Recent Perspectives of Anticancer Histone Deacetylase Inhibitors

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Abstract
Histone deacetylases (HDACs) are common targets for cancer therapy as they are expressed in many forms of cancers; several research studies have been introduced discussing the design of small molecules that target this abnormal epigenetic changes developed by HDACs in chromatin. In the past 10 years, HDAC inhibitors have emerged as important agents of interest in clinical trials for several types of cancers and other diseases. Due to the recent availability of a number of HDACIs into market as effective anticancer agents (like vorinostat, belinostat, panobinostat, romidepsin, chidamide and pracinostat), HDACIs are considered one of the promising targeted anticancer agents. The current review highlights the most recent chemical modifications of HDACIs including different caps, linkers and zinc binding groups and interestingly the dual acting or multi-targeted HDACIs.

Key words
Anticancer, HDACIs, Hydroxamic acids, Multi-targeted HDACIs

1. Histone deacetylases (HDACs)

1.1. HDACs overview
Histone deacetylases (HDACs) have received great attention in the research area of epigenetics. Acetylation by HATs transfers an acetyl group and neutralizes the positive charge of lysine residues in histone tail resulting in loosen histone–DNA interactions and allow access of transcription factors to DNA. The stimulatory effect of HATs on gene expression is reversed by HDACs, which remove an acetyl group from the terminal amino group of lysine residue leaving a positive charge of lysine residues in histone tails and thereby repress transcription and induce gene silencing. [1–3] Thereby, HATs are defined as co-activators and the HDACs are co-repressors. [4] Consequently, a higher level of acetylation induces apoptotic cell death, whereas a higher level of deacetylation promotes gene repression and suppression of tumor suppressor genes (p53, p21, p27) resulting in carcinogenesis. In normal cells, there is a balance between HATs and HDACs activity. On the contrast, there is an imbalance between histone acetylation / deacetylation in cancer cells through over-expression of HDACs or suppression of HATs that result in oncogene activation, tumor progression and tumor suppressor gene inactivation. [1] The aberrant expression and/or activity of HDACs and HATs have been associated with the development of different tumors therefore, HDACs were found to be over-expressed in a wide variety of human cancers. [5,6]

1.2. Classes of HDAC enzymes

Eighteen HDACs have been identified and grouped into four classes (class I, II, III and IV) based on the homology to their yeast Rpd3, Had 1 and Sir2. [7] Classical HDACs include Class I, II, and IV and comprise 11 family members, whereas class III members are called sirtuins. Classical HDACs and sirtuins differ in their catalytic mechanisms; Classical HDACs are Zn²⁺-dependent enzymes with a Zn²⁺ ion as essential co-factor at its active site that can be inhibited by Zn²⁺ chelating histone deacetylase inhibitors. In contrast, class III enzymes that called sirtuins requiring NAD⁺ as an essential cofactor. [8–15] The class I HDACs comprises HDAC1, 2, 3 and 8 that are found to be expressed and localized mainly in the nucleus of the cells and having sequence homology with the yeast Rpd3 (reduced potassium dependency 3). [16] On the other hand, class II HDACs is further divided into two subclasses: class IIa which includes HDACs 4, 5, 7, and 9 and class IIb which includes HDACs 6 and 10. [17] Class II HDACs have homologous similarity with yeast gene Hda 1, they are primarily localized in the cytoplasm but can shuttle between the nucleus and the cytoplasm. [18] Moreover, class III HDACs or sirtuins (SIRT1-7) have homologous similarity with yeast Sir2 (silent information regulator 2). They are localized in nucleus, mitochondria and cytoplasm. [19] The only member of class IV is HDAC11 which is found in both the nucleus and the cytoplasm. [20] It has a structural similarity to class I, II enzymes. [17]

1.3. HDACs and cancer

HDACs are considered as a potential target for regulation of epigenetic aberrance. [21] HDACs reverse the function of HATs by deacetylating core histones. In addition, they regulate non-
histone proteins, including tumor suppressors, signaling mediators and transcription factors. [22] Most importantly, different HDACs are over-expressed in many cancer cell lines and tumor tissues and also have an important role in tumor development. [23] Individual HDACs regulate tumorogenesis via quite diverse mechanisms. Because HDACs induce a range of cellular and molecular effects through modification of histone and nonhistone substrates, HDACs could either repress tumor suppressor gene expression (p21, p53) or regulate the oncogenic cell-signaling pathway. The contribution of HDACs to cancer may not necessarily be related to the level of HDAC expression, because the aberrant activity of HDACs is also common in cancer development. Aberrant expression of classical (class I, II, IV) HDACs has been linked to a variety of malignancies, including solid and hematological tumors. [24] High expression of HDAC1, 2, and 3 are associated with poor prognosis in gastric, ovarian, prostate, lung, breast and colorectal cancers. [25–27] Similarly, over-expression of HDAC-6 and HDAC-7 has also been found in cutaneous T-cell lymphoma and pancreatic adenocarcinoma. [26] Moreover, HDAC6 is over-expressed in mammary tumors, HDAC8 is overexpressed in neuroblastoma cells and HDAC11 mainly in rhabdomyosarcoma. [27,28] According to the above data, the inhibition of HDACs introduces anticancer activities in many cancer cell lines. Inhibition of HDACs promotes cell differentiation, apoptosis, cell cycle arrest and inhibit angiogenesis in many cancer cells. Hence, HDAC inhibitors are considered as promising targets for the development of new anticancer drugs. [29–31]

2. Histone deacetylase inhibitors (HDACIs)

2.1. HDACIs overview

Histone deacetylase inhibitors (HDACIs) have been emerged as a new class of anti-cancer agents that play important roles in epigenetic regulation of gene expression inducing death, apoptosis, and cell cycle arrest in cancer cells. [32–34] A number of HDACIs with much more potent anticancer effects and diverse structures have been identified; they include natural or synthetic products. [35,36] Recently, many HDAC inhibitors have been clinically validated in cancer patients resulting in the approval of five HDACIs, vorinostat, romidepsin, belinostat, panobinostat and pracinostat by the FDA and chidamide by Chinese FDA for the treatment of cutaneous, peripheral T-cell lymphoma (CTCL, PTCL) multiple myeloma (MM) and acute myeloid leukemia (AML) (Figure 1). [37–45] SAHA, belinostat, and panobinostat are pan-HDACIs since they targeting multiple HDAC isoforms, while romidepsin is a selective one. [46,47] Several new HDACIs are in different stages of clinical development for the treatment of hematological malignancies as well as solid tumors. HDACIs have the potential to be used as mono-therapies or in combination with other anticancer therapies. [35,48] Also, great efforts are exerted to discover novel HDACIs for use as anti-cancer drugs alone or in combination and have isoform selectivity are continuing by researchers. [49]

![HDACIs](https://example.com/hdacis.png)

**Figure 1:** FDA approved HDACIs

2.2. Mechanism of action of HDACIs as anticancer

The HDACIs induce acetylation of histones and non-histone proteins and alter gene expression leading to transcriptional activation of certain genes and repression of others. The anticancer activity of HDACIs is stemmed from their ability to regulate the expression of specific proliferative and/or apoptotic genes. HDACIs induce cancer cell cycle arrest, differentiation; reduce angiogenesis and leading to cell death. [27,50,51]

a) Induction of Cell Cycle Arrest

HDACIs increase expression of cell cycle genes such as p21 (CDK inhibitor), and in addition, inhibit CDK activity via down-regulation of cyclins that eventually induce cell cycle arrest and differentiation. [52–54]

b) Anti-angiogenesis

HDACIs can inhibit tumor angiogenesis via suppression of pro-angiogenic factors, including vascular endothelial growth factor (VEGF), hypoxia-inducible factor-1α (HIF-1α), and up-regulation of p53 that inhibit hypoxia-induced angiogenesis. [55–57]

c) Activation of apoptotic pathways

HDACIs induce apoptosis via both the extrinsic and intrinsic pathways. The extrinsic pathway of apoptosis cause up-regulation of TNF-α that induces activation of caspase 8 and 10, resulting in apoptotic cell death. On the other hand, the intrinsic pathway leads to increase expression of pro-apoptotic proteins including Bim, Bak, Bax, and caspase-3, while the expression of anti-apoptotic proteins such as Bcl-2 is decreased. [58,59]

d) Induction of DNA damage and inhibition of DNA repair

HDACIs activate the DNA damage pathway. They alone do not induce double-strand breaks but synergize the activity of DNA damaging agents to inhibit tumor growth. Moreover, HDACIs
reduce the expression of DNA repair proteins. Reactive oxygen species (ROS) are accumulated, leading to DNA damage after treatment with these agents. [25,51,60,61]

2.3. General Pharmacophore of HDACIs

The known HDACIs share a common pharmacophore. This pharmacophoric model consists of three structural parts named a cap group also named surface recognition moiety (SRM), a hydrophobic spacer (HS) or the linker, and a zinc-binding group (ZBG) (Figure 2). [62–64] The cap group linked to a HS through a connection unit (CU). At the end of the HS, a ZBG chelates with the zinc metal and inhibits enzyme activity. Cap group able to interact with the surface of the catalytic tunnel of the enzyme and it is an extremely variable moiety, ranging from a simple benzene ring to a more complex cyclic tetrapeptide. The HS that fits in the narrow hydrophobic channel of the active site is usually with various length linear hydrophobic methylene groups (saturated or unsaturated), aromatic or hetero-aromatic. The ZBG chelates with the zinc metal and the most common ZBGs include hydroxamic acid, [65–72] 2-aminoanilide, [73–75] electrophilic ketones [76] and short chain fatty acids. [77,78] The changes in the cap group, the linker or the ZBG individually can provide selectivity for specific HDAC isofrom. [79]

![Figure 2: Classical pharmacophore of HDACIs and the structure of SAHA.](image)

2.4. Classes of HDACIs

HDACIs are classified into different distinct classes based on their ZBG: hydroxamic acids, 2-aminoanilides (benzamides), cyclic peptides, electrophilic ketones, and short chain fatty acids. [35,46,80] These molecules are more or less specific to one/several HDAC enzymes or HDAC classes. The hydroxamates were the first discovered and still being the most potent and popular HDACIs. Cyclic peptides are the most structurally complex class of HDACIs. Benzamides have lower activity than the corresponding hydroxamic acids and cyclic peptides. The compounds in benzamide class are selective and potent inhibitors of Class I HDACs. The short-chain fatty acids are relatively weak inhibitors of the HDACs. [81–84]

Hydroxamic acids

Hydroxamic acid derivatives represent the most popular and potent class of HDACI drugs [85,86]. According to the x-ray crystal structure of trichostatin A (TSA) and SAHA binding interactions with the active site of histone deacetylase-like protein (HDLP), the hydroxamic acid group chelates zinc ion in a bi-dentate fashion through its carbonyl, and hydroxyl groups. Also, three additional hydrogen bonds arises between the carboxyl, the hydroxyl, and the amino groups of hydroxamic acid derivative and Tyr 297, His 132, His 131 of HDLP (Figure 3). [87]

![Figure 3: Chelation of hydroxamic acid group with zinc ion in the active site of HDLP.](image)

A large number of hydroxamic acid containing HDACIs are known. Among the first known hydroxamates was the natural HDACI trichostatin A (TSA) (Figure 4). [88] TSA was the first HDACI identified and belonging to the hydroxamate group. It is an organic antibiotic produced by Streptomyces hygroscopicus and further studies proved that it has potent HDAC inhibitory activity [88,89] but not entered clinical use due to its toxicity. [90]

![Figure 4: Structure of TSA.](image)

Moreover, SAHA, belinostat, and panobinostat are another hydroxamate HDACIs that approved by the FDA for treatment of CTCL, PTCL and MM. Due to the importance of hydroxamic acid derivatives as a promising anticancer agents that are effective against different cancer types, several research groups have been interested in development of newer more potent and less toxic agents. A series of saccharin hydroxamic acids [91] and 1,3,4-thiadiazole-containing HDACIs [92] have been developed. Among them, compounds 1-5 showed similar or better HDACs inhibitory activity compared with SAHA. Compound 2 specifically exhibited potent anti-proliferative activity against breast cancer cell MDA-MB-231 and prostate cancer cell PC-3.
with IC\textsubscript{50} 4.34 μM and 9.28 μM, respectively. A linker with five carbon units between the ZBG and the saccharin ring as a cap group was optimal for potency. In order to improve potency of compound 4, the phenyl moiety are replaced by its bioisostere thiienyl moiety. The formed thiienyl derivative 5 showed higher activity than SAHA with IC\textsubscript{50} of 310 nM and 416 nM, respectively where the 6-carbon linker afforded the most potent cell growth inhibition.

Wang et al., have reported the synthesis of a series of quinoline HDACIs 5-9. The prepared compounds showed good inhibitory activities against HDACs and potent anti-proliferative activities against some tumor cell lines. Compound 5 exhibited better HDAC inhibitory activity than SAHA with IC\textsubscript{50} value of 85 nM and 161 nM, respectively. Moreover, compound 5 showed better anti-proliferative activity than SAHA. The substituents on the C4 and C6-position of the quinoline ring significantly affected activity. Substituents on the C4-position of quinoline ring decreased the enzyme inhibitory activity. Meanwhile, substituents on C6-position of quinoline ring enhance the enzyme inhibitory activity; it is considered crucial for binding affinities. The best substituent in this position was halogens and by replacing halogen with a phenyl group resulted in a decrease of enzymatic inhibitory activity. Regarding the linker length, the most suitable length was 5 that helped the ZBG to chelates the zinc ion.

Zhang et al., have reported the synthesis of a series of hydroxamic acid-based HDACIs with 4-aminoquinazolinyl moieties as cap groups 10, 11. The derivatives showed good activities against HDAC 1, 2, 3. In particular, the thienopyrimidine derivative 13 exhibited excellent inhibitory activity against HDAC1, HDAC3, and HDAC6 with IC\textsubscript{50} values 29.81 ± 0.52 nM, 24.71 ± 1.16 nM, and 21.29 ± 0.32 nM, respectively. It showed strong anti-proliferative activity against these two cancer cell lines with IC\textsubscript{50} of 0.97 ± 0.072 μM and 1.01 ± 0.033 μM, respectively.

Drug development in hydroxamic acid HDACIs resulted in the introduction of Ricolinostat (ACY-1215) 12, a potent selective HDAC6 inhibitor, it inhibited the HDAC6 with IC\textsubscript{50} of 4.7 nM. This compound is currently under phase II clinical trials. It is effective alone or in combination with dexamethasone and either bortezomib or lenalidomide which gave a synergistic effect against multiple myeloma.

Recently thienopyrimidine based HDACIs 13-17 have been synthesized and evaluated for their anti-proliferative and HDAC inhibitory activity on leukemia cancer cell RMPI-8226 and colon cancer cell HCT 116. These derivatives showed good activities against HDAC 1, 2, 3. In particular, the thienopyrimidine derivative 13 exhibited excellent inhibitory activity against HDAC1, HDAC3, and HDAC6 with IC\textsubscript{50} values 29.81 ± 0.52 nM, 24.71 ± 1.16 nM, and 21.29 ± 0.32 nM, respectively. It showed strong anti-proliferative activity against these two cancer cell lines with IC\textsubscript{50} of 0.97 ± 0.072 μM and 1.01 ± 0.033 μM, respectively.
A novel hydroxamic acids have been designed by Dung et al., that include 2-oxoindoline as a cap group. [97] These hydroxamic acids 18, 19 potently inhibited a class-I HDAC 2 isoform with IC₅₀ values 1.28 μM for 5-Ch₂ and 0.91 for 5-OCH₃. They exhibited cytotoxicity up to 8-fold more potent than SAHA in three human cancer cell lines, including the colon cancer SW620, prostate cancer PC3 and pancreatic cancer AsPC-1. In designing these compounds, they incorporated the triazole moiety to increase hydrogen bonding with the amino acid in the active binding sites of HDAC, and still serving as a part of a linker between the 2-oxoindoline and hydroxamic acid moieties.

Moreover, different SAHA analogs have been synthesized to improve selectivity. The nonselective HDACI SAHA was modified at the C2, C3, C4, C5, and C6 positions of the linker introducing compounds 20-24. [98–102] The C₂-n-hexyl SAHA analog 20 displayed 49-300 fold selectivity for HDAC 6 and 8 over HDAC 1, 2, and 3. Substituents on the C₂ position 21 displayed HDAC 6 selectivity, compared to the broad-spectrum inhibitor SAHA and showing IC₅₀ of 350 nM, which is only four-fold less potent than SAHA. The potency of analogs decreased by increasing the size of C₂ substituent and the methyl substituent displayed the most potent analog. The C₄-benzyl SAHA analog 22 exhibited dual HDAC 6/8 selectivity with 520 to 1300 fold increase for HDAC 6 and HDAC 8 over HDAC 1, 2, and 3, and IC₅₀ values of 48 and 27 nM with HDAC 6 and 8, respectively. Longer substituents at the C₄ position led to greater HDAC 6/8 selectivity. Moreover, C₅-modified SAHA analog 23 also exhibited dual selectivity to HDAC 6 and HDAC 8 over HDAC 1, 2, and 3, with only a modest reduction in potency in HDAC 6 inhibition but enhanced HDAC 8 inhibition compared to SAHA. It showed 8-21 fold increase in selectivity for HDAC 6 and HDAC 8 over HDAC 1, 2, and 3 with IC₅₀ values of 270 and 380 nM for HDAC 6 and HDAC 8, respectively. The size of the substituent has affected selectivity, bulky substituents in the linker region enhanced HDAC 6/8 selectivity due to the wider active site entrance of both HDAC 6 and HDAC 8 compared to HDAC 1, 2 and 3 that exhibited steric clash with the compound entered and leading to a reduction in inhibition of HDAC 1, 2, and 3. Furthermore, C₅-SAHA analogs have been synthesized and the selectivity and potency were measured. C₅-phenyl SAHA 24 analogue was the most potent analog, displaying IC₅₀ values in nanomolar concentrations. It experienced four-fold reduced potency compared to SAHA. In addition, it inhibited HDAC 1, HDAC 3, and HDAC 6. In contrast, the C₆-methyl SAHA analog showed modest selectivity for HDAC 1 and HDAC 3 over HDAC 6 at 500 nM. The aforementioned results revealed that small structural changes in the linker region of SAHA could significantly impart selectivity.

Aboeldahab et al., have designed a new HDACIs that involved spirohydantoin and 1,2,4 triazole groups 25-27. [103] Compound 25 was the most potent growth inhibitor against breast cancer cell line MCF-7, having IC₅₀ of 2.56 μM. Conversely, it showed low activity against hepatic cancer cell line HepG2. In addition, the six carbon linker gave the highest HDAC inhibitory activity with IC₅₀ value comparable to SAHA against HDAC 4 and low selectivity at HDAC 1. Moreover, increasing the linker length increased the HDAC inhibitory activity. In addition, compounds 26 and 27 showed significant HDAC inhibitory activities against the four tested HDAC isoforms. Compound 26 exhibited a potent antiproliferative activity against MCF-7 cell line but compound 27 exhibited a potent antiproliferative activity against HepG2 cell line. Moreover, compound 27 experienced a potent inhibition of TUBb polymerization and, showed higher potency than CA4.
The N-hydroxyacrylamide moiety is considered a promising linker in the design of newer HDACIs. This linker has occurred in the two important FDA approved HDACIs belinostat and panobinostat where the cinnamide moiety is considered as the hydrophobic spacer. [105]

Lu et al., have synthesized a series of HDACIs that involved isofeeric acid derivatives to improve the affinity and activity of the ferulic acid-based HDACIs that was previously synthesized. [106,107] The two compounds 30 and 31 displayed a good HDAC inhibitory activity with IC\textsubscript{50} values of 0.73 ± 0.08 and 0.57 ± 0.16 \(\mu\text{M}\), respectively. Compound 30 exhibited moderate anti-proliferative activity with an IC\textsubscript{50} value of 3.91 ± 0.97 \(\mu\text{M}\) against HeLa cells.

Pompo et al., designed a new HDACI 32 having a cinnamoyl moiety. This compound showed a potent cytotoxicity, which induced apoptosis and arrest development in osteosarcoma cancer stem cells at lower concentrations (0.1 \(\mu\text{M}\)). [108] It is considered a pan-HDACI, efficient in inhibiting all the HDAC isoforms 1-11 in the range 0.08-12 \(\mu\text{M}\).

Moreover, a series of novel N-hydroxyacrylamides have been synthesized 33 and 34. [109] The presence of N-hydroxyacrylamide moiety was associated with significant HDAC inhibitory activity. Compound 30 and 31 showed more potency than FDA approved analogues belinostat and SAHA at HDAC 1, 2, 6 and 8 but didn’t show any HDAC isoenzyme selectivity. The SAR showed that the presence of N-hydroxyacrylamide group at C3 position experienced the most potent anti-proliferative activity, shifting of this moiety from C3 to C4 led to a mild decrease of cellular activity. In addition, biososteric replacement of SO\(_2\) with CO led to a slight decrease in anti-proliferative activity. Moreover, replacement of the quinoline ring with a pyridine led to a decrease in cellular activity.

In addition, a series of HDACIs involving 4,5-indolyl-N-hydroxyphenylacrylamides has been synthesized and evaluated for their anti-proliferative and HDAC inhibitory activity. [110] The research study revealed that 4-Indolyl compounds 36 and 37 have potent inhibitory activity against HDAC1 (IC\textsubscript{50} 1.28 nM and 1.34 nM, respectively) and HDAC 2 (IC\textsubscript{50} 0.90 and 0.53 nM, respectively). The study also showed that para-substitution for N-hydroxyphenylacrylamides moiety was the best position for optimal activity.

Recently, Ling et al., have prepared a series of novel hybrids of \(\beta\)-carboline and N-hydroxycinnamamide as HDACIs to overcome drug-resistant hepatocellular carcinoma. [111] Compound 38 showed strong anti-proliferative activity with IC\textsubscript{50} values of 1.01 \(\mu\text{M}\), 0.41 \(\mu\text{M}\), 0.87 \(\mu\text{M}\), 0.69 \(\mu\text{M}\) human cancer cells SUMM-7721, Hep G2, HCT116, H1299, respectively. In addition, it selectively inhibited HDAC 1/6 with IC\textsubscript{50} of 1.3 \(\mu\text{M}\), 3.1 \(\mu\text{M}\), respectively. This selectivity of 38 for HDAC1/6 may attributed to its active fragment N-hydroxycinnamamide, which form bi-chelation with the zinc ion in the active site through its hydroxamic acid group, also be able to form sandwich-like \(\pi-\pi\) interactions with two parallel phenylalanine residues of HDAC1/6 by its vinyl benzene group. Moreover, it showed high anticancer activity against drug-sensitive HepG2 and Bel7402 cells and drug-resistant Bel7402/5FU cells. Also, compound 39 exhibited high HDAC1 inhibitory activity but still lower than 38 with IC\textsubscript{50} of 2.8 \(\mu\text{M}\).

Furthermore, a series of novel and potent N-hydroxycinnamamide-based HDACIs that involved indole as a cap group have been designed 40-44. [112] All of these compounds exhibited selectivity on HDAC1 with compound 43 being the most one with IC\textsubscript{50} of 0.92 \(\mu\text{M}\). They also showed potent anti-proliferative activity against five cancer cell lines.
On the other hand, HDACIs with phenylimidazolidin-2-one as a new linker was introduced 45-48. [113] In particular, Compound 45 displayed a potent HDAC1 inhibitory activity with IC$_{50}$ of 2.7 nM. In addition, it showed 6-9 fold increase in activity compared to vorinostat, with GI$_{50}$ value of 0.1, 0.086, 0.11 µM against prostate cancer cell PC-3, leukemia cancer cell HL-60 and colon cancer cell HCT-116, respectively. The other compounds 46-48 in this series also exhibited potent HDAC 1 inhibitory activity but lower than 45 with IC$_{50}$ of 5.4, 5.4, and 4 nM, respectively.

Blackburn et al., have prepared series of 1,2,3,4-tetrahydropyrrolo[1,2-a]pyrazine hydroxamic acid derivatives 49 and 50. [114] The pyrrole moiety serves as a cap group that imparts potency and selectivity to the compounds than larger groups like indole. Compound 49 showed a potent HDAC6 inhibitory activity with IC$_{50}$ of 33 nm. This represents 200-fold selectivity versus nuclear extract (HDAC1, 2) and 100-fold selectivity versus HDAC8.

Moreover, Mackwitz et al., have synthesized a series of HDACIs with imidazo[1,2-a]pyridine based cap groups 51-53. [115] Compound 51 having 4-aminophenyl linker is a selective HDAC6 inhibitor, it selectively inhibited HDAC6 with IC$_{50}$ 0.058 µM and selectivity factor 38. Compounds 52 and 53 also potently inhibit HDAC 6 with IC$_{50}$ 0.05 and 0.076 µM, respectively but with lower selectivity factor (3 and 11, respectively).

Moreover, a series of quinazoline 2,4-dione based hydroxamic acids 54-56 experienced a selectivity inhibition toward HDAC 6. [116] Compound 54 experienced the most potent with selectively inhibition of HDAC 6 with IC$_{50}$ 4 nM. IC$_{50}$ of compounds 55 and 56 were 5 and 20 nM, respectively. From the SAR studies, replacement of the phenyl ring with other equivalent rings or changing the position of the hydroxamic acid group reduced the HDAC 6 inhibitory activity.

A series of 2,4-imidazolidinedione derivatives as novel HDAC6 selective inhibitors have been designed 57-59. [117] They exhibited good HDAC 6 inhibitory activity with IC$_{50}$ of 9.7, 4.4, and 7.6 nM, respectively. In addition, they showed potent antiproliferative activity against five cancer cell lines with IC$_{50}$ in the range of 0.23 to 9.45 nM. Compound 58 induced apoptosis in HL-60 cell line by activating caspase 3.

Benzamides or ortho-Aminoanilides

The benzamide HDACIs were the second class of HDACIs that exhibited a good anticancer activity and binded the zinc ion in the HDAC active site through the benzamide moiety. [118,119] They exert more selectivity to specific HDAC isoforms than the hydroxamate class. Several examples of benzamide inhibitors are in clinical studies such as Entinostat (MS-275, phase III), Mocetinostat (MGCD0103, phase II), tacedinaline (CI994, phase II) (Figure 5). [120–124]
A series of HDACIs bearing a bicyclic heterocycle moiety 60-62 have been designed and synthesized based on the lead compound MS275. [125] They exerted a reasonable inhibitory potency against HDAC 1 with IC\textsubscript{50} of 0.118 μM, 0.129 μM and 0.120 μM, respectively that was higher than that of MS275 (IC\textsubscript{50} = 0.273 μM). They also exhibited more potent antiproliferative activities than MS275. From the SAR studies, the introduction of fluoro group at the benzamide ring lowered antitumor activities against three cancer cell lines. The inhibitory activities of bicyclic heterocyclic substituted compounds were better than pyridinyl substituted compounds. When replacing the methyl with ethyl results in reduction of the inhibitory activity.

Cai et al., have synthesized a series of HDACIs that involved 2-aminobenzamide moiety 63 and 64. [126] Compound 60 achieved a significant anti-proliferative activity with IC\textsubscript{50} values of 0.41 and 0.46 μM toward NCI-H661 and U937, respectively, which somewhat better than that of Vorinostat. Moreover, Compound 63 exhibited a higher selectivity for HDAC 1 over HDAC 2 with IC\textsubscript{50} value of 140 nM toward HDAC 1, and 680 nM toward HDAC 2. Compound 64 showed good anti-proliferative activity with IC\textsubscript{50} values of 0.69 and 0.73 μM toward NCI-H661 and U937, respectively. Also, it exerted higher HDAC 1 inhibitory activity with IC\textsubscript{50} of 210 nM.

Mohamed et al., have synthesized a group of novel chalcone derivatives that comprising 2-aminobenzamide group as ZBG.
they showed potent HDAC inhibitory activity against HDAC 1 and 2 with IC$_{50}$ of 0.016 μM and 0.205 μM for 73 and 0.071 μM and 0.144 μM for 74, respectively.

Cyclic peptides

Cyclic peptide HDACIs are the most complex class of all HDACIs such as apicidin, romidepsin (FK228), trapoxin B and chlamydocin (Figure 6). [131–133] The natural product romidepsin (FK-228) which has been isolated from Chromobacterium violaceum is the prototype of this class. Romidepsin is a prodrug that reduced to active form (RedFK) after uptake into the cells (Figure 7). [134–136] This naturally occurring compound exhibit very strong HDAC inhibitory activity than hydroxamate and other HDACIs, even though its thiol ZBG is a weaker group. [137]

New cyclodepsipeptide analog 77 and 78 has been synthesized and evaluated for its inhibitory potency and selectivity towards HDAC 1, HDAC 3 and HDAC 6 isoenzymes. They showed potent HDAC 3 inhibitory activity with IC$_{50}$ of 10.9 μM and 1.4 μM, respectively. Sulfonylhydrazide group serves as ZBG and the two oxygen atoms of sulfonyle group chelates with the zinc ion. [139]

Electrophilic ketones

Electrophilic ketones have been used as a ZBG replacement and included trifluoromethyl ketones. They showed potent HDAC inhibitory activity. [140] This moiety is readily hydrated in aqueous media and binds to the zinc ion in the active site of HDAC enzyme. [141]

Gong et al., have synthesized a series of bisthiazole-based compounds with a trifluoromethyl ketone as the ZBG 79-81. [142] In particular, compound 81 exhibited excellent inhibitory activity against human HDAC 1, 3, 4 and 6, with an IC50 of 20-30 nM. In addition, it showed improved anti-proliferative activity against cancer cell lines RPMI 8226, NCI-H929 with IC50 of 0.08 and 1.23 μM, respectively. The reduction of ketone group in compound 81 reduced the HDAC inhibitory activity as in compound 79. In addition, changing the length of the linker or cyclopropyl group to isobutyl or benzyl reduce the HDAC inhibitory activity of compound 81.

Based on the structure of natural and synthetic compounds such as TSA, SAHA, chlamydocin, and FK228, numerous HDACIs have been developed as potential anticancer agents. Wang et al., have synthesized a series of chlamydocin analogues 75 and 76 that have combined with various ZBGs. Compound 75 exhibited better anti-proliferative effects than TSA against breast cancer cell MCF-7 with an IC$_{50}$ value of 26 nm. Moreover, compound 75 was an effective inhibitor of HDAC 1 and HDAC 4 with IC$_{50}$ of 15 nM and 14 nM, respectively. This hybrid of cyclic peptide class and hydroxamate class termed cyclic hydroxamic acid peptide. On the other hand, compound 76 also showed a good inhibition of HDAC 1 and HDAC 4 with IC$_{50}$ of 14 nM and 19 nM, respectively. [138]
Miscellaneous

There are several HDACIs that include different ZBGs and the most common of them were thiol based HDACIs. Thiols are well-known chelators of zinc-dependent enzymes. Replacement of the hydroxamate group of SAHA with a thiol group produced thiol-based SAHA that has found to be as potent as SAHA with IC_{50} of 0.21 µM. [143]

A series of novel thiol-based HDACIs included 3-phenyl-1H-pyrazole-5-carboxamide scaffold as a cap group were synthesized. [144] Compound 83 showed a potent HDAC inhibitory activity with IC_{50} of 0.08 µM. Moreover, it exhibited more selectivity at HDAC 6 than HDAC 1 with IC_{50} of 0.09 µM and 0.13 µM, respectively due to incorporation of aliphatic carboxylic acid at position 1 in pyrazole ring. Compound 82 was more selective HDAC 6 than compound 83 with IC_{50} of 0.04 µM. Alkyl chain length with 4 carbon atom at N-1 of pyrazole ring was optimal for HDAC inhibitory activity, better than 2 or 3 carbon as in compound 83 that has high inhibitory activity. Addition of methyle group on pyrazole C-4 position diminished the HDAC inhibition activity. Replacement of thiol group with mercaptoacetamides or mercaptopropanamides decreases the inhibitory activity.

Islam et al., have prepared a disulphide SK-658 analogues 85 and 86 that exhibited a higher HDAC inhibitory activity than SAHA and SK-658 against HDAC 1, 4 and 6 with IC_{50} in the range of 2 to 8.2 nM. [145] The disulphide group reduced to sulphydryl group by dithiothreitol (DTT) that has a strong affinity to the active site.

In addition, thioesters derived from the natural compound psammaplin A displayed moderate to high potency in cytotoxic and HDAC inhibitory activity. Thioester compound 87 exhibited potent inhibitory activity against recombinant HDAC1 with IC_{50} value of 5 nM. It also had potent cytotoxic activity against breast cancer cell MCF7 with IC_{50} of 3.2 µM. [146]

3. Multi-targeted HDACIs

The cancer disease depends on more than one receptor or signaling pathway for its initiation and progression as a result the drugs that target a one receptor may be inadequate treatment. To overcome this obstacle problem, multi-targeted therapeutics have been developed that were one of the most effective strategies and advantageous than mono-therapies. This combination therapy gave a synergistic or potentiation effect that led to a significant anticancer activity. [147] A multi-targeted drug is the agent that including two or more different pharmacophores integrated in the same structure to yield a hybrid molecule. Hybrid molecules with HDAC inhibitory activity and an additional activity has become an attractive area of research field and are under development [148]. Several examples in these therapies include HDACIs with topoisomerase inhibitors, antiestrogens, antiandrogens, LSD inhibitors, tubulin inhibitors, VEGFR-2 inhibitors, EGFR/HER2 kinase inhibitors, JAK-2 inhibitors, Bcl-2 inhibitors, BRafE600E inhibitors, and DNMT inhibitors 88-100. [149–161]

4. Conclusion and future prospectives of HDACIs

HDACIs have emerged, in the last years, as potent agents in the treatment of cancer. HDAC inhibitors interfere with HDAC activity and results in cell cycle arrest, differentiation and apoptosis in cancer cells. As a result, HDAC inhibitor-based therapies have gained much attention for cancer treatment. There are several HDACIs in market (vorinostat, romidepsin, belinostat, and panobinostat, pracinostat and chidamide) and others in clinical trials that displaying significant activity in both hematological and solid tumors of various tissues. HDACIs not only play a role in the etiology of cancer but also in the etiology of various clinical disorders such as central nervous diseases, systemic sclerosis, cardiovascular diseases, chronic pain and depression. [162–167] Thus, identification of new multitarget HDACIs to overcome cancer resistance, the development of selective HDACIs and the optimization of HDACIs in combination chemotherapy continue to attract more and more attention to their development by researchers. The search for more selective HDAC isotypes may overcome the toxicity associated with the treatment with HDACIs. In this review, we focused at the recent developments in HDACIs, the multitargeted agents and the effect of HDACIs on cancer cells.
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88: HDAC1 + Tubulin inhibitor
HDAC1 IC_{50} = 0.72 μM
HDAC3 IC_{50} = 0.44 μM

89: HDAC1 + Antiandrogen
HDAC6 IC_{50} = 0.0356 μM
AR binding IC_{50} = <0.03 μM

90: HDAC1 + EGFR/HER2 Inhibitor
HDAC1 IC_{50} = 0.12 nM
HDAC6 IC_{50} = 0.72 nM
EGFR IC_{50} = 3.2 nM

91: HDAC1 + JAK2 Inhibitor
HDAC1 IC_{50} = 83 nM
HDAC6 IC_{50} = 0.25 nM
JAK2 IC_{50} = 0.041 nM

92: HDAC1 + Antiestrogen
HDAC3 IC_{50} = 0.73 μM
HDAC6 IC_{50} = 0.30 μM

93: HDAC1 + VEGFR-2 Inhibitor
VEGFR-2 IC_{50} = 84 nM
HDAC IC_{50} = 2.8 nM

94: HDAC1 + Topoisomerase I Inhibitor
HDAC1 IC_{50} = 37 nM
HDAC6 IC_{50} = 81 nM
HDAC8 IC_{50} = 1046 nM

95: HDAC1 + LSD1 Inhibitor
HDAC1 IC_{50} = 15 nM
HDAC2 IC_{50} = 23 nM
LSD1 IC_{50} = 1.20 μM
References


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C6 Inhibitors.


