Received: 24 July 2023 | Revised: 14 September 2023 | Accepted: 18 September 2023

DOI: 10.1111/cbdd.14366

WILEY

REVIEW

# Discussion on structure classification and regulation function of histone deacetylase and their inhibitor

Han Han[1] | Xue Feng[2] | Ting He[2] | Yingfan Wu[2] | Tianmei He[2] | Ziwen Yue[2] | Weiqiang Zhou[2]

[1]Department of Biochemistry and Molecular Biology, Shenyang Medical College, Shenyang City, P. R. China

[2]Department of Pathogen Biology, Shenyang Medical College, Shenyang City, P. R. China

**Correspondence**
Weiqiang Zhou, Department of Pathogen Biology, Shenyang Medical College, No.146 North Huanghe St, Huanggu Dis, Shenyang City, Liaoning Pro 110034, P. R. China.
Email: zhouwq@hotmail.com

**Funding information**
Natural Science combination Foundation for improving innovation of Liaoning Province (2022-NLTS-14-01)

## Abstract

Epigenetic regulation of genes through posttranslational regulation of proteins is a well-explored approach for disease treatment, particularly in cancer chemotherapy. Histone deacetylases have shown significant potential as effective drug targets in therapeutic studies aiming to restore epigenetic normality in oncology. Besides their role in modifying histones, histone deacetylases can also catalyze the deacetylation of various nonhistone proteins and participate in the regulation of multiple biological processes. This paper provides a review of the classification, structure, and functional characteristics of the four classes of human histone deacetylases. The increasing abundance of structural information on HDACs has led to the gradual elucidation of structural differences among subgroups and subtypes. This has provided a reasonable explanation for the selectivity of certain HDAC inhibitors. Currently, the US FDA has approved a total of six HDAC inhibitors for marketing, primarily for the treatment of various hematological tumors and a few solid tumors. These inhibitors all have a common pharmacodynamic moiety consisting of three parts: CAP, ZBG, and Linker. In this paper, the structure–effect relationship of HDAC inhibitors is explored by classifying the six HDAC inhibitors into three main groups: isohydroxamic acids, benzamides, and cyclic peptides, based on the type of inhibitor ZBG. However, there are still many questions that need to be answered in this field. In this paper, the structure-functional characteristics of HDACs and the structural information of the pharmacophore model and enzyme active region of HDAC is are considered, which can help to understand the inhibition mechanism of the compounds as well as the rational design of HDACs. This paper integrates the structural-functional characteristics of HDACs as well as the pharmacophore model of HDAC is and the structural information of the enzymatic active region, which not only contributes to the understanding of the inhibition mechanism of the compounds, but also provides a basis for the rational design of HDAC inhibitors.

**KEYWORDS**

classification, function, HDACI, HDACs, pharmacodynamic groups, structure-effect relationship

  

## 1 | EPIGENETIC MODIFICATIONS AND TUMORS

In the context of the remarkable progress in molecular biology, there has been a more systematic study of epigenetics at the molecular level. This has led to the discovery of various epigenetic modifications and a better understanding of their complex biological roles. Epigenetic regulation refers to the control of gene expression without altering the DNA sequence. It is based on two molecular mechanisms (Tulsyan et al., 2022): covalent modifications (such as methylation and demethylation) that target DNA, and posttranslational modifications (including acetylation, deacetylation, methylation, demethylation, ubiquitination, deubiquitination, phosphorylation, and dephosphorylation) of histones. Covalent modifications of DNA directly silence the transcription of associated genes, while posttranslational modifications of histones regulate gene expression by influencing the structural transition of chromosomes between euchromatin and heterochromatin (Gil & Vagnarelli, 2019). Epigenetic modifications do not alter the base pair composition or alignment, but they do affect the expression of associated genes through complex DNA–protein and protein–protein interactions. These modifications can result in stable and heritable phenotypic changes.

Numerous enzymes associated with epigenetic modifications have been discovered and named based on their primary functions. These include DNA methyltransferases (DNMT), DNA demethylases (DDM), histone acetyltransferases (HATs), histone deacetylases (HDAC), histone methyltransferases (HMT), histone demethylases (HDM), histone ubiquitin ligases (HUL), histone deubiquitinating enzyme (HUSP), histone phosphorylase, and histone dephosphorylase (DesJarlais & Tummino, 2016). The dysregulation of their expression and function can result in various diseases, with cancer being one of the most perilous conditions affecting human life and health.

Research has revealed that malignant tumor tissues exhibit a higher number of abnormal epigenetic modifications compared to normal tissues. Furthermore, the degree of abnormal epigenetic modifications tends to increase with the level of malignancy. Numerous studies have reported that the acetylation status of histones plays a crucial role in tumorigenesis and evolution. Specifically, it has been observed that tumor cells and tissues often display lower levels of histone acetylation compared to their normal counterparts, while histone deacetylases exhibit a significant overexpression (Lakshmaiah et al., 2014). Recent studies have highlighted the potential of targeting histone deacetylases as an effective strategy in therapeutic interventions aiming to restore epigenetic normality in tumors (Wang et al., 2016).

Researchers have developed a range of inhibitors that exhibit remarkable antitumor activity and tumor selectivity by increasing the acetylation of histones. This indicates that the development of HDAC inhibitors holds promising prospects for application. Additionally, it offers new possibilities for identifying new markers for tumor diagnosis and prevention, as well as for creating novel lead structures and innovative drugs that are highly efficient and have low toxicity.

## 2 | HISTONE DEACETYLASE

Chromatin is a complex composed of DNA and proteins, with nucleosomes being its basic building blocks. Nucleosomes consist of DNA strands that are approximately 147 bp in length wrapped around a histone octamer (Khorasanizadeh, 2004). The structure of chromatin can change in response to epigenetic modifications of the DNA and histone ends, which can in turn activate or repress specific genes. Around 50 years ago, Allfrey and his colleagues discovered the acetylation of histone lysines, revealing the significance of the acetylation of the S-amino group of histone lysine residues in gene expression (Allfrey et al., 1964). Acetylation works by neutralizing the positive charge of the lysine residue and relaxing the chromatin structure. This relaxation allows various transcription factors and co-transcription factors to easily and specifically bind to DNA binding sites, leading to transcriptional activation. Conversely, the deacetylation of histones results in the compaction of nuclear chromatin, leading to the repression of gene transcription. Lysine acetylation also occurs on nonchromosomal proteins, such as transcription factors and cytoplasmic proteins (Li & Zhu, 2014), thereby affecting gene transcription and other cellular processes. In normal human cells, the level of histone lysine acetylation is regulated by two enzymes with opposing roles: histone acetyltransferase and histone deacetylase. The catalytic action of histone acetyltransferase transfers the acetyl group of acetyl coenzyme A to the ε-amino group of histone lysine residues (Bannister & Kouzarides, 2011). Conversely, HDACs carry out deacetylation by hydrolyzing the ε-amino group on the side chain of lysine residues in histones using $Zn^{2+}$ ions and also exhibit catalytic activity (Ramaiah et al., 2021). Mutations and abnormal expression of HDACs contribute to the development of various diseases, particularly tumors, highlighting the significance of HDACs as potential targets for antitumor therapies.

## 2.1 | Classification of histone deacetylase family

All HDAC proteins in the histone deacetylase family have a common ancestor, resulting in shared 3D structure, function, and sequence homology. There are a total of 18 isoforms of human-derived HDACs, which can be categorized into four subfamilies (Class I, Class II, Class III, and Class IV) based on their homology, intracellular localization, and tissue distribution specificity with yeast histone deacetylases (Nalawansha & Pflum, 2017) (Table 1).

Class I subfamilies (HDAC1, HDAC2, HDAC3, and HDAC8) are homologous to yeast RPD3 (potassium dependency-3) and are widely expressed in various tissues (Ma et al., 2012). They are primarily localized in the nucleus, where they function as repressors of gene transcription. HDAC1 and HDAC2 exhibit high homology and are closely associated with cellular processes such as cell proliferation, cell cycle regulation, and apoptosis (Segré & Chiocca, 2011). HDAC3 plays a significant role in the cell cycle and DNA damage response (Bhaskara et al., 2008). HDAC8 is particularly involved in smooth muscle cell differentiation (Kim et al., 2022). The deacetylation of nucleosomal histones by Class I HDACs is predominantly achieved through the formation of enzymatically active complexes (Li & Seto, 2016).

Class II subfamilies (HDAC4, HDAC5, HDAC6, HDAC7, HDAC9, and HDAC10) are homologous to yeast Had l (histone deacetylases). The Class II subfamily can be further divided into two groups: Class IIa (HDAC4, HDAC5, HDAC7, and HDAC9) and Class IIb (HDAC6 and HDAC10) (Kumar et al., 2022). Class IIa, similar to other HDACs, contains only one catalytic domain, while Class IIb members have two catalytic domains (McClure et al., 2018). HDAC4 and HDAC5, which belong to Class IIa, are expressed in the brain, heart, and skeletal muscle (Kee et al., 2022). HDAC7 is expressed in the heart, lung, placenta, pancreas, skeletal muscle, and thymus (Verdin et al., 2004). HDAC9 is mainly expressed in the brain and skeletal muscle (Das & Natarajan, 2020). On the contrary, HDAC6, a member of Class IIb, is expressed in the heart, skeletal muscle, and brain (LoPresti, 2020), while HDAC10 is expressed in the liver, spleen, and kidney (Cheng et al., 2021).

The only Class IV enzyme, HDAC11, is expressed in the brain, heart, kidney, testis, and skeletal muscle, and it is localized in the nucleus. HDAC11 is known for its high catalytic efficiency as a fatty acid acylase and possesses a catalytic activity center that is common to both Class I and Class II enzymes (Chen, Xie, et al., 2022).

The Class III subfamily, known as sirtuins, is named after its strong resemblance to the yeast Sir2 protein. All seven members of this subfamily (SIRTI-7) rely on $NAD^+$ for their catalytic activity and exhibit dual enzymatic activities: ADP-ribosyltransferase and histone deacetylase (Morigi et al., 2018). The SIR2 regulator family is divided into four subclasses: I, II, III, and IV. Subclass I consists of SIRT1, SIRT2, and SIRT3 proteins; subclass II contains SIRT4 protein; subclass III includes SIRT5 protein; and subclass IV comprises SIRT6 and SIRT7 proteins (Chen, Zhou, et al., 2022). The amino acid sequence homology of these seven proteins ranges from 22% to 50% and 27% to 88% in the conserved catalytic structural domain. Among them, Sirt1 exhibits the most significant histone deacetylase activity and has been extensively studied (Chang & Guarente, 2014). SIRT1, SIRT6, and SIRT7 are predominantly located in the nucleus, SIRT2 in the cytoplasm, and SIRT3, SIRT4, and SIRT5 in the mitochondria (Wątroba et al., 2017).

Classes I, II, and IV HDACs can be classified as Rpd3/Had1 deacetylases. They all contain a high homology of the catalytic core structural domain and depend on the participation of $Zn^{2+}$ ions for their catalytic activity (Finnin et al., 1999; Somoza et al., 2004; Wu et al., 2010). The sequences and structures outside the catalytic domain are relatively variable, suggesting different biological functions. On the contrary, Class III HDACs are an atypical family of histone deacetylases known as the $NAD^+$-dependent Sir2 super protein family. They are completely distinct from other HDACs (Blander & Guarente, 2004; Haigis & Sinclair, 2010; Michan & Sinclair, 2007). Although they also bind $Zn^{2+}$ ions and require them for their deacetylase activity, $Zn^{2+}$ ions are not directly involved in the deacetylase reaction (Feldman et al., 2012; Landry et al., 2000; Sauve, 2010).

## 2.2 | Histone deacetylase structure and function

HDAC typically consists of a core structural domain known as the HDAC structural domain, which spans approximately 350 amino acids. This domain comprises two highly conserved isoforms: the HDAC N-terminal structural domain and the HDAC central structural domain (Yoon & Eom, 2016). In contrast, the HDAC C-terminal structural domain is a more diverse region, exhibiting variations in length and amino acid sequences across different types of HDAC (Witt et al., 2009). Additionally, HDAC can form complexes with other proteins, including cell cycle regulatory proteins and transcription factors (de Ruijter et al., 2003). The HDAC structural domain may also contain significant catalytic sites, such as zinc ions and arginine residues, which are essential for its catalytic activity (Ren et al., 2014).

**TABLE 1** Classification of HDAC enzymes.

| | | | | | |
|---|---|---|---|---|---|
| $Zn^{2+}$ Dependent | CLASS I | | HDAC1 |  | Nucleus |
| | | | HDAC2 |  | Nucleus |
| | | | HDAC3 |  | Nucleus/Cytoplasm |
| | | | HDAC8 |  | Nucleus |
| | CLASS II | IIa | HDAC4 |  | Nucleus/Cytoplasm |
| | | | HDAC5 |  | Nucleus/Cytoplasm |
| | | | HDAC7 |  | Nucleus/Cytoplasm |
| | | | HDAC9 |  | Nucleus/Cytoplasm |
| | | IIb | HDAC6 |  | Nucleus/Cytoplasm |
| | | | HDAC10 |  | Nucleus/Cytoplasm |
| | CLASS IV | | HDAC11 |  | Nucleus |
| NAD+ Dependent | CLASS III | CLASS I | SIRT1 |  | Nucleus/Cytoplasm |
| | | | SIRT2 |  | Cytoplasm |
| | | | SIRT3 |  | Mitochondria |
| | | CLASS II | SIRT4 |  | Mitochondria |
| | | CLASS III | SIRT5 |  | Mitochondria |
| | | CLASS IV | SIRT6 |  | Nucleus |
| | | | SIRT7 |  | Nucleus |

*Note*: HDAC catalytic domain Nuclear localization signal Nuclear export signal Zinc finger protein binding domain Sirtuin catalytic domain. Serine binding motif MEF2 binding domain Serine-glutamate tetradecapeptide Leucine rich domain Zinc binding domain. All HDAC proteins of the histone deacetylase family share a common ancestor, resulting in similar 3D structure, function, and sequence homology. There are a total of 18 isoforms of human-derived HDACs, which can be categorized into four subfamilies (Class I, Class II, Class III, and Class IV) based on their homology, intracellular localization, and tissue distribution specificity, similar to yeast histone deacetylases.

### 2.2.1 | Class I HDAC: Deacetylase complexes

The earliest discovered members of the Class I HDAC family include Rpd3 in budding yeast, as well as HDACs 1–3 and 8, which belong to this class. Their catalytic structural domains are located at the N terminus of the protein and show 40%–70% sequence conservation with the catalytic domain of yeast Rpd3 (Yang & Seto, 2008). In addition to the catalytic domain, HDAC1-3 also has C-terminal extensions of varying lengths. These extensions can be modified by phosphorylation to enhance their deacetylase activity and can influence the formation of co-inhibitory complexes (Pflum et al., 2001; Sengupta & Seto, 2004). Unlike HDAC1, HDAC2 is only present in the nucleus, while HDAC3 contains both the NLS (nuclear localization signal) region and the NES (nuclear export-signal) region. The localization of HDAC3 between the nucleus and cytoplasm may vary depending on the cell type and environmental conditions (Yang et al., 2002). In addition, HDACs 1, 2, and 3 isozymes are found in large multiprotein complexes. HDAC 1 and HDAC 2 act as catalytically active subunits in Sin3, NuRD, CoREST, MiDAC, and MIER complexes, while HDAC3 serves as the catalytic subunit of SMRT and N-CoR complexes (Sarkar et al., 2020). All three isozymes play a major role as nuclear deacetylases. Unlike other Class I isozymes, HDAC8 lacks a C-terminal extension region, aside from the catalytic core structural domain. Interestingly, the HDAC8 protein alone exhibits significant histone deacetylase activity and substrate selectivity, suggesting it may function relatively independently (Minucci & Pelicci, 2006).

The crystal structures reveal that the catalytic domain of Class I HDACs consists of approximately 400 amino acid residues. These residues have a similar overall structure, with a core composed of eight parallel β-fold bundles forming a β-fold sheet. Surrounding the core are more than 13 α-helices and long loops extending from the C-terminus of the β-fold, creating a narrow hydrophobic channel (Seto & Yoshida, 2014). In HDAC8, the hydrophobic channel is made up of Phe152, Phe208, His180, Gly151, Met274, and Tyr306. In the other members of the Class I subfamily (HDAC1-3), all amino acid residues are highly conserved, except for Met274, which is replaced by leucine residues (Bondarev et al., 2021). The conserved hydrophobic residues within the channel serve as binding sites for the substrate. The acetylated lysine of the substrate reaches the catalytic core pocket at the bottom of the channel and interacts with the zinc ion bound there. In normal physiological conditions, the hydrophobic channel is occupied by the acetylated lysine side chain containing four methylene groups of the substrate. The $Zn^{2+}$ ion, located at the bottom of the channel, forms a five-tooth chelate with Asp178 and Asp267 of HDAC, His180, the oxygen atom of the acetyl group of the substrate, and the oxygen atom of the water molecule involved in the hydrolysis reaction. In addition to the substrate binding site and the zinc ion binding site, the catalytic domain of HDAC also contains two metal ion binding sites (De & Chatterji, 2015). One site, called Site 1, is located near the zinc ion binding site. The other site, called Site 2, is located at the periphery of the catalytic domain, close to the N-terminal end of the β-fold bundle. The binding of these two metal ions may contribute to stabilizing the overall structure of the enzyme. Additionally, the metal ion at Site 1 may assist in binding the zinc ion and play a role in the deacetylase reaction.

The deacetylation of nucleosomal histones by Class I HDACs primarily occurs through the formation of complexes, in which HDACs serve as the enzymatically active components. These complexes not only directly bind to and regulate the activity and selectivity of HDACs, but more importantly, they are also regulated by other transcription factors. These transcription factors enable the complexes to bind to chromosomes and deacetylate them at specific times and locations. As deacetylation itself leads to the down-regulation of gene transcription, these corepressor complexes typically work in conjunction with transcription factors or suppressors that inhibit gene transcription.

The crystal structures of the HDAC3 complex with the DAD (deacetylase activation domain) in the SMRT complex and the HDAC1/2 complex with the MTA1 (metastasis-associated protein 1) in the NuRD complex reveal that the corepressor protein binds to the end of the HDAC catalytic domain, near the substrate binding channel. This binding significantly enhances the deacetylase activity of HDAC (Millard et al., 2013). Additionally, phosphatidylinositol, a regulator conserved in the Class I HDAC co-inhibitor complex, was found to effectively promote HDAC enzymatic activity. However, it was observed that HDAC enzymatic activity could only be effectively activated in the presence of both co-inhibitors and phosphatidylinositol. In the HDAC3/DAD complex structure, a phosphatidylinositol Ins (1, 4, 5, 6) P4 molecule was bound to the substrate binding channel of HDAC3. In the structure of the HDAC3/DAD complex, a phosphatidylinositol Ins (1, 4, 5, 6) P4 molecule binds between the substrate binding channel of HDAC3 and the DAD structural domain. This binding facilitates the interaction between HDAC3 and the DAD domain. Additionally, Ins (1, 4, 5, 6) P4 binds to the "distorted" α-like helix H1, loop L1, and loop L6 at the substrate binding channel, potentially causing a change in the conformation of the substrate binding channel. These interactions have the potential to modify the conformation of the substrate binding channel, making it easier for the substrate to access the catalytic active site (Li et al., 2023). The significance of polyinositol in this

complex also suggests a potential link between epigenetics and cellular metabolism.

Unlike other Class I isoenzymes, HDAC8 functions independently of the multiprotein complex and typically has a higher catalytic efficiency for acyl lysine substrates than acetyl lysine. As the first crystal structure of a human-derived histone deacetylase to be resolved, the three-dimensional structural map in the PDB database shows that the secondary structure of human HDAC8 contains 11 or 13α-helices and 8 β-folds(Figure 1) (Amin et al., 2018). The PDB Protein Data Bank (PDB) (http://www.rcsb.org/) is a U.S. Brookhaven National Laboratory in 1971, maintained by the Research Collaboratory for Structural Bioinformatics (RCSB). It is the leading collection of 2.5-dimensional (data in two dimensions representing three-dimensions) structures of biomolecules (proteins, nucleic acids, and sugars), and is a database of three-dimensional structures of proteins, polysaccharides, nucleic acids, viruses, and other biomolecules, as determined by experimental means such as x-ray single-crystal diffraction, nuclear magnetic resonance, electron diffraction, and so on. (Westbrook et al., 2022) The PDB database shows that the α-helix H1 of HDAC8 is a regular and typicalα-helix structure, the loop L1 is 2 amino acid residues shorter than the L1 of HDAC3, and the loop L6 contains a proline which makes the loop slightly far away from the catalytic site, so that HDAC8 possesses a relatively open pocket of catalytic activity, which may make it easier for the substrate to approach the catalytic core of HDAC8; moreover, all of the above differences are present in the crystal structure of HDAC1-3, which makes HDAC8 more suitable for the catalytic core of HDAC8, and also makes it easier for the substrates to approach the catalytic core. The above differences are present in the crystal structures of HDAC1-3, making HDAC8 a more independent deacetylase from HDAC1-3 (Watson et al., 2012). The crystal structure analysis of HDAC8 is highly significant for studying the structural analysis and catalytic mechanism of zinc ion-dependent HDACs, particularly the members of the Class I subgroup. This is because HDAC8 shares a 40% amino acid sequence identity with HDAC1, 41% with HDAC2, and 41% with HDAC3. Additionally, the catalytic activity centers of all zinc ion-dependent HDACs are conserved. Therefore, understanding the crystal structure of

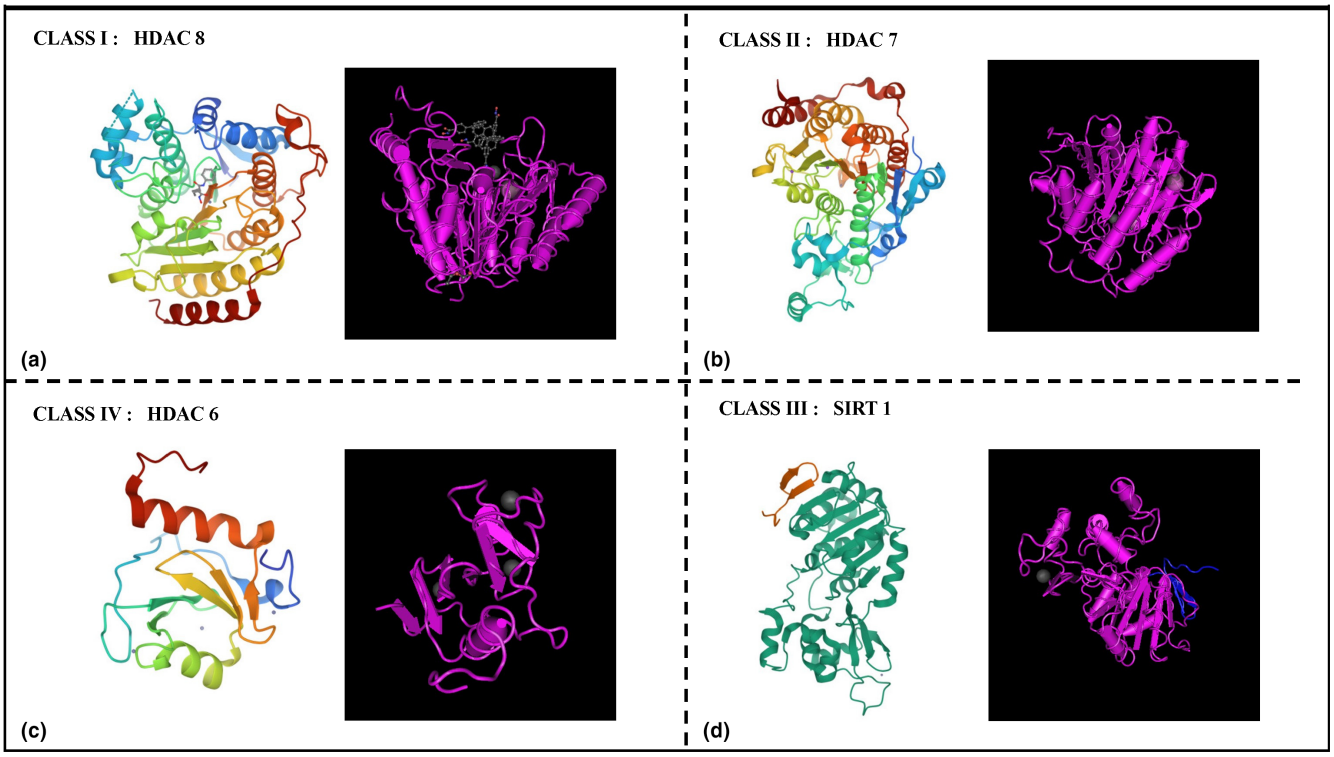


**FIGURE 1** The crystal structure of representative HDACs. The three-dimensional structure of human HDAC8, the first human histone deacetylase to be resolved, consists of 11 or 13 α-helices and 8 β-folds (a). HDAC6 is unique as it is primarily located in the cytoplasm. Its main structural domains include the nuclear localization signal region (NLS), two conserved leucine-rich nuclear export signal regions (NES1, NES2), two tandem deacetylation catalytic regions (DD1, DD2), a serine-glutamate-containing tetradecapeptide repeat region (SE14), and a ubiquitin-binding zinc finger structure (ZnF-UBP) (b). The HDAC7 protein has a distinct structure composed of a conserved catalytic core structural domain at its C-terminus. Additionally, it features a conserved N-terminal extension region that contains multiple binding sites (c). On the contrary, the catalytic structural domain of SIRT1 is elliptical and made up of two large and two small domains, totaling around 270 amino acid residues (d).

HDAC8 can provide valuable insights into this group of enzymes.

### 2.2.2 | Class II HDAC: II A recruiting class I; II b cytoplasmic protein deacetylation

The Class II HDAC family is a homolog of the Hda1 protein found in budding yeast. This family is further divided into two subtypes: Type a and Type b. Type a includes HDAC4, 5, 7, and 9, while Type b includes HDAC6 and the recently discovered HDAC10 (Bantscheff et al., 2011).

*Structure of IIa*

The structural feature of IIa is characterized by a conserved catalytic core structural domain at the C-terminus of the protein. Additionally, it possesses a unique and conserved N-terminal extension region that contains multiple binding sites. For instance, it has the MEF2 (myogenic transcription factor 2) binding site, which binds MEF2 protein to inhibit muscle cell differentiation, as well as two to three phosphorylated serine sites. These phosphorylated serine sites bind to 14-3-3 proteins, regulating the cellular localization of the enzymes and influencing their interactions with tissue-specific transcription factors and co-blockers (Hsu et al., 2017). Furthermore, type IIa HDAC has both an NLS region at the N-terminus and an NES region at the C-terminus, enabling it to shuttle between the nucleus and cytoplasm. Overall, the catalytic domain of type IIa HDAC is similar to that of Type I HDAC (Liu, Dong, et al., 2021). It consists of an α/β domain with several loops forming the substrate binding channel and catalytic active site, with one zinc ion bound at the active site and two potassium/sodium ion binding sites.

Although Class II a HDAC has a highly conserved histone deacetylation domain, the unique catalytic activity of this class remains to be explored. Class I HDAC and Class II b HDAC possess a conserved tyrosine residue, whereas Class II a HDAC has a conserved histidine residue instead (Wright & Menick, 2016). Due to the replacement of the key tyrosine with a histidine at the active site and the shorter histidine side chain compared to tyrosine, Class II a HDAC exhibits limited deacetylation activity (Hess et al., 2022). However, this limitation does not prevent them from functioning as transcriptional repressors by binding to 14-3-3 protein or MEF 2 protein. Therefore, it can be observed that Class II a HDAC exerts epigenetic functions not solely through its deacetylation activity (Li et al., 2022), but rather through its involvement in the recruitment of Class I HDACs after being recruited by other transcription factors. Class IIa deacetylase activity is dependent on its binding to HDAC-polyprotein complexes, such as HDAC3-SMRT/N-CoR. It is speculated that Class IIa HDACs may function as regulators by binding to regulatory factors, such as transcription factors, through their N-terminal binding site. Subsequently, they target the enzymatically active SMRT/NCoR-HDAC3 complex to specific sites through their C-terminal catalytic domain, thus exerting a regulatory effect (Park et al., 2018).

*The structure of IIb*

HDAC6 is primarily located in the cytoplasm and consists of several distinct structural domains, including the nuclear localization signal region (NLS), two conserved leucine-rich nuclear export signal regions (NES1, NES2), two tandem deacetylation catalytic regions (DD1, DD2), a serine-glutamate-containing tetradecapeptide repeat region (SE14), and a ubiquitin-binding zinc finger structure (ZnF-UBP) (Liu, Xiao, et al., 2021). While HDAC6 does contain a nuclear localization signal, it is mainly found in the cytoplasm due to the combined action of NES and SE14 (Yue et al., 2022). The nuclear export signals prevent HDAC6 from binding to nuclear proteins and facilitate its translocation from the nucleus to the cytoplasm, while the SE14 region anchors HDAC6 in the cytoplasm (Bertos et al., 2004). For a long time, the function of HDAC6 in the cytoplasm remained unknown. However, in 2002, it was discovered that HDAC6 is a major histone deacetylase and has various nonhistone substrates, including α-microtubulin, cortactin, Ku70, and HSP90 (Ali et al., 2020; Miyake et al., 2016). The main substrate of HDAC 6 is tubulin, which directly affects the cytoskeleton, intracellular material transport, and cell motility (Kaur et al., 2022). HDAC 6 regulates microtubule assembly and the localization of microtubule motor complexes, which in turn affects microfilament-based cell motility and the interaction of cortical actin with microfilaments (Losson et al., 2020). Inhibition of HDAC 6 leads to hyperacetylation of microtubule proteins and enhanced intracellular vesicular transport, which is associated with neurological disorders like Parkinson's disease and Huntington's disease (English & Barton, 2021). Additionally, HDAC 6 plays a role in important intracellular signaling processes and its extensive substrate deacetylation activity highlights its significance to cells. Another substrate of HDAC 6 is heat shock protein 90 (HSP 90) (Seidel et al., 2016). As a cytoplasmic protein substrate, HSP 90 plays a crucial role as a molecular chaperone in facilitating the structural maturation and functional integrity of various intracellular proteins. The deacetylation of HDAC 6 is vital for the interaction between HSP 90 and the glucocorticoid receptor (GR). Knocking down HDAC 6 leads to the acetylation of HSP 90, which subsequently weakens its interaction with proteins like GR or Bcr-Abl (Karra et al., 2022).

HDAC10 acts as a transcriptional repressor and is capable of shuttling between the nucleus and cytoplasm. It

contains two conserved deacetylation catalytic domains at the N-terminal end. Although the C-terminal region bears some sequence similarity to the N-terminal end, it lacks deacetylase activity. The leucine-rich domain at the C-terminal end is responsible for its localization in the cytoplasm (Herp et al., 2022). HDAC10 can interact with HDAC3, similar to type a HDAC, but what sets HDAC10 apart is its ability to function as a deacetylase on its own. However, the specific substrate of HDAC10 is currently unknown.

### 2.2.3 | Class IV HDAC: Still needs to be explored

Class IV HDAC consists of only one member, HDAC11, which shows less similarity to both Class I and Class II HDACs. HDAC11 is the shortest among all identified HDACs and mainly comprises the core catalytic domain, exhibiting deacetylase activity alone (Liu et al., 2020). It is localized in the nucleus and cytoplasm of cells. HDAC11 exhibits tissue-specific expression, with high levels observed in the kidney, heart, brain, skeletal muscle, testis, and other tissues (Liu et al., 2023). Additionally, HDAC11 has been found to form complexes with HDAC6 in vivo (Yanginlar & Logie, 2018). Furthermore, HDAC11 plays a role in regulating the protein stability of the DNA replication factor CDT1 and the expression of interleukin 10 (Núñez-Álvarez & Suelves, 2022). Being the most recently discovered HDACs, HDAC10, and HDAC11 are among the least studied and understood proteins within the HDAC family.

### 2.2.4 | Class III HDAC: Diversity

In addition to the 11 enzymes of the $Zn^{2+}$ cofactor-based histone deacetylase family mentioned earlier, there are seven specific deacetylases known as Class III HDACs (Blander & Guarente, 2004). These deacetylases belong to the sirtuin protein family and are represented by the yeast Sir2 protein. Unlike the $Zn^{2+}$-dependent HDACs mentioned before, Class III HDACs are a type of $NAD^{+}$-dependent deacetylases that catalyze the deacetylation of both histone and nonhistone substrates. Sirtuin proteins are highly conserved across all organisms, from prokaryotes to eukaryotes, and there are seven family members in humans known as NAD-dependent deacetylases or SIRT 1–7 (Pande & Raisuddin, 2022).

The crystal structures of the catalytic core domains of SIRT1, 2, 3, 5, and 6 in human Sirtuin proteins have all been resolved. These structures exhibit a consistent overall structure, which can be attributed to the evolutionary and sequence conservation of the catalytically active region. The catalytic structural domain is elliptical in shape and comprises two large and two small structural domains, each consisting of approximately 270 amino acid residues. The large structural domain, which is relatively conserved, features a typical Rossmann-fold domain. This domain includes a central β-sheet with six β-strands, surrounded by several α-helices that form pockets capable of accommodating and binding $NAD^{+}$. On the contrary, the small structural domain, which shows more variability, consists of two modules that extend from the large structural domain. These modules include a conserved $Zn^{2+}$ binding element and an α-helix region with relatively high variability. The zinc finger structural domain is composed of three reverse parallel β-strands and one α-helix (Villalba & Alcaín, 2012). It binds to $Zn^{2+}$ through two pairs of cysteine residues in the conserved sequence CysX 2-4 -CysX 15-40-CysX 2-4-Cys. The four loops region that connects the structural domains is highly conserved in the Sirtuin family. This region forms the substrate binding pocket and is the center of catalytic activity. The largest loop, known as the β1-α2 loop or cofactor binding loop, is responsible for part of the NAD+ binding site. It exhibits a highly dynamic structure and plays a crucial role in catalytic reactions (Gasparrini et al., 2022).

In addition to the catalytic domain, certain Sirtuin proteins (such as Sir2, HST1, and SIRT1) also contain N-terminal and/or C-terminal regulatory regions (Chadha et al., 2019). Studies have shown that these regulatory regions not only enhance the enzymatic activity of SIRT1 but also serve as binding sites for other proteins. This allows for protein–protein interactions that can regulate the enzymatic activity of these proteins (Pillus & Rine, 2004).

Studies have shown that SIRT 1–3 exhibit high deacetylating activity, while SIRT 5–7 have low activity (Simó-Mirabet et al., 2017). Notably, SIRT 4 does not demonstrate any relevant deacetylation activity. Several studies also suggest that different SIRTs may have higher activity toward other novel acylations. Specifically, SIRT 1–2 exhibit significant activity against various acylations (Wu et al., 2022). Additionally, SIRT 2 catalyzes the debenzoylation of histone lysine both in vitro and in vivo (Chen & Guarente, 2007). SIRT 5 possesses debenzoylation function and is active against malonylation, butanoylation, and glutarylation. Both SIRT 4 and SIRT 6 also exhibit ADP ribosyltransferase activity, with SIRT 6 specifically demonstrating debenzoylation of long-chain fatty acids (Dong, 2023). The deacetylation activity of SIRT 7 is activated by double-stranded DNA, leading to the deacetylation of histone H3 position 18 lysine (H3K18) in chromatin. Furthermore, the long-chain fatty acylation activity of SIRT 7 can be enhanced by rRNA, possibly surpassing its deacetylation activity (Lucatelli et al., 2022).

The intracellular environment of Sirtuin family deacetylases is well characterized. SIRT 1, which is closely related to yeast Sir 2, has been extensively studied. SIRT 3 is present in both the nucleus and mitochondria (Zhang et al., 2020), while SIRT 4 and SIRT 5 are primarily found in mitochondria (Di et al., 2021). SIRT 6 is exclusively located in the nucleus, and SIRT 7 is specifically found in the nucleolus (Kida & Goligorsky, 2016). Additionally, there are cytoplasmic and nuclear proteins associated with these SIRT enzymes (Yoon & Eom, 2016). Overall, the functions of SIRT 1–7 in cells are complex and diverse (Beegum et al., 2022).

With the increasing abundance of structural information on HDACs, researchers have gradually uncovered the structural differences among subgroups and even subtypes. This knowledge has provided a reasonable explanation for the selectivity of certain HDAC inhibitors. For instance, a series of novel o-phenylenediamine HDAC inhibitors were discovered to only inhibit HDAC1 and HDAC2 (Wang et al., 2022). Further analysis of homologously modeled HDAC1 and HDAC3 revealed that a difference in an amino acid located in the cavity at the base of the catalytically active center (the substitution of Ser113 residue of HDAC1 with Tyr96 residue of HDAC3) may be the structural basis for this selectivity. Additionally, the lack of isoform selectivity in the majority of current HDAC inhibitors can be attributed to the highly conserved amino acid sequence of the catalytic active center of $Zn^{2+}$ dependent HDACs (Morse et al., 2022). In contrast, the amino acid sequence around the entrance of the active center, located on the protein surface, varies significantly among isoforms. In the HDAC8 isoform, the L1 loop near the entrance of the active center is shorter compared to other Class I members. This results in a larger active center entrance and a more flexible protein surface for the HDAC8 isoform. Currently, one effective strategy in this field is to design subtype-selective inhibitors based on the structural differences around the active center inlet of each HDAC8 subtype. Furthermore, a comprehensive analysis and understanding of the structural information of each isoform of HDACs will greatly assist in the design of highly active and selective inhibitors.

## 3 | HISTONE DEACETYLASE INHIBITOR (HDACI)

HDACis are a novel class of targeted anticancer drugs that primarily modify chromatin structure by altering the acetylation levels of histones, thus regulating gene expression (Sanaei & Kavoosi, 2019). In both in vivo and in vitro settings, HDACis have demonstrated the ability to induce growth arrest, differentiation, and apoptosis in tumor cells, making them promising candidates for tumor treatment (Ceccacci & Minucci, 2016). Clinical trials have been conducted for targeted Class I/II HDACis, with the most effective inhibitors being isohydroxamic acids like SAHA and cyclic peptides resembling FK228. However, it is worth noting that most existing HDACis suffer from issues such as low bioavailability, rapid metabolism, irreversible differentiation, and lack of selectivity toward cancer cells. Therefore, it is crucial to investigate the diverse functions of different HDACs and the range of HDAC substrates, in order to develop effective and selective HDACis based on the specific biological effects of individual HDACs.

### 3.1 | Overview of HDACis research

The discovery of HDACis predates the discovery of their targets, HDACs. HDACis have been found to have effects on various cells and genes, indicating that they have multiple antitumor mechanisms (Mottamal et al., 2015). As the close relationship between HDACs and tumors becomes clearer, more and more HDAC inhibitors have shown highly effective antitumor activities in vitro and in vivo, with multiple mechanisms of action. These mechanisms include inducing apoptosis and autophagy (Gilardini et al., 2017), causing tumor cell cycle arrest (Zhang et al., 2013), inhibiting tumor cell angiogenesis (Mottamal et al., 2015), reducing tumor cell motility and migration ability, and enhancing tumor cell sensitivity to radiotherapy and chemotherapy (Chen et al., 2007; Lee et al., 2010). HDACis can activate either the extrinsic pathway (receptor death pathway) or the intrinsic pathway (mitochondrial pathway) for cell death in many cancer cells (Johnstone et al., 2002).

In recent years, a significant number of HDACis have been synthesized or derived from natural sources (Yoshida et al., 2001). Trichostatin A (TSA) was the first natural hydroxamic acid known to inhibit HDACs. Vorinostat (suberoylanilide hydroxamic acid, SAHA), which has a similar structure to TSA, was the first FDA-approved HDAC inhibitor for the treatment of refractory cutaneous T-cell lymphoma (CTCL) (Zhang, Wang, et al., 2018). Currently, the FDA has approved six HDACIs for the treatment of various hematologic tumors and a few solid tumors (Kelly et al., 2005; O'Connor et al., 2006).

### 3.2 | Histone deacetylase inhibitor pharmacophore model

The compound library of $Zn^{2+}$-dependent HDACis has significantly expanded in the past 20 years. While the

structures of these HDACis, whether synthetic or natural, are complex and diverse, the majority of $Zn^{2+}$ HDACis share a common pharmacophore model. This model consists of three components: (1) the Cap structure, also known as the Surface Recognition Domain, which is typically a hydrophobic aromatic group that interacts with the enzyme's surface; (2) a $Zn^{2+}$ binding group (ZBG), such as isohydroxamic acid, carboxylic acid, or benzamide, which chelates the $Zn^{2+}$ at the enzyme's active center; and (3) a Linker, which is a saturated or unsaturated linear or hydrophobic long chain with a ring structure. The Linker connects the Cap structure to the ZBG (Finnin et al., 1999; Miller et al., 2003). Co-crystalline complex studies of isohydroxamic acid HDACis and HDACs demonstrate the interaction between the inhibitor's Cap structure and the amino acids near the entrance of the enzyme's active center, while the ZBG structure chelates with the metal ion at the bottom of the enzyme's active center to form the complex (Lauffer et al., 2013; Vannini et al., 2004). The linker structure, of appropriate length, brings the ZBG group to the bottom of the HDACs active region. It chelates $Zn^{2+}$ and forms hydrogen bonds with histidylic acid and tyrosine, among others. The long linker chain interacts with the amino acid residues in the active region through forces such as van der Waals forces. It occupies the active region, while the cap structure acts as a cover for the entrance to the enzyme's active region, resembling a cap (Drummond et al., 2005). HDACis inhibit enzyme activity by competitively inhibiting the binding of acetyllysine residues of the substrate to the active site of the enzyme, as mentioned earlier.

Changes in all three components can significantly affect the activity or selectivity of HDACis. The functional groups of ZBG include isohydroxamic acid, benzamide, carboxylic acid, sulfhydryl groups, ketones, and epoxides. When comparing the three clinical HDACis SAHA, entinostat (MS275), and valproic acid, which use isohydroxamic acid, benzamide, and carboxylic acid as chelating groups, respectively, it is evident that these compounds exhibit significant differences in their inhibitory activity against HDACs. Among them, isohydroxamic acid demonstrates the strongest ability to chelate zinc ions (Wu et al., 2011).

Linker structures can vary in terms of different chain lengths, saturation, unsaturation, linearity, cyclicality, and modifications. It has been observed that modifying the linker can significantly impact the activity (Rajak et al., 2014). Some common types of linkers include aliphatic chains (e.g., six-carbon chains in SAHA), aromatic rings (e.g., 1,4-phenylene support in MS-275), and vinyl-aromatic rings (e.g., styryl groups in PXD101). Through docking and energy-optimized pharmacophore localization, it has been found that inhibitors with at least one aromatic ring in their linkage region exhibit a higher affinity for the target enzyme, whereas those without aromatic rings tend to be poor binding agents. Additionally, the length of the linkage region also plays a role in determining the activity. The study demonstrated that the most effective enzyme inhibitory activity occurred when the carbon number of the linkage region (n) was 6. Hydrophobic and high-capacity Cap groups (such as phenyl, naphthyl, and thiophene groups) were found to enhance HDAC inhibition. Additionally, greater lipophilicity of the substituent (trifluoromethyl) resulted in stronger HDAC inhibition when methoxy and trifluoromethyl were substituted at the adjacent, inter-, and para-positions of the CAP group. Therefore, lipophilicity promotes a stronger hydrophobic interaction between the surface of the HDAC active site and the HDAC inhibitor, consequently increasing the activity of the HDAC inhibitor (Micelli & Rastelli, 2015).

The integration of the pharmacophore model of HDAC inhibitors and the structural information of the enzymatic active region contributes to understanding the inhibition mechanism and serves as a basis for the rational design of HDAC inhibitors. Consequently, modifying the structure of HDAC inhibitors based on the three components of the pharmacophore is a widely adopted strategy for the rational design and optimization of HDAC inhibitors.

## 3.3 | Classification of histone deacetylase inhibitors

ZBG binds to $Zn^{2+}$ and its surrounding residues, playing a crucial role in the inhibitory activity of HDACis (Bantscheff et al., 2011). Based on the type of inhibitor ZBG, the six HDACIs can be categorized into three main groups (Figure 2): (1) isohydroxamic acids, including SAHA, belinostat (PXD101), and panobinostat (LBH589); (2) benzamide, including mocetinostat (MGCD0103) and chidamide; (3) cyclic peptides, such as romidepsin (FK228) (Heers et al., 2018). Furthermore, several HDACI drugs are currently being studied in preclinical and clinical trials(Rodríguez-Paredes & Esteller, 2011).

### 3.3.1 | Isohydroxamic acid

The isohydroxamic acid HDACI are the most extensively studied and widely used inhibitors. They are considered broad spectrum HDACIs because of their inhibitory effect on almost all $Zn^{2+}$ dependent HDACs of Classes I, II, and IV. However, this also leads to a series of toxic side effects. Despite these side effects, these drugs exhibit potent single-agent antitumor effects and are often

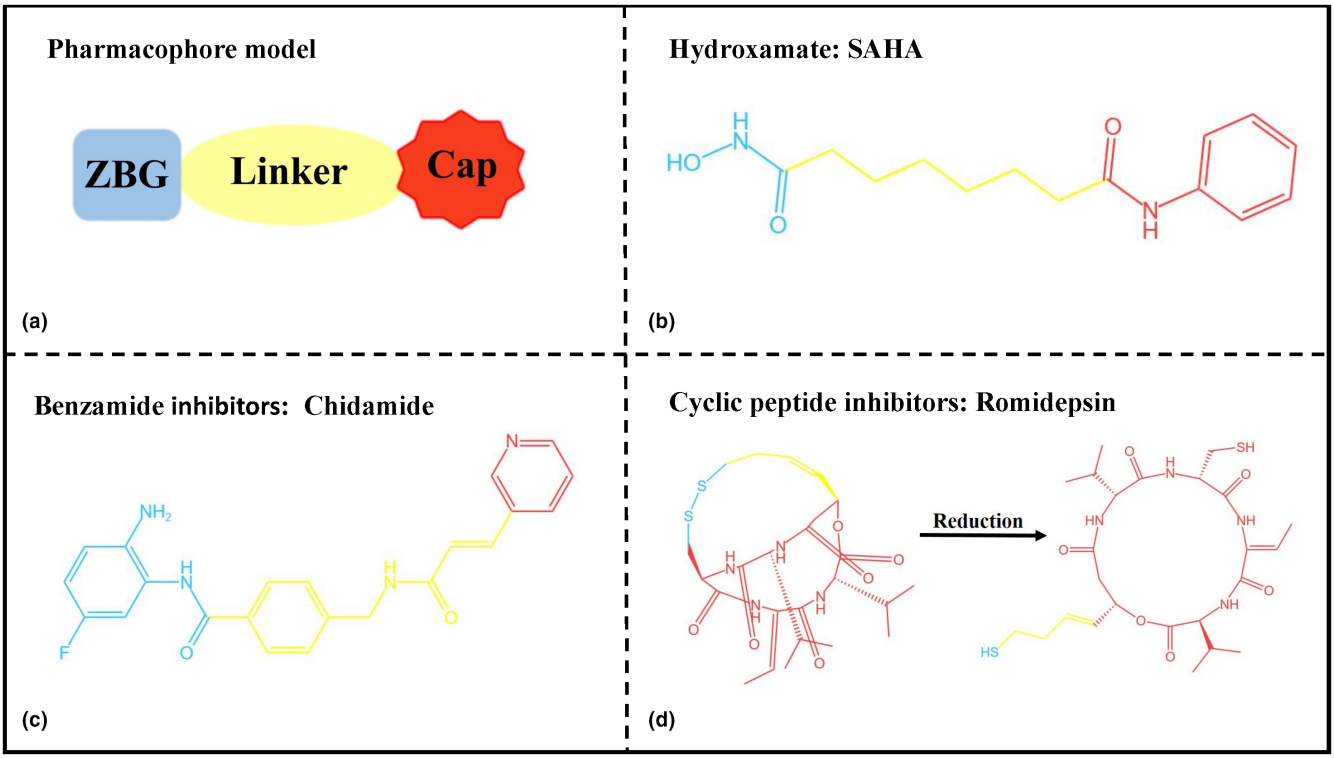


**FIGURE 2** Classification of HDACis and the representative compounds. ZBG binds to $Zn^{2+}$ and its surrounding residues, playing a crucial role in the inhibitory activity of HDAC is (a). Based on the type of inhibitor ZBG, the six HDACIs were categorized into three main groups: (b) isohydroxamic acids, including SAHA, belinostat (PXD101), and panobinostat (LBH589); (c) benzamide, consisting of mocetinostat (MGCD0103) and chidamide; (d) cyclic peptides, represented by romidepsin (FK228).

used in combination with various anticancer drugs. The combination of these drugs not only enhances their antitumor effects but also has irreplaceable clinical value (Li & Seto, 2016).

Under normal physiological function, the hydrophobic channel of HDAC is occupied by the acetyl lysine side chain of the substrate, which contains four methylene groups. At the bottom of the channel, $Zn^{2+}$ forms a five-tooth chelate. In the presence of inhibitors of isohydroxamic acid HDACs, the hydrophobic channel is competitively occupied by the hydrophobic linker of the inhibitor. $Zn^{2+}$ is chelated by the zinc ion chelating group (ZBG) of the inhibitor. The ZBG in the figure represents the widely used isohydroxamic acid group, which has the strongest chelating ability with $Zn^{2+}$. Additionally, the isohydroxamic acid group can form oxygen bonds with H142, H143, and Y306, aside from forming a strong diphthong chelate with $Zn^{2+}$. The isohydroxamic acid group, acting as a ZBG, possesses several advantages including strong binding ability with zinc, good in vitro stability, good solubility, and easy synthesis (Mohammed et al., 2016). However, it is important to note that the binding of isohydroxamic acid groups to other zinc-dependent enzymes such as aminopeptidases, matrix metalloproteinases, and carbonic anhydrases may result in undesirable side effects. Moreover, isohydroxamic acid is prone to hydrolysis and glucuronidation, leading to unfavorable pharmacokinetic properties and reduced efficacy in vivo (Zhang et al., 2015). The linker domain of isohydroxamic acid can have linear or cyclic structures, as well as saturated or unsaturated structures. The cap structure of straight-chain linear linker isohydroxamic acid HDACis is the main site for modifying and optimizing the compounds, which contributes to its diversity. Linear linkers are flexible structures that facilitate interaction between the cap structure and the amino acid residues on the surface during the activity of HDACs. Therefore, more complex cap structures, such as branching caps, are often used in the design of HDACis. The cap structural domains typically consist of hydrophobic groups, particularly aromatic ones (Giannini et al., 2012).

### *SAHA*

This drug, developed by Merck and approved by the FDA in 2006, was the first HDACI used for the treatment of cutaneous T-cell lymphoma (Mann et al., 2007). Recent research has revealed its potential for various clinical

effects, including hematologic tumors. It has shown different efficacy in B-cell lymphomas such as diffuse large B-cell lymphoma, follicular lymphoma, and set of cell lymphoma. In solid tumors like prostate and pancreatic cancers, SAHA has been found to inhibit the Akt/FOXO3a signaling pathway, promoting apoptosis of prostate tumor cells and preventing the occurrence of resistant prostate cancer caused by traditional therapies like androgen deprivation therapy (Shi et al., 2017). Additionally, SAHA helps reduce side effects such as drug resistance and dose toxicity caused by chemotherapeutic drugs like paclitaxel (Wu et al., 2017). Moreover, SAHA is known to play a crucial role in inducing autophagy in tumor cells and preventing acute graft-versus-host disease.

SAHA has demonstrated significant antitumor effects, but it is also associated with considerable toxicity, including fatigue, diarrhea, anorexia, dehydration, bone marrow suppression, and thrombocytopenia, particularly at high doses (Mrakovcic et al., 2017). As a broad-spectrum inhibitor, SAHA simultaneously targets multiple HDAC isoforms, which contributes to its toxic side effects. Therefore, enhancing the selectivity of HDAC inhibitors (HDACIs) or developing novel HDACIs based on the fundamental pharmacodynamic moiety of SAHA holds promise for future applications. Studies have shown that modifying the hydrophobic long chain of SAHA can enhance its selectivity for HDAC. For instance, substituting the hydrogen atom (H) at the C2 position of the hydrophobic long chain of SAHA with aliphatic or aromatic hydrocarbons resulted in the analog C2-R-SAHA, which effectively improved its selectivity for HDAC6 and 8 (Yang et al., 2020).

The molecular docking model revealed that all HDAC isoforms have highly conserved active catalytic regions. Class I HDAC has a narrower hydrophobic channel compared to HDAC6. Substituting aliphatic hydrocarbons on the hydrophobic chain of SAHA can increase the barrier to the catalytic channel, effectively preventing the catalysis of compounds HDAC1, 2, and 3. Furthermore, substituting unsaturated hydrocarbons on aromatic, cyclic, or adjacent isohydroxamic acid groups can enhance the selectivity of HDAC6, as seen in tubastatin A (Brightman et al., 1992). It is important to note that TSA has a similar structure to SAHA, but exhibits much stronger inhibition activity on HDAC. The main difference lies in the TSA linkage region, which contains a diene and an R-type methyl group. However, researchers discovered that these features are not solely responsible for the increased activity. The arylamine ring on the surface recognition region of TSA may also play a key role in its high activity by interacting with amino acid residues of the enzyme capsule (Patel et al., 2022).

### 3.3.2 | Benzamide inhibitors

Benzamide inhibitors are a novel type of HDACI that exhibit reduced side effects due to their enhanced selectivity. These drug molecules contain a distinctive N-(2-aminophenyl) benzamide pharmacodynamic group, which provides them with a stronger selectivity towards HDAC1 and 2 compared to conventional isohydroxamic acid compounds. In a molecular docking study using histone deacetylase-like protein (HDLP) to screen for inhibitors, it was found that benzamides inhibitors bind to HDLP in a manner that may be quite different from that of isohydroxamic acid analogs to HDLP (Bass et al., 2021). The docking results showed that, instead of targeting $Zn^{2+}$ for binding, the former targeted the 2 benzene rings relative to Phe141 and Phe198, the narrowest part of the active pocket, to block the channel of the N-terminal Lys acetylation side chain of histone, the physiological substrate of HDAC, reaching to the catalytic center, where the 2-amino acid forms a hydrogen bond with Tyr91 or Glu92, and the intermediate benzene ring forms a sandwich structure with Phe141 and Phe198 to form a sandwich structure. Because of this mode of binding, benzamide-based inhibitors have significantly better target selectivity than isohydroxamic acid inhibitors with $Zn^{2+}$ as the binding target. There are many $Zn^{2+}$-containing proteins in the object, so the isohydroxamic acid inhibitors are more toxic.

The eutectic structures of HDAC2-inhibitor reveal that the catalytic active center of HDAC2 encompasses not only an approximately 8 Å long hydrophobic channel and the catalytic $Zn^{2+}$ located at the bottom of the channel, but also an adjacent inner cavity (referred to as the foot pocket) of approximately 14 Å. This foot pocket region plays a crucial role in constituting the HDAC2 active center. During drug inhibition, the benzamide inhibitor deeply penetrates the bottom of the active cavity. The o-amino group of the molecule, along with the carbonyl oxygen, participates in the $Zn^{2+}$ chelation. Additionally, one side of the inhibitor molecule's aromatic ring enters the catalytic "foot pocket", causing the repositioning of the two side chains of the "foot pocket" residues to accommodate the aryl part. However, SAHA is unable to enter the catalytic foot pocket due to its own structural properties. This inability is a significant reason why SAHA does not specifically inhibit HDAC2 (Bressi et al., 2010).

The time-dependent effect of benzamide inhibitors is significantly influenced by the intramolecular hydrogen bonding in the compounds. In contrast, SAHA only has a $Zn^{2+}$ chelating group at the top of the molecule, which means that the formation of drug-target complexes does not require extensive protein rearrangement or hydrogen bond breaking of the internal ligand. When SAHA is in close proximity to the protein, isohydroxamic acid can directly bind $Zn^{2+}$ at the bottom of the hydrophobic channel and replace the

bound water. As a result, ligands containing isohydroxamic acid esters usually exhibit rapid binding kinetics. On the contrary, benzamide inhibitors need to break their intramolecular hydrogen bonds before they can chelate with $Zn^{2+}$. Additionally, the large molecular size and curved hydrophobic channels of benzamide inhibitors are important factors that limit their rapid binding to $Zn^{2+}$ (Lauffer et al., 2013).

*Chidamide*

Chidamide, developed and synthesized in China, is the first subtype-selective histone deacetylase oral inhibitor. It has been approved by the State Food and Drug Administration (China Food and Drug Administration, CFDA) for clinical trials (Dong et al., 2012). Chidamide belongs to the class of benzamide histone deacetylase subtype-selective inhibitors and possesses a unique chemical structure. Its chemical name is N-(2-amino-4-fluorophenyl)-4-{N-[(E)-3-(3-pyridyl)acryloyl]aminomethyl}benzamide. Chidamide exhibits high antitumor activity and low cytotoxicity compared to similar drugs. It primarily targets subtypes 1, 2, and 3 of Class I HDAC and subtype 10 of Class II b (Zhang, Tao, et al., 2018). Chidamide has the potential to induce tumor stem cell differentiation and reverse epithelial-mesenchymal phenotypic transformation (EMT) of tumor cells, thereby restoring the sensitivity of drug-resistant tumor cells to drugs and inhibiting tumor metastasis and recurrence. It achieves this by inhibiting relevant HDAC isoforms, increasing the acetylation level of chromatin histones, and triggering chromatin remodeling, which leads to epigenetic alterations that inhibit the tumor cell cycle and induce apoptosis. Additionally, Chidamide exhibits modulatory activity on cellular immunity, enhancing natural killer (NK) and antigen-specific cytotoxic T cell (CTL)-mediated tumor killing.

### 3.3.3 | Cyclic peptide inhibitors

Cyclic peptides are the most structurally complex class of HDACi and can be divided into two groups, that is, cyclic peptides containing the Aoe moiety, such as trapoxin A, trapoxin B, and WF-3161, and cyclic peptides without the Aoe moiety, such as apicidin, and depsipeptide. both of them bind HDACs in a manner similar to that of isohydroxamic acids but with different mechanisms of action differ (Buckton et al., 2021). The spatial orientation of the cyclic tetrapeptide macrocycles of Aoe-containing inhibitors was tested by x-ray crystallography and NMR techniques, and it was found that these macrocycles were arranged with D-amino acids and cycloamino acids, with a spacer region on one side of the amino acids, and a large number of internal hydrogen bonds to generate a restricted 12-membered cyclic structure, which was speculated that the amino acids of the D-configuration were required for the tight binding to the edge of the CAP activation site; some inhibitors of the Aoe structure also required epoxyketones to bind to HDAC. Some Aoe-containing inhibitors also require an epoxy keto group, a large cyclic peptide structure that can bind to the "groove" at the entrance of the duct, a keto carbonyl group that can interact with Zn2+ and polar amino acid residues in the HDAC ribbon duct, and an epoxy group that can alkylate the active site of HDAC and irreversibly inhibit the HDAC enzyme activity. activity, and if the epoxy keto group is replaced with isohydroxamic acid, the inhibition of HDAC shows reversibility (Ramadhani et al., 2022).

Cyclic peptide inhibitors of HDACs mainly use larger cyclic peptide structures as Cap groups, and the most widely studied one is FK228, which does not conform to the classical pharmacophore model of HDAC, and needs to be hydrolyzed in vivo to release the sulfhydryl moiety of the zinc chelating group in order to chelate with zinc metal ions and thus exert the enzyme inhibitory activity effectively. Cyclic peptide HDAC inhibitors exhibit good enzyme inhibitory activity due to their larger Cap group, which enhances their interaction with amino acids on the edge of the active pocket, thereby increasing their affinity for the target (Pojani & Barlocco, 2021). In addition, cyclic peptide inhibitors of HDACs showed some subtype selectivity for the HDAC family, with good selective inhibitory activity for class I HDACs and poor enzyme inhibitory activity for class IIb HDACs (especially HDAC6), which provided a new idea for the design of selective HDAC inhibitors. However, the complexity of the cyclic peptide structure and the poor drug ability caused by the large molecular skeleton and molecular mass are the main challenges for the design and synthesis of these inhibitors.

In 2012, the US FDA approved romidepsin (FK228) for the treatment of cutaneous T-cell lymphomas (CTCL) and peripheral T-cell lymphomas (Ni et al., 2015). The drug, an atypical HDACI, primarily acts as a Class I HDAC. It is produced by Gram-negative pigmented bacillus No. 968 and has a caged bicyclic phenolic peptide structure with rare disulfide bonds. These disulfide bonds are activated in human cells after metabolism (Furumai et al., 2002). FK228 is a precursor drug that is more stable than its reduced form, Red-FK228. The disulfide bond helps the compound diffuse more efficiently across the cell membrane. Once inside the cell, FK228 is activated by glutathione reduction. This activation allows the Red-FK228 free sulfhydryl group to interact with the active site, $Zn^{2+}$, thereby preventing HDAC from binding to the substrate. It is important to note that although FK228 itself does not have HDAC inhibitory activity, its intracellularly active form after glutathione reduction is fully compatible with the pharmacophore model of HDACis.

**TABLE 2** Completed Phase III clinical trial of co-administered HDACIs.

| | HDACi | Drug | Condition | Title |
|---|---|---|---|---|
| 1 | Vorinostat | Bortezomib | Multiple myeloma | Study of Vorinostat (MK-0683) an HDAC Inhibitor, or Placebo in Combination With Bortezomib in Patients With Multiple Myeloma (MK-0683-088 AMN) |
| 2 | Vorinostat | Cytarabine, daunorubicin, hydrochloride, idarubicin | Acute Myeloid leukemia untreated adult acute myeloid leukemia | Cytarabine and daunorubicin Hydrochloride or idarubicin and Cytarabine With or Without Vorinostat in Treating Younger Patients With Previously Untreated Acute Myeloid Leukemia |
| 3 | Vorinostat | Bortezomib | Lymphoma | Vorinostat With or Without bortezomib in treating patients with refractory or recurrent Stage IIB, Stage III, or Stage IV Cutaneous T-cell lymphoma |
| 4 | Chidamide | PD-1, lenalidomide and etoposide Antibody | NK/T-cell lymphoma | PD-1 Antibody, Chidamide, Lenalidomide and Etoposide for Relapsed or Refractory NK/T-cell lymphoma |
| 5 | Chidamide | PD-1 blocking antibody, lenalidomide and gemcitabine | Peripheral T-cell Lymphoma | PD-1 Antibody, Chidamide, lenalidomide and gemcitabine for peripheral T-cell Lymphoma |
| 6 | Panobinostat | Bortezomib, Dexamethasone | Multiple Myeloma | Panobinostat or Placebo With bortezomib and dexamethasone in Patients With Relapsed Multiple Myeloma |
| 7 | Panobinostat | Ruxolitinib tablets or oral pediatric formulation | Primary Myelofibrosis Chronic Idiopathic Myelofibrosis Post Polycythemia Vera Myelofibrosis | CINC424A2X01B Rollover Protocol |

*Note*: Several HDACi-bsased combination drug regimens have entered clinical studies.

### *Limitations of HDACi therapy and strategies for combination drugs*

Significant progress has been made in the study of HDACis, but there are still unresolved issues. First, due to the high sequence similarity of some HDACs, most HDACis are currently broad-spectrum inhibitors. They primarily compete for $Zn^{2+}$ in the enzyme's active site, lacking selectivity for specific isoforms. However, by disrupting specific HDAC activities crucial for protein–protein interactions, some selectivity for HDAC isoforms can be achieved (Sacks et al., 2006). As mentioned earlier, HDAC1, 2, and 3 function as subunits of multiprotein complexes in the nucleus. Removal of HDAC from these complexes significantly reduces enzyme activity. Therefore, disrupting the formation of these complexes can partially inhibit HDAC activity. Inositol phosphate, a conserved regulatory factor in the multi-protein complex, effectively enhances enzyme activity. The interaction between inositol phosphate and arginine residues near the active site entrance plays a pivotal role in complex formation and enzyme activation. Competing or disrupting the interaction of arginine residues with inositol phosphate may specifically enhance the inhibitory effect on HDAC1, 2, and 3 (2). HDAC inhibitors (HDACis) typically utilize $Zn^{2+}$ binding groups such as isohydroxamic acid, thiol, carboxylic acid, ketone, or 2-aminoaniline. However, these functional groups can also strongly bind to other essential metalloenzymes, leading to cytotoxicity and restricting the clinical use of HDACis (Sacks, Lichtenstein, Van, et al., 2006). In addition, the currently identified inhibitors of HDACs show significant therapeutic effects only in hematologic tumors, but are not yet effective as single agents in solid tumors. These inhibitors have also been shown to cause serious side effects in clinical trials. Notably, successful cases of combining HDAC inhibitors with other chemotherapeutic agents in the treatment of solid tumors are not uncommon.

Combination is an important strategy to improve efficacy, reduce the occurrence of adverse effects and overcome drug resistance in tumor treatment. Many studies have investigated the use of HDACi in combination with other drugs, including antimetabolites, antimicrotubule drugs, topoisomerase II inhibitors, DNA cross-linking agents such as cisplatin, HSP90 antagonists, and targeted drugs (Wanczyk et al., 2011). HDACi has also been reported to synergize with the transcriptional regulator all-trans retinoic acid, DNA demethylating agents, and Bcr-Abl kinase inhibitors (Suraweera

et al., 2018). HDACi up-regulates death receptors and/or reduces inhibitory regulators of the death receptor pathway, sensitizing tumor cells to TRAIL. Many kinase inhibitors, all of which enhance the cell-killing effect of HDACi. Taken together, these findings suggest that combination therapy with HDACi may be the optimal therapeutic strategy using these agents. First, HDACi synergistically increases the effectiveness of anticancer therapy in combination with other anticancer regimens. Second, HDACi can overcome the resistance of some tumor cells to chemotherapy or targeted therapy drugs. Again, intervening against the deficiency or cancer-promoting effect of HDACi in anticancer therapy can further enhance the anticancer effect of HDACi.

Currently, several HDACi-based combination drug regimens have entered the clinical study stage (Table 2) (Tasneem et al., 2022). With the continuous development of a large number of novel HDACi, the in-depth understanding of the anticancer mechanism of HDACi and the continuous optimization of the drug combination strategy, HDACi has gradually become a promising new tumor therapeutic drug with broad application prospects in the field of anticancer therapy. In conclusion, conducting a comprehensive study on the conformational relationship of HDACis will aid in the rational design of drugs and the development of effective and innovative treatments. While HDACis are still in the early stages of research, they offer a new perspective for mankind to overcome tumors, making them a promising avenue for tumor treatment. The development of HDACis holds significant clinical and social value.

## ACKNOWLEDGMENTS

The work is supported by the National Science combination Foundation for improving innovation of Liaoning Provice (2022-NLTS-14-01).

## FUNDING INFORMATION

The work is supported by the Natural Science combination Foundation for improving innovation of Liaoning Province (2022-NLTS-14-01).

## CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest, financial or otherwise.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Protein Data Bank (PDB) at http://www.rcsb.org/.

## REFERENCES

Ali, A., Zhang, F., Maguire, A., Byrne, T., Weiner-Gorzel, K., Bridgett, S., O'Toole, S., O'Leary, J., Beggan, C., Fitzpatrick, P., McCann, A., & Furlong, F. (2020). HDAC6 degradation inhibits the growth of high-grade serous ovarian cancer cells. *Cancers*, *12*(12), null.

Allfrey, V. G., Faulkner, R., & Mirsky, A. E. (1964). Acetylation and methylation of histones and their possible role IN the regulation of RNA synthesis. *Proceedings of the National Academy of Sciences of the United States of America*, *51*(5), 786–794.

Amin, S. A., Adhikari, N., & Jha, T. (2018). Structure-activity relationships of HDAC8 inhibitors: Non-hydroxamates as anticancer agents. *Pharmacological Research*, *131*, 128–142.

Bannister, A. J., & Kouzarides, T. (2011). Regulation of chromatin by histone modifications. *Cell Research*, *21*(3), 381–395.

Bantscheff, M., Hopf, C., Savitski, M. M., Dittmann, A., Grandi, P., Michon, A. M., Schlegl, J., Abraham, Y., Becher, I., Bergamini, G., Boesche, M., Delling, M., Dümpelfeld, B., Eberhard, D., Huthmacher, C., Mathieson, T., Poeckel, D., Reader, V., Strunk, K., … Kruse, U. (2011). Chemoproteomics profiling of HDAC inhibitors reveals selective targeting of HDAC complexes. *Nature Biotechnology*, *29*(3), 255–265.

Bass, A. K. A., El-Zoghbi, M. S., Nageeb, E. M., Mohamed, M. F. A., Badr, M., & Abuo-Rahma, G. E. A. (2021). Comprehensive review for anticancer hybridized multitargeting HDAC inhibitors. *European Journal of Medicinal Chemistry*, *209*, 112904.

Beegum, F., Anuranjana, P. V., George, K. T., Divya, K. P., Begum, F., Krishnadas, N., & Shenoy, R. R. (2022). Sirtuins as therapeutic targets for improving delayed wound healing in diabetes. *Journal of Drug Targeting*, *30*(9), 911–926.

Bertos, N. R., Gilquin, B., Chan, G. K., Yen, T. J., Khochbin, S., & Yang, X. J. (2004). Role of the tetradecapeptide repeat domain of human histone deacetylase 6 in cytoplasmic retention. *The Journal of Biological Chemistry*, *279*(46), 48246–48254.

Bhaskara, S., Chyla, B. J., Amann, J. M., Knutson, S. K., Cortez, D., Sun, Z. W., & Hiebert, S. W. (2008). Deletion of histone deacetylase 3 reveals critical roles in S phase progression and DNA damage control. *Molecular Cell*, *30*(1), 61–72.

Blander, G., & Guarente, L. (2004). The Sir2 family of protein deacetylases. *Annual Review of Biochemistry*, *73*, 417–435.

Bondarev, A. D., Attwood, M. M., Jonsson, J., Chubarev, V. N., Tarasov, V. V., & Schiöth, H. B. (2021). Recent developments of HDAC inhibitors: Emerging indications and novel molecules. *British Journal of Clinical Pharmacology*, *87*(12), 4577–4597.

Bressi, J. C., Jennings, A. J., Skene, R., Wu, Y., Melkus, R., Jong, R. D., OConnell, S., Grimshaw, C. E., Navre, M., & Gangloff, A. R. (2010). Exploration of the HDAC2 foot pocket: Synthesis and SAR of substituted N-(2-aminophenyl)benzamides. *Bioorganic & Medicinal Chemistry Letters*, *20*(10), 3142–3145.

Brightman, A. O., Wang, J., Miu, K. M., Sun, I. L., Barr, R., Crane, F. L., & Morré, D. J. (1992). A growth factor- and hormone-stimulated NADH oxidase from rat liver plasma membrane. *Biochimica et Biophysica Acta*, *1105*(1), 109–117.

Buckton, L. K., Rahimi, M. N., & McAlpine, S. R. (2021). Cyclic peptides as drugs for intracellular targets: The next frontier in peptide therapeutic development. *Chemistry*, *27*(5), 1487–1513.

Ceccacci, E., & Minucci, S. (2016). Inhibition of histone deacetylases in cancer therapy: Lessons from leukaemia. *British Journal of Cancer*, *114*(6), 605–611.

Chadha, S., Wang, L., Hancock, W. W., & Beier, U. H. (2019). Sirtuin-1 in immunotherapy: A Janus-headed target. *Journal of Leukocyte Biology*, *106*(2), 337–343.

Chang, H. C., & Guarente, L. (2014). SIRT1 and other sirtuins in metabolism. *Trends in Endocrinology and Metabolism*, *25*(3), 138–145.

Chen, C. S., Wang, Y. C., Yang, H. C., Huang, P. H., Kulp, S. K., Yang, C. C., Lu, Y. S., Matsuyama, S., Chen, C. Y., & Chen, C. S. (2007). Histone deacetylase inhibitors sensitize prostate cancer cells to agents that produce DNA double-strand breaks by targeting Ku70 acetylation. *Cancer Research*, *67*(11), 5318–5327.

Chen, D., & Guarente, L. (2007). SIR2: A potential target for calorie restriction mimetics. *Trends in Molecular Medicine*, *13*(2), 64–71.

Chen, H., Xie, C., Chen, Q., & Zhuang, S. (2022). HDAC11, an emerging therapeutic target for metabolic disorders. *Frontiers in Endocrinology*, *20*(13), 989305.

Chen, Y., Zhou, D., Feng, Y., Li, B., Cui, Y., Chen, G., & Li, N. (2022). Association of sirtuins (SIRT1-7) with lung and intestinal diseases. *Molecular and Cellular Biochemistry*, *477*(11), 2539–2552.

Cheng, F., Zheng, B., Wang, J., Zhao, G., Yao, Z., Niu, Z., & He, W. (2021). Histone deacetylase 10, a potential epigenetic target for therapy. *Bioscience Reports*, *41*(6), BSR20210462.

Das, S., & Natarajan, R. (2020). HDAC9: An inflammatory link in atherosclerosis. *Circulation Research*, *127*(6), 824–826.

De Souza, C., & Chatterji, B. P. (2015). HDAC inhibitors as novel anti-cancer therapeutics. *Recent Patents on Anti-Cancer Drug Discovery*, *10*(2), 145–162.

de Ruijter, A. J., van Gennip, A. H., Caron, H. N., Kemp, S., & van Kuilenburg, A. B. (2003). Histone deacetylases (HDACs): Characterization of the classical HDAC family. *The Biochemical Journal*, *370*(Pt 3), 737–749.

DesJarlais, R., & Tummino, P. J. (2016). Role of histone-modifying enzymes and their complexes in regulation of chromatin biology. *Biochemistry*, *55*(11), 1584–1599.

Di Emidio, G., Falone, S., Artini, P. G., Amicarelli, F., D'Alessandro, A. M., & Tatone, C. (2021). Mitochondrial Sirtuins in reproduction. *Antioxidants (Basel)*, *10*(7), 1047.

Dong, M., Ning, Z. Q., Xing, P. Y., Xu, J. L., Cao, H. X., Dou, G. F., Meng, Z. Y., Shi, Y. K., Lu, X. P., & Feng, F. Y. (2012). Phase I study of chidamide (CS055/HBI-8000), a new histone deacetylase inhibitor, in patients with advanced solid tumors and lymphomas. *Cancer Chemotherapy and Pharmacology*, *69*(6), 1413–1422.

Dong, X. C. (2023). Sirtuin 6-a key regulator of hepatic lipid metabolism and liver health. *Cell*, *12*(4), 633.

Drummond, D. C., Noble, C. O., Kirpotin, D. B., Guo, Z., Scott, G. K., & Benz, C. C. (2005). Clinical development of histone deacetylase inhibitors as anticancer agents. *Annual Review of Pharmacology and Toxicology*, *45*, 495–528.

English, K., & Barton, M. C. (2021). HDAC6: A key link between mitochondria and development of peripheral neuropathy. *Frontiers in Molecular Neuroscience*, *31*(14), 684714.

Feldman, J. L., Dittenhafer-Reed, K. E., & Denu, J. M. (2012). Sirtuin catalysis and regulation. *Journal of Biological Chemistry*, *287*(51), 42419–42427.

Finnin, M. S., Donigian, J. R., Cohen, A., Richon, V. M., Rifkind, R. A., Marks, P. A., Breslow, R., & Pavletich, N. P. (1999). Structures of a histone deacetylase homologue bound to the TSA and SAHA inhibitors. *Nature*, *401*(6749), 188–193.

Furumai, R., Matsuyama, A., Kobashi, N., Lee, K. H., Nishiyama, M., Nakajima, H., Tanaka, A., Komatsu, Y., Nishino, N., Yoshida, M., & Horinouchi, S. (2002). FK228 (Depsipeptide) as a natural prodrug that inhibits class I histone deacetylases. *Cancer Research*, *62*(17), 4916–4921.

Gasparrini, M., Mazzola, F., Cuccioloni, M., Sorci, L., Audrito, V., Zamporlini, F., Fortunato, C., Amici, A., Cianci, M., Deaglio, S., Angeletti, M., & Raffaelli, N. (2022). Molecular insights into the interaction between human nicotinamide phosphoribosyltransferase and toll-like receptor 4. *The Journal of Biological Chemistry*, *298*(3), 101669.

Giannini, G., Cabri, W., Fattorusso, C., & Rodriquez, M. (2012). Histone deacetylase inhibitors in the treatment of cancer: Overview and perspectives. *Future Medicinal Chemistry*, *4*(11), 1439–1460.

Gil, R. S., & Vagnarelli, P. (2019). Protein phosphatases in chromatin structure and function. *Biochimica et biophysica acta. Molecular Cell Research*, *1866*(1), 90–101.

Gilardini, M. S., Granato, M., Santoni, C., Del Porto, P., Merendino, N., DOrazi, G., Faggioni, A., & Cirone, M. (2017). Histone deacetylase inhibitors VPA and TSA induce apoptosis and autophagy in pancreatic cancer cells. *Cellular Oncology (Dordrecht)*, *40*(2), 167–180.

Haigis, M. C., & Sinclair, D. A. (2010). Mammalian sirtuins: Biological insights and disease relevance. *Annual Review of Pathology*, *5*, 253–295.

Heers, H., Stanislaw, J., Harrelson, J., & Lee, M. W. (2018). Valproic acid as an adjunctive therapeutic agent for the treatment of breast cancer. *European Journal of Pharmacology*, *15*(835), 61–74.

Herp, D., Ridinger, J., Robaa, D., Shinsky, S. A., Schmidtkunz, K., Yesiloglu, T. Z., Bayer, T., Steimbach, R. R., Herbst-Gervasoni, C. J., Merz, A., Romier, C., Sehr, P., Gunkel, N., Miller, A. K., Christianson, D. W., Oehme, I., Sippl, W., & Jung, M. (2022). First fluorescent Acetylspermidine Deacetylation assay for HDAC10 identifies selective inhibitors with cellular target engagement. *Chembiochem*, *23*(14), e202200180.

Hess, L., Moos, V., Lauber, A. A., Reiter, W., Schuster, M., Hartl, N., Lackner, D., Boenke, T., Koren, A., Guzzardo, P. M., Gundacker, B., Riegler, A., Vician, P., Miccolo, C., Leiter, S., Chandrasekharan, M. B., Vcelkova, T., Tanzer, A., Jun, J. Q., ... Seiser, C. (2022). A toolbox for class I HDACs reveals isoform specific roles in gene regulation and protein acetylation. *PLoS Genetics*, *18*(8), e1010376.

Hsu, K. C., Liu, C. Y., Lin, T. E., Hsieh, J. H., Sung, T. Y., Tseng, H. J., Yang, J. M., & Huang, W. J. (2017). Novel class IIa-selective histone deacetylase inhibitors discovered using an in Silico virtual screening approach. *Scientific Reports*, *7*(1), 3228.

Johnstone, R. W., Ruefli, A. A., & Lowe, S. W. (2002). Apoptosis: A link between cancer genetics and chemotherapy. *Cell*, *108*(2), 153–164.

Karra, A. G., Sioutopoulou, A., Gorgogietas, V., Samiotaki, M., Panayotou, G., & Psarra, A. G. (2022). Proteomic analysis of the mitochondrial glucocorticoid receptor interacting proteins reveals pyruvate dehydrogenase and mitochondrial 60 kDa heat shock protein as potent binding partners. *Journal of Proteomics*, *257*, 104509.

Kaur, S., Rajoria, P., & Chopra, M. (2022). HDAC6: A unique HDAC family member as a cancer target. *Cellular Oncology (Dordrecht)*, *45*(5), 779–829.

Kee, H. J., Kim, I., & Jeong, M. H. (2022). Zinc-dependent histone deacetylases: Potential therapeutic targets for arterial hypertension. *Biochemical Pharmacology*, *202*, 115111.

Kelly, W. K., O'Connor, O. A., Krug, L. M., Chiao, J. H., Heaney, M., Curley, T., MacGregore-Cortelli, B., Tong, W., Secrist, J. P., Schwartz, L., Richardson, S., Chu, E., Olgac, S., Marks, P. A., Scher, H., & Richon, V. M. (2005). Phase I study of an oral histone deacetylase inhibitor, suberoylanilide hydroxamic acid, in patients with advanced cancer. *Journal of Clinical Oncology*, *23*(17), 3923–3931.

Khorasanizadeh, S. (2004). The nucleosome: From genomic organization to genomic regulation. *Cell*, *116*(2), 259–272.

Kida, Y., & Goligorsky, M. S. (2016). Sirtuins, cell senescence, and vascular aging. *The Canadian Journal of Cardiology*, *32*(5), 634–641.

Kim, J. Y., Cho, H., Yoo, J., Kim, G. W., Jeon, Y. H., Lee, S. W., & Kwon, S. H. (2022). Pathological role of HDAC8: Cancer and beyond. *Cell*, *11*(19), 3161.

Kumar, V., Kundu, S., Singh, A., & Singh, S. (2022). Understanding the role of histone deacetylase and their inhibitors in neurodegenerative disorders: Current targets and future perspective. *Current Neuropharmacology*, *20*(1), 158–178.

Lakshmaiah, K. C., Jacob, L. A., Aparna, S., Lokanatha, D., & Saldanha, S. C. (2014). Epigenetic therapy of cancer with histone deacetylase inhibitors. *Journal of Cancer Research and Therapeutics*, *10*(3), 469–478.

Landry, J., Slama, J. T., & Sternglanz, R. (2000). Role of NAD (+) in the deacetylase activity of the SIR2-like proteins. *Biochemical and Biophysical Research Communications*, *278*(3), 685–690.

Lauffer, B., Mintzer, R., Fong, R., Mukund, S., Tam, C., Zilberleyb, I., Flicke, B., Ritscher, A., Fedorowicz, G., Vallero, R., Ortwine, D. F., Gunzner, J., Modrusan, Z., Neumann, L., Koth, C. M., Lupardus, P. J., Kaminker, J. S., Heise, C. E., & Steiner, P. (2013). Histone deacetylase (HDAC) inhibitor kinetic rate constants correlate with cellular histone acetylation but not transcription and cell viability. *Journal of Biological Chemistry*, *288*(37), 26926–26943.

Lee, J. H., Choy, M. L., Ngo, L., Foster, S. S., & Marks, P. A. (2010). Histone deacetylase inhibitor induces DNA damage, which normal but not transformed cells can repair. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(33), 14639–14644.

Li, J., Lu, L., Liu, L., Ren, X., Chen, J., Yin, X., Xiao, Y., Li, J., Wei, G., Huang, H., Wei, W., & Wong, J. (2023). HDAC1/2/3 are major histone desuccinylases critical for promoter desuccinylation. *Cell Discovery*, *9*(1), 85.

Li, Y., & Seto, E. (2016). HDACs and HDAC inhibitors in cancer development and therapy. *Cold Spring Harbor Perspectives in Medicine*, *6*(10), a026831.

Li, Z., Fang, P., Duan, P., Chen, J., Fang, L., & Xiao, S. (2022). Porcine Deltacoronavirus infection cleaves HDAC2 to attenuate its antiviral activity. *Journal of Virology*, *96*(16), e0102722.

Li, Z., & Zhu, W. G. (2014). Targeting histone deacetylases for cancer therapy: From molecular mechanisms to clinical implications. *International Journal of Biological Sciences*, *10*(7), 757–770.

Liu, L., Dong, L., Bourguet, E., & Fairlie, D. P. (2021). Targeting class IIa HDACs: Insights from phenotypes and inhibitors. *Current Medicinal Chemistry*, *28*(42), 8628–8672.

Liu, P., Xiao, J., Wang, Y., Song, X., Huang, L., Ren, Z., Kitazato, K., & Wang, Y. (2021). Posttranslational modification and beyond: Interplay between histone deacetylase 6 and heat-shock protein 90. *Molecular Medicine*, *27*(1), 110.

Liu, S. S., Wu, F., Jin, Y. M., Chang, W. Q., & Xu, T. M. (2020). HDAC11: A rising star in epigenetics. *Biomedicine & Pharmacotherapy*, *131*, 110607.

Liu, Y., Tong, X., Hu, W., & Chen, D. (2023). HDAC11: A novel target for improved cancer therapy. *Biomedicine & Pharmacotherapy*, *31*(166), 115418.

LoPresti, P. (2020). HDAC6 in diseases of cognition and of neurons. *Cell*, *10*(1), 12.

Losson, H., Schnekenburger, M., Dicato, M., & Diederich, M. (2020). HDAC6-an emerging target against chronic myeloid leukemia? *Cancers*, *12*(2), 318.

Lucatelli, P., De, R., Ungania, S., Rocco, B., De Gyurgyokai, S. Z., Masi, M., Pecorella, I., Cappelli, F., Lai, Q., Catalano, C., & Vallati, G. (2022). Vivo comparison of micro-balloon interventions (MBI) advantage: A retrospective cohort study of DEB-TACE versus b-TACE and of SIRT versus b-SIRT. *Cardiovascular and Interventional Radiology*, *45*(3), 306–314.

Ma, P., Pan, H., Montgomery, R. L., Olson, E. N., & Schultz, R. M. (2012). Compensatory functions of histone deacetylase 1 (HDAC1) and HDAC2 regulate transcription and apoptosis during mouse oocyte development. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(8), E481–E489.

Mann, B. S., Johnson, J. R., Cohen, M. H., Justice, R., & Pazdur, R. (2007). FDA approval summary: Vorinostat for treatment of advanced primary cutaneous T-cell lymphoma. *The Oncologist*, *12*, 12–1252.

McClure, J. J., Li, X., & Chou, C. J. (2018). Advances and challenges of HDAC inhibitors in cancer therapeutics. *Advances in Cancer Research*, *138*, 183–211.

Micelli, C., & Rastelli, G. (2015). Histone deacetylases: Structural determinants of inhibitor selectivity. *Drug Discovery Today*, *20*(6), 718–735.

Michan, S., & Sinclair, D. (2007). Sirtuins in mammals: Insights into their biological function. *The Biochemical Journal*, *404*(1), 1–13.

Millard, C. J., Watson, P. J., Celardo, I., Gordiyenko, Y., Cowley, S. M., Robinson, C. V., Fairall, L., & Schwabe, J. W. (2013). Class I HDACs share a common mechanism of regulation by inositol phosphates. *Molecular Cell*, *51*(1), 57–67.

Miller, T. A., Witter, D. J., & Belvedere, S. (2003). Histone deacetylase inhibitors. *Journal of Medicinal Chemistry*, *46*(24), 5097–5116.

Minucci, S., & Pelicci, P. G. (2006). Histone deacetylase inhibitors and the promise of epigenetic (and more) treatments for cancer. *Nature Reviews Cancer*, *6*(1), 38–51.

Miyake, Y., Keusch, J. J., Wang, L., Saito, M., Hess, D., Wang, X., Melancon, B. J., Helquist, P., Gut, H., & Matthias, P. (2016). Structural insights into HDAC6 tubulin deacetylation and its selective inhibition. *Nature Chemical Biology*, *12*(9), 748–754.

Mohammed, M., Chandrasekar, M. J. N., Jeyapal, G. P., & Nanjan, M. J. (2016). Inhibitors of histone deacetylase as antitumor agents: A critical review. *Bioorganic Chemistry*, *67*, 18–42.

Morigi, M., Perico, L., & Benigni, A. (2018). Sirtuins in renal health and disease. *JASN*, *29*(7), 1799–1809.

Morse, J. S., Sheng, Y. J., Hampton, J. T., Sylvain, L. D., Das, S., Alugubelli, Y. R., Chen, P. C., Yang, K. S., Xu, S., Fierke, C. A., & Liu, W. R. (2022). Phage-assisted, active site-directed ligand evolution of a potent and selective histone deacetylase 8 inhibitor. *Protein Science*, *31*(12), e4512.

Mottamal, M., Zheng, S., Huang, T. L., & Wang, G. (2015). Histone deacetylase inhibitors in clinical studies as templates for new anticancer agents. *Molecules*, *20*(3), 3898–3941.

Mrakovcic, M., Kleinheinz, J., & Fröhlich, L. F. (2017). Histone deacetylase inhibitor-induced autophagy in tumor cells: Implications for p53. *International Journal of Molecular Sciences*, *18*(9), 1883.

Nalawansha, D., & Pflum, M. (2017). Lsdi substrate binding and gene expression are affected by hdac1-mediated deacetylation. *ACS Chemical Biology*, *12*(1), 254–264.

Ni, M., Esposito, E., Raj, V. P., Muzi, L., Zunino, F., Zuco, V., Cominetti, D., Penco, S., & Dal Pozzo, A. (2015). New macrocyclic analogs of the natural histone deacetylase inhibitor FK228; design, synthesis and preliminary biological evaluation. *Bioorganic & Medicinal Chemistry*, *23*, 6785–6793.

Núñez-Álvarez, Y., & Suelves, M. (2022). HDAC11: A multifaceted histone deacetylase with proficient fatty deacylase activity and its roles in physiological processes. *The FEBS Journal*, *289*(10), 2771–2792.

O'Connor, O. A., Heaney, M. L., Schwartz, L., Richardson, S., Willim, R., MacGregor-Cortelli, B., Curly, T., Moskowitz, C., Portlock, C., Horwitz, S., Zelenetz, A. D., Frankel, S., Richon, V., Marks, P., & Kelly, W. K. (2006). Clinical experience with intravenous and oral formulations of the novel histone deacetylase inhibitor suberoylanilide hydroxamic acid in patients with advanced hematologic malignancies. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology*, *24*(1), 166–173.

Pande, S., & Raisuddin, S. (2022). Molecular and cellular regulatory roles of sirtuin protein. *Critical Reviews in Food Science and Nutrition*, 1–19.

Park, S. Y., Kim, G. S., Hwang, H. J., Nam, T. H., Park, H. S., Song, J., Jang, T. H., Lee, Y. C., & Kim, J. S. (2018). Structural basis of the specific interaction of SMRT corepressor with histone deacetylase 4. *Nucleic Acids Research*, *46*(22), 11776–11788.

Patel, P., Wahan, S. K., Vishakha, S., Kurmi, B. D., Gupta, G. D., Rajak, H., & Asati, V. (2022). Recent Progress in histone deacetylase (HDAC) 1 inhibitors as anticancer agent. *Current Cancer Drug Targets*, *3*(1), 47–70.

Pflum, M. K., Tong, J. K., Lane, W. S., & Schreiber, S. L. (2001). Histone deacetylase I phosphorylation promotes enzymatic activity and complex formation. *Journal of Biological Chemistry*, *276*(50), 47733–47741.

Pillus, L., & Rine, J. (2004). SIR1 and the origin of epigenetic states in Saccharomyces cerevisiae. *Cold Spring Harbor Symposia on Quantitative Biology*, *69*, 259–265.

Pojani, E., & Barlocco, D. (2021). Romidepsin (FK228), a histone deacetylase inhibitor and its analogues in cancer chemotherapy. *Current Medicinal Chemistry*, *8*(7), 1290–1303.

Rajak, H., Singh, A., Raghuwanshi, K., Kumar, R., Dewangan, P. K., Veerasamy, R., Sharma, P. C., Dixit, A., & Mishra, P. (2014). A structural insight into hydroxamic acid based histone deacetylase inhibitors for the presence of anticancer activity. *Current Medicinal Chemistry*, *21*(23), 2642–2664.

Ramadhani, D., Maharani, R., Gazzali, A. M., & Muchtaridi, M. (2022). Cyclic peptides for the treatment of cancers: A review. *Molecules*, *27*(14), 4428.

Ramaiah, M. J., Tangutur, A. D., & Manyam, R. R. (2021). Epigenetic modulation and understanding of HDAC inhibitors in cancer therapy. *Life Sciences*, *277*(16), 119504.

Ren, J., Zhang, J., Cai, H., Li, Y., Zhang, Y., Zhang, X., Zhao, D., Li, Z., Ma, H., Wang, J., Gao, Y. E., Xiao, L., Liu, R., Qian, J., Liu, Y., Wei, H., & Li, J. (2014). HDAC as a therapeutic target for treatment of endometrial cancers. *Current Pharmaceutical Design*, *20*(11), 1847–1856.

Rodríguez-Paredes, M., & Esteller, M. (2011). Cancer epigenetics reaches mainstream oncology. *Nature Medicine*, *17*, 330–339.

Sacks, F. M., Lichtenstein, A., Van Horn, L., Harris, W., Kris-Etherton, P., Winston, M., & American Heart Association Nutrition Committee. (2006). Soy protein, isoflavones, and cardiovascular health: An American Heart Association Science Advisory for professionals from the nutrition committee. *Circulation*, *113*, 2943–2946.

Sacks, F. M., Lichtenstein, A., Van Horn, L., Harris, W., Kris-Etherton, P., & Winston, M. (2006). Soy protein, Isoflavones, and cardiovascular health: A summary of a Statement for professionals from the American Heart Association nutrition committee. *Arteriosclerosis Thrombosis and Vascular Biology*, *26*(8), 1689–1692.

Sanaei, M., & Kavoosi, F. (2019). Histone deacetylases and histone deacetylase inhibitors: Molecular mechanisms of action in various cancers. *Advanced Biomedical Research*, *8*, 63.

Sarkar, R., Banerjee, S., Amin, S. A., Adhikari, N., & Jha, T. (2020). Histone deacetylase 3 (HDAC3) inhibitors as anticancer agents: A review. *European Journal of Medicinal Chemistry*, *15*(192), 112171.

Sauve, A. A. (2010). Sirtuin chemical mechanisms. *Biochimica et Biophysica Acta-Proteins and Proteomics*, *1804*(8), 1591–1603.

Segré, C. V., & Chiocca, S. (2011). Regulating the regulators: The post-translational code of class I HDAC1 and HDAC2. *Journal of Biomedicine & Biotechnology*, *2011*, 690848.

Seidel, C., Schnekenburger, M., Mazumder, A., Teiten, M. H., Kirsch, G., Dicato, M., & Diederich, M. (2016). 4-Hydroxybenzoic acid derivatives as HDAC6-specific inhibitors modulating microtubular structure and HSP90$\alpha$ chaperone activity against prostate cancer. *Biochemical Pharmacology*, *99*, 31–52.

Sengupta, N., & Seto, E. (2004). Regulation of histone deacetylase activities. *Journal of Cellular Biochemistry*, *93*(1), 57–67.

Seto, E., & Yoshida, M. (2014). Erasers of histone acetylation: The histone deacetylase enzymes. *Cold Spring Harbor Perspectives in Biology*, *6*(4), a018713.

Shi, X. Y., Ding, W., Li, T. Q., Zhang, Y. X., & Zhao, S. C. (2017). Histone deacetylase (HDAC) inhibitor, Suberoylanilide Hydroxamic acid (SAHA), induces apoptosis in prostate cancer cell lines via the Akt/FOXO3a signaling pathway. *Medical Science Monitor International Medical Journal of Experimental & Clinical Research*, *23*, 5793–5802.

Simó-Mirabet, P., Bermejo-Nogales, A., Calduch-Giner, J. A., & Pérez-Sánchez, J. (2017). Tissue-specific gene expression and fasting regulation of sirtuin family in gilthead sea bream (Sparus aurata). *Journal of Comparative Physiology. B*, *187*(1), 153–163.

Somoza, J. R., Skene, R. J., Katz, B. A., Mol, C., Ho, J. D., Jennings, A. J., Luong, C., Arvai, A., Buggy, J. J., Chi, E., Tang, J., Sang, B. C., Verner, E., Wynands, R., Leahy, E. M., Dougan, D. R., Snell, G., Navre, M., Knuth, M. W., ... Tari, L. W. (2004). Structural snapshots of human HDAC8 provide insights into the class I histone deacetylases. *Structure*, *12*(7), 1325–1334.

Suraweera, A., O'Byrne, K. J., & Richard, D. J. (2018). Combination therapy with histone deacetylase inhibitors (HDACi) for the treatment of cancer: Achieving the full therapeutic potential of HDACi. *Frontiers in Oncology*, *29*(8), 92.

Tasneem, S., Alam, M. M., Amir, M., Akhter, M., Parvez, S., Verma, G., Nainwal, L. M., Equbal, A., Anwer, T., & Shaquiquzzaman, M. (2022). Heterocyclic moieties as HDAC inhibitors: Role

in cancer therapeutics. *Mini Reviews in Medicinal Chemistry*, *22*(12), 1648–1706.

Tulsyan, S., Aftab, M., Sisodiya, S., Khan, A., Chikara, A., Tanwar, P., & Hussain, S. (2022). Molecular basis of epigenetic regulation in cancer diagnosis and treatment. *Frontiers in Genetics*, *24*(8), 10–3389.

Vannini, A., Volpari, C., Filocamo, G., Casavola, E. C., Brunetti, M., Renzoni, D., Chakravarty, P., Paolini, C., De Francesco, R., Gallinari, P., Steinkühler, C., & Di Marco, S. (2004). Crystal structure of a eukaryotic zinc-dependent histone deacetylase, human HDAC8, complexed with a hydroxamic acid inhibitor. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(42), 15064–15069.

Verdin, E., Dequiedt, F., & Kasler, H. (2004). HDAC7 regulates apoptosis in developing thymocytes. *Novartis Foundation Symposium*, *259*, 115–129. discussion 129–31, 163–9.

Villalba, J. M., & Alcaín, F. J. (2012). Sirtuin activators and inhibitors. *BioFactors*, *38*(5), 349–359.

Wanczyk, M., Roszczenko, K., Marcinkiewicz, K., Bojarczuk, K., Kowara, M., & Winiarska, M. (2011). HDACi–going through the mechanisms. *Front Biosci (Landmark Ed)*, *16*(1), 340–359.

Wang, R., Xin, M., Li, Y., Zhang, P., & Zhang, M. (2016). The functions of histone modification enzymes in cancer. *Current Protein & Peptide Science*, *17*(5), 438–445.

Wang, S., Han, S., Cheng, W., Miao, R., Li, S., Tian, X., & Kan, Q. (2022). Design, synthesis, and biological evaluation of 2-Anilino-4-Triazolpyrimidine derivatives as CDK4/HDACs inhibitors. *Drug Design, Development and Therapy*, *16*, 1083–1097.

Wątroba, M., Dudek, I., Skoda, M., Stangret, A., Rzodkiewicz, P., & Szukiewicz, D. (2017). Sirtuins, epigenetics and longevity. *Ageing Research Reviews*, *0*, 11–19.

Watson, P. J., Fairall, L., Santos, G. M., & Schwabe, J. W. (2012). Structure of HDAC3 bound to co-repressor and inositol tetraphosphate. *Nature*, *481*(7381), 335–340.

Westbrook, J. D., Young, J. Y., Shao, C., Feng, Z., Guranovic, V., Lawson, C. L., Vallat, B., Adams, P. D., Berrisford, J. M., Bricogne, G., Diederichs, K., Joosten, R. P., Keller, P., Moriarty, N. W., Sobolev, O. V., Velankar, S., Vonrhein, C., Waterman, D. G., Kurisu, G., … Peisach, E. (2022). PDBx/mmCIF ecosystem: Foundational semantic tools for structural biology. *Journal of Molecular Biology*, *434*(11), 167599.

Witt, O., Deubzer, H. E., Milde, T., & Oehme, I. (2009). HDAC family: What are the cancer relevant targets? *Cancer Letters*, *277*(1), 8–21.

Wright, L. H., & Menick, D. R. (2016). A class of their own: Exploring the nondeacetylase roles of class IIa HDACs in cardiovascular disease. *American Journal of Physiology. Heart and Circulatory Physiology*, *311*(1), H199–H206.

Wu, Q. J., Zhang, T. N., Chen, H. H., Yu, X. F., Lv, J. L., Liu, Y. Y., Liu, Y. S., Zheng, G., Zhao, J. Q., Wei, Y. F., Guo, J. Y., Liu, F. H., Chang, Q., Zhang, Y. X., Liu, C. G., & Zhao, Y. H. (2022). The sirtuin family in health and disease. *Signal Transduction and Targeted Therapy*, *7*(1), 402.

Wu, R., Lu, Z., Cao, Z., & Zhang, Y. (2011). Zinc chelation with hydroxamate in histone deacetylases modulated by water access to the linker binding channel. *Journal of the American Chemical Society*, *133*(16), 6110–6113.

Wu, R. B., Wang, S. L., Zhou, N. J., Cao, Z., & Zhang, Y. (2010). A proton-shuttle reaction mechanism for histone deacetylase 8 and the catalytic role of metal ions. *Journal of the American Chemical Society*, *132*(27), 9471–9479.

Wu, Z., Jing, S., Li, Y., Gao, Y., Yu, S., Li, Z., Zhao, Y., Piao, J., Ma, S., & Chen, X. (2017). The effects of SAHA on radiosensitivity in pancreatic cancer cells by inducing apoptosis and targeting RAD51. *Biomedicine & Pharmacotherapy*, *89*, 705–710.

Yang, F., Zhao, N., Hu, Y., Jiang, C. S., & Zhang, H. (2020). The development process: From SAHA to Hydroxamate HDAC inhibitors with branched CAP region and linear linker. *Chemistry & Biodiversity*, *17*(1), e1900427.

Yang, W. M., Tsai, S. C., Wen, Y. D., Fejer, G., & Seto, E. (2002). Functional domains of histone deacetylase-3. *Journal of Biological Chemistry*, *277*(11), 9447–9454.

Yang, X. J., & Seto, E. (2008). The Rod3/Hal family of lysine deacetylases: From bacteria and yeast to mice and men. *Nature Reviews Molecular Cell Biology*, *9*(3), 206–218.

Yanginlar, C., & Logie, C. (2018). HDAC11 is a regulator of diverse immune functions. *Biochimica et biophysica acta. Gene Regulatory Mechanisms*, *1861*(1), 54–59.

Yoon, S., & Eom, G. H. (2016). HDAC and HDAC inhibitor: From cancer to cardiovascular diseases. *Chonnam Medical Journal*, *2*(1), 1–11.

Yoshida, M., Furumai, R., Nishiyama, M., Komatsu, Y., Nishino, N., & Horinouchi, S. (2001). Histone deacetylase as a new target for cancer chemotherapy. *Cancer Chemotherapy and Pharmacology*, *48*(Suppl 1), S20–S26.

Yue, K., Qin, M., Huang, C., James, C. C., Jiang, Y., & Li, X. (2022). Comparison of three zinc binding groups for HDAC inhibitors–a potency, selectivity and enzymatic kinetics study. *Bioorganic & Medicinal Chemistry Letters*, *70*, 128797.

Zhang, C., Wang, X., Zhang, E., Yang, L., Yuan, H., Tu, W., Zhang, H., Yin, Z., Shen, W., Chen, X., Zhang, Y., & Ouyang, H. (2018). An epigenetic bioactive composite scaffold with well-aligned nanofibers for functional tendon tissue engineering. *Acta Biomaterialia*, *66*, 141–156.

Zhang, J., Xiang, H., Liu, J., Chen, Y., He, R. R., & Liu, B. (2020). Mitochondrial Sirtuin 3: New emerging biological function and therapeutic target. *Theranostics*, *10*(18), 8315–8342.

Zhang, L., Han, Y., Jiang, Q., Wang, C., Chen, X., Li, X., Xu, F., Jiang, Y., Wang, Q., & Xu, W. (2015). Trend of histone deacetylase inhibitors in cancer therapy: Isoform selectivity or multitargeted strategy. *Medicinal Research Reviews*, *35*(1), 63–84.

Zhang, Q., Tao, W., Geng, C., Zhang, Y., Zhang, J., Ning, Z., & Jiang, Z. (2018). Exploratory clinical study of chidamide, an oral subtype-selective histone deacetylase inhibitor, in combination with exemestane in hormone receptor-positive advanced breast cancer. *Chinese Journal of Cancer Research*, *30*(6), 605–612.

Zhang, Z., Hao, C., Wang, L., Liu, P., Zhao, L., Zhu, C., & Tian, X. (2013). Inhibition of leukemic cells by valproic acid, an HDAC inhibitor, in xenograft tumors. *Oncotargets and Therapy*, *6*, 733–740.

**How to cite this article:** Han, H., Feng, X., He, T., Wu, Y., He, T., Yue, Z., & Zhou, W. (2023). Discussion on structure classification and regulation function of histone deacetylase and their inhibitor. *Chemical Biology & Drug Design*, *00*, 1–19. https://doi.org/10.1111/cbdd.14366