EXPLORING STRUCTURAL REQUIREMENT OF NATURAL INHIBITORS AGAINST HDAC1 IN CANCER: A STEP TOWARDS NON-TOXIC ANTICANCER DRUG DEVELOPMENT

Submitted by

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CERTIFICATE OF APPROVAL

This is to certify that ***Sourav Sardar*** (Exam Roll No: **M4PHC23017**, Reg. No: 127792 of 2014-2015) has sincerely carried out the research work on the subject entitled "***Exploring Structural Requirement of Natural Inhibitors Against HDAC1 in Cancer: A Step Towards Non-toxic Anticancer Drug Development***" under the supervision of **Dr. Shovanlal Gayen**, Laboratory of Drug Design and Discovery, Department of Pharmaceutical Technology of Jadavpur University. He has incorporated his findings in this thesis submitted by him in partial fulfillment of the requirements for the degree of **Masters of Pharmacy** (Pharmaceutical Technology) of Jadavpur University. He has carried out the research work independently and sincerely with proper care and attention to our entire satisfaction.

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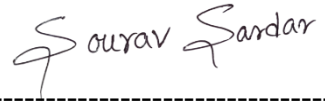

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India.

Declaration of Originality and Compliance

Of Academic Ethics

I hereby declare that this thesis contains a literature survey and original research work performed by me (Sourav Sardar) as a part of my Masters of Pharmacy studies. All the information in this document has been obtained and presented in accordance with academic rules and ethical conduct.

I also declare that, as required by these rules and conduct, I have cited and referenced the materials and results that are not original to this work.

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Signature with Date

Dedicated to

My Parents, Teachers, Seniors

and

Friends

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Preface

For a several decades, "Cancer" is a serious human threat to mankind, which is defined as a group of pathological conditions characterized by uncontrolled and abnormal cell proliferation. In recent times, it ranks among the prime reasons of mortality, with an exponentially increasing number of individuals affected globally. Moreover, the expensive and painful treatments required for cancer patients pose a substantial threat to both our society and economy. The onset and proliferation of cancer cells result from both internal and external factors. Numerous external factors, such as pollution, inadequate nutrition, radiation exposure, and lifestyle choices, contribute to alterations in the biological system, alongside internal factors like genetic mutations and irregular hormone regulation.

Histone deacetylase 1 (HDAC1) stands out as a crucial member within the HDAC family, involved in several pathological and physiological conditions related to various diseases, encompassing neurological disorders, heart-related ailments, and a diverse spectrum of cancers, such as those affecting the several vital organs liver, stomach, colon, kidney, prostate, thyroid, and blood, like leukaemia. Despite the availability of numerous inhibitors targeting HDACs, the lack of selective and potent HDAC1 inhibitors that could effectively combat cancer and other HDAC1-associated conditions remains a notable limitation in their treatment. Due to non-selective or pan-HDAC inhibition the drugs not safe for human body in many cases.

From ancient time, people have a strong believe in nature-derived medicines as an effective and safe remedies. In this regard, it is very important to find some natural source of drugs that will have less toxicity and specific HDAC inhibition. There are lot of natural compounds that shows HDAC1 inhibitory activity. There are very few HDAC inhibitors that have been approved by Food and FDA approval due to the lack of research.

Based on structural aspect, natural HDAC1 inhibitors can be classified as hydroxamate and non-hydroxamate types. These are consisting of a cap group, a linker moiety, and a zinc binding group that the majority of HDAC1 inhibitors share in order to create an effective HDAC1inhibitor. In contrast, the hydroxamic acid moiety has demonstrated to be a more effective zinc binding group for HDAC1 activity. Additionally, the quantitative structure-activity relationship (QSAR) methodology quantifies the biological activity of molecules in relation to their molecular structure.

The goal of this study is to find the significant structural fingerprints of the natural HDAC1 inhibitors that are essential for controlling the inhibitory activity. Some of the important fingerprints of natural HDAC1 inhibitors regulating the activity have discovered in this work. In addition, the molecular docking study was processed for generating the protein-ligand complex which identified some potential amino acid residues for the binding interactions in case of natural inhibitors. Finally, molecular dynamics study establishes the compounds' stability inside the core of HDAC1 receptor. The discovery of HDAC1 inhibitors that are inspired by natural sources can be a topic of extensive investigation in the way of non-toxic anticancer drug development.

(Sourav Sardar)

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Chapter 1: Introduction

1.1. Cancer

Cancer or neoplasmic conditions is regarded as one of the most terrifying medical conditions in the field of medicine and is known to mankind from the ancient era. The word "cancer" itself comes from the Latin word "canker," which means crab (Patrick *et al*., 2013). Numerous biochemical pathways linked to carcinogenesis have been discovered as a result of advances in molecular biology research. However, several genes are concerned to promote cancer; Ras and TP53 factors play significant roles in this context. Ras encodes the Ras protein that promotes over-proliferation of normal cells whereas suppression of TP53 decreases the activity of p53 by altering the mode of cell-cycle regulation, repair as well as the suicide of rouge cells (Patrick *et al*., 2013). On the other hand, the growth of an oncogene from a proto-oncogene also causes cancer. The etiology of cancer demonstrates that a number of factors including Epstein-Barr virus, hepatitis B virus, and HIV, play critical roles in the development of cancer. For example, HIV is linked to lymphoma and Kaposi's sarcoma (Patrick *et al*., 2013). Hepatitis B causes liver cancer and Epstein Barr virus causes Burkitt's lymphoma. There are at least 200 different varieties of cancer, and because of the biochemical complexity of these conditions, it has been noted that treatments that work for one type of cancer disease may not work for another (Patrick *et al*., 2013). Nearly 1.68 million new instances of cancer were identified nationwide in 2016, and it is expected that 60,000 people would pass away from it, making it the second most prevalent cause of death in the country and accounting for 1 in 4 deaths (Becker *et al*., 2012). Recent advances in cancer biology and the development of a growing number of novel medications that target specific vulnerabilities in certain malignancies have made a profound impact on cancer pharmacology. Some lethal cancers, such as testicular cancer, lymphomas, and leukemia, have effective early therapies available. On the other hand, after surgical removal of localized lung, colorectal, and breast cancer, adjuvant chemotherapy and hormone treatment can only prolong overall survival and prevent disease recurrence (Brunton *et al*., 2018). Chemotherapy is utilized in three main clinical contexts nowadays as mentioned below:

1. There are no other successful therapeutic options for a malignancy or an advanced condition. Generally primary induction therapy is used.

2. Neoadjuvant therapy is given to patients who have localized disease and for whom local treatments like surgery, radiotherapy, or both are inadequate.

3. Adjuvant therapy to local method of treatment, such as surgery, radiation therapy, or both, is also used.

1.2. Mechanism of action of Histone deacetylases

In the context of cancer treatment, many biological receptors, as well as enzymes play a significant role in cancer pathogenesis. Over the last several decades, the research community take interest on the chromatin modification *via* histone deacetylation in cancer therapy. The acetylation and deacetylation of histone is balanced by two enzymes named histone acetyltransferases (HAT) and histone deacetylases (HDAC). HATs mainly catalyse the transfer of an acetyl group from acetyl-CoA to the ε-NH_2 group of lysine residues in proteins and HDACs remove the acetyl group from the ε-NH_2 group of lysine residues (Liu *et al*., 2022). Histone acetylation is a critical epigenetic modification that changes the chromatin architecture as well as regulates gene expression by opening or closing the chromatin structure. Chromatin contains nucleosome which consist of an octamer having four histone cores (H2A, H2B, H3, and H4) surrounded by 146 base pairs of DNAs. ε amino groups of lysine residues located at the N-termini is the target for acetylation and facilitates the binding of transcription factors. It loosens the contact between core nucleosome proteins and DNA. As a result, the transcription factor binding sites become more accessible (Ruijter *et al*., 2003). However, these HDACs, not only deacetylate the histones but also some non-histone proteins such as E2F, c-Myc, NF-κB, p53, etc. (Jenke *et al*., 2021). The downregulation of the GATA family of transcriptional factors is also regulated by the HDACs (Caslini *et al*., 2006; Linggi *et al*., 2002). These HDAC enzymes are also involved in leukemogenesis. There are several natural HDAC inhibitors that can be used as anticancer drugs **(Figure 1)**.

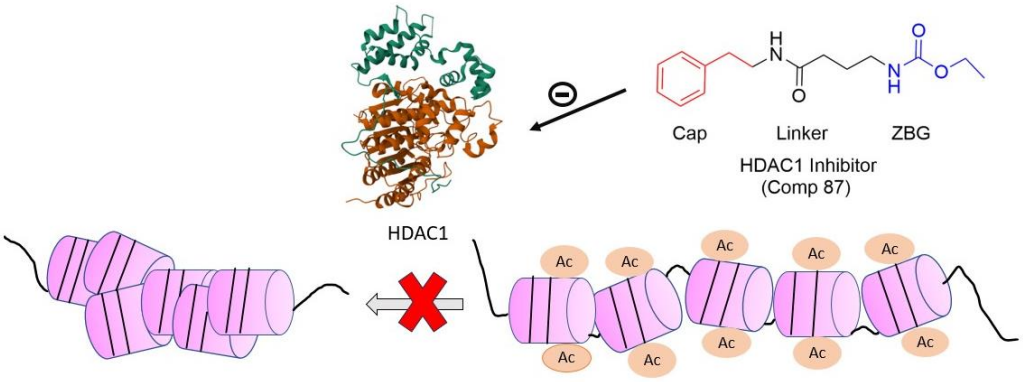

Figure 1: Deacetylation of Histone protein inhibited by HDAC inhibitors

1.3. Classification of HDACs

HDAC enzymes are grouped into four classes like class I, class II, class III and class IV based on their sequence homology to their yeast ortho-logues Rpd3, HdaI and Sir2, respectively. Class I HDAC includes HDAC1, 2, 3 and 8, Class II includes HDAC4, 5, 6, 7, 9 and 10, Class III is represented by SIRT1–7, and Class IV comprises of only HDAC11 (Moinul *et al*., 2022). Enzyme Classes I, II, and IV consist of 11 types of HDACs, which are referred to as ‘‘classical” HDACs. Different HDACs including the classical HDACs are important targets in various diseases. HDAC1 is found in three distinct biochemical complexes: Sin3, NuRD, and CoREST (Ruijter *et al*., 2003). The catalytic activity of Classes I, II, and IV depends on zinc, however Classes III differs from other HDACs in that it depends on NAD+ for its enzymatic activity.

1.4. Role of HDACs in Cancer

In hypoxic cancer cells, HDACs (Histone Deacetylases) are found to be encoded. Within the intracellular environment, two proteins, Runx1 and ETO, undergo fusion to create a chimeric protein known as Runx1-ETO. This chimeric protein, Runx1-ETO, has the ability to recruit HDACs to its location within the cell (Linggi *et al*., 2002; Follows *et al*., 2003). This leads to the activation of matrix metalloproteinases (MMPs), which break down the intercellular matrix in the epithelial cells of blood vessels, resulting in the formation of numerous small vessels that provide an increased oxygen supply to the rapidly multiplying cancerous cells. This phenomenon is referred to as 'Angiogenesis.' (Jenke *et al*., 2021) HDACs play a crucial role in governing the process of epithelial-mesenchymal transition (EMT), which is pivotal in facilitating the invasion and metastasis of cancer cells (Becker *et al*., 2012). However, EMT is described as the loss of epithelial cell markers like epithelial-cadherin (CDH1) and CDH1 along with other transcriptional factors such as Snail, Slug, Twist, ZEB1, and ZEB2. Sirtuin (SIRT) induces cell migration in vitro and metastasis in vivo in the case of prostate cancer in cooperation with ZEB1 to suppress the transcription of CDH1 (Becker *et al*., 2012). The role of autophagy in cancer is quite complicated. Autophagy plays a surveillance role to wipe out undesirable damage to organelles and cellular components. That might prevent the transformation of a normal cell into a cancer cell (Becker *et al*., 2012). Depletion or inhibition of HDAC1 induces autophagy by promoting the accumulation of autophagosomal marker LC3-II (Becker *et al*., 2012). It is concerned that autophagy provides an essential link between autophagy and the ubiquitinproteasome system in the case of neurodegenerative diseases such as Huntington’s disease, Alzheimer’s disease, etc (Becker *et al*., 2012).

Somatic mutation helps the cell to acquire novel capacities and no need to say such capacities are often regulated for a beneficial role in survival (Becker *et al*., 2012). Recent studies say that many HDACs are increased in malignant cells and associated with several cancers **(Table 1)**.

Table 1: Cancer conditions associated with HDAC enzymes

Serial No	HDAC enzymes	Cancer conditions
1	HDAC1	Gastrointestinal, breast, and prostate cancer
2	HDAC2	Uterine, cervical, and gastric cancer
3	HDAC3	Colon, breast cancer, gastric, prostate cancer
4	HDAC8	Leukemia, breast, colon, lung cancer
5	HDAC6	Breast cancer

HDAC expressions get increased in solid tumor and hematological cancers, moreover, both HDAC1 and HDAC2 inhibit apoptosis of cancer cells. On the contrary HDAC3, 4, 5, and 8 tend to inhibit differentiation whereas HDAC4, 6, 9, and 10 are closely associated with cancer angiogenesis (Yoon *et al*., 2016). On the other hand, both HDAC6 and HDAC10 provoke cell motility (Yoon *et al*., 2016). The action of the p21 gene is a potent cell cycle arrester inversely proportional with HDACs. The transcriptional level of p21 declines by up-regulation of HDACs (Yoon *et al*., 2016; Sarkar *et al*.,2020). Aneuploidy is defined as an abnormal number of chromosomes and is identified as a negative prognostic factor for epithelial malignancies (Yoon *et al*., 2016). It is reported that aneuploidy is observed in a majority of solid tumors as well as hematopoietic neoplasia and HDAC2 is highly expressed in the aneuploidy cell line than diploid cell line (Yoon *et al*., 2016).

1.5. Role of HDACs in other diseaseses

1.5.1. Cardiovascular disease

Research on HDACs' involvement in cardiac hypertrophy has revealed that both class I and class II HDACs play a role in the development of this condition (Yoon *et al*., 2016). Cardiac fibrosis, a significant condition resulting from reduced elasticity and inadequate expansion of the contracting chamber during the diastolic phase, is linked to hypertrophy in notable heart disorders (Yoon *et al*., 2016). Fibrosis can be inhibited by HDAC inhibitors that directly regulate transdifferentiation of fibroblast to myofibroblast (Yoon *et al*., 2016). Very recently, the European Society of Cardiology and the American Heart Association issued a warning about the seriousness of HFpEF (Heart failure with preserved ejection function) (Yoon *et al*., 2016). They summarize the clinical outcome of HFpEF patients during the last two diseases that got the conventional regimen for heart failure with reduced ejection fraction. Recent molecular biological research has increasingly implicated HDACs as the one of the reasons behind cardiac arrhythmia, providing more conclusive evidence. Trichostatin A (TSA) dramatically cured atrioventricular conduction abnormalities in mouse hearts which are induced by genetic disruption of Hop X (Yoon *et al*., 2016). Atherosclerosis is a chronic and progressive disease of arteries resulting from abnormal accumulation of lipid droplets, inflammation of multi-factorial cells, generation of a fibrosis cap, and a reactive proliferation of vascular smooth muscle cells. According to several studies HDACs are closely linked with the progression of atherosclerosis (Yoon *et al*., 2016). HDAC inhibitors are beneficial in the treatment of various conditions such as cardiac arrhythmia, cardiac fibrosis, myocardial infarction, and cardiac hypertrophy. Additionally, they show promise in potentially inhibiting the progression of atherosclerosis and the development of vascular calcification.

1.5.2. Diabetes mellitus (DM)

Diabetes Mellitus (DM) is a chronic metabolic disorder distinguished by either a reduced secretion of insulin, impaired insulin function, or both (Wong *et al*., 2009; Chatterjee *et al*., 2014; Damić *et al* 2022; Dehghani *et al* 2023). Hepatic gluconeogenesis plays a significant role in the occurrence of high blood sugar levels in individuals with type 2 diabetes (Chen *et al*. 2017). HDACs regulates glucose homeostasis and plays a significant role in controlling glucose metabolism (Chen *et al*. 2017). discovered that HDACs modulate hepatic gluconeogenesis by controlling the expression of peroxisome proliferator-activated receptor-γ (PPAR- γ), coactivator-1α (PGC-1α) and cyclic AMP-responsive element-binding

protein (CREB) by the deacetylation of forkhead box O1 (FoxO1) transcription factor (Zou *et al*., 2015) CREB, PGC-1α and glucocorticoid receptor (GR) have been identified as novel FoxO1 regulatory targets. Since, HDACs silencing increase acetylation of FOXO1, the FOXO1-HDAC axis may be responsible for HDAC's advantageous impact on gluconeogenesis (Chen *et al*. 2017; Chen *et al*. 2015). Another link between HDACs and diabetes, has been suggested via the regulation of adiponectin levels in the blood, which is secreted by adipocytes (Dehghani *et al* 2023). HDACs has been shown to be selectively down-regulated prior to adipogenic differentiation (Dehghani *et al* 2023; Parra *et al* 2015; Li *et al* 2015) The researchers have discovered that at the E-box region of the C/EBPα gene promoter, HDACs is recruited along with USF1, in preadipocytes. In this instance, HDACs prevents C/EBPα transcription [37]. Also, HDACs overexpression inhibits the transcription of ABCG1, a gene for cholesterol efflux. Important genes involved in adipocyte differentiation, insulin signalling, and glucose uptake are all inhibited by ABCG1 silencing (Khamis *et al* 2020).

1.5.3. Kidney fibrosis

Kidney fibrosis is caused by the abnormal deposition of extracellular matrix (ECM) in the kidney tissue and is thought to be the most common cause of chronic kidney disease (CKD) (Zhang *et al*., 2023) The principal component of the kidney and a key target in the progression of CKD is tubular epithelial cells (TECs) (Sako *et al* 2022; Humphreys *et al* 2008; Li *et al* 2022). The HDAC isoforms responsible for kidney fibrosis and its associated mechanism remains unknown. Recently in 2023, Zhang *et al*. discovered that HDACs expression is dramatically elevated in fibrotic mice kidneys, particularly in the proximal tubules. HDACs cause deacetylation of STAT1 and increases its reactivation, which leads to G2/M arrest of TECs and ultimately lead to tubulointerstitial fibrosis. Therefore, *in vitro* blocking of HDACs may prevent TECs from losing their epithelial phenotype and reduce fibroblast activation by preventing epithelial cell cycle arrest in G2/M phase. Thus, this research suggests that HDACs may be a promising therapeutic target for renal fibrosis (Zhang *et al*., 2023).

1.5.4. Liver fibrosis

Liver fibrosis is described as the abnormal deposition of fibrous connective tissue around the damaged area of the liver (Puche *et al*., 2013; Yang *et al*., 2017; Claveria *et al*., 2020; Moran *et al*., 2017) Chronic liver disease (CLD) has a wide range of aetiologies, including alcoholism, viral infections, metabolic disorders, etc. They all lead to liver fibrosis, which is a typical outcome of practically every CLD (Friedman *et al*., 2008). The most crucial mechanism for hepatic fibrogenesis is reported to be stimulation of hepatic stellate cells (HSCs) (Rippe *et al*., 2004; Li *et al*., 2016) . HDACs plays a vital role in HSC stimulation and liver fibrosis. HSCs are the primary source ECM producing myofibroblasts (Barcena *et al*., 2019). In a healthy liver, these cells also manage the turnover of the ECM and regulate the contraction of the sinusoids (Puche *et al*., 2013) When the liver is injured, HSCs get activated and go through trans-differentiation to form myofibroblasts, which produce ECM (Li *et al*., 2016) When compared to healthy livers, human livers with primary biliary cirrhosis, alcoholic cirrhosis and non-alcoholic liver disease have up-regulated HDACs mRNA and protein levels (Yang *et al*., 2016). It has been reported that HDACs knockdown in LX-2 cell lines, significantly reduces the gene expressions involved in fibrous tissue formation. It was also demonstrated that HDACs suppression inhibit the expression of TGFβ-target genes (α-SMA and COL1A1) in LX2 cells treated with HDACs siRNA (Yang *et al*., 2016). This shows that HDACs is essential for HSC activation and inhibiting it could halt the progression of liver fibrosis. According to a study by Mannaerts *et al.* (Mannaerts *et al*., 2016). expression of HDACs is downregulated during the trans-differentiation of HSCs. Another study investigated that most HDACs (HDAC1, 2, 4, 5, 6, 8) levels are elevated during fibrosis. Yang *et al.* reported that HDACs expression is significantly greater in human liver diseases such as alcoholic cirrhosis and primary biliary cirrhosis (PBC). Due to these discrepancies, more research is needed to determine the changes in HDACs expression during hepatic fibrogenesis.

1.5.5. Obesity

Obesity is significantly influenced by decreased adipose tissue function which is triggered by altered gene expression. *In vitro* and animal model studies suggests that HDACs are a new epigenetic player in the pathogenesis of obesity (Li *et al*., 2022). However, the clinical relevance of HDACs in insulin resistance and obesity research is still not clear. Pour *et al.*

(Jannat *et al*., 2022). examined the mRNA expression of HDACs in visceral adipose tissue (VAT) of obese women (n = 20) and normal-weight women (n = 19). The VAT mRNA levels of HDACs in obese participants were considerably lower than in controls. As a result, the decreased HDACs mRNA expression in adipose tissue may be linked to obesity. However, more research, required to validate this hypothesis (Jannat *et al*., 2022). Recent research has identified a link between insulin resistance/obesity with HDACs. It has been reported that, HDACs are a negative regulator of adipogenic growth, and human pre-adipocytes express less HDACs before differentiating into adipocytes. HDACs deletion improves insulin sensitivity while reducing the detrimental effects of a high-fat diet on weight gain, fatty tissue dysfunction and hepatic steatosis. FOXO1 transcription factor, which regulates phosphoenolpyruvate carboxykinase (PEPCK) and glucose 6-phosphatase (G6PC) enzymes, gets activated as a result of weight gain-induced insulin resistance and leads to gluconeogenesis (Gross *et al*., 2008). In Huh7 cells, overexpression of HDACs results in FOXO1 deacetylation which enhances transcriptional activity, whereas HDACs knockdown causes opposite effect.

1.5.6. Bone diseases

HDACs control osteogenic differentiation and autophagic activity, which make HDACs an appealing target for the management of several bone diseases (Zhang *et al*., 2020). Osteonecrosis is a progressive refractory disease characterised by apoptosis of osteocytes and bone marrow. Human bone marrow mesenchymal stem cells (MSCs) act as seed cells for osteonecrosis (Yan *et al*., 2011). Wang *et al.* investigated that HDACs regulate osteogenic differentiation in MSCs via the MAPK signalling pathway, resulting in osteonecrosis. Inhibition of HDACs may diminish the production of phosphorylated extracellular signal-regulated kinase 1/2 (pERK1/2), reducing osteogenic differentiation of human bone marrow MSCs. Thus, HDACs may serve as a possible target for the treatment of osteonecrosis . In addition to this, HDACs are also associated with osteoporosis (Jin *et al*., 2015). HDACs appear to decrease osteoclast development, which could prevent bone loss by limiting bone resorption. HDACs activity have been linked to PPAR-γ, an essential mediator of osteoclast progression. By promoting MSC bone marrow development, HDACs may reduce the occurrence of age-related bone loss. According to Li *et al*., microRNA-188 (miR-188) specifically target the mRNA for HDAC gene. MiR-188 excessive expression inhibits HDACs expression in bone marrow (Sun *et al*., 2016). whereas, the deletion of miR-188 in mice lowered age-related loss of bone mass, boosted bone formation in the osteoblast and

reduced the build-up of fat in the bone marrow (Li *et al*., 2015). Overall, HDACs appear to prevent osteoporosis by encouraging osteogenesis and suppressing osteoclastogenesis.

1.5.7. Inflammation and immune responses

HDACs play a crucial role in inflammatory processes. Despite their significance, it is uncertain whether and how HDACs are controlled during inflammation (Li *et al*., 2015). HDACs deficiency reduce inflammation and cytokine and chemokine production in the kidney and spleen due to the overexpression of PPAR-γ (Yan *et al*., 2011; Xiao *et al*., 2010). PPAR-γ activation is most likely the cause of suppression of several inflammatory chemokines and cytokines, including IL-12, IFN-γ, MCP1, inducible NOS, etc. In human sebocytes, bacterial substances promote histone acetylation and cytokine expression. Silencing HDACs in these cells promote the production of inflammatory cytokines such as CXCL8 and IL1B. Depletion of HDACs, boost the overall inflammatory response in human sebocytes (Sanford *et al*., 2016; Sanford *et al*., 2019) Thus, HDACs act as an epigenetic opponent of the inflammatory response. HDACs are essential for the proliferation and differentiation of many distinct cell types, particularly regulatory T-cells (Yan *et al*., 2011). However, the biological relevance of HDACs in T-effector cells is unknown. Yan *et al.* in 2011 demonstrated that reduction of lymphoproliferation and autoantibody production are caused by HDACs deficiency. Moreover, HDACs deficiency is also associated with increased GATA3 and roquin and decreased BCL6 gene expression. Additionally, they have a special role in the plasticity of $CD4^+$ T-cells and autoimmune disease. HDACs alter gene expression through a variety of mechanisms such as histone acetylation, non-histone acetylation and protein-protein interaction, however these routes are not always mutually exclusive (Tao *et al*., 2011). Tao *et al.* discovered that higher HDACs expression in Treg cells diminishes their suppressive capacity. HDAC deficient Treg cells grow faster, resulting in greater immunological suppression . Therefore, HDACs act as a potent immune booster (Li *et al*., 2015).

1.6. Pharmacological roles of HDACs

1.6.1. Effects on DNA damage

Due to numerous research studies, it has been demonstrated that HDACs play a significant role in the repair of DNA damage. Both HDAC1 and HDAC2 are recruited to the sites of DNA damage to remove acetyl groups from histones H3K56 and H4K16, thereby facilitating non-homologous end-joining. Class I HDACs not only induce changes in histone deacetylation but also regulate proteins such as ATR, ATM, FUS, and BRCA1, which are involved in the DNA damage response. Class II HDACs (HDAC4,6,9,10) are also involved in DNA damage response processes like HDAC1 that directly stimulate oxoguanineglcosylage1, a repair protein critically involved in base excision in oxidized guanine residue. Hence deacetylation of 8 oxo-guanine in DNA can promote oxidative stress in the case of inhibitors of HDACs. The presence of HDAC inhibitors disrupts the DNA repair process, leading to accumulation of DNA damage (Jenke *et al*., 2021). HDAC inhibitors, when used in combination with DNA damaging chemotherapeutics, show a potential for synergy due to their combined HDAC inhibitory and DNA alkylating properties. In contrast, SIRT is a critical component of the DNA damage response pathway that involved in multiple steps of DDR, including damage sensing, signal transduction, DNA repair, and apoptosis. SIRT1 specifically interacts and removes acetyl groups from proteins like Ku 70, NBS1, APE1, XPA, PARP1, Top BP1, and KAP1. All of them are example of DDR proteins. SIRT1 is crucial for preserving the integrity and stability of the genome (Becker *et al*., 2012).

1.6.2. Hormone signaling

The expression of hormone receptors such as testosterone, progesterone, and oestrogen receptors (ER alpha and ER beta) has been proposed by HDAC inhibitors, which raises concerns. Under hypoxic conditions, ER alpha down-regulation has been seen in response to treatment with HDAC inhibitors, which reverses the proliferation-stimulating impact caused by estradiol via up-regulation of cyclin D. An intact proteosome pathway is crucial for this response. It has now been established that trichostatin A, a drug formerly used to treat schistosomiasis, and raloxifene promote a strong up-regulation of ER beta in ER alpha positive cells while at the same time lowering ER alpha expression. In the case of TNBC cells, entinostat, a class I selective inhibitor, increases the expression of both aromatase and ER alpha. Additionally, individual, or combined inhibition of HDAC1, 2, and 3 promotes

effective ER upregulation over TNBC cells. A key factor in the promotion of cancer cells is hormone regulation (Jenke *et al*.,2021).

1.6.3. Autophagy induction

The term "autophagy" refers to the controlled elimination of undesirable cellular organelles and components by the lysosome. The function of autophagy in cancer is quite complex. An ordinary cell can become a cancerous cell through autophagy. It helps the cancer cell not just with surveillance but also with resistance from chemotherapy. Factor in transcription FOXO-1 is dependable for initiating autophagy, and many HDAC inhibitors have dual anti- and pro-tumor effects. Alteration in the level of some proteins such as LC3-II, p62, and Beclin 1 increase autophagic flux and they are autophagic hallmarks that are responsible for the knockdown of those factors or proteins that regulate the activities of HDAC-mediated apoptosis. It raises concerns that inhibiting HDAC1 results in a formation of autophagy markers such LC3-II and the subsequent induction of autophagy. The ubiquitin-proteosome pathway is inversely proportional to autophagy. Sirtuins, in addition to class I, II, and IV HDACs, also play a role in the control of autophagy. For instance, SIRT1 directly deacetylates the essential autophagy machinery Atg5, Atg7, Atg8, and LC3 to generate starvation-induced autophagy. (Becker *et al*., 2012; Jenke *et al*.,2021).

1.6.4. Apoptosis induction

The Latin word "apoptosis" refers to the "falling of a leaf," and it is a crucial concept in the study of cellular biology (Alberts *et al*.,2002). This implies the energy mediated normal cell death associated with several steps like cell shrinkage, pyknosis, and karyorrhexis. In the case of apoptosis, several phenomena take place like degradation of the cytoskeleton microtubules, caused by caspase, a special sort of proteolytic enzyme that possesses cystine amino acid in its functional domain (Elmore *et al*.,2007). However, cystine binds to the aspartic acid that is present in the functional residue of the caspase target protein where the caspase is activated form of procaspase. Cytochrome found in mitochondria comes out from the inner mitochondrial membrane where the Electron Transport System (ETS) functions. Cytochrome, however, binds with APAF1 to form apoptosome, this very process is followed by the generation of caspase from procaspase which has been aforementioned here (Patrick *et al*., 2013). Apart from the degradation of mitochondria, dissociation of the ribosome, alteration of the function of food vacuole, accumulation or agglomeration of various cellular

components, disruption of the nuclear membrane and chromosomal aberration as well as faulty DNA repair, etc. the entire process related to caspase based cellular degradation causes intrinsic pathway (Elmore *et al*.,2007). Unlike the intrinsic pathway, the extrinsic pathway involves activation of Bcl-x, Bcl-2 TNF/TNFR, TRAIL/TRAIL-R, FAS/APO FAS, and reduction and reduction in cytoplasmic FLIP-like inhibitory protein (c-FLIP) (Zhang *et al*., 2014).

HDAC inhibitors play crucial roles not only by enhancing the tendency of the intrinsic pathway using caspase activation but also cause the up-regulation of death protein vis a vis down-regulation of growth factor receptor, platelet-derived growth factor, and epidermal growth factors concerned in undesired cell proliferation which can be terminated by administration of HDAC inhibitors like SAHA, VPA, TSA, AR 42, etc. HDACs, on the contrary, stimulate and restore the activity of p53 by its acetylation or downregulation of MDM2 and MDM4 (Sonnemann *et al*., 2014). p53 in its dephosphorylated form binds to a transcription factor that passively imposes cell cycle arrest. However, in the case of a normal cell cycle, cyclin-CDK binding can dephosphorylation which is unable to bind with transcription factors (Reed *et al*., 2014). Moreover, the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) play crucial roles in apoptosis. HDAC inhibitors enhance the action of both of them although they also stimulate the KEAP1-NRF2 pathway that regulates central antioxidant molecules (Sajadimajd *et al*., 2018; Paunkov *et al*.,2019; Zhou *et al*.,2019). NRF2 causes up-regulation of cytoprotectant agents which give birth to a contradictory pro-survival of cancer cells (McMahon *et al*.,2014).

Recent studies show that immunotherapy plays a vital role in the treatment of cancer which is often influenced by HDAC inhibitors. Based on cellular context as well as the tumor microenvironment, HDAC inhibitors have an impact on the efficacy of checkpoint inhibitor 24 treatment. Romidepsin and vorinostat induce the mRNA level of chemokines-Ccl5, Ccxl9, and Ccxl10 in KRAS mutant cells. Such type finding was confirmed in xenografts showing increased T cell infiltration upon romidepsin treatment. Romidepsin and anti-PD1 treatment proved to be synergistic depending on IFN-γ, a special type of interferon. On the other hand, anti-CD4 and anti-CD8 antibodies reversed the anti-proliferation effects on T cells (Zheng *et al*.,2016). However, it was obvious that T-cell activation as well as activation of natural killer cells exerts a pivotal role in HDAC inhibitors induced immune response (Zheng *et al*.,2016). On the other hand, Tregs depletion is responsible for improved ICI results upon HDAC inhibitors treatment TNBC in accompany of nucleosome. Peripheral blood mononuclear cell

(PMPC) in a culture medium enriched with FOXP3 positive class I selective HDAC inhibitors dose dependency increases PDL1 expression (Jenke *et al*., 2021).

1.7. FDA-approved HDAC Inhibitors:

Currently, there are numerous HDAC inhibitors under clinical development possessing various chemical groups viz; hydroxamate, benzamide, short chain fatty-acid, cyclic peptides, etc. which can be divided into three groups in terms of specificity:

1. Nonselective HDAC inhibitors such as vorinostat, belinostat, and panobinostat.

2. Selective HDAC inhibitors such as Class I (romidepsin and entinostat) and HDAC6 inhibitors (ricolinostat).

3. Multi-pharmacological HDAC inhibitors: such as CUDC-101 and CUDC-907.

Apart from that, there are several novel HDAC1/2 inhibitors like MRLB 223, BRD 8430 CPD 60, etc. (Jenke *et al*., 2021).

FDA has approved several fruitful pharmacological agents which are briefly described hereunder:

- **I.** Vorinostat (Zolinga)
- **II.** Romidepsin (Istodax)
- **III.** Belinostsat (Belodaq)
- **IV.** Panabinostat (Frydak)
- **V.** Pacinopstat
- **VI.** Chidamide (Epidaza)

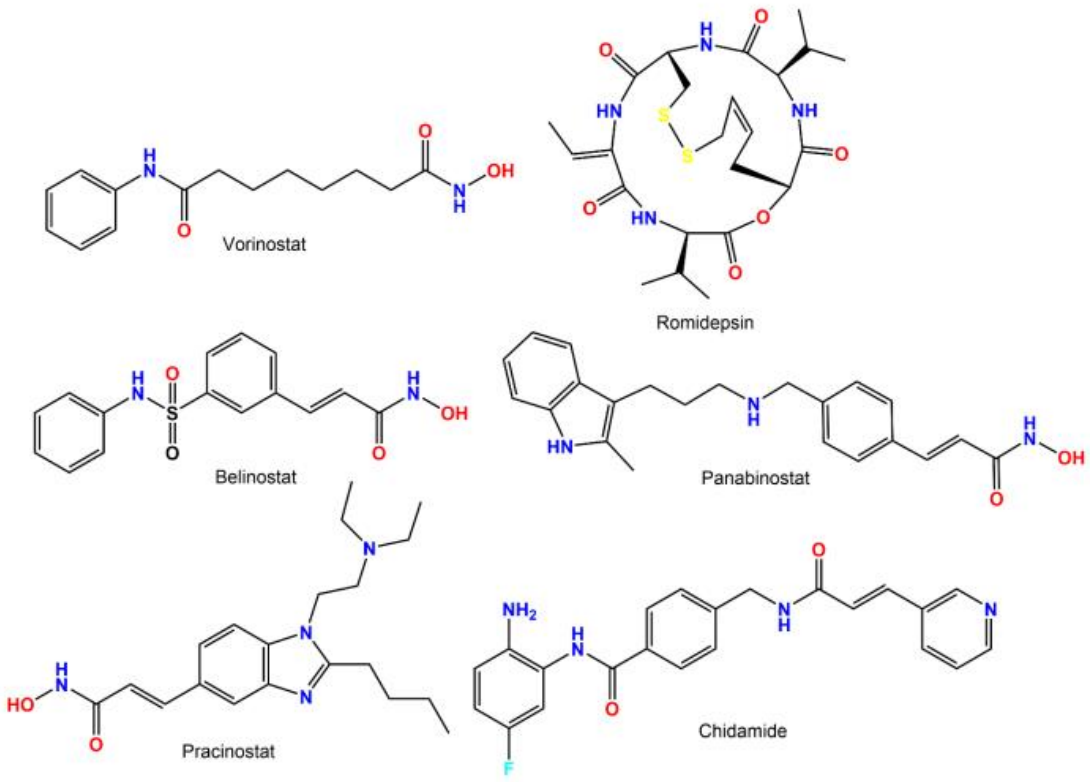

Figure 2: FDA-approved HDAC inhibitors

1.7.1. Vorinostat

Vorinostat (Suberoylanilide hydroxamic acid/SAHA) (**Figure 2**) is an orally active potent HDAC inhibitor used in the treatment of hematological malignancies such as cutaneous Tcell lymphoma and peripheral T-cell lymphoma (Brunton *et al*., 2018). Vorinostat binds to the active site of HDAC and chelates Zn 2+ in the active site; the resulting inhibition of HDACs causes the accumulation of acetylated histones and other acetylated proteins, among which are transcriptional factors crucial for cell differentiation. Vorinostat inhibits the enzymatic activities of HDAC1, 2, 3, and HDAC6 at nanomolar concentrations (IC50 < 100 nM) (Brunton *et al*., 2018). In vitro, Vorinostat induces cell cycle arrest or apoptosis of some cancer cells. Vorinostat is approved for the treatment of patients with cutaneous T-cell lymphoma with persistent or recurrent disease after two systemic therapies. The most common adverse reactions are diarrhea, fatigue, nausea, thrombocytopenia, anorexia, and dysgeusia. Patients with severe diseases should be excluded from treatment. Vorinostat is classified as pregnancy category D: evidence of risk (Brunton *et al*., 2018). The absorption

of vorinostat is slightly improved when taken with a meal. Metabolism is mostly through glucuronidation and hydrolysis. The elimination t1/2 is about 2h (Brunton *et al*., 2018). Impairment of SAHA enhances phosphorylation of LGF, upregulates the expression of PTEM and p21 as well as reduces the level of cyclin D1 and p53 in the case of the type-1 human endothelial cancer cell line. Radio sensitization by SAHA in co-operation with capecitabine inhibits tumor growth in the colorectal carcinoma xenograft model (Yoon *et al*., 2016).

1.7.2. Romidepsin

Romidepsin (**Figure 2**) was isolated from *Chromobacterium violaceum* and was reported in 1994 in the scientific literature performed by a group of researchers from Fujisawa Pharmaceutical company, Japan. It is a cyclic peptide HDAC inhibitor approved in November 2009. US FDA approved it for cutaneous T-cell lymphoma (CTCL) although in November 2011, 26% treatment of peripheral T-cell lymphoma (PTCL) was also concerned (Yoon *et al*., 2016).

1.7.3. Belinostat

Belinostat (**Figure 2**) is a sulphonamide-based hydroxamate primarily metabolized by UGT1A1 as well as CYP2A6, CYP2C9, and CYP3A4 (Brunton *et al*., 2018). The overall response rate was 26% in the treatment of relapsed or refractory PTCL. The effective concentration of belinostat was high enough because of its insufficient blood supply caused by its antiangiogenic effect (Yoon *et al*., 2016).

1.7.4. Panobinostat

Panobinostat is another pan HDAC inhibitor under the treatment of multiple myeloma, the objective response rate was 27%. Similar to vorinostat, it also shows effectiveness against hematological cancer and solid tumors in clinical trials. The side effect of panobinostat is cardiotoxicity and electrolyte abnormality-mediated exacerbation of arrhythmia. Close cardiac monitoring during treatment is recommended. The oral bioavailability of panobinostat is about 21% and is metabolized by CYP3A4. Its elimination half-life is approximately 37h (Brunton *et al*., 2018; Jenke *et al*., 2021; Yoon *et al*., 2016).

1.7.5. Pracinostat

Another hydroxamate-based orally active compound approved by FDA was pracinostat (**Figure 2**) which proved fruitful against acute myeloid leukemia (AML). Although it was not so effective towards other metalloenzymes, unlike HDACs. It accumulates into tumor cells and causes acetylation of histone. Apart from that remodeling of chromatin and transcription of various tumor suppressor genes is carried out by pracinostat (Brunton *et al*., 2018; Jenke *et al*., 2021)

1.7.6. Chidamide

Chidamide (**Figure 2**) was regarded as a benzamide-based orally active drug developed by Chipscreen Bioscience; in the year of 2015 China food and drug administration (CFDA) launched it for combating relapsed or refractory peripheral T-cell lymphoma (PTCL) as well as pancreatic cancer, it has selectivity against HDAC1, 2, 3 and apoptosis and arresting cellular growth and it is, however, treated as genuine epigenetic modulator inducing cellular growth (Brunton *et al*., 2018; Becker *et al*., 2012). A good number of preclinical as well as clinical studies support the use of HDAC inhibitors in combination with other anticancer agents they have been tested in solid tumors in combination with conventional chemotherapeutic agents, for example, taxanes, gemcitabine, fluorouracil, anthracyclines, and platinum compound and last but not least ionizing radiation (Marks *et al*., 2010). Many of such studies are conducted in patients with myelodysplastic syndrome and myeloid leukemia (Ramalingam *et al*.,2009). In several cases, proteasome and HDAC inhibitors are implemented because of not only the beneficial role of HDAC inhibitors in modifying MPR but also the interaction of proteasome and HDAC inhibitors; it is however observed that HDAC inhibitors can decline the function of HSP90 chaperone which enhances the degradation of Bcr-Abl, human epidermal growth factor receptor2/neu and FLT3; these data suggest potential synergy of HDAC inhibitors and FLT3 inhibitors in case driven by amplified or mutated tyrosine kinase (Khan *et al*., 2012). Paclitaxel and carboplatin were administered in combination with vorinostat in first-line therapy in non-small cell lung cancer which is assumed the most encouraging combination clinical study (Gore *et al*., 2006) Although there was a significantly greater incidence of platelet toxicity seen in patients treated with vorinostat (Khan *et al*., 2012). A phase II trial of estrogen receptor antagonist tamoxifen with vorinostat resulted in a 19% resistance in some patients (Munster *et al*.,2011).

Also, some responses were observed in a clinical trial of mocetinostat combined with gemcitabine in patients with solid tumors. Unfortunately, other combinations in solid tumors have no efficacy including two small clinical trials in colorectal cancer and metastatic melanoma where vorinostat is co-administered with 5-fluorouracil and karenitecin (Khan *et al*., 2012). Expression of HR23B encodes a protein that shuttles ubiquitinated cargo proteins to the proteasome and has been validated as a sensitivity determinant for HDAC inhibitor-induced apoptosis. HR23B also governs tumor cell sensitivity to drugs that act directly on the proteasome. The level of HR23B is found to influence the response of tumor cells to HDAC inhibitors-based therapy (Khan *et al*., 2010). other proteins that are known to be induced upon HDAC inhibitor treatment include p21 and HSP90-related proteins such as HSP72 and c-Raf (Stimson *et al*.,2009). The predictive utility of these biomarkers remains to be determined, and no clinical studies have been carried out so far that have utilized biomarkers to select patients to predict response to HDAC inhibitor treatment (Khan *et al*., 2012). All these established HDACis have been extensively studied and have received a fair amount of success in clinical applications. However, these are pan-HDACis i.e. they are non-specific and act on several isoforms. These HDACis require high dose and are still associated with toxicities, which is impeding their development as a clinical treatment. Their non-specificity is a factor in their conflicting effects in many cell types. Therefore, the discovery of isoform-selective natural inhibitors can be a useful weapon in the fight against cancer.

1.8. Natural Products as HDAC Inhibitors

Historically, natural products (NPs) have played an important role in drug discovery, not only for cancer and infectious diseases (Atanasov *et al*., 2021), but also in other therapeutic areas, including cardiovascular diseases, multiple sclerosis etc. (Ruijter *et al*., 2003; Kazanets *et al*.,2016; Bolden *et al*.,2006). In comparison to conventional synthetic molecules, NPs have unique properties that include both advantages and challenges in the drug discovery process. The scaffold diversity and structural complexity of NPs are enormous. Compared to synthetic compound libraries, they generally have a higher molecular mass, lower calculated octanol-water partition coefficients (Lower *cLogP*), a greater number of H-bond acceptors and donors and greater molecular rigidity (Atanasov *et al*., 2021). These distinctions can be useful in guiding alternate lead molecule generation in case of different pharmacological activities (such as HDAC1 inhibition). Recent experiments with small interfering RNA (siRNA) gave an idea that HDAC1 can be a potential target for therapeutic intervention in certain cancers (Glaser *et al*.,2003). HDAC1 is over expressed in hepatocellular cancer (Zhou *et al*.,2019;

Ler *et al*.,2015) and renal cancer cells (Fritzsche *et al*., 2008). It has been proposed that HDAC1 can bind to the promoters of the $p21^{Cip1/WAF1}$ and $p57^{KIP2}$ genes and adversely influence the cell cycle process (Yamaguchi *et al*.,2010). HDAC1 also influenced the early stages of adipocyte differentiation (Wiper-Bergeron *et al*., 2003). Moreover, HDAC1 attracted to the C/EBP- promoter for degradation and dislodgement during adipogenesis (Li *et al*.,2014). It is found to interact with AF-2 domain of ER-α and involved in breast cancer progression because of suppression of ER-α. Over expression of HDAC1 increases tumorigenicity of breast epithelial cells, which leads to breast cancer. A number of studies demonstrate that over and under expression of HDAC1 has major role in promoting of survival and proliferation of colon cell, which lead to colon cancer (Wilson *et al*.,2006). In previous years, a study demonstrated that the necessary amino acids for the binding of several inhibitors (Weerasinghe *et al*.,2008). Several non-selective HDAC inhibitors have been developed, and several of them have gone through various stages of clinical testing (Wagner *et al*.,2010).

SAHA (Suberoyl Hydroxamic Acid) has completed clinical studies and is the first HDAC inhibitor which is approved by the FDA (Richon *et al*.,1998). Because the active site pocket of Class I HDACs is substantially conserved, an isoform specific inhibitor would be critical for gene expression regulation and carcinogenesis (Karagiannis *et al*.,2007). Thus, identification of structural fingerprints among different inhibitors may help in the design of alternative lead molecule for specific HDAC1 inhibition. Here natural inhibitors can play significant role as they are supposed to provide various structural clues for lead generation. In this study different molecular modelling strategies namely Bayesian classification, recursive partioning, molecular docking and molecular dynamics simulations were applied for the identification of structural fingerprints among natural inhibitors. Structurally diverse inhibitors from various scholarly literatures (Qiu *et al*.,2021; Kim *et al*.,2014; Luparello *et al*.,2020; Farooqi *et al*.,2018; Tan *et al*.,2015; Ni *et al*.,2021; Lin *et al*.,2020; Zhao *et al*.,2020; Yang *et al*.,2019; Ling *et al*.,2018; Kim *et al*.,2014; Yao *et al*.,2015) are collected and used in the modelling analysis and the HDAC1 inhibitors have IC_{50} value in the range of 0.11 to 2,19,000 nM.

Chapter 2: Literature Review

Chapter 2: Literature Review

Previously, QSAR studies (Abdizadeh *et al*.,2017; Choubey *et al*.,2016; Zhao *et al*.,2013; Kozikowski *et al*.,2008; Praseetha *et al*.,2016; Tang *et al*.,2009; Chen *et al*.,2008) were conducted using various types of HDAC1 inhibitors, as shown in **Table 2**. However, there is no molecular modelling analysis on natural inhibitors against HDAC1. In this scenario, we want to give more emphasis on drug discovery from natural sources as they may contribute to the development of nontoxic molecule against cancer and also add value to the alternative lead molecule generation which may not be found out from synthetic sources.

2.1. Chen et al. in 2008, have built quantitative pharmacophore models from a training set of 30 hydroxamic acid compounds active as inhibitors of the HDAC1 enzyme. The best pharmacophore consists of five pharmacophore features, including a hydrogen bond donor, a hydrogen bond acceptor, three hydrophobic features, having a correlation coefficient (training set r^2) of 0.924. Besides, this hypothesis is further validated by an external test of 25 compounds (test r^2= 0.896) . The pharmacophore showed distinct chemical features that may be responsible for the activity of the inhibitors. The type and spatial location of the chemical feature agree perfectly with the pattern of enzyme inhibitor interactions identified from crystallography. Therefore, the pharmacophore completely fulfills the requirements for an effective interaction of the inhibitors with the active site of HDAC. The knowledge concerning the pharmacophore which reflects structural requirements for interaction with the target is expected to be useful in identifying and designing inhibitors with greater selectivity toward HDAC1

2.2. Kozikowski et al. in 2008 charecterized 23 biphenyls or phenylthiazoles bearing hydroxamates or mercaptoacetamides against HDACs 1, 2, 8, 10, and 6. They showed that the majority of the variance in the inhibitory activity (expressed as pIC50) of the inhibitors against HDAC1 can be explained with the three different compound classes: biphenylhydroxamates, biphenylmercaptoacetamides, and phenylthiazoles. The negative coefficient of I-NHCOCH2SH indicates that the biphenylmercaptoacetamides are 70-fold (1.844 log units) less potent than the biphenylhydroxamates, and the phenylthiazoles are 9.6-fold (0.983 log units) more potent than the biphenylhydroxamates. The best model generated with an excellent squared correlation coefficient (R^2 = 0.920) and the root mean square error (*RMSE*) is reasonable (0.322). It shows the statistical significance of this QSAR model.

2.3. Tang et al. in 2009 have employed a combinatorial QSAR approach to generate models for 59 chemically diverse compounds tested for their inhibitory activity against HDAC1. The SVM and kNN QSAR methods were used in combination with MolconnZ and MOE descriptors independently to identify the best approach with the highest external predictive power. Highly predictive QSAR models were generated with kNN/ MolconnZ and SVM/MolconnZ approaches. Rigorously validated QSAR models were then used to screen our inhouse database collection of a total of over 9.5 million compounds. This study resulted in 45 consensus hits that were predicted to be potent HDAC1 inhibitors. The validated statistical models were generated by 2D QSAR by using kNN (q^2 = 0.800, r^2 = 0.870) and by using SVM (q^2 = 0.930, r^2 = 0.870). After model generation it was employed for virtual screening. This study illustrates that validated QSAR models have the ability of identifying novel structurally diverse hits by the means of virtual screening. It is believed that the technology described in this study could be used for data analysis and hypothesis generation in many computational drug discovery studies.

2.4 Zhao et al., 2013 developed a two-step modeling approach was employed to study the selectivity and activity of histone deacetylase inhibitors. First, according to the activity difference against HDAC1 and HDAC6, a binary classification model was established to classify two kinds of inhibitors. Then two continuous models were built for each subclass to predict the activity value of HDAC1 and HDAC6 inhibitors. The three models were all built with the GA-kNN method combined with dragon descriptors. They were external validated by using external prediction set and Y-randomization test. The highly predictive models were generated for all three data sets. For the classification model, the classification accuracies of the models were as high as 100% for the external test set. For HDAC1and HDAC6 inhibitor consecutive models, external R^2 values are 0.947 and 0.911, respectively. The results proved the reliability of these models. All models were used to screen 1000 compounds included in PubMed dataset. Virtual screening resulted in 8 and 13 structurally unique consensus hits that were considered novel putative HDAC1 and HDAC6 inhibitors, respectively.

2.5 Choubey et al. in 2016 had performed a multistep framework combining ligand based 3D-QSAR, molecular docking and Molecular Dynamics (MD) simulation studies were performed to explore potential compound with good HDAC1 binding affinity. Four different pharmacophore hypotheses Hypo1 (AADR), Hypo2 (AAAH), Hypo3 (AAAR) and Hypo4 (ADDR) were obtained. The hypothesis Hypo1 (AADR) with two hydrogen bond acceptors

(A), one hydrogen bond donor (D) and one aromatics ring (R) was selected to build 3D-QSAR model on the basis of statistical parameter. The pharmacophore hypothesis produced a statistically significant QSAR model, with co-efficient of correlation r^2 = 0.82 and cross validation correlation co-efficient q^2 = 0.70. External validation result displays high predictive power with r^2 (o) value of 0.88 and r^2 (m) value of 0.58 to carry out further in silico studies. Virtual screening result shows ZINC70450932 as the most promising lead where HDAC1 interacts with residues Asp99, His178, Tyr204, Phe205 and Leu271 forming seven hydrogen bonds. A high docking score (−11.17 kcal/mol) and lower docking energy −37.84 kcal/mol) displays the binding efficiency of the ligand. Binding free energy calculation was done using MM/GBSA to access affinity of ligands towards protein. Density Functional Theory was employed to explore electronic features of the ligands describing intramolecular charge transfer reaction. Molecular dynamics simulation studies at 50 ns display metal ion (Zn)-ligand interaction which is vital to inhibit the enzymatic activity of the protein.

2.6. Praseetha et al., 2016 found that molecular descriptors derived from 3-D Morse and Radial Distribution Function indices were selective in all the models. It contributes significantly in developing statistically important models. These descriptors were very important for potent HDAC inhibition activity of Vorinostat analogues . Statistically fit, stable, and predictive linear (MLR) and non-linear (SVM) QSAR models were produced against HDAC1. In case of HDAC1 activity as end point, linear (R^2 =0.8089, R^2_{CV}=0.7343) and non-linear (R^2 =0.9801, R^2_{CV} =0.8952) QSAR models turned reliable to investigate SAR.

2.7. Abdizadeh et al., 2017 in the present study, 3D-QSAR and molecular docking were used to provide a theoretical basis for finding highly potent anti-tumor drugs. QSAR was used to generate models and predict the HDAC1 inhibitory activity using the Sybyl program (x1.2 version). Biaryl benzamides (n = 73) as selective HDAC1 inhibitors were selected as data set, which was split randomly into training (n =63) and test sets (n = 10). Docking was carried out using the MOE software. Partial least square was used as QSAR model-generation method. External validation and cross-validation (leave-one-out and leave-10-out) were used as validation methods. Both CoMFA (q^2 , 0.663; r_{ncv2} , 0.909) and CoMSIA models (q^2 , 0.628; r_{ncv2} , 0.877) for training set yielded significant statistical results. The predictive ability of the derived models was examined by a test set of 10 compounds and external validation results displayed r_{pred}^2 and r_m^2 values of 0.767 and 0.664

for CoMFA and 0.722 and 0.750 for CoMSIA, respectively. The obtained models showed a good predictive ability in both internal and external validation and could be used for designing new biaryl benzamides as potent HDAC1 inhibitors in cancer treatment. The amido and amine groups of benzamide part as scaffold and the bulk groups as a hydrophobic part were key factors to improve inhibitory activity of HDACIs.

Table 2. Summary of previous QSAR studies of HDAC1 inhibitors.

Sl. No.	Chemical class of inhibitors	QSAR methods	Statistical parameters	n_{train}[a]	n_{test}[b]	Conclusion	Ref
1	Diverse compounds	3D pharmacophore model	training set r^2= 0.924, test r^2= 0.896	30	25	Three hydrophobic features as well as a hydrogen bond acceptor feature and hydrogen bond conor feature are important in HDAC1 inhibition.	Chen *et al*.,2008
2	Substituted Biaryl Hydroxamates and Mercaptoacetamides	2D QSAR with indicator variables	r^2 = 0.920 *RMSE* = 0.322	23	-	The biphenylmercaptoacetamides are not very important than the biphenylhydroxamates in case of HDAC1 inhibition. The phenylthiazoles is also important for HDAC1 inhibition.	Kozikowski *et al*.,2008
3	Diverse compounds	2D QSAR by using kNN 2D QSAR by using SVM	q^2 = 0.800 r^2 = 0.870 q^2 = 0.930 r^2 = 0.870	45 37	5 13	The validated statistical models were generated by both the methods and employed for virtual screening	Tang *et al*., 2009
4	Diverse compound	2D QSAR based KNN continuous Models	r^2 = 0.947 *MAE* = 0.173	90	18	The predictive model was generated for HDAC1 and it is used for virtual screening.	Zhao *et al*.,2013
5	Diverse compound	ligand based Pharmacophore, 3D-QSAR	r^2 = 0.820 and q^2 = 0.700.	20	10	Two hydrogen bond acceptors, one hydrogen bond donor, and one aromatic ring feature are important for HDAC1 inhibition.	Choubey *et al*.,2016
6	Vorinostat Derivatives	2D QSAR based on MLR and SVM	MLR (r^2 =0.809, r^2_{CV} = 0.734) SVM (r^2 =0.980, r^2_{CV}=0.895)	28	-	Consistent appearance of 3-D Morse and Radial distribution functions based on molecular weight and electro-negativities in linear and non-linear QSAR models signifies their importance in HDAC1 inhibition	Praseetha *et al*.,2016
7	Biaryl benzamides	CoMFA CoMSIA	q^2 = 0.663 r^2_{ncv} = 0.909 r^2_{pred} and r^2_m 0.767 and 0.664, respectively q^2 = 0.628 r^2_{ncv} = 0.877 r^2_{pred} and r^2_m 0.722 and 0.750, respectively	63	10	The amido and amine groups of benzamide and the presence of bulky groups in the molecular structure are important factors to improve the HDAC1 inhibition.	Abdizadeh *et al*., 2017

` [a]No of molecules in the training set; [b]No of molecules in the test set; [Ref]Reference

Chapter 3: Rationale Behind the Study

Chapter 3: Rationale Behind the Study

It has been determined that one of the main causes of the development of cancer is an excess of HDACs (Histone Deacetylases). Through the deacetylation of histone protein, it is actively involved in chromatin modification. It involves in the development of numerous small vessels in hypoxic cancer cells, which increases the oxygen supply to the rapidly multiplying cancerous cells (Jenke et al., 2021).There plays very crucial in epithelial-mesenchymal transition that initiate invasion and metastasis of cancer cells.Recent studies say that many HDACs are increased in malignant cells and associated with several cancers i.e Cutaneous T-Cell Lymphoma (CTCL), Multiple Myeloma, Leukemias, Solid Tumors, Colon Cancer, Pancreatic Cancer etc. HDACs are involved in several other diseases like Cardiovascular disease, Diabetes mellitus, Kidney fibrosis, Liver fibrosis etc.

Histone deacetylase (HDAC) inhibitors are a class of drugs that have gained significant attention in the field of cancer research and other medical areas due to their potential to modify gene expression and epigenetic regulation .There are very few FDA approved drugs are available i.e. Vorinostat, Romidepsin, Panobinostat, and Belinostat, Pacinopstat, Chidamide. However, it's important to note that while HDAC inhibitors have shown promise in preclinical and early clinical studies, their efficacy and safety in different cancer types are still being studied, and they may not be a standard treatment for all cancers. They are often used in combination with other cancer therapies, and their specific role in cancer treatment may vary depending on the cancer type and individual patient factors. Majority of HDAC inhibitors are synthetic or semi synthetic because of that it is associated with a range of toxicities that must be carefully considered and managed (Subramanian et al.,2010). Ongoing research aims to develop more selective HDAC inhibitors with reduced toxicity profiles. Additionally, toxicity can be decreased if drug development is based on natural inspired.

Therefore, in this work, the Classification based quantitative structure-activity relationship (QSAR) study has been conducted on a set of 155 Nature based HDAC1 inhibitors. Bayesian classification study and Recursive partitioning study has been done to find important fingerprints for potent HDAC1 inhibitory activity. Previously, a research study revealed the essential amino acids for the binding of HDAC1 inhibitors **(Figure 3)** (Weerasinghe et al.,2008). This research is beneficial for the study of molecular docking of the selected compounds. Following these stages, some of the lead compounds are selected for molecular

dynamics. With the help of Molecular dynamics , stability of the compounds in the receptor is determined.

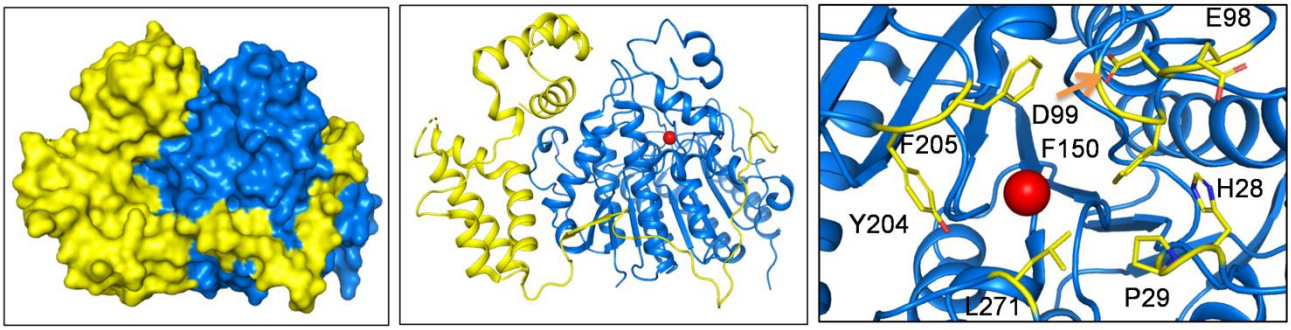

Figure 3. The structure of HDAC1:MTA1 complex (PDB ID: 4BKX). The blue and yellow colour represent MTA1 domain and HDAC1, respectively.

Chapter 4: Materials and Methods

Chapter 4: Materials and Methods

4.1. Dataset

A series of diverse natural HDAC1 inhibitors with various chemical scaffolds and their IC_{50} values were collected from the survey of different literature reports literatures (Qiu *et al*.,2021; Kim *et al*.,2014; Luparello *et al*.,2020; Farooqi *et al*.,2018; Tan *et al*.,2015; Ni *et al*.,2021; Lin *et al*.,2020; Zhao *et al*.,2020; Yang *et al*.,2019; Ling *et al*.,2018; Kim *et al*.,2014; Yao *et al*.,2015). A list of different type of compounds with specific scaffolds taken for the QSAR study is given in **Table 3**. 2D structure of the compounds was drawn by using ChemDraw ultra version 5.0 (Cambridge Soft Corporation, USA, 2010). Representative potent molecules from various classes of natural HDAC1 inhibitors are given in **Figure 4.**

Table 3. Different types of natural HDAC inhibitors taken for QSAR study

Sl. No	Compound Name	No. of compounds	References
1	Depudecin	01	Kim *et al*.,2014
2	Azumamide isoforms	05	Kim *et al*.,2014
3	Thailandepsin B	01	Kim *et al*.,2014
4	Burkholdac B	01	Kim *et al*.,2014
5	Saprorthoquinone	07	Lin *et al*.,2020
6	Beta- Carboline Derivatives	11	Ling *et al* 2018
7	Gymnochrome E	01	Luparello *et al*.,2020
8	Nigranoic acid and Manwuweizic acid derivatives	04	Ni *et al*.,2021
9	Trichostatin	01	Qiu *et al*.,2021
10	Psammaplin	01	Qiu *et al*.,2021
11	Aurones	04	Qiu *et al*.,2021
12	Colchicine Derivatives	11	Qiu *et al*.,2021
13	Chalcone Derivatives	03	Qiu *et al*.,2021
14	Camptothecin Analogues	05	Qiu *et al*.,2021
15	Etoposide and SAHA Hybrids	22	Qiu *et al*.,2021
16	Flavone	01	Qiu *et al*.,2021
17	Largazole analogues	15	Qiu *et al*.,2021; Kim *et al*.,2014
18	Romidepsin and its analogues	10	Qiu *et al*.,2021; Yao *et al*.,2015
19	Cumarin derivatives	41	Qiu *et al*.,2021; Zhao *et al* 2020
20	Santacruzamate A	01	Tan *et al*.,2015
21	Apicidin	01	Tan *et al*.,2015
22	Spiruchostatin isoform	03	Tan *et al*.,2015
23	Chlamydocin	01	Tan *et al*.,2015
24	Trapoxin isoforms	02	Tan *et al*.,2015
25	Bispyridinium diene derivatives	02	Tan *et al*.,2015

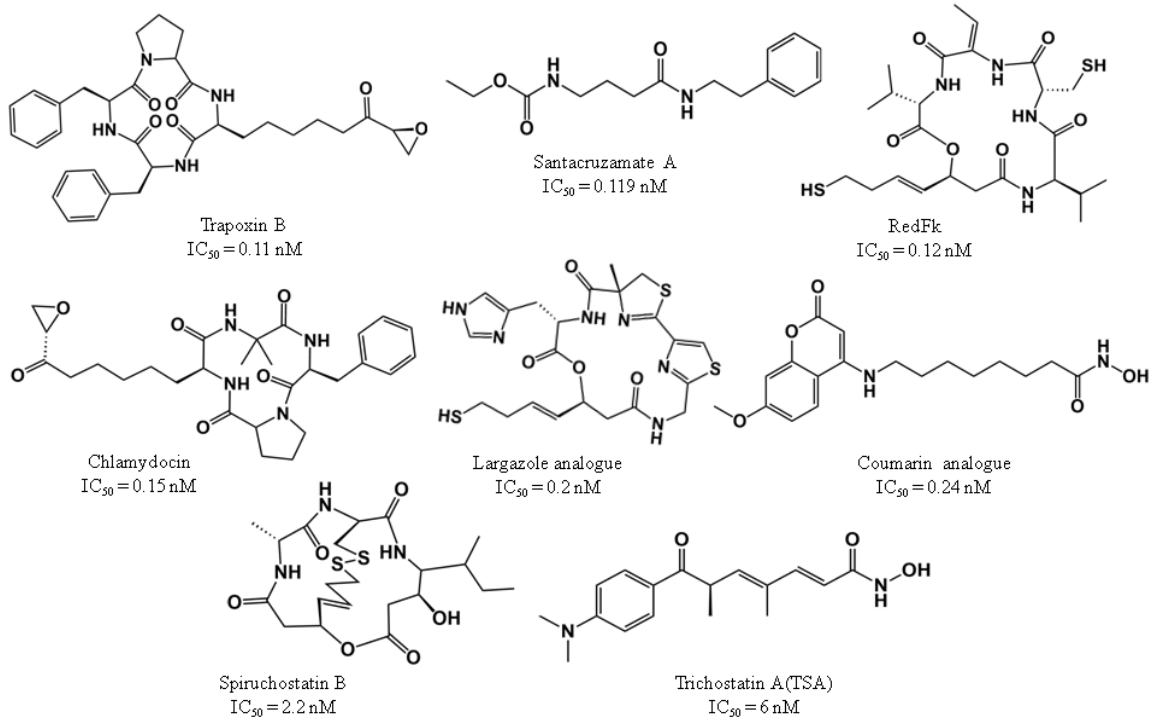

Figure 4. Structure of potent molecules present in the dataset.

The compounds with improper activity scales were discarded from the study. Duplicate molecules were also removed from the list. Ultimately, 155 HDAC1 inhibitors have been taken into consideration for the modelling study. The dataset was divided into the training (**Table 4**) set and test set (**Table 5**) to develop the model and to validate the developed model, respectively. The HDAC inhibitory activities (IC_{50}) were converted to the negative logarithm values (pIC_{50}). Binary method is applied for the development of classification model (Amin *et al*.,2016). According to biological activity these compounds with pIC_{50} value >7.26 nM was assigned as '1' (Higher active) and compounds with the IC_{50} value ≤ 7.26 nM assigned as 0 (Lower active).

Table 4. Training set compounds in SMILES format

Comp. name	SMILES format	pIC 50 value (nM)	Binary
1	N1[C@H](C(=O)O[C@H]2CC(=O)N[C@@H](C(=O)N[C@H](C(=O)N/C(=C\C)/C1=O)CSSCC/C=C/2)C(C)C)C(C)C	7.4437	1
2	c1c(ccc(c1)C(=O)[C@@H](/C=C(/C=C/C(=O)NO)\C)C)N(C)C	8.2219	1
3	C(=O)(/C(=N/[O-])/Cc1cc(c(cc1)O)Br)NCCSSCCNC(=O)/C(=N/[O-])/Cc1ccc(c(c1)Br)O	7.3468	1
4	c1ccc2c(c1)C(=O)/C(=C/c1ccc(cc1)O)/O2	3.8887	0
6	c1ccc2c(c1)C(=O)/C(=C/c1cccc(c1O)O)/O2	4.6778	0
7	c1c(cc2c(c1O)C(=O)/C(=C/c1ccc(c(c1)O)O)/O2)[O-]	4.4202	0
8	S1SCC/C=C/[C@H]2OC(=O)CNC(=O)[C@H](NC(=O)[C@H](NC(=O)[C@H](NC(=O)CC2)C(C)C)C1)C(C)C	8.6778	1
9	S1SCC/C=C/[C@H]2OC(=O)CNC(=O)[C@H](NC(=O)[C@H](NC(=O)[C@H](NC(=O)CC2)C(C)C)C1)C	9.2676	1
10	S1SCC/C=C/[C@H]2OC(=O)CNC(=O)[C@H](NC(=O)[C@H](NC(=O)[C@H](NC(=O)CC2)C(C)C)C1)Cc1ccccc1	9.0177	1
13	N1C(=O)/C(=C\C)/NC(=O)c2[nH+]c(sc2)CNC(=O)C[C@H](OC(=O)[C@@H]1C(C)C)/C=C/CCS	8.5560	1
14	C(C(=O)SCC/C=C/[C@H]1OC(=O)[C@@H](NC(=O)/C(=C\C)/NC(=O)c2[nH+]c(sc2)CNC(=O)C1)C(C)C)CCCCCC	6.80134	0
15	N1C(=O)/C(=C\C)/NC(=O)c2[nH+]c(sc2)CNC(=O)C[C@H](OC(=O)[C@@H]1C(C)C)/C=C/CCSC(=O)C	7.6656	1
16	N1C(=O)/C(=C\C)/NC(=O)[C@@H](NC(=O)[C@H](NC(=O)C[C@H](OC(=O)[C@@H]1C(C)C)/C=C/CCS)C(C)C)CS	9.9208	1
18	N1[C@H](C(=O)O[C@@H](CC(=O)NCc2[nH+]c(C3=N[C@@](CS3)(C1=O)C)cs2)/C=C/CCSSCC/C=C/[C@H]1OC(=O)[C@@H](NC(=O)[C@]2(N=C(c3[nH+]c(sc3)CNC(=O)C1)SC2)C)C(C)C)C(C)C	9.3979	1
19	C(=O)([C@H](CSSCC/C=C/[C@H]1OC(=O)[C@@H](NC(=O)[C@]2(N=C(c3[nH+]c(sc3)CNC(=O)C1)SC2)C)C(C)C)NC(=O)OC(C)(C)C)OC(C)(C)C	9.5229	1
20	N1[C@H](C(=O)O[C@@H](CC(=O)NCc2[nH+]c(C3=N[C@@](CS3)(C1=O)C)cs2)/C=C/CCSSC[C@H](NC(=O)OC(C)(C)C)C(=O)[O-])C(C)C	9.3979	1
21	N1[C@H](C(=O)O[C@@H](CC(=O)NCc2[nH+]c(C3=N[C@](CS3)(C1=O)C)cs2)/C=C/CCSC(=O)CCCCCCC)C(C)C	6.8357	0
22	C1C(=O)Oc2c(C1)ccc(c2CCC(C)C)OCCOc1cc(ccc1)/C=C/C(=O)NO	7.1750	0
23	C1C(=O)Oc2c(C1)ccc(c2CCC(C)C)OCCCOc1cc(ccc1)/C=C/C(=O)NO	7.1891	0
24	C1C(=O)Oc2c(C1)ccc(c2CCC(C)C)OCCOc1ccc(cc1)/C=C/C(=O)NO	7.0798	0
36	C(c1ccc(cc1)c1cc(=O)c2c(o1)cc(cc2[O-])OCCC(=O)NO)C[NH+](C)C	4.4443	0
37	c1(ccc2c(cc1=O)[C@@H](CCc1c2c(c(c(c1)OC)OC)OC)NC(=O)CCC(=O)NO)OC	5.8762	0

38	c1(ccc2c(cc1=O)[C@H](CCc1c2c(c(c(c1)OC)OC)OC)NC(=O)CCCC(=O)NO)OC	5.0187	0
39	c1(ccc2c(cc1=O)[C@H](CCc1c2c(c(c(c1)OC)OC)OC)NC(=O)CCCCC(=O)NO)OC	6.1427	0
40	c1(ccc2c(cc1=O)[C@H](CCc1c2c(c(c(c1)OC)OC)OC)NC(=O)CCCCCC(=O)NO)OC	5.7959	0
41	C(=O)(N[C@@H]1c2c(ccc(c(=O)c2)OC)c2c(CC1)cc(c(c2OC)OC)OC)c1ccc(cc1)C(=O)Cc1ccccc1N	4.7980	0
42	C(=O)(N[C@@H]1c2c(ccc(c(=O)c2)OC)c2c(CC1)cc(c(c2OC)OC)OC)c1ccc(cc1)C(=O)Cc1cc(ccc1N)c1cccs1	5.6498	0
43	C(=O)(N[C@@H]1c2c(ccc(c(=O)c2)OC)c2c(CC1)cc(c(c2OC)OC)OC)c1cc(ccc1)C(=O)Cc1ccccc1N	5.0168	0
45	c1(ccc2c(cc1=O)[C@@H](CCc1c2c(c(c(c1)OC)OC)OC)NCc1ccc(cc1)C(=O)Cc1ccccc1N)OC	4.9031	0
46	c1(ccc2c(cc1=O)[C@@H](CCc1c2c(c(c(c1)OC)OC)OC)NCc1ccc(cc1)C(=O)Cc1cc(ccc1N)c1cccs1)OC	5.4789	0
48	c1(c(c(cc(c1)C(=O)/C=C/c1ccc(c(c1)OCCCCCCCC(=O)NO)OC)OC)OC)OC	7.4401	1
52	O1C(=O)[C@@](c2c(C1)c(=O)n1c(c2)c2c(C1)c(c1c([nH+]2)ccc(c1)OCc1cn(CCCCCCC(=O)NO)nn1)CC)(CC)O	7.3010	1
53	C(c1c2c(c3cc4[C@](C(=O)OCc4c(=O)n3C2)(CC)O)[nH+]c2c1cc(cc2)OCc1cn(CCCCCCCC(=O)NO)nn1)C	6.4330	0
54	O1C(=O)[C@@](c2c(C1)c(=O)n1c(c2)c2c(C1)cc1c([nH+]2)ccc(c1)OCc1cn(CCCC(=O)NO)nn1)(CC)O	6.9355	0
55	O1C(=O)[C@@](c2c(C1)c(=O)n1c(c2)c2c(C1)cc1c([nH+]2)ccc(c1)OCc1cn(CCCCCC(=O)NO)nn1)(CC)O	7.4318	1
56	c12c(cc3c(c1)[C@H]([C@H]1[C@H]([C@@H]3c3cc(c(c(c3)OC)O)OC)C(=O)OC1)Nc1ccc(cc1)NC(=O)CCCCC(=O)NO)OCO2	5.5482	0
57	c12c(cc3c(c1)[C@H]([C@@H]1[C@@H]([C@@H]3c3cc(c(c(c3)OC)O)OC)C(=O)OC1)Nc1ccc(cc1)NC(=O)CCCCCC(=O)NO)OCO2	6.2292	0
59	c12c(cc3c(c1)[C@H]([C@@H]1[C@@H]([C@@H]3c3cc(c(c(c3)OC)O)OC)C(=O)OC1)Nc1cccc(c1)NC(=O)CCCCC(=O)NO)OCO2	5.8447	0
60	c12c(cc3c(c1)[C@H]([C@@H]1[C@@H]([C@@H]3c3cc(c(c(c3)OC)O)OC)C(=O)OC1)Nc1cccc(c1)NC(=O)CCCCCC(=O)NO)OCO2	6.4202	0
61	c12c(cc3c(c1)[C@H]([C@@H]1[C@@H]([C@@H]3c3cc(c(c(c3)OC)O)OC)C(=O)OC1)Nc1cccc(c1)NC(=O)CCCCCCC(=O)NO)OCO2	7	0
62	c12c(cc3c(c1)[C@H]([C@@H]1[C@@H]([C@@H]3c3cc(c(c(c3)OC)O)OC)C(=O)OC1)Nc1ccc(cc1)OC(=O)CCCCCCC(=O)NO)OCO2	6.6144	0
63	c12c(cc3c(c1)[C@H]([C@@H]1[C@@H]([C@@H]3c3cc(c(c(c3)OC)O)OC)C(=O)OC1)Nc1ccc(cc1)OC(=O)CCCCCCCC(=O)NO)OCO2	6.9031	0
64	c12c(cc3c(c1)[C@H]([C@@H]1[C@@H]([C@@H]3c3cc(c(c(c3)OC)O)OC)C(=O)OC1)Nc1ccc(cc1)OC(=O)CCCCCCCCC(=O)NO)OCO2	6.6840	0
65	c12c(cc3c(c1)[C@H]([C@@H]1[C@@H]([C@@H]3c3cc(c(c(c3)OC)O)OC)C(=O)OC1)Nc1ccc(cc1)OC(=O)CCCCCCCCCC(=O)NO)OCO2	7.5376	1
66	c12c(cc3c(c1)[C@H]([C@@H]1[C@@H]([C@@H]3c3cc(c(c(c3)OC)O)OC)C(=O)OC1)Nc1ccc(cc1)OC(=O)CCCCCCCCCCC(=O)NO)OCO2	7.0605	0
67	c12c(cc3c(c1)[C@H]([C@@H]1[C@@H]([C@@H]3c3cc(c(c(c3)OC)O)OC)C(=O)OC1)Nc1cc(ccc1)OC(=O)CCCCCCC(=O)NO)OCO2	6.4145	0
68	c12c(cc3c(c1)[C@H]([C@@H]1[C@@H]([C@@H]3c3cc(c(c(c3)OC)O)OC)C(=O)OC1)Nc1cc(ccc1)OC(=O)CCCCCCCC(=O)NO)OCO2	7.0315	0

70	c12c(cc3c(c1)[C@H]([C@@H]1[C@@H]([C@@H]3c3cc(c(c(c3)OC)O)OC)C(=O)OC1)Nc1cc(ccc1)OC(=O)CCCCCCCCCC(=O)NO)OCO2	7.9586	1
72	c12c(cc3c(c1)[C@H]([C@H]1[C@@H]([C@@H]3c3cc(c(c(c3)OC)O)OC)C(=O)OC1)Nc1c(cccc1)OC(=O)CCCCCCC(=O)NO)OCO2	6.2381	0
74	c12c(cc3c(c1)[C@H]([C@@H]1[C@@H]([C@@H]3c3cc(c(c(c3)OC)O)OC)C(=O)OC1)Nc1c(cccc1)OC(=O)CCCCCCCCC(=O)NO)OCO2	6.8386	0
76	c12c(cc3c(c1)[C@H]([C@@H]1[C@@H]([C@@H]3c3cc(c(c(c3)OC)O)OC)C(=O)OC1)Nc1c(cccc1)OC(=O)CCCCCCCCCCC(=O)NO)OCO2	6.7570	0
77	c12c(cc3c(c1)[C@H]([C@@H]1[C@@H]([C@@H]3c3cc(c(c(c3)OC)O)OC)C(=O)OC1)Nc1ccc(cc1)OC(=O)CCCCCCC(=O)Nc1c(N)cccc1)OCO2	7.8514	1
80	C1(=O)[C@H](NC(=O)[C@H]([C@H](NC(=O)[C@H](NC(=O)[C@H](N1)C)Cc1ccc(cc1)O)C/C=C\CCC(=O)N)C)C(C)C	5.3010	0
82	C1(=O)[C@H](NC(=O)[C@H]([C@H](NC(=O)[C@H](NC(=O)[C@H](N1)C)Cc1cccc cc1)C/C=C\CCC(=O)N)C)C	<5.3010	0
83	C1(=O)[C@H](NC(=O)[C@H]([C@H](NC(=O)[C@H](NC(=O)[C@H](N1)C)Cc1cccc cc1)C/C=C\CCC(=O)[O-])C)C(C)C	7.1739	0
84	[C@@H]12C(=O)N[C@@H]([C@H](CC(=O)O[C@H](CC(=O)N[C@@H](C(=O)N1)CCSC)/C=C/CCSSC2)O)[C@@H](C)CC	5.1249	0
85	N1C(=O)[C@H](NC(=O)C[C@@H]2/C=C/CCSSC[C@H]1C(=O)N[C@H]([C@H](CC(=O)O2)O)[C@H](C)CC)CCCC	5.1135	0
86	c12c3C(=O)c4c(c(c(c5c6c(c(c(c7C(=O)c8c(c(c67)c1c45)c(c(cc8[O-])O)c2c(cc3[O-])O)O)Br)C[C@H](C)O)C[C@H](CCC)O)Br)O	4.9626	0
87	CCOC(=O)NCCCC(=O)NCCc1ccccc1	9.9244	1
88	C1(=O)N[C@H](C(=O)[C@@H]2[C@@H](C(=O)N[C@@H](C(=O)N[C@H]1C[C@H]1c3c([NH+](C1)OC)cccc3)CCCCCC(=O)CC)CCCC2)[C@H](CC)C	1	1
90	C1(=O)[C@H](NC(=O)C[C@H]2/C=C/CCSSC[C@@H](N1)C(=O)N[C@@H]([C@H](CC(=O)O2)O)[C@@H](C)CC)C	8.6576	1
91	C1(=O)[C@H](NC(=O)C[C@@H]2/C=C/CCSSC[C@H](N1)C(=O)N[C@H]([C@H](CC(=O)O2)O)CC(C)C)C	1	1
94	N1[C@H](C(=O)N2[C@@H](C(=O)N[C@H](C(=O)N[C@H](C1=O)Cc1ccccc1)CCCCCC(=O)[C@@H]1CO1)CCC2)Cc1ccccc1	9.9586	1
96	c1c[n+](cc(c1)CCCCCCCCCCCCC[n+]1cc(ccc1)CCCC=C)CCCCCCC=C	5.7258	0
98	C(C[C@]12[C@@]3(CC[C@]4([C@]([C@@H]3CC[C@@H]1C(=C)C)(CC[C@@H]4[C@@H](CC/C=C(/C)\C(=O)[O-])C)C)C)C2)C(=O)[O-]	5.4023	0
100	C(=O)(CC[C@]12[C@@]3(CC[C@]4([C@]([C@@H]3CC[C@@H]1C(=C)C)(CC[C@@H]4[C@@H](CC/C=C(/C)\C(=O)[O-])C)C)C)C2)NO	5.9431	0
102	C1(=Cc2c(C(=O)C1=O)c(c(cc2)C)CCC(=O)Nc1c(cccc1)N)C(C)C	<7	0
103	C1(=Cc2c(C(=O)C1=O)c(c(cc2)C)CCC(=O)Nc1c(ccc(c1)F)N)C(C)C	<7	0
105	C1(=Cc2c(C(=O)C1=O)c(c(cc2)C)CCC(=O)NCCC(=O)Nc1c(cccc1)N)C(C)C	<7	0
106	C1(=Cc2c(C(=O)C1=O)c(c(cc2)C)CCC(=O)NCCCC(=O)Nc1c(cccc1)N)C(C)C	4.4575	0
108	C1(=Cc2c(C(=O)C1=O)c(c(cc2)C)CCC(=O)NCCCCCC(=O)Nc1c(cccc1)N)C(C)C	6.3372	0
110	c1c(cc2c(c1)c(cc(=O)o2)OCCCCCCCCC(=O)NO)OCCCC	9.5086	1
111	c1c(cc2c(c1)c(cc(=O)o2)OCCCCCCCCC(=O)NO)OCCOC	9.5228	1

113	c1c(cc2c(c1)c(cc(=O)o2)OCCCCCCCCC(=O)NO)OCCOCc1ccccc1	9.1135	1
114	c1c(cc2c(c1)c(cc(=O)o2)OCCCCCCCCC(=O)NO)OCC(C)C	9.2219	1
116	c1c(cc2c(c1)c(cc(=O)o2)OCCCCCCCCC(=O)NO)OCc1ccccc1	9.0177	1
117	c1c(cc2c(c1)c(cc(=O)o2)OCCCCCCCCC(=O)NO)OCc1ccccc1[N+](=O)[O-]	8.7986	1
118	c1c(cc2c(c1)c(cc(=O)o2)OCCCCCCCCC(=O)NO)OCc1cccc(c1)[N+](=O)[O-]	8.7055	1
120	c1c(cc2c(c1)c(cc(=O)o2)OCCCCCCCCC(=O)NO)OCc1ccccc1F	9.1249	1
121	c1c(cc2c(c1)c(cc(=O)o2)OCCCCCCCCC(=O)NO)OCc1cccc(c1)F	9.0043	1
122	c1c(cc2c(c1)c(cc(=O)o2)OCCCCCCCCC(=O)NO)OCc1ccc(cc1)F	9.0555	1
123	c1c(cc2c(c1)c(cc(=O)o2)OCCCCCCCCC(=O)NO)OCc1ccccc1Cl	8.6198	1
124	c1c(cc2c(c1)c(cc(=O)o2)OCCCCCCCCC(=O)NO)OCc1cccc(c1)Cl	8.3716	1
125	c1c(cc2c(c1)c(cc(=O)o2)OCCCCCCCCC(=O)NO)OCc1ccc(cc1)Cl	8.5986	1
126	c1c(cc2c(c1)c(cc(=O)o2)OCCCCCCCCC(=O)NO)OCc1ccc(cc1)Br	8.5199	1
127	c1c(cc2c(c1)c(cc(=O)o2)OCCCCCCCCC(=O)NO)OCc1ccc(cc1)C(F)(F)F	8.2596	1
128	c1c(cc2c(c1)c(cc(=O)o2)OCCCCCCCCC(=O)NO)OCc1c(cccc1F)F	9.3010	1
129	c1c(cc2c(c1)c(cc(=O)o2)OCCCCCCCCC(=O)NO)OCc1ccc(cc1F)F	9.1079	1
130	c1c(cc2c(c1)c(cc(=O)o2)OCCCCCCCCC(=O)NO)OCc1ccc(cc1Cl)F	8.4921	1
131	c1c(cc2c(c1)c(cc(=O)o2)OCCCCCCCCC(=O)NO)OCc1ccccc1C	8.8068	1
132	c1c(cc2c(c1)c(cc(=O)o2)OCCCCCCCCC(=O)NO)OCc1cccc(c1)C	8.5301	1
134	c1c(cc2c(c1)c(cc(=O)o2)OCCCCCCCCC(=O)NO)OCc1c(cccc1C)C	8.2848	1
135	c1ccc2c(c1)/c(=N/CCC(=O)NO)/cc(o2)O	<7	0
137	c1c(cc2c(c1)c(cc(=O)o2)NCCCCCC(=O)NO)OC	8.1918	1
138	c1c(cc2c(c1)c(cc(=O)o2)NCCCCCCC(=O)NO)OC	8.6003	1
139	c1c(cc2c(c1)c(cc(=O)o2)NCCCCCCCC(=O)NO)OC	9.6198	1
140	c1c(cc2c(c1)c(cc(=O)o2)OCCCCC(=O)NO)OC	<7	0
141	c1c(cc2c(c1)c(cc(=O)o2)OCCCCCC(=O)NO)OC	7.7120	1
143	c1c(cc2c(c1)c(cc(=O)o2)OCCCCCCCC(=O)NO)OC	8.7328	1
144	c1c(cc2c(c1)c(cc(=O)o2)OCCCCCCCCC(=O)NO)OC	8.1349	1
145	c1c(cc2c(c1)ccc(=O)o2)OCCCCCCCC(=O)NO	8.0595	1
146	c1c(cc2c(c1)c(cc(=O)o2)C)OCCCCCCCC(=O)NO	8.1624	1

147	c1cc2c(cc1)c1c([NH2+]2)cnc(c1)NCc1ccc(cc1)C(=O)NO	7.1487	0
148	c1cc2c(cc1)c1c([NH2+]2)c(nc(c1)NCc1ccc(cc1)C(=O)NO)C	7.3188	1
149	c1cc2c(cc1)c1c([NH2+]2)c(nc(c1)NCc1ccc(cc1)C(=O)NO)CC	6.7520	0
150	c1cc2c(cc1)c1c([NH2+]2)c(nc(c1)NCc1ccc(cc1)C(=O)NO)c1ccccc1	6.6596	0
151	c1cc2c(cc1)c1c([NH2+]2)c(nc(c1)NCc1ccc(cc1)C(=O)NO)c1ccc(cc1)OC	8.0315	1
155	c1cc2c(cc1)c1c([NH2+]2)c(nc(c1)NCc1ccc(cc1)C(=O)NO)c1cccc(c1)[N+](=O)[O-]	7.3872	1
156	c1cc2c(cc1)c1c([NH2+]2)c(nc(c1)NCc1ccc(cc1)C(=O)NO)c1ccc(c(c1)OC)OC	8.0862	1
157	c1cc2c(cc1)c1c([NH2+]2)c(nc(c1)NCc1ccc(cc1)C(=O)NO)c1cc(c(c(c1)OC)OC)OC	8.3279	1
158	[C@@H]1(C(=O)O[C@@H](CC(=O)NCc2scc(C3=N[C@](C(=O)N1)(CS3)C)[nH+]2)/C=C/CCS)C(C)C	9.3979	1
160	[C@@H]1(C(=O)O[C@@H](CC(=O)NCc2scc(C3=N[C@](C(=O)N1)(CS3)C)[nH+]2)/C=C/CCS)Cc1ccc(cc1)O	9.6777	1
161	[C@@H]1(C(=O)O[C@@H](CC(=O)NCc2scc(C3=N[C@](C(=O)N1)(CS3)C)[nH+]2)/C=C/CCS)Cc1c[nH]cn1	9.6990	1
162	[C@@H]1(C(=O)O[C@@H](CC(=O)NCc2scc(C3=N[C@](C(=O)N1)(CS3)C)[nH+]2)/C=C/CCS)CC(=O)[O-]	7.4089	1
163	[C@@H]1(C(=O)O[C@@H](CC(=O)NCc2scc(C3=N[C@](C(=O)N1)(CS3)C)[nH+]2)/C=C/CCS)CC[NH3+]	8.2596	1
164	[C@@H]1(C(=O)O[C@@H](CC(=O)NCc2scc(C3=N[C@](C(=O)N1)(CS3)C)[nH+]2)/C=C/CCS)CCC[NH3+]	8.4814	1
165	[C@@H]1(C(=O)O[C@@H](CC(=O)NCc2scc(C3=N[C@](C(=O)N1)(CS3)C)[nH+]2)/C=C/CCS)CCNC(=O)OC(C)(C)C	8.6382	1

Table 5. Test set compounds in SMILES format

Comp. name	SMILES format	pIC 50 value (nM)	Binary
5	c1ccc2c(c1)C(=O)/C(=C/c1ccc(c(c1)O)O)/O2	4.9431	0
11	N1C(=O)/C(=C\C)/NC(=O)c2[nH+]c(sc2)[C@H](NC(=O)C[C@H](OC(=O)[C@@H]1C(C)C)/C=C/CCS)C(C)C	8.6615	1
12	C(C(=O)SCC/C=C/[C@H]1OC(=O)[C@@H](NC(=O)/C(=C\C)/NC(=O)c2[nH+]c(sc2)[C@H](NC(=O)C1)C(C)C)C(C)C)CCCCCC	6.7825	0
17	N1[C@H](C(=O)O[C@@H](CC(=O)NCc2[nH+]c(c3nn(CC1=O)nn3)cs2)/C=C/CCS)C(C)C	7	0
44	C(=O)(N[C@@H]1c2c(ccc(c(=O)c2)OC)c2c(CC1)cc(c(c2OC)OC)OC)c1cc(ccc1)C(=O)Cc1cc(ccc1N)c1cccs1	5.8239	0
47	c1(ccc2c(cc1=O)[C@@H](CCc1c2c(c(c(c1)OC)OC)OC)NCc1cc(ccc1)C(=O)Cc1cc(ccc1N)c1cccs1)OC	4.5935	0
49	c1(c(c(cc(c1)C(=O)/C(=C/c1ccc(c(c1)OCCCC(=O)NO)OC)/C)OC)OC)OC	6.0017	0
50	c1(c(c(cc(c1)C(=O)/C(=C/c1ccc(c(c1)OCCCCCCCCC(=O)NO)OC)/C)OC)OC)OC	7.8182	1
51	O1C(=O)[C@@](c2c(C1)c(=O)n1c(c2)c2c(C1)c(c1c([nH+]2)ccc(c1)OCc1cn(CCCCCC(=O)NO)nn1)CC)(CC)O	6.8894	0
58	c12c(cc3c(c1)[C@H]([C@@H]1[C@H]([C@@H]3c3cc(c(c(c3)OC)O)OC)C(=O)OC1)Nc1ccc(cc1)NC(=O)CCCCCCC(=O)NO)OCO2	6.6778	0
69	c12c(cc3c(c1)[C@H]([C@@H]1[C@@H]([C@@H]3c3cc(c(c(c3)OC)O)OC)C(=O)OC1)Nc1cc(ccc1)OC(=O)CCCCCCCCCC(=O)NO)OCO2	7.3468	1
71	c12c(cc3c(c1)[C@H]([C@@H]1[C@@H]([C@@H]3c3cc(c(c(c3)OC)O)OC)C(=O)OC1)Nc1cc(ccc1)OC(=O)CCCCCCCCCCC(=O)NO)OCO2	7.4089	1
73	c12c(cc3c(c1)[C@H]([C@@H]1[C@@H]([C@H]3c3cc(c(c(c3)OC)O)OC)C(=O)OC1)Nc1c(cccc1)OC(=O)CCCCCCCC(=O)NO)OCO2	6.4330	0
75	c12c(cc3c(c1)[C@H]([C@@H]1[C@@H]([C@@H]3c3cc(c(c(c3)OC)O)OC)C(=O)OC1)Nc1c(cccc1)OC(=O)CCCCCCCCCC(=O)NO)OCO2	7.1426	0
78	C=C[C@H]([C@H]1[C@@H](/C=C/[C@H]2[C@@H]([C@@H](C)O)O2)O1)O	5.3279	0
79	C1(=O)[C@H](NC(=O)[C@H]([C@H](NC(=O)[C@H](NC(=O)[C@H](N1)C)Cc1ccccc1)C/C=C\CCC(=O)N)C)C(C)C	<5.3010	0
81	C1(=O)[C@H](NC(=O)[C@H]([C@H](NC(=O)[C@H](NC(=O)[C@H](N1)C)Cc1ccc(cc1)O)C/C=C\CCC(=O)[O-])C)C(C)C	7.4948	1
89	C1(=O)[C@H](NC(=O)C[C@H]2/C=C/CCSSC[C@@H](N1)C(=O)N[C@@H]([C@H](CC(=O)O2)O)C(C)C)C	8.4814	1
92	N12C(=O)[C@@H](NC(=O)C(NC(=O)[C@@H](NC(=O)[C@H]1CCC2)CCCCCC(=O)[C@H]1OC1)(C)C)Cc1ccccc1	9.8239	1
93	N1[C@H](C(=O)N2[C@@H](C(=O)N[C@H](C(=O)N[C@H](C1=O)Cc1ccccc1)CCCCCC(=O)[C@@H]1CO1)CCCC2)Cc1ccccc1	9.0861	1
97	c1c[n+](cc(c1)CCCCCCCCCCCCC[n+]1cc(ccc1)CCCCC=C)CCCCCCC=C	6.2757	0
99	C(C[C@@]1(C2=C([C@]3([C@](CC2)([C@H](CC3)[C@@H](CC/C=C(/C)\C(=O)[O-])C)C)C)CC[C@@H]1C(=C)C)C)C(=O)[O-]	5.0640	0
101	C(=O)(CC[C@@]1(C2=C([C@]3([C@](CC2)([C@H](CC3)[C@@H](CC/C=C(/C)\C(=O)[O-])C)C)C)CC[C@H]1C(=C)C)C)NO	5.5784	0
104	C1(=Cc2c(C(=O)C1=O)c(c(cc2)C)CCC(=O)Nc1c(cc(cc1)F)N)C(C)C	<7	0
107	C1(=Cc2c(C(=O)C1=O)c(c(cc2)C)CCC(=O)NCCCCC(=O)Nc1c(cccc1)N)C(C)C	4.4036	0
109	c1c(cc2c(c1)c(cc(=O)o2)OCCCCCCCC(=O)NO)OCCC	9.3188	1
112	c1c(cc2c(c1)c(cc(=O)o2)OCCCCCCCC(=O)NO)OCCCOC	9.4815	1
115	c1c(cc2c(c1)c(cc(=O)o2)OCCCCCCCC(=O)NO)OCC1CCCCC1	8.3002	1
119	c1c(cc2c(c1)c(cc(=O)o2)OCCCCCCCC(=O)NO)OCc1ccc(cc1)[N+](=O)[O-]	8.7645	1
133	c1c(cc2c(c1)c(cc(=O)o2)OCCCCCCCC(=O)NO)OCc1ccc(cc1)C	8.6021	1
136	c1ccc2c(c1)/c(=N/CCCCCC(=O)NO)/cc(o2)O	8.0405	1
142	c1c(cc2c(c1)c(cc(=O)o2)OCCCCCCC(=O)NO)OC	8.2510	1
152	c1cc2c(cc1)c1c([NH2+]2)c(nc(c1)NCc1ccc(cc1)C(=O)NO)c1ccc(cc1)C	8.5528	1
153	c1cc2c(cc1)c1c([NH2+]2)c(nc(c1)NCc1ccc(cc1)C(=O)NO)c1cccc(c1)OC	8.1487	1
154	c1cc2c(cc1)c1c([NH2+]2)c(nc(c1)NCc1ccc(cc1)C(=O)NO)c1ccc(cc1)[N+](=O)[O-]	7.1871	0
159	[C@@H]1(C(=O)O[C@@H](CC(=O)NCc2scc(C3=N[C@](C(=O)N1)(CS3)C)[nH+]2)/C=C/CCS)Cc1ccccc1	9.5376	1

166	[C@@H]1(C(=O)O[C@@H](CC(=O)NCc2scc(C3=N[C@](C(=O)N1)(CS3)C)[nH+]2)/C=C/CCS)CCCNC(=O)OC(C)(C)C	9.0177	1
167	[C@@H]1(C(=O)O[C@@H](CC(=O)NCc2scc(C3=N[C@](C(=O)N1)(CS3)C)[nH+]2)/C=C/CCS)Cc1cn(cn1)C(=O)OC(C)(C)C	9.2076	1

4.2. Identification of molecular fingerprints

4.2.1. Bayesian classification study

This is one of the crucial techniques for finding out important structural feature or fingerprints required for HDAC enzymes inhibitory activity. Discovery Studio 3.0 (DS 3.0, Accelrys Inc., San Diego, USA, 2015) was used to create the Bayesian classifier model (Amin *et al*.,2019; Chen *et al*.,2011; Ghosh *et al*.,2020). The descriptors like Extended-connectivity fingerprints with a diameter of 6 (*ECFP_6*), number of rings (*nR*), number of rotatable bonds (*nBonds*), number of hydrogen bond donors (*nHBDs*), number of hydrogen bond acceptors (*nHBAs*), molecular weight (*MW*), *ALogP*, number of aromatic rings (*nAR*). were taken for model generation. Further for validation purpose the leave-one-out and 5-fold cross-validation were utilized. Moreover, robustness and quality of the model became checked by observing the receiver operating characteristics (*ROC*) plot. The external model prediction was checked via the test set. The predictability power of the model relies on the calculated value of true positive (*TP*), true negative (*TN*), false negative (*FN*), false positive (*FP*), specificity, sensitivity, accuracy, and conductance. The main advantage of Bayesian classification technique is that the structurally diverse molecules can be added for the generation of QSAR model. Other advantages of this methods are, it can handle huge amount of data with high-speed and generate dependable output with minimum noise.

4.2.2. Recursive partitioning study

Recursive partitioning (RP) study is a multivariate statistical method for classification and analysis of the important molecular features for a group of different molecules having a specific pharmacological or biological activity (Amin *et al*., 2021). Decision tree which helps to understand the specific features for different compounds can be generated through this method. In this study, RP approach was used to classify the HDAC1 inhibitors. Here, different molecular properties and fingerprint features were used for generating RP models by means of DS 3.0 (Accelrys Inc., San Diego, USA, 2015). Fivefold cross-validation method was used for validating the developed models. Different models are generated but the RP model with highest discrimination capability was selected for further procedures.

4.3. Validation for the prediction accuracies of the Bayesian and RP models (Model Evaluation)

Quality of the Bayesian and RP models is determined by the *SE*, *SP*, *AC*. The predictability and accuracy of these models is observed by Receiver operating characteristics (*ROC*)-based statistical evaluation.

$$SE = \frac{TP}{TP+FN} \quad (1)$$

$$SP = \frac{TN}{TN+FP} \quad (2)$$

$$AC = \frac{TP+TN}{TP+TN+FP+FN} \quad (3)$$

In the equations (Eq. 1-3), *TP* signifies that active compounds that are correctly predicted, *TN* signifies inactive compounds that are correctly predicted, *FP* means inactive compounds incorrectly predicted as active and *FN* means active compounds that are incorrectly predicted negative. Several additional statistical metrics, such as precision, Matthew's correlation coefficient (*MCC*), positive likelihood ($\rho+$), and negative likelihood ($\rho-$), were also obtained using the following equation.

$$MCC = \frac{(TP*TN)-(FP*FN)}{\sqrt{(TP+FN)(TP+FP)(TN+FN)(TN+FP)}} \quad (4)$$

$$Precision = \frac{TP}{(TP+FP)} \quad (5)$$

$$\rho+ = \frac{Sensitivity}{(1-Specificity)} \quad (6)$$

$$\rho- = \frac{(1-Sensitivity)}{Specificity} \quad (7)$$

4.4. Molecular docking study

Molecular docking studies were performed using Schrodinger molecular modelling software (http://www.Schrodinger.com/Glide) to understand the probable mode of interactions of the promising HDAC1 inhibitors with HDAC1 enzyme (PDB: 4BKX, Chain B, https://www.rcsb.org/structure/4BKX). Compound **70** (Etoposide SAHA hybrid analogue), compound **81** (Azumamide C), compound **87** (Santacruzamate A), compound **94** (Trapoxin B) and compound **132** (Cumarin derivative) belonging to the different structural categories were selected for molecular docking. The 2D structure of the diverse compounds **70**, **81**, **87**, **94** and **132** used for molecular docking is shown in **Figure 11**.

At first, the ligands were prepared using the Schrodinger, *LigPrep* module. The *LigPrep* module can produce the expected ionised forms at significant concentrations corresponding to pH 7.0±2.0, perform verification, generate variations, and optimise the ligand structures. HDAC1's 3D crystal structure was obtained from the Protein Data Bank (PDB ID: 4BKX, Chain B). The protein preparation wizard in the Schrödinger software was used to prepare the receptor before docking into the active site. To generate the grid, hydrogen atoms were added and the active site of the protein was defined. At the active site, the grid box was generated. During grid generation, zinc metal was chosen for its interaction with ligand.

4.5. Molecular dynamic simulation

The molecular dynamics simulation was then carried out utilising the GROMACS 5.1.4 version (Abraham *et al*., 2015) using the GROMOS96 43A2 force field and SPC/E water model as reported previously (Berendsen *et al*., 1987), where topology files and charges for the ligand atoms were generated by the PRODRG server (http://davapc1.bioch.dundee.ac.uk/cgi-bin/prodrg/submit.html). Before energy minimization of the system, it was neutralized by the addition of sufficient counter ions (Na^+). Subsequently, the steepest descent method was used to energy-minimize the solvated system in 50,000 steps for the stability and removal of steric clashes. NVT (at 300 K) and NPT (at 1 bar) ensemble equilibrations were performed for 100 ps, and then each of the equilibrated systems was run for the production simulation of 100 ns. The production simulation data was further used for the enumeration of root mean square deviation (*RMSD*), root mean square fluctuations (*RMSF*) and radius of gyration (*Rg*) for each systems described earlier (Singh & Qureshi, 2022). Furthermore, hydrogen bond was analysed between HDAC1 active site amino acid residues and compounds of interest. After that, simulation trajectories were examined and visualized at different time period through PDBsum (http://www.ebi.ac.uk/thornton-srv/databases/pdbsum/) and PyMOL (https://pymol.org/2/), respectively. To calculate the average binding free energy of selected complexes, a molecular mechanics Poisson-Boltzmann surface area (MM-PBSA) approach was used through MmPbSa-Stat' Python script of g_mmpbsa package of GROMACS. The calculation of the total binding free energy was conducted for stable trajectories of 50-70 ns.

Chapter 5: Results and discussion

Chapter 5: Results and discussion

5.1. Analysis of the dataset

Wide variety of natural HDAC1 inhibitors were collected for the molecular modeling studies. The list of different scaffolds was already mentioned in **Table 3**. The structural diversity was performed to understand the degree of dissimilarity among all the natural compounds. In order to perform the structural diversity analysis, some common molecular properties like *ALogP*, *MW*, *nR*, *nAR*, *nHBDs*, *nHBAs*, number of rotable bonds (*nRB*), molecular fractional polar surface area (*MFPSA*) were calculated by using DS 3.0. Results were plotted by taking an appropriate number of bins in binned scattered plots as shown in **Figure 5**.

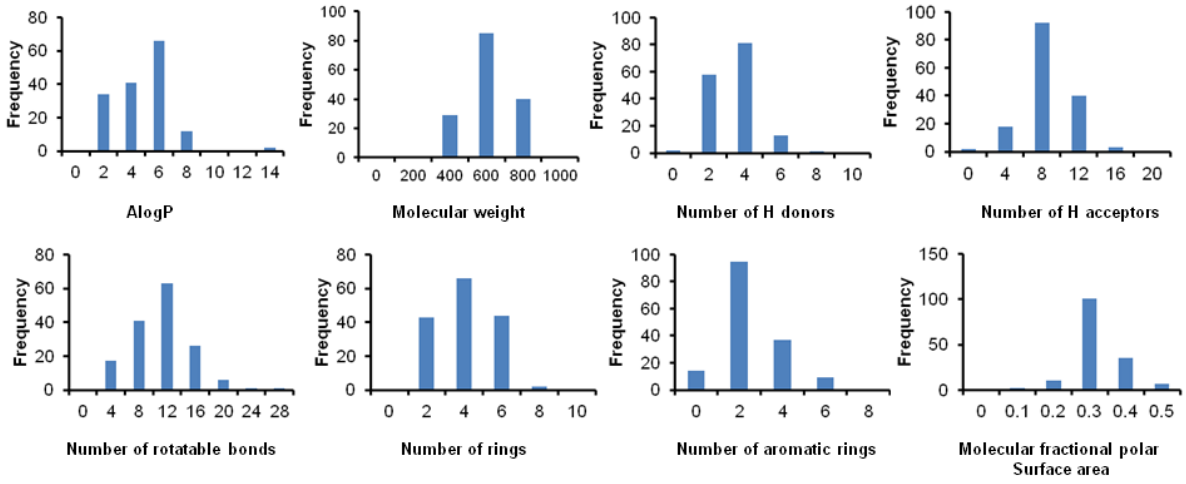

Figure 5. Graphical representation of molecular properties of natural HDAC1 inhibitors.

The analysis of the **Figure 5** clearly shows different variation in properties of the natural inhibitors. For example, there is a variation of *ALogP* from 0 to 14 whereas maximum number of inhibitors were concentrated to a value < 8. As can be seen from the **Figure 5** there is also a variation of *MW* from 0 to 1000. Maximum number of inhibitors was concentrated to value of 600. Number of hydrogen bond donors and acceptors are also important properties. Different variations of hydrogen bond donor and acceptor properties among natural HDAC1 inhibitors were seen from the **Figure 5**. The variation of *nRB* (0-28) clearly indicates the differences in the structures. Similarly, the variation of other parameters like *nR*, *nAR* and *MFPSA* are observed among the natural HDAC1 inhibitors (**Figure 5**).

5.2. Bayesian classification study

Bayesian classification study has been performed for generation of classification model. Biological sorting method was used for splitting of the dataset. A number of 117 molecules

were selected as training set and 38 molecules were selected for test set preparation. Different statistical parameters like sensitivity, specificity and concordance were studied for describing the generated model. **Table 6** summarises the statistical findings for the training sets, which indicate a high sensitivity of 89.1%, specificity of 83.0%, and total accuracy of 86.3%.

Table 6. Statistical parameters of the best Bayesian model

Set	ROC	TP	FN	FP	TN	Sensitivity	Specificity	Concordance
Training	0.844	57	7	9	44	0.891	0.830	0.863
Test	0.847	16	4	6	12	0.800	0.667	0.737

Where, TP =True positive; FN =False negative; FP =False positive; TN =True negative

Table 6 also shows that the test set has a high sensitivity (80.0%), specificity (66.7%), and accuracy (73.7%). The training *ROC* score (0.844) and test set *ROC* score (0.847) indicate that the model has good predictability. *ROC* curves for both the training and test sets depicted in **Figure 6**.

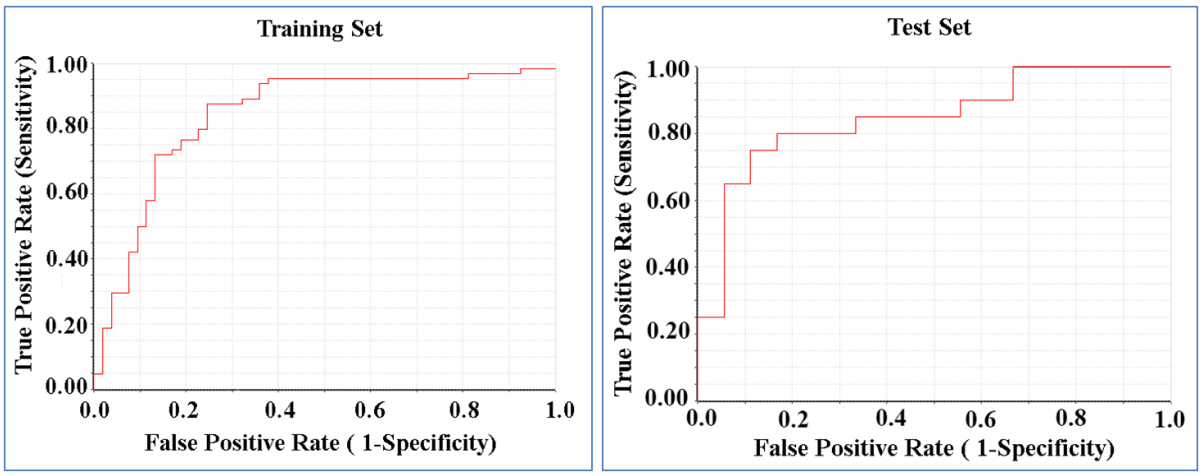

Figure 6. Receiver operating characteristics curve for (**A**) Training set (**B**) Test set as per Bayesian classification model of HDAC1 inhibitors.

The statistics of the training set molecules clearly support the model's predictive capacity, and the greater value of the parameters plainly reflects the model's dependability (*Precision* = 0.864; *AC* = 0.737). The positive likelihood ratio ($+\rho$ = 5.24) exceeds the negative likelihood ratio ($-\rho$ =0.13) suggesting that the model is acceptable. *MCC* is another important parameter that determines the model's predictability. A *MCC* score of 1.0 indicates perfect prediction, whereas the number of -1.0 indicates a very poor model. The *MCC* value of 0.720 in the developed model strongly supports the results. Statistical parameters are also calculated for

the test set to validate the external prediction of the developed model. The statistical value (*Precision* = 0.727; *Accuracy* = 0.737) is highly closed with the values of the training set. The positive likelihood value and the negative likelihood values are calculated as ρ+= 2.40, ρ-=0.30. The higher values of these parameters clearly indicates that the predictive power of the model is statistically significant.

5.2.1. Analysis of fingerprints from the Bayesian Classification Model

Twenty good (G1-G20) and twenty bad (B1- B20) sub-structural fragments are exported from the generated Bayesian model of HDAC1 inhibitors. Good fingerprints (as shown in **Figure 7**) are divided into three clusters depending on the structural similarities of *ECFP_6* fingerprints.

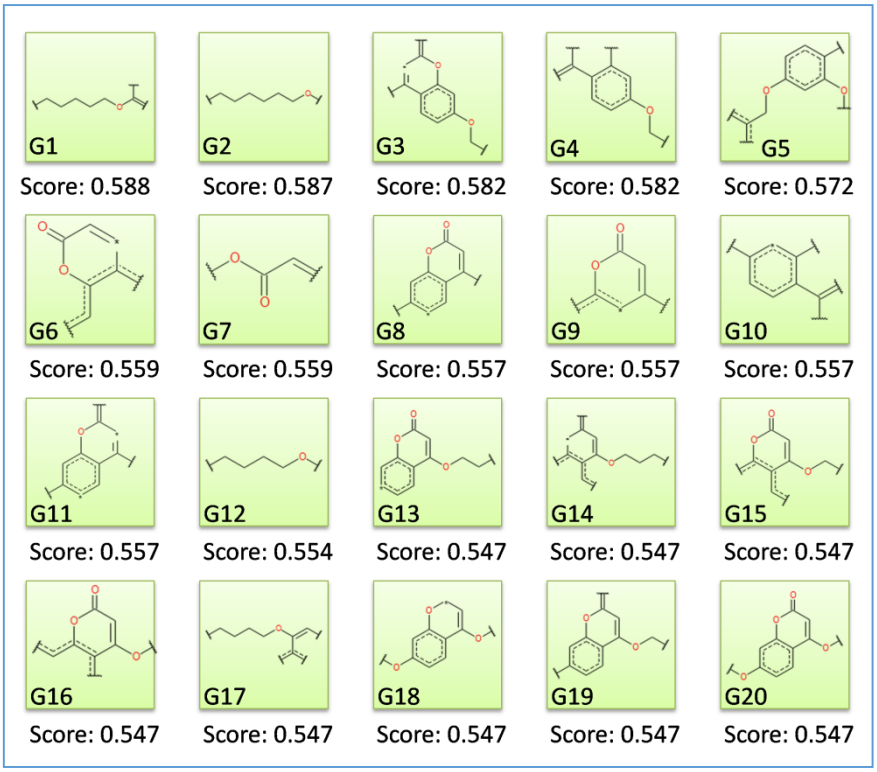

Figure 7. Good fingerprints generated by Bayesian classification study

For G1, G2, G12, G17 good fingerprints, a carbon chain containing an oxygen is very important for its HDAC1 inhibitory activity. For intense, compounds **48** (**Figure 8**) and **50** having the G1 fingerprint possess good HDAC1 inhibition (IC_{50} 36.3 and 15.2 nM, respectively). G5 fingerprint containing 2,4-di methoxy benzene responsible for higher potency in the compounds **131**, **132** and **133** with IC_{50} values of 1.56, 2.95 and 2.5 nM, respectively (**Figure 8**). The fingerprints G3, G4, G6-G11, G13-G16, G18-G20 have structural similarities and the main part of the fingerprints contain 2,4-dimethoxy-2H-

chromen-2-one ring. This feature is an important scaffold for imparting better HDAC1 inhibitory activity to the molecules such as compounds **109** (IC_{50} = 0.48 nM), **128** (IC_{50} = 0.5 nM), **114** (IC_{50} = 0.6 nM).

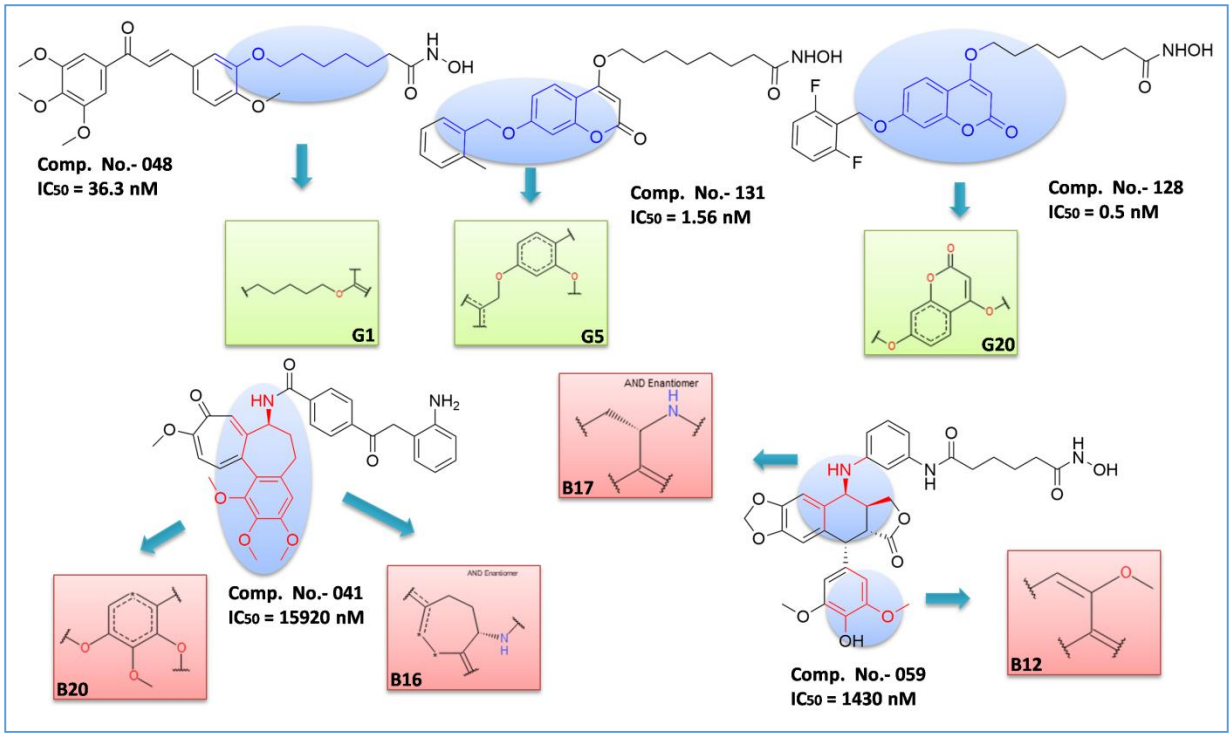

Figure 8. Examples of some compounds having good and bad fingerprints

Twenty bad (B1- B20) sub-structural fragments as depicted in **Figure 9** are also exported from the generated Bayesian model of HDAC1 inhibitors. Bad fingerprint B1 suggests some fused aromatic ring together that may be responsible for lowering the HDAC1 inhibitory activity of the compounds (For example: compound **086** with IC_{50} 10,900 nM). Cyclic or acyclic carbonyl group (Fingerprint B2) in psammaplin analogues or other compounds responsible for showing comparatively poor activities (compound **004**: IC_{50} = 1,29,200 nM). Most of the bad fingerprints contain a seven membered ring fused with or without a six or seven membered ring (B4-B11, B13-B16) which is an indication of lowering inhibition against HDAC1 enzyme. Fingerprints with aromatic benzene ring having multiple substitutions in para and meta position with an ether or hydroxyl functional group are responsible for promoting low activity towards inhibition of HDAC1 enzyme (for example: compound **043** with IC_{50} 9620 nM). B17 fingerprint contains amine group which can be substituted in a ring. This type of fingerprint is responsible for poor activity of various natural as well as semisynthetic HDAC1 inhibition (e.g., compounds **038**, **041**, **045** with IC_{50} 9,580 nM, 15,920 nM and 12,500 nM). B12 is also an example of bad fingerprint containing

carbonyl group which impart low inhibition of concerned enzyme (e.g., compound **036** with IC_{50} 35,950 nM).

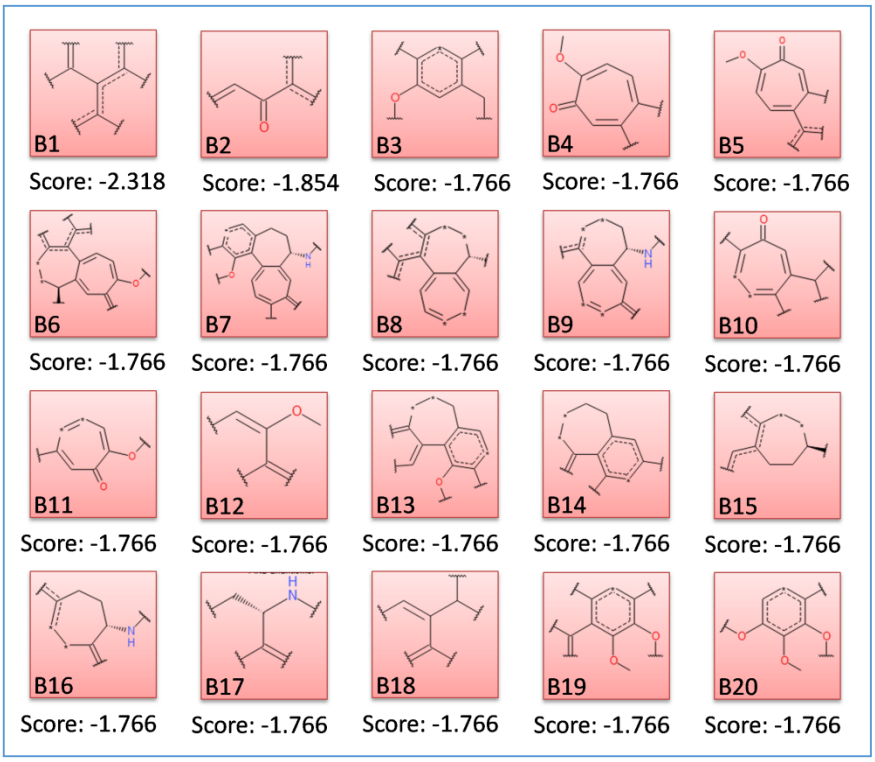

Figure 9. Bad fingerprints generated by Bayesian classification study

5.3. Recursive partitioning study

A fingerprint feature (*FCFP_6*) with different molecular properties have been taken for developing the RP model with the help of DS 3.0 software. The same training set was employed to construct the RP model with 3 trees. The tree 1 with ROC_{Train} value of 0.926 (**Table 7**) and 5-fold cross validated (ROC_{CV}) of 0.821 is considered as the best tree. Also, the tree 1 possesses higher accuracy (*AC* = 0.857) compared to other trees (**Table 8**).

Table 7. The results of the RP model as obtained from the training set HDAC1 inhibitors.

Tree Information	Confusion Matrix			*ROC*	ROC_{CV}
Tree 1: 6 leaves		**1**	**0**	**0.926**	**0.821**
Error Rate (training data): 16.867	**0**	**54**	**10**		
Min alpha: 0	**1**	**7**	**46**		
Tree 2: 3 leaves		1	0	0.845	0.817
Error Rate (training data): 19.954	0	47	17		
Min alpha: 1.508	1	4	49		
Tree 3: 2 leaves		1	0	0.711	0.817
Error Rate (training data): 33.82	0	27	37		

Min alpha: 16.09	1	0	53

Table 8. The results of the RP model as obtained from the test set HDAC1 inhibitors.

Confusion Matrix			Tree ID	ROC Rating
Actual\Pred.	**1**	**0**	**1**	**Accuracy 0.857: Good**
1	**17**	**3**		
0	**5**	**13**		
Actual\Pred.	1	0	2	Accuracy 0.825: Good
1	15	5		
0	3	15		
Actual\Pred.	1	0	3	Accuracy 0.700: Poor
1	8	12		
0	0	18		

5.3.1. Analysis of fingerprints from the Recursive partitioning study

The best decision tree with 6 leaves (as shown in **Figure 10**) exported five structure fragments (FP-1 to FP-5) based on *FCFP_6*. Hence, these fingerprint features are important those play very crucial roles in discriminating *active* and *inactive* HDAC1 inhibitors.

Leaf **#1** contains **FP-1** fingerprint containing a carbon chain with an intact oxygen is a very crucial structure to find some of the very potent molecule like chalcone derivatives (compound **048** with IC_{50} = 36.3 nM), coumarin derivative (compounds **110**, **111**, **128** with IC_{50} = 0.31, 0.3 and 0.5 nM, respectively). Leaf No. **#3** contains **FP-2** fingerprint which is responsible for inserting potency to various molecule like romidepsin analogues (compounds **19**, **18** and **20** with IC_{50} = 0.3, 0.4 and 0.4 nM, respectively), spiruchostatin B, C (compounds **90** with IC_{50} = 2.2 nM), trapoxin B (compound **94** with IC_{50} = 0.11 nM), largazole analogues (compounds **160** and **161** with IC_{50} = 0.21 and 0.2 nM, respectively). Leaf **#7** contains **FP-3** fingerprint which includes heterocyclic rings. This fingerprint found in colchicine derivatives are found to be poor HDAC1 inhibitors (compounds **38**, **41** with IC_{50} = 9,580 and 15,920 nM, respectively). Saprorthoquinones also contain this fingerprint (compounds **102**, **103**: IC_{50} >100 nM).

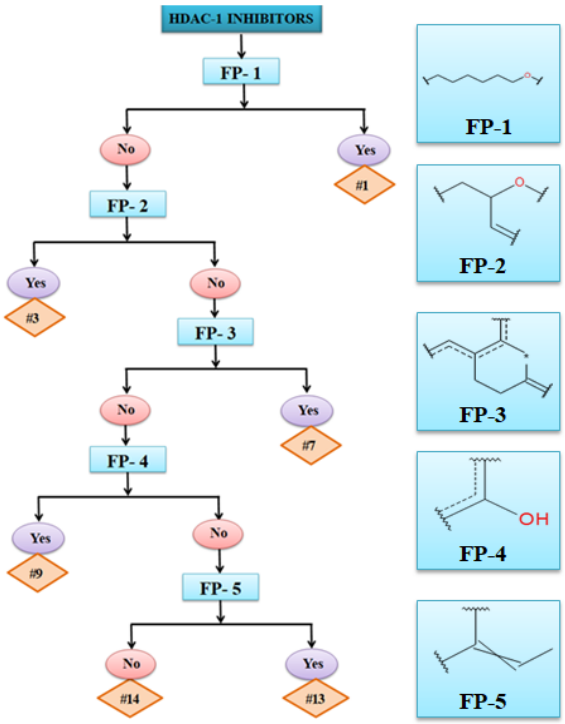

Figure 10. Decision tree generated from recursive partitioning study, along with the important fingerprints are shown below.

Leaf **#9** contains **FP-4** fingerprint which has a hydroxyl group in a cyclic ring. A well-known natural HDAC inhibitor named psammaplin (compound **3** with IC_{50} = 45 nM) contains this fingerprint which is a very potent molecule. Etoposide and SAHA hybrids also contain this fingerprint which impart better potency (compound **70** and **77** with IC_{50} = 11, 14.08 nM, respectively). Gymnochrome E (compound **86**: IC_{50} = 10,900 nM) also contains this fingerprint but it does not impart that much potency in this molecule. Leaf **#13** contains **FP-5** fingerprint which is very important for imparting better potency for molecules such as trichostatin (compound **2** with IC_{50} = 6 nM), camptothecin analogues (compounds **52**, **55** with IC_{50} = 50 and 37 nM, respectively, azumamide E (compound **83**: IC_{50} = 67 nM), coumarin derivatives (compounds 137, 138 and 139 with IC_{50} = 6.43, 2.51 and 0.24 nM, respectively). These fingerprints impart less potency to nigranoic acid and manwuweizic acid derivatives (compound **98** and **100** with IC_{50} = 3,960 and 1,140 nM, respectively). After RP modeling and Bayesian classification result analyses, it can be seen that **FP-1** fingerprint and G2 fingerprint both have similar structure. Hence, this feature is important for potency of natural HDAC1 inhibitor. By this type of classification-based model generation we can have better idea about the fingerprints which are responsible for better activity of a compound.

5.4. Molecular docking study

Molecular docking was performed to check whether the identified structural fingerprints (by Bayesian classification and recursive partitioning studies) have influence on the HDAC1 binding interaction or not. Five potential compounds (including one reference molecule: Trapoxin B or compound **94**) representing diverse classes of HDAC1 inhibitors were selected for molecular docking analysis. The compounds are compound **70** (Etoposide SAHA hybrid analogue), compound **81** (Azumamide C), compound **87** (Santacruzamate A) and compound **132** (Cumarin derivative). The 2D structure of the diverse compounds used for molecular docking is shown in **Figure 11**.

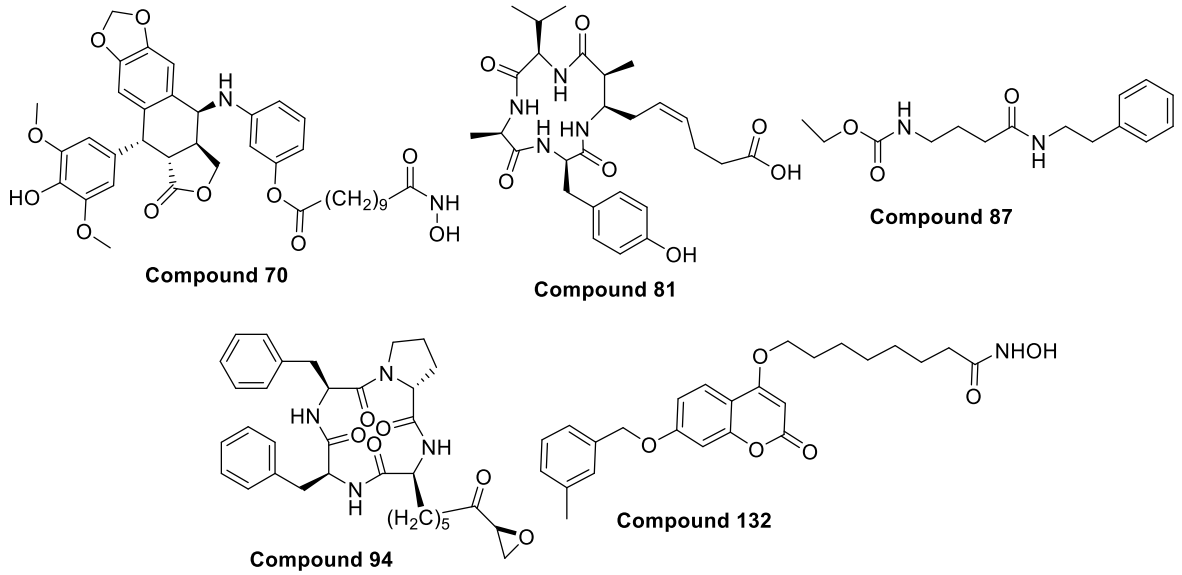

Figure 11. 2D structure of the diverse compounds used for molecular docking.

Table 9. Glide Score of all the docked compounds along with reference inhibitors (compound 94).

S. No	Compounds	Glide Score
1	Compound 94	-6.154
2	Compound 70	-5.895
3	Compound 81	-4.454
4	Compound 87	-3.904
5	Compound 132	-5.950

The selection of compounds for molecular docking study was done since their potential HDAC inhibition and the structural diversity. The glide Score of all the docked compounds

along with the reference compound **94** (Trapoxin B, PubChem CID: 395803) has been provided in **Table 9** for comparison. Meanwhile, compound **94** shows interaction with H140, C151, H141, G301 through the oxirane moity (**Figure 12**). The benzene ring attached to the cyclo tetrapeptide shows interaction with H28 and P29 through π-π stacking and alkyl interaction. It also interacts with different amino acids like D99, F150, H178 etc. Likewise, compound **132** possesses the good fingerprints like G1, G5, G20 (identified by Bayesian classification study) which are important for various interactions with the receptor HDAC1. G1 have interactions with amino acids like F150, H178, L271, F205 by π-sigma and alkyl interactions with the receptor. The fingerprints G5 or G20 interacted with amino acid H28, D99 through carbon hydrogen bond and π-anion interaction (**Figure 12**). Other inhibitors are also showing important interactions with the HDAC1 receptor. Compound **87** has H-bonded interaction with G149 (**Figure 12**).

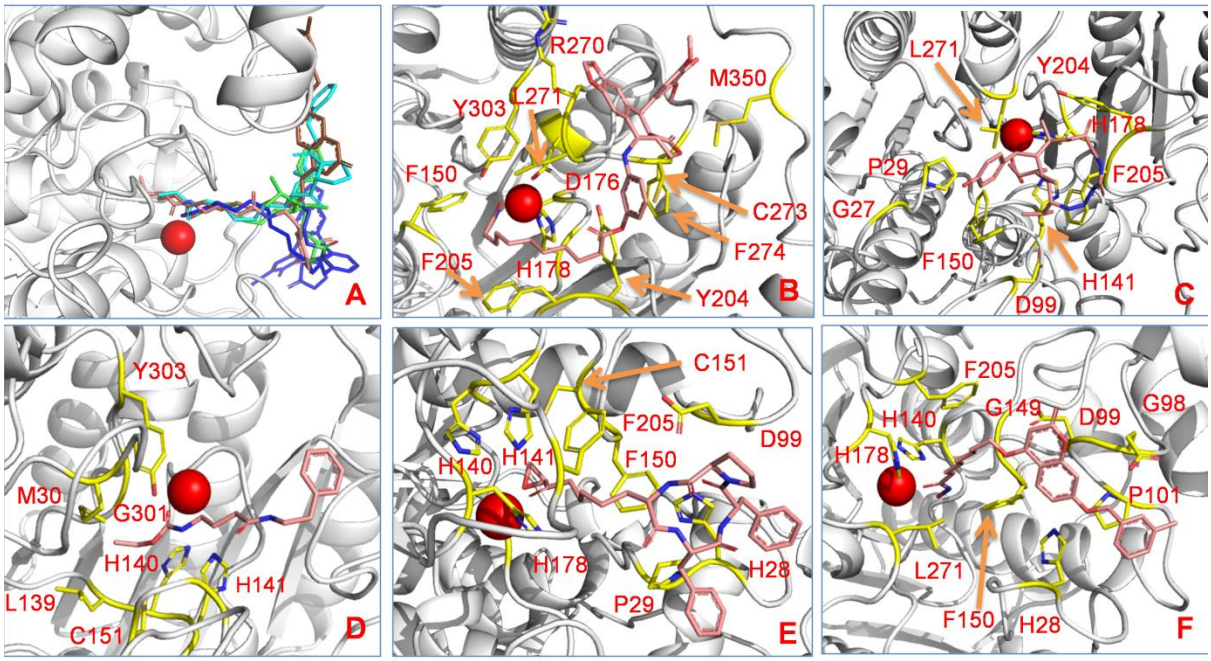

Figure 12. Molecular docking analysis; A denotes five compounds docked with the HDAC1. B-F denote the complex of Compounds **70**, **81**, **87**, **94** and **132** with HDAC1 separately.

The terminal ethoxy group interacted with M30, L139, C151 with the help of alkyl interaction. One of the carbonyl groups is showing metal acceptor interaction with Zinc. H140, H141, Y303 mainly shows carbon hydrogen bond interactions. Despite having a large structure compound **70** successfully interacts with several amino acids through its oxime group. It interacted with zinc, D264, D176 etc. The carbon chain interacted with Y204, L271,

F205, F150 amino acids through carbon hydrogen bond. Benzo dioxol group interacted with R270 through hydrogen bond interaction. There are also others important amino acids like F274, C273, F274 etc showing important interactions with the receptor (**Figure 12**). Compound **81** also has very good interactions through carboxyl group in its structure (**Figure 12**). It binds with zinc and H178, H141. The phenol ring attached to the cyclo tetrapeptide ring has several alkyl interactions with amino acids like L271, P29, and conventional hydrogen bond interaction with G27. The Carbonyl group and amine group also plays important role in several interactions with Y204, F205, D99 etc.

5.5. Molecular dynamic simulation

The B chain of protein was considered for docking studies and the outcome presented that all the compounds are nicely docked into the active site of HDAC1 and they are finally assesessed by MD simulations studies. The average *RMSD* of the apo and its complexes with compounds **70**, **81**, **87**, **94**, and **132** was listed as 0.243, 0.230, 0.223, 0.230, 0.207, and 0.216 nm, respectively. Comparision of *RMSD* has shown that all complexes had significantly lower deviation than the apo form. It suggests that deviation of HDAC1 backbone atoms are restricted when it is bound to compounds. However, *RMSD* values for compound **94**-complexed HDAC1 was lower than that of the other systems (**Figure 13A**). Moreover, all the complex systems achieved equilibration and maintained its consistency till the end of simulation. Further, the comparative analysis of *Rg* data was performed to determine the compactness of HDAC1 before and after interaction with compounds. The mean *Rg* values of 1.909, 1.913, 1.919, 1.891, 1.906 and 1.907 nm were computed for apo and complexes with **70**, **81**, **87**, **94** and **132**, respectively, delineating the compactness of complexes to be quite like that of apo form (**Figure 13B**).

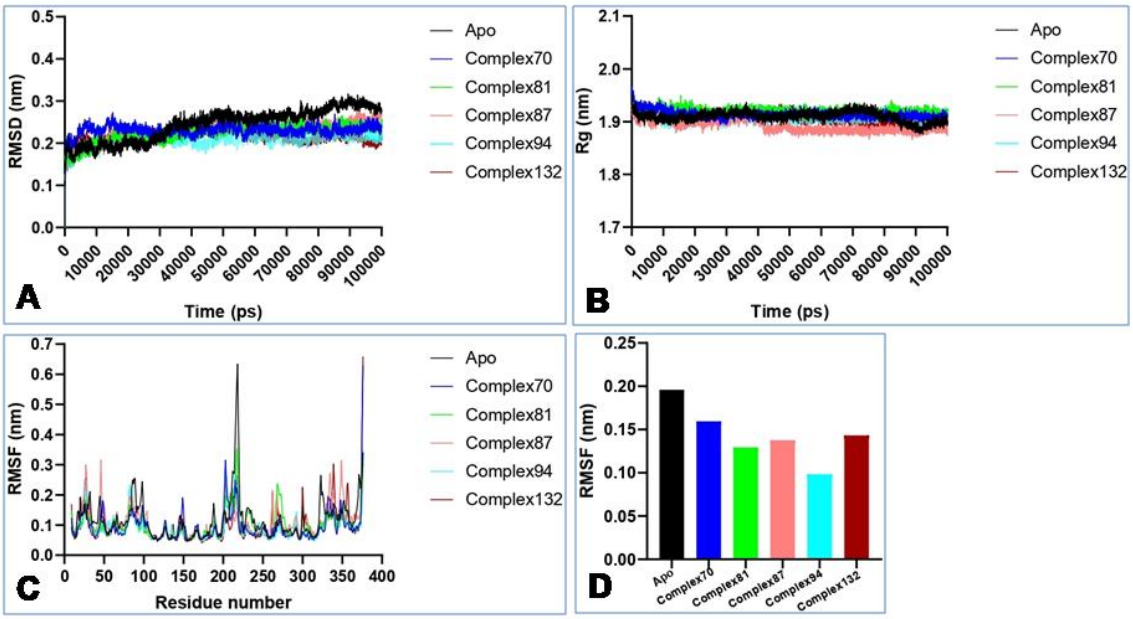

Figure 13. Molecular dynamic simulation analysis: (A) *RMSD*, (B) *Rg* and (C) *RMSF* of the backbone-atoms of the apo HDAC8 and its complexes with **70**, **81**, **87**, **94** and **132** compounds. (D) Comparison of *RMSF* for D99 in apo and complex forms of HDAC1 with **70**, **81**, **87**, **94** and **132** during simulations.

It also reveals that these compounds interact with protein without any noticeable structural expansion or contraction in the dynamic environment except for complex with **87**. Importantly, the complex with **87** had lesser *Rg* value as compared to the other complexes during simulation period of 40 to 100 ns that indicate towards substantial stabilization of the protein after the binding of compound **87**.

Simultaneously, *RMSF* of individual residues were observed in order to evaluate the residual mobility in the ligand-bound and unbound forms of HDAC1. The corresponding average *RMSF* values of backbone atoms were 0.116, 0.094, 0.098, 0.11, 0.093, 0.095 nm for apo protein and its complex with **70**, **81**, **87**, **94** and **132**, respectively. It depicts that fluctuation in the residues were reduced for all complexes as compared to the apo form (**Figure 13C**). Notably, *RMSF* of some ligand-binding residues of these complexes was relatively higher in comparison to the apo form of ptotein that could be due to the weak interactions between the binding residues of HDAC1 and the complexes. It is also observed that flexibility of D99 had decreased after ligand binding in all complex systems, which is considered as a crucial residue for π interaction and hydrogen bond formation (**Figure 13D**). This indicates towards

the existence or formation of bond between compounds and HDAC1 residue during MD simulations.

5.6. Hydrogen bonding and trajectories analysis during MD Simulations

Intermolecular hydrogen bonding between the receptor and ligand complex plays a crucial role in maintaining the conformation stability of the complex. Therefore, it was necessary to determine the total number of hydrogen bonds (*HBs*) present in the complex. The results indicated that complex **70** exhibits the highest average number of hydrogen bonds throughout the 100 ns simulation period when compared to the other complexes (**Figure 14**). It was also found that some of the trajectories retained fewer hydrogen bonds or did not contain hydrogen bonds between the selected molecules and HDAC1, however non-bonded interactions such as hydrophobic or van der Waals' contacts may have contributed to the binding in the absence of HB. The fluctuation in number of hydrogen bonds might be due to the interaction of water molecules at the binding site. Additionally, the snapshots of all complexes were visualized at different time intervals to explore the compound stability within the binding pocket during MD simulation (**Figure 14**). Surprisingly, compound **132** is moving away from the catalytic pocket after 93 ns of simulation period, however all other compounds remained in the binding site till the end of simulation.

A close observation delineated that most of frames either retained old HB or formed new HB. Notably, H178 of HDAC1 was found to interact with all the compounds through hydrogen bond that remained stable throughout the simulation in complex **70**, **87** and **94.** This finding highlights the significance of H178 in the complex structures, as it was also present in the pre-MD structure of complex **70, 81** and **94**. In addition, D176 interacted with all the compounds, remained constant until the end of simulation and was present in the pre-MD structure of complex 70. Another residue (D264) was also present in most of the post-MD structures of all four complexes suggesting that these three amino acid residues (D176, H178 and D264) play a crucial role in effective binding with the compounds. It is worth noting that the analysis of protein-ligand interactions during molecular dynamics simulations was consistent with the docking results. However, the number and type of bonds observed in the two approaches differed slightly that could be due to the dynamic nature of the protein-ligand complex and the conformational changes occurring during the simulation.

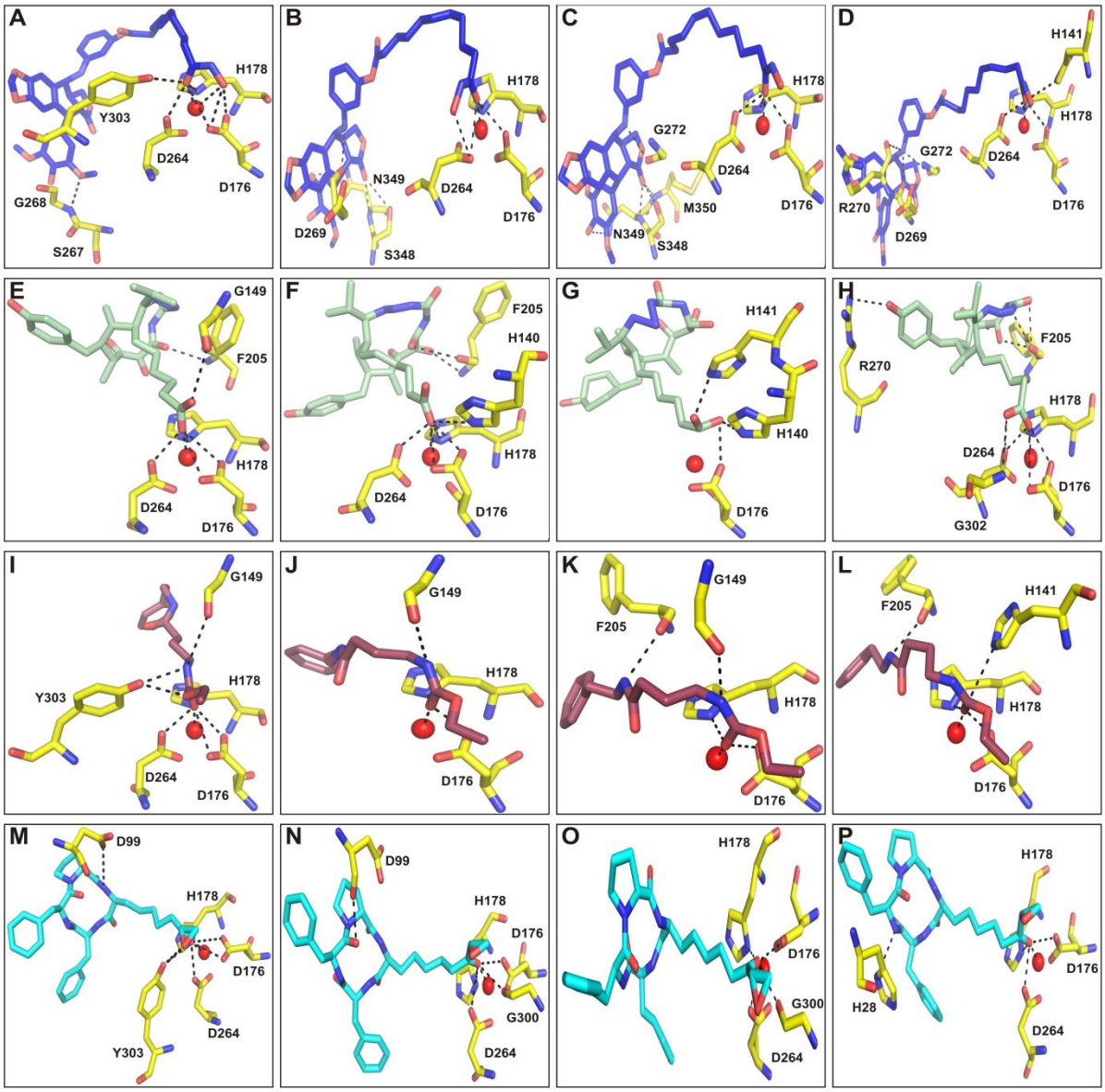

Figure 14. Binding stability of complexes during MD simulation: The complexes of apo HDAC8 and with compounds **70** (A-D), **81** (E-H), **87** (I-L) and **94** (M-P) have been observed at different time points to analyse the stability of complexes. Figures A, E, I and M indicate 0 ns, B, F, J and N display 25 ns, C, G, K and O denote 50 ns, whereas D, H, L and P represent 100 ns of molecular dynamic simulations.

5.7. Binding free energy assessment

The binding free energy of complex **81** and a reference compound-complex **94** (two of the best complexes) were evaluated through MM-PBSA using the simulation trajectories of 50-70 ns. In protein-ligand interactions, non-covalent forces such as hydrophobic forces, hydrogen bonds, electrostatic interactions, and van der Waals forces play a dominant role. These forces can contribute positively or negatively to the overall binding free energy. The

result indicated that in the binding of compounds **81** and **94** to HDAC1 protein, van der Waals interactions were the most significant contributor followed by electrostatic interactions. Additionally, there was a small contribution of Solvent accessible surface area energy (ΔE_{SASA}) in the overall binding of both compounds to protein. However, polar solvation energy was less favourable for the interaction of both ligands with HDAC1 suggesting non-polar solvation energies majorly contributed to the molecular interaction. Moreover, the corresponding total binding free energy of complex **81** and **94** was determined to be -28.91 ± 2.82 and -61.59 ± 1.89 kJ/mol (**Table 10**), respectively, indicating a strong binding of both compounds to HDAC1 protein during the dynamics.

Table 10. Binding free energy ΔE_{BE} (kJ/mol) and its contributing energy terms for the HDAC1-inhibitor complexes

Types of Energy	**Complex 81**	**Complex 94 (Reference)**
Van der Waal energy (ΔE_{vdW})	-219.05 ± 1.11 kJ/mol	-270.72 ± 2.03 kJ/mol
Electrostatic energy (ΔE_{ele})	-121.78 ± 1.25 kJ/mol	-147.80 ± 1.48 kJ/mol
Polar solvation energy (ΔE_{PSE})	337.96 ± 2.96 kJ/mol	380 ± 2.77 kJ/mol
Solvent accessible surface area energy (ΔE_{SASA})	-18.96 ± 0.08 kJ/mol	-23.61 ± 0.15 kJ/mol
Total binding energy (ΔE_{BE})	-28.91 ± 2.82 kJ/mol	-61.59 ± 1.89 kJ/mol

Chapter 6: Conclusion and future perspective

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In this study, we successfully performed Bayesian classification, recursive partitioning (RP), molecular docking and molecular dynamic simulation studies on a set of 155 nature-inspired compounds having HDAC1 inhibitory activities. The Bayesian modelling study reveals fingerprints G1 and G2 as important fingerprints whereas the best decision tree from the RP study supports five structural fragments that are anticipated to play a crucial role in determining the potent compounds for further exploration. The RP study also expresses the importance of oxygen containing carbon chain in FP-1 that correlates with the G2 fingerprint in the Bayesian study. Moreover, G14 and G16 fingerprint from Bayesian study resembles with the FP-3 fingerprint and FP-4 fingerprint of RP study, respectively. Therefore, both the modelling study correlate with each other. However, the RP study is found to be the best model because of its better ROC value. Furthermore, diverse compounds with good inhibitory action against HDAC1 were studied using molecular docking for confirming the effect of good and bad fingerprint on their binding ability with HDAC1. MD simulations is performed to explore their ligand-receptor interactions. The modeling results provide useful quantitative and qualitative information about the structural requirements of an effective HDAC1 inhibitor that is useful for searching compounds containing similar kind of structural features in nature. Overall, this study may also motivate medicinal chemists to design similar type of compounds as HDAC1 inhibitors in future.

Chapter 7: References

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