COMPUTATIONAL APPROACH FOR HDAC1 PREDICTING PROTEIN-LIGAND INTERACTIONS FOR CANCER THROUGH HOMOLOGY MODELLING, VIRTUAL SCREENING AND MOLECULAR DOCKING

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Abstract

Histone Deacetylase 1 (HDAC1) is vital for controlling gene expression, chromatin remodelling, and biological functions like distinction and cellular proliferation by acetylation of histone tail residues. Its abnormal expression is of high significance in inflammatory diseases, cancers and allergic diseases. Thus, considering the structure and function of HDAC1 is very crucial because it may be used as a therapeutic target for cancer, neurologicalillnesses, and other disorders. In this study, the structure of HDAC1 was predicted using homology modelling. First, based on the known crystal structures of template proteins, a high-quality 3D model of HDAC1 was generated using the concepts of homology modelling by Modeller 10.6 software. The correctness and reliability of this model were enhanced using a GalaxyWEB refine tool and for energy minimization YASARA software was put to use.The model obtained after refinement and energy minimization was put for validation on the ProSA-WEB and SAVES server where the results of z-score, ERRAT, VERIFY-3D and Ramachandran plot were obtained and analysed.

The analysed model was then employed to investigate the binding interactions with ligands that may inhibit HDAC1 activity, in molecular docking studies. The steps involved were performed using Schrodinger suites 'Maestro 13.5 included: protein preparation using protein preparation wizard, grid generation using GLIDE and virtual screening employing molecular docking using virtual screening workflow wizard. Therefore, through this study HDAC1's structure was predicted, validated and molecular docking studies were performed on it.

Keywords: *Cancer, HDAC1, Homology modelling, Virtual Screening and Molecular Docking*

I. Introduction

Histone deacetylases (HDAC) will remove the acetyl group from histone. HDAC plays a crucial role in gene regulation. It was classified into 4 classes, they are class 1, class 2,class 3,class 4.In class 1 (HDAC1,2,3,8) were present located in the nucleus. In class 2 (HDAC4,5,6,7,9,10) were present which are located in between the nucleus and cytoplasm. Next class 3 all the sirtuins like (STRT1,2,3,4,5,6,7) were present NAD+ and dependent enzymes were present and in class 4 HDAC 11 was present which shows similarity between class 1 and class 2 HDAC (Ye et al., 2009).

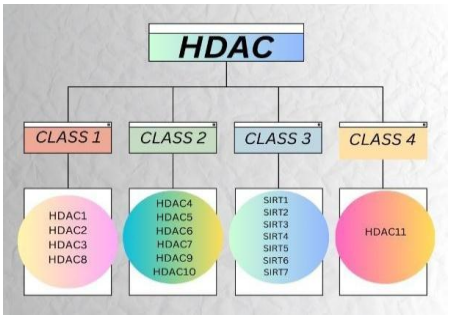


Figure 1 Classification of HDAC in Human Cancer

Histone deacetylases (HDACs) are predominantly found in multi-protein complexes, including the transcriptional corepressors mSin3, N-CoR, and SMRT (Glass et al., 2000). The complexes are directed to the target genomic locations by interactions with DNA-binding factors, including transcription factors, nuclear receptors, and other epigenetic regulators.These regulators are methyl-binding proteins (MBDs), DNA methyltransferases (DNMTs), and histone methyltransferases (HMTs) (Minucci et al., 2006). Of the interactions between these proteins, recruitment of HDACs to methylated DNA by methyl-binding proteins is the best-characterized. A well-studied case is the interaction of HDACs with MBDs, specifically MeCP2, which recruits HDAC-containing complexes to methylated gene promoters and thus mediates transcriptional repression (Jones et al., 1998) (Nan et al., 1998) In addition, HDACs are also associated with DNA methyltransferases.(Nan et al., 1998) For example, in DNMT1-deficient cancer cells, accumulation of acetylated histone H3 and reduction of methylated histone H3 have been reported. These effects are associated with the impaired interaction of HDACs and the heterochromatin protein HP1 with histone H3. These observations strongly suggest that histone hyperacetylation is not necessarily the consequence of reduced HDAC activity but, instead, of the decrease in the site-specific recruitment of HDACs to chromatin.

Specific DNA sequences. Main clarification would be that alterations of Epigenetic mark also result in histone moderation through straight interaction among the enzymes controlling distinct epigenomic changes (Espada et al., 2004). HDAC2 plays a part in the control of adaptation by direct binding to the N-terminal domain of DNMT3b. Preincubation of the pheochromocytoma cell line PC12 with HDAC inhibitors suppresses nerve growth factor- induced differentiation of this cell line, but overexpression of the N- terminal domain of DNMT3b promotes differentiation(Bai et al., 2005). Epigenetics is defined as molecular mechanisms (Mirzaei et al., 2020). Which control Multifaceted molecular changes of DNA and chromatin, including histone acetylation and DNA methylation, participate in gene regulation without a change in the sequence of DNA. (He et al., 2019). The capacity to turn DNA replication on and off, thus significantly contributing to tumorigenesis and cancer, is given to epigenetics (Fyodorov et al., 2018). Out of all the epigenomic mechanisms, the importance of histone modification in carcinogenesis has been illustrated (Shanmugam et al., 2017). Specific enzyme complexes catalyze histone modification by means of ATP-using processes, in turn affecting gene transcription, replication, repair, and recombination (Shanmugam et al., 2017). The acetylation and methylation of amino-terminal (N-terminal)

tail domains of core histones and post-translational DNA-histone crosstalk control recruitment of proteins and transcription factors (Ellmeier et al., 2018). Histone acetylation is reversible modification of the N-terminal lysine of histones and is implicated in multiple cellular processes and disease onset (Adeegbe et al., 2017) (Gil et al., 2017) The chromatin is more open to allow for gene transcription when acetylated lysine occupies the histone tail. Deacetylation, on the other hand (Fullwiley et al., 2018). Strengthens the ionic interaction between the positively charged histones and negatively charged DNA. This deacetylation results in condensed chromatin, thus blocking gene transcription (Draney et al., 2018). They have been shown to exhibit high correlations with gene expression in malignant and non-malignant cell (Barneda-Zahonero et al., 2012). As the gene silencing complexes, HDACs have been shown to be involved in the repression of gene expression through the use of transcription factors such as E2F1. Additionally, acetylation can be reversed from non-histone proteins by HDACs (Yoon et al., 2016). The process by which T cells identify and kill cancer cells is suppressed by HDACs (Burke et al., 2020). But when HDACs are inhibited or knocked down in biological systems, T cells are capable of killing cancer cells.

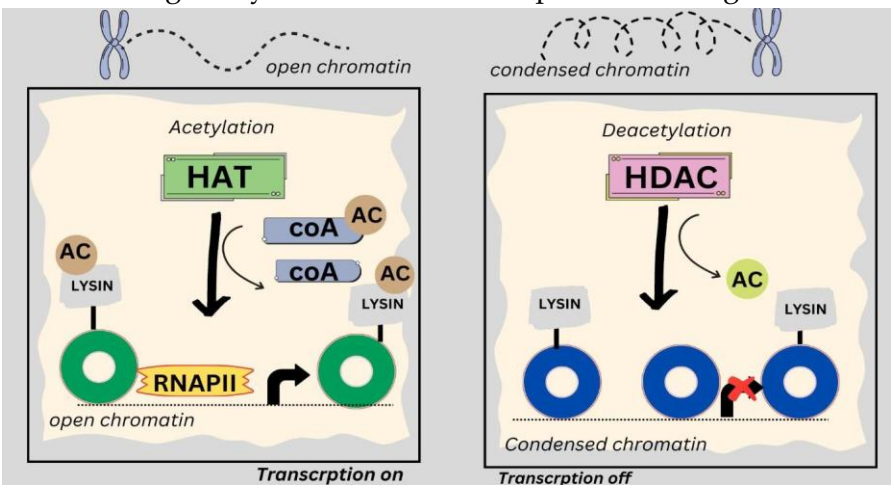


Figure 2 Acetylation by HATs and deacetylation by HDACs influence gene transcriptional activity. HATs and HDACs add or remove acetyl groups at the N- terminus lysine, which leads to an open or condensed state of the chromatin.

II. Methods & Materials

2.1 Template Selection and Sequence Alignment

The Three dimension of HDAC1 protein is not yet reported in PDB byX-ray crystallography, NMR nor Electron spectroscopy (Bhargavi et al., 2017). Of the protein was performed by taking 7JVU as template from BLASTserver (basic local alignment service tool) based on the Query coverage, sequence similarity and statistical E value. Alignment of the query protein (HDAC1) with the template 7JVU protein was conducted in CLUSTALX2 server (Thompson et al., 1994). MODELLER(Zare et al., 2011)10.6 server has been utilized to construct a novel three- dimensional structure, and twenty homology models were created from which the lowest energy model was chosen for further development. MODELLER is an application for homology or comparative protein 3D structure modeling. The user

supplies an alignment of the target sequence versus similar known structures, and MODELLER will produce a model with all the non-hydrogen atoms automatically. It will perform comparative protein structure modeling by fulfilling spatial restraints and provides several other capabilities. These comprise denovo protein loop modeling, optimization of several structural models from a flexible objective function, several sequence and/or structure alignment, clustering, database searching, and protein structure comparison (Fiser et al., 2000).

2.2 Refinement and Energy Minimization:

The least Molpdf model was submitted to GALAXYWEB (ko et al., 2012). Five best models are accessible for viewing and download on the site. GALAXYWEB is a web server for protein structure prediction, refinement, and related methods. This step relies upon the GalaxyRefine algorithm (Heo et al., 2013) (Lee et al., 2016). Later moving to energy minimization the loops regions were automatically refined model energy minimization was performed by utilizing an online energy minimization server YASARA. The 3D structure of the protein was acquired with the help of a template protein, which was stabilized after YASARA energy minimization and checked by quality validation tools such as PROCHECK, Verify3D, ERRAT.

2.3 Validation of 3D Model

UCLA-SAVESv6.1 was used to identify Three dimensional model was validated by PROCHECK stereochemical analysis with Ramachandran plot(Hosen et al., 2024). Dihedral angles φ against ψ of AA suitable conformations in protein structure have been explored in Ramachandran plot(Zhou et al., 2011). ProSA refered as Protein Structure Analysis .Validation of the protein is done with ProSA web server to recognize the likely structural mistakes and z- score of the selected model by differentiate it with the experimentally known proteins in PDB(Wiederstein et al., 2007)

2.4 Active Site Recognition

Active site prediction of HDAC1was carried out from computational tools and literature studies (Dundas et al., 2006). SCF BIO grew up as Supercomputing facility for Bioinformatics and Computational Biology IIT Delhi servers provide the volume and area of the binding cavity of every pocket to foresee the active site (Sarita et al., 2012) PDBsum (Laskowski et al., 2018)is used to identify the binding Site residues of target protein. Protein-ligand docking was performed with the help of Maestro 13.5 software to study the binding and interactions of a ligand with a hypothetical protein, paying attention to differnt AA. The model structure, active site, and binding site were viewed with the SCF BIO.

III .Virtual Screening & Molecular Docking Studies

3.1 Ligand Preparation

Low-energy three-dimensional conformations of every molecule of the aforementioned chemical databases were generated by employing the LigPrep module of the Maestro 13.5

suite Schrödinger. Maestro 13.5 (Lu et al., 2021)Maestro is Schrödinger's efficient gateway to state- of-the-art predictive computational modeling and machine learning protocols for molecular discovery. With an easy-to-use, sophisticated graphical user interface, Maestro gives all users a single point of entry for acquiring new molecular insights to inform their research.(Gorecki et al., 2020)24,000 molecules were submitted to the library to continue the process of LigPrep program and which results in conformers. LigPrep will elaborate the library of the ligands molecules into stable 3D molecules,and minimization was performed using OPLC_2005 force field(Chen et al., 2010).

3.2 Protein Preparation

Before the docking of ligands onto the active site of the protein, the protein was prepared with protein preparation wizard of Schrodinger's molecular docking tool. During this protein preparation all the water molecules and hetero atoms were eliminated. Hydrogen atoms were added to the protein.

3.3 Grid Generation

Grid generation acts as a significant role, targeting the site of protein molecules. Ligand complex are docked into target receptors in an attempt to discover docking parameters using Grid- Based ligand docking with computational tools. A grid was generated on the active site of the protein using the GLIDE Mastero Schrodinger suit was used to generate grid for energy minimised protein. including those needed to specify the correct ionization and tautomeric forms of amino acid residues like GLY 161, PHE 218, MET 284, PRO 283, TYR 316, GLY 314, PHE 163.The active site of the protein was specified for generating the grid .The screened ligands were then docked into the prepared grid, for which "standard precision mode" was selected. No constraints were defined (Firdhouse et al., 2015)

3.4 Virtual Screening:

Virtual screening (VS) is currently an integral part of the drug design and development process. VS is typically considered to be the selection of potential drug candidates from large collections of chemical structures using computational approaches (F Sousa et al., 2010) The generated models were submitted to virtual screening with the help of multiple steps in filtering like HTVS (high throughput virtual screening) SP (standard precision). XP (extra precision), The resultant top ranked molecules were prioritized on Glide score and least energy conformer further studied for Prime MM-GBSA energy calculations.

3.5 Molecular Docking:

Molecular docking is a type of computational modeling, which allows one to predict the preferred binding orientation molecule (e.g. ligand) to another (e.g. Receptor), when both of them interact with each other to produce a stable complex (Mukesh et al., 2011)To further refine the selection, molecular docking was performed followed by the analysis of ADME properties using the Qikprop module of Maestro Additionally the binding free energy (ΔG) was calculated using the prime- MMGBS method to understand and analyse the most favourable inhibitors.

ADME Properties:

The ADME characteristics of all the ligands were computed using the Qikprop module of the Schrödinger package. It's parameters control which molecules get marked as being unlike the other 95% of known drugs. It assists us in examining the pharmacokinetics and pharmacodynamics of the ligands by referring to the drug like attributes Predicted important ADME characteristics such as Glide score, glide energy, molW, DonarHB, AcceptorHB, human absorbent value and prime MMGBSA were checked(Vadivelu et al., 2015).The calculation of dg binding free energy of protein-ligand complex & prediction of ADME properties.

III. Results & Discussion:

The template was choosen from blast with their respective parameters like e value is 5e-107, percent identity is 43.11% and query coverage is 71%. As 7JVU was selected as they had proper ligand interactions and they are with respect to parameters.

After homology modelling using (Modeller 10.6) 20 models were generated for target protein in the logfile. Among these the least mol pdf value was selected as shown in (table 1) These models were constructed by aligning the target sequence with the selected template. In our case, Model 10 had the lowest molpdf value, making it the most optimal candidate.

MODEL NO:	MOLPDF VALUE
01	2460.37939
02	2506.05664
03	2551.02051
04	2545.26904
05	2495.94800
06	2547.35278
07	2627.06982
08	2346.47754
09	2520.06006
10	2403.68530
11	2487.04272
12	2543.25903
13	2735.87109
14	2583.75391
15	2583.75391
16	2492.84692
17	2650.51001
18	2593.29614
19	2712.75610
20	2681.69385

Table 1 generated models after homology modelling

From the generated models to enhance accuracy the least molpdf value was selected and the

structure was shown in (figure 3). That least energy was further refined using galaxy web server as shown in (figure 4) .The minimised model was shown in (Figure 5) 3D structure of protein shown in (figure 6)

Figure 3 least value of molpdf

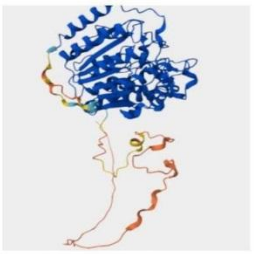


Figure 6 3D structure

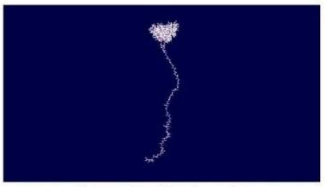


Figure 4 refined model

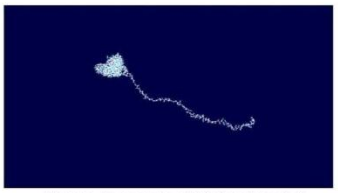


Figure 5 energy minimized model

Figure 3 Refined Model

That refined model was further energy minimized using YASARA as shown in the figure. After energy minimization, the energy values showed a significant improvement. The initial energy was -200,000KJ/Mol after refinement and further minimized to -273,000KJ/Mol following energy optimization. After refinement and energy minimization, the optimized model was further evaluated for structural quality Using ProSA (Protein Structure Analysis), we assessed the Z-score, which indicates the overall quality of a protein structure model. A more negative Z-score signifies a better-quality model. In our case, the Z-score was -6.58, placing our protein within the X-ray crystallography region as shown in (figure 7**)**

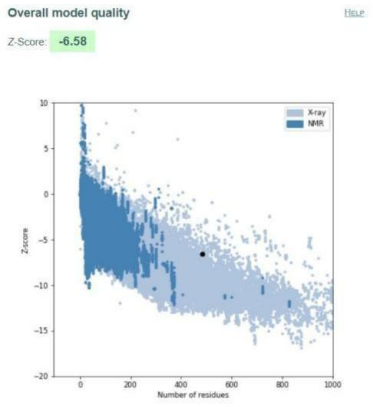


Figure7 Negative z-score(−6.58)is depicting the overall good quality model of HDAC1 protein in comparison with aminoacids deposited in PDB with similar length

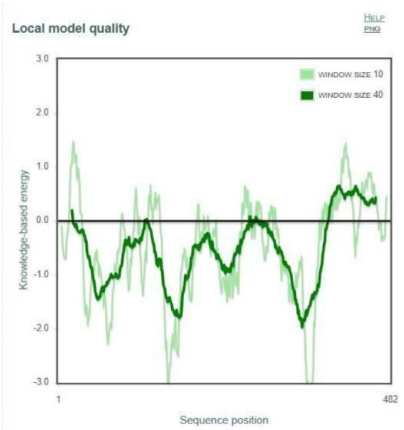


Figure 8 Local model quality of HDAC1 protein evaluated from ProSA energy plot with respect to aminoacid sequence

Next, we analyzed the ProSA plot. In this graph, if the value falls below the baseline (i.e., less than 0), it indicates greater structural stability. Our protein exhibits a more negative

value, confirming its stability as shown in the (figure 8)

Sequence alignment: Template selection for HDAC1 protein obtained from Blast gave 7JVU_A as template with respective parameters as shown in (figure 9)

Figure 9 Representing Pairwise Sequence Alignment of HDAC1 protein and 7J VU using Clustal

Additionally, we used Verify3D to assess the compatibility between the 3D protein structure and its 1D amino acid sequence. For a valid model, the 3D-to-1D ratio should be ≤ 0.1. As shown in (Figure 10), our model meets this criterion, further validating its accuracy.

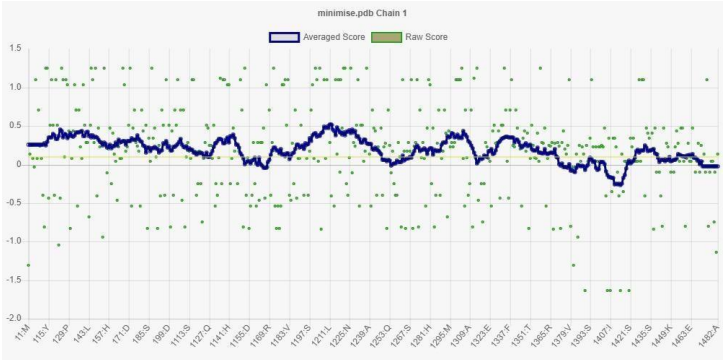


Figure 10 shows the graph of verify 3D obtained from SAVES

Next, we evaluated the ERRAT value to assess the overall quality of the protein model. A quality factor above 80% indicates a good protein structure, as shown in (Figure 11). If amino acids (AAs) are incorrectly positioned, the error value exceeds 99%, resulting in a red line in the graph, which signifies a poor-quality structure. Conversely, a yellow line represents a quality factor above 95%, indicating a well-validated protein model, as depicted in (Figure 12)

Figure 11 shows the overall quality

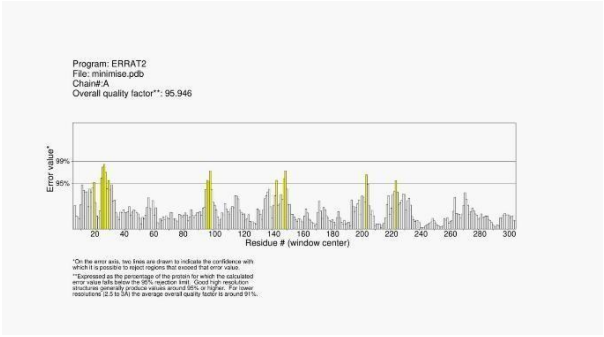
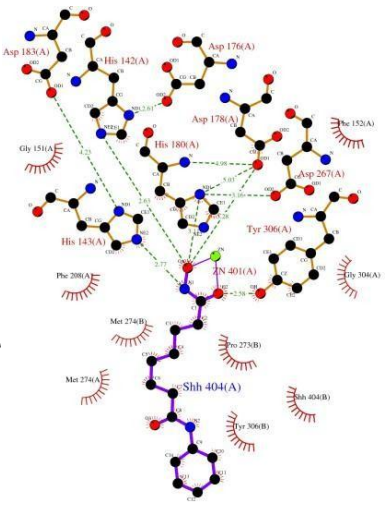


Figure 12 the graph of ERRAT

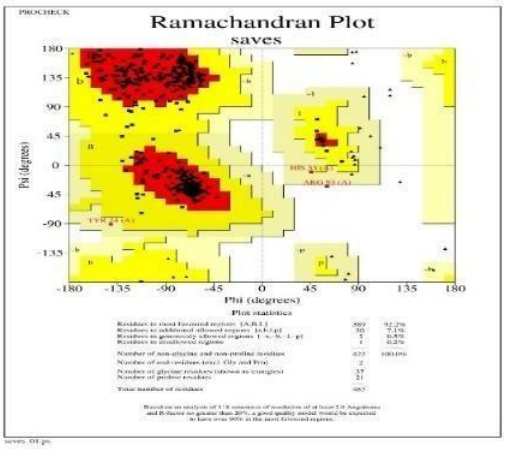
Active site residues of HDAC1 protein identified from template 7JVU using P-L interaction diagram in PDBSum as shown in (figure 13) Total amino acids of hdac1 protein are 482AA.The Amino acids residues in these ligand interactions were further used in protein preparation using mastero 13.5

Moving on to the Ramachandran plot, generated using ProCheck, we analyzed the residue distribution and overall structural quality of our protein. The plot categorizes amino acid residues based on their ϕ (phi) and ψ (psi) backbone angles, which determine protein folding. Red regions represent energetically favored conformations. Dark yellow regions indicate usually allowed conformations. Light yellow regions represent generously allowed conformations. White regions are disallowed conformations, indicating steric clashes. The plot confirms that our protein is stereochemically validated, with 92% of residues falling within the favored region, signifying high model quality. Only 0.2% of residues fall in the disallowed region, reinforcing the reliability of our protein structure as shown in (figure 14)



SHH 404(A)

Figure 13 Ligand Interactions of Protein based on Residues



Molecular docking:

After minimization and validation, we proceeded with virtual screening, followed by molecular docking. In the virtual screening process, 24,000 ligand molecules were submitted. Out of which 1840 ligands were filtered out using HTVS with the parameter of 20% , and again ligands were filtered by SP with the parameter of 20% - 368 ligands were obtained, finally more few ligands were filtered out with 20% by XP and 73 ligands were obtained. After HTVS, SP, and XP filtering, we narrowed them down to 73 ligands that showed potential interactions with our target protein. Next, molecular docking was performed to evaluate the binding interactions. The Glide score was calculated to assess binding affinity, along with Glide energy, ADME properties, and Prime MM-GBSA(ΔG)binding energy, later calculations were performed to prioritise the ligands.

Figure 14 stereochemical analysis of HDAC1 protein by Ramachandran contour plot using PROCHECK server

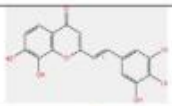
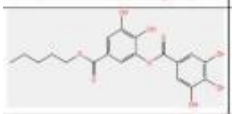
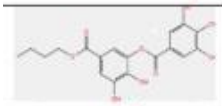
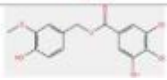
STRUCTURE OF THE LIGAND	GLIDE SCORE	GLIDE ENERGY	MolW	DonorHB	AcceptHB	%HOA	PRIME MMGBSA dG BIND
	-8.750	-54.548	328.273	5.000	6.250	72.499	-26.9906
	-7.901	-60.648	392.357	5.000	8.250	78.975	-5.97611
	-7.775	-56.447	378.330	5.000	8.250	59.694	5.059352
	-7.570	-49.393	306.267	4.000	5.750	63.411	-20.7454

Table 2 shows the results of molecular docking

A Glide score below -6 indicates a strong binding interaction. In our study, the Glide score of our protein was -8, confirming a high-quality binding model (as shown in the table 2). According to Lipinski's Rule of Five, an ideal drug-like molecule should have: Hydrogen bond donors ≤ 5 Hydrogen bond acceptors ≤ 10 For ΔG binding energy, a more negative value signifies greater stability of the protein-ligand complex and MolW should be less than 500, making the inhibitors more effective."

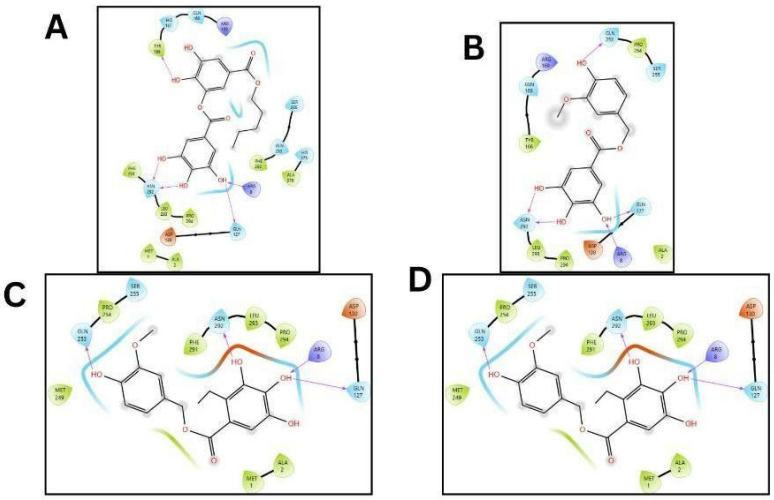


Figure 15 protein ligand interactions

Conclusion: This in silico analysis, through molecular docking, has identified promising inhibitors with the potential to regulate the protein's activity. As all the ligands are having good glide score, glide energy, MolW, DonarHB, AcceptHB, and %HOA Value are with respect to the ADME properties. These findings lay a critical foundation for future drug discovery, paving the way for the development of targeted inhibitors of HDAC. With further research, these inhibitors could revolutionize treatment strategies for cancers such as colorectal and gastric cancer, where abnormal protein expression is a key driver of disease. If successfully developed and approved, these inhibitors have the potential to significantly reduce mortality rates, offering new hope for patients battling these aggressive cancers.

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