINC000028110230	6,047974069	1,441623188	1,765084639	116.061
ZINC000028092696	7,023164953	1,321782609	1,745457163	115.178
ZINC000027653366	2,343669426	1,343164251	1,728328185	112.538
ZINC000003626741	3,407999546	1,001347826	1,733308873	111.668
ZINC000016249755	6,256280667	1,000507246	1,722351118	111.706
ZINC000016249757	5,266255363	1,209227053	1,725999592	112.872
ZINC000043020382	3,403776077	1,15531401	1,719137502	111.031
Ganetespib	1,202213717	1,174700483	1,753448229	115.459

Table 3.4 Investigation of HDAC6 complexes stability average values through molecular dynamics simulations.

Complexes	RMSD (Å)	RMSF (Å)	Rg (nm)	SASA (nm ²)
ZINC000036349281	9,721340901	0,773124646	1,935014737	147.293
ZINC000028110230	2,78207968	0,762886686	1,930051773	144.572
ZINC000028092696	5,141141571	0,796385269	1,938823177	147.729
ZINC000027653366	2,831705611	0,898943343	1,941251756	150.209
ZINC000003626741	7,342020742	0,706427762	1,948048714	152.211
ZINC000016249755	6,440945793	0,697209632	1,93721479	147.629
ZINC000016249757	8,590350416	0,72592068	1,940924645	149.876
ZINC000043020382	6,678753843	0,808583569	1,945030644	149.428
Panobinostat	3,240522667	0,769161473	1,94162513	147.239

In this study, Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), Radius of Gyration (Rg), Solvent-Accessible Surface Area (SASA), number of Hydrogen-Bonds and Molecular Mechanics/Poisson-Boltzmann Surface Area (MM/PBSA) analyses were obtained to successfully assess the quality of candidates. All average values obtained from MD simulations could be seen in table 3.3 and 3.4, for Hsp90 and HDAC6, respectively.

3.2.1 Root Mean Square Deviation (RMSD) of HSP90 and HDAC6

Root Mean Square Deviation (RMSD) analysis is used to quantify structural changes over time by assessing the equilibrium stability and flexibility of proteins and ligands, as well as the distance between the protein's backbone and its atoms. RMSD plots were created for eight hit candidates to evaluate the variation in the protein's backbone atoms between their initial and final conformations. The stability of a protein-ligand complex can be discussed based on their fluctuations observed during simulations, with smaller fluctuations indicating a more stable protein structure. Ganetespib was used as a reference for Hsp90 and panobinostat for HDAC6, and their MD simulations were performed for 300 ns for their target proteins under the same conditions as the other ligands using the same methodology. Figure 3.2 clearly indicates the average RMSD values of the Hsp90-HDAC6 and its complexes separately in two graphs. Across all simulations, the protein RMSD values were consistently slightly elevated when compared with the backbone RMSD values (Figure 3.3 and Figure 3.4). Basically, it reflects the inclusion of both backbone and side chain atoms in the overall protein RMSD calculation. These structural deviations suggest that the protein retained a stable configuration throughout the simulation period. In Figure 3.2A, RMSD plots show that the fluctuations of all ligands do not pass beyond 10 Å and 3 of them do not pass beyond 5 Å for Hsp90 complexes. During a 300 ns simulation, the hit molecules ZINC27653366, ZINC3626741, and ZINC43020382 exhibited exceptional stability within the active site of Hsp90, highlighting their remarkable structural and dynamic stability. The results demonstrated that, in comparison to the reference, ganetespib, RMSD of Hsp90 in complex with ZINC27653366 stabilized immediately after the simulation begin and maintained stability throughout the entire simulation, with an average RMSD value of 2.3 Å (Table

3.4). Similarly, the Hsp90 complexes with ZINC3626741 and ZINC43020382 also exhibited convergence with minimal deviation, and they remained stable over the 300 ns simulation period, with an average RMSD value of 3.4 Å (Figure 3.3). The superimposition of all complexes revealed that the reference molecule, ganetespib, exhibited the highest stability, followed by ZINC27653366, ZINC3626741, and ZINC43020382 (Figure 3.2A). In Figure 3.2B, RMSD trajectories of HDAC6-ligand complexes over 300 ns were observed. The reference molecule panobinostat shows some fluctuations at the beginning of the simulation, reaches stability after 90 ns and remains constant at ~ 1.9 Å from 170 ns to the end of the simulation. It exhibited an average RMSD value of 3.2 Å throughout the entire simulation. For the other complexes, it can be clearly seen that ZINC28092696 and ZINC3626741 show the highest RMSD values (~ 17 Å) at 75 ns and 123 ns, respectively, and also ZINC36349281, ZINC16249755, and ZINC16249757 exceed 10 Å throughout the simulation. From the remaining three candidates, ZINC43020382 shows fluctuations throughout the simulation with the average RMSD value of 6.68 Å, while the last two candidates, ZINC28110230, and ZINC27653366 show RMSD values closest to the reference. Although the ZINC28110230-HDAC6 complex was stable at 2 Å up to 240 ns, it showed a fluctuation after this time value and remained stable at 6 Å for the last 60 ns (Figure 3.4). Despite all this, ZINC27653366 remained stable without any fluctuations at $\sim 2.5 - 3$ Å RMSD values from the beginning to the end of the simulation and its average RMSD value was 2.8 Å (Table 3.4). Consequently, ZINC27653366 formed reasonable stable complexes on both the active site of Hsp90 and HDAC6 over 300 ns of simulation.

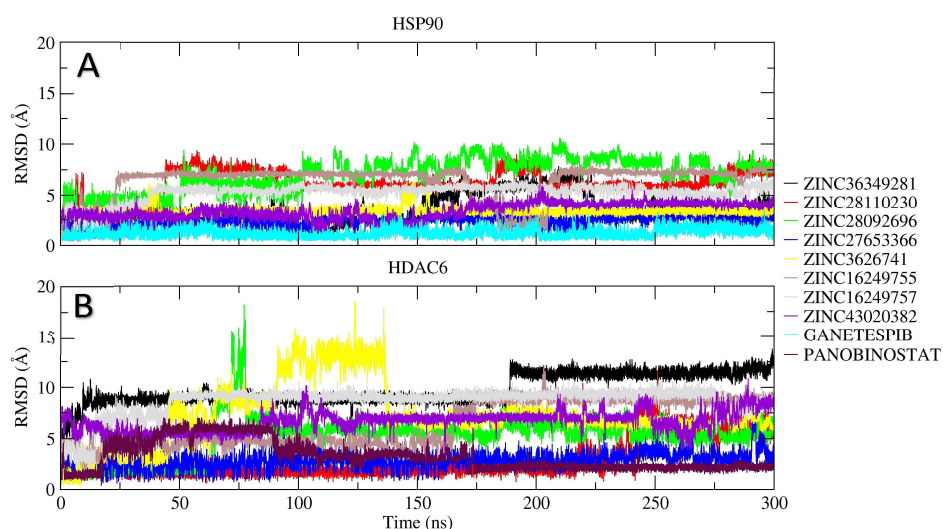


Figure 3.2 RMSD graphs of compounds on both A) Hsp90 and B) HDAC6

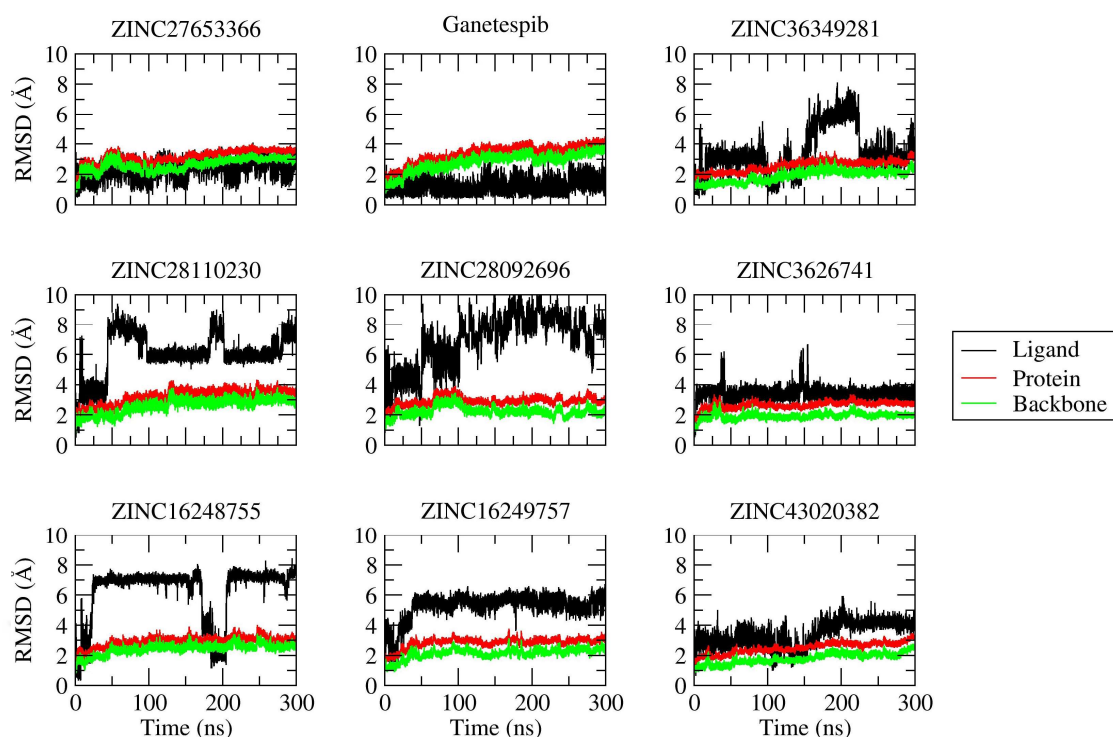


Figure 3.3 RMSD plots of 8 hit and reference compounds during 300 ns simulations separately on Hsp90.

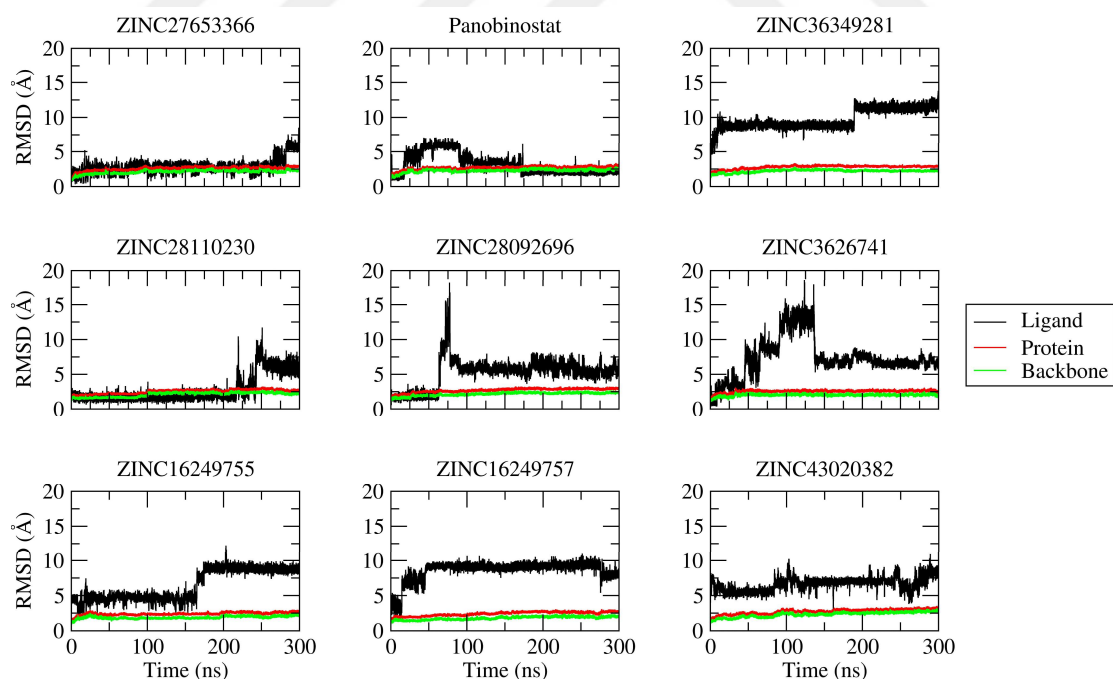


Figure 3.4 RMSD plots of 8 hit and reference compounds during 300 ns simulations separately on HDAC6.

3.2.2 Root-Mean-Square-Fluctuation (RMSF) of HSP90 and HDAC6

Next, the root mean square fluctuation (RMSF) analysis was used to evaluate proteins' stability by looking the average atomic flux of individual amino acids throughout the simulation [190]. When we examine all eight candidate and reference molecule for Hsp90 protein, all of them exhibited similar average values. Also, it can be clearly seen that the fluctuation of amino acids for each protein–ligand complex is mostly under 3 Å (Figure 3.5A). Similarly, in the RMSF analysis performed for the HDAC6 protein, all complexes revealed similar average RMSF values mostly under the 3 Å (Figure 3.5B). These findings suggest that candidate ligands do not have a significant impact on the protein's stability after binding.

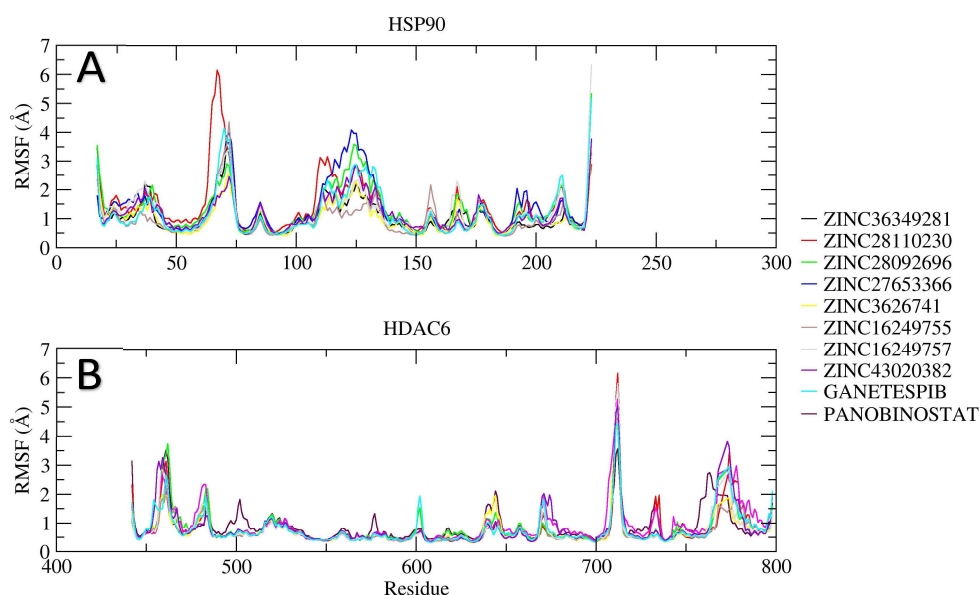


Figure 3.5 RMSF plots of 8 hit and reference compounds during 300 ns simulations on both A) Hsp90 and B) HDAC6.

3.2.3 Radius of Gyration (Rg) Analysis of HSP90 and HDAC6

Furthermore, radius of gyration (Rg) was calculated by using molecular dynamics simulation trajectories. Ligand binding to the target protein can affect the structural stability of the complex and cause conformational changes. Rg values provide insights into the compactness and conformational stability of protein–ligand complexes and they measure the root mean square distance between the protein's centre of mass and its residues. Minimal fluctuations in Rg show that the ligand does not significantly affect the protein's conformation. Rg analysis of the Hsp90 protein with possible candidates demonstrated results similar to HDAC6

and its complexes. The R_g values fluctuated in the range of 1.70 to 1.80 nm (Figure 3.6A). Considering HDAC6 and its complexes, we observe R_g values very similar to the reference molecule and each other. They fluctuate slightly between values of approximately 1.93 nm and 1.97 nm (Figure 3.6B). These results suggest the compactness of complexes on both Hsp90 and HDAC6 during 300 ns simulation.

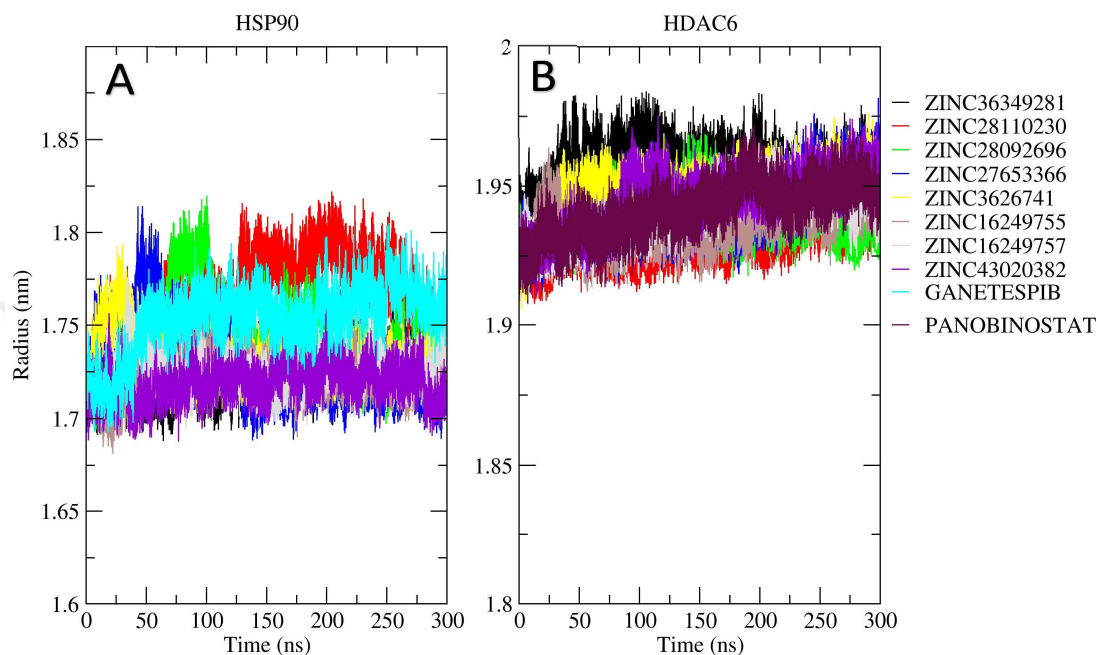


Figure 3.6 Radius of Gyration plots of 8 hit and reference compounds during 300 ns simulations on both A) Hsp90 and B) HDAC6.

3.2.4 Hydrogen Bond analysis of HSP90 and HDAC6

The binding affinity of candidate compounds on both Hsp90 and HDAC6 was evaluated by calculating the total number of hydrogen bonds formed within the protein-ligand complexes again by using MD trajectories since binding strength and the H-bond number are related. Hydrogen bond formation plays a critical role in stabilizing complexes. Figure 3.7 and 3.8 illustrates that all compounds have formed H-bonds with the target amino acids of Hsp90 and HDAC6, respectively, as reported in previously discussed molecular docking analysis. The average H-bond number was four for the Hsp90 reference molecule and two for the HDAC6 reference molecule. The other eight candidates showed similar results over the 300 ns MD simulations. The maximum number of hydrogen bonds was observed in the ZINC43020382 complex, with approximately average 5 and 4 for the Hsp90 and HDAC6, respectively.

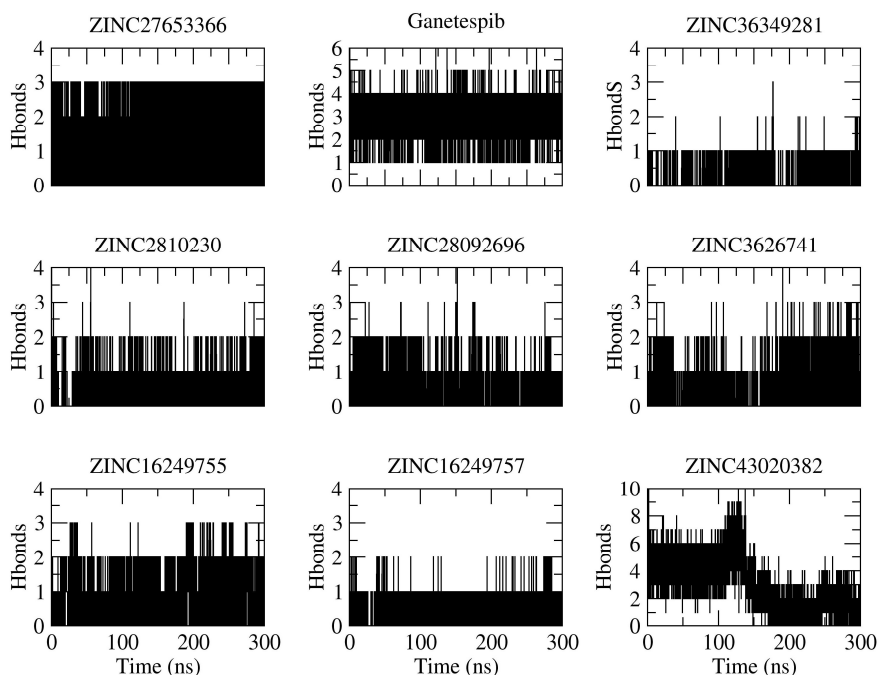


Figure 3.7 Hydrogen bond plots of 8 hit and reference compound ganetesipib during 300 ns simulations on Hsp90.

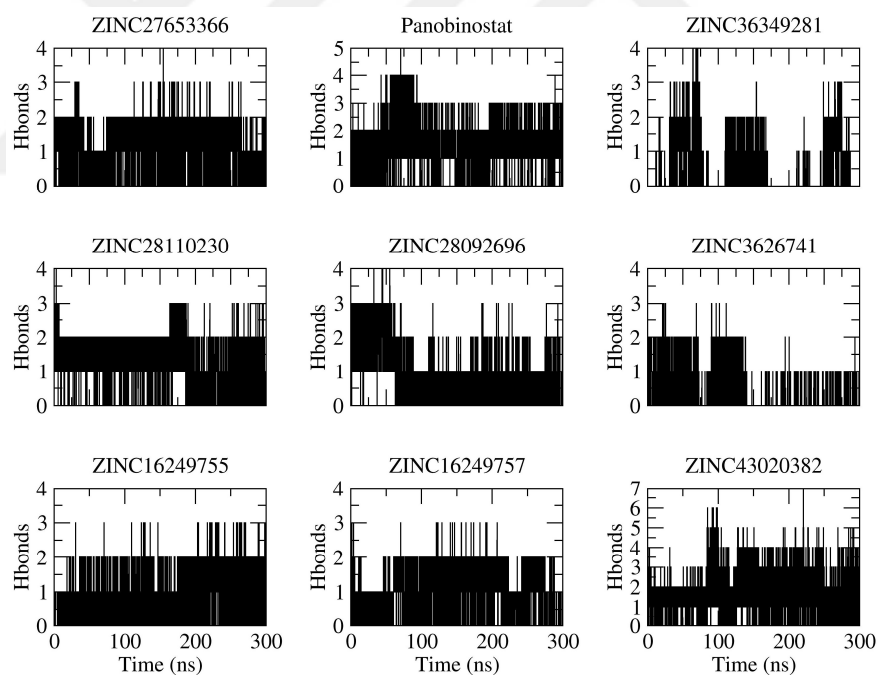


Figure 3.8 Hydrogen bond plots of 8 hit and reference compound panobinostat during 300 ns simulations on HDAC6.

3.2.5 Solvent-Accessible Surface Area (SASA) analysis of HSP90 and HDAC6

Additionally, SASA identification approach was employed to predict the conformational changes in proteins that water molecules would encounter throughout the simulation. The Hsp90-reference complex demonstrated an average SASA value of 115.46 nm². The other complexes exhibited average SASA values of between 111.03 nm² to 116.06 nm². From the Figure 3.9A, it can be seen clearly that all complexes remained relatively stable over the 300 ns MD simulation. It means that the ligands do not make a change in the target proteins. Similarly, HDAC6-candidate complexes show stable SASA graph over the simulation (Figure 3.9B). Panobinostat-HDAC6 complex exhibited an average SASA value of 147.23 nm², while others demonstrated SASA values between 144.572 nm² to 152.211 nm².

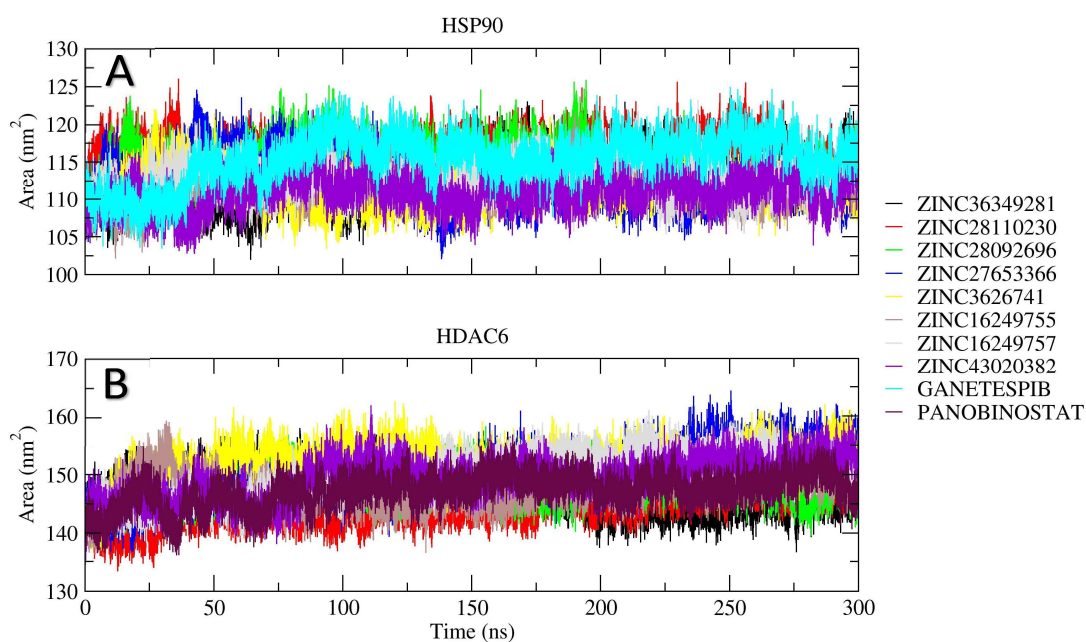


Figure 3.9 Solvent accessible surface area (SASA) plots of 8 hit and reference compounds during 300 ns simulations on both A) Hsp90 and B) HDAC6.

3.2.6 Ligand binding across Molecular Dynamics intervals

Molecular dynamic trajectories of ZINC27653366-Hsp90 and HDAC6, ganetespib-Hsp90, and panobinostat-HDAC6 were analysed at regular time intervals (0, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, and 300 nanoseconds) by superimposing them. It can be seen clearly from the Figure 3.10, ZINC27653366 remained stable at binding sites of both Hsp90 and HDAC6 proteins as expected from the previous analyses. As revealed by the RMSD analysis, there was relatively mild fluctuation during the first 90 ns of the HDAC6-reference MD simulation, which then reached stability. In support of this, the ligands of the complex show very slight differences for the first quarter of the simulation. Broadly, images from various stages of the MD simulation confirmed the stable binding of ZINC27653366 to Hsp90 and HDAC6. In addition to the lead compound, reference compounds, panobinostat and ganetespib, with each target were also analyzed across the MD intervals (Figure 3.11).

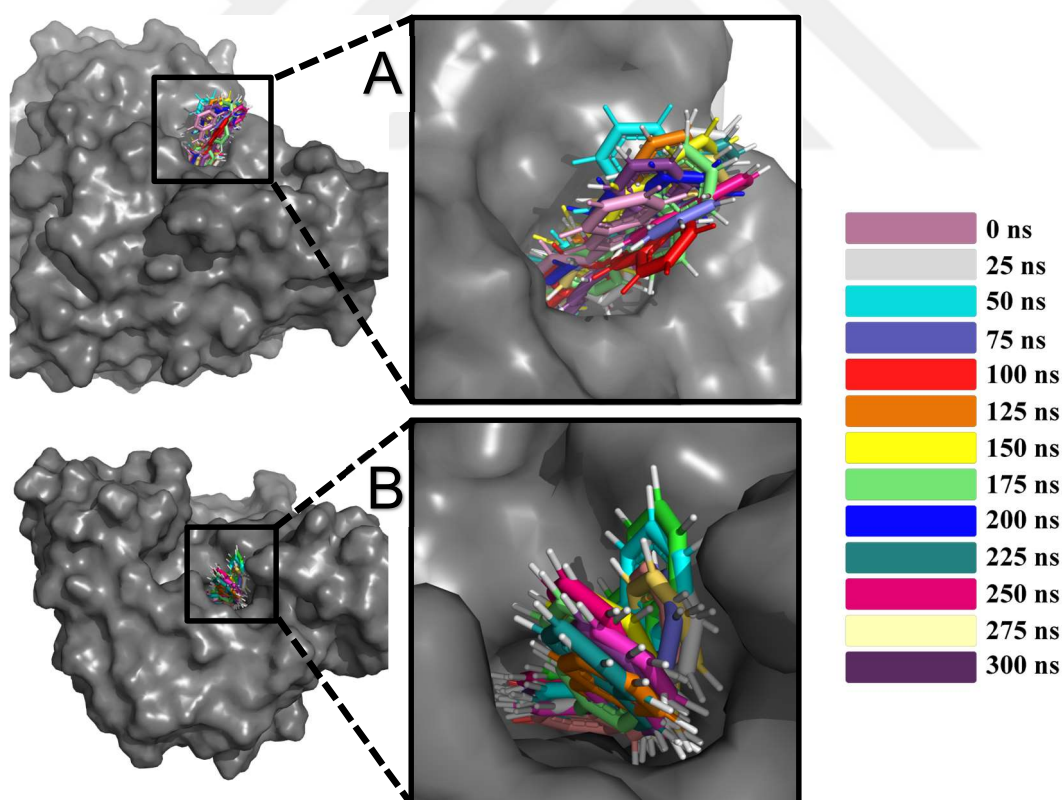


Figure 3.10 Surface of binding site of target protein and superimposition representation of A) ZINC27653366 - HDAC6, and B) ZINC27653366 - Hsp90 proteins over the 300 ns simulations at different time intervals (0, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, and 300 nanoseconds).

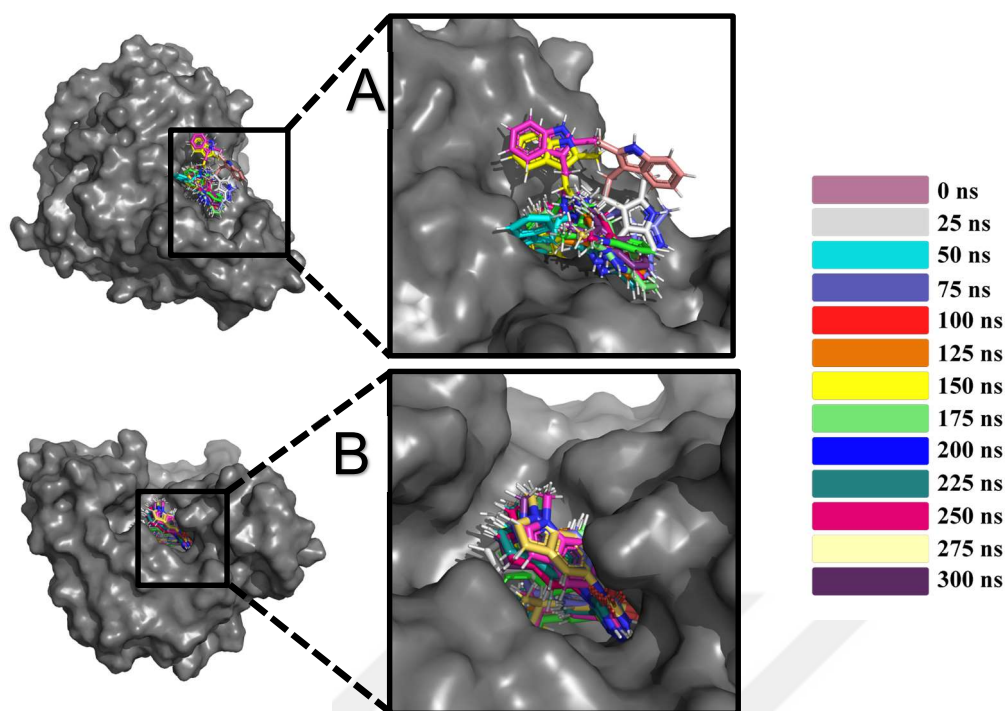


Figure 3.11 Surface of binding site of target protein and superimposition representation of A) Panobinostat - HDAC6, and B) Ganetespib - Hsp90 proteins over the 300 ns simulations at different time intervals (0, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, and 300 nanoseconds).

3.2.7 Molecular Mechanics/Poisson-Boltzmann Surface Area (MM/PBSA) analyses of HSP90 and HDAC6

The binding free energy of the complexes was analyzed using the Molecular Mechanics/Poisson-Boltzmann Surface Area (MM-PBSA) method, as implemented in GROMACS and derived from molecular dynamics trajectories (Figure 3.12). The calculated total binding free energy of 8 candidates and reference compound for Hsp90 protein exhibited similar and good binding free energy. ZINC36349281 has shown the best binding free energy with -29.17 kcal/mol. This was followed by ZINC43020382 with -28.54 kcal/mol and ZINC27653366 with -26.70 kcal/mol. The reference molecule ganetespib showed a value of -29.18 kcal/mol, similar to these results. However, taking into account the HDAC6 side, different and diverse binding energies are observed. Seven out of eight compounds showed negative binding free energy range from -9.41 kcal/mol to -24.70 kcal/mol, whereas the reference compound Panobinostat exhibited -14.25 kcal/mol. Only ZINC28110230 showed very small positive binding energy with 0.02 kcal/mol. ZINC36349281 has shown the best binding free energy with -24.70 and ZINC16249757 has shown the second-best binding free energy with -20.50 kcal/mol. All other results obtained from MM/PBSA analysis, Van der Waals energy, electrostatic

energy, polar solvation energy, SASA energy, and binding free energy for Hsp90 and HDAC6 proteins are shown in Tables 3.5 and 3.6, respectively.

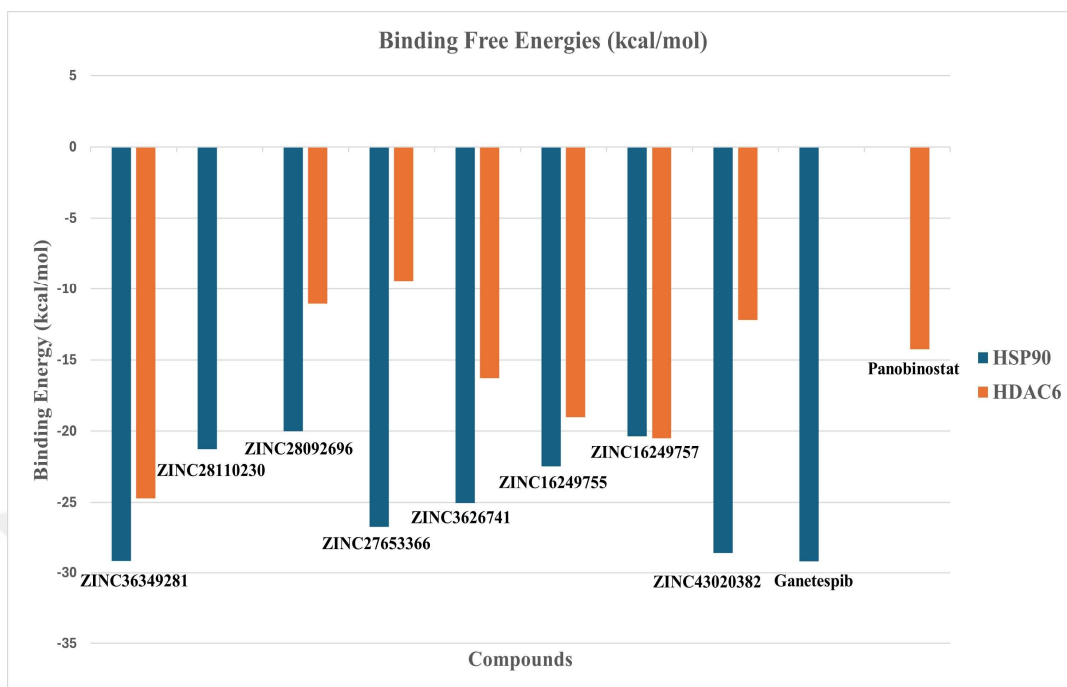


Figure 3.12 Average binding energies of top 8 compounds and reference molecules against both Hsp90 and HDAC6 protein.

Table 3.5 The components of binding energy which were determined using the MM-PBSA method for the Hsp90 protein.

Compound	Van der Waals energy (kJ/mol)	Electrostatic energy (kJ/mol)	Polar solvation energy (kJ/mol)	SASA energy (kJ/mol)	Binding energy (kJ/mol)
ZINC36349281	-194.702 +/- 20.274	-41.226 +/- 14.831	134.578 +/- 29.873	-20.796 +/- 1.919	-122.145 +/- 19.691
ZINC28110230	-120.208 +/- 13.623	-15.577 +/- 10.793	60.409 +/- 19.952	-13.671 +/- 1.338	-89.047 +/- 15.325
ZINC28092696	-110.141 +/- 13.881	-14.529 +/- 9.575	53.827 +/- 19.465	-12.858 +/- 1.495	-83.701 +/- 17.260
ZINC27653366	-151.192 +/- 9.478	-38.296 +/- 8.012	92.875 +/- 13.140	-15.196 +/- 0.830	-111.809 +/- 13.149
ZINC3626741	-186.224 +/- 13.155	-17.405 +/- 13.808	120.507 +/- 17.336	-21.653 +/- 1.096	-104.775 +/- 15.815
ZINC16249755	-143.186 +/- 13.218	-27.911 +/- 15.203	95.141 +/- 23.244	-18.067 +/- 1.470	-94.024 +/- 17.205
ZINC16249757	-143.876 +/- 11.802	-5.294 +/- 10.276	81.622 +/- 20.508	-17.661 +/- 1.427	-85.209 +/- 16.736
ZINC43020382	-166.606 +/- 14.777	-76.024 +/- 37.034	142.240 +/- 34.941	-19.114 +/- 1.558	-119.505 +/- 18.875
Ganetespib	-166.501 +/- 11.729	-81.546 +/- 13.137	143.553 +/- 26.195	-17.701 +/- 0.933	-122.195 +/- 16.766

Table 3.6 The components of binding energy which were determined using the MM-PBSA method for the HDAC6 protein.

Compound	Van der Waals energy (kJ/mol)	Electrostatic energy (kJ/mol)	Polar solvation energy (kJ/mol)	SASA energy (kJ/mol)	Binding energy (kJ/mol)
ZINC36349281	-147.831 +/- 13.712	-50.418 +/- 36.275	112.395 +/- 49.452	-17.580 +/- 1.630	-103.434 +/- 24.238
ZINC28110230	-116.073 +/- 12.743	-33.505 +/- 17.798	161.340 +/- 55.210	-11.658 +/- 1.253	0.104 +/- 49.144
ZINC28092696	-119.933 +/- 19.829	-36.923 +/- 17.002	123.903 +/- 64.040	-13.368 +/- 2.016	-46.322 +/- 51.187
ZINC27653366	-106.133 +/- 9.911	-43.941 +/- 17.293	121.921 +/- 37.791	-11.246 +/- 1.089	-39.399 +/- 30.973
ZINC3626741	-131.736 +/- 26.925	-41.949 +/- 28.147	121.737 +/- 65.111	-16.169 +/- 3.084	-68.117 +/- 58.803
ZINC16249755	-128.829 +/- 16.905	-14.460 +/- 8.939	79.014 +/- 21.381	-15.300 +/- 1.657	-79.575 +/- 21.749
ZINC16249757	-122.070 +/- 17.763	-28.139 +/- 9.834	78.913 +/- 27.911	-14.552 +/- 1.742	-85.847 +/- 22.953
ZINC43020382	-159.559 +/- 25.603	-97.371 +/- 33.103	223.683 +/- 80.968	-17.893 +/- 2.247	-51.141 +/- 57.502
Panobinostat	-142.760 +/- 16.732	-194.857 +/- 31.318	294.424 +/- 40.733	-16.477 +/- 1.319	-59.671 +/- 40.643

3.3 ADME Analysis

Absorption, distribution, metabolism, and excretion (ADME) are critical factors in the process of drug discovery and development [155], [156], [191]. A good drug candidate must not only exhibit robust efficacy against its therapeutic target but also it needs to demonstrate favourable ADME characteristics at therapeutic concentrations [192]. In this study, ADME analyses were performed to complement the molecular dynamics simulations, providing additional support for the selection of promising compounds for further studies. Eight selected candidate compounds and two references were evaluated for their drug-likeness properties using Lipinski's Rule of Five (RO5) as the primary framework. The rule of 5 indicates that poor absorption is more likely to occur when there are more than 5 hydrogen-bond donors, 10 hydrogen-bond acceptors, 10 rotatable bonds, a molecular weight greater than 500 g/mol, and Log P greater than 5 [193], [194]. The results demonstrated that six of eight compounds were found suitable for Lipinski Rule of Five (Table 3.7). ZINC36349281 exhibited deviations with 536.447 g/mol molecular weight and 5.72 logP. Also, in ZINC43020382, logP slightly exceeds the threshold, with the value of 5.053. These deviations suggest the potential challenges in oral bioavailability. The reference compounds,

ganetespib and panobinostat, were used as benchmarks and their profiles did not show any violation of the RO5 criteria. The remaining candidates may fully comply with the Rule of 5. In particular, ZINC27653366, ZINC28110230 and ZINC28092696 have shown very similar results to the references.

When we combined all results, one of eight compounds stood out as promising. To determine this compound, we meticulously analysed all experiments and further focused on studies which have shown differences between ligands.

One of them was RMSD analysis, which is an important component of in-silico molecular dynamics simulation. In the Hsp90-ligand complexes, although some of them exhibited more similar results to the reference compound, there were no significant deviations and differences over the 300 ns simulation time. On the other hand, these differences were much more clearly visible when looking at the HDAC6-ligand complexes. As discussed in the RMSD analysis section, the compound ZINC27653366 showed the most stability and low RMSD value with HDAC6. The same compound also showed more stability in Hsp90 protein compared to other candidates.

Table 3.7 The binding affinities and Lipinski's Rule of Five for the top 8 compounds and references. Abbreviations: MW – molecular weight, HBD – hydrogen bond donor, HBA – hydrogen bond acceptor, RB – rotatable bonds.

ZINC ID	MW	HB	HBA	RB	LogP	Rule of Five
ZINC36349281	536.447	0	4	7	5.720	2
ZINC28110230	237.258	2	2	1	3.112	0
ZINC28092696	237.258	2	2	1	3.112	0
ZINC27653366	263.296	2	2	3	3.626	0
ZINC16249755	382.463	2	2	4	4.368	0
ZINC16249757	382.463	2	2	4	4.368	0
ZINC3626741	443.503	3	4	8	4.208	0
ZINC43020382	465.466	4	6	10	5.053	1
Panobinostat	349.434	4	2	7	3.327	0
Ganetespib	364.405	3	4	3	3.254	0

Furthermore, when we look at binding free energy results, ZINC36349281 was seen as the best compound for both targets with very similar value to the Hsp90

reference and better result than HDAC6 reference. Notably, all ligands, except one, exhibited negative binding values which shows favourable interactions with the target proteins. However, despite its best binding free energy results, ZINC36349281 outlined fluctuations and relatively high RMSD values during MD simulation. This suggests its potential instability in the active site of proteins.

This consideration can also be supported by the ADME analysis. In this study, ZINC36349281 failed to comply with Lipinski's Rule of Five, with 2 violations, high molecular weight and elevated logP values. These properties may limit the oral bioavailability and drug-likeness of candidate.

On the other side, ZINC27653366 consistently showed the most desirable outcomes through several evaluations such as stable RMSD profiles, compliance with the Lipinski's Rule of Five, and competitive binding free energy values underline the strong potential of this compound as a dual inhibitor for Hsp90 and HDAC6 proteins. These assessments could be supported by the MD movie of the complex. This movie gives clear dynamic visualization of protein-ligand complexes and their behaviour.

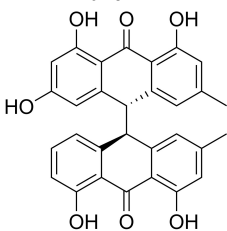
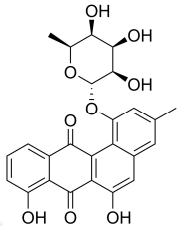
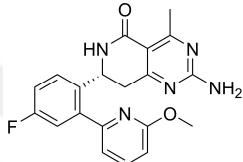
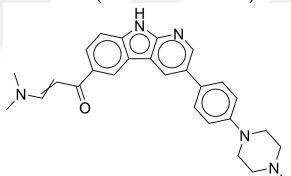
Chapter 4

Investigation of Potential Dual Inhibitors of HSP90 – ALK

4.1 Molecular Docking Evaluation

A total of 361,179 molecules received from COCONUT database according to Lipinski Rule of Five filtration, were docked into the Hsp90 (4W7T) and ALK (8ARJ) active site separately. This step was carried out on the SMINA docking program, the same as the first study, to get the lowest energy conformers of the compounds at the binding site of target proteins. Molecular dynamics (MD) simulations of a period of 150 nanoseconds were conducted starting from high-affinity candidates on both two targets to evaluate the stability and behavior of the protein-ligand complexes in a dynamic environment. Ligands that exhibited high instability during the initial 150 ns MD simulations were excluded from subsequent analyses. Conversely, those with stable and promising MD profiles were subjected to extended simulations and more detailed studies. Ultimately, two potential ligands were identified, and all subsequent analyses focused on these candidates. NVP-HSP990 and NRR were used as reference molecules to confirm methodology and to compare the results for Hsp90 and ALK, respectively. Their COCONUT IDs, chemical structures, and binding affinities can be seen in Table 4.1. These compounds listed as follows for Hsp90 and ALK, respectively: CNP0264442.1 with -13.7 kcal/mol and -10.7 kcal/mol; CNP0371528.1 with -11.2 kcal/mol and -10.7 kcal/mol. NVP-HSP990 (Hsp90 reference) showed -9.6 kcal/mol, and NRR (ALK reference) showed -10.9 kcal/mol binding affinities on target proteins.

Table 4.1 Molecular docking results of selected compounds on both Hsp90 (4W7T) and ALK (8ARJ) proteins and their COCONUT IDs.

COCONUT ID	Binding Affinity (kcal/mol)	
	HSP90	ALK
CNP0264442.1 	-13.7	-10.7
CNP0371528.1 	-11.2	-10.7
NVP-HSP990 (Hsp90 reference) 	-9.6	-
NRR (ALK reference) 	-	-10.9

The binding interaction analysis shows that our candidate ligands CNP0264442.1 and CNP0371528.1 bind effectively to ALK. Their interaction patterns are comparable to our reference ligand NRR and known ALK inhibitors like brigatinib and crizotinib. This suggests that CNP0264442.1 and CNP0371528.1 may serve as effective ALK inhibitors. Table 4.2 summarizes the main interacting residues, distances and interaction types between ALK protein and the selected candidate molecules and references, with corresponding visual representations provided in Figure 4.1. CNP0264442.1 forms strong hydrophobic contacts with several important residues in the ATP-binding site of ALK, such as Leu1122, Val1130, Ala1148, Leu1198, and Leu1256. These residues are known to play a role in the binding of well-characterized inhibitors like brigatinib and entrectinib [195], [196]. In addition, a clear hydrogen bond is observed between CNP0264442.1 and Arg1253. It indicates a stabilizing polar interaction, likely supporting its binding strength and selectivity.

Another candidate, CNP0371528.1, interacts strongly with Val1130 and Ala1148 through hydrophobic Pi-alkyl contacts, similar to those seen in ALK–brigatinib and ALK–ceritinib complexes [196]. A crucial aspect of this ligand's interaction is its hydrogen bond with Met1199. This hinge region residue plays an important role in stabilizing numerous ALK inhibitors via common scaffolds such as 2-aminopyridine or 2-aminopyrimidine. This type of interaction reflects the natural ATP-binding mode. For comparison, brigatinib forms two hydrogen bonds with Met1199, and crizotinib relies on both Met1199 and Glu1197 for hinge region engagement [195], [196].

The reference ligand NRR successfully re-establishes key interactions with Glu1197, Met1199, Ala1200, and Asp1203. It confirms the docking methodology and helps us to evaluate the performance of ligands CNP0264442.1 and CNP0371528.1. Therefore, the binding profiles of the two candidates show similar structural features with the reference compound and the known ALK inhibitors. Both compounds engage key hydrophobic residues, which form stabilizing contacts in the hinge region.

On the other hand, similar to the findings presented in the first part of this study, key residues previously identified as critical for HSP90 inhibition, such as Asn51, were also involved in interactions with the two candidate ligands and the reference (Table 4.2). The corresponding visual representations are provided in Figure 4.1. CNP0264442.1 interacted with Asn51, Gly97, Met98, Leu107, Gly135, and Phe138, with interatomic distances ranging from 2.0 to 4.8 Å. CNP0371528.1 also showed similar interaction patterns, such as with Asn51, Ala55, Met98, and Gly135. Notably, the reference compound NVP-HSP990 displayed all the interactions we explained for the two candidate ligands. These interactions reinforce the role of ligands as a benchmark for evaluating binding performance. These results collectively suggest that both candidate molecules interact effectively with the functional sites of Hsp90, potentially contributing to its inhibition in a manner comparable to that observed in the initial study.

Table 4.2 Molecular docking results and interaction details of top two hit molecules and references against Hsp90 and ALK. (NVP-HSP990 for Hsp90 reference and NRR for ALK reference).

COCONUT ID	HSP90			ALK		
	Main interacting residues	Distance (Å)	Interaction types	Main interacting residues	Distance (Å)	Interaction types
CNP0264442.1	Leu48	4.52	Pi-alkyl	Leu1122	3.83	Pi-alkyl
	Asn51	4.79	Amide-pi stacked	Leu1122	5.49	Pi-alkyl
	Asp93	2.49	Conventional H. bond	Val1130	3.96	Pi-sigma
	Gly97	2.14	Conventional H. bond	Val1130	5.02	Pi-alkyl
	Met98	3.92	Pi-sulfur	Ala1148	4.79	Pi-alkyl
	Leu107	3.96	Pi-alkyl	Leu1198	4.79	Pi-alkyl
	Lys112	2.32	Conventional H. bond	Asp1203	4.12	Pi-anion
	Gly135	2.03	Conventional H. bond	Arg1253	2.01	Conventional H. bond
	Phe138	4.77	Pi-alkyl	Leu1256	3.77	Pi-sigma
	Tyr139	4.28	Pi-alkyl	Leu1256	5.15	Pi-alkyl
	Thr184	2.43	Carbon-Hydrogen bond			
	Val186	4.28	Pi-alkyl			
	Asn51	2.13	Conventional H. bond	Val1130	4.38	Pi-alkyl
	Ala55	4.33	Pi-alkyl	Val1130	4.54	Pi-alkyl
CNP0371528.1	Ala55	4.91	Pi-alkyl	Ala1148	4.33	Pi-alkyl
	Met98	3.99	Pi-sulfur	Met1199	1.63	Unfavorable donor-donor
	Met98	4.36	Pi-alkyl	Met1199	2.34	Conventional H. bond
	Met98	4.60	Pi-alkyl	Met1199	2.36	Conventional H. bond
	Gly135	2.10	Conventional H. bond	Asp1203	3.72	Pi-anion
	Thr184	2.41	Conventional H. bond	Arg1253	1.84	Conventional H. bond
				Arg1253	2.97	Unfavorable donor-donor
				Leu1256	4.71	Pi-alkyl
				Leu1256	4.78	Pi-alkyl
				Asp1270	2.52	Conventional H. bond
Reference	Asn51	2.40	Conventional H. bond	Leu1122	2.30	Conventional H. bond
	Ala55	5.03	Pi-alkyl	Leu1122	3.84	Pi-alkyl
	Lys58	2.59	Carbon-Hydrogen bond	Leu1122	5.03	Pi-alkyl
	Ile96	5.36	Pi-alkyl	Val1130	4.94	Pi-alkyl
	Gly97	2.91	Conventional H. bond	Val1130	4.97	Pi-alkyl
	Met98	5.38	Pi-alkyl	Val1130	5.18	Pi-alkyl
	Asp102	2.61	Conventional H. bond	Ala1148	3.86	Pi-alkyl
	Leu107	5.21	Pi-alkyl	Ala1148	4.41	Pi-alkyl
	Gly135	3.36	Carbon-Hydrogen bond	Ala1148	4.98	Pi-alkyl
	Phe138	4.87	Pi-pi stacked	Lys1150	2.15	Conventional H. bond
				Leu1196	5.44	Pi-alkyl
				Glu1197	2.14	Conventional H. bond
				Met1199	2.01	Conventional H. bond
				Met1199	3.08	Carbon-Hydrogen bond
				Arg1253	3.44	Carbon-Hydrogen bond
				Leu1256	4.39	Pi-alkyl
				Leu1256	4.52	Pi-alkyl
				Leu1256	5.03	Pi-alkyl
				Asp1270	2.54	Carbon-Hydrogen bond

HSP90		ALK	
COCONUT ID & 3D interactions	2D interactions	COCONUT ID & 3D interactions	2D interactions
CNP0264442.1			
CNP0371528.1			
NVP-HSP990 (HSP90 reference)		NRR (ALK reference)	

Figure 4.1 Binding modes of the selected candidates and reference molecules: 3D and 2D interaction diagrams with Hsp90 and ALK.

4.2 Molecular Dynamics Analysis

4.2.1 Root Mean Square Deviation (RMSD) of HSP90 and ALK

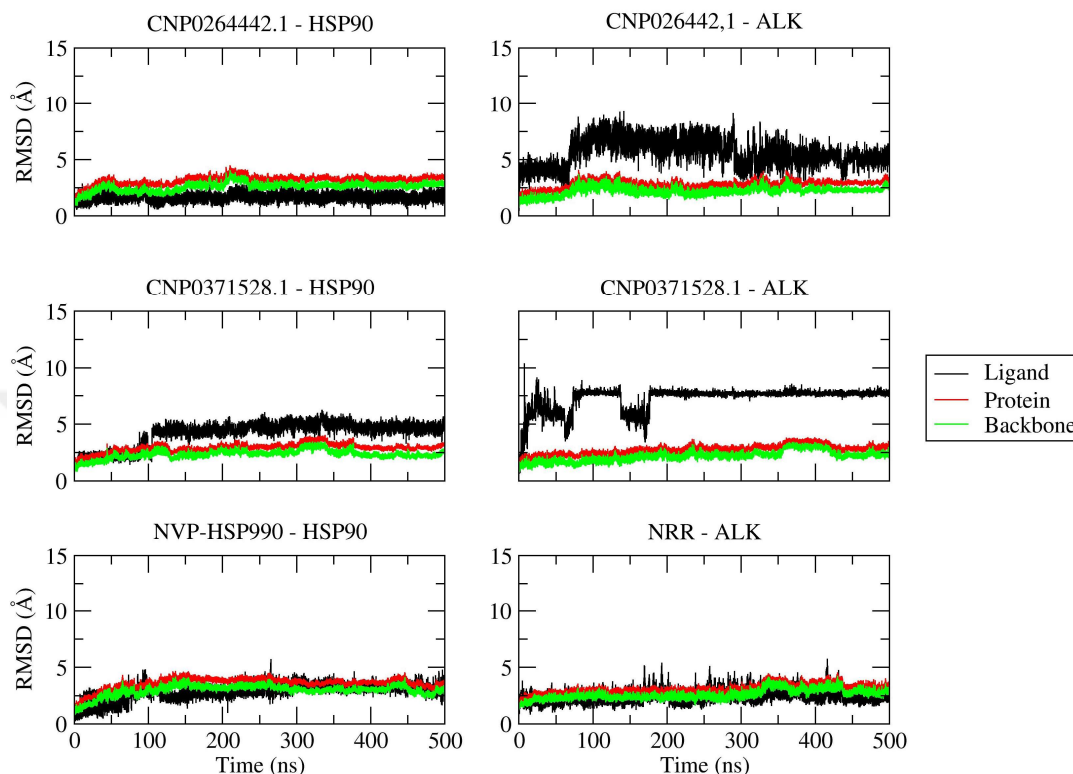


Figure 4.2 Root-mean square deviation (RMSD) plots of selected hit compounds and references during 500 ns simulations.

Molecular dynamics simulations were performed for a period of 500 ns to evaluate the structural stability and binding consistency of two candidate compounds and the respective reference ligands. All average results obtained from MD simulations could be seen in table 4.3 and 4.4, for Hsp90 and ALK, respectively. Root mean square deviation (RMSD) values were calculated for each protein's backbone, the entire protein, and the corresponding ligand to assess the dynamic behavior and complex stability. As shown in Figure 4.2, all six systems remained within acceptable RMSD fluctuation ranges. For Hsp90 complexes, both candidates-maintained ligand RMSD values below ~ 5 Å throughout the simulation, with the protein backbone and overall protein RMSDs remaining stable around 2–3 Å. This indicates strong and stable interactions. For the ALK, the candidate ligand CNP0264442.1 exhibited a relatively stable RMSD profile, with ligand fluctuations generally remaining below 5 Å throughout the 500 ns simulation. In the case of CNP0371528.1, the ligand showed higher initial fluctuations but stabilized

significantly after approximately 175 ns, maintaining a steady RMSD around 7–8 Å for the remainder of the simulation. Despite these ligand-level variations, both ALK systems demonstrated strong structural stability, with the protein and backbone RMSD values staying consistently low and stable. The reference ligand NRR also displayed excellent stability, with ligand RMSD values remaining below 4 Å. Overall, these RMSD results support that the candidate ligands form stable complexes with both targets, with fluctuations within an acceptable range. The data suggests that both CNP0264442.1 and CNP0371528.1 maintain sufficient structural integrity to interact consistently with key residues over the course of extended MD simulation, comparable to known inhibitors.

4.2.2 Root Mean Square Fluctuation (RMSF) and Solvent Accessible Surface Area (SASA) Analysis of HSP90 and ALK

In RMSF analysis part, when we examine both two candidates and reference molecule for Hsp90 protein, all of them exhibited similar average values and also it can be clearly said that the fluctuation of amino acids for each protein–ligand complex mostly under 4 Å. Similarly, in the same analysis performed for the ALK protein, all complexes revealed similar average RMSF values, mainly under the 3 Å (Figure 4.3A). These findings suggest that candidate ligands do not have a significant impact on the protein's stability after binding.

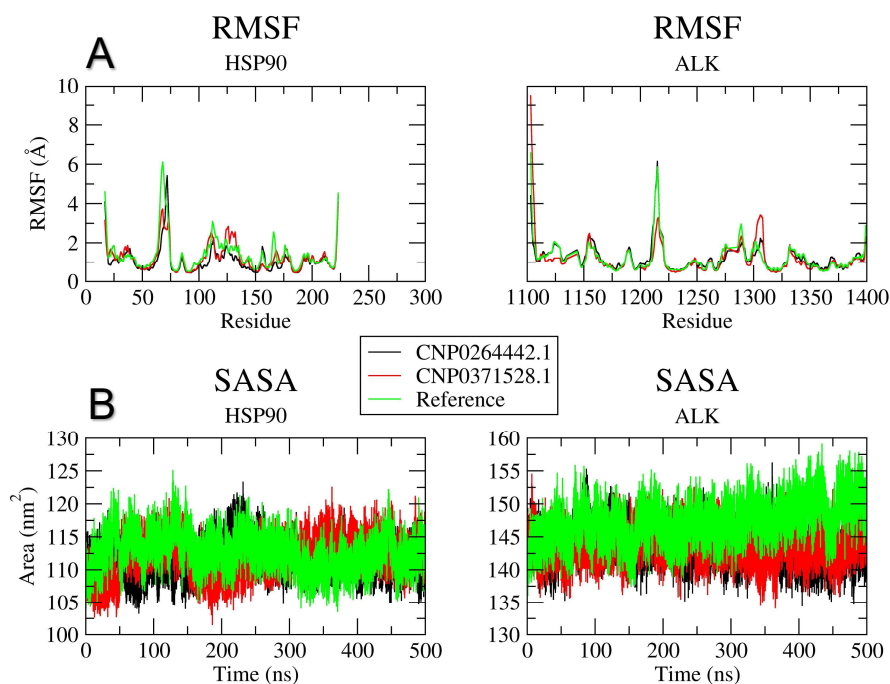


Figure 4.3 A) Root-mean square fluctuation (RMSF) and B) solvent-accessible surface area (SASA) results.

Additionally, SASA identification approach was employed to predict the conformational changes in proteins that water molecules would encounter throughout the simulation. The Hsp90-reference complex and candidates exhibited average SASA values of between $\sim 106 \text{ nm}^2$ to 116 nm^2 . From the Figure 4.3B, it can be seen clearly that all complexes remained relatively stable over the 500 ns MD simulation. It means that the ligands do not make a change in the target proteins. Similarly, ALK-candidate complexes show a stable SASA graph over the simulation. NRR-ALK complex exhibited an average SASA value of $\sim 146 \text{ nm}^2$, while others demonstrated SASA values between $\sim 142 \text{ nm}^2$ to $\sim 152 \text{ nm}^2$.

4.2.3 Hydrogen Bond and Radius of Gyration Analysis of HSP90 and ALK

The binding affinity of candidate compounds on both Hsp90 and ALK was evaluated by calculating the total number of hydrogen bonds formed within the protein-ligand complexes again by using MD trajectories since binding strength and the H-bond number are related. Hydrogen bond formation plays a critical role in stabilizing complexes, as we explained before. Figure 4.4A illustrates that all compounds have formed H-bonds with the target amino acids of Hsp90 and ALK, as supposed from the molecular docking analysis discussed. The average H-bond number was three for the Hsp90 reference molecule and four for the ALK reference molecule. The other two candidates showed similar results over the 500 ns MD simulations. The maximum number of hydrogen bonds was observed in the CNP0371528.1 complex, with approximately an average of 4 and 5 for the Hsp90 and ALK, respectively.

Further, to support these results, the radius of gyration (Rg) was calculated again by using MD simulation trajectories. Rg values provide insights into the compactness and conformational stability of protein-ligand complexes, and they measure the root mean square distance between the protein's center of mass and its residues. Rg analysis of Hsp90 protein with possible candidates demonstrated similar fluctuations. We observed Rg values in the range 1.70 to 1.76 nm. When we look at ALK - and its complexes, we observe Rg values very similar to the reference molecule and each other. They fluctuate slightly between values of approximately 2.0 nm and 2.07 nm (Figure 4.4B). These results suggest the compactness of complexes on both Hsp90 and ALK during 500 ns simulation.

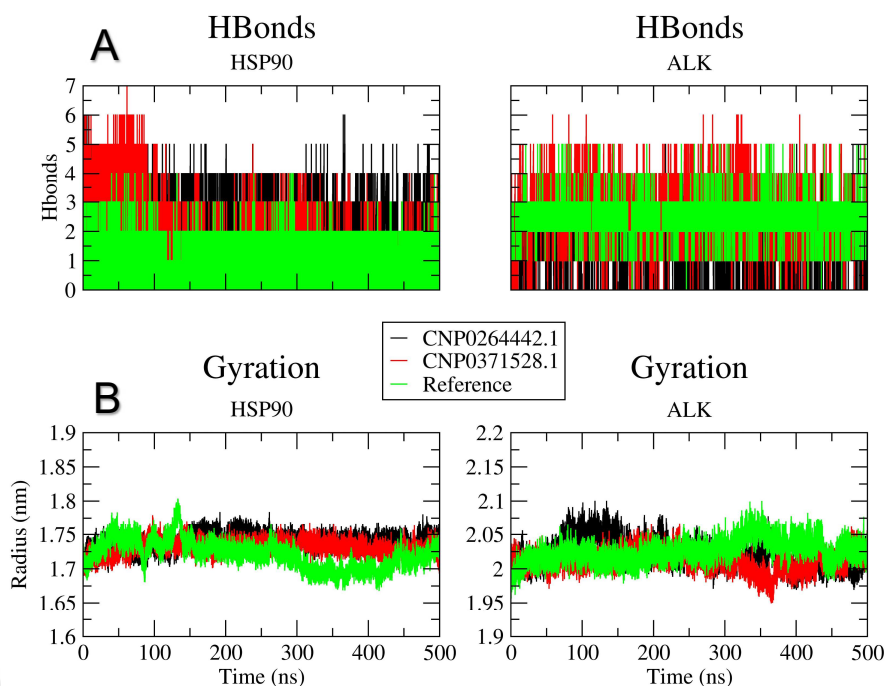


Figure 4.4 A) Hydrogen bond and B) Radius of gyration analysis.

4.2.4 Ligand Binding across Molecular Dynamics intervals

Molecular dynamic trajectories- of CNP0264442.1-Hsp90 and ALK, NVP-HSP990-Hsp90, and NRR-ALK were analyzed at regular time intervals by superimposing them. (0, 50, 100, 150, 200, 250, 300, 350, 400, 450, and 500 nanoseconds). It can be seen clearly from Figure 4.5 that CNP0264442.1 remained stable at the binding sites of both Hsp90 and ALK proteins, as expected from the previous analyses. As revealed by the RMSD analysis, there were no any big fluctuations during the MD simulation time. Precisely, snapshots from various stages of the MD simulation confirmed the stable binding of CNP0264442.1 to Hsp90 and ALK.

Table 4.3 Investigation of Hsp90 complexes stability average values through molecular dynamics simulations.

Complexes	RMSD (Å)	RMSF (Å)	Rg (nm)	SASA (nm ²)
CNP0264442.1	1.67	1.10	1.739	111.670
CNP0371528.1	4.19	1.26	1.731	111.953
NVP-HSP990	2.86	1.40	1.720	112.707

Table 4.4 Investigation of ALK complexes stability average values through molecular dynamics simulations.

Complexes	RMSD (Å)	RMSF (Å)	Rg (nm)	SASA (nm ²)
CNP0264442.1	5.65	1.20	2.022	143.614
CNP0371528.1	7.26	1.15	2.012	143.860
NRR	2.37	1.19	2.025	147.176

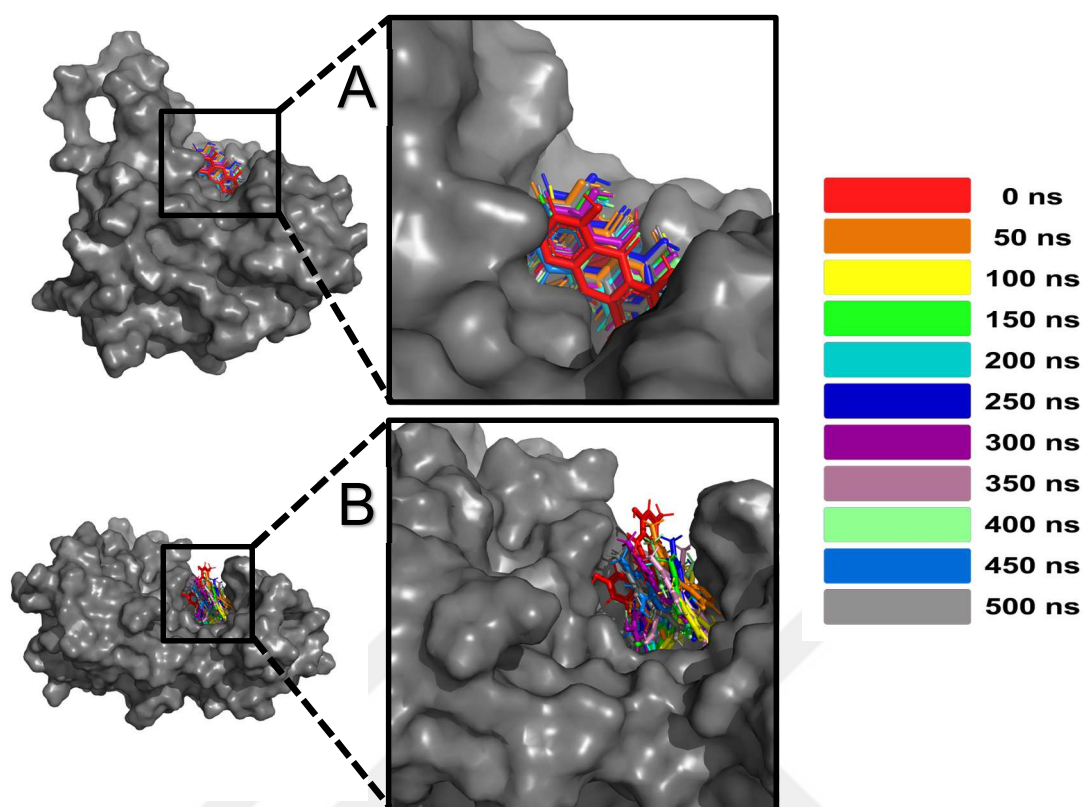


Figure 4.5 Surface of binding site of target protein and superimposition representation of A) CNP0264442.1 – Hsp90, and B) CNP0264442.1 - ALK proteins over the 500 ns simulations at different time intervals (0, 50, 100, 150, 200, 250, 300, 350, 400, 450 and 500 nanoseconds).

4.2.5 MM/PBSA Analysis of HSP90 and ALK

Next, the binding free energies of the ligands were calculated by using MM/PBSA analysis technique. In Figure 4.6, the bar plots clearly demonstrated that all ligands have presented negative binding free energy in a range of -11,7476 kcal/mol to -17,1472 kcal/mol. Our potential candidate CNP0264442.1 has shown the best binding free energy on both Hsp90 and ALK with -17,1472 kcal/mol and -16,5988 kcal/mol, respectively. Moreover, these results were even better than the reference compounds' binding free energies on their corresponding targets. CNP0371528.1 has shown similar binding free energy with -11,7476 kcal/mol and -12,4248 kcal/mol on Hsp90 and ALK, respectively. In addition to the average binding free energy values, their fluctuation throughout the 500 ns MD simulations is presented in Figure 4.7. It can be clearly seen that free energy trajectories remained consistently negative throughout the simulations on both Hsp90 and ALK, with most values fluctuating between approximately -10 and -20 kcal/mol. These results indicate that all ligands maintained stable binding to their respective protein targets

over time. All other results obtained from MM/PBSA analysis, Van der Waals energy, electrostatic energy, polar solvation energy, SASA energy, and binding free energy for Hsp90 and ALK proteins can be seen in Tables 4.5 and 4.6, respectively.

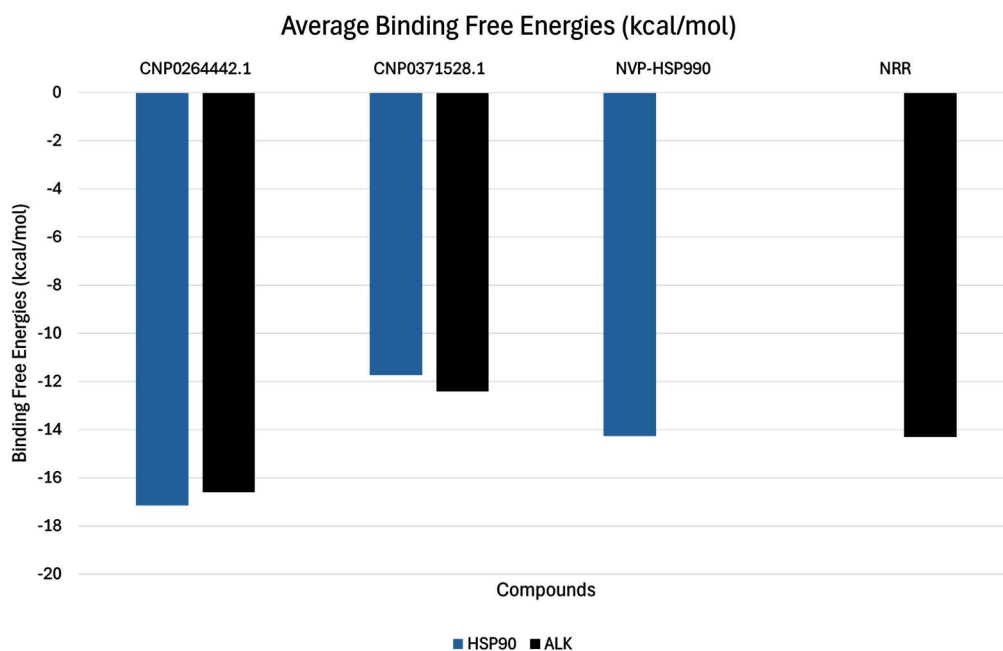


Figure 4.6 Average binding energies of selected and reference molecules against both Hsp90 and ALK.

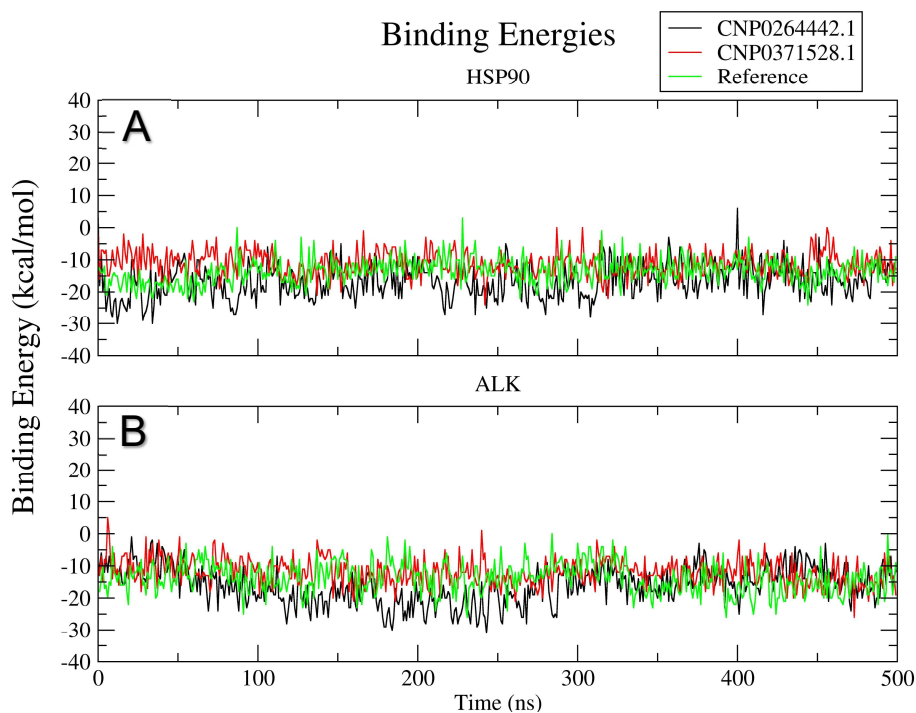


Figure 4.7 MM/PBSA binding free energies of top two compounds and references on A) Hsp90 and B) ALK.

Table 4.5 The components of binding energy which were determined using the MM-PBSA method for the Hsp90.

Compound	Van der Waals energy (kJ/mol)	Electrostatic energy (kJ/mol)	Polar solvation energy (kJ/mol)	SASA energy (kJ/mol)	Binding energy (kJ/mol)
CNP0264442.1	-185.361 +/- 12.333	-92.142 +/- 20.053	226.176 +/- 34.155	-20.464 +/- 1.204	-71.792 +/- 22.263
CNP0371528.1	-160.515 +/- 11.910	-46.322 +/- 14.945	177.073 +/- 24.054	-19.421 +/- 1.189	-49.185 +/- 16.449
NVP-HSP990	-155.221 +/- 16.545	-15.255 +/- 11.929	129.989 +/- 26.961	-18.270 +/- 1.362	-58.757 +/- 16.487

Table 4.6 The components of binding energy which were determined using the MM-PBSA method for the ALK.

Compound	Van der Waals energy (kJ/mol)	Electrostatic energy (kJ/mol)	Polar solvation energy (kJ/mol)	SASA energy (kJ/mol)	Binding energy (kJ/mol)
CNP0264442.1	-146.864 +/- 15.820	-28.087 +/- 19.516	122.381 +/- 32.582	-16.925 +/- 1.347	-69.496 +/- 23.776
CNP0371528.1	-134.363 +/- 15.154	-68.849 +/- 22.657	168.617 +/- 34.552	-17.426 +/- 1.469	-52.020 +/- 17.592
NRR	-160.369 +/- 17.656	-57.559 +/- 18.416	177.811 +/- 28.906	-19.762 +/- 1.669	-59.879 +/- 19.740

4.3 ADME Analysis

In this study, ADME analyses were performed to complement the molecular dynamics simulations, providing additional support for the selection of promising compounds for further studies. Selected candidate compounds and two references were evaluated for their drug-likeness properties using Lipinski's Rule of Five (RO5) as the primary framework. The Rule of 5 criteria has been explained in the first study. As an initial screening step, compounds were filtered based on Lipinski's Rule of Five, and candidate molecules were subsequently selected. As expected, given this prior filtering, all final candidates comply with the Rule of Five. (Table 4.7). The reference compounds, NVP-HSP990 and NRR, were used as benchmarks, and their profiles did not show any violation of the RO5 criteria.

Table 4.7 The binding affinities and Lipinski's Rule of Five for the selected compounds and references. Abbreviations: MW – molecular weight, HBD – hydrogen bond donor, HBA – hydrogen bond acceptor, RB – rotatable bonds.

COCONUT ID	MW	HBD	HBA	RB	LogP	Rule of Five
CNP0264442.1	494.49	5	7	1	4.33	0
CNP0371528.1	466.44	5	9	2	1.53	0
NVP-HSP990	379.39	2	6	3	2.49	0
NRR	439.55	1	3	5	3.67	0

Chapter 5

Conclusions and Future Prospects

5.1 Conclusions

In this thesis study, dual in silico drug discovery approaches were conducted to target Heat Shock Protein 90 (Hsp90), which is a molecular chaperone that plays a critical role in cancer progression. The primary objective was to identify unique inhibitors that are capable of simultaneously disrupting Hsp90's interactions, first with Histone Deacetylase 6 (HDAC6) and subsequently with Anaplastic Lymphoma Kinase (ALK). Computational techniques were utilized to evaluate dual inhibitor candidates, including molecular docking, molecular dynamics (MD) simulations, and binding free energy calculations. For the HDAC6–Hsp90 study, 791 compounds were selected from the ZINC database with similarity-based filtering. In the Hsp90–ALK section, a total of 361,179 compounds that meet Lipinski's Rule of Five criteria were screened. Eight potential candidates for the HDAC6 - Hsp90 and two for the Hsp90 - ALK with the best binding affinity and interactions with critical residues were selected for further study. Subsequently, molecular dynamics (MD) simulations, binding free energy analysis, and ADME predictions were carried out for the selected ligands in complex with their respective targets. Specifically, 300 ns MD simulations for the HDAC6–Hsp90 complex and 500 ns simulations for the Hsp90–ALK complex were conducted. The results were analyzed in detail to assess the stability, binding interactions, and dynamic behavior of the ligand-protein systems.

At the end of all experiments, a comprehensive evaluation was performed to identify the most promising dual inhibitors. Among the screened compounds, ZINC27653366 demonstrated the strongest inhibitory potential within the Hsp90–HDAC6 complex by showing stable binding profiles throughout the 300 ns simulation, favorable interaction profiles with key active site residues, and negative binding free energies on both targets. Similarly, in the Hsp90–ALK complex, CNP0264442.1 emerged

as the top-performing candidate by exhibiting consistent conformational stability during the 500 ns MD simulation, optimal pharmacokinetic properties, and favorable interactions at the binding site of both targets. These findings indicate that these two compounds ZINC27653366, and CNP0264442.1 hold strong potential as lead dual inhibitors targeting HDAC6 - Hsp90 and Hsp90 - ALK, respectively. In conclusion, although additional *in vitro* and *in vivo* studies are required, *in-silico* evaluation of these compounds may position them as promising candidates for the treatment of cancer in the future.

5.2 Societal Impact and Contribution to Global

Sustainability

This research project focuses on the identification of novel dual inhibitors that target Hsp90 together with HDAC6 (in the first part of the study) or ALK (in the second part of the study), using *in-silico* drug discovery techniques including molecular docking, molecular dynamics, MM/PBSA and ADME. As of today, cancer still continues to be a major health problem around the worldwide, and unfortunately it causes millions of deaths each year. Unarguably, there is a significant need for new therapeutic options that are both more effective and less toxic than current treatments. This study aims to accelerate the identification of novel, potent, and selective dual inhibitors that could inhibit two key cancer-related proteins at the same time, which can improve treatment outcomes and reduce the risk of drug resistance.

From a societal perspective, the success of dual-target therapies offers a more efficient and generally less toxic approach than conventional single-target treatment techniques because dual inhibitors can simultaneously disrupt multiple cancer-associated signaling pathways. This may lead to improved patient outcomes, reduced side effects, and lower recurrence rates. Furthermore, dual inhibitors can help to overcome complex resistance mechanisms like in aggressive or late-stage cancers. This thesis study focuses mainly on Hsp90, which is a central chaperone protein involved in the stabilization of numerous oncogenic client proteins. The study emphasizes its importance across a wide range of cancer types.

In terms of global sustainability, this study was conducted in a computational environment from start to end, and this research promoted responsible consumption of

resources by reducing the need for extensive wet lab experimentation in the early-stage drug discovery process. Clearly, the time and material costs are significantly minimized by using in-silico studies. For example, screening a massive ligand library to select a number of potential candidates would be both time-consuming and costly without these types of studies. Additionally, the use of in silico tools aligns with the principles of green chemistry and sustainable pharmaceutical development. It basically limits chemical waste and energy-intensive processes.

To sum up, this thesis study encourages collaboration between different scientific fields, such as computational biology, medicinal chemistry, and cancer research. It helps build knowledge and skills that are essential for future developments in health science.

5.3 Future Prospects

The findings of this study provide a strong foundation for the future development of dual inhibitors targeting Hsp90 in combination with HDAC6 or ALK. As a next step, the most promising candidate compounds identified in both two studies (ZINC0265345 from the first study for Hsp90 and HDAC6 and CNP0264442.1 from the second study for Hsp90 and ALK) through in-silico analyses can be subjected to *in-vitro* and *in-vivo* tests to evaluate their biological activity and pharmacokinetic properties. In the long term, this research may contribute to the development of new and more effective treatment options against cancer.

BIBLIOGRAPHY

- [1] J. S. Brown, S. R. Amend, R. H. Austin, R. A. Gatenby, E. U. Hammarlund, and K. J. Pienta, "Updating the Definition of Cancer," *Molecular Cancer Research*, vol. 21, no. 11, pp. 1142–1147, Nov. 2023, doi: 10.1158/1541-7786.MCR-23-0411.
- [2] R. L. Siegel, T. B. Kratzer, A. N. Giaquinto, H. Sung, and A. Jemal, "Cancer statistics, 2025," *CA Cancer J Clin*, vol. 75, no. 1, pp. 10–45, Jan. 2025, doi: 10.3322/caac.21871.
- [3] D. Hanahan and R. A. Weinberg, "Hallmarks of Cancer: The Next Generation," *Cell*, vol. 144, no. 5, pp. 646–674, Mar. 2011, doi: 10.1016/j.cell.2011.02.013.
- [4] T. C. Dakal *et al.*, "Oncogenes and tumor suppressor genes: functions and roles in cancers," *MedComm (Beijing)*, vol. 5, no. 6, Jun. 2024, doi: 10.1002/mco2.582.
- [5] S. G. Almalki, "The pathophysiology of the cell cycle in cancer and treatment strategies using various cell cycle checkpoint inhibitors," *Pathol Res Pract*, vol. 251, p. 154854, Nov. 2023, doi: 10.1016/j.prp.2023.154854.
- [6] P. Anand *et al.*, "Cancer is a Preventable Disease that Requires Major Lifestyle Changes," *Pharm Res*, vol. 25, no. 9, pp. 2097–2116, Sep. 2008, doi: 10.1007/s11095-008-9661-9.
- [7] R. Sharman, Z. Harris, B. Ernst, D. Mussallem, A. Larsen, and K. Gowin, "Lifestyle Factors and Cancer: A Narrative Review," *Mayo Clin Proc Innov Qual Outcomes*, vol. 8, no. 2, pp. 166–183, Apr. 2024, doi: 10.1016/j.mayocpiqo.2024.01.004.
- [8] F. Bray *et al.*, "Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries," *CA Cancer J Clin*, vol. 74, no. 3, pp. 229–263, May 2024, doi: 10.3322/caac.21834.
- [9] H. M. Bizuayehu *et al.*, "Global Disparities of Cancer and Its Projected Burden in 2050," *JAMA Netw Open*, vol. 7, no. 11, p. e2443198, Nov. 2024, doi: 10.1001/jamanetworkopen.2024.43198.
- [10] K. M. Kamal *et al.*, "A Systematic Review of the Effect of Cancer Treatment on Work Productivity of Patients and Caregivers," *J Manag Care Spec Pharm*, vol. 23, no. 2, pp. 136–162, Feb. 2017, doi: 10.18553/jmcp.2017.23.2.136.
- [11] T. A. Kitaw, B. D. Tilahun, A. B. Zemariam, A. Getie, M. A. Bizuayehu, and R. N. Haile, "The financial toxicity of cancer: unveiling global burden and risk factors – a systematic review and meta-analysis," *BMJ Glob Health*, vol. 10, no. 2, p. e017133, Feb. 2025, doi: 10.1136/bmjgh-2024-017133.
- [12] S. E. Wan Puteh, Y. R. Abdullah, and A. N. Aizuddin, "Catastrophic Health Expenditure (CHE) among Cancer Population in a Middle Income Country with Universal Healthcare Financing," *Asian Pacific Journal of Cancer Prevention*, vol. 24, no. 6, pp. 1897–1904, Jun. 2023, doi: 10.31557/APJCP.2023.24.6.1897.
- [13] L. Doshmangir, E. Hasanpoor, G. J. Abou Jaoude, B. Eshtiagh, and H. Haghparast-Bidgoli, "Incidence of Catastrophic Health Expenditure and Its Determinants in Cancer Patients: A Systematic Review and Meta-analysis," *Appl Health Econ Health Policy*, vol. 19, no. 6, pp. 839–855, Nov. 2021, doi: 10.1007/s40258-021-00672-2.
- [14] S. Chen *et al.*, "Estimates and Projections of the Global Economic Cost of 29 Cancers in 204 Countries and Territories From 2020 to 2050," *JAMA Oncol*, vol. 9, no. 4, p. 465, Apr. 2023, doi: 10.1001/jamaoncol.2022.7826.

- [15] K. Hristova-Panusheva, C. Xenodochidis, M. Georgieva, and N. Krasteva, "Nanoparticle-Mediated Drug Delivery Systems for Precision Targeting in Oncology," *Pharmaceuticals*, vol. 17, no. 6, p. 677, May 2024, doi: 10.3390/ph17060677.
- [16] L. F. Méndez-López, "Revisiting Epithelial Carcinogenesis," *Int J Mol Sci*, vol. 23, no. 13, p. 7437, Jul. 2022, doi: 10.3390/ijms23137437.
- [17] M. A. Duncan and M. A. Lautner, "Sarcomas of the Breast," *Surgical Clinics of North America*, vol. 98, no. 4, pp. 869–876, Aug. 2018, doi: 10.1016/j.suc.2018.03.013.
- [18] S. Sharma, T. K. Kelly, and P. A. Jones, "Epigenetics in cancer," *Carcinogenesis*, vol. 31, no. 1, pp. 27–36, Jan. 2010, doi: 10.1093/carcin/bgp220.
- [19] C. M. Perou *et al.*, "Molecular portraits of human breast tumours," *Nature*, vol. 406, no. 6797, pp. 747–752, Aug. 2000, doi: 10.1038/35021093.
- [20] E. Orrantia-Borunda, P. Anchondo-Nuñez, L. E. Acuña-Aguilar, F. O. Gómez-Valles, and C. A. Ramírez-Valdespino, "Subtypes of Breast Cancer," in *Breast Cancer*, Exon Publications, 2022, pp. 31–42. doi: 10.36255/exon-publications-breast-cancer-subtypes.
- [21] R. Dienstmann, L. Vermeulen, J. Guinney, S. Kopetz, S. Tejpar, and J. Tabernero, "Consensus molecular subtypes and the evolution of precision medicine in colorectal cancer," *Nat Rev Cancer*, vol. 17, no. 2, pp. 79–92, Feb. 2017, doi: 10.1038/nrc.2016.126.
- [22] Y. Hasin, M. Seldin, and A. Lusis, "Multi-omics approaches to disease," *Genome Biol*, vol. 18, no. 1, p. 83, Dec. 2017, doi: 10.1186/s13059-017-1215-1.
- [23] R. D. Neal *et al.*, "Is increased time to diagnosis and treatment in symptomatic cancer associated with poorer outcomes? Systematic review," *Br J Cancer*, vol. 112, no. S1, pp. S92–S107, Mar. 2015, doi: 10.1038/bjc.2015.48.
- [24] A. N. Wilkinson, "Cancer diagnosis in primary care," *Canadian Family Physician*, vol. 67, no. 4, pp. 265–268, Apr. 2021, doi: 10.46747/cfp.6704265.
- [25] G. Şahin *et al.*, "Optimizing Final Pathology Determination in Endometrial Cancer: The Role of PET/CT, MRI, and Biopsy in Serous, Mixed Cell, Clear Cell, and Grade 3 Endometrioid Subtypes," *Diagnostics*, vol. 15, no. 6, p. 731, Mar. 2025, doi: 10.3390/diagnostics15060731.
- [26] J. Barentsz *et al.*, "Commonly Used Imaging Techniques for Diagnosis and Staging," *Journal of Clinical Oncology*, vol. 24, no. 20, pp. 3234–3244, Jul. 2006, doi: 10.1200/JCO.2006.06.5946.
- [27] H. R. Saeidnia, F. Firuzpour, M. Kozak, and H. S. majd, "Advancing cancer diagnosis and treatment: integrating image analysis and AI algorithms for enhanced clinical practice," *Artif Intell Rev*, vol. 58, no. 4, p. 105, Jan. 2025, doi: 10.1007/s10462-025-11117-w.
- [28] A. Pulumati, A. Pulumati, B. S. Dwarakanath, A. Verma, and R. V. L. Papineni, "Technological advancements in cancer diagnostics: Improvements and limitations," *Cancer Rep*, vol. 6, no. 2, Feb. 2023, doi: 10.1002/cnr2.1764.
- [29] L. Ma *et al.*, "Liquid biopsy in cancer: current status, challenges and future prospects," *Signal Transduct Target Ther*, vol. 9, no. 1, p. 336, Dec. 2024, doi: 10.1038/s41392-024-02021-w.
- [30] Y. Zhou *et al.*, "Tumor biomarkers for diagnosis, prognosis and targeted therapy," *Signal Transduct Target Ther*, vol. 9, no. 1, p. 132, May 2024, doi: 10.1038/s41392-024-01823-2.
- [31] V. T. DeVita and E. Chu, "A History of Cancer Chemotherapy," *Cancer Res*, vol. 68, no. 21, pp. 8643–8653, Nov. 2008, doi: 10.1158/0008-5472.CAN-07-6611.

- [32] D. T. Debela *et al.*, “New approaches and procedures for cancer treatment: Current perspectives,” *SAGE Open Med*, vol. 9, Jan. 2021, doi: 10.1177/20503121211034366.
- [33] P. Sharma *et al.*, “Immune checkpoint therapy—current perspectives and future directions,” *Cell*, vol. 186, no. 8, pp. 1652–1669, Apr. 2023, doi: 10.1016/j.cell.2023.03.006.
- [34] A. Haslam and V. Prasad, “Estimation of the Percentage of US Patients With Cancer Who Are Eligible for and Respond to Checkpoint Inhibitor Immunotherapy Drugs,” *JAMA Netw Open*, vol. 2, no. 5, p. e192535, May 2019, doi: 10.1001/jamanetworkopen.2019.2535.
- [35] Y. Li *et al.*, “Recent advances in therapeutic strategies for triple-negative breast cancer,” *J Hematol Oncol*, vol. 15, no. 1, p. 121, Aug. 2022, doi: 10.1186/s13045-022-01341-0.
- [36] R. Oun, Y. E. Moussa, and N. J. Wheate, “The side effects of platinum-based chemotherapy drugs: a review for chemists,” *Dalton Transactions*, vol. 47, no. 19, pp. 6645–6653, 2018, doi: 10.1039/C8DT00838H.
- [37] J. Boshuizen and D. S. Peeper, “Rational Cancer Treatment Combinations: An Urgent Clinical Need,” *Mol Cell*, vol. 78, no. 6, pp. 1002–1018, Jun. 2020, doi: 10.1016/j.molcel.2020.05.031.
- [38] J. Wang, N. Seebacher, H. Shi, Q. Kan, and Z. Duan, “Novel strategies to prevent the development of multidrug resistance (MDR) in cancer,” *Oncotarget*, vol. 8, no. 48, pp. 84559–84571, Oct. 2017, doi: 10.18632/oncotarget.19187.
- [39] B. Al-Lazikani, U. Banerji, and P. Workman, “Combinatorial drug therapy for cancer in the post-genomic era,” *Nat Biotechnol*, vol. 30, no. 7, pp. 679–692, Jul. 2012, doi: 10.1038/nbt.2284.
- [40] A. Doostmohammadi, H. Jooya, K. Ghorbanian, S. Gohari, and M. Dadashpour, “Potentials and future perspectives of multi-target drugs in cancer treatment: the next generation anti-cancer agents,” *Cell Communication and Signaling*, vol. 22, no. 1, p. 228, Apr. 2024, doi: 10.1186/s12964-024-01607-9.
- [41] E. Seto and M. Yoshida, “Erasers of Histone Acetylation: The Histone Deacetylase Enzymes,” *Cold Spring Harb Perspect Biol*, vol. 6, no. 4, pp. a018713–a018713, Apr. 2014, doi: 10.1101/cshperspect.a018713.
- [42] G. Milazzo *et al.*, “Histone Deacetylases (HDACs): Evolution, Specificity, Role in Transcriptional Complexes, and Pharmacological Actionability,” *Genes (Basel)*, vol. 11, no. 5, p. 556, May 2020, doi: 10.3390/genes11050556.
- [43] S. Bahl and E. Seto, “Regulation of histone deacetylase activities and functions by phosphorylation and its physiological relevance,” *Cellular and Molecular Life Sciences*, vol. 78, no. 2, pp. 427–445, Jan. 2021, doi: 10.1007/s00018-020-03599-4.
- [44] S.-Y. Park and J.-S. Kim, “A short guide to histone deacetylases including recent progress on class II enzymes,” *Exp Mol Med*, vol. 52, no. 2, pp. 204–212, Feb. 2020, doi: 10.1038/s12276-020-0382-4.
- [45] A. G. Kazantsev and L. M. Thompson, “Therapeutic application of histone deacetylase inhibitors for central nervous system disorders,” *Nat Rev Drug Discov*, vol. 7, no. 10, pp. 854–868, Oct. 2008, doi: 10.1038/nrd2681.
- [46] S. Minucci and P. G. Pelicci, “Histone deacetylase inhibitors and the promise of epigenetic (and more) treatments for cancer,” *Nat Rev Cancer*, vol. 6, no. 1, pp. 38–51, Jan. 2006, doi: 10.1038/nrc1779.
- [47] A. J. M. de RUIJTER, A. H. van GENNIP, H. N. CARON, S. KEMP, and A. B. P. van KUILENBURG, “Histone deacetylases (HDACs): characterization of the

- classical HDAC family,” *Biochemical Journal*, vol. 370, no. 3, pp. 737–749, Mar. 2003, doi: 10.1042/bj20021321.
- [48] C. Moreno-Yruela *et al.*, “Class I histone deacetylases (HDAC1–3) are histone lysine deacetylases,” *Sci Adv*, vol. 8, no. 3, Jan. 2022, doi: 10.1126/sciadv.abi6696.
 - [49] X.-J. Yang and S. Grégoire, “Class II Histone Deacetylases: from Sequence to Function, Regulation, and Clinical Implication,” *Mol Cell Biol*, vol. 25, no. 8, pp. 2873–2884, Apr. 2005, doi: 10.1128/MCB.25.8.2873-2884.2005.
 - [50] E. Verdin, F. Dequiedt, and H. G. Kasler, “Class II histone deacetylases: versatile regulators,” *Trends in Genetics*, vol. 19, no. 5, pp. 286–293, May 2003, doi: 10.1016/S0168-9525(03)00073-8.
 - [51] Y. Wen *et al.*, “Histone deacetylase (HDAC) 11 inhibits matrix metalloproteinase (MMP) 3 expression to suppress colorectal cancer metastasis,” *J Cancer*, vol. 13, no. 6, pp. 1923–1932, 2022, doi: 10.7150/jca.66914.
 - [52] Y. Li and E. Seto, “HDACs and HDAC Inhibitors in Cancer Development and Therapy,” *Cold Spring Harb Perspect Med*, vol. 6, no. 10, p. a026831, Oct. 2016, doi: 10.1101/cshperspect.a026831.
 - [53] G. Li, Y. Tian, and W.-G. Zhu, “The Roles of Histone Deacetylases and Their Inhibitors in Cancer Therapy,” *Front Cell Dev Biol*, vol. 8, Sep. 2020, doi: 10.3389/fcell.2020.576946.
 - [54] K. J. Falkenberg and R. W. Johnstone, “Histone deacetylases and their inhibitors in cancer, neurological diseases and immune disorders,” *Nat Rev Drug Discov*, vol. 13, no. 9, pp. 673–691, Sep. 2014, doi: 10.1038/nrd4360.
 - [55] A. C. West and R. W. Johnstone, “New and emerging HDAC inhibitors for cancer treatment,” *Journal of Clinical Investigation*, vol. 124, no. 1, pp. 30–39, Jan. 2014, doi: 10.1172/JCI69738.
 - [56] Z. Li and W.-G. Zhu, “Targeting Histone Deacetylases for Cancer Therapy: From Molecular Mechanisms to Clinical Implications,” *Int J Biol Sci*, vol. 10, no. 7, pp. 757–770, 2014, doi: 10.7150/ijbs.9067.
 - [57] W. Yang *et al.*, “PKM2 Phosphorylates Histone H3 and Promotes Gene Transcription and Tumorigenesis,” *Cell*, vol. 150, no. 4, pp. 685–696, Aug. 2012, doi: 10.1016/j.cell.2012.07.018.
 - [58] N. R. Bertos, A. H. Wang, and X.-J. Yang, “Class II histone deacetylases: Structure, function, and regulation,” *Biochemistry and Cell Biology*, vol. 79, no. 3, pp. 243–252, 2001, doi: 10.1139/bcb-79-3-243.
 - [59] J. J. Kovacs *et al.*, “HDAC6 Regulates Hsp90 Acetylation and Chaperone-Dependent Activation of Glucocorticoid Receptor,” *Mol Cell*, vol. 18, no. 5, pp. 601–607, May 2005, doi: 10.1016/j.molcel.2005.04.021.
 - [60] C. Hubbert *et al.*, “HDAC6 is a microtubule-associated deacetylase,” *Nature*, vol. 417, no. 6887, pp. 455–458, May 2002, doi: 10.1038/417455a.
 - [61] A. Iwata, B. E. Riley, J. A. Johnston, and R. R. Kopito, “HDAC6 and Microtubules Are Required for Autophagic Degradation of Aggregated Huntingtin,” *Journal of Biological Chemistry*, vol. 280, no. 48, pp. 40282–40292, Dec. 2005, doi: 10.1074/jbc.M508786200.
 - [62] C. Boyault, K. Sadoul, M. Pabion, and S. Khochbin, “HDAC6, at the crossroads between cytoskeleton and cell signaling by acetylation and ubiquitination,” *Oncogene*, vol. 26, no. 37, pp. 5468–5476, Aug. 2007, doi: 10.1038/sj.onc.1210614.
 - [63] A. Valenzuela-Fernández, J. R. Cabrero, J. M. Serrador, and F. Sánchez-Madrid, “HDAC6: a key regulator of cytoskeleton, cell migration and cell-cell

- interactions,” *Trends Cell Biol*, vol. 18, no. 6, pp. 291–297, Jun. 2008, doi: 10.1016/j.tcb.2008.04.003.
- [64] P. Bali *et al.*, “Inhibition of Histone Deacetylase 6 Acetylates and Disrupts the Chaperone Function of Heat Shock Protein 90,” *Journal of Biological Chemistry*, vol. 280, no. 29, pp. 26729–26734, Jul. 2005, doi: 10.1074/jbc.C500186200.
- [65] G. I. Aldana-Masangkay and K. M. Sakamoto, “The Role of HDAC6 in Cancer,” *Biomed Res Int*, vol. 2011, no. 1, Jan. 2011, doi: 10.1155/2011/875824.
- [66] Y.-S. Lee *et al.*, “The Cytoplasmic Deacetylase HDAC6 Is Required for Efficient Oncogenic Tumorigenesis,” *Cancer Res*, vol. 68, no. 18, pp. 7561–7569, Sep. 2008, doi: 10.1158/0008-5472.CAN-08-0188.
- [67] S. Kaur, P. Rajoria, and M. Chopra, “HDAC6: A unique HDAC family member as a cancer target,” *Cellular Oncology*, vol. 45, no. 5, pp. 779–829, Oct. 2022, doi: 10.1007/s13402-022-00704-6.
- [68] M. S. Finnin *et al.*, “Structures of a histone deacetylase homologue bound to the TSA and SAHA inhibitors,” *Nature*, vol. 401, no. 6749, pp. 188–193, Sep. 1999, doi: 10.1038/43710.
- [69] T. Eckschlager, J. Plch, M. Stiborova, and J. Hrabeta, “Histone Deacetylase Inhibitors as Anticancer Drugs,” *Int J Mol Sci*, vol. 18, no. 7, p. 1414, Jul. 2017, doi: 10.3390/ijms18071414.
- [70] M. Duvic and J. Vu, “Vorinostat: a new oral histone deacetylase inhibitor approved for cutaneous T-cell lymphoma,” *Expert Opin Investig Drugs*, vol. 16, no. 7, pp. 1111–1120, Jul. 2007, doi: 10.1517/13543784.16.7.1111.
- [71] C. M. Grozinger, C. A. Hassig, and S. L. Schreiber, “Three proteins define a class of human histone deacetylases related to yeast Hda1p,” *Proceedings of the National Academy of Sciences*, vol. 96, no. 9, pp. 4868–4873, Apr. 1999, doi: 10.1073/pnas.96.9.4868.
- [72] Y. Li, D. Shin, and S. H. Kwon, “Histone deacetylase 6 plays a role as a distinct regulator of diverse cellular processes,” *FEBS J*, vol. 280, no. 3, pp. 775–793, Feb. 2013, doi: 10.1111/febs.12079.
- [73] C. Simões-Pires, V. Zwick, A. Nurisso, E. Schenker, P.-A. Carrupt, and M. Cuendet, “HDAC6 as a target for neurodegenerative diseases: what makes it different from the other HDACs?,” *Mol Neurodegener*, vol. 8, no. 1, p. 7, Dec. 2013, doi: 10.1186/1750-1326-8-7.
- [74] T. Li *et al.*, “Histone deacetylase 6 in cancer,” *J Hematol Oncol*, vol. 11, no. 1, p. 111, Dec. 2018, doi: 10.1186/s13045-018-0654-9.
- [75] M. Brindisi, A. P. Saraswati, S. Brogi, S. Gemma, S. Butini, and G. Campiani, “Old but Gold: Tracking the New Guise of Histone Deacetylase 6 (HDAC6) Enzyme as a Biomarker and Therapeutic Target in Rare Diseases,” *J Med Chem*, vol. 63, no. 1, pp. 23–39, Jan. 2020, doi: 10.1021/acs.jmedchem.9b00924.
- [76] S. Pulya, Sk. A. Amin, N. Adhikari, S. Biswas, T. Jha, and B. Ghosh, “HDAC6 as privileged target in drug discovery: A perspective,” *Pharmacol Res*, vol. 163, p. 105274, Jan. 2021, doi: 10.1016/j.phrs.2020.105274.
- [77] P. Poonia, M. Sharma, P. Jha, and M. Chopra, “Pharmacophore-based virtual screening of ZINC database, molecular modeling and designing new derivatives as potential HDAC6 inhibitors,” *Mol Divers*, vol. 27, no. 5, pp. 2053–2071, Oct. 2023, doi: 10.1007/s11030-022-10540-3.
- [78] Y. Sixto-López, J. A. Gómez-Vidal, N. de Pedro, M. Bello, M. C. Rosales-Hernández, and J. Correa-Basurto, “In silico design of HDAC6 inhibitors with neuroprotective effects,” *J Biomol Struct Dyn*, vol. 40, no. 24, pp. 14204–14222, Dec. 2022, doi: 10.1080/07391102.2021.2001378.

- [79] A. Shamsi, M. Shahwan, A. Zuberi, and N. Altwajry, "Identification of Potential Inhibitors of Histone Deacetylase 6 Through Virtual Screening and Molecular Dynamics Simulation Approach: Implications in Neurodegenerative Diseases," *Pharmaceuticals*, vol. 17, no. 11, p. 1536, Nov. 2024, doi: 10.3390/ph17111536.
- [80] S. Lindquist and E. A. Craig, "THE HEAT-SHOCK PROTEINS," *Annu Rev Genet*, vol. 22, no. 1, pp. 631–677, Dec. 1988, doi: 10.1146/annurev.ge.22.120188.003215.
- [81] C. Hu *et al.*, "Heat shock proteins: Biological functions, pathological roles, and therapeutic opportunities," *MedComm (Beijing)*, vol. 3, no. 3, Sep. 2022, doi: 10.1002/mco2.161.
- [82] L. Whitesell and S. L. Lindquist, "HSP90 and the chaperoning of cancer," *Nat Rev Cancer*, vol. 5, no. 10, pp. 761–772, Oct. 2005, doi: 10.1038/nrc1716.
- [83] F. U. Hartl, A. Bracher, and M. Hayer-Hartl, "Molecular chaperones in protein folding and proteostasis," *Nature*, vol. 475, no. 7356, pp. 324–332, Jul. 2011, doi: 10.1038/nature10317.
- [84] B. Bukau, J. Weissman, and A. Horwich, "Molecular Chaperones and Protein Quality Control," *Cell*, vol. 125, no. 3, pp. 443–451, May 2006, doi: 10.1016/j.cell.2006.04.014.
- [85] H. Jee, "Size dependent classification of heat shock proteins: a mini-review," *J Exerc Rehabil*, vol. 12, no. 4, pp. 255–259, Aug. 2016, doi: 10.12965/jer.1632642.321.
- [86] K. Richter, M. Haslbeck, and J. Buchner, "The Heat Shock Response: Life on the Verge of Death," *Mol Cell*, vol. 40, no. 2, pp. 253–266, Oct. 2010, doi: 10.1016/j.molcel.2010.10.006.
- [87] D. P. Easton, Y. Kaneko, and J. R. Subjeck, "The Hsp110 and Grp170 stress proteins: newly recognized relatives of the Hsp70s," *Cell Stress Chaperones*, vol. 5, no. 4, p. 276, 2000, doi: 10.1379/1466-1268(2000)005<0276:THAGSP>2.0.CO;2.
- [88] J. G. Facciponte, X. Wang, and J. R. Subjeck, "Hsp110 and Grp170, members of the Hsp70 superfamily, bind to scavenger receptor-A and scavenger receptor expressed by endothelial cells-I," *Eur J Immunol*, vol. 37, no. 8, pp. 2268–2279, Aug. 2007, doi: 10.1002/eji.200737127.
- [89] S. K. Calderwood, M. A. Khaleque, D. B. Sawyer, and D. R. Ciocca, "Heat shock proteins in cancer: chaperones of tumorigenesis," *Trends Biochem Sci*, vol. 31, no. 3, pp. 164–172, Mar. 2006, doi: 10.1016/j.tibs.2006.01.006.
- [90] H. M. Beere, "The stress of dying: the role of heat shock proteins in the regulation of apoptosis," *J Cell Sci*, vol. 117, no. 13, pp. 2641–2651, Jun. 2004, doi: 10.1242/jcs.01284.
- [91] G. Jago, A. Hazoumé, R. Seigneuric, and C. Garrido, "Targeting heat shock proteins in cancer," *Cancer Lett*, vol. 332, no. 2, pp. 275–285, May 2013, doi: 10.1016/j.canlet.2010.10.014.
- [92] D. R. Ciocca and S. K. Calderwood, "Heat shock proteins in cancer: diagnostic, prognostic, predictive, and treatment implications," *Cell Stress Chaperones*, vol. 10, no. 2, p. 86, 2005, doi: 10.1379/CSC-99r.1.
- [93] J. Trepel, M. Mollapour, G. Giaccone, and L. Neckers, "Targeting the dynamic HSP90 complex in cancer," *Nat Rev Cancer*, vol. 10, no. 8, pp. 537–549, Aug. 2010, doi: 10.1038/nrc2887.
- [94] L. H. Pearl and C. Prodromou, "Structure and Mechanism of the Hsp90 Molecular Chaperone Machinery," *Annu Rev Biochem*, vol. 75, no. 1, pp. 271–294, Jun. 2006, doi: 10.1146/annurev.biochem.75.103004.142738.

- [95] M. M. Biebl and J. Buchner, "Structure, Function, and Regulation of the Hsp90 Machinery," *Cold Spring Harb Perspect Biol*, vol. 11, no. 9, p. a034017, Sep. 2019, doi: 10.1101/cshperspect.a034017.
- [96] F. H. Schopf, M. M. Biebl, and J. Buchner, "The HSP90 chaperone machinery," *Nat Rev Mol Cell Biol*, vol. 18, no. 6, pp. 345–360, Jun. 2017, doi: 10.1038/nrm.2017.20.
- [97] A. Donnelly and B. Blagg, "Novobiocin and Additional Inhibitors of the Hsp90 C-Terminal Nucleotide-binding Pocket," *Curr Med Chem*, vol. 15, no. 26, pp. 2702–2717, Nov. 2008, doi: 10.2174/092986708786242895.
- [98] J. Li, K. Richter, and J. Buchner, "Mixed Hsp90-cochaperone complexes are important for the progression of the reaction cycle," *Nat Struct Mol Biol*, vol. 18, no. 1, pp. 61–66, Jan. 2011, doi: 10.1038/nsmb.1965.
- [99] G. Chiosis, C. S. Digwal, J. B. Trepel, and L. Neckers, "Structural and functional complexity of HSP90 in cellular homeostasis and disease," *Nat Rev Mol Cell Biol*, vol. 24, no. 11, pp. 797–815, Nov. 2023, doi: 10.1038/s41580-023-00640-9.
- [100] Z.-N. Li and Y. Luo, "HSP90 inhibitors and cancer: Prospects for use in targeted therapies (Review)," *Oncol Rep*, vol. 49, no. 1, p. 6, Nov. 2022, doi: 10.3892/or.2022.8443.
- [101] S. Rastogi *et al.*, "An update on the status of HSP90 inhibitors in cancer clinical trials," *Cell Stress Chaperones*, vol. 29, no. 4, pp. 519–539, Aug. 2024, doi: 10.1016/j.cstres.2024.05.005.
- [102] K. Jhaveri, T. Taldone, S. Modi, and G. Chiosis, "Advances in the clinical development of heat shock protein 90 (Hsp90) inhibitors in cancers," *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, vol. 1823, no. 3, pp. 742–755, Mar. 2012, doi: 10.1016/j.bbamer.2011.10.008.
- [103] G. Augello *et al.*, "Targeting HSP90 with the small molecule inhibitor AUY922 (luminespib) as a treatment strategy against hepatocellular carcinoma," *Int J Cancer*, vol. 144, no. 10, pp. 2613–2624, May 2019, doi: 10.1002/ijc.31963.
- [104] G. S. Hobbs *et al.*, "Hsp90 inhibition disrupts JAK-STAT signaling and leads to reductions in splenomegaly in patients with myeloproliferative neoplasms," *Haematologica*, vol. 103, no. 1, pp. e5–e9, Jan. 2018, doi: 10.3324/haematol.2017.177600.
- [105] W. Ying *et al.*, "Ganetespib, a Unique Triazolone-Containing Hsp90 Inhibitor, Exhibits Potent Antitumor Activity and a Superior Safety Profile for Cancer Therapy," *Mol Cancer Ther*, vol. 11, no. 2, pp. 475–484, Feb. 2012, doi: 10.1158/1535-7163.MCT-11-0755.
- [106] B. Lamottke *et al.*, "The novel, orally bioavailable HSP90 inhibitor NVP-HSP990 induces cell cycle arrest and apoptosis in multiple myeloma cells and acts synergistically with melphalan by increased cleavage of caspases," *Eur J Haematol*, vol. 88, no. 5, pp. 406–415, May 2012, doi: 10.1111/j.1600-0609.2012.01764.x.
- [107] D. L. Menezes *et al.*, "The Novel Oral Hsp90 Inhibitor NVP-HSP990 Exhibits Potent and Broad-spectrum Antitumor Activities *In Vitro* and *In Vivo*," *Mol Cancer Ther*, vol. 11, no. 3, pp. 730–739, Mar. 2012, doi: 10.1158/1535-7163.MCT-11-0667.
- [108] M. Ouassaf, L. Bourougaa, S. H. Al-Mijalli, E. M. Abdallah, A. R. Bhat, and S. M. A. Kawsar, "Marine-Derived Compounds as Potential Inhibitors of Hsp90 for Anticancer and Antimicrobial Drug Development: A Comprehensive *In Silico* Study," *Molecules*, vol. 28, no. 24, p. 8074, Dec. 2023, doi: 10.3390/molecules28248074.

- [109] B. Chandrasekaran *et al.*, “Synthesis, Anticancer Screening, and In Silico Evaluations of Thieno[2,3-c]pyridine Derivatives as Hsp90 Inhibitors,” *Pharmaceuticals*, vol. 18, no. 2, p. 153, Jan. 2025, doi: 10.3390/ph18020153.
- [110] M. A. Lemmon and J. Schlessinger, “Cell Signaling by Receptor Tyrosine Kinases,” *Cell*, vol. 141, no. 7, pp. 1117–1134, Jun. 2010, doi: 10.1016/j.cell.2010.06.011.
- [111] T. R. Webb *et al.*, “Anaplastic lymphoma kinase: role in cancer pathogenesis and small-molecule inhibitor development for therapy,” *Expert Rev Anticancer Ther*, vol. 9, no. 3, pp. 331–356, Mar. 2009, doi: 10.1586/14737140.9.3.331.
- [112] R. Roskoski, “Anaplastic lymphoma kinase (ALK): Structure, oncogenic activation, and pharmacological inhibition,” *Pharmacol Res*, vol. 68, no. 1, pp. 68–94, Feb. 2013, doi: 10.1016/j.phrs.2012.11.007.
- [113] C. M. Della Corte *et al.*, “Role and targeting of anaplastic lymphoma kinase in cancer,” *Mol Cancer*, vol. 17, no. 1, p. 30, Dec. 2018, doi: 10.1186/s12943-018-0776-2.
- [114] R. T. Bossi *et al.*, “Crystal Structures of Anaplastic Lymphoma Kinase in Complex with ATP Competitive Inhibitors,” *Biochemistry*, vol. 49, no. 32, pp. 6813–6825, Aug. 2010, doi: 10.1021/bi1005514.
- [115] G. E. Stoica *et al.*, “Midkine Binds to Anaplastic Lymphoma Kinase (ALK) and Acts as a Growth Factor for Different Cell Types,” *Journal of Biological Chemistry*, vol. 277, no. 39, pp. 35990–35998, Sep. 2002, doi: 10.1074/jbc.M205749200.
- [116] C. C. Lee *et al.*, “Crystal structure of the ALK (anaplastic lymphoma kinase) catalytic domain,” *Biochemical Journal*, vol. 430, no. 3, pp. 425–437, Sep. 2010, doi: 10.1042/BJ20100609.
- [117] M. Soda *et al.*, “Identification of the transforming EML4–ALK fusion gene in non-small-cell lung cancer,” *Nature*, vol. 448, no. 7153, pp. 561–566, Aug. 2007, doi: 10.1038/nature05945.
- [118] B. Hallberg and R. H. Palmer, “Mechanistic insight into ALK receptor tyrosine kinase in human cancer biology,” *Nat Rev Cancer*, vol. 13, no. 10, pp. 685–700, Oct. 2013, doi: 10.1038/nrc3580.
- [119] E. L. Kwak *et al.*, “Anaplastic Lymphoma Kinase Inhibition in Non-Small-Cell Lung Cancer,” *New England Journal of Medicine*, vol. 363, no. 18, pp. 1693–1703, Oct. 2010, doi: 10.1056/NEJMoa1006448.
- [120] R. Katayama *et al.*, “Mechanisms of Acquired Crizotinib Resistance in ALK-Rearranged Lung Cancers,” *Sci Transl Med*, vol. 4, no. 120, Feb. 2012, doi: 10.1126/scitranslmed.3003316.
- [121] A. T. Shaw *et al.*, “Crizotinib versus Chemotherapy in Advanced *ALK* -Positive Lung Cancer,” *New England Journal of Medicine*, vol. 368, no. 25, pp. 2385–2394, Jun. 2013, doi: 10.1056/NEJMoa1214886.
- [122] D. R. Camidge *et al.*, “Brigatinib versus Crizotinib in *ALK* -Positive Non-Small-Cell Lung Cancer,” *New England Journal of Medicine*, vol. 379, no. 21, pp. 2027–2039, Nov. 2018, doi: 10.1056/NEJMoa1810171.
- [123] B. J. Solomon *et al.*, “Lorlatinib in patients with *ALK*-positive non-small-cell lung cancer: results from a global phase 2 study,” *Lancet Oncol*, vol. 19, no. 12, pp. 1654–1667, Dec. 2018, doi: 10.1016/S1470-2045(18)30649-1.
- [124] Y. O. Bouone, A. Bouzina, and N.-E. Aouf, “In Silico Investigation of a New 4-Hydroxyquinolone Analogue as an Anaplastic Lymphoma Kinase (ALK) Inhibitor: Molecular Docking and ADMET Prediction,” in *ECSOC 2023*, Basel Switzerland: MDPI, Nov. 2023, p. 83. doi: 10.3390/ecsoc-27-16139.

- [125] J. E. Faya Castillo, R. J. Zapata Dongo, P. A. Wong Chero, and S. F. Infante Varillas, "Mitoxantrone and abacavir: An ALK protein-targeted in silico proposal for the treatment of non-small cell lung cancer," *PLoS One*, vol. 19, no. 2, p. e0295966, Feb. 2024, doi: 10.1371/journal.pone.0295966.
- [126] Y.-W. Wu *et al.*, "A novel dual HDAC and HSP90 inhibitor, MPT0G449, downregulates oncogenic pathways in human acute leukemia in vitro and in vivo," *Oncogenesis*, vol. 10, no. 5, p. 39, May 2021, doi: 10.1038/s41389-021-00331-0.
- [127] R. R. Ramsay, M. R. Popovic-Nikolic, K. Nikolic, E. Uliassi, and M. L. Bolognesi, "A perspective on multi-target drug discovery and design for complex diseases," *Clin Transl Med*, vol. 7, no. 1, Dec. 2018, doi: 10.1186/s40169-017-0181-2.
- [128] B. Ravikumar and T. Aittokallio, "Improving the efficacy-safety balance of polypharmacology in multi-target drug discovery," *Expert Opin Drug Discov*, vol. 13, no. 2, pp. 179–192, Feb. 2018, doi: 10.1080/17460441.2018.1413089.
- [129] H. Y. Chae *et al.*, "Design, synthesis, and biological evaluation of bifunctional inhibitors against Hsp90-HDAC6 interplay," *Eur J Med Chem*, vol. 240, p. 114582, Oct. 2022, doi: 10.1016/j.ejmech.2022.114582.
- [130] O. H. Krämer, S. Mahboobi, and A. Sellmer, "Drugging the HDAC6–HSP90 interplay in malignant cells," *Trends Pharmacol Sci*, vol. 35, no. 10, pp. 501–509, Oct. 2014, doi: 10.1016/j.tips.2014.08.001.
- [131] L. Pinzi, R. Benedetti, L. Altucci, and G. Rastelli, "Design of Dual Inhibitors of Histone Deacetylase 6 and Heat Shock Protein 90," *ACS Omega*, vol. 5, no. 20, pp. 11473–11480, May 2020, doi: 10.1021/acsomega.0c00559.
- [132] T.-Y. Wu *et al.*, "Rational design of synthetically tractable HDAC6/HSP90 dual inhibitors to destroy immune-suppressive tumor microenvironment," *J Adv Res*, vol. 46, pp. 159–171, Apr. 2023, doi: 10.1016/j.jare.2022.06.009.
- [133] P. Liu *et al.*, "Posttranslational modification and beyond: interplay between histone deacetylase 6 and heat-shock protein 90," *Molecular Medicine*, vol. 27, no. 1, p. 110, Dec. 2021, doi: 10.1186/s10020-021-00375-3.
- [134] X. Lu, L. Xiao, L. Wang, and D. M. Ruden, "Hsp90 inhibitors and drug resistance in cancer: The potential benefits of combination therapies of Hsp90 inhibitors and other anti-cancer drugs," *Biochem Pharmacol*, vol. 83, no. 8, pp. 995–1004, Apr. 2012, doi: 10.1016/j.bcp.2011.11.011.
- [135] C. Li, J. Tu, G. Han, N. Liu, and C. Sheng, "Heat shock protein 90 (Hsp90)/Histone deacetylase (HDAC) dual inhibitors for the treatment of azoles-resistant *Candida albicans*," *Eur J Med Chem*, vol. 227, p. 113961, Jan. 2022, doi: 10.1016/j.ejmech.2021.113961.
- [136] S. H. Kim *et al.*, "The heat shock protein 90 inhibitor SNX5422 has a synergistic activity with histone deacetylase inhibitors in induction of death of anaplastic thyroid carcinoma cells," *Endocrine*, vol. 51, no. 2, pp. 274–282, Feb. 2016, doi: 10.1007/s12020-015-0706-7.
- [137] N. Abdelmoniem *et al.*, "Identification of Novel Natural Dual HDAC and Hsp90 Inhibitors for Metastatic TNBC Using e-Pharmacophore Modeling, Molecular Docking, and Molecular Dynamics Studies," *Molecules*, vol. 28, no. 4, p. 1771, Feb. 2023, doi: 10.3390/molecules28041771.
- [138] R. Garcia-Carbonero, A. Carnero, and L. Paz-Ares, "Inhibition of HSP90 molecular chaperones: moving into the clinic," *Lancet Oncol*, vol. 14, no. 9, pp. e358–e369, Aug. 2013, doi: 10.1016/S1470-2045(13)70169-4.
- [139] R. Katayama *et al.*, "Therapeutic strategies to overcome crizotinib resistance in non-small cell lung cancers harboring the fusion oncogene EML4-ALK,"

- Proceedings of the National Academy of Sciences*, vol. 108, no. 18, pp. 7535–7540, May 2011, doi: 10.1073/pnas.1019559108.
- [140] J. Sang *et al.*, “Targeted Inhibition of the Molecular Chaperone Hsp90 Overcomes ALK Inhibitor Resistance in Non–Small Cell Lung Cancer,” *Cancer Discov*, vol. 3, no. 4, pp. 430–443, Apr. 2013, doi: 10.1158/2159-8290.CD-12-0440.
 - [141] L. V. Sequist *et al.*, “Activity of IPI-504, a Novel Heat-Shock Protein 90 Inhibitor, in Patients With Molecularly Defined Non–Small-Cell Lung Cancer,” *Journal of Clinical Oncology*, vol. 28, no. 33, pp. 4953–4960, Nov. 2010, doi: 10.1200/JCO.2010.30.8338.
 - [142] E. Normant *et al.*, “The Hsp90 inhibitor IPI-504 rapidly lowers EML4–ALK levels and induces tumor regression in ALK-driven NSCLC models,” *Oncogene*, vol. 30, no. 22, pp. 2581–2586, Jun. 2011, doi: 10.1038/onc.2010.625.
 - [143] K. Geng *et al.*, “Design, synthesis and pharmacological evaluation of ALK and Hsp90 dual inhibitors bearing resorcinol and 2,4-diaminopyrimidine motifs,” *Eur J Med Chem*, vol. 152, pp. 76–86, May 2018, doi: 10.1016/j.ejmech.2018.04.019.
 - [144] P. Sharma, K. Sharma, and M. Nandave, “Computational approaches in drug discovery and design,” in *Computational Approaches in Drug Discovery, Development and Systems Pharmacology*, Elsevier, 2023, pp. 53–93. doi: 10.1016/B978-0-323-99137-7.00009-5.
 - [145] B. K. Shoichet, “Virtual screening of chemical libraries,” *Nature*, vol. 432, no. 7019, pp. 862–865, Dec. 2004, doi: 10.1038/nature03197.
 - [146] D. B. Kitchen, H. Decornez, J. R. Furr, and J. Bajorath, “Docking and scoring in virtual screening for drug discovery: methods and applications,” *Nat Rev Drug Discov*, vol. 3, no. 11, pp. 935–949, Nov. 2004, doi: 10.1038/nrd1549.
 - [147] A. Lavecchia and C. Giovanni, “Virtual Screening Strategies in Drug Discovery: A Critical Review,” *Curr Med Chem*, vol. 20, no. 23, pp. 2839–2860, Jun. 2013, doi: 10.2174/09298673113209990001.
 - [148] C. Gorgulla *et al.*, “An open-source drug discovery platform enables ultra-large virtual screens,” *Nature*, vol. 580, no. 7805, pp. 663–668, Apr. 2020, doi: 10.1038/s41586-020-2117-z.
 - [149] L. Ferreira, R. Dos Santos, G. Oliva, and A. Andricopulo, “Molecular Docking and Structure-Based Drug Design Strategies,” *Molecules*, vol. 20, no. 7, pp. 13384–13421, Jul. 2015, doi: 10.3390/molecules200713384.
 - [150] G. Madhavi Sastry, M. Adzhigirey, T. Day, R. Annabhimoju, and W. Sherman, “Protein and ligand preparation: parameters, protocols, and influence on virtual screening enrichments,” *J Comput Aided Mol Des*, vol. 27, no. 3, pp. 221–234, Mar. 2013, doi: 10.1007/s10822-013-9644-8.
 - [151] E. M. Terefe and A. Ghosh, “Molecular Docking, Validation, Dynamics Simulations, and Pharmacokinetic Prediction of Phytochemicals Isolated From *Croton dichogamus* Against the HIV-1 Reverse Transcriptase,” *Bioinform Biol Insights*, vol. 16, Jan. 2022, doi: 10.1177/11779322221125605.
 - [152] X.-Y. Meng, H.-X. Zhang, M. Mezei, and M. Cui, “Molecular Docking: A Powerful Approach for Structure-Based Drug Discovery,” *Current Computer Aided-Drug Design*, vol. 7, no. 2, pp. 146–157, Jun. 2011, doi: 10.2174/157340911795677602.
 - [153] N. S. Pagadala, K. Syed, and J. Tuszynski, “Software for molecular docking: a review,” *Biophys Rev*, vol. 9, no. 2, pp. 91–102, Apr. 2017, doi: 10.1007/s12551-016-0247-1.
 - [154] C. A. Lipinski, F. Lombardo, B. W. Dominy, and P. J. Feeney, “Experimental and computational approaches to estimate solubility and permeability in drug

- discovery and development settings,” *Adv Drug Deliv Rev*, vol. 23, no. 1–3, pp. 3–25, Jan. 1997, doi: 10.1016/S0169-409X(96)00423-1.
- [155] J. Tibbitts, D. Canter, R. Graff, A. Smith, and L. A. Khawli, “Key factors influencing ADME properties of therapeutic proteins: A need for ADME characterization in drug discovery and development,” *MAbs*, vol. 8, no. 2, pp. 229–245, Feb. 2016, doi: 10.1080/19420862.2015.1115937.
- [156] H. Komura, R. Watanabe, and K. Mizuguchi, “The Trends and Future Prospective of In Silico Models from the Viewpoint of ADME Evaluation in Drug Discovery,” *Pharmaceutics*, vol. 15, no. 11, p. 2619, Nov. 2023, doi: 10.3390/pharmaceutics15112619.
- [157] J. A. McCammon, B. R. Gelin, and M. Karplus, “Dynamics of folded proteins,” *Nature*, vol. 267, no. 5612, pp. 585–590, Jun. 1977, doi: 10.1038/267585a0.
- [158] S. A. Hollingsworth and R. O. Dror, “Molecular Dynamics Simulation for All,” *Neuron*, vol. 99, no. 6, pp. 1129–1143, Sep. 2018, doi: 10.1016/j.neuron.2018.08.011.
- [159] M. De Vivo, M. Masetti, G. Bottegoni, and A. Cavalli, “Role of Molecular Dynamics and Related Methods in Drug Discovery,” *J Med Chem*, vol. 59, no. 9, pp. 4035–4061, May 2016, doi: 10.1021/acs.jmedchem.5b01684.
- [160] K. Lindorff-Larsen *et al.*, “Improved side-chain torsion potentials for the Amber ff99SB protein force field,” *Proteins: Structure, Function, and Bioinformatics*, vol. 78, no. 8, pp. 1950–1958, Jun. 2010, doi: 10.1002/prot.22711.
- [161] M. J. Abraham *et al.*, “GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers,” *SoftwareX*, vol. 1–2, pp. 19–25, Sep. 2015, doi: 10.1016/j.softx.2015.06.001.
- [162] O. M. H. Salo-Ahen *et al.*, “Molecular Dynamics Simulations in Drug Discovery and Pharmaceutical Development,” *Processes*, vol. 9, no. 1, p. 71, Dec. 2020, doi: 10.3390/pr9010071.
- [163] J. S. E. Loo, A. Y. Y. Yong, and Y. N. Yong, “The effect of multiple simulation parameters on MM/PBSA performance for binding affinity prediction of CB1 cannabinoid receptor agonists and antagonists,” *Chem Biol Drug Des*, vol. 96, no. 5, pp. 1244–1254, Nov. 2020, doi: 10.1111/cbdd.13733.
- [164] P. A. Kollman *et al.*, “Calculating Structures and Free Energies of Complex Molecules: Combining Molecular Mechanics and Continuum Models,” *Acc Chem Res*, vol. 33, no. 12, pp. 889–897, Dec. 2000, doi: 10.1021/ar000033j.
- [165] S. Genheden and U. Ryde, “The MM/PBSA and MM/GBSA methods to estimate ligand-binding affinities,” *Expert Opin Drug Discov*, vol. 10, no. 5, pp. 449–461, May 2015, doi: 10.1517/17460441.2015.1032936.
- [166] E. Wang *et al.*, “End-Point Binding Free Energy Calculation with MM/PBSA and MM/GBSA: Strategies and Applications in Drug Design,” *Chem Rev*, vol. 119, no. 16, pp. 9478–9508, Aug. 2019, doi: 10.1021/acs.chemrev.9b00055.
- [167] H. K. Garcha *et al.*, “High Efficacy and Drug Synergy of HDAC6-Selective Inhibitor NN-429 in Natural Killer (NK)/T-Cell Lymphoma,” *Pharmaceutics*, vol. 15, no. 11, p. 1321, Oct. 2022, doi: 10.3390/ph15111321.
- [168] M. Bruncko *et al.*, “N-Aryl-benzimidazolones as novel small molecule HSP90 inhibitors,” *Bioorg Med Chem Lett*, vol. 20, no. 24, pp. 7503–7506, Dec. 2010, doi: 10.1016/j.bmcl.2010.10.010.
- [169] C. M. McBride *et al.*, “Design, Structure–Activity Relationship, and in Vivo Characterization of the Development Candidate NVP-HSP990,” *J Med Chem*, vol. 57, no. 21, pp. 9124–9129, Nov. 2014, doi: 10.1021/jm501107q.

- [170] L. Mologni *et al.*, “Discovery of Novel α -Carboline Inhibitors of the Anaplastic Lymphoma Kinase,” *ACS Omega*, vol. 7, no. 20, pp. 17083–17097, May 2022, doi: 10.1021/acsomega.2c00507.
- [171] E. F. Pettersen *et al.*, “UCSF Chimera—A visualization system for exploratory research and analysis,” *J Comput Chem*, vol. 25, no. 13, pp. 1605–1612, Oct. 2004, doi: 10.1002/jcc.20084.
- [172] T. Sterling and J. J. Irwin, “ZINC 15 – Ligand Discovery for Everyone,” *J Chem Inf Model*, vol. 55, no. 11, pp. 2324–2337, Nov. 2015, doi: 10.1021/acs.jcim.5b00559.
- [173] M. Sorokina, P. Merseburger, K. Rajan, M. A. Yirik, and C. Steinbeck, “COCONUT online: Collection of Open Natural Products database,” *J Cheminform*, vol. 13, no. 1, p. 2, Dec. 2021, doi: 10.1186/s13321-020-00478-9.
- [174] N. M. O’Boyle, M. Banck, C. A. James, C. Morley, T. Vandermeersch, and G. R. Hutchison, “Open Babel: An open chemical toolbox,” *J Cheminform*, vol. 3, no. 1, p. 33, Dec. 2011, doi: 10.1186/1758-2946-3-33.
- [175] K. Vanommeslaeghe *et al.*, “CHARMM general force field: A force field for drug-like molecules compatible with the CHARMM all-atom additive biological force fields,” *J Comput Chem*, vol. 31, no. 4, pp. 671–690, Mar. 2010, doi: 10.1002/jcc.21367.
- [176] D. R. Koes, M. P. Baumgartner, and C. J. Camacho, “Lessons Learned in Empirical Scoring with smina from the CSAR 2011 Benchmarking Exercise,” *J Chem Inf Model*, vol. 53, no. 8, pp. 1893–1904, Aug. 2013, doi: 10.1021/ci300604z.
- [177] O. Trott and A. J. Olson, “AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading,” *J Comput Chem*, vol. 31, no. 2, pp. 455–461, Jan. 2010, doi: 10.1002/jcc.21334.
- [178] M. Şansaçar, C. Sarı, M. S. Yücel, E. B. Gencer Akçok, and İ. Akçok, “Discovery of New Candidates Targeting the SH2 Domains of Spleen Tyrosine Kinase (Syk) Through In Silico Studies,” *ChemistrySelect*, vol. 10, no. 22, Jun. 2025, doi: 10.1002/slct.202500547.
- [179] P. Mark and L. Nilsson, “Structure and Dynamics of the TIP3P, SPC, and SPC/E Water Models at 298 K,” *J Phys Chem A*, vol. 105, no. 43, pp. 9954–9960, Nov. 2001, doi: 10.1021/jp003020w.
- [180] J. Lemkul, “From Proteins to Perturbed Hamiltonians: A Suite of Tutorials for the GROMACS-2018 Molecular Simulation Package [Article v1.0],” *Living J Comput Mol Sci*, vol. 1, no. 1, 2019, doi: 10.33011/livecoms.1.1.5068.
- [181] N. Homeyer and H. Gohlke, “Free Energy Calculations by the Molecular Mechanics Poisson–Boltzmann Surface Area Method,” *Mol Inform*, vol. 31, no. 2, pp. 114–122, Feb. 2012, doi: 10.1002/minf.201100135.
- [182] R. Kumari, R. Kumar, and A. Lynn, “g_mmpbsa —A GROMACS Tool for High-Throughput MM-PBSA Calculations,” *J Chem Inf Model*, vol. 54, no. 7, pp. 1951–1962, Jul. 2014, doi: 10.1021/ci500020m.
- [183] A. Daina, O. Michielin, and V. Zoete, “SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules,” *Sci Rep*, vol. 7, no. 1, p. 42717, Mar. 2017, doi: 10.1038/srep42717.
- [184] J. He *et al.*, “Synthesis and Biological Evaluation of HDAC Inhibitors With a Novel Zinc Binding Group,” *Front Chem*, vol. 8, Apr. 2020, doi: 10.3389/fchem.2020.00256.
- [185] P. M. Lombardi, K. E. Cole, D. P. Dowling, and D. W. Christianson, “Structure, mechanism, and inhibition of histone deacetylases and related metalloenzymes,”

- Curr Opin Struct Biol*, vol. 21, no. 6, pp. 735–743, Dec. 2011, doi: 10.1016/j.sbi.2011.08.004.
- [186] H. P. Nguyen *et al.*, “Anti-multiple myeloma potential of resynthesized belinostat derivatives: an experimental study on cytotoxic activity, drug combination, and docking studies,” *RSC Adv*, vol. 12, no. 34, pp. 22108–22118, 2022, doi: 10.1039/D2RA01969H.
- [187] Y. Miyake *et al.*, “Structural insights into HDAC6 tubulin deacetylation and its selective inhibition,” *Nat Chem Biol*, vol. 12, no. 9, pp. 748–754, Sep. 2016, doi: 10.1038/nchembio.2140.
- [188] S. Rezvani, A. Ebadi, and N. Razzaghi-Asl, “In silico identification of potential Hsp90 inhibitors via ensemble docking, DFT and molecular dynamics simulations,” *J Biomol Struct Dyn*, vol. 40, no. 21, pp. 10665–10676, Dec. 2022, doi: 10.1080/07391102.2021.1947383.
- [189] A. M. Magwenyane, S. C. Ugbaja, D. G. Amoako, A. M. Somboro, R. B. Khan, and H. M. Kumalo, “Heat Shock Protein 90 (HSP90) Inhibitors as Anticancer Medicines: A Review on the Computer-Aided Drug Discovery Approaches over the Past Five Years,” *Comput Math Methods Med*, vol. 2022, pp. 1–20, May 2022, doi: 10.1155/2022/2147763.
- [190] E. B. Gencer Akçok, H. Güner, and İ. Akçok, “Determination of promising inhibitors for N-SH2 domain of SHP2 tyrosine phosphatase: an in silico study,” *Mol Divers*, vol. 28, no. 5, pp. 3393–3407, Oct. 2024, doi: 10.1007/s11030-024-10880-2.
- [191] N. A. Durán-Iturbide, B. I. Díaz-Eufracio, and J. L. Medina-Franco, “*In Silico* ADME/Tox Profiling of Natural Products: A Focus on BIOFACQUIM,” *ACS Omega*, vol. 5, no. 26, pp. 16076–16084, Jul. 2020, doi: 10.1021/acsomega.0c01581.
- [192] L. Guan *et al.*, “ADMET-score – a comprehensive scoring function for evaluation of chemical drug-likeness,” *Medchemcomm*, vol. 10, no. 1, pp. 148–157, 2019, doi: 10.1039/C8MD00472B.
- [193] R. Roskoski, “Properties of FDA-approved small molecule protein kinase inhibitors,” *Pharmacol Res*, vol. 144, pp. 19–50, Jun. 2019, doi: 10.1016/j.phrs.2019.03.006.
- [194] C. A. Lipinski, “Lead- and drug-like compounds: the rule-of-five revolution,” *Drug Discov Today Technol*, vol. 1, no. 4, pp. 337–341, Dec. 2004, doi: 10.1016/j.ddtec.2004.11.007.
- [195] J. Tu, L. T. Song, R. R. Liu, H. L. Zhai, J. Wang, and X. Y. Zhang, “Molecular inhibitory mechanism study on the potent inhibitor brigatinib against four crizotinib-resistant ALK mutations,” *J Cell Biochem*, vol. 120, no. 1, pp. 562–574, Jan. 2019, doi: 10.1002/jcb.27412.
- [196] S. Y. Cheon and S. Kwon, “Molecular Anatomy of the EML4-ALK Fusion Protein for the Development of Novel Anticancer Drugs,” *Int J Mol Sci*, vol. 24, no. 6, p. 5821, Mar. 2023, doi: 10.3390/ijms24065821.

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PUBLICATIONS AND PRESENTATIONS

J1) M. Şansaçar, C. Sarı, M.S. Yücel, E.B.G. Akçok, & İ. Akçok, Discovery of new candidates targeting the SH2 domains of spleen tyrosine kinase (SYK) through in silico studies published in ChemistrySelect (June 2025)

J2) M.S. Yücel, İ. Akçok, Identification of Potential Dual HDAC6 and HSP90 Inhibitors for the Treatment of Cancer using Molecular Docking, Molecular Dynamics and MM/PBSA Studies: A Comprehensive In Silico Study published in Medicinal Chemistry (July. 2025).

C1) M.S. Yücel. (2023, 8-9 November). In-silico Design of Dual Inhibitors of Histone Deacetylase 6 and Heat Shock Protein 90 for Triple- Negative Breast Cancer Using Virtual Screening, Molecular Docking, and Molecular Dynamics Studies Poster Presentation in Anatolian Universities Association 2nd R&D project market, Tokat, Turkey (November 2023).

C2) M. Şansaçar, C. Sarı, M.S. Yücel, E.B.G. Akçok, İ. Akçok "In silico evaluation of potential natural product inhibitors from COCONUT database targeting SH2 domains of Spleen Tyrosine Kinase (Syk)" 13th Drug Chemistry Congress,6-9 February 2025, Antalya, Turkey.