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(Review)

Recent advances in dual-targeting HDAC inhibitors for cancer treatment

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ABSTRACT

Histone deacetylases, often known as HDACs, are a class of enzymes that remove acetyl from the ε-N-acetyl lysine histones, thereby enabling histones to surround DNA with greater fidelity. HDACs are involved in a wide variety of biological activities, including gene regulation, transcription, cell proliferation, angiogenesis, migration, differentiation, and metastasis, among others. As a consequence, HDACs constitute an outstanding target for the discovery of anti-cancer drugs. The search for histone deacetylase inhibitors (HDACIs) has been expanded over the past decade with several B, and some have been offered. However, the HDACIs that are currently accessible are predominantly non-isoform selective and suffer from a number of problems, including limited efficacy, drug resistance, and toxicities. As a result, isoform-selective HDACIs and HDACIs with dual targeting capabilities have garnered a lot of attention from both academia and industry over the past five years. As a result, significant progress has been made in this field. In this paper, we summarize recent developments on HDACIs with dual targeting capabilities and their potential application in cancer treatment.

Keywords: HDAC; dual target; HDACIs.

Introduction

Cancer arises from alterations in the genetic material and variations in gene expression, including DNA methylation and histone modifications, which can impact the chromatin structure. [1],[2]. The nucleosome is the primary structural unit of chromatin, with a histone core that is coiled around it. A nucleosome consists of a pair of core histones, specifically H2A, H2B, H3, and H4 [3]. The main types of histone modifications include histone acetylation, phosphorylation, and methylation. Of these processes, histone acetylation is the predominant epigenetic process disrupted in cancer. [4]. The dysregulation of histone acetylation is intricately linked to the onset and progression of several malignancies. Typically, the process of acetylation of histones promotes the separation of DNA from histone octamers in nucleosomes, resulting in a more open and relaxed conformation. This allows for the binding of several transcription factors and cooperative transcription factors to DNA, resulting in the activation of specific genes that are engaged in different subsequent transcription processes [5]. Histone acetylation and histone deacetylation in the nucleus are regulated by histone acetyltransferases (HATs) and histone deacetylases (HDACs), respectively, to provide a stable and dynamic equilibrium. Histone acetyltransferases (HATs) catalyze the transfer of the acetyl group from acetyl-CoA to a specific lysine residue located at the amino terminus of histones. HDACs are enzymes that facilitate the removal of acetyl groups from histones, which have a strong affinity for negatively charged DNA. This leads to the condensation of chromatin and hinders the process of gene transcription. In addition, HDACs also specifically influence several non-histone substrates, including hormone receptors, chaperone proteins, and cytoskeleton proteins, to control essential physiological processes such as cell growth and cell apoptosis. Figure 2 illustrates the physiological roles of HDACs. HDACs are commonly acknowledged as transcriptional repressors because they can stabilize and condense chromosomes, hence reducing their accessibility to transcription factors [6]. In addition, HDACs act as suppressors of programmed cell death, whereas the administration of HDAC inhibitors boosts the synthesis of proteins that promote cell death, such as Bad, and decreases the amounts of proteins that prevent cell death, such as Bcl-2. HDAC inhibitors induce programmed cell death (apoptosis) in cancer cells through both internal (intrinsic) and external (extrinsic) routes [7]. In addition, HDACs have essential functions in DNA damage pathways since they act as central regulators of chromatin remodeling and the acetylation status of proteins involved in DNA damage. HDACs play a crucial role in regulating the cell cycle by influencing key components such as cyclin, CDK,

and nuclear material replication [8]. Recent research has confirmed that the process of autophagy is controlled by acetylation, specifically through the actions of HDAC6, which can trigger autophagy. Research has confirmed the connection between autophagy and the ubiquitin-proteasome system [9],[10]. HDACs are often classified into four major categories: The protein HDAC 1 belongs to the class I. The categorization of histone deacetylases (HDACs) can be delineated into the subsequent classifications: The HDAC enzymes are categorized into four distinct classes: class I (HDAC 1, 2, and 8), class II (HDAC 4, 5, 6, 7, 9, and 10), class III (comprising of seven members: sirt1-sirt7, which rely on nicotinamide adenine dinucleotide (NADþ)), and class IV (HDAC 11) [11],[12]. Class I and II HDACs are commonly referred to as "classic" HDACs. Class I HDACs are situated within the nucleus and possess the capacity to influence both histone and non-histone substrates. Class II histone deacetylases (HDACs) can be categorized into two subgroups: class IIa (comprising HDAC 4, 5, 7, and 9) and class IIb (consisting of HDAC 6 and 10). Class II HDACs have significant downstream effects. Divergent functions compared to class I HDACs [13]. Moreover, numerous studies have shown a clear link between the abnormal expression of HDACs and the development of particular cancerous tumors [14]. HDAC1 was discovered to be overexpressed in gastric [15], prostate [16], lung [17], and breast cancer [18]. Additionally, HDAC6 was increased specifically in breast cancer. In addition, there was an excessive presence of HDAC2 and HDAC3 in colorectal cancer [19],[20]. In cases of acute promyelocytic leukemia (PML), the target genes are inappropriately accessed by HDAC enzymes due to the presence of PML-RARα (retinoic acid receptor-α) [21].

1 Compounds that inhibit histone deacetylase (HDAC) enzymes.

In recent years, a significant number of HDAC inhibitors have proven to be highly effective in treating cancer. Here are the names of five medications: The following are the names of five drugs: Vorinostat (SAHA), Romidepsin (FK228), Panobinostat (LBH-589), Chidamide (CS-055), and Belinostat (PXD101) (fig.1) I have been granted permission to provide medical treatment for hematological malignancies. HDAC inhibitors generally follow a traditional three-motif pharmacophore paradigm, consisting of a "Cap" group (an aromatic amide surface recognition group), a ZBG (zinc-binding group), and a linker that connects the ZBG and Cap. HDACIs can be categorized into four main groups based on their chemical structures: hydroxamic acids (such as Trichostatin A and Vorinostat), cyclic tetrapeptides (such as Romidepsin), benzamides (such as

Entinostat), and fatty acids (such as Valproic acid and Phenyl butyrate). TSA is a hydroxamic acid that is generated from the microorganisms Streptomyces hygroscopicus naturally. The US Food and Drug Administration (FDA) has officially approved SAHA, the first histone deacetylase (HDAC) inhibitor, exclusively for the treatment of cutaneous T cell lymphoma. Hydroxamates are commonly thought to have the capacity to affect all four types of HDAC enzymes. Fk228 is a tricyclic peptide derived from Bacillus violaceus and categorized as a natural hydroxamic acid. Entinostat (MS-275) is a benzamide HDAC inhibitor that has a prolonged duration of action and a high affinity for class I HDACs. Phenylbutyrate and valproic acid (VPA) are examples of HDAC inhibitors derived from fatty acids. Despite the efficacy of HDAC inhibitors in treating different cancer types, their utilization is restricted due to the incidence of adverse effects such as diarrhea, myelosuppression, and cardiovascular harm. The adverse consequences might be ascribed to the impact of HDACs on several cellular pathways, and the existing HDAC inhibitors primarily lack specificity for specific isoforms, sometimes known as pan-HDAC inhibitors. Hence, it is imperative to devise HDAC inhibitors that exhibit enhanced efficacy and selectively target certain isoforms. Isoform-specific HDAC inhibitors can mitigate the adverse effects linked to broad-spectrum HDAC inhibitors. However, even HDAC inhibitors that specifically target a molecule can still encounter resistance in cancer treatments due to the activation of other pathways like AKT and CDK. Hence, it is crucial to develop HDAC inhibitors with the capability to selectively target several sites (known as dual-acting inhibitors) to successfully combat drug resistance caused by single-target HDAC inhibitors. During the last ten years, multiple evaluations have been conducted on medications that focus on two key regions related to HDAC. However, each study focuses on specific aspects of the dual inhibitors. Papavassiliou et al.[22] provided expert advice on the integration of HDAC inhibitors with other anti-tumor pharmacophores to create innovative tools for cancer treatment. Musso et al. provided a thorough summary of the progress made in the field of HDAC-based dual inhibitors up until 2015 [22]. In addition, they emphasized the significance of the pharmacological foundation for the logical progression of hybrid bifunctional drugs. Duan and his colleagues have performed a comprehensive analysis of dual-target inhibitors that are based on HDAC. Their review focused on analyzing the theoretical principles that underlie the creation of dual-target medications using HDAC, as well as the molecular interactions between HDAC and other target proteins. Furthermore, they investigated the methods by which compounds interact with their target proteins, yielding crucial insights into the relationships between the

structure and function of these agents that target several locations. Given the ongoing advancements in the creation of dual-acting inhibitors that specifically target HDAC, it is imperative to provide a thorough summary of the latest accomplishments in this area from several angles [22].

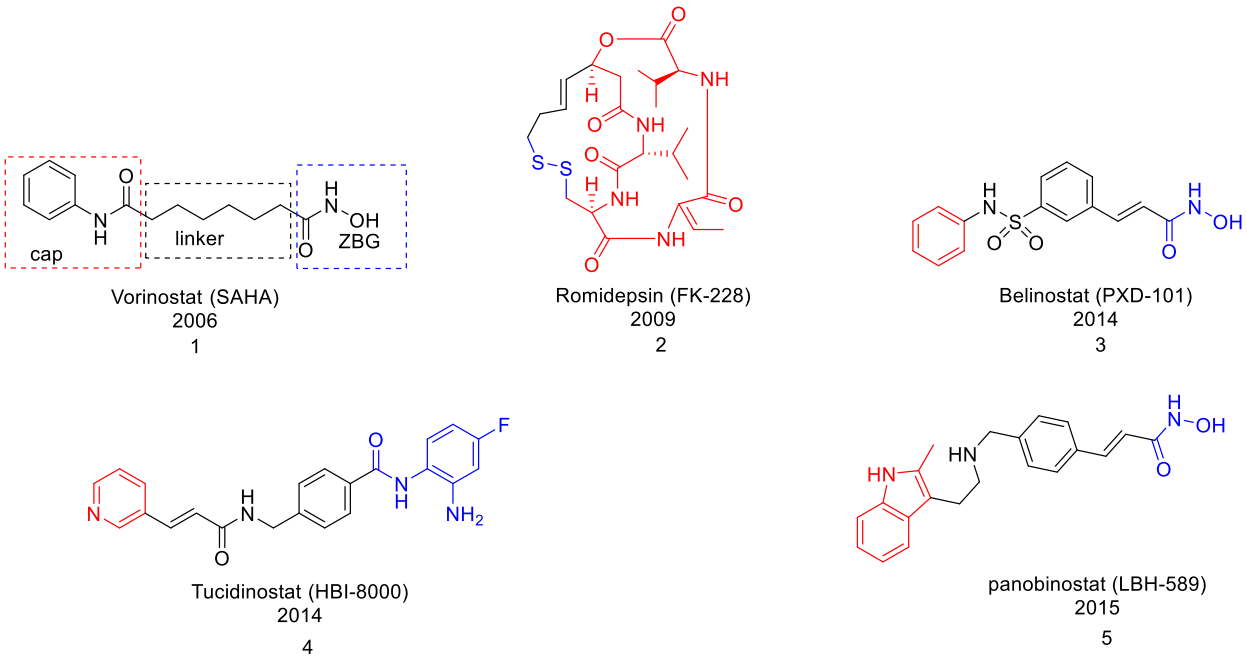
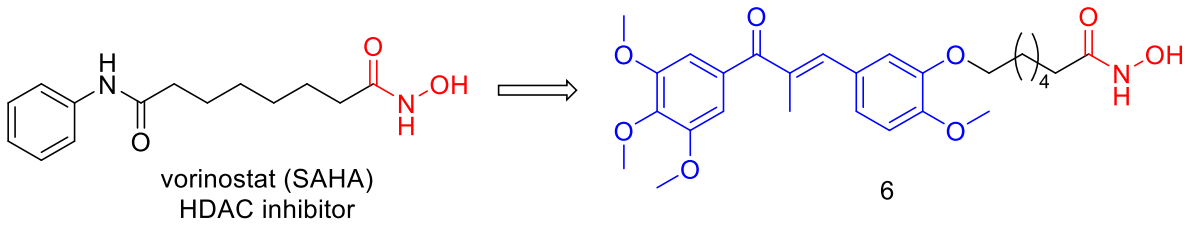


Figure 1. The approved anticancer drugs targeting HDACs [22].

2 Categorization of HDAC inhibitors with multifaceted targeting capabilities

2.1 Dual Tubulin/HDAC Inhibitors

Microtubules, which are made up of tubulins, have a crucial function in preserving cellular structure and enabling intracellular transportation. They are regarded as a prominent target for anti-cancer drugs. Studies have shown that the simultaneous use of tubulin inhibitors, such as taxanes and vincristine, along with HDAC inhibitors can result in synergistic effects. This discovery provides evidence for the advancement of dual tubulin/HDAC inhibitors [23]. **Wang et al.** found the chalcone molecule as a dual inhibitor of tubulin and HDAC. Compound 6 demonstrated greater inhibitory efficacy against A459 cells (IC_{50} value of 0.55 μM) and HeLa cells (IC_{50} value of 0.89 μM) compared to SAHA. Furthermore, it induced apoptosis and G2/M cell cycle arrest in A549 cells in a way that depended on the dosage [24].

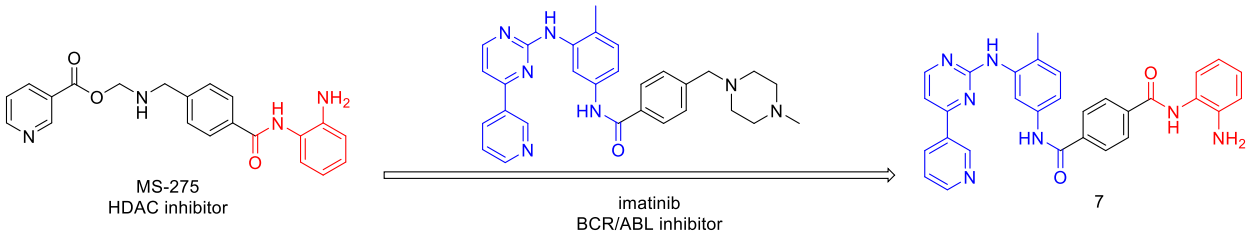


2.2 Combination inhibitors that target both protein tyrosine kinase (PTK) and histone deacetylase (HDAC) enzymes.

Protein tyrosine kinases (PTKs) catalyze the transfer of the γ-phosphate group from adenosine triphosphate (ATP) to the hydroxyl group on the tyrosine residues of substrate proteins. Protein tyrosine kinases (PTKs) exert a substantial influence on the processes of cell division, differentiation, and apoptosis. Protein tyrosine kinases (PTKs) have been a prominent area of interest in the field of cancer therapeutic research during the past two decades. HDAC inhibitors, such as the Cap and linker components, have a high degree of flexibility for structural modifications. Therefore, the scientific community has recently displayed substantial interest in the progress of dual PTK-HDAC inhibitors, as elaborated below [25].

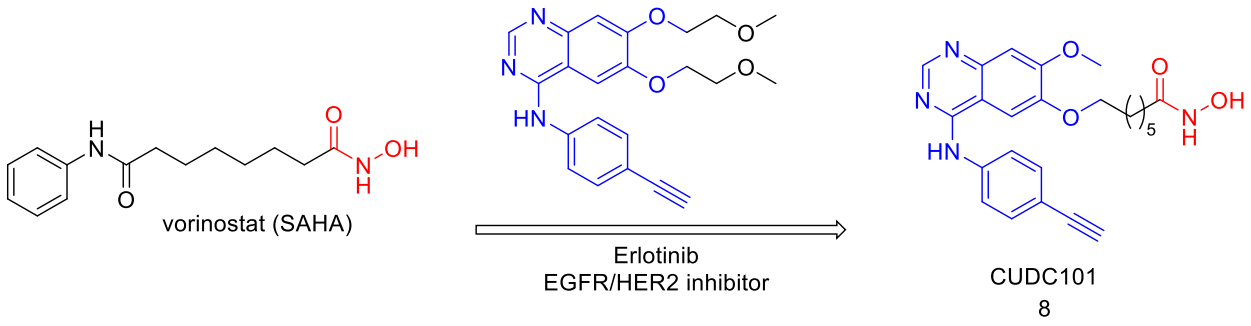
2.2.1 Dual BCR/ABL and HDAC inhibitors

Individuals diagnosed with chronic myelogenous leukemia (CML) are typically treated with tyrosine-kinase inhibitors, specifically Bcr-Abl TKIs, as the first-line therapy. Imatinib is an orally administered tyrosine kinase inhibitor (TKI) that selectively inhibits the activity of BCR/ABL kinase. Imatinib acts by competitively binding to the ATP site, inducing the enzyme to assume a closed or self-suppressed conformation. Consequently, the enzyme's activity is hindered. Compound 7 was created by Thomas et al. as a dual inhibitor of BCR/ABL and HDAC. This was achieved by mixing Imatinib with MS-275. This compound demonstrates strong inhibitory activity against HDAC1, with an IC_{50} value of 77nM. Furthermore, compound 7 exhibited similar inhibitory effects on cell proliferation as MS-275 and Imatinib when evaluated on HeLa cells [26].



2.2.2 EGFR-HDAC dual inhibitors

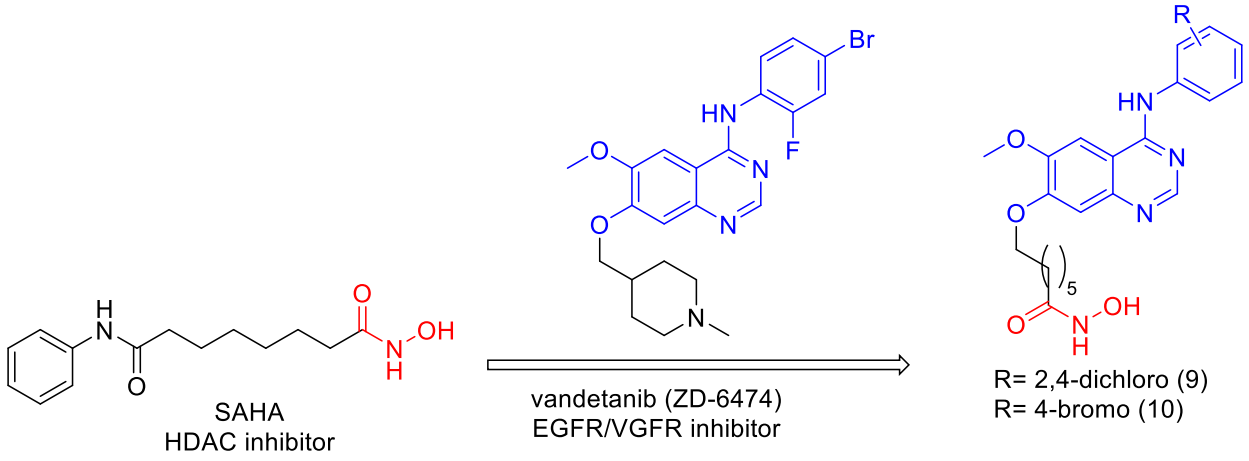
EGFR is a prominent target for PTK inhibitors, and there has been substantial progress in developing dual EGFR HDAC inhibitors by utilizing the pharmacophores of various EGFR inhibitors and the HDAC inhibitor SAHA [27]. Cai et al. combined the basic pharmacophores of erlotinib and SAHA to create a class of medicines that had strong anticancer properties. Among all the compounds, compound 8 (CUDC101) exhibited the highest level of activity, with IC_{50} values of 4.4 and 2.4nM for HDAC and EGFR, respectively [28].



2.2.3 Combination of drugs that simultaneously target vascular endothelial growth factor receptor (VEGFR) and histone deacetylase (HDAC)

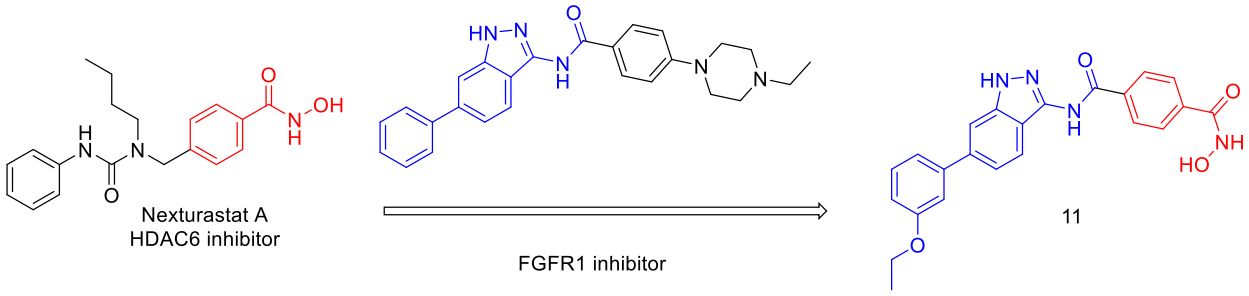
Anti-angiogenic therapy has been extensively utilized in the management of cancer. Angiogenesis is controlled by a range of angiogenic factors, with VEGFR being the primary target for anti-angiogenic therapy that has been extensively studied. Over 10 monoclonal antibodies and tiny compounds that specifically target VEGFR have been approved for the treatment of solid tumors. Nevertheless, the emergence of drug resistance has impeded the efficacy of VEGFR inhibitors in medical therapies, leading to the exploration of multi-targeted inhibitors derived from VEGFR. Peng et al. synthesized compounds 9 and 10 by merging the pharmacophore components of SAHA and Vandetanib. These compounds have dual inhibitory activity against VEGFR and

HDAC. Compounds 9 and 10 demonstrated exceptional inhibitory efficacy, displaying an IC_{50} value of less than 3 nM for HDAC and less than 85 nM for VEGFR-2 [29, 30].



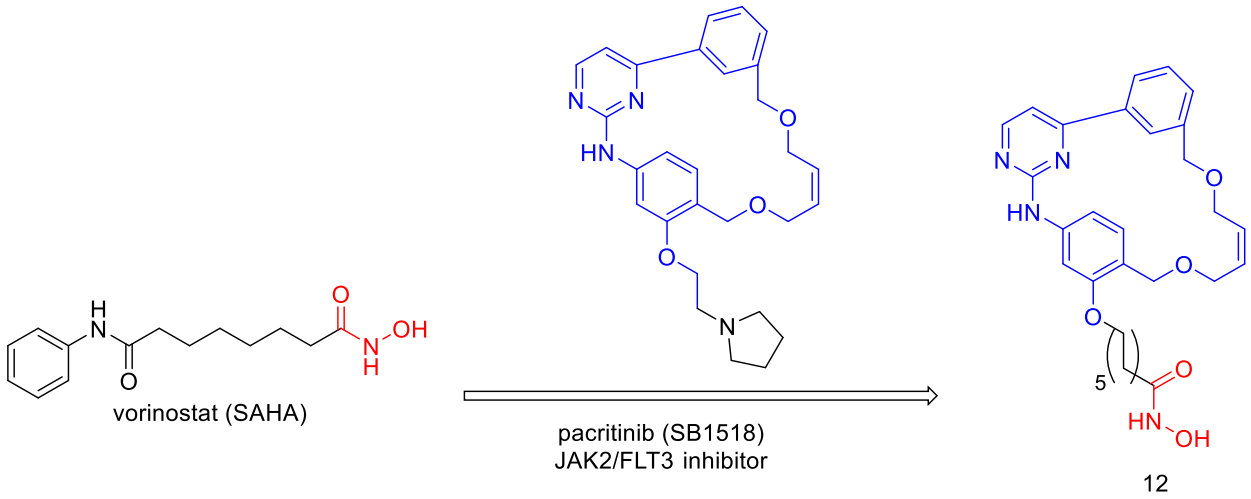
2.2.4 Combination of FGFR and HDAC inhibitors

The fibroblast growth factor (FGF) family, along with their four receptor tyrosine kinases (FGFR1, FGFR2, FGFR3, FGFR4), have a vital function in various physiological processes. FGFR overexpression has been detected in several malignancies, including breast cancer, gastric cancer, and ovarian cancer. Therefore, FGFR has become a pivotal target for cancer therapy. Liu et al. synthesized compound 11, which functions as a dual inhibitor of FGFR1 and HDAC. Compound 11 exhibited potent inhibitory activity against HDAC6, demonstrating an IC_{50} value of 34 nM. Furthermore, compound 11 exhibited similar anti-proliferative effects as SAHA (IC_{50} = 2.7 μM) and Nexturastat A (IC_{50} = 1.4 μM) on MCF-7 cells [31].



2.2.5 Compounds that inhibit both Janus kinase (JAK) and histone deacetylase (HDAC) simultaneously.

Janus kinases (JAKs) are frequently upregulated in many types of cancer and are considered a promising target for cancer therapy. Yang et al. synthesized Compound 12 by merging the pharmacophores of pacritinib and SAHA. This drug acts as a dual inhibitor of JAK and HDAC. The IC50 values for HDAC6 and JAK2 are 2.1 nM and 1.4 nM, respectively [32].

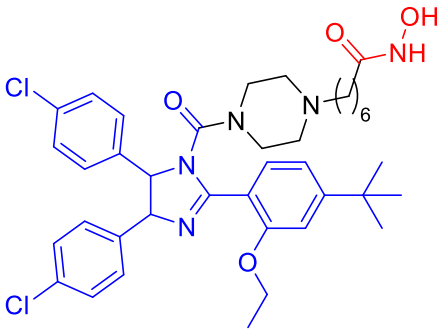


2.3 Compounds that simultaneously inhibit HDAC and other targets

Over the past few years, several chemicals that can block the activity of both HDAC and other targets have been developed. This section will specifically concentrate on the dual inhibitors that are being studied.

2.3.1 Dual inhibitors of MDM2 and HDAC

Murine Double Minute 2 (MDM2) regulates the anti-tumor activity of p53, a gene that suppresses tumor growth, by binding to the p53 protein and keeping its levels low. Compound 13 is a potent dual inhibitor that efficiently inhibits the activity of MDM2 and HDAC6 enzymes, with IC_{50} values of 0.11μM and 17.5 nM, respectively [33].

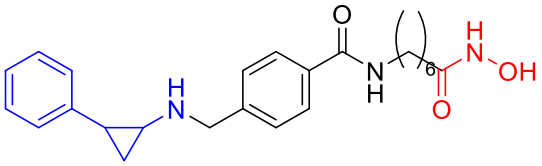


MDM2/HDAC dual inhibitor

13

2.3.2 Dual inhibitors of LSD1/HDAC

LSD1 was the first histone demethylase to be discovered. It selectively eliminates methyl groups from histones H3 lysine 4 (H3K4) and H3 lysine 9 (H3K9) that are mono or di-methylated, hence either inhibiting or enhancing gene transcription [34, 35]. Duan et al. identified a cluster of tranylcypromine derivatives, particularly compound 15, which had exceptional inhibitory efficacy. The IC_{50} values for HDAC1, HDAC2, and LSD1 were determined to be 15 nM, 23 nM, and 1.2 μM, respectively [36]. Nitric oxide (NO) is an essential signaling molecule that has a vital role in the management of cancer and inflammation [37].

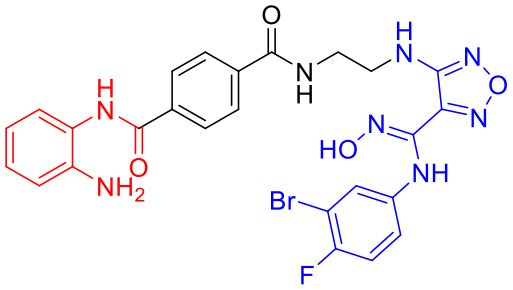


LSD1/HDAC dual inhibitor

14

2.3.3 Dual inhibitors IDO/HDAC

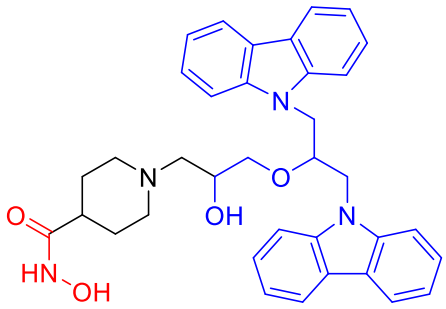
Indoleamine2,3-dioxygenase (IDO) is an enzyme that regulates the speed at which tryptophan is broken down. It exerts a beneficial influence on the proliferation of certain cancer cells via modulating the immune system [38]. Fang et al. synthesized compound 15 as a dual inhibitor that effectively targets both IDO1 and HDAC. The compound has IC_{50} values of 69.0 nM and 66.5 nM for IDO1 and HDAC, respectively [39].



IDO1/HDAC inhibitor
15

2.3.4 Dual inhibitors DNMT/HDAC

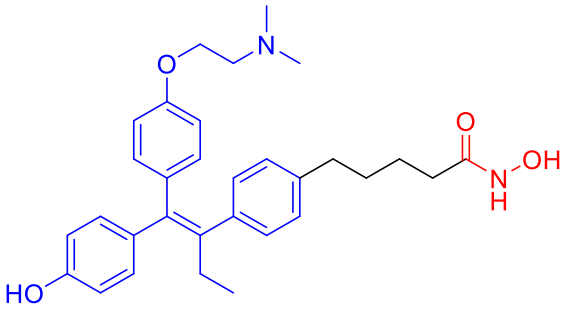
DNA methyltransferase (DNMT) is the enzymatic catalyst responsible for the addition of methyl groups to DNA. Overexpression of DNMT can lead to the suppression of tumor suppressor genes. A study conducted by Yuan et al. discovered that compound 16, which consists of NSC-319745 and SAHA, exhibits dual inhibition of DNMT and HDAC. Compound 16 exhibited potent inhibition of these enzymes, with IC_{50} values of 2.02, 0.93, and 4.16 μM for DNMT1/3A and HDAC1, respectively [40].



DNMT/HDAC inhibitor
16

2.3.5 Dual inhibitors ER/HDAC

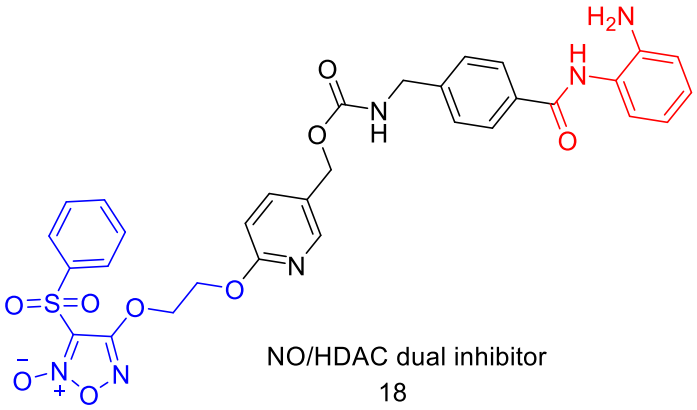
The estrogen receptor (ER) is a transcription factor that is controlled by ligands. It has been discovered that it is over-expressed in around 80% of breast cancers. Compound 17 had a notable inhibitory effect against HDAC3/6 and ERs, with IC_{50} values of 0.734, 0.3, and 0.82 μM, respectively [41].



ERs/HDAC dual inhibitor
17

2.3.6 Dual Inhibitors of NO/HDAC

They synthesized a series of compounds that function as dual inhibitors of nitric oxide (NO) and histone deacetylase (HDAC) by connecting the NO donor component to the α-position of the pyridine ring in MS-275. The consequences of enzyme inhibition. Compound 18 showed higher levels of inhibition against HDAC1, 2, and 3 in comparison to MS-275, as indicated by the results. Compound 18 had IC_{50} values of 1.02, 0.37, and 1.19 µM for HDAC1, 2, and 3, respectively. In comparison, MS-275 had IC_{50} values of 1.07, 0.74, and 1.23 µM for the same HDAC enzymes [42].

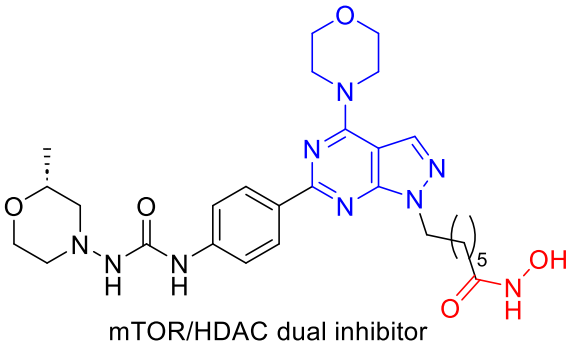


NO/HDAC dual inhibitor
18

2.3.7 Dual Inhibitors of mTOR/HDAC

mTOR, the mammalian target of rapamycin, is a vital protein kinase that plays a pivotal role in integrating signals from both the external environment and within the cell. It is responsible for overseeing cell growth and a range of other physiological tasks [43]. Compound 19 was created by merging the pharmacophores of an HDAC inhibitor with a mTOR inhibitor, resulting in a dual

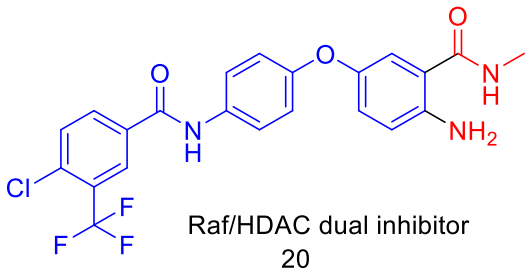
inhibitor of both targets. It exhibited potent biological action by efficiently suppressing both HDAC1/10 and mTOR, with an IC_{50} in the nanomolar range [44].



mTOR/HDAC dual inhibitor
19

2.3.8 Dual inhibitors of Ras/Raf MAPK/HDAC

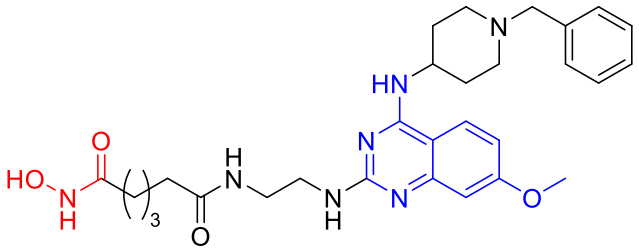
The Ras/Raf mitogen-activated protein kinase (MAPK) pathway is strongly associated with the development of tumors. Compound 20 was created by combining the properties of the Raf inhibitor sorafenib and the HDAC inhibitor Chidamide. This resulted in a dual inhibitor that can target both Raf and HDAC. Compound 20 showed significant inhibitory effects against HDAC1 (with an IC_{50} value of 1.17 µM) and B-RafV600E (with an IC_{50} value of 0.073 µM), as demonstrated by the enzyme inhibition assay [33].



Raf/HDAC dual inhibitor
20

2.3.9 Dual Inhibitors of HMT/HDAC

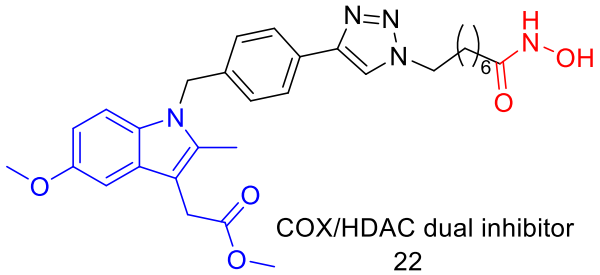
G9a is a methyltransferase enzyme that catalyzes the addition of a methyl group to the lysine residue on the histone protein H3. Methylation specifically targets lysine 9 on histone H3 and is involved in regulating gene expression by suppressing transcription. Zanget et al. have developed a novel pharmaceutical compound, 21, that acts as a dual inhibitor of G9a/HDAC. This compound combines the quinazoline core of a G9a inhibitor with the hydroxamic acid moiety of an HDAC inhibitor. It effectively inhibits the function of both HDAC and G9a enzymes, with IC_{50} values of 5.7 and 7.136 µM, respectively [45].



G9a/HDAC dual inhibitor
21

2.3.10 Dual Inhibitors of COX/HDAC

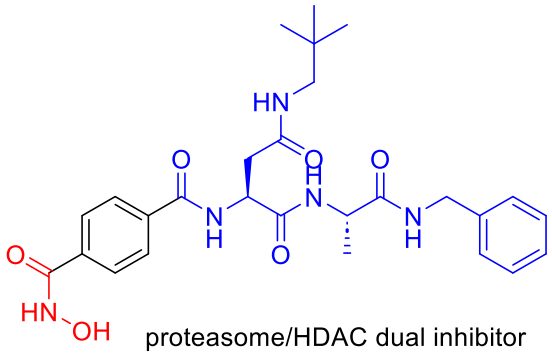
Cyclooxygenase (COX) is divided into two different subtypes: COX-1 and COX-2. COX-2 is essential for the growth of cancer cells as it consistently supplies prostaglandin E2 (PGE2) to these cells [46]. The synthesized compound 22 by employing SAHA and indomethacin. This medication exhibited strong suppression of HDAC6 activity, with an IC_{50} value of 5 nM [47].



COX/HDAC dual inhibitor
22

2.3.11 Dual inhibitors of proteasome/HDAC

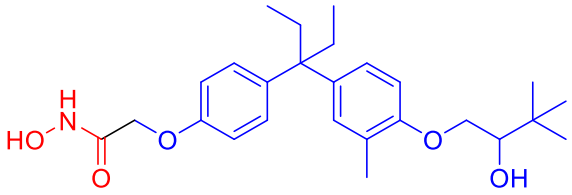
The proteasome, operating as a multi-enzyme complex, possesses the capacity to eradicate misfolded or unfolded proteins that pose a threat to cells [48]. RTX-V5 (24) is a potent dual inhibitor that efficiently blocks the activity of HDAC6 and HDAC8 enzymes. It achieves this by exhibiting an IC_{50} value of 0.27 µM for HDAC6 and 0.53 µM for HDAC8 [49].



proteasome/HDAC dual inhibitor
23

2.3.12 Dual Inhibitors of VDR/HDAC

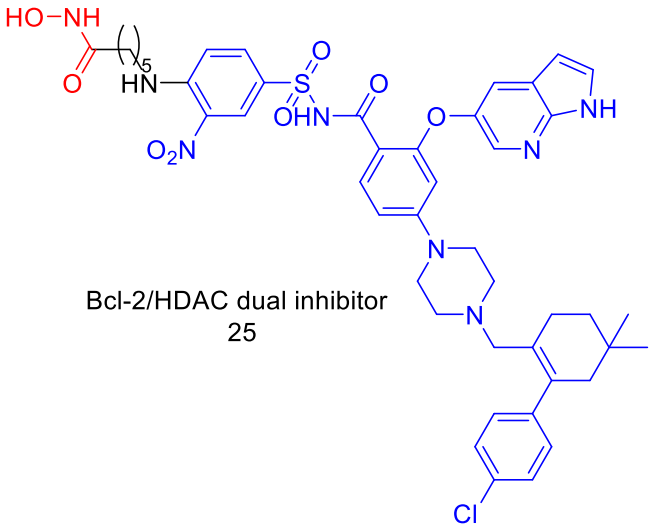
The Vitamin D receptor (VDR) is a nuclear receptor regulated by ligands. VDR, acting as a gene transcription factor, plays a crucial role in controlling the cell cycle and differentiation. Compound 24 is a highly effective inhibitor of both the vitamin D receptor (VDR) and histone deacetylase (HDAC). The compound demonstrates notable inhibitory effects on cell proliferation, with an IC_{50} value of 10 µM against 4T1 cells [50].



VDR/HDAC dual inhibitor
24

2.3.13 Dual Inhibitors of Bcl-2/HDAC

The B-cell lymphoma-2 (Bcl-2) gene acts as an oncogene and can inhibit apoptosis. Compound 25 showed the highest level of enzymatic inhibition against HDAC6 (IC_{50} = 28 nM) and Bcl-2 (IC_{50} = 0.23 µM) [51].

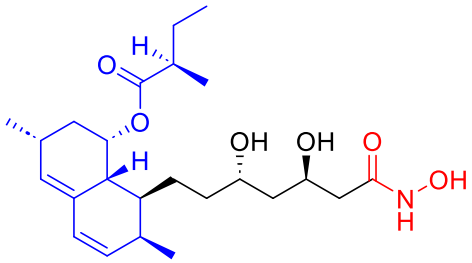


Bcl-2/HDAC dual inhibitor
25

2.3.14 Dual Inhibitors of HMGR/HDAC

Compound 26 was specifically engineered to target both HDAC and HMGR (3-hydroxy-3-methylglutaryl coenzyme A reductase) by connecting the hydroxamate group with the essential structural components of statin, allowing it to bind to both proteins. Compound 26 demonstrated

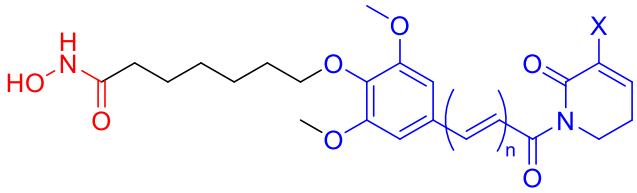
strong inhibitory activity against HDACs and HMGR, with IC_{50} values in the nanomolar range. Furthermore, compound 26 efficiently decreased the activity of HMGR and facilitated the acetylation of histone and tubulin in cancer cells. Remarkably, these effects were observed without causing any harm to normal cells [52].



HMGR/HDAC dual inhibitor
26

2.3.15 Dual inhibitors of prototype PL/HDAC

In 2016, the discovery of that a hybrid compound 27, which contains SAHA and PL, had a strong ability to cause programmed cell death in AML cells by working together beneficially. The IC_{50} values of 27 in different AML cell lines, including aggressive and chemotherapy-resistant cell types, were much lower than the IC_{50} values of PL, SAHA alone, or their combination. Both PL and SAHA were demonstrated to synergistically promote DNA damage in U937 cells. This hybrid molecule serves as a beginning stage in the investigation of more potent and precise chemicals that selectively attack tumor cells. PL-HDAC is utilized for the treatment of hematological malignancies [53].

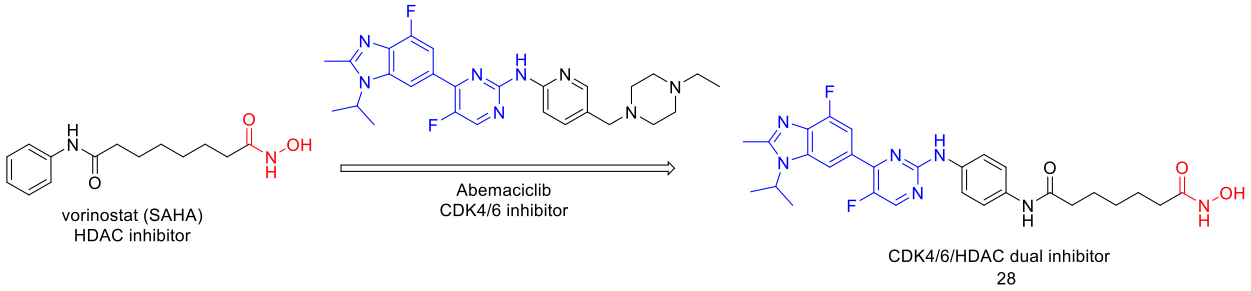


X= H/Cl
n=H
piperlongumine/HDAC dual inhibitor
27

2.4 Dual cyclin-dependent kinases (CDKs) and HDAC inhibitors

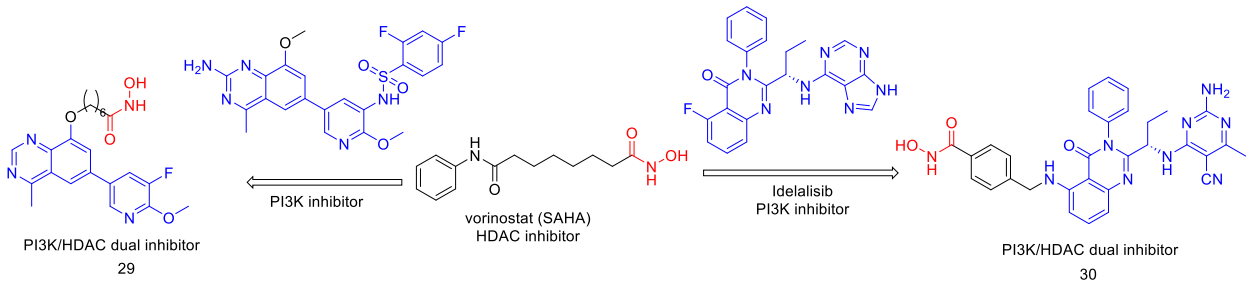
Cyclin-dependent kinases (CDKs) can regulate the progression of the cell cycle and induce apoptosis, proliferation, and cellular expansion [54, 55]. Due to the excessive activation of CDKs in numerous cancers, researchers have created small molecule inhibitors to specifically target

CDKs in cancer treatment. Zhao et al. significantly inhibited HDAC1 (with an IC_{50} of 17 nM) and CDK4 (with an IC_{50} of 1.2 nM) by employing SAHA and abemaciclib, resulting in the synthesis of compound 28 [56].



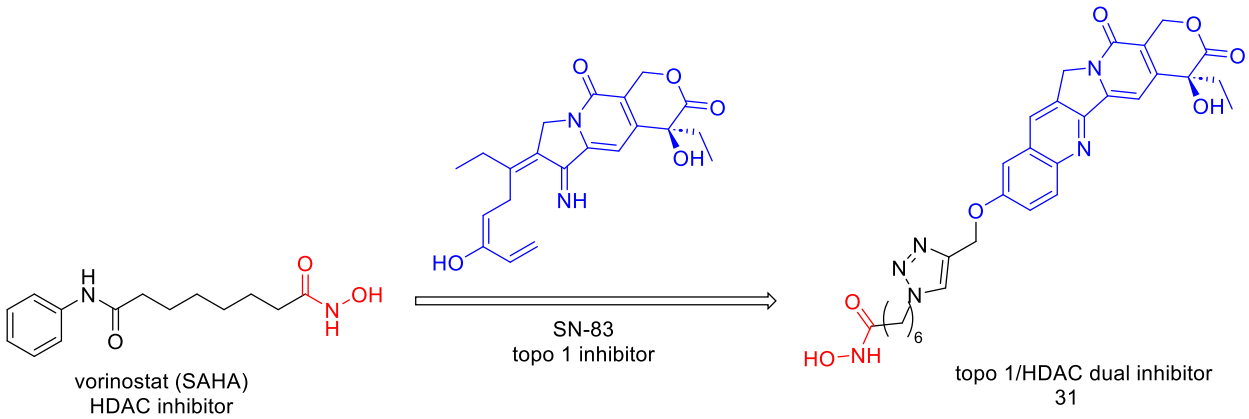
Dual inhibitors of phosphatidylinositol 3-kinase (PI3K) and histone deacetylase (HDAC)

Studies have shown that cancer cells exhibit atypical amounts of phosphatidylinositol 3-kinase (PI3K) expression [57], Multiple PI3K inhibitors are currently being developed to treat solid tumors and hematological malignancies. Chen et al. have created compound 29, which acts as a dual inhibitor of HDAC and PI3K. It has demonstrated strong inhibitory activity against HDAC1 and PI3Ka, with IC_{50} values of 2.7 and 50 nM, respectively [58]. Grewal and his team have created a novel drug named Inhibitor 30. This chemical combines the HDAC hydroxamate pharmacophore with Idelalisib, a PI3K inhibitor, utilizing a meticulously constructed linker (-benzyl). Compound 30 demonstrated strong inhibitory effects on many cancer cell lines and displayed remarkable selectivity and effectiveness (IC50 < 10 nM) against the PI3Kg, d, and HDAC6 enzymes. Crucially, compound 30 did not demonstrate any deleterious impacts on healthy cells, including PBMCs, NIH3T3, and HEK293. Nevertheless, it caused necrosis in numerous cell lines, including mutant and FLT3-resistant AML cells, as well as raw blasts obtained from AML patients [59].



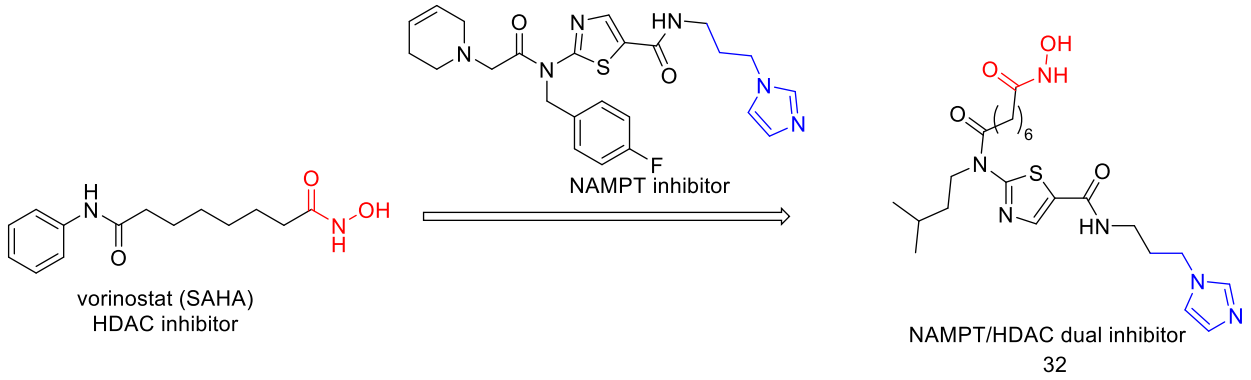
2.5 Dual topoisomerase/HDAC inhibitors

Topoisomerase, often known as topo, is a frequently occurring ribozyme that can manipulate the structure of DNA's superhelix by creating reversible covalent complexes with the DNA [60, 61]. Several topoisomerase inhibitors, such as camptothecin and doxorubicin, have been specifically targeted targeting topoisomerase due to its vital biological functions in the proliferation of cancer cells. SAHA and the topo I inhibitor SN-38 served as the basis for the development of the dual-acting HDAC-topoisomerase I inhibitor 31, as reported by Oyelere and colleagues. Based on biological testing, 31 demonstrated strong suppression of cancer cell proliferation and maintained its effectiveness against HDAC (with IC_{50} values of 37 nM and 81 nM for HDAC1/6) and Topo I [62].



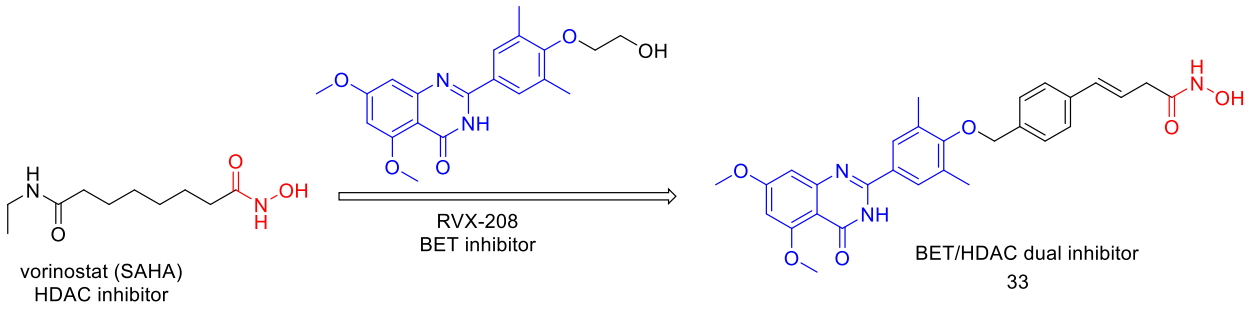
2.6 Dual nicotinamide phosphoribosyl transferase (NAMPT)/HDAC Inhibitors

Nicotinamide adenine dinucleotide (NAD^+), an essential signaling molecule, is involved in the redox processes of several cells [63]. Nicotinamide phosphoribosyl transferase (NAMPT) controls the synthesis of NAD^+. Recent research has discovered that cancer cells have a substantial dependency on NADP. Thus, by blocking NAMPT, the production of NAD^+ in cancer cells can be limited, thereby affecting the growth and division of cancer cells [64]. Chen et al. synthesized Compound 32 by adding the hydroxamate group to the NAMPT inhibitor, resulting in a dual inhibitor of NAMPT and HDAC. Compound 32 demonstrated potent inhibitory activity against both HDAC1 and NAMPT, with IC_{50} values of 2 and 15 nM, respectively [64].



Dual inhibitors of bromodomain and extra terminal (BET) proteins and histone deacetylases (HDACs)

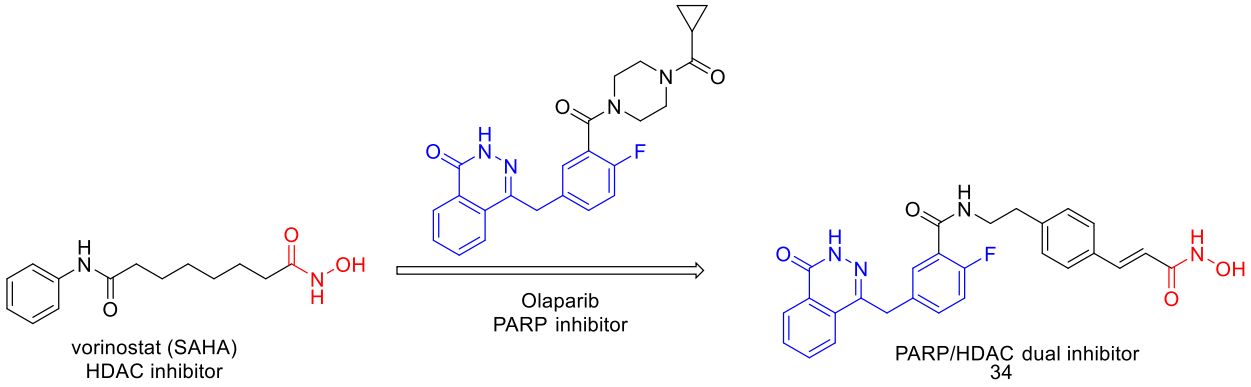
The BET family, consisting of bromodomain and extra terminal proteins, is a prominent set of epigenetic regulators that control gene transcription and impact cell proliferation [65]. HDACs exert influence on gene transcription by participating in several epigenetic mechanisms. Considering the similar biological effects of HDACs and BETs, it is possible to construct a combination of both, known as dual BET/HDAC. Shao et al. developed Compound 33 as a dual inhibitor of BET and HDAC, using RVX-208 and SAHA as the basis. Compound 33 exhibited potent inhibitory effects on HDAC1 and BRD4/BD2, with IC_{50} values of 0.2 and 0.4 μM, respectively [66].



2.7 Dual poly (ADP-ribose) polymerase (PARP)/HDAC inhibitors

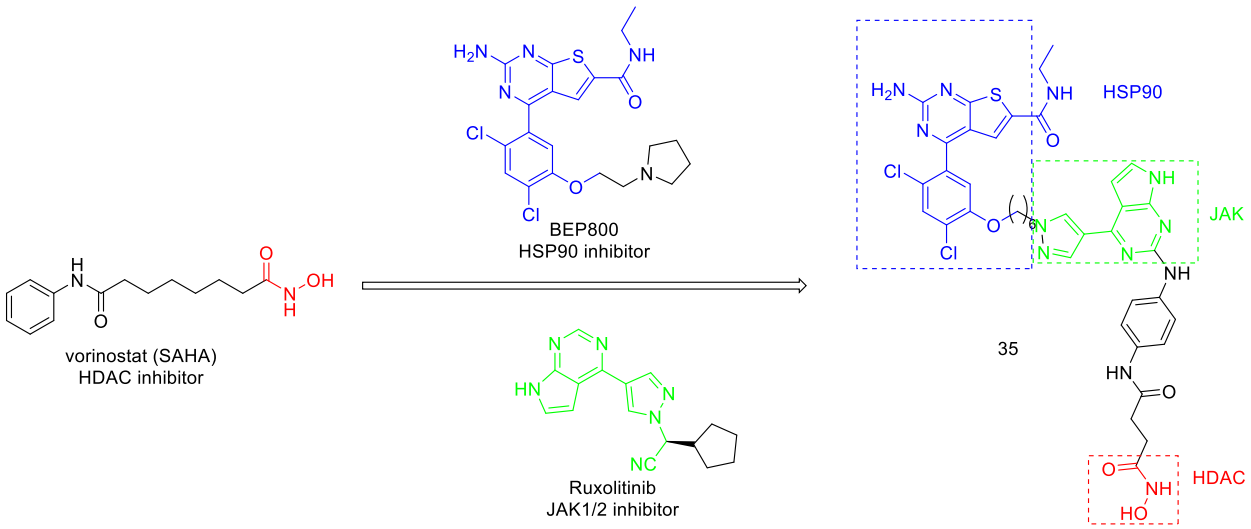
The DNA repair enzyme poly (ADP-ribose) polymerase (PARP) is essential for DNA replication, chromatin remodeling, and apoptosis [67, 68]. PARP is overexpressed in numerous solid and hematological malignancies [69]. Studies have shown that HDAC inhibitors and PARP inhibitors can enhance the responsiveness of other anticancer drugs [70, 71]. The development of dual inhibitors targeting both PARP and HDAC holds promise for cancer therapy. Yuan and

coworkers have created compound 34, which exhibits dual inhibitory effects for PARP and HDAC. Compound 34 has an IC_{50} value of 8.21 nM for HDAC6 and an IC_{50} value of 5.02 nM for PARP2.



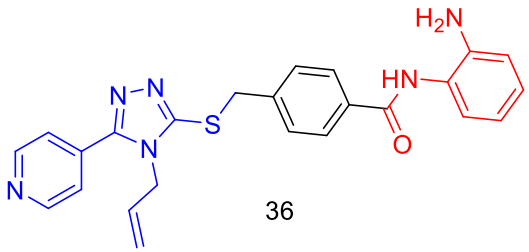
2.8 Dual inhibitors of HSP90/HDAC

Heat shock protein 90 (Hsp90) is an ATP-dependent molecular chaperone that regulates the folding of oncoproteins. Studies have shown that the presence of HSP90 is elevated in cancer cells [72] Furthermore, HDAC6 specifically acts on HSP90 in a subsequent step. Dual inhibitors of HDAC and Hsp90 have a strong basis in the chaperone activity of Hsp90, which can be influenced by the reduction of HDAC6 [73]. Yao et al. revealed that Compound 35, which combines the pharmacophores of roxolitinib, BEP800, and SAHA, has a powerful inhibitory effect on JAK2, HDAC6, and HSP90. The IC_{50} values for these inhibitory activities are 3.76 μM, 6.30 μM, and 20.2 μM, respectively [74].



2.9 HDAC/FAK drugs that target both HDAC and FAK simultaneously

Focal adhesion kinase (FAK) is an intracellular enzyme that belongs to the class of non-receptor tyrosine kinases [75]. It plays a pivotal role in the processes of cancer cell proliferation, survival, and migration. FAK inhibitors that specifically target cancer cells have emerged as a promising treatment option for various types of malignancies. Research indicates that the combination of FAK and HDAC inhibitors has a synergistic effect in preventing cell growth, and HDAC inhibitors promote the deactivation of FAK caused by FAK inhibitors [76, 77]. Mustafa et al. discovered new compounds that can inhibit both HDAC2 and FAK. These compounds are based on 5-pyridyl-1,2,4-triazoles. Compound 36, which has the o-aminobenzamide group as a ZBG, has been found as a possible inhibitor of HDAC2 with an IC_{50} value of 1.28 μM and FAK with an IC_{50} value of 15.6 nM [78].

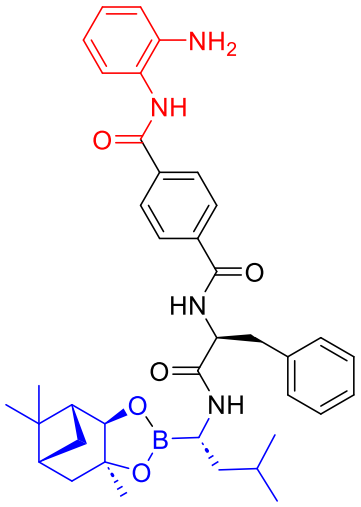


36

2.10 Dual targeting HDAC/proteasome inhibitors

The proteasome is a crucial enzyme in the ubiquitin-proteasome system (UPS) that is responsible for regulating and breaking down cellular proteins [79]. Inhibiting the proteasome has a significant impact on several signaling pathways within the cell. Currently, five known categories of drugs have been recognized as proteasome inhibitors. However, only a small number of compounds, like bortezomib, have progressed to the clinical development stage. This is because of issues related to metabolic instability, problems with effectiveness, or a lack of selectivity. In 2020, Zhou et al. developed and created new HDAC/Proteasome inhibitors using the fundamental pharmacodynamic structures of MS-275 and bortezomib [80]. Compound 37, which has the o-aminobenzamide group as its ZBG, demonstrated a remarkable inhibitory impact on the proteasome with an IC_{50} of 1.1 nM. Additionally, it exhibited effective targeting of HDAC1 with an IC_{50} of 0.255 μM. In comparison to bortezomib, the molecule has shown greater effectiveness in inhibiting the growth of bortezomib-resistant multiple myeloma (MM) cell line KM3/BTZ. The

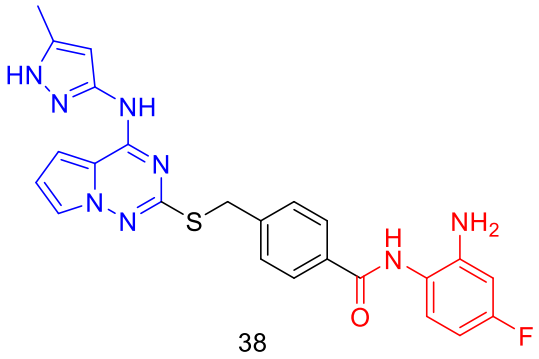
IC_{50} values for the compound were 8.98 nM, whereas for bortezomib it was 226 nM. This difference was statistically significant ($P < 0.01$).



37

2.11 Dual targeting HDAC/Snail inhibitors

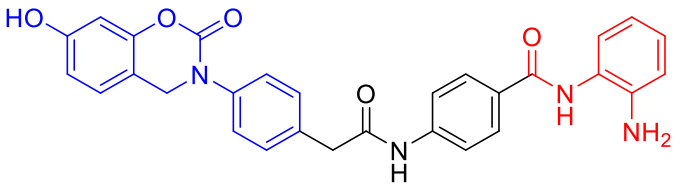
Snail, a zinc finger transcription factor [81], is the primary catalyst of epithelial-mesenchymal transition (EMT) and effectively suppresses the production of E cadherin. Research has demonstrated that Snail can interact with chromatin remodeling elements through its distinct domain (SNAG), hence performing its function of inhibiting transcription [82]. Founded Snail interacts with the histone complex (Sin3A-HDAC1/2) through this domain to deacetylate histone H3 and H4. This process inhibits the production of E-cadherin. To enhance the effectiveness of cancer treatment, Cui et al. developed a range of HDAC/Snail dual inhibitors, taking advantage of the significant influence that the combined application of Snail and HDACs has on cancer treatment [83]. One of the compounds, compound 38, with o-aminobenzamide as its ZBG, demonstrated the most potent inhibitory effects against HDAC1 (IC_{50} = 0.405 μM) and Snail (K_d = 0.180 μM).



38

2.12 Dual targeting HDAC/MIF inhibitors

Macrophage migration inhibitory factor (MIF), often referred to as glycosylation inhibitory factor (GIF), is a cytokine with pro-inflammatory properties. It has a significant function in regulating the first stages of both innate and adaptive immune responses. Studies indicate that the MIF signal can enhance cell survival via the Akt pathway and hinder cell apoptosis by suppressing the tumor suppressor protein P53 [84]. Furthermore, MIF is abundantly present in several types of cancers such as gastric cancer, pancreatic cancer, and lung cancer, among others [85, 86]. There is significant potential to create small compounds that can simultaneously inhibit both HDACs and MIFs. In 2021, Cao et al. published a study introducing novel small molecule inhibitors that can simultaneously target HDAC/MIF [87]. Compound 39 showed outstanding inhibitory activity against MIF, with an IC_{50} value of 0.18 μM. It also demonstrated effective inhibition of HDAC, with IC_{50} values of 0.2 μM, 1.1 μM, and 0.6 μM for HDAC1, HDAC2, and HDAC3, respectively.



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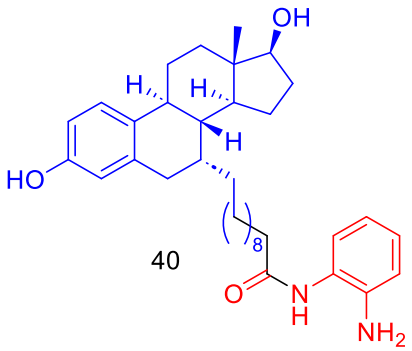
2.13 Dual targeting HDAC/estrogen inhibitors

Estrogen is a lipid-soluble steroid hormone that exists in three forms in the human body: estrone (E1), 17β-estradiol (E2), and estriol (E3). Multiple investigations have demonstrated a strong correlation between endogenous estrogen and the development of breast cancer [88]. Estrogen imbalances have the potential to lead to diseases including breast cancer and ovarian cancer. Gastric cancer and other types of cancer can result in the inhibition of important estrogen signaling.

This blockage, caused by chemotherapy, might lead to death in cancer patients due to toxicity-related complications [89]. The combination of the HDAC inhibitor Vorinostat and fulvestrant is more efficient than fulvestrant alone in controlling the expression of cyclin and promoting estrogen oogenesis. The down-regulation of the receptor α (Erα) and the inhibition of its transcription is achieved. Mendoza Sanchez et al. [90] created and produced estrogen/HDAC inhibitors that target two areas simultaneously by merging the structural features of ICI-164384 and MS-275. One of the compounds, compound 40, had potent anti-estrogen activity (IC_{50} = 0.21 μM) and outstanding HDAC3 inhibitory activity with an IC_{50} value of 3.18 μM.



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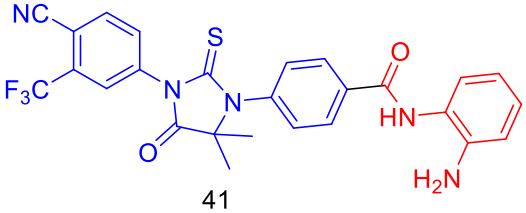
2.14 Dual targeting HDAC/androgen inhibitors

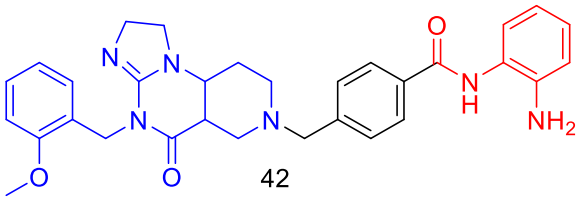
Prostate cancer, often known as PCa, is the predominant form of cancer found in males. The prevalence and development of this phenomenon are intricately linked to androgen, primarily testosterone, and are propelled by the androgen signaling system [90]. The androgen receptor (AR), which is a transcription factor activated by ligands, is currently a primary focus for therapeutic interventions in prostate cancer [91]. Due to the rapid increase in the number of prostate cancer cells, Barrett et al. created, produced, and assessed a range of anti-androgen/HDAC hybrids [92]. Out of all the compounds examined, those based on o-aminobenzamide exhibited superior performance. These compounds have greater inhibitory efficacy compared to hydroxamic acid-based drugs. Compound 41 demonstrated strong inhibitory effects against androgen and HDAC, with IC_{50} values of 0.98μM and 1.33μM, respectively.



41

2.15 Dual targeting TRAIL/HDAC inhibitors

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a type II transmembrane protein that belongs to the tumor necrosis factor superfamily [93]. Research has demonstrated that the concurrent use of histone deacetylases (HDACs) and TRAIL agonists can enhance the sensitivity of TRAIL to colon cancer, liver cancer, breast cancer, and other types of solid tumors [94-96]. Cui et al. developed and created a range of dual-target inhibitors for TRAIL/HDAC by including potent fragments and merging them with the HDAC inhibitor Chidamide [97]. One of the compounds is compound 42. The compound demonstrated potent inhibitory activity against HDAC1, with an IC_{50} value of 151 nM. Additionally, it displayed increased sensitivity towards the production of the TRAIL protein.



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Current challenges and future perspectives

Treatments that target a single agent or single target are often deemed ineffective in the setting of cancer due to the presence of redundant disease pathways and the development of drug resistance. Therefore, to improve the combined effectiveness and reduce the development of drug resistance, many drugs are used in several anti-cancer treatment processes [98]. An alternative strategy could be developing a singular medicine that has customized numerous pharmacodynamics. Several studies have been conducted on the utilization and incorporation of multi-target pharmacology in anti-tumor medications, including both small molecules and protein biologics [99, 100]. The notion of synergistic pharmacology involves targeting two enzymes simultaneously using a single complex or small molecule inhibitor. These enzymes are known to work together as epigenetic modulators, and by targeting them, gene expression can be effectively suppressed [101]. The discovery of dual inhibitors that selectively target different epigenetic modulators could offer a novel strategy for the advancement of epigenetic medications. Dual-targeting medications offer several potential advantages over conventional therapies, such as single-target or combination therapy. These advantages include more predictable pharmacokinetics, increased patient compliance, lower dosage requirements for delivery, and

enhanced potency with reduced toxicity. Nevertheless, there are numerous difficulties and obstacles involved in effectively implementing the concept of simultaneously targeting HDAC and other epigenetic regulators in laboratory experiments. The primary obstacle is the problem of optimizing the combined therapeutic effectiveness of the medicine within a living organism while also maximizing its safety profile. The issue arose in achieving a balance between the potencies of the medication and the desired levels of epigenetic modulators. The drug's in vitro activity should preferably be within a range of one order of magnitude. Attaining this objective is challenging, as it may ultimately compromise the effectiveness of either target. The development of dual epigenetic inhibitors that have a balanced effect on living organisms is made more complex by differences in how the inhibitors bind to their targets, how they are distributed throughout the body, and the levels of enzymes in different tissues. Additionally, the timing of when the inhibitors start to block their targets is also a factor [102]. The second obstacle is to achieve a satisfactory pharmacokinetic profile without compromising the actions of inhibitors. Most dual epigenetic inhibitors that are now known are created using either a pharmacophore-linked or -fused design technique. However, this approach often leads to the production of compounds with high molecular weight and unfavorable pharmacokinetic properties. The observed failure of certain dual epigenetic inhibitors is likely due to their suboptimal pharmacokinetics. This additional loophole must be promptly resolved by thoroughly investigating the SAR (Structure-Activity Relationship) of the parent target, while also considering all the advantages and disadvantages of the pharmacophore-merged and fragment-based screening techniques. The objective is to develop a small molecule dual epigenetic inhibitor with favorable pharmacokinetics. Dual action inhibitors intelligently aim to achieve equilibrium in inhibiting both enzymes simultaneously at the cellular level, while also overcoming the substantial regulatory obstacles posed by the two parent molecules. Efficiently arranged for a clinical trial. The robust correlation between histones and other epigenetic modulators, along with the demonstrated effectiveness of dual epigenetic inhibitors in treating cancer, will drive the exploration of dual epigenetic target treatments as a prominent area of research in the next years. It is expected that the continued examination of cancer's epigenetic networks will soon result in the discovery of inhibitors that target two epigenetic targets simultaneously[103].

Conclusion

As more intricate mechanisms are unveiled, it is fascinating to observe that epigenetic processes play a vital role not only in regular cellular activities but also in the formation of malignancies. The alteration in The manifestation of the epigenetic state becomes increasingly evident at the onset of cancer, so rendering therapy that focuses on the epigenome a possible avenue for cancer treatment. However, the main treatment strategy for malignancies revolves around methods that cause DNA damage. Nevertheless, the development of resistance and the lack of response impede the efficacy of the treatment. After the approval of vorinostat, belinostat, panobinostat, romidepsin, and chidamide, HDAC inhibitors (HDACI) have become generally suggested as a standard therapeutic method for treating a growing number of cancers. The concurrent targeting of HDAC inhibitors (HDACI) and medicines that specifically target other epigenetic pathways, such as BET, DNA/DNMT, G9a, LSD1, and EZH2, holds great potential. While many medications that specifically target these epigenetic pathways are currently undergoing clinical trials, their interactions with HDACi have not been studied yet. The simultaneous utilization of epigenetic regulators and HDACi as dual inhibitors has exhibited substantial synergy in both preclinical and clinical investigations. This synergistic combination has significant potential to advance the utilization of HDAC inhibitors in the fight against cancer.

- **Conflict of interest**

  The Authors declare no conflict of interest

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