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COMPUTATIONAL IDENTIFICATION OF HDAC6-TARGETED INHIBITOR CANDIDATES FOR CANCER THERAPY

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Abstract

Cancer continues to impact millions of people worldwide and incur significant economic costs despite advances in modern therapeutics. Precision medicine offers a promising approach to overcome the harmful off-target impacts on healthy tissue of conventional therapies. In particular, HDAC6 is an attractive therapeutic target due to its pivotal role in facilitating tumor growth. This study employs a comprehensive computational framework to identify potential HDAC6 inhibitor candidates to accelerate early-stage drug discovery. First, binding sites were identified within the HDAC6 structure to determine its suitability for binding with small molecules. Then, pharmacophore modeling identified molecular features required for favorable interactions with the HDAC6 binding pocket. The resulting compounds underwent molecular docking to determine their predicted binding affinities. Then, the pharmacokinetic properties and toxicity of the remaining compounds were analyzed to identify the compounds that best balanced favorable interactions with the HDAC6 binding site, suitable pharmacokinetic properties, and minimal predicted safety liabilities. Based upon this workflow, Molport-023-219-174 was determined to be the lead compound and Molport-035-800-274 was a promising backup compound. The integration of these computational processes streamlined the early-stage drug discovery pipeline, enabling the efficient identification of promising HDAC6 inhibitors and providing a foundation for drug-discovery workflows.

Keywords: *Drug discovery, HDAC6, cancer therapies, virtual screening, molecular docking*

Introduction

Cancer is one of the most devastating global health challenges, accounting for approximately 19.3 million new cases and nearly 10 million deaths worldwide in 2020 (Sung et al., 2021). In fact, by 2050, global cancer diagnoses are projected to increase to 30.5 million and deaths will increase to 18.6 million (GBD 2023 Cancer Collaborators, 2025),

demonstrating the inherent danger of cancer despite advancements in diagnostic procedures and therapeutics. Additionally, cancer incurs significant economic costs with the global economic cost from 2020 to 2050 estimated to be approximately US \$25.2 trillion due to factors including healthcare expenses, productivity losses, and premature mortality (Chen et al., 2023). Beyond its clinical im-

pact, cancer significantly burdens healthcare systems, patients, families, and economies, highlighting the urgent need for effective therapeutics to mitigate its impact.

Researchers have attempted to find effective cancer therapies, contributing to the wide array of modern treatments available. Currently, the most pervasive cancer therapies include surgery, chemotherapy, radiation therapy, and hormonal therapy; while each of these treatment options has demonstrated clinical efficacy in certain cancer types, stages, and conditions, they are often limited by factors such as drug resistance, treatment-related toxicity, limited precision, and reduced long-term efficacy (Kouidhi et al., 2024). A prevalent concern about current cancer therapies, especially radiation and hormone therapy, is that they frequently suffer from poor targeting precision, resulting in damage to healthy tissue and hindering patient recovery (Zafar et al., 2025). In particular, chemotherapy has empirically constituted negative side-effects such as damage to healthy tissues and suppression of healthy tissue due to the non-discriminate nature of its treatment mechanism (Zafar et al., 2025). Overall, while current treatment methods have significantly decreased the mortality rate of cancer, their off-target effects often complicate treatment and negatively impact patients' health.

Due to these clinical complications, researchers have invested significant time and resources into the field of precision medicine in an attempt to discover therapeutics that selectively target malignant cells. Targeted therapies have received attention in academia due to their potential to improve treatment efficacy while minimizing damage to healthy cells (Meslamani, 2023). For example, the ROME trial revealed that initially conducting Comprehensive Genomic Profiling (CGP) in patients helped identify an effective drug that specifically targeted genetic mutations perpetuating the spread of cancer (Marchetti et al., 2025). Precision medicine adopts a paradigm in which cancer is not solely evaluated by the tissue where it originates but also based on molecular abnormalities (Tsimberidou et al., 2020). This approach ensures that cancer therapies directly interfere with biological pathways causing tumor growth rather than unnecessarily impacting surrounding tissue.

Within the field of precision medicine, there has been particular interest in inhibiting the enzyme HDAC6. HDAC6 is a histone deacetylase enzyme located in the cytoplasm, characterized by its ability to deacetylate several non-histone proteins involved in cell signaling, protein degradation, and cytoskeletal organization (Chen and Zhang, 2026). HDAC6 has been shown to promote cancer cell growth by deacetylating key targets such as HSP90, thereby maintaining the growth and function of cancer-causing proteins (Chen and Zhang, 2026). Additionally, HDAC6 deacetylates structural proteins such as α -Tubulin and Cortactin, significantly modifying cellular adhesion and mobility, increasing the risk of cancer growth and metastasis (Huang et al., 2024). Due to these properties of HDAC6, researchers have sought methods to inhibit it and consequently hinder its cancer-growth properties. Several investigational compounds have emerged to inhibit HDAC6, including Ricolinostat and SW-100; however, a common limitation with these therapies is that they may also inadvertently target other HDAC isoforms, such as HDAC8 (Ho et al., 2020). Candidates that inadvertently target other HDAC isoforms have been associated with adverse medical consequences, including thrombocytopenia and neutropenia (Subramanian et al., 2010). As a result of these off-target impacts, current inhibitors also have limited therapeutic windows, as higher doses risk severe bodily harm (Ho et al., 2020). Despite the discovery of several potential therapies, they face many limitations and require further research to address. It is necessary for researchers to continue developing HDAC6 inhibitors that effectively suppress tumor-promoting pathways while minimizing off-target interactions.

Therefore, the aim of the study was to find suitable molecular candidates that could inhibit HDAC6. This was achieved through a sequential computational screening workflow that integrated binding-site analysis, pharmacophore-based virtual screening, molecular docking, Absorption, Distribution, Metabolism, and Excretion (ADME) prediction, and toxicity assessments. Candidate compounds were progressively prioritized through each stage of experimentation, ultimately resulting in a lead and backup com-

pound based upon predicted binding affinity, drug-like properties, and safety profiles.

Materials and methods

Identification of HDAC6 Binding Sites Via Geometric and Machine Learning Analysis

The process of finding those candidates began with an analysis of the binding sites of HDAC6 in order to determine which types of small molecules would contain the strongest binding potential. Several computational methods were used to identify binding sites within the HDAC6 protein, including a geometric analysis and a machine learning analysis. First, the geometric analysis was conducted through the DoGSiteScorer, a computational tool that identifies and characterizes potential ligand-binding pockets on a protein's three-dimensional structure and evaluates their druggability based on geometric and physicochemical properties. The PDB ID 6V79 representing the crystal structure of the HDAC6 catalytic domain was used to render a visual representation of the protein and its binding sites.

Within the DoGSite Scorer, the ligand QQD, a known inhibitor of HDAC6, was used as a reference to identify a binding site where QQD and other potential inhibitors could have favorable interactions with the protein. DoGSiteScorer generated predicted binding pockets and reported quantitative descriptors including pocket volume, surface area, and druggability score. Additionally, to better assess the viability of the HDAC6 binding sites, a machine learning analysis was conducted using the Prankweb platform. Prankweb analyzes the structural and physicochemical properties of proteins and compares them to hundreds of protein-ligand combinations to identify regions that are most likely to bind small molecules. From Prankweb, a visual representation of binding sites was collected, along with other data points that ranked the binding sites' viability, such as a drug score and number of residues.

Pharmacophore-based Virtual Screening of the Molport Compound Library

After confirming that the HDAC6 protein contains binding sites suitable for small

molecules, a compound library was screened against pharmacophore models, maps of essential chemical features a molecule needs to bind to a biological target, to find small molecules that could favorably bind to the HDAC6 binding sites. These models were generated from the HDAC6 crystal structure in Pharmit, a web-based platform for constructing pharmacophore models.

Various interactions were omitted or added to the model to determine the most optimal combinations of chemical features (e.g. hydrogen-bond donors, acceptors, hydrophobic regions, and aromatic features) that could represent the binding requirements of the HDAC6 binding site. Then, each pharmacophore model was screened with the Molport chemical library, which is a repository of thousands of commercially available small molecules. All of the molecules within Molport were evaluated using their Root Mean Square of Deviation (RMSD), where molecules with lower RMSD values indicate a greater likelihood of matching the pharmacophore.

After several pharmacophore models were created, three models were selected for further screening based on their ability to maintain chemical diversity while containing a manageable number of candidate molecules. Chemical diversity was assessed by visual inspection of the molecular structures of each pharmacophore model. The three resulting pharmacophores were screened to remove duplicate candidates. After this filter was applied, each of the three pharmacophores contained a significantly reduced number of chemical hits. From the remaining options, five were selected from each pharmacophore based on their low RMSD values and structural diversity. This resulted in fifteen compounds being considered as potential chemical inhibitors of HDAC6 and they were selected for docking in the next stage of the study.

Molecular Docking to the HDAC6 Catalytic Domain

Docking of these compounds was conducted in Swissdock, a web-based molecular docking platform that predicts the binding affinity of ligands within a target protein. Each compound was computationally docked into the HDAC6 catalytic domain to predict its preferred binding orientation and es-

timate the strength of its interaction with the protein. The metric used to analyze the binding strength of each compound in various locations of the protein was the SwissParam score, where more negative values indicate more energetically favorable predicted protein-ligand interactions and therefore a stronger binding affinity. Of the fifteen compounds identified through pharmacophore screening, the ten compounds with the most favorable SwissParam scores were selected for further analysis.

Prediction of Pharmacokinetic and Drug-likeness Properties

The next stage of the experiment was to analyze the ADME of the selected compounds to determine their suitability as potential drug candidates. The ADME of these compounds was analyzed using the SwissADME platform, where key pharmacokinetic and drug-likeness parameters were predicted to determine whether a compound's molecular weight, hydrogen bond donors and acceptors, and lipophilicity (LogP) would comply with Lipinski's Rule of Five.

Lipinski's Rule of Five was applied because it is a widely accepted guideline for identifying drug-like molecules, filtering out compounds based upon empirically proven metrics associated with poor absorption or permeability (Lipinski et al., 2001). Additionally, the predicted water solubility of each of the compounds through the Estimated Solubility (ESOL) framework and gastrointestinal absorption (GI) were assessed, adding a further layer of analysis of the viability of these candidates. For water solubility, the ESOL metric was specifically analyzed because it is a widely used quantitative model predicting solubility for small molecules especially in a pharmaceutical context.

Predicted Toxicity and Safety Profiling

Five compounds were selected to advance to the next stage of screening due to both their favorable pharmacokinetic properties, as validated through the ADME analysis, and their predicted binding affinity from molecular docking. The ProTox online platform was used to analyze each of the compounds' predicted toxicity class, LD₅₀ values, and potential toxicity targets. The

predicted toxicity class was used to estimate the overall acute toxicity of each compound, with higher classes indicating lower toxicity. LD₅₀ values were evaluated to estimate the dose required to cause mortality in 50% of a test population, with higher values indicating lower acute toxicity. Additionally, potential toxicity targets were analyzed to predict interactions with biological systems and targets in order to accurately measure safety risks. The toxicity radar chart from ProTox provided information about each compound's predicted toxicity profile compared to FDA-set benchmarks.

Results and Discussion

Binding Site Analysis

To initially determine whether HDAC6 contains suitable binding spots for potential druggable small molecules, both geometric and machine learning analyses were performed. DoGSiteScorer accounted for factors including surface area, volume, and shape to identify sites with favorable geometric characteristics that could facilitate interactions between HDAC6 and potential small molecules. Along with a geometric analysis, Prankweb was used to validate these sites by using machine learning to predict which HDAC6 regions were most likely to bind ligands.

An initial geometric analysis of binding sites was conducted in DoGSiteScorer, which used the ligand QQD as a reference to locate binding sites within the HDAC6 protein. There were twelve potential binding sites. However, only the site with a volume of 873.07 Å³ (P-0) was further analyzed because it contained the greatest volume out of the sites, indicating it contains the greatest capacity to accommodate potential small molecules. This result is shown in Table 1.

All remaining binding sites had substantially smaller predicted volumes, which could limit interactions between small molecules and the HDAC6 protein and were therefore omitted from consideration. Additionally, Table 1 demonstrates that P-0 tied with the highest druggability score of 0.78, further corroborating the site's suitability for small-molecule binding based upon features including the site's volume, shape, surface properties, and hydrophobicity.

Table 1. *Do GSite Scorer-predicted HDAC6 binding pockets, ranked by volume, with corresponding druggability scores*

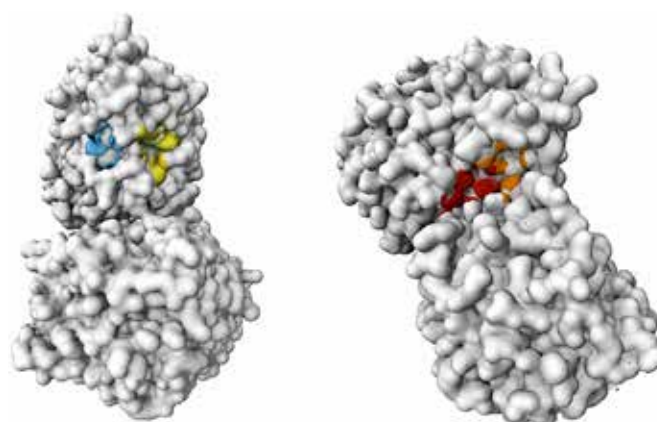
Binding Site	Volume (Å ³)	Druggability Score
P_0	873.07	0.78
P_1	466.15	0.78
P_10	165.55	0.23
P_11	64.86	0.32
P_2	351.67	0.59
P_3	483.48	0.70
P_4	271.75	0.51
P_5	88.71	0.55
P_6	294.25	0.60
P_7	249.31	0.24
P_8	131.95	0.44
P_9	151.29	0.28

These results were complemented by further analysis of the binding sites in Prankweb, which demonstrated that there are four potential sites within HDAC6, as shown in Table 2.

Table 2. *Prankweb-predicted HDAC6 ligand-binding sites, ranked by probability, with associated residue counts*

Rank	Probability	Number of Residues
1	0.890	20
2	0.817	14
3	0.041	10
4	0.014	8

Figure 1. *Prankweb-rendered visualization of predicted HDAC6 binding sites, showing two orientations of the protein structure with potential binding pockets highlighted as colored regions*



The site ranked first had a probability of 0.890, indicating high confidence that the site represents a ligand-binding site. The second highest ranked site had a probability of 0.817,

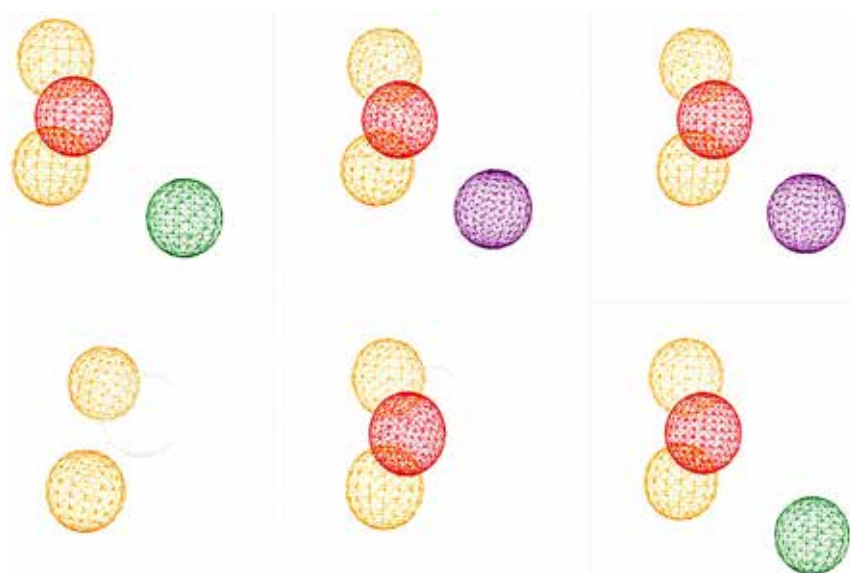
similarly corresponding to a relatively high level of confidence that this site represents a ligandbinding site. These high prediction scores suggest that the identified sites possess

structural and physicochemical characteristics similar to those of experimentally validated ligand-binding sites. Additionally, an analysis of residues demonstrated that these sites had the potential to facilitate ligand binding, as residues (amino acids) provide the necessary side chains essential for hydrogen bonding and other types of ligand interactions. The sites with the highest probabilities predicted by Prankweb contained 20 and 14 residues, respectively, further highlighting their potential to facilitate efficient ligand interactions. Additionally, Prankweb generated images highlighting potential binding sites on the HDAC6 protein, as shown in Figure 1. Overall, both the geometric and machine-learning analyses confirm that HDAC6 contains structurally and biologically favorable binding sites for small-molecule inhibitors. These findings justify the selection of HDAC6 for pharmacophore analysis and virtual screening in subsequent procedures of the study.

Pharmacophore-Based Virtual Screening

The subsequent pharmacophore analysis and virtual screening further indicated the potential to develop HDAC6 inhibitors. To test how different compounds interact with the binding sites of the protein, researchers typically employ both physical screening and virtual screening. The advantage of physical screening is that it directly measures the binding activity of compounds in real-life conditions, providing highly reliable evidence of their inhibitory potential. However, this process is often extremely time-, money-, and resource-intensive because thousands of compounds must be individually synthesized and experimentally tested. Therefore, researchers also employ virtual screening, which uses computational methods to rapidly evaluate large chemical libraries and identify compounds that have the potential to bind to the target protein.

Figure 2. Six pharmacophore models generated in Pharmit with varying combinations of chemical features. Colored spherical regions denote different chemical interaction features used to define each model. Naming convention: Pharmacophore 1, 2, 3 (top), Pharmacophore 4, 5, 6 (bottom)



This process significantly decreases the number of compounds tested and makes the overall drug discovery process more efficient and cost-effective. In this study, virtual screening was conducted with a pharmacophore model from Pharmit. The pharmacophore was edited to add or omit several interactions to determine chemical features that best represented a suitable combination of

chemical characteristics of the binding requirements of the HDAC6 active site. In Pharmit, only the QQD molecule was used to generate a pharmacophore map because it is a known HDAC6 inhibitor; using it as a reference ensured that the pharmacophore represented the essential features that allow for ligand binding. The Pharmit application identified thirteen chemical features that

allowed for ligand binding. Several iterations of the pharmacophore were created with 4 to 6 different features to determine which model most accounted for the binding requirements of HDAC6 while also being relatively selective. Six different pharmacophores were created, with varying combinations of chemical features, as shown in Figure 2.

After each map was created, it was screened with the Molport dataset of compounds to find suitable compounds that could bind to the HDAC6 protein under the chemical conditions within the map. The resulting compounds were evaluated according to their Root Mean Square Deviation (RMSD), which measures how closely a molecule's chemical features align with the pharmacophore. Of the six pharmacophore models created, only three were selected for further screening because they provided the best balance between chemical interaction diversity and the selectivity of potential hits. Some of the models produced an excessively large number of hits, indicating that the chemical constraints were unlikely to identify specific HDAC6 inhibitors. Moreover, while some of the models produced a more constrained list of chemical hits, they contained little to no variety in chemical interactions within the models themselves. Specifically, pharmacophores 3, 5, and 6 were screened further.

To decrease the number of hits to a viable set of candidate molecules, only compounds with an RMSD of less than 0.1 were considered. This threshold was used in this study as it prioritized compounds that demonstrated the closest resemblance to the pharmacophore model. Out of the remaining options, 5 com-

pounds were chosen from each pharmacophore. The selection of these compounds was not only based upon low RMSD values but also on structural diversity to avoid overrepresentation of compounds sharing the same chemical structure. This approach ensured that analogous compounds with similar binding characteristics were not selected for docking.

Molecular Docking to HDAC6

After fifteen suitable compounds were selected, molecular docking was conducted to determine whether the compounds that were chosen from the pharmacophores were capable of finding favorable binding locations within HDAC6. While pharmacophore screening can identify molecules that satisfy the chemical requirements of HDAC6, it cannot predict the viability of three-dimensional interactions between the compound and HDAC6. Therefore, docking provides an additional computational assessment of whether the selected compounds can adopt energetically favorable predicted binding poses. Docking provided an estimate of the SwissParam score for each protein-ligand interaction, allowing for a comprehensive analysis of potential inhibitors based upon their predicted binding affinity. The SwissParam scores represent the thermodynamic favorability of a binding interaction, with more negative values indicating a more energetically favorable protein-ligand complex, leading to a greater binding affinity. For each docking experiment, the lowest SwissParam score was collected to represent the most favorable interaction for each compound. The values are displayed in Table 3.

Table 3. *SwissParam scores for compounds associated with pharmacophore models 3, 5, and 6*

Pharmacophore Number	Compound	Swiss Param Score (kcal/mol)
3	Molport-004-327-550	-6.3968
3	Molport-006-013-429	-6.1620
3	Molport-027-636-748	-7.3761
3	Molport-035-907-729	-6.5961
3	Molport-002-985-507	-6.2479
5	Molport-004-336-101	-6.5282
5	Molport-000-478-723	-6.0724

Pharmacophore Number	Compound	Swiss Param Score (kcal/mol)
5	Molport-004–331–688	-6.2312
5	Molport-023–219–174	-7.2671
5	Molport-004–917–109	-6.6884
6	Molport-004–338–175	-6.2658
6	Molport-046–683–866	-6.3438
6	Molport-002–464–756	-6.9570
6	Molport-035–800–274	-6.7653
6	Molport-004–336–041	-6.1219

ADME profiling

Then, the ten compounds with the lowest SwissParam scores were selected for the next set of experiments. At this point of the study, all of these compounds had demonstrated favorable predicted binding affinities, highlighting their potential as candidate HDAC6 inhibitors. The consequent experiment conducted was the evaluation of these candidates' ADME properties because compounds with favorable docking scores may exhibit poor absorption, rapid metabolism, or other pharmacokinetic limitations that reduce their therapeutic effectiveness. ADME performance was measured through Lipinski's rule to determine if these compounds con-

tained physicochemical properties associated with oral bioavailability. Of the 10 compounds analyzed, all satisfied Lipinski's Rule of Five, indicating that their physicochemical properties were consistent with established criteria for drug-likeness. Additionally, all of the compounds contained a water solubility metric of "high" or "very high," and GI absorption for all of the compounds was high. These findings highlight that all of the candidate molecules exhibited pharmacokinetic properties consistent with effective absorption and oral drug-likeness. The findings from this analysis are displayed in detail in Table 4.

Table 4. *Physicochemical and pharmacokinetic properties of the selected lead compounds predicted using SwissADME. MW = molecular weight (g/mol); HBD = hydrogen-bond donors; HBA = hydrogen-bond acceptors; ESOL = estimated water solubility; GI = gastrointestinal absorption*

Compound	MW	HBD	HBA	LogP	ESOL	GI
Molport-027–636–748	298.09	2	4	0.60	Soluble	High
Molport-023–219–174	296.32	2	3	2.17	Soluble	High
Molport-002–464–756	239.27	2	4	1.80	Soluble	High
Molport-035–800–274	243.26	2	3	2.19	Soluble	High
Molport-004–917–109	196.16	4	4	-0.28	Very Soluble	High
Molport-035–907–729	207.27	2	2	2.66	Soluble	High
Molport-004–336–101	202.21	2	3	1.49	Soluble	High
Molport-004–327–550	187.19	2	2	2.01	Soluble	High
Molport-046–683–866	221.13	2	6	2.03	Soluble	High
Molport-004–338–175	167.16	2	3	0.66	Very Soluble	High

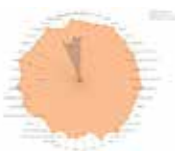
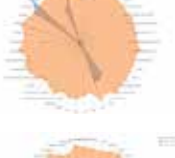
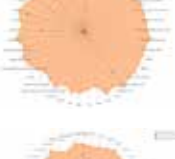
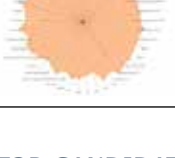
To continue experimentation with the compounds with the greatest potential for HDAC6 inhibition, 5 compounds were selected for further evaluation. Particularly, Molport-027–636–748 was an especially promising candidate at this stage because it contains the lowest SwissParam score (–7.3761) out of the 10 compounds, indicating the greatest predicted binding affinity toward the HDAC6 active site. The compound also fully complies with Lipinski's Rule and contains a high predicted solubility and GI absorption, validating its potential as a suitable drug candidate. Molport-023–219–174 was selected because it exhibited the second-most favorable docking score (–7.2671), also highlighting its binding potential. Its predicted logP of 2.17 was also consistent with the physicochemical criteria used to evaluate oral drug-likeness due to its indicated moderate lipophilicity. Molport-002–464–756 and Molport-035–800–274 also produced suitable SwissParam scores and contained LogP values

(1.80 and 2.19, respectively) that effectively balance aqueous compatibility and lipophilicity. Finally, Molport-004–327–550 was selected as the final compound for the next experimental stage due to it containing the 5th lowest SwissParam score and exhibiting a LogP value of 2.01, suggesting an effective balance between aqueous compatibility and lipophilicity. Overall, these five compounds demonstrated the strongest combination of predicted HDAC6 binding affinity, ADME metrics, and drug-like properties, justifying their inclusion in subsequent toxicity evaluation.

Toxicity screening and final candidate selection

Afterwards, extensive toxicity screening and analysis was conducted upon these five compounds. This was to assess their predicted safety profiles and determine if any concerns about toxicity hinder further evaluation. The results of the toxicity screening are displayed in Table 5.

Table 5. Predicted toxicity profiles of the selected compounds. LD_{50} values are reported in mg/kg

Compound	Toxicity Class	LD_{50} (mg/kg)	Cluster-Associated Toxicities	Toxicity Radar Chart
Molport-027–636748	3	200	BBB, neurotoxicity, clinical toxicity, mutagenicity, DILI, nephrotoxicity, respiratory toxicity	
Molport-023–219174	4	1250	BBB, clinical toxicity, mutagenicity, DILI, nephrotoxicity, respiratory toxicity, carcinogenicity	
Molport-002–464756	4	2000	BBB, immunotoxicity, clinical toxicity, mutagenicity, nephrotoxicity, respiratory toxicity, MIE-TR α , MIE-TR β , MIE-TTR	
Molport-035–800-274	4	2000	BBB, clinical toxicity, mutagenicity, DILI, nephrotoxicity, carcinogenicity, MIE-TTR	
Molport-004–327-550	4	1160	BBB, clinical toxicity, mutagenicity, DILI, carcinogenicity, CYP1A2 interaction, MIE-TTR	

All of the compounds contained a toxicity class of 3 or greater, indicating they were predicted to exhibit moderate to relatively low predicted acute toxicity according to the externally validated Globally Harmonized System (GHS) classification. However, as Molport-027–636–748 was the only compound to exhibit a toxicity of class 3, it exhibited the highest predicted acute toxicity among the selected compounds, warranting caution for its continued evaluation. Molport-035800–274 and Molport-002–464–756 jointly exhibited the greatest potential LD_{50} value (2000 mg/kg) out of all of the compounds, indicating that they contained the lowest predicted toxic potency relative to the other compounds. For the rest of the compounds, LD_{50} values ranged from 200 to 1160, showcasing variation in predicted acute toxicity. Analysis of the cluster-associated toxicity endpoints further revealed differences in the predicted toxicological profiles of the selected compounds. For all of the compounds, blood-brain barrier permeability, clinical toxicity, and mutagenicity were predicted, while drug-induced liver injury (DILI), nephrotoxicity, carcinogenicity, respiratory toxicity, immunotoxicity, CYP1A2 interaction, and thyroid-related molecular initiating events (MIE-TTR, MIE-TR α , and MIE-TR β) were all factors that varied in each of the compounds. These differences in the toxicity profiles of the compounds suggest that while the compounds may share some similar toxicological liabilities, they also differ in specific combinations of interactions and predicted toxicity endpoints, necessitating further review of specific toxicological conditions. Therefore, the toxicity radar chart was analyzed for each compound to determine if these toxicological liabilities truly hindered the compound's effectiveness. The toxicity radar charts, inserted in Table 5, provide a visual representation of the level of toxicity for each biological target or system for each compound as compared to similar compounds present in drugs containing FDA approval.

As a result of this final experiment, Molport-027–636–748 was eliminated from consideration due to its low predicted LD_{50} value (200 mg/kg), demonstrating a higher predicted acute toxicity than the remaining candidate compounds. Additionally,

Molport-002–464–756 was also excluded from further consideration as a suitable inhibitor due to its predicted elevated levels of immunotoxicity, as shown in the toxicity radar chart, raising concerns about its overall safety profile. Consequently, Molport-023–219–174 was determined to be the most promising candidate due to its comparatively favorable docking affinity (SwissParam score = -7.2671) among the rest of the compounds, suggesting a stronger predicted binding interaction with the HDAC6 binding site. Additionally, this compound also contains favorable pharmacokinetic properties, complies with Lipinski's Rule of Five, and contains a toxicity profile that is more favorable relative to the other candidates, further validating its selection as the lead candidate for further experimentation. Molport-035–800–274 emerged as a promising backup compound due to it containing the second-highest docking affinity (SwissParam score = -6.7653) as well as its favorable ADME profile and the absence of significant toxicity liabilities.

Conclusion

As HDAC6 promotes tumor progression by deacetylating regulatory enzymes and structural proteins, it has become a compelling target for inhibition and consequent therapeutic intervention. This study analyzed the potential of a computational drug discovery workflow to identify novel HDAC6 inhibitors. Initially, the computational platforms DoGSiteScorer and Prankweb were employed to identify suitable binding sites within the HDAC6 structure. Then, pharmacophore modeling was conducted through Pharmit to identify the molecular features necessary for favorable interactions with the HDAC6 binding pocket, which allowed for the selection of candidate compounds with the highest predicted binding affinity. Fifteen compounds were specifically selected from this process, and they underwent docking in SwissDock to evaluate their predicted binding affinities. Subsequently, the ADME of ten of these compounds was evaluated by determining their compliance with Lipinski's Rule of Five as well as their water solubility and GI absorption. Finally, based on both ADME results and binding affinity, five

compounds were selected for toxicity analysis, where metrics including LD₅₀, predicted toxicity class, and a toxicity profile were used to determine a lead compound that exhibited the most favorable balance of predicted binding affinity, pharmacokinetic properties, and safety profile. After this comprehensive workflow, Molport-023–219–174 was determined to be the lead compound due to its favorable predicted binding affinity (–7.2671), pharmacokinetic properties, and toxicity profile, while Molport-035–800–274 was recognized as a promising backup candidate. These compounds have the potential to be utilized in further experimental validation and may contribute to the development of precise and effective HDAC6 inhibition therapeutics for cancer treatment.

However, several opportunities remain for future research. The study only used computational methods to determine potential inhibitor candidates for HDAC6, which may limit evaluation of these candidates under biological conditions. Future studies should evaluate the inhibitory potential of the lead and backup compounds through in vitro and

in vivo experiments to assess their therapeutic efficacy. Furthermore, studies that expand the scope of compounds assessed for binding affinity, favorable ADME, and acceptable toxicity thresholds can provide additional insight into potential inhibitors and the common pharmacokinetic traits these inhibitors may possess. Overall, this study presents a promising drug discovery pipeline through the integration of various computational methods, accelerating the identification of early-stage drug candidates by reducing the time and resources necessary for experimental validation. By enabling the efficient identification of suitable HDAC6 inhibitor candidates, this proposed framework has the potential to assist in the development of more selective cancer therapeutics.

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