REGULATION OF HYPOXIA INDUCIBLE FACTORS VIA HISTONE DEACETYLASE 3 INHIBITOR DRUGS AND A COMPARISON BETWEEN THEIR INTERACTIONS

A DISSERTATION

SUBMITTED IN THE PARTIAL FULFILMENT OF THE

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MASTER OF SCIENCE

IN

BIOTECHNOLOGY

Submitted by:

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I, Yami Garg, Roll No., 2K20/MSCBIO/37, student of M.Sc. Biotechnology, hereby declare that the Dissertation project titled "**Regulation of Hypoxia Inducible Factors via Histone Deacetylase Inhibitor 3 Drugs and a Comparison between their Interactions**" which is submitted by me to the Department of Biotechnology, Delhi Technological University, Delhi in partial fulfilment of the requirements for the award of the degree of Master of Science, is original and not extracted from any source without proper citation. This 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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I hereby certify that the dissertation project titled "**Regulation of Hypoxia Inducible Factors via Histone Deacetylase 3 Inhibitor Drugs and a Comparison between their Interactions**" which is submitted by Yami Garg, 2K20/MSCBIO/37, Department of Biotechnology, Delhi Technological University, Delhi in partial fulfilment of requirement for the award of the degree of Master of Science, is a record of work carried out by the student under my supervision. To the best of my knowledge, this work has not been submitted in part of any Degree or Diploma to this University or elsewhere.

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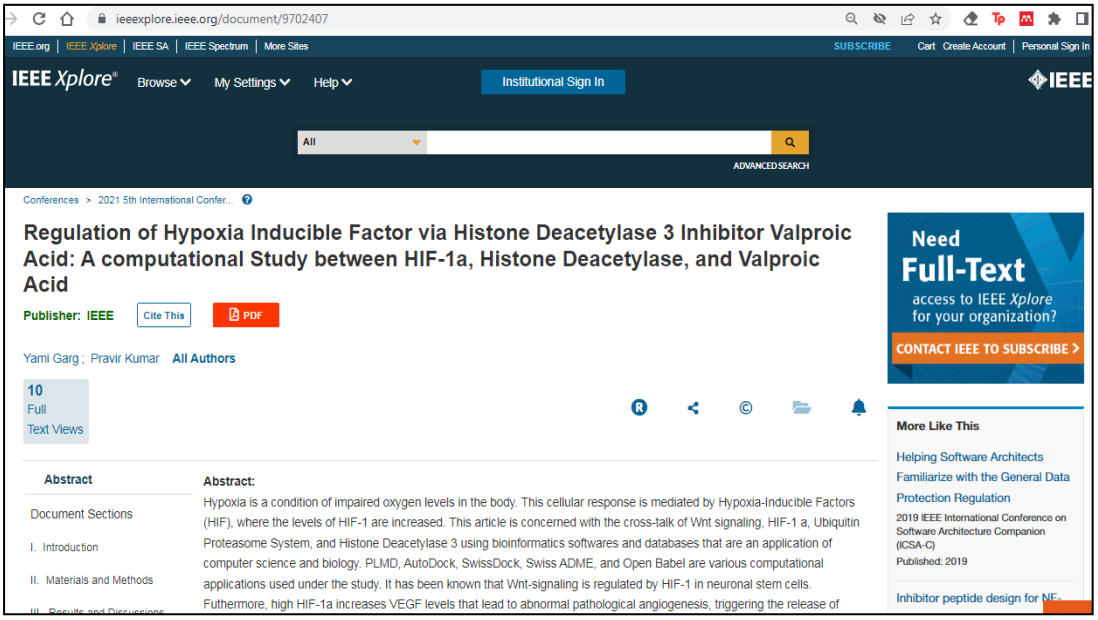

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Abstract

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I. Introduction

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Abstract:

Hypoxia is a condition of impaired oxygen levels in the body. This cellular response is mediated by Hypoxia-Inducible Factors (HIF), where the levels of HIF-1 are increased. This article is concerned with the cross-talk of Wnt signaling, HIF-1 a, Ubiquitin Proteasome System, and Histone Deacetylase 3 using bioinformatics softwares and databases that are an application of computer science and biology. PLMD, AutoDock, SwissDock, Swiss ADME, and Open Babel are various computational applications used under the study. It has been known that Wnt-signaling is regulated by HIF-1 in neuronal stem cells. Futhermore, high HIF-1a increases VEGF levels that lead to abnormal pathological angiogenesis, triggering the release of

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YAMI GARG

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ABSTRACT

Hypoxia is a condition of impaired oxygen levels in the body. This cellular response is mediated by Hypoxia-Inducible Factors (HIF), where the levels of HIF-1 are increased. This article is concerned with the cross-talk of Wnt signaling, HIF-1 α, Ubiquitin Proteasome System, and Histone Deacetylase 3 using bioinformatics softwares and databases that are an application of computer science and biology. PLMD, AutoDock, SwissDock, Swiss ADME, and Open Babel are various computational applications used under the study. It has been known that Wnt-signaling is regulated by HIF-1 in neuronal stem cells. Futhermore, high HIF-1α increases VEGF levels that lead to abnormal pathological angiogenesis, triggering the release of TGFs which leads to the accumulation of AβPP and secretion of neurotoxic peptides. This research refers to a study where valproic acid, an HDAC has been known to restore the functions of NEP and loss of the memory that had been caused by prenatal hypoxia in an adult human neuroblastoma cell line. Moreover, the other drugs,i.e. vorinostat, pracinostat, entinostat, and mocetinostat, are known to inhibit Histone deacetylase 3 activitieswere analysed using blind docking. These drugs are primarily involved in treating different types of advanced cancers like breast cancer, lymphoma, acute myelogenous leukemia, T-cell lymphoma etc. Therefore, under this study, the interaction between HIF-1α and HDAC 3 has been analyzed using PPI Network Analysis followed by molecular docking of HDAC 3 and the drugs under comparison. The results have shown Valproic acid is involved in treating neurological disorders previously but mocetinostat shows better inhibition against HDAC 3, and thus HIF-1α. Hence, these drugs under study can be used as a putative drug in the treatment of Alzheimer's disease. The findings direct future prospects towards laboratory experiments between valproic acid, vorinostat, pracinostat, entinostat, and mocetinostat, and its inhibitory effect on HDAC 3 to prevent Alzheimer's disease.

Keywords—*Hypoxia-inducible factor-1 alpha; Histone deacetylase 3; HDAC 3 inhibitors; Alzheimer's disease; Wnt-signaling; Swiss Dock*

CONTENT

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LIST OF SYMBOLS AND ABBREVIATIONS

S.NO.	ABBREVIATION	FULL FORM
1.	AD	Alzheimer's disease
2.	HIF-1 α	Hypoxia-Inducible Factor 1 alpha
3.	HIF-β	Hypoxia-Inducible Factor beta
4.	UPS	Ubiquitin Proteosome System
5.	HDAC	Histone Deacetylase
6.	PLMD	Protein Lysine Modification Database
7.	ADME	Absorption, Digestion, Metabolism, and Excretion
8.	AβPP	Amyloid beta precursor protein
9.	VEGF	Vascular Endothelial Growth Factor
10.	TGF	Transformation Growth Factor
11.	TNF	Tumor Necrosis Factor
12.	NEP	Neural Endopeptide
13.	PPI	Protein-Protein Interaction
14.	BBB	Blood-Brain Barrier
15.	EOAD	Early-onset Alzheimer's disease
16.	LOAD	Late-onset Alzheimer's disease
17.	GSK	Glycogen Synthase Kinase
18.	DNA	Deoxyribonucleic acid
19.	LRP	Lipoprotein Receptor-related Protein
20.	Dvl	Dishevelled protein
21.	SAHA	Suberoylanilide Hydroxamic Acid
22.	ΔG	Free energy

CHAPTER – 1

INTRODUCTION

1.1 BACKGROUND

Alzheimer's disease is one of the most common neurodegenerative disorders that is multifactorial and has no known effective treatment to date and the reason might be the single amyloid pathway directed studies [1]. It can lead to approximately 80% of dementia cases in elder people [2].

Alzheimer's disease is identified by amyloid plaques along with the neurofibrillary tangles and can be a result of genetic or epigenetic mechanisms. Most of the studies are directed towards these plaques and very few focus on other inducible factors. Hypoxia is one of them.

Hypoxia is a low oxygen condition that can be classified as acute, intermittent, and chronic depending upon the severity. It is known that prolonged hypoxia is involved in the pathogenesis of Alzheimer's disease by inducing the formation of amyloid-beta peptides via increasing the mitochondrial ROS (reactive oxygen species) and altering the enzymes involved in the production or degradation of the proteins [3]. Hypoxia, under normal and abnormal conditions, is regulated by Hypoxia-inducible factors that consist of two subunits, names HIF-α and HIF-β. An elevated level of these factors is reported under hypoxic conditions. Among the HIF-α isoforms, HIF-1α is known to be involved in cellular adaptations of the cell and thus becomes an important target for the studies in neuroprotective roles[4].

1.2 NEUROPROTECTION VIA HDAC 3 INHIBITION

The latest research has demonstrated the role of Histone Deacetylase in chromatin compaction, and transcription repression that leads to damaged DNA response, metabolic dysfunction, autophagy, disrupted cell cycle, etc. which leads to neurodegenerative disorders like Alzheimer's [5]. HDAC3, involved in Alzheimer's disease, promotes amyloid beta induced cell death. Thus, HDAC3 inhibition plays a vital role in neuroprotection. The recent studies have shown that valproic acid acts on HDAC3 to improve the transcriptional regulation, along with the inhibition of neural apoptosis. Valproic acid works by binding its carboxylic acid group to the metal ion and inhibits HDAC3, and the histone acetylation is enhanced [6]. Therefore, this study proves the efficacy and efficiency of the binding of valproic acid to HDAC3 via molecular docking using Swiss Dock. The other known HDAC3 inhibitors include Vorinostat, Pracinostat, Entinostat and Mocetinostat. These drugs are commonly used in cancer treatment where they bind to the catalytic sites of HDAC 3 and leads to its inhibition. Blind docking of these ligands against HDAC 3 was performed in order to verify their interaction and HDAC 3 inhibition.

1.3 COMPUTATIONAL AND BIOINFORMATICS' APPROACH FOR ESTABLISHING A LINK

The entire study is concerned with the establishment of the links between HIF-1α, HDAC3, and Histone Deacetylase inhibitor valproic acid using bioinformatics databases and softwares. The primary tool used for the molecular docking is "Swiss Dock". Auto Dock and Swiss Dock tools forms the core of this study. EADock DSS forms the basis of Swiss Dock. Its algorithm works on the principle of generating high affinity binding structures of the target and ligand, further calculating their CHARMM energies using grid formation. Then, the most favored structures are selected and the clusters with the appropriate energies are selected for the evaluation.

Additionally, PLMD has been used for data mining for the putative interaction between HDAC3 and HIF-1α. Besides PLMD, Swiss ADME, an online tool has been used which has been developed using several *in-silico* methods and analysis to develop a database that can predict ADME parameters.

1.4 OBJECTIVES OF THE STUDY

- Cross-talk between Wnt signaling, HIF-1 alpha, Ubiquitin Proteasome System, and Histone Deacetylase
- Finding a putative HDAC for reduced HIF-1 alpha activity (i.e. HDAC 3)
- Identifying the ligands that interact with HDAC 3 and constitutes its inhibition
- Molecular docking of HDAC 3 and drugs that are HDAC 3 inhibitors (i.e. Valproic acid, Vorinostat, Pracinostat, Entinostat, and Mocetinostat)
- ADME analysis of the drugs under study
- Comparison between the interaction potency of different HDAC 3 inhibitors i.e. drugs with HDAC 3.

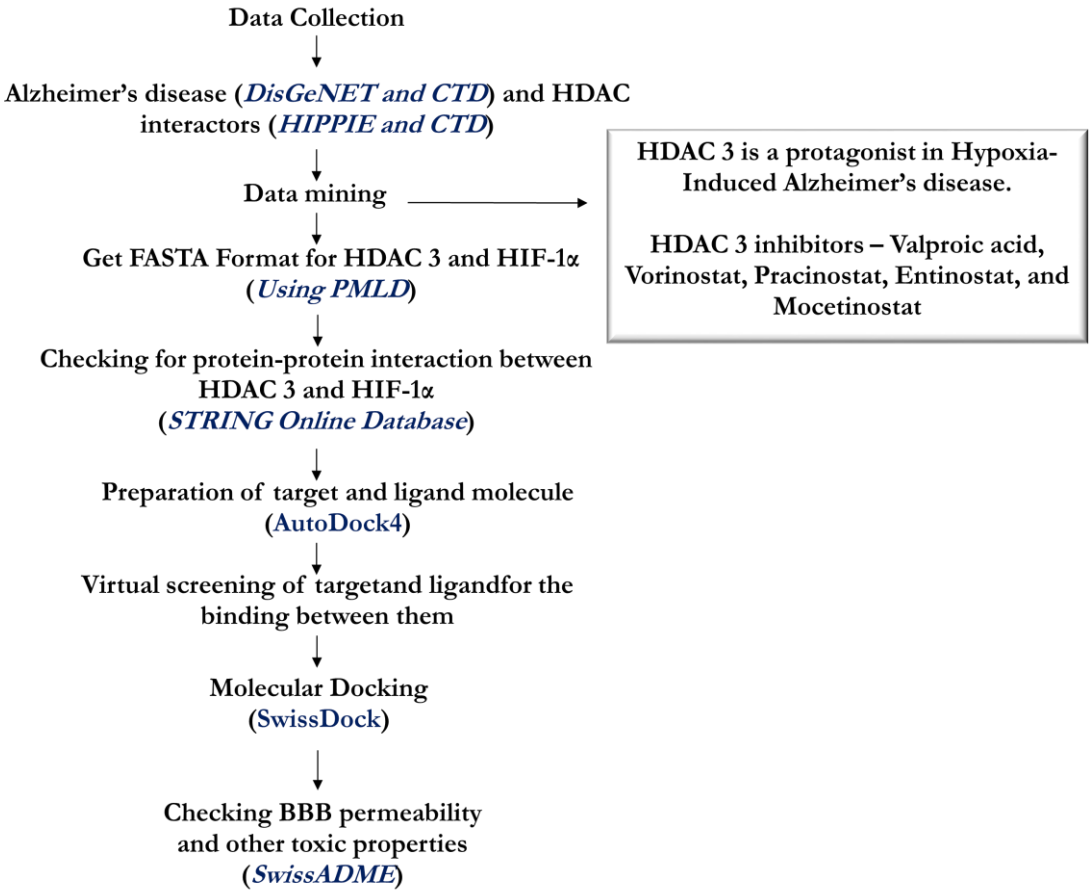


Fig. 1.1 Flowchart of the methodology followed for the study of interaction between the target protein HDAC 3 and HDAC inhibitor drugs

CHAPTER 2

LITERATURE REVIEW

2.1 ALZHEIMER'S DISEASE AND FACTORS INVOLVED

Alzheimer's disease is a neuropathological slowly progressive disorder [7,8] that is the most common type of dementia. The symptoms include loss of memory and thinking ability, degradation of neurons [9], and the loss of connection between two neurons. Based on the onset, the disease is divided into two categories – Early Onset Alzheimer's Disease (EOAD), whereas the other one is Late Onset Alzheimer's disease (LOAD). The commencement of EOAD and LOAD is mid-60s, and between mid-30s and 60s respectively.The brain is characterised by abnormal lumps i.e. amyloid plaques and entangled fibre bundles like neurofibrillary or tau. AD usually damages entorhinal cortex along withhippocampus initially; and when the disease enters the later stages, it affects cerebral cortex as well.

There are two pathophysiological indications of Alzheimer's disease i.e. beta-amyloid plaques that are present extracellularly and neurofibrillary tangles that are intracellular [7,10,11]. As it is a multifactorial disease, the other causes are tau hyperphosphorylation, inflammation, and cholinergic receptors [12]. It is known that Amyloid Precursor Protein (APP) undergoes cleavage via alpha-secretase, followed by processing using beta- and gamma-secretases that results in an imbalance between producting and clearing of amyloid beta peptides that had been accumulated [13]. This leads to an aggregation of soluble oligomers; the fibrils are then converted into beta-sheet type of conformation and eventually leads to a deposition of senile plaques [14]. Additionally, GSK3 beta and CDK5 also contribute to the pathogenesis of Alzheimer's disease [15].

2.2 HYPOXIA AND ALZHEIMER'S DISEASE

Most of the cases of AD are sporadic and are of late-onset type that is somehow not related to Amyloid Precursor Protein or gene mutations. This is the condition where epigenetic mechanisms and environmental factors come into picture contributing to etiopathogenesis of AD [16,17]. Cerebral ischemia and stroke that occur under hypoxia conditions play a major role in AD under such conditions and make the patient more susceptible to the disease. Recent researches have proved that hypoxia influences the pathological complications of AD by increased accumulation of amyloid beta peptides, decreased degradation of amyloid beta peptides and its clearance, and accelerated tau hyperphosphorylation. This leads to impared blood-brain barrier functions and neuronal degradation [18]. Additionally, hypoxia leads to induction ofneuroinflammatory responses. Thus, cerebral hypoxia directly influences Alzheimer's disease.

The pathogenesis of hypoxia is controlled by Hypoxia-Inducible Factors that majorly consists of two subunits i.e. alpha and beta [19]. According to the previously established studies, HIF-1α is the protagonist in regulating the activity of HIF and thus hypoxia. Futhermore, the previous researches evidences the fact that HIF-1α is involved in altering the activity of Histone deacetylase 3 and inhibiting HDAC 3 would repressHIF-1α as well [20].

2. 3 HISTONE DEACETYLASE AND ALZHEIMER'S DISEASE

Epigenetics is the study of gene expression where there is no change in the DNA sequence of a gene but instead various modifications such as histone acetylation and DNA methylation affect the pathology or the epidemiology of any disease [21]. This epigenetic regulation and alteration provide the basis for understanding AD better. Previous studies have shown the importance of DNA methylation in characterising Alzheimer's disease and various recent studies have been established describing the role of histone acetylation as well in the etiological studies of Alzheimer's disease [22, 23].The catalysis of histone acetylase and deacetylase is done via histone acetyltransferases and histone deacetylases respectively [24].Chromatin condensation and the transcription of genes is regulated by histone acetylation and histone

acetyltransferases play an important role in this process [25].Whereas histone deacetylases are involved in regulation of histone acetylation, and they have an effect on downstreaming of the gene expression. Alzheimer's disease is marked by anomalous acetylation of histones that contributes to its pathology.

Studies have shown that inhibition of histone deacetylases has recovered memory and cognitive thinking abilities in mice model of Alzheimer's disease [24]. In that study, a specified downregulation of histone H4 lysine 12 acetylation had been reported that led to impaired gene expression associated with hippocampus. However, when treated with vorinostat, the acetylation had been restored [26].Further different studies have proved that histone H4 is associated with the pathology of Alzheimer's disease [27, 28].

2.4 HYPOXIA-INDUCIBLE FACTOR 1 ALPHA AND ITS ROLE IN NEURODENEGERATION

2.4.1 HYPOXIA AND UBIQUITIN PROTEOSOME PATHWAY

Under normal oxygen tension, ubiquitination and proteasomal degradation pathways induce HIF disruption. HIF-1α, during normoxia, is subjected to certain propyl-4-hydroxylases that hydrolyses its prolyl residues present in its stabilization domain [29]. The hydroxylated HIF-1α undergoes polyubiquitination via Ubiquitin E3, leading to its degradation. Under hypoxic conditions, this hydroxylation is deterred, which leads to stabilization and maintenance of the active state of HIF-1α. HIF-1α then binds to the core hypoxia response elements and leads to neurogenesis that shows a progression towards AD[30].

2.4.2 HYPOXIA AND WNT-SIGNALING

In normal Wnt signaling, the Wnt ligand interacts with F2 receptors and Low density lipoprotein receptor-Related Protein (LRP) 5/6 and translates its signal through Dishevelled protein (Dvl), thus inactivating Glycogen Synthase Kinase (GSK)-3 beta. This leads to the accumulation of beta-catenin in the cell and later enters into the nucleus. Herein the T-Cell Factor/Lymphoid Enhancer Factor

transcription factor activation takes place. Hypoxia instigates β-catenin function redirection. Under normal conditions, β-catenin activates T-Cell Factor 4 and elevates the proliferation of cells by enhancing Wnt-signaling. When under hypoxic conditions, HIF-1α competes with T-Cell Factor 4 for the binding of beta-catenin, and thus Hypoxia Inducible Factor-1alpha is stabilized and activated. Hence, TCF-4 activity is downregulated leading to a quiescence stage. In the mean-time, HIF-1-mediated transcription takes place by the interaction between HIF-1α and β-catenin. This mechanism induces tumor progression and neurogenesis [31].

2.4.3 HYPOXIA AND VASCULAR ENDOTHELIAL GROWTH FACTOR

HIF-1α induces the increase in the VEGF levels that leads to abnormal vessel branching i.e., pathological angiogenesis by endothelial cell proliferation [32]. This, in turn, stimulates the release of Transforming Growth Factor (TGF), VEGF, and Tumor Necrosis Factor (TNF) [33]. Thus, the accumulation of amyloid-beta peptides and the secretion of neurotoxic peptides take place.

Therefore, a link has been established between hypoxia and hypoxia-induced Alzheimer's disease and the various factors contributing to it.

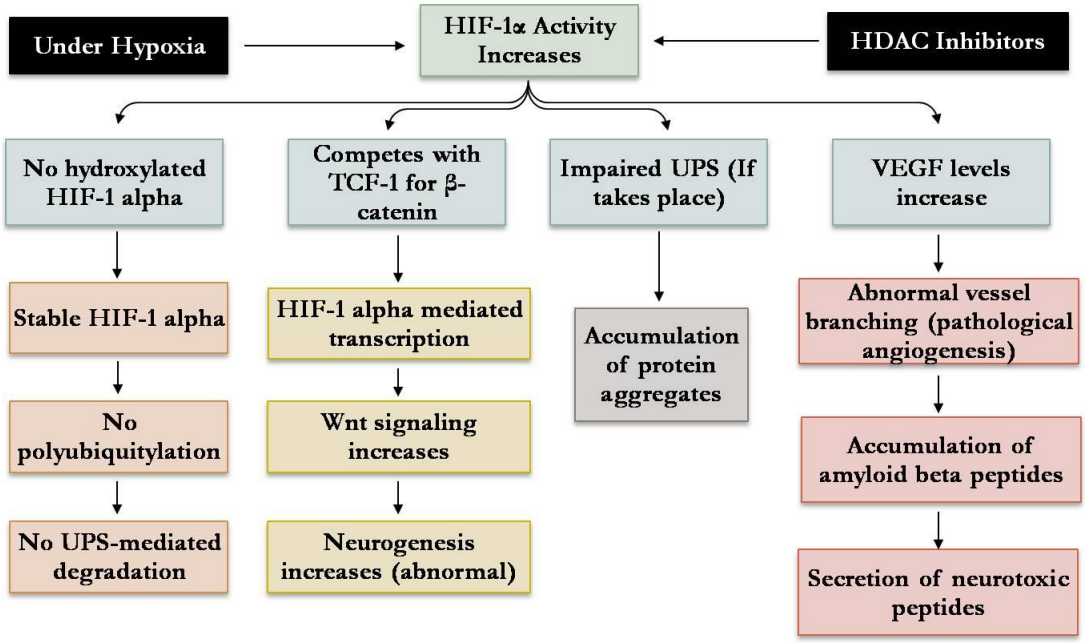


Fig 2.1. Cross-talk among HIF-1α, UPS, and Wnt-signaling(Under hypoxia, HIF-1α levels are increased which results in disruption of polyubiquitination, abnormal neurogenesis, accumulation of protein aggregates, and secretion of neurotoxic peptides leading to Alzheimer's disease)

2.5 INTERACTION OF HDAC 3 AND DRUGS UNDER STUDY

2.5.1HISTONE DEACETYLASE 3 AND VALPROIC ACID

Histone Deacetylase 3, a class I HDAC, is an enzyme that is encoded by the HDAC3 gene that regulates the levels of gene expression of histone and non-histone deacetylation. Histone deacetylases function by removing the acetyl group from lysine residues of histone and non-histone proteins [34]. HDAC 3 is located in the cytoplasm and nucleus both [35] and is a critical negative regulator of long-term memory formation [36]. Histone deacetylases are known to provide a putative therapeutic target for the treatment of AD. Various studies have been done to establish the interaction between HDAC 1, 4, 9, etc but a few are focused on HDAC 3.

2.5.2 HISTONE DEACETYLASE 3 AND VORINOSTAT

The chemical name of vorinostat is suberoylanilide hydroxamic acid (SAHA) and it is an orally available drug [37].Vorinostat acts broadly on HDAC activities and it can inhibit class I and II of HDAC [38, 39].Histone deacetylase enzyme has a catalytic site consisting of a zinc atom and it is known that vorinostat binds to that zinc atom via phenyl ring that is present in the structure of vorinostat. This leads to the projection of the catalytic domain on the surface of histone deacetylase enzyme [40]. When vorinostat binds to HDAC enzyme, the accumulated acetylated proteins like that of histones can be seen that can contribute to a variety of cell effects [41, 42]. Additionally, these effects can include transcriptional and non-transcriptional activity modifications [43, 44].

2.5.3 HISTONE DEACETYLASE 3 AND PRACINOSTAT

Pracinostat is a hydroxymate, acid-based histone deacetylase inhibitor (i.e. HDAC i) and it can act on class I, II, and IV type of HDAC. Moreover, it has superior ADME properties, than that of SAHA type of drugs i.e. vorinostat, like pharmaceutical, physicochemical, and pharmacokinetics [45] with 100 times affinity [46].A previous research by Kim SH et al has established that in breast cancer, pracinostat has partial efficacy that corresponds to metastasis towards the brain [47]. In a previous study, it was found that pracinostat constitutes a variety of hydrophobic and hydrogen bonds along with salt bridges at ASP 92 and ASP 93 [48]. Futhermore, when HDAC activity is inhibited, the acetyl groups start accumulating on histone lysine residues that leads to activation of transcription.

2.5.4 HISTONE DEACETYLASE 3 AND ENTINOSTAT

Entinostat is an orally taken drug and is a benzamide derivative [49] that is synthetic and is a zinc-binding ligand. Entinostat is a selective histone deacetylase 3 inhibitor [50] and is a member of substituted pyridylcarbamate class of HDAC-inhibiting compounds [51]. A recently published study has confirmed that MS-275 i.e. entinostat reduces neuroinflammation and the load of amyloid beta plaques, hence it is considered effective in the treatment of pathological symptoms of Alzheimer's disease [52].In tumor cells, entinostat has been known to increase histone hyperacetylationthat permits the activation of transcription process and kinase protein expression that is signal-induced and present extracellularly [53, 54].

2.5.5 HISTONE DEACETYLASE 3 AND MOCETINOSTAT

It is also a benzamide derivative [49] and an isotype-selective histone deacetylase 3 inhibitor. According to a previously established study, mocetinostat had recovered 85% of locomotor ability when different assessment assays were done [55]. Moreover, the study has shown recovered exonal transport phenotype [55].Mocetinostat is available orally, synthetic, and small molecule type of histone deacetylase inhibitor [56]. It has a variety of non-histone targets like mitochondrial

pathways and its efficiency increases when combined with preoteosomal degraders [57-60].

This study has been focused on the establishment of the potent interaction between HDAC 3 and HIF-1α via Protein-Protein Interacting Network Analysis studies and it has been argued that HDAC 3 inhibitors would affect the activity of HIF-1α and therefore would eventually help as a treatment strategy for Alzheimer's disease.

Valproic acid is one of the drugs that have been known to interact and inhibit HDACs but the studies are focused on HDAC 4, 9, etc. This study has been conducted in order to institute the relationship between valproic acid and HDAC 3 and validate this interaction using molecular docking. Moreover, this study focuses on establishing a comparison between different HDAC 3 inhibitor drugs in order to study the interaction and effects of the mentioned drug in regulating the activity of HDAC 3.

CHAPTER 3

MATERIALS AND METHODS

3.1 INTEGRATION OF PROTEIN-PROTEIN INTERACTION OF HDAC 3 AND HIF-1A.

The FASTA format of the two proteins HDAC 3 and HIF-1α were extracted from PMLD (http://plmd.biocuckoo.org/) to study the interaction between HDAC 3 and HIF-1α. Then, the Protein-Protein Interaction clustering was done in order to perform the network exploration of proteins. This task was accomplished using the STRING online database (https://string-db.org/).

3.2 PROTEIN AS WELL AS LIGAND PREPARATION FOR DOCKING

The .pdb file of the protein HDAC 3 was downloaded from Protein Data Bank database. The protein found was bound to corepressor and inositol tetraphosphate. Thus, the ligands attached to it were removed using Autodock4. The database showed that A and B chain represented HDAC 3, whereas C and D chain was nuclear receptor corepressor 2. Therefore, the C and D chains were removed to get pure HDAC 3 molecule. For ligands, .pdb file was downloaded from Drug Bank.

The target protein HDAC 3 and the ligands i.e. Valproic acid, Vorinostat, Pracinostat, Entinostat, and Mocetinostat were prepared using Autodock4. The redundancy because of water molecules and heteroatoms was removed manually. The Kollman charges were applied to all of them i.e. the target and the ligands. And the .pdb file for the target and the ligand was saved for docking using SwissDock-an online docking tool. SwissDock requires a .mol2 file for the ligand, which was converted using OpenBabel.

3.3 PROTEIN-LIGAND DOCKING USING SWISSDOCK.

SwissDock is an online service for molecular docking between a protein and a ligand. The .pdb file for the target and .mol2 file for the ligand was uploaded to the server (http://www.swissdock.ch/) and blind docking was performed for each of the above mentioned drug to study their comparison. After uploading the files were analysed for their competencies and structural precision for improved docking results. The ligand file was prepared with all the hydrogen atoms and 3D coordinates.

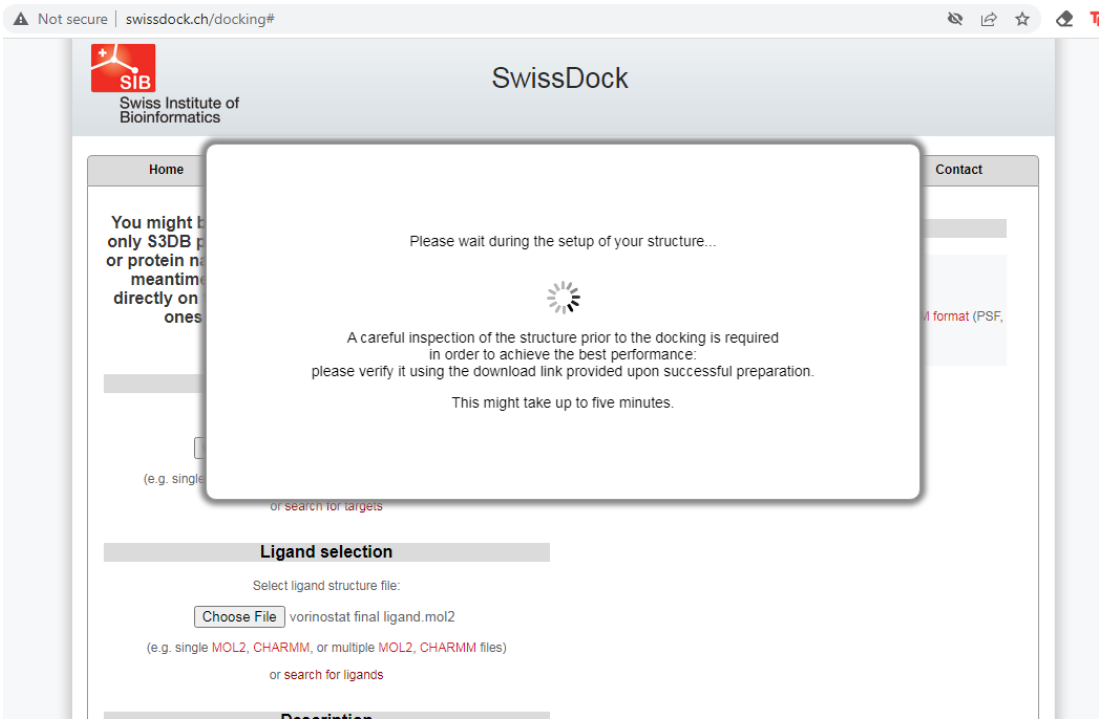


Fig. 3.1. Uploading target and checking its structure for better performance

3.4 STRUCTURAL ANALYSIS OF THE DOCKED PROTEIN-LIGAND COMPLEX USING UCSF CHIMERA.

For structural analysis, UCSF Chimera software was downloaded. The results were downloaded from SwissDock and .chimera file was analyzed for the structural interaction between the protein HDAC 3 and the drugs i.e. Valproic acid, Vorinostat, Pracinostat, Entinostat, and Mocetinostat using UCSF Chimera software and an interactive ribbon structure is made for better visualisation.

3.5 ADME ANALYSIS FOR THE LIGAND DRUGS USING SWISSADME

ADME stands for Absorption, Distribution, Metabolism, and Excretion. These parameters were analyzed in order to study the physicochemical properties, pharmacokinetics, likeliness, lipophilicity, and water solubility of the drug. These analyses provide the evidence for the efficacy and the potency of the drugs that were under study.

SwissADME (http://www.swissadme.ch/) is an online tool to evaluate these criteria of small ligand molecules. Here, each drug i.e. Valproic acid, Vorinostat, Pracinostat, Entinostat, and Mocetinostat was observed. For this analysis, the .pdb format of the ligand was converted to smiles format using OpenBabel software and the data was entered in the SwissADME tool. The scores for different parameters were obtained to determine the effectiveness of the drugs based on previously available research and studies.

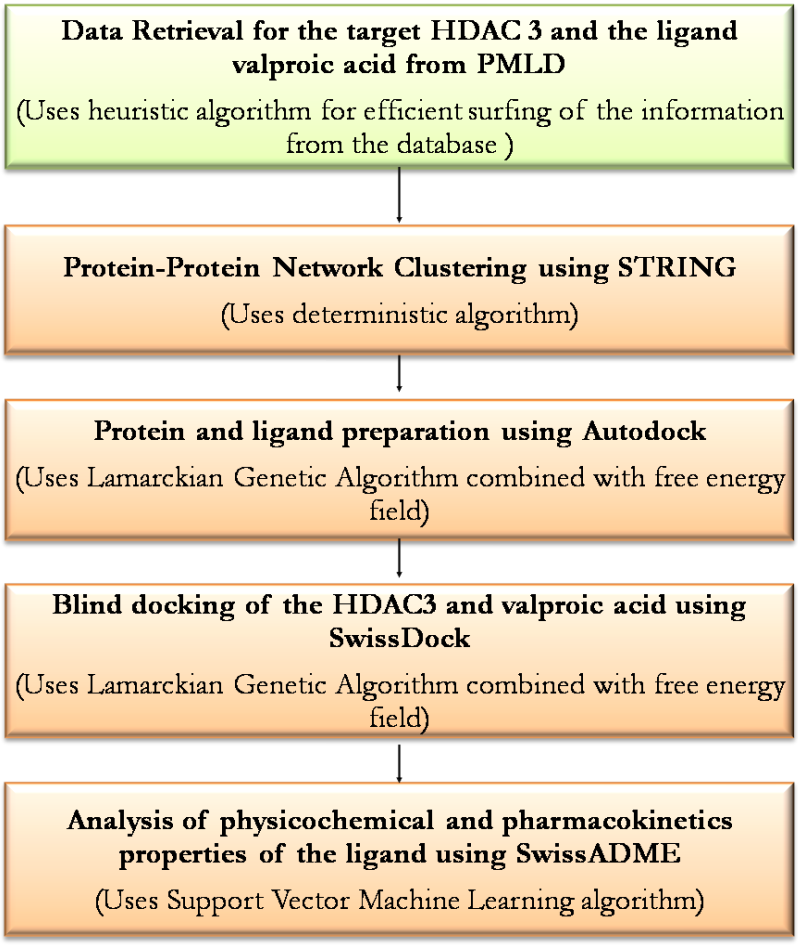


Fig 3.2. Softwares and databases(describing the algorithms of the functional activity of the computational tools used under the study)

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 INTERACTION BETWEEN HDAC 3 AND HIF-1A.

The PPI network of the HDAC 3 and HIF-1α has shown significant interactions. The PPI enrichment p-value was calculated to be 0.0265 by the network stats analysis of the STRING database. The average local clustering coefficient was determined to be 0.8 for the given proteins. The edges represent the protein-protein association [See Fig. 2]. The pink edges represent the experimentally determined interaction while the yellow edges represent the interactions established via data mining. Additionally, the results show that there is enough interaction for the HDAC 3 inhibitors to act on HIF-1α and cause its inhibition.

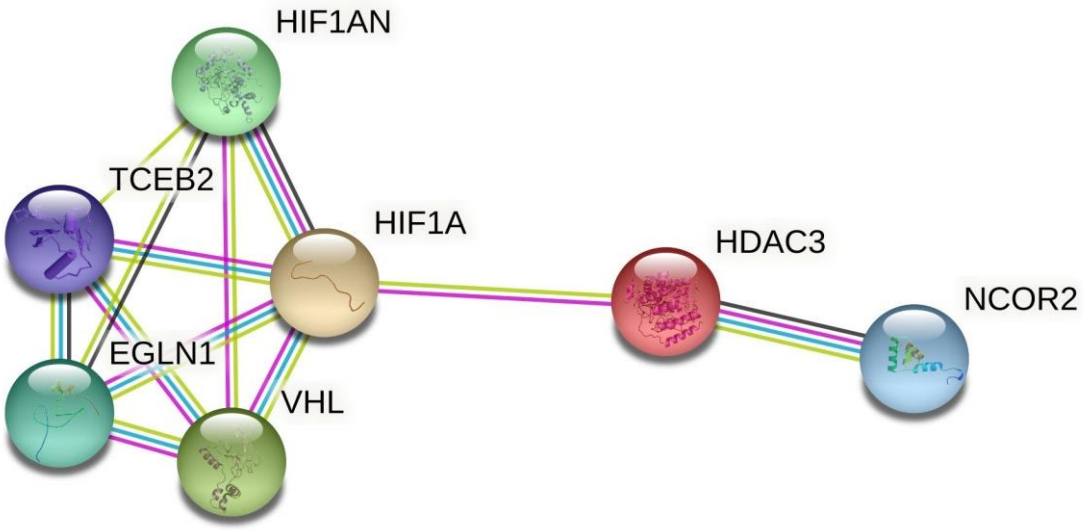


Fig. 4.1. Interaction between HIF-1α and HDAC 3 (STRING database). (HDAC 3 is a 428 amino acid protein whereas HIF-1α is an 850 amino acid sequence. The STRING database has confirmed the interrelation between the two)

TABLE I. **ROLE OF STRING NETWORK PROTEINS IN ALZHEIMER'S**

Name of the protein	Role[a]
HDAC3	Directly phosphorylated by GSK-3β and contributes to strong cellular selective neurotoxicity [61].
HIF-1α	Increases amyloid-beta peptides generation via β/γ-secretases promotion and inhibition of α-secretases, thus inducing microglial death and neuroinflammation [62].

[a]·The table indicates the role and importance of the major proteins involved in this study of Alzheimer's disease. STRING database has scored and incorporated the protein-protein interaction information between them.

4.2 INTERACTION BETWEEN HDAC 3 AND VALPROIC ACID

4.2.1 Docking results of HDAC 3 and Valproic acid

After blind docking was performed using SwissDock, the results came out to be positive showing a high degree of interaction between HDAC 3 and Valproic acid. The docking scores show that Valproic acid binds to significant receptor sites of the Histone deacetylase 3. The scores are given below in table 1.

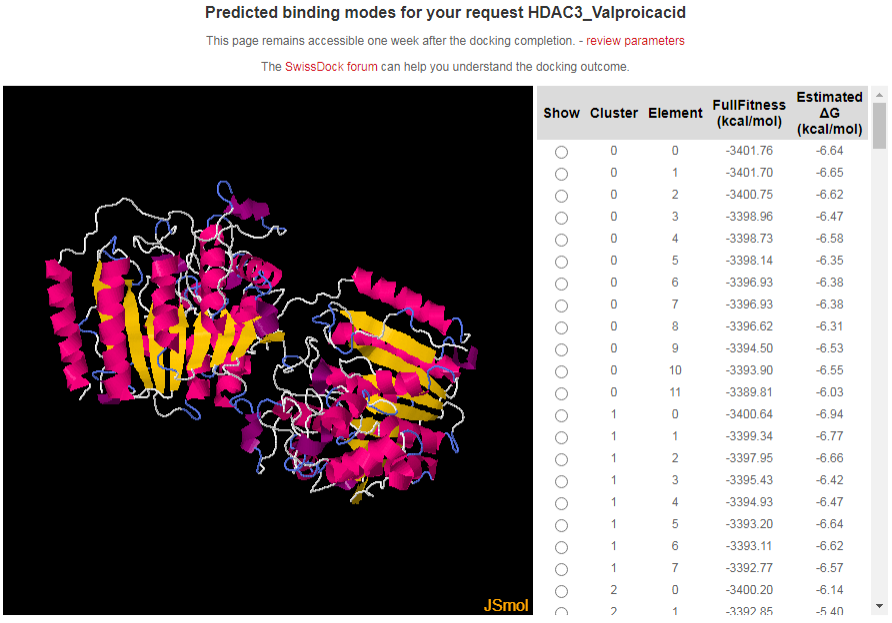


Fig. 4.2. Docking results for Valproic acid

TABLE II. **SWISSDOCK SCORES FOR MOST EFFECTIVE CLUSTERS OF VALPROIC ACID**

Cluster	Element	Full fitness[a] (kcal/mol)	EstimatedΔG[b] (kcal/mol)
0	0	-3020.27	-6.92
0	1	-3015.84	-6.47
0	2	-3015.80	-6.47
0	3	-3014.18	-6.37
0	4	-3013.55	-6.28

[a.] Full fitness (the average of the most favored energies of the elements in order to reduce the risk of effects caused by few complexes present in the cluster [63])

[b.] Estimated ΔG (provides the crude idea of a preferred pose of the structures that are docked)

The energy of the full fitness and estimated ΔG shows that the clustering and interaction of the docked molecules are crucial. The more negative energy, the more stable interaction between the molecules is established. Therefore, the cluster with full fitness of the most negative energy i.e., -3020.27, and the estimated ΔG i.e., -6.92 shows the highest interaction between HDAC 3 and Valproic acid. The structure of the interaction of the molecules was analyzed using UCSF Chimera as shown in Fig. 3.

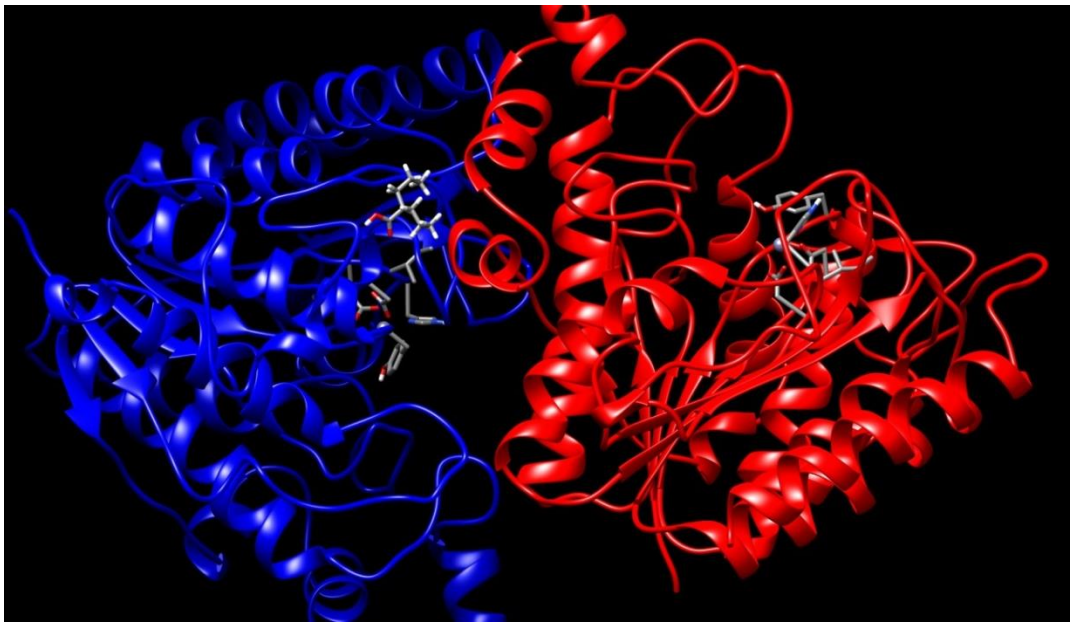


Fig. 4.3. Interaction between HDAC 3 and Valproic acid (UCSF Chimera). (The image shows the ligand i.e. valproic acid sitting in the receptor pockets of the target protein i.e. HDAC 3)

The structure shows the target protein i.e., HDAC 3 in the form of blue and red ribbons. The blue and the red ribbons represent the A and the B chains, respectively, of the HDAC 3 protein whereas the ball and the line structures in between the ribbons are representative of the putative ligand i.e., Valproic acid. The structure shows the interaction of the ligand with the receptor site of the target protein.

It has been known that valproic acid is an HDAC inhibitor and the results of this study establishes that the valproic acid is an interactor of, especially, HDAC 3 protein and thus it is concluded that valproic acid has shown its inhibitory properties against HDAC 3 protein, which in turn would interact with HIF-1α, inhibiting Alzheimer's. In the due course, it can be used a putative target for the treatment of Hypoxia-induced Alzheimer's disease.

4.2.2 ADME analysis of the ligand Valproic acid

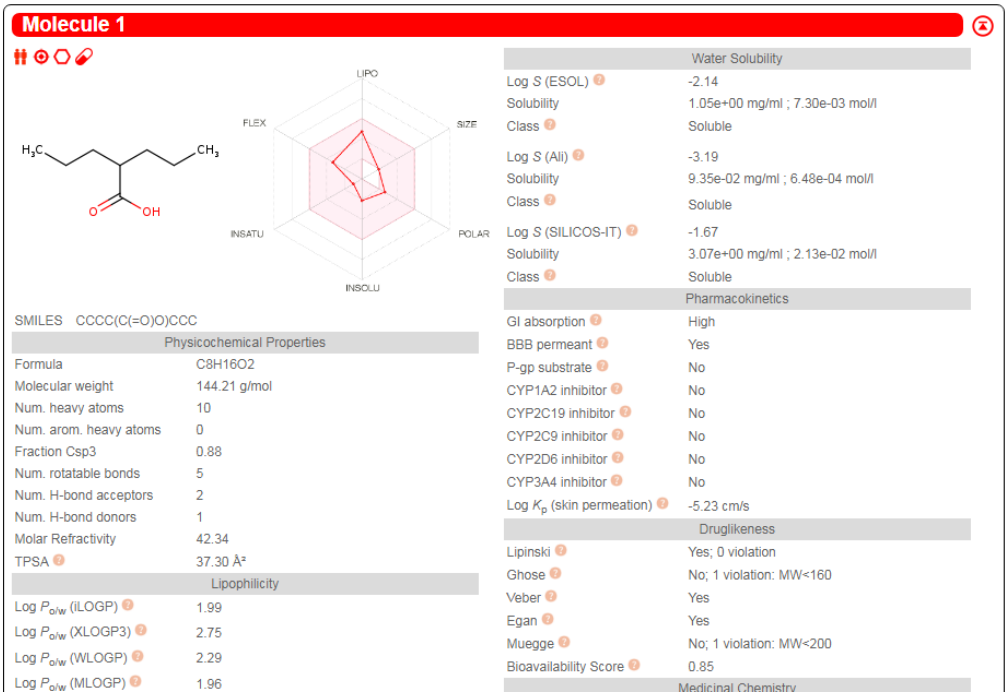


Fig. 4.4. ADME analysis of valproic acid showing its structure and efficacy.

The pharmacokinetics results suggest that valproic acid has high gastrointestinal absorption and a value of Log K_p (skin permeation) of -5.23 cm/s. It has shown the blood-brain barrier permeability and no inhibition towards CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4. It duly follows Lipinski's rule and has shown solubility in water under Log *S* (ESOL), Log *S* (Ali), and Log *S* (SILICOS-IT) category with a value of -2.14, -3.19, and -1.67 respectively. The lipophilicity of valproic acid was found to be 2.75 for the analysis through Log $P_{o/w}$ (XLOGP3) and the bioavailability score of 0.85.

4.3 INTERACTION BETWEEN HDAC 3 AND VORINOSTAT

4.3.1 Docking results of HDAC 3 and Vorinostat

When vorinostat was docked against HDAC 3, the docking scores show high feasibility between the target and ligand. Thus, it can be said that there is a significant interaction between HDAC 3 and vorinostat; and hence, it can be concluded that vorinostat sits well in the pockets of HDAC 3 protein.

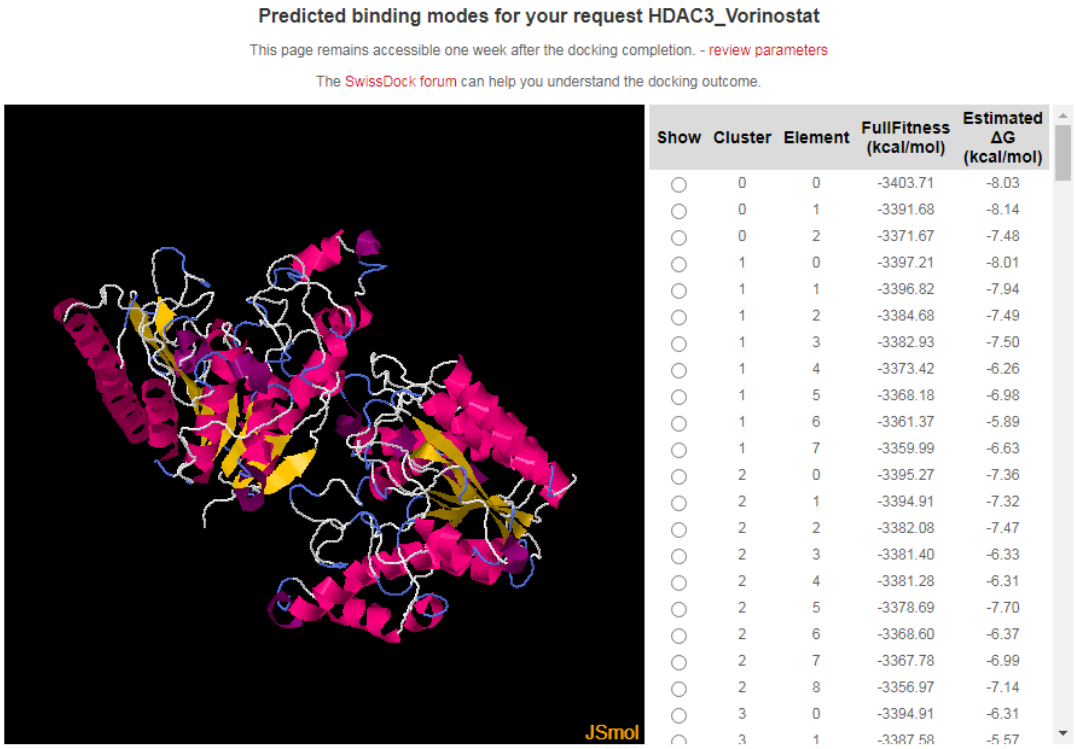


Fig. 4.5. Interaction of HDAC 3 with vorinostat using SwissDock.

The scores for docking are as follows:

TABLE III. **SWISSDOCK SCORES FOR THE MOST FAVOURED CLUSTER OF VORINOSTAT**

Cluster	Element	Full fitness (kcal/mol)	Estimated ΔG (kcal/mol)
0	0	-3403.71	-8.03
0	1	-3391.68	-8.14
0	2	-3371.67	-7.48
1	0	-3397.21	-8.01
1	1	-3396.82	-7.94

The cluster 0 and element 1 has the highest negative ΔG and thus this clustering shows the maximum amount of interaction and stable bonding. The full fitness score for this cluster is -3391.68, which means that it has the minimal effects of the nearby complexes. The analysis for its structure had been carried out with UCSF Chimera and ligand can be seen interacting with the target i.e. HDAC 3.

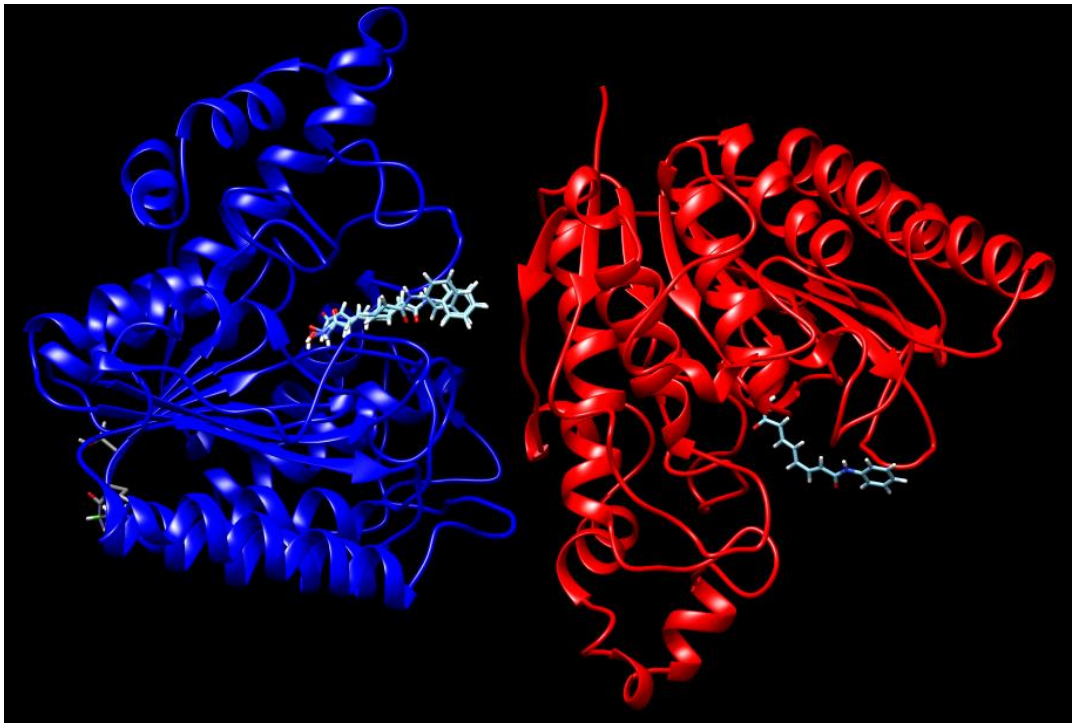


Fig. 4.6. Ligand Vorinostat has been shown interacting with HDAC 3 (UCSF Chimera)

Vorinostat can be seen to have better binding with blue ribbons. As the blue ribbons represent A chain, thus it can deduce that vorinostat shows higher interaction with chain A in comparison to chain B. Previous researches show that vorinostat is an HDAC inhibitor and on comparing its interaction against HDAC 3, it can be seen to have high affinity for it. Therefore, it can be inferred that vorinostat can be used in the treatment of Alzheimer's disease as its inhibitory nature would downregulate HDAC 3, which in turn would reduce hypoxic conditions and eventually the deteriorating effects of AD.

4.3.2 ADME analysis of vorinostat

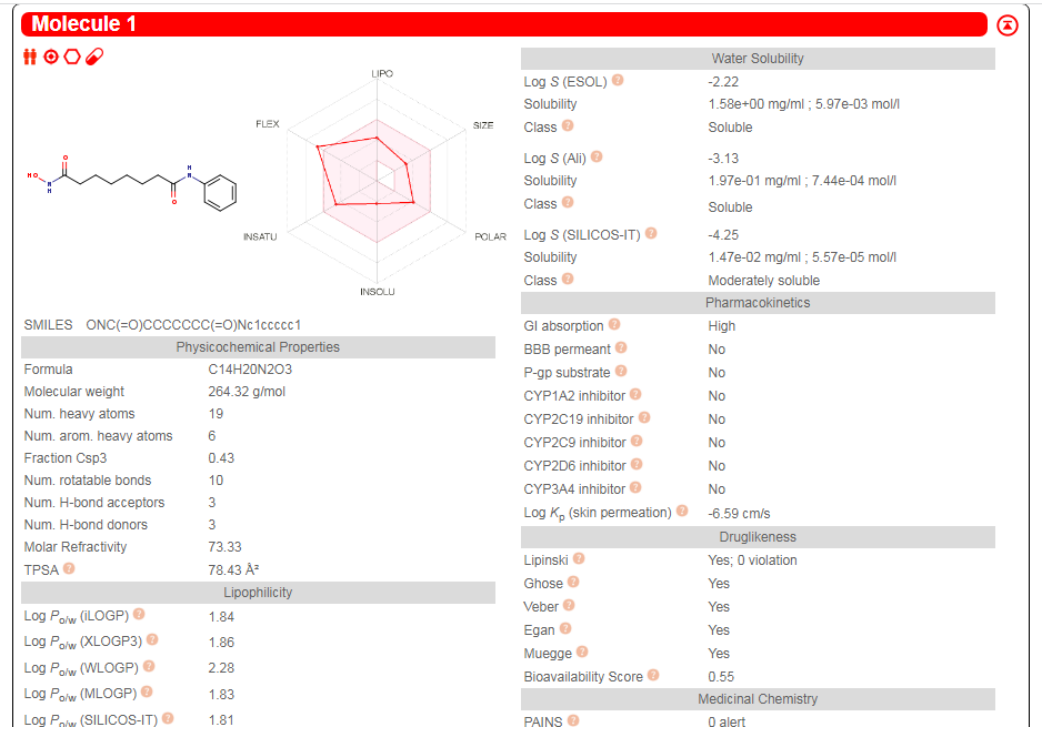


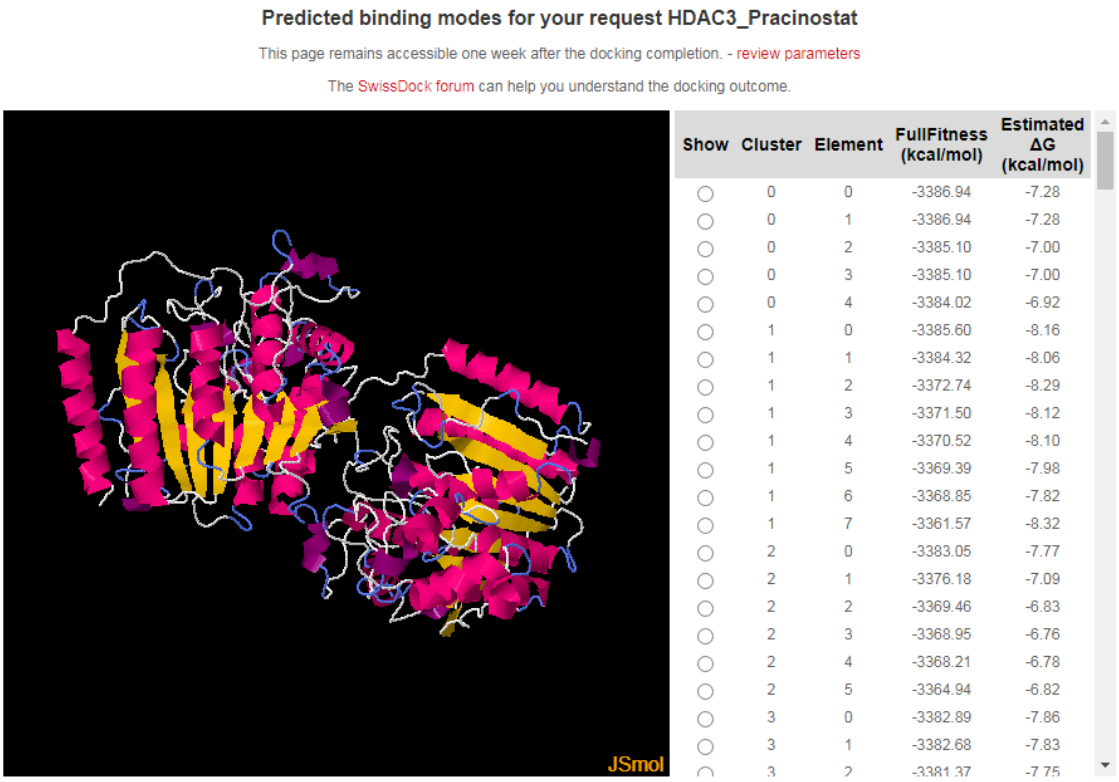
Fig. 4.7. Analysing efficacy of vorinostat using SwissADME.

The drug Vorinostat shows high gastrointestinal absorption without blood-brain barrier permeability. Additionally, vorinostat is not a CYP1A2 inhibitor, CYP2C19 inhibitor, CYP2C9 inhibitor, CYP2D6 inhibitor, CYP3A4 inhibitor. Furthermore, the value for Log K_p (skin permeation) is -6.59 cm/s. It follows all the rules including Lipinski, Ghose, Veber, Egan, and Muegge without any violation. Moreover, its bioavailability score is 0.55. The Synthetic accessibility score for vorinostat is 1.91. This means that is very easily synthetically accessible because the scale considers 1 as the easiest while 10 as the most difficultly accessible. According to the analysis for water solubility, the drug is soluble under Log *S* (ESOL) study. Its value is -2.22 and the value of solubility is 1.58e+00 mg/ml; 5.97e-03 mol/l. It is also soluble under Log *S* (Ali) analysis while moderately soluble under Log *S* (SILICOS-IT) scoring parameter. In addition, the lipophilicity scores are 1.84 for Log $P_{o/w}$ (iLOGP), 1.83 for Log $P_{o/w}$ (MLOGP), 1.92 for Consensus Log $P_{o/w}$, 1.86 for Log $P_{o/w}$ (XLOGP3), etc.

4.4 INTERACTION BETWEEN HDAC 3 AND PRACINOSTAT

4.4.1 Docking results of HDAC 3 and Pracinostat

The results obtained after docking pracinostat against HDAC 3, the interaction achieved showed a high level of binding between the target and the ligand. Pracinostat is known to suppress breast cancer in females and it is a potent HDAC inhibitor. While the docking results with our target molecule i.e. HDAC 3, it shows an improved binding and interaction with the target.



Show	Cluster	Element	FullFitness (kcal/mol)	Estimated ΔG (kcal/mol)
○	0	0	-3386.94	-7.28
○	0	1	-3386.94	-7.28
○	0	2	-3385.10	-7.00
○	0	3	-3385.10	-7.00
○	0	4	-3384.02	-6.92
○	1	0	-3385.60	-8.16
○	1	1	-3384.32	-8.06
○	1	2	-3372.74	-8.29
○	1	3	-3371.50	-8.12
○	1	4	-3370.52	-8.10
○	1	5	-3369.39	-7.98
○	1	6	-3368.85	-7.82
○	1	7	-3361.57	-8.32
○	2	0	-3383.05	-7.77
○	2	1	-3376.18	-7.09
○	2	2	-3369.46	-6.83
○	2	3	-3368.95	-6.76
○	2	4	-3368.21	-6.78
○	2	5	-3364.94	-6.82
○	3	0	-3382.89	-7.86
○	3	1	-3382.68	-7.83
○	3	2	-3381.37	-7.75

Fig. 4.8. Blind docking results of Pracinostat analysed using SwissDock

The table given below shows the docking scores against the cluster and element, along with full fitness and estimated ΔG for the drug pracinostat.

TABLE IV. **SWISSDOCK SCORES FOR THE HIGHEST ENERGY CLUSTER OF PRACINOSTAT**

Cluster	Element	Full fitness (kcal/mol)	Estimated ΔG (kcal/mol)
0	0	-3386.94	-7.28
0	2	-3385.10	-7.00

0	4	-3384.02	-6.92
1	0	-3385.60	-8.16
1	2	-3372.74	-8.29

In this interaction, cluster 1 with the element 2 shows the maximum negative ΔG and thus shows the highest interaction. This interaction corresponds to a full fitness score of -3372.74 which indicates a potent energy that is favoured and required for the molecules to interact.

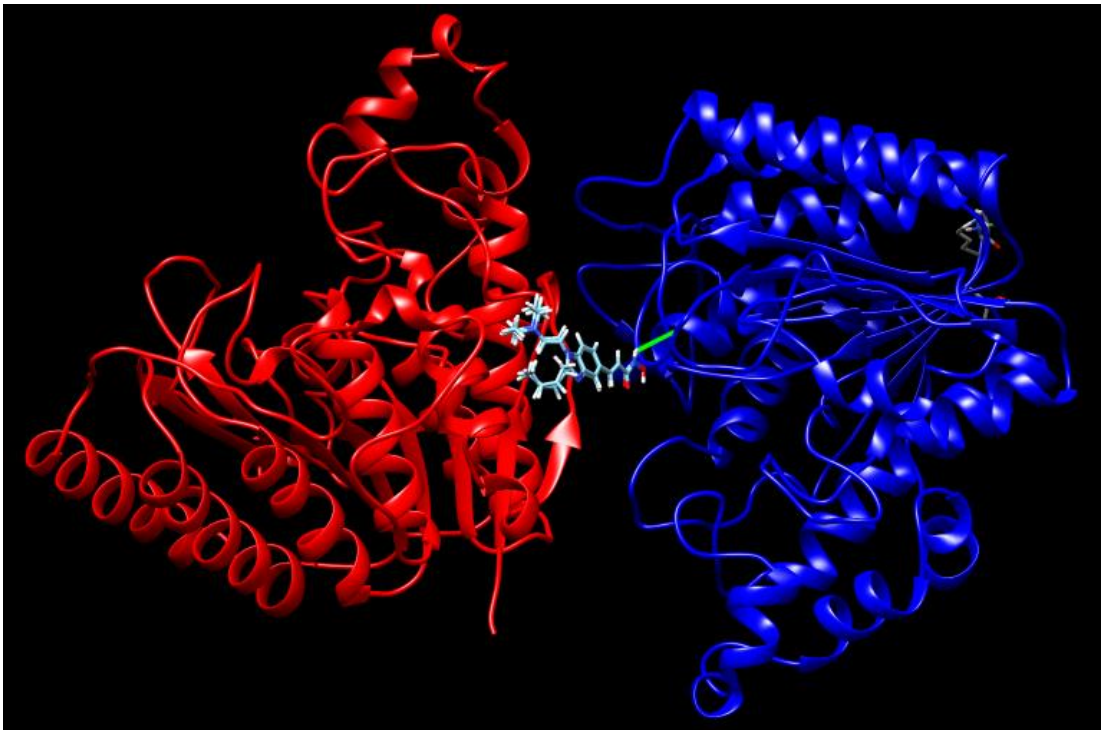


Fig. 4.9. Pracinostat – Structural analysis of chain A and B of HDAC 3 with the drug

According to the structural observations made using UCSF Chimera, the drug, pracinostat can be seen interacting well between both of the chain. This shows that the receptor for pracinostat in HDAC 3 is present between chain A and B. Therefore, it can be concluded that pracinostat can also be used for inhibiting HDAC 3 and hence can be used against Alzheimer's disease as well. It has shown its potential against breast cancer effectively and thus should work well with AD too. The clinical

experiments should be carried out for this interaction in order to verify its efficiency against AD.

4.4.2 ADME analysis of Pracinostat

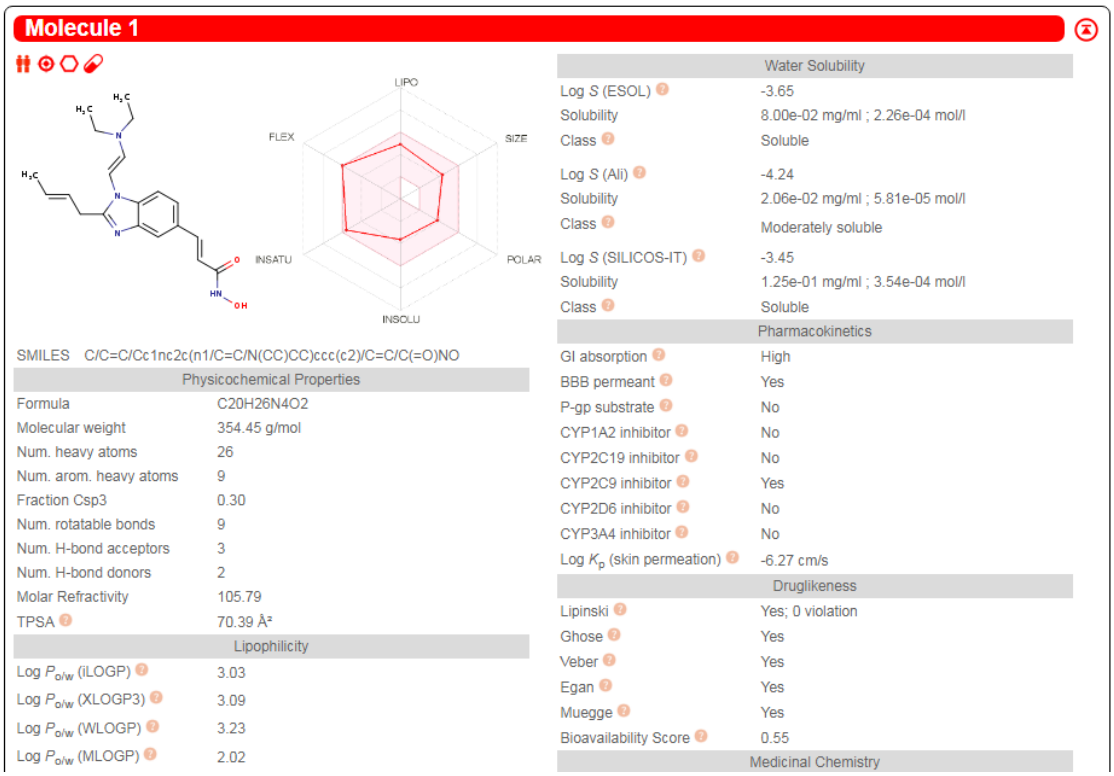


Fig. 4.10.Pracinostat drug observations using ADME analysis

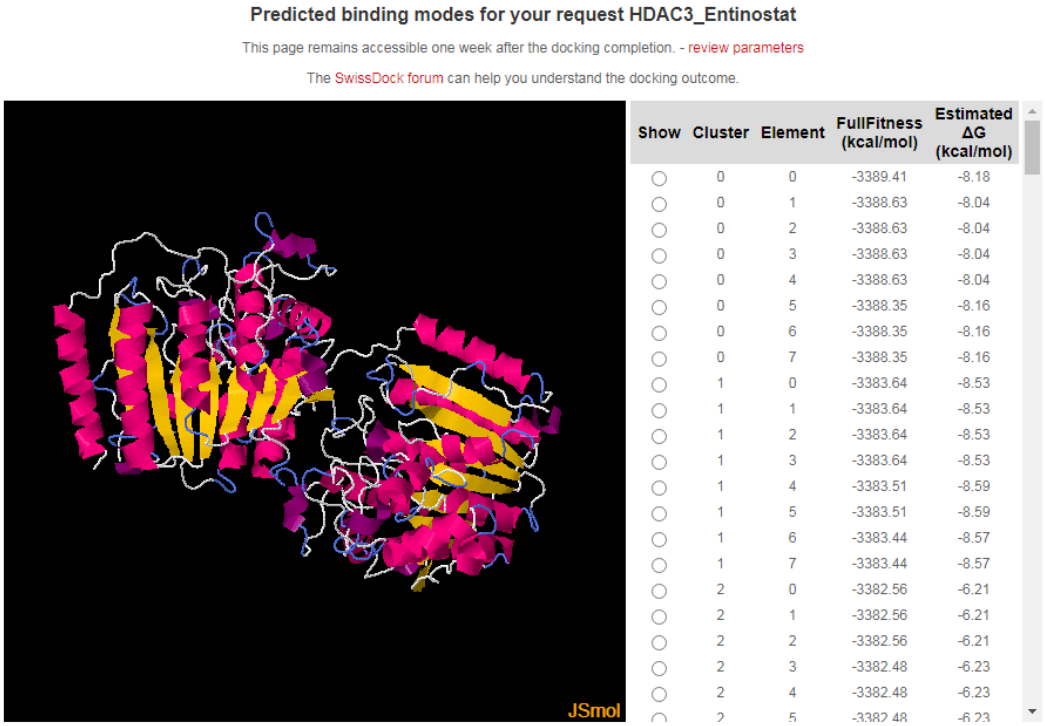
Pracinostat has been made to under ADME analysis using SwissADME. The observations show that the drug shows high gastrointestinal absorption and it has blood-brain barrier permeability. The drug is known to be a CYP2C9 inhibitor while it is not a CYP1A2 inhibitor, CYP2C19 inhibitor, CYP2D6 inhibitor, CYP3A4 inhibitor. Besides this, the Log K_p (skin permeation) score for pracinostat came out to be -6.27 cm/s. Additionally, the drug shows a bioavailability score of 0.55 and it follows all the parameters under druglikeness, for instance, Lipinksi, Ghose, Veber, Egan, and Muegge with no violation. The studies involving water solubility shows that it is soluble under Log *S* (ESOL) with a value of -3.65 and solubility score as 8.00e-02 mg/ml ; 2.26e-04 mol/l. Moreover, it is soluble under Log *S* (SILICOS-IT) with a score of -3.45 and solubility as 1.25e-01 mg/ml ; 3.54e-04 mol/l. However it is moderately soluble under Log *S* (Ali) and have a score of -4.24. Furthermore, the

lipophilicity score for Log $P_{o/w}$ (iLOGP) is 3.03, for Log $P_{o/w}$ (WLOGP) it is 3.23, and Consensus Log $P_{o/w}$ is 2.79, etc.

4.5 INTERACTION BETWEEN HDAC 3 AND ENTINOSTAT

4.5.1 Docking results of HDAC 3 and drug Entinostat

The predicted models for the highest binding interaction between HDAC 3 and drug entinostat was analysed using SwissDock. The docking scores provide the evidence for admissible and allowable binding between the target and the ligand. An immense amount of interactions had been predicted by SwissDock, and the clusters with more negative ΔG had been selected for further visualisation of the structures.



Show	Cluster	Element	FullFitness (kcal/mol)	Estimated ΔG (kcal/mol)
○	0	0	-3389.41	-8.18
○	0	1	-3388.63	-8.04
○	0	2	-3388.63	-8.04
○	0	3	-3388.63	-8.04
○	0	4	-3388.63	-8.04
○	0	5	-3388.35	-8.16
○	0	6	-3388.35	-8.16
○	0	7	-3388.35	-8.16
○	1	0	-3383.64	-8.53
○	1	1	-3383.64	-8.53
○	1	2	-3383.64	-8.53
○	1	3	-3383.64	-8.53
○	1	4	-3383.51	-8.59
○	1	5	-3383.51	-8.59
○	1	6	-3383.44	-8.57
○	1	7	-3383.44	-8.57
○	2	0	-3382.56	-6.21
○	2	1	-3382.56	-6.21
○	2	2	-3382.56	-6.21
○	2	3	-3382.48	-6.23
○	2	4	-3382.48	-6.23
○	2	5	-3382.48	-6.23

Fig.4.11. SwissDock scores for the interaction performed for HDAC 3 and Entinostat

The most effective interactions had been selected from the docking scores and the below given table had been prepared, showing the most preferred structural energies.

TABLE V. **SWISSDOCK SCORES FOR THE HIGHEST ENERGY CLUSTER OF ENTINOSTAT**

Cluster	Element	Full fitness (kcal/mol)	Estimated ΔG (kcal/mol)
0	0	-3389.41	-8.03
0	1	-3388.63	-8.04
0	5	-3388.35	-8.16
1	4	-3383.51	-8.59
1	6	-3383.44	-8.57

The most crucial interaction is seen where ΔG is most negative. It can be seen in cluster 1 with element 4. It has shown ΔG of -8.59 which is considered to be a significant energy. The full fitness of this cluster is found to be -3383.51 kcal/mol. The other cluster that can be taken into consideration is the one with a value of 1 and element 6, which has a full fitness score of -3383.44 kcal/mol and ΔG as -8.57.

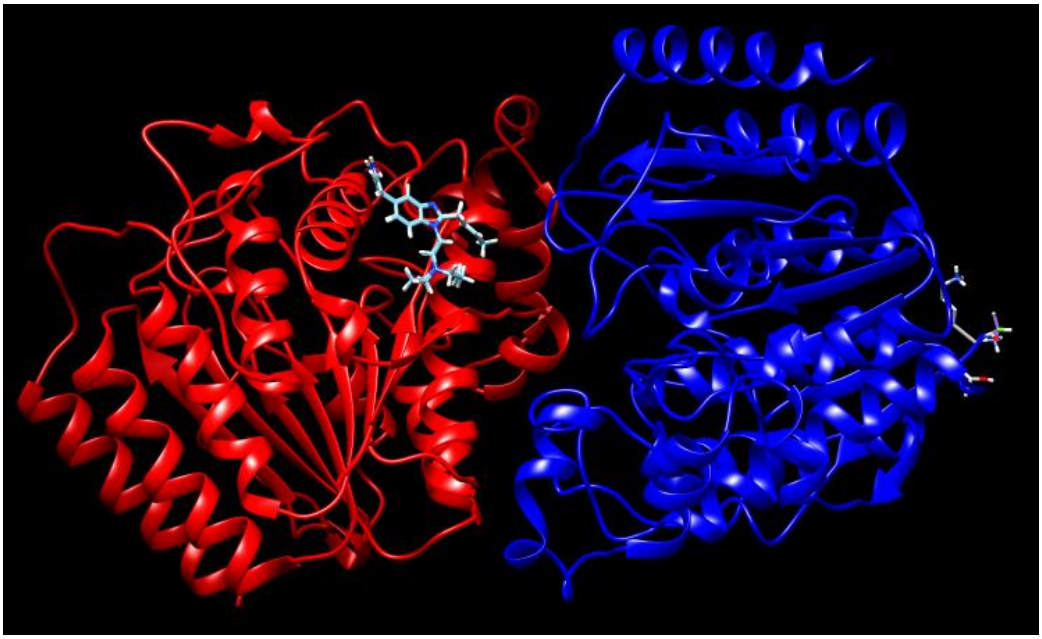


Fig. 4.12. Entinostat interacting with A and B chain of HDAC 3

The drug entinostat is primarily involved in the treatment of advanced breast cancer. The visualisation provided in Fig. 15 shows that this drug interacts better with B chain and has its receptor on A chain. The interaction between the ligand and target protein HDAC 3 has evinced entinostat sits well in the pockets on chain A of HDAC 3.Thus, the feasibility of this computational analysis can be substantiated via laboratory experiments. If the drug is found to be effective then it can be sent for clinical trials. This study would help to generate the better targets and drugs for the treatment of AD.

4.5.2 ADME analysis of Entinostat

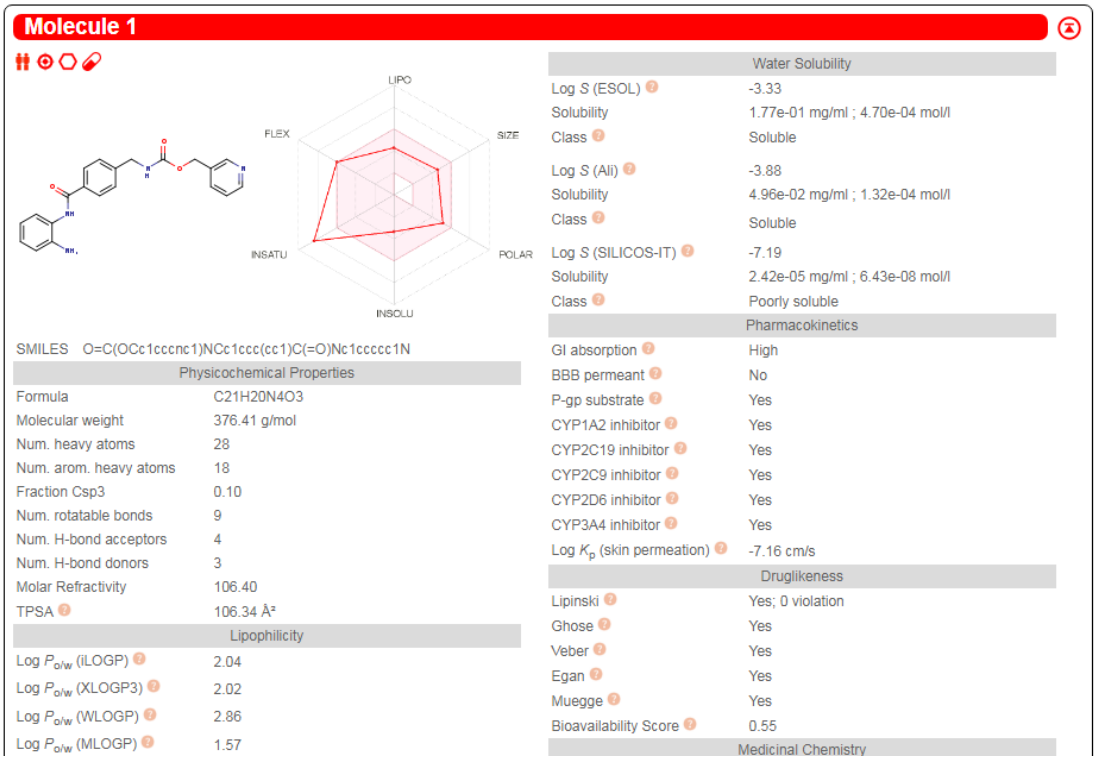


Fig. 4.13. SwissADME analysis of drug Entinostat

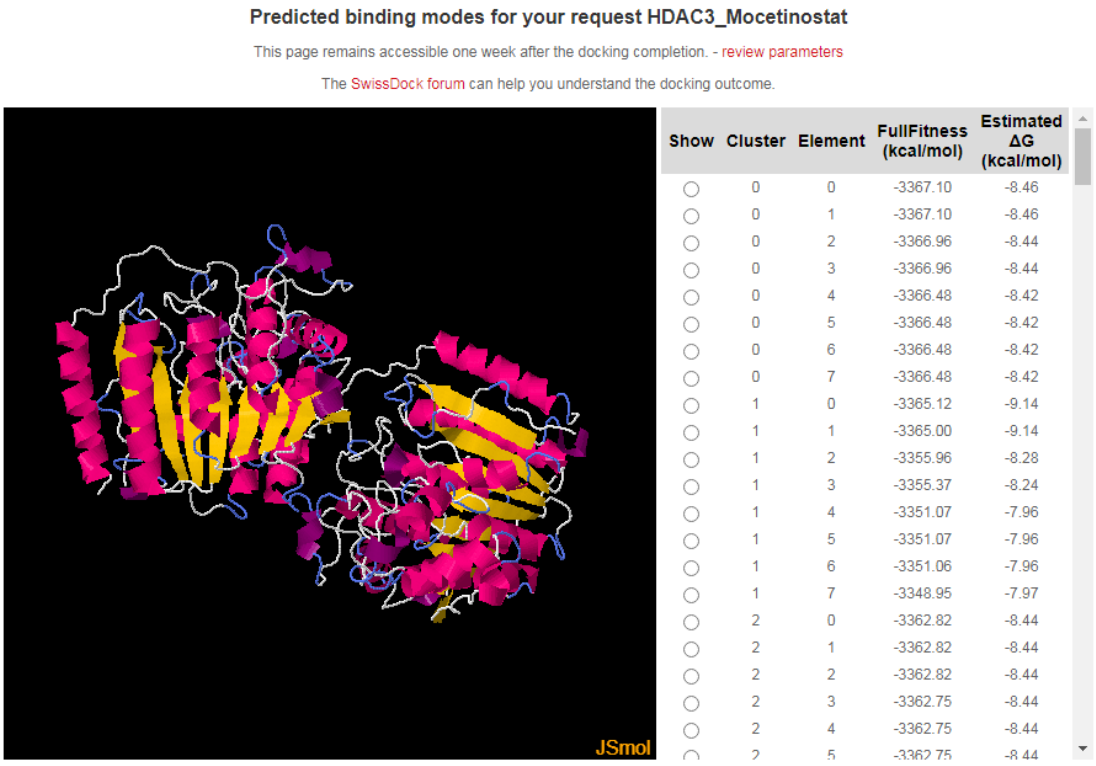
The ADME analysis of Entinostat shows that it has high gastrointestinal absorption and it is not a blood-brain barrier permeant. It is known to be a CYP1A2 inhibitor, CYP2C19 inhibitor, CYP2C9 inhibitor, CYP2D6 inhibitor, CYP3A4 inhibitor. Additionally, it has a Log K_p (skin permeation) value as -7.16 cm/s. It follows all the parameters included in druglikeness such as Lipinksi, Ghose, Veber, Egan, and Muegge. It's bioavailability score is found to be 0.55. Furthermore, for water solubility, it has values as -3.33 for Log *S* (ESOL) and the Solubility as 1.77e-01 mg/ml ; 4.70e-04 mol/l. Under these conditions, the drug is known to be water soluble. The drug is also observed and studied under Log *S* (Ali) and Log *S* (SILICOS-IT); and

was found to be water soluble under these conditions as well. The drug's lipophilicity scores are as follows: 2.04 for Log $P_{o/w}$ (iLOGP), 2.35 for Log $P_{o/w}$ (SILICOS-IT), 2.17 for Consensus Log $P_{o/w}$, etc.

4.6 INTERACTION BETWEEN HDAC 3 AND MOCETINOSTAT

4.6.1 Docking results for HDAC 3 and Mocetinostat

The molecular blind docking for HDAC 3 and mocetinostat has shown significant interaction with each other. The docking scores provide the evidence for the feasible and favoured interaction between the target protein and the ligand drug. The clusters with the most significant interactions had been selected for further visualisation and better observation.



Show	Cluster	Element	FullFitness (kcal/mol)	Estimated ΔG (kcal/mol)
○	0	0	-3367.10	-8.46
○	0	1	-3367.10	-8.46
○	0	2	-3366.96	-8.44
○	0	3	-3366.96	-8.44
○	0	4	-3366.48	-8.42
○	0	5	-3366.48	-8.42
○	0	6	-3366.48	-8.42
○	0	7	-3366.48	-8.42
○	1	0	-3365.12	-9.14
○	1	1	-3365.00	-9.14
○	1	2	-3355.96	-8.28
○	1	3	-3355.37	-8.24
○	1	4	-3351.07	-7.96
○	1	5	-3351.07	-7.96
○	1	6	-3351.06	-7.96
○	1	7	-3348.95	-7.97
○	2	0	-3362.82	-8.44
○	2	1	-3362.82	-8.44
○	2	2	-3362.82	-8.44
○	2	3	-3362.75	-8.44
○	2	4	-3362.75	-8.44
○	2	5	-3362.75	-8.44

Fig.4.14. Predicted structures for the binding of HDAC 3 and Mocetinostat

The SwissDock scores have provided the best interaction for mocetinostat drug and the critical and substantial clusters have been indicated in table 6. Comparing these scores against ΔG and full fitness provided the best possible outcomes. The cluster 1 with the element 0 has shown the maximum fitness with a ΔG score of -9.14. It corresponds to the full fitness energy of -3365.12 kcal/mol. This

favourability of this interaction is followed by first cluster i.e. with a number 0 and element number 0. It has shown the ΔG of -8.46 and the full fitness of -3367.10 kcal/mol. The scores show that there is a high level of interaction between HDAC 3 and the drug mocetinostat.

TABLE VI. **SWISSDOCK SCORES FOR THE MOST FAVOURED CLUSTER OF MOCETINOSTAT**

Cluster	Element	Full fitness (kcal/mol)	Estimated ΔG (kcal/mol)
0	0	-3367.10	-8.46
0	2	-3366.96	-8.44
0	4	-3366.48	-8.42
1	0	-3365.12	-9.14
1	2	-3355.37	-8.28

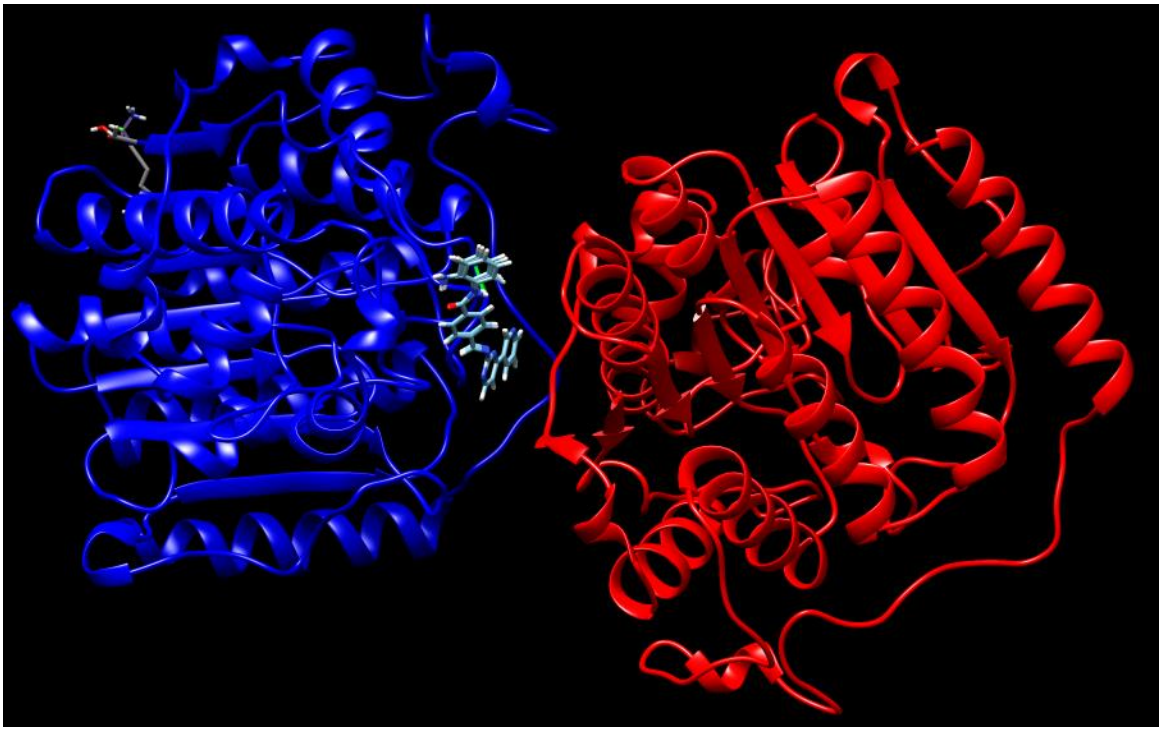


Fig. 4.15. Interaction of A and B chains of HDAC 3 with the drug Mocetinostat

From the visualisation provided in Fig. 18. via UCSF Chimera, it can be concluded that mocetinostat drug interacts better with A chain and is better fitted in the receptors of A chain present in HDAC 3 for mocetinostat. Mocetinostat is a known HDAC inhibitor and his interaction proves that mocetinostat can inhibit the action of HDAC 3 which plays a major role in Alzheimer's disease. Thus, this study can be used for further investigation of the feasibility of this reaction via hypothesis building and testing in laboratories. Mocetinostat would inhibit HDAC 3, which in turn would prevent the action of hypoxia-inducible factors and thus hypoxic conditions would be under control. And therefore, mocetinostat can be an acknowledged inhibitor in AD.

4.6.2 ADME analysis of the ligand drug i.e.Mocetinostat

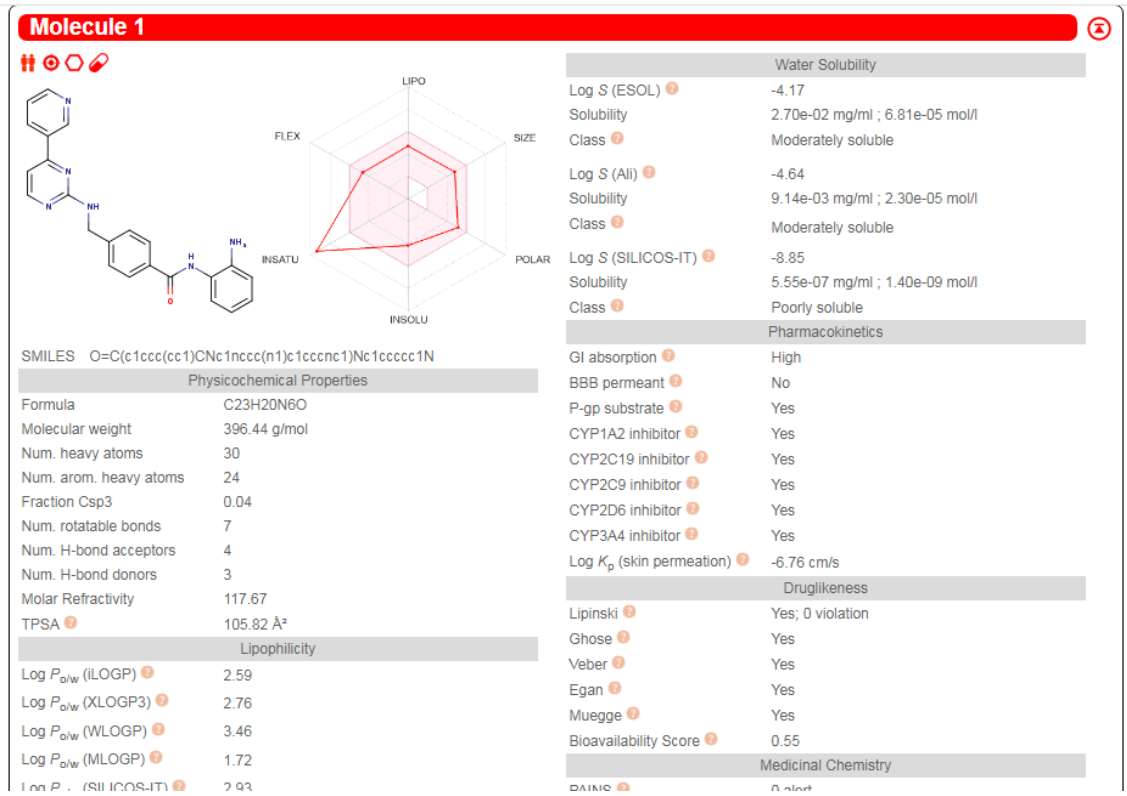


Fig. 4.16. ADME analysis of the drug Mocetinostat using SwissADME

The pharmacokinetics analysis of mocetinostat has shown that it has high gastrointestinal absorption with no blood-brain barrier permeability in ADME analysis. It is known to be an inhibitor of CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4. Also, it is a P-gp substrate. In addition to this, the Log K_p (skin permeation) value for mocetinostat drug is -6.76 cm/s. The drug follows all the

druglikeness rules i.e.Lipinksi, Ghose, Veber, Egan, and Muegge without any violation. And the bioavailability score is found to be 0.55. As for the medicinal chemistry the synthetic availability score for mocetinostat drug is 2.93. For water solubility, the Log *S* (ESOL) score is found to be -4.17 corresponding to the water solubility of 2.70e-02 mg/ml; 6.81e-05 mol/l. Thus, it is moderately soluble under this parameter. The studies involving Log *S* (Ali), the score is found to be-4.64 along with the solubility of 9.14e-03 mg/ml; 2.30e-05 mol/l which means that it is moderately soluble. Under Log *S* (SILICOS-IT) analysis, the score is -8.85, which indicates that it is poorly/insoluble in water.The lipophilicity scores for mocetinostat are as follows, 2.59 for Log $P_{o/w}$ (iLOGP), 3.46 for Log $P_{o/w}$ (WLOGP), 2.69 for Consensus Log $P_{o/w}$, and 2.76 for Log $P_{o/w}$ (XLOGP3), etc.

CHAPTER 5

COMPARISON AND INFERENCE

According to the different docking scores that were obtained after blind docking of HDAC 3 and the drugs i.e. Valproic acid, Vorinostat, Pracinostat, Entinostat, and Mocetinostat; it can be inferred that all of the drug interaction with the target protein is at par and that these drugs can be used to inhibit the action of Histone Deacetylase 3. HDAC 3 being an important contributor in Hypoxia-induced Alzheimer's disease needs to be regulated in order to control the action of HIF-1α, because HIF-1α is directly influenced under the action of HDAC 3. Thus, inhibiting HDAC 3 would serve as a putative target in controlling Alzheimer's disease and these drugs can pave the path for it.

TABLE VII. **COMPARISON BETWEEN DIFFERENT DRUGS FOR THEIR POTENTIAL AGAINST HDAC 3 INHIBITION**

Drug	Full fitness (kcal/mol)	Estimated ΔG (kcal/mol)
Valproic acid	-3020.27	-6.92
Vorinostat	-3391.68	-8.14
Pracinostat	-3372.74	-8.29
Entinostat	-3383.51	-8.59
Mocetinostat	-3365.12	-9.14

When the free energies of these drugs that were obtained during blind docking were compared against each other, they have shown a significant difference in their interaction and binding with HDAC 3. The docking scores of these drugs are analysed for better comparison of the potential interaction of these drugs.

It can be seen in table 7 that mocetinostat drug has the most negative ΔG with a comparable score of full fitness i.e. -3365.12 kcal/mol. It is followed by ΔG of entinostat, pracinostat, vorinostat, and valproic acid in that order. Thus, it can be concluded that mocetinostat interacts the best with Histone deacetylase 3 amongst all of the studied drugs whereas valproic acid has the least efficiency in interacting with a ΔG score of -6.92.

CHAPTER 6

CONCLUSION AND FUTURE PROSPECTS

Alzheimer's is a major neurodegenerative disorder that is marked by progressive deterioration of neuronal cells and leads to cognitive memory deficiency. Hypoxia, a known cause of Alzheimer's, under prolonged and severe conditions can result in a critical motor neuronal death. The attributed principle is the lack of the ability to meet the glucose and oxygen requirement of the motor neurons[64]. Hypoxia is directly linked with the increased levels of HIF-1α. And this study has established a link between various factors contributing to the increased neuronal degradation under hypoxic conditions. Ubiquitin-proteosome pathway, Wnt- signaling, the absence of polyubiquitination, and the increased levels of VEGF, all contribute to the increased neuron degradation, secretion of toxic peptides, and abnormal neurogenesis.

This study's main focus was to signify the interaction between HIF-1α, HDAC 3, and Valproic acid. Further, the study has provided the evidence for the significant interconnection between HIF-1α and HDAC 3 via the STRING database providing the PPI p-value as 0.0265. Moreover, the docking analysis has evinced the noteworthy association between HDAC 3 and different drugs with an estimated ΔG value to be -9.14 kcal/mol for mocetinostat. These results verify that the drugs under study inhibit HDAC 3, which in turn can inhibit HIF-1α. The inhibition of HIF-1α would eventually release the hypoxic stress and can be a putative approach for the therapeutics in Hypoxia-induced Alzheimer's disease. The ADME analysis has verified the safety of using analysed ligands as the drug against the disease.

The analysed drugs i.e. vorinostat, pracinostat, entinostat, and mocetinostat are most commonly used in the treatment of different types of cancer till date whereas valproic acid has been used for of bipolar disorders, epilepsy, and mood/mental conditions which are somehow related to brain and neurodegeneration. Thus, it would be interesting to find out the efficacy of the following drugs in the treatment of a neurodegenerative disorder like Alzheimer's disease. Looking at the interaction that

each of the drug shows against HDAC 3 inhibition, it is certain that they can be used for treating neuronal degradation. Some of the drugs like mocetinostat are still under clinical trials but this study directs their laboratory experiments that must be carried out carried out in order to study and observe their binding precisely.

Moreover, this study directs the future investigation of the particular amino acids of HDAC 3 that interact with different drugs and thus can be experimentally proven in the clinical laboratories so that it can be used as a putative drug in Alzheimer's disease. But further studies can constitute valproic acid, vorinostat, pracinostat, entinostat, and mocetinostat for Alzheimer's therapeutics as well.

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LIST OF PUBLICATION

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Respected Sir/Madam,

I, **Yami Garg**, final year student of **M.Sc. Biotechnology**, Roll no. **2K20/MSCBIO/37**, Department of Biotechnology, Delhi Technological University, hereby declare that the work which is presented in the Dissertation thesis entitled "**Regulation of Hypoxia Inducible Factors via Histone Deacetylase Inhibitor 3 Drugs and a Comparison between their Interactions**" in the fulfilment of the requirement for the award of Degree of Masters of Science in Biotechnology is an authentic record of my own work done under the supervision of **Prof. Pravir Kumar**.

The work attached to this declaration complies with the University's requirements and is my own work. The overall similarity is 32%, out of which 27% is from my own publications. The plagiarism report has been attached. Further, the work attached to this declaration has not been submitted in full or partial satisfaction of an academic award in another university.

Yours Sincerely,
Yami Garg
2K20/MSCBIO/37
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CANDIDATE'S DECLARATION

I, Yami Garg, Roll No., 2K20/MSCBIO/37, student of M.Sc. Biotechnology, hereby declare that the Dissertation project titled "**Regulation of Hypoxia Inducible Factors via Histone Deacetylase Inhibitor 3 Drugs and a Comparison between their Interactions**" which is submitted by me to the Department of Biotechnology, Delhi Technological University, Delhi in partial fulfilment of the requirements for the award of the degree of Master of Science, is original and not extracted from any source without proper citation. This 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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SUPERVISOR CERTIFICATE

I hereby certify that the dissertation project titled "**Regulation of Hypoxia Inducible Factors via Histone Deacetylase 3 Inhibitor Drugs and a Comparison between their Interactions**" which is submitted by Yami Garg, 2K20/MSCBIO/37, Department of Biotechnology, Delhi Technological University, Delhi in partial fulfilment of requirement for the award of the degree of Master of Science, is a record of work carried out by the student under my supervision. To the best of my knowledge, this work has not been submitted in part of any Degree or Diploma to this University or elsewhere.

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