Bioinformatic Investigation of the Effects of Romidepsin and Tamoxifen Compounds in Inhibiting Histone Deacetylase Enzyme for Cancer Treatment

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ABSTRACT

Cancer is a complex group of diseases, and the transformation of a normal cell into a cancerous one is a complicated, multi-step process resulting from abnormal gene expression and regulation. This study aims to conduct a bioinformatic investigation of the effects of romidepsin and tamoxifen compounds in inhibiting the human histone deacetylase (HDAC) enzyme. HDACs play a crucial role in gene expression regulation by remodeling chromatin structure. An imbalance in histone acetylation, caused by dysregulated HDAC expression and activity, is known to contribute to cancer progression in various malignancies. Therefore, this research seeks to target HDAC inhibition as a therapeutic strategy against cancer.
The studied compounds were downloaded from the PubChem database. The target enzyme was also obtained from the PDB database, optimized using Chimera and Discovery software, and subjected to molecular docking via the HDock server. Finally, the results were analyzed and evaluated using the two aforementioned software tools and the PDBsum Generate website.
The studied compounds were capable of occupying the enzymes' active sites, with binding energy levels of -144.17 kcal/mol for tamoxifen and -140.42 kcal/mol for romidepsin.
Given the efficacy of these compounds in the bioinformatic analysis, further investigations under in vitro and in vivo conditions are recommended to validate their therapeutic potential.

Keywords: *Histone deacetylase, Cancer, Romidepsin, Tamoxifen, Molecular Docking*

1. INTRODUCTION

Cancer is a complex set of diseases, and the transformation of a normal cell into a cancerous one is a intricate, multi-step process. Cancer is a disease caused by abnormal gene expression and manifestation. It is the third leading cause of death in Iran and the second worldwide, as well as the second largest group of chronic and non-communicable diseases linked to modern lifestyle habits. Factors such as increased tobacco use, population growth, aging demographics, etc., are underlying causes of cancer. For years, cancer has affected countless individuals and is the second leading global cause of death after cardiovascular diseases. Cancer is a highly complex genetic, epigenetic, and environmental disease with significant diversity at tissue, tumor, and cellular levels. This heterogeneity can lead to inappropriate treatments [1].
Aging is the primary cause of the rising incidence of cancer worldwide. As people age, the regulatory mechanisms controlling cell division gradually deteriorate, contributing to cancer development.
Chemical factors are among the cancer-causing agents. Carcinogenic chemicals can induce cancer through various mechanisms, such as sodium saccharin, which acts as a promoter by accelerating the cell cycle. Tobacco and cigarette smoke, containing polycyclic hydrocarbons and nicotine (a producer of nitrosamines), cause lung, laryngeal, pharyngeal, esophageal, prostate, and other cancers. Alcohol consumption, interacting with smoking, leads to oral and esophageal cancers due to its synergistic effect with tobacco. Liver tissue damage from alcohol also causes liver cancer. Dietary factors, such as nitrates and salt in preserved foods, are directly linked to stomach and esophageal cancers, while a high-fat, low-fiber diet is

associated with colon cancer. Aflatoxins, cooking and frying methods, artificial colorings, preservatives, sweeteners, etc., are also dietary contributors to cancer.
Biological factors account for about 15% of all cancers, including those caused by infections such as hepatitis B and C, which lead to liver cancer. Some cancers are associated with specific infections, such as stomach cancer with H. pylori and bile duct cancers with certain parasites. Viral factors, such as sexually transmitted HPV, play a role in cervical cancer in women [2].
The most common cancers worldwide are lung, stomach, and breast cancer. In Iran, after skin cancer, the most prevalent cancers are breast cancer in women, stomach cancer in men, bladder cancer, colorectal cancer, and esophageal cancer. A 2009 study in China identified lung, stomach, and colorectal cancers as the most common. A 2012 European study listed breast cancer in women, colorectal cancer, prostate cancer, and lung cancer as the most frequent [3].
Romidepsin is a prodrug that becomes active upon entering cells. Its active metabolite contains a reactive thiol group that interferes with zinc ions in the active sites of class 1 and 2 HDAC enzymes, inhibiting their enzymatic activity. Romidepsin is a histone deacetylase inhibitor (HDACi) approved by the U.S. FDA for treating cutaneous T-cell lymphoma (CTCL). Although multiple mechanisms have been proposed for its effects—including induction of cell cycle regulatory genes, acetylation of cytoplasmic proteins, and direct apoptosis induction—the exact mechanism of romidepsin and other HDACis in CTCL remains unclear. Romidepsin induces long-term responses, with a side-effect profile similar to other HDACis, including fatigue, nausea, and thrombocytopenia. Managing CTCL patients requires vigilance against skin infections and monitoring of potassium and magnesium levels, as electrolyte imbalances are common. Electrocardiographic (ECG) changes are frequent but not associated with myocardial damage. In a phase II trial of romidepsin involving 61 patients, treatment response correlated with sustained histone H3 acetylation, indicating drug exposure is crucial for efficacy.
Future studies aim to identify combination strategies to enhance efficacy in resistant CTCL and solid tumors and to discover biomarkers for patient selection [4].
Romidepsin is a cyclic molecule that inhibits histone deacetylases. It is FDA-approved for cutaneous and peripheral T-cell lymphoma, but its precise mechanism against malignant T-cells remains unknown [5].
Tamoxifen is a non-steroidal, synthetic derivative of trans-triphenylethylene and the hormonal treatment of choice for breast cancer. It has proven efficacy in breast cancer patients, with adjuvant use significantly reducing tumor recurrence and mortality. In estrogen receptor-positive patients, tamoxifen improves long-term survival and reduces breast cancer incidence in high-risk women. Optimal use—maximizing benefits while minimizing adverse effects—is still under investigation. In postmenopausal women, tamoxifen reduces fatal myocardial events, increases bone mineral density, and lowers contralateral breast cancer risk by 40–50%. Adverse effects include thromboembolic events and endometrial cancer. Its long-term safety, particularly in prevention, was questioned after it acted as a hepatic carcinogen in rats [6].
Tamoxifen is a non-steroidal anti-estrogen used in all stages of breast cancer, improving overall and relapse-free survival. It also prevents secondary breast cancers in high-risk individuals. Due to its estrogenic effects on bone, endometrium, breast tissue, and the vascular system, it can cause genital tissue changes [7].
Histone deacetylases (HDACs) are enzymes that remove acetyl groups from lysine residues. Aberrant HDAC activity is observed in many cancers, including colorectal cancer, making HDAC inhibitors potential anticancer agents. Developing drugs with low IC50 and minimal side effects is crucial. Since early HDAC inhibitors had short-lived effects, newer research focuses on long-acting pharmacodynamic agents [8].
HDACs influence chromatin condensation and gene expression by deacetylating lysine residues, restricting transcriptional access. Dysregulated HDAC activity promotes cancer stem cells (CSCs), metastasis, and cell migration by suppressing apoptosis-related genes. HDAC family members play key roles in cancer progression. Recent studies on HDAC gene expression and inhibitor effects have opened new therapeutic avenues [9].
Restoring histone acetylation via HDAC inhibitors can regulate chromatin protein homeostasis and potentially reverse cancer initiation and progression. These inhibitors bind zinc ions in the HDAC active site, blocking its function. Due to cancer cells' heightened apoptotic sensitivity, HDACis effectively eliminate them. Studies show that quisinostat , an HDACi, significantly inhibits cell migration and prevents metastasis [10].

2. METHODS

In this study, molecular docking was performed using the H Dock server. Ligand and protein files were uploaded to the website, and docking results were analyzed.
The research investigated compounds with potential pharmacological properties. The structures of target compounds were obtained from http://pubchem.ncbi.nlm.nih.gov, with names and structural details presented in Table 1. A suitable crystalline structure of the enzyme containing the central catalytic domain was selected and downloaded from https://www.rcsb.org/pdb (PDB ID: 1T64, resolution: 1.90 Å).
Preparation of histone deacetylase protein and ligands for docking:
3D structures of target ligands were downloaded from PubChem and converted to PDB format. Non-protein components (e.g., water molecules) were removed using Discovery Studio software. The structures were then fully optimized for molecular docking using Chimera.
Protein and ligand files were uploaded and submitted to the H Dock server. The top 10 docking models were downloaded, with the model showing the most negative binding energy selected for detailed analysis.
Visualization and analysis of docking results:
Post-docking analysis included:
Evaluation of Most Favored and Disallowed regions, Ligand binding energies, Ligand-protein interactions: Hydrogen bonds, Hydrophobic interactions, Pi interactions, Interactions with copper ions in the enzyme's active site
Data analysis was performed using Discovery Studio software and two servers: HDOCK and PdbSum Generate.

Table 1. Names and Structural Details of the Studied Compounds

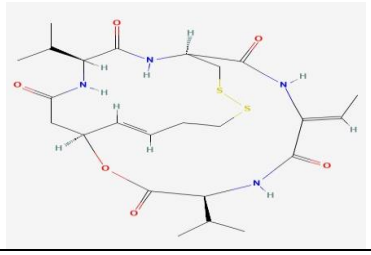
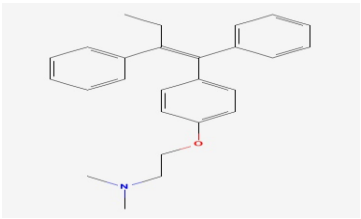
Compound name and CID code	2D Structures
Romidepsin 5352062	
Tamoxifen 2733526	

Figure 1, Tamoxifen in the active site of the enzyme

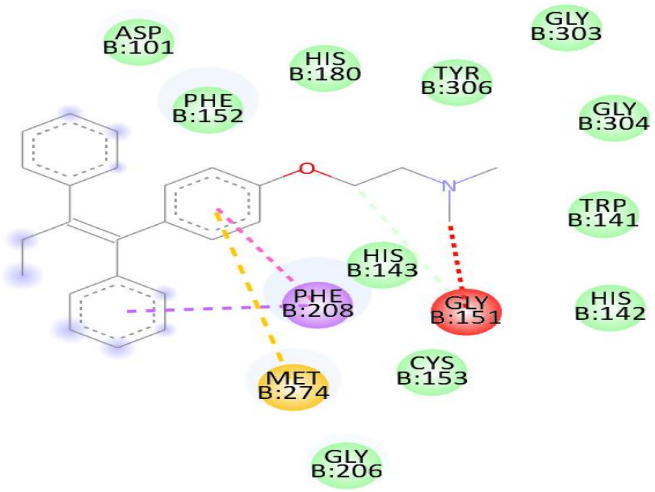
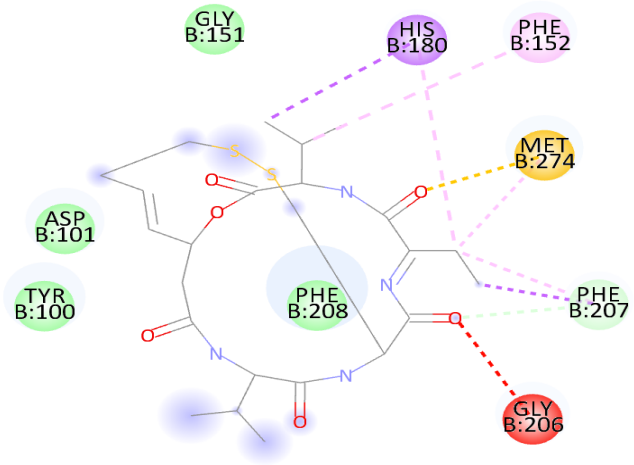


Figure 2, Romidepsin in the active site of the enzyme



3. RESULTS

In this study, both compounds were successfully docked into the enzyme's active site with favorable binding energies and a good number of interactions. Tamoxifen exhibited a binding energy of -144.17 kcal/mol and interacted with three amino acids (GLY151, PHE208, MET274), while romidepsin showed a binding energy of -140.42 kcal/mol and formed interactions with five amino acids (HIS180, PHE152, MET274, PHE207, GLY206) in the enzyme's active site.

4. CONCLUTION

Sixto-López and colleagues conducted a molecular docking study in 2019-2020 to investigate HDAC8 inhibition mechanisms and identify key active site amino acid interactions. Their research examined several compounds including tubacin which interacted with HIS180, HIS143, PHE152 and TYR306; vorinostat that bound to PHE207, PHE208, TYR306 and PHE152; APHA showing

interactions with PHE152, LEU179, GLY151, HIS143, HIS180, ASP267, TYR306 and ASN307; and valproic acid which engaged with ARG37, ASP101, TYR111, TRP141, GLY151, PHE152, TYR154, LEU179, HIS180, PHE208, ASP267, ASP272, SER276, TYR306 and ASN307. In our comparative studies, we observed tamoxifen interacting with GLY151, PHE208 and MET274 while romidepsin formed bonds with HIS180, PHE152, MET274, PHE207 and GLY206, revealing novel interaction patterns particularly involving the MET274 residue that were not reported in the previous study. All these interactions occurred at the enzyme's catalytic site, demonstrating distinct binding profiles for each inhibitor compound [11].

A research team led by David Ebuka Arthur from the Universities of Maiduguri, Ahmadu Bello, and BAZE in Nigeria conducted a comprehensive study in 2023 on various HDAC inhibitor compounds (PDB ID: 6FYZ). Among their findings, several compounds including LIG1, LIG2, LIG1B, LIG2C, LIG1E, and LIG1F demonstrated interesting interaction patterns with the enzyme's active site.

The LIG1 compound interacted with amino acids GLY811, HIS842, HIS802, and PHE812. LIG2 showed binding with a different set of residues: ASP904, GLN1006, LEU908, TYR955, ALA905, and ARG1008. LIG1B was observed to form bonds with GLY811, PHE812, CYS813, HIS802, HIS803, and PHE871, while LIG2C exhibited more complex interaction patterns involving PHE812, GLY975, CYS813, ASP934, HIS842, TYR814, and PHE871.

The remaining two compounds, LIG1E and LIG1F, each displayed unique binding profiles. LIG1E interacted with GLY811, PHE812, and ASP934, whereas LIG1F bound to GLY811, PHE812, GLY975, and ASP934.

Notably, certain amino acids like GLY811 and PHE812 repeatedly appeared in interactions with most compounds, while others such as PHE871 and CYS813 showed specificity for particular inhibitors. In our parallel research, the compound tamoxifen interacted with GLY151, PHE208, and MET274, while romidepsin bound to HIS180, PHE152, MET274, PHE207, and GLY206 [12].

In 2020, Bernardina Scafuri and colleagues published a study titled "Molecular Docking Simulations on Histone Deacetylases (HDAC)-1 and -2 to Investigate Flavone Binding." The research examined the inhibitory effects of three flavonoid compounds (flavone, luteolin, and apigenin) on HDAC1 and HDAC2 enzymes. Molecular docking simulations demonstrated that these compounds effectively bind to the enzymes' active sites.

Flavone interacts with zinc ion (Zn^{2+}) and key amino acids (Asp176/181, His178/183, Phe150/155, and Tyr303/308) through its carbonyl oxygen. Luteolin forms additional interactions via its hydroxyl groups with supplementary amino acid residues. Apigenin similarly binds to Zn^{2+} along with Asp176/181, His178/183, and Phe150/155. Notably, both flavone and apigenin exhibited superior binding energies compared to the standard inhibitor vorinostat (which interacts with Zn^{2+}, Asp176/181, His178/183, and His140/145). These findings suggest that natural flavonoids may serve as potent HDAC inhibitors with significant anticancer potential.

In our parallel research, we observed that tamoxifen interacts with GLY151, PHE208, and MET274, while romidepsin binds to HIS180, PHE152, MET274, PHE207, and GLY206 in the enzyme's active site [13].

5. REFERENCES

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