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Design, Molecular Docking and Molecular Dynamics Simulation Studies of Novel Pyridinecarboxamide Derivatives as Potent HDAC6 inhibitors

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HDAC6,
Molecular Docking,
MD Simulations.

Abstract: Histone deacetylases (HDACs) are a family of enzymes which play vital roles in the regulation of gene expression and cellular processes. HDACs are classified into four main classes based on their homology, cellular localization, and structural characteristics. HDAC6 enzyme which is one of the class IIb enzyme has important functions in variety of physiological processes such as cell migration, immune responses, and neuronal function. Dysregulation of HDAC6 activity has been linked to the accumulation of toxic protein aggregates in neurodegenerative diseases, while its overexpression or altered activity in cancer cells can contribute to metastasis and tumorigenesis. In this study, potential HDAC6 inhibitors were designed and their inhibitory activities were investigated using in silico protocols, including molecular docking, molecular dynamics simulations and MM-PBSA calculations. Among the designed molecules, IA64 showed the best binding profile against HDAC6 enzyme, and could be considered as a lead molecule for further studies.

Potansiyel HDAC6 İnhibitörleri Olarak Yeni Piridinkarboksamid Türevlerinin Tasarım, Moleküler Kenetlenme ve Moleküler Dinamik Simülasyon Çalışmaları

Anahtar Kelimeler
İlaç tasarımı
Kanser,
HDAC6,
Moleküler Kenetlenme,
MD simulasyonu

Öz: Histon deasetilazlar (HDACs), gen ifadesinin ve hücresel süreçlerin düzenlenmesinde önemli rol oynayan bir enzim ailesidir. HDAC enzimleri homolojilerine, hücresel lokalizasyonlarına ve yapısal özelliklerine göre dört ana sınıfa ayrılır. Sınıf IIb enzimlerinden biri olan HDAC6 enzimi, hücre göçü, bağışıklık tepkileri ve nöronal işlev gibi çeşitli fizyolojik süreçlerde önemli işlevlere sahiptir. HDAC6 aktivitesinin düzensizliği, nörodejeneratif hastalıklarda toksik protein agregatlarının birikimiyle ilişkilendirilmiştir; kanser hücrelerinde aşırı ifadesi veya değişen aktivitesi ise metastaz ve tümör oluşumuna sebep olabilmektedir. Bu çalışmada, potansiyel HDAC6 inhibitörleri tasarlanmış ve bu moleküllerin inhibisyon potansiyelleri, moleküler kenetlenme, moleküler dinamik simülasyonları ve MM-PBSA hesaplamalarını içeren in silico protokolleri kullanılarak araştırılmıştır. Tasarlanan moleküller arasında IA64, HDAC6 enzimine karşı en iyi bağlanma profilini göstermiştir ve ileri çalışmalar için öncü bir molekül olarak görülebilir.

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1. Introduction

Histone deacetylases (HDACs) are a family of enzymes which play crucial roles in the regulation of gene expression and cellular processes [1]. This process occurs by the removal of acetyl groups from histones and non-histone proteins which is known as deacetylation, typically leads to chromatin condensation and transcriptional repression [2]. Studies had shown that, there are 18 isoforms of HDAC that are classified into four main classes (I, II, III, and IV) based on sequence similarity to yeast HDACs, cellular localization, and structural characteristics. Three of these classes contain zinc-dependent histone deacetylases (class I, II and IV) and one of them (class III) NAD+-dependent histone deacetylases (also known as sirtuins). HDAC6 belongs to class IIb and is mostly localized in the cytoplasm [3,4].

HDAC6 has important functions in variety of physiological processes such as cell migration, immune responses, and neuronal function [5]. Dysregulation of HDAC6 activity has been linked to the accumulation of toxic protein aggregates in neurodegenerative diseases, while its overexpression or altered activity in cancer cells can contribute to metastasis and tumorigenesis [6]. Its involvement in neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease, and cancers has prompted growing interest in HDAC6 as a therapeutic target [7]. Regarding the role of HDACs on cancer, depending on the tissue context, HDACs can either inhibit or promote tumor progression. For example, HDAC1 and HDAC2 was found to be highly expressed and correlated with metastasis, differentiation, proliferation, and poor prognosis in many types of cancer. On the other hand, class IIb HDACs mainly play critical role in angiogenesis and migration [8].

Most of the pharmacophore structure of the HDAC inhibitors, including HDAC6, consist of three parts: Zinc binding group (ZBG), linker, and cap region. Zinc binding groups (such as hydroxamic acid group) makes a chelation with Zn^{2+} at the inner part of the active site, and this could be considered as the major interaction with the catalytic site. Cap group interacts with the amino acids found in the outer domain of the active site. Linker interacts with hydrophobic channels at the active site and combines the ZBG to the cap group. There are five pan-HDAC inhibitors are approved for use in clinics. These are vorinostat (SAHA), belinostat, panobinostat, chidamide (tucidinostat) and romidepsin [9-13]. Among these inhibitors, chidamide is not approved by United State Food and Drug Administration (FDA), however it is approved by China for the treatment of it was approved for the treatment relapsed or refractory adult T-cell leukemia-lymphoma. Up to date, no selective HDAC6 inhibitors were approved in clinics, however three of the candidates (ricolinostat, citarinostat and KA2507) have been in clinical trials for the treatment for different cancer cells, such as breast cancer, non-small cell lung cancer and melanoma [14-16].

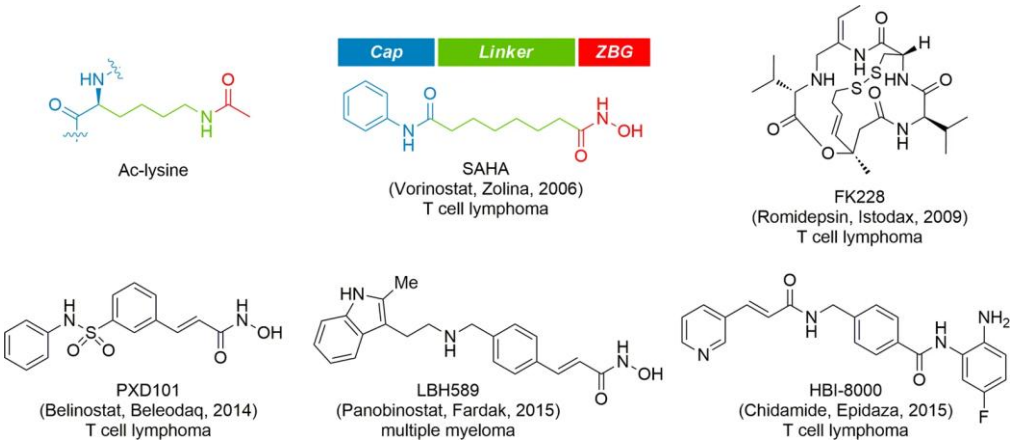


Figure 1. General Pharmacophore Model for HDAC Inhibitors and the Chemical Structures of Approved HDAC inhibitors [17]

Although various numbers of HDAC6 inhibitors and pan-HDAC inhibitors have been developed, it was observed that these candidates had limited success in clinical trials. HDAC6 inhibitors showed anticancer activity at high concentrations. This high concentration administration of HDAC6 inhibitors, however, have been demonstrated to reduce their HDAC6 selectivity, which potentially leading to off-target effects. However, the non-selectivity of pan-HDAC inhibitors has limited their clinical application due to side effects and off-target toxicity. When we consider all these reasons above, design and development of novel and selective HDAC6 inhibitors are crucial to overcome the potential issues.

When we consider the structures of the HDAC inhibitors, it is seen that, most of them (e.g. SAHA, belinostat and panobinostat) consist of hydroxamic acid moiety as Zn^{2+} binding group. On the other hand, chidamide and similar

compounds consist of amino-substituted benzamide moiety. In this study, it is aimed to design of a series of novel HDAC6 inhibitors and to investigate their binding properties against HDAC6 by using in silico protocols, including molecular docking, molecular dynamics simulations and MM-PBSA calculations. To create a suitable HDAC6 inhibitor skeleton, here it was designed novel HDAC6 inhibitors comprising pyridinecarboxamide "zinc binding group" (ZBG), phenyl linker, and naphthyl "cap group". Pyridinecarboxamide moiety, plays a crucial role in this study, due to its potential interaction with Zn^{2+}. Since the pyridinecarboxamide structural motif consist of both carbonyl group and nitrogen containing aromatic ring, it is expected to observe a chelation between pyridine nitrogen-Zn^{2+} and carbonyl oxygen-Zn^{2+}. Chemical structures of designed molecules (**IA61-IA72**) are listed in Figure 2.

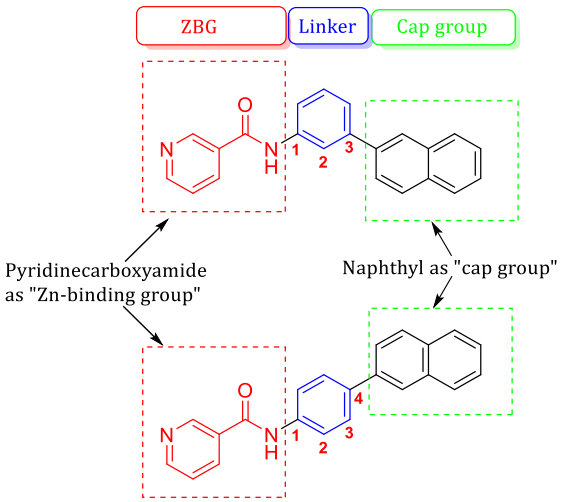


Figure 2. Design scheme of the potential HDAC6 inhibitors

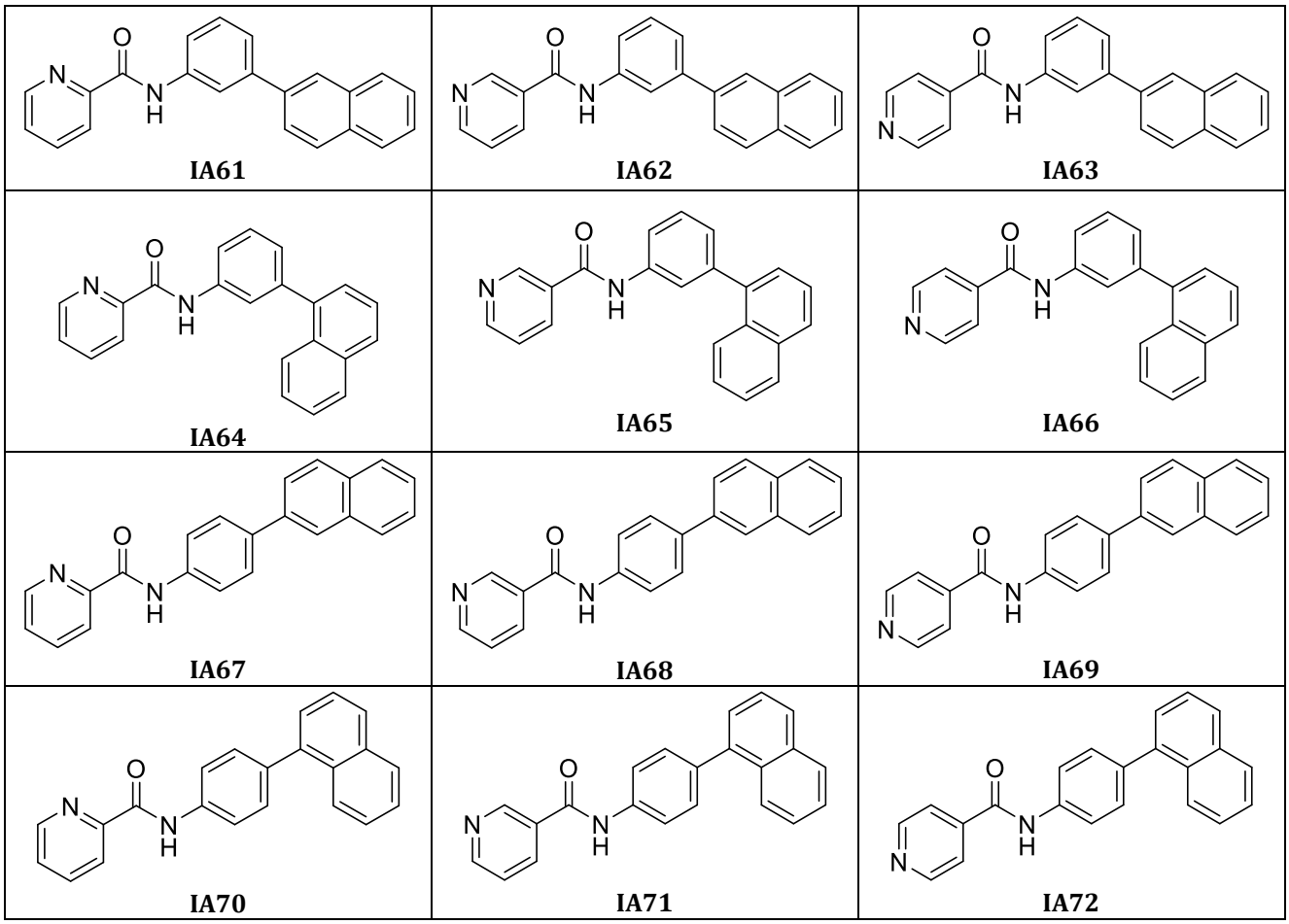


Figure 3. Chemical structures of the designed molecules **(IA61-IA72)**

2. Material and Method

2.1. Target protein and ligand preparation

X-ray crystal crystallography model of the target protein HDAC6 (PDB ID: 5EDU) was retrieved from RCSB Protein Data Bank (www.rcsb.org) web site. PDBFixer was used to prepare the protein for docking studies. It was cleaned off the existing water molecules, and USCF Chimera's DockPrep tool was used to add the missing residues and hydrogens to the protein.

For ligand preparation, molecules were drawn using ChemDraw. Structures were minimized by utilizing MMFF94 force, 3D coordinates generated, and converted to ready-to-dock format by using OpenBabel [18,19] The ligand topology and parameters files of designed compounds for use in the MD simulations were generated using SwissParam web service [20].

2.2. Molecular Docking

Smina [21], a fork of Autodock Vina[22], was used in molecular docking studies. Interactions between docked ligands and receptor were visualized by BIOVIA, Dassault Systèmes, Discovery Studio Visualizer, v21.1.0.20298, San Diego: Dassault Systèmes, 2021 and PyMOL, The PyMOL Molecular Graphics System, Version 3.0 Schrödinger, LLC. Furthermore, one of them according to their residue interactions and as well as binding affinity was selected for MD analysis.

2.3. Molecular Dynamics (MD) Simulations

Molecular dynamics simulation was used for determining stability of the ligand in the active site of the target protein in over time, which coordinates for it was provided by the molecular docking. MD simulation was computed using GROMACS version 2021[23,24]. The same methodology was used described in our previous work[25].

2.4. MM-PBSA Calculations

To clearly assess the binding of the selected compounds with the active site of the protein, MM-PBSA (Molecular Mechanics Poisson-Boltzmann Surface Area) calculations were performed using the *g_mmpbsa* package of GROMACS. Binding averages of the whole trajectory have been utilized for each compound at every 100 ps, resulting in 1000 frames in total. Furthermore, the last 10 ns while using every frame available, meaning last 1000 frames, 90-100 ns, interval also calculated to provide additional information regarding the overall stability of the compounds in the simulation.

3. Results

3.1. Molecular Docking Studies

Molecular docking studies are important for discovery of novel drug candidates, since the experimental binding mechanism and affinity can be predicted by this method, and it provides indispensable information about the possible interactions. Docking scores give us information about the binding energy between the ligands and the target protein. A very negative score corresponds to a strong binding. Thus, smaller the docking score, better the affinity.

When we check the docking scores between the designed molecules and HDAC6 protein, PDB ID: 5EDU, **IA64** shows the best binding energy with -8.8 kcal/mol. Other ligands were also shown good binding energies between 8.1 – 8.6 kcal/mol. When we examine the best candidate's (IA64) interactions in the active site, it is observed that it has a closest interaction with Zn^{2+} (grey sphere) with the expected zinc binding group (ZBG) interaction (Figure 4). Main interactions between the ligands and the 5EDU were mainly observed as Π-Π (pi-pi) interactions with Phe620, Phe679 and Phe680, H bond interaction with His651, Π-alkyl interactions with Leu749, and Π-cation interaction with Arg673 (see the 3D and 2D representations at Figure 4, 5 and 6).

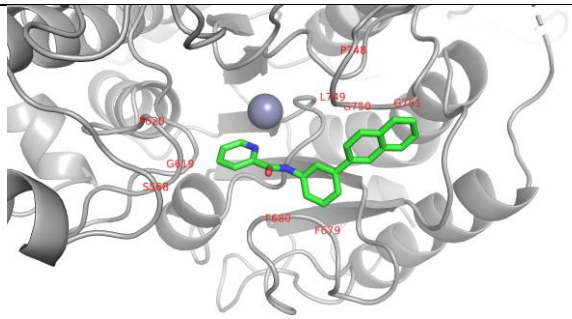
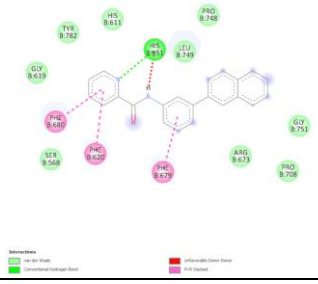
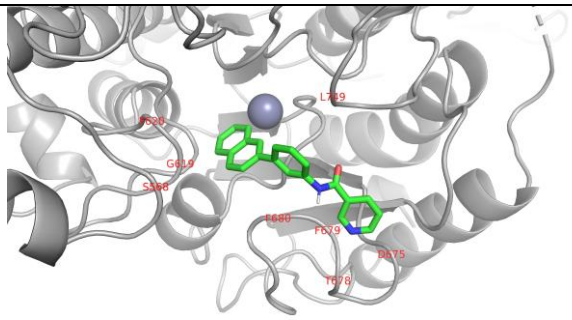
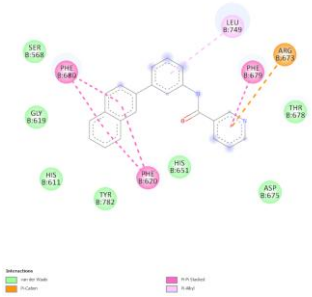
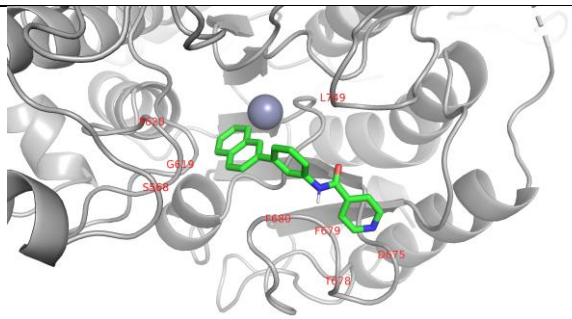
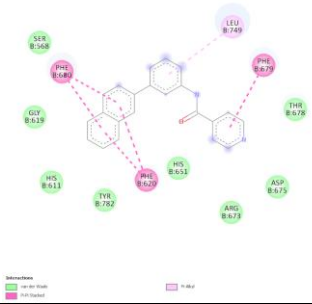
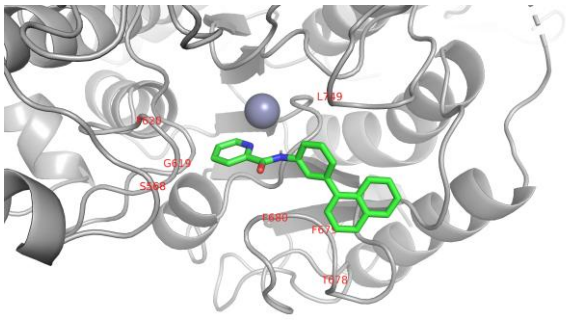
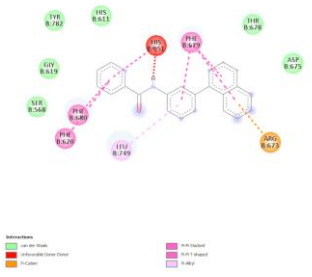
Compound ID	Docking Score (kcal/mol)	3D representation of ligand-protein interactions	2D ligand-protein interactions
IA61	-8.4		
IA62	-8.1		
IA63	-8.1		
IA64	-8.8		

Figure 4. 2D and 3D representations of HDAC6 (5EDU) and ligands interactions **(IA61-IA64)**.

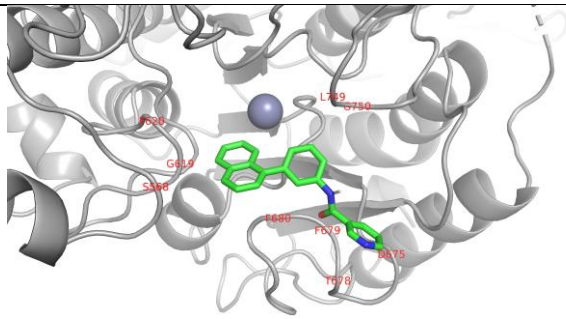
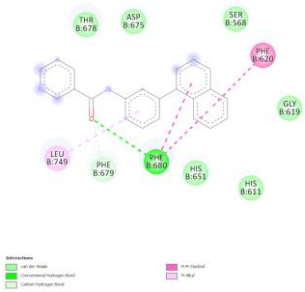
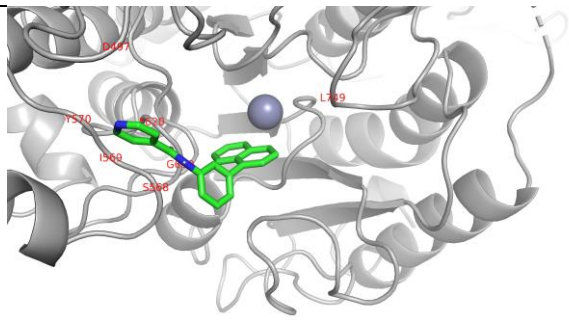
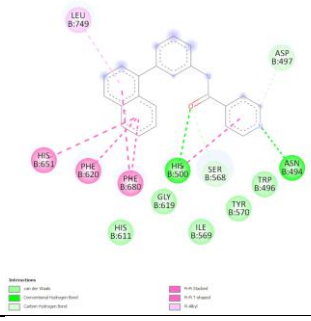
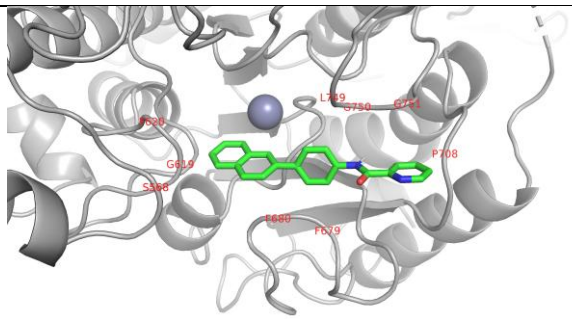
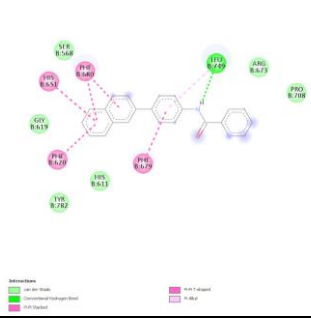
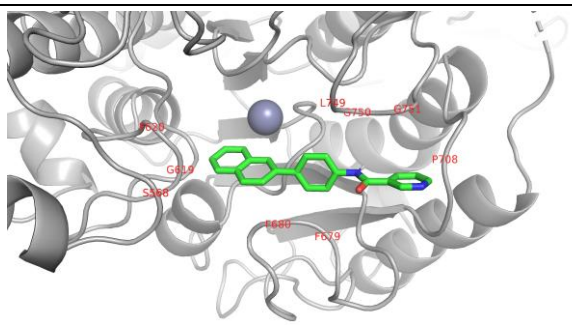
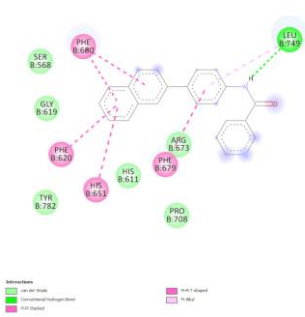
Compound ID	Docking Score (kcal/mol)	3D representation of ligand-protein interactions	2D ligand-protein interactions
IA65	-8.6		
IA66	-8.2		
IA67	-8.3		
IA68	-8.2		

Figure 5. 2D and 3D representations of HDAC6 (5EDU) and ligands interactions **(IA65-IA68)**.

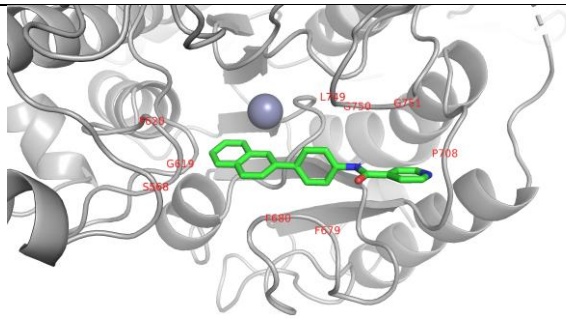
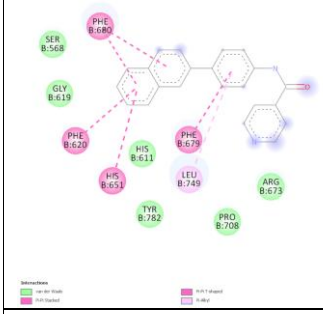
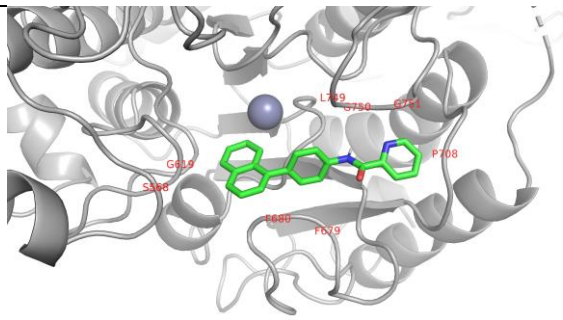
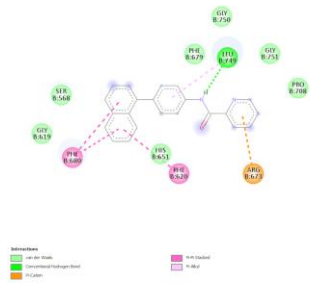
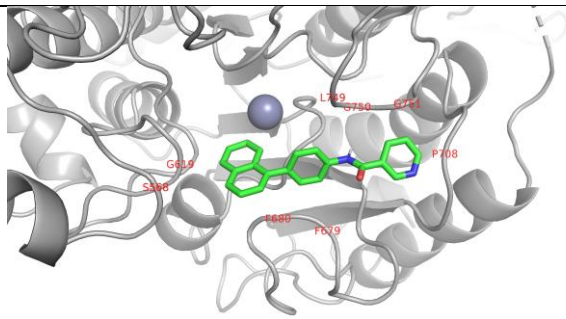
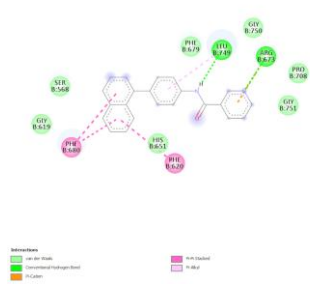
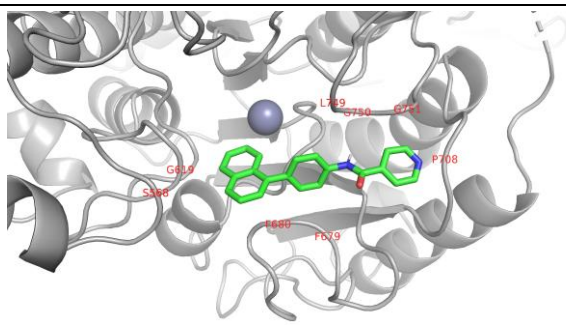
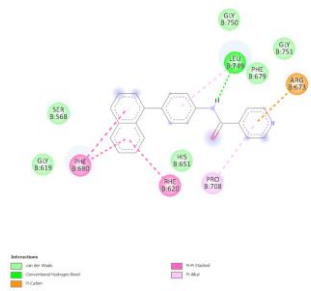
Compound ID	Docking Score (kcal/mol)	3D representation of ligand-protein interactions	2D ligand-protein interactions
IA69	-8.2		
IA70	-8.3		
IA71	-8.5		
IA72	-8.4		

Figure 6. 2D and 3D Representations of HDAC6 (5EDU) and Ligands Interactions **(IA69-IA72)**.

3.2. Molecular Dynamics (MD) Simulations & MM-PBSA Calculations

MD simulations give us crucial information about the stability of the ligand-protein complex. In this study, docking pose of the molecule **IA64**, which has the best docking score, has been selected for further studies. MD simulation studies have been performed for the evaluation of the **IA64-5EDU** protein complex. For this purpose, 100 nanoseconds (ns) molecular dynamic simulation was applied. There are a lot of information which can be obtained from MD simulations. Hereby, it is presented the root mean square deviation (RMSD) plot, H-bond plot, distance between the center of mass (COM) of the ligand and COM of the amino acid residues in active site of the protein.

The RMSD plot of shows us the difference between the protein's backbone atoms from its initial conformation to its final conformation. The stability of the protein is inversely correlated with fluctuations observed during the simulations. For more stable the protein structures, we observe smaller deviations. In Figure 7A, RMSD plot shows

that the deviation of the protein and backbone atoms do not exceed 3Å. On the other hand, although IA64 shows a fluctuation at the beginning (0-17 ns) and last period of the simulation, its RMSD values are smaller than 5Å at the most of the period of the simulation. Especially, between 40 ns and 90 ns period, RMSD value is around 3Å (Figure 7A).
During the simulation, the number of the H-bond interaction is observed as one H-bond. Although there is no H-bond interaction at beginning of the simulation, between 18 ns and 80 ns a constant H-bond interaction is detected (Figure 7B).

The Contact Heatmap Plot (Figure 7C) shows the distance (in Å) between the center of mass (COM) of the ligand and COM of the amino acid residues in active site of the protein. Phe680, Phe679, Leu749, Gly750 are observed as the closest amino acid residues during the simulation. Besides, the distance between the COM of the ligand and Zn^{2+} is observed as 5Å to 10Å after the 20 ns of the simulation. By using MM-PBSA calculation methodology, average binding energy was calculated as –19.2 kcal/mol (Figure 7B).

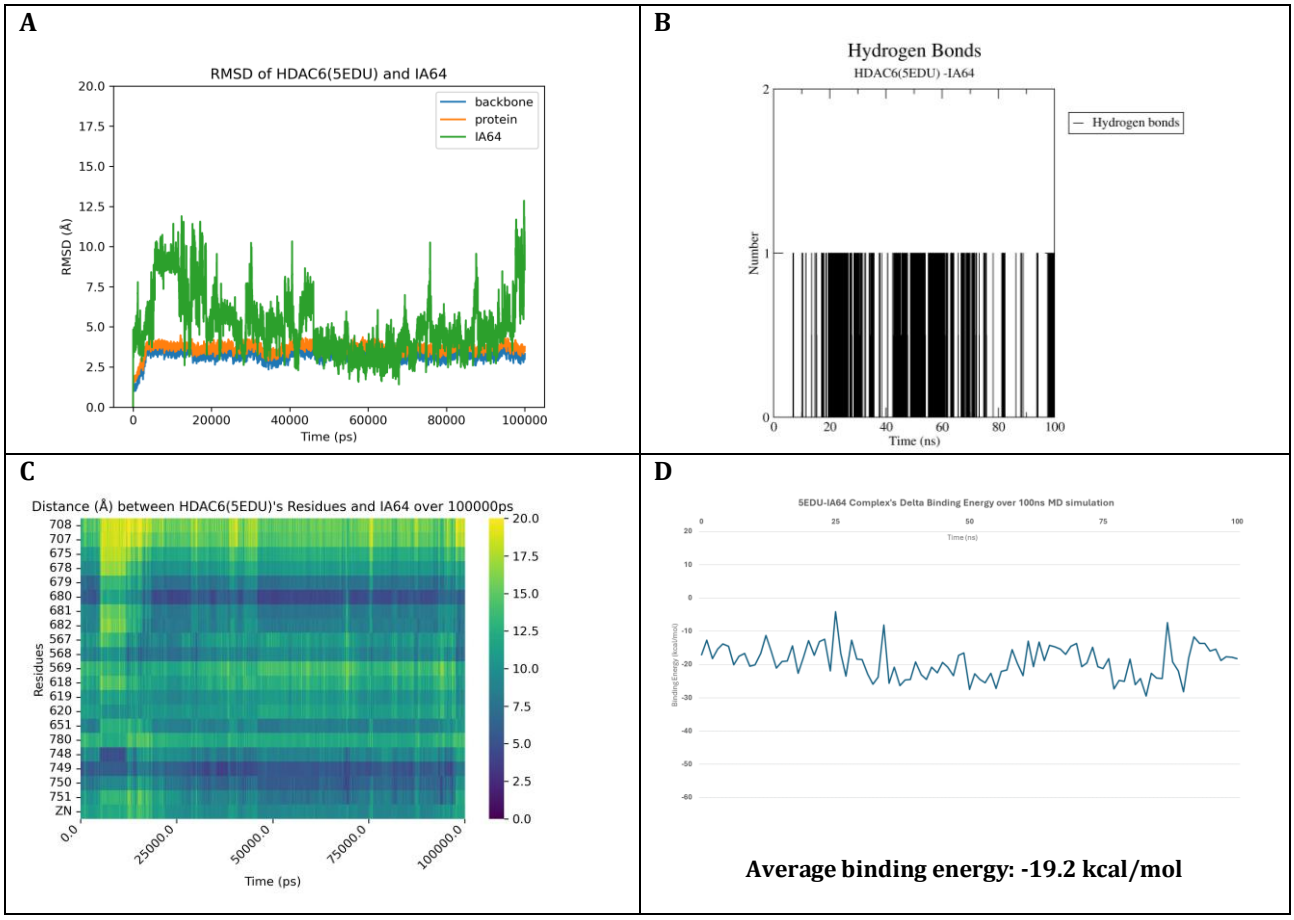


Figure 7. A) RMSD Plot, **B)** H-bond Plot, **C)** Contact Heatmap Plot, and **D)** MM-PBSA Analysis of **IA64-5EDU** Complex During 100 ns Simulation.

4. Discussion and Conclusion

In this study, a series of novel HDAC6 inhibitors were designed and their binding properties were evaluated against HDAC6 by using in silico protocols, including molecular docking and MD simulation. Amide functional group is commonly used fragment in the HDAC inhibitors. Amide group can make a number of hydrogen bonds in the active site of the HDAC6. In the design of the molecules, pyridinecarboxamide moiety was focused, since pyridine ring has a nitrogen atom and it may form additional hydrogen bond with the side chains, and it has also potential to make chelation with Zn^{2+}. Different pyridines (2-pyridyl, 3-pyridyl and 4-pyridyl groups) were used in the design of the potential inhibitors according to the position of the nitrogen atom on pyridine. As a linker, phenyl is chosen because of its potential Π-Π (pi-pi) interactions with phenylalanine (Phe) side chains, and Π-alkyl interactions with hydrophobic leucine (Leu) side chains in the active site of the protein. We can see such a phenyl linker in many HDAC inhibitors, such as belinostat, panobinostat and chidamide. For the cap, naphthyl group was chosen considering the ability to close the "cap region" of the active site with its size. In order to reveal another structure-activity relationship, naphthyl groups was bonded to the phenyl linker on either carbon 3 or carbon 4.

According to results, the most promising candidate was determined as IA64, which has 2-pyridinecarboxamide group as ZBG, and naphthyl group bonded to carbon 3 of phenyl linker. When we check the interactions between the most promising candidate IA64 and HDAC6 complex, the close interaction of 2-pyridinecarboxamide group with Zn^{2+} and histidine side chain (His651) which has a coordination with Zn^{2+} was clearly seen. Other important interactions were observed as Π-Π interactions of Phe620, Phe679 and Phe680 residues with phenyl linker. MD simulation data proves the consistent interactions of these Phe residues with molecule IA64. These results are consistent with our expectations.

Considering the results of this in silico study, among the designed molecules, IA64 could be considered as a potential HDAC6 inhibitor candidate. Besides, the ease of the synthesis of the candidate molecule is always one of the important parameters in drug design process. On the other hand, although in silico studies show promising results, in vitro and in vivo validations are always needed. As a result, in this study, it was proposed the design of a new inhibitor candidate against HDAC6.

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