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Computational Discovery of New HDAC2 Inhibitors by High Throughput Virtual Screening, Molecular Docking, MM-GBSA, and Molecular Dynamic Simulation

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Abstract: The research is currently centered on investigating how FDA approved medications can be used as blockers for HDAC2. The study was started by gathering more than 4,000 drugs that have been approved by the FDA from the DrugBank database. These compounds were virtually screened to find inhibitors for HDAC2. After that the molecules were analyzed for molecular docking and those, with acceptable docking scores were underwent energy calculations using a molecular mechanics with generalized Born and surface area solvation (MM-GBSA) method. Compounds that showed decent results have been chosen for the molecular dynamic simulation studies to validate their binding affinity. Various parameters such as RMSD, RMSF, and protein ligand contacts were analyzed over a 25 ns simulation period. Encouraged by the results from the docking investigation and MM GBSA analysis two complexes vilazodone-HDAC2 and atenolol-HDAC2 were chosen for a 25 ns Desmond Schrodinger simulation of molecular dynamics (MD). The simulations showcased the stability of the receptor ligand interactions throughout the course of MD simulation.

Keywords: HDAC2, molecular docking, MMGBSA, molecular dynamic simulation and RMSD.

1. Introduction

Cancers that are on the rise in terms of both occurrence and death rates have become a concern posing a threat to public health. This underscores the significance of developing and identifying chemotherapy drugs [1]. Despite advancements in cancer treatments, the effectiveness of antitumor drugs is often hindered by drug resistance and the harmful side effects that restrict their use in settings [2,3,4]. The process of tumor formation evolves due to changes in gene expression associated with mutation, loss and rearrangement which add to the intricacy of the phenomenon. [5]. Most of the time its challenging to address these alterations with therapy [6,7]. The continuous progress in mechanisms suggests a connection between the disturbance of gene activity and the development and advancement of tumors [8,9,10,11], This connection can be thoroughly explored as an approach, to developing anticancer drugs. Epigenetic alterations encompass modifications in gene function that are independent of DNA sequence alteration. Research in the field of epigenetics has seen a rise in interest regarding the examination of histone acetylation carried out by enzymes such as histone acetyltransferases (HATs) and histone deacetylases (HDACs) [12,13,14]. Unlike changes in DNA sequence, epigenetic modifications can be reversed, offering opportunities for targeting gene regulation through epigenetics in the search for anticancer treatments. Histone deacetylases (HDACs) are a set of zinc enzymes comprising eighteen forms that play a part in influencing epigenetic modifications.

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Researchers have identified 18 forms of HDACs (1-18) which are classified into four groups (I- IV) based on their similarities, variations and sequences. Class I includes HDACs (1, 2 ,3 and 8) class II is divided into (IIa :4, 5 7 and 9 , IIb :6 and 10) all of which're zinc dependent. Class IV consists of HDAC11. The Rpd3/Hda1 family is also known as the reduced potassium dependency 3/Histone deacetylase -a1 family. Class III comprises NAD+ dependent enzymes called sirtuins (SIRT1 -7). [16]. Researchers have discovered that when the function of HDAC enzymes is suppressed it impacts the balance between HATs and HDACs causing a shift towards increased activity. This results in the stimulation of acetylation in both histone and non-histone proteins. Therefore, genes can be affected by either activating or deactivating genes that impact the function of transcription factors and specific genes related to cell growth or cell death [17]. This process triggers cell death by activating pathways that cause cancer cells to stop dividing undergo differentiation, reduce blood vessel growth, induce DNA fragmentation, and prevent DNA repair [18, 19]. Many studies have indicated that HDAC inhibitors (HDACIs) have promising potential as agents and are considered a category of therapies for cancer [20].

HDAC2 is a part of class I isoforms known for its conservation. It tends to be overexpressed in hematological tumors showing a strong association with poorer prognosis. Interestingly, it is absent in resting cells and normal organs (21). Hence a significant emphasis in cancer treatment has been placed on targeting the HDAC2 isoform to inhibit its functions directly (21).

In the field of drug development virtual methods have played a role. They have been instrumental in finding uses for existing drugs cutting down on costs and time required for discovery. Different computational methods such, as systems biology, virtual screening and molecular dynamics simulations have played a role in identifying drug targets, for addressing various diseases [22,23,24,25,26]. Numerous methods in computing can be used for repurposing drugs. While the outcomes may not match the precision of in studies the cost effectiveness and reasonable accuracy make these approaches worthwhile. Assessing 1,000 drugs for repurposing through means is significantly quicker compared to conducting in studies on the same number of drugs [27,28]. This study leverages the benefits of repurposing drugs to pinpoint targets for HDAC2 by exploring a collection of 4,371 compounds sourced from the drug bank database. The primary objective was to identify inhibitors of HDAC2 through screening. We tested drugs on a protein and selected potential candidates based on their docking scores. Furthermore, a dynamics simulations study was carried out to evaluate the stability of proteins and the drug-protein complex.

2. Computational Method

In silico study was carried out using Schrodinger suite (2022), including (Glide, Desmond, prime, and QikProp software), Figure 1 demonstrates the in silico workflow.

2.1 Ligand library preparation

Molecular A set of 4,371 medication compounds was acquired from the drug bank website [29], Then analyzed through molecular docking against the HDAC2 protein using the Schrödinger Maestro software in 2022. Additionally, a recognized HDAC2 inhibitor called SAHA was included in the dataset for comparison purposes.

2.2 Protein structure

The protein target was obtained from the structure stored in the Protein Data Bank with the (PDB IDs; 4XLZ). The protein structure was prepared for screening using the Protein Preparation Wizard. The preparation phase included adding hydrogen atoms and removing molecules to create an environment, for docking simulations. Furthermore, in cases where the protein structures had bound ligands, such as SAHA, additional steps were taken. These included minimizing the protein-ligand complexes, likely to ensure that the bound ligands were in energetically favorable conformations within the protein binding site. This step is essential for accurately simulating ligand binding interactions and assessing binding affinities. Molecular docking grids were generated using the co-crystallized bound ligands as references, grid box parameters were 25.71, -15.82, 1.12. The grids were specifically designed to identify the attachment locations within the sites of

the protein of interest. This process plays a role in directing the docking simulations.

2.3 Virtual screening

A virtual screening was conducted to find inhibitors for HDAC2 by examining a ligand library. The depicted procedure in figure 2 included the docking of established compounds from the DrugBank database into the HDAC2 receptor site through throughput screening (HTVS) and flexible docking mode in Glide. These compounds were then re docked with precision (SP) for increased accuracy. The compounds were re-docked with precision (XP) for further refinement. The resulting compounds underwent screening according to Lipinskis rule of five and other factors such, as surface area (PSA) absorption rate, CNS score and interactions, between receptors and ligands were evaluated. In the end only the compounds that successfully cleared all screening criteria are elaborated upon in this research (Figure 2) [30].

The ligands were selected based on an evaluation of the protein ligand complexes binding energy followed by an analysis using MM GBSA and molecular dynamics simulations.

2.4 GBSA/MM Study

The research involved energy calculations utilizing the Prime module within the Schrödinger model software package, applying GBSA/MM to estimate the binding energy of the material with the HDAC2 receptor in the docking score.

The force response to fluid simulation is achieved through force fields, which mathematically describe molecular interactions to predict binding forces accurately, considering the influence of solvent molecules on the ligand-receptor binding energy.

The solution model considers solvent molecules in the calculations to gain insight into the thermodynamic aspects of ligand binding, particularly the free energy of binding, crucial for evaluating the strength of ligand-receptor interactions and their potential as drug candidates. One method to calculate the binding free energy is through a specific equation [32].

$$\Delta G(bind) = \Delta G(solv) + \Delta E(MM) + \Delta G(SA)$$

where:

The ΔGsolv value indicates the difference, in solvation energy between the complex of the PIK3CA inhibitor and the combined solvation energies of PIK3CA without a ligand and the inhibitor.

The ΔEMM value shows the difference in energy levels when comparing the PIK3CA inhibitor complex to the energies of unbound PIK3CA and inhibitor.

The term ΔGSA represents the variance in surface area energies comparing the complex with the surface area energies of PIK3CA and inhibitor.

The Prime MM GBSA technique calculates the energy levels of three elements: the energetically optimized receptor alone, the energetically optimized ligand alone, and the complex formed when the ligand binds to the receptor. In addition, it calculates the ligands strain energy by simulating the ligand in a solution generated automatically using the VSGB 2.0 package. The Prime Energy Visualizer tool is then used to provide a visual representation of these energy calculations, aiding in the interpretation and analysis of energy-related data.

2.5 Molecular dynamic simulation

The proteins stability and how it interacts with ligands in conditions were evaluated using simulations. In this research we used substances with their binding energies to carry out MD simulations. Unlike docking which provides a view of how ligands bind to proteins molecular dynamics simulations monitor the motion of atoms as time progresses by applying Newtons equations of motion. The study involved using the Schrodinger's Desmond module (2023) to analyze two compounds binding energy scores during a 25-nanosecond simulation period with the OPLS4 force field. To evaluate the ligand protein interactions, we created an environment for our simulations. The Maestro Protein Preparation Wizard was used to get the ligand protein complexes ready before putting them into a box filled with water molecules. Sodium and chloride ions were included to balance the charges and keep the system neutral. The pressure was kept at 1.0132 bar, temperature, at 300K all while making sure the box volume was minimized. The stability of the simulation was evaluated by measuring the root mean deviation (RMSD) across all paths. [33,34].

3. Results and discussion

3.1 Molecular Docking analysis

Molecular docking Studies can provide insights into how various active chemical compounds bind to molecular targets [35–42]. In this research 4,371 substances were tested against the HDAC2 protein. The top 10 compounds were ranked according to their docking scores. By re-docking the co-crystallized ligand (SAHA) against the HDAC2 active site, the docking approach was verified. The validation step established the applicability of the protocol, as evidenced by the small RMSD (1. 9157 Å) between the co-crystallized pose and the re-docked pose. Table 1 presents the binding energies of the top 10 compounds compared to the drug (SAHA) indicating that all these compounds exhibit significant virtual binding energies. Our attention in this investigation was primarily focused on the two leading compounds with binding affinities of -10.714 and -10.945 kcal/mol.

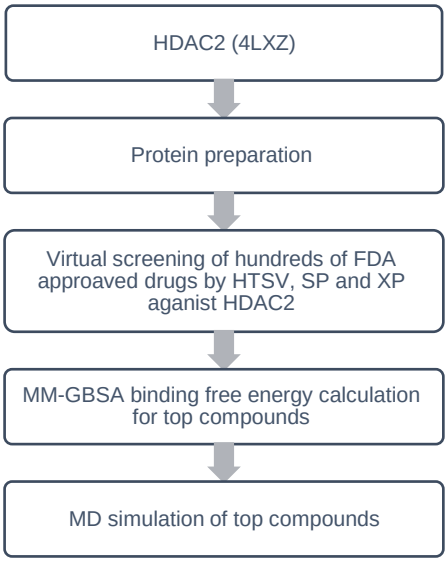
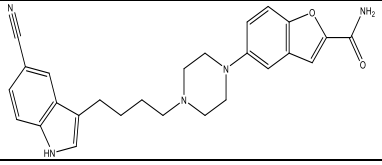
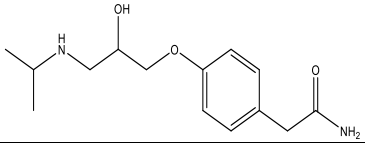
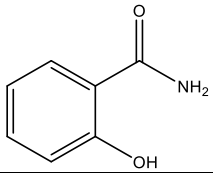
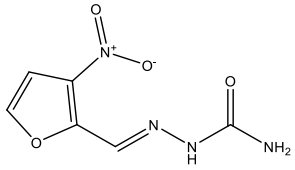
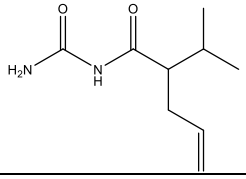
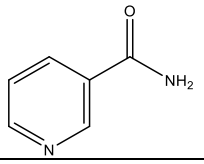
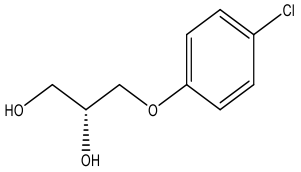
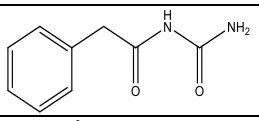
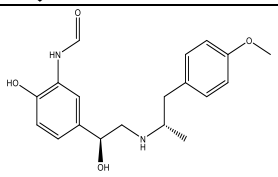
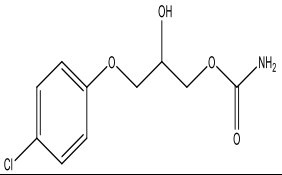


Figure 1. Overall computational workflow of the study

Table 1. Docking scores of top 10 hit FDA-approved drugs

DRUGBANK ID	Compound name	2D structure	HDAC-2 Docking score (Kcal/mole)
DB06684	Vilazodone		-10.945
DB00335	Atenolol		-10.714
DB08797	Salicylamide		-9.951
DB00336	Nitrofural		-8.685

DB13221	Apronalide		-8.628
DB02701	Niacinamide		-8.606
DB00856	Chlorphenesin		-8.491
DB01121	Phenacemide		-8.468
DB00983	Formoterol		-8.287
DB14656	Chlorphenesin carbamate		-8.136
Vorinostat	-----		-10.182

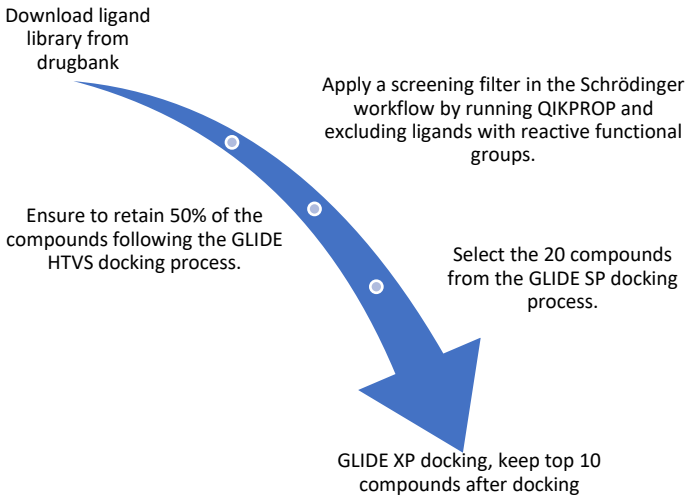


Figure 2. The flowchart of virtual screening.

Analysis was conducted on Vilazodone and Atenolol to study how they interact with proteins at a level. The focus was on examining the locations where these substances bind and the amino acids

that play a role in forming bonds with them. This investigation aimed to compare these interactions with those of Vorinostat (also known as SAHA) an HDAC2 (Figure 3). Visualizing the bonds formed when interacting with the receptors site for the suggested compounds includes H bonds, pi-pi stacking and engagements with the zinc ion within the area. Docking score indicate energy involved when ligand interact with the active site, considering flexible ligand docking. Visual assessment of the fitness and the type of bond involved, and Glide energy. Visual examinations of these elements can anticipate the suitability to the site despite high docking scores. It is possible that a compound might not engage with the site with elevated scores. Both Vilazodone and Atenolol showed comparable interaction pattern to the reference(SAHA).

Table 2. GBSA/MM values of top 2 FDA approved drugs and standard cocrystallized compound (Vorinostat) in HDAC2 receptor (PDB ID: 4XLZ).

Comp.	Prime Energy	ΔG bind (Kcal/mol)	ΔG bind Coulom	ΔG bind Covalent	ΔG bindVander	ΔG bind Lipophilic	ΔG bind H Bond
Vilazodone	-17473.56	-52.68	-36.58	10.03	-33.21	-24.34	-1.80
Atenolol	-17458.37	-49.98	-33.25	5.70	-27.18	-28.94	-2.00
Vorinostat	-17001.6	-30.61	-78.03	2.13	-32.27	-16.96	-3.07

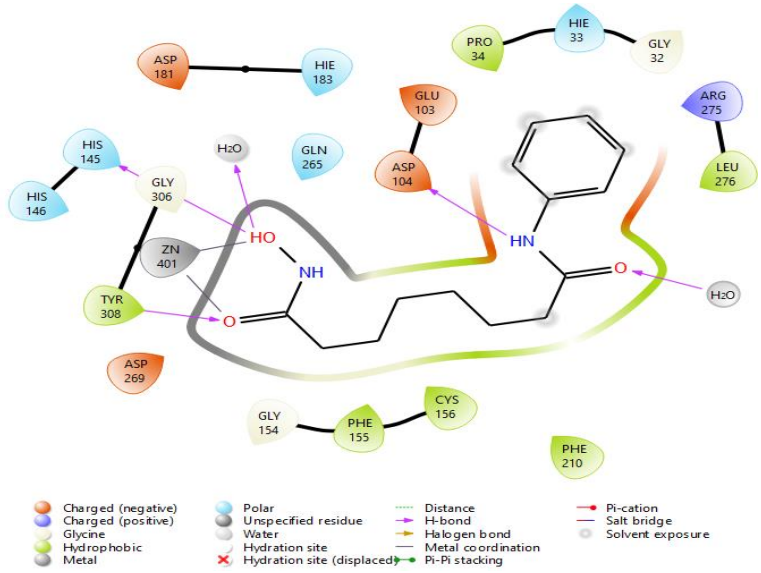


Figure 3. 2D interaction diagram illustrating the connection between vorinostat and HDAC2.

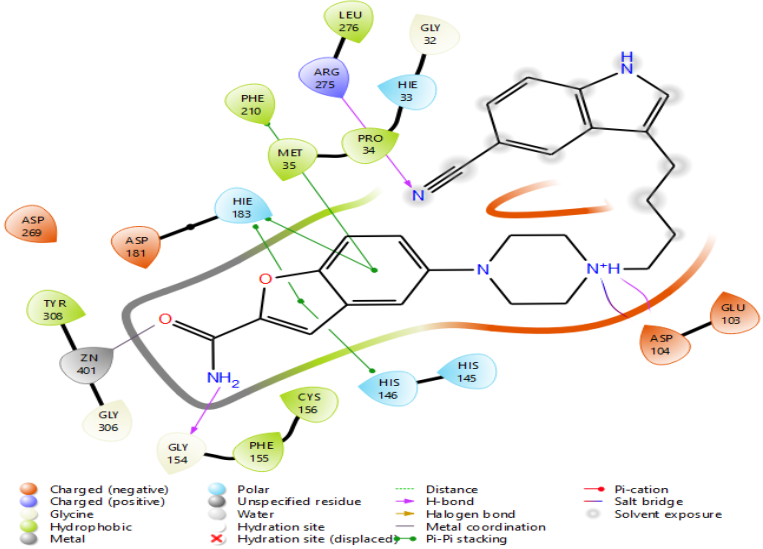


Figure 4. The 2D interaction between Vilazodone and HDAC2.

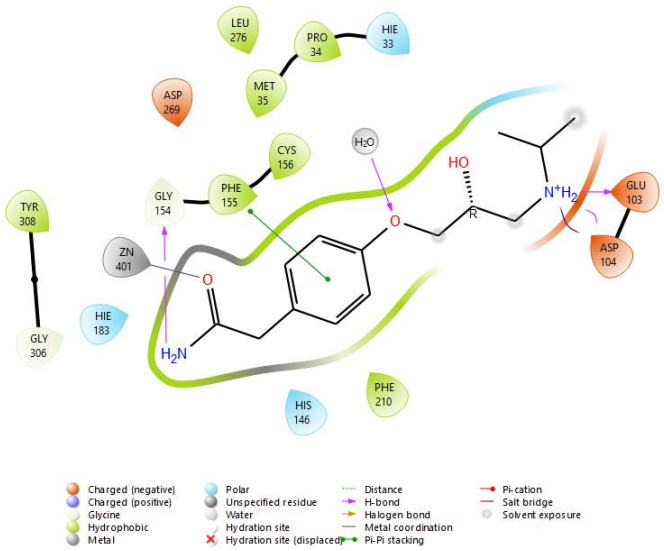


Figure 5. The 2D interaction diagram of Atenolol with HDAC2

Vilazodone showed the highest docking score within the HDAC-2 receptor and had good binding interactions with the receptor backbone. These interactions included favorable hydrophobic interactions with HIS145, HIS146, CYS156, and MET35, along with hydrogen bonding interactions involving GLY154, ASP104 and ARG275. In addition, there are zinc binding interaction and pi-pi stacking between this drug and HIE183, PHE210 and HIS146 amino acids in HDAC enzyme as shown in figure 4.

Atenolol showed a promising docking score with similar interactions within the catalytic domain of the HDAC-2 receptor (Figure 5). These interactions included favorable hydrophobic interactions with PHE210, PHE155 and CYS156, along with hydrogen bonding interactions involving GLY154, ASP104 and GLU103. In addition, there are zinc binding interaction and pi-pi stacking between this drug and PHE155 amino acid in HDAC enzyme as shown in figure 5.

3.2 GBSA/MM study

The docking results were examined using MMGBSA to determine the binding energy associated with the scoring method for the HDAC2 target (PDB ID; 4XLZ).

The durability of ligand receptor connections is mainly evaluated by calculating the Prime MM/GBSA, which is recognized for its precision. When calculating the MM/GPSA different factors are taken into account that can influence the stability of these interactions. One crucial factor is the impact of solvent on the system. As detailed in Table 2, we have determined the Prime - MM/GBSA values for HDAC2 protein when bound to (Vorinostat) a control and the top 2 FDA approved drugs through molecular docking scores. The chosen ligands exhibit binding energies indicating a fit with the HDAC2 receptor. Vilazodone shows the binding energy -52.68 kcal/mol when binding to COX 2 compared to Vorinostat having a ΔG value of 30.6 kcal/mol is considered a benchmark, for pharmaceuticals.

The results of the MM GBSA test show that Van der Waals energy (ΔGvdW) and nonpolar solvation (ΔGLipo) play roles in facilitating ligand binding within the binding site of HDAC2 evident from the high negative values observed across all compounds. Conversely covalent energy (ΔGCov) and hydrogen bonding (ΔGHbond) energies do not significantly contribute to receptor binding.

3.3 Molecular dynamic simulation

3.3.1 RMSD analysis

The alterations in the protein ligand complex, during a 25-nanosecond simulation were identified using root mean deviation (RMSD). The RMSD was used to evaluate the equilibration, flexibility of the protein and the average distance between atoms in the protein. In Figure 6 there is a graph depicting the root mean deviation that shows the structure of the complexes. The system of complexes remained stable during the simulations performed along a 25-

nanosecond trajectory. In the Vilazodone-HDAC2 complex the ligands RMSD was calculated to be 2.0 Å on the side while the proteins RMSD in the complex state was less than 1.5 Å on the left side. For Atenolol -HDAC2 the average ligands and proteins RMSD values were determined to be 3.2 Å and 1.5 Å. Low RMSD values indicated system stability [43]. The results suggested that there were interactions and a suitable range observed for the analyzed complexes throughout the MD simulations.

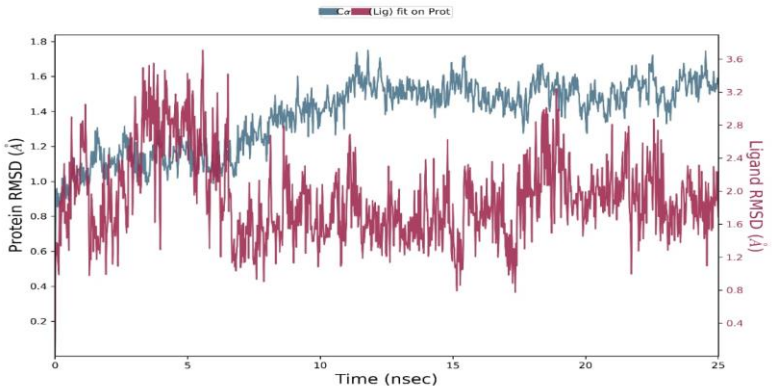


Figure 6. The RMSD pattern of the Vilazodone HDAC2 compound complex.

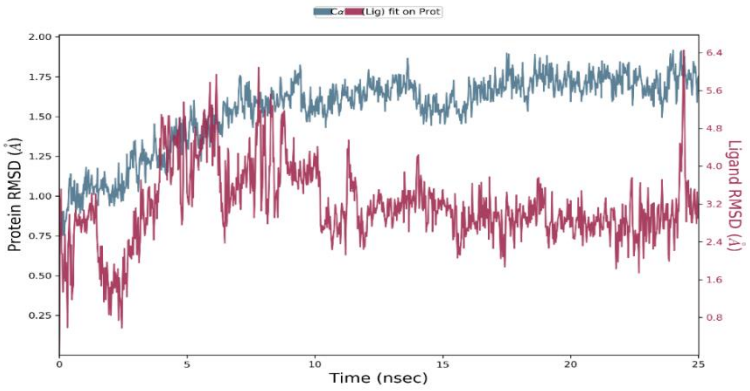


Figure 7. The RMSD profile of the Atenolol-HDAC2 complex

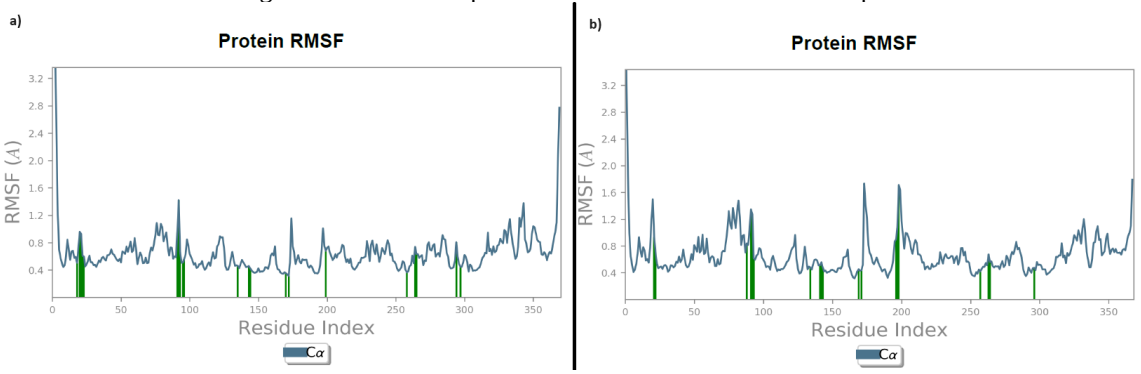


Figure 8: The arrangement of acids in the HDAC2 protein within the examined system: a) HDAC2 Vilazodone combination b) HDAC2 Atenolol combination.

3.3.2 RMSF analysis

Root mean square fluctuation analysis commonly referred to as RMSF is used to study the areas of proteins that show fluctuations compared to the protein structure. This technique computes how atoms behave given temperature and pressure settings.

A low RMSF value typically indicates a system while a high value suggests flexibility during molecular dynamics simulations. A chart showing

root mean deviation was created to study the variations in the HDAC2 protein structures during a 25-nanosecond period. The flexibility of the amino acid residues in the proteins was analyzed effectively (Figure 8). Both structures displayed fluctuation indicating a condition as evidenced by their RMSF values

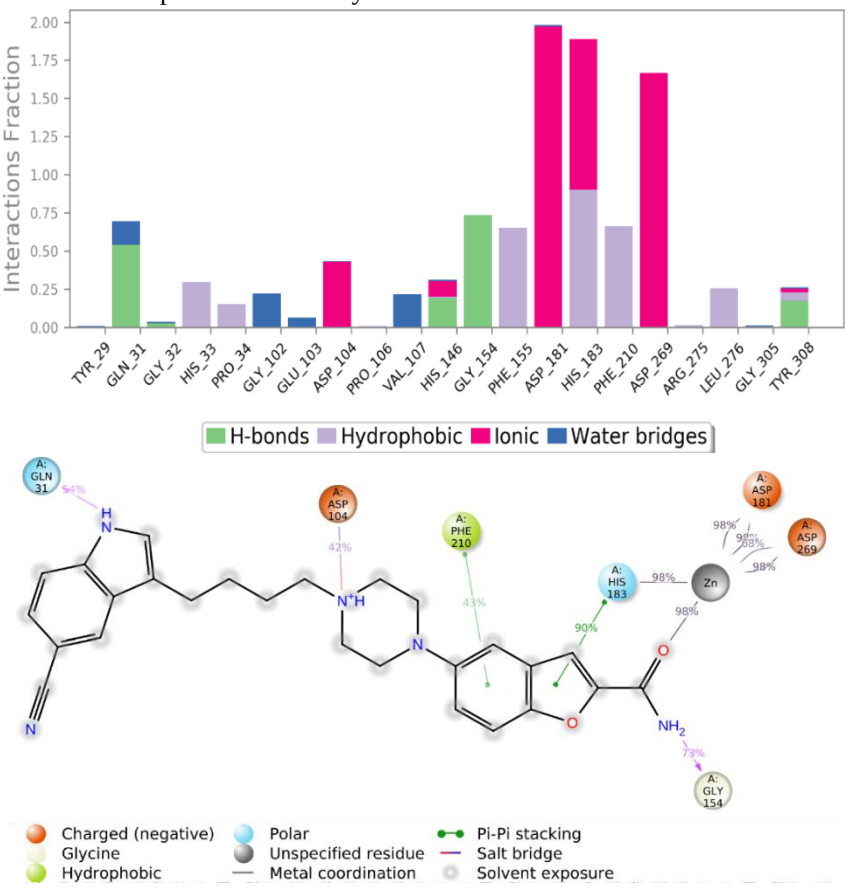
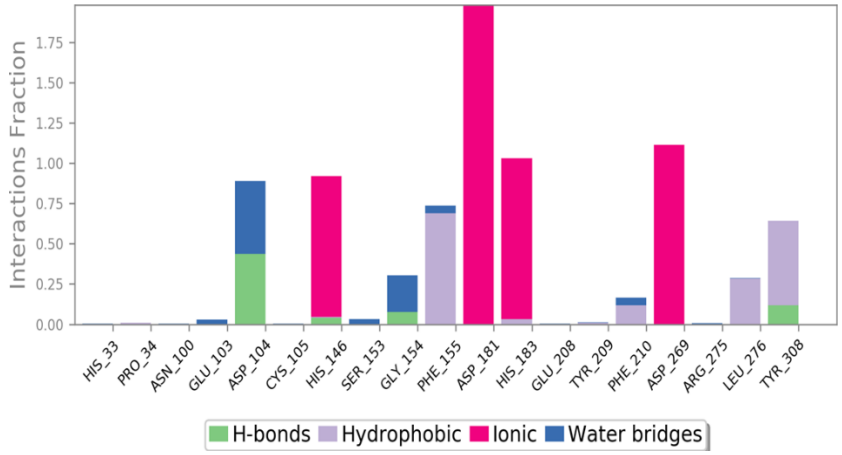


Figure 9. HDAC2 and Vilazodone Hydrogen bonds contacts During over 30% of the simulation time.



3.3.3 Hydrogen bond analysis

Hydrogen bonds also known as H bonds are crucial in the process of binding ligands. It's important to consider the properties related to hydrogen bonding when designing drugs as they greatly impact factors such as drug specificity, metabolism, and absorption [44].

In our research we concentrated on the interactions that occurred for than 30.0% of the simulation duration within the designated trajectory (0.00, to 25.00 nanoseconds). In the simulation Figure 9 and 10 show the count of Hydrogen bonds established between the ligands Vilazodone and Atenolol with HDAC2. During the simulation it was found that

Vilazodone formed a hydrogen bond with the GLY154 residues at the HDAC2 site based on the analysis of the results. The positioning of Vilazodone in the site led to interactions, such as essential metal coordination with ZN and additional low energy interactions like Hydrophobic interaction (HIS33, PRO34, PHE155, HIS183, PHE210, LEU276) and multiple Water bridges interactions. These interactions play a role in stabilizing ligand binding within the proteins site. On the hand Atenolol only exhibited a metal coordination bond without any Hydrogen bonding interaction. This outcome underscores the inhibition of HDAC2 by these repurposed inhibitors.

3.3.4 Total Protein-Ligand Contacts

Figures 11 and 12 illustrate a depiction of the connections and interactions such as hydrogen bonds, hydrophobic interactions, ionic bonds and water bridges. The upper section illustrates the total count of interactions between the HDAC2 protein and the Vilazodone and Atenolol ligands throughout a molecular dynamic's simulation. The lower section indicates which amino acid residues engage with the ligands at each point in the simulation. Some specific residues such as ASP181, HIS183 and ASP269 interact with both ligands shown in a shade of orange on the scale positioned to the right of the graph

4. Conclusions

The study presented a technique to discover HDAC2 blockers by employing computer methods such as screening, molecular docking, MM GBSA and molecular dynamics simulation.

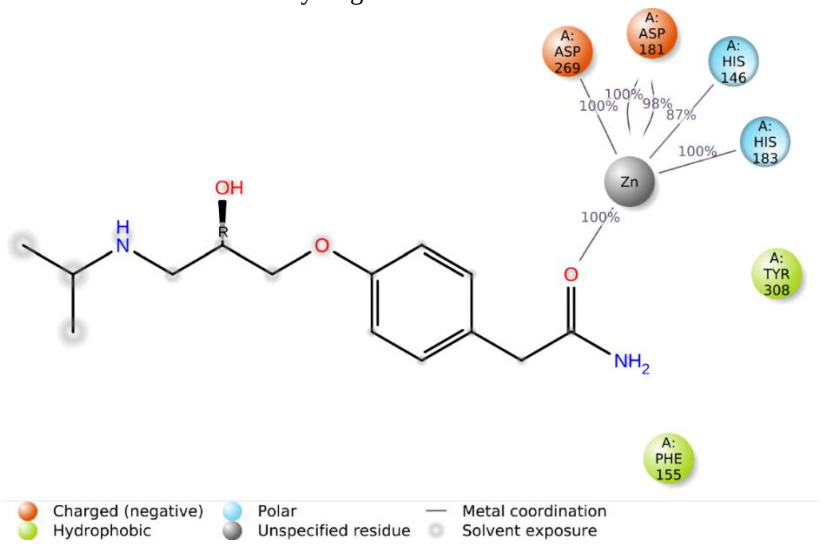


Figure 10. HDAC2 and Atenolol Hydrogen bonds contacts During over 30% of the simulation time.

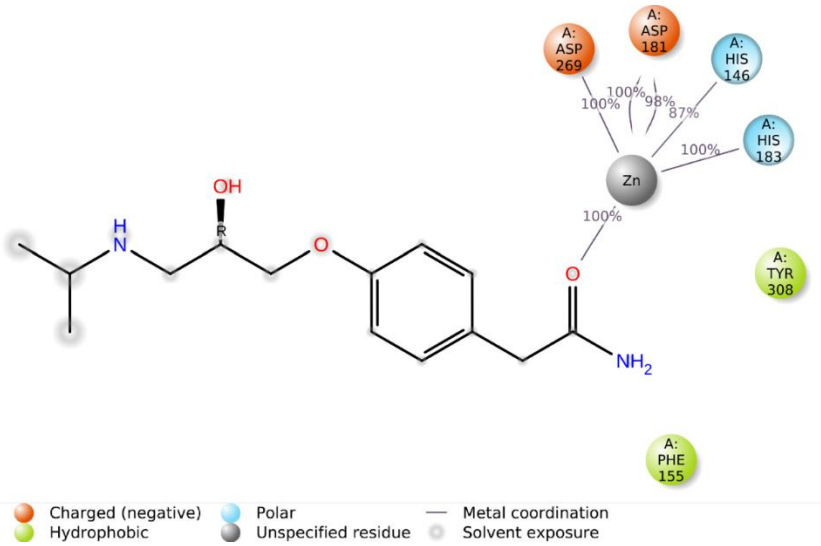


Figure 11. Total HDAC2-Vilazodone Contacts derived from the dynamics simulation trajectories.

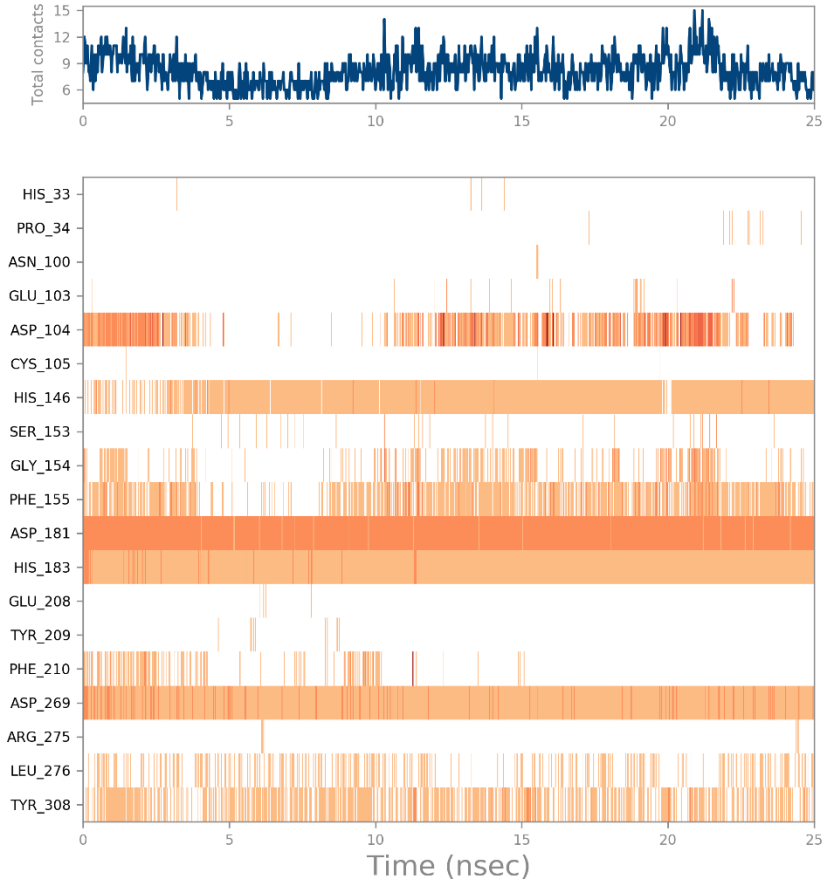


Figure 12. Total HDAC2-Vilazodone Contacts derived from the dynamics simulation trajectories.

The aim was to investigate the effectiveness of FDA approved drugs as inhibitors of HDAC2 for treating cancer types.

After analyzing than 4,000 FDA approved medications using virtual screening techniques and conducting docking assessments followed by MM GBSA calculations we discovered two potential compounds Vilazodone and Atenolol with strong binding affinities to HDAC2. Subsequent molecular dynamics simulations verified the stability of these compounds when interacting with HDAC2 over a 25-nanosecond trajectory.

The examination of how proteins and ligands interact showed bonding relationships such as hydrogen bonds and metal coordination. This highlights the potential of Vilazodone and Atenolol as promising inhibitors for HDAC2.

The results of the study indicate that using FDA approved medications to inhibit HDAC2 could present opportunities for creating cancer fighting drugs. This comprehensive computer-based method demonstrates how virtual techniques can speed up the drug discovery process and pinpoint drug options which can help advance cancer treatment. Moreover, conducting tumor tests on cell lines is necessary to validate these virtual findings.

Acknowledgement

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Declaration of Competing Interest

There are no conflicts to declare.

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