Octahedron Drug Research 5 (2024) 22-50 ISSN: 2812-6351
Online ISSN: 2812-636X

OCTAHEDRON Drug Research Vol. 5

Review Article


# HDAC as a cancer target: Various scaffolds based on HDAC inhibitors and an overview of their advances

**Abrar Abdel-raheem Mortada[1*], Omar M Aly[2], Aliaa M. Mohassab[3], Mamdouh F. A. Mohamed[4]**

[1] Medicinal Chemistry Department, Faculty of Pharmacy, Egyptian Russian University, Badr 11829, Egypt.
[2] Medicinal Chemistry Department, Faculty of Pharmacy, Port Said University, Port Said, Egypt.
[3] Medicinal Chemistry Department, Faculty of Pharmacy, Minia University, Minia 61519, Egypt.
[4] Medicinal Chemistry Department, Faculty of Pharmacy, Sohag University, Sohag 82524, Egypt.
Correspondence: abrar-mortada@eru.edu.eg

**ARTICLE INFO**

Article history :
Received 20 March 2024
Received in revised form
1 May 2024
Accepted 2 May 2023
Available online 1 June 2024

***Keywords***: Cancer; HDAC; HDACI; SAR.



**ABSTRACT**

Epigenetic enzymes HDACs enzymatically remove acetyl groups from ε-N-acetylated lysine residues in many protein substrates, including histones and non-histones. Different histone deacetylases (HDACs) have different biological functions and are recruited to certain genomic regions. HDACs are clinically authorized cancer treatments with major biological roles. These chemicals are also being studied for treating Alzheimer's disease (AD), metabolic disorders, viral infections, and multiple sclerosis. Around thirty HDAC inhibitors are being researched in clinical trials, in addition to the five authorized drugs. This review discusses HDAC inhibitor drug discovery progress. It analyzes the logical design of these inhibitors based on structure, isoform selectivity, pharmacology, and toxicity. The goal is to update medicinal chemistry researchers and speed up medication discovery. Cancer is multidimensional, thus targeting two components with a single drug is an effective and beneficial method. Given its role in cell proliferation, metastasis, and death, histone deacetylase (HDAC) has been intensively researched as a prospective cancer treatment target. Clinically licensed HDAC inhibitors include vorinostat and panobinostat. Low efficacy, lack of selectivity, drug resistance, and toxicity limit their efficacy. HDACs that can target two entities are therefore popular. Combining a histone deacetylase (HDAC) inhibitor with other anticancer drugs has been shown to improve non-selectivity and drug resistance in single-target therapies.

## Introduction

### Cancer

Cancer, a potentially fatal illness, is a leading cause of death worldwide [1]. India is the third highest country in terms of the prevalence of cancer cases. According to the National Cancer Registry Program Report, more than 1.3 million individuals in India are affected by cancer annually. As per a report published by the Indian Council of Medical Research (ICMR), the projected number of

cancer cases is likely to rise to 29.8 million by the year 2025. The most prevalent therapies for cancer are chemotherapy, targeted therapy, radiation therapy, immunotherapy, and stem cell therapy. Although there have been significant advancements in biotechnologies and diagnosis, the creation of new anticancer medications remains crucial for effectively treating cancer patients. This is a significant hurdle for scientists in their pursuit of successfully curing cancer. To improve cancer treatment and mitigate the toxicity and negative side effects of the medicine involved, scientists are currently focusing on targeted drug therapy. This approach involves the use of a single molecule that effectively targets a specific site with great selectivity [2]. Tamoxifen was the inaugural pharmaceutical agent employed in targeted therapy for breast cancer, namely by selectively targeting estrogen receptors [3]. While the "one drug, one target" approach is a powerful and targeted treatment for cancer that avoids unintended side effects, it is not achieving considerable success due to drug resistance, limited effectiveness in the body, and poor patient adherence. Due to the intricate nature of cancer, it is imperative to design a new therapy to achieve favorable long-term results. Consequently, a fresh approach to discovering cancer drugs is required. Therefore, the combination therapy method has gained significant prominence in the field of cancer medication research to optimize effectiveness and reduce the development of drug resistance. In pharmacological combination therapy, drug cocktails consist of physical combinations of two or more substances that are utilized together [4]. Despite the notable achievements of combination therapy, it is plagued by several issues including medication solubility, drug resistance, and drug interactions. Furthermore, the likelihood of drug-drug interactions and side effects is growing, necessitating dose adjustment to prevent drug toxicity. Many research organizations focus on molecular hybridization to produce a dual-targeting medicine instead of a pharmacological combination to address the limitations of combination therapy. Therefore, combination therapy has shifted towards developing dual-target ligands, which are single molecules capable of simultaneously targeting multiple sites. This approach results in synergistic effects and can potentially decrease drug-drug interactions, and drug resistance, and improve pharmacokinetics when compared to physical mixtures of drug molecules. capable of targeting multiple sites simultaneously. This approach results in synergistic effects and has the potential to decrease drug-drug interactions, and drug resistance, and improve pharmacokinetics when compared to physical mixtures of drug molecules [5].

## 1. Histone deacetylases (HDACs)

## 2. Overview

The functional regulation of the interaction levels between histones and DNA in eukaryotic gene expression is carried out by two sets of crucial enzymes, namely histone acetyltransferases (HATs) and histone deacetylases (HDACs). Histone acetyltransferases (HATs) can counteract a positive charge on the surface of histones by adding acetyl groups to the histone tails. This leads to a decrease in the level of interaction, a less condensed chromatin structure, and an increase in the accessibility of RNA polymerases. Those modifications would ultimately result in heightened gene expression. Moreover, acetylation can serve as a chemical cue to identify bromodomains, which are components involved in protein-protein interactions and are commonly seen in transcriptional activators [6]. HDACs, however, can remove acetyl groups from histone lysine residues, causing a more compact and tightly bound chromatin structure, ultimately leading to decreased gene expression. HATs (histone acetyltransferases) and HDACs (histone deacetylases) regulate the equilibrium between these two conflicting activities in healthy cells, guaranteeing sufficient efficiency in both transcriptional and post-translational modifications[7].

## 3. Classes of HDAC enzymes

So far, researchers have discovered eighteen different types of human HDAC enzymes. These enzymes are categorized into four groups based on their resemblance to HDACs found in yeast. Among the various classes of HDAC enzymes, classes I, II, and IV are categorized as metalloenzymes that rely on zinc ions for their biological function [8].

## 4. Class I HDAC enzymes

The members of this family are HDAC1, HDAC2, HDAC3, and HDAC8. HDAC1, HDAC2, and HDAC3 are exclusively located in the nucleus, whereas HDAC8 is found in both the nucleus and the cytoplasm. HDAC1, HDAC2, and HDAC3 are constituents of multi-protein complexes that play a role in transcriptional regulation. The homology models of the HDAC isoforms explained the mutagenesis observed in HDAC1. These models were also important in the development of pharmacophore and inhibitor design. The crystal structure of HDAC1 contains an active site that includes a hydrophobic channel measuring 11 Å and a metal ion with catalytic activity. Additionally, there is an internal cavity measuring 14 Å located at the bottom of the active site. In the absence of a ligand, the interior cavity plays a role in the deacetylation reaction and facilitates the release of the acetate byproduct by promoting potential ionic interactions [9]. The

structure of human HDAC2 is composed of eight β sheets and thirteen $\alpha$ helices within a single $\alpha$/β domain, with the β sheets positioned between the $\alpha$ helices. The active site channel is a pathway that links the protein surface with the active site. The $Zn^{2+}$ ion exhibits a strong affinity for lipids and has a hydrophobic nature. It has a size of around 11 Å and is surrounded by specific amino acid residues, namely Gly154, Phe155, Phe210, and His183, which bind to the $Zn^{2+}$ ion. In addition to the zinc binding site, there is a hydrophobic cavity in the enzyme that measures 14 Å [10]. This cavity, known as the foot pocket, extends towards the core of the enzyme and contains numerous amino acids, namely Met35, Phe114, and Leu144, which are responsible for defining the foot pocket (fig.2) [11]. The interior cavity of HDAC8 is accessible to the external environment, suggesting the malleability of the surface channel of the protein. The internal cavity harbors a conserved Arginine residue, with the central arginine residue of the R37 side chain being the most critical component. This study focuses on enhancing the catalytic activity and acetate affinity of HDAC8. Additionally, it serves as a protective mechanism for the channel involved in the transportation of water or acetate through the active site of HDAC8. The strong interaction between G139 and G303 inhibits the movement of water across the active site. Consequently, a significant structural rearrangement of R37 and the loop is necessary to open the active site and allow the movement of water or acetate ions through the internal channel [12].

**Class II HDAC enzymes**

Class II HDACs exhibit tissue-specific expression patterns and play a crucial role in the differentiation and development of skeletal, cardiac, smooth muscle, bone, immune system, vascular system, and brain tissues through their ability to suppress transcription [8]. The members of HDACs are divided into two sub-categories: Class IIa, which includes HDACs 4, 5, 7, and 9, and Class IIb, which includes HDACs 6 and 10. The Class IIa HDACs consist of a persistent deacetylase domain that includes a conserved N-terminal region like HDAC1. This N-terminal domain controls DNA binding by facilitating the movement of molecules between the nucleus and cytoplasm. The phosphorylation of the serine residue in the regulatory N-terminal domain of Class IIa HDACs is reliant on the signal. This phosphorylation regulates whether the HDACs should remain in the nucleus or leave and affects their ability to function as transcriptional co-repressors in the nucleus. HDAC4 suppresses the activity of RunX2 and controls the process of endochondral bone formation and bone hypertrophy [13],[14]. HDAC7 plays a role in controlling programmed cell death in thymocytes, a kind of immune cell. It enhances the process of transcribing MEF2D and hinders the activity of the Nur77 receptor. Despite the minimal presence of HDAC5 in the human thymus, it plays a crucial role in suppressing Nur77. Within the endothelial cells, it inhibits the expression of FGF2 and Slit2 genes, which are involved in the process of angiogenesis [15]. The Class II2b consists of HDAC6 and HDAC 10. Both enzymes share a similar structure. The two catalytic domains of HDAC6 connect and both domains are oriented outward from the protein, allowing them to be accessible for the substrate. The crystal structure of HDAC6 tandem catalytic domains has been unveiled, as depicted below. The enzyme-dependent actions of HDAC6 rely on its second catalytic domain (CD2), which is essential. However, the first catalytic domain (CD1) is also enzymatically active. CD1 of HDAC6 removes acetyl groups from lysine residues located at the C termini of substrates, while CD2 mostly removes acetyl groups from $\alpha$-tubulin [16]. While HDAC10 is not exclusively located in the nucleus, it is less significant compared to HDAC6. The structure includes an additional deacetylase domain that interacts with particular substrates, although their identity remains unknown. The process involves the control of vascular endothelial growth factor receptor (VEGFR)[17] degradation by proteasomes through the acetylation and deacetylation of HSP-90 [18].

**Class III HDACs**

The extensive presence of sirtuins ins in cells renders them suitable for a myriad of biological processes, including aging, DNA repair, management of oxidative stress, and regulation of metabolism. Similar to a coin, sirtuins exhibit dual characteristics, with one side demonstrating a pro-oncogenic action and the other side playing a role as a tumor suppressor in carcinomas. In cancer, there is a change in the control of sirtuins, in addition to the abnormal expression of the traditional HDACs. **SIRT1** has been discovered to be increased in acute myeloid leukemia (AML), prostate cancer, and non-melanoma skin cancer [19],[20], while decreased in colon cancers [21]. In breast cancer, the expression of **SIRT3** and **SIRT7** is increased, while in gliomas and gastric carcinoma, the expression of **SIRT2** is decreased. SIRT2 deficiency results in the malfunction of the mitotic checkpoint [22], which in turn causes genomic instability and the development of tumors. The upregulation of P-glycoprotein induced by exogenous SIRT1 confers drug tolerance to cancer cells, namely to medicines like doxorubicin. However, this effect can be reversed by downregulating SIRT1 using silencing RNA [23]. SIRT3 provides cellular defense against apoptosis in fibro sarcoma cells and its

upregulation reverses the growth arrest induced by p53 in bladder cancer cells [24]. Suppression of the same in colon cancer cells decreases the amounts of reactive oxygen species (ROS), hypoxia-inducible factor 1-α (HIF-1α) [25], and its target genes. SIRT7 has been found as a highly selective deacetylase of H3K18Ac that is related to promoters. The depletion of this substance reduces the likelihood of tumor formation in mice that have been transplanted with human cancer cells [20].

**Class IV HDACs**

There is less information available regarding the involvement of HDAC11 in cancer. HDAC11 has been identified as a participant in the development of Hodgkin lymphoma (HL)[26]. Philadelphia-negative chronic myelo-proliferative neoplasms (CMPNs) exhibit elevated levels of HDAC11[27].

**The study of the biological functions and characteristics of HDAC enzymes.**

The regulation of gene expression is controlled by the post-translational changes of the histones. These processes encompass acetylation, phosphorylation, methylation, ubiquitination, and SUMOylation [28]. The non-histone proteins identify a certain pattern displayed by the alterations made on the histones, and then form complexes that govern the process of gene transcription. HDACs can be found either as standalone proteins or as complexes with other proteins, such as the transcriptional co-repressors mSin3, N-CoR, and SMRT (fig3) [29]. After formation, the complexes are transported to genomic regions through interactions with specific DNA-binding factors, such as transcription factors, nuclear receptors, and other genes involved in epigenetic modifications, such as methyl-binding proteins (MBDs), DNA methyl transferases (DNMTs), and histone methyl transferases (HMTs). Epigenetic regulation is shown by the process of chromatin remodeling through histone acetylation and deacetylation [30]. The discovery of lysine acetylation of histone proteins was made approximately fifty years ago by *Vincent Allfrey* and his colleagues. The significance of acetylation of the ε-amino group of lysine residues on histone proteins in gene expression was demonstrated [31]. It has been determined that maintaining a balance between the activities of histone acetyltransferases (HATs) and histone deacetylases (HDACs) is crucial for the process of activating and deactivating gene transcription [32]. HDACs were initially designated as such due to their initial discovery of removing acetyl groups from lysine residues located on the N-terminal tails of histones. Additionally, HDACs have been found to not only remove acetyl groups from histone proteins but also to have an impact on various other proteins that are not histones. These proteins include transcription factors such as RUNX3 [33], p53 [34], E2F [35], c-Myc [36], nuclear factor kBNFkB [37], hypoxia-inducible factor 1-α (HIF-1α) [38], estrogen receptor α (ERα), androgen receptor (AR) [39], MyoD [40], chaperone (HSP90) [41], signaling mediator (Stat3 [42] and Smad7 [43]), and repair proteins (Ku70) [44]. Additionally, a key transcription factor involved in bone growth has been included in the list of HDAC substrates [45].

**Role of HDACs in cancer**

Mammalian cancer is characterized by DNA dysregulation and post-translational changes of the histone tails. The most often reported modifications are the removal of acetylated Lys16 (K16-H4) and the addition of three methyl groups to Lys20 (K20-H4) of histone H4 [46]. The primary indicator of cancer initiation and progression is the absence of acetylated and trimethylated forms of histone H4, which are commonly observed in the early stages of cancer (fig.4). Furthermore, it has been observed that histone deacetylation is also responsible for tumor invasion and metastasis[47]. Colon cancer samples exhibit heightened levels of HDAC2 and HDAC3 proteins. In addition, gastric cancer has elevated levels of HDAC1, while lung cancer shows decreased levels of HDAC5. Furthermore, HDAC10 has been associated with a negative prognosis. HDAC4 mutations have been detected in breast cancer samples discovered in a large-scale sequencing research. In addition, modifications to the HDAC2 protein have been observed in both human epithelial cancer cell lines and colon cancer cell lines. However, a microarray analysis indicated that HDAC inhibition had an impact on only 2-5% of genes [48]. Additionally, the study demonstrated that a similar number of genes were both activated and suppressed [30]. The significance of regulating the transcriptional effects of HDAC on cellular activities, such as the regulation of acetylation in various non-histone HDAC substrate proteins, is extremely valuable. The complex network of physiological pathways depends on the activity of various transcription factors, chaperones, and structural proteins. These components are influenced by their acetylation state, such as the ATP binding enhancer HSP-90. The formation of functional HSP-90 chaperone complexes is facilitated by the deacetylation of HSP-90 by HDAC6 [49]. HDAC6 served as a conduit between EGF and Wnt signaling pathways in tumor growth, suggesting that disabling HDAC6 could be beneficial in cancers with disrupted signaling. HDACs 1 and 2 participate in Wnt signaling by disrupting the link between b-catenin and TCF. In addition, during the G1 phase of the cell cycle, the activity of histone deacetylase is responsible for inhibiting

transcription by the E2FeRb complex. The lysine residues, located adjacent to the DNA-binding domain, exhibit a high degree of conservation, and serve as sites for acetylation of E2F1. Acetylation of these residues enhances the DNA-binding activity, transactivation potential, and stability of E2F1[35].

**Histone deacetylase inhibitors**

**HDACIs overview**

HDACIs, or histone deacetylase inhibitors, are potent agents that inhibit cell proliferation and induce apoptosis in cell-based studies. Consequently, scientists worldwide are diligently striving to develop these compounds as effective anti-cancer medicines and as agents that can limit the actions of HDAC [50]. Multiple studies have

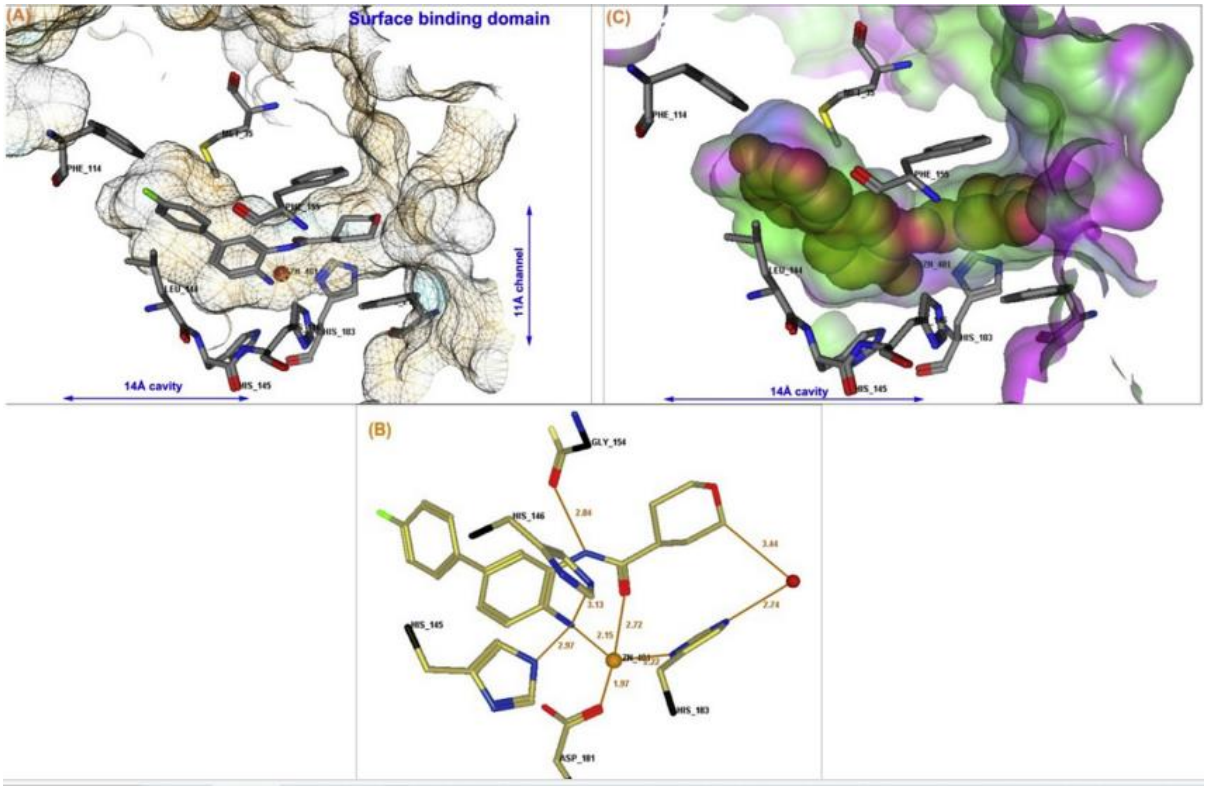



**Figure 1. (**A) BRD4884 bound to h-HDAC2; (B) ligand -$Zn^{+2}$ coordination sphere with key interacting amino acids; (C) view of the 14 Å cavity: BRD4884, tightly fits the hydrophobic 14 Å cavity [51].

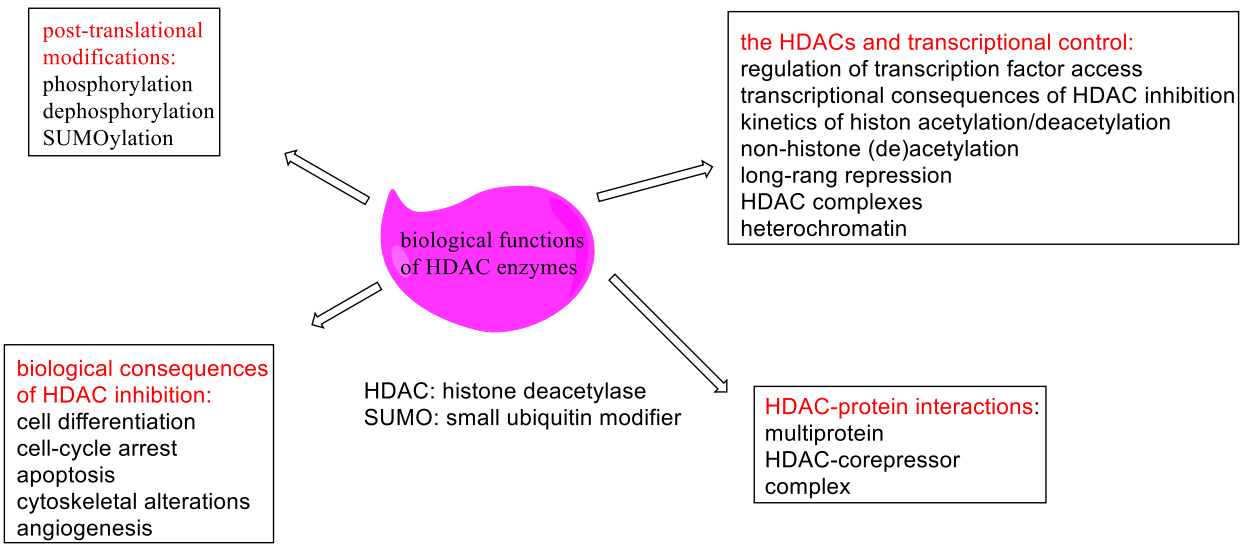



**Figure 2**. Biological functions of HDAC enzymes

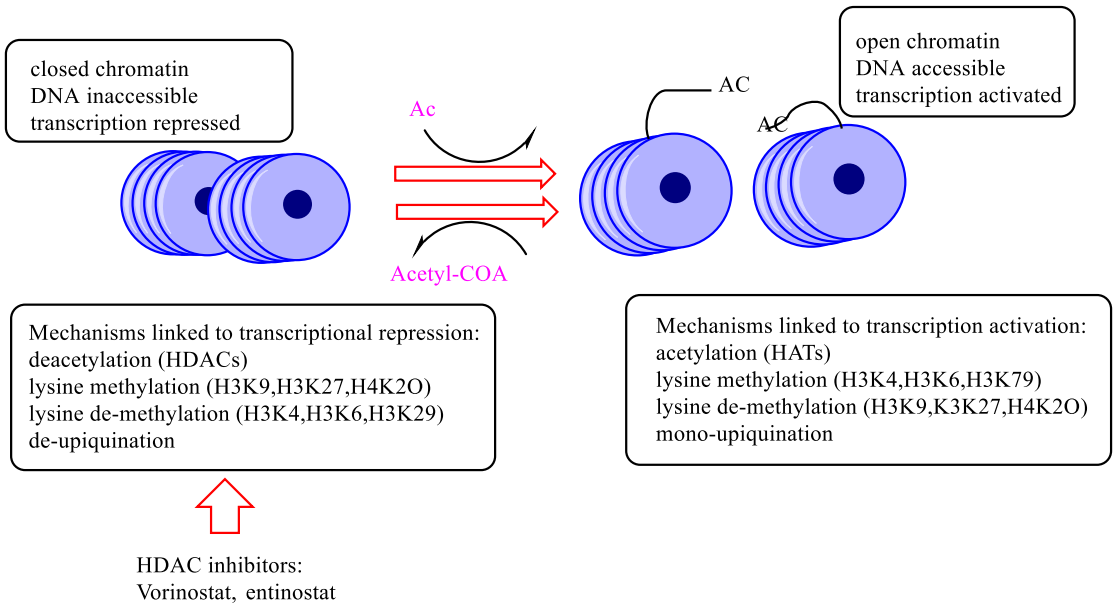



**Figure 3.** Mechanism of histone acetylase and histone deacetylase enzyme

demonstrated that HDAC inhibitors enhance the effectiveness of pre-existing therapeutically active frameworks in fighting cancer and would have a substantial effect when used in combination with other anticancer treatment strategies such as ionizing radiation and/or chemotherapy. The therapeutic application of HDAC inhibitors has significantly enhanced their usefulness in treating cancer and a few other illnesses. Due to the essential biological functions of HDACs, research in this field has led to significant advancements and valuable findings during the past few decades. HDACs have been clinically validated for cancer therapy and are currently being studied for the treatment of various other conditions, including Alzheimer's disease, metabolic disease, viral infection, and multiple sclerosis, among others (fig.5) [52].

**Mechanism of action of histone deacetylase inhibitors as anticancer**

Certain genes are transcriptionally activated while others are transcriptionally repressed because of HDACI-induced histone and non-histone protein acetylation and gene expression changes. The ability of HDACIs to alter the expression of specific proliferative and/or apoptotic genes accounts for part of their anticancer activity. HDACIs promote differentiation, angiogenesis decrease, cell death, and halt of the cancer cell cycle (fig.5), (fig.6) [53],[54],[55].

**Activation of apoptotic pathways**

HDACIs induce apoptosis using both intrinsic and extrinsic pathways. TNF-$\alpha$ overexpression, which follows the extrinsic mode of apoptosis, activates caspases 8 and 10, ultimately leading to apoptotic cell death. Conversely, the intrinsic pathway results in a decrease in the expression of anti-apoptotic proteins like Bcl-2 and an increase in the production of pro-apoptotic proteins like Bim, Bak, Bax, and caspase-3 [56],[57].

**Induction of cell cycle arrest**

HDACIs cause cell cycle arrest and differentiation by upregulating cyclins, which inhibits CDK activity, and by increasing the expression of cell cycle genes including p21 (a CDK inhibitor) [58],[59].

**Induction of DNA damage and inhibition of DNA repair**

HDACIs cause the DNA damage pathway to become active. Although they don't create double strand breaks on their own, they work in concert with medicines that damage DNA to inhibit the growth of tumors [55],[60], ,[61],[62].

**Anti-angiogenesis**

By inhibiting proangiogenic factors like VEGF, hypoxia-inducible factor-1 (HIF-1), and overexpression of p53, which prevent hypoxia-induced angiogenesis, HDACIs can reduce tumor angiogenesis. Moreover, HDACIs inhibit DNA repair protein expression. After receiving these medications, reactive oxygen species (ROS) accumulate and damage DNA [63].

**General pharmacophore of HDACIs**

Multiple histone deacetylase (HDAC) inhibitors from diverse structural categories are now undergoing clinical development. (fig.7) Demonstrate the process by which metalloenzymes bind to inhibitors [64]. In computational

approaches, this contact is represented only by electrostatic or van der Waals interaction, disregarding the covalent nature of metal coordination [65]. Furthermore, it is common for a fundamental residue (such as histidine in HDACs or glutamate in MMPs) to be located near the zinc ion and potentially attract an acidic proton. The crystallographic analysis of HDAC and subsequent docking studies with various ligands yield a plethora of valuable insights. The data was utilized to derive a conclusion on the characteristic features of an HDACIs. The composition should include: **(a)** a cap group with the ability to interact hydrophobically with the peripheral binding site next to the zinc ion; **(b)** a linker group that connects the hydrophobic cap group and the zinc-binding group; and **(c)** a zinc binding group ZBG that binds to the zinc ion by forming coordinate bonds within the active site(fig.8).

**Classes of HDACIs**

HDACIs are classified into five different types based on their ZBG: hydroxamic acids, cyclic peptides, 2-aminoanilides (benzamides), electrophilic ketones, and short chain fatty acids [66],[67],[68]. These substances exhibit some degree of selectivity towards specific HDAC enzymes or classes of HDACs. Having been the first to be found, hydroxamates are still the most popular and efficient HDACIs. Cyclic peptides are the class of HDACIs with the most complex structures. Compared to cyclic peptides and hydroxamic acids, benzamides have less activity. Class I HDACs are selectively and potently inhibited by the benzamide class of compounds. Generally speaking, short-chain fatty acids are poor HDAC inhibitors [69],[70],[71],[72].

*5. Hydroxamic acids*

Hydroxamic acid derivatives are the most widely used and potent class of HDACI drugs. [73],[74]. The x-ray crystal structure of trichostatin A (TSA) and SAHA binding interactions with the active site of histone deacetylase-like protein (HDLP) indicates that the hydroxamic acid group chelates zinc ion in a bi-dentate manner through its carbonyl and hydroxyl groups. Furthermore, three additional hydrogen bonds are formed between the HDLP Tyr 297, His 132, and His 131 and the amino, carbonyl, and hydroxyl groups of the hydroxamic acid derivative [63, 75](fig.7) . SAHA effectively suppresses the activity of the majority of the 11 HDAC isoforms that rely on metal ions for their function.

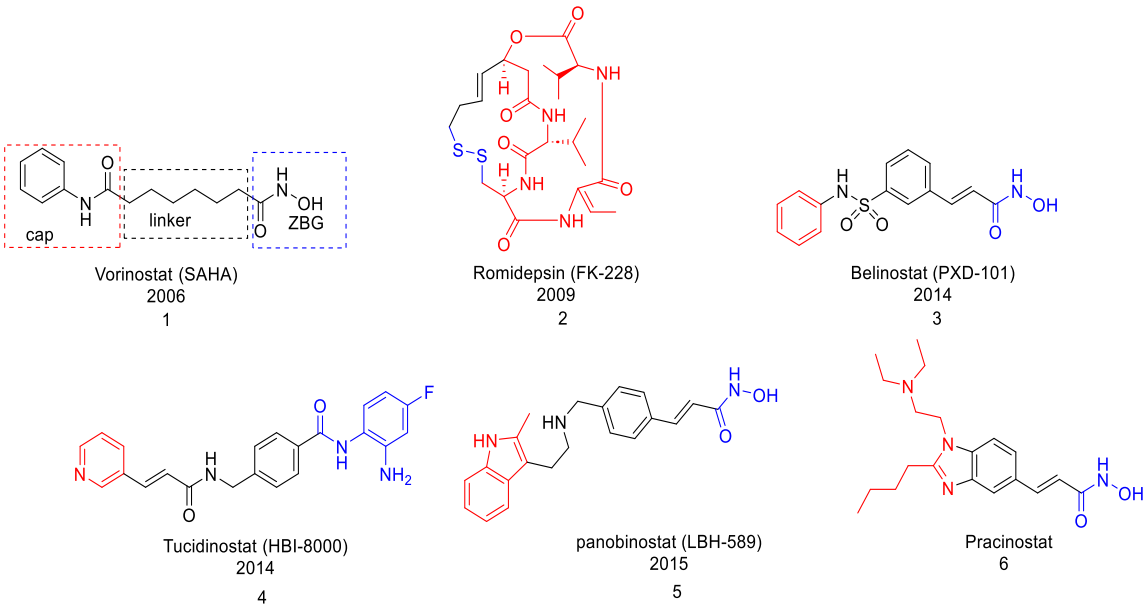
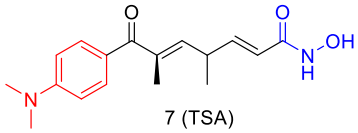



Figure 4. *FDA-approved* HDACIs drugs

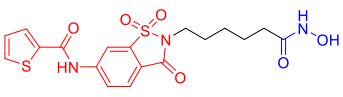
The presence of nonspecific inhibition may explain the occurrence of many adverse effects during treatment, ranging from minor to severe, such as dehydration, thrombocytopenia, anorexia, and cardiac arrhythmia. There is growing concern about reducing the side effects of HDACI, which are caused by its continuous use. One way to minimize these side effects is by modifying the cap group using substances like benzimidazole, purine, pyrimidine, lactam, and various linkers such as substituted aliphatic, triazole, hydroxyl cinnamamide, and aryl (fig.10). Targeting specific HDAC isoforms may offer a more efficient therapeutic approach for some diseases, while minimizing potential side effects. Enhancing selectivity for particular HDAC isoforms can be accomplished by independently modifying the surface recognition center, linker, and ZBG [51].

**TSA 7** was the first HDACI identified and belonging to the hydroxamate group [76]. Research has shown that Streptomyces hygroscopic produces this organic antibiotic, which has strong HDAC inhibitory action [77] but did not enter clinical use due to its toxicity [78].

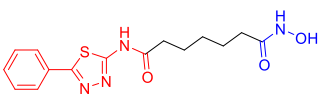


7 (TSA)

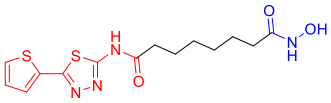
Additionally, panobinostat, belinostat, and SAHA have been approved by the FDA as hydroxamate HDACIs for the treatment of MM, PTCL, and CTCL (fig.5). Many research groups have been interested in creating newer, more potent, and less toxic chemicals because hydroxamic acid derivatives are significant as promising anticancer drugs that are effective against numerous cancer types [63]. There were compounds with comparable or better HDAC inhibitory efficacy than SAHA. With $IC_{50}$ values of 4.34 μM and 9.28 μM, respectively, **compound 8** showed strong antiproliferative activity against the prostate cancer cell PC-3 and the breast cancer cell MDA-MB-231. For maximum efficacy, a cap group with five carbon units between the saccharin ring and the ZBG performed well. The bioisostere thienyl moiety of **compound 9** is used in place of the phenyl moiety to boost its potency. With $IC_{50}$ values of 310 nM and 416 nM, respectively, the produced thienyl derivative **compound 10** was more active than SAHA, and the 6-carbon linker offered the most inhibition of cell growth.



8

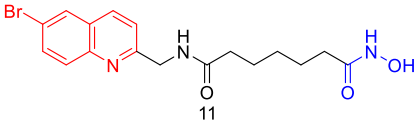


9



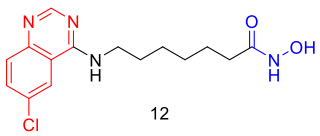
10

The synthesis of a series of quinoline HDACIs **11 [79]** demonstrated good inhibitory activity against HDACs as well as powerful antiproliferative activity against various tumor cell lines. **Compound 11** had an $IC_{50}$ value of 85 nM and 161 nM, respectively, which indicated that it inhibited HDAC more efficiently than SAHA. Moreover, **compound 11** demonstrated superior antiproliferative effectiveness compared to SAHA. Activity was significantly impacted by the substituents on the C4 and C6 locations of the quinoline ring. The C4 position of the quinoline ring's substitutes decreased the inhibitory effect of the enzyme. In the meantime, substituents on the quinoline ring's C6-position raised the inhibitory activity of the enzyme; this is assumed to be significant for binding affinities. The most efficient substitution at this location was a halogen; the enzyme inhibitory efficacy decreased when a phenyl group was substituted for a halogen. The zinc ion was chelated by the ZBG because a linker length of five was the most suitable.

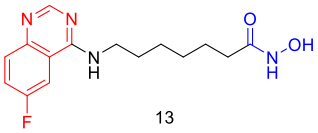


11

produced several HDACIs based on hydroxamic acid that have cap groups made of 4-aminoquinazolinyl moieties **12**, **13**. These medications more successfully inhibited HDACs than SAHA did [80]. Moreover, they were safe for human cells and specifically inhibited HDAC1,2 rather than HDAC8. The compound's $IC_{50}$ values are 3.08 ± 0.19 nM, whereas the 6-F derivative's is 8.17 ± 1.15 nM. When compared to a comparable 6-F, the addition of 6-Cl can increase potency, with a 10-fold increase in activity over SAHA.

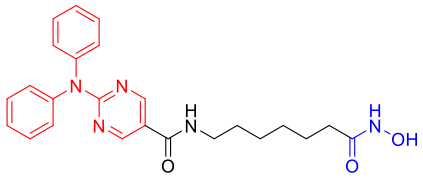


12



13

The introduction of ricolinostat **14 [81]**, a potent, selective HDAC6 inhibitor that started out in pharmacological research on hydroxamic acid HDACIs and has an $IC_{50}$ of 4.7 nM. Phase II clinical trials are now being conducted on this drug. It has a synergistic effect on multiple myeloma when used alone or in conjunction with dexamethasone, bortezomib, or lenalidomide.

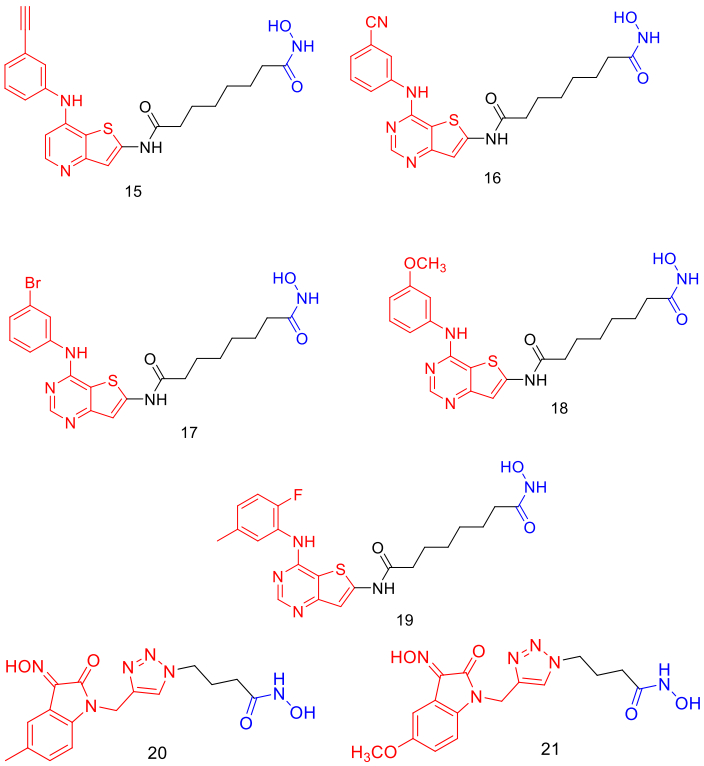


Ricolinostat

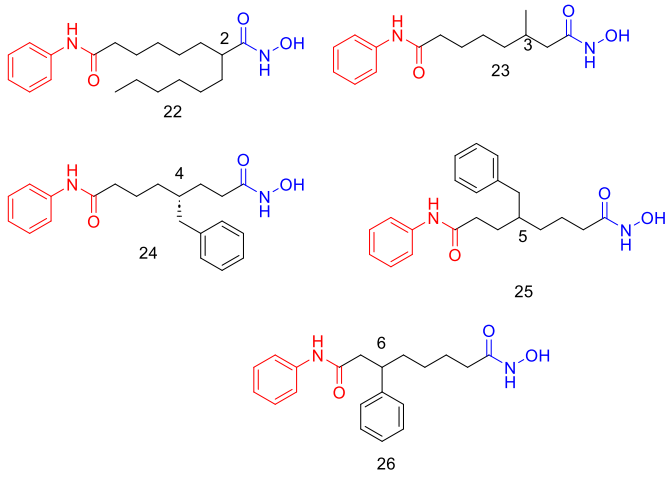
14

Thienopyrimidine-based HDACIs **15-19** were recently synthesized and tested for antiproliferative and HDAC inhibitory action on leukemia cancer cell RMPI-8226 and colon cancer cell HCT116 [82]. These substances showed promise against HDAC1, 2, and 3. Compound 19, specifically the thienopyrimidine derivative, exhibited strong inhibitory action against HDAC1, HDAC3, and HDAC6, as evidenced by its respective $IC_{50}$ values of 29.81 ± 0.52 nM, 24.71 ± 1.16 nM, and 21.29 ± 0.32 nM. It showed strong antiproliferative activity against these two cancer cell lines, with $IC_{50}$ values of 1.01 ± 0.033 μM in each case and 0.97± 0.072 μM and 0.97± 0.072 μM, respectively. created a new class of hydroxamic acids with 2-oxoindoline as a cap group [83]. These hydroxamic acids **20**, **21** inhibited a class-I HDAC2 isoform with $IC_{50}$ values of 1.28 μM for 5-$CH_3$ and 0.91 μM for 5- $OCH_3$. They were up to eight times more harmful than SAHA in three

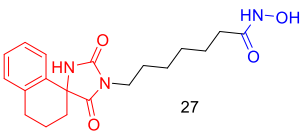
human cancer cell lines, including colon cancer SW620, prostate cancer PC3, and pancreatic cancer AsPC-1. The triazole moiety was introduced into these compounds to improve hydrogen bonding with the amino acid in the active binding sites of HDAC while also functioning as a linker between the 2-oxoindoline and hydroxamic acid moieties.

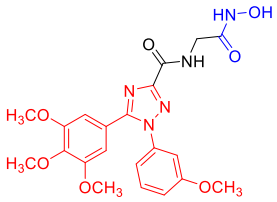


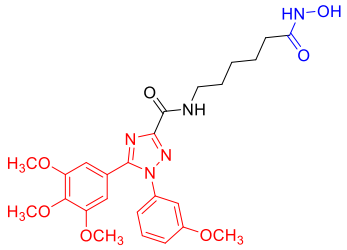

Furthermore, various SAHA analogues have been developed to improve selectivity. Compounds **22** to **26** were inserted into the nonselective HDACI SAHA linker at the C2, C3, C4, C5, and C6 sites [84],[85],[86],[87],[88]. The C2-n-hexyl SAHA analogue **22** preferred HDAC6 and 8 over HDAC1, 2, and 3. Substituents on C3 position **23** showed HDAC6 selectivity when compared to the broad-spectrum inhibitor SAHA, with an $IC_{50}$ of 350 nM, just fourfold less effective. Analog efficacy decreased as the size of the C3 substituent rose, with the methyl substituent having the most potent analog. The C4-benzyl SAHA analog **24** exhibited dual HDAC6/8 selectivity, with $IC_{50}$ values of 48 and 27 nM for HDAC6 and 8, respectively, and a 520 to1300-fold increase for HDAC6 and HDAC8 over HDAC1, 2, and 3. Longer C4 substituents enhanced HDAC6/8 selectivity. Furthermore, C5-modified SAHA analog **25** showed dual selectivity for HDAC6 and HDAC8 over HDAC1, 2, and 3, with only a slight decrease in HDAC6 inhibitory activity but an increase in HDAC8 inhibition compared to SAHA. It showed an 8-21fold increase in selectivity for HDAC6 and HDAC8 over HDAC1, 2, and 3, with $IC_{50}$ values of 270 and 380 nM for HDAC6 and HDAC8. Bulky substituents in the linker region improved HDAC6/8 selectivity because of the larger active site entry of both HDAC6. The most effective counterpart was C6-phenyl SAHA **26**, which had $IC_{50}$ values in nanomolar dosages. When compared to SAHA, its potency was fourfold lower. It also inhibited HDACs 1, 3, and 6. At 500 nM, the C6-methyl SAHA counterpart showed modest selectivity for HDAC1 and HDAC3 over HDAC6. The findings revealed that small structural changes to SAHA's linker region could significantly boost selectivity.



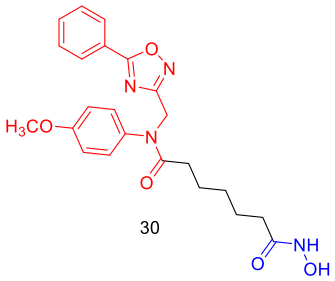

produced a novel HDACI using spirohydantoin and 1,2,4 triazole groups **27–29 [**89**]**. Compound **27** had the highest growth inhibition against the MCF-7 breast cancer cell line, with an $IC_{50}$ of 2.56 μM. In contrast, it has no effect on the liver cancer cell line HepG2. Furthermore, the six-carbon linker had the highest HDAC inhibitory effectiveness, with an $IC_{50}$ value like SAHA against HDAC4 and low selectivity against HDAC1. Furthermore, increasing the linker length increased the inhibitory effect of HDAC. Furthermore, compounds **28** and **29** showed substantial HDAC inhibitory activity against the four HDAC isoforms tested. Compound **28** exhibited substantial antiproliferative activity against the MCF-7 cell line, whereas compound **29** demonstrated strong antiproliferative activity against the HepG2 cell line. Furthermore, compound **29** reduced TUBb polymerization more effectively than CA4 and shown greater potency.

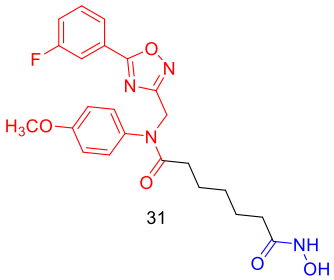

27


28

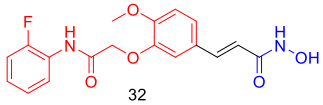

29

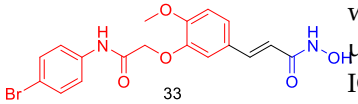
Another class of HDACIs comprising 1,2,4-oxadiazole has been created [90]. HepG2 cells were more susceptible to apoptosis induced by compounds 30 and 31 than by SAHA; their $IC_{50}$ values were 1.07 μM, 1.03 μM, and 4.50 μM, respectively. Additionally, they inhibit the HDAC1 enzyme with corresponding $IC_{50}$ values of 8.2 nM and 10.5 nM.


30

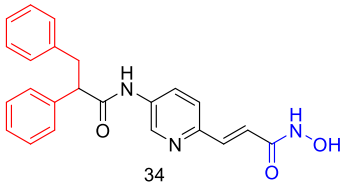

31

The N-hydroxyacrylamide moiety is viewed as a viable linker in the construction of more recent HDACIs. The cinnamide moiety of this linker functions as a hydrophobic spacer in belinostat and panobinostat, the two main HDACIs that have received FDA approval [91]. A series of HDACIs employing isoferulic acid derivatives were created to improve the affinity and activity of previously created ferulic acid-based HDACIs [92],[93]. Compounds **32** and **33** demonstrated significant HDAC inhibitory action, as evidenced by their respective $IC_{50}$ values of 0.73 ± 0.08 and 0.57 ± 0.16 μM. Against HeLa cells, compound **32** showed a moderate antiproliferative effect, with an $IC_{50}$ value of 3.91 ± 0.97 μM.

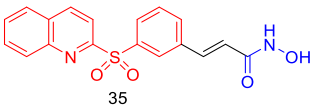

32

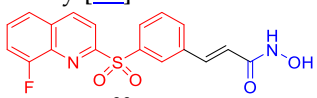

33

A new HDACI 34 was generated with a cinnamoyl moiety. At lower doses (0.1 μM), this chemical-induced apoptosis and inhibited cell proliferation in osteosarcoma cancer stem cells [94]. It is classified as a pan-HDACI since it inhibits all HDAC isoforms 1-11 in the range 0.08-12 μM.

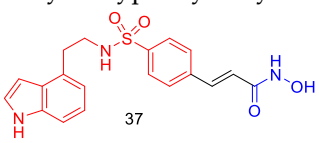

34

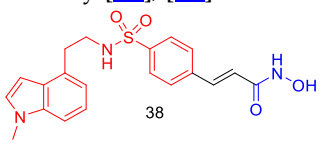
Furthermore, a number of novel N-hydroxyacrylamides **35** and **36** have been created. The presence of the N-hydroxyacrylamide moiety was found to be a significant HDAC inhibitor. Compounds **35** and **36** outperformed the FDA-approved equivalents belinostat and SAHA at HDAC1, 2, 6, and 8, but lacked HDAC isoenzyme selectivity. The SAR revealed that the presence of the N-hydroxyacrylamide group at the C3 position had the greatest antiproliferative effect but shifting this moiety from C3 to C4 resulted in a minor decrease in cellular activity. Furthermore, bioisosteric substitution of $SO_2$ with CO resulted in a little decrease in antiproliferative activity. Furthermore, replacing the quinoline ring with a pyridine caused a decrease in cellular activity [95].


35


36

In addition, 4,5-indolyl-N-hydroxyphenylacrylamide-based HDACIs were produced and evaluated for antiproliferative and HDAC inhibitory properties. The results show that 4-Indolyl compounds 37 and 38 have strong inhibitory effects against HDAC1 ($IC_{50}$ 1.28 nM and 1.34 nM, respectively) and HDAC2 ($IC_{50}$ 0.90 and 0.53 nM, respectively). The study also discovered that the best position for optimal activity was para-substitution of the N-hydroxyphenyl acrylamides moiety [96], [97].


37


38

To treat drug-resistant hepatocellular carcinoma, researchers recently produced many novel carboline and N-hydroxycinnamamide hybrids as HDACIs [97]. Compound **39** effectively inhibited the growth of human cancer cells SUMM-7721, Hep G2, HCT116, and H1299, with $IC_{50}$ values of 1.01 μM, 0.41 μM, 0.87 μM, and 0.69 μM. Additionally, it effectively inhibited HDAC1/6 with $IC_{50}$ values of 1.3 μM and 3.1 μM, respectively. The selectivity of compound **39** for HDAC1/6 may be attributed to its active fragment N-hydroxycinnamamide, which can form sandwich-like interactions with two parallel phenylalanine residues of HDAC1/6 via its vinyl benzene group and bi-chelation with the zinc ion in the active site via its hydroxamic acid group. It also showed strong antitumor activity against drug sensitive HepG2 and Bel7402 cells.

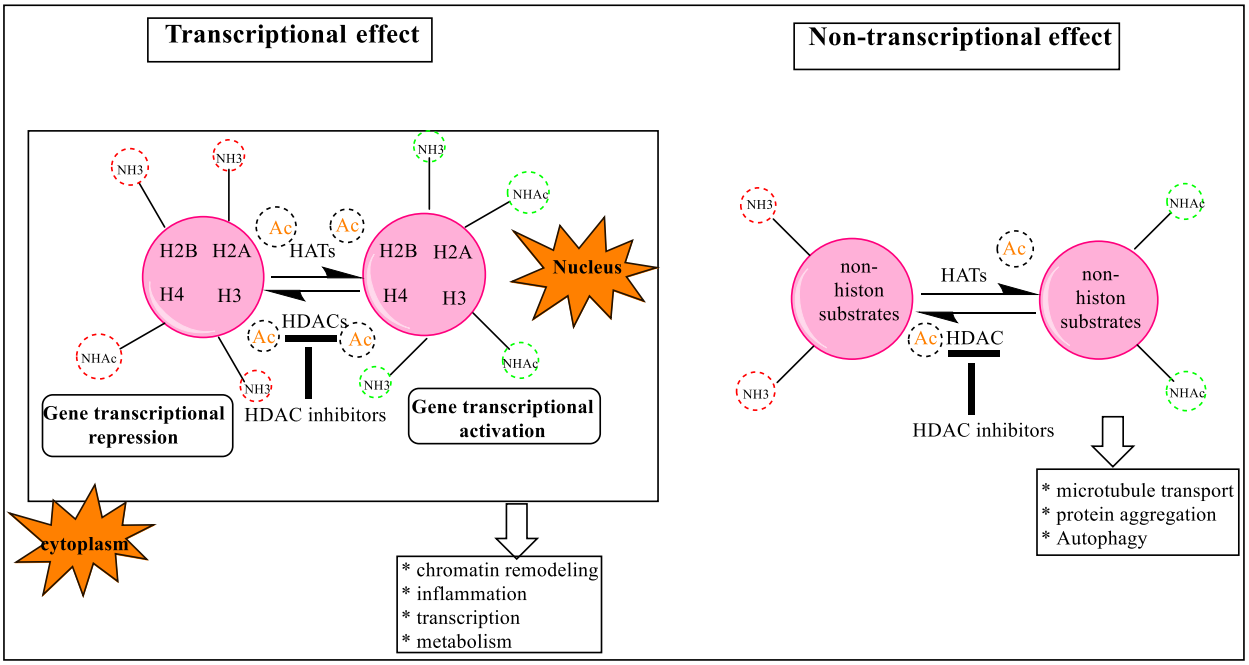



Figure 5.Mechanisms of action of HDACs inhibitors. Abbreviations: Ac = acetyl group; HDACs = histone deacetylases; HATs = histone acetyl transferases; H2B = Histone H2B; H4 = Histone 4; H2A = Histone H2A; H3 = Histone H3.

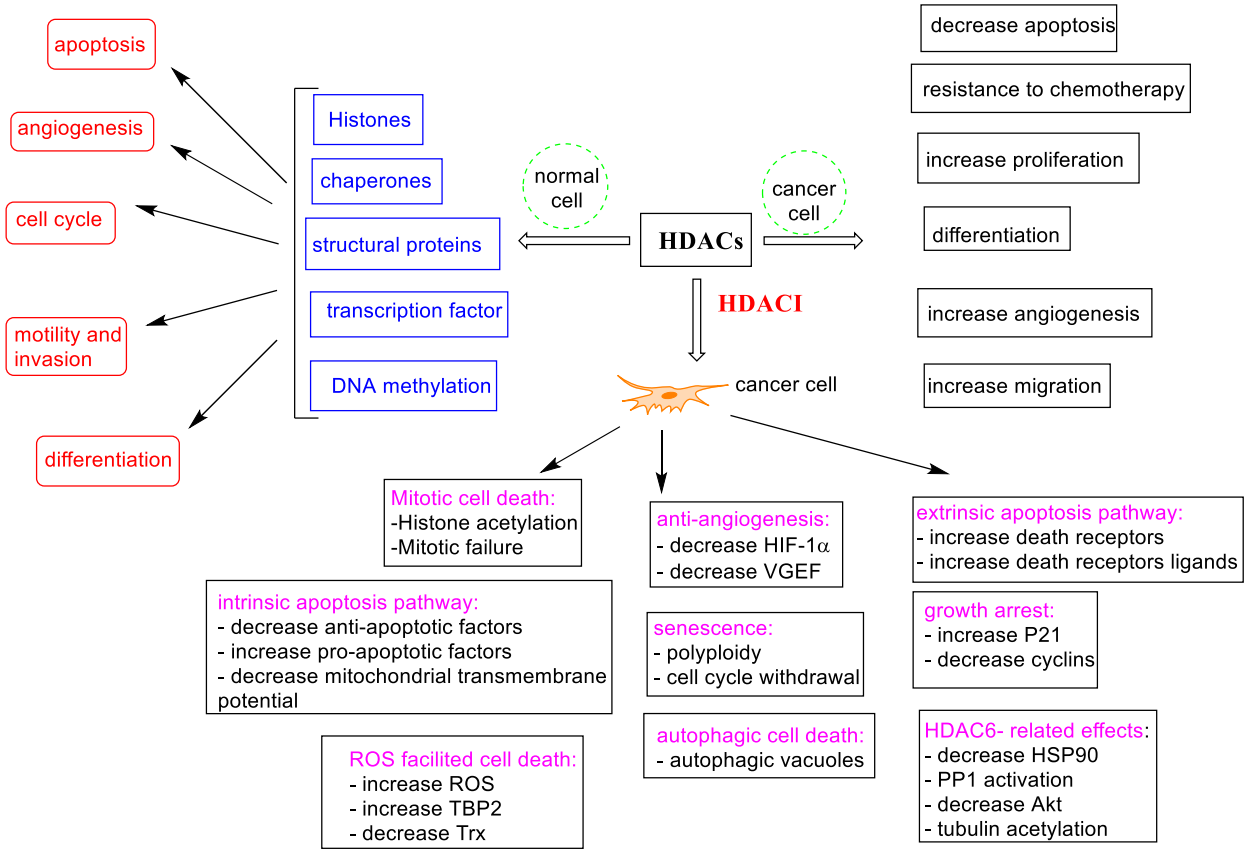



**Figure 6**. mechanism of HDACI

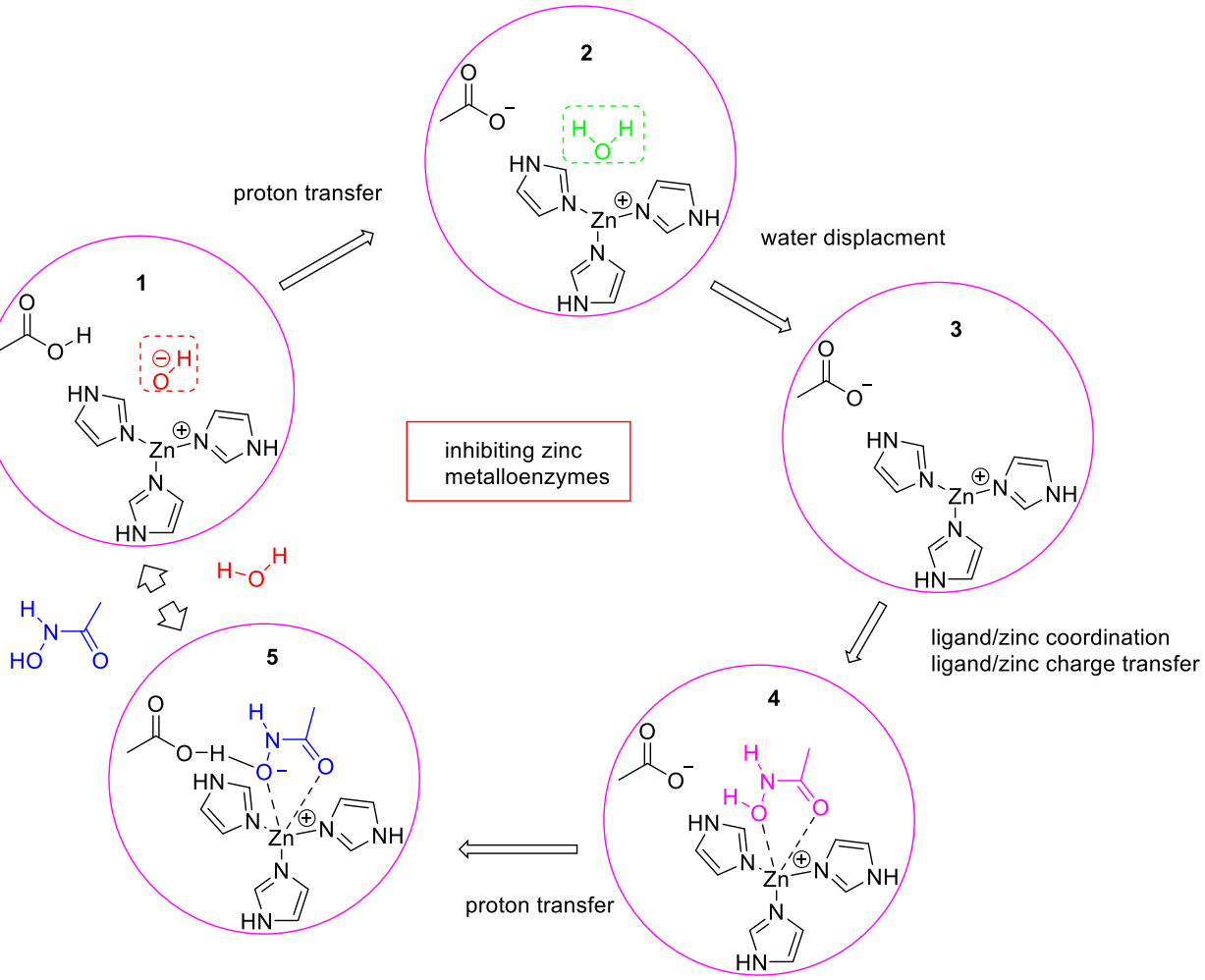



**Figure 7.** binding of HDACIs to metalloenzymes

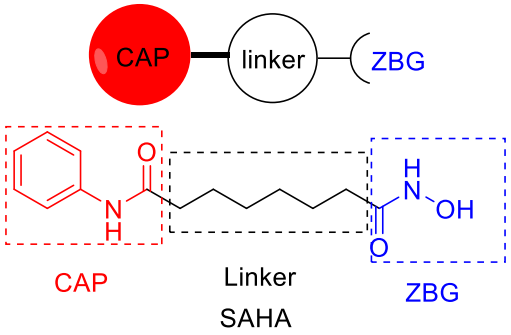



**Figure 8.** Pharmacophoric structure of HDAC inhibitor

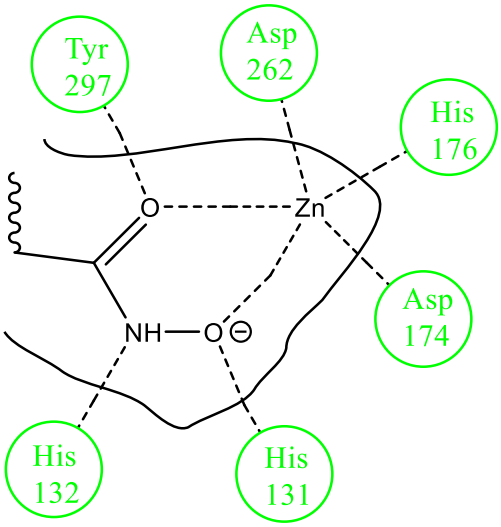



**Figure 9**. Binding of TSA and SAHA with HDLP (HDAC8)

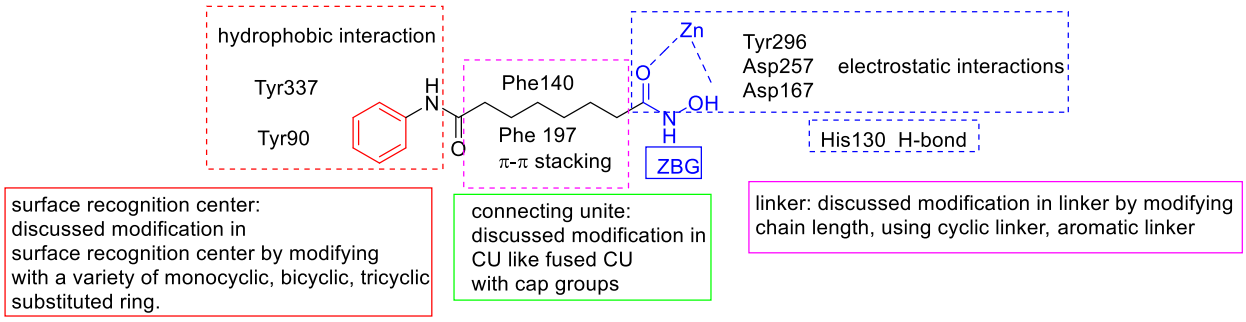



**Figure 10**. Basic pharmacophore framework representation

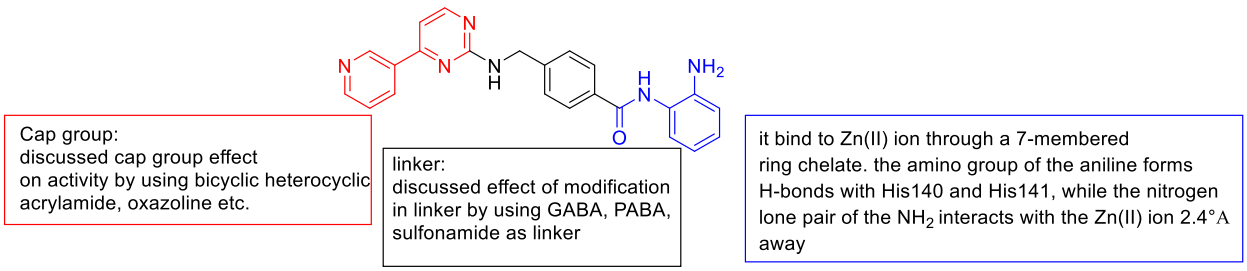
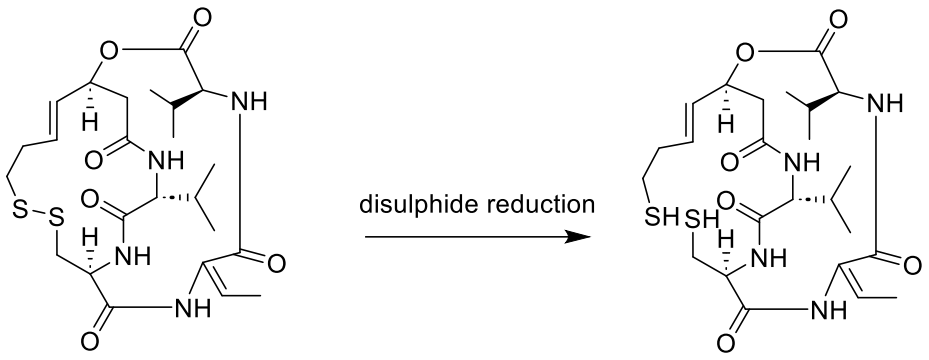



**Figure 11.** basic pharmacophore model of benzamide derivative MS-275




Romidepsin
77

Red FK
78

Figure 12. Metabolism of romidepsin

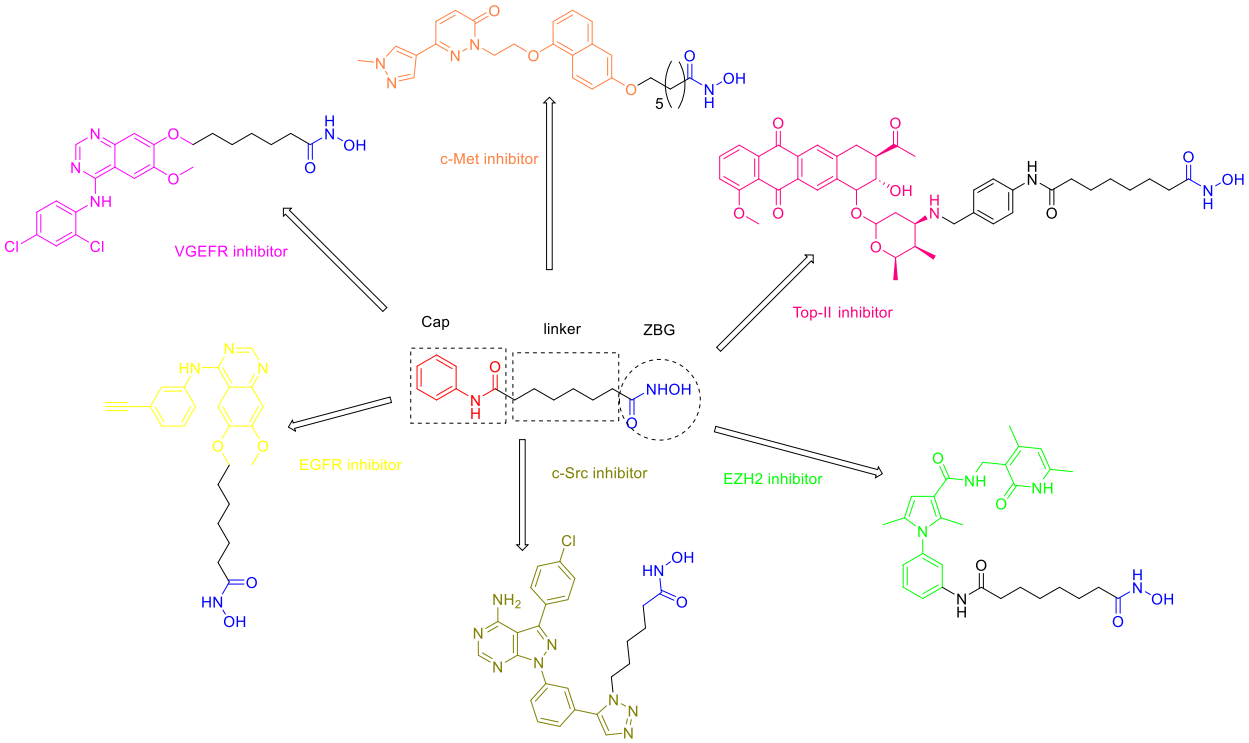



Figure 13. Examples for designing of dual target drugs

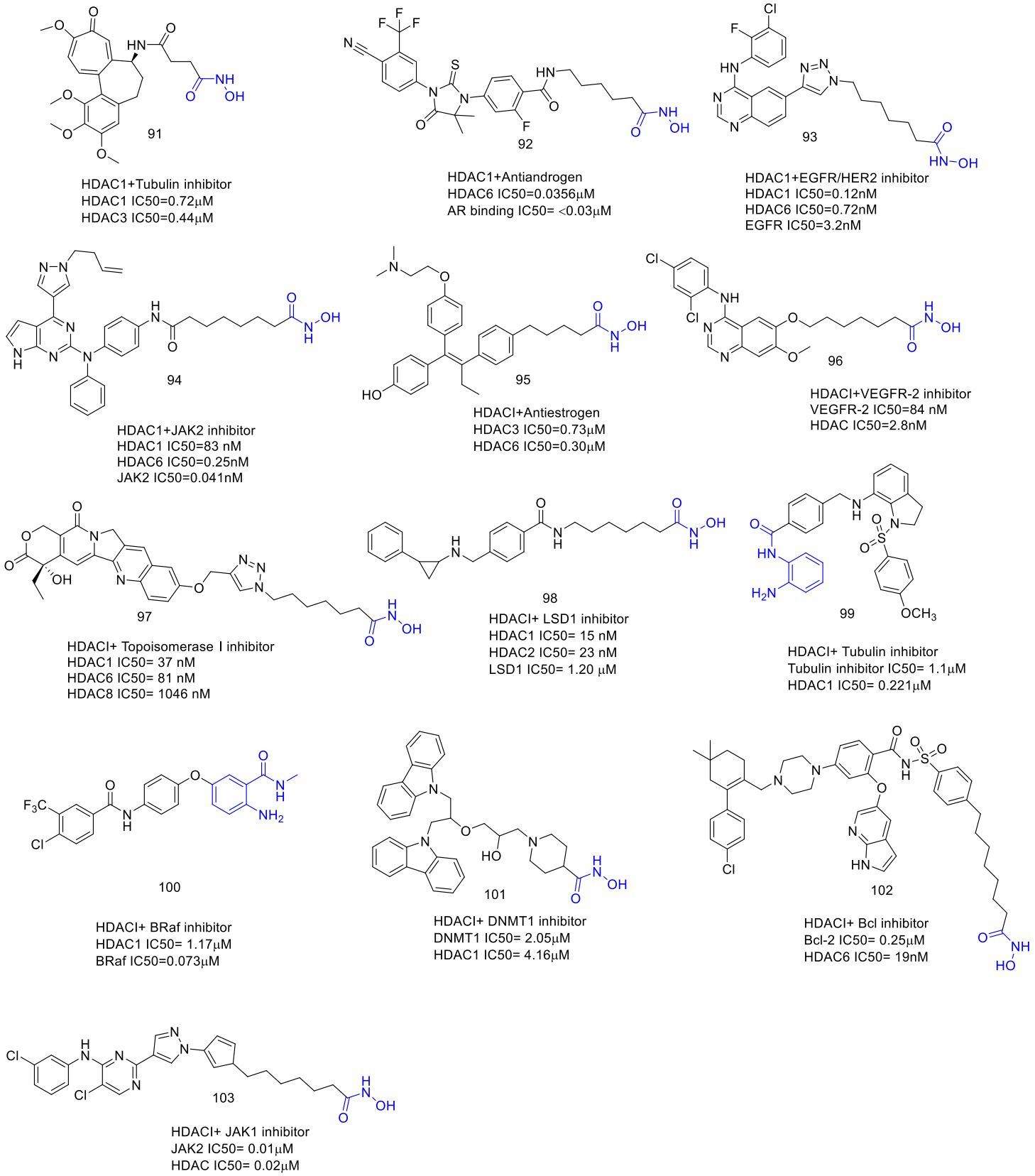



**Figure 14.** Hydroxamic acid-based HDAC inhibitors targeting mechanisms

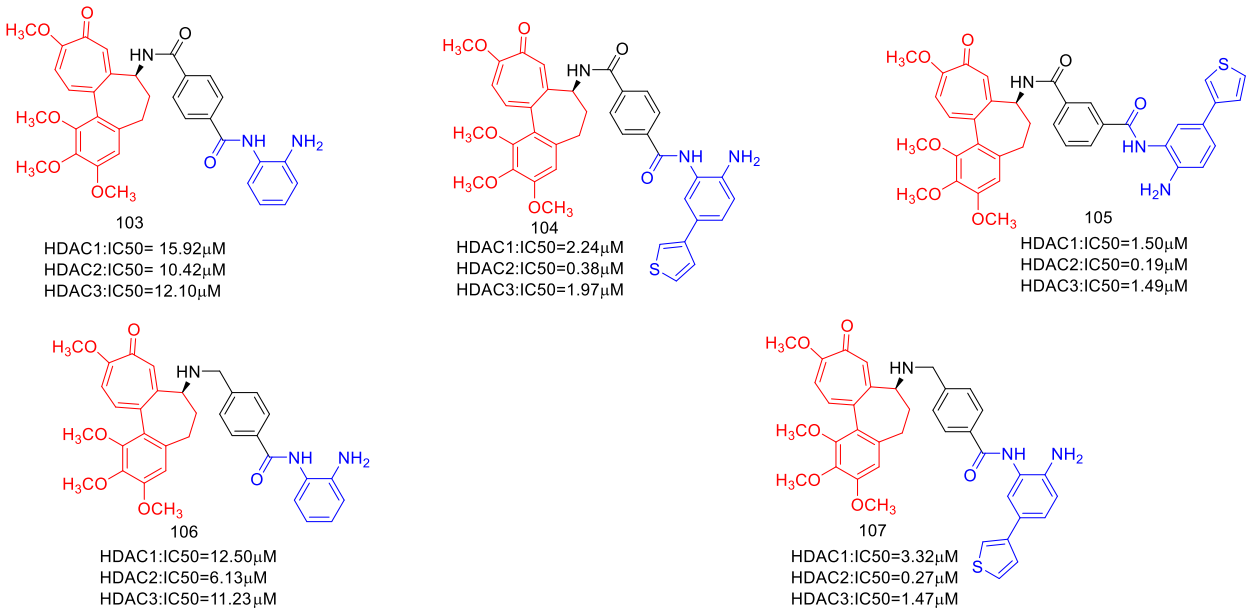



**Figure 15.** Benzamide based-HDAC inhibitors targeting mechanisms

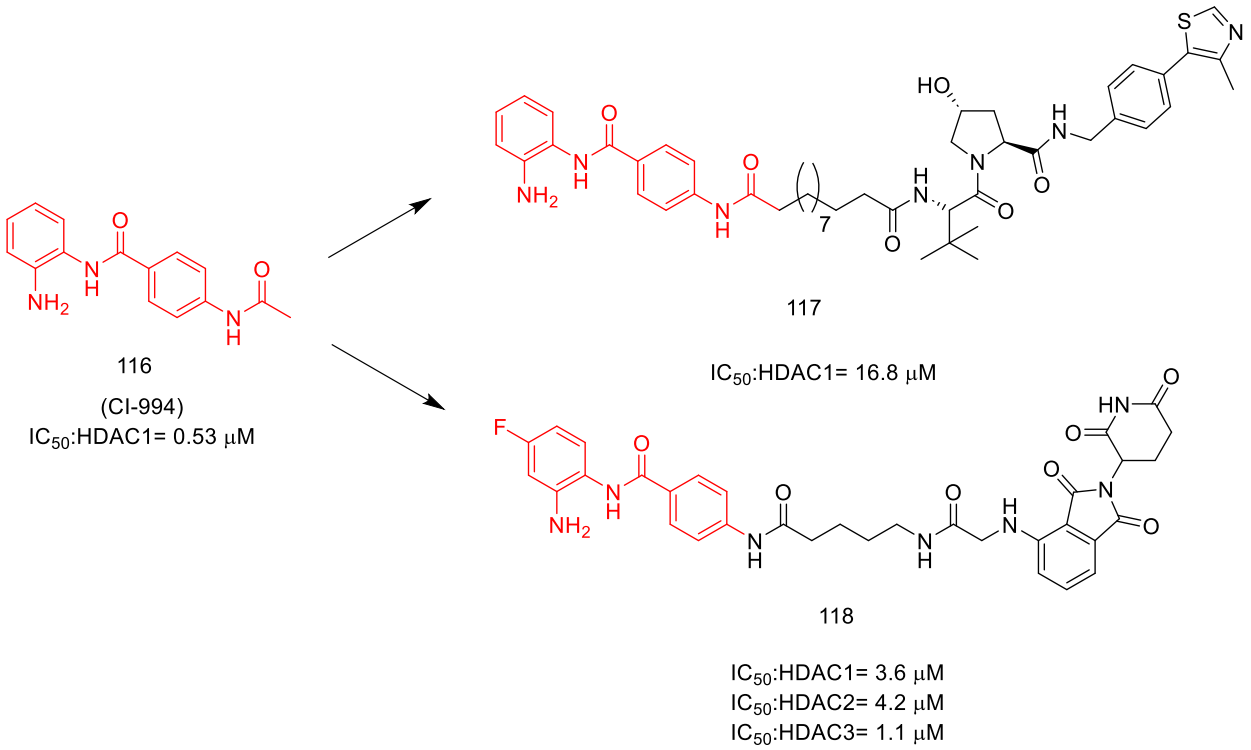



**Figure 16.** HDAC-specific PROTACs

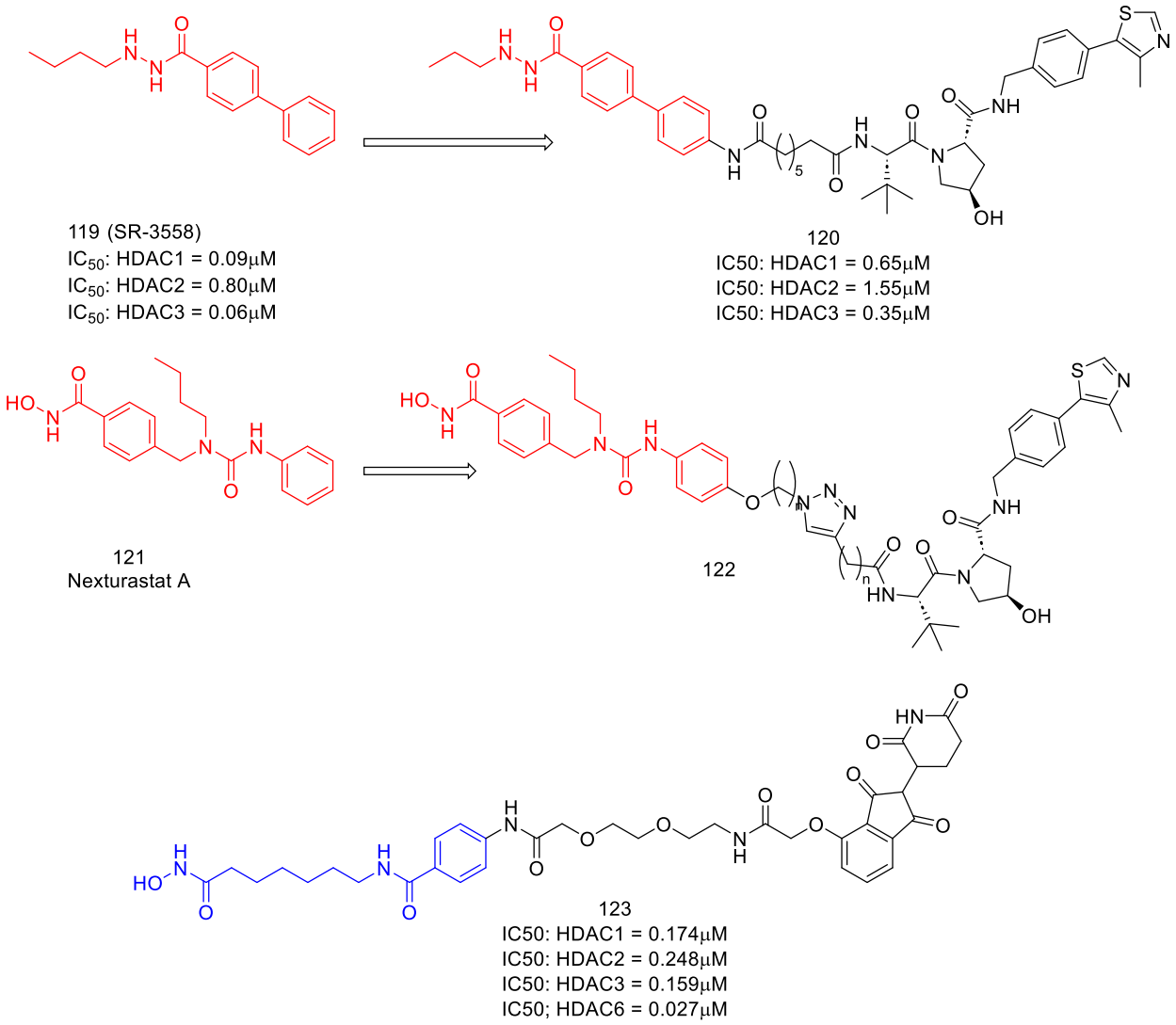
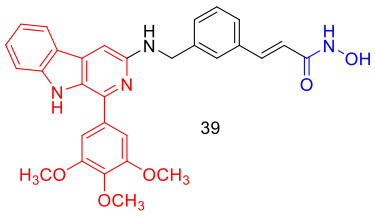
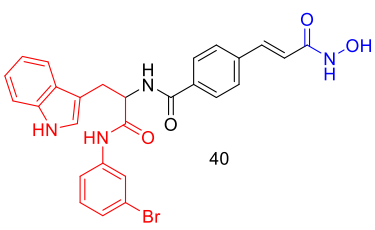


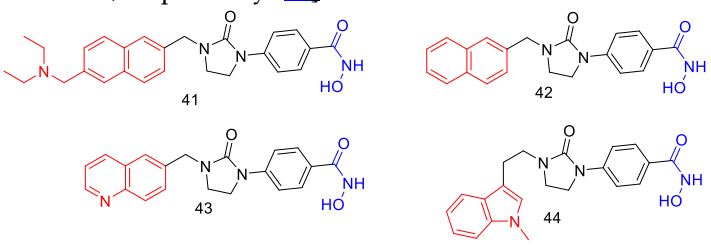

**Figure 17. HDAC specific -PROTACs**




Furthermore, compound **40**, is a new and powerful N-hydroxycinnamamide-based HDACIs with indole as a cap group have been developed [98]. All these compounds showed HDAC1 selectivity, with compound **40** having the highest $IC_{50}$ of 0.92 μM. They also shown significant antiproliferative efficacy against five cancer cell lines.

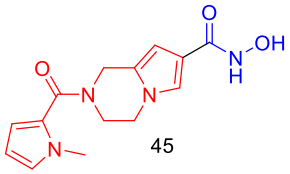


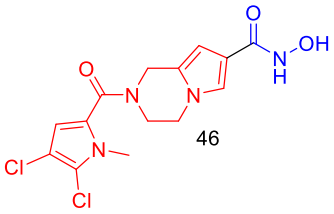

HDACIs using phenylimidazolidin-2-one as a new linker, on the other hand, have been introduced (41-44). Compound **41** showed substantial HDAC1 inhibitory activity, with an $IC_{50}$ of 2.7 nM. In comparison to vorinostat, it demonstrated a 6- to 9-fold increase in activity, with $GI_{50}$ values of 0.1, 0.086, and 0.11 μM against prostate cancer cell PC-3, leukemia cancer cell HL-60, and colon cancer cell HCT-116, respectively. The remaining compounds in this series, **42-44**, also inhibited HDAC1 but at lower concentrations than **41**, with $IC_{50}$ values of 5.4, 5.4, and 4 nM, respectively [91].



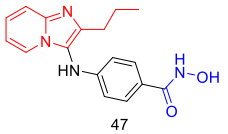

produced the hydroxamic acid derivatives **45** and **46** of 1,2,3,4-tetrahydropyrrolo[1,2-a] pyrazine. Compared to larger groups like indole, the pyrrole moiety functions as a cap group, giving the compounds more potency and

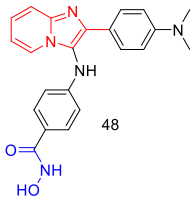
selectivity. Compound **45** showed an $IC_{50}$ of 33 nM, substantially inhibiting HDAC6. This corresponds to a 200-fold selectivity against nuclear extract (HDAC1, 2) and a 100-fold selectivity against HDAC8 [92]. **[99]**

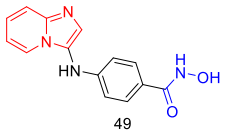


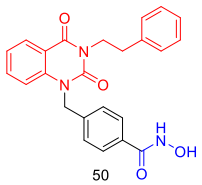

In addition, a series of HDACIs containing imidazo[1,2-a] pyridine-based cap groups **47–49** was developed. Compound 47, with a 4-aminophenyl linker, is a selective HDAC6 inhibitor, having an $IC_{50}$ of 0.058 μM and a selectivity factor of **47**. Compounds **48** and **49** inhibit HDAC 6 with $IC_{50}$ values of 0.05 and 0.076 μM, respectively, but have lower selectivity factors (3 and 11) **[99]**.

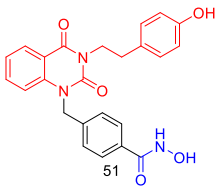


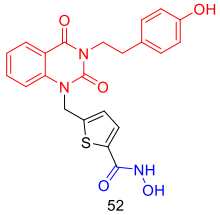



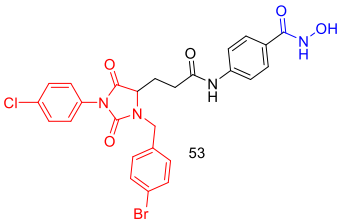

Moreover, the selectivity of HDAC6 was reduced by a series of hydroxamic acids **50–52** based on quinazoline 2,4-dione. With an $IC_{50}$ of 4 nM, compound **50** showed the strongest specific inhibition of HDAC6. The $IC_{50}$ values of compounds **51** and **52** were 5 and 20 nM, respectively. The HDAC6 inhibitory activity was shown to be reduced by moving the hydroxamic acid group or substituting alternative rings for the phenyl ring, according to the SAR research [100].

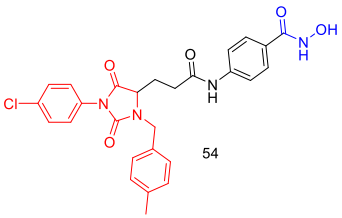


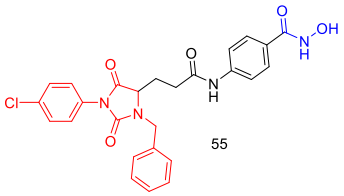




As novel HDAC6 selective inhibitors, a number of 2,4-imidazolinedione compounds have been developed **53–55**. With $IC_{50}$ values of 9.7, 4.4, and 7.6 nM, respectively, they effectively inhibited HDAC6. Additionally, they showed significant antiproliferative activity ($IC_{50}$ values ranging from 0.23 to 9.45 nM) against five cancer cell lines. Compound **54** induced apoptosis in the HL-60 cell line by activating caspase-3**[101]**.

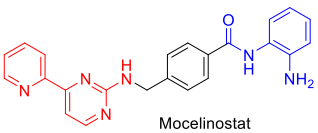


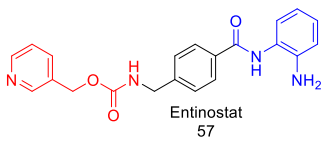



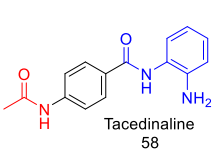




### 6. *Benzamides or ortho-Aminoanilides*

The second class of HDACIs with good anticancer effectiveness were benzamide HDACIs, which bind to the zinc ion in the HDAC active site via the benzamide moiety [102],[103]. They have a higher affinity for specific HDAC isoforms than the hydroxamate class. Several benzamide inhibitors are under investigation in clinical studies, including mocetinostat **56** (MGCD0103, phase II), entinostat **57** (MS-275, phase III), and tacedinaline **58** (CI994, phase II) [104],[105],[106],[107],[108]. MS-275 displays hazardous effects as a result of its inherently unstable structure. Various adjustments can be made at different locations indicated in Figure 91. In order to mitigate the harmful impact of benzamides, numerous compounds, which are briefly outlined in this review, have been created with a more stable group in comparison to MS-275. Furthermore, a significant number of them exhibit a minor improvement in their activity when tested in a laboratory setting, but their pharmacokinetic profile is considered undesirable (fig.11).

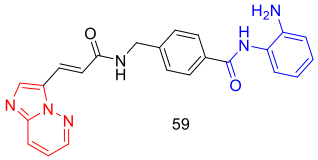


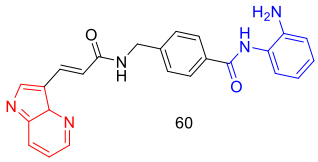



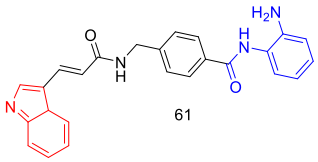




A series of HDACIs, compounds **59-61** having bicyclic heterocycle moieties, have been designed and synthesized based on the primary drug entinostat **57** [109] . With $IC_{50}$ values of 0.118 μM, 0.129 μM, and 0.120 μM, they exhibited a respectable inhibitory efficacy against HDAC1, surpassing that of entinostat **57** ($IC_{50}$ = 0.273 μM). Moreover, they possessed more potent antiproliferative qualities than **57**. The SAR studies indicate that the

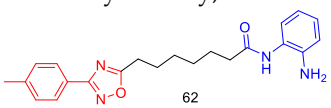
anticancer activity against three cancer cell lines is decreased when a fluoro group is added to the benzamide ring. Bicyclic heterocyclic substituted compounds exhibited superior inhibitory effects compared to those substituted with pyridinyl. The inhibitory action is reduced when ethyl is used in place of methyl. With $IC_{50}$ values of 0.41 and 0.46 μM, respectively, compound **59** demonstrated a significant antiproliferative effect against NCI-H661 and U937. This [110] action was considerably greater than vorinostat.

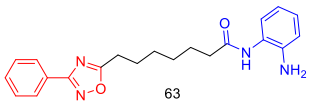

59


60

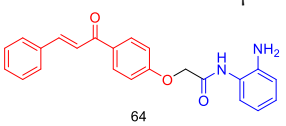

61

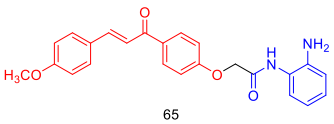
Compound **62** showed a greater degree of selectivity for HDAC1 over HDAC2, with $IC_{50}$ values of 140 nM for HDAC1 and 680 nM for HDAC2[110] . Compound **63** demonstrated a substantial antiproliferative activity against NCI-H661 and U937, with $IC_{50}$ values of 0.69 and 0.73 μM, respectively. It also shows stronger HDAC1 inhibitory activity, with an $IC_{50}$ of 210 nM.


62

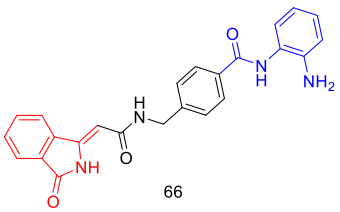

63

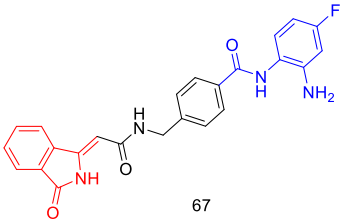
created a new class of chalcone derivatives known as ZBG that have a 2-aminobenzamide group. HDAC inhibitory activity was significant for compounds **64** and **65**. Compound **65** showed $IC_{50}$ values larger than 100 μM and modest anticancer activity against three cancer cell lines: MCF-7, HepG2, and HCT-116. Conversely, compound 64 showed similar antiproliferative activity to SAHA, with an $IC_{50}$ of 3.02-12.99 μM. [111].


64

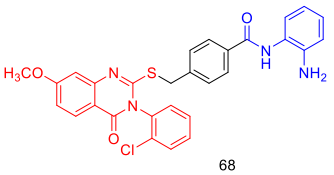

65

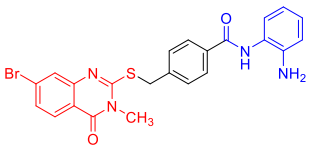
based on isoindolinones, developed a novel class of HDACIs. HL-60 and K562 cell lines were suppressed by compounds **66** and **67**, respectively, with $IC_{50}$ values ranging from 193 to 450 nM. They also demonstrated superior HDAC1 inhibition than chidamide, with $IC_{50}$ values of 65.6 nM, 65.1 nM, and 296 nM, in that order. The SAR study found that adding methyl or chlorine substituents to compound **67** decreased HDAC1 inhibitory action, probably as a result of interference at this position [112].

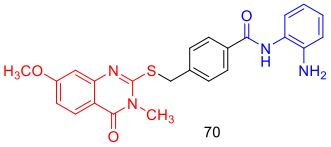

66

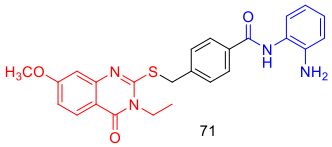

67

A range of novel 2-aminobenzamide compounds including thioquinazolinone have been produced and are designated as HDACIs **68–71** [113]. Regarding their antiproliferative efficacy against the three cancer cell lines A375, A549, and SMMC7721, they fared better than CS055, MS-275, and CI994. The compounds 68–71 exhibited remarkable inhibitory activity against HDAC1, as evidenced by their respective $IC_{50}$ values of 0.38 μM, 0.29 μM, 0.01 μM, and 0.1 μM. Additionally, they showed inhibition of HDAC2 with $IC_{50}$ values of 0.61 μM, 0.53 μM, 0.16 μM, and 0.18 μM, in that order. Compound **79** exhibited superior antiproliferative efficacy against three distinct[114] cancer cell lines, as seen by its $IC_{50}$ values of 0.98 μM, 0.75 μM, and 0.03 μM.

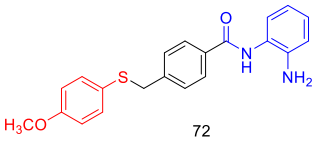

68

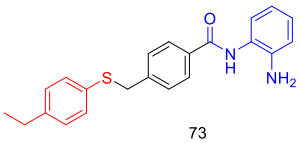

69


70


71

further produced a number of 2-aminobenzamide HDACIs based on thioethers [114]. With $IC_{50}$ values ranging from 1.64 to 3.80 μM, compounds **72** and **73** showed significant antiproliferative action against five cancer cell lines. Additionally, they showed potent HDAC inhibitory action against HDAC1 and 2, with $IC_{50}$ values for **72** and **73** being 0.016 μM and 0.205 μM and 0.071 μM and 0.144 μM, respectively.

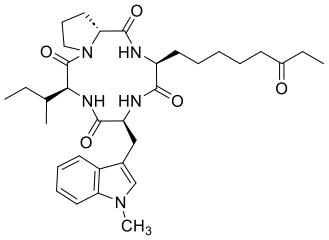

72


73

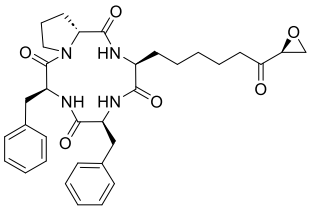
## 7. *Cyclic peptides*

Cyclic-peptide HDACIs (**74**, **75**, **76**, and **77**) constitute the most sophisticated class of HDACIs [115],[116],[117]. Romidepsin (FK-228) **77**, a natural chemical derived from Chromobacterium violaceum, serves as the prototype for this class. Romidepsin is a prodrug that is transformed into the active form **78** (RedFK) upon entering cells (fig.8) [118],[119]. Even though its thiol ZBG is a weaker group, this naturally occurring compound has far stronger

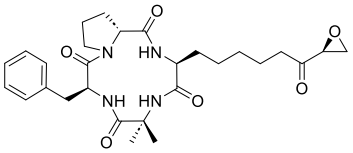
HDAC inhibitory activity than hydroxamate and other HDACIs **[**120**]**.



Apicidin
74

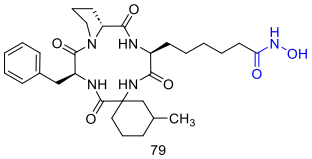


Trapoxin B
75

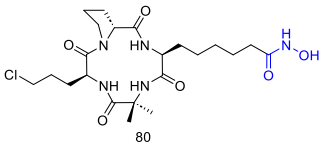


Chlamydocin A
76

Several HDACIs have been developed as potential anticancer drugs based on the structures of natural and synthetic compounds such as TSA, SAHA, chlamydocin, and FK228. Compound **79**, a chlamydocin analog, exceeded TSA in antiproliferative activity against the breast cancer cell MCF-7. Furthermore, compound **79** inhibited HDAC1 and HDAC4 with $IC_{50}$s of 15 nM and 14 nM, respectively. This cyclic hydroxamic acid peptide combines the cyclic peptide and hydroxamate classes. Compound **80** inhibited HDAC1 and HDAC4 with $IC_{50}$ values of 14 and 19 nM, respectively[121].

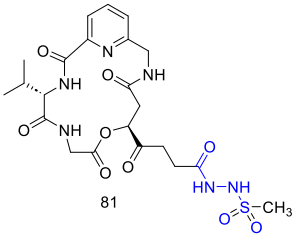


79

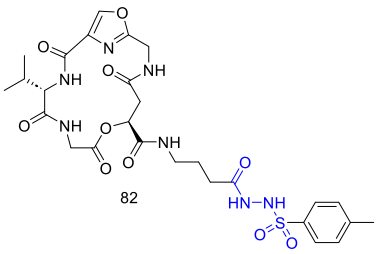


80

The inhibitory efficacy and specificity of the novel cyclodepsipeptide analogs **81** and **82** against HDAC1, HDAC3, and HDAC6 isoenzymes were investigated. They effectively inhibited HDAC3 with $IC_{50}$ values of 10.9 μM and 1.4 μM, respectively. The sulfonylhydrazide group acts as the ZBG, and its two oxygen atoms chelate with the zinc ion [122].



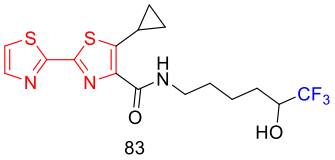
81



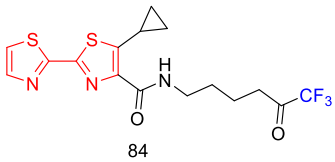
82

### *Electrophilic ketones*

Electrophilic ketones, such as trifluoromethyl ketones, have been used as ZBG substitutes. They had substantial HDAC inhibitory activity [123]. This moiety hydrates easily in water and binds to the zinc ion in the active site of the HDAC enzyme [124]. compounds **83 and 84,** which are bisthiazole-based derivatives, with a trifluoromethyl ketone moeity [125]. Compound **84**, in particular, showed strong inhibitory activity against human HDAC1, 3, 4, and 6, with an $IC_{50}$ of 20–30 nM. Additionally, it showed enhanced antiproliferative activity against cancer cell lines RPMI 8226 and NCI-H929, with $IC_{50}$ values of 0.08 and 1.23 μM, respectively. The ketone group in compound **87**, like compound **83**, diminished its HDAC inhibitory action. Furthermore, changing the length of the linker or cyclopropyl group to isobutyl or benzyl decreases compound **84**'s HDAC inhibitory activity.



83

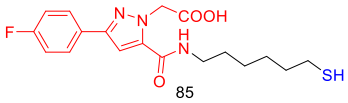


84

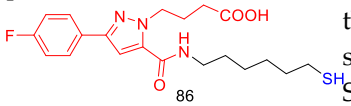
### *Miscellaneous*

There are numerous HDACIs with varying ZBGs, the most common of which are thiol-based HDACIs. Thiols are widely used zinc-dependent enzyme chelators. After replacing the hydroxamate group of SAHA with a thiol group, the $IC_{50}$ of the thiol-based SAHA was found to be 0.21 μM, making it just as effective [63]. Several novel thiol-based HDACIs with a cap group of 3-phenyl-1H-pyrazole-5-carboxamide were produced [126]. Compound **86** suppressed HDAC efficiently ($IC_{50}$ = 0.08 μM). The presence of an aliphatic carboxylic acid at position 1 in the pyrazole ring made it more selective for HDAC6 than HDAC1, with $IC_{50}$ values of 0.09 μM and 0.13 μM, respectively. Compound **85** has a higher specificity for HDAC6 ($IC_{50}$ = 0.04 μM) compared to compound **86**. The alkyl chain length of 4 carbon atoms at N-1 of the pyrazole ring was optimum for HDAC inhibitory activity, outperforming compounds with 2 or 3 carbon atoms, such as compound **86**, which has strong inhibitory action. The addition of a methyl group to the pyrazole C-4 position decreased the HDAC inhibitory

activity. The inhibitory activity is diminished when the thiol group is replaced by mercaptoacetamides .

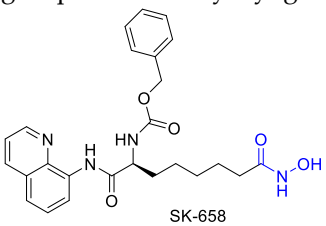



85

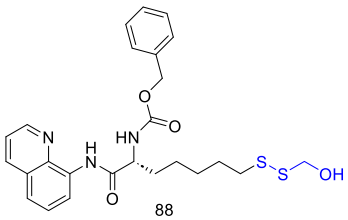



86

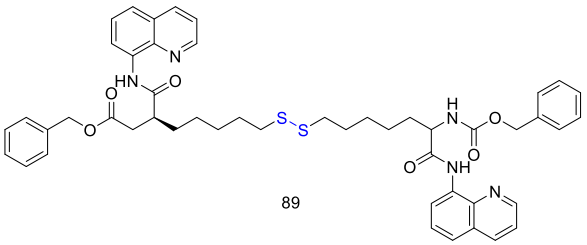
Compounds **88** and **89**, which are disulfide SK-658 87 analogs, have higher HDAC inhibitory activity than SAHA and SK-658 against HDACs 1, 4, and 6. The $IC_{50}$ values range from 2 to 8.2 nM. Dithiothreitol (DTT), which binds strongly to the active site, converts the disulfide group to the sulfhydryl group [4].




SK-658
87

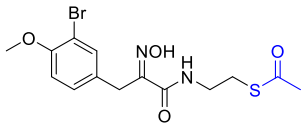



88




89

Furthermore, thioesters derived from the natural material psammaplin A have demonstrated moderate to high cytotoxic and HDAC inhibitory activity. Thioester compound **90** inhibited recombinant HDAC1 efficiently, with an $IC_{50}$ of 5 nM. It demonstrated high cytotoxic activity against the breast cancer cell MCF-7, with an $IC_{50}$ of 3.2 μM [127].




90

### *Multi-targeted HDACIs*

An individual inhibitor can selectively hinder a specific target molecule. Consequently, a solitary inhibitor specifically targets cancer cells while excluding normal cells, resulting in great selectivity and effectiveness and little toxicity. Initially, while clinicians may be enthusiastic, they quickly encounter the issue of cancer cells developing resistance to the medicine following repeated administration. Consequently, patients have a limited life expectancy. The resistance mostly arises from the epigenetic modification of the target, rendering the drug molecule incapable of binding to the target and thus the targeted therapy ineffective. Due to the intricate nature of cancer, manipulating a single target can be easily done, leading to therapy failure. Subsequently, the practice of utilizing a combination of pharmaceuticals has superseded the use of a single targeted pharmacological therapy. This involves administering two or more drugs simultaneously to improve their effectiveness. Simultaneously, the use of numerous pharmaceuticals might heighten the likelihood of drug-drug interactions and the occurrence of toxicities that restrict the dosage. Despite the initial success of combination therapy in cancer treatment, it is no longer beneficial due to these drawbacks. Hence, a singular medication possessing the capacity to simultaneously target two specific objectives could function as an efficacious approach in the realm of cancer therapies [128]. The development of dual-targeting inhibitors is based on the goal of overcoming medication resistance and reducing the toxicity associated with single-targeted inhibitors. A recent report has shown that dual-targeting inhibitors have yielded greater positive results in the treatment of cancer. As an example, erlotinib, a medication that targets a specific site, is the primary treatment for non-small-cell lung cancer (NSCLC). However, its effectiveness is restricted because of drug resistance. The combination of vorinostat (SAHA) and erlotinib produces a synergistic anticancer effect in non-small cell lung cancer (NSCLC). This observation prompted the quest for a novel and potent treatment for human lung cancer. Combination therapy enhances effectiveness by administering two individual drugs simultaneously. However, this approach has disadvantages such as drug–drug interactions, toxicity, and drug resistance. However, the combination of SAHA with erlotinib as a single molecule result in effective outcomes due to reduced toxicity and drug-drug interactions, as well as improved efficacy.

**Importance of dual-target drugs**

The utilization of dual-targeting inhibitors has been employed since the inception of modern pharmacology and has demonstrated notable efficacy in clinical settings [129]. A dual-targeting inhibitor concurrently affects two molecular targets. Dual-target pharmaceuticals, also known as chimeric medications, have gained significant interest in the last 10 years. This field of research has had rapid growth and is currently a focal point for medicinal chemists. By simultaneously inhibiting two anticancer agents that contribute to disease progression, medication resistance is enhanced. In addition, dual-target therapies can address the drawbacks associated with single-targeted medications, such as drug–drug interactions, inadequate safety, a low therapeutic index value, limited effectiveness, and various adverse effects [130]. In this review, we examine a bifunctional single medication that is composed of two established inhibitors. This drug

targets two particular areas and offers the benefits of increased anticancer activity and decreased adverse effects. To improve the attraction and effectiveness in comparison to their separate original substances, two molecules are merged to create novel hybrid compounds. Dual inhibitors have several advantages over their separate parent compounds. They exhibit higher activity, lower toxicity, and enhanced therapeutic index values and bioavailability. Currently, there are a limited number of dual-targeting inhibitors undergoing clinical studies for the treatment of cancer. CUDC-101, a dual-targeting inhibitor of epidermal growth factor receptor (EGFR) and histone deacetylase (HDAC), and CUDC-907, a dual inhibitor of phosphoinositide 3-kinase (PI3K) and HDAC, have completed phase I and phase II clinical trials for the treatment of solid tumors and relapsed/refractory diffuse large B-cell and high-grade B-cell lymphoma, respectively. Hence, the advancement of dual-targeting inhibitors is receiving considerable interest in the search for anticancer medications that offer superior pharmacokinetic benefits in comparison to traditional cancer treatments [131].

**Approach for Developing a Dual-Targeted Inhibitor**

A single compound combines the two known inhibitors to function as a dual-targeting inhibitor. The inhibitors are linked or fused to create a single bifunctional molecule that can inhibit two targets simultaneously. In addition, computational-based design has been crucial in the field of medication development, which is both costly and time-consuming. The use of computational chemistry, combined with high-throughput screening tools, has expedited the drug discovery process. Hence, this computational methodology can also be applied while developing a dual-targeting inhibitor. Computer-aided drug design encompasses several approaches such as target identification, docking-based virtual screening, scoring functions, molecular similarity calculation, virtual library creation, and sequence-based drug design. The next section provides a comprehensive explanation of the two methodologies (fig.13) [132].

**Hydroxamic acid-based HDAC inhibitor with dual targeting capabilities**

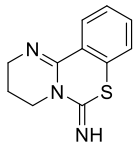
Because cancer develops and progresses through multiple receptors or signaling pathways, drugs that target only one receptor may be unsuccessful. To overcome this hurdle, multi-targeted treatments were developed, which were one of the most effective approaches and preferred above monotherapies. This combined therapy generated a synergistic or potentiating effect, resulting in significant anticancer activity [133]. A multi-targeted drug integrates two or more distinct pharmacophores into a single structure to create a hybrid molecule. Hybrid compounds with HDAC inhibitory activity and an additional activity have been an intriguing topic of research and are currently being produced [134]. HDACIs with topoisomerase inhibitors, antiestrogens, antiandrogens, LSD inhibitors, tubulin inhibitors, VEGFR-2 inhibitors, EGFR/HER2 kinase inhibitors, JAK-2 inhibitors, Bcl-2 inhibitors, BRafV600E inhibitors, and DNMT inhibitors include compounds **91-103**(fig.10)[135],[136],[137],[138],[139],[140],[141],[142].

**Benzamide-based HDAC inhibitor with dual targeting capabilities**

Compounds **103-108** were produced and evaluated for efficacy as tubulin-HDAC dual inhibitors. A benzamide zinc binding group was added to hybrids to alter the inhibitory action of HDAC and tubulin. In contrast to hybrids with unsubstituted benzamide ZBG, their counterparts with bis(aryl)-type ZBG were more effective against HDAC1-3 (**103** against **104**, **106** versus **107**). Compound **105** was a potent HDAC-tubulin dual inhibitor with cytotoxicity similar to colchicine. Compound **106** inhibited tubulin had moderate anti-HDAC activity and had the highest cytotoxicity. Compound **103** had high anti-HDAC activity against HDAC1 ($IC_{50}$ = 1.50 μM), HDAC2 ($IC_{50}$ = 0.19 μM), and HDAC3 ($IC_{50}$ = 1.49 μM), as well as mild antiproliferative activity against the A549 cell line ($IC_{50}$ = 2.790 μM). The differential in cytotoxicity was caused by changes in cell membrane penetration (fig.11) [143].

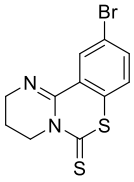
**Non-canonical HDACs inhibitors**

Due to the potential mutagenicity, it's necessary to search other ZBGs as replacement. Meyer-Almes' was discovered as dimethylarginine dimethylaminohydrolase 1 (DDAH1) inhibitor PD-404,182 **108** is a highly selective HDAC8 non-hydroxamate inhibitor with $IC_{50}$ value 0.011μM. Compound **108**, decomposes rapidly in the presence of glutathione (GSH) [144].Over other HDACs, the representative compound **109** demonstrated double-digit nanomolar potency and significant HDAC8 selectivity with $IC_{50}$ value 0.26μM but less potent than **108** due to the presence of Br [145].



108

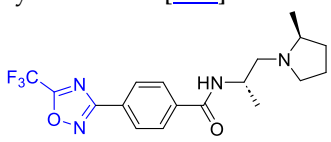
(PD-404,182)
HDAC8 IC50=0.011μM
HDAC1 IC50=3.6μM
HDAC6 IC50=8.6μM



109

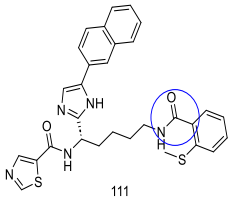
HDAC8 IC50=0.26μM
HDAC1 IC50=>50μM
HDAC6 IC50=>50μM

Compound **110** was identified as a CNS-penetrant 5-(trifluoromethyl)-1,2,4-oxadiazole derivative with PK characteristics and CNS exposure suited for the research of Huntington's disease in mice. Compound**110** had a larger unbound exposure in the mouse brain than the existing hydroxamate-based inhibitors, indicating the potential for Class IIa HDAC targeted treatments for Huntington's disease (HDAC4:$IC_{50}$ = 10nM). It should be mentioned that 5-(trifluoromethyl)-1,2,4-oxadiazole could be used as a pharmacophore for HDACs instead of standard ZBG hydroxamate [146].



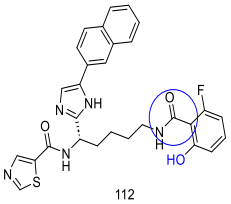
110

To quickly synthesize substituted benzamide libraries, the use of a parallel medicinal chemistry method. They discovered a series of drugs with 2-substituted benzamide functional groups that appeared to particularly target HDAC3.The example compound **111** is a strong and selective HDAC3 inhibitor ($IC_{50}$ of 30 nM and greater than 300-fold selectivity against other HDACs). Interestingly, its close analogue **112** was shown to retain HDAC3 potency while losing all selectivity over HDAC1 and HDAC2[147]. The identification of a variety of new HDAC inhibitors missing ZBG, which exhibited good selectivity primarily for diverse subtypes such as HDAC1, HDAC2, HDAC3, HDAC10, and HDAC11. In biochemical and cell-based studies, compound **113** exhibits remarkable potency against HDACs, comparable to the marketed HDACs medicines Belinostat **(3)** and Vorinostat **(1).** This chemical belongs to a new class of HDAC inhibitors that lack electrophilic and mutagenic structural features like hydroxamate [148].



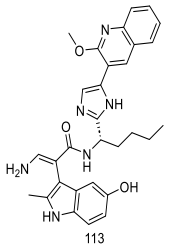
111

HDAC3 IC50=29 nM
HDAC1 IC50=20000 nM
HDAC2 IC50=31000 nM



112

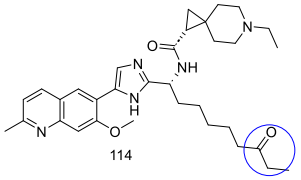
HDAC3 IC50=7.6 nM
HDAC1 IC50=9.7 nM
HDAC2 IC50=29 nM



113

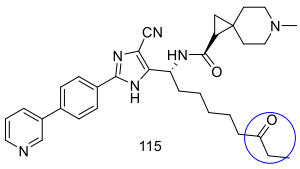
HDAC3 IC50=5 nM
HDAC1 IC50=2 nM
HDAC2 IC50=5 nM

They used ethyl ketone as a ZBG in HDAC inhibitors that specifically target HDAC1-3. These inhibitors, which include a variety of substituted heterocycles on the imidazole or oxazole scaffold, exhibit good enzymatic activity against HDAC1-3 while being highly selective against HDAC6 and HDAC8. Compound **114** is a potent HDAC1-3 inhibitor that exhibits remarkable selectivity over HDAC6 and HDAC8. It also shows lower hERG activity (cardiovascular liabilities). Compound **115** maintains HDAC1-3 inhibitory efficacy while dramatically improved selectivity over HDAC6, HDAC8, and PK characteristics [149].



114

IC50:HDAC1<0.30 nM; IC50:HDAC2<0.40 nM
IC50:HDAC3<0.30nM
IC50:HDAC6=350 nM; IC50:HDAC8=63000 nM



115

IC50:HDAC1=0.2 nM; IC50:HDAC2=1.7 nM
IC50:HDAC3=0.3nM
IC50:HDAC6=9466nM
IC50:HDAC8=12620 nM

### *HDAC-specific PROTACs*

The Proteolysis Targeting Chimera (PROTAC) is a method that exploits the natural ubiquitin-proteasome system (UPS) to break down a specific protein of interest (POI). PROTAC, a groundbreaking tool in drug discovery, possesses numerous benefits including strong effectiveness, prolonged duration of impact, and the potential to selectively target certain tissues or cell types [150]. PROTACs were created through the formation of a covalent bond between a small molecule inhibitor of the protein of interest (POI) and a ligand for E3 ubiquitin ligase. These two components are joined together by a linker of varying lengths. Recently, some HDAC-specific PROTACs have been described. Although numerous HDAC inhibitors have been reported, only a limited number of them have been used as ligands for POI in the development of HDAC targeted PROTACs. The primary ligands for E3 ligases are cereblon (CRBN) and Von Hippel-Lindau (VHL), which facilitate the recruitment of E3 ligase activity to the target protein. Smalley et al. developed four PROTACs targeting Class I HDACs. The primary degrader **117** comprises a benzamide HDAC inhibitor CI-994 (**116**), an alkyl linker, and the VHL E3 ligand. At a dose of 1 μM, there is a reported degradation

of approximately 50% for HDAC1, HDAC2, and HDAC3. HDAC1 and HDAC2 do not undergo degradation when CI-994 is connected to the inactive diastereoisomer VHL ligand. This confirms that the degradation is happening through a pathway mediated by VHL, which is an E3-ubiquitin ligase [151]. Knocking down HDAC3 using siRNA can enhance the expression of the anti-inflammatory cytokine interleukin-10 (IL-10) gene [152]. they create a new PROTACs, inspired by this, to break down HDAC3. They achieved this by linking o-aminoanilide-based inhibitors to the CRBN ligand pomalidomide. The degrader **118** exhibited significant activity in degrading HDAC3, with a $DC_{50}$ value of 0.32 μM, indicating potential degradation effects. Nevertheless, the administration of **118** did not result in any notable impact on the expression of the IL-10 gene. Additional bioassays demonstrated that the administration of either **118** or pomalidomide led to the suppression of NF-kB p65, a transcription factor that activates IL-10. The utilization of pomalidomide as a CRBN E3 ligase ligand in PROTACs may elucidate the reason behind the lack of success in enhancing IL-10 gene transcription by compound **118** (fig.16). Previously discovered that SR-3558 is a powerful and specific inhibitor of Class I HDACs [153]. The researchers created innovative PROTACs by utilizing SR-3558 as the weapon, which effectively targets and breaks down HDAC3. There is no text provided. The compound **120** was shown to be the most effective in degrading HDAC3 in a manner that depended on both the dose and time in MDA-MB-468 cells. The concentration required for 50% protein degradation, known as the $DC_{50}$, was determined to be 42 nM. No notable alterations in the protein levels of HDAC1, HDAC2, and HDAC6 were detected. Studies on the mechanism of action confirmed that the degradation caused by this PROTAC was actually facilitated by both the VHL E3 ligase and the UPS [154]. Previously, Yang *et al*. found that CRBN-based HDAC6 degraders can cause the degradation of unwanted neo-substrates such as IKZFs. However, the binding of pomalidomide to CRBN can also trigger this degradation. Therefore, they utilized Nexturastat A **121** as the explosive device to create a novel category of HDAC6 degraders that employed VHL instead of CRBN as the E3 ubiquitin ligase. The linker length necessary for VHL-ligand based degraders may exceed that of CRBN-ligand based degraders. Compound **123** was shown to be the most powerful contender within this group of HDAC6 degraders. There was no apparent decline in the functioning of other HDACs when **123** achieved its maximum impact on the deterioration of HDAC6 at a concentration of approximately 100 nM. Experimental investigations revealed that 123 specifically targeted HDAC6 for degradation by the proteasome, without causing the degradation of IKZF1/3 [155]. Sinatra et al. devised a highly effective method using hydroxamic acids immobilized on resins (HAIRs) as stable and adaptable components for creating functionalized inhibitors of HDACs through a solid-phase-supported protocol. Using this approach, they created the HDACs degrader **124** by linking SAHA to the CRBN ligand pomalidomide. The investigation conclusively demonstrated that a concentration-dependent manner was able to specifically degrade HDAC6 and HDAC1. Exposing HL60 cells to 124 resulted in a notable increase in the acetylation of histone H3 and a-tubulin [156]. Fischer et al. conducted a detailed investigation into the accessibility and degradability of HDACs. They created a collection of 48 degrader molecules that target multiple HDACs by combining four different pan-HDAC binding warheads (SAHA, dacinostat, TMP269, and NVS-HD1) with the recruiters of three different ubiquitin E3 ligases: CRBN, von VHL, and inhibitor of apoptosis proteins (IAP). The researchers extensively investigated the chemical space surrounding four pan-HDAC inhibitors to identify target-binding ligands. Their goal was to construct a compound matrix that could accurately map the degradability of the HDACs family. Through the application of chemo-proteomics, researchers successfully identified the substances responsible for breaking down HDAC1-8 and HDAC10. They discovered a considerable number of these substances that are capable of causing the reduction of certain components within the HDAC complex. These findings offer a significant resource for informing the design and development of innovative HDAC-specific PROTACs in the future (fig.17) [157].

**Concluding Remarks and Future Prospects of Histone Deacetylase Inhibitors (HDACIs)**

HDAC inhibitors have gained significant attention in recent decades and are now considered one of the most promising categories of anticancer medicines. They have been both validated and are now being investigated in clinical settings. Presently, clinical studies encompass both mono-treatment and combination therapy, and they encompass a wide range of indications, including chronic lymphocytic leukemia (CLL) and many forms of solid malignancies. Recently, there has been an increase in the discovery of new synthetic compounds that are generated from varied structural cores such as indole, coumarin, quinoline, azoles, triazine, thienopyridine, pyrimidine, etc. These molecules have been found to effectively block HDACs and exhibit different levels of selectivity. In preclinical models, the combination of HDACs and other antineoplastic therapy demonstrates greater efficacy

compared to standard therapy. However, additional research is needed to see if these findings can be applied to clinical practice. Most recently reported inhibitors of HDACs primarily consist of hydroxamic acid ZBG. Recently, other ZBGs such as o-aminoanilide, carboxylazide, ketone, and isoxazole have been identified. Throughout the timeframe examined in this study, numerous newly developed drugs demonstrate strong inhibitory activity against HDACs and exceptional selectivity for certain subtypes. Hydroxamic acid is susceptible to hydrolysis and glucuronidation, leading to rapid metabolism and elimination. This has a significant impact on the pharmacokinetic parameters and in vivo effectiveness. The potential for mutagenicity is a significant problem when it comes to hydroxamic acid-based HDAC inhibitors, which restricts their application to cancer therapy alone. Hence, to create the upcoming class of HDAC inhibitors that possess optimal pharmacokinetic properties and selectivity towards specific subtypes, it is imperative to persist in the exploration of novel chemical entities and new ZBGs. Simultaneously, the effectiveness of PROTAC design has been proven as a valuable approach to hinder and specifically break down protein targets. Researchers have tried to overcome this difficulty by developing and creating HDAC-specific PROTACs. Currently, the HDAC medications available on the market are all classified as pan-HDAC inhibitors, meaning they target almost all subtypes without specificity. The observed side effects, such as weariness, nausea/vomiting, and cardiotoxicity, may be attributed to the broad inhibitory actions of the substance in question. The tolerated adverse effects and the accompanying optimal ranges of therapeutic effectiveness could potentially hinder the maximizing of the anticancer potency of these medicines that inhibit pan-HDACs. Recent scientific research indicates that inhibitors that target certain subtypes selectively may have the potential to be safer than pan-HDAC inhibitors. There will be an increasing number of scholarly papers and patents focused on selectively inhibiting a certain subtype of HDACs. With the advancement of our understanding and the accumulation of research findings, it is strongly expected that selective HDAC inhibitors would soon receive official approval for therapeutic use. The identification of these recently discovered HDAC inhibitors has also sparked interest in exploring new applications, including intestinal fibrosis, autoimmune and inflammatory illnesses, metabolic abnormalities, viral infections, and various other conditions. The therapeutic potential of HDAC-targeting drugs for various disorders is now more evident than ever. Moreover, the advancement of the next iteration of HDAC inhibitors can serve not only as a novel therapeutic medication but also as a molecular tool to better investigate the physiological and pathological impacts of HDACs.

## References


[1] Dhuguru, J. and O.A. Ghoneim, *Quinazoline based HDAC dual inhibitors as potential anti-cancer agents.* Molecules, 2022. **27**(7): p. 2294.

[2] A Baudino, T., *Targeted cancer therapy: the next generation of cancer treatment.* Current drug discovery technologies, 2015. **12**(1): p. 3-20.

[3] Craig Jordan, V., *The role of tamoxifen in the treatment and prevention of breast cancer.* Current Problems in Cancer, 1992. **16**(3): p. 134-176.

[4] Mokhtari, R.B., et al., *Combination therapy in combating cancer.* Oncotarget, 2017. **8**(23): p. 38022.

[5] Anighoro, A., J. Bajorath, and G. Rastelli, *Polypharmacology: Challenges and Opportunities in Drug Discovery.* Journal of Medicinal Chemistry, 2014. **57**(19): p. 7874-7887.

[6] Cochran, A.G., A.R. Conery, and R.J. Sims III, *Bromodomains: a new target class for drug development.* Nature Reviews Drug Discovery, 2019. **18**(8): p. 609-628.

[7] Vahid, F., et al., *The role dietary of bioactive compounds on the regulation of histone acetylases and deacetylases: a review.* Gene, 2015. **562**(1): p. 8-15.

[8] New, M., H. Olzscha, and N.B. La Thangue, *HDAC inhibitor-based therapies: Can we interpret the code?* Molecular Oncology, 2012. **6**(6): p. 637-656.

[9] Wambua, M.K., et al., *Mutagenesis Studies of the 14 Å Internal Cavity of Histone Deacetylase 1: Insights toward the Acetate-Escape Hypothesis and Selective Inhibitor Design.* Journal of Medicinal Chemistry, 2014. **57**(3): p. 642-650.

[10] Bressi, J.C., et al., *Exploration of the HDAC2 foot pocket: Synthesis and SAR of substituted N-(2-aminophenyl)benzamides.* Bioorganic & Medicinal Chemistry Letters, 2010. **20**(10): p. 3142-3145.

[11] Wagner, F.F., et al., *Kinetic and structural insights into the binding of histone deacetylase 1 and 2 (HDAC1, 2) inhibitors.* Bioorganic & medicinal chemistry, 2016. **24**(18): p. 4008-4015.

[12] Haider, S., et al., *On the function of the internal cavity of histone deacetylase protein 8: R37 is a crucial residue for catalysis.* Bioorganic & Medicinal Chemistry Letters, 2011. **21**(7): p. 2129-2132.

[13] Sun, X., et al., *HDAC4 Represses Vascular Endothelial Growth Factor Expression in Chondrosarcoma by Modulating RUNX2 Activity*.* Journal of Biological Chemistry, 2009. **284**(33): p. 21881-21890.

[14] Vega, R.B., et al., *Histone Deacetylase 4 Controls Chondrocyte Hypertrophy during Skeletogenesis.* Cell, 2004. **119**(4): p. 555-566.

[15] Urbich, C., et al., *HDAC5 is a repressor of angiogenesis and determines the angiogenic gene expression pattern of endothelial cells.* Blood, 2009. **113**(22): p. 5669-5679.

[16] Hai, Y. and D.W. Christianson, *Histone deacetylase 6 structure and molecular basis of catalysis and inhibition.* Nature chemical biology, 2016. **12**(9): p. 741-747.

[17] Park, J.-H., et al., *Class II histone deacetylases play pivotal roles in heat shock protein 90-mediated proteasomal degradation of vascular endothelial growth factor receptors.* Biochemical and biophysical research communications, 2008. **368**(2): p. 318-322.

[18] Kotian, S., et al., *Histone Deacetylases 9 and 10 Are Required for Homologous Recombination*.* Journal of Biological Chemistry, 2011. **286**(10): p. 7722-7726.

[19] Bradbury, C.A., et al., *Histone deacetylases in acute myeloid leukaemia show a distinctive pattern of expression that changes selectively in response to deacetylase inhibitors.* Leukemia, 2005. **19**(10): p. 1751-1759.

[20] Stünkel, W., et al., *Function of the SIRT1 protein deacetylase in cancer.* Biotechnology Journal: Healthcare Nutrition Technology, 2007. **2**(11): p. 1360-1368.

[21] Özdağ, H., et al., Differential expression of selected histone modifier genes in human solid cancers. BMC genomics, 2006. 7: p. 1-15.

[22] Inoue, T., et al., SIRT2, a tubulin deacetylase, acts to block the entry to chromosome condensation in response to mitotic stress. Oncogene, 2007. 26(7): p. 945-957.

[23] Chu, F., et al., Control of multidrug resistance gene mdr1 and cancer resistance to chemotherapy by the longevity gene sirt1. Cancer research, 2005. 65(22): p. 10183-10187.

[24] Li, S., et al., p53-induced growth arrest is regulated by the mitochondrial SirT3 deacetylase. PloS one, 2010. 5(5): p. e10486.

[25] Kim, H.-S., et al., SIRT3 Is a Mitochondria-Localized Tumor Suppressor Required for Maintenance of Mitochondrial Integrity and Metabolism during Stress. Cancer Cell, 2010. 17(1): p. 41-52.

[26] Buglio, D., et al., HDAC11 plays an essential role in regulating OX40 ligand expression in Hodgkin lymphoma. Blood, 2011. 117(10): p. 2910-2917.

[27] Skov, V., et al., Increased gene expression of histone deacetylases in patients with Philadelphia-negative chronic myeloproliferative neoplasms. Leukemia & lymphoma, 2012. 53(1): p. 123-129.

[28] Bannister, A.J. and T. Kouzarides, Regulation of chromatin by histone modifications. Cell research, 2011. 21(3): p. 381-395.

[29] Sengupta, N. and E. Seto, Regulation of histone deacetylase activities. Journal of cellular biochemistry, 2004. 93(1): p. 57-67.

[30] Xu, W.-S., R.B. Parmigiani, and P.A. Marks, Histone deacetylase inhibitors: molecular mechanisms of action. Oncogene, 2007. 26(37): p. 5541-5552.

[31] Gershey, E.L., G. Vidali, and V.G. Allfrey, Chemical Studies of Histone Acetylation: The Occurrence of ε-N-Acetyllysine in the f2a1 Histone. Journal of Biological Chemistry, 1968. 243(19): p. 5018-5022.

[32] Haberland, M., R.L. Montgomery, and E.N. Olson, The many roles of histone deacetylases in development and physiology: implications for disease and therapy. Nature Reviews Genetics, 2009. 10(1): p. 32-42.

[33] Jin, Y.-H., et al., Transforming Growth Factor-β Stimulates p300-dependent RUNX3 Acetylation, Which Inhibits Ubiquitination-mediated Degradation*. Journal of Biological Chemistry, 2004. 279(28): p. 29409-29417.

[34] Ito, A., et al., MDM2–HDAC1-mediated deacetylation of p53 is required for its degradation. The EMBO journal, 2002.

[35] Martínez-Balbás, M.A., et al., Regulation of E2F1 activity by acetylation. The EMBO journal, 2000. 19(4): p. 662-671.

[36] Patel, J.H., et al., The c-MYC oncoprotein is a substrate of the acetyltransferases hGCN5/PCAF and TIP60. Molecular and cellular biology, 2004. 24(24): p. 10826-10834.

[37] Chen, L.-f., et al., Duration of nuclear NF-κB action regulated by reversible acetylation. Science, 2001. 293(5535): p. 1653-1657.

[38] Kim, S.-H., et al., Regulation of the HIF-1α stability by histone deacetylases. Oncology reports, 2007. 17(3): p. 647-651.

[39] Gaughan, L., et al., Tip60 and Histone Deacetylase 1 Regulate Androgen Receptor Activity through Changes to the Acetylation Status of the Receptor*. Journal of Biological Chemistry, 2002. 277(29): p. 25904-25913.

[40] Sartorelli, V., et al., Acetylation of MyoD Directed by PCAF Is Necessary for the Execution of the Muscle Program. Molecular Cell, 1999. 4(5): p. 725-734.

[41] Bali, P., et al., Inhibition of Histone Deacetylase 6 Acetylates and Disrupts the Chaperone Function of Heat Shock Protein 90: A NOVEL BASIS FOR ANTILEUKEMIA ACTIVITY OF HISTONE DEACETYLASE INHIBITORS*. Journal of Biological Chemistry, 2005. 280(29): p. 26729-26734.

[42] Yuan, Z.-l., et al., Stat3 dimerization regulated by reversible acetylation of a single lysine residue. Science, 2005. 307(5707): p. 269-273.

[43] Simonsson, M., et al., The Balance between Acetylation and Deacetylation Controls Smad7 Stability*. Journal of Biological Chemistry, 2005. 280(23): p. 21797-21803.

[44] Cohen, H.Y., et al., Acetylation of the C Terminus of Ku70 by CBP and PCAF Controls Bax-Mediated Apoptosis. Molecular Cell, 2004. 13(5): p. 627-638.

[45] Jeon, E.-J., et al., Bone Morphogenetic Protein-2 Stimulates Runx2 Acetylation*. Journal of Biological Chemistry, 2006. 281(24): p. 16502-16511.

[46] Fraga, M.F., et al., Loss of acetylation at Lys16 and trimethylation at Lys20 of histone H4 is a common hallmark of human cancer. Nature genetics, 2005. 37(4): p. 391-400.

[47] Yasui, W., et al., Histone acetylation and gastrointestinal carcinogenesis. Annals of the New York Academy of Sciences, 2003. 983(1): p. 220-231.

[48] Glaser, K.B., et al., Gene expression profiling of multiple histone deacetylase (HDAC) inhibitors: defining a common gene set produced by HDAC inhibition in T24 and MDA carcinoma cell lines. Molecular cancer therapeutics, 2003. 2(2): p. 151-163.

[49] Kovacs, J.J., et al., HDAC6 Regulates Hsp90 Acetylation and Chaperone-Dependent Activation of Glucocorticoid Receptor. Molecular Cell, 2005. 18(5): p. 601-607.

[50] Khan, O., et al., HR23B is a biomarker for tumor sensitivity to HDAC inhibitor-based therapy. Proceedings of the National Academy of Sciences, 2010. 107(14): p. 6532-6537.

[51] Sangwan, R., R. Rajan, and P.K. Mandal, HDAC as onco target: Reviewing the synthetic approaches with SAR study of their inhibitors. European journal of medicinal chemistry, 2018. 158: p. 620-706.

[52] Ho, T.C., A.H. Chan, and A. Ganesan, Thirty years of HDAC inhibitors: 2020 insight and hindsight. Journal of medicinal chemistry, 2020. 63(21): p. 12460-12484.

[53] Eckschlager, T., et al., Histone deacetylase inhibitors as anticancer drugs. International journal of molecular sciences, 2017. 18(7): p. 1414.

[54] Su, H., L. Altucci, and Q. You, Competitive or noncompetitive, that's the question: research toward histone deacetylase inhibitors. Molecular Cancer Therapeutics, 2008. 7(5): p. 1007-1012.

[55] Carew, J.S., F.J. Giles, and S.T. Nawrocki, Histone deacetylase inhibitors: mechanisms of cell death and promise in combination cancer therapy. Cancer letters, 2008. 269(1): p. 7-17.

[56] Xu, W., R. Parmigiani, and P. Marks, Histone deacetylase inhibitors: molecular mechanisms of action. Oncogene, 2007. 26(37): p. 5541-5552.

[57] Saelens, X., et al., Toxic proteins released from mitochondria in cell death. Oncogene, 2004. 23(16): p. 2861-2874.

[58] Chun, P., Histone deacetylase inhibitors in hematological malignancies and solid tumors. Archives of pharmacal research, 2015. 38: p. 933-949.

[59] Bi, G. and G. Jiang, The molecular mechanism of HDAC inhibitors in anticancer effects. Cellular & molecular immunology, 2006. 3(4): p. 285-290.

[60] Li, Y. and E. Seto, HDACs and HDAC inhibitors in cancer development and therapy. Cold Spring Harbor perspectives in medicine, 2016. 6(10).

[61] Bose, P., Y. Dai, and S. Grant, Histone deacetylase inhibitor (HDACI) mechanisms of action: emerging insights. Pharmacology & therapeutics, 2014. 143(3): p. 323-336.

[62] Sinha, S., et al., Mechanistic insights into the binding of class IIa HDAC inhibitors toward spinocerebellar ataxia type-2: a 3D-QSAR and pharmacophore modeling approach. Frontiers in Neuroscience, 2017. 10: p. 606.

[63] Abbass, S.A., et al., Recent prospectives of anticancer histone deacetylase inhibitors. Journal of advanced Biomedical and Pharmaceutical Sciences, 2019. 2(4): p. 135-151.

[64] Santos-Martins, D., et al., AutoDock4Zn: an improved AutoDock force field for small-molecule docking to zinc metalloproteins. Journal of chemical information and modeling, 2014. 54(8): p. 2371-2379.

[65] Mendoza-Sanchez, R., et al., Design, synthesis and evaluation of antiestrogen and histone deacetylase inhibitor molecular hybrids. Bioorganic & medicinal chemistry, 2015. 23(24): p. 7597-7606.

[66] Ververis, K., et al., Histone deacetylase inhibitors (HDACIs): multitargeted anticancer agents. Biologics: Targets and Therapy, 2013: p. 47-60.

[67] Yu, C., et al., Structure optimization and preliminary bioactivity evaluation of N-hydroxybenzamide-based HDAC inhibitors with Y-shaped cap. Bioorganic & Medicinal Chemistry, 2018. 26(8): p. 1859-1868.

[68] Dokmanovic, M., C. Clarke, and P.A. Marks, Histone deacetylase inhibitors: overview and perspectives. Molecular cancer research, 2007. 5(10): p. 981-989.

[69] Hervouet, E., The Promising Role of New Generation HDACis in Anti-Cancer Therapies. EBioMedicine, 2018. 32: p. 6-7.

[70] Kachhadia, V., et al., Orally available stilbene derivatives as potent HDAC inhibitors with antiproliferative activities and antitumor effects in human tumor xenografts. European Journal of Medicinal Chemistry, 2016. 108: p. 274-286.

[71] Musso, L., et al., Investigation on the ZBG-functionality of phenyl-4-yl-acrylohydroxamic acid derivatives as histone deacetylase inhibitors. Bioorganic & medicinal chemistry letters, 2015. 25(20): p. 4457-4460.

[72] Dung, P.T.P., et al., Exploration of novel 5′(7′)-substituted-2′-oxospiro [1, 3] dioxolane-2, 3′-indoline-based N-hydroxypropenamides as histone deacetylase inhibitors and antitumor agents. Arabian Journal of Chemistry, 2017. 10(4): p. 465-472.

[73] Suzuki, T. and N. Miyata, Non-hydroxamate histone deacetylase inhibitors. Current medicinal chemistry, 2005. 12(24): p. 2867-2880.

[74] Sternson, S.M., et al., Synthesis of 7200 small molecules based on a substructural analysis of the histone deacetylase inhibitors trichostatin and trapoxin. Organic letters, 2001. 3(26): p. 4239-4242.

[75] Vigushin, D.M., et al., Trichostatin A is a histone deacetylase inhibitor with potent antitumor activity against breast cancer in vivo. Clinical Cancer Research, 2001. 7(4): p. 971-976.

[76] Hoshino, I. and H. Matsubara, Recent advances in histone deacetylase targeted cancer therapy. Surgery today, 2010. 40: p. 809-815.

[77] Han, L., et al., Design, synthesis and preliminary bioactivity studies of 1, 2-dihydrobenzo [d] isothiazol-3-one-1, 1-dioxide hydroxamic acid derivatives as novel histone deacetylase inhibitors. Bioorganic & Medicinal Chemistry, 2014. 22(5): p. 1529-1538.

[78] Guan, P., et al., Improved antiproliferative activity of 1, 3, 4-thiadiazole-containing histone deacetylase (HDAC) inhibitors by introduction of the heteroaromatic surface recognition motif. Bioorganic & medicinal chemistry, 2014. 22(21): p. 5766-5775.

[79] Santo, L., et al., Preclinical activity, pharmacodynamic, and pharmacokinetic properties of a selective HDAC6 inhibitor, ACY-1215, in combination with bortezomib in multiple myeloma. Blood, The Journal of the American Society of Hematology, 2012. 119(11): p. 2579-2589.

[80] Wang, J., et al., Design, synthesis and biological evaluation of thienopyrimidine hydroxamic acid based derivatives as structurally novel histone deacetylase (HDAC) inhibitors. European Journal of Medicinal Chemistry, 2017. 128: p. 293-299.

[81] Dung, D.T.M., et al., Novel hydroxamic acids incorporating 1-((1 H-1, 2, 3-Triazol-4-yl) methyl)-3-hydroxyimino-indolin-2-ones: synthesis, biological evaluation, and SAR analysis. Journal of Chemical Sciences, 2018. 130: p. 1-13.

[82] Negmeldin, A.T., J.R. Knoff, and M.K.H. Pflum, The structural requirements of histone deacetylase inhibitors: C4-modified SAHA analogs display dual HDAC6/HDAC8 selectivity. European journal of medicinal chemistry, 2018. 143: p. 1790-1806.

[83] Negmeldin, A.T. and M.K.H. Pflum, The structural requirements of histone deacetylase inhibitors: SAHA analogs modified at the C5 position display dual HDAC6/8 selectivity. Bioorganic & medicinal chemistry letters, 2017. 27(15): p. 3254-3258.

[84] Negmeldin, A.T., et al., Structural requirements of HDAC inhibitors: SAHA analogues modified at the C2 position display HDAC6/8 selectivity. ACS Medicinal Chemistry Letters, 2017. 8(3): p. 281-286.

[85] Choi, S.E., S.V. Weerasinghe, and M.K.H. Pflum, The structural requirements of histone deacetylase inhibitors: Suberoylanilide hydroxamic acid analogs modified at the C3 position display isoform selectivity. Bioorganic & medicinal chemistry letters, 2011. 21(20): p. 6139-6142.

[86] Choi, S.E. and M.K.H. Pflum, The structural requirements of histone deacetylase inhibitors: Suberoylanilide hydroxamic acid analogs modified at the C6 position. Bioorganic & medicinal chemistry letters, 2012. 22(23): p. 7084-7086.

[87] Aboeldahab, A.M., et al., Spirohydantoins and 1, 2, 4-triazole-3-carboxamide derivatives as inhibitors of histone deacetylase: Design, synthesis, and biological evaluation. European journal of medicinal chemistry, 2018. 146: p. 79-92.

[88] Yang, F., et al., Development of hydroxamate-based histone deacetylase inhibitors containing 1, 2, 4-oxadiazole moiety core with antitumor activities. Bioorganic & Medicinal Chemistry Letters, 2019. 29(1): p. 15-21.

[89] Giannini, G., et al., N-Hydroxy-(4-oxime)-cinnamide: A versatile scaffold for the synthesis of novel histone deacetilase (HDAC) inhibitors. Bioorganic & medicinal chemistry letters, 2009. 19(8): p. 2346-2349.

[90] Lu, W., et al., Search for novel histone deacetylase inhibitors. Part II: design and synthesis of novel isoferulic acid derivatives. Bioorganic & Medicinal Chemistry, 2014. 22(9): p. 2707-2713.

[91] Wang, F., et al., Development of novel ferulic acid derivatives as potent histone deacetylase inhibitors. Bioorganic & medicinal chemistry, 2013. 21(22): p. 6973-6980.

[92] Di Pompo, G., et al., Novel histone deacetylase inhibitors induce growth arrest, apoptosis, and differentiation in sarcoma cancer stem cells. Journal of medicinal chemistry, 2015. 58(9): p. 4073-4079.

[93] Lee, H.-Y., et al., 2-(Phenylsulfonyl) quinoline N-hydroxyacrylamides as potent anticancer agents inhibiting histone deacetylase. European Journal of Medicinal Chemistry, 2016. 122: p. 92-101.

[94] Mehndiratta, S., et al., 4-Indolyl-N-hydroxyphenylacrylamides as potent HDAC class I and IIB inhibitors in vitro and in vivo. European Journal of Medicinal Chemistry, 2017. 134: p. 13-23.

[95] Ling, Y., et al., β-Carboline and N-hydroxycinnamamide hybrids as anticancer agents for drug-resistant hepatocellular carcinoma. European Journal of Medicinal Chemistry, 2019. 168: p. 515-526.

[96] Zhang, Q., et al., Design, synthesis and activity evaluation of indole-based double–Branched HDAC1 inhibitors. Bioorganic & Medicinal Chemistry, 2019. 27(8): p. 1595-1604.

[97] Cheng, J., et al., Design, synthesis and evaluation of novel HDAC inhibitors as potential antitumor agents. Bioorganic & Medicinal Chemistry Letters, 2014. 24(19): p. 4768-4772.

[98] Blackburn, C., et al., Histone deacetylase inhibitors derived from 1, 2, 3, 4-tetrahydropyrrolo [1, 2-a] pyrazine and related heterocycles selective for the HDAC6 isoform. Bioorganic & medicinal chemistry letters, 2014. 24(23): p. 5450-5454.

[99] Liang, T., et al., Design, synthesis, and biological evaluation of 2, 4-imidazolinedione derivatives as HDAC6 isoform-selective inhibitors. ACS medicinal chemistry letters, 2019. 10(8): p. 1122-1127.

[100] Li, Y., et al., Design, synthesis and bioevalution of novel benzamides derivatives as HDAC inhibitors. Bioorganic & medicinal chemistry letters, 2013. 23(1): p. 179-182.

[101] Xie, R., et al., Design, synthesis and biological evaluation of novel 2-aminobenzamides containing dithiocarbamate moiety as histone deacetylase inhibitors and potent antitumor agents. European Journal of Medicinal Chemistry, 2018. 143: p. 320-333.

[102] Gao, S., et al., Design, synthesis and anti-tumor activity study of novel histone deacetylase inhibitors containing isatin-based caps and o-phenylenediamine-based zinc binding groups. Bioorganic & medicinal chemistry, 2017. 25(12): p. 2981-2994.

[103] Abdizadeh, T., et al., Design, synthesis and biological evaluation of novel coumarin-based benzamides as potent histone deacetylase inhibitors and anticancer agents. European journal of medicinal chemistry, 2017. 132: p. 42-62.

[104] Xie, R., et al., Design, synthesis and biological evaluation of novel hydroxamates and 2-aminobenzamides as potent histone deacetylase inhibitors and antitumor agents. European Journal of Medicinal Chemistry, 2017. 134: p. 1-12.

[105] Trivedi, P., et al., Design, synthesis and biological screening of 2-aminobenzamides as selective HDAC3 inhibitors with promising anticancer effects. European Journal of Pharmaceutical Sciences, 2018. 124: p. 165-181.

[106] Li, X., et al., Selective HDAC inhibitors with potent oral activity against leukemia and colorectal cancer: Design, structure-activity relationship and anti-tumor activity study. European journal of medicinal chemistry, 2017. 134: p. 185-206.

[107] Li, Y., et al., Design, synthesis and antiproliferative activities of novel benzamides derivatives as HDAC inhibitors. European Journal of Medicinal Chemistry, 2015. 100: p. 270-276.

[108] Cai, J., et al., Discovery, bioactivity and docking simulation of Vorinostat analogues containing 1, 2, 4-oxadiazole moiety as potent histone deacetylase inhibitors and antitumor agents. Bioorganic & Medicinal Chemistry, 2015. 23(13): p. 3457-3471.

[109] Mohamed, M.F., et al., Design, synthesis, docking studies and biological evaluation of novel chalcone derivatives as potential histone deacetylase inhibitors. Bioorganic chemistry, 2017. 72: p. 32-41.

[110] Chen, X., et al., Design, synthesis and biological evaluation of novel isoindolinone derivatives as potent histone deacetylase inhibitors. European Journal of Medicinal Chemistry, 2019. 168: p. 110-122.

[111] Cheng, C., et al., Design, synthesis and biological evaluation of novel thioquinazolinone-based 2-aminobenzamide derivatives as potent histone deacetylase (HDAC) inhibitors. European Journal of Medicinal Chemistry, 2019. 173: p. 185-202.

[112] Yun, F., et al., Thioether-based 2-aminobenzamide derivatives: Novel HDAC inhibitors with potent in vitro and in vivo antitumor activity. European Journal of Medicinal Chemistry, 2019. 176: p. 195-207.

[113] Itazaki, H., et al., Solation and structural elucidation of new cyclotetrapeptides, trapoxins A and B, having detransformation activities as antitumor agents. The Journal of antibiotics, 1990. 43(12): p. 1524-1532.

[114] Qin, H.-T., H.-Q. Li, and F. Liu, Selective histone deacetylase small molecule inhibitors: recent progress and perspectives. Expert opinion on therapeutic patents, 2017. 27(5): p. 621-636.

[115] Huang, D., et al., Synthesis, evaluation and molecular modeling of cyclic tetrapeptide histone deacetylase inhibitors as anticancer agents. Journal of Peptide Science, 2012. 18(4): p. 242-251.

[116] Furumai, R., et al., FK228 (depsipeptide) as a natural prodrug that inhibits class I histone deacetylases. Cancer research, 2002. 62(17): p. 4916-4921.

[117] Prince, H.M., M. Dickinson, and A. Khot, Romidepsin for cutaneous T-cell lymphoma. Future Oncology, 2013. 9(12): p. 1819-1827.

[118] Ni, M., et al., New macrocyclic analogs of the natural histone deacetylase inhibitor FK228; design, synthesis and preliminary biological evaluation. Bioorganic & Medicinal Chemistry, 2015. 23(21): p. 6785-6793.

[119] Wang, S., et al., Discovery of potent HDAC inhibitors based on chlamydocin with inhibitory effects on cell migration. ChemMedChem, 2014. 9(3): p. 627-637.

[120] Leteve, M., et al., Unexpected effect of cyclodepsipeptides bearing a sulfonylhydrazide moiety towards histone deacetylase activity. Bioorganic chemistry, 2018. 81: p. 222-233.

[121] Osada, S., et al., Fluoroalkene modification of mercaptoacetamide-based histone deacetylase inhibitors. Bioorganic & medicinal chemistry, 2010. 18(2): p. 605-611.

[122] Madsen, A.S. and C.A. Olsen, A potent trifluoromethyl ketone histone deacetylase inhibitor exhibits class-dependent mechanism of action. MedChemComm, 2016. 7(3): p. 464-470.

[123] Gong, C.-J., et al., Design, synthesis and biological evaluation of bisthiazole-based trifluoromethyl ketone derivatives as potent

HDAC inhibitors with improved cellular efficacy. European Journal of Medicinal Chemistry, 2016. 112: p. 81-90.

[124] Suzuki, T., et al., Thiol-based SAHA analogues as potent histone deacetylase inhibitors. Bioorganic & medicinal chemistry letters, 2004. 14(12): p. 3313-3317.

[125] Wen, J., et al., Novel thiol-based histone deacetylase inhibitors bearing 3-phenyl-1H-pyrazole-5-carboxamide scaffold as surface recognition motif: Design, synthesis and SAR study. Bioorganic & Medicinal Chemistry Letters, 2016. 26(2): p. 375-379.

[126] Baud, M.G., et al., Thioester derivatives of the natural product psammaplin A as potent histone deacetylase inhibitors. Beilstein Journal of Organic Chemistry, 2013. 9(1): p. 81-88.

[127] Musso, L., S. Dallavalle, and F. Zunino, Perspectives in the development of hybrid bifunctional antitumour agents. Biochemical Pharmacology, 2015. 96(4): p. 297-305.

[128] Giordano, S. and A. Petrelli, From Single- to Multi-Target Drugs in Cancer Therapy: When Aspecificity Becomes an Advantage. Current Medicinal Chemistry, 2008. 15(5): p. 422-432.

[129] Raghavendra, N.M., et al., Dual or multi-targeting inhibitors: The next generation anticancer agents. European Journal of Medicinal Chemistry, 2018. 143: p. 1277-1300.

[130] Talevi, A., Multi-target pharmacology: possibilities and limitations of the "skeleton key approach" from a medicinal chemist perspective. Frontiers in pharmacology, 2015. 6: p. 156790.

[131] Roy, R., et al., Single inhibitors versus dual inhibitors: role of HDAC in cancer. ACS omega, 2023. 8(19): p. 16532-16544.

[132] Shaker, B., et al., In silico methods and tools for drug discovery. Computers in Biology and Medicine, 2021. 137: p. 104851.

[133] Lai, M.-J., et al., 1-Arylsulfonyl indoline-benzamides as a new antitubulin agents, with inhibition of histone deacetylase. European Journal of Medicinal Chemistry, 2019. 162: p. 612-630.

[134] Duan, Y.-C., et al., Design and synthesis of tranylcypromine derivatives as novel LSD1/HDACs dual inhibitors for cancer treatment. European Journal of Medicinal Chemistry, 2017. 140: p. 392-402.

[135] Zhou, R., et al., Design, synthesis, and bioactivity evaluation of novel Bcl-2/HDAC dual-target inhibitors for the treatment of multiple myeloma. Bioorganic & Medicinal Chemistry Letters, 2019. 29(3): p. 349-352.

[136] Yuan, Z., et al., Development of a versatile DNMT and HDAC inhibitor C02S modulating multiple cancer hallmarks for breast cancer therapy. Bioorganic Chemistry, 2019. 87: p. 200-208.

[137] Liang, X., et al., Discovery of novel janus kinase (JAK) and histone deacetylase (HDAC) dual inhibitors for the treatment of hematological malignancies. Journal of medicinal chemistry, 2019. 62(8): p. 3898-3923.

[138] Geng, A., et al., Discovery of novel phenoxybenzamide analogues as Raf/HDAC dual inhibitors. Bioorganic & Medicinal Chemistry Letters, 2019. 29(13): p. 1605-1608.

[139] Palermo, A.F., et al., Incorporation of histone deacetylase inhibitory activity into the core of tamoxifen–A new hybrid design paradigm. Bioorganic & medicinal chemistry, 2018. 26(15): p. 4428-4440.

[140] Yao, L., et al., Merging of ruxolitinib and vorinostat leads to highly potent inhibitors of JAK2 and histone deacetylase 6 (HDAC6). Bioorganic & Medicinal Chemistry Letters, 2018. 28(15): p. 2636-2640.

[141] Ding, C., et al., Synthesis and investigation of novel 6-(1, 2, 3-triazol-4-yl)-4-aminoquinazolin derivatives possessing hydroxamic acid moiety for cancer therapy. Bioorganic & medicinal chemistry, 2017. 25(1): p. 27-37.

[142] De Simone, A. and A. Milelli, Histone deacetylase inhibitors as multitarget ligands: new players in Alzheimer's disease drug discovery? ChemMedChem, 2019. 14(11): p. 1067-1073.

[143] Zhang, X., et al., Design, synthesis and biological evaluation of colchicine derivatives as novel tubulin and histone deacetylase dual inhibitors. European Journal of Medicinal Chemistry, 2015. 95: p. 127-135.

[144] Okazaki, S., et al., Investigations of possible prodrug structures for 2-(2-mercaptophenyl) tetrahydropyrimidines: Reductive conversion from anti-HIV agents with pyrimidobenzothiazine and isothiazolopyrimidine scaffolds. Organic & Biomolecular Chemistry, 2015. 13(16): p. 4706-4713.

[145] Wolff, B., et al., Synthesis and structure-activity relationship of 1, 3-benzo-thiazine-2-thiones as selective HDAC8 inhibitors. European Journal of Medicinal Chemistry, 2019. 184: p. 111756.

[146] Stott, A.J., et al., Evaluation of 5-(Trifluoromethyl)-1, 2, 4-oxadiazole-based class IIa HDAC inhibitors for Huntington's disease. ACS Medicinal Chemistry Letters, 2021. 12(3): p. 380-388.

[147] Liu, J., et al., Discovery of Highly Selective and Potent HDAC3 Inhibitors Based on a 2-Substituted Benzamide Zinc Binding Group. ACS Medicinal Chemistry Letters, 2020. 11(12): p. 2476-2483.

[148] Beshore, D.C., et al., Redefining the Histone Deacetylase Inhibitor Pharmacophore: High Potency with No Zinc Cofactor Interaction. ACS Medicinal Chemistry Letters, 2021. 12(4): p. 540-547.

[149] Yu, W., et al., Discovery of Ethyl Ketone-Based Highly Selective HDACs 1, 2, 3 Inhibitors for HIV Latency Reactivation with Minimum Cellular Potency Serum Shift and Reduced hERG Activity. Journal of Medicinal Chemistry, 2021. 64(8): p. 4709-4729.

[150] Pettersson, M. and C.M. Crews, *PROteolysis TArgeting Chimeras (PROTACs) — Past, present and future.* Drug Discovery Today: Technologies, 2019. **31**: p. 15-27.

[151] James, K., W. John, and S. MichaeláCowley, *PROTAC-mediated degradation of class I histone deacetylase enzymes in corepressor complexes.* Chemical Communications, 2020. **56**(32): p. 4476-4479.

[152] Leus, N.G.J., et al., *HDAC 3-selective inhibitor RGFP966 demonstrates anti-inflammatory properties in RAW 264.7 macrophages and mouse precision-cut lung slices by attenuating NF-κB p65 transcriptional activity.* Biochemical Pharmacology, 2016. **108**: p. 58-74.

[153] Wang, Y., et al., *Identification of Histone Deacetylase Inhibitors with Benzoylhydrazide Scaffold that Selectively Inhibit Class I Histone Deacetylases.* Chemistry & Biology, 2015. **22**(2): p. 273-284.

[154] Xiao, Y., et al., *Discovery of histone deacetylase 3 (HDAC3)-specific PROTACs.* Chemical Communications, 2020. **56**(68): p. 9866-9869.

[155] Yang, K., et al., *Development of Selective Histone Deacetylase 6 (HDAC6) Degraders Recruiting Von Hippel–Lindau (VHL) E3 Ubiquitin Ligase.* ACS Medicinal Chemistry Letters, 2020. **11**(4): p. 575-581.

[156] Sinatra, L., et al., *Hydroxamic Acids Immobilized on Resins (HAIRs): Synthesis of dual-targeting HDAC inhibitors and HDAC degraders (PROTACs).* Angewandte Chemie International Edition, 2020. **59**(50): p. 22494-22499.

[157] He, X., et al., *Medicinal chemistry updates of novel HDACs inhibitors (2020 to present).* European Journal of Medicinal Chemistry, 2022. **227**: p. 113946.