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"Repurposing of Anti-viral compounds against HDAC6 in Alzheimer's Therapeutics

A DISSERTATION

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Submitted by:

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CANDIDATE'S DECLARATION

I, NEHA, bearing Roll No.: 2K21/MSCBIO/29, hereby declare that the Major Project titled "Repurposing of anti-viral compounds against HDAC6 in Alzheimer's therapeutics" presented in fulfillment of the requirements for the Degree of Masters of Science in Biotechnology at the Department of Biotechnology, Delhi Technological University, Delhi is an authentic representation of my own work. This research was conducted under the guidance and supervision of Prof. Pravir Kumar, during the period from 10 July 2022to 0101 May 2023I confirm that the content presented in this thesis has not been submitted by me for the purpose of obtaining any other degree from this university or any other institution. I hereby assert the originality and integrity of this research and take full responsibility for the accuracy and authenticity of the information presented. Additionally, the findings and results of this work have been communicated in Scopus-indexed journals and conferences, details of which are as follows:

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CERTIFICATE

This certificate verifies that the research project titled "Repurposing of anti-viral compounds against HDAC6 in Alzheimer's therapeutics" submitted by Neha (2K21/MSCBIO/29) as part of the requirements for the degree of Master of Technology at Delhi Technological University, is a genuine representation of the candidate's work conducted under my supervision. To the best of my understanding, this research has not been submitted, either partially or in its entirety, for any other degree or diploma at this university or any other institution.

30/05/23

Place: Delhi
Date: 30/05/23

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Neha

ABSTRACT

There are many types of neurodegenerative disease and Alzheimer's disease (AD) is one of them that increasingly disturbs brain function and is distinguished by the buildup of abnormal proteins, neuroinflammation, and synaptic dysfunction, all of which contribute to cognitive loss. Effective therapeutic strategies for AD are still difficult to come by despite intensive research efforts. Histone deacetylase 6 (HDAC6), an enzyme intricate in protein deprivation and cellular transport procedures, has been suggested as an ideal target for AD treatment in recent investigations. Due to our limited understanding of their intricate activities and unintended side effects, the progress of particular HDAC6 inhibitors has encountered difficulties.

In this context, repurposing existing anti-viral compounds as potential HDAC6 inhibitors emerge as a promising strategy. Anti-viral compounds, originally designed to combat viral infections by targeting viral replication pathways, exhibit pleiotropic effects and have been shown to modulate cellular processes beyond their primary antiviral activity. This repurposing approach provides a unique opportunity to leverage the existing knowledge and safety profiles of these compounds, expediting the translation of HDAC6-targeting therapeutics for AD treatment.

The rationale behind repurposing anti-viral compounds against HDAC6 in AD lies in the shared molecular pathways implicated in both viral infections and neurodegeneration. Elimination of misfolded proteins occurs through the HDAC6, regulating immune responses, and modulating neuronal plasticity, which is dysregulated in AD. Moreover, emerging evidence suggests that viral infections can exacerbate neurodegeneration, highlighting the interplay between viral pathogenesis and neuroinflammatory processes. By repurposing anti-viral compounds as HDAC6 inhibitors, we can potentially harness their dual antiviral and neuroprotective properties, thereby mitigating the detrimental effects of viral-induced neuroinflammation in AD.

In this review, we assess the potential of repurposing anti-viral drugs as inhibitors of HDAC6 in the therapy of AD. We deliver a comprehensive summary of preclinical studies investigating these drugs' efficacy in AD animal models. Additionally, we highlight the opportunities and challenges involved in advancing these compounds into clinical trials for AD therapy. Furthermore, we emphasize the position of continued exploration to optimize

the safety and efficiency of anti-viral drugs such as HDAC6 inhibitors in the context of AD treatment.
Keywords—Alzheimer’s disease, ACY-1215, Maraviroc, Histone deacetylase 6, AutoDock Vina, Drug re-purposing

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LIST OF ABBREVIATIONS

AD	Alzheimer's Disease
NFT	Neurofibrillary Tangles
PS1	Presenile 1
HDAC	Histone Deacetylase
Aβ	Amyloid β
HAT	Histone Acetyltransferase
PS2	Presenile 2
NEP	Neprilysin
VPA	Valproic acid
MRFs	Myogenic Regulatory Factors
APP	Amyloid Peptide Protein
GSK3β	Glycogen Synthase Kinase 3 Beta
MAPK	Mitogen-activated protein kinase
CREB	cAMP-Response Element-Binding Protein
MeCP2	Methyl CpG Binding Protein 2
ROS	Reactive oxygen species
TF	Transcription Factor

CHAPTER 1
INTRODUCTION

Alzheimer's disorder is a destructive neurological ailment affecting neurons and causes diminished memory, cognitive decline, and behavioral changes as time goes on. It is the primary cause of dementia. The disease typically manifests initially as mild memory impairment and difficulty recollecting recent events. Over time, individuals may experience confusion, disorientation, language and communication difficulties, mood swings, and behavioral changes. In advanced stages, they may lose the capacity to perform routine tasks and necessitate full-time care. The exact causes of Alzheimer's disease remain incompletely understood, despite the fact that a number of factors related to genes, the environment, and lifestyle are believed to play a role. The accumulation of specific proteins in the brain is a characteristic feature that disrupts normal brain function and leads to the death of brain cells. Despite getting older is the key risk factor, a lot of cases affect those over the median age of 65, other factors include family history, certain genetic mutations, previous head trauma, cardiovascular conditions, and lifestyle choices like smoking, obesity, and insufficient physical and mental activity[1].

The primary characteristic of AD is the loss of neurons, which in turn help in the accumulation of NFT and Aβ plaques [2]. There are many types of hypotheses given by scientists on AD origin but the most widely accepted is widely Aβ plaques pathway hypothesis, the buildup of Aβ peptide triggers a sequence of events that ultimately lead to neuronal apoptosis and the development of AD [3], [4]. Familial AD has been linked to genetic modifications, such as excessive production of Aβ peptide, associated with genes like Aβ precursor protein, PS1, PS2, and apolipoprotein E [4], [5].

Epigenetics, which combines advanced genetics and proteomics, has offered valuable insights into sporadic and familial AD through the involvement of histone proteins, with a particular emphasis on sporadic cases [5]. With the advanced progress in functional genomics, scientists saw the best effects of HAT and HDAC on the development of AD and other neurodegenerative disorders have been illuminated [6]. The investigation of post-translational modifications has emerged as a best area of study for comprehending the underlying mechanisms of AD[6].

Prior research has emphasized the impact of post-translational modification in the case of AD to help in memory impairment and the restoration of learning and cognitive abilities.[7].

Two crucial enzymes involved in these processes are HATs and HDACs. HATs catalyze the acetylation of histone proteins, while HDACs facilitate their deacetylation, ultimately influencing the structure and function of double-stranded DNA [8], [9]. Acetylation of histones that occur through HATs, promotes the relaxation of chromatin by loosening the compact arrangement of histones and DNA. This relaxation opens the DNA and allows particular TF to attach to gene promoters, leading to transcriptional activation and gene expression[10], [11] Deacetylation, on the other hand, allows nucleosomes to compact, inhibiting the capacity of TF to bind to promoters and resulting in regulated gene transcription. Dysregulation of HAT and HDAC activity can disrupt normal gene expression, leading to various human disorders, including PD, AD, cancer, neuropathy, diabetes, and neoplasms[12],[13].

Extensive investigations using mouse models have yielded robust evidence supporting the potential therapeutic use of HDAC inhibitors in enhancing cognitive functions and treating neurodegenerative disorders. HDAC inhibitors have shown effectiveness in addressing neurological conditions through two primary neuroprotective mechanisms. The first mechanism involves the targeted activation of genes associated with the disease, enabling control over gene expression. The second mechanism involves the modulation of histone acetylation homeostasis, which plays a critical role in maintaining cellular balance [14]. According to this outcome, HDAC inhibitors hold the possibility to treat neurodegenerative diseases through improvements in cognition and learning. HDAC inhibitors might be beneficial in treating AD, according to research. For instance, neprilysin (NEP), an enzyme that regulates the breakdown of A, was found to be less abundant after the administration of an individual HDAC6 inhibitor.

Furthermore, administering MS-275 to a mouse model with amyloid precursor protein (APP) pathology resulted in decreased Aβ deposition. These findings provide greater provision for any possible medical success of HDAC inhibitors in the medical management of AD [15], [16]. Research has revealed the potential effects of specific HDAC inhibitors on various aspects related to neurodegenerative disorders. For example, it emerged that inhibiting HDAC2 increased dendritic spine quantity growth and decreased Aβ deposition[17], [18]. Conversely, blocking HDAC3 decreases neural transmission induced by amyloid oligomers[19]. In another study, inhibiting the activity of HDAC6 was shown to facilitate the clearance of both Aβ and tau proteins, while also promoting the restoration of α-tubulin acetylation and enhancing the axonal transport system[20], [21]. Additionally, when Swedish APP751 is transfected in HEK293 cell cultures the formation

of Aβ peptide may be blocked by Valproic acid (VPA) and lithium, as demonstrated by studies, suggesting their potential therapeutic value[22]. Numerous prior research findings have consistently indicated the vital involvement of HDACs in the guideline of acetylation. Through the inhibition of HDAC activity. This has been shown to improve memory and learning abilities while also protecting neuronal cells from death[23]. Additionally, three proteins—Aβ, GSK3β, and tau— have been widely acknowledged as important contributions to ailments, and their activity can be modulated by the application of HDAC inhibitors[24].

An existing medication known as ACY-1215 serves as an HDAC6 inhibitor. This medication has been proven to improve microtubule stability in the context of AD and reduce dementia in AD mice by raising tubulin acetylation. In the process of lowering the concentrations of A and hyperphosphorylated tau, increasing tubulin acetylation likewise renders it easier for these proteins to be removed by autophagy. When hyperphosphorylated tau proteins are released from microtubules, they have a propensity to accumulate in cells and form NFTs. These NFTs aid in the dissection and disintegration of the intracellular transport system.

When contrast to a conventional healthy brain, the hippocampus and cerebellum of a mouse model of ailments had much higher levels of HDAC6 expression [25]. The compound Tubacin, known for its selective inhibition of HDAC6, demonstrates effective suppression of the excessive phosphorylation of tau protein. This phosphorylation event triggers abnormal movement of mitochondria within neurons by activating the GSK3β pathway [26]

CHAPTER 2
REVIEW OF LITERATURE

2.1 Overview of Alzheimer's Disease

Alzheimer's disease (AD) is a neurological ailment that negatively impacts people's ability to acquire information and remember things. It frequently occurs with aging and mostly affects the cortex of the brain. The production of Aβ protein in the cerebral cortex as well as the hippocampus area of the mind is assumed to be the primary cause of AD. This protein disintegrates into a neurotoxic peptide, which in effect results in neuronal apoptosis and dying cells. The prevalence of AD significantly increases after the age of 70-75, with the data indicating that this percentage may reach up to 50% in the population above 85 years old. The possibility of developing AD is increased by several risk variables, such as diabetes, high blood pressure, and cardiovascular disease. There is an extra risk factors including high blood cholesterol levels, a lack of formal education (less than eight years), and a history of severe head injury, particularly in individuals with the apoE4 gene [27]. Families of those who have AD frequently exhibit early symptoms that worsen as the disease progresses over time. Memory loss, trouble with routine tasks, issues with temporal and space judgment, a tendency to lose things frequently, and personality, attitude, and behavior changes are all signs of AD. All these symptoms are linked to memory impairment and difficulties in learning, which are key characteristics of AD[28]. The onset of AD is typically gradual and progressively worsens with age. The condition may further deteriorate with the onset of cognitive decline, which represents the second stage of the disease. Early-stage AD is marked by short-term memory problems, behavioral changes, neglecting tasks such as switching off buttons and closing doors, and social withdrawal. Over time, the disease progresses to the second stage, characterized by difficulties in mental thinking abilities and various cerebral functions. There are two primary explanations for the Pathological mechanisms of AD: -

1. Amyloid hypothesis

The amyloid hypothesis postulates that a number of enzymes responsible for degrading this amyloid beta progenitor polypeptide (APP) are to be responsible for a buildup of Aβ plaques in the brain. Together α-, β-, and γ secrtases work to process this material. Additionally, elevated β-secretase 1 (BACE1) activity is thought to play a vital part in AD,

as it promotes the creation of Aβ plaques by allowing Aβ molecules to aggregate into oligomers. Enzymes cleaves APP, generating a fragment called C99, which is further processed by γ-secretase. This processing generates Aβ monomers, specifically Aβ40 and Aβ42, which are prone to aggregation and contribute to plaque formation. Interestingly, APP may additionally be digested by α-secretase. at different sites, resulting in a beneficial reduction in Aβ monomer production. By cleaving APP in this manner, α-secretase limits Aβ generation and helps prevent the gathering of Aβ. In essence, the amyloid hypothesis suggests that abnormal processing of APP by enzymes such as BACE1 and γ-secretase leads to the production and aggregation of Aβ, leading to the characteristic plaques observed in AD. However, the activity of α-secretase serves as a protective mechanism by reducing Aβ production through alternative cleavage of APP [29].

2. Tau hypothesis

The Tau hypothesis is widely accepted as an explanation for the development of NFTs observed in those with Alzheimer's disease's minds. Tau, a well-known protein associated with microtubules that maintain cellular structure. In AD, Tau undergoes phosphorylation by different kinases, leading to its accumulation in a hyperphosphorylated state. This accumulation of hyperphosphorylated Tau primarily occurs in axons and neuronal cell bodies, resulting in the formation of NFTs and Tau pathology. Recent research indicates that small aggregates of Tau, called Tau oligomers, may contribute to neuropathology and potentially initiate molecular events in AD. Furthermore, there is evidence suggesting a molecular connection between the deposition of Aβ plaques and NFT formation. Aβ has been found to hinder the transport of mitochondria along microtubules. As a result, the downstream outcome of Aβ polymerization might therefore correspond to Tau neurotoxicity, potentially responsible for the harmful effects associated with Tau. It is worth noting, however, that experimental validation is still required to confirm the validity of this hypothesis and gain a comprehensive understanding of the intricate relationship between Aβ deposition, Tau pathology, and neurotoxicity in AD[30], [32].

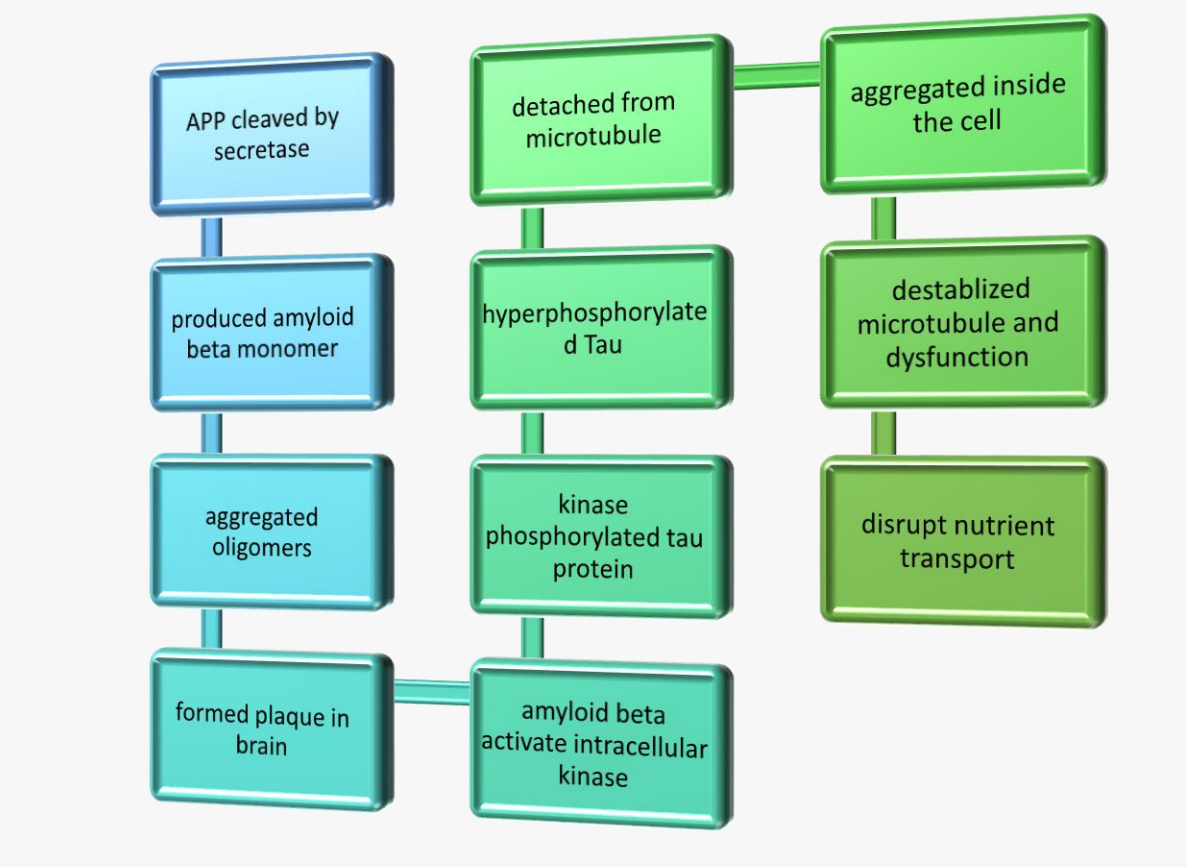

Figure 2.1. Signaling pathways of Aβ formation and Tau protein hyperphosphorylation.

2.2 Histone Proteins in AD Pathogenesis

Regulation of genes and expression during brain growth and memory improvement depends heavily on chromatin remodeling and modifications in the double-stranded DNA structure. Treatment for AD involves a lot of importance in altering histone proteins at the level of the cells. Nucleosome acetylation, which involves offering the protein histone an acetyl group, is a significant epigenetic mechanism implicated in this change. In decomposing brain tissues, It is currently shown that during the course of AD, this lysine mechanism is crucial for cells to grow and survival.

The crystalline structures of eukaryotic cells include a collection of highly conserved proteins known as histone proteins. They are vital for the management and wrapping of Genes, aiding in the packing of the lengthy DNA molecules into the chromatin structure. The nucleosome, which is formed up of eight histone proteins and DNA, is the basic part of chromatinH2B, H2A, H3, and H4 are among the four distinct kinds of nucleosome

proteins—each have two copies of these histone proteins. The DNA forms approximately 1.65 revolutions over this histone octamer in a left-handed superhelical route.

Histones have a globular domain known as the histone fold, which is a conserved structural motif. This fold allows the histone proteins to interact with each other, forming the octameric structure and providing stability to the nucleosome. These modifications may affect the structure of chromosomal and regulate how genes are expressed by either encouraging or preventing DNA accessibility to transcription factors and other proteins that regulate gene expression.

Compared to other neurological conditions, the transcriptional mechanisms underpinning the pathogenesis of AD are still poorly known. However, evidence has found a substantial link between AD and histone deacetylases (HDACs). An important protein termed APP is first broken down in brain tissues by the enzymes -secretase and -secretase, which causes a generation of -amyloid and intracellular tail fragments. These amyloid fragments grow up and interfere with gene function and expression, ultimately causing the death of the cell and apoptosis.

Potassium and glutamate levels in the brain that get not in balance may permit HDAC4 to go into neurons, alleviate the transcription of MEF2 and CREB-associated genes, and ultimately cause death in neurons. The latest study has shown that HDAC6 activates the system known as ubiquitin-proteasome and mechanisms including apoptosis for the detoxification of highly aggregated proteins.

HDAC1 was found to have a protective role in the p25/cyclin-dependent kinase 5 (CDK5)-induced mutant mouse model. In this mouse model, neurodegeneration is caused by stimulation of the p25 gene expression, and the procedure is aided by an immediate link between p25 and an enzyme domain of HDAC1. Reduced HDAC1 activity causes strange production of several cell cycle proteins, DNA damage, and eventually, the death of neuronal cells.

The effects of the class I HDAC transcription on neuronal cells have been examined in the cerebral part of the brain. It was found that expressed HDAC1 in these neurons protected against p25-induced neurotoxicity, but not a mutant with a catalytically inactive enzyme. Furthermore, it was discovered that the coexpression of HDAC1 did not increase the level of protection provided by pharmacological suppression of histone acetyltransferase (HAT) activity against p25-induced neurotoxicity.

These results suggest that histone deacetylation promotes the beneficial action of HDAC. By regulating the acetylation status of histones, HDAC1 plays a crucial role in protecting neurons from the toxic effects induced by p25/CDK5 activation [33], [34].

HDAC4's role in managing neurodegeneration is still little understood and needs to be thoroughly investigated. Initially, studies suggested that overexpressing HDAC4 in cerebellar granule neurons in vitro caused apoptosis and cell death. However, further research discovered that HDAC4 activity can help improve neurological function by safeguarding neurons against cell death brought on by low potassium levels. Furthermore, it has been found that HDAC4 safeguards against the toxicity brought on by 6-hydroxydopamine and homocysteine acid in cultured cortical neurons.

2.3 Types of Histone Deacetylase

Mammals include 18 confirmed histone deacetylases (HDACs), which are separated into two families: Families of histone deacetylases and sirtuin inhibitors. They are additionally separated into four classes based on their structural and sequence similarities that exist with yeast deacetylases. HDAC2, HDAC1, HDAC3, and HDAC8 are all found in Class I, then followed by Class IIa (HDAC5, HDAC7, HDAC4, and HDAC9), Class IIb (HDAC10, and HDAC6), Class III (Sir1–Sir7), and Class IV (HDAC11). The arginase/deacetylase superfamily, which contains enzymes with amidohydrolase and histone deacetylase movement, is made up of classes I, II (IIa and IIb), and IV. On the reverse hand, the sirtuins family has connections to the NAD/FAD-binding motif complex and includes enzymes with central pyruvate oxidase, carboxy-terminal, and decarboxylase districts.

While Class II HDACs (IIa and IIb) like the yeast protein Hda1, Class I HDACs (IIa and IIb) resembled the yeast protein Rpd3. Though considerably less researched than other HDACs. Class I and Class II HDACs have shared structural characteristics with HDAC11. Sirtuins, a subclass of class III HDACs, contain sequence similarities with the yeast protein Sir2 due to the simple fact that transcription factors employ them as corepressors that cling to DNA, class I HDACs are referred to as transcription repressors. Both the cytoplasm and the nucleus contain them, and they are essentially expressed when carrying out particular tasks in many tissues. For instance, the deacetylation-dependent efficiency of the MAP kinase phosphatase (MKP1) is altered by HDAC1, HDAC2, and HDAC3, and this modification of histones [35].

Class III HDACs are constituents of the Sir2 regulatory family, while Class I, II, and IV HDACs are components of the arginase/deacetylase subfamily. The deacetylase activity of class I HDACs varies. The most prevalent HDAC is HDAC1, while HDAC2 (also called mRPD3), which shares a significant amount of sequence similarity with yeast Rpd3, was originally identified as a transcriptional repressor protein. By researching DNA and protein databases, it was shown that HDAC3 and HDAC1, and HDAC2 have similar sequences. By comparing its protein sequence to those of HDAC1, 2, and 3, HDAC8, which parallels yeast Rpd3 HDAC, was discovered.

Class I HDACs have sequence similarity that varies between 45% to 95% and typically function in the nucleus or through the body[36], [37]. Using a Silencing Mediator for Retinoid or Thyroid Hormone Receptor (SMRT) silencing involves the three receptor subunits in responsible for transcriptional suppression. HDAC7 was the initially identified member of Class IIa HDACs to be discovered. HDAC9 has a conserved deacetylase domain and several alternatively spliced isoforms. Class IIb HDACs, mainly found in the cytoplasm, including HDAC6 and HDAC10, have a 55% similarity in their amino acid sequences. However, only 25% to 80% of the putative regions of HDAC6 and HDAC10 exhibit sequence homology.

The Sir2 family of proteins, consisting of the class III HDACs, SIRT1-SIRT7, are involved in yeast transcriptional repression or silencing. These enzymes are NAD+-dependent. The Sir2 protein served as the initial basis for the discovery of SIRT1-SIRT5 utilizing the GenBank database. Lexical sequence analogies in SIRT1 and SIRT7 range from 22% to 50%, with SIRT1 exhibiting excellent conserved compared to yeast Sir2. Class I, II, and IV HDACs use a central zinc atom for their active action. To explain the catalytic processes of Class I and II HDACs, two models have been put forth. The HDLP (HDAC-like protein) structure model and the HDAC8 structure model illustrate the catalytic mechanisms. Class III HDACs (sirtuins) depend on NAD+

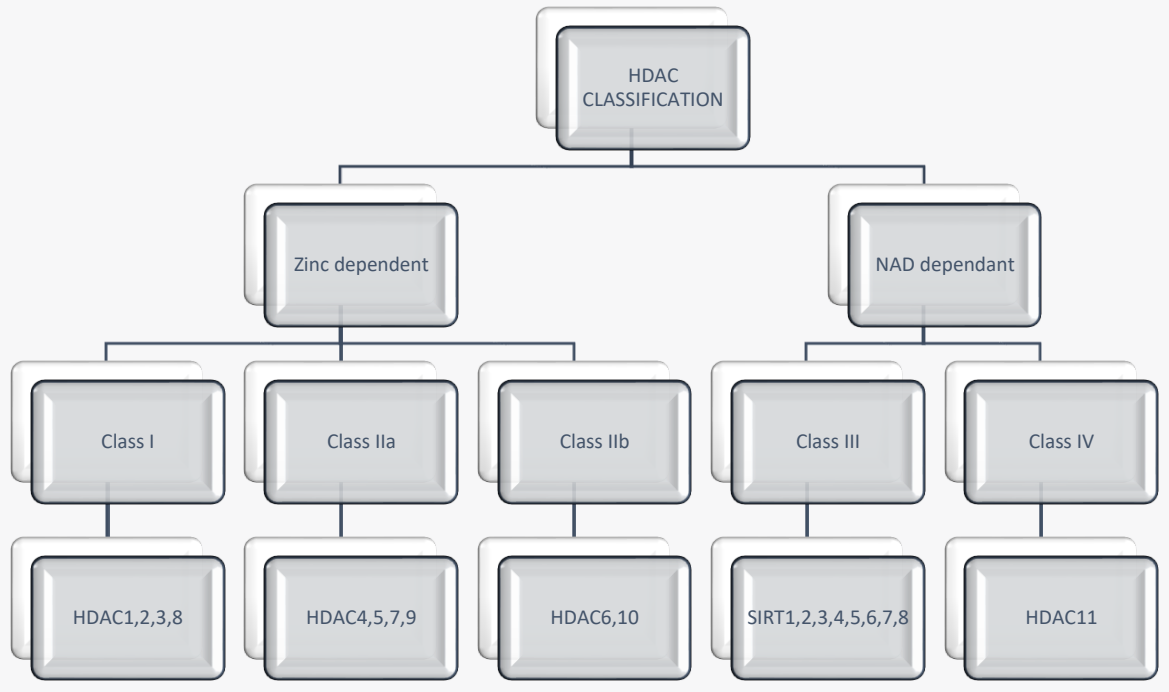

Figure 2.2. flow chart of classification of HDAC superfamily

2.4 Role of HDAC in AD

HDACs influence the degree to which acetylation of histone takes place and drugs known as HDAC inhibitors promote histone acetylation, boosting memory and recall. Aβ, GSK-3, and protein tau are a few examples that are particular proteins with important roles in Alzheimer's disease (AD) that these inhibitors also have an impact on. A crucial enzyme in the phosphorylation of tau, GSK-3, is connected to numerous pathways hypothesized to contribute to AD. Emerging evidence suggests that Aβ triggers excessive phosphorylation of tau by activating GSK-3β [38], [39]. Therefore, preventing the deficits in histone acetylation and the hyperphosphorylation of tau induced by Aβ is beneficial for AD treatment. HDAC inhibitors reduce the activity of HDACs, resulting in increased histone acetylation. One specific enzyme, HDAC6, primarily located in the cytoplasm, is responsible for removing acetyl groups from various proteins. Recent findings have revealed that HDAC6 deacetylates tubulin, a protein involved in the structure of microtubules. HDAC6 can independently remove acetyl groups from both individual tubulin molecules and assembled microtubules. In individuals with AD, the levels of HDAC6 protein are significantly elevated in the cortex and hippocampus compared to those with normal brain function [40]. It has been demonstrated that using the HDAC6 inhibitors tubacin causes less phosphorylated of tau at specific locations, supporting the theory that HDAC6 is involved

in AD. The targeted inhibition of HDAC6 has a profound effect on enhancing the movement of mitochondria in hippocampal neurons. It is possible that GSK-3β, through a phosphorylation pathway, actively regulates HDAC6, potentially contributing to the misregulation of HDAC6 and subsequent abnormal mitochondrial transport in AD[41]. Additionally, An increase in oxidative is a hallmark of AD, and specific inhibition of HDAC6 has shown to be preventive against neurodegeneration carried on by oxidative stress. Furthermore, it promotes the growth of neurites in cortical neurons. HDAC6's operation in AD: -

• Tau Pathology: Tau is governed to some extent by HDAC6, a protein that forms abnormal tangles in AD. In AD, tau becomes hyperphosphorylated, leading to its aggregation into neurofibrillary tangles. Inhibiting HDAC6 reduces tau phosphorylation, preventing abnormal aggregation and potentially mitigating tau-related pathology in AD.

• Elimination of Misfolded Proteins: HDAC6 is recognized for its role in mechanisms that regulate the quality of proteins. It facilitates the removal of misfolded or clustered proteins, which include tau and A, another protein linked to AD. ITotransfer protein aggregates to cellular breakdown divisions like aggresomes or autophagosomes, HDAC6 interacts with the aggregates. Dysregulation of HDAC6 could impede this process. Impaired clearance processes can lead to the buildup of hazardous protein aggregates in AD.

• Cellular Transport and Mitochondrial Function: The deacetylation of tubulin is facilitated by HDAC6, a component of microtubules that are crucial for cellular transport. AD is associated with abnormal mitochondrial transport and dysfunction. Inhibiting HDAC6 enhances the movement of mitochondria in neurons, potentially improving mitochondrial dynamics and function in AD.

• Oxidative Stress: The formation of reactive oxygen species (ROS) development and the defense system that produces antioxidants are in a state of harmony throughout oxidative stress, which is an essential element of AD pathogenesis. HDAC6 is implicated in the regulation of pathways involved in responding to oxidative stress. Selectively inhibiting HDAC6 has shown protective effects against neurodegeneration induced by oxidative stress.

Figure 2.3. Different types of roles of HDAC6 in AD

CHAPTER 3
METHODOLOGY

3.1 Source

The database used: PubMed, Drugbank, ChEMBL, PubChem, and Protein Data Bank (PDB).

Software used: SwissDock, Biovia Discovery Studio Visualizer, pyRx, CB ligand

3.2 WORKFLOW

From the literature survey, HDAC6 was identified as a potential target molecule for repurposing an antagonist that could specifically target the abnormal indirect signaling of HDAC6 in Alzheimer's disease (AD). To find suitable candidates, a list of FDA-approved anti-viral drugs was obtained from Drugbank. Those of these medications that might penetrate the blood-brain barrier (BBB) were chosen as ligands for further investigation in the study. The flowchart of the protocol has been depicted in Fig. 3.1.

3.3 Data Extraction

From the RCSB (PDB official website) the 3-D structure of HDAC6 (6CGP) (https://www.rcsb.org/). A total of 90 FDA-approved anti-viral drugs were collected from the Drugbank database. The structures of these drugs were retrieved from PubChem. Additionally, the structures of two well-known HDAC6 inhibitors, Tubacin and ACY-1215, were also extracted. These inhibitors were identified using the BioGRID database (https://thebiogrid.org/). Tubacin and ACY-1215 were selected as control drugs for the HDAC6 study. The ChEMBL database was utilized to assess various properties of the ligands, such as molecular weight. From the initial set of 90 drugs, a subset was chosen for further molecular docking studies.

3.4 BBB Permeability Analysis

Out of the 90 drugs, only 30 drugs cross the BBB. BBB permeability analysis was conducted using an online tool specifically designed for predicting BBB permeability. (https://www.cbligand.org/BBB/mainpage.php).

3.5 Molecular Docking

The receptor-ligand interaction has been examined using molecular docking on a Pyrx web server. The following procedures were carried out afterward:

a. Preparation of the Target Receptors

HDAC6's three-dimensional structures were retrieved in. pdb file format from the RCBS PDB. Then, the Biovia Discovery Studio Visualizer was applied to open this.PDB file. By removing additional water and hetatm molecules, structures had been altered and redefined. The altered files were then stored in. pdb format.

b. Preparation of the Ligand Molecules

Through PubChem, the 3-D structures of ligands have been generated. SDF files. We acquire the SDF file with the 30 ligands' structure.

c. Molecular Docking Studies

Once the receptor and ligand structures were prepared, molecular docking analysis was conducted using PyRx and SwissDock web servers. The interactions between the receptor and ligands were visualized and assessed using Biovia Discovery Studio Visualizer.

d. SwissADME-based ADME assessment for the Ligand

Finally, ADMET analysis and calculations of pharmacokinetic properties were performed using SwissADME and ChEMBL. This step is vital in identifying lead molecules as it provides information on the pharmacokinetics and drug-likeness of the compounds

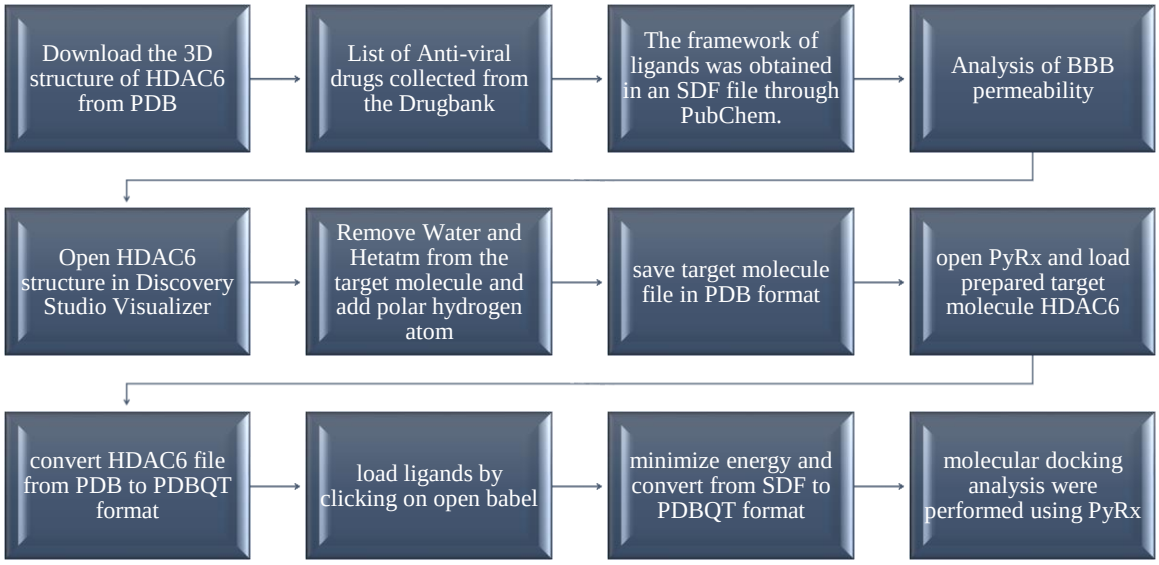

figure.3.1. flowchart of steps involved in Molecular Docking.

CHAPTER 4
RESULTS

4.1 Structure of Target protein [HDAC6]

The novel 3-D structure without energy minimize and after energy minimized and removing water molecules and hetatm were depicted in Fig. 4.1, 4.2.

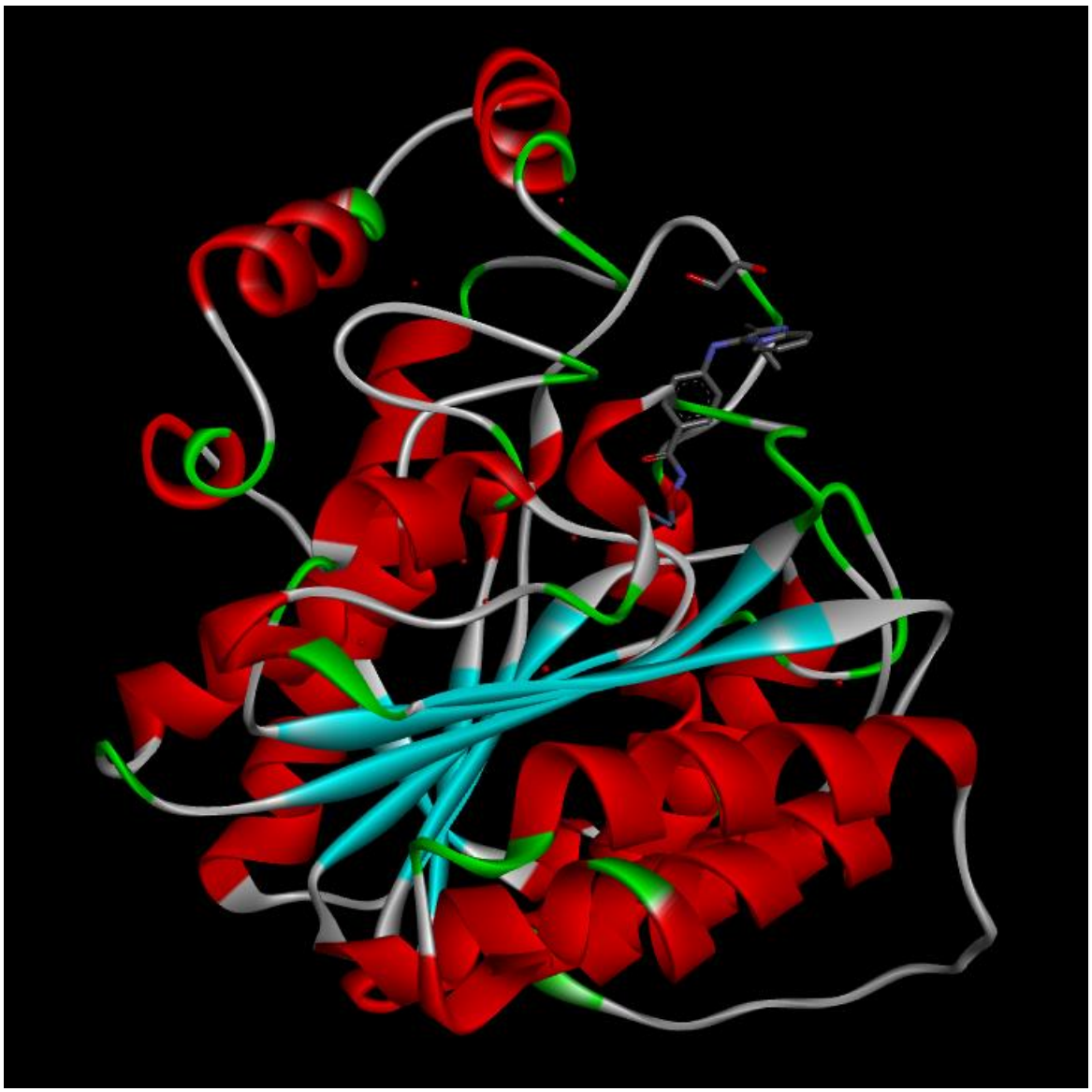

Figure 4.1. The 3-D structure of HDAC6 [target protein] without removing Hetatm and water molecules.

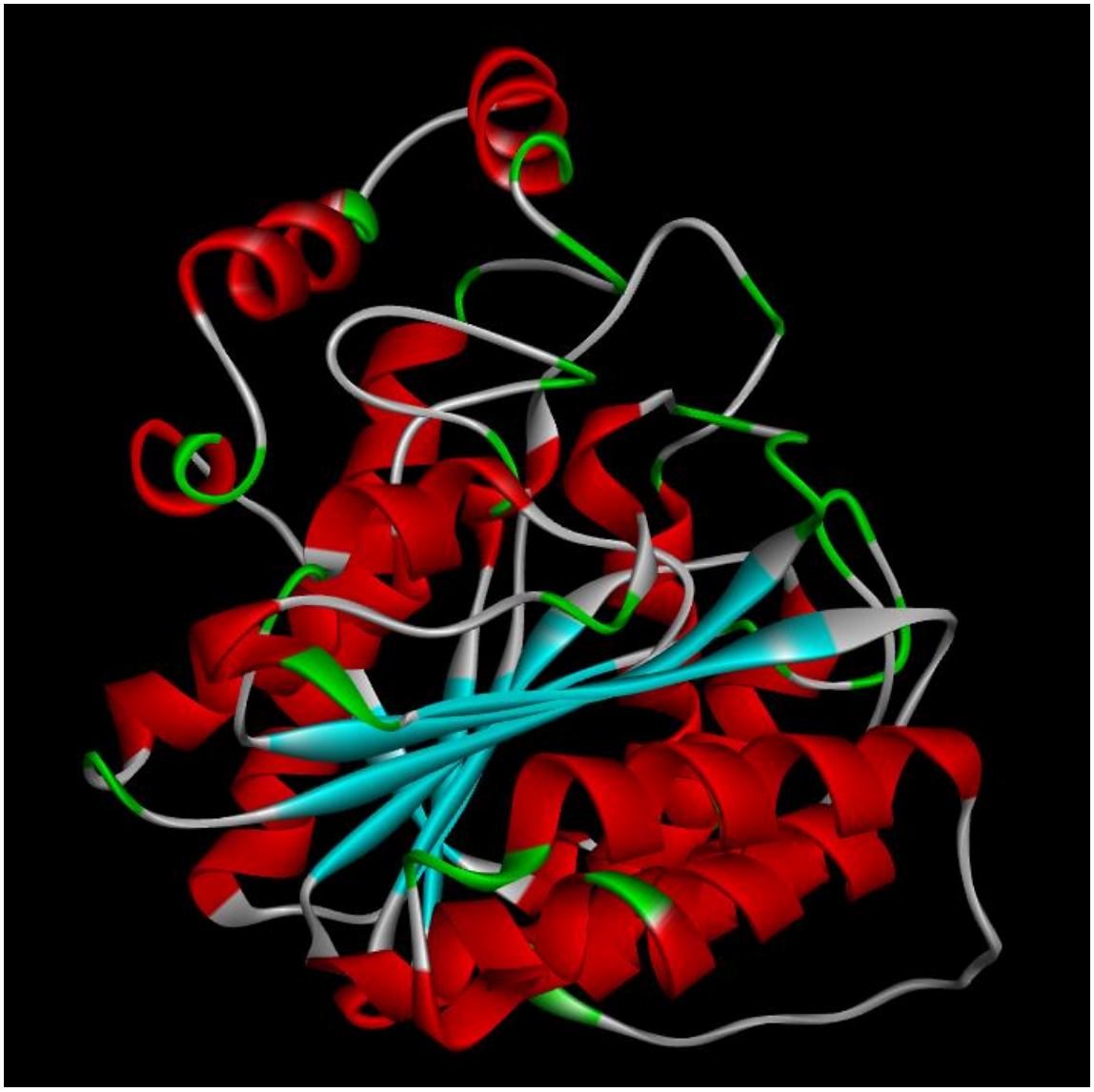

Figure 4.2. The 3-D structure of HDAC6 [target protein] after removing Hetatm and water molecules.

4.2 BBB Permeability Analysis

The anti-viral drugs included in the analysis were sourced from the DrugBank database and their structures were retrieved from PubChem. Subsequently, the drugs underwent analysis to determine their permeability through the Cbligand (BBB). Thirty medicines among an overall of 90 had the opportunity to pass through the BBB.

Table 4.1. List of BBB cross anti-viral drugs

Entecavir	Emtricitabine	Amprenavir	Dolutegravir	Famciclovir
Foscarnet	Nevirapine	Fosamprenavir	Abacavir	Penciclovir
Didanosine	Delavirdine	Tipranavir	Lamivudine	Valgancicloir
Stavudine	Efavirenz	Imiquimod	Maraviroc	Amantadine
Lamivudine	Etravirine	Raltegravir	Acyclovir	Rimantadine
Abacarvir	Rilpivirine	Elvitegravir	Gancidovir	Podofilox

4.3 Analysis of Receptor-Ligand Interaction

Among the 90 FDA-approved antiviral drugs, only 30 can penetrate the blood-brain barrier. Out of these 30 drugs, only 5 meet the required criteria of having an RMSD value of less than 1 Å and binding energy higher than -8 kcal/mol. Following the analysis, Maraviroc emerged as the top-ranked drug based on its RMSD value of 0.0 Å and binding energy of -9.4 kcal/mol, making it the clear winner.

Table 4.2. Binding energy (in kcal/mol) of 30 selected ligands

Ligand	**Ligand**	**rmsd/ub**	**rmsd/lb**
HDAC6PROTEIN_49850262_uff_E=486.15	-10	0	0
HDAC6PROTEIN_3002977_uff_E=751.35	**-9.4**	0	0
HDAC6PROTEIN_54726191_uff_E=465.06	**-9**	0	0
HDAC6PROTEIN_6451164_uff_E=557.73	**-8.9**	0	0
HDAC6PROTEIN_53340666_uff_E=406.00	**-8.6**	0	0
HDAC6PROTEIN_54671008_uff_E=553.49	**-8.6**	0	0
HDAC6PROTEIN_193962_uff_E=459.89	**-8.5**	0	0
HDAC6PROTEIN_65016_uff_E=806.98	-7.6	0	0
HDAC6PROTEIN_54682461_uff_E=818.62	-7.6	0	0
HDAC6PROTEIN_5277135_uff_E=362.75	-7.6	0	0
HDAC6PROTEIN_10607_uff_E=825.29	-7.4	0	0
HDAC6PROTEIN_64139_uff_E=1370.89	-7.2	0	0
HDAC6PROTEIN_131536_uff_E=1156.58	-7.2	0	0
HDAC6PROTEIN_5625_uff_E=1203.06	-7.1	0	0

HDAC6PROTEIN_441300_uff_E=1759.65	-6.8	0	0
HDAC6PROTEIN_4463_uff_E=1702.40	-6.6	0	0
HDAC6PROTEIN_57469_uff_E=442.70	-6.6	0	0
HDAC6PROTEIN_5071_uff_E=155.08	-6.4	0	0
HDAC6PROTEIN_135398508_uff_E=503.82	-6.3	0	0
HDAC6PROTEIN_135413535_uff_E=452.35	-6.3	0	0
HDAC6PROTEIN_18283_uff_E=190.05	-6.1	0	0
HDAC6PROTEIN_3324_uff_E=386.77	-6.1	0	0
HDAC6PROTEIN_135398739_uff_E=491.96	-6	0	0
HDAC6PROTEIN_60825_uff_E=198.09	-5.7	0	0
HDAC6PROTEIN_135398740_uff_E=421.15	-5.7	0	0
HDAC6PROTEIN_60877_uff_E=192.79	-5.6	0	0
HDAC6PROTEIN_135398513_uff_E=387.01	-5.6	0	0
HDAC6PROTEIN_135398748_uff_E=381.11	-5.5	0	0
HDAC6PROTEIN_2130_uff_E=115.62	-5	0	0
HDAC6PROTEIN_3415_uff_E=283.60	-4.2	0	0

TABLE 4.3. LIST OF BEST POTENT ANTIVIRAL DRUGS AND THEIR BINDING ENERGY

S.NO	CHEMBLID	NAME	BINDING AFFINITY (KCAL/MOL)
1	CHEMBEL1201187	MARAVIROC	-9.4
2	CHEMBEL3526034	DOLUTEGRAVIR	-9
3	CHEMBEL175691	RILPIVIRINE	-8.9
4	CHEMBEL254316	RALTEGRAVIR	-8.6
5	CHEMBL308954	ETRAVIRINE	-8.5

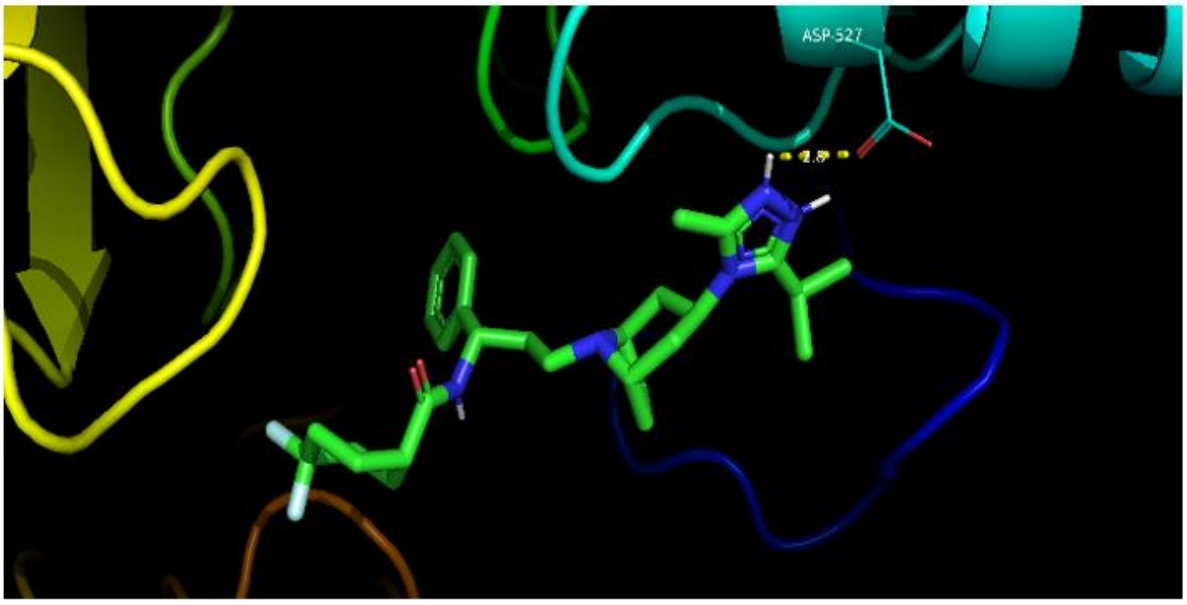

Figure 4.1. 3-D Interaction diagram between Target protein HDAC6 and Maraviroc.

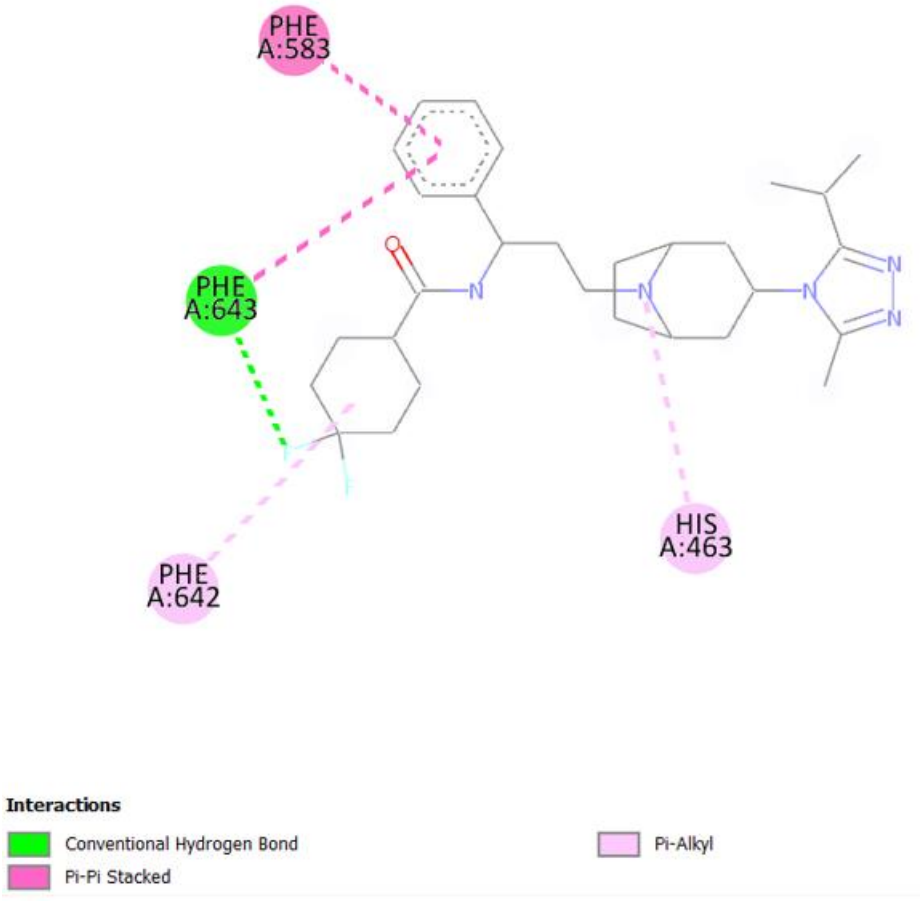

Figure 4.2. Diagram of the 2-D relationship among Maraviroc and the target protein HDAC6.

Maraviroc, marketed under the brand names Celsentri and Selzentry, is a highly potent drug with a strong binding affinity (-9.4 kcal/mol). It is classified as a small molecule type of medication, and its DrugBank Accession Number is DB04832. Maraviroc is an antiviral drug classified as a CCR5 antagonist. It is primarily utilized to treat human immunodeficiency virus (HIV) infection. The medication functions by blocking the CCR5 receptor, a protein present in certain immune cells. This prevents HIV from entering and infecting these cells. CCR5 is one of the entry points for HIV into CD4+ T-cells, which are vital immune cells. By inhibiting the CCR5 receptor, maraviroc reduces the virus's presence in the body and slows down HIV progression. Maraviroc is frequently employed along with other antiretroviral drugs as part of a highly active antiretroviral therapy (HAART) regimen rather than being used alone for HIV therapy. To enhance the effectiveness of the treatment, HAART employs a number of medications from diverse classes to attack the virus at multiple stages in its life cycle. Like any medication, maraviroc may have side effects. Common ones include cough, fever, dizziness, rash, and abdominal pain.

Dolutegravir is an antiretroviral drug used to treat HIV infection by inhibiting the integrase enzyme necessary for HIV replication. It falls under the class of drugs called integrase strand transfer inhibitors (INSTIs). When combined with other antiretroviral medications, dolutegravir has been proven effective in suppressing viral replication, alleviating HIV-related symptoms, and improving immune function. It is commonly prescribed as a first-line treatment for both treatment-naive individuals and those switching from other regimens. Dolutegravir offers several advantages as an HIV medication. It has a high barrier to resistance, meaning that the development of drug-resistant HIV strains is less likely compared to some other antiretrovirals. With its long half-life, once-daily dosing is possible. Additionally, dolutegravir is typically tolerated well, and usual negative reactions include headaches, sleeplessness, and digestive problems. However, it is crucial to emphasize that dolutegravir should only be used under medical supervision due to specific precautions and considerations. It may interact with other medications, and caution is advised during pregnancy, as there have been concerns about potential neural tube defects when used in the first trimester. In summary, dolutegravir has emerged as a vital component of HIV treatment regimens, offering efficacy, tolerability, and a high barrier to resistance. The best treatment strategy for people with Aids should, however, always be chosen after assessing each patient's unique circumstances and healthcare requirements.

As an antimicrobial medicine, Rilpivirine is used to treat contracting a viral infection. It falls under the category of non-nucleoside reverse transcriptase inhibitors (NNRTIs). Rilpivirine

effectively suppresses viral replication, alleviates HIV-related symptoms, and enhances immune function. As a first-line treatment for HIV/AIDS, it is frequently prescribed when combined with various other antiretroviral drugs both treatment-naive individuals and those switching from other regimens. Rilpivirine offers several advantages as an HIV medication. It exhibits high potency against HIV and has the convenience of once-daily oral tablet dosing, resulting in a low pill burden. Additionally, rilpivirine is generally well-tolerated, with common side effects such as headache, dizziness, and insomnia. However, it is essential to highlight that rilpivirine should only be used under the supervision of a healthcare professional due to specific precautions and considerations. It may interact with other medications, and caution is advised for individuals with certain liver conditions. In summary, rilpivirine has become a crucial component of HIV treatment regimens, demonstrating effectiveness, tolerability, and convenience with its once-daily dosing. Nevertheless, individual circumstances and medical factors should always be taken into account when determining the most suitable treatment approach for individuals living with HIV.

Raltegravir is an antiretroviral drug used to treat HIV infection. In combination therapy, raltegravir has proven effective in suppressing viral replication, alleviating HIV-related symptoms, and enhancing immune function. It is commonly prescribed alongside other antiretroviral medications as a first-line treatment for both treatment-naive individuals and those transitioning from other regimens. Raltegravir offers several advantages as an HIV medication. Its high barrier to resistance reduces the likelihood of developing drug-resistant strains compared to other antiretrovirals. Additionally, raltegravir provides convenient dosing options, with formulations available for twice-daily or once-daily administration. However, it is crucial to note that raltegravir should be used under medical supervision due to specific precautions and considerations. It may interact with other medications, and caution is advised in individuals with certain kidney conditions. In summary, raltegravir has become a significant component of HIV treatment regimens, demonstrating effectiveness, resistance reduction, and flexible dosing options. Nonetheless, individual circumstances and medical factors should always be considered when determining the most appropriate treatment approach for individuals living with HIV.

Limited information is available regarding the direct effects of etravirine on HDAC6 inhibition. Although the suppression of HDAC6 is being investigated as an alternative therapy for neurological conditions including Alzheimer's, the specific interaction between etravirine and HDAC6 is not well-studied. The main way that etravirine works is by

blocking its own reverse transcribing enzyme, which is crucial to the transmission of HIV. Its role, if any, in HDAC6 inhibition would likely be secondary or incidental. It's critical to reiterate that additional study is required to completely understand any possible effects of etravirine on HDAC6 activity and its relevance to neurodegenerative conditions. For more comprehensive and up-to-date information on this topic, consulting recent scientific literature or seeking advice from experts in the field of HIV therapeutics or neurodegenerative diseases is recommended.

4.4 SwissADME-based ADME assessment for the Ligand

ADME, which stands for Absorption, Distribution, Metabolism, and Excretion, refers to a set of factors that are analyzed to gain insights into a drug's physicochemical composition, pharmacokinetics, lipophilicity, water solubility, and potential effectiveness. Evaluating these factors provides valuable information about the drug's performance and efficiency. SwissADME is an online program (http://www.swissadme.ch/) commonly utilized to assess these characteristics for small-molecule ligands. In this program, data in the form of SMILES format, representing the ligand-protein interactions, is loaded. The SMILES data used in SwissADME is typically collected from PubChem.

Table 4.4. **Physiochemical properties of selected ligands**

Properties	**Drug name**				
	Maravioc	**Dolutegravir**	**Rilpivirine**	**Raltegravir**	**Etravirine**
Molecular weight (g/mol)	513.67	419.38	366.42	444.42	435.28
Hydrogen-bond donors	1	2	2	3	2
Hydrogen-bond acceptors	6	7	4	9	5
Molar refractivity	145.84	104.48	110.41	109.03	109.56
Topological polar surface area (Å2)	63.05	100.87	97.42	152.24	120.64
Lipinski's rule of five	No, 2 violation	Yes, 0 violation	Yes, 0 violation	Yes, 0 violation	Yes, 0 violation
Bioavailability score	0.17	0.55	0.55	0.55	0.55
Gastro-Intestinal absorption	High	high	high	low	low
Log P [SILICOS-IT]	4.78	1.85	4.04	1.81	3.72
BBB permeant	YES	YES	YES	YES	YES
Solubility	-7.00	-4.47	-7.78	-5.83	-7.81

DISCUSSION

According to recent studies, a relationship between the antiviral medication Maraviroc and the Medical advantages of HDAC6 protein in the cure of AD. HDAC6 inhibitors are chemicals that control the transcription of genes by preventing HDAC6 from performing its normal functions, which include transporting and decomposing proteins in the body. Due to their capacity to enhance cognitive performance and lessen the clinical symptoms connected with the disease, these drugs are at present under consideration as an option for Alzheimer's therapy.

Maraviroc, primarily used for treating HIV infections, has also shown promise in the context of Alzheimer's treatment. Studies have indicated that Maraviroc can reduce neuroinflammation and enhance cognitive function in animal models of the disease. Interestingly, both HDAC6 inhibitors and Maraviroc have an impact on a Tau protein. Tau is in charge of retaining nerve cells mechanically intact, but in Alzheimer's, it can accumulate abnormally and disrupt brain function.

Animal models of Alzheimer's have revealed that the combination of HDAC6 inhibitors and Maraviroc can decrease the accumulation of tau, suggesting that their synergistic action may alleviate tau pathology and improve cognitive function. This finding has sparked interest in further exploring the potential of this drug combination as a therapeutic approach for Alzheimer's.

In summary, current research indicates that the interaction between HDAC6 inhibitors and Maraviroc might be effective in treating dementia. Both drugs have demonstrated the ability to impact tau protein accumulation, a hallmark of the disease, and improve cognitive function in preclinical studies. For a review of their reliability and efficacy in human beings, as well as the processes that lie behind their communication, more research is however required.

As of my last knowledge update in September 2021, there were ongoing preclinical trials investigating the potential of antiviral drugs in Alzheimer's disease, but no conclusive results were available at that time. The aim of these trials is to determine if certain viral infections, such as HSV-1 and CMV, contribute to the development or progression of Alzheimer's disease. Antiviral drugs developed to target specific viral infections are being evaluated for their safety and efficacy in laboratory settings or animal models. It's important to note that

preclinical trials are just the initial stage of research and do not guarantee the effectiveness or safety of a drug in humans. The outcomes of preclinical trials inform the decision to proceed to human clinical trials, where the drugs are further evaluated for their safety, dosing, and effectiveness. For the most up-to-date information on preclinical trials of antiviral drugs in Alzheimer's disease, I recommend consulting recent scientific literature, and clinical trial databases, or reaching out to relevant research institutions or organizations specializing in Alzheimer's research.

CONCLUSION

The current lack of treatment options for neurodegenerative diseases (NDDs) emphasizes the need to explore alternative approaches. In this context, in silico studies, which involve computer simulations and virtual experiments, have emerged as valuable tools for developing new treatments for various NDDs. One promising approach is drug repurposing, which involves identifying existing drugs that may be effective in treating NDDs. Although initial findings from these studies are encouraging, further research is necessary to fully understand the potential benefits and risks of combining HDAC6 inhibitors and Maraviroc as a treatment for Alzheimer's disease.

HDAC6 inhibitors are medications that can modify gene expression, and Maraviroc is primarily used to treat HIV infections. Both drugs have the potential to cause side effects and interact with other medications, necessitating careful monitoring and dosing if they are used together clinically. During an investigation, researchers discovered a novel HDAC6 inhibitor that exhibits significantly higher binding affinity compared to well-known inhibitors. This finding opens up a promising avenue of research into the interaction between HDAC6 inhibitors and Maraviroc for Alzheimer's treatment.

If successful, this research could lead to the development of innovative and effective therapies for Alzheimer's disease, a devastating condition. However, it is important to note that further research is needed to fully comprehend the potential benefits and risks associated with combining HDAC6 inhibitors and Maraviroc. This ongoing effort aims to provide novel and efficient therapies to combat the debilitating effects of neurodegenerative diseases. In summary, the lack of effective treatments for neurodegenerative diseases necessitates exploring alternative approaches. In silico studies and drug repurposing offer hope for discovering new therapeutic strategies. Investigating the interaction between HDAC6 inhibitors and Maraviroc for Alzheimer's treatment shows great potential, but additional research is required to fully understand its benefits and risks. The ultimate goal of these endeavors is to provide innovative and effective therapies to address the challenges posed by neurodegenerative diseases.

To sum up, repurposing antiviral medicines that target HDAC6 as an intervention for Alzheimer's condition has huge potential. HDAC6, an enzyme involved in cellular processes, has been linked to protein clearance and neuroinflammation regulation, both

important factors in Alzheimer's pathology. By repurposing antiviral drugs that can modulate HDAC6 activity, researchers aim to leverage their known safety profiles and mechanisms of action to target the underlying molecular pathways involved in the disease. Preclinical studies have shown promising outcomes, demonstrating the ability of these repurposed antiviral drugs to enhance the clearance of abnormal protein aggregates and reduce neuroinflammation in animal models. This approach offers advantages such as a potentially faster and more cost-effective drug development process, given that these drugs have already undergone extensive testing for their antiviral properties. Repurposing existing drugs also reduces the risks associated with developing novel drugs. However, it's important to acknowledge that the translation of preclinical findings to effective clinical treatments is still ongoing. Further research is necessary to establish the optimal safety profiles, dosages, and long-term efficacy of these repurposed antiviral drugs in human clinical trials. In conclusion, the repurposing of antiviral drugs targeting HDAC6 represents an innovative and promising avenue in Alzheimer's therapeutics. Continued exploration and investigation in this field may provide novel treatment options that can modify the disease's progression and ultimately improve the lives of individuals affected by Alzheimer's disease.

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