A quantitative structural analysis of hydroxamate-based HDAC1 inhibitors: Identification of promising structural contributors for the development of effective anticancer agent

Submitted by

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EXAM ROLL NO.: M4PHC22003

CLASS ROLL NO.: 002011402013

REG. NO.: 154274

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Session- ***2020-2022***

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Thesis submitted in partial fulfilment of the requirements for the Degree of Master of Pharmacy
Department of Pharmaceutical Technology
Faculty of Engineering and Technology
Jadavpur University, Kolkata

2022

Jadavpur University

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CERTIFICATE OF APPROVAL

This is to certify that ***Ritabrata Kundu*** (Exam Roll No. - , Reg. No. – of 2020-2021) has sincerely carried out the research work on the subject entitled **"*A quantitative structural analysis of hydroxamate-based HDAC1 inhibitors: Identification of promising structural contributors for the development of effective anticancer agent*"** under the supervision of **Prof. Tarun Jha,** Professor, Natural Science Laboratory, Department of Pharmaceutical Technology of Jadavpur University. He has incorporated his findings in this thesis submitted by him in partial fulfilment of the requirements for the degree of **Master in 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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Acknowledgement

The final outcome of this thesis required a lot of guidance and assistance from many people. I am extremely fortunate to have these all along the completion of my work. Whatever I have done is only due to such guidance and assistance and I would not forget to thank them.

I am highly obliged and like to express my deep gratitude and profoundness to my reverend mentor Prof. Tarun Jha of Department of Pharmaceutical Technology, Jadavpur University, Kolkata for his excellent and constant guidance and help, endless encouragement, thoughtful and freedom and stupendous co-operation throughout the term paper till its successful completion. I am greatly indebted to his motivation, fruitful suggestions and inspirations.

I owe my deep respect to Prof. Sanmoy Karmakar, Head of the Department, Department of Pharmaceutical Technology, Jadavpur University, Kolkata for all the necessary help and encouragement. I would like to convey my sincere gratitude to AICTE and Jadavpur University for their financial and equipmental support for my M. Pharm course.

I am both extremely honoured and grateful to Dr. Nilanjan Adhikari, Mr. Sandip Kr. Baidya, Mr. Sk. Abdul Amin, Mr. Sanjib Das, Mr. Suvankar Banerjee for their priceless guidance and support which assisted me to gather knowledge about the different aspects of this work.

Finally, I would like to express my deep respect to my father and mother and my friends and relatives for their continuous help, love, encouragement and moral support throughout the period of my work.

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Declaration of Originality and Compliance Of Academic Ethics

I hereby declare that this thesis contains literature survey and original research work performed by me (Ritabrata Kundu) as a part of my Master of Pharmacy studies. All the information in this document have 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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(Ritabrata Kundu)

Signature with Date

Dedicated to
My Parents, Teachers, Seniors
and
Friends

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Preface

One of the most important diseases to mankind in the modern era is "cancer," which refers to a collection of ailments that cause abnormal and unchecked cellular proliferation in living things. In this day and age, cancer is one of the leading causes of death, and the number of people affected by it is growing quickly. Additionally, cancer patients require expensive and complicated treatments, which puts our society and economy in danger.

There are several internal and external variables that contribute to the development and spread of cancer. Numerous external variables, such as pollution, malnutrition, radiation, lifestyle choices, etc are linked to the transformation of the biological system in addition to internal causes such genetic mutation, improper hormone regulation, etc.

Histone deacetylase 1 (HDAC1) is a significant member of the HDAC family that plays a role in the pathogenesis of a number of illnesses, including neurological conditions, cardiovascular diseases, and a wide range of cancers, including liver, stomach, colorectal, renal, prostate, squamous thyroid, and leukemia. Although there are many HDAC1 inhibitors available, the absence of selective HDAC1 inhibitors that are effective anticancer agents is a significant drawback for the treatment of malignancies and other disorders linked to HDAC1.

The inclusion of a cap group, a linker moiety, and a zinc binding group are three pharmacophoric properties 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 HDAC.

Additionally, the quantitative structure-activity relationship (QSAR) methodology quantifies the biological activity of molecules in relation to their molecular structure through the development of mathematical correlation in an effort to predict the key structural factors

influencing the activity of these compounds. As a result, a comparative molecular modelling technique is used in this study to examine a set of AR-42 derived HDAC inhibitors that have a wide range of HDAC1 inhibitory activity and contain the hydroxamate group as the zinc binding motif.

The goal of this study is to investigate the significant structural characteristics of the potent HDAC1 inhibitors that are essential for controlling the inhibitory activity. Some of the key structural and molecular variables for the HDAC1 inhibitors regulating the activity were discovered in this work.

(Ritabrata Kundu)

Chapter 1: Introduction

Cancer or neoplasmic conditions is regarded as one of the most dreadful diseases in the field of medicine and is known to mankind from the ancient era. 'Cancer', the very term is obtained from the Latin word '*Canker*' which means crab [**1**]. With the advancement of molecular biological research, various biochemical pathways associated with carcinogenesis are disclosed to date. 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 [**1**]. On the contrary transformation of proto-oncogene to oncogene also induces cancer. The etiology of cancer shows that various factors like Epstein-Bar virus, hepatitis B virus, papillomavirus, and HIV play crucial roles in the development of cancer, such as HIV being associated with lymphoma and Kaposi's sarcoma [**1**]. Hepatitis B causes liver cancer and Epstein Bar virus causes Burkitt's lymphoma. There are at least 200 types of cancer and due to their biochemical complexity, it is observed that medicine that is fruitful for one type of cancer condition may fail for another one [**1**]. In the year 2016, nearly 1.68 million new cancer cases were diagnosed throughout the USA and approximately 60,000 individuals are expected to die from cancer, becoming the second most common cause of death in the United States, accounting for 1 in 4 deaths [**2**]. Cancer pharmacology has changed dramatically in the recent past with the improved understanding of cancer biology and an ever-expanding set of newly developed drugs that target vulnerabilities in individual cancers. Effective early treatments have been developed for some fatal malignancies, including testicular cancer, lymphomas, and leukemia. On the contrary adjuvant chemotherapy and hormonal therapy can only extend overall survival and prevent disease recurrence following surgical resection of localized breast, colorectal, and lung cancer [**3**]. Nowadays, chemotherapy is used in three main clinical settings [**2**]:

1. Primary induction treatment for advanced disease or for cancers for which there are no other effective treatment approaches.

2. Neo-adjuvant treatment for patients who present with localized disease, for which local forms of therapy such as surgery or radiation or both, are inadequate by themselves.

3. Adjuvant treatment to local methods of treatment including surgery, radiation therapy, or both.

Again, in the context of cancer treatment, many biological receptors, as well as enzymes play a significant role in cancer pathogenesis. The histone deacetylases (HDACs) are one of those significant groups of enzymes that have direct and indirect contributions toward cancer development and progression. The HDAC enzymes are responsible for the deacetylation of ε-NH_2 of lysine of the histone proteins, consequently regulating the epigenetic mechanisms [**4, 5**]. The acetylated form of the aforementioned ε-NH_2 function is required for the binding of positively charged histone to the negatively charged DNA molecule. Therefore, deacetylation at that position of lysine dissociates the histone from DNA. Due to such activity, the HDACs are also known as Lysine deacetylase (KDACs) [**6**].

However, these HDACs, not only deacetylate the histones but also some non-histone proteins such as E2F, c-Myc, NF-κB, p53, etc. [**7**]. The downregulation of the GATA family of transcriptional factors is also regulated by the HDACs [**8, 9**]. These HDAC enzymes are also involved in leukemogenesis.

There is a total of 18 different HDAC isoforms present in the HDAC family which can be categorized into four principal subgroups based on their structural homology i.e., class I (HDAC1, HDAC2, HDAC3, HDAC8), class II (HDAC4-7, HDAC9, HDAC10), class III (Sirtuins) and class IV (HDAC11) [**5**]. Among these four classes of HDACs, all isoforms from class I, class II, and class IV are dependent on a catalytic Zn^{2+} ion present in their

catalytic domain for their deacetylation function. On the other hand, only the class III sirtuins (SIRT1-SIRT-7) are dependent on the nicotinamide adenine dinucleotide (NAD^+) for their activity [**6**]. Furthermore, among these four classes of the HDAC family, the former two classes (Class I and class II) are regarded as classical HDACs [**8, 9**].

It is observed that HDACs are encoded in the hypoxic conditions of the cancer cell. Two intracellular proteins, Runx1 and ETO fuse to generate a chimeric protein called Runx1-ETO which recruits HDACs [**9, 10**]. This further causes the upregulation of matrix metalloproteinases (MMPs) that degrade the intercellular matrix of epithelial cells of the blood vessels and form several small vessels to flow sufficient oxygen to the ever-increasing malignant cells. This very process is known as 'Angiogenesis' [**7**].

HDACs regulate epithelial-mesenchymal transition (EMT), playing a vital role in cancer cell invasion and metastasis [**4**]. 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. The role of SIRT1 in EMT regulation relies on the type of human [**4**]. 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 [**4**].

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 [**4**]. Depletion or inhibition of HDAC1 induces autophagy by promoting the accumulation of autophagosomal marker LC3-II [**4**]. It is concerned that autophagy provides an essential link between autophagy and the ubiquitin-proteasome system in the case of neurodegenerative diseases such as Huntington's disease, Alzheimer's disease, etc [**4**]. There is some autophagy machinery that comes under conditions like autophagy-related gene (Atg) 5, 7, 8, and LC3 [**4**].

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 [**4**]. 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 [**11**]. On the other hand, both HDAC6 and HDAC10 provoke cell motility [**11**]. 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 [**6, 11**]. Aneuploidy is defined as an abnormal number of chromosomes and is identified as a negative prognostic factor for epithelial malignancies [**11**]. 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 [**11**].

- ***Role of HDACs in cardiovascular disease***

Studies related to the role of HDACs in cardiac hypertrophy elucidated that both class I and class II HDACs are associated with the development of cardiac hypertrophy. On the contrary, genetic ablation of HDAC2 results in resistance to various hypertrophic stimuli activated

CK2 alpha 1 increases the intrinsic activity in response to hypertrophic stimuli [**11**]. Cardiac fibrosis, another major disease caused due to loss of elasticity and insufficient dilation of the contractile chamber in the diastolic phase is associated with hypertrophy in notable cardiac diseases [**11**]. Fibrosis is directly inhibited by HDAC inhibitors that directly regulate trans-differentiation of fibroblast to myofibroblast [**11**]. Very recently, the European Society of Cardiology and the American Heart Association alert the severity of HFpEF (Heart failure with preserved ejection function) [**11**]. 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. More direct evidence that HDACs are responsible for cardiac arrhythmia has been concerned now a day through molecular biological research. Trichostatin A (TSA) dramatically corrected atrioventricular conduction abnormalities in mouse hearts which are induced by genetic disruption of Hop X [**11**].

Atherosclerosis is a chronic and progressive disease of arteries caused by 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 and HDAC inhibitors by significantly increasing the transcription of $p^{WAF1/Cip1}$present the progression of atherosclerosis [**11**]. Moreover, HDAC inhibitors are beneficial in several other outcomes like cardiac arrhythmia, cardiac fibrosis, myocardial infarction, and cardiac hypertrophy. Also, HDAC inhibitors may reduce the growth of atherosclerosis and vascular calcification (**Figure 1**).

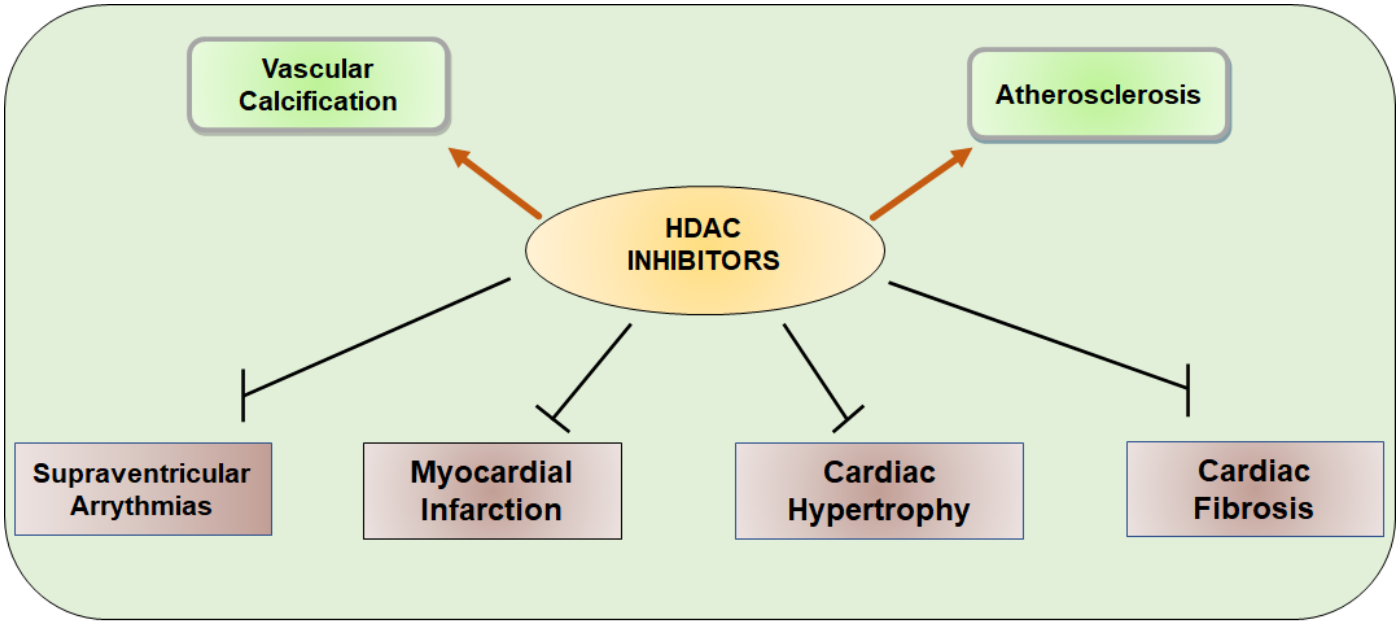


Figure 1: Therapeutic potential of HDAC inhibitors in cardiovascular diseases. Arrow: Stimulation; Bar: Suppression

❖ **Role of HDACs in miscellaneous diseases**

Apart from carcinogenesis and cardiovascular diseases, HDACs have been studied in inflammatory diseases, osteoporosis, and neurodegenerative diseases like Alzheimer's disease, Huntington's disease, and ischemic stroke, on the contrary, the Danish group has tested HDAC inhibitors in HIV treatment [**11**].

➢ ***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. [**7**].

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)

A brief description of these FDA approved HDAC inhibitors are provided below:

- **Vorinostat**

Vorinostat (Suberoyl anilide hydroxamic acid/SAHA) (**Figure 2**) is an orally active potent HDAC inhibitor used in the treatment of hematological malignancies such as cutaneous T-cell lymphoma and peripheral T-cell lymphoma [**3**]. 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 (IC_{50} < 100 nM) [**3**]. *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 [**3**]. The absorption of vorinostat is slightly improved when taken with a meal. Metabolism is mostly through glucuronidation and hydrolysis. The elimination $t_{1/2}$ is about 2h [**3**]. 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 [**11**].

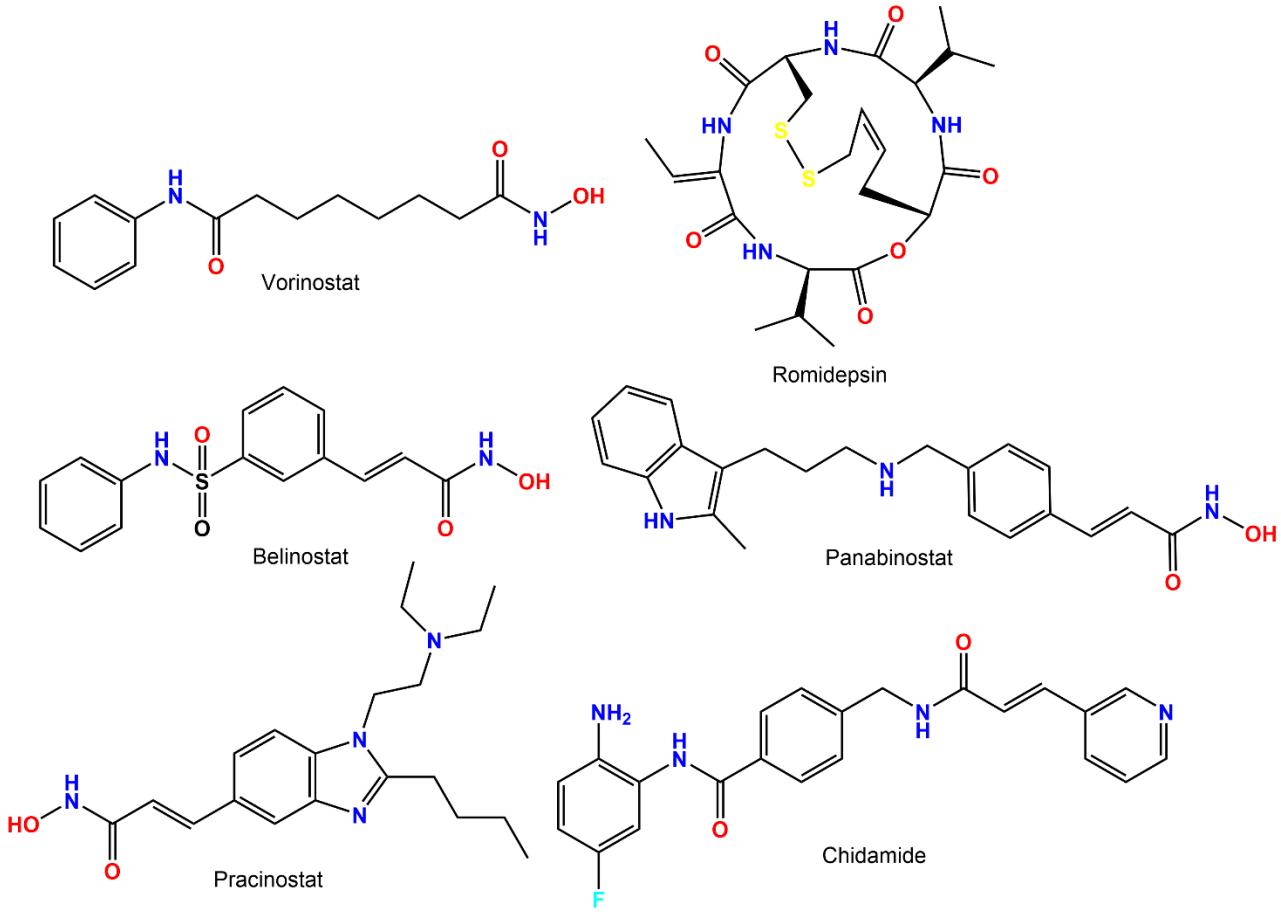


Figure 2: FDA-approved HDAC inhibitors

- **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 [**11**].

- **Belinostat**

Belinostat (**Figure 2**) is a sulphonamide-based hydroxamate primarily metabolized by UGT1A1 as well as CYP2A6, CYP2C9, and CYP3A4 [**3**]. 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 anti-angiogenic effect [**11**].

- **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 [**3, 7, 11**].

- **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 [**3, 7**].

- **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 [**3**, **4**].

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 [**12**]; many of such studies are conducted in patients with myelodysplastic syndrome and myeloid leukemia [**13**]. 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 [**14**]. 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 [**15**]. Although there was a significantly greater incidence of platelet toxicity seen in patients treated with vorinostat [**14**]. A phase II trial of estrogen receptor antagonist tamoxifen with vorinostat resulted in a 19% resistance in some patients [**16**]. 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 [**14**].

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 [**17**]. other proteins that are known to be induced upon HDAC inhibitor treatment include p^{21} and HSP90-related proteins such as HSP72 and c-Raf [**18**]. 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 [**14**].

- **Pharmacological roles of HDAC inhibitors*:***
  - ***Effects on DNA damage*:** On account of numerous studies, it has been shown that HDACs have a significant role in DNA damage repair; both HDAC1 and HDAC2 are recruited to DNA damage sites to deacetylate histones H3K56 and H4 K16 and facilitate non-homologues end-joining. Class I HDACs not only cause alter histone deacetylation and but also regulate proteins like ATR, ATM, FUS, BRAC 1 which are involved in the DNA damage response. Among class II HDACs (HDAC4,6,9,10) are 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 oxoguanine in DNA can promote oxidative stress in the case of inhibitors of HDACs. Interference with the DNA repair mechanism by HDAC inhibitors results in the accumulation of DNA damage [**7**]. HDAC inhibitors suggest a synergism upon the combination with DNA damaging

chemotherapeutics provide combine HDAC inhibiting and DNA alkylating properties, on the contrary SIRT is a critical component of the DNA damage response pathway that regulates multiple steps of DDR, including damage sensing, signal transduction, DNA repair, and apoptosis. SIRT1 interacts and deacetylates Ku 70, NBS1, APE1, XPA, PARP1, Top BP1, and KAP1. All of them have come under the consideration of DDR proteins. SIRT1 plays an essential role in maintaining genome integrity and stability [**4**].

- ***Hormone signaling*:** It is concerned that HDAC inhibitors proposed the expression of hormone receptors like androgen, progesterone, and estrogen receptors (ER alpha and ER beta). Proliferation stimulating effect provoked by estradiol viz up-regulation of cyclin D which are efficiently abrogated upon vorinostat treatment under hypoxic condition more pronounced ER alpha down-regulation under HDAC inhibitors treatment has been observed. This response was highly dependent on an intact proteosome pathway. Now it has been shown that trichostatin A, the compound, which was formally used in the treatment of schistosomiasis, and raloxifene induces a potent ER beta up-regulation in ER alpha positive cell while simultaneously reducing β ER alpha expression. Entinostat, the class I selective inhibitor, enhance the expression of both aromatase as well as ER alpha in the case of TNBC cell. Moreover, knockdown of HDAC1, 2, and 3 alone or in combination promote efficient ER upregulation over TNBC cell. Hormone regulation plays a pivotal role in promoting cancer cells [**7**].
- ***Autophagy induction*:** The term Autophagy implies the removal of unwanted cellar components and organelles from the cell through lysosome mediated regulated mechanism. The role of autophagy in cancer is quite complicated. Autophagy presents the transformation of a normal cell into a neoplastic cell. In the cancer cell, it is not

only beneficial for surveillance but also provides resistance to chemotherapy. Transcription factor FOXO-1 is reliable for induction of autophagy; many HDAC inhibitors act a dual role as a pro and anti-tumor agent. Alter 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 promote the activities of HDAC-mediated apoptosis. It is concerned that depletion or inhibition of HDAC1 causes accumulation of autophagy markers like LC3-II followed by induction of autophagy. The ubiquitin proteosome system is inversely proportional to autophagy. Additionally, apart from class I-II and IV HDACs, sirtuins also participate in regulating autophagy. For example, SIRT1 causes starvation-induced autophagy through direct deacetylation of critical autophagy machinery viz. Atg5, Atg7, Atg8, and LC3 [**4**, **7**].

- ***Apoptosis induction:*** 'Apoptosis' is a Latin word that means "falling of leaf" the term in the case of cellular biology is very significant [**19**]. 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 [**20**]. 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 [**1**]. 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 [**20**]. 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) [**21**].

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 [**22, 23**]. 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 [**24**].

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 [**25-30**]. NRF2 causes up-regulation of cytoprotectant agents which give birth to a contradictory pro-survival of cancer cells [**31**].

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

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 [**32**]. 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 [**32**].

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 [**7**].

Chapter 2: Literature Review

Cancer pathology consists of a number of receptors, enzymes as well as a transcription factors. Among them HDAC enzymes play a crucial role, to understand what the HDACs are can be termed as histone protein. Histone proteins are positively charged macromolecules (comprised of five domains such as H1, H2A, H2B, H3, and H4) bind strictly with negatively charged DNA molecules found in some specific regions of nucleosome. The binding mode is characterized by acetylation of the ε-NH_2 group of lysine found in the functional domain of histone [**13**]. On the eve of DNA replication, deacetylation must be performed to dissociate histone from the chromosome, there play two different types of enzymes have opposite role-histone acetylate (HAT) and histone deacetylase (HDAC) and they are responsible for maintaining the equilibrium between two states of the chromosome. Histone deacetylase, as it causes deacetylation of the ε-NH_2 group of lysine, also known as Lysine deacetylase (KDACs) [**13**]. There are nearly eighteen HDAC enzymes all of them come under the consideration of only four classes such as class I (HDAC1, 2, 3, and 8); class II (HDAC4-7, 9, and 10); class III (SIRTs), and class IV (HDAC11). Among these classes of HDACs, class I and class II are regarded as classical HDACs [**4**]. However, HDAC1 has also come as a target for lead molecules/drugs such as trichostatin A, trapoxin, valproic acid, FK228 (romidepsin), SAHA (suberoylanilide hydroxamic acid: vorinostat, belinostat, panobinostat) [**9**] and most recently AR 42 and some of their analogs. All of them possess a specific pharmacophore, i.e., they consist of three parts such as:

i One hydrophobic cap group

ii One linker motif

iii One zinc binding group (ZBG)

Unlike HDAC III all other classes of HDAC possess a Zn^{2+} ion in the molecular keyhole, where the zinc ion helps deacetylation. HDAC1 causes upregulation of oxoguanoglycosylase

1 which can excision of oxoguano residue of the chromosome. Apart from that, HDAC inhibitors causes upregulation of FOXO-1 which induces apoptosis.

When cancer cells over-proliferate, they suffer from a hypoxic state due to the lack of proper oxygen supply. In that situation, two factors Runx and ETO fuse to form a chimeric protein Runx-ETO. However, this stimulates the expression of HDAC1, 2, and 3 [**10**]. Here it should be mentioned that class I HDACs cause upregulation of another metal enzyme like matrix metalloproteinase (MMP). There are nearly 28 MMPs, among them, MMP-2 is highly significant as it has a direct relation to cancer pathogenesis. Other MMPs such as MMP-8, -9, -12, and -14 act as anti-targets of cancer means their inactivation by exogenous compound cause cancer [**33**]. All the MMPs have come under the consideration of gelatinase enzyme possess a Zn^{2+} ion as metallic atom MMP degrade the extracellular matrix which is composed of two types of sugar says glycosaminoglycan and proteoglycan as well as three types of proteins fibronectin, collagenase, and elastase by degradation of the extracellular matrix of vascular endothelial cells of blood vessel [**34**]. HDACs and MMPs promote angiogenesis, although two factors-hypoxia-induced factor (HIF) and vascular epithelial growth factor (VEGF) released from cells with a hypoxic state plays a crucial role in cancer [**33**].

HDAC1 has regarded as a target for cancer pathogenesis and several drugs were developed likes SAHA, FK228, CHAP, VPA, AR-42, pyroxamide, chidamide, etc. As aforementioned, the pharmacophore of them must possess three parts: A hydrophobic cap, a linker, and most significantly a zinc-binding group. There are many groups concerned as zinc-binding groups for example carboxylate, hydroxamate, electrophilic ketone, cyclic tetra peptides, oximes, β-lactum, benzamide, etc. This group blocks the class I HDACs by forming a chelating complex with Zn^{2+} ions [**35**].

Trichostatin A isolated from *Streptomyces hygroscopious* formally administered in the treatment of schistosomiasis [**36, 37**] also acts as a potent HDAC inhibitor (with Ki value of 3.4 nM). Trichostatin A is a chiral compound and the S configuration is more active than the R configuration. SAR of trichostatin A shows that the presence of methyl and two double bonds reduce the activity by 2.3 and 33-fold respectively in comparison to linear alkyl chain [**35**]. Oxamflatin and scriptaid are two other compounds concerned as potent HDAC inhibitors although their potency is lower than trichostatin A (TSA), oxamflatin was prepared by Shinogi Laboratory in the year of 1996 and its IC_{50} was reported at 15.9 nM on the other hand TSA was found having IC_{50} of 1.44 nM. Scriptaid was identified via high-throughput screening for transcriptional activation series of a tricyclic compound are related scriptaid and most potent analog among them have IC_{50} = 10 nM [**35**].

AR-42 chemically known as N-hydroxy-4(3-methyl 2-phenyl butanamido)-benzamide is also regarded as a potent HDAC1 inhibitor-basically a chiral compound where the S enantiomer is 5-fold more potent than R configuration the IC_{50} of S form is 16 nM whereas the R configuration 82 nM [**38, 39**]. AR-42 is administered orally 3 times per week for three weeks of a 28-day cycle [**40**]. It is an orally active anti-proliferative against cancer cells *in vitro* and *in vivo*. In the solid tumor, osteosarcoma, vestibular schwannoma, and meningioma [**41**] and other malignancies (likes myeloma, lymphoma, and leukemia) [**42**]. It is reported that AR-42 comes under consideration in phase I for the treatment of advanced or relapsed multiple myeloma, lymphomas, or chronic lymphocytic leukemia [**43**].

The suberoylanilide hydroxamic acid (SAHA) group of drugs is a very popular drug of choice in the treatment of cancer as they are regarded as pan-HDAC inhibitors. Drugs likes-vorinostat (Zolinza) [FDA01], belinostat (Belodaq) [FDA03], panobinostat (Farydak) [FDA04] pracinostat [FDA05] have come under the consideration of SAHA and they all are US-FDA approved drugs. Vorinostat-orally active potent HDAC inhibitor and used in the

treatment of hematological malignancies of such as cutaneous T-cell lymphoma and peripheral T-cell lymphoma (CTCL+PTCL). Belinostat possesses a sulphonamide-based ligand structure which is chemically known as (2E)N-hydroxy-3[3(phenyl sulphomoyl)prop-2-enamide. It is principally metabolized by UGT1A1 as well as CYP2A6, CYP2C9, and CYP3A4. The overall response rate was 26% in the treatment of relapsed or refractory PTCL. The effective concentration of belinostat was high enough because of an insufficient blood supply caused by its anti-angiogenic effect [**11, 6**].

Pracinostat is used in the treatment of acute myeloid leukemia. One interesting fact about pracinostat is it has no such effect on the zinc-dependent metal enzyme. After accumulation inside neoplastic cells, it causes acetylation of histone, remodeling of histone of chromatin, and transcription of various tumor suppressor genes [**11, 6**].

Trapoxin is a fungal metabolite and considers an irreversible HDAC inhibitor, chemically they possess cyclic tetrapeptide-trapoxin A (TpxA and TpxB) both come in the treatment of cancer. Trapoxin (Tpx) induces a morphological reversion of vis-a-vis transformed NIH 313 cells. Tpx contains 2-amino-8-oxo-9, 10-epoxy decanoic acid, a bizarre amino acid that mimics acetylated lysine structure. Moreover, the epoxyketone group of Aoe helps in forming a covalent bond between Tpx and HDAC enzyme [**35**]. Romidepsin is another cyclic peptide launched in November 2009. Its earlier name was FK228 and it is depsipeptide in nature. The overall repression rate was 34% for CTCL and 25% for PTCL [**11**]. SAHA on the other hand increase phosphorylation of LGF, up-regulate the expression of PTEM and p21 as well as reduce the level of cyclin D and p53 in type 1 human endothelial cancer cell line, radio sensitization in SAHA in cooperation with capecitabine inhibits tumor growth in the colorectal carcinoma xenograft model.

In the year of 1999, Suzuki and his co-workers found MS275 -a synthetic benzamide-based HDAC inhibitor. SAR of the functionality assessed that 2'-amino or 2'-hydroxy moiety is

essential in partially purified histone deacetylase inactivation [**35**]. Synthesis of 3',4' and 5' position attenuate enzyme activity increased steric interaction. CI994 is concerned an acetylated derivative of cardinalin. Initially, it was developed for the treatment of epilepsy or convulsion. Although the exact mechanism of CI 994 is not clear to date [**7**]. However, it is predicted that the drug can modulate the activity of HDAC asses that SAR study of the carboxylate class shows that the carboxyl group in the metal binding domain possesses poor HDAC inhibitory activity. The activity of CHAP carboxylic acid analog put forward the surface recognizing domain to HDAC inhibitory activity. In the clinic, these agents have been studied for the treatment of cancer at high concentration doses. For example, butyric acid is a millimolar HDAC inhibitors. Butyric acid is produced in the human body through the metabolism of fatty acid (particularly in gluconeogenesis and ketone body formation) as well as bacterial fermentation of fiber in the colon. Other short-chain fatty acids, apart from butyric acid like sodium phenylbutyrate, sodium phenylacetate, and valproic acid are also concerned as HDAC inhibitor anti-proliferative agents [**35**].

In the field of drug design and drug discovery poly-pharmacology is now a days concerned as an emerging discipline. The ultimate goal of poly-pharmacology is to minimize the downside of polypharmacy by designing a single drug interacting with multiple targets. The fundamental difference between polypharmacy and poly-pharmacology is that the latter is performed for an undesirable reduction in cost and effort throughout the preclinical development process followed by clinical trials. It is a challenging job to design a selective drug for multiple targets considering the highly distinguished shapes of biological targets.

There is various sort of kinase found in human and animal bodies such as Janus kinase, cyclin-dependent kinase, phosphoinositide-3 kinase, receptor tyrosine kinase, etc. The fimepinostat is a drug of choice possess HDAC pharmacophore, i.e., a hydroxamate zinc binding group (ZBG), a pyrimidine linker and a cap group having phosphoinositide-3 kinase

inhibition property (pictilisib) CUDC-101 shows potent inhibitory activity against EGFR, HER2, and HDACs evaluated in clinical trials in the treatment of advanced solid tumor, viz, head and neck, breast, liver, gastric and non-small cell lung cancer whereas CUDC-907 was found to inhibit both HDACs and phosphoinositide 3 kinase (PI3K) in treatment of lymphoma, myeloid myeloma and advanced or solid tumor. The aforementioned US-FDA-approved drug romidepsin was also concerned with having dual inhibition of HDACs and PI3K [**7**].

Tinostamustine, another promising dual agent, functions as a hybrid of vorinostat (pan HDAC inhibitors) and bendamustine (alkylating agent) and has entered phase I/II trials against lung cancer, brain tumor, and hematological malignancies. On the other hand, chlorambucil/ vorinostat hybrid vorambucil resemble its parent compounds in term of both HDAC inhibition and anti-proliferative potential in four cancer cell lines. Chlordinaline possesses an amino-anilide-based HDAC binding site of tacedinaline linking with chlorambucil scaffold displaying HDAC3 preferential inhibition along with DNA damaging properties *in vivo.* Remnostat believes it impairs the formation of the epigenetic eraser lysine-specific demethylase that takes part in CoREST complex formation alongside HDAC1 and 2 unexpected inhibition of tubulin polymerization performed by HDAC inhibitors. The drug possesses clinical trials in phase I and phase II hematological and gastrointestinal cancer [**7**].

Anthracycline glycoside is known as a popular drug of choice in the treatment of cancer. Doxorubicin, daunorubicin, epirubicin, and idarubicin have come under the consideration of anthracycline glycoside. They are isolated from a microbe called *Streptomyces peuceticus*.

Anthracycline glycoside plays a crucial role in combating cancer pathology in several ways:

1. They bind and inactivate DNA-helicase and prevent replication fork formation.
2. Bind DNA polymerase and terminate the nucleotide polymerization which is an essential step to carry out daughter DNA strand formation.

3. Doxorubicin forms a complex with Fe^{2+} that further binds with DNA (Doxorubicin-Fe^{2+}-DNA complex) and promotes intra-strand formation that is unfit for replication.

4. Last but not least they bind topoisomerase I and topoisomerase II to terminate the uncoiling of double-strand and single-strands [**1**].

Previous studies asses that histone acetylation is reliable for binding positively charged histone with negatively charged DNA and causing chromosomal relaxation, four bromo, and extra terminal domain proteins-BRD2, BRD3, BRD4, and BRDT that acetylated histone. Hence, they are known as epigenetic readers [**44**]. Due to presumed association with super-enhancer that assemble transcription factors near oncogenes and boost cancer progression, it can be easily hypothesized that the most promising synergism concerns HDAC1, HDAC2, and specifically BRD4 could be inhibited to disturb the transcription machinery of super-enhancer by the help of Atkinson et al (2014) on DUAL946 several examples of BRD4/HDAC inhibitors have been disclosed [**44-47**]. Interestingly, He et al. [**48**] glorified their work as the pioneer to merge (+) JQ1, the first BET inhibitor. Along with phenyl linker and hydroxamate ZBG of HDAC inhibitors into their hit compound which possessed superior antitumor activity compared to their parent compound in a Capan-1 human pancreatic cancer Xenograft model [**48**].

Another novel approach to combat cancer pathogenesis is the so-called proteolysis targeting chimeras (PROTACs). PROTACs are bi-functional small molecules consisting of ubiquitin ligase recognition motifs as well as a ligand for the protein of interest. Due to their bi-functional nature, they can form a ternary complex, says E3 ligase, PROTACs, PO1, and as a consequence hijack the cellular protein degradation system by inducing poly-ubiquitinylation and subsequent proteasomal degradation of the po1. Moreover, they are preferable to classical small molecule inhibition in several ways:

i Catalytic mode of action ARV-471(estrogen receptor degrader) have ene,

ii Avoidance or resistance due to up-regulation of PO1

iii Possibility of drugging, currently un-druggable target by target degradation

iv Removal of all possible functions i.e. enzymatic, scaffolding, regulatory, etc.

Very recently ARV-110 (androgen receptor degrader) and ARV-471 (estrogen receptor degrader have entered clinical phase I trial as the first PROTACs and have shown promising early data in terms of tolerability, safety, and efficacy [**49**].

The efficacy of HDAC inhibitors tested in a clinical trial has been largely restricted to hematological malignancies with positive therapeutic responses in leukemia, lymphoma, and multiple myeloma, why HDAC are more effective in hematological malignancy not clear properly although it is presumed that poor pharmacokinetic properties of some HDAC inhibitors for example short half-life that restrict them to distribute to solid tumors, however, selective and HDAC inhibitors utilizing accurate drug delivery like Novel drug delivery system (NDDS) may assist to overcome inefficient bioavailability [**4**]. Another obstacle that limits the use of HDAC inhibitors is their side effects and toxicity, the common toxicity related to vorinostat, belinostat, and romidepsin were nausea, vomiting, anorexia, and fatigue. HDAC inhibitors have a broad effect on chromatin and can reverse the aberrant epigenetic changes in cancer. Although the second generation of HDAC inhibitors has been developed with improved pharmacokinetic and pharmacodynamic values [**4**].

HDAC1 has been implicated in cell cycle progression, the growth ability of HDAC1 cells to that of control cells. It was found that there was no effect of deleting HDAC1 or HDAC2 or colony formation when plating ES cells at low density or on their population time. Loss of MBD3, a central component of the NuRD complex, or treatment with HDAC inhibitor has been demonstrated to inhibit ES cell differentiation [**50**].

Dynamic transmission between different conformational states are well-known phenomena in enzymology-such motions are considered intrinsic enzymatic properties but they can also be a consequence of external perturbation such as ligand or protein binding [**11**].

Class I HDAC contain an N-terminal catalytic domain having 400 amino acids, 39 of crystallographic human HDACs (isoform 1, 2, 3, 4, 7, and 9) and HDAC homology from bacteria (HDLP and HDAH) have been solved. Among class I HDACs a 14Å tunnel located perpendicular to the 11 Å channel bottom is also conserved. This is water filled foot pocket in the catalytic domain. This is suggested to be an aggressive route for the acetate product and potentially an additional target area for selective structure-based inhibitor design. An aromatic wall formed by face-to-face Phe150/Phe155 and Phe205/Phe210 residue lines. The 11 Å channel entrance of HDAC1 and HDAC2 respectively [**51**].

The overall fold of zinc-dependent HDAC comprises a single compound having an α/β-domain composed of central eight standard parallel β sheets flanked by several α-helix on both sides. The binding site architecture is almost all for all sorts of HDAC. The walls of the channel are lined mainly by hydrophobic residue Pro542, Gly678, Phe679, Phe738, and Leu810. The His-Asp arrangement is typical of serine protease where the aspartic acid. Carboxylate oxygen accepts a hydrogen bond from Nδ-1 and polarizes the imidazole Nε-2 increasing its basicity. Mutagenesis studies say that histidine and aspartic acid residue of the buried charged is necessary to achieve the effective enzymatic activity, the H150A mutation of RPD3 lost HDAC activity D174N in HDAC1 leads to an approximately12 times drop in HDAC activity compared with wild-type class I and class II have two different metal binding site-1 and site-2 [**51**].

Chapter 3: Rationale Behind the Study

Rationale Behind Selection of HDAC1 inhibitors

HDACs are essential group of enzymes that not only promote tumorigenesis but are also associated with several diseases like-metabolic default, inflammation, cardiovascular disorder, rheumatoid arthritis, etc [**43**]. To combat all these diseases (such as cancer) in terms of human benefit there are a number of drugs have been launched to date and administered either alone or in combination with earlier established anti-neoplastic agents like cisplatin, doxorubicin, vinca alkaloids, podophyllotoxin, etc [**7**]. There are 18 HDAC enzymes all of them come under the consideration of nearly four groups, i.e., class I, class II, class III, and class IV. Among them class I and class II are regarded as classical HDACs. However, the nomenclature assigned on behalf of their discovery which started with HDAC1 first come to light in the year 1996 [**51**]. However, both class I and class II are containing metal-enzyme where Zn^{2+} lies between L4 and L7 loops [**51**]. Overall, fold zinc dependent HDAC comprises central eight standard parallel β sheets flanked by several α heli on both sides. There is a great similarity in binding site architecture for all sort of HDACs-walls of the channel is lined principally by hydrophobic residues Pro542, Gly678, Phe679, Phe738, and Leu810. In the case of class I HDACs, a 14 Å tunnel located perpendicularly to the 11 Å channel bottom- this is regarded as the catalytic domain of HDAC also known as 'foot pocket' and filled with a water molecule. The 'foot pocket' is an aggressive route for acetate products as well as an additional target area for selective structure-based inhibitors. The aromatic wall is formed by face-to-face Phe180/Phe185. Mutagenesis theory assigned that mutation of D174N in HDAC1 can lose activity of deacetylation that plays a vital role in the dissolution of positively charged histone from negatively charged DNA replication [**51**]. HDAC1 directly stimulate oxoguanine glycosylase OGG1, a base repair of oxidized guanine residue. It is observed that many HDAC inhibitors are effective in hematological malignancies- although the exact cause is still not clear properly. It can be assessed that poor

pharmacokinetic properties of some HDAC inhibitors, for example, short half-life that restrict them to distribute to solid tumors. With the help of NDDS selective and accurate drug delivery of HDAC inhibitors can overcome the efficiency of bioavailability a good number of the drug has been launched to decrease the activity of HDAC1 [**4, 7**].

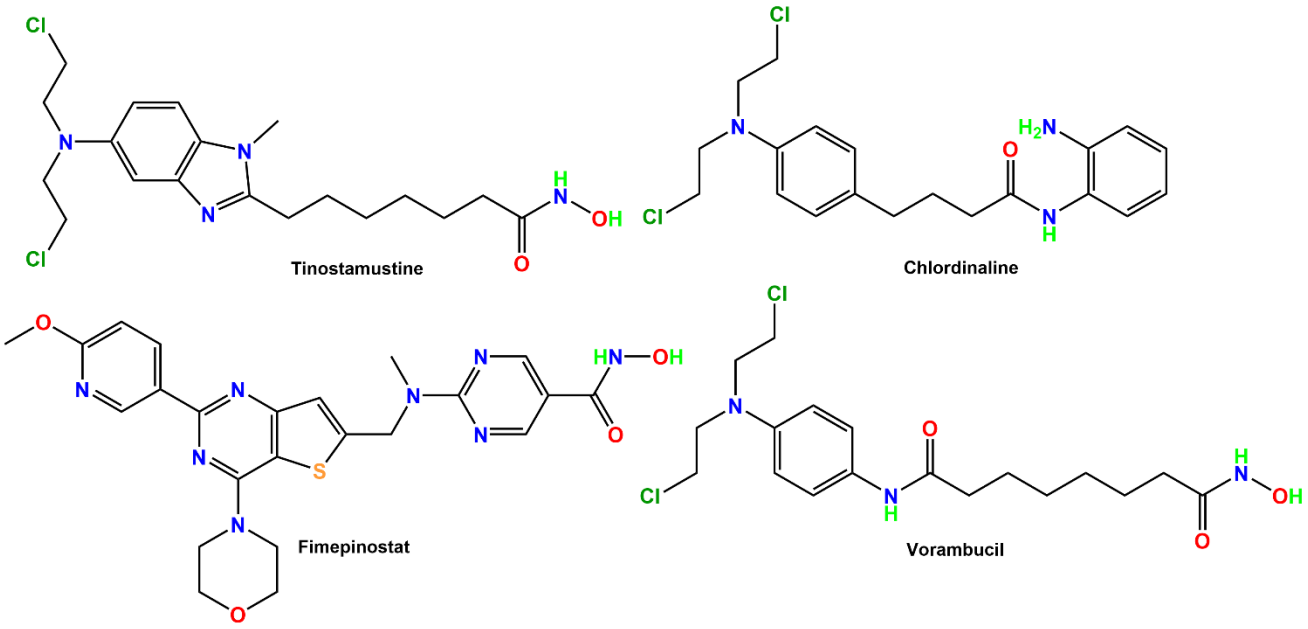


Figure 3: Chimeric compound used in poly-pharmacology

Trichostatin A, an earlier compound used in the treatment of schistosomiasis also proved beneficial effect in the termination of cancer. Trichostatin A has a hydroxamate group as a zinc-binding group (ZBG). Suberoylanilide hydroxamic acid (SAHA) was also designed based on trichostatin A because the double alkene group and an extra methyl group can reduce the activity of HDAC1 [**35**]. Another novel approach is poly-pharmacology which is utilized nowadays to decline dose-dependent side-effect of polypharmacy where the combination of drugs shows synergistic additive property, for example, fimepinostat (**Figure 3**) is used to block both HDAC and kinase enzyme (*viz,* phosphoinositide3K, tyrosine kinase, etc).

Tinostamustine (**Figure 3**) serves as a chimeric compound having properties both of bendamustine and vorinostat, vorambucil (**Figure 3**) also poses pharmacological activity by blocking HDAC and intercalation of DNA molecules [**7**].

In this landscape, the development of potential HDAC1 inhibitors can be the prime solution for the treatment of HDAC1-related pathophysiological conditions.

Furthermore, quantitative structure-activity relationships (QSARs), are one of the most widely used methods to correlate the molecular features of compounds with their biological activity via mathematical means [**52**]. These statistics-derived techniques help to important molecular structural features by correlating them with their biological activities which can guide the lead identification and optimization to design newer molecules to ease the anti-cancer drug development process [**52**].

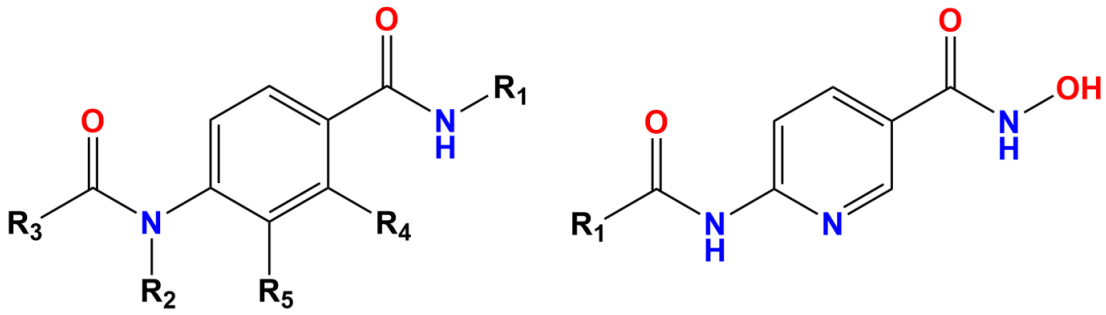
Therefore, in this work, the quantitative structure-activity relationship (QSAR) study has been conducted on a set of 66 HDAC1 inhibitors [**53-54**] with a wide range of HDAC1 inhibitory activity which has elucidated several important structural aspects of these HDAC1 inhibitors that will assist the design and development of potential HDAC1 inhibitor for the treatment of HDAC1-related disease conditions.

Chapter 4: Materials and Methods

➢ ***Dataset selection and preparation***

A total of 66 hydroxamate-containing AR-42 derivatives (**Table 2**) having a wide range of *in vitro* HDAC1 inhibitory activity (IC_{50} value ranging from 0.72 nM - 8,680 nM) were mustered together from the literature [**53**-**54**]. To maintain the uniformity of the dataset, the mean HDAC1 inhibitory activity (IC_{50} in nM) values were transformed into their negative logarithmic scale. A set of 1,444 2D molecular descriptors along with 881 PubChem fingerprint descriptors were calculated for each compound using PaDEL descriptor software [**55**] followed by the dataset pre-treatment technique to remove the highly correlated descriptors. The dataset division was carried out using the Kennard-Stone (*KS*) method using DTC Lab software [**56**] where a 3:1 ratio was preserved for the training and the test sets (N_{Train} = 49, N_{Test} = 19).

Table 2: Common structures, SMILES notation, and HDAC1 inhibitory activity of 66 AR-42 analogs



Cpd No	*SMILES*	IC_{50}	pIC_{50}
1*	c1ccc(cc1)[C@@H](C(=O)Nc1ccc(cc1)C(=O)NO)C(C)C	25	7.602
2	c1c(ccc(c1)C(=O)Nc1ccccc1N)NC(=O)[C@H](c1ccccc1)C(C)C	231	6.636
3	c1c(ccc(c1)C(=O)NO)N(C(=O)[C@@H](C(C)C)c1ccccc1)C	439	6.358
4	c1c(ccc(c1)C(=O)NO)C(=O)N[C@H](c1ccccc1)C(C)C	2116	5.674
5	c1c(ccc(c1)C(=O)NO)NC(=O)Cc1ccccc1	75	7.125
6	c1c(ccc(c1)C(=O)NO)NC(=O)C(c1ccccc1)c1ccccc1	250	6.602
7	c1c(ccc(c1)C(=O)NO)NC(=O)C(CCC)CCC	738	6.132
8	c1c(cc(c(c1)C(=O)NO)Cl)NC(=O)[C@H](c1ccccc1)C(C)C	8680	5.061
9*	c1c(c(cc(c1)C(=O)NO)Cl)NC(=O)[C@@H](c1ccccc1)C(C)C	1065	5.973
10	c1c(ncc(c1)C(=O)NO)NC(=O)[C@H](c1ccccc1)C(C)C	22	7.658

11*	c1c(ccc(c1)C(=O)NO)NC(=O)C(c1ccccc1)(C)C	218	6.662
12	c1c(ccc(c1)C(=O)NO)NC(=O)C1(c2ccccc2)CC1	93	7.032
13	c1c(ccc(c1)C(=O)NO)NC(=O)C(c1ccccc1)(CC)CC	430	6.367
14	c1c(ccc(c1)C(=O)NO)NC(=O)C1(c2ccccc2)CCCC1	28	7.553
15	c1c(ccc(c1)C(=O)NO)NC(=O)C1(c2ccccc2)CCCCC1	15	7.824
16	c1c(ccc(c1)C(=O)NO)NC(=O)C1(c2ccccc2)CCOCC1	28	7.553
17	c1c(ccc(c1)C(=O)NO)NC(=O)C1(c2ccccc2)CCNCC1	12	7.921
18	c1c(ncc(c1)C(=O)NO)NC(=O)C1(c2ccccc2)CCCC1	132	6.879
19*	c1c(ccc(c1)C(=O)NO)NC(=O)C1(c2ccc(cc2)F)CCCC1	41	7.387
20	c1c(ccc(c1)C(=O)NO)NC(=O)C1(c2ccc(cc2)Cl)CCCC1	12	7.921
21	c1c(ccc(c1)C(=O)NO)NC(=O)C1(c2ccc(cc2)Br)CCCC1	7	8.155
22	c1c(ccc(c1)C(=O)NO)NC(=O)C1(c2ccc(c(c2)F)C(F)(F)F)CCCC1	227	6.644
23	c1c(ccc(c1)C(=O)NO)NC(=O)C1(c2ccc(cc2)N(C)C)CCCC1	4	8.398
24	c1c(ccc(c1)C(=O)NO)NC(=O)C1(c2ccc(cc2)c2ccccc2)CCCC1	16	7.796
25*	c1c(ccc(c1)C(=O)NO)NC(=O)C1(c2ccc(c(c2)F)c2ccccc2)CCCC1	42	7.377
26	c1c(ccc(c1)C(=O)NO)NC(=O)C1(c2ccc3c(c2)cccc3)CCCC1	11	7.959
27	c1c(ccc(c1)C(=O)NO)NC(=O)C1(CCCC1)c1c2c([nH]c1)cccc2	23	7.638
28	c1c(ccc(c1)C(=O)NO)NC(=O)C1(CCCC1)c1csc(n1)c1ccccc1	12	7.921
29*	c1c(ccc(c1)C(=O)NO)NC(=O)C1(CCCC1)c1csc(n1)c1cc(ccc1)OC	15	7.824
30	c1c(ccc(c1)C(=O)NO)NC(=O)C1(CCCC1)c1csc(n1)c1ccccc1Oc1ccccc1	431	6.366
31	c1c(ccc(c1)C(=O)NO)NC(=O)C1(c2c[nH]c(n2)c2ccccc2)CCCC1	3	8.523
32	c1c(ccc(c1)C(=O)NO)NC(=O)C1(CCCC1)c1c[nH]c(n1)c1ccc(cc1)Br	10	8.000
33	c1c(ccc(c1)C(=O)NO)NC(=O)C1(CCCC1)c1c[nH]c(n1)c1ccc(cc1)[N+](=O)[O-]	6	8.222
34	c1c(ccc(c1)C(=O)NO)NC(=O)C1(CCCC1)c1ccc(cc1)c1cn[nH]c1	3	8.523
35	c1c(ccc(c1)C(=O)NO)NC(=O)C1(CCCC1)c1ccc(cc1)c1cc[nH]n1	4	8.398
36	c1c(ccc(c1)C(=O)NO)NC(=O)C1(c2ccc(cc2)c2cn[nH]c2C)CCCC1	5	8.301
37	c1c(ccc(c1)C(=O)NO)NC(=O)C1(CCCC1)c1ccc(cc1)c1c(n[nH]c1C)C	8	8.097
38	c1c(ccc(c1)C(=O)NO)NC(=O)C1(CCCC1)c1ccc(cc1)c1ccsc1/C(=N/O)/C	43	7.367
39	c1c(ccc(c1)C(=O)NO)NC(=O)C1(CCCC1)c1ccc(cc1)c1ccccc1NC(=O)C	0.94	9.027
40	c1c(ccc(c1)C(=O)NO)NC(=O)C1(CCCC1)c1ccc(cc1)c1cccc(c1)N	4	8.398
41*	c1c(ccc(c1)C(=O)NO)NC(=O)C1(CCCC1)c1ccc(cc1)c1cccc(c1)NC(=O)C	5	8.301
42*	c1c(ccc(c1)C(=O)NO)NC(=O)C1(CCCC1)c1ccc(cc1)c1cccnc1	5	8.301
43	c1c(ccc(c1)C(=O)NO)NC(=O)C1(CCCC1)c1ccc(cc1)c1ccc(nc1)OCc1ccccc1	240	6.620
44*	c1c(ccc(c1)C(=O)NO)NC(=O)C1(CCCC1)c1ccc2c(c1)cc[nH]2	8	8.097
45*	c1c(ccc(c1)C(=O)NO)NC(=O)C1(CCCC1)c1ccc(cc1)c1cccc(c1)C(=O)O	2	8.699
46	c1c(ccc(c1)C(=O)NO)NC(=O)C1(c2ccc(c(c2)OC)OC)CCCCC1	29	7.538
47	c1c(ccc(c1)C(=O)NO)NC(=O)C1(c2ccc3c(c2)OCO3)CCCCC1	42	7.377
48*	c1c(ccc(c1)C(=O)NO)NC(=O)C1(c2ccc(c(c2)Br)OC)CCCCC1	31	7.509
49	c1c(ccc(c1)C(=O)NO)NC(=O)C1(c2ccc(c(c2)c2cccc(c2)N)OC)CCCCC1	10	8.000
50	c1c(ccc(c1)C(=O)NO)NC(=O)C1(c2ccc(c(c2)c2ccccc2NC(=O)C)OC)CCCCC1	9	8.046
51	c1c(ccc(c1)C(=O)NO)NC(=O)C1(c2ccc(c(c2)c2cn[nH]c2)OC)CCCCC1	3	8.523
52	c1c(ccc(c1)C(=O)NO)NC(=O)C1(c2ccc(cc2)c2cc(ccc2NC(=O)C)N)CCCCC1	10	8.000
53	c1c(ccc(c1)C(=O)NO)NC(=O)C1(c2ccc(cc2)c2cn[nH]c2)CCOCC1	2	8.699

54*	c1c(ccc(c1)C(=O)NO)NC(=O)C1(c2ccc(cc2)c2cn[nH]c2)CCCCC1	2	8.699
55*	c1(ccc(cc1)C1(C(=O)Nc2ccc(cc2)C(=O)NO)CCOCC1)Br	2	8.699
56	c1c(ccc(c1)C(=O)NO)NC(=O)C1(c2ccc(cc2)Br)CCCCC1	63	7.201
57	c1c(ccc(c1)C(=O)NO)NC(=O)C1(c2ccc(cc2)[N+](=O)[O-])CCOCC1	9	8.046
58	c1c(ccc(c1)C(=O)NO)NC(=O)C1(c2ccc(cc2)N)CCOCC1	22	7.658
59	c1c(ccc(c1)C(=O)NO)NC(=O)C1(c2ccc(c(c2)Cl)Cl)CCOCC1	6	8.222
60	c1c(ccc(c1)C(=O)NO)NC(=O)C1(c2ccc3c(c2)cccc3)CCOCC1	0.72	9.143
61	c1c(ccc(c1)C(=O)NO)NC(=O)C1(c2ccc3c(c2)cccc3)CCCCC1	43	7.367
62*	c1c(c(cc(c1)C(=O)NO)F)NC(=O)C1(c2ccc(cc2)Cl)CCCC1	774	6.111
63	c1c(c(cc(c1)C(=O)NO)F)NC(=O)C1(c2ccc(cc2)F)CCCC1	204	6.690
64*	c1(ccc(cc1)C1(C(=O)Nc2ccc(cc2)C(=O)NO)CCNCC1)Br	3	8.523
65*	c1c(c(cc(c1)C(=O)NO)F)NC(=O)C1(c2ccccc2)CCOCC1	73	7.137
66*	c1c(c(cc(c1)C(=O)NO)F)NC(=O)C1(c2ccccc2)CCCC1	181	6.742

** marked molecules are considered as the test set instances*

- ***Regression-based multiple linear regression (MLR) model development***

When correlating the relationship between a scalar answer and one or more explanatory variables in statistics, linear regression is a linear method (also having dependent and independent variables). Simple linear regression is used when there is only one explanatory variable, and multiple linear regression is used when there are numerous variables. [**57**] As opposed to multivariate linear regression, which predicts several correlated dependent variables as opposed to a single scalar variable, this phrase is more general [**58**].

In linear regression, the relationships are modeled using linear predictor functions, and the model's unobserved parameters are inferred from the data. These models are referred to as linear models. [**59**] The conditional mean of the response is typically considered to be an affine function of the values of the explanatory variables (or predictors); the conditional median or another quantile is occasionally employed. In common with all other types of regression analysis, linear regression concentrates on the conditional probability distribution

of the response given the values of the predictors rather than the joint probability distribution of all these variables, which is the purview of multivariate analysis.

In the larger category of evolutionary algorithms, the genetic algorithm (GA) is a metaheuristic that draws inspiration from the process of natural selection (EA). Utilizing biologically inspired operators such as mutation, crossover, and selection, genetic algorithms are frequently employed to produce high-quality solutions to optimization and search problems [**60**]. Here, the correlation between chemical descriptors (independent variables) and biological activity (dependent variable) can be achieved by multiple linear regression (MLR) analysis [**61**]. Here, the multiple linear regression model was developed by the genetic algorithm-based best subset selection method.

- ***Classification-based linear discriminant analysis (LDA) model development***

Fisher's linear discriminant analysis is a technique used in statistics and other fields to identify a linear combination of features that distinguishes between two or more classes of objects or events. Its generalizations include linear discriminant analysis (LDA), normal discriminant analysis (NDA), and discriminant function analysis. The resulting mixture can be applied as a linear classifier or, more frequently, to reduce the dimensionality before a subsequent classification. Regression analysis and analysis of variance (ANOVA), which both aim to express one dependent variable as a linear mixture of other traits or measures, are closely connected to LDA [**62-63**]. Discriminant analysis, on the other hand, utilizes continuous independent variables and a categorical dependent variable while ANOVA employs categorical independent variables and a continuous dependent variable (i.e., the class label) [**64**].

Linear discriminant analysis (LDA) is a generalization of Fisher’s Linear Discriminant technique which is defined as a statistical pattern recognition and coefficient-based machine

learning method used to find a linear contribution of features/variables which can be able to classify objects in two or more difficult groups, LDA is a binary classification based QSAR technique. In LDA, similar to MLR, it tries to correlate the independent variables with the response. An LDA equation (*equation 1*) can be written as:

$$DF = C_1 * X_1 + C_2 * X_2 + \ldots\ldots + C_n * X_n + a \qquad \textbf{Equation (1)}$$

In the above equation, the discriminant function is described as *DF*, C represents the discriminant constant, *X* refers to the independent variable, *n* is the number of predictors, and a signifies the constant value.

Being a pattern recognition method, LDA provides a classification model based on the combination of descriptors that may predict the groups on category best to which a given compound belongs [**61**]. Here, to perform the LDA study, the biological activity of the 66 AR-42 analogs (Compound **1**-**66**, **Table 2**) was transformed into a binary manner by calculating their average pIC_{50} value (mean pIC_{50} = 7.588). Therefore, considering the pIC_{50} value of 7.600 as the threshold for the binary categorization, the compounds having a pIC_{50} value of 7.600 or above were considered as '*higher active*' inhibitors (*N* = 37), and the compounds with a pIC_{50} value less than 7.600 was considered as '*lower active*' inhibitors (*N* = 29). The LDA model has been developed in a forward stepwise feature selection method with a $F_{Inclusion}$ of 4.0 and $F_{Exclusion}$ of 3.9 [**61**].

- ***Statistical metrics for model evaluation***

- *Statistical parameters for the regression-based model evaluation*

The developed MLR model was evaluated for this performance and reliability based on statistical parameters that include the correlation coefficient (*R*), squared correlation

coefficient (R^2), adjusted R^2 (R^2_{Adj}), variation ratio (F) at a specified degree of freedom (df) and standard error estimate (SEE). The SEE for the residue was calculated by *equation 2*:

$$SEE=\sqrt{\frac{\sum(Yobs-Ycalc)\hat{}2}{n-p-1}} \qquad \textbf{\textit{Equation (2)}}$$

Where Y_{obs} and Y_{calc} are observed and predicted activities of these AR-42 analogs predicted by the MLR model for the training set, where n is the number of instances in the training set and p represents the number of variables in the model.

Fischer value is a measure of the portability of the QSAR equation onto other sets of data that suggest the level of statistical significance; simultaneously the p (acceptable value $p < 0.5$) values are checked and considered for the descriptor is a measure for coincidental correlation. A p-value suggests the highly significant MLR model. The predictivity of any regression-based QSAR model can be judged primarily based on its R^2 internally cross-validated R^2 (Q^2) value.

Moreover, the predicted residual sum of squares (*PRESS*) was calculated to judge the internal predictive ability further of the QSAR equation. The external predictive ability of the QSAR equation was checked by external validated R^2 (R^2_{pred}) value for test set compounds

- *Statistical parameters for the linear discriminant analysis (LDA) evaluation*

The equality of the LDA model was evaluated using Wilk's parameter (λ) which was obtained by a multivariable analysis of variance that judged the equality [54].

- *Betterment of fit and quality measurements*: Wilk's lambda (*equation 3*) was used to observe the betterment of fit of the LDA method. It is a distance-based scaler transformation calculation that evaluates the fitting performance of the LDA model.

$$Wilk's\ lambda(\lambda) = \frac{Wittickgroupofsumofsquare}{total\ no\ of\ square} \qquad \textbf{Equation (3)}$$

The value of $\lambda = 0$ determines minimum difference values between the classes/groups signifies good discrimination whereas $\lambda = 1$ implies no difference between groups. Therefore, the lower value of λ indicates better the discrimination capability of the model. Also, the canonical correlation coefficient (R_C), chi-square (χ^2), squared Mahalanobis distance (D_M^2) and the F value were also determined to justify the reliability of the LDA model [**61**].

- *Measurement of predictive capability*: For the evaluation of the predictability of the LDA model statistical properties such as sensitivity (*Se*), specificity (*Sp*), precision (*Pr*), accuracy (*Acc*), F_1 measure, and Matthews's correlation coefficient (*MCC*) were computed by the following equations (*equation 4* to *equation 9*):

$$Sensitivity = \frac{TP}{(TP+FN)} \qquad \textbf{Equation (4)}$$

$$Specificity = \frac{TN}{(TN+FP)} \qquad \textbf{Equation (5)}$$

$$Precision = \frac{TP}{(TP+FP)} \qquad \textbf{Equation (6)}$$

$$Accuracy = \frac{(TP+TN)}{(TP+FP+TN+FN)} \qquad \textbf{Equation (7)}$$

$$F_1 = \frac{2TP}{(2TP+FP+FN)} \qquad \textbf{Equation (8)}$$

$$\text{MCC}=\frac{(TP*TN)-(FP*FN)}{\sqrt[2]{(TP+FP)(TP+TN)(TN+FP)(TN+FN)}} \qquad \textbf{\textit{Equation (9)}}$$

Here, *TP* is the true positive or the number of known '*active*' compounds that appeared as '*active*' in the model, *FN* indicates the false negative which is known as an '*active*' compound predicted as '*inactive*' whereas *TN* is the true negative that is the number of known '*inactive*' molecules predicted as 'inactive', and *FP* represents false positive that is the number of known '*inactive*' predicted as '*active*' [**61**].

Chapter 5: Results and Discussion

- ***Regression-based MLR model***

The final MLR model (*equation 3*), consisted of 6 descriptors was selected based on their Q^2 and R^2_{pred} values. The selected MLR model is provided below-

HDAC1 pIC50 = 4.91659 (+/- 0.35329) - 1.47059 (+/- 0.43252) ***PubchemFP261*** - 0.22761 (+/- 0.04096) ***MDEC-33*** + 0.79897 (+/- 0.23129) ***MLFER_E*** + 0.29416 (+/- 0.0936) ***nHBAcc*** -0.07764 (+/- 0.03676) ***ATSC8p*** + 2.16325 (+/- 0.95294) ***VE1_Dzv*** ***Equation (10)***

N_{train} = 49, N_{test} = 17, R = 0.883, R^2 = 0.779, R^2_A = 0.748, Q^2 = 0.719, *SEE* = 0.436, *PRESS* = 7.998, F (6, 42) = 24.692, *Avg.* $r_m^2{}_{LOO}$ = 0.611, cR^2_p = 0.720, R^2_{pred} = 0.721; *Avg.* $r_m^2{}_{test}$ = 0.652, $p< 0.05$.

The above statistics depicts that the MLR model (*equation 10*) clearly explained 74.8% and predicted 71.9% variance in the inhibitory activity of these molecules for HDAC1. Additionally, the MLR model (*equation 10*) also passed the Golbraikh and Tropsha model acceptability criteria [**65**] (**Table 3**). The observed *vs* predicted activity plot for the constructed MLR model is given in **Figure 4A**. Also, the applicability domain of the MLR model (*equation 10*) was checked for the model using the Euclidean-distance-based normalized mean distance values for each of the dataset molecules for the MLR model (**Figure 4B**) [**66**]. Additionally, none of the compounds from the model exhibited a higher normalized mean distance (>1.0).

Table 3. Golbraikh and Tropsha criteria's for MLR model

Parameter	***Threshold***	***MLR Model (Equation 10)***
Q^2	$Q^2>0.5$	0.719
r^2	$r^2>0.6$	0.743
r_0^2-$r_0'^2$	$\|r0^2-r'0^2\| < 0.3$	0.053
k	$0.85 < k < 1.15$	1.017
k'	$0.85 < k' < 1.15$	0.981
$(r^2-r_0^2)/r^2$	$[(r^2-r0^2)/r^2] < 0.1$	0.003

$(r^2-r_0'^2)/r^2$	*[(r^2-r'0^2)/r^2] < 0.1*	0.074

r^2, Squared correlation coefficient between observed *vs* predicted response of the test set compounds; r_0^2, the values for regression through origin (observed *vs* predicted); $r_0'^2$, The values for regression through origin (predicted *vs* observed); *k*, Slope of the regression lines through the origin for observed vs predicted; *k'*, Slope of the regression lines through the origin for predicted vs observed.

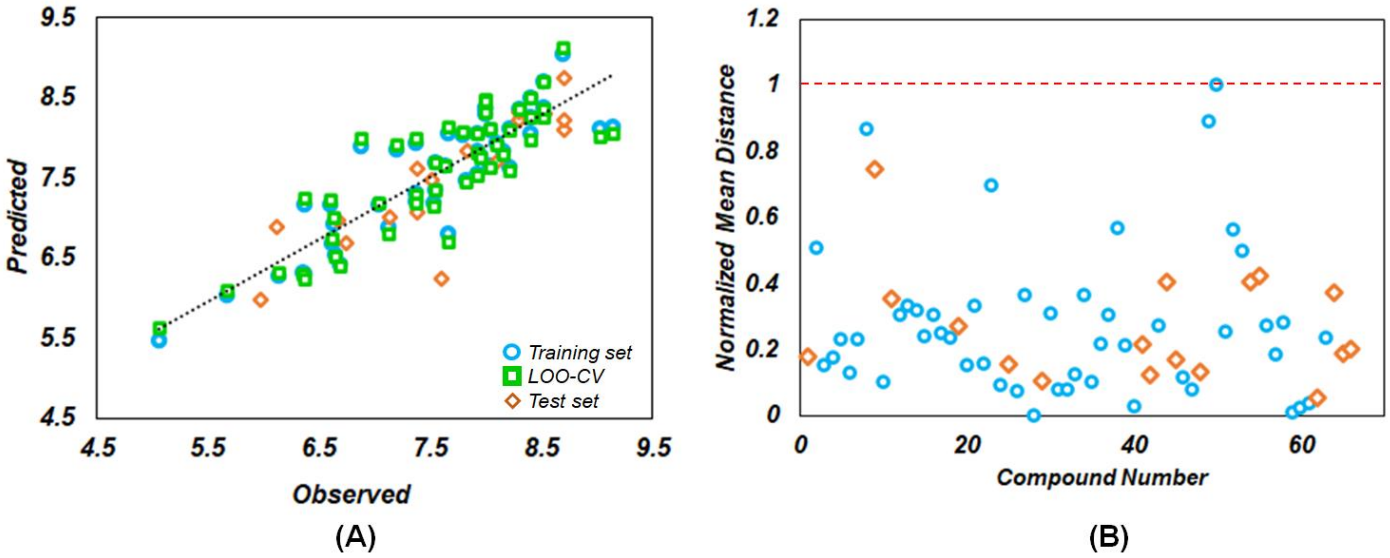


Figure. **4. (A)** The observed *vs* predicted activity of the training set, *LOO-CV* and the test set from the MLR model; **(B)** Normalized mean distance values *vs* compound numbers for the MLR model for the investigation of applicability domain (AD).

Furthermore, the description of the equation 10 variables along with the observed and the MLR predicted activities as well as the values of the individual predictor variables are also provided in **Table 4** and **Table 5**.

Table 4. Description of the different descriptors used for the LDA analysis

Sl No.	*Descriptor*	*Description*	*Contribution*
1	*PubchemFP261*	Presence of 4 aromatic rings	Negative
2	*MDEC-33*	Molecular distance edge between all tertiary carbons	Negative

3	*MLFER_E*	Excessive molar refraction	Positive
4	*nHBAcc*	Number of hydrogen bond acceptors	Positive
5	*ATSC8p*	Centered Broto-Moreau autocorrelation - lag 8 / weighted by polarizabilities	Negative
6	*VE1_Dzv*	Coefficient sum of the last eigenvector from Barysz matrix / weighted by van der Waals volumes	Positive

Table 5. Observed and predicted values with the descriptors used to develop *equation 10*

Cpd No	***PubchemFP261***	***MDEC-33***	***MLFER_E***	***nHBAcc***	***ATSC8p***	***VE1_Dzv***	***Observed***	***Predicted***
1*	0	6.596306	1.901	3	-0.13204	0.19437	7.602	6.247
2	0	8.850397	2.827	5	-0.78212	0.104577	6.636	6.919
3	0	6.596306	1.951	3	-0.53187	0.192525	6.358	6.314
4	0	6.470167	1.847	3	-0.07223	0.106681	5.674	6.038
5	0	2.812801	1.931	3	-1.23899	0.037111	7.125	6.878
6	0	6.596306	2.540	3	-1.51917	0.332969	6.602	7.165
7	0	3.193226	1.292	3	-1.32234	0.035765	6.132	6.285
8	0	8.839331	2.036	3	2.637415	0.123134	5.061	5.475
9*	0	8.988959	2.036	3	-4.06611	0.132447	5.973	5.982
10	0	6.596306	2.064	4	-1.05983	0.227094	7.658	6.814
11*	0	2.812801	1.910	3	0.959098	0.170002	6.662	6.978
12	0	2.812801	2.118	3	0.655587	0.172316	7.032	7.173
13	0	2.812801	1.910	3	0.807249	0.254858	6.367	7.174
14	0	2.812801	2.118	3	0.768178	0.262704	7.553	7.360
15	0	2.812801	2.118	3	-0.11318	0.285349	7.824	7.477
16	0	2.812801	2.131	4	0.795351	0.272886	7.553	7.684
17	0	2.812801	2.256	4	0.350123	0.27539	7.921	7.824
18	0	2.812801	2.281	4	0.193405	0.296105	6.879	7.901
19*	0	3.451531	2.000	3	0.939785	0.251816	7.387	7.083
20	0	3.451531	2.253	3	-1.83039	0.274036	7.921	7.548
21	0	3.451531	2.426	3	-3.52147	0.278892	8.155	7.828
22	0	4.935486	1.708	3	0.482743	0.247279	6.644	6.538
23	0	3.451531	2.368	4	-5.60312	0.197998	8.398	8.063
24	0	4.536698	2.990	3	-2.18394	0.332535	7.796	8.044
25*	0	6.418872	2.872	3	-2.28822	0.372508	7.377	7.616
26	0	4.935486	2.854	3	-0.06356	0.376899	7.959	7.776
27	0	5.525066	2.688	4	1.971746	0.378638	7.638	7.649
28	0	4.935486	2.948	4	-1.08662	0.299822	7.921	8.058
29*	0	6.027659	2.970	4	-2.54853	0.259225	7.824	7.853
30	1	7.641185	3.594	4	-1.71071	0.194606	6.366	6.309
31	0	4.935486	2.802	5	-2.02973	0.285948	8.523	8.279
32	0	5.760675	3.110	5	-1.52712	0.281292	8.000	8.288

33	0	5.760675	3.083	5	-2.43164	0.175949	8.222	8.109
34	0	4.536698	2.924	5	-4.14167	0.314342	8.523	8.693
35	0	4.536698	2.924	5	-2.03922	0.299544	8.398	8.497
36	0	5.884996	2.932	5	-3.23558	0.328704	8.301	8.353
37	0	7.62755	2.940	5	-2.27764	0.350006	8.097	7.934
38	0	7.422541	3.336	3	2.042289	0.320795	7.367	7.310
39	0	7.041611	3.329	5	-0.94683	0.278312	9.027	8.120
40	0	5.543473	3.265	4	-1.49076	0.327683	8.398	8.265
41*	0	6.542936	3.329	5	-2.70589	0.21203	8.301	8.227
42*	0	4.536698	2.968	4	-2.80922	0.313216	8.301	8.328
43	1	5.994186	3.614	4	-3.3164	0.126676	6.620	6.677
44*	0	4.935486	2.688	4	2.206923	0.35104	8.097	7.706
45*	0	6.852351	3.110	5	-1.36574	0.31486	8.699	8.100
46	0	4.935486	2.162	3	-2.52275	0.270527	7.538	7.184
47	0	4.935486	2.368	3	0.047645	0.294258	7.377	7.201
48*	0	4.935486	2.448	3	-2.74322	0.290396	7.509	7.473
49	0	7.935643	3.287	4	-6.03677	0.449528	8.000	8.354
50	0	9.808318	3.351	5	-4.89734	0.412729	8.046	8.105
51	0	6.601578	2.946	5	-3.10871	0.414941	8.523	8.378
52	0	8.571831	3.604	6	-1.38324	0.304925	8.000	8.377
53	0	4.536698	2.937	6	-4.32907	0.329767	8.699	9.045
54*	0	4.536698	2.924	5	-4.36683	0.335093	8.699	8.755
55*	0	3.451531	2.439	4	-4.2306	0.29269	8.699	8.218
56	0	3.451531	2.426	3	-3.10583	0.303553	7.201	7.850
57	0	3.451531	2.412	4	0.531966	0.214784	8.046	7.658
58	0	3.451531	2.406	5	0.728288	0.275138	7.658	8.063
59	0	4.935486	2.401	4	-0.54445	0.324912	8.222	7.633
60	0	4.935486	2.867	4	-0.39166	0.391489	9.143	8.138
61	0	4.935486	2.854	3	-1.39276	0.398685	7.367	7.927
62*	0	5.375078	2.135	3	-1.62887	0.222216	6.111	6.889
63	0	5.375078	1.882	3	1.099334	0.199666	6.690	6.426
64*	0	3.451531	2.564	4	-3.92638	0.294867	8.523	8.299
65*	0	4.770542	2.013	4	0.985002	0.221498	7.137	7.018
66*	0	4.770542	2.000	3	0.930566	0.212209	6.742	6.698

** marked molecules are considered as the test set instances*

Interestingly, from the contributions of the selected features in the MLR model (equation 10), it is observed that PubChem fragment feature *PubchemFP261* (Presence of 4 aromatic rings), *MDEC-33* (Molecular distance edge between all tertiary carbons), and *ATSC8p* (Centered Broto-Moreau autocorrelation - lag 8 / weighted by polarizabilities) contributed negatively

with the HDAC1 activity, suggesting their negative contribution for higher HDAC1 inhibition for these hydroxamate-based AR-42 derivatives. On the other hand, the descriptors *MLFER_E* (Excessive molar refraction), *nHBAcc* (Number of hydrogen bond acceptors), and *VE1_Dzv* (Coefficient sum of the last eigenvector from Barysz matrix / weighted by van der Waals volumes) showed positive contribution toward HDAC1 inhibition. It was noticed that the compound **30** and **43** with four aromatic rings (*Pubchem261*) was comparatively lower active in nature. Whereas, it is also observed, that most of the AR-42 derivatives having a hydrogen bond acceptor count of 5 are highly potent HDAC1 inhibitors.

- ***Classification-based Linear discriminant analysis (LDA) model***

From the LDA analysis, equation 11 was developed with three descriptors. The discrimination capability of the *actives* and the *inactive* HDAC1 inhibitors has been observed. Definitions of these descriptors are shown in **Table 6**.

$$\boldsymbol{DF} = 21.258 + 7.762 \times \boldsymbol{VE1\_Dzp} - 2.505 \times \boldsymbol{mindO} + 1.217 \times \boldsymbol{nHBAcc} \qquad \textbf{Equation (11)}$$

N_{Train} = 49, Wilk's Lambda (λ) = 0.438, R_C = 0.749, D_M^2 = 5.016, F(3, 45) = 19.211, p < 0.000, χ^2 = 37.515, MCC_{Train} = 0.673, $AUROC_{Train}$ = 0.946; N_{Test} = 17, MCC_{Test} = 0.653, $AUROC_{Test}$ = 0.903

Table 6. Description of the different descriptors used for the LDA analysis

Sl No.	***Descriptor***	***Description***	***Contribution***
1	*VE1_Dzp*	Coefficient sum of the last eigenvector from Barysz matrix / weighted by polarizabilities	Positive
2	*mindO*	Minimum atom-type E-State: =O	Negative
3	*nHBAcc*	Number of hydrogen bond acceptors	Positive

The LDA model displays significant discrimination function (*DF*) that is characterized by a low *Wilk's lambda* (λ = 0.438) value and a high canonical correlation coefficient (R_c =

0.844). The statistical significance reflects that the actives and inactive classes are distinctly separated as evidenced by the high value of χ^2 (37.515). However, as per the LDA model, 23 *active* compounds present in the training set are hypothesized as truly active (*TP*). Similarly, for the inactive class, 18 inactive molecules of the training set are hypothesized as truly *inactive* (*TN*). Only 3 molecules were falsely hypothesized as *active* (*FP*) whereas 5 *active* molecules were hypothesized as *inactive* (*FN*) in the training set. It was observed that the LDA model has hypothesized 7 out of 9 *actives* from the test set as truly actives (TP) while 2 molecules were falsely hypothesized as *inactives* (*FN*). Also, 7 out of 8 test set *inactives* were hypothesized as truly *inactive* (TN). Only one molecule was falsely hypothesized as *active* (*FP*). Different qualitative statistical analyses were determined to judge the internal and external predictive quality of the LDA model. Different qualitative statistical parameters were determined to judge the internal and external predictive quality of the LDA model (**Table 7**). Moreover, the *ROC* plots for the LDA model (*equation 11*) are shown in **Figure 5**. Additionally, the observed and predicted activities classes along with the posterior probabilities are given in **Table 8**.

Table 7. Statistical performance of the classification based LDA models

Set	***ROC***	***TP***	***TN***	***FP***	***FN***	***Se***	***Sp***	***Pr***	***Acc***	***F_1***	***MCC***
Training	0.946	23	18	3	5	0.821	0.857	0.885	0.837	0.821	0.673
Test	0.903	7	7	1	2	0.778	0.875	0.875	0.824	0.824	0.653

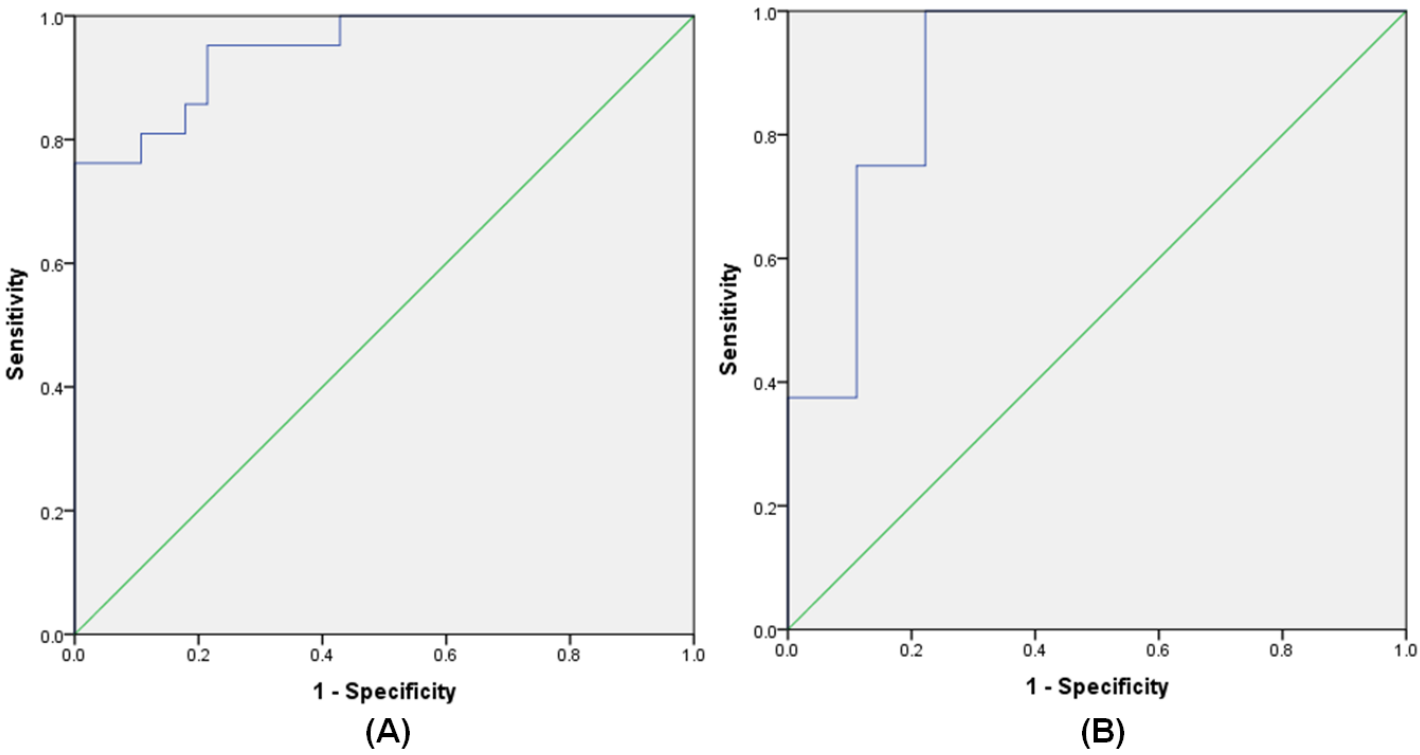


Figure 5. *ROC* plots for (**A**) the training and (**B**) test set for the LDA model (*Equation 11*)

Table 8. Actual and predicted classes along with the posterior probabilities and the descriptors used to develop *equation 11*

Cpd No	*VE1_Dzp*	*nHBAcc*	*mindO*	*Actual Class*	*Predicted Class*	*Posterior Probabilities*	
						Group-(0)	*Group-(1)*
1*	0.275525	3	11.28102	1	0	0.997331	0.002669
2	0.127106	5	12.42834	0	0	0.945194	0.054806
3	0.275785	3	11.36935	0	0	0.985443	0.014557
4	0.169907	3	11.28501	0	0	0.988385	0.011615
5	0.109836	3	11.13965	0	0	0.590662	0.409338
6	0.420871	3	11.37672	0	0	0.996903	0.003097
7	0.04538	3	11.17706	0	0	0.994112	0.005888
8	0.195771	3	11.52803	0	0	0.210174	0.789826
9*	0.207223	3	11.45142	0	0	0.933276	0.066724
10	0.317562	4	11.24102	1	1	0.827229	0.172771
11*	0.248042	3	11.24274	0	0	0.815368	0.184632
12	0.250199	3	11.25274	0	0	0.776157	0.223843
13	0.338537	3	11.33538	0	0	0.267829	0.732171
14	0.345855	3	11.34365	0	0	0.221383	0.778617
15	0.372095	3	11.38184	1	0	0.099056	0.900944
16	0.340223	4	11.36795	0	1	0.786390	0.213610

17	0.356958	4	11.3749	1	1	0.767203	0.232797
18	0.388617	4	11.30365	0	1	0.985107	0.014893
19*	0.292298	3	11.34052	0	0	0.615819	0.384181
20	0.36627	3	11.37447	1	0	0.656267	0.343733
21	0.373101	3	11.3759	1	0	0.480016	0.519984
22	0.194803	3	11.35802	0	0	0.058831	0.941169
23	0.270602	4	11.41558	1	0	0.263682	0.736318
24	0.436045	3	11.47364	1	0	0.989558	0.010442
25*	0.447503	3	11.47004	0	0	0.032907	0.967093
26	0.470463	3	11.45076	1	1	0.034383	0.965617
27	0.463513	4	11.43506	1	1	0.023595	0.976405
28	0.372461	4	11.46406	1	1	0.011551	0.988449
29*	0.28251	4	11.49651	1	0	0.017510	0.982490
30	0.089472	4	11.58146	0	0	0.009755	0.990245
31	0.34694	5	11.4512	1	1	0.007418	0.992582
32	0.351375	5	11.47303	1	1	0.823302	0.176698
33	0.178427	5	10.86806	1	1	0.046256	0.953744
34	0.408185	5	11.45048	1	1	0.130951	0.869049
35	0.383736	5	11.44994	1	1	0.823640	0.176360
36	0.424793	5	11.47149	1	1	0.972741	0.027259
37	0.447467	5	11.49251	1	1	0.932136	0.067864
38	0.399094	3	11.51816	0	0	0.057029	0.942971
39	0.350884	5	11.52659	1	1	0.012709	0.987291
40	0.42912	4	11.48532	1	1	0.007129	0.992871
41*	0.285274	5	11.36644	1	1	0.002524	0.997476
42*	0.40716	4	11.46973	1	1	0.000743	0.999257
43	0.256683	4	11.56305	0	0	0.717146	0.282854
44*	0.437367	4	11.42599	1	1	0.157238	0.842762
45*	0.400443	5	11.27168	1	1	0.024195	0.975805
46	0.267225	3	11.47243	0	0	0.135571	0.864429
47	0.315797	3	11.45276	0	0	0.059026	0.940974
48*	0.336366	3	11.46133	0	0	0.425568	0.574432
49	0.514113	4	11.58596	1	1	0.945182	0.054818
50	0.461399	5	11.6326	1	1	0.913453	0.086547
51	0.467217	5	11.54657	1	1	0.989013	0.010987
52	0.380091	6	11.57646	1	1	0.932108	0.067892
53	0.417689	6	11.47479	1	1	0.916733	0.083267
54*	0.432739	5	11.48867	1	1	0.605228	0.394772
55*	0.372438	4	11.40021	1	1	0.672368	0.327632
56	0.400512	3	11.4141	0	0	0.058190	0.941810
57	0.228813	4	10.90275	1	1	0.168223	0.831777
58	0.343856	5	11.38523	1	1	0.085580	0.914420
59	0.410432	4	11.43455	1	1	0.004879	0.995121
60	0.476222	4	11.47507	1	1	0.909742	0.090258
61	0.495444	3	11.48896	0	1	0.009360	0.990640
62*	0.336182	3	11.36003	0	0	0.200248	0.799752
63	0.261832	3	11.32607	0	0	0.851362	0.148638

64*	0.387124	4	11.40715	1	1	0.167831	0.832169
65*	0.310481	4	11.35351	0	1	0.361316	0.638684
66*	0.31656	3	11.3292	0	0	0.871412	0.128588

** marked molecules are considered as the test set instances*

From the *equation 11*, it was noticed that the descriptors *VE1_Dzp* (Coefficient sum of the last eigenvector from Barysz matrix / weighted by polarizabilities) and *nHBAcc* (Number of hydrogen bond acceptors) both were correlated positively whereas the descriptor *mindO* (Minimum atom-type E-State: =O) showed the negative contribution with discrimination factor. This suggests that the descriptor *VE1_Dzp* and *nHBAcc* can be used to discriminate the active HDAC1 inhibitors from the higher ones. Also, these contributions of *VE1_Dzp*, *mindO* and *nHBAcc* suggests that the polarizability and the number of hydrogen bond acceptor groups can be used to discriminate the active HDAC1 inhibitors from the inactive ones.

Chapter 6: Conclusion & Future Perspective

This current study revealed that drug or compound showing HDAC inhibitor property can possess several functional groups; short chain fatty acid, benzamide, cyclic peptide, electrophilic ketone, etc. Most of them are applicable for hematological and solid tumor conditions but their pharmacokinetic profile is quite poor. This problem can be bypassed by the development of Novel Drug Delivery System (NDDS), target specific as well as sustained release formulations that can be achieved avoid the shortcomings associate with the efficacy of HDAC inhibitors.

Moreover, HDAC inhibitors discovered till date are found administered with radiotherapy as well as DNA alkylating agents which showed synergistic effect, but due to some disadvantage of polypharmacy a novel approach 'Polypharmacology' comes under consideration in combating cancer pathology where chimeric drugs are designed likes vorambucil which shows both the property of vorinostat as well as chlorambucil (DNA alkylating agent); fimepinostat, tinostamustine, chlordinaline are also come under consideration of such kind of chimeric drug.

Additionally, the HDACs as well as HDAC1 associated not only cancer pathology itself but also associated with various other diseases likes metabolic disorder, neurological disorders (such as Alzheimer's disease), inflammatory diseases, cardiovascular disorder etc. In this context, though a good number of compounds came under consideration in treatment of cancer as well as several FDA approved HDAC inhibitor such as vorinostat, belinostat, panobinostat, pracinostat, and chidamide are being used for HDAC-related cancer treatments. Although, the pan-HDAC selective nature of these approved inhibitors is one of the major shortcomings of these drugs for the treatment of HDAC1-related disorders due to their off-target binding. Therefore, it is necessary to develop HDAC1-specific inhibitors to combat such scenario.

In this context, the quantitative structure-activity relationship study of the 66 AR-42 derived HDAC1 inhibitors, it was quite noticeable that the polarizability (*ATSC8p, VE1_Dzp*), molecular refractivity (*MLFER_E*), number of aromatic rings (*Pubchem261*), number of hydrogen bond acceptor groups (*nHBAcc*), van der Waal volume (*VE1_Dzv*), carboxnyl groups (*mindO*) are the crucial factors for the development of potent HDAC1 inhibitors. It was interesting to note that, both the regression-based MLR and the classification-based LDA models suggested the importance of molecular polarizability as well as higher number of hydrogen bond acceptor groups for HDAC1 inhibitory activity. It was also observed that, most of the HDAC1 inhibitors having a hydrogen bond acceptor group of five or more were highly potent HDAc1 inhibitors. This may suggest that the presence of higher number of hydrogen bond acceptor groups may deliver higher hydrogen bond interactions inside the HDAC1 active site, consequently leading to a higher HDAC1 inhibition.

Nonetheless, from this study, it is quite clear that the development of the selective HDAC1 specific inhibitors is required for the treatment of HDAC1-related pathophysiological conditions including cancer. Therefore, this study of HDAC1 inhibitors can be useful for the development of such specific inhibitors that can be utilized effectively for the treatment of HDAC1-associated pathophysiology.

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