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REVIEW

Targeting histone deacetylases for cancer therapy: Trends and challenges

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Abstract Dysregulation of histone deacetylases (HDACs) is closely related to tumor development and progression. As promising anticancer targets, HDACs have gained a great deal of research interests and two decades of effort has led to the approval of five HDAC inhibitors (HDACis). However, currently traditional HDACis, although effective in approved indications, exhibit severe off-target toxicities and low sensitivities against solid tumors, which have urged the development of next-generation of HDACi. This review investigates the biological functions of HDACs, the roles of HDACs in oncogenesis, the structural features of different HDAC isoforms, isoform-selective inhibitors, combination therapies, multitarget agents and HDAC PROTACs. We hope these data could inspire readers with new ideas to develop novel HDACi with good isoform selectivity, efficient anticancer effect, attenuated adverse effect and reduced drug resistance.

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1 Epigenetics and histone deacetylation

Epigenetics refers to a reversible or inheritable process that regulates gene expression without altering DNA nucleotide sequence[1]. There are several primary epigenetic mechanisms: DNA/RNA methylation, RNA editing, noncoding RNA-mediated regulation, histone variant, histone modification and chromatin remodeling[2−4]. Histone modification, especially acetylation/deacetylation on histone mediated by histone deacetylases (HDACs) and histone acetyltransferases (HATs), is a major epigenetic mechanism. This dynamic process confers unique structural properties to histones and nucleosomes, thus regulating gene expression[5,6].

As the subunit of eukaryotic chromatin, nucleosome consists of DNA coiled around a well-protected histone core[7]. HDACs and HATs modulate gene transcription by altering the structure of nucleosome. Briefly, HDACs deacetylate the ϵ-NH_2 group of lysine in nucleosome histone, which tauts the charged association of histone tails with DNA and prevents the binding of transcription co-factors to DNA, thereby repressing gene transcription. Conversely, HATs acetylate the ϵ-NH_2 group and relax the charged interaction, followed by greater accesses of transcription co-factors to DNA, thus promoting gene transcription (Fig. 1)[6,8,9].

Under physiological conditions, HDACs together with HATs regulate the dynamic equilibrium of deacetylation/acetylation on histones/nonhistone proteins and maintain gene expression, homeostasis and ontogeny. However, in pathological conditions, external stimuli or gene mutation may result in the overexpression of HDACs. Aberrant HDACs activity disrupts the balance of acetylation−deacetylation process and causes a more compact nucleosome structure, thus downregulating tumor suppressor genes, followed by tumorigenesis[10,11]. Aside from tumor, aberrant HDACs are also associated with a wide range of diseases (Table 1), which make HDACs promising targets.

2. The classification and function of HDACs

2.1. *Classification of HDACs*

According to the cofactors and primary homologous structures, 18 HDAC members phylogenetically fall into Zn^{2+}-dependent HDACs and NAD^+-dependent HDACs. Zn^{2+}-dependent HDACs are also known as classical HDACs (mainly containing class I, II, IV) and they catalyze the deacetylation of histones relying on zinc cation (Fig. 2). On the other hand, NAD^+-dependent enzymes refer to class III (Sirt 1−7), which employ NAD^+ as a cofactor[39]. Notably, Sirt 1−7 are structurally and evolutionarily unrelated to the classical HDACs and the term "HDACs" usually refers to classical HDACs.

Generally, class I HDACs share a large degree of similarities in structures as well as functions, and their superior reactivities confer them the major intracellular deacetylation functions[40]. Of note, class I HDACs are ubiquitously expressed by diverse cells, predominantly reside in the nucleus and participate in multiple biological events through their enzymic functions or formation of multiprotein corepressor complexes (Tables 2 and 3)[41]. In addition, many studies revealed that overexpression of class I HDACs is common in multiple malignancies and class I HDACs are closely involved in cancer progression[42].

Class IIa HDACs and class IIb HDACs share high homology sequences respectively, but the additional catalytic domain of HDAC6 leads to an overall low homology similarity of the class II HDACs[43]. Although class IIa HDACs share 57% homology with class I HDACs in the catalytic domains, their reactivities are significantly lower than that of class I HDACs[44]. Moreover, class II HDACs are located in cytoplasm and nucleus, where they can deacetylate histone proteins and nonhistone proteins[44,45].

As the sole member of class IV HDACs, HDAC11 is the smallest histone deacetylase, and the catalytic domain accounts for more than 80% of its sequence. HDAC11 was firstly characterized in 2002 and is somewhat more distantly related to other HDAC family members[39]. Interestingly, HDAC11 removes acetyl group from lysine with lower efficacy, but it is the most proficient fatty acid deacetylase in HDAC family[46].

2.2. *The biological functions of HDACs*

HDACs are able to participate in multiple biological processes by their deacetylase activities or forming protein complexes with various partners. As shown in Fig. 3, HDACs are involved in following biological cascades: (1) Gene transcription. HDACs

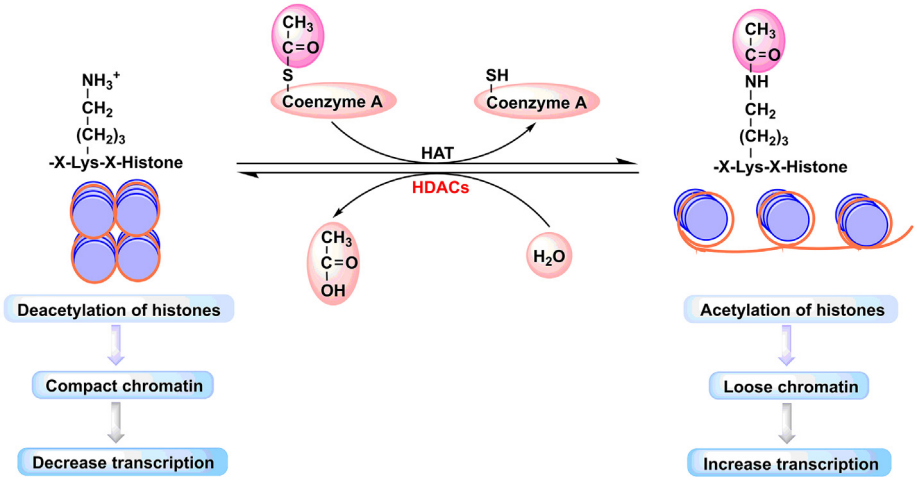


Figure 1 The regulation of gene transcription mediated by HDACs and HATs.

Table 1 Diseases involved in HDACs.

HDACs	Diseases	Ref.
HDAC1	Cancers, cardiovascular diseases, allergic diseases, memory and learning, neurodegenerative disorders, viral infections, osteoarthritis, allergic rhinitis.	12−17
HDAC2	Cancers, liver diseases, memory and learning, neurodegenerative disorders, viral infections, osteoarthritis, cognitive deficits, diabetes mellitus, airway diseases, chronic obstructive pulmonary disease, cardiac hypertrophy.	13−16,18−23
HDAC3	Cancers, obesity, diabetes mellitus, neurodegenerative disorders, rheumatoid arthritis, inflammations, cardiovascular diseases, memory and learning, osteoarthritis, allergic rhinitis, cognitive deficits, lung and kidney diseases.	16,17,20,24,25
HDAC8	Cancers, cornelia de lange syndrome (CdLS), X-linked disorders, infectious diseases, duchenne muscular dystrophy (DMD), cardiovascular diseases, liver fibrosis, pulmonary diseases, hepatic diseases, myopathy, renal fibrosis, neuroinflammation-mediated diseases.	26,27
HDAC4	Cognitive disorders, neurodegenerative disorders.	28,29
HDAC5	Vascular hypertrophy, diabetes mellitus, schizophrenia, neurodegenerative disorders.	20,30
HDAC7	Cancers, osteoarthritis, autoimmune diseases, inflammatory bowel diseases, systemic sclerosis, peyronie's diseases, type 2 diabetes mellitus, hepatic steatosis, liver fibrosis, primary sclerosing cholangitis, respiratory diseases, cystic fibrosis, acute lung injury, huntington's diseases.	31
HDAC9	Cerebrovascular diseases, osteoporosis, autoimmune diseases, cancers, obesity, liver fibrosis.	32
HDAC6	Cancers, neurodegenerative disorders, cardiovascular diseases, inflammatory diseases, autoimmune diseases, viral infections, cognitive deficits.	20,33−35
HDAC10	Cancers, schizophrenia, HIV infection, intracerebral hemorrhage and immunoglobulin A nephropathy.	36
HDAC11	Allergic rhinitis, neurological and mental disorders, metabolic disorders, cancers, age-related macular degeneration.	17,37,38

regulate transcription by altering the structure of nucleosome or the association of transcription factors (ETS, RUNX and SMAD7) to DNA[47−50]. (2) Protein stability. HDACs and HATs can affect protein stability (such as that of p53 and CDT1) since lysine residues are subjected to both acetylation and ubiquitylation[51,52]. (3) Protein translocation. When Ku70 (a DNA damage-associated protein) is acetylated by HATs, Bax protein is released from the "Ku70−Bax" complex, followed by Bax mitochondrial localization[53−55]. Moreover, reversible acetylation also influences STAT3 subcellular localization. (4) Protein−protein interaction. For example, acetylation could intervene in the p53−MDM2 interaction[56,57]. (5) Protein import and export. HDACs together with HATs modulate importin protein acetylation, thereby regulating the nuclear import and export of HMGB1 or transcription factors[58]. (6) Immunomodulation. Class I HDACs participate in innate immunity by regulating the production of inflammatory cytokines, while class IIa HDACs take part in specific immunity by influencing antigen presentation[59]. (7) Cell motility and migration. HDAC6 removes the acetyl group from its substrates (α-tubulin, HSP90 and cortactin), and low acetylation promotes

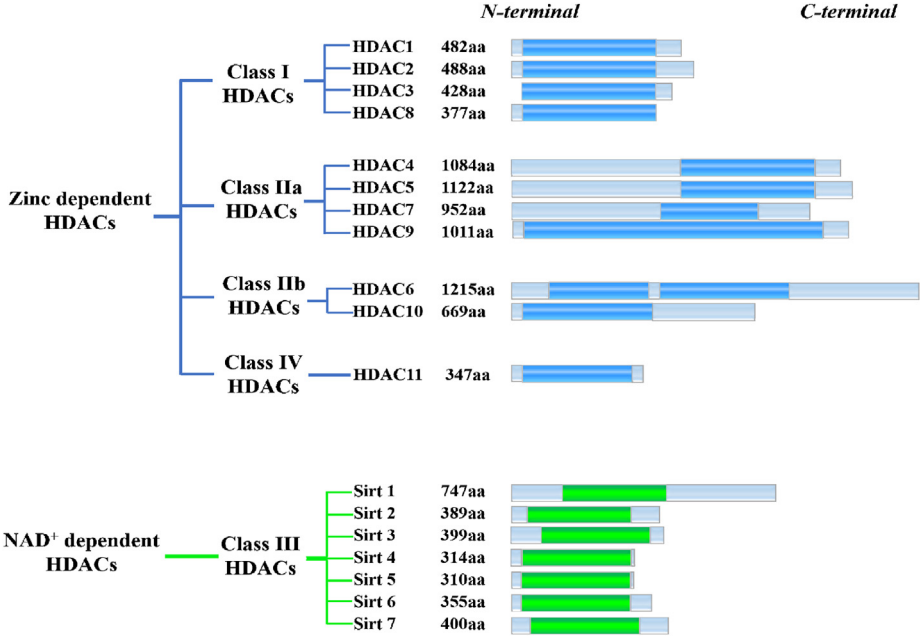


Figure 2 Schematic depiction of HDAC isoforms. Catalytic domains are shown as bright blue column (zinc-dependent HDACs) and green column (NAD^+-dependent HDACs).

Table 2 The key features of HDAC family members[25].

HDACs	Gene	Homology	Size (aa)	Catalytic domain[a]	Localization	Substrate
HDAC1	1p34	Yeast Rpd3	482	9−321	N[b]	Histones, YY1, p53, SHP, DNMT1, E2F-1, ER, AR, ATM, STAT3, pRb, GATA, MeCP2, MEF2, MyoD, NF-κB.
HDAC2	6q21	Yeast Rpd3	488	9−322	N	Histones, YY1, STAT3, BRCA1, pRb, GATA2, NF-κB, Bcl-6, HOP.
HDAC3	5q31	Yeast Rpd3	428	3−316	C/N	Histones, pRb, SHP, MEF2D, YY-1, STAT3, NF-κB, GATA1, RelA.
HDAC8	Xq13	Yeast Rpd3	377	14−324	C/N	Histones, SMC3, p53, ERRα, HSP70.
HDAC4	2q37.3	Yeast HDA1	1084	655−1084	C/N	Histones, p21, HP1, p53, HIF-1α, FOXO, GATA1, SRF, Runx 2, SUV39H1, GCMa.
HDAC5	17q21	Yeast HDA1	1122	684−1028	C/N	CaM, YY1, HP1, MEF2, GCMa, Runx 2, Smad 7.
HDAC7	12q13.1	Yeast HDA1	952	518−865	C/N	FLAG1, FLAG2.
HDAC9	7p21.1	Yeast HDA1	1011	631−978	C/N	Histones, CaM, MEF2, HIF-1α, PML, Runx 2.
HDAC6	Xp11.23		1215	87−404, 482−800	C[c]	α-Tubulin, Tau, SHP, HSP90, HSF-1, Runx 2, Smad 7, cortactin.
HDAC10	22q13.31		669	1−323	C	HSP90, LcoR.
HDAC11	3p25.1	Class I, II HDACs	347	14−326	N/C	Histones (longer chain acyllysine residues), Cdt 1.

[a]Catalytic domain: amino acid sequences.
[b]N: Nuclear.
[c]C: Cytoplasm.

cell motility and migration[60−62]. Moreover, HDACs can also increase the expression of MMPs which degrade the intact basement membrane and extracellular matrix, followed by migration and invasion[63,64]. (8) Degradation of misfolded protein. HDAC6 can recruit misfolded protein to the aggresome, followed by degradation *via* ubiquitin−proteasome system[65]. (9) Apoptosis. HDACs promote cell survival and evade apoptosis by modulating the intrinsic or extrinsic apoptosis pathway[66−69]. (10) Autophagy. Upon HDACs inhibition, autophagy is initiated by inactivating mTOR, upregulating ATG, Beclin-1 and LC3[70,71]. (11) Cell cycle. The protein−protein interaction of cell-cycle regulator (CCR) modulates cell cycle progression and HDACs are able to change the expression or function of CCR to regulate cell cycle progression[71]. (12) Angiogenesis. HDACs not only increase the expression of pro-angiogenesis factors including VEGF, HIF-1α and CXCR4, but also downregulate p53 and VHL protein, thus stimulating angiogenesis[72−74]. (13) DNA synthesis. HDACs inhibition represses the expression of thymidylate synthetase and CTP synthase, which reduces DNA synthesis[25]. (14) DNA repair. HDACs are required for the function or gene expression of DNA repair proteins, such as Ku70, Ku86, BRCA1, BRCA2 and RAD51[75−78].

In addition to abovementioned cascades, HDACs are able to complex with some proteins to achieve a number of biological functions. Table 3 illustrates the partners interacting with HDACs and related functions.

3. HDACs and cancer

3.1. *The expression of HDACs in different tumors*

A considerable number of studies have proven that HDAC overexpression is closely related to tumor progression and 13 out of 15 tumors (breast cancer, colon cancer, gastric cancer, kidney cancer, liver cancer, lung cancer, ovarian cancer, lymphoma, pancreatic cancer, prostate cancer, medulloblastoma, neuroblastoma and chronic lymphocytic leukemia) overexpress HDACs[47,81,82]. Accumulating evidence revealed that HDACs are required for tumor-related functions, such as cell growth/proliferation, cell differentiation, cell cycle, cell motility/migration, drug resistance, prognosis, angiogenesis, autophagy and apoptosis[83−86]. Given the overexpression and tumor-related functions, HDACs therefore have emerged as promising anti-cancer targets.

3.2. *HDACs in cancer pathogenesis*

HDACs play multiple roles in tumor progression (Fig. 4). In tumorigenesis, overexpressed HDACs transcriptionally silence tumor suppressor genes and promote tumorigenesis. In tumor development, overexpressed HDACs (1) induce angiogenesis *via* hypoxia inducible factor-1 (HIF-1) and provide nutrients for tumor growth and metabolism; (2) disrupt the cell cycle progression and promote the proliferation of tumor cells; (3) improve cell infiltration and increase the metastasis as well as invasion of tumor cells; (4) reduce the sensitivity of tumor cells to apoptosis signals or chemical drugs, resulting in apoptosis escape or drug resistance.

3.2.1. *HDACs in angiogenesis*

Hypoxia is a significant feature of the tumor microenvironment (TME) and tumor cells can induce angiogenesis in TME *via* HIF-1α. In malignant cells, the activity of HIF-1α is regulated by HDACs through several pathways. Functionally, acetylation at Lys-532 leads to the ubiquitin-dependent degradation of HIF-1α, whereas HDAC1 and HDAC4 are capable of inducing the deacetylation of HIF-1α and repressing ubiquitination and degradation processes[87,88]. Moreover, HDAC5 and HDAC6 promote the maturation and stabilization of HIF-1α by regulating the deacetylation of the chaperones HSP70 and HSP90 rather than directly regulating the acetylation of HIF-1α. Upon HDAC5 and HDAC6 inhibition, hyperacetylation of HSP70 and HSP90 renders

Table 3 Partners interacting with HDACs and their related functions[79,80].

HDAC isoform	Partners	Functions
HDAC1	Form complex with LSD1, CoREST 1 and HDAC2. Interact with HDAC2, NuRD, MTA1-3, RBBP4/7, CHD3-5, GATAD2A/B, CDK2AP1 and MBD2/3. Component of MiDAC complex including HDAC2, MIDEAS and DNTTIP1. Form a mSin3A multiprotein complex containing HDAC1-2, SUDS3/SAP45, ARID4B/SAP180, SIN3A and SAP130. Bind to APBB1 and TSHZ3. Form complex with HDAC2, RBBP4, 7.	It regulates stem cell fate and mediates cell plasticity in cancer and vascular diseases. It modulates cell cycle progression, chromatin remodeling and gene expression. It works with MTA2 to regulate cell growth and apoptosis *via* p53. It suppresses circadian genes expression.
HDAC2	Component of SMRT complex with HDAC3/4/7, SMRT, GPS2 and TBL1X. Found in a complex including HDAC1, HDAC2, RBBP4,7. Form a HDAC/KDM1A/GFI/RCOR complex. Form a BHC-HDAC heterodimer. Bind to unphosphorylated DAXX, DEK and histones. Interact with ATR and CHD4. Form mSin3A−HDAC complex. Form a HDAC1, 2, EHMT2 and TRIM28 complex. Bind to CDYL, MIER1 and MIER2.	It is involved in lipid metabolism, fibroblast cell cycle, circadian rhythms, macrophage polarization and inflammation. It interacts with YY1 to form the transcriptional repressor complexes. It is associated with gene expression and ontogeny. HDAC2 knockout causes aberrant transcription.
HDAC3	Forms a complex with YY1. Bind to NCOR1 and SMRT. Form N-CoR/HDAC3 co-repressor complex. Interacts with APEX1, BCL6, BCOR, BEND3, BTBD14B, CCAR2, DACH1, DAXX, GLIS2, HDAC7, HDAC9, HDAC10, INSM1, MAPK14, MEF2D, MJD2A/JHDM3A, NRIP1, NR2C1, PRDM6, SRY, SMRT/NCOR2, XBP1 isoform 1 and ZMYND15.	It may take part in the modulation of transcription by binding to YY1. HDAC3 is considered a potential tumor suppressor gene. HDAC3 can repress the function of p53, thereby regulating cell growth and apoptosis.
HDAC8	Interacts with alpha-SMA, CBFA2T3, PEPB2-MYH11 and phosphorylated SMG5/EST1B.	HDAC8 regulates the release of cohesion complexes from chromatin.
HDAC4	Interacts with 14-3-3 chaperone protein, AHRR, ANKRA, BTBD14B, EP300, HDAC7, KDM5B, MEF2C, MYOCD, MORC2 and NR2C1.	HDAC4 binds to DNA indirectly *via* MEF2C and MEF2D.
HDAC5	Interacts with HDAC7, HDAC9, 14-3-3 protein, AHRR, BAHD1, BCOR, CTBP1, EP300, GRK5, KDM5B, MEF2C, SMRT, NRIP1 and PHB2 at least.	HDAC5 binds to MEF2, leading to the repression of MEF2-dependent gene. It may form multicomplex with HDAC3. It is involved in tumor progression.
HDAC7	Interacts with the 14-3-3 protein, EDNRA, FOXP3, HDAC1, HDAC2, HDAC3, HDAC4, HDAC5, KAT5, KDM5B, MBD3, MEF2A, MEF2B, MEF2C, MTA1L1, NCOR1, NCOR2, PML, RBBP4, RBBP7, SIN3A, SIN3B, SAP30 YWHAE and ZMYND15.	In muscle differentiation, HDAC7 allows the expression of myocyte enhancer factors. Its gene encodes splicing transcript variants of different isoforms.
HDAC9	Homodimer. Interacts with the 14-3-3 protein, BCL6, CTBP1, ETV6, HDAC1, HDAC3, FOXP3, MEF2, MAPK10 and NCOR1.	HDAC9 inhibits the activity of MEF2 *via* the formation of CtBP−HDACs complexes. It participates in hematopoiesis, skeletal myogenesis inhibition and heart development. It prevents neuronal apoptosis.
HDAC6	Bind to LIMK1 and TPPP1. Form complex with APOBEC3G. Interact with FAT10.	It promotes drug resistance. It is involved in HIV-1 infection. It is required for misfolded protein degradation.
HDAC10	Bind to SMRT, HDAC2 and HDAC3.	It suppresses cell growth and also serves as prognostic factor. Regulates DNA mismatch repair and G2/M transition. HDAC10 upregulation may mediate autophagy and growth decline.
HDAC11	Interact with HDAC6.	HDAC11 participates in immune regulation *via* IL-10 expression. It also mediates the stability of CDT1 protein.

them a higher affinity for HIF-1α, which causes the attenuated nuclear accumulation and accelerated degradation of HIF-1α[89]. HDAC4, HDAC5 and HDAC7 increase the transcription of HIF-1α by triggering the association of HIF-1α with CBP/p300[87,90,91]. Aside from HIF-1α, HDAC9 hyperexpression results in endothelial cell germination and angiogenesis by blocking the antiangiogenic microRNA-17-92 cluster[92].

Angiogenesis has great significance for TME and HDACs are able to regulate angiogenesis through multiple pathways. Therefore, HDACs can be potential anti-angiogenesis targets in tumor therapy[93].

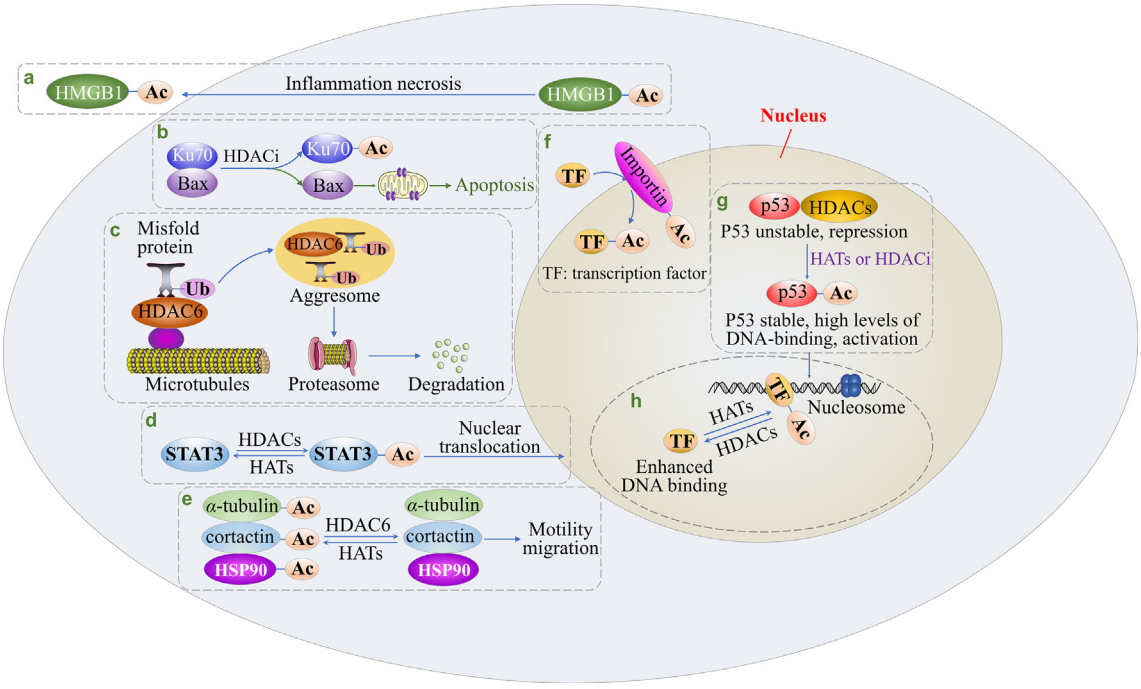


Figure 3 A schematic view of HDACs biological functions. (a) Nuclear export of HMGB1; (b) Bax translocation and the intrinsic apoptosis; (c) Degradation of misfolded protein; (d) STAT3 nuclear translocation; (e) Cell motility and migration; (f) The acetylation and function of importin; (g) p53 protein stability; (h) Association of transcription factors with DNA.

3.2.2. HDACs in cell cycle progression

Cell cycle is a complex and orchestrated cellular process, whose regulation involves in various cell-cycle regulators (CCRs), including cyclins, cyclin-dependent kinases (CDKs) and CDK inhibitors (CDKIs). In the network interactions of CCRs, the activities of CDKs are positively regulated by cyclins, but negatively modulated by CDKIs. The CIP/KIP family of CDKIs consist of $p21^{WAF1/CIP1}$, $p27^{KIP1}$ as well as p57, and HDACs can change their expression or function to regulate the cell cycle.

HDAC1 and HDAC2 are recruited to the promoters of $p21^{WAF1/CIP1}$, $p27^{KIP1}$ and $p57^{KIP2}$ genes and repress CDKIs (p21, p27 and p57), thereby shortening the cell cycle and enhancing unrestricted proliferation[94,95]. HDAC3 directly deacetylates cyclin A and regulate the stability of cyclin A. HDAC3 inhibition induces PCAF/GCN5-mediated acetylation of cyclin A and leads to proteasome-dependent degradation of cyclin A, followed by S and G2/M phases arrest[96]. Knockdown of HDAC5 upregulates the CDK inhibitory protein p21 and simultaneously downregulates

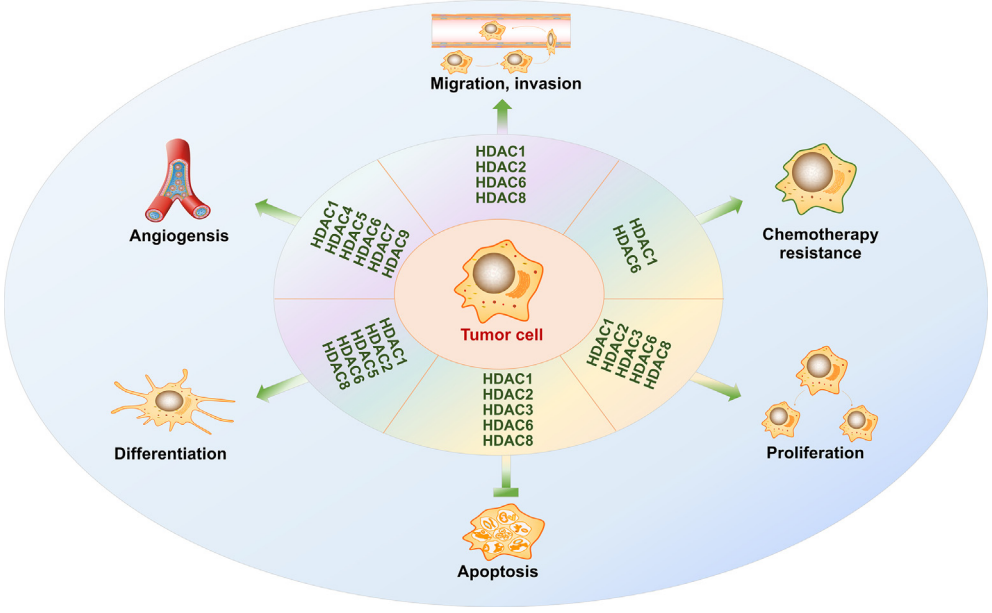


Figure 4 The role of HDACs in cancer pathogenesis.

CDK2/4/6 as well as cyclin D1, causing G1 phase arrest[97]. Moreover, HDAC10 regulates the G2/M transition *via* a pathway mediated by let-7/HMGA2/cyclin A2[98].

3.2.3. *HDACs in apoptosis*

HDACs inhibition can directly regulate apoptosis through intrinsic and extrinsic apoptosis pathways. In extrinsic apoptosis pathway, HDACs inhibition can prime malignancies for TRAIL-induced apoptosis by upregulating cell surface death receptors, reducing c-FLIP and enhancing the formation of death inducing signal complex (DISC)[99]. Moreover, HDACs inhibition also directly induces the activation of TNFS10 gene promoter, followed by TRAIL expression and apoptosis[99].

In intrinsic apoptotic pathways, the main characteristics of HDACs regulating apoptosis is elevated levels of antiapoptotic proteins and reduced expression of proapoptotic proteins (Fig. 5)[100,101]. For example, HDAC1 inhibition increases the ratio of Bax/Bcl-2[69]. Knockdown of HDAC2 selectively upregulates proapoptotic proteins, including Bax, Apaf-1 and AIF, while downregulating antiapoptotic protein Bcl-2[102]. Additionally, inhibition of HDAC3 upregulates the expression of pro-apoptotic protein PUMA by promoting the binding of p53 to its promoter[103]. Moreover, HDAC8 together with HDAC1 interacts with the Bcl-2-modifying factor (BMF) gene and establishes a pro-survival scenario *via* the repression of BMF protein expression[104]. Notably, the knockout of HDAC8 can effectively activate the transcription of BMF and promote BMF-mediated apoptosis[104]. As for HDAC6, it interferes Bax/Ku70 interaction. In the absence of HDAC6, Ku70 is acetylated by CREB-binding protein (CBP) and then Bax is released from "Bax−Ku70" complex, followed by Bax conformation activation and intrinsic apoptosis initiation[55].

3.2.4. *HDACs in cell metastasis and invasion*

Downregulation of E-cadherin is a significant feature of epithelial-mesenchymal transition (EMT), and HDACs play a pivotal role in this process. In prostate cancer cells, the transcriptional repressor complex containing Snail, HDAC1 and HDAC2, can mediate the silencing of E-cadherin and accelerate EMT process, causing cell metastasis and invasion[105].

On the other hand, an intact basement membrane and extracellular matrix are barriers that repress the metastasis and invasion of malignant cells. However, the upregulation of matrix metalloproteinases (MMPs) mediated by HDACs helps malignant cells to degrade and cross the barrier. For example, in breast cancer cells, overexpression of HDAC1, 6 and 8 increased cellular metastasis by upregulating the expression of MMP-9[63], while upregulation of MMP-2 mediated Sirt-1 promotes cellular metastasis and invasion in prostate cancer cells[64].

3.2.5. *HDACs in cell proliferation, differentiation and drug resistance*

Many studies have revealed that HDACs control cell proliferation by regulating the cell cycle, whereas inhibition or knockout of HDACs induces apoptosis or cell cycle arrest. For example, class I HDACs can shorten the cell cycle of smooth muscle cells and accelerate cell proliferation. However, knockout of class I HDACs arrests the cell cycle in G1/S phase and significantly reduces cellular proliferative activity[41]. In glioma cells, HDAC4 promotes cell proliferation by downregulating CDK inhibitory proteins p27 and p21, whereas knockdown of HDAC4 induces G0/G1 cell cycle arrest and apoptosis[106]. Moreover, HDAC4 also represses the expression of proapoptotic genes, such as BIK and PMAIP1, thereby enhancing drug resistance[107]. HDAC5 is able to enhance cell proliferation by upregulating Six1 in human hepatoma cells and downregulating HDAC5 significantly inhibits the proliferation of human hepatoma cells[108]. In addition, both HDAC7 in HeLa cells and HDAC9 in retinoblastoma cells can shorten the cell cycle and promote cell proliferation[109,110]. For class IIb HDACs, HDAC6 overexpression promotes glioblastoma cell proliferation and induces temozolomide resistance.

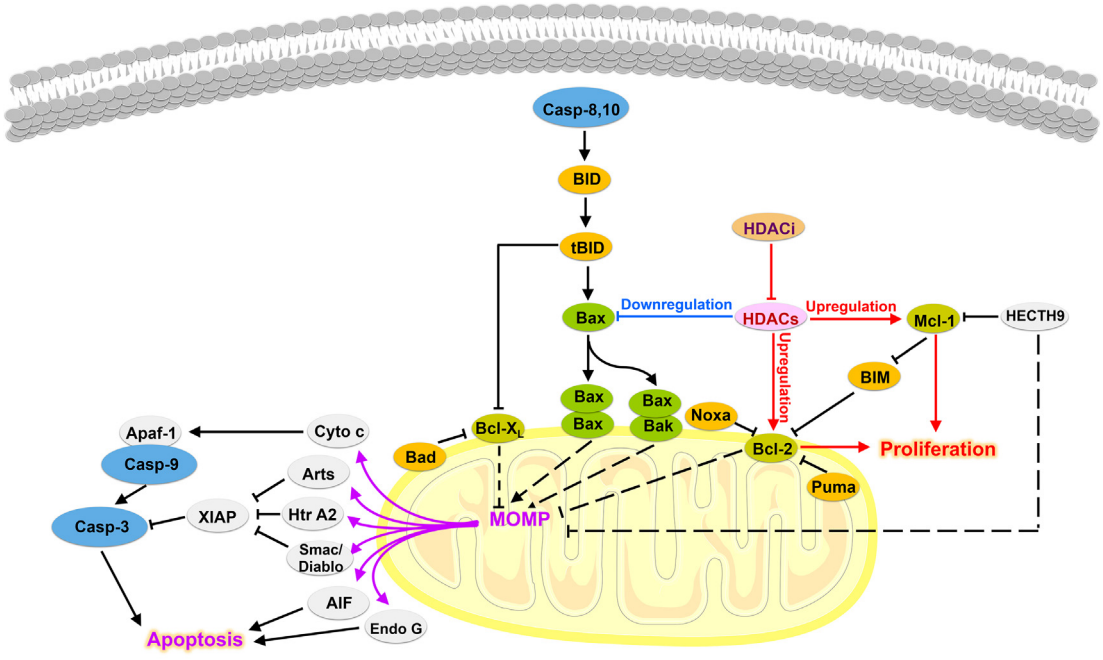


Figure 5 The intrinsic apoptotic pathway regulated by HDACs.

However, HDAC6 knockdown or HDAC6 isoform-selective inhibitor overcomes temozolomide resistance and significantly suppresses cell proliferation[111].

Notably, HDACs have great significances for ontogeny and are widely involved in the differentiation of osteoblasts, nerve cells, skeletal muscle cells, colorectal cancer cells and glioma stem cells[112−118].

Given the crucial role of HDACs in tumorigenesis, development, proliferation, apoptosis, metastasis, invasion and drug resistance, developing HDACi for the therapeutic intervention of aberrant HDACs is still an attractive field in medicinal chemistry.

4. Small molecular HDAC inhibitor (HDACi)

4.1. General pharmacophore model for HDACi

Zinc ion-dependent HDACs possess a highly conserved and homologous catalytic domain: a catalytic channel, a zinc cation and some secondary pockets. To achieve good inhibition of HDACs, various HDACis were designed according to the unique structure of the target enzymes. HDACi generally possesses three common pharmacophore models: surface binding region (cap), linker and zinc binding group (ZBG), whereas there are also a few HDACis with only two functional fragments of linker and ZBG[119].

Cap group binds to the pockets in the rim of HDACs channel, which can increase the affinity of HDACi for target enzyme and block the entry of substrate into HDAC catalytic channel. The linker is responsible for delivering ZBG to chelate zinc cation. A suitable linker is capable of helping HDACi achieve good inhibitory activity. A short linker cannot allow ZBG to interact with zinc ion well, resulting in poor binding, while a long linker may affect the interaction of the cap group with the pocket in the rim of the catalytic channel and reduce the affinity of HDACi for target enzyme. ZBG, another key group for HDACi, can chelate zinc ion in the catalytic center and inhibit the catalytic activity of HDACs. Common ZBGs with strong chelating effect are hydroxamic acid and anilinobenzamide; weak ZBGs include trifluoromethyl ketone, thiocarbonate, hydrazide, carboxylic acid, boric acid, mercaptoacetamide, etc.[120].

4.2. The indications and drawbacks of approved HDACis

4.2.1. Indications and clinical phases of HDACis

Up to date, two-decade efforts have led to the approval of five HDACis. Among them, vorinostat (SAHA), romidepsin (FK228), belinostat (PXD101) and panobinostat (LBH589) were approved by US Food and Drug Administration (FDA) or European Medicines Agency for the treatment of cutaneous T-cell lymphoma (CTCL), peripheral T-cell lymphoma (PTCL) and multiple myeloma (MM). Chidamide (CS055), approved by China Food and Drug Administration (CFDA), was used to treat PTCL and advanced (metastatic or locally recurrent, inoperable) breast cancer (Fig. 6).

In addition to approved HDACis, some clinical candidate HDACis show good profiles concerning HDACs inhibitory activities and isoform selectivities. Herein, we investigated their chemical structures, HDACs inhibitory activities and clinical status (Fig. 6).

4.2.2. The drawbacks of approved HDACis

4.2.2.1. Adverse effects. Vorinostat (SAHA), a pan-HDACi with hydroxamic acid, was approved for CTCL therapy. In phase I trial, vorinostat was proven to be effective against a defined subset of hematological malignancies as intravenous and oral preparations, whereas it showed limited efficacy on solid tumors. Vorinostat displayed good efficacy with overall response rates of 24%−30% at a dosage of 400 mg/day in the treatment of refractory advanced CTCL in phase II development[125]. Despite the good efficacy toward hematological malignancies, a series of adverse effects have also been discovered. The common mild adverse effects are fatigue, nausea, diarrhea, anorexia, dysgeusia as well as thrombocytopenia, and the severe adverse effect is thrombosis[125,126].

In 2009, romidepsin (FK-228) was approved for relapsed/refractory PTCL therapy. Romidepsin serves as a pan-HDACi but shows preferential inhibition toward class I HDACs and modest inhibition against class II HDACs. In a phase II clinical trial enrolling 131 patients with PTCL, romidepsin exhibited significant therapeutic effects with an objective response rate of 25%, a median duration of response of 28 months and the longest response of 56 months[127,128]. Notably, romidepsin is well tolerated in older patients, and the common adverse effects are similar, including anemia, fatigue, fever, thrombocytopenia, leukopenia and neutropenia[129].

Belinostat (PXD-101) is also a pan-HDACi and was approved for relapsed/refractory PTCL therapy. In a phase II clinical study of belinostat in 129 patients with PTCL, the objective response rate is 25.8% and the median duration of response is 13.6 months[130]. Of note, 96.9% of patients treated with belinostat experienced moderate treatment-emergent adverse events, such as vomiting, nausea, pyrexia, fatigue, anemia, neutropenia, dyspnea, thrombocytopenia and pneumonia[130,131].

Chidamide (CS055), an orally potent benzamide-derived HDACi, was first approved for PTCL therapy in 2014[132]. Unlike the aforementioned approved pan-HDACis, chidamide favors the inhibition of class I HDACs as well as IIb HDACs, and exhibits modest inhibition against class IV HDAC. Moreover, chidamide possesses the better properties in terms of anticancer effects and adverse effects relative to other approved HDACis. Upon treatment with chidamide, the objective response rate of patients was 28%. The good class I HDAC selectivity of chidamide seems to significantly decrease its clinical adverse events, and its frequent adverse events are thrombocytopenia, leukopenia and neutropenia[133]. Therefore, developing isoform-specific HDACi is supposed to be a promising approach to attenuate adverse effects or off-target toxicities.

Panobinostat (LBH-589) is a potent broad-spectrum HDACi developed by Novartis and it displays very good inhibitory activity against all HDAC enzymes. In 2015, panobinostat was approved in combination with dexamethasone and bortezomib for the treatment of relapsed MM, but its clinical toxicity and side effects are relatively severe. In the phase II clinical trial of panobinostat enrolling 35 patients, all patients experienced adverse events, with most (71.4%) reporting at least one grade 3 adverse events and 4 patients experiencing grade 4 toxicity[134]. The most common adverse events included fatigue (62.9%), nausea (51.4%), and thrombocytopenia (45.7%)[135].

Currently conventional approved HDACis, although effective in specific indications, exhibit severe adverse effects and off-target toxicities due to the limited isoform selectivity[136]. Considering the tumor-related functions, class I HDACs selective inhibitor may be an alternative to pan-HDACi because of the good anticancer effect and less off-target toxicity[137−139]. Notably, HDAC6 selective inhibitors are well tolerated and they can be effective in the treatment of neurodegenerative diseases as monotherapy or various cancers in combination with other anti-cancer agents[140,141].

4.2.2.2. Limited efficiency in solid tumors. Despite the great progress of approved HDACi in the therapy for a defined subset of

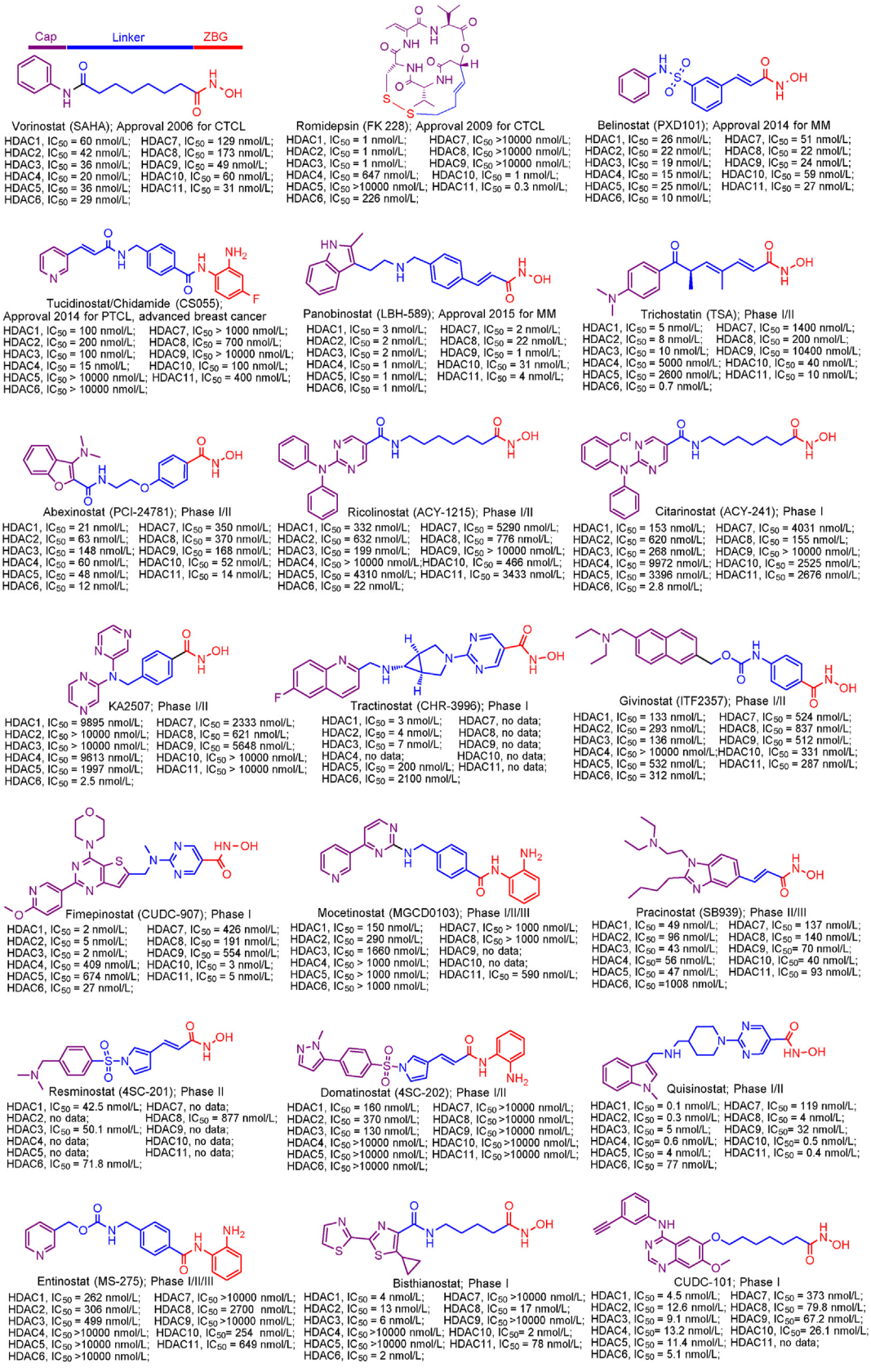


Figure 6 Chemical structures, HDACs inhibitory activities of approved HDACis and clinical candidate HDACis[25,121–124].

hematological malignancies, their poor therapeutic efficacy as monotherapy in solid tumors has emerged as an obstacle to be overcome. As illustrated in Fig. 7, vorinostat was effective against a defined subset of hematological malignancies, whereas it displayed limited efficacy toward solid tumors[142]. Although some more potent HDACis (romidepsin, belinostat, chidamide and panobinostat) were subsequently developed, their efficacies toward solid tumors remain unsatisfactory, probably due to their ineffectively low concentrations[143]. Moreover, available data revealed that most of the known HDACis exhibit very modest benefits in almost all solid tumors, such as prostate cancer, breast cancer and renal cancer[144].

Accumulating evidence indicated that combining HDACi with anticancer drugs in synergy with HDACi is capable of providing breakthroughs in therapeutic effects and expansion of indications *via* synergistic or additive effects[136]. Therefore, combination therapy or multitarget agents-based HDACi opens the door to the effective therapy of solid tumor. Up to date, some combination regimens involved in HDACi[141,145–147] and multitarget agents-based HDACi[124,148–150] exhibit encouraging anticancer effects and superior safety profiles, which make one believe that combination therapy and multitarget agent can be effective approaches to sensitize solid tumors.

The drawbacks of conventional HDACis have motivated the development of next-generation HDACi. Isoform-specific inhibitors, combination therapies, multitarget agents and HDAC PROTACs have emerged as the trends of next-generation HDACi. In this manuscript, we will introduce the trends and challenges of next-generation HDACi.

5. The trends of next-generation HDACi

5.1. *Isozyme-specific HDACi*

A central theme on HDACi is isoform selectivity or lack of specificity because non-selective pan-HDACi is involved in a broad spectrum of adverse effects. An investigation showed that vorinostat, romidepsin, belinostat and panobinostat are non-selective HDACi that exhibit good inhibitory activities toward almost all HDAC isoforms (Fig. 6)[121]. Despite chidamide exhibits preferential inhibition of class I HDACs, its good inhibitory activities against HDAC10 and HDAC11 indicates that its isoform-selectivity remains to be further improved.

5.1.1. *Advantages and challenges of isozyme-specific HDACi*

It is well accepted that good isoform selectivity confers compound superior therapeutic efficacy and pivotal safety profile in the treatment of some diseases, especially for chronic diseases (obesity, fibrosis and inflammation)[121]. Moreover, isozyme-specific HDACi can be more effective anticancer agent or powerful tool to explore the biology as well as cellular distribution of individual HDAC isozyme in both physiological and pathological settings[43]. Therefore, HDAC isoform selective inhibitor can be an alternative to pan-HDACi and is a promising trend in the future[80].

Despite some potent HDAC isoform selective inhibitors have been developed, there are some challenges for the development of isozyme-specific HDACi: (1) HDAC enzymes share a conserved tertiary structure with only minor differences, which makes it difficult to develop one compound targeting specific isoform; (2) The structures of several HDAC isoforms remain to be largely unknown due to lack of crystal structure; (3) We know relatively little about the enzymatic activities and biological functions of several HDAC isoforms, such as HDAC10 and HDAC11. Therefore, isozyme-specific HDACi has a long way to go.

5.1.2. *Protein structure of different HDAC isoforms*

The key to success in the rational design of HDAC isoform-selective inhibitor is comprehension and deeper knowledge of the structure of HDAC isoforms. Therefore, we investigated the structural features of different HDAC isoforms, and hope these data could benefit readers in the design of isozyme-specific HDACi.

The common tube-like binding channel of HDAC can be divided into rim, wall, and bottom regions (Fig. 8). In addition to above common structures, some HDAC isoforms also possess secondary pockets, such as side pocket, lower pocket, foot pocket and potential pocket (Supporting Information Fig. S1). These

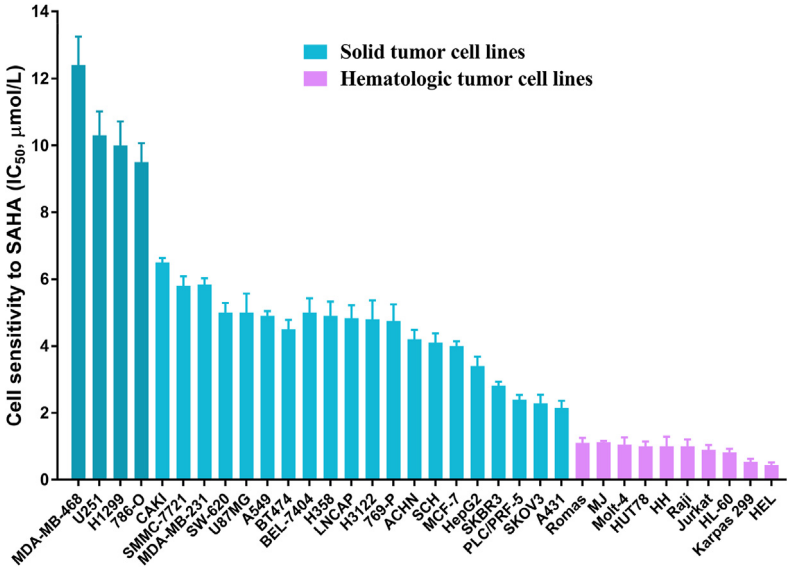


Figure 7 The therapeutic effects of vorinostat against solid tumors and hematomas[142].

secondary pockets have great significance for the design of isoform-selective HDACi (see Figs. 9−14).

HDAC1−3 have a deep and narrow binding channel, and there is an additional internal cavity domain called foot pocket at the bottom region, which is thought to be used to enter water and remove acetic acid[80]. Introducing a fragment into the ZBG group to accommodate the foot pocket of HDAC1-3 can improve the binding affinity and isoform selectivity (Fig. 8A)[151]. To be noted that the small Ser113 residues in HDAC1/2 confer them the large foot pocket while HDAC3 has a smaller foot pocket due to the presence of bulkier Tyr96 residue[152]. Additionally, HDACi with anilinobenzamide or trifluoromethyl ketone as ZBG appears to preferentially inhibit HDAC1 and HDAC2.

Moreover, HDAC8 possesses a side pocket consisting of L1 and L6 loops on the rim of channel and binding to the side pocket results in potent L-shaped HDAC8-selective inhibitor PCI-34051 (Fig. 8B)[153].

The foot pocket and side pocket seem not to exist in class II HDACs, whereas class IIa HDACs have a lower pocket, and the occupation of the lower pocket with linker-aside phenyl leads to some potent U-shaped HDACis (Fig. 8C)[154,155].

For class IIb HDACs, their shallow and wide binding channel prefers inhibitor with short and bulky linker. Of note, there are some potential binding pockets in the rim region (Fig. 8D) and the occupation of these potential pockets using large and extended cap groups is in favor of achieving high potency in class IIb HDACs inhibition and selectivity[156].

To date, the structure of HDAC11 remains largely unknown due to a lack of crystal structure. However, some studies reveal that HDAC11 serves as a defattyacetylase instead of a deacetylase owing to its efficient defatty-acylation activity and low deacetylase activity[46]. According to its physiological function, an activity-guided design approach was applied in the development of HDAC11 selective inhibitors with long-chain fatty acyl groups[157].

5.1.3. *Observations/phenotypes of HDACs knockout*

Apart from studying the structural features of HDAC isoforms, investigating their biological functions is also pivotal for the development of isozyme-specific HDACi. Therefore, this review summarizes the observations/phenotypes of HDACs knockout in mouse models (Table 4).

Global silence of HDAC1 results in embryonic lethality before E10.5 because of severe defects in proliferation as well as retardation in development, while upregulating HDAC2 and HDAC3 cannot compensate for HDAC1 loss[158]. HDAC2-deficient mice survive until the perinatal period, but they succumb to some cardiac defects, such as bradycardia, apoptosis of cardiomyocytes and obliteration of the lumen of the right ventricle[159]. Moreover, HDAC1/HDAC2 double knockout exhibited enhanced cell apoptosis and cell cycle arrest compared with HDAC1 or HDAC2 individual knockout[160]. Similarly, HDAC3 is required for early embryonic development and ablation of HDAC3 causes embryonic lethality at E9.5−10.5 due to gastrulation defects. In addition, cell cycle arrest, DNA breakage as well as apoptosis of mouse embryonic fibroblasts were observed in HDAC3-deficient cells[161]. Additionally, HDAC8 is associated with the morphogenesis of the skull by repressing a subset of transcription factors, and global deletion of HDAC8 results in skull instability, followed by perinatal lethality[162].

Interestingly, disruption of HDAC4 selectively induces mitotic arrest and chromosome segregation defects, followed by caspase-dependent apoptosis in tumor cells, whereas it shows no detectable effect in embryonic fibroblasts, myelopoietic progenitors or normal human dermal fibroblasts (NHDFs). Of note, HDAC4 disruption influences the proliferation of both p53-wild-type and p53-null carcinoma cells but defect segregation was discovered only in p53-null cells[163]. Therefore, HDAC4 selective inhibitor may be such an agent with higher efficiency and less adverse effects in the treatment of p53-deficient tumors.

As suppressors of cardiac hypertrophy, HDAC5 and HDAC9 regulate a specific subset of cardiac stress signal pathways and play redundant roles in cardiac development[164]. Although mice lacking HDAC5 or HDAC9 can survive at birth with profoundly enlarged hearts, HDAC5/HDAC9 double deletion leads to death during embryogenesis or the perinatal period owing to ventricular septal defects (VSD) and thin ventricular walls[164,165]. HDAC7 is required for blood vessel development and the association of HDAC7 to myocyte enhancer factor-2 (MEF2) represses MMP-10

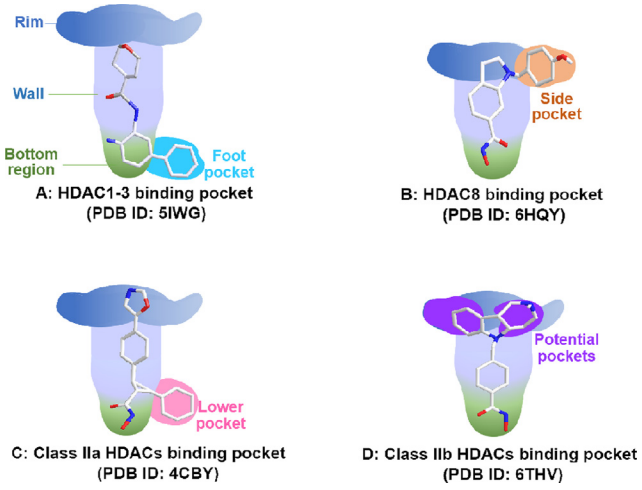


Figure 8 Schematic view of different HDAC isoform structures with selective inhibitors.

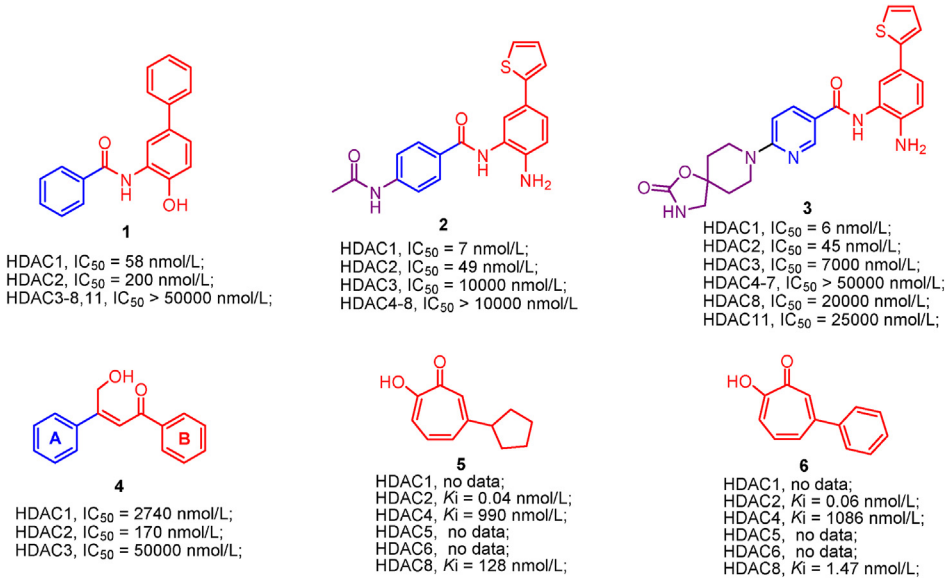


Figure 9 Examples of HDAC1 and HDAC2 selective inhibitors.

gene transcription, thus maintaining vascular integrity. Ablation of HDAC7 leads to embryonic lethality because of a failure in endothelial cell−cell adhesion and consequent blood vessels dilatation as well as rupture[166].

Unlike the knockout of above HDAC isoforms, HDAC6 knockout does not lead to lethal or teratogenic effects. In the absence of HDAC6, mice develop normally with moderate impact on the bone mineral density and immune response[167]. Notably, a variety of HDAC6 selective inhibitors have been developed and applied in antitumor field, where they exhibit good antiproliferation activities with few adverse effects[168−170]. To date, several HDAC6 selective inhibitors including citarinostat (ACY-241), ricolinostat (ACY-1215), monohydrochloride (KA2507) and purinostat mesylate are under clinical development, and they achieve good anticancer effects with less toxicities compared with pan-HDACi when used alone or in combination (www.clinicaltrials.gov). These results indicated that HDAC6 selective inhibitors are well tolerated and could avoid the on-target adverse effects of some molecules.

Recently, Li et al.[171] revealed that HDAC10 might serve as a presumed tumor suppressor and cells isolated from HDAC10 knockout mice showed highly tumorigenic and stem-like properties. Similar to HDAC6 disruption, HDAC10-deficient mice are viable and fertile without developmental defects.

In neural stem/precursor cells, inactivation of HDAC11 exhibits no significant effect on proliferation and differentiation[172]. Moreover, an additional study revealed that HDAC11 knockdown selectively affects malignant cells instead of normal cells[173].

Class I HDACs are required for cell proliferation as well as differentiation, and disruption or inhibition of class I HDACs is able to significantly reduce cell proliferation rate *via* cell cycle retardation or apoptosis[159]. However, class I HDACs inhibition appears to cause a spectrum of adverse effects in clinical settings[136].

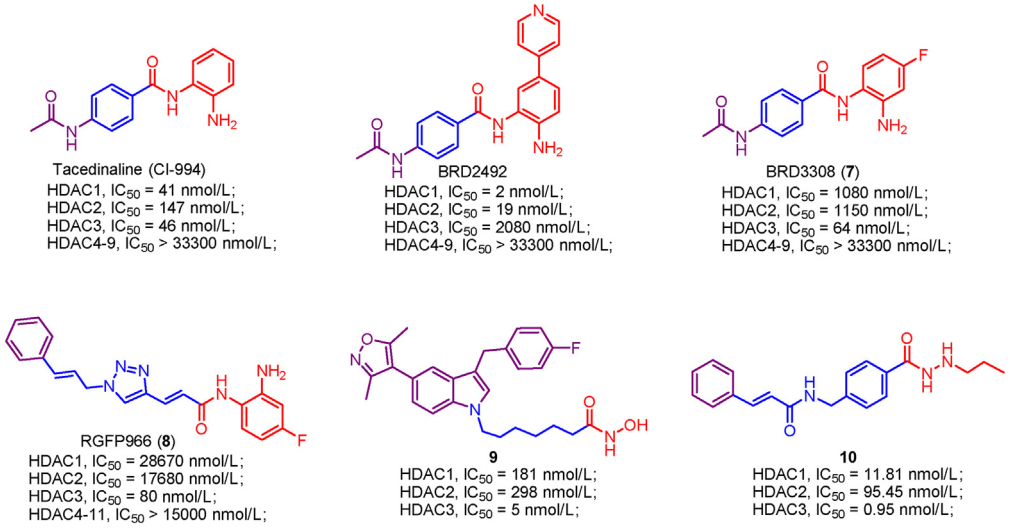
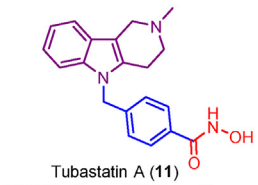
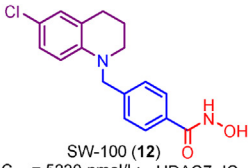


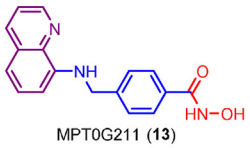
Figure 10 Examples of HDAC3 selective inhibitors.



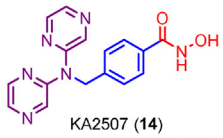
Tubastatin A (**11**)
HDAC1, IC_{50} = 16400 nmol/L;
HDAC2-5, 7, 9-11, IC_{50} > 30000 nmol/L;
HDAC6, IC_{50} = 15 nmol/L;
HDAC8, IC_{50} = 854 nmol/L;



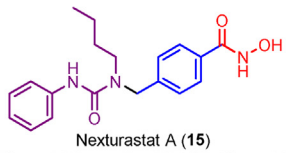
SW-100 (**12**)
HDAC1, IC_{50} = 5230 nmol/L; HDAC7, IC_{50} = 4550 nmol/L;
HDAC2, IC_{50} = 32800 nmol/L; HDAC8, IC_{50} = 3720 nmol/L;
HDAC3, IC_{50} = 29500 nmol/L; HDAC9, IC_{50} = 3460 nmol/L;
HDAC4, IC_{50} = 10900 nmol/L; HDAC10, IC_{50} = 26200 nmol/L;
HDAC5, IC_{50} = 4070 nmol/L; HDAC11, IC_{50} = 5720 nmol/L;
HDAC6, IC_{50} = 2.3 nmol/L;



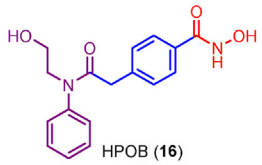
MPT0G211 (**13**)
HDAC1, IC_{50} = 9550 nmol/L; HDAC7, IC_{50} = 709 nmol/L;
HDAC2, IC_{50} = 12500 nmol/L; HDAC8, IC_{50} = 1190 nmol/L;
HDAC3, IC_{50} = 7750 nmol/L; HDAC9, IC_{50} = 1530 nmol/L;
HDAC4, IC_{50} = 4438 nmol/L; HDAC10, IC_{50} = 9791 nmol/L;
HDAC5, IC_{50} = 3112 nmol/L; HDAC11, IC_{50} = 376 nmol/L;
HDAC6, IC_{50} = 0.29 nmol/L;



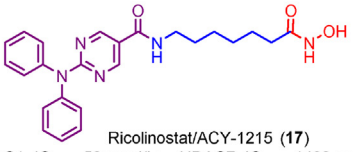
KA2507 (**14**)
HDAC1, IC_{50} = 9895 nmol/L; HDAC7, IC_{50} = 2333 nmol/L;
HDAC2, IC_{50} > 10000 nmol/L; HDAC8, IC_{50} = 621 nmol/L;
HDAC3, IC_{50} > 10000 nmol/L; HDAC9, IC_{50} = 5648 nmol/L;
HDAC4, IC_{50} = 9613 nmol/L; HDAC10, IC_{50} > 10000 nmol/L;
HDAC5, IC_{50} = 1997 nmol/L; HDAC11, IC_{50} > 10000 nmol/L;
HDAC6, IC_{50} = 2.5 nmol/L;



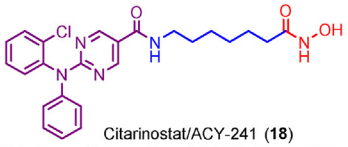
Nexturastat A (**15**)
HDAC1, IC_{50} = 3020 nmol/L; HDAC7, IC_{50} = 4460 nmol/L;
HDAC2, IC_{50} = 6920 nmol/L; HDAC8, IC_{50} = 954 nmol/L;
HDAC3, IC_{50} = 6680 nmol/L; HDAC9, IC_{50} > 6720 nmol/L;
HDAC4, IC_{50} = 9390 nmol/L; HDAC10, IC_{50} = 7570 nmol/L;
HDAC5, IC_{50} = 11700 nmol/L; HDAC11, IC_{50} = 5140 nmol/L;
HDAC6, IC_{50} = 5 nmol/L;



HPOB (**16**)
HDAC1, IC_{50} = 2900 nmol/L; HDAC7, IC_{50} > 10000 nmol/L;
HDAC2, IC_{50} = 4400 nmol/L; HDAC8, IC_{50} = 2800 nmol/L;
HDAC3, IC_{50} = 1700 nmol/L; HDAC9, IC_{50} > 10000 nmol/L;
HDAC4, IC_{50} > 10000 nmol/L; HDAC10, IC_{50} = 3000 nmol/L;
HDAC5, IC_{50} > 10000 nmol/L; HDAC11, IC_{50} > 10000 nmol/L;
HDAC6, IC_{50} = 56 nmol/L;



Ricolinostat/ACY-1215 (**17**)
HDAC1, IC_{50} = 58 nmol/L; HDAC7, IC_{50} = 1400 nmol/L;
HDAC2, IC_{50} = 48 nmol/L; HDAC8, IC_{50} = 100 nmol/L;
HDAC3, IC_{50} = 51 nmol/L; HDAC9, IC_{50} > 10000 nmol/L;
HDAC4, IC_{50} = 7000 nmol/L; HDAC11, IC_{50} > 10000 nmol/L;
HDAC5, IC_{50} = 5000 nmol/L;
HDAC6, IC_{50} = 4.7 nmol/L;



Citarinostat/ACY-241 (**18**)
HDAC1, IC_{50} = 153 nmol/L; HDAC7, IC_{50} = 4031 nmol/L;
HDAC2, IC_{50} = 620 nmol/L; HDAC8, IC_{50} = 155 nmol/L;
HDAC3, IC_{50} = 268 nmol/L; HDAC9, IC_{50} > 10000 nmol/L;
HDAC4, IC_{50} = 9972 nmol/L; HDAC10, IC_{50} = 2525 nmol/L;
HDAC5, IC_{50} = 3396 nmol/L; HDAC11, IC_{50} = 2676 nmol/L;
HDAC6, IC_{50} = 2.8 nmol/L;

Figure 11 Examples of HDAC6 selective inhibitors.

Class II HDACs participate in development, where HDAC5/HDAC9 are associated with cardiac development and HDAC7 is involved in blood vessel development. Disruption of HDAC5/HDAC7/HDAC9 in mice causes dysplastic hearts or vessels, followed by perinatal lethality.

Conversely, deletion of HDAC4 and HDAC6 does not result in lethal or teratogenic effects, and mice are viable and fertile with no developmental defects. Notably, silence of HDAC4 induces segregation defects in p53-null carcinoma cells, and inactivation of HDAC6 suppresses the proliferation of tumor cells without harming normal-tissue cells. Therefore, the selective inhibitors of HDAC4 and HDAC6 may be efficient with attenuated adverse effects in the treatment of specific subsets of tumors.

Although the ablation of HDAC10 or HDAC11 shows no impact on development progression in mice, it does not affect cell proliferation. Thus, it seems to be an unwise choice to develop HDAC10 or HDAC11 isoform-selective inhibitors for cancer therapy in terms of anti-proliferation activity.

5.1.4. Known isozyme-specific HDACi and dual HDACi

There are several promising isoform selectivity patterns: (1) Target transcription-related nuclear enzymes (HDAC1, HDAC2 and HDAC3); (2) Focus on cytoplasmic HDAC6; (3) Selectively inhibit non-acetyllysine enzymes (HDAC8, HDAC10 and HDAC11); (4) Develop class IIa HDACs (HDAC4, HDAC5, HDAC7 and HDAC9) selective inhibitors[121]. This manuscript summarizes some representative selective inhibitors of aforementioned HDAC isoforms (see Figs. 9–15).

5.1.4.1. HDAC1 selective inhibitors. Employing high throughput screening, Methot et al.[152] discovered hydroxybiphenyl benzamide **1**, which demonstrated preferential inhibition of HDAC1 in the absence of cap group. Further structural

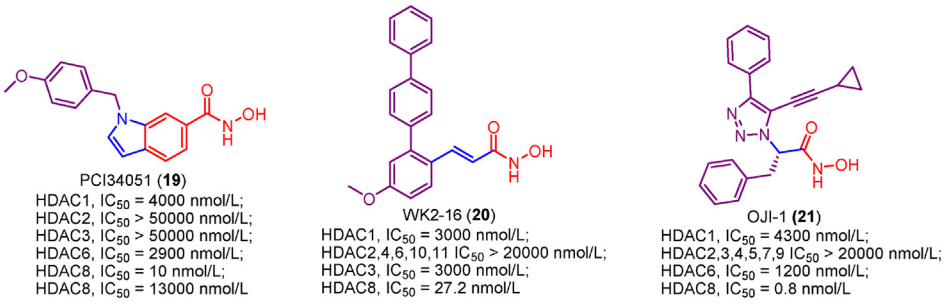


Figure 12 Examples of HDAC8 selective inhibitors.

optimization of compound **1** led to a more potent and selective thiophene analogs **2**, which is active against HDAC1/2 but is inactive toward other HDAC isoforms. Aside from enzymes inhibition, thiophene analog **2** also exhibits good anti-proliferation activity (HCT-116, GI_{50} = 0.11 μmol/L), druggability and pharmacokinetics[152]. In addition, Methot et al.[174] subsequently identified spirocyclic carbamate nicotinamides **3** as HDAC1 selective inhibitor by targeting the foot pocket (an internal cavity domain) and it displays good profiles in terms of HDAC1 inhibition, HDAC1 selectivity and *in vivo* tumor growth inhibition.

5.1.4.2. HDAC2 selective inhibitors. β-Hydroxymethyl-chalcone **4**, although lacks cap group, it preferentially inhibits HDAC2 (IC_{50} = 170 nmol/L) with moderate inhibition over HDAC1 (IC_{50} = 2740 nmol/L) and mild inhibition toward HDAC3 (IC_{50} = 50,000 nmol/L)[175]. QM/MM MD simulations revealed that the extended B-ring of compound **4** is able to occupy the large foot pocket of HDAC2 while the bulkier Tyr96 residue of HDAC3 blocks its access. The selective occupation of HDAC2 foot pocket may be responsible for HDAC2 selectivity. Despite the simple structure, α-hydroxy-ketone tropolone compound **5** not only well chelates zinc ion and inhibit enzymic reactivity, but also suppresses T cell lymphocytes growth[176]. Compound **6**, whose phenyl ring attached to α-hydroxy-ketone tropolone, shares a similar structure to compound **5** but its HDAC2 selectivity is less than that of compound **5**[176].

5.1.4.3. HDAC3 selective inhibitors. Tacedinaline (CI-994) is a potent inhibitor against HDAC1/2/3, whereas addition of the C-4 fluorine gave a remarkable improvement in HDAC3 selectivity. BRD3308 (**7**), the 4-fluorine derivative of tacedinaline (CI-994), exhibits more than 17-fold HDAC3 selectivity compared with HDAC1/2 and it inhibits HDAC3 with an IC_{50} value of 64 nmol/L[177]. Conversely, 4-pyridyl ring substituents at C-5 of tacedinaline (CI-994) produced BRD2492, a potent HDAC1/2 selective inhibitor (HDAC1, IC_{50} = 2 nmol/L; HDAC2, IC_{50} = 19 nmol/L; HDAC3, IC_{50} = 2080 nmol/L)[177]. That BRD3308 (**7**) bearing C-4 fluorine substituent and BRD2492 with C-5 4-pyridyl substituent show preferential inhibition toward distinct HDAC isoform, further confirmed the different topography of the foot pocket in HDAC1, 2 and 3 (HDAC1/2 have a large foot pocket and HDAC3 possesses the small one).

Moreover, RGFP966 (**8**) shows high HDAC3 inhibitory activity and selectivity without inhibiting other isoforms[13]. As a molecular tool, RGFP966 (**8**) with the ability to cross the blood brain barrier is used to investigate the biological function of HDAC3 and is widely applied in various *in vivo* disease models.

Compound **9** is an hydroxamic acid-based HDAC3 selective inhibitor (IC_{50} = 5 nmol/L) and shows over 36-fold selectivity for HDAC3 compared with HDAC1 (IC_{50} = 181 nmol/L) and HDAC2 (IC_{50} = 298 nmol/L). SAR gathered to date suggested that removing the fluorine attached to the benzyl group or reducing the linker led to the decreased HDAC3 inhibitory activity[178].

In addition to hydroxamic acid derivatives and *ortho*-aminoanilide derivatives, some hydrazide derivatives were also identified as HDAC3 selective inhibitors. Compound **10**, a hydrazide-derived HDAC3 selective inhibitor, displays low nanomolar

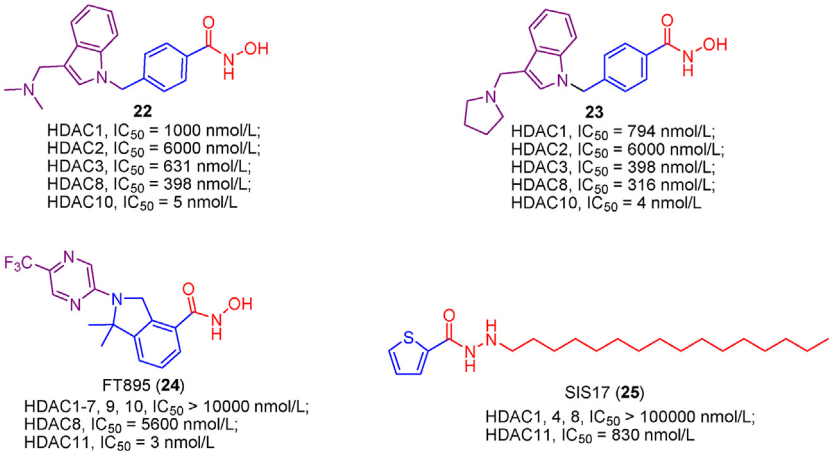


Figure 13 Examples of HDAC10 and HDAC11 selective inhibitors.

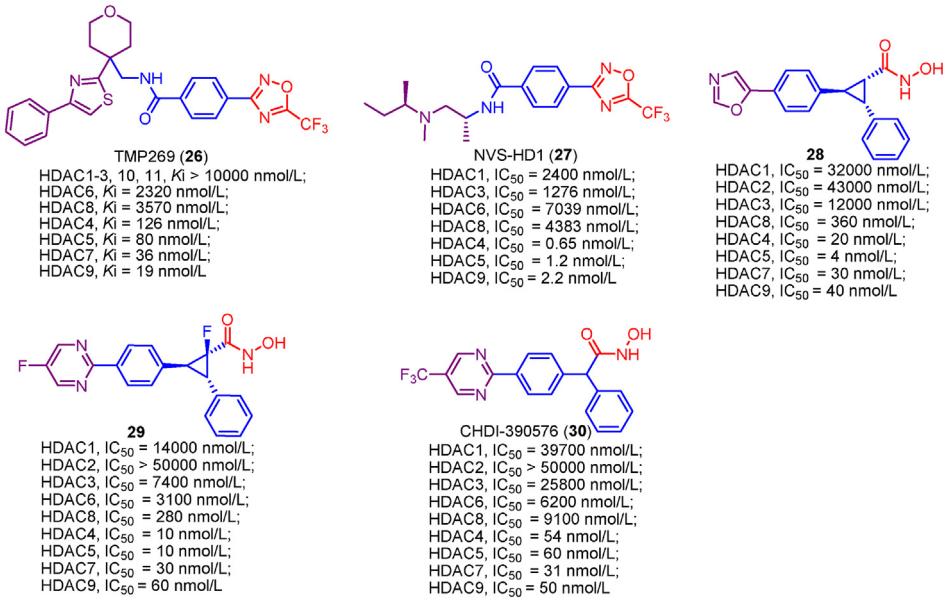


Figure 14 Examples of class IIa HDACs selective inhibitors.

HDAC3 inhibitory activity (IC_{50} = 0.95 nmol/L) and 12-fold HDAC3-selectivity over HDAC1 (IC_{50} = 11.81 nmol/L) and HDAC2 (IC_{50} = 95.45 nmol/L)[179]. SAR analysis indicated that suitable alkyl (three to four carbon units) substitution at the hydrazide group is in favor of achieving HDAC3 inhibitory activity and HDAC3-selectivity, but shorter alkyl (two carbon units) or longer alkyl (more than four carbon units) impairs HDAC3 inhibitory activity and selectivity.

HDAC1 and HDAC2 have larger foot pocket, which prefers a ZBG group containing big fragment (phenyl or thienyl). However, there is a bulky Tyr96 residue in the foot pocket of HDAC3, which limits the access of big fragment. Therefore, introducing small substituent (alkyl or fluorine) into ZBG is a good choice to achieve HDAC3 selectivity.

5.1.4.4. HDAC6 selective inhibitors. Compared to HDAC1, 2 and 3, HDAC6 possesses the shallower and wider channel, which prefers inhibitor with shorter, bulkier linker and extended, larger cap. Considering the structure features of HDAC6, Butler et al.[180] developed a potent HDAC6 selective inhibitor Tubastatin A (**11**), a tetrahydro-γ-carboline derivative with *N*-hydroxybenzamide. The short and bulky *N*-hydroxybenzamide of Tubastatin A can fit well into HDAC6 channel and its large cap is well tolerated by the potential binding pockets in the rim of channel. Therefore, Tubastatin A exhibits good potency in HDAC6 inhibition (IC_{50} = 15 nmol/L) and selectivity (more than 1000-fold selectivity over other HDACs excluding HDAC8)[180]. Despite the high potency in HDAC6 inhibition and selectivity, the modest ratio of brain to plasma levels of Tubastatin A (AUC_{brain}/AUC_{plasma} = 0.18) would impair its therapeutic potency in central nervous system (CNS) disorders. Therefore, Kozikowski et al.[181] subsequently identified the other potent HDAC6 selective inhibitors, SW-100 (**12**). Tetrahydroquinoline-based inhibitor SW-100 (**12**) demonstrates good brain penetrability, low-nanomolar HDAC6 inhibitory activity (IC_{50} = 2.3 nmol/L) and more than 1769-fold HDAC6 selectivity against other HDACs. In Fragile X Syndrome (FXS) mouse model, SW-100 (**12**) ameliorates several memory performances through its HDAC6 selective inhibition[181].

Lee and co-workers identified a class of bicyclic arylamino/heteroarylamino hydroxamic acids as novel HDAC6 selective inhibitors[168]. As the most potent molecule in their study, MPT0G211 (**13**) displays remarkable potency in HDAC6 inhibition (IC_{50} = 0.29 nmol/L) and over 4000-fold selectivity against other HDAC isoforms[168]. It has been proved that MPT0G211 (**13**)

Table 4 Observations/phenotypes of the different HDACs knockout.

HDACs knockout	Observation/phenotype
HDAC1	Lethal at E9.5−10.5 due to embryonic stem cell proliferation defects, apoptosis and cell cycle arrest.
HDAC2	Perinatal lethal due to cardiac defects, cell cycle delay and apoptosis.
HDAC3	Lethal at E9.5−10.5 due to gastrulation defects, cell cycle arrest, DNA damage and apoptosis.
HDAC8	Perinatal lethal due to craniofacial defects.
HDAC4	Segregation defects in p53-null carcinoma cell with no detectable effects in normal cells.
HDAC5	Profoundly enlarged hearts.
HDAC7	Embryonic lethality owing to blood vessels dilatation and rupture.
HDAC9	Cardiac hypertrophy.
HDAC6	Normal development with slight effects on bone density and immune response.
HDAC10	Normal development with no defects.
HDAC11	No significant effect on growth and differentiation.

is able to suppress the proliferation of NCI-H929, U266 and RPMI 8226 cells with no impact on normal cells. Moreover, MPT0G211 (**13**) also demonstrates significant *in vivo* antitumor activity when used alone (%TGI = 60.4%) or in combination with bortezomib (%TGI = 86.2%) in human multiple myeloma RPMI 8226 xenograft models.

KA2507 (**14**), a potent HDAC6 selective inhibitor under clinical investigation, delivers low nanomolar HDAC6 inhibitory activity (IC_{50} = 2.5 nmol/L) with little effect on other HDAC isoforms[122]. In preclinical models, KA2507 (**14**) play a crucial role in the antitumor response modulation. In a phase I study, KA2507 (**14**) exhibited good safety profiles and it was well tolerated at the oral dose of 800 mg. In the future clinical studies, KA2507 (**14**) may be used alone or combined with other immuno-oncology agents for the better antitumor efficacy[122].

Nexturastat A (**15**) and HPOB (**16**) are examples of the "Y-shaped" HDAC6 selective inhibitor and they achieve high potency in HDAC6 inhibition and selectivity[182,183]. The cap groups of Nexturastat A (**15**) and HPOB (**16**) contain two fragments, which were thought to interact with the potential pockets in the rim of HDAC6 channel.

Ricolinostat/ACY-1215 (**17**) and citarinostat/ACY-241 (**18**) are well-known potent HDAC6 inhibitors and they are aliphatic hydroxamic acid derivatives[140,184]. Structural modification of ricolinostat gave an improvement in HDAC6 inhibitory activity and produced the more potent citarinostat/ACY-241 (**18**). Generally, ricolinostat and citarinostat share similar structures and prefer HDAC6 to other HDACs. Up to date, ricolinostat and citarinostat have undergone several clinical trials and they used as single agent or in combination achieve good anticancer therapeutic potency[140,141].

5.1.4.5. HDAC8 selective inhibitors. PCI-34051 (**19**), a well-known HDAC8 specific inhibitor, was widely used as a tool molecule to explore the functions of HDAC8[153]. PCI-34051 (**19**) shows high potency in HDAC8 inhibition (IC_{50} = 10 nmol/L) and is free from significant class I, II as well as IV HDAC inhibitory activity. In subsequent biological evaluations, PCI-34051 (**19**) is able to induce caspase-dependent apoptosis in T-cell lymphomas or leukemias[185].

WK2-16 (**20**) is another cinnamoyl terphenylhydroxamic acid-based HDAC8 specific inhibitor and it was developed by knowledge-based design combined with structural modeling techniques[186]. In addition to HDAC8 inhibitory activity comparable to PCI34051, WK2-16 (**20**) also exhibited good anti-proliferation activity against several human lung cancer cell lines with no cytotoxicity for normal IMR-90 cells.

Efforts of the structural optimization identified the most potent HDAC8 selective inhibitor OJI-1 (**21**) (IC_{50} = 0.8 nmol/L). In the future, OJI-1 (**21**) can be the best probe molecule to study the cellular function of HDAC8[187].

5.1.4.6. HDAC10 selective inhibitors. Using a TR-FRET and BRET displacement assay, Géraldy and co-workers found that Tubastatin A, an HDAC6 inhibitor, strongly binds to HDAC10 instead of HDAC6. Subsequently, Géraldy et al.[188] synthesized a library of Tubastatin A derivatives and SAR analysis revealed that a basic amine in the cap group was responsible for high HDAC10 potency. Docking study further confirmed that the cap group nitrogen forms a favorable hydrogen bond interaction with the gatekeeper residue Glu272. As the Tubastatin A analogues, compounds **22** and **23** displayed higher binding affinities and selectivities for HDAC10[188].

5.1.4.7. HDAC11 selective inhibitors. Recently, a novel class of *N*-hydroxy-2-arylisoindoline-4-carboxamides were identified as the first-in-class HDAC11 selective inhibitors and FT895 (**24**) (HDAC11, IC_{50} = 3 nmol/L) exhibited the highest potency in HDAC11 inhibitory activity and selectivity[189]. Notably, the superior pharmacokinetic properties and cellular activities make FT895 (**24**) powerful tool to study the biological functions of HDAC11.

SIS17 (**25**), developed by an activity-guided rational design approach, was the most selective HDAC11 inhibitor, which is free from significant other HDACs inhibitory activity[157]. Interestingly, in HPLC-based assay with myristoyl-H3K9 peptide as substrate, SIS17 (**25**) are slightly less potent than FT895 (**24**). However, in cellular assay, SIS17 (**25**) performs better anti-proliferation activity compared with FT895 (**24**) because of the increased metabolic stability and permeability. Of course, SIS17 (**25**) can be a useful probe to investigate the biology and therapeutic potential of HDAC11.

5.1.4.8. HDAC4, 5, 7 and 9 selective inhibitors. Class IIa HDACs remains largely unknown due to lack of pharmacological tools to probe their biology, whereas TMP269 (**26**), a potent and selective class IIa inhibitor, fills this void. TMP269 (**26**) was firstly developed by Lobera et al.[190] using trifluoromethyloxadiazole (TFMO) as ZBG. The crystal structure of TMP269 (**26**) bound to HDAC7 revealed that TMP269 (**26**) in a U-shaped conformation directly chelates zinc ion using TFMO group (fluorine and oxygen atoms) through weak electrostatic interactions. To be noted that TFMO play a critical role in class IIa HDACs selectivity of TMP269 (**26**) because the bulky TFMO is able to fit well into the roomier catalytic pockets of class IIa HDACs but cannot be accommodated by class I or IIb HDACs.

NVS-HD1 (**27**) shares the same ZBG (trifluoromethyloxadiazole, TFMO) with TMP269 (**26**), but it is more potent and selective than TMP269 (**26**). As the most potent and selective class IIa HDACs inhibitor, NVS-HD1 (**27**) displayed very high potency in class IIa HDACs inhibition (HDAC4, IC_{50} = 0.65 nmol/L; HDAC5, IC_{50} = 1.2 nmol/L and HDAC9, IC_{50} = 2.2 nmol/L) and showed over 200-fold selectivity for class IIa HDACs against class I HDACs (HDAC1, IC_{50} = 2400 nmol/L; HDAC3, IC_{50} = 1276 nmol/L; HDAC8, IC_{50} = 4383 nmol/L) and class IIb HDAC (HDAC6, IC_{50} = 7039 nmol/L)[191]. The good profiles concerning class IIa HDACs inhibition and selectivity make NVS-HD1 (**27**) a suitable tool molecule to investigate the enzymic activity and biological functions of class IIa HDACs.

Targeting the lower pocket, a unique structure of class IIa HDACs, is in favor of yielding class IIa HDACs inhibitory activity and selectivity. Recently, Burli et al.[154] identified a class of trisubstituted diarylcyclopropanehydroxamic acid derivatives as class IIa HDACs inhibitors by exploiting the lower pocket and compound **28** exhibited the best profiles concerning the potency and selectivity against class IIa HDACs. The crystal structure of compound **28** bound to HDAC4 revealed that the linker-aside phenyl is well accommodated by the lower pocket and forms edge-to-face π-stacking interactions with Arg681 and Phe812. Subsequently, the structural optimization led to more potent as well as selective

compound **29** and CHDI-390576 (**30**), which achieved improved pharmacokinetic profiles and CNS properties[192,193].

5.1.4.9. Dual HDAC1/3, HDAC3/6 and HDAC6/8 selective inhibitors. Efforts of structural optimization resulted in a potent dual HDAC1/3 selective inhibitor **31**, characterized by a *N*-hydroxycinnamamide linker, capped with an indole group[194]. Docking studies indicated that introducing *p*-chlorophenyl conferred compound **31** a boost potency in HDAC1/3 inhibition because the lipophilic *p*-chlorophenyl forms van der Waals interactions with HDACs protein. Further biological evaluations revealed the excellent carcinoma cells growth suppression activity and U937 xenograft tumor inhibition ability of compound **31**. In addition, subsequent structural modification of compound **31** caused some other potent *ortho*-aminoanilide-based HDAC1/3 dual inhibitors and they showed good oral antitumor activity against HCT116 human tumor xenografts[195].

Soumyanarayanan et al.[196] employed a pharmacophore merging strategy to serendipitous identified a series of novel potent HDAC3/6 dual selective inhibitors. Among these molecules, compound **32** stands out due to its low nanomolar potency toward HDAC3/6 inhibition (34 and 2.6 nmol/L, respectively) and more than 26-fold HDAC3/6 selectivity over other HDAC isoforms. SAR studies indicated that the structure of compound **32** appears to fit well into HDAC6 binding pockets and any alterations in pharmacophore resulted in reduced HDAC6 inhibitory activity. Preliminary biological evaluations illustrated that these HDAC3/6 dual selective inhibitors exhibited low micromolar antiproliferation activity and potent compound **32** is able to induce apoptosis in MDA-MB-231 cells to exert antiproliferation activity.

CUDC-101 is a first-in-class EGFR/HER2/HDAC multitarget compound with good antitumor efficacy[149]. To yield a boost potency in HDACs inhibition, Yang et al.[197] employed the bioisosterism principle to modify the structure of CUDC-101 by replacing the quinazoline scaffold of CUDC-101 with isoquinoline fragment, and developed a library of potent isoquinoline-based HDAC1/3/6 selective inhibitors, including compound **33**. Moreover, aside from potency in HDACs inhibition, above soquinoline-based inhibitors demonstrated submicromolar antiproliferation activity against several tumor cell lines (HCT-116, RPMI-8226 and HepG2 cells) with no effect on hERG channel.

The introduction of substituents into the linker of non-selective HDACs inhibitors seems to improve HDAC6/8 selectivity. SAHA is a well-known pan-HDAC inhibitor targeting most of 11 HDAC isoforms without HDAC6/8 selectivity. However, Negmeldin et al.[198−200] modified the linker of SAHA at C_2, C_4 as well as C_8 position and conferred SAHA-modified analogs HDAC6/8 selectivity. Briefly, modification at C_2 and C_5 position (compounds **34** and **35**) led to decreased potency in HDAC inhibition with slight improvement in HDAC6/8 selectivity, whereas C_4-modified SAHA analog (**36**) gave a boost in HDAC6/8 selectivity. The SAR analysis of SAHA analogues indicated that extended cap and short linker appear to fit well into the catalytic pockets of HDAC6 and 8.

Recently, Rodrigues et al.[201] identified a novel series of *N*-acylhydrazone (NAH) derivatives as HDAC6/8 dual inhibitors based on the structure of trichostatin A (TSA). Briefly, they replaced CH moieties of TSA with nitrogen atom, replaced unsaturated double bond with *N*-hydroxybenzamide and explored the impact of alkylation at nitrogen atom. In their current study, compounds **37** and **38** exhibited the best potencies in HDAC6/8 inhibition and the modification gave an improvement in HDAC6/8 selectivity compared with TSA. Further evaluations revealed that compounds **37** and **38** are able to induce growth suppression, cell migration inhibition, apoptosis and cell cycle arrest. These results indicated that HDAC6/8 can be regarded as potential anti-tumor targets.

5.2. Combination therapies and multitarget agents

Clinical trials revealed that known HDACis are effective as monotherapies in a defined subset of hematological tumors, whereas they exhibit very modest effects against solid tumors[144]. Therefore, combination therapies with HDACi and HDACi-based multitarget agents came into being due to the poor therapeutic effect of HDACi in solid tumors[202].

5.2.1. Combination therapies with HDACi

5.2.1.1. Advantages and challenges of combination therapies. HDACs are widely involved in various biological events by regulating the acetylation of substrates or interacting with various partners, which endows HDACi with the ability to synergize with a series of anticancer agents. Combining HDACi with anticancer drugs in synergy with HDACi can provide breakthroughs in therapeutic effects and expansion of indications *via* synergistic or additive effects, while reducing adverse effects or resistance by administering lower drug doses[136]. The development of combination therapy generally follows pragmatic guidelines: (i) both drugs should have antitumor activity and elicit synergistic effects; (ii) each drug should have different mechanisms of action or nonoverlapping patterns of resistance; (iii) the combination should be tolerable with reduced dosage[203].

Combination therapies have been widely applied in a broad spectrum of dreadful disease therapies, especially acquired immunodeficiency syndrome (AIDS) and more difficult-to-treat solid tumors that are only infrequently cured at present, due to the flexible dosing schedules, improved efficiency profiles, reduced drug resistance and broad indications[204−206]. However, there are several issues to be addressed, such as poor patient compliance, complex clinical trial design and potential drug−drug interactions (DDIs). In addition, the different metabolic rates of each drug in combination therapy produce unpredictable pharmacokinetics (PK)/pharmacodynamic (PD) relationships, followed by necessitating extensive and expensive clinical studies[207,208]. Although cocktails or multicomponent drugs whereby multiple agents are coformulated in a single tablet improves patient compliance, other problems remain to be solved. Moreover, experience indicated that there may be more toxicities or adverse effects in drug combinations, since multiple drugs would be administered instead of a single compound[208]. Given the principal considerations above, multitarget agent, a single compound with multiple desired properties, may provide an appealing and cost-effective alternative to combination therapy[209,210].

5.2.1.2. Known combination therapies with HDACi. Given the advantages of combination therapy, it is extensively explored and yields clinical benefits in the treatment of some dreadful diseases. Nowadays, the drugs in combination with HDACi include chemotherapy drugs, kinase inhibitors, protease inhibitors, immune checkpoint inhibitors (PD-1/PD-L1, CTLA-4), monoclonal antibodies as well as other agents (Fig. 16)[140,211−215]. For example, a phase III clinical trial (NCT02482753) of chidamide plus exemestane for patients with advanced, hormone receptor-positive breast cancer revealed that the combination regimen

was well tolerated and significantly improved progression-free survival compared to exemestane plus placebo. Briefly, the median progression-free survival was 7.4 months in combination therapy group and 3.8 months in the placebo group[145]. In addition, chidamide plus carboplatin and paclitaxel (NCT01836679) in advanced non-small cell lung cancer[146], citarinostat plus paclitaxel in advanced solid tumors[141] and citarinostat plus nivolumab in advanced non-small cell lung cancer[147] exhibit encouraging anti-solid tumor efficacy and superior safety profiles. Aforementioned clinical trials revealed that combination therapy is promising, beneficial and effective in the treatment of solid tumors, especially difficult-to-treat solid tumors and it opens the door to the effective therapy of solid tumor.

Notably, this review also summarizes the drugs in combination with approved HDACis in clinical trials (Supporting Information Table S1). We hope readers can gain inspiration from these drug combination schedules and develop new drug combination regimens or multitarget drugs.

5.2.2. *HDACi-based multitarget agents*

5.2.2.1. Advantages and challenges of multitarget agents. Different from combination therapy, multitarget agent possesses a series of superior profiles, such as enhanced patient compliance, simple clinical trial design and predictable pharmacokinetics (PK)/pharmacodynamic (PD) relationships. Despite the advantages of multitarget agent, the rational design of multitarget agent with desired profiles is rather challenging because multitarget agent needs to match potencies at two completely different targets with a single chemical entity. Upon the design of multitarget agent, we need to consider all aspects of the factors, such as the structures of different targets, the structure-activity relationship of ligands bound to different targets and the ratio of activity level at the different targets. Fortunately, two main strategies for rationally designed multitarget ligands have been developed: (i) knowledge-based methods and (ii) screening methods[210]. Knowledge-based methods focus on existing data from the literature, classical molecules or clinical trials, whereas screening methods are based on computational chemistry.

5.2.2.2. Known HDACi-based multitarget agents. Accumulated efforts have identified various multitarget agents and the targets hybridizing to HDAC include kinases, receptors, DNA, enzymes, transcriptional regulators, apoptosis related proteins and some other proteins. Multitarget agents, the promising multifunctional molecules, exhibit superior properties concerning safety, growth suppression, anti-invasion, antiangiogenic activity, etc. This review investigates some known HDACi-based multitarget agents and related targets (Tables 5−11). Readers who are interested in these multitarget agents can refer to the literatures in Tables 6−12 for more details.

Up to now, four HDACi-based multitarget agents (EDO-S101, CUDC-907, 4SC-202 and CUDC-101) are under clinical investigation and the results suggested that the above HDACi-based multitarget agents exhibit better properties in terms of antitumor efficacy, safety and indications compared with single drug[124,148−150] (Table 12).

Although combination therapy is more extensively explored in the clinic than multitarget agents, both methods possess potential advantages in relation to monotherapy and are able to obtain enhanced efficacy, improved safety and reduced drug resistance. Therefore, combination therapy and multitarget agents will likely provide blueprints for the safe and effective treatment of diverse diseases, especially solid tumor. In either case, the key to success depends on the selection of appropriate targets or related signaling pathways that need to be concurrently regulated with the drug. Therefore, sufficient preliminary investigation is necessary for the development of combination therapy and multitarget agent.

5.3. *PROTAC*

5.3.1. *General concept of PROTAC*

Proteolysis-targeting chimera (PROTAC), a revolutionary technology in drug discovery, employs ubiquitin-proteasome system (UPS) to degrade target protein. As a heterobifunctional molecule, PROTAC consists of three pharmacophores: a target protein binding ligand, a E3 ligase binding ligand and a linker connecting above two ligands[277]. Different from traditional inhibitors inhibiting the activities of enzymes or blocking part of functions of target proteins at relatively high concentrations, PROTACs degrade target proteins and eliminate all the functions of target proteins (enzymatic and non-enzymatic functions) at low exposures due to the unique catalytic mode of action of PROTACs[278]. Nowadays, PROTAC has emerged as a promising approach in drug development, especially in “undruggable target” owing to its unique advantages.

5.3.2. *Advantages and challenges of PROTAC*

As a revolutionary technology, PROTACs have attracted great attentions because of their advantages: (1) Overcome drug resistance; (2) Eliminate non-enzymatic functions of kinase; (3) Target undruggable protein; (4) Reversible and fast protein knockdown; (5) Superior safety profiles; (6) Substoichiometric catalytic[279]. A library of advantages enables PROTACs to be powerful tools or agents in the treatment of cancer, inflammation/immune diseases and neurodegenerative disorders[278,280]. Aside from above advantages, there are some challenges: (1) Complicated evaluation of degradation activity; (2) Poor druggability and unpredictable pharmacokinetics (PK) and pharmacodynamics (PD) properties; (3) Few successes in undruggable targets; (4) Few E3 ligase ligands for PROTACs; (5) Unpredictable degradation of off-target proteins; (6) It is challenging to screen target protein ligands for PROTACs; (7) It is difficult to design PROTACs rationally[278]. Given the challenges, we therefore need to devote more efforts to PROTACs.

5.3.3. *HDAC PROTACs*

Up to date, PROTACs have been widely applied in the drug development and attempts to degrade HDACs with PROTACs have been made. This manuscript investigates some examples of HDAC PROTACs (see Figs. 17−20).

Smalley et al.[281] developed four HDAC PROTACs by introducing the linker from the terminal acetyl group of CI-994, a class I HDACs selective inhibitor. In their PROTACs development, they explored the length of linker (six carbon and twelve carbon) and different E3 ligase ligands (VHL ligand and CRBN ligand). Interestingly, PROTACs with longer linker including JSP004 (**39**)

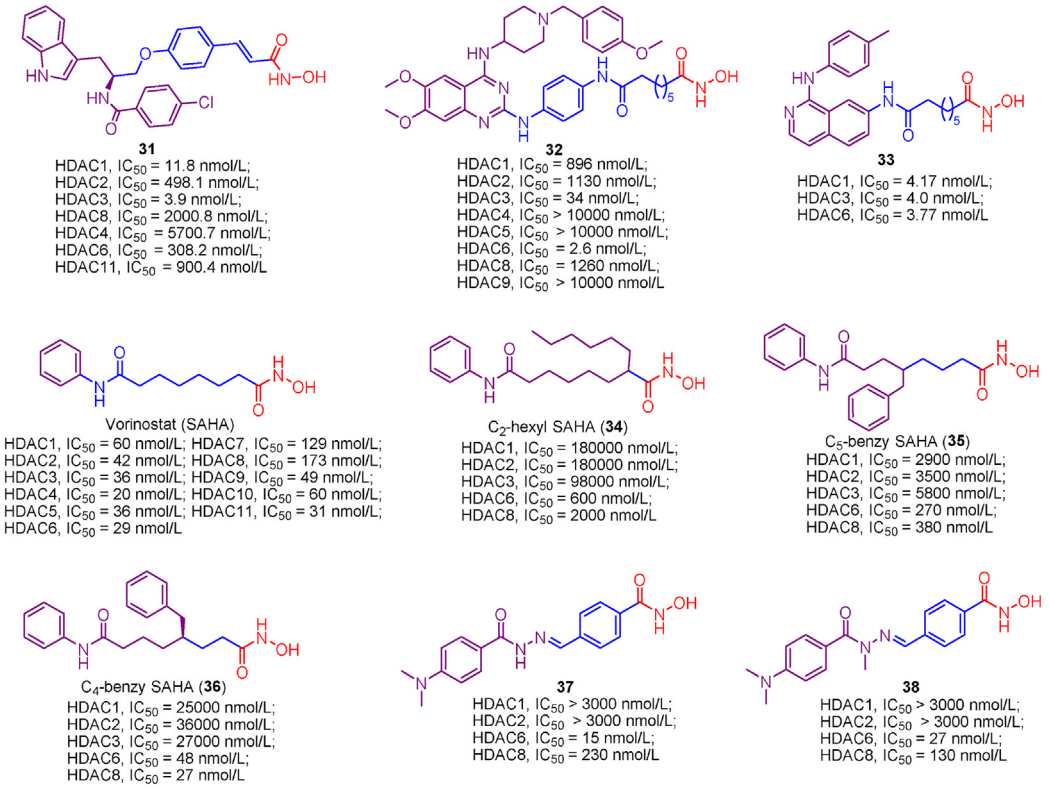


Figure 15 Examples of HDAC dual selective inhibitors.

are less potent but they are more effective in histone acetylation induction. The poor HDACs binding affinity and the increased histone acetylation indicated that these PROTACs with longer linker are likely to degrade rather than inhibit HDACs. Quantitative Western blot revealed that VHL-based JSP004 (**39**) demonstrated better degradation efficacy in relation to CRBN-

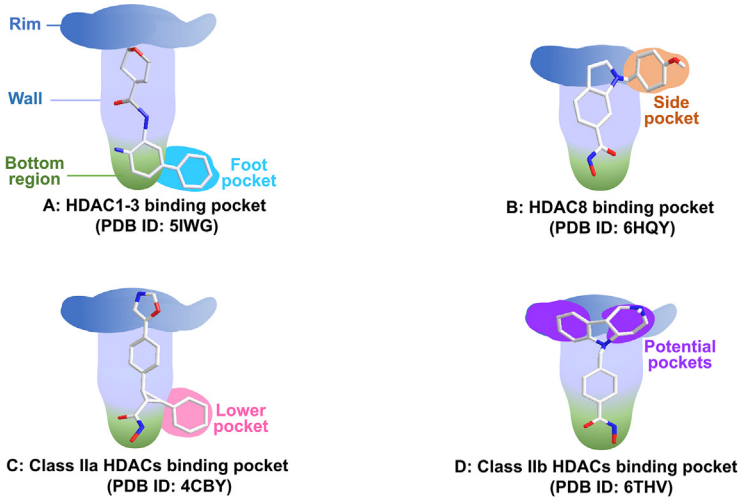


Figure 16 Promising synergistic targets with HDACs.

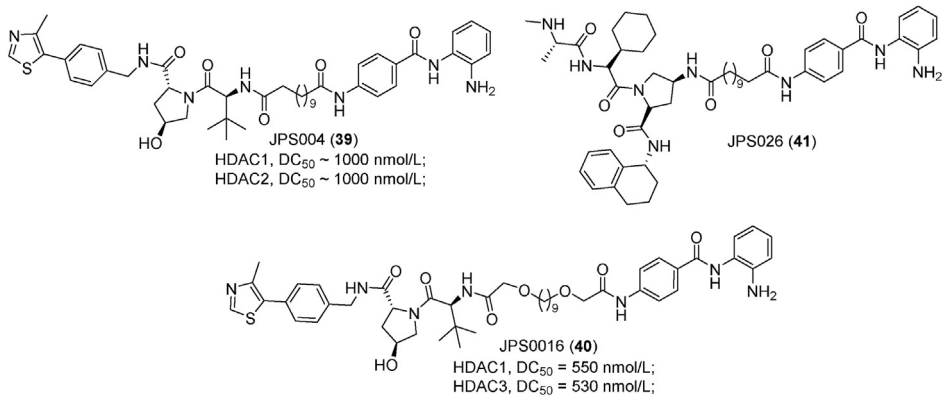


Figure 17 Examples of PROTACs **39**−**41**.

based PROTACs and it is able to degrade approximately 50% HDAC1, 2 at 1000 nmol/L[281]. Subsequently, Smalley et al.[282] explored the impacts of linker length (8−15 atoms) and linker composition (alkyl, alkoxy, alkyl incorporating piperazine and poly ethylene glycol (PEG)) on the HDACs degradation ability of PROTACs. In this study, they identified a more potent degrader JSP016 (**40**) (HDAC1, DC_{50} = 550 nmol/L; HDAC2, DC_{50} = 530 nmol/L), which showed the highest potency in sub-G1 phase arrest among their PROTACs. Then they further developed two additional PROTACs (JPS026 (**41**) and JPS027), which employ IAP as E3 ligases ligand[283]. In addition to HDAC1/2 degradation, JSP026 (**41**) induced apoptosis as well as DNA replication repression and exhibited higher potency in cell death induction compared with JSP004 (**39**).

Recently, SR-3558, a potent class I HDACs selective inhibitor, was converted into HDAC3-specific PROTAC XZ9002 (**42**) using VH032 (VHL) as E3 ligase ligand[284]. Docking studies revealed that the terminal phenyl of SR-3558 was exposed to solvent and the occupation of foot pocket using the alkyl tail in the hydrazide was responsible for its class I HDACs selectivity. Considering HDACs binding affinity, the linker was attached to the terminal phenyl moiety. Upon treatment with XZ9002 (**42**) for 14 h, HDAC3 was specifically degraded with a DC_{50} value of 42 nmol/L without affecting HDACs 1, 2 and 6 in MDA-MB-468 cells[284]. Notably, the use of VHL ligand or a proteasome inhibitor blocking XZ9002 (**42**)-mediated HDAC3 degradation further confirmed that the HDAC3 degradation mediated by XZ9002 (**42**) is required for VHL E3 ligase and UPS. In the future, compound **42** can be a powerful tool to explore the biology of HDAC3.

Yang et al.[285] firstly developed zinc-dependent HDAC degraders by connecting CRBN ligand to a non-selective HDAC inhibitor crebinostat. Surprisingly, dHDAC6 (**43**) derived from crebinostat preferentially degrades HDAC6 (DC_{50} = 34 nmol/L) instead of all HDAC enzymes. Upon treatment with 10 μmol/L dHDAC6 (**43**), 70.5% HDAC6 degradation was observed without "Hook effect". Subsequently, they employed a general cell-based target engagement assay to identify a cell-permeable CRBN E3 ligase ligand, which was used to develop two series of potent PROTACs, including the most potent molecule YZ167 (**44**) (HDAC6, DC_{50} = 1.94 nmol/L)[286].

Employing solid-phase synthesis, Sinatra et al.[287] synthesized two classes of HDAC6 PROTACs. SAHA-based degrader A6 (**45**) exhibited the highest potency in HDAC6 degradation (DC_{50} = 3.46 nmol/L) with no effects on HDAC1 and HDAC4. Biological evaluations revealed the promising anti-tumor therapeutic potential of degrader A6 (**45**) and it was able to induce caspase-dependent apoptosis and sub-G1 phase arrest. In addition, a library of potent HDAC6 PROTACs derived from Nexturastat A have been developed, such as NP8 (**46**)[288], compounds **47**[289],

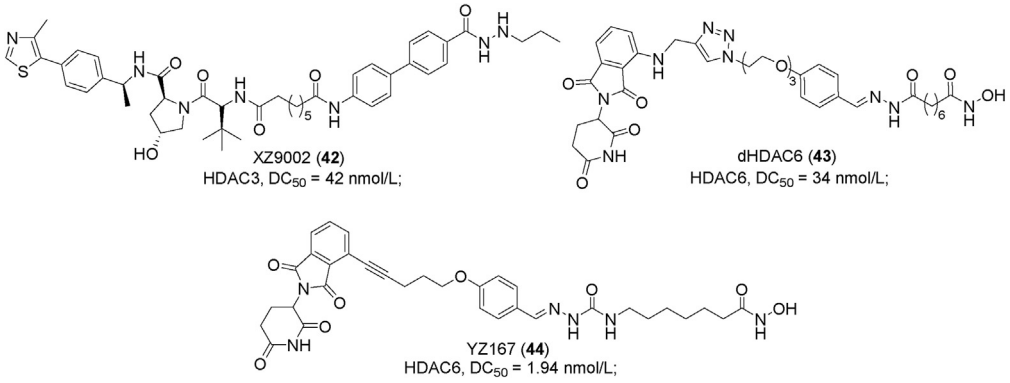


Figure 18 Examples of PROTACs **42**−**44**.

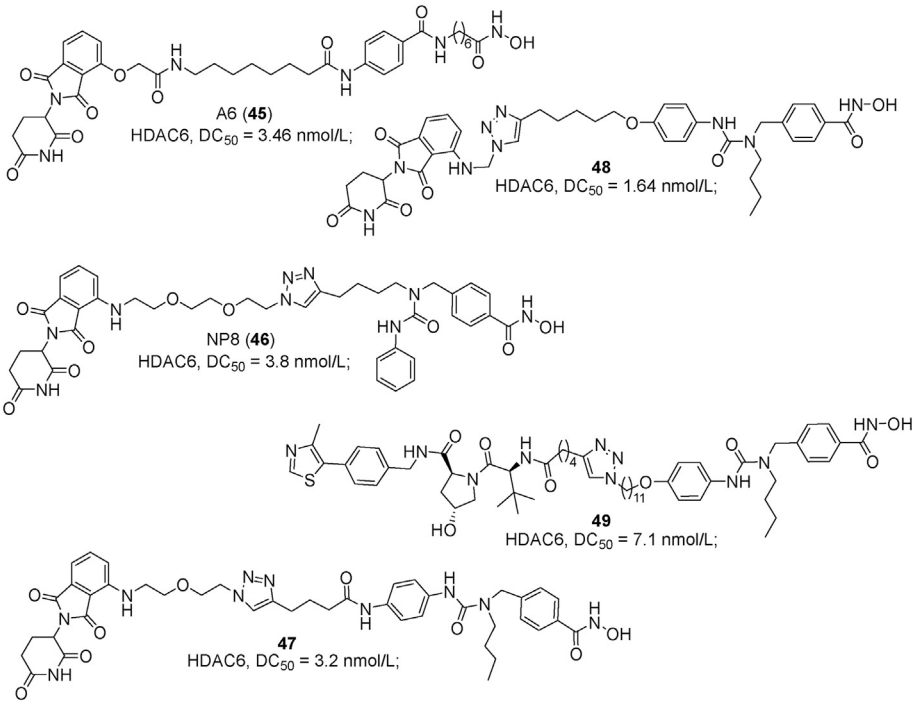


Figure 19 Examples of PROTACs **45**–**49**.

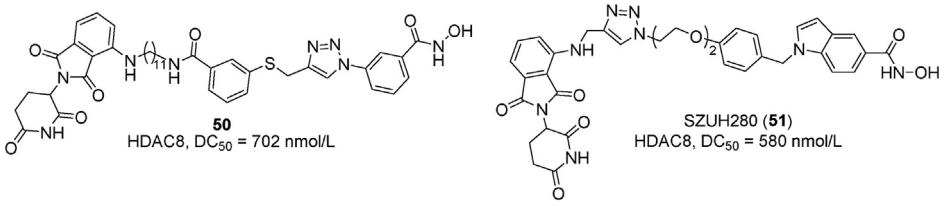
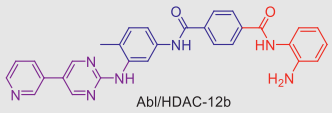
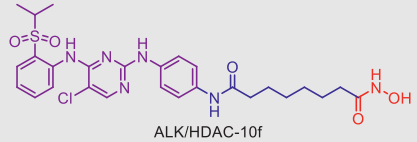


Figure 20 Examples of PROTACs **50**–**51**.

Table 5 Kinase and HDAC dual inhibitors.

Target	Name	Structure/Activity	Ref.
Abl/HDAC	Abl/HDAC-12b	 Abl/HDAC-12b HDAC1, IC_{50} = 270 nmol/L; EOL-1 cell, IC_{50} = 0.007 μmol/L; HDAC6, IC_{50} > 100000 nmol/L; K562 cell, IC_{50} = 0.76 μmol/L; Abl, IC_{50} = 5300 nmol/L; HeLa cell, IC_{50} = 1.97 μmol/L;	216
ALK/HDAC	ALK/HDAC-10f	 ALK/HDAC-10f HDAC1, IC_{50} = 7.9 nmol/L; HepG2 cell, IC_{50} = 0.64 μmol/L; HDAC2, IC_{50} = 9.3 nmol/L; A549 cell, IC_{50} = 0.46 μmol/L; HDAC6, IC_{50} = 4.4 nmol/L; H2228 cell, IC_{50} = 0.13 μmol/L; ALK^{wt}, IC_{50} = 2.1 nmol/L; SK-N-BE2 cell, IC_{50} = 0.05 μmol/L;	217

(*continued on next page*)

Table 5 (*continued*)

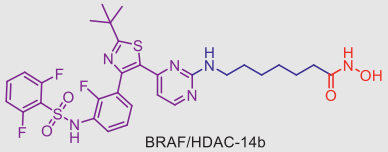
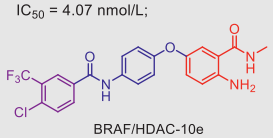
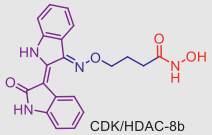
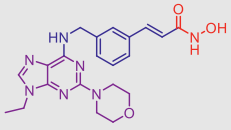
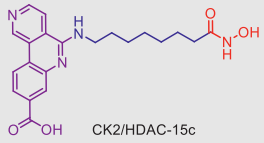
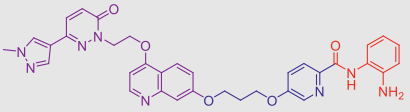
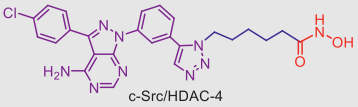
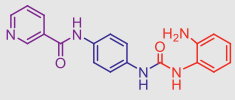
Target	Name	Structure/Activity	Ref.
BRAF/HDAC	BRAF/HDAC-14b	 BRAF/HDAC-14b HDAC1, IC_{50} = 493.95 nmol/L; HDAC6, IC_{50} = 49.20 nmol/L; HCT-116 cell, IC_{50} = 1.68 μmol/L; $BRAF^{V600E}$, IC_{50} = 1.71 nmol/L; HT-29 cell, IC_{50} = 0.31 μmol/L; $BRAF^{wt}$, IC_{50} = 4.07 nmol/L;	218
	BRAF/HDAC-10e	 BRAF/HDAC-10e HDAC1, IC_{50} = 1170 nmol/L; K562 cell, IC_{50} = 2.73 μmol/L; $BRAF^{V600E}$, IC_{50} = 73 nmol/L; MV4-11 cell, IC_{50} = 1.12 μmol/L; ARAF, IC_{50} = 183 nmol/L; HepG2 cell, IC_{50} = 1.33 μmol/L; CRAF, IC_{50} = 92 nmol/L; MDA-MB-468 cell, IC_{50} = 0.57 μmol/L;	219
CDK/HDAC	CDK/HDAC-8b	 CDK/HDAC-8b HDAC6,IC_{50} = 128.6 nmol/L; CDK2, IC_{50} = 60.9 nmol/L; HeLa cell, IC_{50} = 0.8 μmol/L; CDK4, IC_{50} = 276 nmol/L; K562 cell, IC_{50} = 2.5 μmol/L; CDK6, IC_{50} = 27.2 nmol/L; A549 cell, IC_{50} = 2.0 μmol/L;	220
	CDK/HDAC-6d	 CDK/HDAC-6d HDAC1, IC_{50} = 5.8 nmol/L; A549 cell, IC_{50} = 1.49 μmol/L; CDK2, IC_{50} = 56 nmol/L; HepG2 cell, IC_{50} = 0.77 μmol/L; CAL-148 cell, IC_{50} = 1.14 μmol/L;	221
CK2/HDAC	CK2/HDAC-15c	 CK2/HDAC-15c HDAC1, IC_{50} = 3.3 nmol/L; PC3 cell, IC_{50} = 40.4 μmol/L; HDAC6, IC_{50} = 13 nmol/L; MCF-7 cell, IC_{50} = 52.4 μmol/L; CK2, IC_{50} = 1.7 nmol/L; A549 cell, IC_{50} = 104.7 μmol/L;	222
c-Met/HDAC	c-Met/HDAC-2m	 c-Met/HDAC-2m HDAC1, IC_{50} = 38 nmol/L; EBC-1 cell, IC_{50} = 0.058 μmol/L; c-Met, IC_{50} = 0.71 nmol/L; HCT-116 cell, IC_{50} = 1.3 μmol/L;	223
c-Src/HDAC	c-Src/HDAC-4	 c-Src/HDAC-4 HDAC1, IC_{50} = 86 nmol/L; KM12 cell, IC_{50} = 0.47 μmol/L; HDAC2, IC_{50} = 231 nmol/L; MCF-7 cell, IC_{50} = 0.35 μmol/L; HDAC3, IC_{50} = 19 nmol/L; U251 cell, IC_{50} = 0.28 μmol/L; HDAC6, IC_{50} = 2.7 nmol/L; DU-145 cell, IC_{50} = 0.39 μmol/L; c-Src, K_I = 138 nmol/L; HCT-116 cell, IC_{50} = 0.22 μmol/L;	224
EphA2/HDAC	EphA2/HDAC-5a	 EphA2/HDAC-5a HDACs, IC_{50} = 940 nmol/L; K562 cell, IC_{50} = 8.36 μmol/L; EphA2, IC_{50} = 680 nmol/L; MCF-7 cell, IC_{50} = 15.38 μmol/L;	225

Table 5 (*continued*)

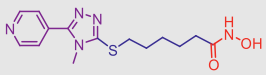
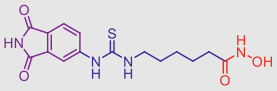
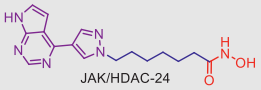
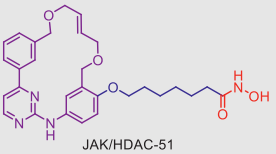
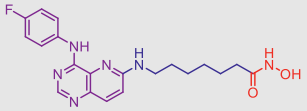
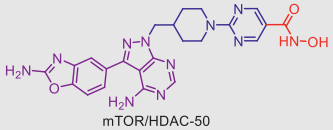
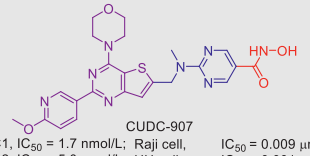
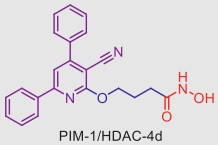
Target	Name	Structure/Activity	Ref.
FAK/HDAC	FAK/HDAC-6a	 FAK/HDAC-6a HDAC1, IC_{50} = 12310 nmol/L; HDAC2, IC_{50} = 90 nmol/L; A-498 cell, IC_{50} = 0.95 μmol/L; HDAC6, IC_{50} = 3410 nmol/L; Caki-1 cell, IC_{50} = 1.23 μmol/L; FAK, IC_{50} = 12.59 nmol/L;	226
GSK-3β/HDAC	GSK-3β/HDAC-11	 GSK-3β/HDAC-11 HDAC1, IC_{50} = 12780 nmol/L; HDAC6, IC_{50} = 3190 nmol/L; GSK-3β, IC_{50} = 2690 nmol/L;	227
JAK/HDAC	JAK/HDAC-24	 JAK/HDAC-24 HDAC1, IC_{50} = 6.9 nmol/L; MDA-MB-231 cell, IC_{50} = 0.79 μmol/L; HDAC2, IC_{50} = 5.8 nmol/L; MCF-7 cell, IC_{50} = 0.84 μmol/L; HDAC3, IC_{50} = 3.9 nmol/L; HCT-116 cell, IC_{50} = 2.32 μmol/L; HDAC6, IC_{50} = 1.4 nmol/L; PC-36 cell, IC_{50} = 2.41 μmol/L; JAK1, IC_{50} = 17 nmol/L; HL-60 cell, IC_{50} = 7.36 μmol/L; JAK2, IC_{50} = 75 nmol/L; Jurkat cell, IC_{50} = 0.47 μmol/L; JAK3, IC_{50} = 569 nmol/L; HEL92.1.7 cell, IC_{50} = 1.33 μmol/L;	228
	JAK/HDAC-51	 JAK/HDAC-51 HDAC1, IC_{50} = 222 nmol/L; PC-3 cell, IC_{50} = 1.7 μmol/L; HDAC2, IC_{50} = 49 nmol/L; MCF-7 cell, IC_{50} = 1.47 μmol/L; HDAC6, IC_{50} = 2.1 nmol/L; MAD-MB-231 cell, IC_{50} = 1.43 μmol/L; JAK1, 8.7% @ 0.1 μmol/L; HCT-116 cell, IC_{50} = 2.23 μmol/L; JAK2, IC_{50} = 1.4 nmol/L;	229
Mnk/HDAC	Mnk/HDAC-A12	 Mnk/HDAC-A12 HDACs, IC_{50} = 780 nmol/L; Mnk1, 54% @ 1 μmol/L; PC3 cell, IC_{50} = 2.91 μmol/L; Mnk2, 70% @ 1 μmol/L;	230
mTOR/HDAC	mTOR/HDAC-50	 mTOR/HDAC-50 HDAC1, IC_{50} = 0.91 nmol/L; A549 cell, IC_{50} = 2.16 μmol/L; mTOR, IC_{50} = 0.49 nmol/L; MV4-11 cell, IC_{50} = 1.74 μmol/L; HCT-116 cell, IC_{50} = 3.85 μmol/L;	231
PI3K/HDAC	CUDC-907	 CUDC-907 HDAC1, IC_{50} = 1.7 nmol/L; Raji cell, IC_{50} = 0.009 μmol/L; HDAC2, IC_{50} = 5.0 nmol/L; HH cell, IC_{50} = 0.001 μmol/L; HDAC3, IC_{50} = 1.8 nmol/L; Molt-4 cell, IC_{50} = 0.001 μmol/L; HDAC6, IC_{50} = 27 nmol/L; HL-60 cell, IC_{50} = 0.002 μmol/L; PI3Kα, IC_{50} = 19 nmol/L; K562 cell, IC_{50} = 0.031 μmol/L; PI3Kβ, IC_{50} = 54 nmol/L; RPMI-8226 cell, IC_{50} = 0.002 μmol/L; PI3Kγ, IC_{50} = 39 nmol/L; HCT-116 cell, IC_{50} = 0.007 μmol/L; PI3Kδ, IC_{50} = 311 nmol/L; A549 cell, IC_{50} = 0.01 μmol/L;	148
PIM-1/HDAC	PIM-1/HDAC-4d	 PIM-1/HDAC-4d HDAC1, IC_{50} = 63.65 nmol/L; HL-60 cell, IC_{50} = 1.81 μmol/L; HDAC6, IC_{50} = 62.39 nmol/L; Molt-4 cell, IC_{50} = 1.22 μmol/L; PIM-1, IC_{50} = 343.87 nmol/L; A549 cell, IC_{50} = 2.2 μmol/L; U251 cell, IC_{50} = 1.69 μmol/L;	232

Table 6 Receptor and HDAC dual inhibitors.

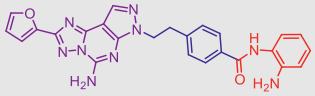
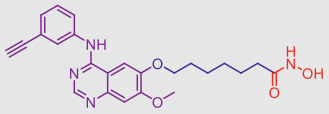
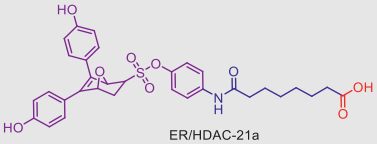
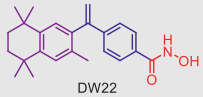
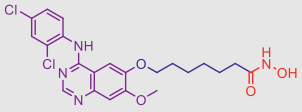
Target	Name	Structure/Activity	Ref.
A2AR/HDAC	A2AR/HDAC-24e	 A_{2A}R/HDAC-24e HDAC1, IC_{50} = 80.2 nmol/L; MC38 cell, IC_{50} = 6.2 μmol/L; $A_{2A}R$, K_i = 2.2 nmol/L; CT26 cell, IC_{50} = 6.1 μmol/L;	233
EGFR/HER2/HDAC	CUDC-101	 CUDC-101 HDAC, IC_{50} = 4.4 nmol/L; H358 cell, IC_{50} = 0.4 μmol/L; EGFR, IC_{50} = 2.4 nmol/L; HepG2 cell, IC_{50} = 0.13 μmol/L; HER2, IC_{50} = 15.7 nmol/L; Sk-Br-3 cell, IC_{50} = 0.04 μmol/L;	149
ER/HDAC	ER/HDAC-21a	 ER/HDAC-21a HDAC1, IC_{50} = 107 nmol/L; HDAC6, IC_{50} = 8340 nmol/L; ERα, K_i = 25 nmol/L; MCF-7 cell, IC_{50} = 19.1 μmol/L; ERβ, K_i = 772.73 nmol/L; DU-145 cell,IC_{50} = 34.8 μmol/L;	234
RXR/HDAC	DW22	 DW22 HDACs, IC_{50} = 6700 nmol/L; HGC-27 cell, IC_{50} = 8.6 μmol/L; RXR, IC_{50} = 19.5 nmol/L; DU-145 cell, IC_{50} = 14.7 μmol/L; Hep-3B cell, IC_{50} = 17.4 μmol/L;	235
VEGFR/HDAC	VEGFR/HDAC-6l	 VEGFR/HDAC -6l HDAC1, IC_{50} = 3.5 nmol/L; HDAC2, IC_{50} = 6.8 nmol/L; MCF-7 cell, IC_{50} = 1.2 μmol/L; HDAC6, IC_{50} = 25.5 nmol/L; HCT-116 cell, IC_{50} = 1.8 μmol/L; HDAC8, IC_{50} = 5.2 nmol/L; HepG2 cell, IC_{50} = 2.7 μmol/L; VEGFR-2, IC_{50} = 84 nmol/L; A549 cell, IC_{50} = 5.4 μmol/L;	236

Table 7 Transcriptional regulator and HDAC dual inhibitors.

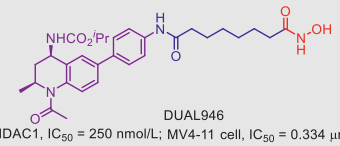
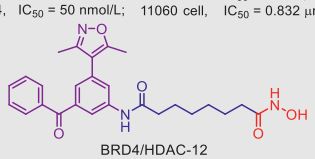
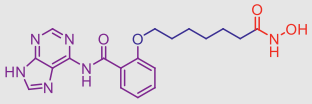
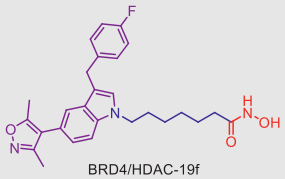
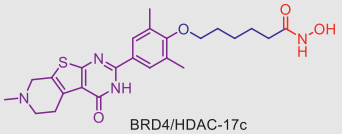
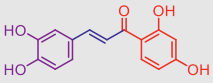
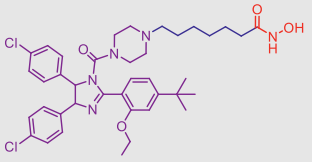
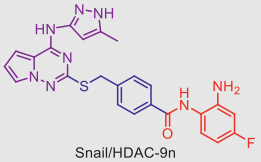
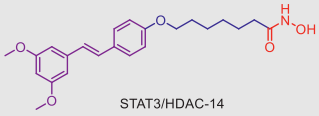
Target	Name	Structure/Activity	Ref.
BRD4/HDAC	DUAL946	 DUAL946 HDAC1, IC_{50} = 250 nmol/L; MV4-11 cell, IC_{50} = 0.334 μmol/L; HDAC6, IC_{50} = 420 nmol/L; HL-60 cell, IC_{50} = 0.764 μmol/L; BRD4, IC_{50} = 50 nmol/L; 11060 cell, IC_{50} = 0.832 μmol/L;	237
	BRD4/HDAC-12	 BRD4/HDAC-12 HDAC1, IC_{50} = 150 nmol/L; MV4-11 cell, IC_{50} = 0.91 μmol/L; BRD4, IC_{50} = 670 nmol/L; K562 cell, IC_{50} = 1.86 μmol/L;	238
	BRD4/HDAC-5d	 BRD4/HDAC-5d HDAC, IC_{50} = 260 nmol/L; HL-60 cell, IC_{50} = 4.4 μmol/L; BRD4, IC_{50} = 2700 nmol/L; T-47D cell, IC_{50} = 2.3 μmol/L;	239

Table 7 (*continued*)

Target	Name	Structure/Activity	Ref.
	BRD4/HDAC-19f	 BRD4/HDAC-19f HDAC1, IC_{50} = 181 nmol/L; HDAC2, IC_{50} = 298 nmol/L; THP-1 cell, IC_{50} = 15.5 μmol/L; HDAC1, IC_{50} = 5 nmol/L; BRD4, 88% @ 10 μmol/L;	178
	BRD4/HDAC-17c	 BRD4/HDAC-17c HDAC1, IC_{50} = 460 nmol/L; HDAC2, IC_{50} = 58 nmol/L; SW620 cell, IC_{50} = 1.78 μmol/L; HDAC6, IC_{50} = 73 nmol/L; HCT-116 cell, IC_{50} = 0.45 μmol/L; BRD4, IC_{50} = 710 nmol/L; DLD1 cell, IC_{50} = 2.11 μmol/L;	240
NF-κB/HDAC	NF-κB/HDAC-12	 NF-κB/HDAC-12 HDAC1, IC_{50} = 60000 nmol/L; NF-κB, IC_{50} = 38000 nmol/L;	241
P53/MDM2/HDAC	P53/MDM2/HDAC-14d	 P53/MDM2/HDAC-14d HDAC1, IC_{50} = 821 nmol/L; HDAC2, IC_{50} = 420.5 nmol/L; A549 cell, IC_{50} = 0.91 μmol/L; HDAC3, IC_{50} = 178.5 nmol/L; HCT-116 cell, IC_{50} = 1.08 μmol/L; HDAC6, IC_{50} = 17.5 nmol/L; MCF-7 cell, IC_{50} = 4.34 μmol/L; MDM2, K_i = 110 nmol/L; NCIH1299 cell, IC_{50} = 4.16 μmol/L;	242
Snai/HDAC	Snai/HDAC-9n	 Snail/HDAC-9n HDAC1, IC_{50} = 405 nmol/L; HL-60 cell, IC_{50} = 0.26 μmol/L; Snail, K_d = 180 nmol/L; K562 cell, IC_{50} = 0.048 μmol/L;	243
STAT3/HDAC	STAT3/HDAC-14	 STAT3/HDAC-14 HDAC1, IC_{50} = 683 nmol/L; HDAC6, IC_{50} = 104 nmol/L; MDA-MB-231 cell, IC_{50} = 0.78 μmol/L; STAT3, K_D = 33.61 nmol/L; HCT-116 cell, IC_{50} = 1.07 μmol/L;	244

48[290] and **49**[291]. They all selectively degrade HDAC6 with nanomolar DC_{50} values.

Recently, PROTAC **50**[292] and SZUH280 (**51**)[293] were identified as potent HDAC8 selective PROTACs. PROTAC **50** with CRBN as E3 ligase ligand was derived from HDAC8 selective inhibitor NCC-149 and it showed good growth inhibition against Jurkat cells[292]. Different from PROTAC **50**, SZUH280 (**51**) was PCI-34051-based degrader, which induced rapid HDAC8 degradation. Notably, SZUH280 (**51**) blocked DNA damage repair and demonstrated good *in vivo* antitumor activity against solid tumor xenograft model (A549)[293]. This study further confirmed the anticancer therapeutic potential of HDAC PROTACs.

Xiong et al.[294] constructed a library of 48 HDAC PROTACs to systematically map the degradability of different HDAC isoforms. In their current study, SAHA (a pan-HDACs inhibitor), dacinostat (class I and IIb HDACs selective inhibitor), TMP269 (class IIa selective inhibitor) and NVS-HD1 (class IIa selective inhibitor) were chosen to target HDACs. Pomalidomide (CRBN ligand), VHL ligand, VH032 analog and LCL161(IAP ligand) were used to recruit E3 ligases. Alkyl or alkoxy linkers with diverse lengths were employed to conjugate the HDAC inhibitor and E3 ligase ligand. Their study identified HDAC3/6/8 as the most degradable HDACs, HDAC1/2/4 as infrequently degraded HDACs, HDAC9 as the least degraded HDAC and these results

Table 8 Enzyme and HDAC dual inhibitors.

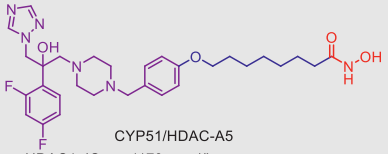
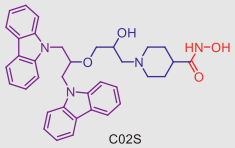
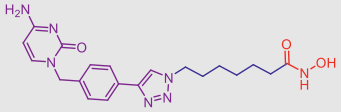
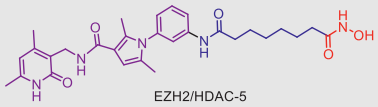
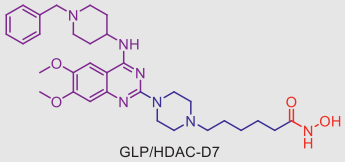
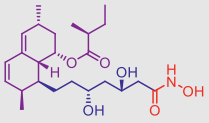
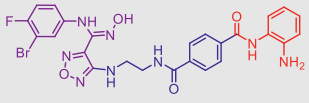
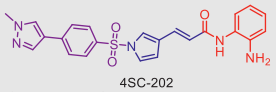
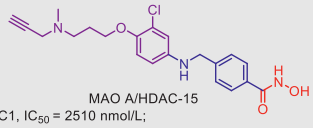
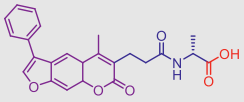
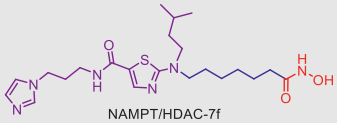
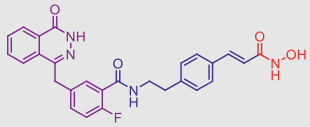
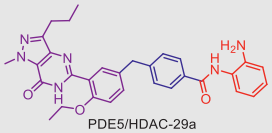
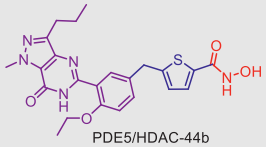
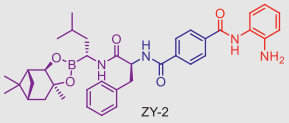
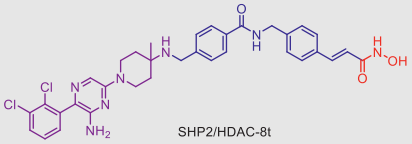
Target	Name	Structure/Activity	Ref.
CYP51/HDAC	CYP51/HDAC-A5	 CYP51/HDAC-A5 HDAC1, IC_{50} = 1170 nmol/L; *C. tropicalis*, MIC_{50} = 0.5 μg/mL; *C. neoformans*, MIC_{50} = 0.5 μg/mL; *C. tropicalis* whole cell HDAC, IC_{50} = 2.38 μmol/L; *C. neoformans* whole cell HDAC, IC_{50} = 12.59 μmol/L;	245
DNMT/HDAC	C02S	 C02S HDAC1, IC_{50} = 4160 nmol/L; DNMT1, IC_{50} = 2020 nmol/L; MCF-7 cell, IC_{50} = 1.88 μmol/L; DNMT3A3L, IC_{50} = 930 nmol/L; A549 cell, IC_{50} = 3.92 μmol/L; DNMT3B3L, IC_{50} = 1320 nmol/L; MDA-MB-231 cell, IC_{50} = 4.65 μmol/L;	246
	DNMT/HDAC-204	 DNMT/HDAC-204 HDAC1, IC_{50} = 2.68 nmol/L; K562 cell, IC_{50} = 11.46 μmol/L; HDAC6, IC_{50} = 8.37 nmol/L; U937 cell, IC_{50} = 7.10 μmol/L; DNMT1, 94.13% @ 50 μmol/L; HCT-116 cell, IC_{50} = 31.53 μmol/L;	247
EZH2/HDAC	EZH2/HDAC-5	 EZH2/HDAC-5 HDAC1, IC_{50} = 430 nmol/L; HDAC2, IC_{50} = 1330 nmol/L; RH4 cell, IC_{50} = 13 μmol/L; HDAC3, IC_{50} = 450 nmol/L; U937 cell, IC_{50} = 9 μmol/L; HDAC6, IC_{50} = 5 nmol/L; THP-1 cell, IC_{50} = 12 μmol/L; HDAC8, IC_{50} = 110 nmol/L; U87 cell, IC_{50} = 20 μmol/L; EZH2/PRC2, IC_{50} = 7370 nmol/L; SH-N-SK cell, IC_{50} = 25 μmol/L;	248
GLP/HDAC	GLP/HDAC-D7	 GLP/HDAC-D7 HDAC1, IC_{50} = 89 nmol/L; A431 cell, IC_{50} = 2.38 μmol/L; HDAC6, IC_{50} = 13 nmol/L; HeLa cell, IC_{50} = 3.34 μmol/L; GLP, IC_{50} = 1.3 nmol/L; K562 cell, IC_{50} = 2.6 μmol/L; HepG2 cell, IC_{50} = 2.74 μmol/L;	249
HMGR/HDAC	JMF3086	 JMF3086	250
IDO1/HDAC	IDO1/HDAC-10	 IDO1/HDAC-10 HDAC1, IC_{50} = 66.5 nmol/L; HCT-116 cell, IC_{50} = 5.12 μmol/L; IDO1, IC_{50} = 69 nmol/L; HT-29 cell, IC_{50} = 11.71 μmol/L;	251
LSD1/HDAC	4SC-202	 4SC-202 HDAC1, IC_{50} = 160 nmol/L; HDAC2, IC_{50} = 370 nmol/L; RT-112 cell, IC_{50} = 0.40 μmol/L; HDAC3, IC_{50} = 130 nmol/L; SW-1710 cell, IC_{50} = 0.47 μmol/L; HDAC4-11, IC_{50} > 15000 nmol/L; 5637 cell, IC_{50} = 0.15 μmol/L; KDM1A/LSD1, IC_{50} = 600 nmol/L; T24 cell, IC_{50} = 0.36 μmol/L;	124

Table 8 (*continued*)

Target	Name	Structure/Activity	Ref.
MAO A/HDAC	MAO A/HDAC-15	 MAO A/HDAC-15 HDAC1, IC_{50} = 2510 nmol/L; HDAC2, IC_{50} = 4750 nmol/L; HDAC6, IC_{50} = 10 nmol/L; GL-26 cell, IC_{50} = 2.41 μmol/L; HDAC8, IC_{50} = 1170 nmol/L; U251S cell, IC_{50} = 10.08 μmol/L; MAO A, IC_{50} = 0.4 nmol/L; U251R cell, IC_{50} = 7.12 μmol/L;	252
MMP-2/HDAC	STOCK1N-46177	 STOCK1N-46177	253
NAMPT/HDAC	NAMPT/HDAC-7f	 NAMPT/HDAC-7f HDAC1, IC_{50} = 2 nmol/L; HDAC2, IC_{50} = 6.8 nmol/L; HDAC3, IC_{50} = 0.67 nmol/L; A549 cell, IC_{50} = 5.2 μmol/L; HDAC6, IC_{50} = 0.64 nmol/L; HepG2 cell, IC_{50} = 24 μmol/L; NAMPT, IC_{50} = 15 nmol/L; HCT-116 cell, IC_{50} = 30 μmol/L;	254
PARP/HDAC	PARP/HDAC-P1	 PARP/HDAC-P1 HDAC1, IC_{50} = 27.26 nmol/L; T47D cell, IC_{50} = 2.14 μmol/L; HDAC6, IC_{50} = 8.21 nmol/L; HCC827 cell, IC_{50} = 1.79 μmol/L; PARP1, IC_{50} = 68.15 nmol/L; MCF-7 cell, IC_{50} = 3.55 μmol/L; PARP2, IC_{50} = 5.02 nmol/L; K562 cell, IC_{50} = 5.55 μmol/L;	255
PDE5/HDAC	PDE5/HDAC-29a	 PDE5/HDAC-29a HDAC1, IC_{50} = 673 nmol/L; PDE5, IC_{50} = 114 nmol/L;	256
	PDE5/HDAC-44b	 PDE5/HDAC-44b HDAC1, IC_{50} = 345 nmol/L; HDAC2, IC_{50} = 3720 nmol/L; HDAC6, IC_{50} = 15 nmol/L; HDAC3, IC_{50} = 2090 nmol/L; PDE5, IC_{50} = 11 nmol/L;	257
Proteasome/HDAC	ZY-2	 ZY-2 HDAC1, IC_{50} = 255 nmol/L; RPMI-8226 cell, IC_{50} = 6.66 nmol/L; Proteasome, IC_{50} = 1.1 nmol/L; U266 cell, IC_{50} = 4.31 nmol/L;	258
SHP2/HDAC	SHP2/HDAC-8t	 SHP2/HDAC-8t HDAC1, IC_{50} = 25 nmol/L; MV4-11 cell, IC_{50} =0.07 μmol/L; HDAC2, IC_{50} = 79 nmol/L; BxPC3 cell, IC_{50} = 1.65 μmol/L; HDAC3, IC_{50} = 233 nmol/L; SW1990 cell, IC_{50} = 3.92 μmol/L; HDAC6, IC_{50} = 27 nmol/L; AsPC-1 cell, IC_{50} = 2.31 μmol/L; SHP2, IC_{50} = 20.4 nmol/L; KYSE520 cell, IC_{50} = 1.95 μmol/L;	259

(*continued on next page*)

Table 8 (*continued*)

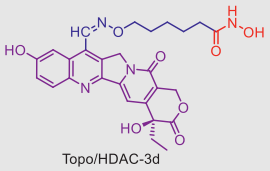
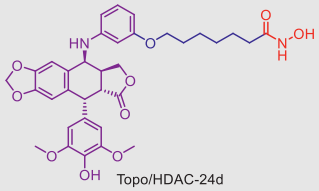
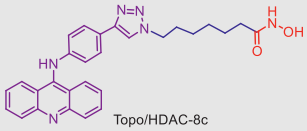
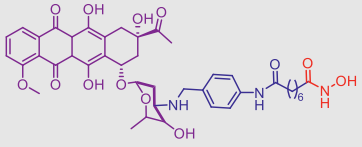
Target	Name	Structure/Activity	Ref.
Topo/HDAC	Topo/HDAC-3d	 Topo/HDAC-3d HDAC1, IC_{50} = 5260 nmol/L; U937 cell, IC_{50} = 0.18 μmol/L; HDAC2, IC_{50} = 9330 nmol/L; THP-1 cell, IC_{50} = 0.22 μmol/L; HDAC6, IC_{50} = 156 nmol/L; RAJI cell, IC_{50} = 0.077 μmol/L;	260
	Topo/HDAC-24d	 Topo/HDAC-24d HDAC1, IC_{50} = 11 nmol/L; HDAC3, IC_{50} = 9.6 nmol/L; HCT-116 cell, IC_{50} = 3.3 μmol/L; HDAC6, IC_{50} = 5.6 nmol/L;	261
	Topo/HDAC-8c	 Topo/HDAC-8c HDAC1, IC_{50} = 3.9 nmol/L; U937 cell, IC_{50} = 0.9 μmol/L; HDAC6, IC_{50} = 2.9 nmol/L; HCT-116 cell, IC_{50} = 2.11 μmol/L; MAD-MB-231 cell, IC_{50} = 7.09 μmol/L;	262
	Topo/HDAC-7	 Topo/HDAC-7 HDAC1, IC_{50} = 47 nmol/L; DU-145 cell, IC_{50} = 0.13 μmol/L; HDAC6, IC_{50} = 20 nmol/L; SK-MES-1 cell, IC_{50} = 0.47 μmol/L; HDAC8, IC_{50} = 220 nmol/L; MCF-1 cell, IC_{50} = 0.99 μmol/L;	263

Table 9 DNA/HDAC dual-targeting agents.

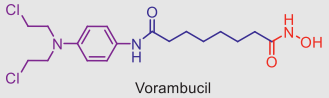
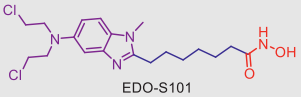
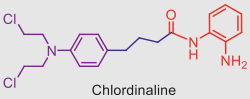
Target	Name	Structure/Activity	Ref.
DNA/HDAC	Vorambucil	 Vorambucil HDAC1, IC_{50} = 270 nmol/L; A549 cell, IC_{50} = 4.4 μmol/L; HDAC2, IC_{50} = 240 nmol/L; HepG2 cell, IC_{50} = 6.1 μmol/L; HDAC6, IC_{50} = 620 nmol/L; HCT-116 cell, IC_{50} = 3.2 μmol/L;	264
	EDO-S101	 EDO-S101 HDAC1, IC_{50} = 17 nmol/L; HDAC2, IC_{50} = 10 nmol/L; HDAC3, IC_{50} = 25 nmol/L; NCI60 cell, IC_{50} = 2.2 μmol/L HDAC4, IC_{50} = 6.4 nmol/L; HDAC6, IC_{50} = 72 nmol/L;	150
	Chlordinaline	 Chlordinaline HDAC2, IC_{50} = 32,900 nmol/L; H460 cell, IC_{50} = 3.1 μmol/L; HDAC3, IC_{50} = 9520 nmol/L; H1299 cell, IC_{50} = 5.5 μmol/L; HDAC8, IC_{50} = 75,200 nmol/L; SMMC7721 cell, IC_{50} = 4.0 μmol/L;	265

Table 9 (*continued*)

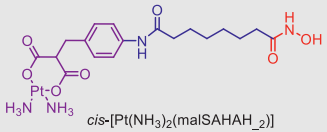
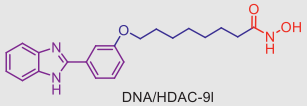
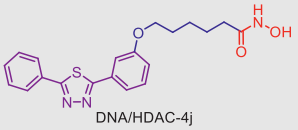
Target	Name	Structure/Activity	Ref.
	cis-[PtII(NH_3)$_2$ (malSAHAH_$_2$)]	 *cis*-[Pt(NH_3)$_2$(malSAHAH_$_2$)] HDAC1, IC_{50} = 1143 nmol/L; A2780P cell, IC_{50} = 9 μmol/L; A2780cisR cell, IC_{50} = 70 μmol/L;	266
	DNA/HDAC-9l	 DNA/HDAC-9l HDAC1, IC_{50} = 24 nmol/L; HEL cell, IC_{50} = 0.02 μmol/L; HDAC2, IC_{50} = 46 nmol/L; KG1 cell, IC_{50} = 0.19 μmol/L; HDAC6, IC_{50} = 47 nmol/L; K562 cell, IC_{50} = 0.29 μmol/L; HDAC8, IC_{50} = 5980 nmol/L; PC-3 cell, IC_{50} = 2.7 μmol/L;	267
	DNA/HDAC-4j	 DNA/HDAC-4j HDAC1, IC_{50} = 15 nmol/L; HCT-116 cell,IC_{50} = 0.47 μmol/L; HDAC6, IC_{50} = 20 nmol/L; HeLa cell, IC_{50} = 0.78 μmol/L; HDAC8, IC_{50} = 3025 nmol/L; MC38 cell, IC_{50} = 0.43 μmol/L;	268

Table 10 Multifunctional molecules targeting protein and HDAC.

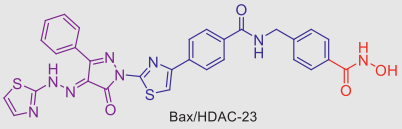
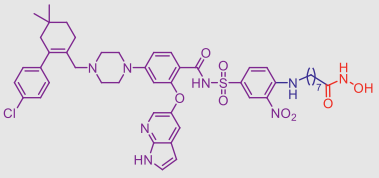
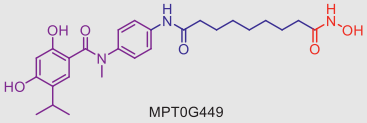
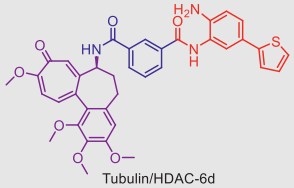
Target	Name	Structure/Activity	Ref.
Bax/HDAC	Bax/HDAC-23	 Bax/HDAC-23 HDAC1, IC_{50} = 62.8 nmol/L; HDAC2, IC_{50} = 197.9 nmol/L; HeLa cell, IC_{50} = 0.86 μmol/L; Bax, EC_{50} = 392.9 nmol/L;	269
Bcl-2/HDAC	Bcl-2/HDAC-7g	 Bcl-2/HDAC-7g HDAC1, IC_{50} = 281 nmol/L; HDAC6, IC_{50} = 19 nmol/L; RPMI-8226 cell, IC_{50} = 0.2 μmol/L; Bcl-2, IC_{50} = 250 nmol/L; U266 cell, IC_{50} = 8.7 μmol/L;	270
HSP90/HDAC	MPT0G449	 MPT0G449 HDAC1, IC_{50} = 6060 nmol/L; A549 cell, IC_{50} = 0.44 μmol/L; HDAC2, IC_{50} = 4910 nmol/L; HCT-116 cell, IC_{50} = 1.06 μmol/L; HDAC3, IC_{50} = 1440 nmol/L; H1975 cell, IC_{50} = 0.40 μmol/L; HDAC6, IC_{50} = 316 nmol/L; HL60 cell, IC_{50} = 0.19 μmol/L; HSP90α, IC_{50} = 77.21 nmol/L; Molt-4 cell, IC_{50} = 0.11 μmol/L;	271,272
Tubulin/HDAC	Tubulin/HDAC-6d	 Tubulin/HDAC-6d HDAC1, IC_{50} = 1500 nmol/L; A549 cell, IC_{50} = 2.79 μmol/L; HDAC2, IC_{50} = 190 nmol/L; HCT-116 cell, IC_{50} = 0.039 μmol/L; HDAC3, IC_{50} = 1490 nmol/L; HepG2 cell, IC_{50} = 0.02 μmol/L;	273

Table 11 Other HDACi-based multitarget agents.

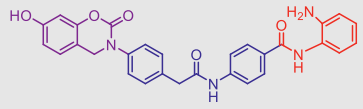
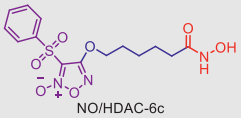
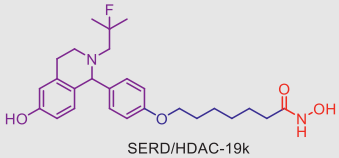
Target	Name	Structure/Activity	Ref.
MIF/HDAC	MIF/HDAC-6a	 MIF/HDAC-6a HDAC1, IC_{50} = 200 nmol/L; HDAC2, IC_{50} = 1100 nmol/L; HDAC3, IC_{50} = 600 nmol/L; MIF, IC_{50} = 180 nmol/L;	274
NO/HDAC	NO/HDAC-6c	 NO/HDAC-6c HDAC1, IC_{50} = 241 nmol/L; HCT-116 cell, IC_{50} = 1.51 μmol/L; HDAC2, IC_{50} = 380.5 nmol/L; HEL cell, IC_{50} = 0.38 μmol/L; HDAC3, IC_{50} = 532.0 nmol/L; HeLa cell, IC_{50} = 1.55 μmol/L; HDAC4, IC_{50} = 30 nmol/L; ES-2 cell, IC_{50} = 1.39 μmol/L; HDAC6, IC_{50} = 7.4 nmol/L; U937 cell, IC_{50} = 3.1 μmol/L; HDAC8, IC_{50} = 343 nmol/L; KG1 cell, IC_{50} = 1.75 μmol/L;	275
SERDs/HDAC	SERDs/HDAC-19k	 SERD/HDAC-19k HDAC1, IC_{50} = 582.4 nmol/L; HDAC6, IC_{50} = 62.8 nmol/L; MCF-7 cell, IC_{50} = 0.17 μmol/L; ERα, IC_{50} = 90.8 nmol/L;	276

Table 12 Clinical trials of HDACi-based multitarget agents.

Compound	Indications	Phase	Trail number[a]
EDO-S101	Malignant melanoma.	I	NCT03903458
	Glioblastoma, GMT-unmethylated glioblastoma, gliosarcoma.	I	NCT03452930
	Cutaneous T-cell lymphoma, hematological malignancies, hodgkin's lymphoma, multiple myeloma.	I	NCT02576496
	Multiple myeloma in relapse, multiple myeloma progression, multiple myeloma with failed remission.	I, II	NCT03687125
	Endometrial cancer, ovarian cancer, small-cell lung cancer, soft tissue sarcoma, triple-negative breast cancer.	I, II	NCT03345485
CUDC-907	Brain tumor, lymphoma, neuroblastoma, solid tumor.	I	NCT02909777
	High-grade serous ovarian cancer, NUT midline carcinoma, triple-negative breast cancer.	I	NCT02307240
	Diffuse intrinsic pontine glioma, recurrent anaplastic astrocytoma, recurrent glioblastoma, recurrent malignant glioma, recurrent medulloblastoma.	I	NCT03893487
	Lymphoma, double-hit lymphoma, double-expressor lymphoma, high-grade B-cell lymphoma, relapsed/refractory lymphoma, relapsed/refractory diffuse large B-cell lymphoma, triple-hit lymphoma.	I	NCT01742988
	Relapsed and/or refractory diffuse large B-cell lymphoma.	II	NCT02674750
	Differentiated thyroid cancer, poorly differentiated and undifferentiated thyroid cancer, thyroid neoplasms.	II	NCT03002623
4SC-202	Advanced hematologic malignancies.	I	NCT01344707
	Malignant melanoma.	I, II	NCT03278665
	Prostate cancer.	I, II	NCT02913131
	Merkel cell carcinoma.	II	NCT04874831
	GI cancer.	II	NCT03812796
CUDC-101	Head and neck cancer.	I	NCT01384799
	Breast cancer; gastric cancer, head and neck cancer, liver cancer, non-small cell lung cancer.	I	NCT01171924

[a]Data was obtained from www.clinicaltrials.gov.

explain why non-selective HDAC inhibitor-based PROTACs are able to selectively degrade a defined subset of HDACs[294]. Notably, their studies also revealed that CRBN-based PROTACs prefer to degrade HDAC6/8, IAP recruiting PROTACs preferentially degrade HDAC6 and PROTACs with VHL exhibit preference for HDAC3 in KELLY cells[294]. Aside from E3 ligase ligand, both linker and cell identity affect the degradability of HDAC family proteins.

Different from traditional HDAC inhibitors, the catalytic mode of action confers PROTACs a series of advantages and it can be an effective approach to overcome drug resistance, does limited toxicity, "undruggable" target protein or the isoform selectivity limitation of traditional inhibitor. Some degraders under clinical investigation strongly proved that PROTACs is a promising technology in drug development[295,296]. However, keep in mind that there are still some problems and challenges alongside with PROTACs and we still need to devote more efforts to address these issues.

6. Conclusions

HDAC enzymes, as critical modulators, regulate the deacetylation/acetylation of histones/nonhistone proteins and maintain homeostasis as well as ontogeny. However, available evidence revealed that the overexpression of HDACs has been purchased as an oncogenesis biomarker and therapeutic intervention of aberrant HDACs with inhibitor is able to yield good antitumor effects. Such multifaceted features of HDACs make one believe that HDACs are promising anticancer targets. To date, decades of efforts have caused five approved HDACis, including vorinostat, romidepsin, belinostat, panobinostat and chidamide. Currently traditional HDACis, although effective in approved indications, display severe adverse effects, off-target toxicities and poor therapeutic effects on solid tumors, which hamper their clinical applications. Therefore, it is urgent to develop the next-generation novel HDACi, including isozyme-specific HDACi, combination therapy, multitarget agent and HDAC PROTAC. Isozyme-specific HDACi, an alternative to pan-HDACi, will turn into very valuable drugs since they can be used as probes for the functional investigation of individual HDAC isozymes or as effective anticancer agents with superior therapeutic efficacy and safety profile. However, it is challenging for the development of isozyme-specific HDACi due to the highly conserved catalytic domain, lack of crystal structure and little known about the enzymatic activities, biological functions of several HDAC isoforms. Combination therapy and multitarget agent are able to well address the poor therapeutic efficacy on solid tumors and drug resistance *via* synergistic or additive effects, which make them the promising trends for solid tumor therapy, especially difficult-to-treat solid tumors. Moreover, PROTAC, a novel technology in drug discovery, exhibits some advantages, whereas there are still several challenges and problems alongside with PROTAC. In summary, isozyme-specific HDACi, combination therapy, multitarget agent and HDAC PROTAC will likely be the trends of next-generation HDACi, whereas more efforts need to be devoted to addressing current challenges.

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Author contributions

Tao Liang conceptualized and wrote the manuscript. Reham M Elhassan and Xiaolei Tang revised the manuscript. Fengli Wang and Yongmei Cheng drew figures. Wengang Chen, Xuben Hou and Hao Fang reviewed the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

Appendix A. Supporting information

Supporting data to this article can be found online at https://doi.org/10.1016/j.apsb.2023.02.007.

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