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"***Machine Learning-assisted Drug Repurposing for Identification of Potential HDAC6 Inhibitors***"

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CANDIDATE'S DECLARATION

I, Shubham Kumar Shrivastav, 2K21/BIO/06 student of M. Tech Bioinformatics, hereby declare that the project Dissertation titled "**Machine Learning-assisted Drug Repurposing for Identification of Potential HDAC6 Inhibitors**" which is submitted by me to the department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Technology, is original and not copied from any source without proper citation. The work has not previously formed the basis for the award of any Degree, Diploma Associateship, Fellowship or other similar title or recognition.

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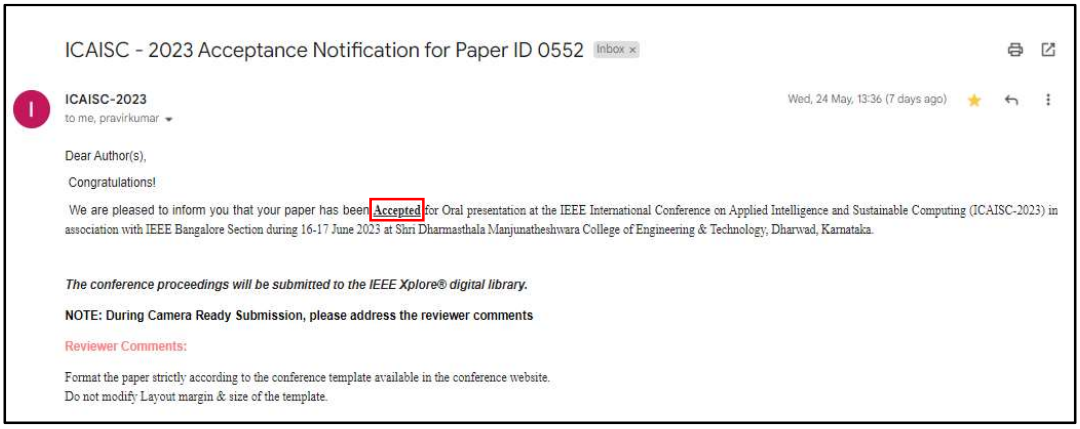

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I hereby certify that the Project Dissertation titled "**Machine Learning-assisted Drug Repurposing for Identification of Potential HDAC6 Inhibitors**" which is submitted by Shubham Kumar Shrivastav, 2K21/BIO/06, Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Technology, is a record of the project work carried out by the student under my supervision. To the best of my knowledge this work has not been submitted in part of full for any Degree or Diploma to this University or elsewhere.

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ABSTRACT

Together, the DNA and epigenome tightly regulate neuronal function and differentiation. The abnormal functioning of the genome and epigenome that results from the epigenetic alterations that occur in the face of environmental input leads to neurodegeneration. Histone deacetylases or HDAC constitute a class of proteins or cofactors that need Zn2+ and contribute to the transcription and operation of cells. The overexpression of these proteins, which is prevalent in the development of diverse anomalies in the brain tissues, leads to the dysregulation of several target proteins involved in cell formation and growth associated with Alzheimer's disease, which impairs memory and learning ability. Although several strategies have been used to regulate the greater expression of HDACs using diverse chemical inhibitors, very limited success has been achieved. In the given study we have used machine learning approach to extract drug inhibitor data and target inhibitors. Algorithms such as Random Forest and Support Vector Machine have been used to preprocess data and add required additional parameters like rotatable bonds, canonical smiles, molecular weight, number of atoms, etc. models were trained and evaluation of the models were performed the prediction of data. Eventually molecular docking was done and a list of top 10 novel compounds were retrieved based on their binding affinities with HDAC6. The best binding drug was Bicalutamide, which was an anti-cancerous drug and can be used to treat AD by inhibiting HDAC6.

Keywords: Alzheimer’s disease, Histone deacetylase 6, post translational modification, Molecular docking, machine learning.

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LIST OF ABBREVIATIONS

AD	Alzheimer's Disease
Aβ	Amyloid β
NFT	Neurofibrillary Tangles
HAT	Histone Acetyltransferase
HDAC	Histone Deacetylase
NDDs	Neurodegenerative diseases

CHAPTER 1

INTRODUCTION

"The investigation of mitotically (and possibly meiotically) heritable changes in gene expression that aren't triggered by changes in DNA sequence" is how epigenetics are defined. Some epigenetic definitions, however, go beyond this and may not necessarily include the necessity for heredity [1]. For instance, the US National Institutes of Health (2009) states that "epigenetics refers to both secure, in the long run, modifications in the transcriptional capacity of a cell that are not generally transferred to next generation and inherited alterations to gene function as well as expression [2]. Expression of genes is a complex process with many steps. Transcribing a molecule of DNA into an identical RNA copy is the first stage of gene expression. RNA polymerase attaches to the promoter section of the DNA to start transcription by transcribing to a strand of mRNA that is equivalent to one of the DNA strands. It is ready, the mRNA may engage the ribosomes and start the translation process. Polypeptides are produced from the N to C terminus, during translation in three distinct phases[3][4]. The mRNA then defines the three sets of nucleotides (codons) from the DNA code that will be read after the initialization. The chosen amino acids are subsequently combined during the elongation phase and linked through a protein transferase reaction, resulting in the formation of a peptide bond and extension of the peptide chain [5] Translation halts when either of the end codons signifies the release of a complete polypeptide chain. The ribosome separates from the mRNA and the subunits of the ribosomal membrane when it is time for the cycle to begin again. The protein may undergo a number of post-translational modifications before being used in its intended function [6].

The buildup of aggregated and/or dysfunctional polypeptides in the biological milieu is one of the defining characteristics of neurodegenerative diseases (NDDs). In the etiology of NDDs, or post-translational modifications (PTMs), are a key regulator of the aggregation of inactive proteins [7]. Any alteration to the post-translational process and the protein quality control system, including the ubiquitin proteomsome protein, autophagy-lysosomal degradation route, misfolded protein accumulation, molecular chaperone, and, increases the accumulation of misfolded protein, ubiquitin-proteasome system, which leads in nervous system dysfunction. [8]. Post-translational modification has a variety of effects on

protein synthesis, aggregation formation, and disease-causing toxic metabolite breakdown. PTMs control protein homeostasis, which controls protein structure, functions, and the tendency for aggregation. PTMs include acetylation, SUMOylation, glycosylation, nitration, phosphorylation, ubiquitination, palmitoylation, and oxidation [9]. Additionally, growing data points to the possibility of targeting certain PTMs with tiny chemical compounds, which function as a suppressor or activator, reverse the buildup of misfolded proteins and so improve neuroprotection [10].

Inhibitors of HDAC also known as Histone Deacetylase are shown to be advantageous in experimental systems of neurological disorders. These type of findings were primarily linked to the chromatin deacetylation-induced epigenetic regulation brought on by HDACs, particularly those from class I [11]. Since each HDAC might play a unique role in the neurodegenerative cascades, additional mechanisms may also be involved in the neuroprotective impact of HDAC inhibitors [12]. HDAC6 is one such example, for which the contribution to neurodegeneration has so far only been partially understood. There is ongoing debate regarding the best approach to take when developing medicines that target HDAC6 [13]. Specific inhibitors work to enhance axonal transport, particularly is typically compromised in neurodegenerative diseases, by raising the levels of acetylation of -tubulin. On the other side, a putative induction of HDAC6 might support the breakdown of protein aggregates that are indicative of several NDD, including Alzheimer's, Parkinson's, and Huntington's diseases [14].

In the given thesis and the literature work, we have majorly discussed post-translational modifications specifically acetylation and deacetylation. For the research part of the thesis, we chose a segment of Histone deacetylase 6 from the literature review for drug repurposing using a machine learning approach. Inhibition of HDAC6 results in the stoppage of the progression of Alzheimer’s Disease or AD.

CHAPTER 2

REVIEW OF LITERATURE

2.1 REGULATION OF HDAC 6 IN POST-TRANSLATIONAL MODIFICATION

A cortactin-dependent, actin-remodeling mechanism that is recruited by HDAC6 assembles an F-actin network which induces the involvement of autophagosomes along with lysosomes and the destruction of substrates [15]. Autophagy malfunctions as a result of HDAC6 loss or dysregulation, which delays the breakdown of protein aggregates. In juvenile-onset PD, Kufor-Rakeb syndrome is brought on by ATP13A2 mutations [16]. To encourage autophagosome-lysosome fusion and the destruction of protein aggregates, ATP13A2 recruits HDAC6 to the lysosome where it deacetylates cortactin and tubulin. Following spinal cord injury, HDAC6 overexpression causes microtubule deacetylation and decreased stability, which inhibits autophagy and causes damage (fig. 1) [17]. P62 elevates HDAC6 levels in prostate cancer and decreases microtubule stability and acetylation of -tubulin, which impairs autophagy flux and promotes EMT. In order to prevent HDAC6 from deacetylating -tubulin, restoring autophagy and preventing the loss of subcutaneous fat [18]. It is notable that SIRT2 has also been engaged in this process, which controls the acetylation of Tau and -tubulin to alter autophagy vesicular flow and cargo clearance, in addition to HDAC6-mediated cytoskeleton protein acetylation [19].

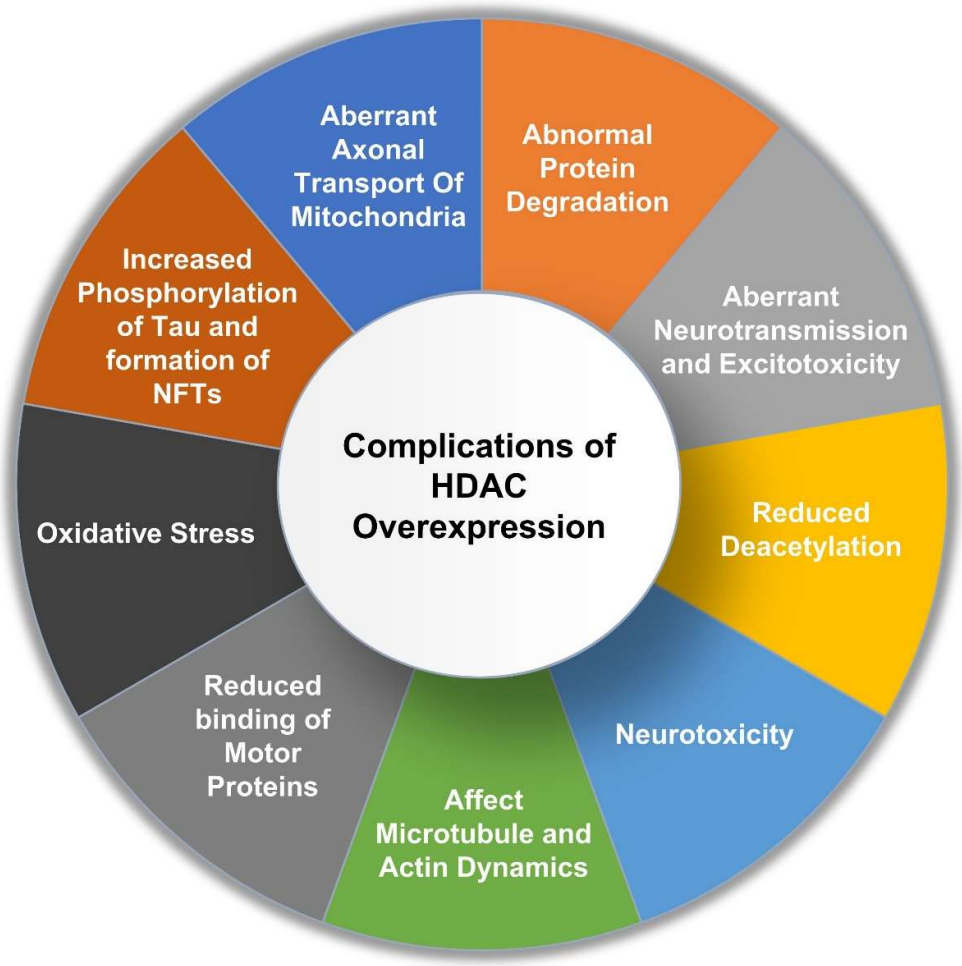


Figure 1: Complications caused due to the overexpression of HDAC 6

2.2 HDAC 6 IN ALZHEIMER'S DISEASE

The area of the hippocampus, entorhinal cerebral cortex, as well as amygdala are the primary brain regions impacted by AD[20]. A characteristic intraneuronal pathology in AD called neurofibrillary tangles develops in a specific way. The entorhinal area, a nearby part of the hippocampus, is where tau protein buildup first affects brain areas, which then gradually extend. The hippocampus, which is crucial for developing memories (memory training) and more especially for declarative or explicit memory—the remembering of events—is therefore affected by tau disease [21]. Therefore, it is important to pay attention to HDAC6 expression in the hippocampus. When compared to young, healthy brains, AD cortex HDAC6 protein levels were 52% higher and 91% higher in AD hippocampus. The amount of HDAC6 protein in the brains of AD patients and age-matched normal brains was compared to demonstrate that the HDAC6 protein has been elevated in AD [22].

Proteasome restriction is a well-known aspect of AD and appears to enhance the interaction between HDAC6 and tau. Such a relationship might be seen in cells, AD patients' brains, and in vitro [23]. Regardless of Histone deacetylase's ability to deacetylate tubulin, HDAC6 along with tau co-localized inside a compartment. Although tau phosphorylation could be reduced by therapy with tubacin or in vivo HDAC6 knockdown, the relationship between HDAC6 and tau was unaffected [24]. The post-mortem analysis of the Alzheimer patients' brains also showed a small amount of alpha-tubulin along with high levels of tubulin acetylation. The majority of these activities were seen in neurons with neurofibrillary tangles. Tau boosted tubulin acetylation through interacting with HDAC6 to block the deacetylase activity [25]. Additionally, human cells overexpressing tau protein showed the same rise. A surplus of tau worked as an HDAC6 blocker and stopped cells' ability to induce autophagy and then inhibit the proteasome. Accordingly, tau can function as an inhibitor of the aggresome pathway as well as the deacetylase function of HDAC6, dependent on the HDAC6 interaction with polyubiquitinated proteins [24]. Even if HDAC6 up-regulation aids in the confinement of ubiquitinated aggregates of proteins and the attraction of autophagic components in AD brains, according to Ding's theory, it would ultimately be harmful to cell survival because of dropped tubulin acetylation as well as increased tau phosphorylation [14]. Finally, combining HDAC6 suppression mice with an experiment for extensive amyloid disease has recently shown the beneficial effects of HDAC6 depletion on cognition. Loss of HDAC6 could reverse the abnormalities while also improving the condition of association and spatial memory formation. These results point to HDAC6 suppression as a potential AD target [20].

2.3 HDAC6 AS A THERAPEUTIC AGENT IN NEURODEGENERATION

HDAC6 is without a doubt implicated in several neurodegenerative cascade events and varies from other HDACs not just in terms of structure but also in terms of subcellular location [26]. Common characteristics of many NDs include impaired mitochondria transport and removal of protein aggregates, which are related to HDAC6's deacetylase as well as ubiquitin ligase activity. But it appears that the outcomes of specifically inhibiting HDAC6 in neurological models cannot be generalized to other diseases [27]. One argument is that various illnesses involve certain proteins, and in the case of HDAC6, protein-protein interactions (PPI) should not be disregarded. One such is the

relationship between HDAC6 with tau, which inhibits HDAC6 and prevents it from participating in autophagy. In the instance of NDs, some of the PPI mechanisms ought to be investigated in both the HDAC6 and class I HDAC contexts [28]. In fact, depending on the illness and the targeted isoform, class I HDAC inhibition was successful in enhancing cognition, memory, and academic performance in animal models (fig. 2). Even though HDAC1 and HDAC2 are quite similar to one another, different effects have been seen when each enzyme has been overexpressed. This could be connected to how PPI affects the regulation of HDAC activity [29]. As a result, HDAC1 may contribute to neurodegeneration by processes other than epigenetics, similar to those of HDAC2, such as nuclear export, association with CRM-1, which is a nuclear factor, and the production of kinesin complexes to interfere with mitochondrial transport [30]. Even though tau buildup in general cell tests and mitochondrial transport impairment in NDs could both be prevented by specifically inhibiting HDAC6, the involvement of HDAC6 in protein aggregation removal was emphasized. Additionally, WT-161, which is a specific HDAC6 inhibitor, did not enhance mental capacity in an animal memory test.

Furthermore, there was convincing evidence that tau in AD inhibited HDAC6. Recently, an animal model of AD that has been crossed with an HDAC6 deletion showed improved cognitive function [28]. Given that tau eventually inhibits overexpressed HDAC6, even though HDAC6 appears to be required for aggregate removal by autophagy, which an induction may fall short of reversing the pathogenic circumstances that characterize AD. Further research on PPI among HDAC6 and additional proteins implicated in certain neurodegenerative diseases would be interesting in this area [26]. PPI inhibitors have attracted a lot of attention in the drug development process because they make it possible to interact with a particular enzyme pathway without affecting the enzyme activity required for other processes. Additionally, tiny molecules may modify PPI, which is a desired property to enable oral delivery and blood-brain barrier permeability. As a result, it appears that studying PPI that underlies HDAC6 processes is a viable strategy for modifying HDAC6 function in the setting of neurodegeneration [30].

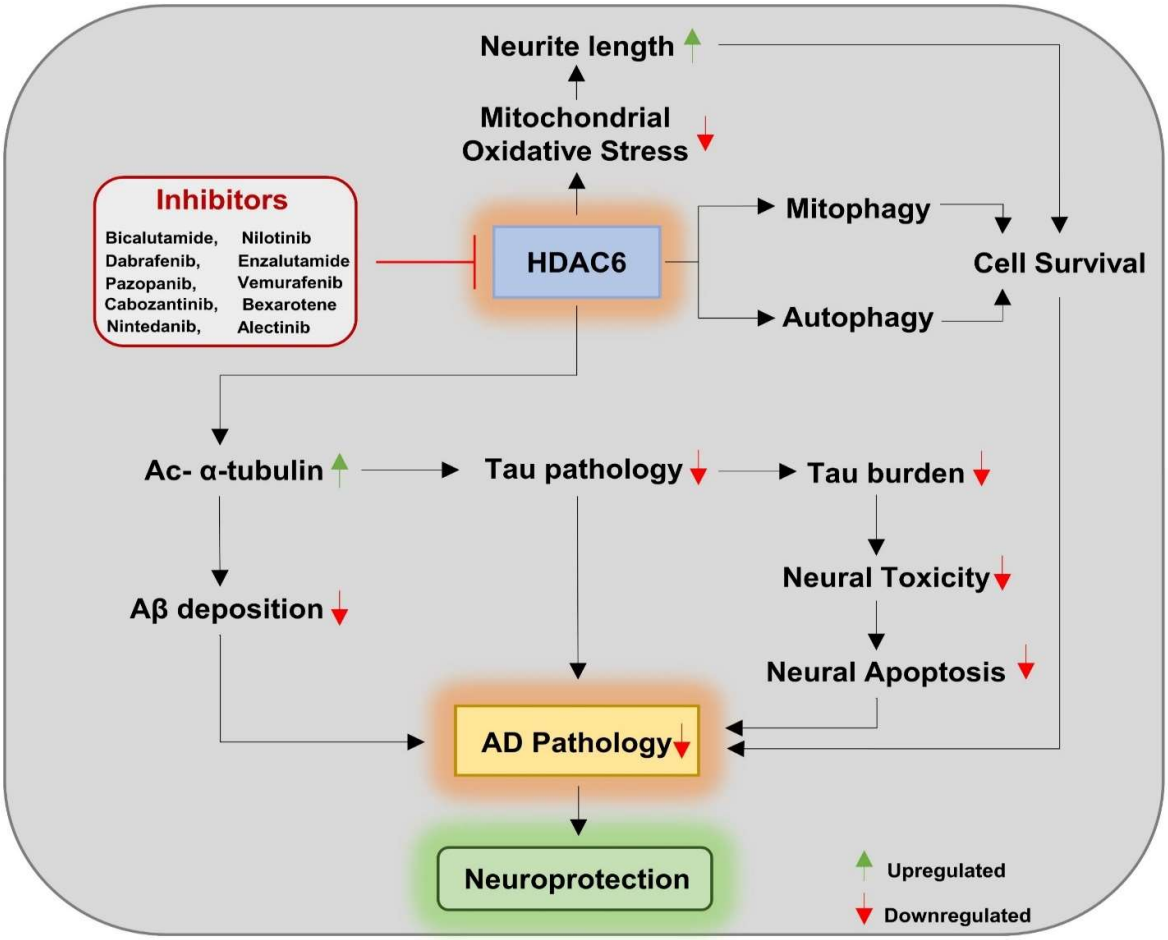


Figure 2: The reduced activity of the HDAC6 deacetylase, promotes the acetylation of alpha-tubulin, which aids in lowering toxic amyloid segregation, tau pathology, and AB deposition, all of which serve in reduced AD pathology and promote neuroprotection.

CHAPTER 3

METHODOLOGY

Initially, to understand the theoretical concepts of HDAC6 and neurodegeneration, the information was summarized from the literature work presented in literature databases like PubMed which is accessible through https://pubmed.ncbi.nlm.nih.gov/, Google Scholar which is easily accessible through https://scholar.google.com/. For the study, three classes of FDA-approved drugs were shortlisted based on diseases like anti-cancer, anti-diabetes, and anti-hypertension. As AD is a multifactorial disease, it has been observed that factors like diabetes, hypertension increased oxidative stress and cancer also have an important part in adding to the progression of AD, on this evidence these classes of drugs were taken in for study as the drugs repurposed in this study will act as multi-target compounds.

3.1Data Collection

Initially, a list of approximately 500 FDA-approved three major classes namely antihypertension, anticancer, and antidiabetics was retrieved from the drug bank. The Spatial Data File (SDF) was downloaded from CHEMBL and PubChem for all the compounds and merged into a single SDF file.

The protein structure of HDAC 6 was retrieved from the Protein Data Bank repository. The crystal structure of the human HDAC6 zinc finger domain with PDB ID: 3C5K was selected for the study. The selected structure had a crystal structure modeled using the X-RAY Crystallography method at a resolution of 1.55 Å. The 3D coordinate file of the structure was saved with the .pdb extension.

3.2 Blood Brain Barrier Prediction

To target HDAC 6 as therapeutics for Alzheimer's disease a drug must be transported to the brain and for a compound to reach the brain it must cross the Blood-brain barrier. An online open-access tool namely CB ligand. This tool utilizes support vector machine (SVM) and LiCABEDS algorithms to make predictions by applying them to four different types of fingerprints (MACCS; OpenBabel (F2P); MolPrint 2D; PubChem) from 1593 reported chemical compounds. Among all, the SVM algorithm and the PubChem fingerprints were used to predict the compounds for BBB+ or BBB-.

3.3 Utilizing machine learning to predict the bioactivity of Compounds

In order to retrieve data and train a ML model Google Colab an online IDE was used. Users can write, execute, and collaborate on Python code online using Google Colab, a cloud-based integrated development environment (IDE) offered by Google. It is based on Jupyter Notebook and gives users access to a hosted environment where they can run code, create and store notebooks, and employ sophisticated computational resources, such as GPU acceleration, all through a web browser.

Training and prediction using ML all were done by writing Python-based codes on collab.

3.3.1 Package Installation

The code starts by installing the necessary packages. The "rdkit" package, which is a set of cheminformatics and machine learning technologies, is first installed. It allows you to manipulate and analyze chemical data. The "pandas" package, a popular data manipulation and analysis toolkit, is then installed. It provides data structures and methods for managing and processing structured data effectively. Finally, the "sklearn" package, which is a machine-learning library with numerous methods and tools for data analysis and modeling, is installed.

3.3.2 Importing Packages

The required packages are first installed, and then the code imports them to use them. Importing the "pandas" package will be used to manipulate and analyze data. It is necessary to load the "rdkit" package, which will be used to manage chemical data and carry out cheminformatics activities. Imported is the "sklearn" package, which will be used to build and hone machine learning models. The "chembl_webresource_client" package's "chembl_webresource_client" module is also imported. This module enables access to the web service for the ChEMBL database.

3.3.3 Searching for Target Protein

The program makes use of the ChEMBL web service to look for a certain target protein. To communicate with the ChEMBL database, it uses the "new_client" module from "chembl_webresource_client". In this instance, "HDAC6" is the target protein

being looked for. The pandas DataFrame "targets" is used to retrieve and store the search results.

3.3.4 Retrieving Bioactivity Data for a Target Protein

The algorithm then retrieves bioactivity information for the chosen target protein from the ChEMBL database after finding it through a search for the target protein. Based on the ChEMBL ID of the chosen target protein, the "activity" module from "chembl_webresource_client" is used to query the bioactivity data. In particular, the "IC50" values for bioactivity data that are presented in nanomolar (nM) units are filtered. A pandas data frame named "df" is then used to store the retrieved data.

3.3.5 Handling Missing Data

The code handles missing data to ensure the accuracy and completeness of the data. The "dropna()" method from pandas is used to remove any rows in the DataFrame "df" that have missing values in the columns "standard_value" and "canonical_smiles." This makes sure that the subsequent analysis and modeling processes only employ entire data points.

3.3.6 Data Pre-processing

depending on their standard values, substances are classified as active, inactive, or intermediate depending on the bioactivity data. The'standard_value' column in the DataFrame "df" is used for this by applying a lambda function to it. The labels "active," "inactive," and "intermediate" are used for compounds with values of less than or equal to 1000, higher than or equal to 10,000, and values in the middle. RDKit functions are used to calculate additional molecular characteristics, including the number of atoms, heteroatoms, rotatable bonds, and molecular weight. The DataFrame "df" gains new columns for these descriptors.

3.3.7 Feature Matrix and Target Variable

A subset of the DataFrame "df" with the columns "num_atoms," "num_heteroatoms," "num_rotatable_bonds," and "molecular_weight" is the feature matrix, denoted as "X." The input features that will be utilized to train the machine learning model are represented by these columns. The 'bioactivity_class' column in the DataFrame is designated as the target variable, indicated as "y". It stands for the bioactivity prediction task's class labels.

3.3.8 Model Training

The "RandomForestClassifier()" class from the "sklearn.ensemble" module is used to build a random forest classifier. Both classification and regression tasks can be handled by this classifier. The constructed model is trained using the "fit()" technique utilizing the feature matrix "X" and the goal variable "y".

3.3.9 Predicting Bioactivity for a Given SMILES

For predicting the bioactivity of a given SMILES string, the method "predict_bioactivity()" is defined. The following actions are carried out by the function after receiving the SMILES string as input: converts the SMILES string to a molecule object using RDKit's "Chem.MolFromSmiles()" function, computes the necessary molecular descriptors (number of atoms, heteroatoms, rotatable bonds, and molecular weight), creates a DataFrame with the input features, makes predictions using the trained model (using the "predict()" and "predict_proba()" methods), and prints the predicted class and probabilities.

3.3.10 Fetching and Predicting Bioactivity for Multiple ChEMBL IDs

The program shows how to retrieve and forecast bioactivity for various ChEMBL IDs. The "read_csv()" function is used to read ChEMBL IDs from a CSV file into a pandas DataFrame. The "molecule.get()" method is used to retrieve the compound information from ChEMBL for each ChEMBL ID in the data frame. The trained model and calculated molecular descriptors are used to estimate bioactivity after the canonical SMILES are extracted from the compound information. The outcomes are kept in a collection of dictionaries. The output is then saved as a CSV file and transformed into a data frame. The predicted results DataFrame is printed as output.

In conclusion, the python-based code executes several operations, such as installing packages, obtaining and processing data, training models, and predicting bioactivity. For cheminformatics operations, data processing, and machine learning activities, it makes use of programs like RDKit, pandas, and scikit-learn. The code demonstrates

retrieving and predicting bioactivity for several compounds based on ChEMBL IDs as well as bioactivity prediction for a specific chemical structure.

3.3.11 Molecular Docking

As a next step, the retrieved FDA-approved drugs were subjected to molecular docking

3.3.12 Preparation of target protein

The target protein must first be ready for docking. Any ligands, water molecules, or co-crystallized small molecules are eliminated in order to prepare the protein structure was done using the Biovia Discovery Studio. After that, bond order is determined, any irregular residues or heteroatoms are eliminated, any missing hydrogen atoms are added and Kolmann charges were finally added using the Autodock Tools.

3.3.13 Preparation of ligands

The ligand molecules will then be prepared, and any essential hydrogen atoms and correct bonding order will be added. Using the OpenBabel command line, all of the ligands were identically produced and transformed into 3D conformational space. Furthermore, using the OpenBabel command line, the ligands were stored in a PDBQT file format.

3.3.14 Setting up the docking parameters

The parameters for the docking simulation were decided at this stage. The coordinates and measurements of a grid box were decided as that surrounds the binding site are used to establish the search space, the grid mapping was done for HDAC6 and the dimensions were 5.669, -4.987, 12.262, and the size was 34.86 x 39.07 x 40.61. The exhaustiveness was increased to 16 from a default value of 8, It signifies how many dockings poses per ligand should be generated, as well as how comprehensive the search method should be. Higher exhaustiveness values improve docking accuracy while lengthening computation time.

3.3.15 Performing molecular docking

The molecular docking simulation is carried out using AutoDock Vina extension in the PyRx tool with the prepared target protein, ligands, and docking parameters. To identify the optimal binding poses, the computer methodically investigates the ligand conformations and orientations inside the specified search space. Based on an

evaluation of the interactions between the ligand and protein, AutoDock Vina determines the binding affinity (predicted binding energy) for each docking position. We used an RMSD threshold of 1Å and free energy of binding cutoff of -9.0kcal/mol to filter for promising findings. The hits were then sorted from lowest to highest binding free energy.

PyRx is a computational tool used to screen the compound libraries against a specific receptor and is an in-silico approach for drug discovery and development. It also comprises of docking wizard and visualization engine used to perform virtual screening and molecular docking. It uses AutoDock Vina as a docking software. AutoDock vina utilizes a scoring function based on empirical force fields to estimate the binding affinity between a ligand and the protein. The scoring function considers various energetic contributions, such as van der Waals interactions, electrostatic interactions, and de-solvation effects, to evaluate the binding affinity. The underlying mathematical model in AutoDock Vina involves algorithms for global optimization, specifically an algorithm called iterated local search. This algorithm performs a systematic exploration of the search space to find the most energetically favorable binding conformations of the ligand within the protein's binding site. Open Babel for the import of files in SDF format. Its programming language is Python and matplotlib is used for 2D plotting.

3.3.16 Visualization and analysis of docking results

Once the docking simulation is complete, the data are examined to determine which ligand poses have the highest binding affinities. The optimal drug-binding conformations have been stored in a discrete PDB file. Biovia Discovery Studio and Pymol were utilized to graphically represent the ligand-receptor protein interaction. Hydrogen bonds, hydrophobic interactions, and electrostatic interactions between ligands and proteins were evaluated by visualizing the docking postures. Binding affinity scores are used to rank the ligands, with the highest-scoring ligands being chosen for further study or experimental verification.

Finally, publication-quality images of docking poses were rendered using Pymol’s ray-tracing feature.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Model Evaluation

Upon predictions by the Random Forest algorithm the performance of the model was evaluated and the following scores were obtained:

Table 1: Performance Parameters of the Random Forest ML Model

Performance Parameters	Value
Accuracy	0.8
Precision	0.666666667
Recall	1
F1-Score	0.8
AUC-ROC	0.833333333

The unique problem, the parameters, and the intended degree of performance all influence whether the ratings for a model are excellent or bad. However, a broad analysis based on acknowledged standards or thresholds is given below:

Accuracy: A score of 0.8 means that 80% of the samples were accurately predicted by the model. An accuracy of over 70% is typically seen as being good.

Precision: The model correctly detects about 66.67% of the positive predictions, with a precision score of 0.666666667. There are fewer false positives the better the precision. Although there is no universal cutoff for good precision scores, a score of at least 0.6 is generally regarded as acceptable.

Recall: A recall score of 1 indicates that all positive samples were accurately identified by the model. This shows that the model is highly capable of identifying all positive events. In many situations, a recall score of 1 is ideal.

F1-Score: The harmonic mean and precision has been denoted by an F1-score of 0.8. It offers a balanced measurement that takes into account both recall and precision. A good F1-score is typically one above 0.7, but the significance varies depending on the issue.

AUC-ROC: The model appears to have a decent ability to distinguish between positive and negative samples, according to the AUC-ROC score of 0.833333333. A good score is one that is above 0.8 and shows that the model has a strong ability to discriminate.

In conclusion, the model appears to have performed reasonably well based on the presented scores, especially in terms of recall, F1-score, and AUC-ROC. It's crucial to remember that the specific problem and the environment in which the model is being used determine whether these ratings are good or bad.

Later after classification of algorithms all the three categories of drugs (anti-hypertension, anti-cancer, and anti-diabetes) were classified in the categories namely Active Inactive and intermediate, assuming the model's performance and precision were significant. The downloaded data frame of 280 FDA-approved drugs were classified as 273 of the totals were classified as active 7 as intermediate and none as inactive.

4.2 Binding Affinity Analysis and Protein Target Interaction

Upon classification all the FDA- approved drugs were subjected to molecular docking. To evaluate the performance of auto dock vina various parameters and algorithms are used (**TABLE 2).**

TABLE 2: Table summarizing the parameters, algorithms, and their working in AutoDock Vina

Parameter/Algorithm	Description	Working
Scoring Function	Estimates binding affinity	AutoDock Vina employs a scoring function based on empirical force fields. It considers van der Waals interactions, electrostatic interactions, and de-solvation effects to evaluate the binding affinity between a ligand and the protein.

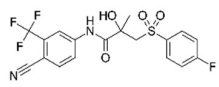
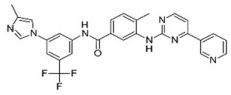
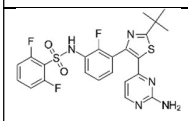
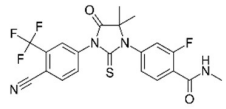
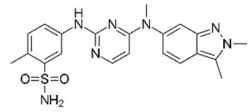
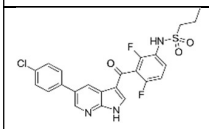
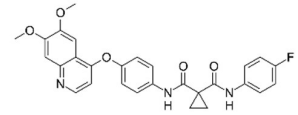
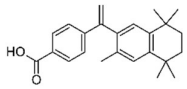
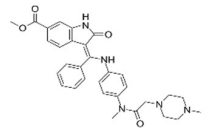
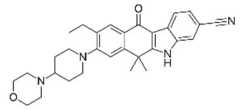
Iterated Local Search (ILS)	Algorithm for global optimization	ILS is utilized in AutoDock Vina to perform a systematic exploration of the search space. It aims to find the most energetically favorable binding conformations of the ligand within the protein's binding site. ILS combines local search (examining local neighborhoods) with random perturbations to escape local minima and explore the entire search space.
Grid-based Docking	Defines the search space	AutoDock Vina utilizes a grid-based approach to define the search space for molecular docking. The grid is generated around the protein's binding site, and its dimensions and spacing can be specified by the user. The grid maps the interaction potential of the ligand with the protein.
Exhaustiveness	Controls the thoroughness of the search	The exhaustiveness parameter determines the thoroughness of the docking search. Higher values increase the number of conformations sampled during docking, resulting in a more exhaustive search. However, it also increases the computational time required for docking. Users can adjust this parameter based on the desired balance between accuracy

		and computational efficiency.
Binding Energy	Quantifies ligand-protein interaction strength	The binding energy is a measure of the strength of the interaction between the ligand and the protein. AutoDock Vina calculates the binding energy using its scoring function. Lower binding energy indicates a stronger binding affinity between the ligand and the protein.
Root Mean Square Deviation (RMSD)	Measures structural similarity	RMSD is a measure of the structural deviation between two molecular structures. In molecular docking, it is used to evaluate the similarity between the predicted ligand conformation and the experimental or reference conformation. Lower RMSD values indicate a closer match between the predicted and reference conformations.

4.3 Best Binding Compounds

After virtually screening all the retrieved compounds against HDAC6, a list of best-binding drugs were obtained. **(TABLE 3)**

Table 3.- Best binding compounds with HDAC6 after the virtual screening.

Drug Structure	ChEMBL ID	Compound	Binding Affinity (Kcal/Mol)	Blood-Brain Barrier
	CHEMBL409	Bicalutamide	-9	+
	CHEMBL255863	Nilotinib	-8.8	+
	CHEMBL2028663	Dabrafenib	-8.8	+
	CHEMBL1082407	Enzalutamide	-8.6	+
	CHEMBL477772	Pazopanib	-8.6	+
	CHEMBL1229517	Vemurafenib	-8.5	+
	CHEMBL2105717	Cabozantinib	-8.3	+
	CHEMBL1023	Bexarotene	-8.3	+
	CHEMBL502835	Nintedanib	-8.3	+
	CHEMBL1738797	Alectinib	-8.2	+

Bicalutamide, an anti-cancerous drug, resulted as the novel drug which shows the best binding that can be repurposed to inhibit HDAC6 and limit the progression of Alzheimer's Disease. The ChEMBL ID for the drug is ChEMBL409. The calculated binding affinity of Bicalutamide was -9kcal/mol and the IC50 value was 470 nM. The active site residues of HDAC6 interacting with the bicalutamide are GLU-33, ARG-47, and TYR-81 with a bond length of 3.3 Å, 1.8 Å, and 2.5 Å respectively (Figure 3). This drug passed the test of Blood Brain-Barrier permeability as well and can be considered for further pre-clinical and clinical trials. Promising candidates who can inhibit HDAC6 and slow the course of AD were found using the performance metrics, including binding affinity, docking accuracy, and computational efficiency.

Bicalutamide is a drug used for the treatment of prostate cancer. It is an androgen receptor inhibitor. The brand name under which this drug is sold is Casodex. The Drug Bank accession number is DB01128. Bicalutamide is an FDA- approved drug that is always taken in oral form. The trace remains of the drugs are easily excreted through urine and fecal matter [16]. Bicalutamide is a non-steroidal drug but when consumed can cause side effects like weakness, dizziness, swelling in various parts of the body, pain, constipation, nausea, fever, high blood pressure, frequent urination, etc. It should be stored at room temperature and should be consumed only when prescribed by medical experts. Bicalutamide is a multi-purpose drug that can be repurposed to treat neurological disorders like Alzheimer's Disease.

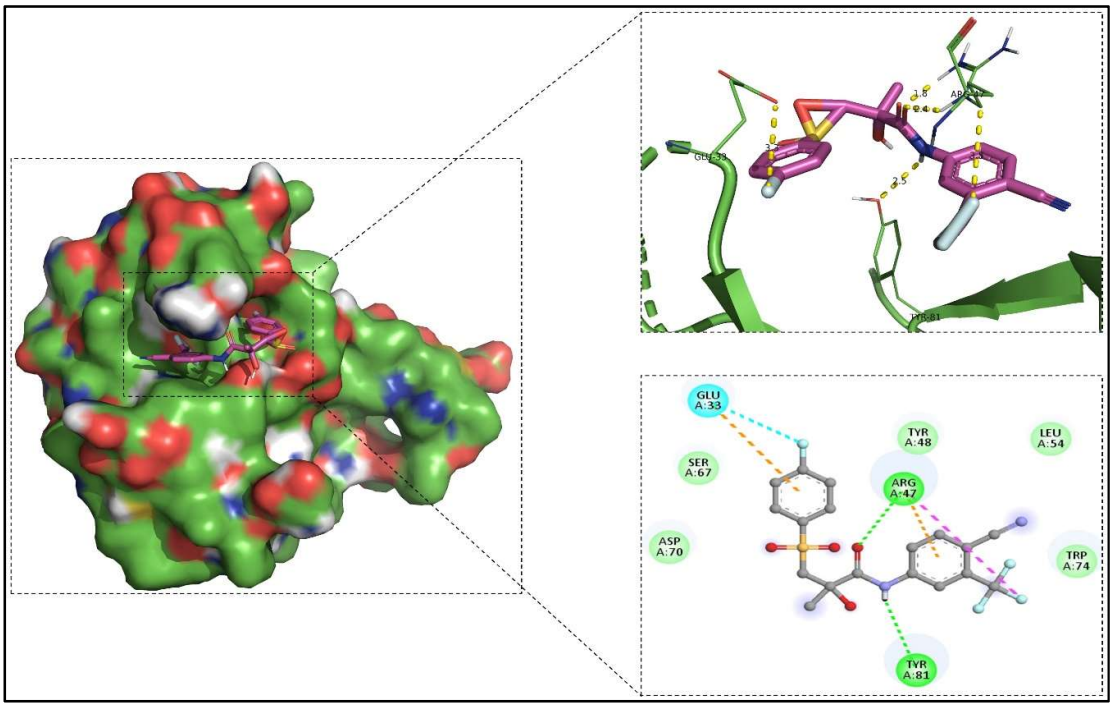


FIGURE 3 – Binding of Histone Deacetylates 6 (Zinc finger domain) (3c5k) with Bicalutamide, active site interacts with GLU-33, ARG-47, and TYR-81.

With the induction of dynein and kinesin motor complexes, inhibition of HDAC6 with an inhibitor reverses oxidative stress-induced neuronal cell death and triggers neurite extension, enhances microtubule stability, and mitochondrial transport enhances cognitive and memory conditions associated with diseases like Alzheimer's and neurological impairments both in animal-based experiments as well as cell. In the R6/2 mouse model, genetically inhibiting HDAC6 has a twofold impact on the development of the illness and motor deficits. This research collectively demonstrated the identification of HDAC6 inhibitors as therapeutics for neurodegenerative disorders. The identified inhibitors were found to be traditionally used for multiple other diseases such as cancer, hypertension, and diabetes.

The points mentioned below highlight the contribution of the Insilco drug repurposing research for the treatment of AD.

- This insilico drug repurposing study finds novel uses for already approved drugs for the inhibition of overexpressed HDAC6 for the treatment of AD by studying the pharmaceutical-target protein molecular interactions.

- Drug repurposing speeds up medication development for neurological disorders like Alzheimer's Disease, Parkinson's Disease, etc. In silico tools can swiftly screen large chemical libraries against many target proteins to uncover potential hits and lead compounds for further investigation. Drug repurposing helps us by saving time and money by using existing data and knowledge.

- Existing drugs that are repurposed for new indications can offer a more comprehensive safety profile than newly developed compounds. Due to the fact that the safety profiles of repurposed pharmaceuticals have already been established through clinical use, the risks associated with adverse effects and toxicity can be better comprehended.

- In silico drug repurposing initiatives provide valuable insights into the action mechanisms and target interactions of existing drugs. This information can aid rational drug design by guiding the modification of existing compounds or the creation of new derivatives with enhanced efficacy or fewer adverse effects. By comprehending the structural and functional properties of repurposed pharmaceuticals, researchers can rationally enhance their therapeutic potential to treat AD.

- Drug repurposing avoids unnecessary animal testing by using approved drugs' safety and knowledge. Repurposed medications have undergone significant preclinical and clinical testing; thus, animal testing is unnecessary.

4.5 Performance Matric Analysis of AutoDock Vina

TABLE 4- Performance metric of AutoDock Vina of the study conducted.

Performance Metric	Description	Evaluation Method	Results
Binding Affinity	Measures the strength of ligand-protein interaction	Calculation of binding affinity scores	Average binding affinity (top 10): -8.54 kcal/mol
Docking Accuracy	Evaluates the accuracy of docking predictions	Comparison of predicted binding poses with experimental data	RMSD less than 1.0 Å
Computational Efficiency	Measures the speed and scalability	Calculation of the time required for	Average docking time: 2 seconds

		docking calculations	

CHAPTER 5

CONCLUSION

FDA-approved drugs which are responsible for the treatment of a variety of diseases were taken for the inhibition of HDAC6 whose over-expression in the brain can cause Alzheimer's disease. Multiple bioinformatics tools and machine learning algorithms were used to predict the bioactivity of various compounds. Various techniques along with online databases were used to conduct the study based on the drug-repurposing approach. Bicalutamide, an anti-cancerous drug resulted to be a potent drug for the inhibition of HDAC6 and curing of AD.

Computational approaches can be one of the best ways to research in the field of life sciences and healthcare which can be cost-effective, and time-saving. At the same time, the only limitation of computational work is its findings need to be approved by clinical trials and further research for human administration. As technology advances, it can be helpful to figure out precise treatments for NDDs for which treatments are yet unknown.

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